Inhibitors of Bacterial and Mammalian Hyaluronidases: Design, Synthesis and Structure-Activity Relationships with Focus on Human Enzymes

Dissertation

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Chapter 1
Introduction

1.1 *Hyaluronic acid*

1.1.1 Structure and physicochemical properties

Hyaluronic acid (HA, also termed hyaluronan or hyaluronate) was first isolated and purified from bovine vitreous humour by Meyer and Palmer in 1939[1]. It took another 20 years until its structure was solved[2]. It is a linear polysaccharide which consists of repeating disaccharides composed of β-1→3 connected *N*-acetylglucosamine (GlcNAc) and glucuronic acid (GlcUA) which are connected by a β-1→4 glycosidic bond (Figure 1.1).

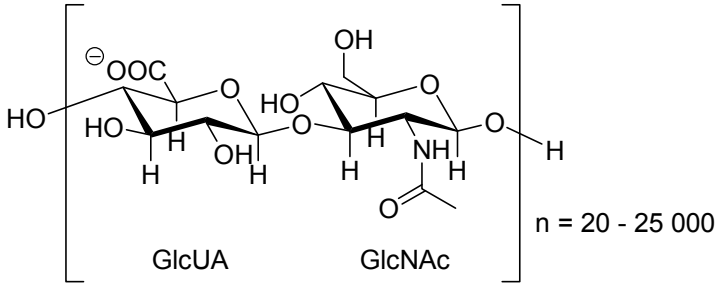

Figure 1.1. Chemical structure of hyaluronic acid.

The 20 to 25000 disaccharide units form a polysaccharide with molecular weights typically between 2×10^5 to 10×10^7 Da and an overall length of the molecule up to 2-25 µm. The carboxyl groups of glucuronic acid which are deprotonated under physiological conditions (pk_a = 3-4)[3] cause the highly negative charge of this

biopolymer. Hyaluronan is a member of the glucosaminoglycanes (GAG) which are generally composed of repeating disaccharides of an uronic acid and an amino sugar. But compared to other GAGs like chondroitin, chondroitin sulfate, keratin, dermatan sulfate, heparin and heparin sulfate, hyaluronan possesses a unique position in this group of macromolecules: HA, which is noticeably larger in size, is the only family member not covalently modified during synthesis, thus it lacks sulfation and it is not linked to a protein core. Additionally, hyaluronic acid differs in the location of its synthesis: whereas the other GAGs are synthesized by resident Golgi enzymes, hyaluronan is synthesized at the inner surface of the plasma membrane and is then extruded[3-5].

Until the end of the last century, it was assumed that hyaluronan forms random coils in solution. It was Scott who first found hints for a defined shape of hyaluronan by use of chemical, X-ray or NMR experiments[6]. Based on these findings, the model of a tape-like, two-fold helix was established (Figure 1.2): Every disaccharide unit is twisted by 180 degrees compared to the disaccharide behind and ahead of the chain. This conformation is stabilized by internal hydrogen-bond formation and interactions with the solvent[7-9].

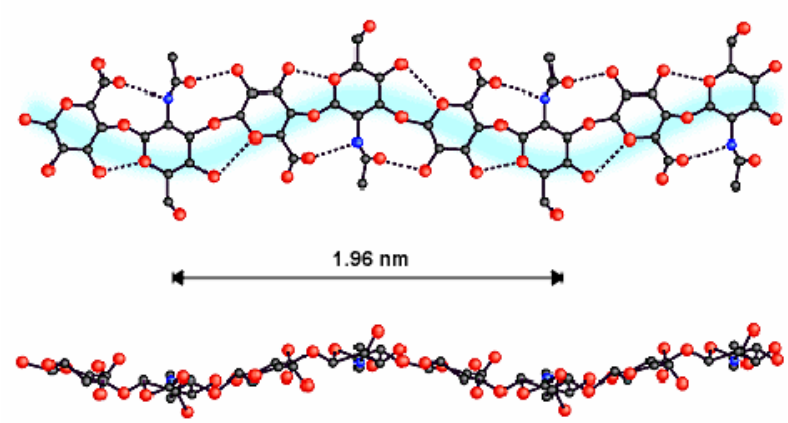

Figure 1.2. Projections of the extended two-fold hyaluronan helix. Dotted lines represent hydrogen bonds. The models are shown at right angels to each other. Modified from Scott[6].

As a result of this specific arrangement, lipophilic patches occur in the secondary structures which are formed by the accumulation of axial hydrogens from 3 carbohydrate units. These hydrophobic characteristics together with the hydrophilic nature of the carbohydrate hydroxyl and carboxyl residues lead to an amphiphilic nature of hyaluronan.

The tertiary structure of hyaluronic acid in solution is based on hydrophobic interactions and hydrogen bonds on the one hand, and the electrostatic repulsion of

the carboxylate residues on the other hand. Although NMR and rotary shadowing electron microscopy data revealed a honeycomb like meshwork with neighboring HA chains arranged in an antiparallel fashion in very diluted solutions[6, 10], a specific tertiary structure is not formed under physiological conditions. Hyaluronan in solution can best be described as porous, dynamic network of interpenetrating chains[11]. The tertiary structure resembles an expanded random coil with reversible formation and breakdown of interactions between the hyaluronan strains. This porous network is able to store huge amounts of water which is the basis for the highly viscoelastic and hydrating properties of hyaluronan. Thus, by binding water, the volume of HA increases by about 1000-fold compared to the non-hydrated state[3]. This hydrated network forms a diffusion barrier *in vivo* and therefore exhibits important functions in the transport of various substances like electrolytes or proteins through the intercellular space[12].

1.1.2 Hyaluronan in the organism

1.1.2.1 Occurrence

All vertebrates produce hyaluronan as major component of the extracellular matrix. It also occurs on the surface of certain pathogenic *Streptococcus* or *Pasteurella* bacteria. In mammals, highest levels can be found in umbilical cord (3 mg/ml), synovial fluid (3-4 mg/ml), skin (0.5 mg/ml) and the vitreous humour (0.2 mg/ml). Increased concentrations of HA are found within the matrix produced by the cumulus cells around the oocyte (0.5 mg/ml). Exceedingly high levels can also be found in rooster comb (7.5 mg/ml). For comparison, the concentration of hyaluronic acid in serum is 0.01-0.1 mg/ml. The overall amount of hyaluronic acid in men is approximately 15 g (for a 70 kg individual), with the largest portion (ca. 50 %) being found in the dermis and epidermis of the skin[13].

1.1.2.2 Metabolism

The biosynthesis of HA is regulated by the three different glycosyltransferases HAS1, HAS2 and HAS3. Possessing two different enzymatic components (glycosyl-transferases), one which is responsible for the addition of glucuronic acid and the other one for addition of *N*-acetylglucosamine, these isoenzymes are unique[14]. The active site of these transmembrane enzymes protrudes from the inner face to the plasma membrane, and the HA is extruded during synthesis trough the cell membrane onto the cell surface or into the extracellular matrix (ECM)[15]. This form of synthesis clearly differs from that of the other GAGs which are synthesized in the Golgi body.

The synthesized hyaluronan is finally released from the synthases e.g. by radical reaction or dissociation[16], but stays (at least partly) in contact with the plasma membrane *via* interaction with cell surface receptors like CD44, RHAMM, LYVE-1, HARE, LEC-receptor, TLR-4[5, 17-22] or possibly the membrane-anchored hyaluronidase Hyal-2. A mechanism for the enzymatic degradation of hyaluronan was suggested by Csoka et al.[23]: In a first step, high molecular weight HA is degraded into fragments of ca 20 kDa by the membrane-bound Hyal-2. After internalization of the fragments, further degradation occurs in the acidified surrounding of the lysosomes *via* another hyaluronidase, Hyal-1, and two exoglycosidases, β-*N*-acetylglucosaminidase and β-glucuronidase. Finally, the monosaccharides (and perhaps oligosaccharides) are able to diffuse out of the lysosome and are used again, e.g., for the biosynthesis of hyaluronan. Hyaluronan is rapidly metabolized with one third of the whole hyaluronan present in the human body being turned-over every day[24]. In addition to enzymatic degradation of hyaluronan, the polymer is also cleaved under physiological conditions by radicals, especially reactive oxygen species like the hydroxyl radical[25, 26]. Thus, the depolymerization of hyaluronan in synovial fluids during the early onset of inflammatory arthritis is believed to be caused by reactive oxygen species rather than by the action of hyaluronidases[27].

1.1.2.3 (Patho)physiological role of hyaluronan

Several functions of hyaluronan are connected with its unique physicochemical properties: It functions as lubricant and shock absorber, regulates water homeostasis and it is an important structure-forming molecule especially in joint fluids, the vitreous eye or Wharton´s jelly. Besides these general functions, hyaluronan is known to interact with various different molecules, called hyaladherins[28]. This heterogeneous group of proteins can be divided into three classes: first, the extracellular hyaladherins, a group of HA-binding proteoglycanes like aggrecan, neurocan, or brevican constitute a gene family which is termed hyalectins[14]. HA stabilizes the ECM by interaction with proteoglycanes like aggrecan and the link protein and thus acts as a scaffold[29]. HA cell-surface receptors are termed cellular hyaladherins. The most prominent representatives are CD44[30] and RHAMM[31]. Interactions with cellular hyaladherins mediate three important processes, namely signal transduction, the formation of pericellular coats and receptor-mediated internalization[14]. Recently, interactions of intracellular hyaluronic acid[32] with the corresponding hyaladherins like the intracellular variant of RHAMM, P-32, Cdc37 or IHABP4 gain attention[33].
The biological activities of HA are diverse. It is known to be involved in embryological development and general processes like migration, proliferation, adhesion, and differentiation of cells[21, 34, 35]. Furthermore, it plays a role in the immune surveillance, inflammation and wound healing[36-38] or in angiogenesis[39] and tumor progression[40, 41]. The effects are strongly dependent on the size of the HA fragments: molecules of different size may even exhibit opposite effects. For instance, high molecular weight hyaluronan is anti-angiogenic and anti-inflammatory, whereas smaller hyaluronan fragments are angiogenic and inflammatory[24, 36, 37, 42], respectively.

1.2 *Hyaluronidases*

The hyaluronidases were first discovered in the beginning of the last century by Duran-Reynals in extracts of mammalian testis und were identified as “spreading factors” due to their ability to facilitate the diffusion of antiviral vaccines, dyes and toxins[43]. With the isolation of hyaluronan by Meyer et al.[1] and the identification of a bacterial enzyme that cleaves hyaluronan[44] it became clear that the “spreading

factor" discovered in mammals is also an enzyme that degrades hyaluronan[45]. The term hyaluronidase was finally introduced by Meyer in 1940[46] but it is a kind of misnomer as this class of enzymes is also able to degrade other GAGs like chondroitin (sulfate). Meanwhile hyaluronidases, which are ubiquitously found in the animal kingdom, were identified and/or isolated from a large number of organs like liver, kidney, spleen, testis, uterus and placenta, or from the venom of lizards, fish, bees, wasps, scorpions and spiders. Additionally, hyaluronidases were found in bacteria or pathogenic fungi. The discovered enzymes differ in their molecular weight, substrate specificity or pH-optimum[47-49]. This group of neglected enzymes[47] was barely investigated since the discovery of the hyaluronidases in the last century due to their instability and other problems associated with isolation, purification and activity assays[14]. In the meantime much data is accumulating rapidly, in part due to the human genome project and the EST (expressed sequence tag) data bank[26].

1.2.1 Classification of hyaluronidases

Based on biochemical experiments and the characterization of degradation products, Meyer first classified the hyaluronidases into three groups (Figure 1.3)[50].

The first group (EC 3.2.1.35), represented by the mammalian or testis type hyaluronidases are endo-β-*N*-acetylhexosaminidases and cleave the β-1→4 glycosidic bonds of hyaluronan, chondroitin and certain chondroitin sulfates. The major products of these hyaluronidases are tetra- and hexasaccharides with *N*-acetylglucosamine at the reducing end. Besides hydrolysis, these hyaluronidases also possess transglycosidase activity[51, 52].

The second group of hyaluronidases (EC 3.2.1.36) is commonly termed leech-hyaluronidases and represents hyaluronate-3-glycanohydrolases that cleave the β-1→3 linkages of the substrate hyaluronan. These enzymes from leeches and hook worms[53], in contrast to the mammalian hyaluronidases, do not cleave other GAGs. The products of hyaluronan degradation are again tetra- and hexasaccharides but with glucuronic acid at the reducing end.

Microbial hyaluronidases (EC 4.2.2.1) also termed β-eliminases or lyases, catabolize hyaluronan *via* β-elimination of the β-1→4 glycosidic bond. As a result Δ4-5 unsaturated oligosaccharides are obtained. Thus, they are clearly separated from the other two groups which utilize a hydrolase mechanism.

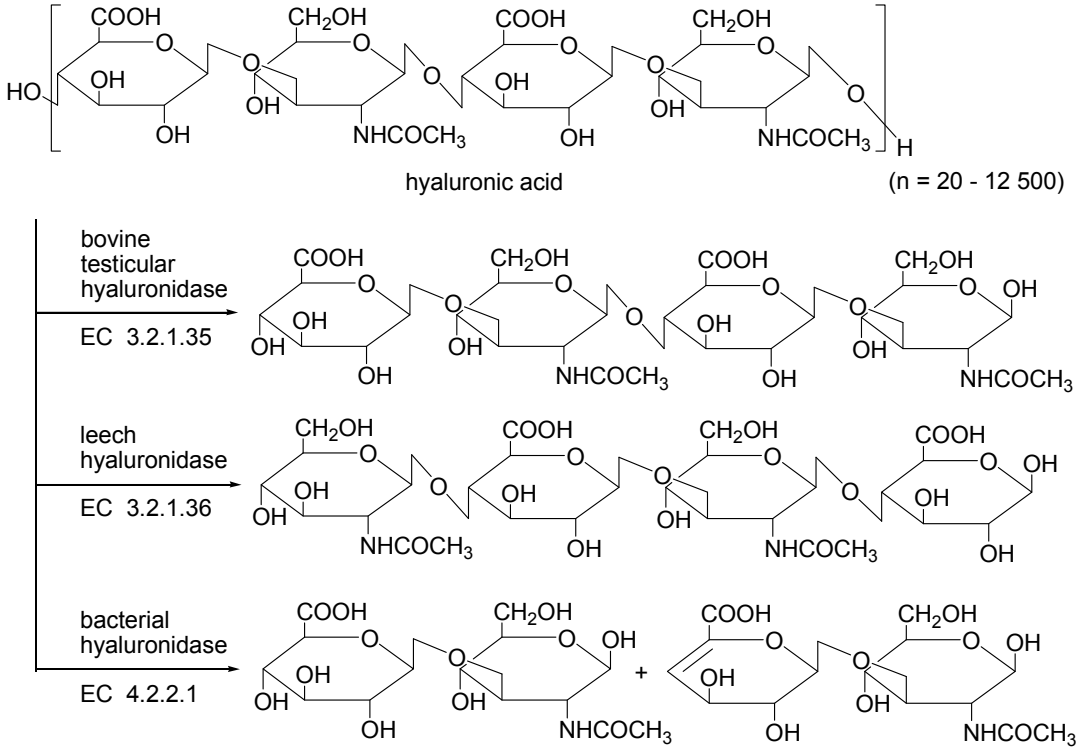

Figure 1.3. Classification of hyaluronidases according to Meyer[50]. Adopted from Muckenschnabel et al.[54]

Alternatively, based on their sequence homology it is possible to classify the hyaluronidases into two major groups: hyaluronidases from eukaryotes and prokaryotes[23, 49]. Additionally, a rough classification in acid- and neutral-active hyaluronidases is possible due to their different pH activity profiles[14].

1.2.2 Hyaluronidases from eukaryotes

1.2.2.1 Mammalian hyaluronidases

The human genome contains six hyaluronidase-like sequences*: hyal1*, *hyal2*, and *hyal3*, which are clustered on chromosome 3p21.3, and *hyal4*, *hyalp1* (a pseudogene which is transcribed but not translated) and *ph20 (spam1),* which are clustered on chromosome 7q31.3. The six genes are known to share about 40 % of their identity with one another. They encode for Hyal-1, Hyal-2, Hyal-3, Hyal-4 and PH-20 and according to the carbohydrate active enzyme database (CAZY)[55] they belong to the glycoside hydrolase family 56. In this chapter, the five human hyaluronidases as well as bovine testicular hyaluronidase are briefly introduced.

Hyal-1

Hyal-1 is also termed LUCA-1 due to the location of the gene on a putative tumor suppressor site[56]. It was first isolated from human plasma where it is the predominant hyaluronidase found in concentrations of about 60 ng/ml [57]. It degrades hyaluronan predominantly to tetrasaccharide fragments with the octasaccharide being the minimal substrate[58]. It also can be found in high levels in liver, kidney, spleen and heart. A second processed form can be found in urine[23]. Together with Hyal-2 it is the major hyaluronidase in somatic tissue. This acid active enzyme is localized in the lysosome and is thus responsible for the degradation of intracellular hyaluronan[57]. Recent studies provided evidence for the dependency of intracellular degradation of HA on expression of the surface receptor CD44 which is required to bind and internalize the substrate[59]. Mutation of the *hyal1* gene leads to catalytically inactive enzyme which causes mucopolysaccharidosis IX, which manifests in periarticular soft tissue masses and a short stature together with somatic disorders[60]. Very recently Harada and Takahashi[59] proposed an enzyme replacement therapy due to observations where catalytically active Hyal-1 was incorporated into cells and thus was able to restore intracellular degradation of hyaluronan. The role of Hyal-1 in tumor formation is still under investigation: on the one hand this enzyme is a candidate tumor suppressor gene, e.g. in tabacco related cancers[61], on the other hand it was designated as a tumor marker, e.g. for bladder and prostate cancer[62, 63].

Hyal-2

Hyal-2 can be found in virtually all human tissues except the adult brain[64] and it was characterized to be lysosomal or GPI-anchored to the plasma membrane[64-68]. It is supposed to act in concert with Hyal-1 degrading hyaluronan. Despite being considerably less active compared to Hyal-1 and PH-20, the reported optima range from acidic to nearly neutral pH values[64, 67, 69, 70]. In several cases no activity was determinable at all[68, 71]. Hyal-2 seems to have a unique substrate specificity and was described to degrade high molecular weight HA to fragments of ca 20 kD (50-60 disaccharide units)[72]. Meanwhile also a very slow degradation to smaller fragments has been reported[69]. Despite its catalytic function, Hyal-2 serves as a receptor for the Jaagsiekte sheep retrovirus (JSRV) and the enzootic nasal tumor virus (ENTV)[68, 73]. The function as a receptor for JSRV seems to be independent from its hyaluronidase activity[70]. Interactions with the cell surface receptor CD44 seem to play an important

role in the function of Hyal-2[59, 74]. Like Hyal-1, Hyal-2 is involved in tumor formation and can act as an oncogene[75, 76] as well as a tumor suppressor gene product[77].

Hyal-3

There is only little data on Hyal-3. It is known to be widely expressed, e.g. in chondrocytes, testis and bone marrow as well as in breast cancer lines[76], but until today, it has been impossible to determine enzymatic activity[23, 24, 59].

Hyal-4

Hyal-4 was shown to be a GPI-anchored protein[78], its expression is restricted to placenta and skeletal muscle[23]. Although no details about the enzymatic activity are known, Hyal-4 was reported to be specific for chondroitin and chondroitin sulfate, thus this enzyme would represent the first chondroitinase identified in vertebrates[24, 78, 79].

PH-20

PH-20, also termed SPAM1 (sperm adhesion molecule) was first described as a protein binding to the *zona pellucida* of the ovum[80]. It is GPI-anchored on the membrane of the sperm head[81, 82] and facilitates the penetration of the egg *via* digestion of the HA-rich cumulus extracellular matrix[83]. It can be found in two different forms, on the one hand as GPI anchored enzyme, on the other hand as soluble protein which is released during the acrosome reaction[84, 85]. Thus, by its hyaluronidase activity on the one side and by its receptor activities on the other side, this protein possesses an important role during fertilization. Meanwhile PH-20 has been also identified in the epididymis, seminal vesicles, prostate, female genital tract, breast, placenta and fetal tissues and was also found in certain malignancies[86-89]. PH-20 is catalytically active under neutral as well as under acidic conditions[82, 85, 90, 91] and cleaves hyaluronan predominantly to small oligosaccharides with the octasaccharide being the minimal substrate[58]. In addition to its hydrolase activity, human PH-20 catalyzes transglycosylation reactions; this was also observed for bovine testicular hyaluronidase[51, 91] which exhibits a sequence identity of 65 % compared to the human homolog.

Bovine testicular hyaluronidase (BTH)

Extracts from bovine testis are known to possess hyaluronidase activity[45] and preparations containing purified BTH like Hylase® "Dessau", Neopermease® and Wydase® were therapeutically used for a long time[34, 92]. It has been shown that the major soluble hyaluronidase present in bull testis extracts is a fragment of membrane bound PH-20[93]. This well known representative of the mammalian hyaluronidases cleaves hyaluronan predominantly to tetra- and disaccharides[52] and degrades to a minor extent the other GAGs chondroitin, chondroitin-4- and -6-sulfate. In contrast to human PH-20, the hyaluronan hexasaccharide is accepted as minimal substrate[51, 58, 94]. Additionally, transglycosylation is catalyzed, depending on the pH value and the salt content of the incubation buffer[58, 91, 95].

1.2.2.2 Venom hyaluronidases

Hyaluronidases are found as components of all types of animal venoms. Examples are the venoms from snakes[96, 97], bees[80, 98], wasps[47, 99], scorpions[100], spiders[101, 102] or lizards[103]. In all these cases the hyaluronidase may contribute to local tissue damage and act as a spreading factor facilitating the diffusion of toxic components of the venom[96, 104]. Bee venom hyaluronidase (BVH) was the first eukaryotic hyaluronidase for which a crystal structure was determined. As a member of the family 56 of glycosyl hydrolases[105], BVH has a sequence homology of about 30 % with that of mammalian hyaluronidases[80]. Compared to mammalian hyaluronidases BVH lacks a C-terminal domain of ca 120-150 amino acid residues. The analysis of the crystal structure revealed an unusual overall fold, a $(\beta/\alpha)_7$ barrel instead of a regular $(\beta/\alpha)_8$-TIM-barrel. The HA binding grove is situated at the C-terminus of the enzyme. The active site is located in the middle of the hyaluronan binding site and is encompassed with highly conserved amino acids i.e. the catalytically active Glu113 and amino acids like Asp111, Tyr184, Tyr227 and Trp301, which are supposed to be involved in substrate positioning are also present in the mammalian hyaluronidases[98, 106]. Meanwhile the X-ray structure of a hyaluronidase from wasp venom with similar structural characteristics has been published (PDB code 2atm)[99].

Due to the co-crystallized hyaluronan tetramer within the BVH protein (PDB code 1fcv[98]) it was possible to elucidate the catalytic mechanism for HA degradation. The unique double-displacement substrate-assisted mechanism is depicted in Figure

1.3[107]. In the first step, the hyaluronan substrate binds to the hyaluronidase and the carbonyl oxygen nucleophile of the *N*-acetyl group is positioned next to the glycosidic bond which will be cleaved. Thereby, the GlcNAc residue adapts an unusual $^4C_1$ boat conformation (**1**). The carbonyl oxygen attacks the C1 carbon which leads to the cleavage of the glycosidic bond and the formation of the oxazolinium ion intermediate **2** under inversion of the configuration at C1. At the same time the single catalytic amino acid Glu113 protonates the leaving part of the hyaluronan chain. In the next step, a water molecule attacks C1 which leads to the hydrolysis of the intermediate **2** under inversion of the configuration and reprotonation of Glu113 (**3**). The remaining HA product is finally released from the active site. Thus, by the release of the hyaluronan fragments after cleavage and subsequent binding of a new fragment for the next cleavage, the enzyme follows an endolytic random bite mode of action[78].

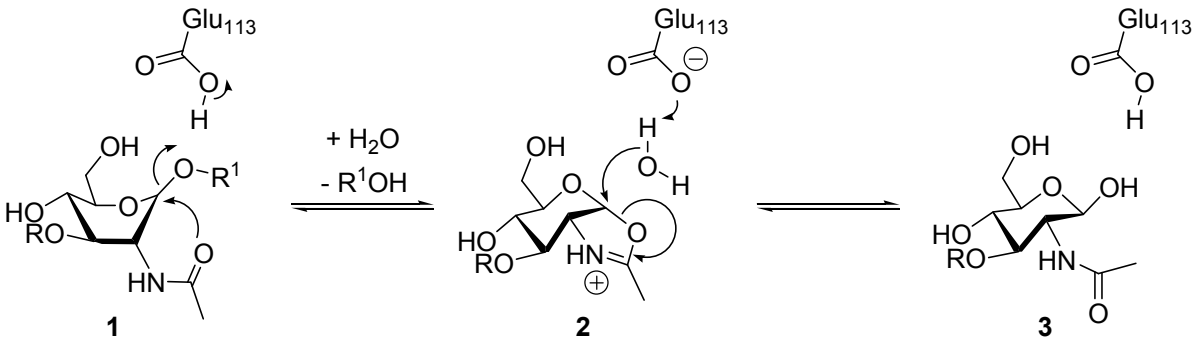

Figure 1.3. Double-displacement substrate-assisted mechanism of bee venom hyaluronidase. The saccharide in subsite[108] -1* (**1**) binds in boat conformation, and catalysis is proposed to occur *via* a formation of a covalent oxazolinium ion intermediate **2** to the product **3**. Adapted from Markovic-Housley and Schirmer[107].

Due to the highly conserved amino acids which are involved in catalysis and substrate binding, the same mechanism for substrate degradation is assumed for the mammalian hyaluronidases[106]. The crystal structure of human Hyal-1, which was elucidated very recently (PDB code 2pe4)[79], further supports this hypothesis due to the high structural similarity found for the active site of Hyal-1 compared to BVH.

* By convention, the sugar residue subsites are labelled from –n to +n, with –n at the non-reducing end and +n at the reducing end of the substrate. Cleavage occurs between the -1 and +1 subsites.

1.2.3 Hyaluronidases from prokaryotes

The hyaluronidases from prokaryotes cleave HA *via* an elimination reaction and must therefore clearly be separated from the mammalian hyaluronidases due to the different catalytic mechanism. The term hyaluronate lyases accommodates for this mechanism. The microbial lyases are important virulence factors involved in pathogeneses and in the disease progression caused by the pathogen. Thus by degradation of hyaluronan-rich tissues of the host, the bacterial hyaluronidases facilitate the invasion of the pathogen. Additionally, the hyaluronan oligomers created by the enzymes are potent inflammatory agents and promote a microbial-friendly environment[109]. Many gram-positive organisms like *Streptococcus*, *Staphylococcus*, *Streptomyces* as well as gram-negative bacteria like *Aeromonas*, *Vibrio*, *Treponema pallidum* produce hyaluronate lyases[14]. The hyaluronidase activity is associated with various disease conditions like meningitis, synovitis, hyperplasia, nephritis, mycoplasmosis, periodontal disease, mastitis, pneumonia, syphilis and toxic shock syndrome[109-113]. Among the bacterial lyases, the enzymes from *S. pneumoniae* and *S. agalactiae* are well characterized. The X-ray structures of these enzymes in complex with various hyaluronan oligosaccharides provided insight into the mechanism of substrate degradation[110, 114-119]. A longish, highly positively charged cleft was identified, which is capable of binding the anionic substrate polymers and is lined with lipophilic amino acids. Two important parts of the active site were identified. Firstly, His479, Tyr488 and Asn429 were identified as the catalytic amino acids (*Sag*Hyal$_{3502}$ numbering) which are responsible for substrate degradation and, secondly, an aromatic patch consisting of Trp371, Trp372 and Phe423 was identified which is responsible for substrate positioning. Based on the X-ray data and site-directed mutagenesis studies[120], a proton acceptance and donation mechanism was suggested for the bacterial hyaluronate lyases (Figure 1.4). Firstly, hyaluronic acid binds to the positively charged enzymatic cleft where three disaccharide units can be accommodated (only two are shown in Figure 1.4). Secondly, HA1 and HA2 are precisely positioned by the aromatic patch providing the optimal position for degradation. Hydrogen formation of Asn429 and the carboxylate group of the HA1 glucuronic moiety leads to acidification of the C5 hydrogen. In the third step His479 withdraws a proton from the glucuronic acid C5 which leads to sp^2 hybridization, and simultaneously Tyr488 protonates the glycosidic oxygen (O4) and thus breaks the

glycosidic bond between HA1 and HA2. HA1 glucuronic C4 is thus rehybridized from sp^3 to sp^2 similarly to the C5 carbon which results in the formation of the double bond. In the final step the catalytic residues are deprotonated (His479) or protonated (Tyr488) by a water molecule. A negative patch (Glu488, Asp478 and Thr480) is probably responsible for the repelling of the negatively charged product.

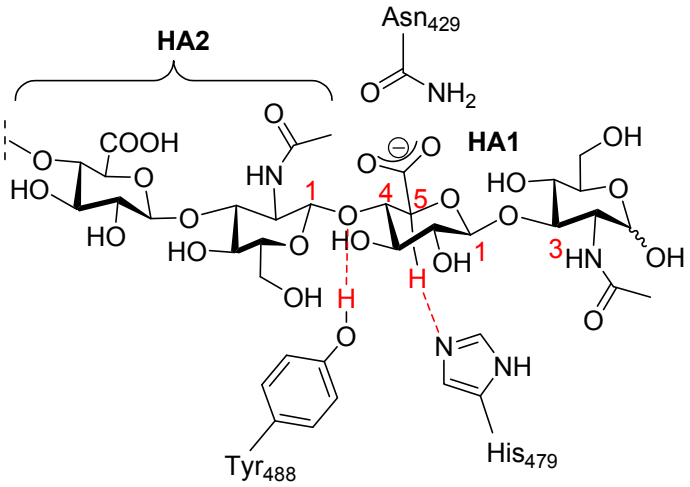

Figure 1.4. Mechanism of hyaluronan degradation by hyaluronate lyase from *S. agalactiae* (*Sag*Hyal$_{3502}$) according to Li and Jedrzejas[116]. Schematic presentation of hyaluronic acid with HA1 and HA2 as disaccharide units and the position of the sidechains of Tyr488, His479 and Asn429 relative to the substrate. Modified from Li and Jedrzejas[116].

The hyaluronidases from *S. pneumoniae* and *S. agalactiae*, respectively, are supposed to degrade hyaluronan *via* a processive mode of action[110, 116]. Thus, the enzyme binds randomly to the hyaluronan molecule and cleaves it into two pieces (initial endolytic cleavage). The obtained unsaturated HA fragment then leaves the binding site, whereas the other fragment remains in the cleft where it is translocated by one disaccharide unit towards the reducing end. After cleavage and release of the unsaturated disaccharide, the remaining HA fragment is translocated again and the next disaccharide is removed from the hyaluronan chain (exolytic processive degradation). Based on the observation that at all stages of digestion a mixture of oligosaccharides of different size was present also a nonprocessive mode of action was proposed for *Sag*Hyal$_{4755}$[121].

1.3 *Medical applications of hyaluronan and hyaluronidases*

The hydroscopic and viscoelastic nature and the high biocompatibility makes hyaluronan suitable for various medical applications. It is in use as vitreous humor supplement/replacement during eye surgery since the middle of last century[122]. In general, it can be used in surgical procedures to support regenerative processes of surgical wounds and it is used as anti-adhesion and anti-scar drug. Additionally, it appears that it promotes corneal, diabetic foot, tendon, bone, nasal mucosal, and venous leg ulcer wound healing[14]. The use of HA preparations for the treatment of osteoarthritis has been intensively studied[123-126]. Injected hyaluronan was found to suppress the cartilage degeneration, protects the surface of articular cartilage, normalizes the properties of the synovial fluid and even reduces pain perception. Increased HA levels are observed during periods of rapid cell turnover or in early wound healing[4, 127]. Furthermore, a change in the amounts of circulating HA is directly connected with events like blood loss, ischaemic stroke, septicaemia after a massive trauma, shock, major surgical procedures or extensive burns, and is indicative for liver cirrhosis, liver fibrosis, knee osteoarthritis and rejection following liver transplantation[14, 38, 128, 129]. Thus, hyaluronan can serve as a biomarker for various diseases. Hyaluronan also can be used in drug delivery where on the one hand it enhances the partitioning of drugs like diclofenac or ibuprofen into human skin and on the other hand enhances its retention in the epidermis[14]. Beneficial effects of direct conjugates of hyaluronan fragments and cytotoxic agents were reported[130] as well as the use of implantable synthetic polymers to provide long-term delivery of antibiotic or anti-inflammatory drugs[14]. Furthermore, hydrogels based on crosslinked or otherwise chemically modified hyaluronan are potentially useful biomaterials for soft-tissue engineering applications[14, 131]. For a few years, hyaluronan can also be found in cosmetics as a “rejuvenation agent”[132, 133].

The clinical use of hyaluronidases which is based on the spreading effect was already described by Breu in 1952[134]. Thus, hyaluronidases can be used to increase the speed of absorption, to promote resorption of excess fluids and to increase the effectiveness of local anesthesia. Furthermore, the application of hyaluronidases leads to diminished tissue destruction after subcutaneous and intramuscular injection of fluids[48, 135]. It is a necessity for every derma-surgeon to treat side effects after injection of hyaluronic acid[136, 137]. Hyaluronidases can also be used to decrease

mycardinal infarction size[138] and they are widely used in fields like orthopaedia, surgery, ophthalmology, internal medicine, oncology, dermatology and gynaecology[34, 139-141].

Hyaluronidase from bovine testis has been broadly used. However, there was a shortage in supply of BTH preparations due to the BSE risk. As a consequence, a number of cases of iatrogenic strabismus have been observed after cataract surgeries[142]. Thus, these bovine preparations should be displaced with bacterial or ovine hyaluronidases[87, 143] or recombinant human enzymes[20, 144, 145]. As animal hyaluronidase preparations always contain a certain degree of impurities and treatment with BTH may cause adverse effects such as allergic reactions and skin irritation reactions[146, 147], recombinant human enzymes should be advantageous.

Hyaluronidase was also investigated as an additive to chemotherapeutic drugs for augmentation of anticancer activity and there is evidence that hyaluronidase itself may have intrinsic anticancer effects[92, 148, 149]. Furthermore, Zahalka et al. reported that they were able to block lymph node invasion by tumor cells in an animal model by treatment with hyaluronidase[150]. Nevertheless, the relevance of hyaluronidases in the development of cancer is discussed controversially: on the one hand human hyaluronidases are candidate tumor suppressor gene products, on the other hand there is evidence for an oncogenic potential of this class of enzymes.

In general, the role of hyaluronan and the hyaluronidases in many (patho)physiological processes is far from being understood. For a long period of time the hyaluronidases have been a group of poorly characterized, neglected enzymes[47]. Additionally, potent and selective inhibitors are not known to date. The inhibitors encountered frequently are even more neglected than the hyaluronidases itself[151]. Such compounds are required as pharmacological tools for the better characterization and understanding of the hyaluronidases. Moreover, hyaluronidase inhibitors might be of therapeutic value, for example, as additives in the treatment of bacterial infections[109], as anti-venom/toxin[14] and anti-tumor agents[152], to promote wound healing or as additives in the treatment of arthroses or gingivitis[152]. Additionally, it is conceivable that hyaluronidase inhibitors have a potential as contraceptives[153] and for the development of new antiallergic drugs[154].

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Chapter 2
Scope and objectives

Potent and selective inhibitors of hyaluronidases are not known to date. Such compounds are necessary as pharmacological tools for the characterization and the investigation of the (patho)physiological role of the hyaluronidases. Moreover, there is a therapeutic potential of hyaluronidase inhibitors in the treatment of various diseases[1] (cf. chapter 1.3).

The scope of this thesis was the design, synthesis and characterization of hyaluronidase inhibitors with focus on recombinantly expressed human enzymes. The project was mainly based on previous results and experience gained from the work of our research group on inhibitors for the bacterial hyaluronate lyase from *S. agalactiae* strain 4755 (*Sag*Hyal$_{4755}$)[2-4]. The *de novo* design of inhibitors of bacterial hyaluronan lyases, supported by x-ray structures in complex with inhibitors resulted in lead compounds with IC_{50} values in the low micromolar range. These compounds were inactive or only weakly active on the bovine testicular enzyme, a prototypal mammalian hyaluronidase, which was considered an appropriate model for the development of inhibitors for the corresponding human enzymes at the beginning. In the course of this work, in a complementary PhD project, the human enzymes Hyal-1 and PH-20 were recombinantly expressed, purified and characterized with respect to biochemical and catalytic properties[5-7]. Therefore, it was possible to investigate the synthesized compounds for inhibition of three mammalian hyaluronidases. Additionally, inhibition of *Sag*Hyal$_{4755}$ as reference enzyme for the class of bacterial hyaluronate lyases was investigated.

The purification of the two recombinantly expressed human hyaluronidases is briefly described as the procedure was a prerequisite for this thesis. Additionally, the

investigation of reference hyaluronidase inhibitors under the assay conditions used in this work is reported with respect to the comparison of the inhibitory potencies of known inhibitors with those of the newly synthesized compounds.

In a first synthetic approach glucurono-6,3-lactone, which is structurally related to the monosaccharide glucuronic acid, served as a scaffold for the synthesis of bacterial and mammalian hyaluronidase inhibitors.

To identify new chemical entities for the development of inhibitors of human hyaluronidases, various compounds synthesized in our workgroup were investigated for inhibition. Based on those results, ascorbic acid was considered as lead structure. Thus, the synthesis of novel vitamin C derivatives and their inhibitory activity represent a major part of this work.

When the chemical work of this thesis was finished, the first crystal structure of a human hyaluronidase was published[8]. The 3D-structure of Hyal-1 was used to perform docking experiments with selected potent ascorbic acid derivatives in order to gain insights into potential protein – inhibitor interactions and to suggest putative binding modes.

Indoles were already identified as inhibitors of bacterial hyaluronidases in our workgroup[3, 4, 9] and the indole based compound indomethacin is also known to inhibit hyaluronidases *in vivo*[10]. Therefore, the indole scaffold looks promising for the development of inhibitors. The synthesis of indole based compounds and the study of the SAR is the aim of another sub-project of this thesis.

The synthesis and the investigation of the inhibitory activity of a diketopiperazine, a compound which was derived as potential inhibitor from previous molecular modeling studies[2] and the preparation and investigation of nonsulfated glycosides as hyaluronidase inhibitors, inspired by promising results obtained for sulfated glycosides[11], as well as the investigation of inhibitory activities of compounds which show structural characteristics related to the synthesized inhibitors are summarized in another chapter.

In the last part, various bioanalytical investigations such as determination of plasma protein binding and hemolytic acitivity were carried out to characterize selected inhibitors in more detail with respect to further usage *in vitro* and *in vivo.*

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Chapter 3
Methods for the determination of hyaluronidase activity

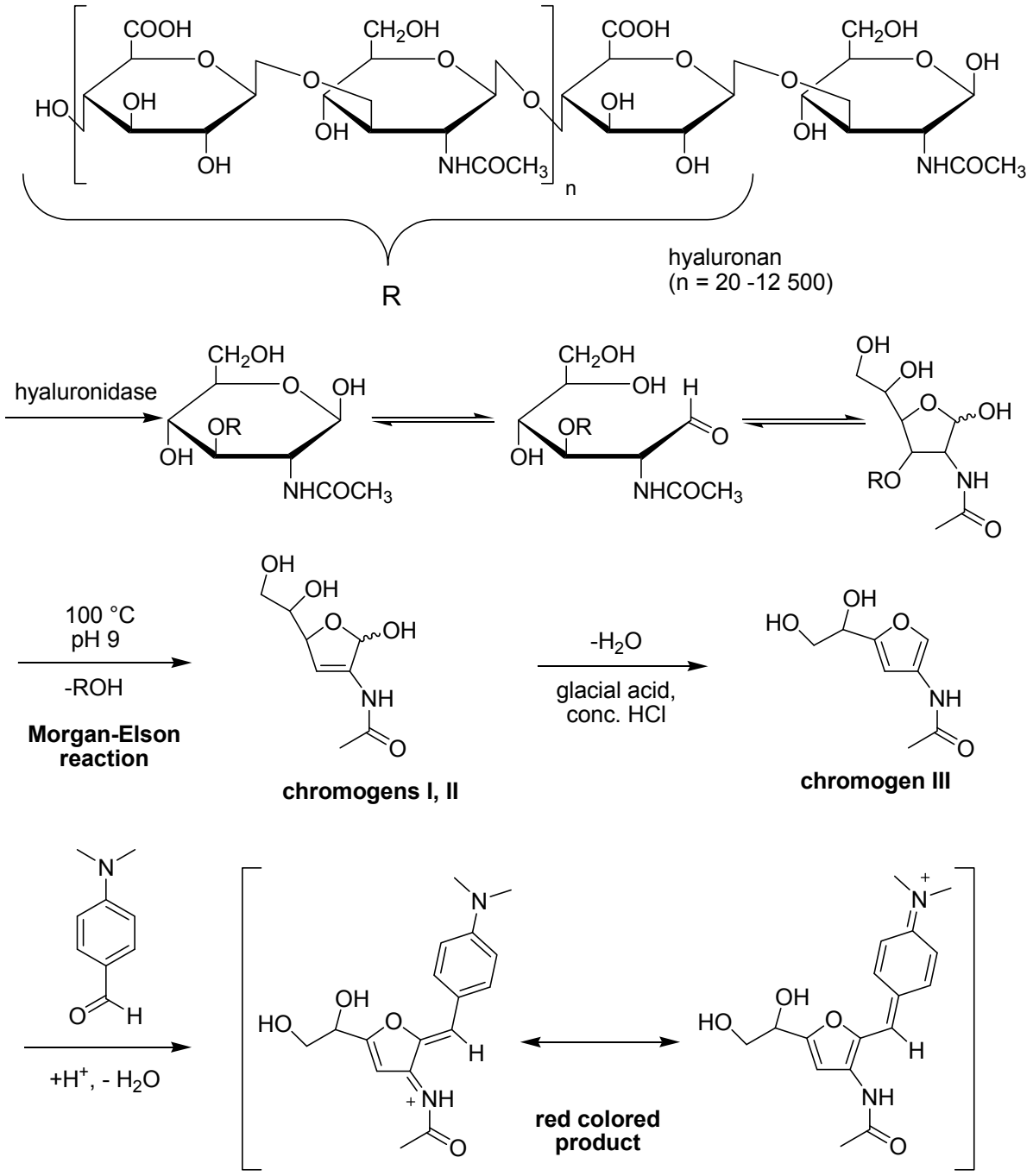
3.1 *Introduction*

Since the discovery of the hyaluronidases by Duran–Reynals[1] in the beginning of the last century, various methods for the determination of hyaluronidase activity have been developed. An overview of the existing methods is given by Hynes and Ferretti[2] who have grouped the methods based on the type of assay performed. Methods based upon spectrophotometric, radiochemical, fluorogenic, enzymoimmunological, plate (solid media), chemical, physicochemical and zymographic analysis are known. Although many different assays are described, they appear to be rarely used today and discussed only briefly[2]. In many cases the early developed assays lack sensitivity or the procedures are rather cumbersome. The colorimetric assay developed by Reissig et al. or assays based on changes in viscosity or turbidity had been the most widely employed hyaluronidase assays[3, 4]. Recently, assays based on techniques like substrate gel analysis, ELISA or fluorescence were developed [5-8].
In this thesis two assays were used to determine the activity of the recombinantly expressed human hyaluronidases Hyal-1 and PH-20, an enzyme preparation from bovine testis (formerly commercially available as Neopermease®) and a bacterial hyaluronate lyase from *Streptococcus agalactiae* strain 4755 (*Sag*Hyal$_{4755}$). The principles of both assays are briefly explained.

3.2 *Morgan-Elson assay*

The Morgan-Elson assay is a colorimetric or, according to the classification given by Hynes and Ferretti[2], a chemical assay which is based on the reaction between reducing *N*-acetyl-D-glucosamine residues of the substrate with Ehrlich´s reagent (*p*-dimethylaminobenzaldehyde) described by Gacessa[9] and Reissig[10]. The reaction, which forms a red colored product that can be detected photometrically at 586 nm was investigated and described in detail by Muckenschnabel et al.[11] Thus, hyaluronidase activity is determined by quantitation of the *N*-acetyl-D-glucosamine residues at the reducing end of hyaluronic acid fragments produced by enzymatic degradation.

The suggested mechanism of this reaction according to Muckenschnabel et al.[11] is depicted in Scheme 3.1. Hyaluronic acid is cleaved by the hyaluronidases during incubation. Then the reducing *N*-acetyl-D-glucosamine residues are cleaved under the basic assay conditions (pH 9, 100 °C) resulting in an anomeric mixture of chromogen I (α-configuration) and chromogen II (β-configuration) that are transformed under acid catalysis (HOAc and HCl) to chromogen III *via* elimination of water. The final red colored product (λ_{max} = 545 nm and 586nm) is subsequently obtained after reaction with *p*-dimethylaminobenzaldehyde.

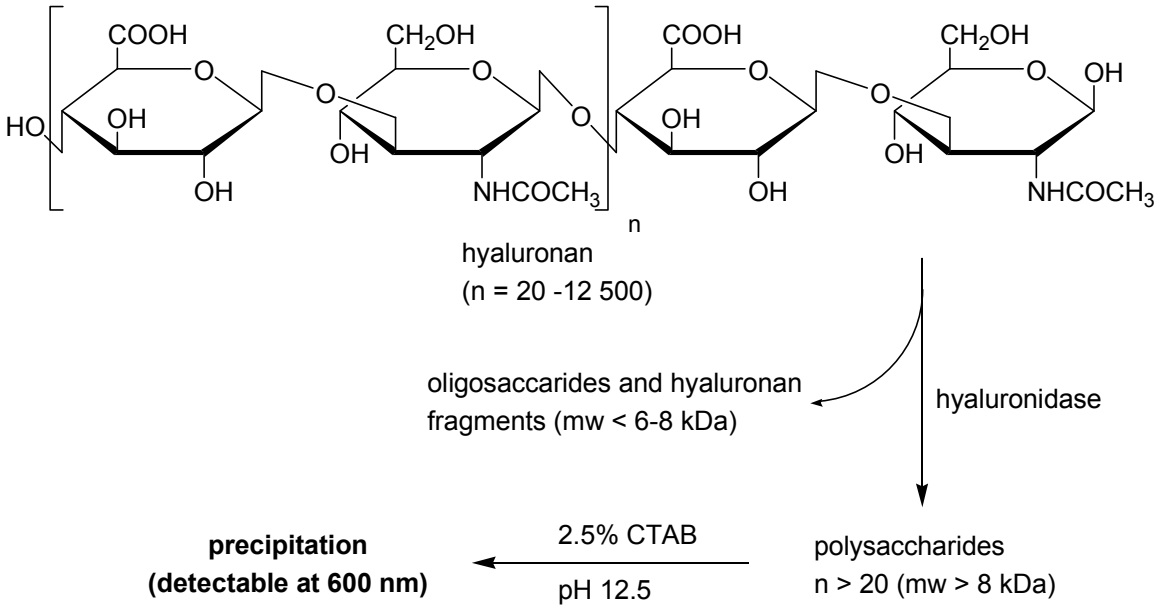


Scheme 3.1. Mechanism of the Morgan-Elson reaction. Modified from Muckenschnabel et al.[11]

3.3 *Turbidimetric assay*

According to Hynes and Ferretti[2] the turbidimetric assay is a representative member of the physicochemical assays. It is based on the method of Di Ferrante[12] who utilized the formation of insoluble complexes between cetyltrimethylammonium bromide (CTAB) and the residual high molecular weight substrate (mw > 8 kDa) after

incubation with enzyme to determine hyaluronidase activity by quantification of the resulting turbidity using photometric detection at 600 nm (Scheme 3.2).



Scheme 3.2. Principle of the turbidimetric assay based on the method of Di Ferrante[12]. Scheme modified from Braun[13].

Due to the fact that this assay is highly reproducible, fast and can be easily performed using microtiter plates, this protocol was chosen as standard method for the investigation of hyaluronidase inhibitory activities of the synthesized compounds.

3.4 *Influence of the pH value on enzymatic activity*

The activities of the four hyaluronidases from different sources are dependent on the pH value. The pH dependent activities of the investigated enzymes are shown in Figure 3.1. It is not possible to select a pH where all investigated enzymes show sufficiently high activity. Highest activity for the bacterial hyaluronate lyase was found at pH 5.0. To obtain comparable results concerning the IC_{50} values of the investigated inhibitors which also depend on pH, the human and bovine PH-20 enzymes were tested at pH 5.0 where both enzymes show considerably high activity in the turbidimetric assay which is routinely used in this work. Thus, the obtained IC_{50} values are comparable to results from previous investigations performed by Salmen and Braun in our workgroup[13-19].

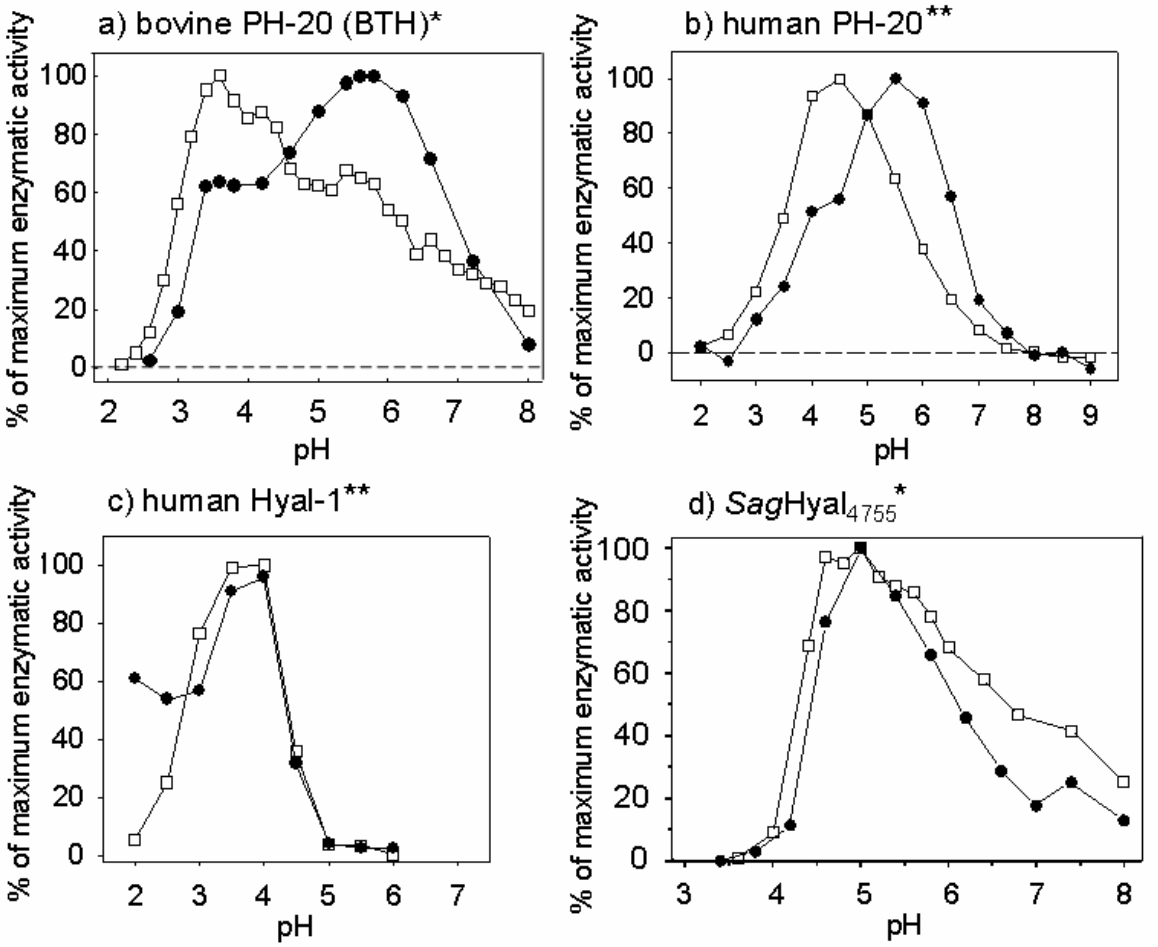


Figure 3.1. pH dependent activity of the investigated hyaluronidases as determined in the turbidimetric assay (black circles) and the Morgan Elson Assay (open squares); a) bovine testicular hyaluronidase (BTH), b) recombinantly expressed human PH-20, c) recombinantly expressed human Hyal-1, d) bacterial hyaluronate lyase from *S. agalactiae* strain 4755 (*Sag*Hyal$_{4755}$); *modified from Hoechstetter[20]; **modified from Hofinger[21].

Since human Hyal-1 is only very weakly active at pH 5.0, the turbidimetric assay was performed at pH 3.5 where enzymatic activity is nearly maximal. Thus, it is possible to compare the IC_{50} values of the new inhibitors investigated in this project with data at acidic pH previously determined in our workgroup[13, 14].

3.5 *Experimental procedures*

3.5.1 Materials and methods

Hyaluronan (hyaluronic acid) from *Streptococcus zooepidemicus* was purchased from Aqua Biochem (Dessau, Germany). Bovine serum albumin (BSA) was obtained

from Serva (Heidelberg, Germany). The investigated hyaluronidases were enzyme preparations from different sources. Stabilized hyaluronate lyase, *i.e.* 200,000 units (according to the supplier, 0.572 mg from *S. agalactiae* strain 4755 plus 2.2 mg of BSA and 37 mg of Tris-HCl per vial) of lyophilized hyaluronate lyase, was kindly provided by id-Pharma (Jena, Germany). Lyophilized hyaluronidase from bovine testis (Neopermease®) (200,000 units, according to the supplier; 4 mg plus 25 mg of gelatine per vial) was a gift from Sanabo (Vienna, Austria). Enzyme solutions containing recombinantly expressed human Hyal-1 and PH-20 were prepared as previously described (see chapter 4)[21-23]. All other chemicals were of analytical grade and were received from Merck or Sigma.

3.5.2 Morgan-Elson assay

The test compounds, dissolved in DMSO (18 µl), were incubated at 37 °C in an incubation mixture containing 200 µl of buffer (if not otherwise indicated: McIlvaine's buffer: solution A: 0.2 M Na_2HPO_4, 0.1 M NaCl, solution B: 0.1 M citric acid, 0.1 M NaCl; solution A and B were mixed in appropriate portions to adjust the required pH), 50 µl of BSA solution (0.2 mg/ml in water), 50 µl of HA solution (5 mg/ml in water), 82 µl of water and 50 µl of an enzyme solution. The final DMSO concentration was 3.9 % (v/v). After incubation at 37 °C, the enzyme reaction was stopped by addition of 110 µl of alkaline borate solution and subsequent heating for 4.5 min at 100 °C. The alkaline borate solution was prepared immediately before use from a borate solution (17.3 g H_3BO_4 and 7.8 g KOH in 100 ml water) and a potassium carbonate solution (8.0 g K_2CO_3 in 10 ml water). After cooling on ice for 2 min 1250 µl of *N*,*N*-dimethylaminobenzaldehyde (20.0 g *N*,*N*-dimethylaminobenzaldehyde dissolved in 25 ml of concentrated HCl and 75 ml of glacial acetic acid; the solution was diluted with 4 volumes of glacial acetic acid immediately before use) was added and the mixture was incubated at 37 °C for 20 min. The samples were transferred into acryl cuvettes and the absorbance of the colored product was measured photometrically at a wavelength of 586 nm using an Uvikon 930 UV spectrophotometer (Kontron, Eching, Germany). As reference for 100 % enzyme activity the formation of the red colored product of a sample without inhibitor (18 µl of DMSO was used instead) was quantified. In absence of both enzyme and inhibitor (50 µl of BSA solution and 18 µl of DMSO, respectively, was used instead) a reference for 0 % enzyme activity could

be measured. In the cases where only quantification of the enzyme activity without inhibitor was necessary, the inhibitor solution was replaced by water (18 µl).
The enzyme activity was calculated from the formation of the red colored product measured at 586 nm. The effect of the test compounds on the enzymatic activity was calculated according to the equation:

$$\text{relative activity [in \%]} = \frac{(A_{sample} - A_{enzyme})}{(A_{inhibitor} - A_{both})} \cdot 100\ \%$$

where A_{enzyme} is the absorbance of the reference sample without enzyme (50 µl of BSA (0.2 mg/ml) solution was used instead), $A_{inhibitor}$ is the absorbance of the reference sample without inhibitor (7 µl of DMSO was used instead) and A_{both} is the absorbance without both, enzyme and inhibitor.

3.5.3 Turbidimetric assay

The turbidimetric hyaluronidase activity assay described by Di Ferrante[12] was modified to allow for the performance in 96-well plates as recently described[22]:
Incubation mixtures contained the following compounds: 31 µL incubation buffer, 8 µL BSA (0.2 mg/mL), 8 µL HA (2 mg/ml), 13 µl H_2O. For the investigation of the test compounds 3 µl of the inhibitor solution in dimethylsulfoxide (DMSO) were added to the incubation mixture. The final concentration of DMSO in the incubation mixture was 4 % which is well tolerated by BTH and *Sag*Hyal$_{4755}$ as demonstrated previously[13, 14, 24]. The decrease in enzymatic activity by DMSO was also found to be acceptable when the recombinant human enzymes were investigated. If not indicated otherwise McIlvaine's buffer was used as incubation buffer (see above). The enzymatic reaction was started by addition of 10 µL enzyme solution (see Table 3.1 for prepared enzyme solutions). After incubation at 37 °C (incubation times see Table 3.1) the enzymatic reaction was stopped by addition of 200 µl of alkaline cetyltrimethylammonium bromide (CTAB) solution (2.5 % (w/v) CTAB in 0.5 M NaOH), and the plates were incubated for 20 min at room temperature. The turbidity was quantified by measurement of the optical density (OD) at 580 nm in a microplate reader (Tecan Deutschland GmbH, Crailsheim, Germany). The plate was shaken in the reader for 10 s, then the OD was measured after 2 s of settling time by 5 flashes at the centre of each well.

For the investigation of potential inhibitors, samples without inhibitor and without both inhibitor and enzyme were taken as references. The activities were plotted against the logarithm of the inhibitor concentration, and IC_{50} ± SEM values were calculated by curve fitting of the experimental data with Sigma Plot 8.0 (SPSS Inc., Chicago, USA).

IC_{50} values for BTH (Neopermease®) and *Sag*Hyal$_{4755}$ generally were determined using the turbidimetric assay performed in acryl cuvettes as described previously[19].

As poor solubility of the compounds could lead to false positive results in the turbidimetric assay, the terminal solubility was determined prior to the investigation of hyaluronidase inhibition. To determine the solubility of the test compounds, a sample containing 600 µl of citrate-phosphate buffer, 396 µl of water, 300 µl of BSA solution (0.2 mg/ml in water) and 54 µl of a solution of the respective test compound (dissolved in DMSO) at various concentrations was measured at 600 nm. A cuvette filled with water served as reference. Compounds were tested for inhibitory activity at concentrations, where no turbidity was measured.

In Table 3.1 the enzymatic activities, pH values and incubation times employed in the turbidimetric assay are summarized. The incubation periods were adjusted to obtain comparable substrate degradation.

Table 3.1. Enzymatic activities, pH and incubation times employed in the turbidimetric assay.

Enzyme	Enzyme solution prepared [mU/ml][a]	Enzyme solution added to incubation mixture [µl]	Final enzymatic activity in the incubation mixture [mU][a]	pH	Incubation time [h]
Hyal-1[b]	20	10	0.2	3.5	0.3
PH-20[b]	1.0	10	0.01	5.0	4.5
BTH[c]	9.0	30	0.27	5.0	0.5
*Sag*Hyal$_{4755}$[c]	97	30	2.9	5.0	0.5

[a]According to the definition of the International Union of Biochemistry 1 unit (U) of hyaluronidase catalyzes the liberation of 1 µmol *N*-acetyl-D-glucosamine (NAG) at the reducing ends of sugars per minute under specific conditions. Enzymatic activity was calculated from the formation of the red-colored product per unit time, using standards with known NAG concentration[11]; [b]tested using the turbidimetric assay performed in microtiter plates; [c]tested using the turbidimetric assay using cuvettes as described previously[19].

BTH and *Sag*Hyal$_{4755}$ were used at equiactive concentrations as described by Braun and Salmen[13, 14]. The enzymatic activity of Hyal-1 used in the turbidimetric assay was in the same range as that of BTH. As small modifications in the enzyme concentration only result in negligible changes of the IC_{50} values, the obtained IC_{50}

values for Hyal-1, BTH and *Sag*Hyal$_{4755}$ are comparable and conclusions about selectivity can be made. Due to economic reasons, the recombinantly expressed human PH-20 had to be used at significantly lower concentrations possibly leading to lower IC_{50} values. Thus, for selectivity considerations, representative compounds were re-investigated at equiactive concentrations (chapter 10.3.5).

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Chapter 4
Purification of human hyaluronidases Hyal-1 and PH-20

The purification of recombinant Hyal-1 and PH-20 which were used in this thesis was done under the supervision of Dr. E. Hofinger according to methods previously described by our workgroup[1-3]. Thus, the purification is only briefly described here. More detailed information and experimental protocols for the construction of expression vectors as well as the expression and purification of the recombinant enzymes are included in a doctoral thesis and articles by Hofinger et al.[1-3].

4.1 *Recombinant human Hyal-1*

The expression of Hyal-1 in *Drosophila* Schneider-2 (DS-2) cells transfected with the vector pMT/Hygro/*hyal1* was induced by addition of $CuSO_4$ to the suspension culture. 10 d after induction the cells were harvested by centrifugation, and the medium was used for isolation of Hyal-1. To reduce sample volume, Hyal-1 was concentrated by ammoniumsulfate precipitation. Triton X-100 was added to the buffers to avoid adhesion of Hyal-/DS-2 to column materials as well as to centrifugal devices. Purification of the enzyme was achieved by cation exchange chromatography and subsequent Ni-IMAC. Cu^{2+} ions from the cell culture medium were chelated using EDTA prior to the chromatography steps as they complex the His-tag from binding to the Ni-IMAC. The identity and activitiy of the purified protein with a molecular mass of 52 kDa was confirmed by western blot analysis and the Morgan-Elson activity assay.

4.2 *Recombinant human PH-20*

Human PH-20 was also expressed as a soluble protein in hygromycin resistant DS-2 cells with the C-terminal GPI-anchoring signal peptide missing after induction with $CuSO_4$. After 10 days PH-20 was partially purified in the presence of Triton X-100 by chelating IMAC utilizing the Cu^{2+} ions present in the culture medium. The identity and activitiy of the purified protein with a molecular mass of 56 kDa was confirmed by western blot analysis and the Morgan-Elson activity assay.

4.3 *References*

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Chapter 5
Inhibitors of hyaluronidases: reference compounds investigated under standardized conditions

5.1 *Hyaluronidase inhibitors reported in the literature*

First inhibitors of hyaluronidases were described in the middle of the last century. Since that time inhibitors were identified in various classes of compounds. For instance, the inhibitory activity of diverse metal ions was described, among them iron, cadmium, copper or zinc salts[1-3]. The existence of a highly potent endogenous inhibitor, characterized as a thermolabile, magnesium dependent glycoprotein, in human serum and tissues was reported early[4, 5]. Meanwhile, newer technologies allowed to identify a hyaluronidase inhibitor, probably a protein belonging to the inter-alpha-inhibitor family, in murine and human serum[6].

On the basis of substrate similarity, several polysaccharides were identified as hyaluronidase inhibitors. Heparin and heparan sulfate as well as sulfated, nitrated or acetylated hyaluronic acid were characterized as (non competitive) inhibitors[1, 7-9], whereas dextran sulfate and sulfated hyaluronic acid oligosaccharides are supposed to act, at least partly, in a competitive manner[10, 11]. Chitosan was also characterized as an inhibitor, with direct correlation between molecular weight and inhibitory potency of the polysaccharide[12]. Synthetic polymers like poly mandelic acid[13] or sodium-polystyrene-4-sulfonate are reported to be (irreversible) inhibitors[14, 15].

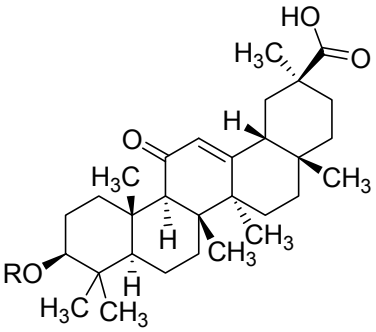
Among the described nonpolymeric inhibitors are compounds from different classes of natural compounds, for instance, flavones and flavone-analogs like silybin, apigenin, luteolin and kaempferol[16-21], saponins and sapogenins like glycyrrhizin and glycyrrhetinic acid[22, 23] or polyphenols such as tannic acid[20], aristolochic acid, the alkaloid reserpine[24] as well as plant extracts[22, 25-30]. Other substances known to (weakly) inhibit hyaluronidase activity are antioxidants like ascorbic acid[31] or propyl-gallate[24], various fatty acids[32], L-arginine derivatives or guanidine hydrochloride[33], representative anti-inflammatory agents such as indomethacin, which is regarded as an inhibitor *in vivo*, phenylbutazone, oxyphenbutazone and salicylates[12, 24, 28, 34, 35]. Finally antiallergic drugs like disodium cromoglycate (DSCG), aurothiomalate, tranilast or traxanox[17, 36, 37] are claimed to possess hyaluronidase inhibitory activities. However, the inhibitory potency was in most cases low, that is in the high micromolar and millimolar range, in the case of low molecular weight substances. Thus, no potent and selective inhibitors are known to date.

5.2 *Inhibitory activities of selected reference compounds as determined in the turbidimetric assay*

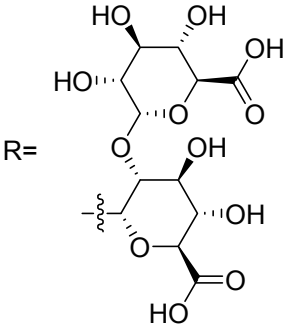
In the case of hyaluronidases the exact determination of K_i values of inhibitors is extremely difficult. On the one hand, the substrate hyaluronan is difficult to quantify as the used preparations are not homogeneous but consist of a mixture of different HA molecules with different molar weights. Additionally, HA is cleaved by the action of hyaluronidases and the resulting hyaluronan fragments are again substrates for another catalytic reaction which affects the substrate concentration. On the other hand, the competing transglycosylation reaction also influences the substrate concentration, a necessary parameter for the calculation of K_i values. Therefore, the determination of IC_{50} values is a viable approach to collect comparable inhibitor data for libraries of compounds with respect to the elucidation of structure-activity relationships. As IC_{50} values are strongly dependent on the method and the assay conditions (e.g. incubation conditions, enzymes, enzyme concentrations, substrate concentrations, pH value), huge discrepancies can occur, so that a direct comparison of data from different laboratories is impossible in most cases, For example, DSCG is designated as one of the most potent inhibitors of bovine testicular hyaluronidase

with an IC_{50} value of 29 µM[17]. When this compound was investigated upon BTH inhibition under the assay conditions applied in this work, the compound proved to be much less potent: no inhibition of the enzyme was detectable at concentrations below 2 mM at pH 5.0 in the turbidimetric assay, and an IC_{50} value as high as 1.24 mM was determined in the colorimetric Morgan Elson assay[38] by Salmen[39].

Therefore, several reference compounds described in the literature were investigated for inhibition of the hyaluronidases under standardized conditions in the turbidimetric activity assay based on the method of Di Ferrante[40] (see chapter 3) to gain reliable and comparable data with respect to the classification of the synthesized inhibitors in terms of potency. The structures of the tested reference compounds are depicted in Figure 5.1, the obtained IC_{50} values are summarized in Table 5.1.



Glycyrrhizic acid / Glycyrrhizin:

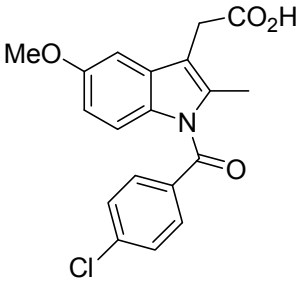


Glycyrrhetinic acid / Glycyrrhetin:

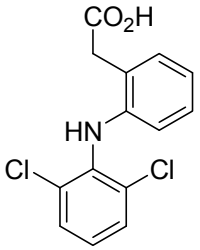
R = H

Carbenoxolone:

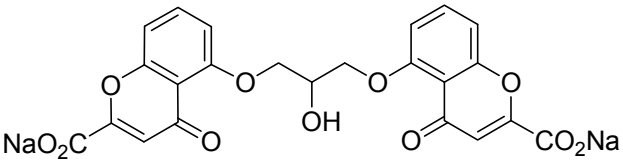
R = $CO(CH_2)_2CO_2H$



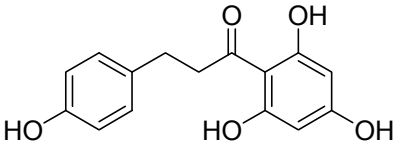
Indomethacin



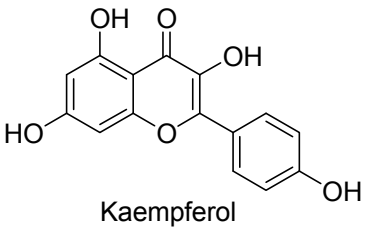
Diclofenac



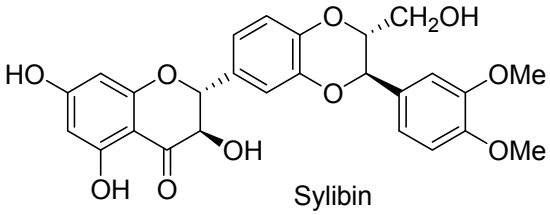
DSCG



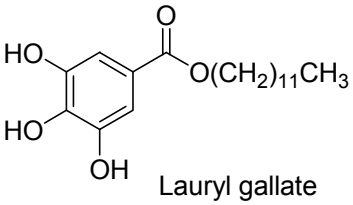
Phloretin



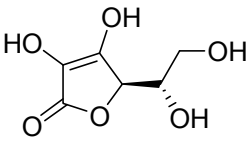
Kaempferol



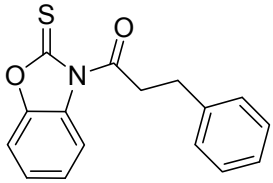
Sylibin



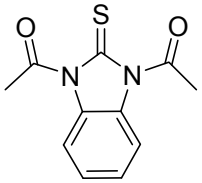
Lauryl gallate



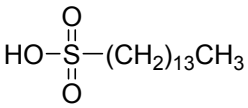
L-Ascorbic acid



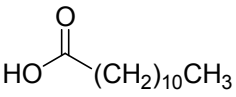
3-Phenyl-1-(2-thioxobenzo[*d*]oxazol-3(2*H*)-yl)propan-1-one



1,3-Diacetylbenzimidazole-2-thione



Tetradecanesulfonic acid



Lauric acid

Figure 5.1. Structures of investigated small molecule hyaluronidase inhibitors.

Table 5.1. Inhibitory activities of reference compounds on four investigated hyaluronidases,

Compound	Hyal-1	PH-20	BTH	*Sag*Hyal$_{4755}$
	IC_{50} [µM][a]			
Glycyrrhizic acid	26 ± 1	17 ± 1	737 ± 30	46 ± 2
Glycyrrhetinic acid	> 100	6.9 ± 0.7	> 100	54 ± 3
Carbenoxolone	> 130	3.2 ± 0.5	134 ± 7	20 ± 1
Indomethacin	390 ± 30	82 ± 9	540[b]	350[c]
Diclofenac	> 500	68 ± 7	> 1000	161 ± 11
DSCG	474 ± 75	> 900	> 2000	1208 ± 81
Phloretin	358 ± 49	200 ± 12	> 1400	213 ± 8
Kaempferol	> 1000	288 ± 23	> 1000	237 ± 15
Sylibinin	> 1000	111 ± 10	> 1000[b]	296 ± 18
Lauryl gallate	> 100	20 ± 3	> 100	13 ± 1
L-Ascorbic acid	> 60000	7564 ± 883	> 100000[d]	6100 ± 100[d]
1,3-Diacetylbenzimidazole-2-thione	> 1000	> 1000	> 1000[b]	160[c]
3-Phenyl-1-(2-thioxobenzo[*d*]oxazol-3(2*H*)-yl)propan-1-one	> 100	> 100	> 100[d]	15 ± 1[d]
Tetradecane sulfonic acid	21 ± 1	7.6 ± 0.3	93 ± 5	12 ± 1
Lauric acid	> 100	25 ± 2	> 150	> 150
Tannic acid	3.6 ± 0.5	1.1 ± 0.1	> 160	8.9 ± 0.6

[a] inhibition of enzyme expressed as IC_{50} (µM), mean values ± SEM; IC_{50} values determined at pH 5.0 (PH-20, BTH, *Sag*Hyal$_{4755}$) or 3.5 (Hyal-1) in the turbidimetric assay; [b]as determined by Salmen[39] at pH 3.6; [c]as determined by Salmen[39]; [d]as determined by Braun[41].

It is obvious that broadly accepted inhibitors like kaemferol, DSCG or indomethacin only weakly inhibit the investigated mammalian hyaluronidases and the bacterial hyaluronate lyase from *Streptococcus agalactiae* strain 4755. Among the investigated inhibitors, strongest inhibition of Hyal-1 was found for the detergent tetradecane sulfonic acid and for glycyrrhizic acid with IC_{50} values of 21 µM and 26 µM, respectively. The related compound carbenoxolone together with tetradecane sulfonic acid were found to be the most potent inhibitors of recombinantly expressed PH-20. The weak inhibitor DSCG was found to be outstanding due to the fact that certain selectivity for Hyal-1 *versus* human PH-20 was obtained, whereas the opposite was found for all other reference compounds. The substances were only very weakly active or inactive on BTH, whereas the bacterial enzyme was inhibited by most compounds in the (sub)millimolar range. The most potent inhibitors of the bacterial enzyme were the antioxidant lauryl gallate and the detergent tetradecane sulfonic acid with IC_{50} values of 13 µM and 12 µM, respectively. 1,3-Diacetylbenzimidazole-2-thione and 3-phenyl-1-(2-thioxobenzo[*d*]oxazol-3(2*H*)-yl)propan-1-one, which were recently identified as potent inhibitors of the bacterial

hyaluronate lyase in our workgroup[39, 41] proved to be inactive when tested on the mammalian enzymes.

Tannic acid, which is commonly used for the immobilization of enzymes[42], is composed of gallic acid moieties attached to a glucose core und must therefore be clearly separated from the other nonpolymeric low molecular weight inhibitors which are subject of this work. Other sulfated polymers like sodium-polystyrene-4-sulfonate (PSS) also possess inhibitory potency in the (sub)micromolar range as reported by Isoyama et al.[15], but it should be stressed that the molecular weight of those derivatives is extremely high (e.g. 519,000 g/mol for PSS990000). Thus, IC_{50} values in the same range as those of low molecular weight inhibitors are obtained when calculated on a weight concentration basis. Although the mentioned polymers or detergents like tetradecane sulfonic acid are rather potent hyaluronidase inhibitors, these compounds are far from being druglike and their utility as pharmacological tools for *in vivo* investigations is limited. Thus, the search for more potent and selective low molecular weight inhibitors is necessary.

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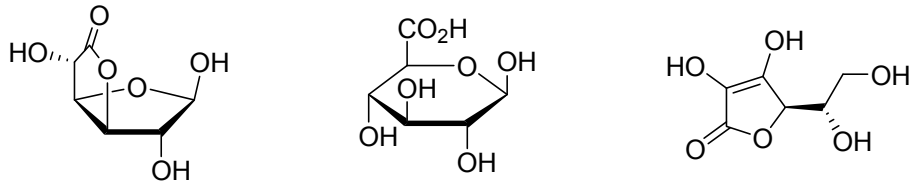
Chapter 6
Derivatives of the glucurono-6,3-lactone as hyaluronidase inhibitors

6.1 *Introduction*

In the synthesis of a hyaluronan disaccharide fragment (see chapter 9) as potential inhibitor of hyaluronidases, monosaccharides were prepared as intermediates. D-glucurono-6,3-lactone (Figure 6.1) is often used as a precursor for the synthesis of uronic acid derivatives[1, 2]. On the one hand this lactone shares structural similarities with ascorbic acid, which is a known hyaluronidase inhibitor[3], on the other hand the carbohydrate-like structure is related to D-glucuronic acid which is part of hyaluronan. This prompted us to synthesize various D-glucurono-6,3-lactones and related compounds as potential inhibitors of hyaluronidase.

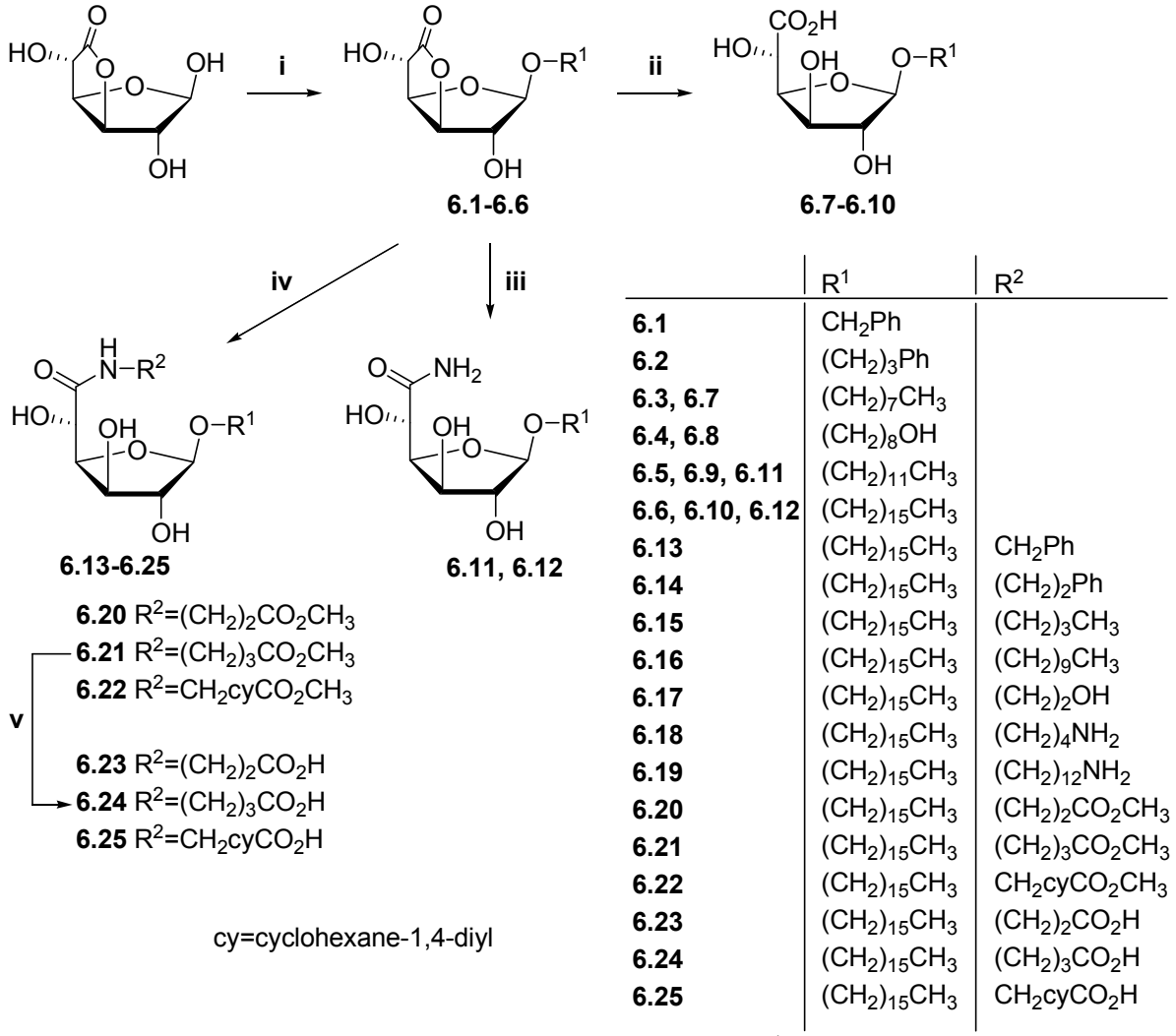


D-Glucurono-6,3-lactone D-Glucuronic acid L-Ascorbic acid

Figure 6.1. Structures of D-Glucurono-6,3-lactone, D-glucuronic acid and L-ascorbic acid.

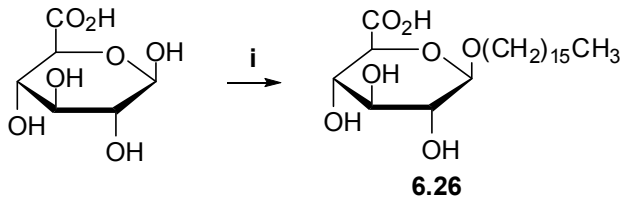
6.2 *Chemistry*

The synthesis of 1-*O*-alkyl-β-D-glucofuranosidurono-6,3-lactones **6.1-6.6** was possible without any protection groups according to the method of Bertho et al.[4] using D-glucurono-6,3-lactone and alcohols in presence of BF_3Et_2O (Scheme 6.1). The β-anomers were formed in high excess and were easily separated from the corresponding α-anomeric side products by flash chromatography. Saponification of the lactone[4] yielded the corresponding 1-*O*-alkyl-β-D-glucofuranosidic acids **6.7-6.10**. Aminolysis of **6.6** using NH_3/MeOH or alkyl amines[5, 6] in alcohol yielded the pertinent amides **6.11-6.22**. To obtain the carboxylic acids **6.23-6.25**, the methyl alkylamino-carboxylates **6.20a-6.22a** were synthesized according to known procedures[7-10]. In those cases the esters of **6.20-6.22** had to be cleaved after aminolysis of the lactone using aqueous LiOH to obtain **6.23-6.25**. 1-*O*-Hexadecyl-β-D-glucuronic acid (**6.26**) was synthesized using the same conditions as employed for the synthesis of **6.1-6.6** (Scheme 6.2).



	R^1	R^2
6.1	CH_2Ph	
6.2	$(CH_2)_3Ph$	
6.3, 6.7	$(CH_2)_7CH_3$	
6.4, 6.8	$(CH_2)_8OH$	
6.5, 6.9, 6.11	$(CH_2)_{11}CH_3$	
6.6, 6.10, 6.12	$(CH_2)_{15}CH_3$	
6.13	$(CH_2)_{15}CH_3$	CH_2Ph
6.14	$(CH_2)_{15}CH_3$	$(CH_2)_2Ph$
6.15	$(CH_2)_{15}CH_3$	$(CH_2)_3CH_3$
6.16	$(CH_2)_{15}CH_3$	$(CH_2)_9CH_3$
6.17	$(CH_2)_{15}CH_3$	$(CH_2)_2OH$
6.18	$(CH_2)_{15}CH_3$	$(CH_2)_4NH_2$
6.19	$(CH_2)_{15}CH_3$	$(CH_2)_{12}NH_2$
6.20	$(CH_2)_{15}CH_3$	$(CH_2)_2CO_2CH_3$
6.21	$(CH_2)_{15}CH_3$	$(CH_2)_3CO_2CH_3$
6.22	$(CH_2)_{15}CH_3$	$CH_2cyCO_2CH_3$
6.23	$(CH_2)_{15}CH_3$	$(CH_2)_2CO_2H$
6.24	$(CH_2)_{15}CH_3$	$(CH_2)_3CO_2H$
6.25	$(CH_2)_{15}CH_3$	CH_2cyCO_2H

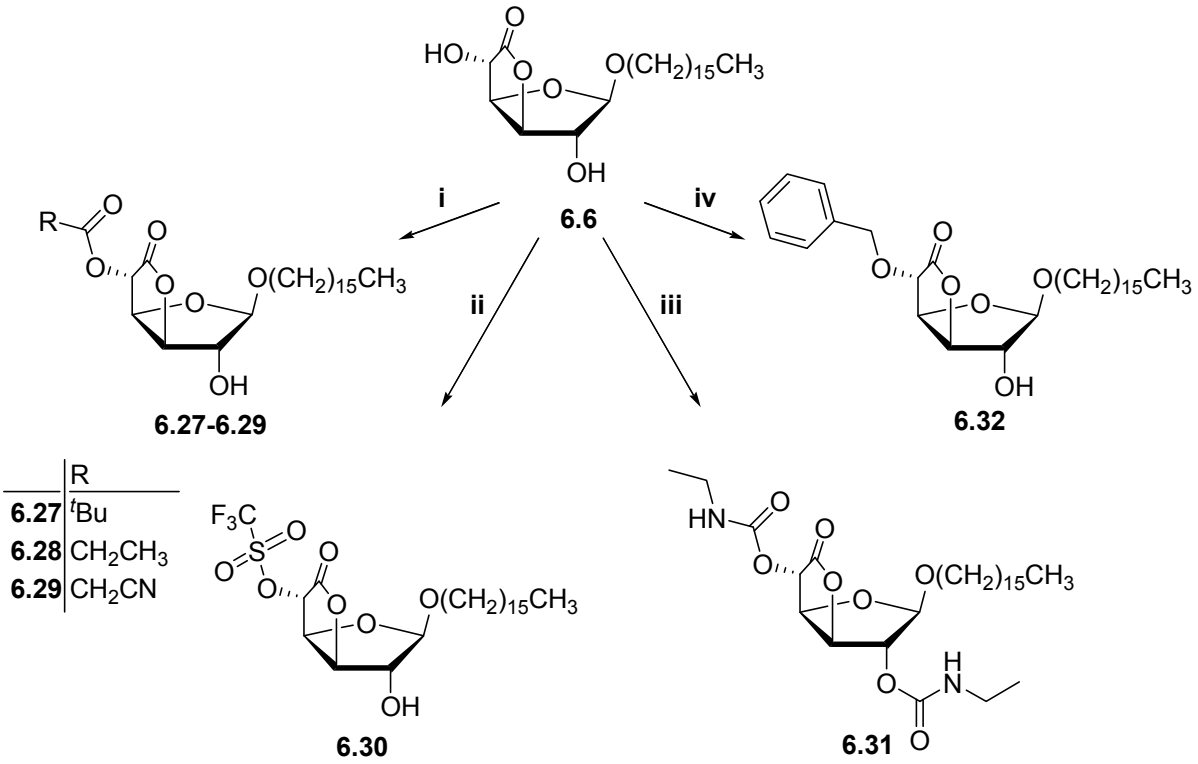
Scheme 6.1. Synthesis of **6.1-6.25**. Reagents and conditions: (i) R^1OH (1-2 eq), BF_3Et_2O (2 eq), THF, 60-70 °C, 1 h; (ii) NaOH, acetone, 0 °C to RT, 2 h; (iii) NH_3/MeOH, 0 °C, 2 h; (iv) NHR^2 (1-3 eq), MeOH, RT overnight; (v) 0.5 N LiOH (2 eq), THF, RT overnight.



Scheme 6.2. Synthesis of **6.26**. Reagents and conditions: (i) Hexadecan-1-ol (2 eq), BF_3Et_2O (2 eq), THF, 60-70 °C, 1 h.

Regioselective reactions were needed for the modification of the 3-OH or 5-OH of the lactone scaffold. Acylation predominantly takes place at position *O*-5 of the 1-*O*-alkylated lactone as already described[11, 12] if one equivalent of acid chloride or otherwise activated carboxylic acid is used (Scheme 6.3). The reaction of **6.6** with equimolar amounts of sulfonic acid chloride or anhydride also leads to the formation of 5-OH-monosulfonylated derivatives as major product[13, 14]. Monocarbamoylation using isocyanates was unsuccessful. Whereas **6.6** did not react with ethyl isocyanate

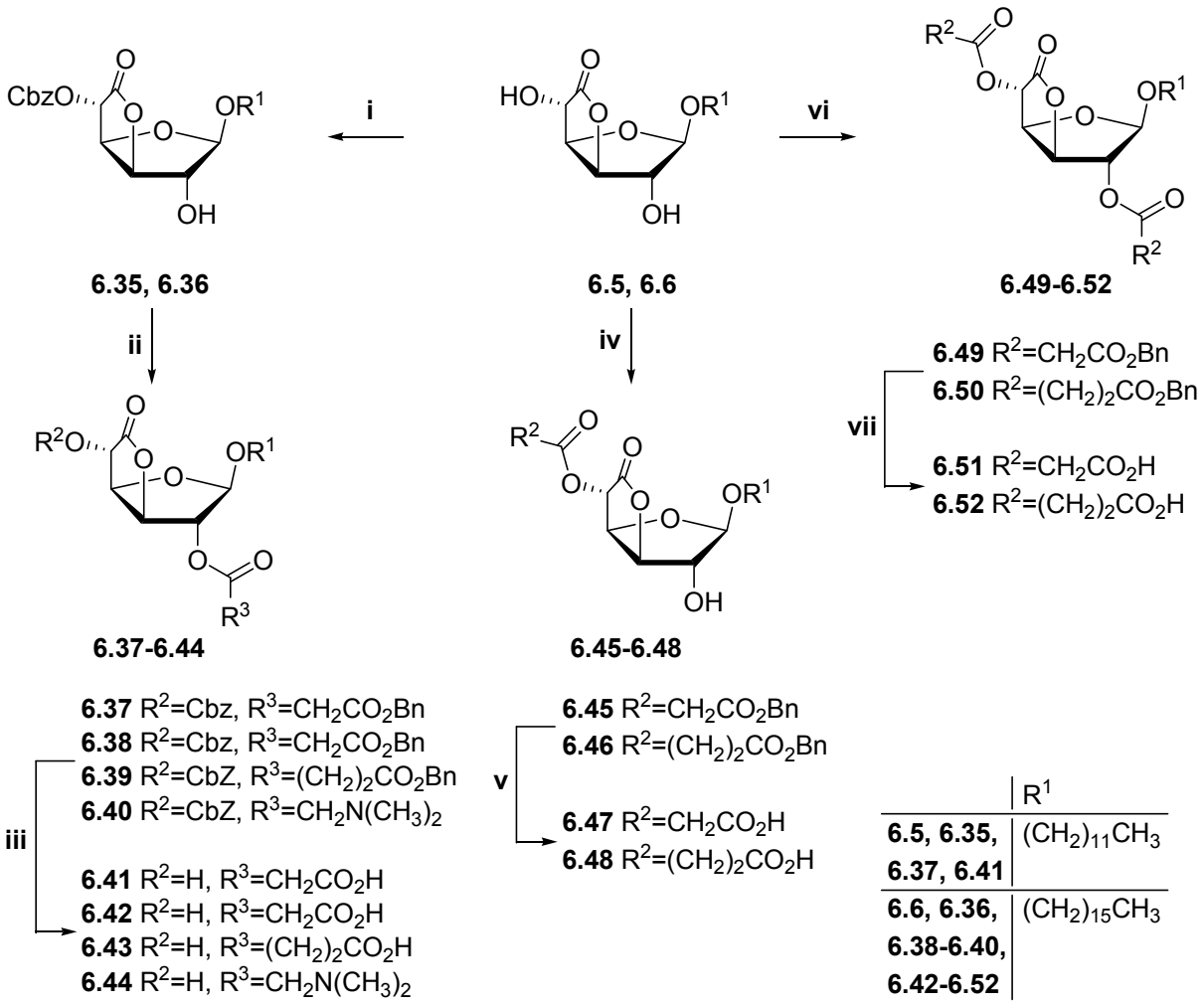
at room temperature, the addition of copper(I)-chloride[15] resulted nearly exclusively in the dicarbamoylated product **6.31**. Alkylation using alkyl halides and base is not possible in the presence of the sensitive lactone ring. Therefore, reactions avoiding strong nucleophiles are needed to leave the lactone ring intact. The monobenzylation of **6.6** was successful using benzyl bromide in presence of Ag_2O[16]. However, this synthetic pathway was unsuccessful with other alkyl halides. Due to the large variety of commercially available carboxylic acids and acid chlorides, acylation was chosen as the method of choice for the modification of the lactone core.



Scheme 6.3. Synthesis of the 1-*O*-alkyl-β-D-glucofuranosidurono-6,3-lactone derivatives **6.27-6.32**. Reagents and conditions: (i) RCOCl (1 eq), pyridine/THF, 0 °C, 1 h; (ii) Tf_2O (1 eq), CH_2Cl_2/pyridine, -30 °C, 30 min; (iii) EtNCO (1 eq), Cu(I)Cl (1 eq), DMF, RT, 1 h; (iv) BnBr (1 eq), Ag_2O (1.5 eq), EtOAc, 40-50 °C, 48 h.

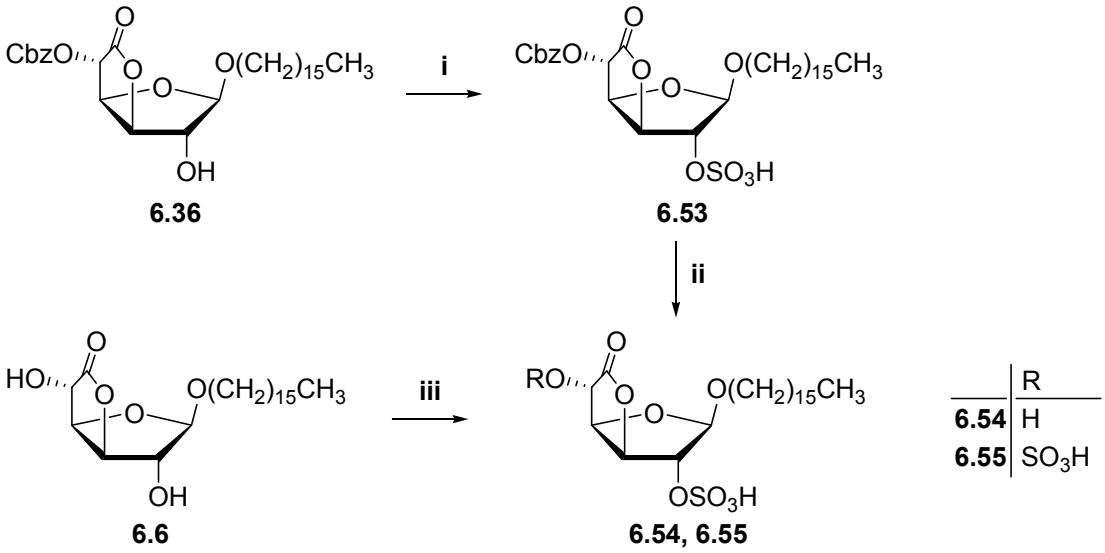
Compounds **6.5** or **6.6** were acylated as depicted in Scheme 6.4. After selective Cbz-protection of O-5 the 2-OH was acylated using carboxylic acids together with DCC and DMAP as coupling reagents. Monobenzyl malonate (**6.33**) and monobenzyl succinate (**6.34**) which were needed for the introduction of a carboxylic acid residue in the amide sidechain were synthesized according to literature conditions[17, 18]. After acylation the protecting groups (Cbz and benzyl esters) were cleaved to obtain the 2-*O*-acylated 1-*O*-alkyl-β-D-glucofuranosidurono-6,3-lactones **6.39-6.42**. The 5-O-acylated derivatives **6.45** and **6.46** were synthesized by treating **6.6** with nearly equimolar amounts of acid chloride or carboxylic acid and DCC followed by

hydrogenolytic cleavage of the benzyl ester. Finally diacylation of **6.6** was performed under similar conditions using an excess of carboxylic acid and activating reagents to obtain **6.51** and **6.52** after removal of the protecting groups.



Scheme 6.4. Synthesis of the acylated 1-*O*-alkyl glucurono-6,3-lactones **6.41-6.44, 6.47, 6.48**, **6.51** and **6.52**. Reagents and conditions: (i) Cbz-Cl (1 eq), pyridine, -20 °C, 20 min; (ii) R^3CO_2H (1.1 eq), DCC (1.1 eq), DMAP (0.1 eq), EtOAc, RT overnight; (iii) 10 % Pd/C (cat.), H_2 (1 atm), EtOAc, RT overnight; (iv) R^2CO_2H (1 eq), DCC (1.1 eq), DMAP (0.1 eq), EtOAc, RT overnight, or: R^2COCl (1.1 eq), THF/pyridine, -20 °C, 10 min; (v) 10 % Pd/C (cat.), H_2 (1 atm), EtOAc, RT overnight; (vi) R^2CO_2H (2.4 eq), DCC (2.4 eq), DMAP (0.2 eq), EtOAc, RT overnight; (vii) 10 % Pd/C (cat.), H_2 (1 atm), EtOAc, RT overnight.

Sulfation of **6.6** or the monoprotected derivative **6.36** using an SO_3-pyridine complex[19] and, if present, removal of the Cbz group yielded the mono- or di-sulfates **6.54** and **6.55** (Scheme 6.5).



6.36 **6.53** **6.6** **6.54, 6.55**

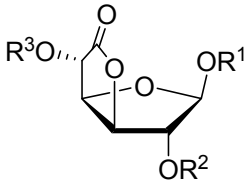
Scheme 6.5. Synthesis of sulfates **6.53** - **6.55**. Reagents and conditions: (i) SO_3-pyridine complex (3 eq), pyridine, RT overnight; (ii) 10 % Pd/C (cat.), H_2 (1 atm), EtOAc, RT overnight; (iii) SO_3-pyridine complex (4 eq), pyridine, RT, overnight.

6.3 *Inhibition of hyaluronidases: results and discussion*

6.3.1 Inhibition of hyaluronidases by 1-*O*-alkyl-glucurono-6,3-lactones

The hyaluronidase inhibitory activities of the synthesized 1-*O*-alkyl glucurono-6,3-lactones are summarized in Table 6.2 (for substitution patterns see Table 6.1).

Table 6.1. Substitution patterns for glucurono-6,3-lactones **6.1-6.6, 6.27, 6.28, 6.41-6.48** and **6.51-6.55**.



6.1-6.6, 6.27, 6.28, 6.41-6.48, 6.51-6.55

Compound	R^1	R^2	R^3
D-Glucurono-6,3-lactone	H	H	H
6.1	CH_2Ph	H	H
6.2	$(CH_2)_3Ph$	H	H
6.3	$(CH_2)_7CH_3$	H	H
6.4	$(CH_2)_8OH$	H	H
6.5	$(CH_2)_{11}CH_3$	H	H
6.6	$(CH_2)_{15}CH_3$	H	H

Table 6.1 (continued)

6.27	$(CH_2)_{15}CH_3$	H	$COC(CH_3)_3$
6.28	$(CH_2)_{15}CH_3$	H	$COCH_2CH_3$
6.41	$(CH_2)_{12}CH_3$	$COCH_2CO_2H$	H
6.42	$(CH_2)_{15}CH_3$	$COCH_2CO_2H$	H
6.43	$(CH_2)_{15}CH_3$	$CO(CH_2)_2CO_2H$	H
6.44	$(CH_2)_{15}CH_3$	$COCH_2N(CH_3)_2$	H
6.47	$(CH_2)_{15}CH_3$	H	$COCH_2CO_2H$
6.48	$(CH_2)_{15}CH_3$	H	$CO(CH_2)_2CO_2H$
6.51	$(CH_2)_{15}CH_3$	$COCH_2CO_2H$	$COCH_2CO_2H$
6.52	$(CH_2)_{15}CH_3$	$CO(CH_2)_2CO_2H$	$CO(CH_2)_2CO_2H$
6.54	$(CH_2)_{15}CH_3$	SO_3H	H
6.55	$(CH_2)_{15}CH_3$	SO_3H	SO_3H

Table 6.2. Inhibitory activity of investigated glucurono-6,3-lactones.

Compound	**Hyal-1**	**PH-20**	**BTH**	***Sag*Hyal$_{4755}$**
	IC_{50} [µM][a]			
D-Glucurono-6,3-lactone	> 60000	> 50000	> 20000	> 20000
6.1	> 4000	> 4000	> 4000	> 4000
6.2	> 1400	478 ± 31	>1400	920 ± 103
6.3	> 1000	1114 ± 137	> 1000	171 ± 10
6.4	> 1400	> 1400	> 1400	683 ± 62
6.5	> 400	69 ± 13	> 400	29 ± 2
6.6	> 30	> 30	> 30	9.5 ± 0.7
6.27	> 30	16 ± 2	> 30	> 30
6.28	> 30	> 30	> 30	> 30
6.41	99 ± 4	6.1 ± 0.5	373 ± 20	22 ± 2
6.42	47 ± 4	1.8 ± 0.1	78 ± 1	2.9 ± 0.1
6.43	> 100	6.4 ± 0.3	142 ± 6	3.7 ± 0.1
6.44	> 30	> 30	> 30	16 ± 2
6.47	57 ± 4	3.1 ± 0.2	107 ± 3	3.9 ± 0.1
6.48	> 100	6.0 ± 0.2	118 ± 2	2.3 ± 0.2
6.51	33 ± 1	2.3 ± 0.1	49 ± 1	2.3 ± 0.1
6.52	> 100	5.7 ± 0.6	53 ± 1	2.6 ± 0.1
6.54	21 ± 3	1.7 ± 0.1	53 ± 1	3.5 ± 0.2
6.55	14 ± 2	1.4 ± 0.1	31 ± 1	3.1 ± 0.1

[a] inhibition of enzyme expressed as IC_{50} (µM), mean values ± SEM (N = 2, experiments performed in duplicate), maximal concentrations of the test compounds were selected with respect to individual terminal solubilities in the incubation mixture; IC_{50} values determined at pH 5.0 (PH-20, BTH, *Sag*Hyal$_{4755}$) or 3.5 (Hyal-1) in the turbidimetric assay.

Whereas D-glucurono-6,3-lactone and the 1-*O*-benzylated compound **6.1** were inactive on all of the investigated hyaluronidases, the compounds **6.2, 6.3** and **6.5**, which are more hydrophobic than the unsubstituted lactone, significantly inhibited the human PH-20 and the bacterial *Sag*Hyal$_{4755}$ in a dose dependent manner (Figure 6.2). The determination of an IC_{50}-value of **6.3** for PH-20 was impossible due to the poor solubility of this compound (maximum concentration: about 1 mM). However, this derivative causes 50 % inhibition at 1 mM. In general, the elongation of the chain leads to an increase in inhibition on those two enzymes. A considerable further increase in inhibition is observed comparing **6.5** with **6.3**: Lenghtening of the chain by 4 methylene groups leads to a 6- to 16-fold inhibition of the two enzymes. 1-*O*-Hexadecyl-β-D-glucurono-6,3-lactone (**6.6**) was inactive on PH-20 at the concentrations used, whereas the bacterial lyase was inhibited with an IC_{50} value of 9.5 µM.

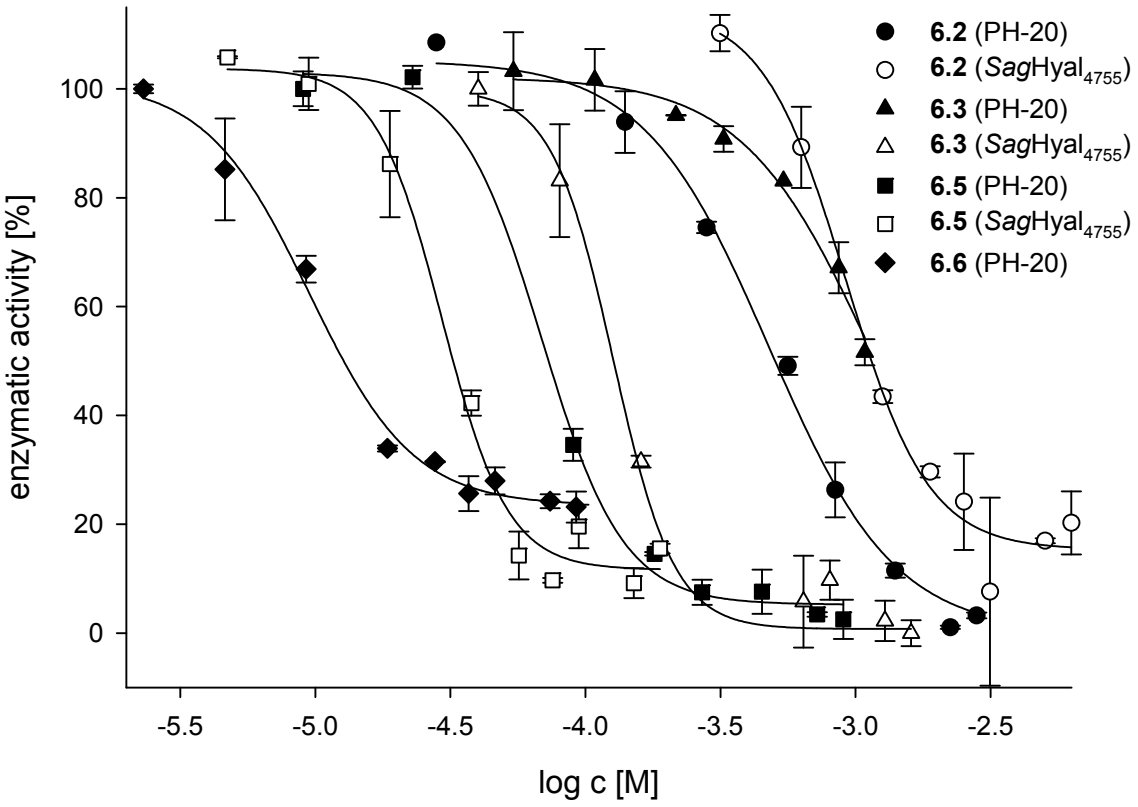


Figure 6.2. Enzymatic activity of human PH-20 (black symbols) and bacterial hyaluronidase from *S. agalactiae* strain 4755 (open symbols) in the presence of compounds **6.2, 6.3**, **6.5** and **6.6**. The different shape of the inhibition curve in the presence of high concentrations of **6.6** most probably results from the limited water solubility of this compound.

The inhibitory activity decreased significantly when an additional hydroxyl group was introduced at the terminal position of the hydrophobic chain (compound **6.4**). The human Hyal-1 and the BTH were not inhibited by the 1-*O*-alkylated derivatives **6.1-6.6**. The compounds **6.27** and **6.28** bear additional hydrophobic substituents at position 2 of the lactone moiety and possess very limited water solubility. These compounds did not show inhibition of Hyal-1, BTH and the bacterial hyaluronate lyase. The results were different for both compounds on human PH-20. Whereas the compound **6.27** with the bulky pivaloyl residue was more potent (IC_{50} 18 µM) than 1-*O*-hexadecyl glucurono-6,3-lactone (**6.6**), no inhibition was observed for the propanoyl substituted lactone **6.28** at highest investigated concentrations. This may be interpreted as a hint to a binding site of PH-20 where a more bulky hydrophobic residue is favourable to increase the affinity of the inhibitor. To increase the water solubility, carboxy residues with different spacer lengths were introduced at different positions of the glucuronolactone moiety (**6.41-6.43, 6.47, 6.48** and **6.51, 6.52**). The carboxyethanoyl substituted derivatives **6.41, 6.42, 6.47** and **6.51** showed distinctly increased inhibitory potencies on all four investigated enzymes compared to the corresponding unsubstituted derivatives (**6.5** and **6.6**). Figure 6.3 illustrates the inhibitory activity of 1-*O*-hecadecyl glucurono-6,3-lactones bearing additional carboxylic acid residues in position *O*-2 and/or *O*-3.

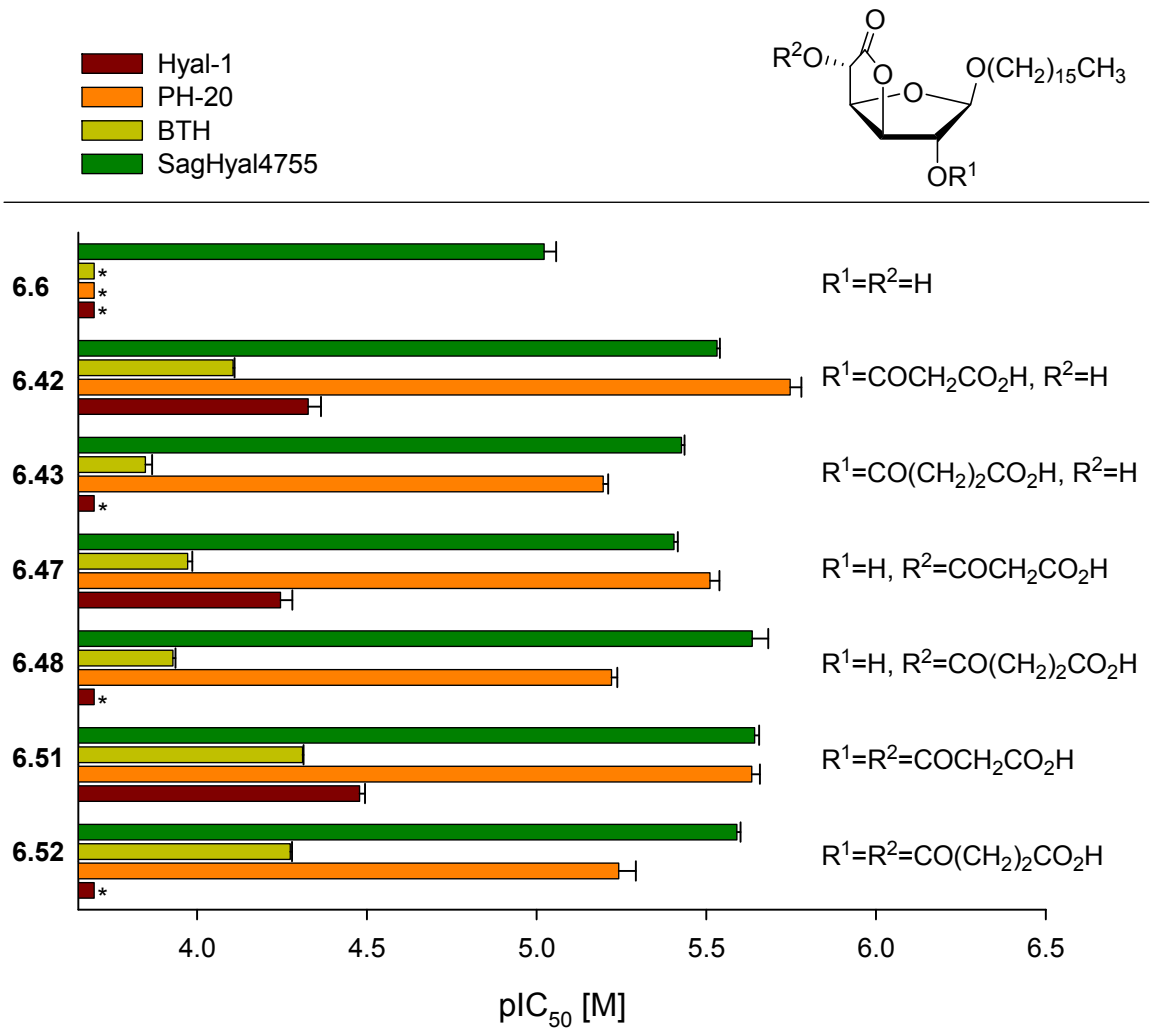


Figure 6.3. Inhibitory activity of 1-*O*-hecadecyl glucurono-6,3-lactones bearing additional carboxylic acid residues in position *O*-2 and / or *O*-3. Compounds marked with * did not show inhibitory activity of the investigated enzyme; therefore the length of the bar was arbitrarily chosen for illustrative reasons.

Comparing compounds **6.42** and **6.47** it is obvious that the IC_{50} values are in the same range regardless of the different positions of substitution. Disubstitution (compound **6.51**) is tolerated but results only in a weak increase in inhibition of Hyal-1 and the bovine testicular hyaluronidase. The potencies on PH-20 and the bacterial enzyme remain rather unaffected by the different structural modifications of the inhibitors. The concentration-dependent inhibition of the investigated hyaluronidases by **6.51** is shown in Figure 6.4.

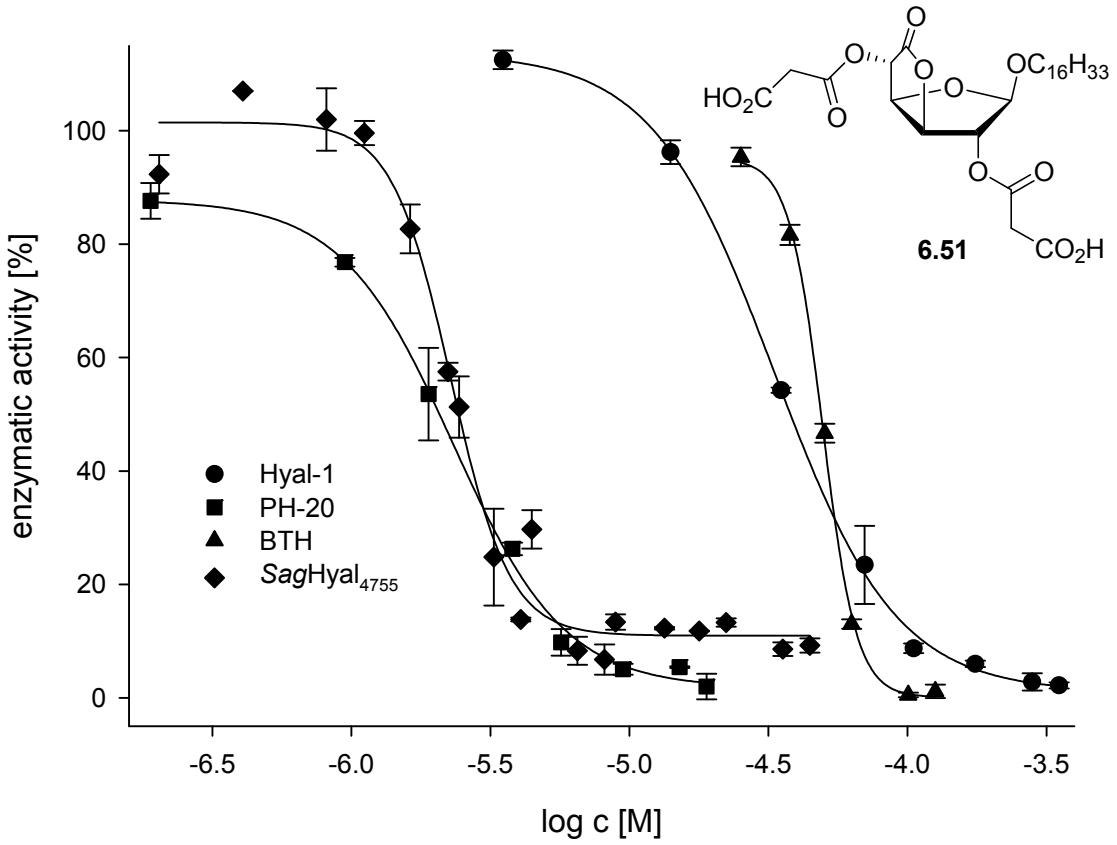


Figure 6.4. Enzymatic activity of the investigated hyaluronidases in the presence of **6.51.**

When **6.41**, bearing a 1-*O*-dodecyl residue, is compared with the hexadecyl derivative **6.42**, the lower homologue is superior by a factor of 2-3 with regard to the two human enzymes, whereas the elongation of the chain by four carbon atoms leads to an 8-fold increase in inhibition of BTH and *Sag*Hyal$_{4755}$. Although, generally, the hydrophobicity of the substituents appear to be the key to high affinity of the inhibitors, it becomes clear that the structure-activity relationships are different for the four investigated hyaluronidases. Obviously, the four terminal carbon atoms of the hexadecyl chain confer additional affinity in the case of BTH and *Sag*Hyal$_{4755}$ but do not in case of the human enzymes. When the carboxypropanoyl substituted 1-*O*-hexadecyl-β-D-glucurono-6,3-lactones **6.43, 6.48** and **6.52** are compared with the corresponding derivatives bearing the shorter alkyl spacer between carboxyl moiety and the glucuronolactone core (**6.42, 6.47** and **6.51**), a minor decrease in inhibitory potency becomes obvious for PH-20, BTH and *Sag*Hyal$_{4755}$, whereas the inhibition of Hyal-1 is completely lost. The solubility decreases when the carboxyl group in position *O*-2 is replaced by a *N,N*-dimethylamino residue (compare **6.42** and **6.44**); therefore, the determination of IC_{50} values was impossible for Hyal-1 and BTH where

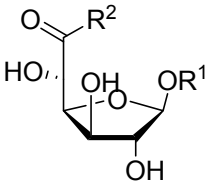
those values are generally higher than for PH-20 and the bacterial enzyme. However, it is obvious that the potency is significantly reduced by the basic functional group as the IC_{50} value of **6.44** drops to 16 µM for *Sag*$Hyal_{4755}$ compared to 2.9 µM for the carboxylic acid **6.42**. Inhibition of human PH-20 is also decreased by at least a factor of 16.

Previous studies revealed that sulfated carbohydrates are potent inhibitors of BTH, BVH and *Sag*$Hyal_{4755}$ with IC_{50} values in the (lower) micromolar range[20]. Hence, the lactone moiety was sulfated to investigate if this increase in acidity of 1-*O*-alkyl-glucurono-6,3-lactones also correlates with an increase in inhibition of the human enzymes. The mono- and di-sulfated derivatives **6.54** and **6.55** show a clearly enhanced inhibitory activity compared to the nonsulfated parent molecule **6.6**, with the disulfated compound **6.55** being slightly more potent than the monosulfated analogue **6.54** on all the four investigated hyaluronidases. The disulfated derivative **6.55** thus represents the most potent inhibitor of the investigated mammalian hyaluronidases in this series with IC_{50} values of 14 µM, 1.4 µM and 31 µM determined for Hyal-1, PH-20 and BTH respectively.

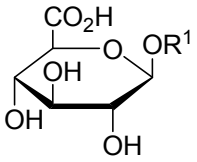
6.3.2 1-*O*-Alkyl-glucuronic acids and 1-*O*-alkyl-glucuronic acid amides

Due to the fact that on one hand long hydrophobic alkyl chains are needed to obtain hyaluronidase inhibitors with reasonable affinity and on the other hand a polar acidic group seems to be essential to gain potency, the lactone group was saponified to obtain the corresponding free carboxylic acids, which are combining both structural features. As shown in Table 6.3, the potency of compounds **6.7-6.10** is more or less in the same range compared to the corresponding lactone derivatives discussed above. Inhibition of PH-20 and the bacterial hyaluronat lyase increases with the length of the aliphatic chain. Again the lengthening of the dodecyl chain to 16 carbon atoms results in a more drastic increase in potency of the compound for *Sag*$Hyal_{4755}$ than for PH-20. 1-Dodecyl-β-D-glucofuranosiduronic acid (**6.9**) also inhibits Hyal-1 and BTH in a concentration-dependent manner (Figure 6.5) but for Hyal-1 the inhibition at terminal solubility was only 40-50 %, therefore, in this case the determination of exact IC_{50} values was impossible.

Table 6.3. Inhibitory activities of synthesized glucuronic acid derivatives **6.7-6.19** and **6.23-6.26**.



6.7-6.19, 6.23-6.25



6.26

No	R^1	R^2	Hyal-1	PH-20	BTH	*Sag*Hyal$_{4755}$
			IC_{50} [µM][a]			
6.7	$(CH_2)_7CH_3$	OH	> 1200	304 ± 78	> 1200	> 1200
6.8	$(CH_2)_8OH$	OH	> 1600	> 1600	> 1600	1170 ± 70
6.9	$(CH_2)_{11}CH_3$	OH	≥ 500	12 ± 2	259 ± 5	36 ± 5
6.10	$(CH_2)_{15}CH_3$	OH	> 50	3.0 ± 0.3	> 50	4.7 ± 0.1
6.11	$(CH_2)_{11}CH_3$	NH_2	> 200	> 200	> 100	82 ± 4
6.12	$(CH_2)_{15}CH_3$	NH_2	> 50	> 50	> 50	> 50
6.13	$(CH_2)_{15}CH_3$	$NHCH_2Ph$	> 50	> 50	> 50	> 50
6.14	$(CH_2)_{15}CH_3$	$NH(CH_2)_2Ph$	> 40	> 40	> 40	> 40
6.15	$(CH_2)_{15}CH_3$	$NH(CH_2)_3CH_3$	> 40	> 40	> 40	> 40
6.16	$(CH_2)_{15}CH_3$	$NH(CH_2)_9CH_3$	> 20	> 20	> 20	> 20
6.17	$(CH_2)_{15}CH_3$	$NH(CH_2)_2OH$	> 50	> 50	> 50	> 50
6.18	$(CH_2)_{15}CH_3$	$NH(CH_2)_4NH_2$	> 50	> 50	> 50	60 ± 5
6.19	$(CH_2)_{15}CH_3$	$NH(CH_2)_{12}NH_2$	> 20	14 ± 7	> 20	> 20
6.23	$(CH_2)_{15}CH_3$	$NH(CH_2)_2CO_2H$	> 90	2.9 ± 0.2	> 90	2.1 ± 0.1
6.24	$(CH_2)_{15}CH_3$	$NH(CH_2)_3CO_2H$	> 70	7.1 ± 1.6	> 70	4.2 ± 0.1
6.25	$(CH_2)_{15}CH_3$	$NHCH_2cyCO_2H$	> 50	9.1 ± 0.8	> 50	> 50
6.26	$(CH_2)_{15}CH_3$	-	> 60	> 60	> 60	> 60

[a] inhibition of enzyme expressed as IC_{50} (µM), mean values ± SEM (N = 2, experiments performed in duplicate), maximal concentrations of the test compounds were selected with respect to individual terminal solubilities in the incubation mixture; IC_{50} values determined at pH 5.0 (PH-20, BTH, *Sag*Hyal$_{4755}$) or 3.5 (Hyal-1) in the turbidimetric assay.

Interestingly, the shapes of the curves are different: whereas steep curves were received for BTH and the bacterial enzyme, the curves of the two human enzymes are clearly flattened. Therefore, it may be speculated about different binding modes or modes of action depending on the considered enzymes.

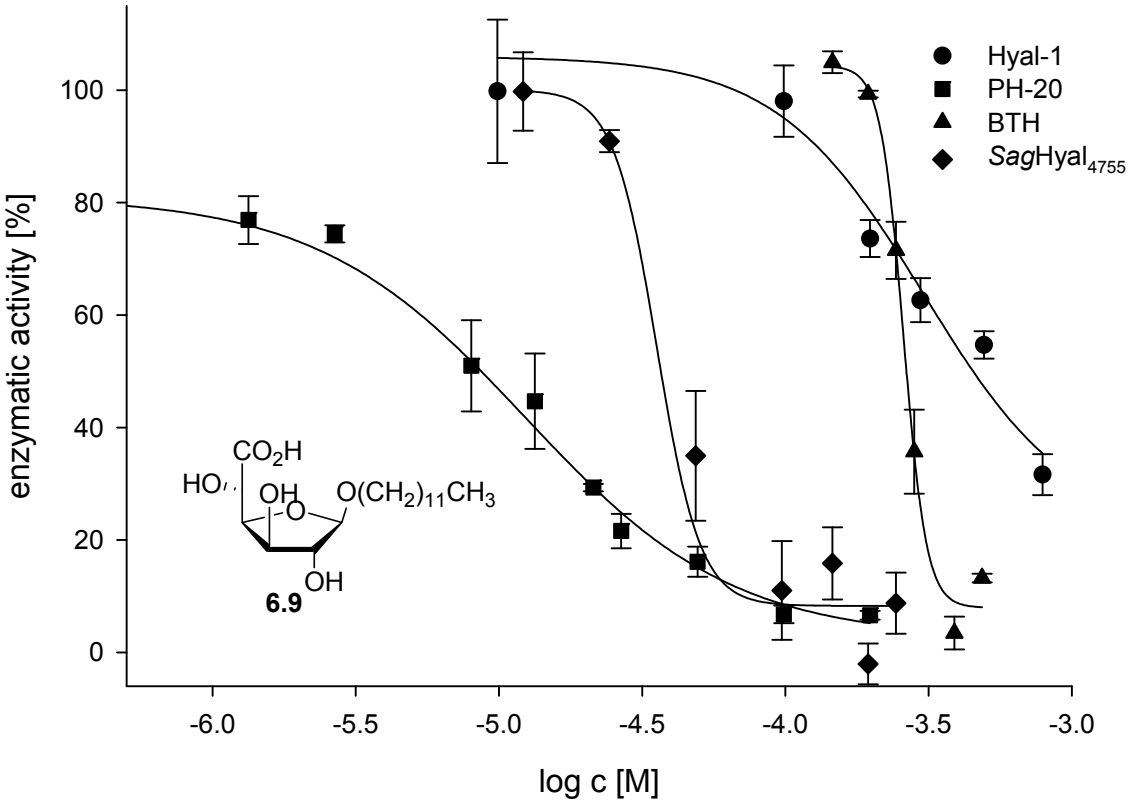


Figure 6.5. Enzymatic activity of the investigated hyaluronidases in presence of compound **6.9**.

The inhibitory activity towards the mammalian hyaluronidases is completely lost when the carboxyl moiety of **6.9** and **6.10** is replaced by an amide group (compounds **6.11** and **6.12**) indicating the importance of the carboxylic acid moiety for inhibition of the hyaluronidases. An IC_{50} value of 82 µM was calculated for the inhibition of the bacterial lyase by the amide derivative **6.11** corresponding to a drop in potency by a factor of about two compared to the carboxylate **6.9** (IC_{50} 36 µM**)**. In this case solubility problems impairing the determination of IC_{50} values can be excluded; the solubility of the amides and the carboxylic acid derivatives lies in a comparable range. However, the elaboration of SAR was impossible for the amides bearing additional hydrophobic substituents (**6.13-6.16**) due to poor solubility of the compounds in the test system. No inhibition of the enzymes was found up to the terminal solubility of the compounds. Both **6.18** and **6.19** bearing a terminal amino residue at the hydrophobic sidechain are inhibitors of *Sag*Hyal$_{4755}$. The solubility of **6.19** was too low for the determination of the IC_{50} value; at a concentration of 20 µM the activity of the enzyme was inhibited by 40 % (data not shown). Interestingly, **6.18** bearing the shorter spacer between terminal amino group and the amide group was

inactive towards PH-20 at the highest achievable concentrations, whereas an IC_{50} of 14 µM was determined for the analogue with the longer chain, compound **6.19**. It is conceivable that another hydrophobic area located in a certain distance from the binding site of the lactone moiety is addressed or that there is an energetically favored interaction of the terminal amino residue or both. The inhibition of PH-20 and *Sag*Hyal$_{4755}$ is again increased when a short alkanoic acid moiety instead of an alkyl residue is attached to the amide group (compare **6.15** with **6.23** and **6.24**). On both enzymes the inhibitory potency of these compounds is in the lower micromolar range and comparable to that of the nonsubstituted carboxylic acid **6.10**. The carboxyethyl derivative **6.23** is more potent than the higher homologue **6.24**. Surprisingly, unlike the alkanoic acid moieties in compounds **6.23** and **6.24** the cyclohexanecarboxylic acid in **6.25** caused enzyme selectivity. The bulkier cyclohexylene spacer is tolerated by PH-20, resulting in an IC_{50} of 9.1 µM which is comparable to the value for **6.24**, but the inhibitory activity towards *Sag*Hyal$_{4755}$ is completely lost within the range of solubility. Although human PH-20 and *Sag*Hyal$_{4755}$ belong to different classes of hyaluronidases and share only very low structural similarity, the investigation of the synthesized compounds usually revealed similar orders of potency on these enzymes, whereas there were pronounced differences in SARs between PH-20 and the related other mammalian hyaluronidases. **6.25** is unique as this compound is capable of discriminating between PH-20 and the bacterial enzyme. This reflects structural differences between these enzymes: obviously, in contrast to PH-20, the binding site of bacterial hyaluronidases is too narrow to allow binding of the cyclohexylene spacer. Hyal-1 as well as BTH were not inhibited by the amide derivatives **6.11-6.19** and **6.23-6.25** in the investigated concentration ranges. The discrepancy between the results obtained for the three mammalian enzymes is extremely suprising, in particular, when human PH-20 and BTH are compared, as the latter has been considered a suitable model for the human homologue, PH-20.

The potencies of representative glucofuranosides are illustrated by bars in Figure 6.6.

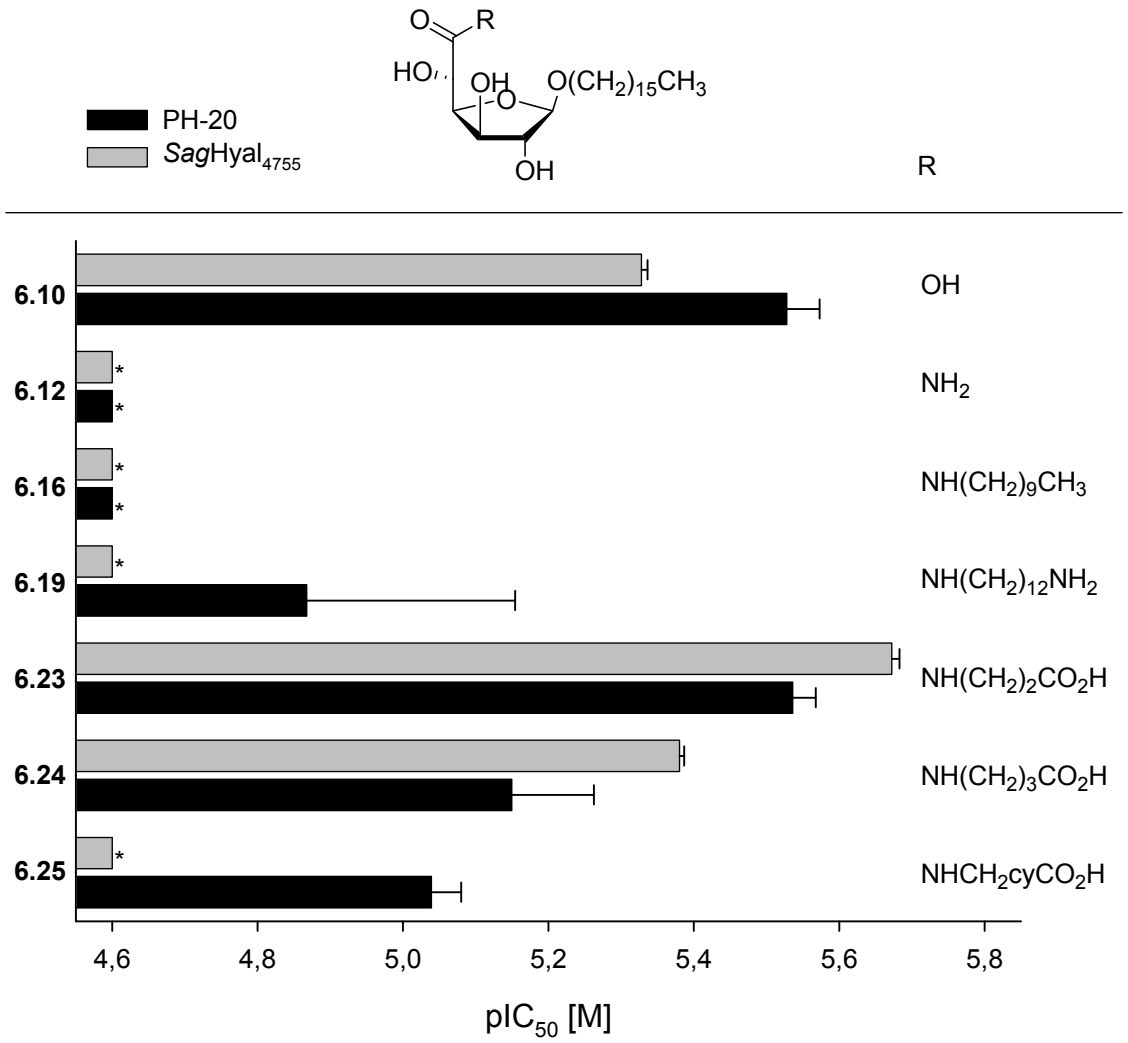


Figure 6.6. Inhibition of human PH-20 and *Sag*Hyal$_{4755}$ by various 1-*O*-hexadecyl-glucofuranosides. Compounds marked with * did not show inhibitory activity of the investigated enzyme at the highest tested concentrations (solubility limited); therefore the length of the bar was arbitrarily chosen for illustrative reasons.

Interestingly, the 1-*O*-alkylated glucuronic acid **6.26** does neither inhibit the bacterial hyaluronate lyase nor the mammalian hyaluronidases. This suggests that the five membered heterocyclic system is superior because inhibitory potency resides in the glucofuranosides presented in this chapter, as well as in the ascorbic acid derivatives presented in chapter 7. Thus, although hydrophobic interactions of the alkyl substituents play a major role in increasing the affinity of the inhibitors, distinct interactions of the lactone-scaffold with the active site of the hyaluronidases exist.

6.4 *Summary*

Generally, the presented derivatives of glucurono-6,3-lactone proved to be more potent when investigated upon inhibition of human PH-20 and the bacterial hyaluronate lyase from *Streptococcus agalactiae* compared to Hyal-1 and BTH. Especially the selectivity for human PH-20 *versus* the bovine homologue, which was proven by testing at equiactive enzyme concentrations (see chapter 10.3.5), is surprising. Whereas the parent compound, D-glucurono-6,3-lactone, does not inhibit human PH-20 and the bacterial hyaluronidase in the investigated concentration range, the inhibition of both enzymes is strongly increased by hydrophobic substituents in position *O*-1 (**6.1-6.6**). No inhibition of Hyal-1 and hyaluronidase from bovine testis was detectable for the 1-*O*-alkyl-glucurono-6,3-lactones **6.1-6.6**. Nevertheless those two enzymes are inhibited as soon as acidic moieties like carboxyl or sulfate groups are added to the glucuronolactone scaffold. Variation of the place of the substitution (*O*-2, *O*-5 or disubstitution; **6.42**, **6.47** and **6.51**) causes only minor changes in biological activity. By contrast, the inhibitory potency is decreased (PH-20, BTH and *Sag*Hyal$_{4755}$) or even lost (Hyal-1) when the spacer connecting carboxyl group and lactone core is elongated (**6.43**, **6.48** and **6.52**). The disulfated 1-*O*-hexadecyl-glucurono-6,3-lactone (**6.55**) is the most potent inhibitor of the investigated mammalian enzymes in this series with IC_{50} values of 14 µM, 1.4 µM and 31 µM determined for Hyal-1, PH-20 and BTH respectively. Interestingly, the introduction of another hydrophobic residue in position *O*-5 of the glucuronolactone (**6.27** and **6.28**) does not result in increased inhibitory potency except for **6.27** (bearing a bulky pivaloyl substituent) when tested upon inhibition of human PH-20. Saponification of the lactone moiety led to glucuronates (**6.7-6.10**) which possess significantly increased inhibitory activity especially for human PH-20. A distinct loss in potency is observed when the carboxyl group is replaced with an amide function (**6.11** and **6.12**) indicating the importance of this structural motive. The increase in hydrophobicity as in **6.13-6.16** is not paralleled by a rise in inhibitory potency. However, those compounds are barely soluble in the aqueous incubation mixture, thus, the determination of IC_{50} above 20-50 µM is considerable impaired or impossible. Whereas the amino-substituted compounds **6.18** and **6.19** clearly emphasize the different nature of the bacterial hyaluronate lyase and the human hyaluronidase PH-20, the SARs are similar for **6.23** and **6.24** bearing carboxyl

residues introduced *via* aminolysis of the lactone scaffold: both the inhibition of PH-20 and *Sag*Hyal$_{4755}$ increases when the spacer connecting carboxyl and amide group is reduced in lenghth. 1-*O*-Hexadecyl-*N*-(3-carboxypropyl)-β-D-glucofuranosiduronamide (**6.24**) with an IC_{50} value of 2.1 µM is the most potent inhibitor of the bacterial enzyme described in this chapter. Taken together, derivatives of D-glucurono-6,3-lactone are potent inhibitors of recombinantly expressed human hyaluronidases, hyaluronidase from bovine testis and the bacterial hyaluronate lyase *Sag*Hyal$_{4755}$. The IC_{50} values are lower than those determined for reference compounds (see chapter 5). Thus, the D-glucurono-6,3-lactones are among the most potent inhibitors of mammalian and bacterial hyaluronidases known to date. The results suggest that the lactone scaffold is mimicking a substructure of the substrate hyaluronan and binding to the active site. Hydrophobic substituents are conferring additional affinity by addressing hydrophobic areas of the hyaluronidases. This structural feature was found to be essential for inhibition of the enzymes. The hydrophobic alkyl chains might mimic the hydrophobic patches of hyaluronic acid[21] as it was found in the crystal structure of a bacterial hyaluronate lyase in complex with ascorbic acid 6-palmitate[22]. The structural requirements are very similar for the mammalian-type hyaluronidases. However, additional acidic functional groups are the key to potent inhibitors, especially in the case of Hyal-1 and BTH. It is conceivable that those negatively charged groups mimic the carboxylates of glucuronic acid moieties in hyaluronan and interact with polar amino acids which are present in the active site of the mammalian and bacterial hyaluronidases.

6.5 *Experimental Section*

6.5.1 General conditions

Chemicals were purchased from the following suppliers: Acros Organics (Geel, Belgium), IRIS Biotech GmbH (Marktredwitz, Germany), Alfa Aesar GmbH & Co. KG (Karlsruhe, Germany), Merck KGaA (Darmstadt, Germany), and Sigma-Aldrich Chemie GmbH (Munich, Germany). Deuterated solvents for NMR spectroscopy were from Deutero GmbH (Kastellaun, Germany). All solvents were of analytical grade or distilled prior to use. If moisture-free conditions were required, reactions were

performed in dried glassware under inert atmosphere (argon or nitrogen); DMF (H_2O < 0.01 %) was purchased from Sigma-Aldrich Chemie GmbH. Nuclear Magnetic Resonance (^{1}H-NMR and ^{13}C-NMR) spectra were recorded on an Avance-300 NMR spectrometer from Bruker BioSpin GmbH (Rheinstetten, Germany). Tetramethyl-silane was added as internal standard (chemical shift δ = 0 ppm) to all samples. Multiplicities are specified with the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), bs (for broad singulet), as well as combinations thereof. The multiplicity of carbon atoms (^{13}C-NMR) were determined by DEPT 135 and DEPT 90 (distortionless enhancement by polarization transfer): "+" primary and tertiary carbon atom (positive DEPT 135 signal), "-" secondary carbon atom (negative DEPT 135 signal), "quat" quaternary carbon atom. Mass spectrometry analysis (MS) was performed on a Finnigan MAT 95 (PI-EIMS 70 eV, HR-MS), Finnigan SSQ 710A (CI-MS (NH_3)) and on a Finnigan ThermoQuest TSQ 7000 (ESI-MS) spectrometer. The peak-intensity in % relative to the strongest signal is indicated in parenthesis. Melting points (mp) were measured on a BÜCHI 530 using open capillaries and are uncorrected. Merck Silica Gel 60 (particle size 0.040–0.063 mm) was used for flash column chromatography. Reactions were routinely monitored by thin layer chromatography (TLC) on Merck silica gel 60 F_{254} aluminum sheets and spots were visualized with UV light at 254 nm, and/or iodine vapor or ammonium molybdate/cerium(IV) sulfate solution.

6.5.2 Chemistry

6.5.2.1 General method for the synthesis of 1-O-Alkyl-β-D-glucofuranosid-urono-6,3-lactones 6.1-6.6

A suspension of D-glucuronic acid or D-glucurono-6,3-lactone (1 eq), the pertinent alkanol (1-2 eq) and BF_3Et_2O (2 eq) in anhydrous THF was stirred at 60-70 °C under an atmosphere of argon for 1 h. After removal of volatiles the raw product was subjected to flash chromatograpy.

1-*O*-Benzyl-β-D-glucofuranosidurono-6,3-lactone (6.1): The title compound was prepared from D-glucurono-6,3-lactone (5 mmol, 0.88 g), benzyl alcohol (10 mmol, 1.0 ml) and BF_3Et_2O (10 mmol, 1.3 ml) in anhydrous THF (20 ml) according to general procedure 6.5.2.1 and was obtained after flash chromatography (PE/EtOAc

40/60 v/v) as colorless semisolid substance (0.52 g, 39 %). ^{1}H-NMR (DMSO-d_6) δ 4.11 (s, 1H, CH_2Ph), 4.32 (d, 1H, ^{2}J = 11.4 Hz, CH_2Ph), 4.50 (dd, 1H, ^{3}J = 4.8 Hz, ^{3}J = 4.8 Hz, H-5), 4.74 (d, 1H, ^{3}J = 2.4 Hz, H-2), 4.76 (d, 1H, ^{3}J = 4.4 Hz, H-3), 4.84 (dd, 1H, ^{3}J = 4.6 Hz, ^{3}J = 6.4 Hz, H-4), 5.01 (s, 1H, H-1), 5.73 (d, 1H, ^{3}J = 3.5 Hz, 2-OH), 6.00 (d, 1H, ^{3}J = 5.0 Hz, 5-OH), 7.30 (m, 5H). ^{13}C-NMR (DMSO-d_6) δ 68.40 (-, $\underline{C}H_2Ph$), 68.63 (+, C-5), 76.92 (+,C-2), 78.20 (+,C-4), 82.58 (+,C-3), 107.78 (+, C-1), 127.32 (+, Ph-C), 127.95 (+, Ph-C), 128.09 (+, Ph-C), 137.40 (quat, Ph-C), 174.95 (quat, lactone CO). EI-MS (70eV) *m/z (%)*: 266 (66) [$M^{+\cdot}$]. $C_{13}H_{14}O_6$ (266.25).

1-*O*-(3-Phenylpropyl)-β-D-glucofuranosidurono-6,3-lactone (6.2): The title compound was prepared from D-glucurono-6,3-lactone (5 mmol, 0.88 g), 3-phenylpropan-1-ol (10 mmol, 1.3 ml) and BF_3Et_2O (10 mmol, 1.3 ml) in anhydrous THF (20 ml) according to general procedure 6.5.2.1 and was obtained after flash chromatography (PE/EtOAc 30/70 v/v) as a colorless oil (1.10 g, 75 %). ^{1}H-NMR (DMSO-d_6) δ 1.70 (m, 2H, $C\underline{H}_2CH_2Ph$), 2.54 (t, 2H, ^{3}J = 7.8 Hz, $C\underline{H}_2Ph$), 3.24 (td, 1H, ^{3}J = 6.6 Hz, ^{2}J = 9.2 Hz, OCH_2), 3.72 (td, 1H, ^{3}J = 6.3 Hz, ^{2}J = 9.2 Hz, OCH_2), 4.09 (s, 1H, H-2), 4.46 (d, 1H, ^{3}J = 6.3 Hz, H-5), 4.72 (d, 1H, ^{3}J = 4.6 Hz, H-3), 4.77 (dd, 1H, ^{3}J = 4.6 Hz, ^{3}J = 6.2 Hz, H-4), 4.92 (s, 1H, H-2), 5.69 (bs, 2H, 2-OH, 5-OH), 7.22 (m, 5H, Ph-C). ^{13}C-NMR (DMSO-d_6) δ 30.44 (-, CH_2), 31.56 (-, CH_2), 66.31 (-, OCH_2), 68.63 (+, C-5), 76.81 (+, C-2), 77.88 (+, C-4), 82.52 (+, C-3), 108.49 (+, C-1), 125.50 (+, Ph-C), 128.11 (+, Ph-C), 128.18 (+, Ph-C), 141.84 (quat, Ph-C), 174.95 (quat, lactone CO). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 312 (100) [$M+NH_4$]$^{+}$. Anal. ($C_{15}H_{18}O_6 \cdot 0.8H_2O$) C, H. $C_{15}H_{18}O_6$ (294.30).

1-*O*-(Octan-1-yl)-β-D-glucofuranosidurono-6,3-lactone[4] (6.3): The title compound was prepared from D-glucurono-6,3-lactone (5 mmol, 0.88 g), 1-octanol (10 mmol, 1.6 ml) and BF_3Et_2O (10 mmol, 1.3 ml) in anhydrous THF (20 ml) according to general procedure 6.5.2.1 and was obtained after flash chromatography (PE/EtOAc 30/70 v/v) as a white solid (1.03 g, 77 %). mp: 65 °C (ref.[23]: 72-73 °C); ^{1}H-NMR (DMSO-d_6) δ 0.86 (t, 3H, ^{3}J = 6.7 Hz, CH_3), 1.15 – 1.59 (m, 12H, $(C\underline{H}_2)_6CH_3$), 3.18 (td, 1H, ^{3}J = 6.8 Hz, ^{2}J = 9.1 Hz, OCH_2), 3.67 (td, 1H, ^{3}J = 6.6 Hz, ^{2}J = 9.3 Hz, OCH_2), 4.04 (s, 1H, H-2), 4.42 (d, 1H, ^{3}J = 6.3 Hz, H-5), 4.69 (d, 1H, ^{3}J = 4.6 Hz, H-3), 4.75 (dd, 1H, ^{3}J = 4.6 Hz, ^{3}J = 6.2 Hz, H-4), 4.89 (s, 1H, H-1). ^{13}C-NMR (DMSO-d_6) δ 13.86 (+, CH_3), 21.99 (-, CH_2), 25.42 (-, CH_2), 28.54 (-, CH_2), 28.57 (-, CH_2), 28.76 (-, CH_2), 31.16 (-, CH_2), 66.77 (-, OCH_2), 68.61 (+, C-5), 76.82 (+, C-2), 77.79

(+, C-4), 82.50 (+, C-3), 108.38 (+, C-1), 174.80 (quat, lactone CO). CI-MS (NH_3) *m/z (%)*: 306 (100) $[M+NH_4]^+$. Anal. ($C_{14}H_{24}O_6$) C, H. $C_{14}H_{24}O_6$ (288.34).

1-*O*-(8-Hydroxyoctan-1-yl)-β-D-glucofuranosidurono-6,3-lactone (6.4): The title compound was prepared from D-glucurono-6,3-lactone (10 mmol, 0.88 g), octan-1,8-diol (20 mmol, 2.9 ml) and BF_3Et_2O (20 mmol, 2.5 ml) in anhydrous THF (30 ml) according to general procedure 6.5.2.1 and was obtained after flash chromatography (PE/EtOAc 30/70 v/v) as a white solid (1.77 g, 58 %). ^{1}H-NMR (DMSO-d_6) δ 1.28 (m, 12H, $OCH_2(C\underline{H}_2)_6$), 3.18 (m, 2H, OCH_2, $CH_2O\underline{H}$), 3.37 (t, 2H, ^3J = 6.5 Hz, $C\underline{H}_2OH$), 3.67 (td, 1H, ^3J = 6.6 Hz, ^2J = 9.2 Hz, OCH_2), 4.04 (s, 1H, H-2), 4.42 (d, 1H, ^3J = 6.3 Hz, H-5), 4.69 (d, 1H, ^3J = 4.5 Hz, H-3), 4.75 (dd, 1H, ^3J = 4.6 Hz, ^3J = 6.2 Hz, H-4), 4.89 (s, 1H, H-1). ^{13}C-NMR (DMSO-d_6) δ 25.37 (-, CH_2), 28.53 (-, CH_2), 28.82 (-, CH_2), 28.85 (-, CH_2), 32.43 (-, CH_2), 60.61 (-, CH_2OH), 66.77 (-, OCH_2), 68.61 (+, CH), 76.81 (+, CH), 77.79 (+, CH), 82.50 (+, CH), 108.37 (+, C-1), 174.81 (quat, lactone CO). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 322 (100) $[M+NH_4]^+$. $C_{14}H_{24}O_7$ (304.34).

1-*O*-(Dodecan-1-yl)-β-D-glucofuranosidurono-6,3-lactone[23] (6.5): The title compound was prepared from D-glucurono-6,3-lactone (20 mmol, 3.52 g), dodecan-1-ol (20 mmol, 3,73 g) and BF_3Et_2O (40 mmol, 5.1 ml) in anhydrous THF (40 ml) according to general procedure 6.5.2.1 and was obtained after flash chromatography (Et_2O) as a white solid (3.64 g, 53 %). mp: 78-80 °C (ref.[23]: 98-99 °C); ^{1}H-NMR ($CDCl_3$) δ 0.87 (t, 3H, ^3J = 6.7 Hz, CH_3), 1.25 (m, 18H, $(C\underline{H}_2)_9CH_3$), 1.52 (m, 2H, $OCH_2C\underline{H}_2$), 3.41 (td, 1H, ^3J = 6.9 Hz, ^2J = 9.3 Hz, OCH_2), 3.67 (td, 1H, ^3J = 6.8 Hz, ^2J = 9.3 Hz, OCH_2), 4.38 (d, 1H, ^3J = 6.9 Hz, H-5), 4.43 (s, 1H, H-2), 4.88 (d, 1H, ^3J = 4.9 Hz, H-3), 5.02 (dd, 1H, ^3J = 4.9 Hz, ^3J = 6.8 Hz, H-4), 5.10 (s, 1H, H-1). ^{13}C-NMR ($CDCl_3$) δ 14.13 (+, CH_3), 22.70 (-, CH_2), 25.89 (-, CH_2), 29.17 (-, CH_2), 29.37 (-, CH_2), 29.56 (-, CH_2), 29.65 (-, CH_2), 29.68 (-, CH_2), 31.93 (-, CH_2), 69.07 (+, C-5), 69.32 (-, OCH_2), 76.98 (+, C-2), 77.53 (+, C-4), 83.17 (+, C-3), 109.09 (+, C-1), 174.55 (quat, lactone CO). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 362 (100) $[M+NH_4]^+$. $C_{18}H_{32}O_6$ (344.44).

1-*O*-(Hexydecan-1-yl)-β-D-glucofuranosidurono-6,3-lactone (6.6): The title compound was prepared from D-glucurono-6,3-lactone (25 mmol, 4.4 g), hexadecan-1-ol (50 mmol, 12.1 g) and BF_3Et_2O (50 mmol, 6.3 ml) in anhydrous THF (50 ml) according to general procedure 6.5.2.1 and was obtained after flash chromatography (PE/Et_2O 20/80 v/v) as a white solid (6.60 g, 66 %). mp: 87-88 °C; ^{1}H-NMR (DMSO-

d$_6$) δ 0.85 (t, 3H, ^3J = 6.7 Hz, CH_3), 1.24 (m, 26H, $(C\underline{H}_2)_{13}CH_3$), 1.38 (m, 2H, $OCH_2C\underline{H}_2$), 3.18 (td, 1H, ^3J = 6.7 Hz, ^2J = 9.1 Hz, OCH_2), 3.67 (td, 1H, ^3J = 6.6 Hz, ^2J = 9.1 Hz, OCH_2), 4.03 (d, 1H, ^3J = 4.0 Hz, H-2), 4.42 (dd, 1H, ^3J = 6.4 Hz, ^3J = 6.4 Hz, H-5), 4.69 (d, 1H, ^3J = 4.6 Hz, H-3), 4.75 (dd, 1H, ^3J = 4.6 Hz, ^3J = 6.2 Hz, H-4), 4.89 (s, 1H, H-1), 5.66 (d, 1H, ^3J = 4.1 Hz, 2-OH), 5.80 (d, 1H, ^3J = 6.4 Hz, 5-OH). ^{13}C-NMR (DMSO-*d*$_6$) δ 13.85 (+, CH_3), 22.00 (-, CH_2), 25.43 (-, CH_2), 28.55 (-, CH_2), 28.61 (-, CH_2), 28.83 (-, CH_2), 28.96 (+, CH_2), 31.20 (-, CH_2), 66.76 (-, OCH_2), 68.61 (+, C-5), 76.82 (+, C-2), 77.79 (+, C-4), 82.49 (+, C-3), 108.37 (+, C-1), 174.79 (quat, lactone CO). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 418 (100) $[M+NH_4]^+$. Anal. ($C_{22}H_{40}O_6$) C, H. $C_{22}H_{40}O_6$ (400.55).

6.5.2.2 General method for the synthesis of 1-*O*-alkyl-β-D-glucofuranosid-uronic acids 6.7-6.10 *via* saponification of 1-*O*-alkyl-D-glucurono-6,3-lactones

To a solution of the pertinent D-glucurono-6,3-lactone (1 eq) in acetone was added 0.5 M NaOH (1 eq) dropwise at 0 °C. After stirring for 2 h at room temperature, ether was added and the mixture was washed twice with water. After adding EtOAc, the volatiles were removed under reduced pressure and the remaining raw product was subjected to flash chromatography.

1-*O*-(Octan-1-yl)-β-D-glucofuranosiduronic acid sodium salt[23] (6.7): The title compound was prepared from **6.3** (3.0 mmol, 0.87 g), 0.5 M (3 mmol, 6.0 ml) in acetone (40 ml) according to general procedure 6.5.2.2 and was obtained after flash chromatography ($CHCl_3$/MeOH 80/20 v/v) as a yellow solid (0.46 g, 47 %). mp: 30 °C; ^{1}H-NMR (CD_3OD) δ 0.90 (t, 3H, ^3J = 6.7 Hz, CH_3), 1.32 (m, 10H, $(C\underline{H}_2)_3CH_3$), 1.61 (m, 2H, $OCH_2C\underline{H}_2$), 3.46 (td, 1H, ^3J = 7.0 Hz, ^2J = 9.5 Hz, OCH_2), 3.81 (td, 1H, ^3J = 6.7 Hz, ^2J = 9.6 Hz, OCH_2), 4.10 (s, 1H, H-2), 4.19 (m, 1H, H-3), 4.38 (d, 1H, ^3J = 2.1 Hz, H-5), 4.56 (dd, 1H, ^3J = 2.4 Hz, ^3J = 5.3 Hz, H-4), 4.86 (d, 1H, ^3J = 1.9 Hz, H-1). ^{13}C-NMR (CD_3OD) δ 14.52 (+, CH_3), 23.80 (-, CH_2), 27.25 (-, CH_2), 30.53 (-, CH_2), 30.68 (-, CH_2), 30.70 (-, CH_2), 33.11 (-, CH_2), 70.17 (-, OCH_2), 74.20 (+, C-5), 77.79 (+, C-3), 81.92 (+, C-2), 83.45 (+, C-4), 109.52 (+, C-1), 178.62 (quat, CO_2H). ES-MS (MeOH + NH_4OAc) *m/z (%)*: 324 (100) $[M+NH_4]^+$. Anal. ($C_{14}H_{25}NaO_7 \cdot 0.6H_2O$) C, H. $C_{14}H_{25}NaO_7$ (328.33).

1-*O*-(8-Hydroxyoctan-1-yl)-β-D-glucofuranosiduronic acid sodium salt (6.8): The title compound was prepared from **6.4** (3.0 mmol, 0.91 g), 0.5 M (3 mmol, 6.0 ml) in acetone (40 ml) according to general procedure 6.5.2.2 and was obtained after flash

chromatography ($CHCl_3$/MeOH 80/20 v/v) as a white solid (0.40 g, 41 %). mp: 123-125 °C; ^{1}H-NMR (CD_3OD) δ 1.29 – 1.64 (m, 8H, $OCH_2(C\underline{H}_2)_6$), 3.41 (td, 1H, ^3J = 6.8 Hz, ^2J = 8.3 Hz, OCH_2), 3.53 (t, 2H, ^3J = 6.6 Hz, $C\underline{H}_2OH$), 3.79 (td, 1H, ^3J = 6.6 Hz, ^2J = 9.5 Hz, OCH_2), 4.05 (s, 1H, H-2), 4.13 (dd, 1H, ^3J = 2.7 Hz, ^3J = 5.5 Hz, H-3), 4.34 (d, 1H, ^3J = 2.5 Hz, H-5), 4.53 (dd, 1H, ^3J = 2.7 Hz, ^3J = 5.6 Hz, H-4), 4.84 (d, 1H, ^3J = 1.6 Hz, H-1). ^{13}C-NMR (DMSO-d_6) δ 25.36 (-, CH_2), 25.51 (-, CH_2), 28.84 (-, CH_2), 28.90 (-, CH_2), 28.96 (-, CH_2), 32.43 (-, CH_2), 60.59 (-, CH_2OH), 66.89 (-, OCH_2), 71.08 (+, C-5), 75.81 (+, C-3), 80.93 (+, C-2), 83.07 (+, C-4), 107.54 (+, C-1), 173.50 (quat, CO_2H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 321 (100) $[M-H]^-$. $C_{14}H_{25}NaO_8$ (344.33).

1-*O*-(Dodecan-1-yl)-β-D-glucofuranosiduronic acid sodium salt[23] (6.9): The title compound was prepared from **6.5** (3.0 mmol, 0.87 g), 0.5 M (3 mmol, 6.0 ml) in acetone (40 ml) according to general procedure 6.5.2.2 and was obtained after flash chromatography ($CHCl_3$/MeOH 85/15 v/v) as a white solid (0.44 g, 40 %). mp: 83-85 °C; ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H, ^3J = 6.6 Hz, CH_3), 1.24 (s, 18H, $(C\underline{H}_2)_9CH_3$), 1.44 (m, 2H, $OCH_2C\underline{H}_2$), 3.20 (td, 1H, ^3J = 6.9 Hz, ^2J = 9.4 Hz, OCH_2), 3.55 (td, 1H, ^3J = 6.5 Hz, ^2J = 9.2 Hz, OCH_2), 3.71 (s, 1H, H-2), 3.77 (d, 1H, ^3J = 2.9 Hz, H-3), 3.92 (s, 1H, H-5), 4.23 (d, 1H, ^3J = 3.8 Hz, H-4), 4.60 (d, 1H, ^3J = 1.6 Hz, H-1), 5.18 (d, 1H, ^3J = 3.9 Hz, 2-OH). ^{13}C-NMR (DMSO-d_6) δ 13.92 (+, CH_3), 22.05 (-, CH_2), 25.63 (-, CH_2), 28.68 (-, CH_2), 28.96 (-, CH_2), 28.99 (-, CH_2), 29.05 (-, CH_2), 31.26 (-, CH_2), 66.93 (-, OCH_2), 71.03 (+, C-5), 75.90 (+, C-3), 81.09 (+, C-2), 83.20 (+, C-4), 107.65 (+, C-1), 173.29 (quat, CO_2H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 380 (100) $[M+NH_4]^+$. Anal. ($C_{18}H_{33}NaO_7 \cdot 0.6H_2O$) C, H. $C_{18}H_{33}NaO_7$ (384.44).

1-*O*-(Hexadecan-1-yl)-β-D-glucofuranosiduronic acid (6.10): The title compound was prepared from **6.6** (1.0 mmol, 0.87 g), 0.5 M (3 mmol, 6.0 ml) in acetone (40 ml) according to general procedure 6.5.2.2 and was obtained after acidification using an acidic ion exchanger (Amberlite® CG-120, protonated prior to use) and recrystallisation from EtOH as a yellow solid (0.25 g, 59 %). mp: 90-91 °C; ^{1}H-NMR (CD_3OD) δ 0.90 (t, 3H, ^3J = 6.7 Hz, CH_3), 1.28 (s, 26H, $(C\underline{H}_2)_{13}CH_3$), 1.54 (m, 2H, $OCH_2C\underline{H}_2$), 3.36 (td, 1H, ^3J = 5.8 Hz, ^3J = 9.4 Hz, OCH_2), 3.78 (td, 1H, ^3J = 5.8 Hz, ^3J = 9.4 Hz, OCH_2), 4.03 (s, 1H, C<u>H</u>OH), 4.13 (m, 1H, C<u>H</u>OH), 4.40 (m, 2H, C<u>H</u>OH), 4.85 (m, 1H under solvent peak, C<u>H</u>OH). ^{13}C-NMR (DMSO-d_6) δ 13.84 (+, CH_3), 21.99 (-, CH_2), 25.56 (-, CH_2), 28.61 (-, CH_2), 28.79 (-, CH_2), 28.96 (-, CH_2), 31.20 (-, CH_2), 66.96 (-, OCH_2), 69.11 (+, C-5), 74.87 (+, C-3), 80.10 (+, C-2), 81.82 (+, C-4),

108.41 (+, C-1), 173.90 (quat, CO_2H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 417 (100) $[M-H]^-$. $C_{22}H_{42}O_7$ (418.56).

6.5.2.3 General procedure for the synthesis of 1-*O*-Alkyl-β-D-glucofuranosid-uronamides 6.11 and 6.12

A solution of the pertinent 1-O-Alkyl-β-D-glucofuranosidurono-6,3-lactone (1 eq) in 7 N NH_3 in methanol was stirred at 0 °C for 2 h. The product was obtained after removal of the solvent.

1-*O*-(Dodecan-1-yl)-β-D-glucofuranosiduronamide (6.11): The title compound was prepared according to general procedure 6.5.2.3 using **6.5** (1 mmol, 0.34 g) in 7 N NH_3 in methanol (10 ml) and was obtained as a white solid (0.36 g, quantitative). mp: 80-82 °C; ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H, ^3J = 6.7 Hz, CH_3), 1.24 (m, 18H, $(C\underline{H}_2)_9CH_3$), 1.46 (m, 2H, $OCH_2C\underline{H}_2$), 3.25 (td, 1H, ^3J = 6.7 Hz, ^3J = 9.5 Hz, OCH_2), 3.62 (td, 1H, ^3J = 6.6 Hz, ^3J = 9.4 Hz, OCH_2), 3.79 (s, 1H, H-2), 3.88 (dd, 1H, ^3J = 4.0 Hz, ^3J = 4.0 Hz, H-3), 4.15 (m, 2H, H-5, H-4), 4.70 (s, 1H, H-1), 5.25 (m, 3H, OH), 7.25 (d, 2H, ^3J = 17.7 Hz, NH_2). ^{13}C-NMR (DMSO-d_6) δ 13.83 (+, CH_3), 21.98 (-, CH_2), 25.55 (-, CH_2), 28.61 (-, CH_2), 28.83 (-, CH_2), 28.96 (-, CH_2), 31.19 (-, CH_2), 67.11 (-, OCH_2), 70.21 (+, C-5), 75.58 (+, C-3), 80.25 (+, CH, C-2), 82.14 (+, C-4), 108.29 (+, C-1), 174.39 (quat, $CONH_2$). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 362 (100) $[M+H]^+$. Anal. ($C_{18}H_{35}NO_6$) C, H, N. $C_{18}H_{35}NO_6$ (361.47).

1-*O*-(Hexadecan-1-yl)-β-D-glucofuranosiduronamide (6.12): The title compound was prepared according to general procedure 6.5.2.3 using **6.6** (1 mmol, 0.40 g) in 7 N NH_3 in methanol (10 ml) and was obtained as a white solid (0.42 g, quantitative). mp: 77-78 °C; ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H, ^3J = 6.7 Hz), 1.24 (m, 24H, $(C\underline{H}_2)_{13}CH_3$), 1.46 (m, 2H, $OCH_2C\underline{H}_2$), 3.25 (td, 1H, ^3J = 6.7 Hz, ^3J = 9.4 Hz, OCH_2), 3.62 (td, 1H, ^3J = 6.6 Hz, ^3J = 9.4 Hz, OCH_2), 3.79 (s, 1H, H-2), 3.88 (t, 1H, ^3J = 4.6 Hz, H-3), 4.12 (dd, 1H, ^3J = 5.6 Hz, ^3J = 5.6 Hz, H-5), 4.18 (m, 1H, H-4), 4.70 (s, 1H, H-1), 5.18 (d, 1H, ^3J = 6.0 Hz, 2-OH), 5.30 (dd, 2H, ^3J = 4.6 Hz, ^3J = 11.7 Hz, OH), 7.26 (d, 2H, ^3J = 18.1 Hz, NH_2). ^{13}C-NMR (DMSO-d_6) δ 13.84 (+, CH_3), 21.99 (-, CH_2), 25.56 (-, CH_2), 28.60 (-, CH_2), 28.84 (-, CH_2), 28.95 (-, CH_2), 31.19 (-, CH_2), 67.10 (-, OCH_2), 70.20 (+, C-5), 75.56 (+, C-3), 80.25 (+, C-2), 82.15 (+, C-4), 108.29 (+, C-1), 174.39 (quat, $CONH_2$). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 418 (100) $[M+H]^+$. $C_{22}H_{43}NO_6$ (417.58).

6.5.2.4 General procedure for the synthesis of 1-*O*-alkyl-*N*-alkyl-β-D-glucofuranosiduronamides 6.13-6.22

A solution of **6.6** (1 eq) and the pertinent amine (1-3 eq) in methanol were stirred at room temperature overnight. The solvent was evaporated and the remaining residue was subjected to flash chromatography.

1-*O*-(Hexadecan-1-yl)-*N*-benzyl-β-D-glucofuranosiduronamide (6.13): The title compound was prepared from **6.6** (0.7 mmol, 0.28 g) and benzylamine (1.05 mmol, 0.12 ml) in MeOH (5 ml) according to general procedure 6.5.2.4 and was obtained after flash chromatography (PE/EtOAc 70/30 v/v) as a white solid (0.18 g, 51 %). mp: 68 °C; ^{1}H-NMR (CD_3OD) δ 0.89 (t, 3H, ^{3}J = 6.1 Hz, CH_3), 1.28 (m, 26H, $(C\underline{H}_2)_{13}CH_3$), 1.54 (m, 2H, $OCH_2C\underline{H}_2$), 3.34 (s, 1H, H-2), 3.71 (td, 1H, ^{3}J = 6.7 Hz, ^{2}J = 9.4 Hz, OCH_2), 4.01 (s, 1H, H-3), 4.09 (td, 1H, ^{3}J = 7.2 Hz, ^{2}J = 8.6 Hz, OCH_2), 4.44 (m, 4H, $C\underline{H}_2$Ph, H-4, H-5), 4.86 (s, 1H, H-1), 7.27 (m, 5H, Ph-H). ^{13}C-NMR (CD_3OD) δ 14.52 (+, CH_3), 23.05 (-, CH_2), 23.81 (-, CH_2), 27.26 (-, CH_2), 30.55 (-, CH_2), 30.62 (-, CH_2), 30.67 (-, CH_2), 30.86 (-, CH_2), 33.14 (-, CH_2), 43.76 (-, $\underline{C}H_2$Ph), 69.55 (-, OCH_2), 72.38 (+, C-5), 77.62 (+, C-3), 81.87 (+, C-2), 84.06 (+, C-4), 109.99 (+, C-1), 128.17 (+, Ph-C), 128.45 (+, Ph-C), 129.51 (+, Ph-C), 139.87 (quat, Ph-C), 174.74 (quat, CONH). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 508 (100) $[M+NH_4]^+$. Anal. ($C_{29}H_{49}NO_6$) C, H, N. $C_{29}H_{49}NO_6$ (507.70).

1-*O*-(Hexadecan-1-yl)-*N*-(2-phenylethyl)-β-D-glucofuranosiduronamide (6.14): The title compound was prepared from **6.6** (0.7 mmol, 0.28 g) and 2-phenylethanamine (1.05 mmol, 0.13 ml) in MeOH (5 ml) according to general procedure 6.5.2.4 and was obtained after flash chromatography (PE/EtOAc 70/30 v/v) as a white solid (0.17 g, 47 %). mp: 45 °C; ^{1}H-NMR (CD_3OD) δ 0.90 (t, 3H, ^{3}J = 6.7 Hz, CH_3), 1.28 (m, 26H, $(C\underline{H}_2)_{13}CH_3$), 1.56 (m, 2H, $OCH_2C\underline{H}_2$), 2.82 (t, 2H, ^{3}J = 7.5 Hz, $C\underline{H}_2$Ph), 3.34 (m, 1H, OCH_2), 3.46 (t, 2H, ^{3}J = 7.3 Hz, $NHC\underline{H}_2$), 3.72 (td, 1H, ^{3}J = 6.7 Hz, ^{2}J = 9.4 Hz, OCH_2), 4.00 (s, 1H, H-2), 4.07 (m, 1H, H-3), 4.33 (d, 1H, ^{3}J = 5.2 Hz, H-5), 4.43 (dd, 1H, ^{3}J = 5.0 Hz, ^{3}J = 5.0 Hz, H-4), 4.84 (s, 1H, H-1), 7.23 (m, 5H, Ph-H). ^{13}C-NMR (CD_3OD) δ 14.51 (+, CH_3), 23.80 (-, CH_2), 27.32 (-, CH_2), 30.55 (-, CH_2), 30.68 (-, CH_2), 30.69 (-, CH_2), 30.85 (-, CH_2), 33.14 (-, CH_2), 36.66 (-, $\underline{C}H_2$Ph), 41.87 (-, $NHCH_2$), 69.51 (-, OCH_2), 72.34 (+, C-5), 77.72 (+, C-3), 81.84 (+, C-2), 84.12 (+, C-4), 109.95 (+, C-1), 127.44 (+, Ph-C), 129.58 (+, Ph-C), 129.88 (+, Ph-C), 140.47 (quat, Ph-C), 174.65 (quat, lactone CO). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 522 (100) $[M+NH_4]^+$. Anal. ($C_{30}H_{51}NO_6$) C, H, N. $C_{30}H_{51}NO_6$ (521.73).

1-*O*-(Hexadecan-1-yl)-*N*-butyl-β-D-glucofuranosiduronamide (6.15): The title compound was prepared from **6.6** (1.0 mmol, 0.40 g) and butan-1-amine (2 mmol, 0.20 ml) in MeOH (5 ml) according to general procedure 6.5.2.4 and was obtained after flash chromatography (PE/EtOAc 90/10 v/v) as a white solid (0.36 g, 76 %). mp: 50 °C; ^{1}H-NMR (CD_3OD) δ 0.92 (m, 6H, CH_3), 1.27 – 1.63 (m, 32H, $NHCH_2(C\underline{H}_2)_2CH_3$, $(C\underline{H}_2)_{13}CH_3$), 3.24 (m, 2H, $NHCH_2$), 3.36 (td, 1H, ^{3}J = 6.8 Hz, ^{2}J = 9.5 Hz, OCH_2), 3.75 (td, 1H, ^{3}J = 6.7 Hz, ^{2}J = 9.4 Hz, OCH_2), 3.99 (s, 1H, H-2), 4.07 (dd, 1H, ^{3}J = 1.7 Hz, ^{3}J = 4.8 Hz, H-3), 4.34 (d, 1H, ^{3}J = 5.2 Hz, H-5), 4.42 (dd, 1H, ^{3}J = 5.0 Hz, ^{3}J = 5.0 Hz, H-4), 4.85 (s, 1H, H-1). ^{13}C-NMR (CD_3OD) δ 14.19 (+, CH_3), 14.49 (+, CH_3), 21.10 (-, CH_2), 23.78 (-, CH_2), 27.30 (-, CH_2), 30.52 (-, CH_2), 30.67 (-, CH_2), 30.84 (-, CH_2), 32.62 (-, CH_2), 33.12 (-, CH_2), 39.95 (-, $NHCH_2$), 69.54 (-, OCH_2), 72.38 (+, CH), 77.71 (+, CH), 81.89 (+, CH), 84.05 (+, CH), 109.96 (+, C-1), 174.52 (quat, lactone CO). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 475 (100) $[M+H]^+$. Anal. ($C_{30}H_{51}NO_6$) C, H, N. $C_{26}H_{51}NO_6$ (473.69).

1-*O*-(Hexadecan-1-yl)-*N*-(decan-1-yl)-β-D-glucofuranosiduronamide (6.16): The title compound was prepared from **6.6** (0.7 mmol, 0.28 g) and decan-1-amine (1.05 mmol, 0.21 ml) in MeOH (5 ml) according to general procedure 6.5.2.4 and was obtained after flash chromatography (PE/EtOAc 70/30 v/v) as a pale yellow solid (0.12 g, 31 %). mp: 45 °C; ^{1}H-NMR (CD_3OD) δ 0.87 (t, 6H, ^{3}J = 6.6 Hz, CH_3), 1.25 (m, 40H, $(C\underline{H}_2)_{13}CH_3$, $(C\underline{H}_2)_7CH_3$), 1.54 (m, 4H, $OCH_2C\underline{H}_2$, $NHCH_2C\underline{H}_2$), 3.28 (m, 2H, $NHC\underline{H}_2$), 3.45 (td, 1H, ^{3}J = 6.7 Hz, ^{2}J = 9.4 Hz, OCH_2), 3.72 (td, 1H, ^{3}J = 6.8 Hz, ^{3}J = 9.3 Hz, OCH_2), 4.19 (s, 1H, H-2), 4.24 (d, 1H, ^{3}J = 3.8 Hz, H-3), 4.32 (d, 1H, ^{3}J = 6.7 Hz, H-5), 4.46 (dd, 1H, ^{3}J = 5.0 Hz, ^{3}J = 6.2 Hz, H-4), 4.98 (s, 1H, H-1), 6.89 (t, 1H, ^{3}J = 5.7 Hz, CONH). ^{13}C-NMR (CD_3OD) δ 14.12 (+, CH_3), 22.70 (-, CH_2), 26.10 (-, CH_2), 26.92 (-, CH_2), 29.34 (-, CH_2), 29.38 (-, CH_2), 29.45 (-, CH_2), 29.54 (-, CH_2), 29.59 (-, CH_2), 29.72 (-, CH_2), 31.94 (-, CH_2), 39.55 (-, $NHCH_2$), 68.99 (-, OCH_2), 70.31 (+, C-5), 77.10 (+, C-3), 80.16 (+, C-2), 82.38 (+, C-4), 108.44 (+, C-1), 171.99 (quat, CONH). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 557 (100) $[M-H]^-$. Anal. ($C_{32}H_{63}NO_6$) C, H, N. $C_{32}H_{63}NO_6$ (557.85).

1-*O*-(Hexadecan-1-yl)-*N*-(2-hydroxyethyl)-β-D-glucofuranosiduronamide (6.17): The title compound was prepared from **6.6** (0.7 mmol, 0.28 g) and 2-aminoethanol (1.05 mmol, 63 µl) in MeOH (5 ml) according to general procedure 6.5.2.4 and was obtained after flash chromatography ($CHCl_3$/MeOH 95/5 v/v) as a pale yellow solid (0.23 g, 71 %). mp: 65-66 °C; ^{1}H-NMR (CD_3OD) δ 0.90 (t, 3H, ^{3}J = 6.7 Hz, CH_3),

1.29 (m, 26H, ($C\underline{H}_2$)$_{13}CH_3$), 1.58 (m, 2H, $OCH_2C\underline{H}_2$), 3.37 (m, 3H, OCH_2, $NHC\underline{H}_2$), 3.62 (dd, 2H, ^3J = 5.8 Hz, ^3J = 5.8 Hz, $C\underline{H}_2OH$), 3.77 (td, 1H, ^3J = 6.6 Hz, ^2J = 9.4 Hz, OCH_2), 4.00 (s, 1H, H-2), 4.08 (dd, 1H, ^4J = 1.8 Hz, ^3J = 4.9 Hz, H-3), 4.37 (d, 1H, ^3J = 5.1 Hz, H-5), 4.46 (dd, 1H, ^3J = 5.0 Hz, ^3J = 5.0 Hz, H-4), 4.86 (s, 1H, H-1). ^{13}C-NMR (CD_3OD) δ 14.51 (+, CH_3), 23.81 (-, CH_2), 27.31 (-, CH_2), 30.55 (-, CH_2), 30.66 (-, CH_2), 30.70 (-, CH_2), 30.84 (-, CH_2), 30.86 (-, CH_2), 33.14 (-, CH_2), 42.59 (-, $NHCH_2$), 61.56 (-, CH_2OH), 69.51 (-, OCH_2), 72.46 (+, C-5), 77.79 (+, C-3), 81.81 (+, C-2), 84.01 (+, C-4), 109.90 (+, C-1), 174.99 (quat, CONH). ES-MS (MeOH + NH_4OAc) *m/z (%)*: 462 (100) $[M+H]^+$. Anal. ($C_{24}H_{47}NO_7$) C, H, N. $C_{24}H_{47}NO_7$ (461.63).

1-*O*-(Hexadecan-1-yl)-*N*-(4-aminobutyl)-β-D-glucofuranosiduronamide (6.18): The title compound was prepared from **6.6** (0.7 mmol, 0.28 g) and butan-1,4-diamine (1.05 mmol, 92 mg) in MeOH (5 ml) according to general procedure 6.5.2.4 and was obtained after flash chromatography (MeOH/aqueous NH_3 90/10 v/v) as a yellow solid (0.20 g, 58 %). mp: 140-142 °C; ^{1}H-NMR (CD_3OD) δ 0.90 (t, 3H, ^3J = 6.0 Hz, CH_3), 1.29 (m, 26H, ($C\underline{H}_2$)$_{13}CH_3$), 1.55 (m, 6H, $OCH_2C\underline{H}_2$, $NHCH_2(C\underline{H}_2)_2$), 2.67 (t, 2H, ^3J = 6.8 Hz, $C\underline{H}_2NH_2$), 3.26 (t, 2H, ^3J = 6.6 Hz, $CONHC\underline{H}_2$), 3.37 (td, 1H, ^3J = 6.7 Hz, ^2J = 9.4 Hz, OCH_2) 3.76 (td, 1H, ^3J = 6.7 Hz, ^2J = 9.5 Hz, OCH_2), 3.99 (s, 1H, H-2), 4.09 (m, 1H, H-3), 4.34 (d, 1H, ^3J = 5.1 Hz, H-5), 4.45 (t, 1H, ^3J = 5.0 Hz, H-4), 4.85 (s, 1H, H-1). ^{13}C-NMR (CD_3OD) δ 14.51 (+, CH_3), 27.33 (-, CH_2), 27.81 (-, CH_2), 30.54 (-, CH_2), 23.80 (-, CH_2), 30.68 (-, CH_2), 30.86 (-, CH_2), 33.14 (-, CH_2), 39.82 (-, NCH_2), 42.04 (-, NCH_2), 69.50 (-, OCH_2), 72.53 (+, C-5), 77.77 (+, C-3), 81.88 (+, C-2), 83.91 (+, C-4), 109.93 (+, C-1), 174.67 (quat, CONH). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 489 (100) $[M+NH_4]^+$. $C_{26}H_{52}N_2O_6$ (488.70).

1-*O*-(Hexadecan-1-yl)-*N*-(12-aminododecan-1-yl)-β-D-glucofuranosiduronamide (6.19): The title compound was prepared from **6.6** (0.7 mmol, 0.28 g) and dodecan-1,12-diamine (1.05 mmol, 0.21 g) in MeOH (8 ml) according to general procedure 6.5.2.4 and was obtained after flash chromatography (MeOH/aqueous NH_3 95/5 v/v) as a yellow solid (0.16 g, 38 %). mp: 66 °C; ^{1}H-NMR ($CDCl_3$) δ 0.87 (t, 3H, ^3J = 6.6 Hz, CH_3), 1.17 – 1.61 (m, 48H, ($C\underline{H}_2$)$_{14}CH_3$, $NHCH_2(C\underline{H}_2)_{10}$), 2.69 (t, 2H, ^3J = 6.6 Hz, $C\underline{H}_2NH_2$), 3.29 (m, 2H, $CONHC\underline{H}_2$), 3.45 (td, 1H, ^3J = 6.7 Hz, ^2J = 9.1 Hz, OCH_2), 3.72 (td, 1H, ^3J = 6.8 Hz, ^2J = 9.2 Hz, OCH_2), 4.17 (s, 1H, H-2), 4.23 (d, 1H, ^3J = 3.6 Hz, H-3), 4.31 (d, 1H, ^3J = 6.6 Hz, H-5), 4.44 (dd, 1H, ^3J = 4.9 Hz, ^3J = 6.1 Hz, H-4), 4.96 (s, 1H, H-1), 6.90 (t, 1H, ^3J = 5.6 Hz, CONH). ^{13}C-NMR ($CDCl_3$) δ 14.14 (+, CH_3), 22.71 (-, CH_2), 26.12, (-, CH_2) 26.55 (-, CH_2), 26.61 (-, CH_2), 28.89 (-, CH_2),

29.03 (-, CH_2), 29.07 (-, CH_2), 29.10 (-, CH_2), 29.12 (-, CH_2), 29.24 (-, CH_2), 29.38 (-, CH_2), 29.45 (-, CH_2), 29.57 (-, CH_2), 29.61 (-, CH_2), 29.69 (-, CH_2), 29.72 (-, CH_2), 31.94 (-, CH_2), 32.66 (-, CH_2), 39.36, (-, NCH_2), 41.74 (-, NCH_2), 68.92 (-, OCH_2), 70.26 (+, C-5), 77.23 (+, C-3), 79.96 (+, C-2), 82.52 (+, C-4), 108.67 (+, C-1), 172.09 (quat, CONH). ES-MS (MeOH + NH_4OAc) *m/z (%)*: 602 (100) $[M+H]^+$. $C_{34}H_{68}N_2O_6$ (600.91).

6.5.2.5 General procedure for the synthesis of methyl esters 6.20a-6.22a

To a solution of carboxylic acid (1eq) in anhydrous methanol under nitrogen atmosphere was added chlorotrimethylsilane (2.2 eq) at 0 °C. The mixture was stirred at reflux overnight, cooled, and evaporated under reduced pressure.

Methyl 3-aminopropanoate hydrochloride[10] (6.20a): The title compound was prepared from β-alanine (40 mmol, 3.56 g) and TMSCl (88 mmol, 11.1 ml) in anhydrous MeOH (50 ml) according to general procedure 6.5.2.5 and was obtained as a white solid (5.58 g, quantitative). mp: 65 °C; ^{1}H-NMR (D_2O) δ 2.76 (t, 2H, ^3J = 6.5 Hz, $C\underline{H}_2CO_2CH_3$), 3.23 (t, 2H, ^3J = 6.4 Hz, $C\underline{H}_2NH_2$), 3.68 (s, 3H, CO_2CH_3). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 104 (100) $[M+H]^+$. $C_4H_{10}ClNO_2$ (139.58).

1-*O*-(Hexadecan-1-yl)-*N*-(2-methoxycarbonylethyl)-β-D-glucofuranosiduron-amide (6.20): The title compound was prepared from **6.6** (1 mmol, 0.40 g), **6.20a** (3 mmol, 0.42 g) and NEt_3 (3 mmol, 0.42 ml) in MeOH (10 ml) according to general procedure 6.5.2.4 and was obtained after flash chromatography (EtOAc) as yellow semisolid substance (0.33 g, 66 %). ^{1}H-NMR ($CDCl_3$) δ 0.87 (t, 3H, ^3J = 6.7 Hz, CH_3), 1.25 (m, 26H, $(C\underline{H}_2)_{13}CH_3$), 1.58 (m, 2H, $OCH_2C\underline{H}_2$), 2.57 (t, 2H, ^3J = 6.0 Hz, $C\underline{H}_2CO_2CH_3$), 3.43 – 3.67 (m, 3H, OCH_2, $NHC\underline{H}_2$), 3.69 (s, 3H, OCH_3), 3.76 (td, 1H, ^3J = 6.8 Hz, ^2J = 9.5 Hz, OCH_2), 4.21 (d, 1H, ^3J = 1.5 Hz, H-2), 4.25 (dd, 1H, ^3J = 1.8 Hz, ^3J = 4.8 Hz, H-3), 4.32 (d, 1H, ^3J = 6.7 Hz, H-5), 4.48 (dd, 1H, ^3J = 4.9 Hz, ^3J = 6.6 Hz, H-4), 4.99 (s, 1H, H-1), 7.34 (t, 1H, ^3J = 6.1 Hz, CONH). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 505 (100) $[M+H]^+$. Anal. ($C_{26}H_{49}NO_8$) C, H, N. $C_{26}H_{49}NO_8$ (503.67).

Methyl 4-aminobutanoate hydrochloride[9] (6.21a): The title compound was prepared from 4-aminobutyric acid (40 mmol, 4.13 g) and TMSCl (88 mmol, 11.1 ml) in anhydrous MeOH (50 ml) according to general procedure 6.5.2.5 and was obtained as a white solid (6.14 g, quantitative). mp: 94 °C; ^{1}H-NMR (D_2O) δ 1.85 (m, 2H, $COCH_2C\underline{H}_2$), 2.42 (t, 2H, ^3J = 7.3 Hz, $COCH_2$), 2.94 (t, 2H, ^3J = 7.7 Hz, $C\underline{H}_2NH_2$), 3.60 (s, 3H, $CO_2C\underline{H}_3$). CI-MS (NH_3) *m/z (%)*: 118 (100) $[M+H]^+$. $C_5H_{12}ClNO_2$ (153.61).

1-*O*-(Hexadecan-1-yl)-*N*-(3-methoxycarbonylpropyl)-β-D-glucofuranosiduronamide (6.21): The title compound was prepared from **6.6** (1 mmol, 0.40 g), **6.21a** (3 mmol, 0.47 g) and NEt_3 (3 mmol, 0.42 ml) in MeOH (10 ml) according to general procedure 6.5.2.4 and was obtained after flash chromatography (EtOAc) as yellow semisolid (0.31 g, 60 %). ^{1}H-NMR (DMSO-d_6) δ 0.90 (t, 3H, ^3J = 6.7 Hz, CH_3), 1.29 (s, 26H, $(C\underline{H}_2)_{13}CH_3$), 1.58 (m, 2H, $OCH_2C\underline{H}_2$), 1.82 (m, 2H, $NHCH_2C\underline{H}_2$), 2.39 (t, 2H, ^3J = 7.5 Hz, $C\underline{H}_2CO_2CH_3$), 3.27 (m, 2H, $NHC\underline{H}_2$), 3.36 (td, 1H, ^3J = 6.7 Hz, ^2J = 9.5 Hz, OCH_2), 3.66 (s, 3H, OCH_3), 3.76 (td, 1H, ^3J = 6.7 Hz, ^2J = 9.4 Hz, OCH_2), 3.99 (s, 1H, H-2), 4.07 (d, 1H, ^3J = 5.0 Hz, H-3), 4.34 (d, 1H, ^3J = 5.4 Hz, H-5), 4.42 (t, 1H, ^3J = 5.1 Hz, H-4), 4.85 (s, 1H, H-1). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 519 (100) $[M+H]^+$. Anal. ($C_{27}H_{51}NO_8$) C, H, N. $C_{27}H_{51}NO_8$ (517.70).

Methyl *trans*-4-(aminomethyl)cyclohexanecarboxylate[8] (6.22a): The title compound was prepared from *trans*-4-(aminomethyl)cyclohexanecarboxylic acid (10 mmol, 1.57 g) and TMSCl (22 mmol, 2.8 ml) in anhydrous MeOH (10 ml) according to general procedure 6.5.2.5 and was obtained as a white solid (2.1 g, quantitative). mp: 164 °C; ^{1}H-NMR (D_2O) δ 0.99 (m, 2H, cy-H), 1.32 (m, 2H, cy-H), 1.57 (m, 1H, cy-H), 1.77 (m, 2H, cy-H), 1.92 (m, 2H, cy-H), 2.30 (m, 1H, cy-H), 2.78 (d, 2H, ^3J = 7.0 Hz, $C\underline{H}_2NH_2$), 3.59 (s, 3H, CO_2CH_3). EI-MS (70eV) *m/z (%)*: 171 (10) $[M^{+\cdot}]$.

1-*O*-(Hexadecan-1-yl)-*N*-(trans-4-methoxycarbonylcyclohexylmethyl)-β-D-glucofuranosiduronamide (6.22): The title compound was prepared from **6.6** (1 mmol, 0.40 g), **6.22a** (3 mmol, 0.62 g) and NEt_3 (3 mmol, 0.42 ml) in MeOH (10 ml) according to general procedure 6.5.2.4 and was obtained after flash chromatography (EtOAc) as a white solid (0.38 g, 66 %). mp: 66 °C; ^{1}H-NMR (CD_3OD) δ 0.90 (t, 3H, ^3J = 6.7 Hz, CH_3), 1.01 (m, 2H, cy-H), 1.41 (m, 10 H, $(C\underline{H}_2)_{13}CH_3$, cy-H, $OCH_2C\underline{H}_2$), 1.86 (m, 2H, cy-H), 1.97 (m, 2H, cy-H), 2.27 (m, 1H, cy-H), 3.10 (m, 2H, $C\underline{H}_2NH$), 3.36 (td, 1H, ^3J = 6.8 Hz, ^2J = 9.5 Hz, OCH_2), 3.64 (s, 3H, CO_2CH_3), 3.75 (td, 1H, ^3J = 6.7 Hz, ^2J = 9.4 Hz, OCH_2), 3.99 (s, 1H, H-2), 4.07 (dd, 1H, ^3J = 1.7 Hz, ^3J = 4.7 Hz, H-3), 4.34 (d, 1H, ^3J = 5.5 Hz, H-5), 4.41 (dd, ^3J = 5.1 Hz , ^3J = 5.1 Hz, 1H, H-4), 4.85 (s, 1H, H-1). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 573 (100) $[M+H]^+$. Anal. ($C_{31}H_{57}NO_8$) C, H, N. $C_{31}H_{57}NO_8$ (571.79).

6.5.2.6 General procedure for the synthesis of carboxylic acids 6.23-6.25 by hydrolysis of the pertinent methyl esters

To a solution of methyl ester (1 eq) in THF was added 0.5 N LiOH (2 eq) and the mixture was stirred overnight at room temperature. After diluting with water, acidification with 1 N HCl to pH <2 and extraction three times with EtOAc, the combined organic phases were washed with brine, dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The raw product was purified using flash chromatography.

1-*O*-(Hexadecan-1-yl)-*N*-(2-carboxyethyl)-β-D-glucofuranosiduronamide (6.23): The title compound was prepared from **6.20** (0.48 mmol, 0.25 g) and 0.5 N LiOH (0.96 mmol, 1.9 ml) in THF (10 ml) according to general procedure 6.5.2.6 and was obtained after flash chromatography as a white solid (0.1 g, 53 %). mp: 97 °C; ^{1}H-NMR (CD_3OD) δ 0.90 (t, 3H, ^3J = 6.7 Hz, CH_3), 1.28 (m, 26H, $(C\underline{H}_2)_{13}CH_3$), 1.58 (m, 2H, $OCH_2C\underline{H}_2$), 2.52 (t, 2H, ^3J = 6.7 Hz, $C\underline{H}_2CO_2H$), 3.37 (td, 1H, ^3J = 6.7 Hz, ^2J = 9.4 Hz, OCH_2), 3.49 (t, 2H, ^3J = 6.7 Hz, $NHC\underline{H}_2$), 3.76 (td, 1H, ^3J = 6.6 Hz, ^2J = 9.4 Hz, OCH_2), 3.99 (s, 1H, H-2), 4.08 (dd, 1H, ^3J = 1.8 Hz, ^3J = 4.9 Hz, H-3), 4.34 (d, 1H, ^3J = 5.2 Hz, H-5), 4.43 (dd, 1H, ^3J = 5.0 Hz, ^3J = 5.0 Hz , H-4), 4.85 (s, 1H, H-1). ^{13}C-NMR (CD_3OD) δ 14.50 (+, CH_3), 23.79 (-, CH_2), 27.31 (-, CH_2), 30.53 (-, CH_2), 30.66 (-, CH_2), 30.69 (-, CH_2), 30.85 (-, CH_2), 33.13 (-, CH_2), 36.12 (-, CH_2), 69.53 (-, OCH_2), 72.38 (+, C-5), 77.77 (+, C-3), 81.82 (+, C-2), 83.99 (+, C-4), 109.89 (+, C-1), 174.71 (quat, CONH). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 491 (100) $[M+H]^+$. Anal. ($C_{25}H_{47}NO_8 \cdot 0.2H_2O$) C, H, N. $C_{25}H_{47}NO_8$ (489.64).

1-*O*-(Hexadecan-1-yl)-*N*-(3-carboxypropyl)-β-D-glucofuranosiduronamide (6.24): The title compound was prepared from **6.21** (0.42 mmol, 0.22 g) and 0.5 N LiOH (0.84 mmol, 1.7 ml) in THF (15 ml) according to general procedure 6.5.2.6 and was obtained after flash chromatography as a white solid (0.1 g, 48 %). ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H, ^3J = 6.6 Hz, CH_3), 1.24 (m, 26H, $(C\underline{H}_2)_{13}CH_3$), 1.46 (m, 2H, $OCH_2C\underline{H}_2$), 1.64 (m, 2H, $NHCH_2C\underline{H}_2$), 2.14 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2CO_2H$), 3.09 (m, 2H, $NHC\underline{H}_2$), 3.23 (td, 1H, ^3J = 6.6 Hz, ^3J = 9.4 Hz, OCH_2), 3.58 (td, 1H, ^3J = 6.6 Hz, ^3J = 9.4 Hz, OCH_2), 3.79 (s, 1H, H-2), 3.88 (dd, 1H, ^3J = 0.8 Hz, ^3J = 3.9 Hz, H-3), 4.17 (m, 2H, H-5, H-4), 4.68 (s, 1H, H-1), 7.93 (t, 1H, ^3J = 5.7 Hz, $CH_2N\underline{H}$). ^{13}C-NMR (DMSO-d_6) δ 13.85 (+, CH_3), 21.98 (-, CH_2), 24.79 (-, CH_2), 25.53 (-, CH_2), 28.59 (-, CH_2), 28.84 (-, CH_2), 28.95 (-, CH_2), 31.18 (-, CH_2), 37.89 (-, $NHCH_2$), 67.12 (-, OCH_2), 70.37 (+, C-5), 75.43 (+, C-3), 80.32 (+, C-2), 82.26 (+, C-4), 108.32 (+, C-1),

171.87 (quat, CO). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 504 (100) $[M+H]^+$. $C_{26}H_{49}NO_8$ (503.67).

1-*O*-(Hexadecan-1-yl)-*N*-(trans-4-carboxycyclohexylmethy)-β-D-glucofuranosid-uronamide (6.25): The title compound was prepared from **6.22** (0.32 mmol, 0.18 g) and 0.5 N LiOH (0.64 mmol, 1.3 ml) in THF (10 ml) according to general procedure 6.5.2.6 and was obtained after flash chromatography as a white solid (0.12 g, 67 %). mp: 105-107 °C; ^{1}H-NMR (DMSO-d_6) δ 0.88 (m, 5H, CH_3, cy-H), 1.17 – 1.48 (m, 29H, $(C\underline{H}_2)_{13}CH_3$, cy-H), 1.74 (m, 2H, cy-H), 1.87 (m, 2H, cy-H), 2.09 (m, 1H, cy-H), 2.86 (ddd, 1H, ^3J = 6.7 Hz, ^3J = 6.7 Hz, ^2J = 13.2 Hz, $C\underline{H}_2NH$), 3.02 (ddd, 1H, ^3J = 6.7 Hz, ^3J = 6.7 Hz, ^2J = 13.2 Hz, $C\underline{H}_2NH$), 3.23 (td, 1H, ^3J = 6.9 Hz, ^2J = 9.3 Hz, OCH_2), 3.57 (td, 1H, ^3J = 6.7 Hz, ^2J = 9.3 Hz, OCH_2), 3.78 (s, 1H, H-2), 3.86 (s, 1H, H-3), 4.15 (m, 2H, H-5, H-4), 4.68 (s, 1H, H-1), 5.20 (s, 1H, OH), 5.28 (s, 1H, OH), 5.38 (s, 1H, OH), 7.87 (t, 1H, ^3J = 5.9 Hz, $CH_2N\underline{H}$), 11.97 (s, 1H, CO_2H). ^{13}C-NMR (CD_3OD) δ 13.84 (+, CH_3), 21.98 (-, CH_2), 25.51 (-, CH_2), 28.24 (-, CH_2), 28.59 (-, CH_2), 28.82 (-, CH_2), 28.94 (-, CH_2), 29.21 (-, CH_2), 31.18 (-, CH_2), 36.86 (+, $\underline{C}HCH_2NH$), 42.47 (+, $\underline{C}HCO_2H$), 44.23 (-, CH_2NH), 67.21 (-, OCH_2), 70.09 (+, C-5), 75.34 (+, C-3), 80.35 (+, C-2), 82.37 (+, C-4), 108.39 (+, C-1), 171.84 (quat, CO), 176.64 (quat, CO). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 559 (100) $[M+H]^+$. Anal. ($C_{30}H_{55}NO_8 \cdot 0.8H_2O$) C, H, N. $C_{30}H_{55}NO_8$ (557.76).

1-*O*-(Hexadecan-1-yl)-β-D-glucuronic acid[24] (6.26): The title compound was prepared from D-glucuronic acid (10 mmol, 1.91 g), hexadecan-1-ol (20 mmol, 4.85 g) and BF_3Et_2O (20 mmol, 2.5 ml) in anhydrous THF (40 ml) by analogy with general procedure 6.5.2.1 and was obtained after flash chromatography (PE/EtOAc 50/50 v/v) as a white solid (1.10 g, 75 %). mp: 49-50 °C; ^{1}H-NMR (CD_3OD+D_2O) δ 0.89 (t, 3H, ^3J = 6.7 Hz, CH_3), 1.28 (s, 26H, $(C\underline{H}_2)_{13}CH_3$), 1.49 (m, 2H, $OCH_2C\underline{H}_2$), 3.30 (td, 1H, ^3J = 6.6 Hz, ^3J = 9.2 Hz, OCH_2), 3.79 (td, 1H, ^3J = 6.6 Hz, ^3J = 9.2 Hz, OCH_2), 4.21 (s, 1H, CH), 4.50 (d, 1H, ^3J = 6.5 Hz, CH), 4.83 (d, 1H, ^3J = 4.6 Hz, CH), 4.92 (m, 1H, under solvent peak, CH), 5.02 (s, 1H, CH). ^{13}C-NMR (DMSO-d_6) δ 13.88 (+, CH_3), 22.04 (-, CH_2), 25.47 (-, CH_2), 28.58 (-, CH_2), 28.65 (-, CH_2), 28.87 (-, CH_2), 29.00 (-, CH_2), 31.24 (-, CH_2), 66.79 (-, OCH_2), 68.64 (+, CH), 76.85 (+, CH), 77.82 (+, CH), 82.52 (+, CH), 108.40 (+, C-1), 174.80 (quat, CO_2H). $C_{22}H_{42}O_7$ (418.56).

6.5.2.7 General procedure for the synthesis of 2-*O*-acyl-1-*O*-alkyl-β-D-gluco-furanosidurono-6,3-lactones 6.27-6.29

To a solution of the pertinent glucuronolactone (1 eq) and pyridine (3 eq) in anhydrous THF was added the acid chloride (1 eq) dropwise at 0 °C under an atmosphere of nitrogen. After stirring for 30 min, the solution was poured on icewater and the mixture was extracted three times with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The remaining residue was then subjected to flash chromatography.

1-*O*-(Hexadecan-1-yl)-5-*O*-(2,2-dimethylpropanoyl)-β-D-glucofuranosidurono-6,3-lactone (6.27): The title compound was prepared from **6.6** (0.7 mmol, 0.28 g), pyridine (2.1 mmol, 0.17 ml) and pivaloyl chloride (0.7 mmol, 86 µl) in THF (5 ml) according to general procedure 6.5.2.7 and was obtained after flash chromatography as a white solid (0.16 g, 47 %). mp: 40-41 °C; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.7 Hz, CH_3), 1.25 (m, 26H, $(C\underline{H}_2)_{13}CH_3$), 1.31 (s, 9H, $C(CH_3)_3$), 1.53 (m, 2H, $OCH_2C\underline{H}_2$), 3.33 (td, 1H, ^3J = 7.1 Hz, ^2J = 9.0 Hz, OCH_2), 3.80 (td, 1H, ^3J = 6.8 Hz, ^2J = 9.0 Hz, OCH_2), 4.44 (s, 1H, CHO), 4.90 (d, 1H, ^3J = 4.8Hz, CHO), 5.05 (s, 1H, CHO), 5.13 (dd, 1H, ^3J = 4.9 Hz, ^3J = 6.7 Hz, CHO), 5.29 (d, 1H, ^3J = 6.8 Hz, CHO). ^{13}C-NMR ($CDCl_3$) δ 14.15 (+, CH_3), 22.72 (-, CH_2), 25.97 (-, CH_2), 26.97 (-, CH_2), 27.14 (+, $C(\underline{C}H_3)_3$), 28.91 (-, CH_2), 29.39 (-, CH_2), 29.46 (-, CH_2), 29.61 (-, CH_2), 29.65 (-, CH_2), 29.69 (-, CH_2), 29.73 (-, CH_2), 31.95 (-, CH_2), 38.91 (quat, $\underline{C}(CH_3)_3$), 68.54 (-, OCH_2), 69.10 (+, CH), 75.59 (+, CH), 83.34 (+, CH), 108.53 (+, C-1), 170.32 (quat, lactone CO), 177.38 (quat, $\underline{C}O_2C(CH_3)_3$). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 502 (100) $[M+NH_4]^+$. Anal. ($C_{27}H_{48}O_7$) C, H. $C_{27}H_{48}O_7$ (484.67).

1-*O*-(Hexadecan-1-yl)-5-*O*-propanoyl-β-D-glucofuranosidurono-6,3-lactone (6.28): The title compound was prepared from **6.6** (0.35 mmol, 0.14 g), pyridine (1.05 mmol, 84 µl) and propanoyl chloride (0.35 mmol, 31 µl) in THF (5 ml) according to general procedure 6.5.2.7 and was obtained after flash chromatography as a white solid (90 mg, 56 %). mp: 30 °C; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.7 Hz, CH_3), 1.20 – 1.30 (m, 29H, $(C\underline{H}_2)_{13}CH_3$, $CH_2C\underline{H}_3$), 1.54 (m, 2H, $OCH_2C\underline{H}_2$), 2.52 (m, 2H, $C\underline{H}_2CH_3$), 3.32 (td, 1H, ^3J = 7.1 Hz, ^2J = 9.0 Hz, OCH_2), 3.76 (td, 1H, ^3J = 6.8 Hz, ^2J = 9.0 Hz, OCH_2), 4.43 (s, 1H, CHO), 4.92 (d, 1H, ^3J = 5.0 Hz, H-3), 5.05 (s, 1H, CHO), 5.17 (dd, 1H, ^3J = 5.0 Hz, ^3J = 7.0 Hz, H-2), 5.27 (d, 1H, ^3J = 7.0 Hz, H-1). ^{13}C-NMR ($CDCl_3$) δ 8.89 (+, $COCH_2\underline{C}H_3$), 14.15 (+, CH_3), 22.72 (-, CH_2), 26.01 (-, CH_2), 26.95

(-, CH_2), 28.85 (-, CH_2), 29.39 (-, CH_2), 29.46 (-, CH_2), 29.62 (-, CH_2), 29.68 (-, CH_2), 29.73 (-, CH_2), 31.95 (-, CH_2), 68.74 (-, OCH_2), 69.01 (+, CH), 75.25 (+, CH), 83.71 (+, CH), 108.47 (+, C-1), 170.55 (quat, lactone CO), 173.16 (quat, CO_2). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 474 (100) $[M+NH_4]^+$. $C_{24}H_{44}O_7$ (444.60).

1-*O*-(Hexadecan-1-yl)-5-*O*-cyanomethylcarbonyl-β-D-glucofuranosidurono-6,3-lactone (6.29): The title compound was prepared from **6.6** (3 mmol, 1.20 g), pyridine (3 mmol, 0.24 ml) and cyanoacetic acid chloride which was prepared according to known procedures[25] (3 mmol, 0.31 g) in THF (10 ml) according to general procedure 6.5.2.7 and was obtained after flash chromatography as a pale yellow solid (0.50 g, 36 %). ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.7 Hz, CH_3), 1.25 (m, 26H, $(C\underline{H}_2)_{13}CH_3$), 1.53 (m, 2H, $OCH_2C\underline{H}_2$), 3.35 (td, 1H, ^3J = 6.9 Hz, ^2J = 9.2 Hz), 3.60 – 3.79 (m, 3H, OCH_2, CH_2CN), 4.44 (s, 1H, CHO), 4.95 (d, 1H, ^3J = 4.9 Hz, CHO), 5.08 (s, 1H, CHO), 5.18 (dd, 1H, ^3J = 4.9 Hz, ^3J = 6.8 Hz, CHO), 5.35 (d, 1H, ^3J = 6.9 Hz, CHO). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 485 (100) $[M+NH_4]^+$. Anal. ($C_{25}H_{41}NO_7$) C, H, N. $C_{25}H_{41}NO_7$ (467.60).

1-*O*-(Hexadecan-1-yl)-5-*O*-trifluormethansulfonyl-β-D-glucofuranosidurono-6,3-lactone (6.30): To a solution of **6.6** (1 mmol, 0.40 g) in anhydrous CH_2Cl_2 (9 ml) and anhydrous pyridine (1 ml) was added trifluorsulfonic acid anhydride (1 mmol, 0.17 ml) in anhydrous CH_2Cl_2 (2 ml) dropwise at -30 °C under an atmosphere of argon. After stirring for 30 min, the mixture was washed with saturated $NaHCO_3$, dried over $MgSO_4$, filtered and evaporated to dryness. The raw product was purified by flash chromatography (PE/Et_2O 20/80 v/v) to obtain the title compound as a brown solid (0.30 g, 56 %). mp: 45-47 °C; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.7 Hz, CH_3), 1.25 (m, 26H, $(C\underline{H}_2)_{13}CH_3$), 1.50 (m, 2H, $OCH_2C\underline{H}_2$), 3.36 (td, 1H, ^3J = 7.0 Hz, ^2J = 9.1 Hz, OCH_2), 3.77 (td, 1H, ^3J = 6.8 Hz, ^3J = 9.1 Hz, OCH_2), 4.45 (s, 1H, CHO), 4.97 (d, 1H, ^3J = 5.0 Hz, CHO), 5.12 (s, 1H, CHO), 5.13 (dd, 1H, ^3J = 5.0 Hz, ^3J = 7.2 Hz, CHO), 5.23 (d, 1H, ^3J = 7.2 Hz, CHO). CI-MS (NH_3) *m/z (%)*: 550 (100) $[M+NH_4]^+$. Anal. ($C_{25}H_{41}NO_7$) C, H. $C_{23}H_{39}F_3O_8S$ (532.61).

1-*O*-(Hexadecan-1-yl)-2,5-di-*O*-ethylcarbamoyl-β-D-glucofuranosidurono-6,3-lactone (6.31): To a mixture of **6.6** (0.35 mmol, 0.14 g) and Cu(I)Cl (0.35 mmol, 35 mg) in anhydrous DMF (2 ml) was added ethylisocyanate (0.35 mmol, 27 µl) and the suspension was stirred for 1 h at room temperature. After dilution with diethylether, the mixture was washed with water and brine. The organic layer then was dried over $MgSO_4$, filtered and evaporated. The title compound was obtained after flash

chromatography (PE/EtOAc 80/20 to 40/60 v/v) as colorless oil (70 mg, 37 %). ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.7 Hz, CH_3), 1.14 – 1.30 (m, 32H, $(C\underline{H}_2)_{13}CH_3$, $CH_2C\underline{H}_3$), 1.54 (m, 2H, $OCH_2C\underline{H}_2$), 3.29 (m, 5H, $C\underline{H}_2CH_3$, OCH_2), 3.73 (td, 1H, ^3J = 6.8 Hz, ^2J = 8.8 Hz, OCH_2), 4.70 – 5.24 (m, 7H, NH, CHO). ^{13}C-NMR ($CDCl_3$) δ 14.16 (+, CH_3), 15.05 (+, CH_3), 15.11 (+, CH_3), 22.72 (-, CH_2), 26.03 (-, CH_2), 28.90 (-, CH_2), 29.39 (-, CH_2), 29.50 (-, CH_2), 29.62 (-, CH_2), 29.72 (-, CH_2), 31.95 (-, CH_2), 36.12 (-, $NHCH_2$), 36.34 (-, $NHCH_2$), 68.74 (-, OCH_2), 69.57 (+, CH), 76.11 (+, CH), 78.53 (+, CH), 81.69 (+, CH), 106.68 (+, C-1), 154.14 (quat, CONH), 154.40 (quat, CONH), 170.50 (quat, lactone CO). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 560 (100) $[M+NH_4]^+$. $C_{28}H_{50}N_2O_8$ (542.71).

1-*O*-(Hexadecan-1-yl)-5-*O*-benzyl-β-D-glucofuranosidurono-6,3-lactone (6.32): To a solution of **6.6** (2.0 mmol, 0.80 g) and Ag_2O (3.0 mmol, 0.70 g) in EtOAc (15 ml) was added benzyl bromide (2.0 mmol, 0.24 ml) dropwise and the suspension was stirred for 48 h at 40-50 °C. After removal of solids by filtration the solvent was evaporated and the remaining raw product was subjected to flash chromatography (PE/EtOAc 80/20 to 40/60 v/v) to obtain the title compound as a white solid (0.20 g, 20 %). mp: 60 °C; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.7 Hz, CH_3), 1.25 (m, 26H, $(C\underline{H}_2)_{13}CH_3$), 1.51 (m, 2H, $OCH_2C\underline{H}_2$), 2.69 (d, 1H, ^3J = 8.9 Hz, OH), 3.37 (td, 1H, ^3J = 6.9 Hz, ^3J = 9.3 Hz, OCH_2), 3.64 (td, 1H, ^3J = 6.8 Hz, ^3J = 9.3 Hz, OCH_2), 4.16 (s, 1H, CHO), 4.35 (dd, 1H, ^3J = 6.9 Hz, ^3J = 8.6 Hz, CHO), 4.65 (s, 2H, $C\underline{H}_2$Ph), 4.90 (d, 1H, ^3J = 4.9 Hz, CHO), 4.97 (dd, 1H, ^3J = 5.0 Hz, ^3J = 6.7 Hz, CHO), 5.14 (s, 1H, CHO), 7.36 (m, 5H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 508 (100) $[M+NH_4]^+$. Anal. ($C_{25}H_{41}NO_7$) C, H. $C_{29}H_{46}O_6$ (490.67)

Monobenzyl malonate (6.33)[26]: To a solution of malonic acid (50 mmol, 5.2 g) and benzyl alcohol (50 mmol, 2.6 ml) in anhydrous EtOAc (50 ml) was added a solution of DCC (25 mmol, 5.2 g) in anhydrous EtOAc (10 ml) at 0 °C under an atmosphere of argon. The mixture was stirred overnight at room temperature and filtered. After washing with water, the organic layer was extracted with a soloution of 2.1 g $NaHCO_3$ in water (40 ml). The aqueous layer was washed with EtOAc, acidified with 2 N HCl to pH<2 and extracted with EtOAc. After washing with water, the organic layer was dried over $MgSO_4$, filtered and evaporated to dryness to obtain the title compound as a white solid (2.4 g, 25 %). mp: 30 °C; ^{1}H-NMR ($CDCl_3$) δ 3.49 (s, 2H, $C\underline{H}_2CO_2H$), 5.22 (s, 2H, $C\underline{H}_2$Ph), 7.37 (m, 5H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 212 (100) $[M+NH_4]^+$. $C_{10}H_{10}O_4$ (194.18).

Monobenzyl succinate (6.34)[17]: A solution of succinic anhydride (50 mmol, 5.0 g) and benzyl alcohol (0.25 mol, 25.9 ml) in anhydrous toluene (100 ml) was stirred at reflux overnight under an atmosphere of argon. After evaporation of the solvent the remaining residue was taken up in EtOAc and water and the pH was adjusted to 8 using 6 N NaOH. The organic phase was discarded and the aqueous layer was washed twice with ether. After acidification of the aqueous layer to pH <2 with 2 N HCl and extraction with ether, the organic layer was dried over $MgSO_4$, filtered and evaporated to yield the title compound as a white solid (4.3 g, 41 %). mp: 50 °C; ^{1}H-NMR (DMSO-d_6) δ 2.54 (m, 4H, CO(C$\underline{H}_2$)$_2$), 5.10 (s, 2H, C$\underline{H}_2$Ph), 7.36 (m, 5H, Ph-H), 12.25 (s, 1H, CO_2H). EI-MS (70eV) *m/z (%)*: 208 (5) [$M^{+\cdot}$]. $C_{11}H_{12}O_4$ (208.21).

6.5.2.8 General procedure for the synthesis of 5-*O*-Cbz-protected 1-*O*-alkyl glucurono-6,3-lactones 6.35 and 6.36

To a solution of 1-*O*-alkyl glucuronolactone (1 eq) in anhydrous pyridine was added Cbz-chloride (1 eq) at -20 °C over a period of 20 min under an atmosphere of argon. The reaction was quenched by adding water and the mixture was extracted three times with $CHCl_3$. After washing the combined organic layers with saturated $NaHCO_3$, saturated $KHSO_4$ and water, the organic phase was dried over $MgSO_4$, filtered and evaporated to dryness. Flash chromatography of the crude material yielded the title 5-*O*-protected derivatives.

1-*O*-(Dodecan-1-yl)-5-*O*-benzyloxycarbonyl-β-D-glucofuranosidurono-6,3-lactone (6.35): The title compound was prepared from **6.5** (5 mmol, 1.7 g), Cbz-chloride (5 mmol, 0.71 ml) in anhydrous pyridine (10 ml) according to general procedure 6.5.2.8 and was obtained after flash chromatography (Et_2O) as a white solid (1.55 g, 65 %). ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.7 Hz, CH_3), 1.23 (m, 18H, (C$\underline{H}_2$)$_9$CH$_3$), 1.50 (m, 2H, OCH$_2$C$\underline{H}_2$), 3.27 (td, 1H, ^3J = 7.1 Hz, ^2J = 9.0 Hz, OCH_2), 3.78 (td, 1H, ^3J = 6.9 Hz, ^2J = 9.0 Hz, OCH_2), 4.41 (s, 1H, CHO), 4.92 (d, 1H, ^3J = 4.6 Hz, H-3), 5.06 (s, 1H, CHO), 5.16 (m, 2H, CHO), 5.24 (s, 2H, C$\underline{H}_2$Ph), 7.37 (m, 5H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 496 (100) [M+NH_4]$^+$. $C_{26}H_{38}O_8$ (478.58).

1-*O*-(Hexadecan-1-yl)-5-*O*-benzyloxycarbonyl-β-D-glucofuranosidurono-6,3-lactone (6.36): The title compound was prepared from **6.6** (4 mmol, 1.6 g), Cbz-chloride (4 mmol, 0.57 ml) in anhydrous pyridine (10 ml) according to general procedure 6.5.2.8 and was obtained after flash chromatography (Et_2O) as a white solid (1.4 g, 65 %). mp: 55 °C; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.7 Hz, CH_3), 1.23

(m, 26H, ($C\underline{H}_2$)$_{13}CH_3$), 1.50 (m, 2H, $OCH_2C\underline{H}_2$), 3.27 (td, 1H, ^3J = 7.1 Hz, ^2J = 9.0 Hz, OCH_2), 3.79 (td, 1H, ^3J = 6.8 Hz, ^2J = 9.0 Hz, OCH_2), 4.41 (s, 1H, CHO), 4.92 (d, 1H, ^3J = 4.8 Hz, H-3), 5.05 (s, 1H, CHO), 5.15 (d, 1H, ^3J = 7.1 Hz, H-1), 5.19 (dd, 1H, ^3J = 4.8 Hz, ^3J = 7.1 Hz, H-2), 5.25 (s, 2H, $C\underline{H}_2$Ph), 7.37 (m, 5H). EI-MS (70eV) *m/z (%)*: 534 (20) [$M^{+\cdot}$]. Anal. ($C_{30}H_{46}O_8$) C, H. $C_{30}H_{46}O_8$ (534.68).

6.5.2.9 General procedure for the DCC mediated synthesis of mono- or di-acylated glucuronolactones 6.37-6.39, 6.45, 6.49 and 6.50

To a solution of glucuronolactone derivative (1 eq), the pertinent carboxylic acid (1-2.4 eq) and DMAP (0.1-0.2 eq) in anhydrous EtOAc was added a solution of DCC (1.1-2.4 eq) in anhydrous EtOAc dropwise at 0 °C under an atmosphere of argon. After stirring overnight at room temperature solids were removed by filtration and the organic layer was washed with saturated $NaHCO_3$ and water. Drying over $MgSO_4$, filtration and evaporation of the solvent gave the crude product which was subjected to flash chromatography.

1-*O*-(Dodecan-1-yl)-5-*O*-benzyloxycarbonyl-2-*O*-(2-benzyloxycarbonylethanoyl)-β-D-glucofuranosidurono-6,3-lactone (6.37): The title compound was prepared from **6.35** (0.8 mmol, 0.38 g), **6.33** (0.88 mmol, 0.17 g), DMAP (0.08 mmol, 10 mg) and DCC (0.88 mmol, 0.18 g) in anhydrous EtOAc (10 ml) according to general procedure 6.5.2.9 and was obtained after flash chromatography (PE/Et_2O 40/60 v/v) as colorless oil (0.40 g, 76 %). ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.7 Hz, CH_3), 1.23 (m, 18H, ($C\underline{H}_2$)$_9CH_3$), 1.50 (m, 2H, $OCH_2C\underline{H}_2$), 3.25 (td, 1H, ^3J = 7.1 Hz, ^3J = 8.9 Hz, OCH_2), 3.47 (s, 2H, $COC\underline{H}_2CO_2$), 3.75 (td, 1H, ^3J = 6.8 Hz, ^3J = 8.9 Hz, OCH_2), 4.82 (d, 1H, ^3J = 5.2 Hz, CHO), 4.95 (dd, 1H, ^3J = 5.3 Hz, ^3J = 7.0 Hz, CHO), 5.06 (m, 2H, CHO), 5.14 (d, 1H, ^2J = 12.0 Hz, $C\underline{H}_2$Ph), 5.21 (d, 1H, ^2J = 12.1 Hz, $C\underline{H}_2$Ph), 5.25 (s, 3H, $C\underline{H}_2$Ph, CHO), 7.37 (m, 10H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 673 (100) [$M+NH_4$]$^+$. $C_{36}H_{46}O_{11}$ (654.74).

1-*O*-(Hexadecan-1-yl)-5-*O*-benzyloxycarbonyl-2-*O*-(2-benzyloxycarbonyl-ethanoyl)-β-D-glucofuranosidurono-6,3-lactone (6.38): The title compound was prepared from **6.36** (0.8 mmol, 0.43 g), **6.33** (0.88 mmol, 0.17 g), DMAP (0.08 mmol, 10 mg) and DCC (0.88 mmol, 0.18 g) in anhydrous EtOAc (10 ml) according to general procedure 6.5.2.9 and was obtained after flash chromatography (PE/Et_2O 30/70 v/v) as colorless oil (0.42 g, 74 %). ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.7 Hz, CH_3), 1.23 (m, 26H, ($C\underline{H}_2$)$_{13}CH_3$), 1.50 (m, 2H, $OCH_2C\underline{H}_2$), 3.25 (td, 1H, ^3J = 7.1 Hz, ^2J = 8.9 Hz, OCH_2), 3.47 (s, 2H, $COC\underline{H}_2CO_2$), 3.75 (td, 1H, ^3J = 6.8 Hz, ^2J = 8.9 Hz,

OCH_2), 4.82 (d, 1H, ^3J = 5.2 Hz, CHO), 4.95 (dd, 1H, ^3J = 5.3 Hz, ^3J = 7.0 Hz, CHO), 5.18 (m, 7H, CHO, C$\underline{H}_2$Ph), 7.37 (m, 10H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 729 (100) $[M+NH_4]^+$. $C_{40}H_{54}O_{11}$ (710.85).

1-*O*-(Hexadecan-1-yl)-5-*O*-benzyloxycarbonyl-2-*O*-(3-benzyloxycarbonyl-propanoyl)-β-D-glucofuranosidurono-6,3-lactone (6.39): The title compound was prepared from **6.36** (0.8 mmol, 0.43 g), **6.34** (0.88 mmol, 0.18 g), DMAP (0.08 mmol, 10 mg) and DCC (0.88 mmol, 0.18 g) in anhydrous EtOAc (10 ml) according to general procedure 6.5.2.9 and was obtained after flash chromatography (PE/EtOAc 50/50 v/v) as colorless oil (0.31 g, 53 %). ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.7 Hz, CH_3), 1.24 (m, 26H, $(C\underline{H}_2)_{13}CH_3$), 1.50 (m, 2H, $OCH_2C\underline{H}_2$), 2.68 (m, 4H, $CO(C\underline{H}_2)_2$), 3.27 (td, 1H, ^3J = 7.1 Hz, ^2J = 8.9 Hz, OCH_2), 3.77 (td, 1H, ^3J = 6.9 Hz, ^2J = 8.9 Hz, OCH_2), 4.85 (d, 1H, ^3J = 4.9 Hz, CHO), 5.14 (m, 8H, CHO, C$\underline{H}_2$Ph), 7.36 (m, 10H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 743 (100) $[M+NH_4]^+$. $C_{41}H_{56}O_{11}$ (724.88).

1-*O*-(Dodecan-1-yl)-5-*O*-benzyloxycarbonyl-2-*O*-(2-dimethylaminoethanoyl)-β-D-glucofuranosidurono-6,3-lactone (6.40): To a solution of **6.36** (0.8 mmol, 0.43 g), *N,N*-dimethylglycine (1.2 mmol, 0.12 g), DMAP (1.2 mmol, 0.15 g) in anhydrous DMF (10 ml) was added EDAC (1.2 mmol, 0.23 g) under an atmosphere of argon and the mixture was stirred overnight at room temperature. After dilution with water and extraction three times with EtOAc, the combined organic layers were washed with 1 N HCl and water, dried over $MgSO_4$, filtered and evaporated to dryness. The crude residue was subjected to flash chromatography ($CHCl_3$/MeOH 95/5 v/v) to obtain the title compound as pale yellow oil (0.32 g, 64 %). ^{1}H-NMR ($CDCl_3$) δ 0.87 (t, 3H, ^3J = 6.7 Hz, CH_3), 1.23 (m, 26H, $(C\underline{H}_2)_{13}CH_3$), 1.51 (m, 2H, $OCH_2C\underline{H}_2$), 2.37 (s, 6H, $N(CH_3)_2$), 3.24 (s, 2H, $COCH_2N$), 3.29 (td, 1H, ^3J = 7.1 Hz, ^2J = 8.9 Hz, OCH_2), 3.78 (td, 1H, ^3J = 7.1 Hz, ^2J = 9.3 Hz, OCH_2), 5.00 (d, 1H, ^3J = 4.7 Hz, CHO), 5.09 (s, 1H, CHO), 5.15 (m, 2H, CHO), 5.24 (s, 2H, C$\underline{H}_2$Ph), 5.26 (s, 1H, CHO), 7.37 (m, 5H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 620 (100) $[M+H]^+$. $C_{34}H_{53}NO_9$ (619.79).

6.5.2.10 General procedure for the synthesis of carboxylic acids and alcohols 6.41-6.44, 6.47, 6.48, 6.51, 6.52 and 6.54 by hydrogenolytic deprotection

The pertinent benzyl-protected compound was dissolved EtOAc and a catalytic amount of palladium on activated charcoal (10 % Pd) was added. After

hydrogenolysis (1 atm) overnight, insoluble material was filtered off, and the solvent was evaporated to yield the target compound.

1-*O*-(Dodecan-1-yl)-2-*O*-(2-carboxyethanoyl)-β-D-glucofuranosidurono-6,3-lactone (6.41): The title compound was prepared according to general procedure 6.5.2.10 using **6.37** (0.46 mmol, 0.30 g) and 10 % Pd/C (0.30 g) in EtOAc (10 ml) and was obtained as a white solid (0.19 g, 96 %). mp: 57 °C; ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H, ^{3}J = 6.7 Hz, CH_3), 1.24 (m, 18H, $(C\underline{H}_2)_9CH_3$), 1.40 (m, 2H, $OCH_2C\underline{H}_2$), 3.25 (td, 1H, ^{3}J = 6.8 Hz, ^{2}J = 9.0 Hz, OCH_2), 3.72 (td, 1H, ^{3}J = 6.6 Hz, ^{2}J = 8.9 Hz, OCH_2), 4.49 (dd, 1H, ^{3}J = 6.0 Hz, ^{3}J = 6.0 Hz, H-5), 4.79 (dd, 1H, ^{3}J = 4.9 Hz, ^{3}J = 6.5 Hz, H-4), 4.92 (d, 1H, ^{3}J = 4.8 Hz, H-3), 4.99 (s, 1H, CH), 5.07 (s, 1H, CHO), 5.99 (d, 1H, ^{3}J = 6.1 Hz, 5-OH), 12.95 (bs, 1H, CO_2H). ^{13}C-NMR (DMSO-d_6) δ 13.85 (+, CH_3), 21.99 (-, CH_2), 25.31 (-, CH_2), 28.38 (-, CH_2), 28.61 (-, CH_2), 28.77 (-, CH_2), 28.92 (-, CH_2), 31.20 (-, CH_2), 41.11 (-, $CO\underline{C}H_2$), 67.11 (-, OCH_2), 68.11 (+, CH), 77.85 (+, CH), 78.60 (+, CH), 79.85 (+, CH), 105.25 (+, C-1), 165.80 (quat, $\underline{C}OCH_2CO_2H$), 167.59 (quat, CO_2H, 174.41 (quat, lactone CO). ES-MS (MeOH + NH_4OAc) *m/z (%)*: 448 (100) $[M+NH_4]^+$. Anal ($C_{21}H_{34}O_9 \cdot 0.3H_2O$) C, H. $C_{21}H_{34}O_9$ (430.49).

1-*O*-(Hexadecan-1-yl)-2-*O*-(2-carboxyethanoyl)-β-D-glucofuranosidurono-6,3-lactone (6.42): The title compound was prepared according to general procedure 6.5.2.10 using **6.38** (0.52 mmol, 0.37 g) and 10 % Pd/C (0.37 g) in EtOAc (10 ml) and was obtained as a white foam (0.22 g, 87 %). mp: 58-60 °C; ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H, ^{3}J = 6.7 Hz, CH_3), 1.23 (m, 26H, $(C\underline{H}_2)_{13}CH_3$), 1.40 (m, 2H, $OCH_2C\underline{H}_2$), 3.24 (td, 1H, ^{3}J = 6.8 Hz, ^{2}J = 9.0 Hz, OCH_2), 3.47 (s, 2H, $COC\underline{H}_2CO_2$), 3.72 (td, 1H, ^{3}J = 6.6 Hz, ^{2}J = 8.9 Hz, OCH_2), 4.49 (m, 1H, H-5), 4.79 (dd, 1H, ^{3}J = 4.9 Hz, ^{3}J = 6.6 Hz, H-4), 4.92 (d, 1H, ^{3}J = 4.8 Hz, H-3), 4.99 (s, 1H, CHO), 5.07 (s, 1H, CHO), 5.99 (s, 1H, 5-OH), 12.97 (s, 1H, CO_2H). ^{13}C-NMR (DMSO-d_6) δ 13.85 (+, CH_3), 22.00 (-, CH_2), 25.32 (-, CH_2), 28.39 (-, CH_2), 28.61 (-, CH_2), 28.79 (-, CH_2), 28.96 (-, CH_2), 31.19 (-, CH_2), 41.12 (-, $CO\underline{C}H_2CO_2H$), 67.10 (-, OCH_2), 68.10 (+, CH), 77.84 (+, CH), 78.58 (+, CH), 79.84 (+, CH), 105.23 (+, C-1), 165.79 (quat, $\underline{C}OCH_2CO_2H$), 167.60 (quat, CO_2H), 174.42 (quat, lactone CO). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 504 (100) $[M+NH_4]^+$. Anal ($C_{25}H_{42}O_9$) C, H. $C_{25}H_{42}O_9$ (486.60).

1-*O*-(Hexadecan-1-yl)-2-*O*-(3-carboxypropanoyl)-β-D-glucofuranosidurono-6,3-lactone (6.43): The title compound was prepared according to general procedure 6.5.2.10 using **6.39** (0.36 mmol, 0.26 g) and 10 % Pd/C (0.26 g) in EtOAc (10 ml) and

was obtained as a pale yellow solid (0.15 g, 83 %). mp: 94 °C; ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H, ^{3}J = 6.7 Hz, CH_3), 1.24 (s, 26H, $(C\underline{H}_2)_{13}CH_3$), 1.40 (m, 2H, $OCH_2C\underline{H}_2$), 2.56 (m, 4H, $CO(C\underline{H}_2)_2CO_2H$), 3.23 (td, 1H, ^{3}J = 6.8 Hz, ^{2}J = 9.0 Hz, OCH_2), 3.71 (td, 1H, ^{3}J = 6.6 Hz, ^{2}J = 8.9 Hz, OCH_2), 4.48 (dd, 1H, ^{3}J = 6.4 Hz, ^{3}J = 6.4 Hz, H-5), 4.80 (dd, 1H, ^{3}J = 4.9 Hz, ^{3}J = 6.5 Hz, H-4), 4.90 (d, 1H, ^{3}J = 4.8 Hz, H-3), 4.96 (s, 1H, CHO), 5.05 (s, 1H, CHO), 5.97 (d, 1H, ^{3}J = 6.4 Hz, 5-OH), 12.28 (bs, 1H, CO_2H). ^{13}C-NMR (DMSO-d_6) δ 13.85 (+, CH_3), 21.99 (-, CH_2), 25.32 (-, CH_2), 28.40 (-, CH_2), 28.48 (-, CH_2), 28.61 (-, CH_2), 28.78 (-, CH_2), 28.95 (-, CH_2), 31.19 (-, CH_2), 67.06 (-, OCH_2), 68.17 (+, CH), 77.84 (+, CH), 78.16 (+, CH), 79.96 (+, CH), 105.40 (+, C-1), 171.08 (quat, CO_2), 173.20 (quat, CO_2H), 174.44 (quat, lactone CO). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 518 (100) $[M+NH_4]^+$. Anal. ($C_{26}H_{44}O_9$) C, H. $C_{26}H_{44}O_9$ (500.62).

1-*O*-(Hexadecan-1-yl)-2-*O*-(2-dimethylaminoethanoyl)-β-D-glucofuranosidurono-6,3-lactone (6.44): The title compound was prepared according to general procedure 6.5.2.10 using **6.40** (0.32 mmol, 0.20 g) and 10 % Pd/C (0.20 g) in EtOAc (10 ml) and was obtained as a colorless solid (0.15 g, 96 %). ^{1}H-NMR (CD_3OD) δ 0.90 (t, 3H, ^{3}J = 6.7 Hz, CH_3), 1.28 (m, 26H, $(C\underline{H}_2)_{13}CH_3$), 1.51 (m, 2H, $OCH_2C\underline{H}_2$), 2.36 (s, 6H, $N(CH_3)_2$), 3.34 (m, 3H, $COCH_2N$, OCH_2), 3.84 (td, 1H, ^{3}J = 6.5 Hz, ^{2}J = 9.1 Hz, OCH_2), 4.50 (d, 1H, ^{3}J = 6.7 Hz, CHO), 4.91 (dd, 1H, ^{3}J = 4.8 Hz, ^{3}J = 6.6 Hz, CHO), 5.00 (d, 1H, ^{3}J = 4.7 Hz, CHO), 5.13 (s, 1H, CHO), 5.16 (s, 1H, CHO). ^{13}C-NMR (CD_3OD) δ 14.49 (+, CH_3), 23.79 (-, CH_2), 27.19 (-, CH_2), 30.32 (-, CH_2), 30.53 (-, CH_2), 30.62 (-, CH_2), 30.66 (-, CH_2), 30.79 (-, CH_2), 30.85 (-, CH_2), 33.13 (-, CH_2), 45.30 (+, $N(CH_3)_2$), 69.29 (-, CH_2), 69.57 (-, CH_2), 70.55 (+, CH), 78.62 (+, CH), 79.37 (+, CH), 84.81 (+, CH), 110.51 (+, C-1), 177.20 (quat, lactone CO). CI-MS (NH_3) *m/z (%)*: 486 (100) $[M+H]^+$. $C_{26}H_{47}NO_7$ (485.65).

1-*O*-(Hexadecan-1-yl)-5-*O*-(2-benzyloxycarbonylethanoyl)-β-D-glucofuranosid-urono-6,3-lactone (6.45): The title compound was prepared from **6.6** (1 mmol, 0.40 g), **6.33** (1 mmol, 0.19 g), DMAP (0.1 mmol, 12 mg) and DCC (1.1 mmol, 0.23 g) in anhydrous EtOAc (10 ml) according to general procedure 6.5.2.9 and was obtained after flash chromatography (PE/EtOAc 60/40 v/v) as a colorless oil (0.24 g, 42 %). ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^{3}J = 6.7 Hz, CH_3), 1.25 (m, 26H, $(C\underline{H}_2)_{13}CH_3$), 1.49 (m, 2H, $OCH_2C\underline{H}_2$), 2.33 (d, 1H, ^{3}J = 5.4 Hz, OH), 3.26 (td, 1H, ^{3}J = 7.0 Hz, ^{2}J = 9.1 Hz, OCH_2), 3.65 (m, 3H, OCH_2, $COCH_2CO_2$), 4.41 (d, 1H, ^{3}J = 4.8 Hz, CHO), 4.92 (d, 1H, ^{3}J = 4.9 Hz, CHO), 5.02 (s, 1H, CHO), 5.16 – 5.27 (m, 4H, CHO, CH_2Ph), 7.37

(m, 5H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 595 (100) $[M+NH_4]^+$. $C_{32}H_{48}O_9$ (576.72).

1-*O*-(Hexadecan-1-yl)-5-*O*-(3-benzyloxycarbonylpropanoyl)-β-D-glucofuranosid-urono-6,3-lactone (6.46): To a solution of **6.34** (1.1 mmol, 0.23 g) in anhydrous CH_2Cl_2 (20 ml) was added oxalylchloride (5.6 mmol, 0.94 ml) and a drop of DMF at 0 °C. After stirring for 30 min the solvent was evaporated and traces of HCl or oxalylchloride were co-evaporated with toluene. The remaining crude acid chloride was taken up in anhydrous THF (20 ml) and added dropwise to a solution of **6.6** (1 mmol, 0.40 g) in anhydrous pyridine (7 ml) at -20 °C under an atmosphere of argon. After stirring for 10 min the reaction was quenched by adding saturated $NaHCO_3$ and the mixture was extracted three times with $CHCl_3$. After washing the combined organic layers with saturated $NaHCO_3$, saturated $KHSO_4$ and water, the organic phase was dried over $MgSO_4$, filtered and evaporated to dryness. Flash chromatography (PE/Et_2O 20/80 v/v) of the crude product yielded the title compound as colorless oil (0.39 g, 66 %). ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.7 Hz, CH_3), 1.25 (m, 26H, $(C\underline{H}_2)_{13}CH_3$), 1.53 (m, 2H, $OCH_2C\underline{H}_2$), 2.23 (d, 1H, ^3J = 5.6 Hz, OH), 2.68 – 2.91 (m, 4H, $CO(C\underline{H}_2)_2$), 3.31 (td, 1H, ^3J = 7.0 Hz, ^2J = 9.1 Hz, OCH_2), 3.74 (td, 1H, ^3J = 6.7 Hz, ^3J = 9.1 Hz, OCH_2), 4.41 (d, 1H, ^3J = 5.1 Hz, H-4), 4.90 (d, 1H, ^3J = 5.0 Hz, H-3), 5.03 (s, 1H, H-5), 5.14 (dd, 1H, ^3J = 4.9 Hz, ^3J = 6.9 Hz, H-2), 5.16 (s, 2H, $C\underline{H}_2Ph$), 5.25 (d, 1H, ^3J = 7.0 Hz, H-1), 7.36 (m, 5H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 609 (100) $[M+NH_4]^+$. $C_{33}H_{50}O_9$ (590.74).

1-*O*-(Hexadecan-1-yl)-5-*O*-(2-carboxyethanoyl)-β-D-glucofuranosidurono-6,3-lactone (6.47): The title compound was prepared according to general procedure 6.5.2.10 using **6.45** (0.35 mmol, 0.20 g) and 10 % Pd/C (0.20 g) in EtOAc (10 ml) and was obtained as a colorless semisolid substance (0.15 g, 88 %). mp: 59-60 °C; ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H, ^3J = 6.6 Hz, CH_3), 1.23 (m, 26H, $(C\underline{H}_2)_{13}CH_3$), 1.39 (m, 2H, $OCH_2C\underline{H}_2$), 3.24 (td, 1H, ^3J = 6.4 Hz, ^3J = 9.2 Hz, OCH_2), 3.49 (s, 2H, $C\underline{H}_2CO_2H$), 3.60 (td, 1H, ^3J = 6.5 Hz, ^3J = 9.0 Hz, OCH_2), 4.08 (d, 1H, ^3J = 3.0 Hz, CHO), 4.84 – 5.00 (m, 3H, CH), 5.49 (d, 1H, ^3J = 6.9 Hz, H-1), 5.75 (d, 1H, ^3J = 3.8 Hz, OH), 12.93 (s, 1H, CO_2H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 504(100) $[M+NH_4]^+$. Anal. ($C_{25}H_{42}O_9$) C, H. $C_{25}H_{42}O_9$ (486.60).

1-*O*-(Hexadecan-1-yl)-5-*O*-(3-carboxypropanoyl)-β-D-glucofuranosidurono-6,3-lactone (6.48): The title compound was prepared according to general procedure 6.5.2.10 using **6.46** (0.49 mmol, 0.29 g) and 10 % Pd/C (0.29 g) in EtOAc (10 ml) and

was obtained as a colorless solid (0.22 g, 90 %). mp: 75-76 °C; ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H, ^{3}J = 6.7 Hz, CH_3), 1.23 (s, 26H, $(C\underline{H}_2)_{13}CH_3$), 1.41 (m, 2H, $OCH_2C\underline{H}_2$), 2.38 (t, 2H, ^{3}J = 6.8 Hz, CH_2CO_2), 2.59 (m, 2H, CH_2CO_2), 3.25 (td, 1H, ^{3}J = 6.7 Hz, ^{2}J = 8.9 Hz, OCH_2), 3.62 (td, 1H, ^{3}J = 6.7 Hz, ^{2}J = 8.8 Hz, OCH_2), 4.08 (s, 1H, CHO), 4.84 (d, 1H, ^{3}J = 5.0 Hz, H-3), 4.91 (s, 1H, CHO), 4.95 (dd, 1H, ^{3}J = 5.1 Hz, ^{3}J = 6.8 Hz, H-2), 5.43 (d, 1H, ^{3}J = 6.9 Hz, H-1). ^{13}H-NMR (DMSO-d_6) δ 13.85 (+, CH_3), 21.99 (-, CH_2), 25.40 (-, CH_2), 28.51 (-, CH_2), 28.61 (-, CH_2), 28.81 (-, CH_2), 28.91 (-, CH_2), 28.96 (-, CH_2), 30.02 (-, CH_2), 31.19 (-, CH_2), 67.07 (-, OCH_2), 69.16 (+, CH), 75.52 (+, CH), 76.57 (+, CH), 83.94 (+, CH), 108.33 (+, C-1), 170.72 (quat, CO_2), 170.91 (quat, CO_2), 172.91 (quat, CO_2). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 518 (100) $[M+NH_4]^+$. Anal. ($C_{26}H_{44}O_9$) C, H. $C_{26}H_{44}O_9$ (500.62).

1-*O*-(Hexadecan-1-yl)-2,5-di-*O*-(2-benzyloxycarbonylethanoyl)-β-D-gluco-furanosidurono-6,3-lactone (6.49): The title compound was prepared from **6.6** (1 mmol, 0.40 g), **6.33** (2.4 mmol, 0.47 g), DMAP (0.2 mmol, 24 mg) and DCC (2.4 mmol, 0.50 g) in anhydrous EtOAc (15 ml) according to general procedure 6.5.2.9 and was obtained after flash chromatography (PE/Et_2O 30/70 v/v) as pale yellow oil (0.44 g, 58 %). ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^{3}J = 6.7 Hz, CH_3), 1.24 (m, 26H, $(C\underline{H}_2)_{13}CH_3$), 1.47 (m, 2H, $OCH_2C\underline{H}_2$), 3.23 (td, 1H, ^{3}J = 7.0 Hz, ^{2}J = 9.0 Hz, OCH_2), 3.47 (s, 2H, $COCH_2CO_2$), 3.58 – 3.68 (m, 3H, OCH_2, $COCH_2CO_2$), 4.80 (d, 1H, ^{3}J = 5.1 Hz, CHO), 4.92 (dd, 1H, ^{3}J = 5.2 Hz, ^{3}J = 6.9 Hz, CHO), 5.02 (s, 1H, CHO), 5.19 (m, 6H, CHO, $C\underline{H}_2$Ph), 7.37 (m, 10H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 770 (100) $[M+NH_4]^+$. $C_{42}H_{56}O_{12}$ (752.89).

1-*O*-(Hexadecan-1-yl)-2,5-di-*O*-(3-benzyloxycarbonylpropanoyl)-β-D-gluco-furanosidurono-6,3-lactone (6.50): The title compound was prepared from **6.6** (1 mmol, 0.40 g), **6.34** (2.4 mmol, 0.50 g), DMAP (0.2 mmol, 24 mg) and DCC (2.2 mmol, 0.46 g) in anhydrous EtOAc (15 ml) according to general procedure 6.5.2.9 and was obtained after flash chromatography (PE/Et_2O 30/70 v/v) as colorless oil (0.70 g, 90 %). ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^{3}J = 6.7 Hz, CH_3), 1.25 (s, 26H, $(C\underline{H}_2)_{13}CH_3$), 1.53 (m, 2H, $OCH_2C\underline{H}_2$), 2.75 (m, 8H, $CO(C\underline{H}_2)_2$), 3.30 (td, 1H, ^{3}J = 7.0 Hz, ^{2}J = 8.9 Hz, OCH_2), 3.73 (td, 1H, ^{3}J = 6.7 Hz, ^{2}J = 8.9 Hz, OCH_2), 4.84 (d, 1H, ^{3}J = 5.2 Hz, CHO), 5.03 (m, 2H, CHO), 5.13 (d, 2H, ^{2}J = 2.2 Hz, $C\underline{H}_2$Ph), 5.15 (s, 2H, $C\underline{H}_2$Ph), 5.20 (d, 1H, ^{3}J = 7.1 Hz, CHO), 5.22 (s, 1H, H-1), 7.35 (m, 10H, PhH). ES-MS (MeOH + NH_4OAc) *m/z (%)*: 799 (100) $[M+NH_4]^+$. $C_{44}H_{60}O_{12}$ (780.94).

1-*O*-(Hexadecan-1-yl)-2,5-di-*O*-(2-carboxyethanoyl)-β-D-glucofuranosidurono-6,3-lactone (6.51): The title compound was prepared according to general procedure 6.5.2.10 using **6.49** (0.34 mmol, 0.16 g) and 10 % Pd/C (0.16 g) in EtOAc (10 ml) and was obtained as a pale yellow semisolid substance (0.11 g, 90 %). mp: 68 °C; ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H, ^{3}J = 6.7 Hz, CH_3), 1.23 (m, 26H, $(C\underline{H}_2)_{13}CH_3$), 1.42 (m, 2H, $OCH_2C\underline{H}_2$), 3.48 (s, 2H, $COC\underline{H}_2CO_2H$), 3.30 (td, 1H, ^{3}J = 6.6 Hz, ^{2}J = 8.9 Hz, OCH_2), 3.51 (s, 2H, $COC\underline{H}_2CO_2H$), 3.64 (td, 1H, ^{3}J = 6.6 Hz, ^{2}J = 8.9 Hz, OCH_2), 5.02 (dd, 1H, ^{3}J = 5.2 Hz, ^{3}J = 6.6 Hz, H-2), 5.03 (s, 1H, CHO), 5.07 (d, 1H, ^{3}J = 5.2 Hz, H-3), 5.10 (s, 1H, CHO), 5.55 (d, 1H, ^{3}J = 6.8 Hz, H-1), 12.94 (s, 2H, CO_2H). ^{13}C-NMR (DMSO-d_6) δ 13.85 (+, CH_3), 21.99 (-, CH_2), 25.26 (-, CH_2), 28.36 (-, CH_2), 28.60 (-, CH_2), 28.78 (-, CH_2), 28.90 (-, CH_2), 28.95 (-, CH_2), 31.19 (-, CH_2), 40.71 (-, $\underline{C}H_2CO_2H$), 41.09 (-, $\underline{C}H_2CO_2H$), 67.57 (-, OCH_2), 69.33 (+, CH), 75.48 (+, CH), 78.18 (+, CH), 81.29 (+, CH), 105.34 (+, C-1), 165.55 (quat, CO_2), 165.76 (quat, CO_2), 167.02 (quat, CO_2), 167.56 (quat, CO_2), 170.03 (quat, CO_2). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 590 (100) $[M+NH_4]^+$. Anal. ($C_{28}H_{44}O_{12}\cdot 1.8H_2O$) C, H. $C_{28}H_{44}O_{12}$ (572.64).

1-*O*-(Hexadecan-1-yl)-2,5-di-*O*-(3-carboxypropanoyl)-β-D-glucofuranosidurono-6,3-lactone (6.52): The title compound was prepared according to general procedure 6.5.2.10 using **6.50** (0.71 mmol, 0.56 g) and 10 % Pd/C (0.56 g) in EtOAc (15 ml) and was obtained after flash chromatography ($CHCl_3$/MeOH 95/5 v/v) as a colorless semisolid substance (0.29 g, 67 %). ^{1}H-NMR (CD_3OD) δ 0.90 (t, 3H, ^{3}J = 6.7 Hz, CH_3), 1.29 (m, 26H, $(C\underline{H}_2)_{13}CH_3$), 1.54 (m, 2H, $OCH_2C\underline{H}_2$), 2.61 – 2.79 (m, 8H, $CO(C\underline{H}_2)_2$), 3.36 (td, 1H, ^{3}J = 6.8 Hz, ^{2}J = 9.0 Hz, OCH_2), 3.79 (td, 1H, ^{3}J = 6.6 Hz, ^{2}J = 9.0 Hz, OCH_2), 5.09 (m, 3H, CHO), 5.14 (s, 1H, CHO), 5.46 (d, 1H, ^{3}J = 6.5 Hz, CHO). ^{1}H-NMR (CD_3OD) δ 14.48 (+, CH_3), 23.78 (-, CH_2), 27.16 (-, CH_2), 29.61 (-, CH_2), 29.68 (-, CH_2), 29.89 (-, CH_2), 30.19 (-, CH_2), 30.52 (-, CH_2), 30.64 (-, CH_2), 30.74 (-, CH_2), 30.80 (-, CH_2), 30.81 (-, CH_2), 30.85 (-, CH_2), 33.12 (-, CH_2), 69.72 (-, OCH_2), 70.67 (+, CH), 77.42 (+, CH), 79.93 (+, CH), 83.42 (+, CH), 107.71 (+, C-1), 172.59 (quat, CO_2), 172.66 (quat, CO_2), 172.80 (quat, CO_2), 175.52 (quat, CO_2), 175.89 (quat, CO_2). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 618 (100) $[M+NH_4]^+$. Anal. ($C_{30}H_{48}O_{12}\cdot H_2O$) C, H. $C_{30}H_{48}O_{12}$ (600.69).

6.5.2.11 General procedure for the synthesis of the sulfates 6.53 and 6.55 using SO_3-pyridine complex

To a solution of the pertinent glucuronolactone (1 eq) in anhydrous pyridine was added SO_3-pyridine complex (3-4 eq) and the mixture was stirred at room temperature overnight. The reaction was quenched by adding MeOH, the solvent was evaporated and the remaining residue was subjected to flash chromatography.

1-*O*-(Hexadecan-1-yl)-5-*O*-benzyloxycarbonyl-β-D-glucofuranosidurono-6,3-lactone-2-sulfate (6.53): The title compound was prepared according to general procedure 6.5.2.11 using **6.36** (0.8 mmol, 0.43 g) and SO_3-pyridine complex (2.4 mmol, 0.38 g) in anhydrous pyridine (5 ml) and was obtained after flash chromatography (CHCl3/MeOH 90/10 v/v) as yellow solid (0.3 g, 61 %). ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H, ^3J = 6.7 Hz, CH_3), 1.20 (m, 26H, $(C\underline{H}_2)_{13}CH_3$), 1.37 (m, 2H, $OCH_2C\underline{H}_2$), 3.22 (td, 1H, ^3J = 6.9 Hz, ^2J = 8.9 Hz, OCH_2), 3.59 (td, 1H, ^3J = 6.8 Hz, ^2J = 9.0 Hz, OCH_2), 4.48 (s, 1H, CHO), 5.00 (dd, 1H, ^3J = 5.1 Hz, ^3J = 6.9 Hz, CHO), 5.10 (s, 1H, CHO), 5.17 (d, 1H, ^3J = 5.0 Hz, CHO), 5.24 (s, 2H, CH_2Ph), 5.46 (d, 1H, ^3J = 6.9 Hz, CHO), 7.40 (m, 5H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 614 (100) $[M-H]^-$. $C_{30}H_{46}O_{11}S$ (614.74).

1-*O*-(Hexadecan-1-yl)-β-D-glucofuranosidurono-6,3-lactone-2-sulfate (6.54): The title compound was prepared according to general procedure 6.5.2.10 groups using **6.53** (0.41 mmol, 0.25 g) and 10 % Pd/C (0.25 g) in EtOAc (10 ml) and was obtained as a white solid (0.18 g, 91 %). mp: 140-141 °C; ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H, ^3J = 6.7 Hz, CH_3), 1.24 (m, 26H, $(C\underline{H}_2)_{13}CH_3$), 1.39 (m, 2H, $OCH_2C\underline{H}_2$), 3.19 (td, 1H, ^3J = 6.8 Hz, ^2J = 8.9 Hz, OCH_2), 3.67 (td, 1H, ^3J = 6.8 Hz, ^2J = 8.9 Hz, OCH_2), 4.45 (m, 2H, CH), 4.72 (dd, 1H, ^3J = 4.7 Hz, ^3J = 6.4 Hz, H-5), 4.99 (d, 1H, ^3J = 4.6 Hz, H-4), 5.07 (s, 1H, CH), 5.86 (d, 1H, ^3J = 6.4 Hz, 5-OH). ^{13}C-NMR (DMSO-d_6) δ 13.86 (+, CH_3), 22.00 (-, CH_2), 25.40 (-, CH_2), 28.52 (-, CH_2), 28.61 (-, CH_2), 28.85 (-, CH_2), 28.96 (-, CH_2), 31.19 (-, CH_2), 66.83 (-, OCH_2), 68.47 (+, C-5), 77.84 (+, CH), 80.44 (+, CH), 80.81 (+, CH), 106.43 (+, C-1), 174.71 (quat, lactone CO). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 479 (100) $[M-H]^-$. $C_{22}H_{40}O_9S$ (480.61).

1-*O*-(Hexadecan-1-yl)-β-D-glucofuranosidurono-6,3-lactone-2,5-disulfate (6.55): The title compound was prepared according to general procedure 6.5.2.11 using **6.6** (1 mmol, 0.4 g) and SO_3-pyridine complex (4.0 mmol, 0.64 g) in anhydrous pyridine (10 ml) and was obtained after flash chromatography ($CHCl_3$/MeOH 80/20 v/v) as a yellow solid (0.48 g, 86 %). mp: 171-73 °C; ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H, ^3J =

6.6 Hz), 1.23 (m, 26H), 1.37 (m, 2H), 3.18 (td, 1H, ^3J = 6.8 Hz, ^2J = 9.0 Hz, OCH_2), 3.63 (td, 1H, ^3J = 6.8 Hz, ^2J = 9.0 Hz, OCH_2), 4.44 (s, 1H, CHO), 4.79 (dd, 1H, ^3J = 4.7 Hz, ^3J = 6.2 Hz, CHO), 4.91 – 5.04 (m, 3H, CHO). ^{1}H-NMR (DMSO-d_6) δ 13.85 (+, CH_3), 21.99 (-, CH_2), 25.45 (-, CH_2), 28.55 (-, CH_2), 28.60 (-, CH_2), 28.90 (-, CH_2), 28.96 (-, CH_2), 31.18 (-, CH_2), 66.81(-, OCH_2), 71.65 (+, CH), 76.95 (+, CH), 80.38 (+, CH), 81.28 (+, CH), 106.33 (+, C-1), 171.63 (quat, lactone CO). ES-MS (MeOH + NH_4OAc) *m/z (%)*: 581 (100) $[M-2H+Na]^-$. $C_{22}H_{40}O_{12}S_2$ (560.68).

6.6 *References*

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Chapter 7
Ascorbic acid derivatives as potent inhibitors of bacterial and mammalian hyaluronidases

7.1 *Introduction*

Ascorbic acid (vitamin C) is a six-carbon lactone which is synthesized from glucose in the liver of most mammalian species except primates, guinea pigs, Indian fruit bats and some insects. In those exceptions the biosynthesis is not possible due to the lack of the enzyme gulonolactone oxidase which is essential for the synthesis of an ascorbic acid precursor[1, 2]. Therefore, humans must ingest ascorbic acid with the diet where vitamin C is present usually in quantities of 10-100 mg / 100 g [3]. An ascorbic acid deficiency is manifested in scurvey which may lead to death if untreated, indicating the enormous importance of this vitamin for survival. In economy this fine chemical is one of the most important specialty chemical manufactured with worldwide consumption of over 50000 tons[4]. The 3-OH of ascorbic acid is remarkably acidic (pK_a = 4.2), thus it is completely dissociated at neutral pH. Among the most important biological functions of vitamin C are the antioxidant[5], free radical scavenger[6], neuroprotectant and neuromodulator[7] properties. The basis of all these physiological and biochemical actions is the electron donor capability of ascorbic acid. It is implicated in host defense mechanisms[8] and probably involved in the prevention of various diseases[9], thus it may play a role in the prevention and treatment of cancer or viral infections[10, 11]. Eight different enzymes need ascorbic

acid as an electron donor in humans[1], three of them participate in collagen hydroxylation[12-14] and two of them are involved in carnitine synthesis[15, 16]. Until now only little information is available about the detailed interactions of vitamin C with proteins or enzymes at the molecular level. The first published crystal structure of a protein in complex with ascorbic acid was that of D-xylose isomerase (PDB code 1xid)[17].

Recently, the crystal structure of a bacterial hyaluronidase from *Streptococcus pneumoniae* (*Spn*Hyal) in complex with ascorbic acid, which was described to inhibit bovine testicular hyaluronidase[18], was elucidated (PDB code 1f9g)[2]. X-ray analysis verified that ascorbic acid binds to the active site and suggested that additional interactions with a hydrophobic patch might increase the potency. Therefore, L-ascorbic acid-6-*O*-hexadecanoate (vitamin C palmitate, Vcpal) was investigated in our workgroup for the inhibition of bacterial hyaluronan lyases, enzymes from *S. pneumoniae* (*Spn*Hyal) and *S. agalactiae* strain 4755 (*Sag*Hyal$_{4755}$), and bovine testicular hyaluronidase (BTH). Furthermore, *Spn*Hyal was crystallized in complex with Vcpal and the x-ray structure was determined[19].

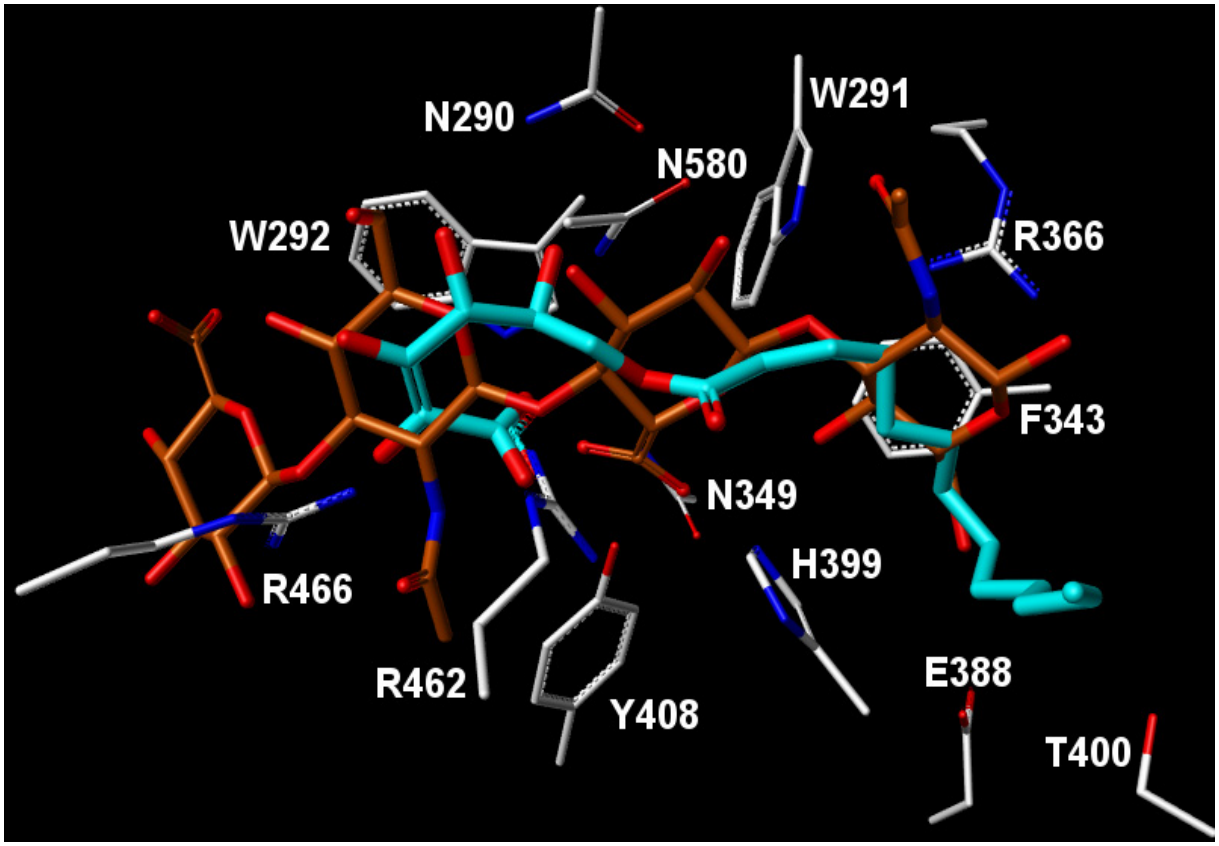


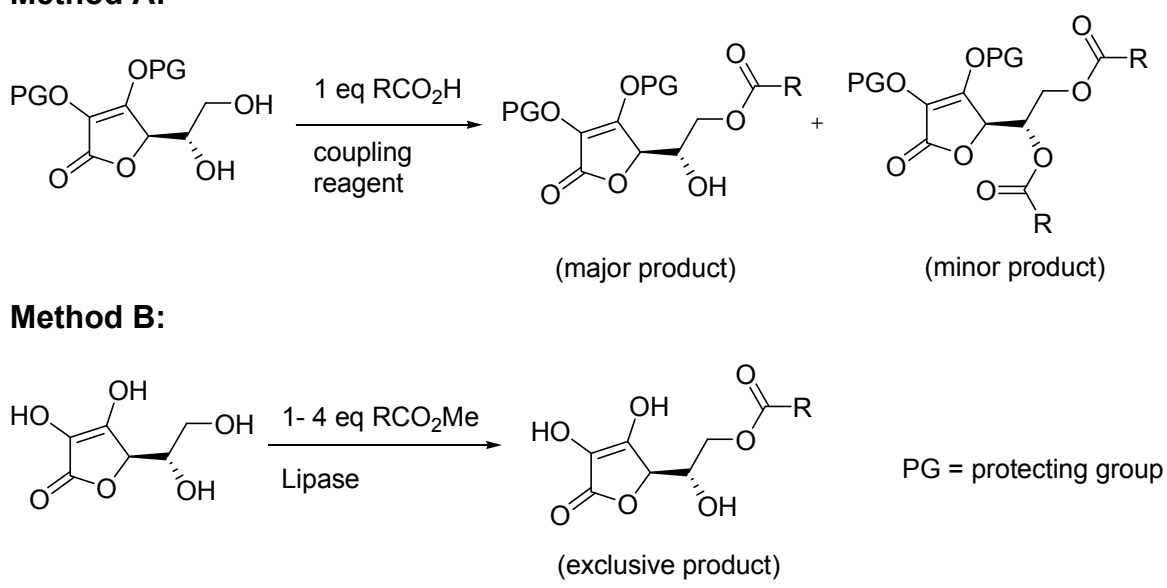
Figure 7.1. Binding mode of Vcpal (C atoms cyan) in the active site of *Spn*Hyal (PDB code 1w3y). The inhibitor was found to bind in a ring opened form. Missing electron density did not permit modeling of the last three carbon atoms of the palmitoyl chain; The hyaluronic acid fragment (C atoms brown) is taken from the complex PDB code 11oh, for clearity only the tetrasaccharide is shown.

Compared to vitamin C, Vcpal is up to 1500 times more potent as an inhibitor of bacterial and bovine hyaluronidases. As shown in Figure 7.1, Vcpal binds in the same region of the catalytic cleft of *Spn*Hyal as a hexasaccharide substrate[20]. In addition to hydrogen bonds of the carboxylate group of the inhibitor (the ring opened form of ascorbic acid was found in the crystal structure) with Tyr408 and Arg462 and of the hydroxy groups at C-4 and C-5 with Asn290, extensive interactions with a hydrophobic patch (Trp291, Trp292, Phe343) are evident. The hydrophobic face of the vitamin C moiety stacks with the indole ring of Trp292, and the palmitoyl group interacts with Trp291 and Phe343, but also with His399 and Thr400.

These results prompted us to synthesize various ascorbic acid derivatives with increased lipophilicity and to investigate these compounds for inhibition of the bacterial hyaluronate lyase from *S. agalactiae* strain 4755 (*Sag*Hyal$_{4755}$), the hyaluronidase from bovine testis (BTH) and the recombinantly expressed human enzymes Hyal-1 and PH-20[21, 22].

7.2 *Chemistry*

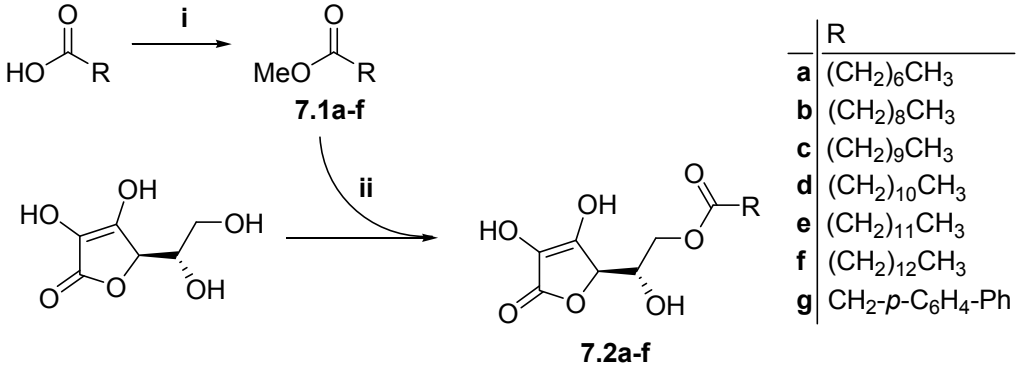
6-*O*-Acylation of ascorbic acid was achieved using two different synthetic strategies as depicted in Scheme 7.1.



Scheme 7.1. Strategies for the 6-*O*-acylation of ascorbic acid (for details see below).

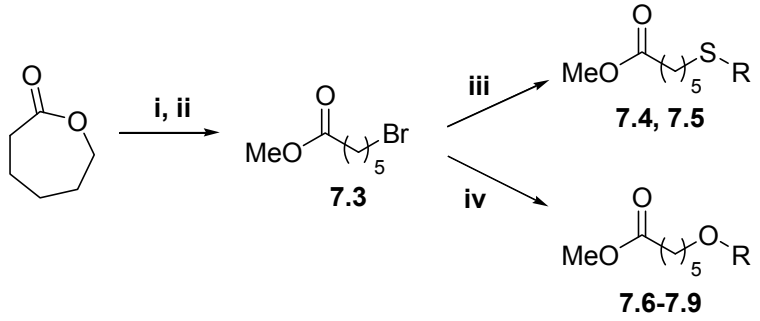
Method A uses standard coupling reagents for the 6-*O*-acylation of 2,3-di-*O*-protected ascorbic acid. This method is compatible with all kind of modification at *O*-2 and *O*-3 and there are no limitations concerning the carboxylic acid used. As a broad range of carboxylic acid activating reagents is commercially available, this synthetic procedure represents the method with the broadest range of applications. A disadvantage of this approach is the longer synthetic pathway because the preparation of the 2,3-di-*O*-protected building block. After the acylation at *O*-6, the removal of the protecting groups (PG) is required. Moreover, although *O*-6 is more reactive than *O*-5, 5,6-di-*O*-acylated compounds are obtained as byproducts which have to be separated by chromatography. Method B utilizes L-ascorbic acid together with methyl esters in presence of lipase from *Candida antarctica* immobilized on acrylic resin (Novozyme 435®) to achieve the 6-*O*-acylation[23, 24]. This enzymatic transesterification is highly regioselective - the protection of free hydroxyl groups is unnecessary – and the required methyl esters are easily accessible. This very short synthetic pathway comes to its limitations when the ascorbic acid moiety was modified prior to the acylation or when methyl esters other than linear fatty acid esters, e. g. branched or aryl substituted alkanoates, are used. Probably such compounds are not accepted as substrate by the enzyme. In these cases the "classical" pathway according to method A was applied in this work.

The 6-*O*-acylated ascorbic acid derivatives **7.2a-f** were synthesized from ascorbic acid and methyl alkanoates using Novozyme 435® in *tert*-amyl alcohol at 70 °C under reduced pressure[23, 24]. The required methyl esters **7.1a-f** were prepared from the pertinent commercially available carboxylic acids using TMSCl in MeOH[25] (Scheme 7.2).



Scheme 7.2. Synthesis of 6-*O*-acylated ascorbic acid derivatives **7.2a-f**. Reagents and conditions: (i) TMSCl (2.2 eq), MeOH, reflux overnight; (ii) Ascorbic acid (1 eq), RCO_2Me (1.5 – 4 eq), Novozyme 435® (50 mg/mmol), *tert*-amyl alcohol, 60 °C, 200 mbar, 16-24 h.

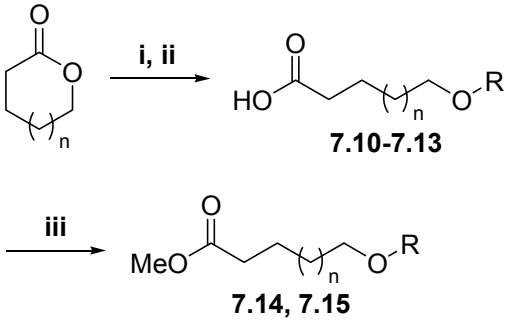
Etherification was chosen as the method of choice to introduce various residues at the end of the linear alkyl chain of carboxylic acids or esters which were then used in the aforementioned acylation reactions. In a first attempt ε-caprolactone was halogenated followed by esterification to yield methyl 6-bromohexanoate[26] (**7.3**) which was then used to alkylate phenols[27] or thiols[28, 29] (Scheme 7.3).



	R
7.4	Ph
7.5	CH_2Ph
7.6	Ph
7.7	*p*-C_6H_4-C_2H_5
7.8	*p*-C_6H_4-Ph
7.9	*p*-C_6H_4-OCH_2Ph

Scheme 7.3. Synthesis of ether and thioether building blocks **7.4-7.9**. Reagents and conditions: (i) HBr/HOAc, reflux 6 h; (ii) Addition of MeOH, RT overnight; (iii) R = aryl: RSH (1.1 eq), DIEA (1.1 eq), THF, RT overnight or R = alkyl: RSH (1 eq), K_2CO_3 (2 eq), THF, reflux overnight; (iv) ROH (1.5 eq), K_2CO_3 (3 eq), acetone, reflux overnight.

The building blocks **7.12-7.15** were synthesized *via* alkylation of the corresponding lactone using alkyl halides in presence of potassium hydroxide[30] (Scheme 7.4) to yield the carboxylic acids **7.10-7.13** which were either transformed to the corresponding methyl esters employed in the enzymatic transesterification procedure (compounds **7.14** and **7.15**) or used as free carboxylic acids (compounds **7.12** and **7.13**) to acylate 2,3-di-*O*-protected ascorbic acids according to standard coupling protocols as described below.

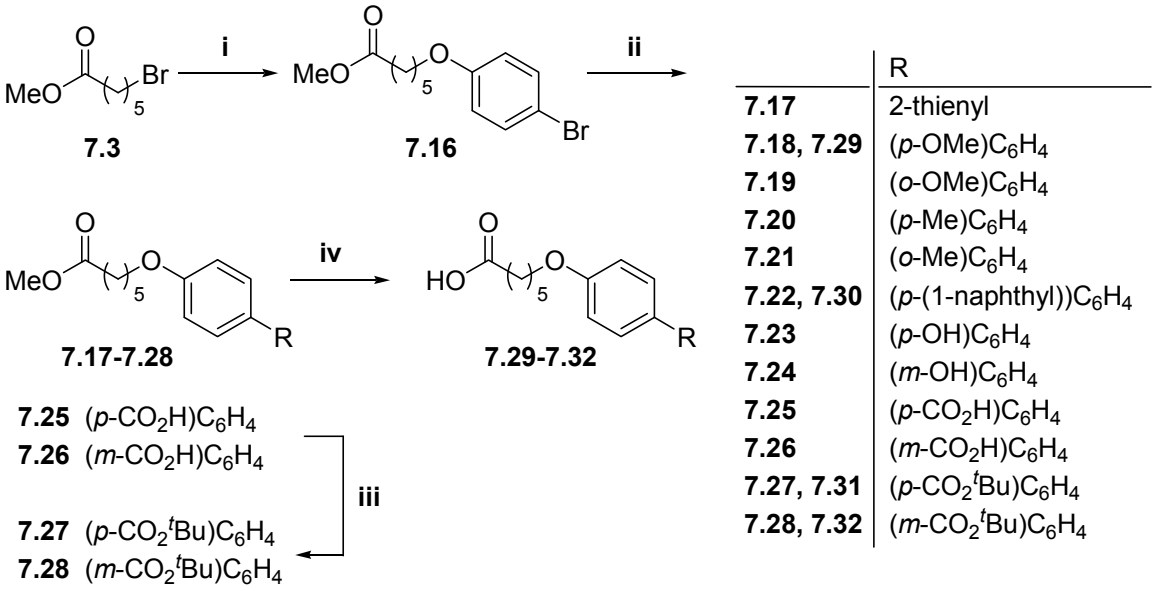


	n	R
7.10, 7.14	1	CH_2Ph
7.11, 7.15	1	CH_2-*p*-C_6H_4-Ph
7.12	0	$(CH_2)_6CH_3$
7.13	1	$(CH_2)_5CH_3$

Scheme 7.4. Synthesis of the intermediates **7.10-7.15**. Reagents and conditions: (i) RHal (2 eq), KOH (3 eq), toluene, reflux overnight; (ii) 1 N HCl; (iii) TMSCl (2.2 eq), MeOH, reflux overnight.

The biaryl derivatives **7.17-7.26** were synthesized from **7.16** according to Scheme 7.5 employing Suzuki cross coupling for the C-C-bond formation[31, 32]. The necessary boronic acids were commercially available or synthesized according to known procedures[33, 34]. Because the enzymatic acylation approach using **7.19, 7.22, 7.27** and **7.28** did not yield the desired target compounds, these esters had to be

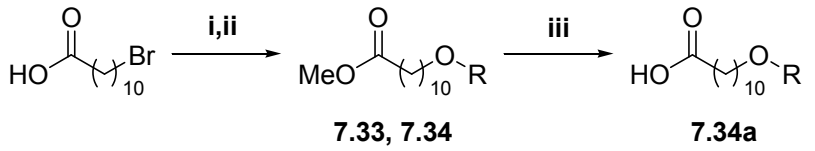
transformed into the corresponding carboxylic acids **7.29-7.32** to be used in the acylation procedure using standard coupling reagents. The aromatic carboxyl residues of **7.25** and **7.26** were protected using TBTA[35] to form *tert*-butyl esters prior to the saponification step.



	R
7.17	2-thienyl
7.18, 7.29	(*p*-OMe)C_6H_4
7.19	(*o*-OMe)C_6H_4
7.20	(*p*-Me)C_6H_4
7.21	(*o*-Me)C_6H_4
7.22, 7.30	(*p*-(1-naphthyl))C_6H_4
7.23	(*p*-OH)C_6H_4
7.24	(*m*-OH)C_6H_4
7.25	(*p*-CO_2H)C_6H_4
7.26	(*m*-CO_2H)C_6H_4
7.27, 7.31	(*p*-$CO_2{}^t$Bu)C_6H_4
7.28, 7.32	(*m*-$CO_2{}^t$Bu)C_6H_4

Scheme 7.5. Synthesis of biaryl building blocks. Reagents and conditions: (i) *p*-Bromophenol (1.5 eq), K_2CO_3 (3 eq), acetone, reflux overnight; (ii) $RB(OH)_2$ (1.5 eq), $Pd(PPh_3)_4$ (1.5mol %), 2 N Na_2CO_3 (1-2 eq), H_2O / MeOH / toluene, 80 °C 48 h; (iii) TBTA (2 eq), BF_3Et_2O (cat), THF, RT overnight; (iv) LiOH (1.5 eq), H_2O / MeOH / THF, RT overnight; (v) 1 N HCl.

The alkylation of 11-bromoundecanoic acid using phenols in presence of KOH yielded building blocks with increased spacer length[36]. As the purification of the carboxylic acids proved to be difficult, the crude carboxylic acids were first esterified to obtain the building blocks **7.33** and **7.34**, which were easily purified using flash chromatography. **7.34a** was then obtained by cleavage of the methyl ester (Scheme 7.6).

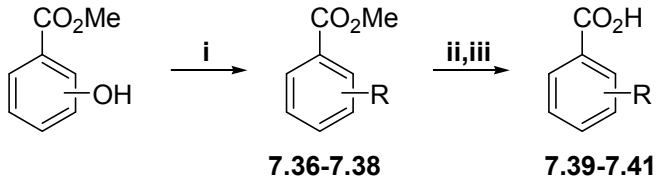


	R
7.33	Ph
7.34, 7.34a	*p*-C_6H_4-Ph

Scheme 7.6. Synthesis of building blocks **7.33**, **7.34** and **7.34a**. Reagents and conditions: (i) ROH (1.1 eq), KOH (2.2 eq), toluene, reflux overnight; (ii) 1 N HCl; (iii) TMSCl (2.2 eq), MeOH, reflux overnight; (iii) LiOH (1.5 eq), H_2O / MeOH / THF, RT overnight.

With respect to the preparation of vitamin C esters bearing a side chain carboxylic group dodecanedioic acid was monoprotected using an equivalent of ^{t}BuOH in presence of DCC and DMAP according to known procedures to obtain dodecanoic acid mono *tert*-butyl ester[37] (**7.35**).

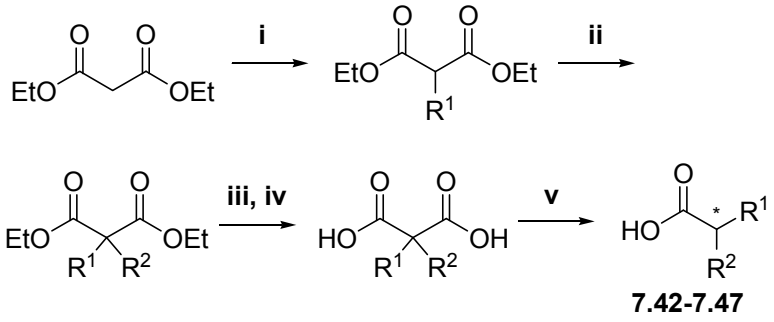
To introduce aromatic residues next to the ascorbic acid moiety, the carboxylic acids **7.39-7.41** were prepared from the pertinent methyl hydroxybenzoates and 1-bromodecane according to Scheme 7.7.



	R
7.36, 7.39	*o*-O$(CH_2)_9CH_3$
7.37, 7.40	*m*-O$(CH_2)_9CH_3$
7.38, 7.41	*p*-O$(CH_2)_9CH_3$

Scheme 7.7. Synthesis of building blocks **7.39-7.41**. Reagents and conditions: (i) 1-Bromodecane (1.5 eq), K_2CO_3 (3 eq), acetone, reflux overnight; (ii) LiOH (1.5 eq), H_2O / MeOH / THF, RT overnight; (iii) 1 N HCl.

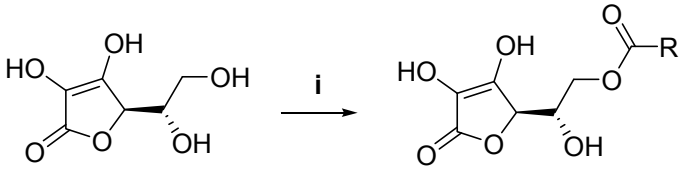
The α-branched alkanoic acids **7.42-7-47** were synthesized according to standard malonic ester synthesis[38] from diethyl malonate and alkyl halides using NaH as base. These carboxylic acids were obtained in racemic form after hydrolysis of the ethyl ester and decarboxylation (Scheme 7.8).



	R^1	R^2
7.42	$(CH_2)_9CH_3$	$(CH_2)_2CH_3$
7.43	$(CH_2)_9CH_3$	$(CH_2)_5CH_3$
7.44	$(CH_2)_9CH_3$	$(CH_2)_9CH_3$
7.45	$(CH_2)_9CH_3$	$(CH_2)_2Ph$
7.46	$(CH_2)_9CH_3$	CH_2(*p*-OMe)C_6H_4
7.47	$(CH_2)_{17}CH_3$	$(CH_2)_5CH_3$

Scheme 7.8. Synthesis of α-branched carboxylic acids **7.42-7.47**. Reagents and conditions: (i) NaH (1 eq), R^1Hal, THF, RT then reflux overnight; (ii) NaH (1 eq), R^2Hal, RT then reflux overnight; (iii) KOH (10 eq), EtOH / H_2O, reflux overnight; (iv) 2 N HCl; (v) 170 – 200 °C, 5 h.

As explained above, the obtained methyl ester building blocks were used for the synthesis of **7.48-7.63** applying the enzymatic approach (Scheme 7.9). In several cases the enzymatic acylation was not successful. An example is the reaction of methyl 6-[4-(naphthalen-1-yl)phenoxy]hexanoate (**7.22**) or methyl 6-[4-(4-methoxyphenyl)phenoxy]hexanoate (**7.18**) with vitamin C in the presence of Novozyme 435®. TLC analysis revealed that only traces of the 6-*O*-acyl ascorbic acid were formed whereas the mixture contained mainly starting material and, due to enzymatic cleavage of the methyl ester, the corresponding carboxylic acids. In the case of methyl 6-[4-(2-methoxyphenyl)phenoxy]hexanoate (**7.19**) the acylated ascorbic acid **7.57** was isolated by preparative HPLC with a yield as low as 4 %.

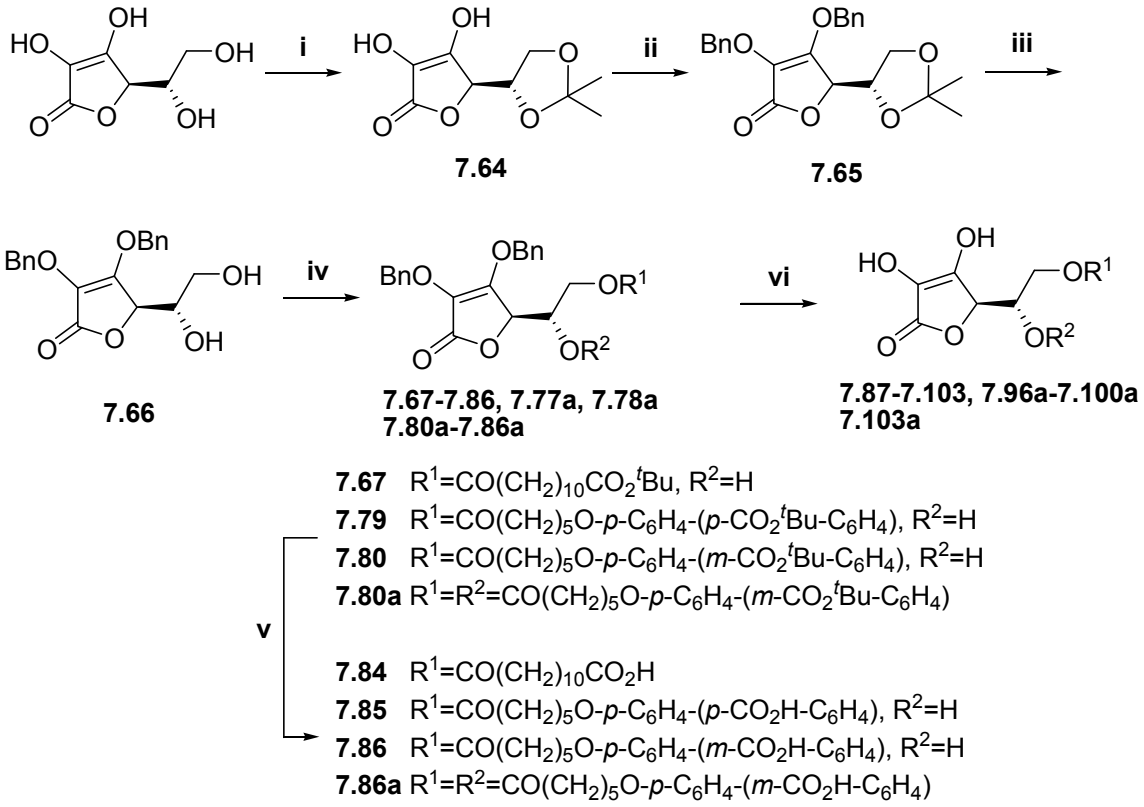


7.48-7.63

	R
7.48	$(CH_2)_5SPh$
7.49	$(CH_2)_5SCH_2Ph$
7.50	$(CH_2)_5OPh$
7.51	$(CH_2)_5O$-*p*-C_6H_4-C_2H_5
7.52	$(CH_2)_5O$-*p*-C_6H_4-Ph
7.53	$(CH_2)_5O$-*p*-C_6H_4-OCH_2Ph
7.54	$(CH_2)_5OCH_2Ph$
7.55	$(CH_2)_5OCH_2$-*p*-C_6H_4-Ph
7.56	$(CH_2)_5O$-*p*-C_6H_4-2-thienyl
7.57	$(CH_2)_5O$-*p*-C_6H_4-(*o*-OMe-C_6H_4)
7.58	$(CH_2)_5O$-*p*-C_6H_4-(*p*-Me-C_6H_4)
7.59	$(CH_2)_5O$-*p*-C_6H_4-(*o*-Me-C_6H_4)
7.60	$(CH_2)_5O$-*p*-C_6H_4-(*p*-OH-C_6H_4)
7.61	$(CH_2)_5O$-*p*-C_6H_4-(*m*-OH-C_6H_4)
7.62	$(CH_2)_{10}OPh$
7.63	$(CH_2)_{10}O$-*p*-C_6H_4-Ph

Scheme 7.9. Synthesis of 6-*O*-acylated ascorbic acid derivatives **7.48-7.63** using Novozyme 435®. Reagents and conditions: (i) RCO_2Me (1.5 – 4 eq), Novozyme 435® (50mg/mmol), *tert*-amyl alcohol, 60 °C, 200 mbar, 16-24 h.

Due to this shortcoming the 6-*O*-acylated ascorbic acid derivatives **7.87–7.103** were synthesized from the obtained carboxylic acid building blocks using standard reagents for the activation and esterification. However, the enediol system of vitamin C had to be protected to make the use of standard coupling reagents possible in the acylation step. Benzyl ethers were chosen as protecting groups, because they can be cleaved easily by mild hydrogenolysis which leaves the sensitive ester bond and the lactone ring of the ascorbic acid moiety intact. The double bond of ascorbic acid was not affected under these conditions. Additionally the benzyl protecting group is orthogonal to the *tert*-butyl esters which were used for the protection of side chain functionalities. Therefore 2,3-di-*O*-benzyl-L-ascorbic acid (**7.66**) was synthesized according to known procedures[39, 40] and acylated followed by removal of the protecting groups present (Scheme 7.10). Compound **7.89** was synthesized from commercially available 2-hexyldecanoic acid. In some cases the 5,6-di-*O*-acylated byproducts from the acylation reaction (**7.77a, 7.78a, 7.80a-7.83a**) were isolated and deprotected to yield the 5,6-di-*O*-acylated ascorbic acids **7.96a-7.100a** and **7.103a**. The substitution pattern for Scheme 7.10 is given in Table 7.1.



Scheme 7.10. Synthesis of 6-*O*-acylated ascorbic acid derivatives **7.87-7.103, 7.96a-7.100a** and **7.103a**. Reagents and conditions: (i) abs. acetone, AcCl (cat); (ii) K_2CO_3 (1.1 eq), BnBr (2.2 eq), DMF, 40-60 °C; (iii) 50 % HOAc, MeOH, 80 °C; (iv) RCO_2H (1 eq), EDAC (1.1 eq) DMAP (1.2 eq), DMF, RT overnight; (v) TFA / CH_2Cl_2, RT 6h; (vi) 10 % Pd/C (cat.), H_2, 4 bar, EtOH / EtOAc.

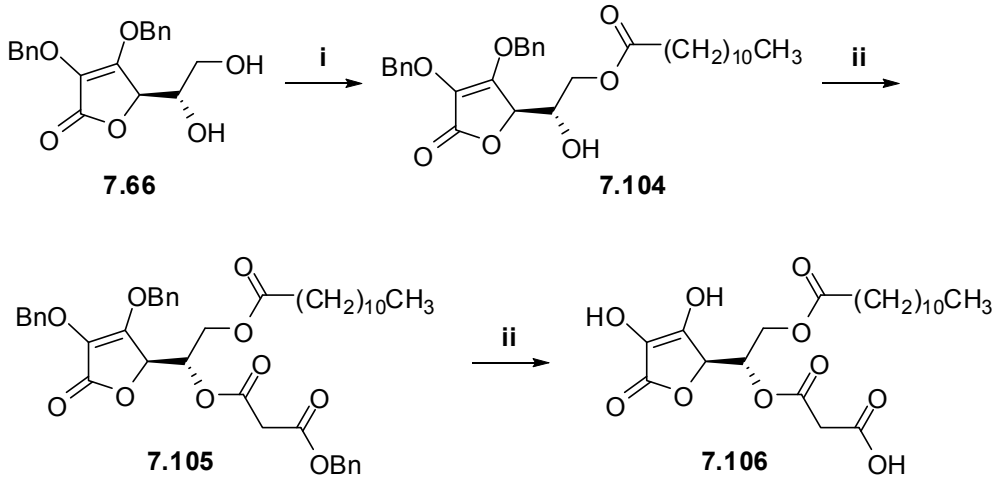
Table 7.1. Substitution patterns for Scheme 7.10.

No	R^1	R^2
7.67	$CO(CH_2)_{10}CO_2{}^tBu$	H
7.68, 7.87	CO-*o*-$C_6H_4O(CH_2)_9CH_3$	H
7.69, 7.88	CO-*m*-$C_6H_4O(CH_2)_9CH_3$	H
7.70, 7.89	$COCH((CH_2)_5CH_3)(CH_2)_7CH_3$	H
7.71, 7.90	$COCH((CH_2)_2CH_3)(CH_2)_9CH_3$	H
7.72, 7.91	$COCH((CH_2)_5CH_3)(CH_2)_9CH_3$	H
7.73, 7.92	$COCH((CH_2)_9CH_3)(CH_2)_9CH_3$	H
7.74, 7.93	$COCH((CH_2)_2Ph)(CH_2)_9CH_3$	H
7.75, 7.94	$COCH(CH_2$-*p*-MeO-$C_6H_4)(CH_2)_9CH_3$	H
7.76, 7.95	$COCH((CH_2)_5CH_3)(CH_2)_{15}CH_3$	H
7.77, 7.96	$CO(CH_2)_5O$-*p*-C_6H_4-(*p*-OMe-C_6H_4)	H
7.77a, 7.96a	$CO(CH_2)_5O$-*p*-C_6H_4-(*p*-OMe-C_6H_4)	$=R^1$
7.78, 7.97	$CO(CH_2)_5O$-*p*-C_6H_4-(1-naphthyl)	H
7.78a, 7.97a	$CO(CH_2)_5O$-*p*-C_6H_4-(1-naphthyl)	$=R^1$
7.79	$CO(CH_2)_5O$-*p*-C_6H_4-(*p*-CO_2tBu-C_6H_4)	H
7.80	$CO(CH_2)_5O$-*p*-C_6H_4-(*m*-CO_2tBu-C_6H_4)	H
7.80a	$CO(CH_2)_5O$-*p*-C_6H_4-(*m*-$CO_2{}^t$Bu-C_6H_4)	$=R^1$
7.81, 7.98	$CO(CH_2)_4O(CH_2)_6CH_3$	H

Table 7.1 (continued)

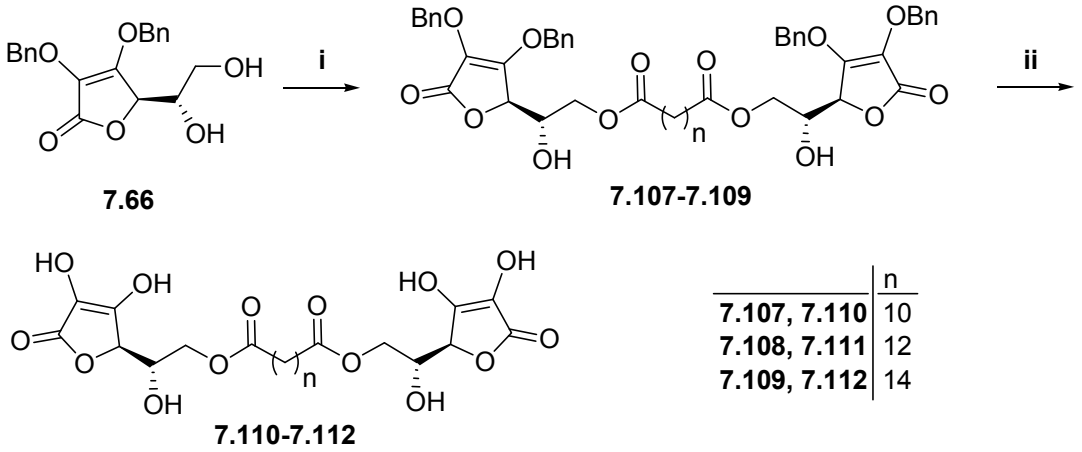
7.81a, 7.98a	$CO(CH_2)_4O(CH_2)_6CH_3$	$=R^1$
7.82, 7.99	$CO(CH_2)_5O(CH_2)_5CH_3$	H
7.82a, 7.99a	$CO(CH_2)_5O(CH_2)_5CH_3$	$=R^1$
7.83, 7.100	CO-*p*-$C_6H_4O(CH_2)_9CH_3$	H
7.83a, 7.100a	CO-*p*-$C_6H_4O(CH_2)_9CH_3$	$=R^1$
7.84, 7.101	$CO(CH_2)_{10}CO_2H$	H
7.85, 7.102	$CO(CH_2)_5O$-*p*-C_6H_4-(*p*-CO_2H-C_6H_4)	H
7.86, 7.103	$CO(CH_2)_5O$-*p*-C_6H_4-(*m*-CO_2H-C_6H_4)	H
7.86a, 7.103a	$CO(CH_2)_5O$-*p*-C_6H_4-(*m*-CO_2H-C_6H_4)	$=R^1$

Compound **7.106** was synthesized *via* stepwise acylation of **7.66** and removal of the benzyl groups as shown in Scheme 7.11.



Scheme 7.11. Synthesis of **7.106**. Reagents and conditions: (i) Lauric acid (1 eq), EDAC (1.1 eq), DMAP (1.2 eq), DMF, RT overnight; (ii) Monobenzyl malonate (**6.33**) (1 eq), EDAC (1.1 eq) DMAP (1.2 eq), DMF, RT overnight; (ii) 10 % Pd/C (cat.), H_2, 4 bar, EtOH / EtOAc.

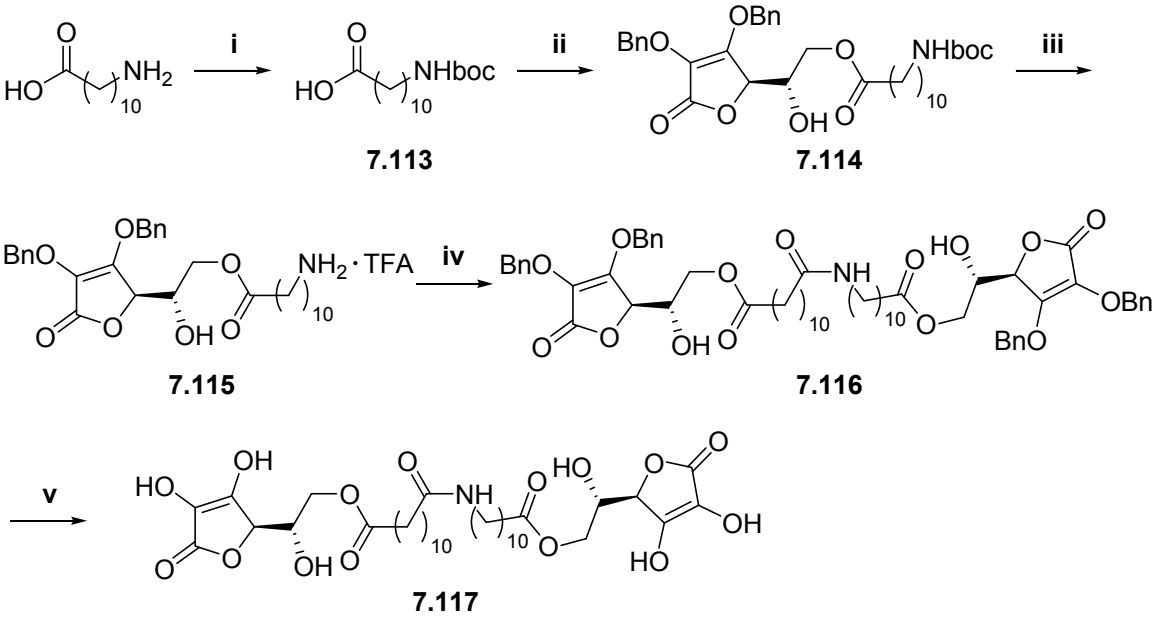
The "bivalent compounds" **7.110-7.112** were accessible from the reaction of the pertinent dicarboxylic acids with two equivalents of **7.66** and subsequent deprotection according to Scheme 7.12.



	n
7.107, 7.110	10
7.108, 7.111	12
7.109, 7.112	14

Scheme 7.12. Synthesis of "bivalent" derivatives **7.110-7.112**. Reagents and conditions: (i) $HO_2C(CH_2)_nCO_2H$ (0.5 eq), EDAC (1.1 eq) DMAP (1.2 eq), DMF, RT overnight; (ii) 10 % Pd/C (cat.), H_2, 4 bar, EtOH / EtOAc.

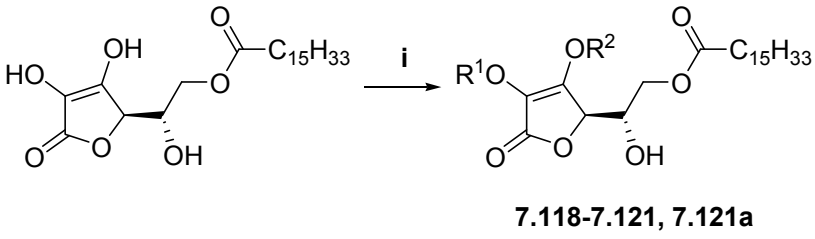
With respect to the variation of chain length and the functional groups of the spacer in "bivalent enzyme inhibitors", 11-aminoundecanoic acid was *N*-boc-protected[41] and treated with **7.66** to give the ester **7.114** which was *N*-deprotected and coupled with the carboxyalkanoyl-L-ascorbic acid **7.84**. Subsequent hydrogenolysis yielded the bivalent compound **7.117** (Scheme 7.13).



Scheme 7.13. Synthesis of **7.117**. Reagents and conditions: (i) Boc_2O (1.1 eq), NaOH (2.2 eq) THF / MeOH, RT overnight; (ii) **7.66** (1 eq), EDAC (1.1 eq) DMAP (1.2 eq), DMF, RT overnight; (iii) TFA / CH_2Cl_2, RT, 6h (iv) **7.84** (1 eq), EDAC (1.1 eq) DMAP (1.2 eq), DMF, RT overnight; (v) 10 % Pd/C (cat.), H_2, 4 bar, EtOH / EtOAc.

Ascorbic acid 6-palmitate was 2-*O*-acylated[42-44] with equimolar amounts of cyclic anhydrides, which were accessible according to described procedures[45], or acid

chlorides to yield **7.118-7.121** without using any protecting groups; **7.121a** was isolated as a byproduct in the monoacylation reaction (Scheme 7.14).

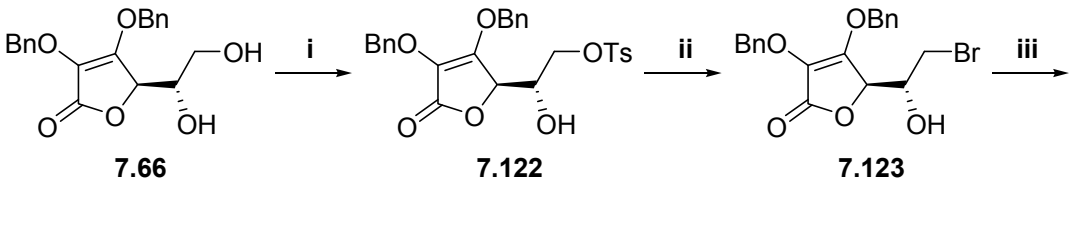


7.118-7.121, 7.121a

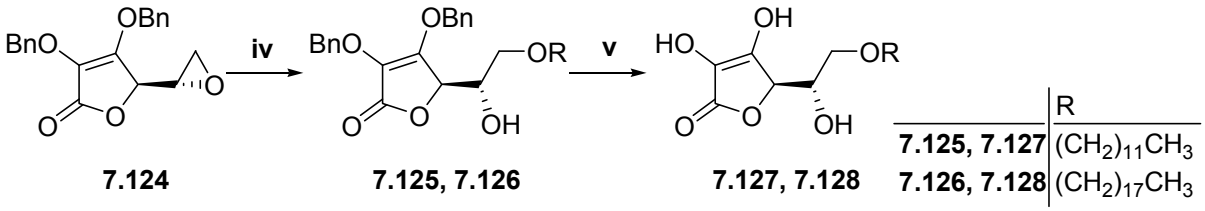
	R^1	R^2
7.118	$CO(CH_2)_2Ph$	H
7.119	$CO(CH_2)_2CO_2H$	H
7.120	$CO(CH_2)_3CO_2H$	H
7.121	$CO(CH_2)_4CO_2H$	H
7.121a	$CO(CH_2)_4CO_2H$	$=R^1$

Scheme 7.14. Synthesis of 2-*O*- and 2,3-di-*O*-acylated ascorbic acid 6-palmitates **7.117-7.120** and **7.120a**. Reagents and conditions: (i) Cyclic anhydride (1 eq), DMAP (cat.), CH_2Cl_2 / THF, RT overnight, or RCOCl (1 eq), DMAP (cat.), NEt_3 (1 eq), CH_2Cl_2 / THF, RT, overnight.

For the 6-*O*-alkylation of ascorbic acid a modified procedure described by Sanders and Dallacker[40, 46, 47] was chosen (Scheme 7.15). First **7.66** was selectively 6-*O*-tosylated followed by a Finkelstein-like exchange with bromine. Finally **7.123** was transformed into the corresponding epoxide which can be opened to form the desired 6-alkylethers. Neither attempts to substitute the tosylate **7.122** or the bromo-derivative **7.123** with alcohols in the presence of a base nor ring-opening of the epoxide with alcohols under basic conditions were successful. Therefore, alkylation of **7.124** was performed under acidic conditions to obtain **7.125** and **7.126** which yielded **7.127** and **7.128** after hydrogenolysis.



7.66 **7.122** **7.123**



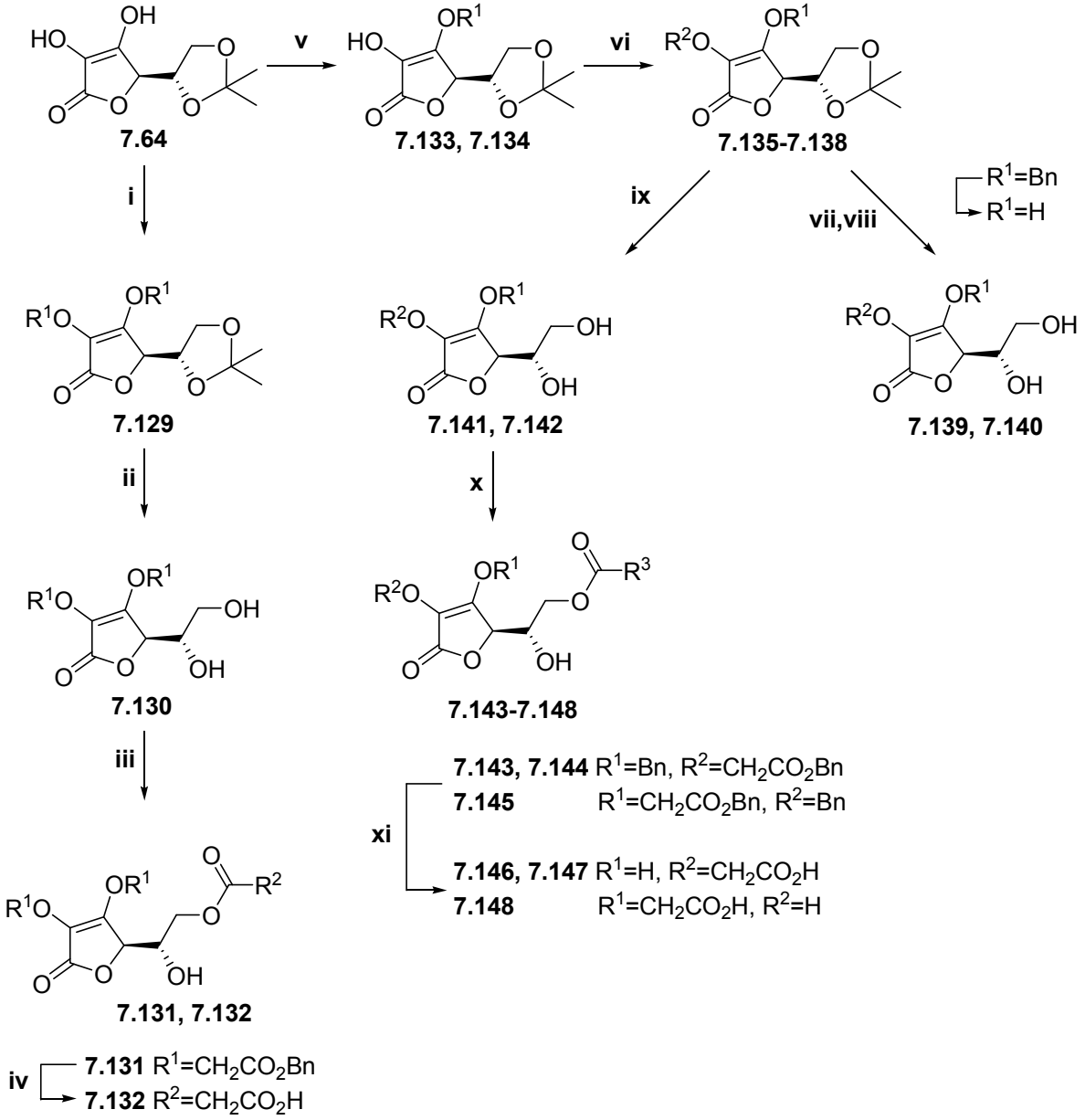
7.124 **7.125, 7.126** **7.127, 7.128**

	R
7.125, 7.127	$(CH_2)_{11}CH_3$
7.126, 7.128	$(CH_2)_{17}CH_3$

Scheme 7.15. Synthesis of 6-*O*-alkylascorbic acids **7.127** and **7.128**. Reagents and conditions: (i) TsCl, pyridine, 0 °C – RT overnight; (ii) NaBr, acetone, 120 °C overnight; (iii) KOH, NBu_4I (cat.), CH_2Cl_2 / H_2O; (iv) ROH, BF_3Et_2O (cat.), THF, RT overnight; (v) 10 % Pd/C (cat.), H_2, 4 bar, EtOH / EtOAc, RT overnight.

The synthetic pathway to 2-*O*-/3-*O*-alkylascorbic acids is depicted in Scheme 7.16 (see Table 7.2 for the definition of the residues). Two equivalents of benzyl bromoacetate were used to alkylate **7.64** in the presence of K_2CO_3. Cleavage of the

acetal, acylation of *O*-6 and cleavage of the benzyl esters gave compound **7.132**. Selective 3-*O*-alkylation was possible when **7.64** was treated with one equivalent of alkyl halide[39]. Subsequent alkylation of *O*-2[48] yielded the precursors **7.135-7.138** which were then deprotected[39] to obtain the 2-*O*-alkyl ascorbates **7.139** and **7.140**. Alternatively, only the acetal was cleaved to enable the acylation in position *O*-6 of the vitamin C moiety. After acylation of **7.141** or **7.142** the benzyl groups were removed to obtain **7.146-7.148**.

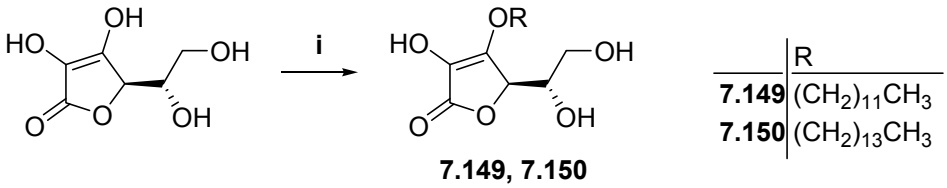


Scheme 7.16. Synthesis of 2-*O*-/3-*O*-alkylated ascorbic acid derivatives **7.132, 7.135, 7.136** and **7.146-7.148**. Reagents and conditions: (i) K_2CO_3 (1.1 eq), R^1Br (2.2 eq), DMF, 40-60 °C; (ii) 2 N HCl, MeOH / THF, RT, 6h; (iii) R^2CO_2H (1 eq), EDAC (1.1 eq) DMAP (1.2 eq), DMF, RT overnight; (iv) 10 % Pd/C (cat.), H_2, 4 bar, EtOH / EtOAc; (v) K_2CO_3 (1 eq), R^1Hal (1 eq), THF / DMF, RT, 4h; (vi) K_2CO_3 (1.1 eq), R^2Hal (1.1 eq), THF / DMSO, RT, 4h; (vii) 2 N HCl, MeOH / THF, RT, 6h; (viii) 10 % Pd/C (cat.), H_2, 4 bar, EtOH / EtOAc; (ix) 2 N HCl, MeOH / THF, RT, 6h; (x) R^3CO_2H (1 eq), EDAC (1.1 eq) DMAP (1.2 eq), DMF, RT overnight; (xi) 10 % Pd/C (cat.), H_2, 4 bar, EtOH / EtOAc.

Table 7.2. Substitution pattern for Scheme 7.16.

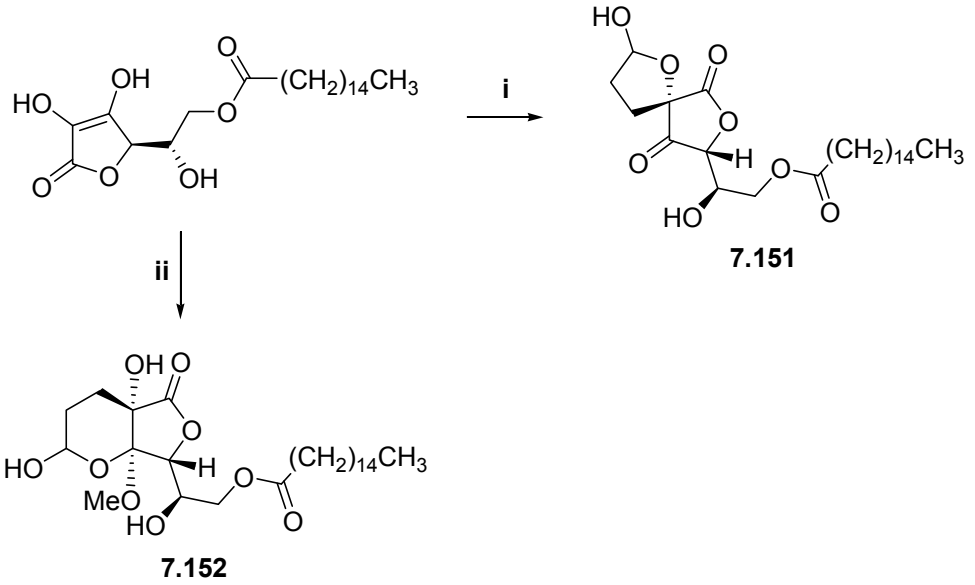
No	R^1	R^2	R^3
7.129, 7.130	CH_2CO_2Bn	-	-
7.131	CH_2CO_2Bn	-	$(CH_2)_{10}CH_3$
7.132	CH_2CO_2H	-	$(CH_2)_{10}CH_3$
7.133	Bn	-	-
7.134	CH_2CO_2Bn	-	-
7.135	Bn	$(CH_2)_{11}CH_3$	-
7.136	Bn	$(CH_2)_{15}CH_3$	-
7.137, 7.141	Bn	CH_2CO_2Bn	-
7.138, 7.142	CH_2CO_2Bn	Bn	-
7.139	H	$(CH_2)_{11}CH_3$	-
7.140	H	$(CH_2)_{15}CH_3$	-
7.143	Bn	CH_2CO_2Bn	$(CH_2)_{10}CH_3$
7.144	Bn	CH_2CO_2Bn	$(CH_2)_{10}O$-*p*-C_6H_4-Ph
7.145	CH_2CO_2Bn	Bn	$(CH_2)_{10}CH_3$
7.146	H	CH_2CO_2H	$(CH_2)_{10}CH_3$
7.147	H	CH_2CO_2H	$(CH_2)_{10}O$-*p*-C_6H_4-Ph
7.148	CH_2CO_2H	H	$(CH_2)_{10}CH_3$

Selective 3-*O*-alkylation of unprotected ascorbic acid was performed under Mitsunobu conditions according to a known procedure[49] to obtain **7.149** and **7.150** in one step (Scheme 7.17).



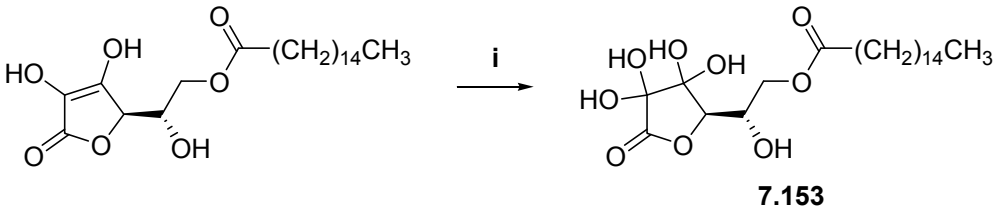
Scheme 7.17. Synthesis of **7.149** and **7.150** under Mitsunobu conditions. Reagents and conditions: (i) PPh_3 (1.14 eq), DIAD (1.12 eq), ROH (1.25 eq), THF / DMF, -78 °C – RT overnight.

Using ascorbic acid 6-palmitate and acrolein as Michael donor and acceptor, respectively, resulted in the spirane **7.151** or the bicyclic compound **7.152** depending on which solvent was used as described by Witt et al.[50] (Scheme 7.18).



Scheme 7.18. Synthesis of **7.151** and **7.152**. Reagents and conditions: (i) Acrolein (1.2 eq), ^{t}BuOH, 70 °C, 48 h. (ii) (1.2 eq), MeOH, 70 °C, 48 h.

The oxidized compound **7.153** was synthesized from ascorbic acid 6-palmitate as described by Okolotowicz et al.[51](Scheme 7.19).



Scheme 7.19. Oxidation of ascorbic acid 6-palmitate. Reagents and conditions: (i) I_2 (1 eq), EtOH, RT, 30 min.

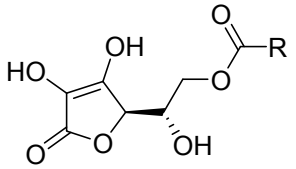
7.3 *Inhibition of hyaluronidases: results and discussion*

All synthesized L-ascorbic acid derivatives were investigated for inhibition of recombinant human hyaluronidases PH-20 and Hyal-1, the bovine testicular enzyme BTH (Neopermease®) and the bacterial hyaluronan lyase *Sag*Hyal$_{4755}$ in a modified turbidimetric assay based on the method of Di Ferrante[52] as described in chapter 3.4.3.

7.3.1 6-*O*-Acylated ascorbic acid derivatives

The IC_{50}-values determined for the 6-*O*-acylated L-ascorbic acid derivatives are summarized in Table 7.3.

Table 7.3. Inhibitory activity of 6-*O*-acylated ascorbic acid derivatives.



Compd.	R^1	Hyal-1	PH-20	BTH	*Sag*Hyal$_{4755}$
		IC_{50} [µM][a]			
Vitamin C	H	> 60000	7564 ± 883	> 100[b]	6100 ± 100[b]
Vcpal	$(CH_2)_{14}CH_3$	> 50	5.0 ± 0.2	57 ± 1[b]	4.2 ± 0.1[b]
7.2a	$(CH_2)_6CH_3$	> 1000	232 ± 46	>2000	772 ± 19
7.2b	$(CH_2)_8CH_3$	378 ± 12	131 ± 26	1380 ± 49	102 ± 5
7.2c	$(CH_2)_9CH_3$	143 ± 4	33 ± 2	580 ± 21	72 ± 2
7.2d	$(CH_2)_{10}CH_3$	76 ± 3	25 ± 2	208 ± 4	47 ± 2
7.2e	$(CH_2)_{11}CH_3$	50 ± 4	14 ± 2	96 ± 5	14 ± 1
7.2f	$(CH_2)_{12}CH_3$	> 70	5.8 ± 0.3	71 ± 1	8.4 ± 0.2
7.2g	CH_2-*p*-C_6H_4-Ph	753 ± 89	156 ± 10	2006 ± 40	358 ± 15
7.48	$(CH_2)_5SPh$	890 ± 42	98 ± 7	> 1500	607 ± 58
7.49	$(CH_2)_5SCH_2Ph$	900 ± 100	189 ± 30	> 1000	461 ± 19
7.50	$(CH_2)_5OPh$	> 1000	370 ± 53	> 1500	759 ± 45
7.51	$(CH_2)_5O$-*p*-C_6H_4-C_2H_5	895 ± 29	184 ± 38	> 1200	280 ± 9
7.52	$(CH_2)_5O$-*p*-C_6H_4-Ph	90 ± 6	20 ± 1	188 ± 3	61 ± 1
7.53	$(CH_2)_5O$-*p*-C_6H_4-OCH_2Ph	> 200	33 ± 1	210 ± 3	76 ± 1
7.54	$(CH_2)_5OCH_2Ph$	> 800	229 ± 53	> 1000	437 ± 77
7.55	$(CH_2)_5OCH_2$-*p*-C_6H_4-Ph	124 ± 5	40 ± 2	543 ± 15	102 ± 2
7.56	$(CH_2)_5O$-*p*-C_6H_4-2-thienyl	≥ 80	28 ± 1	146 ± 3	54 ± 2
7.57	$(CH_2)_5O$-*p*-C_6H_4-(*o*-OMe-C_6H_4)	122 ± 16	42 ± 4	> 300	58 ± 6
7.58	$(CH_2)_5O$-*p*-C_6H_4-(*p*-Me-C_6H_4)	> 200	13 ± 1	87 ± 4	24 ± 1
7.59	$(CH_2)_5O$-*p*-C_6H_4-(*o*-Me-C_6H_4)	63 ± 5	31 ± 2	232 ± 2	67 ± 2
7.60	$(CH_2)_5O$-*p*-C_6H_4-(*p*-OH-C_6H_4)	> 300	53 ± 3	152 ± 1	66 ± 1
7.61	$(CH_2)_5O$-*p*-C_6H_4-(*m*-OH-C_6H_4)	> 300	60 ± 9	> 300	125 ± 3
7.62	$(CH_2)_{10}OPh$	> 200	12 ± 1	105 ± 1	31 ± 1
7.63	$(CH_2)_{10}O$-*p*-C_6H_4-Ph	> 150	1.3 ± 0.4	37 ± 1	7.5 ± 0.2
7.87	*o*-$C_6H_4O(CH_2)_9CH_3$	40 ± 2	6.8 ± 1.1	158 ± 5	21 ± 1
7.88	*m*-$C_6H_4O(CH_2)_9CH_3$	65 ± 4	6.1 ± 0.5	86 ± 1	5.4 ± 0.2
7.89	$CH((CH_2)_5CH_3)(CH_2)_7CH_3$	23 ± 2	5.1 ± 0.5	104 ± 2	9.3 ± 0.7
7.90	$CH((CH_2)_2CH_3)(CH_2)_9CH_3$	24 ± 3	8.2 ± 1.3	139 ± 8	13 ± 1
7.91	$CH((CH_2)_5CH_3)(CH_2)_9CH_3$	21 ± 1	5.5 ± 0.7	135 ± 6	2.7 ± 0.1

Table 7.3 (continued)

7.92	$CH((CH_2)_9CH_3)(CH_2)_9CH_3$	28 ± 1	5.0 ± 0.3	135 ± 4	6.4 ± 0.2
7.93	$CH((CH_2)_2Ph)(CH_2)_9CH_3$	24 ± 1	6.9 ± 1.3	197 ± 12	6.8 ± 0.2
7.94	$CH(CH_2$-*p*-MeO-$C_6H_4)(CH_2)_9CH_3$	22 ± 2	7.8 ± 0.4	176 ± 6	5.3 ± 0.3
7.95	$CH((CH_2)_5CH_3)(CH_2)_{15}CH_3$	17 ± 1	5.5 ± 0.4	99 ± 2	2.2 ± 0.2
7.96	$(CH_2)_5O$-*p*-C_6H_4-(*p*-OMe-C_6H_4)	> 200	11 ± 1	69 ± 1	37 ± 1
7.97	$(CH_2)_5O$-*p*-C_6H_4-(1-naphthyl)	17 ± 1	8.9 ± 0.4	316 ± 25	11 ± 1
7.98	$(CH_2)_4O(CH_2)_6CH_3$	390 ± 30	93 ± 9	> 500	257 ± 9
7.99	$(CH_2)_5O(CH_2)_5CH_3$	449 ± 32	74 ± 9	> 500	221 ± 20
7.100	*p*-$C_6H_4O(CH_2)_9CH_3$	69 ± 2	7.4 ± 1.0	64 ± 2	8.3 ± 0.3
7.101	$(CH_2)_{10}CO_2H$	> 400	32 ± 3	> 700	124 ± 6
7.102	$(CH_2)_5O$-*p*-C_6H_4-(*p*-CO_2H-C_6H_4)	> 400	18 ± 2	129 ± 4	60 ± 2
7.103	$(CH_2)_5O$-*p*-C_6H_4-(*m*-CO_2H-C_6H_4)	> 400	39 ± 2	> 400	94 ± 8

[a] inhibition of enzyme expressed as IC_{50} (μM), mean ± SEM (N = 2, experiments performed in duplicate), maximal concentrations of the test compounds were selected with respect to individual terminal solubilities in the incubation mixture; IC_{50} values determined at pH 5.0 (PH-20, BTH, *Sag*$Hyal_{4755}$) or 3.5 (Hyal-1) in the turbidimetric assay; [b]as previously shown[53].

In general, lower IC_{50} values were obtained for human PH-20 and the bacterial enzyme compared to Hyal-1 and BTH. When the compounds **7.2a-7.2f** are compared with ascorbic acid and ascorbic acid 6-palmitate it is obvious that the inhibition of human PH-20, the corresponding bovine enzyme and *Sag*$Hyal_{4755}$ increases with the length of the aliphatic chain. Vitamin C itself is only a very weak inhibitor of *Sag*$Hyal_{4755}$ and human PH-20 with IC_{50} values of 6.1 mM and 7.6 mM, respectively, and does not inhibit Hyal-1 and BTH in the investigated concentration range. By contrast, the potency of the synthesized derivatives increased by several orders of magnitude, for instance IC_{50} values of 5.0 μM, 58 μM and 4.2 μM on human PH-20, BTH and *Sag*$Hyal_{4755}$, respectively, were determined for Vcpal. The situation is different for the inhibition of Hyal-1 by the alkyl derivatives of vitamic C: ascorbic acid lacking a hydrophobic alkyl chain did not inhibit this human enzyme and inhibition first increased with the length of the attached alkyl chain, but in this case a maximum in potency was found for L-ascorbic acid tridecanoate (**7.2e**) with an IC_{50} of 50 μM. Further lengthening of the chain (compounds **7.2f** and Vcpal) resulted in a significant decrease in inhibitory activity. Whereas **7.2f** caused 50 % inhibition (determined at a concentration of 100 μM, corresponding to terminal solubility), no significant inhibition was detectable for Vcpal. Therefore, Vcpal is the compound with the highest selectivity for human PH-20, BTH and the bacterial enzyme *versus* human Hyal-1 in

this series of ascorbic acid derivatives. The concentration dependent enzymatic activity of Hyal-1 in presence of **7.2b-7.2f** is depicted in Figure 7.2.

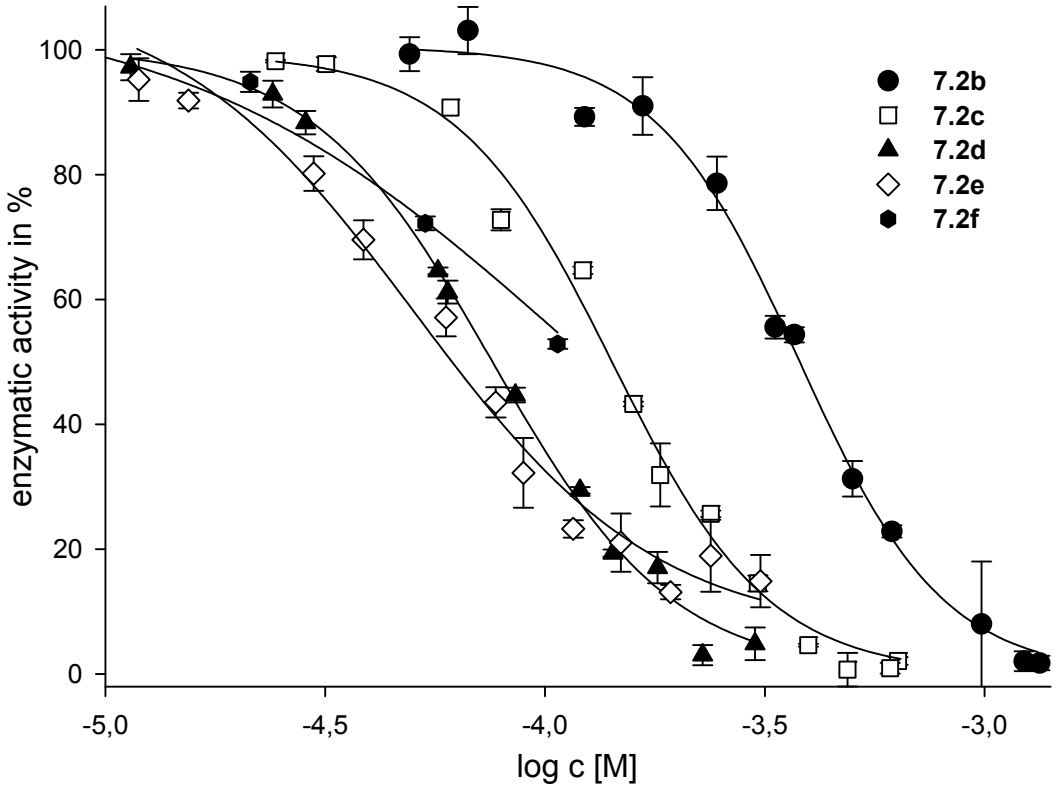


Figure 7.2. Enzymatic activity of Hyal-1 in the presence of **7.2b-7.2f**.

The prolongation of the alkyl chain selectively affects the inhibitory potencies on the investigated enzymes (see Figure 7.3). In this figure the average shifts of the pIC_{50} values per C-atom are presented. These quantities are related to the free energy of interaction and calculated by the expression

$$(pIC_{50(n+z)} - pIC_{50(n)}) / z$$

where $pIC_{50(n)}$ applies to the ascorbic acid derivative esterified with a carboxylic acid having n C-atoms (reference), and $pIC_{50(n+z)}$ to the adjacent derivative bearing z more carbon atoms. Thus, the term $pIC_{50(n)}$-$pIC_{50(n+z)}$ is the difference between the two pIC_{50} values of neighboring compounds in Table 7.3. The highest gain in inhibitory potency on Hyal-1 is observed around derivative **7.2d** referring to ascorbic acid 6-dodecanoate. Further extension of the chain beyond 13 carbon atoms results in a decrease in potency. The other enzymes behave differently: increasing the chain length leads to increased potency of the inhibitor. Regarding human PH-20, highest increase in potency is observed for the derivatives **7.2c** and **7.2f** bearing 11- and 14-

membered alkyl chains. The results suggest that the terminal carbon atoms in both compounds are important for the inhibition of PH-20, i. e. there appear to be especially favored interactions between those two positions of the alkyl chain and amino acids of the enzyme. By contrast, the inhibition of BTH, the bovine homolog of human PH-20, constantly rises between ascorbic acid 6-undecanoate (**7.2c**) and –tridecanoate (**7.2e**). The biggest gain in inhibitory potency on the bacterial enzyme is found for compound **7.2e**.

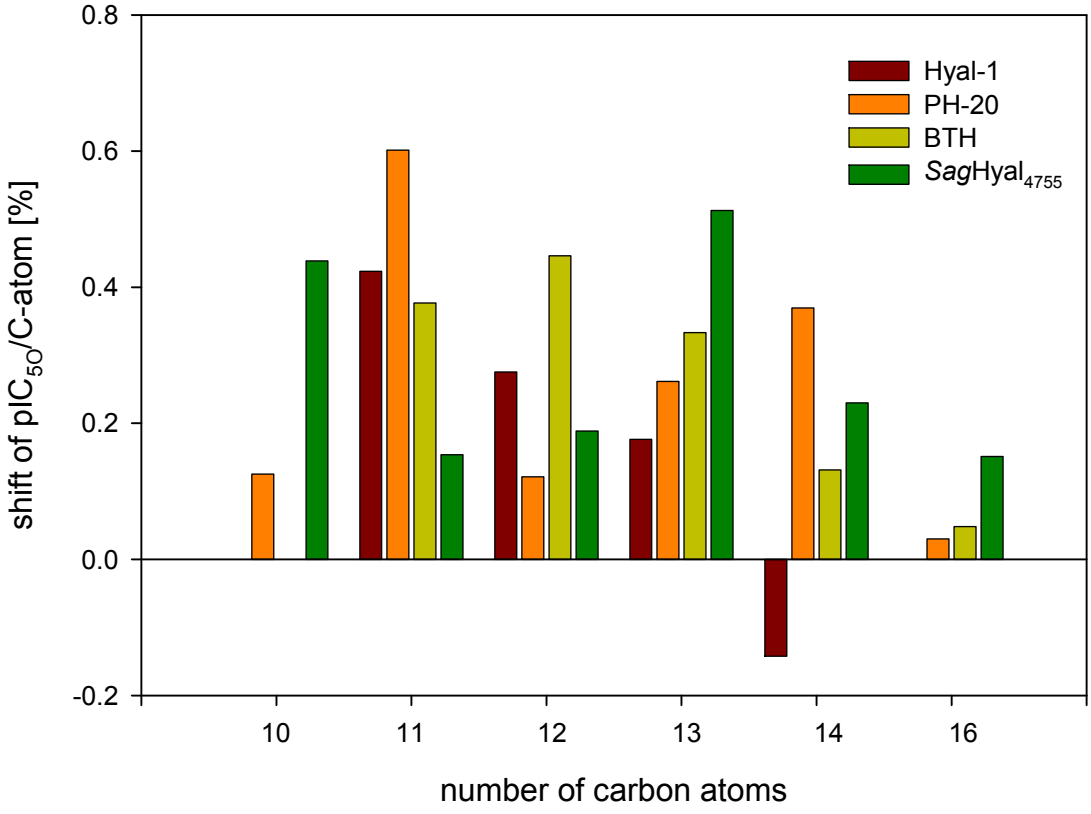


Figure 7.3. Plot of the increase / decrease in inhibition of hyaluronidases *versus* number of carbon atoms used to increase hydrophobicity of the 6-*O*-acylated ascorbic acid derivatives.

The solubility of the ascorbic acid derivatives under the assay conditions is strongly impaired depending on the hydrophobic character of the substituents. As lengthening of the alkyl chain beyond 14 carbon atoms resulted only in a weak increase in the inhibition of PH-20, BTH and *Sag*Hyal$_{4755}$ and virtual inactivity on Hyal-1, additional substances bearing hydrophobic residues with 12-13-membered carbon chain were synthesized. This compromise should yield more potent compounds which are sufficiently soluble in aqueous medium to enable the determination of IC_{50} values at reasonable inhibitor concentrations.

The potencies of derivative **7.2g** bearing a biphenyl residue are similar as those of the linear alkyl chain derivative **7.2a** indicating that the more rigid aromatic residue is also tolerated by the enzymes. Encouraged by these results additional derivatives with aromatic residues in a certain distance to the ascorbic acid moiety were synthesized. The synthetic approach using a (thio-)ether linkage to introduce aromatic residues made such compounds easily accessible. Moreover, the ether group is capable of forming H-bonds in the active site by analogy with the substrate hyaluronan. Compared to the ether derivatives **7.50** and **7.54** the corresponding thioethers **7.48** and **7.49** are more potent on Hyal-1 and PH-20, suggesting that the 6-*O*-acyl residue covers a highly hydrophobic area of the mammalian enzymes similar to the orientation of palmitoyl residue found in the crystal structure of the hyaluronidase from *S. pneumoniae* in complex with ascorbic acid 6-palmitate[19]. Para-substitution of the terminal phenyl residue in **7.50** with ethyl or phenyl residues (**7.51, 7.52**) leads to significant increases in potency. In the case of the biphenyl derivative **7.52**, the IC_{50} values on all the investigated hyaluronidases are one order of magnitude lower than for the unsubstituted derivative **7.50**. Whereas compounds **7.52** and **7.53** are nearly equipotent inhibitors of PH-20, BTH and *Sag*Hyal$_{4755}$, inhibition of Hyal-1 is lost when the terminal phenyl residue of **7.52** is replaced by a benzyloxy moiety. When an additional methylene group is introduced between the biphenyl moiety and the ether oxygen (**7.55**), the potency on all enzymes is approximately halved.

The potency profile of **7.52** is most similar to that of the ascorbic acid 6-tridecanoate derivative **7.2d**. As suitable replacement of flexible alkyl chains, the semirigid biphenyl moiety of **7.52** was modified to further investigate the role of this moiety (compounds **7.56-7.61, 7.96, 7.97, 7.102** and **7.103** in Table 7.3). The bioisosteric replacement of the phenyl with a thiophenyl residue (**7.52** vs. **7.56**) resulted in nearly equipotent activity of the compounds on all four hyaluronidases. Due to the poor solubility of **7.56** no IC_{50} value was calculated for Hyal-1, however, a 50 % inhibition at a concentration of 80 µM was determined which is comparable to the IC_{50} of 90 µM determined for **7.52**. Biphenyl derivatives bearing relatively small para-substituents did not inhibit human Hyal-1 in the investigated concentration ranges regardless of the chemical nature of the substituent: compared to the unsubstituted reference compound **7.52** neither rather hydrophobic residues like methyl (**7.58**) or methoxy (**7.96**) nor polar groups like hydroxy (**7.60**) or carboxy (**7.102**) were

tolerated. Polar meta-substitution as in the case of **7.61** and **7.103** also led to inactivity. By contrast, ortho-substitution of the terminal phenyl residue (**7.57** and **7.59**) was tolerated. An additional ortho-methyl residue (**7.59**) even led to an increase in potency. These findings suggest that the biphenyl moiety of derivative **7.52** fits well to a hydrophobic pocket of Hyal-1. However, this pocket is not yet fully exploited since compound **7.97** bearing an additional 1-naphthyl moiety, additionally aligning with the ortho- and meta position of the terminal phenyl group of **7.52**, proved to be the most potent inhibitor of Hyal-1 among the biaryl-series.

In contrast to the results for Hyal-1, para-methyl and –methoxy substitution (**7.58**, **7.96**) increased the inhibition of PH-20, BTH and the bacterial enzyme. In the case of the bulky 1-naphthyl residue of **7.97**, the inhibition of PH-20 and the bacterial enzyme rose by at least a factor of two compared to the biphenyl derivative **7.52**, whereas the inhibitory activity at BTH decreased. When polar para-hydroxy or -carboxy residues were introduced (**7.60** and **7.102**), the potency of the compounds at the bovine enzyme increased slightly. The human homolog behaves somewhat differently: whereas **7.60** is a less potent inhibitor of PH-20 than the unsubstituted derivative, the inhibition of this enzyme is unchanged when the para-carboxy substituted derivative **7.102** is compared to **7.52**. Para-hydroxy or -carboxy substitution also does not lead to remarkable changes in inhibition of BTH and the bacterial hyaluronate lyase. Nevertheless, these derivatives are superior to the compounds with hydrophobic substituents due to their increased water solubility. Ortho or meta substitution of the terminal phenyl ring of **7.52** leads to a certain decrease in inhibition of human and bovine PH-20, whereas the inhibition of *Sag*Hyal$_{4755}$ remains unaffected in the case of ortho substituents. A significant decrease in potency regarding the latter enzyme is observed for the derivatives bearing meta-hydroxy (**7.61**) or -carboxy substituents (**7.103**). The results obtained for the biaryl series indicate characteristic, but not very distinctive differences in the SAR of the investigated hyaluronidases. Relatively small modifications of the biaryl moiety caused moderate changes in potency and selectivity of the compounds, suggesting slightly different binding modes and/or differences in the binding pockets of the enzymes. Figure 7.4 illustrates the different SAR of synthesized biaryl derivatives upon inhibition of the investigated hyaluronidases.

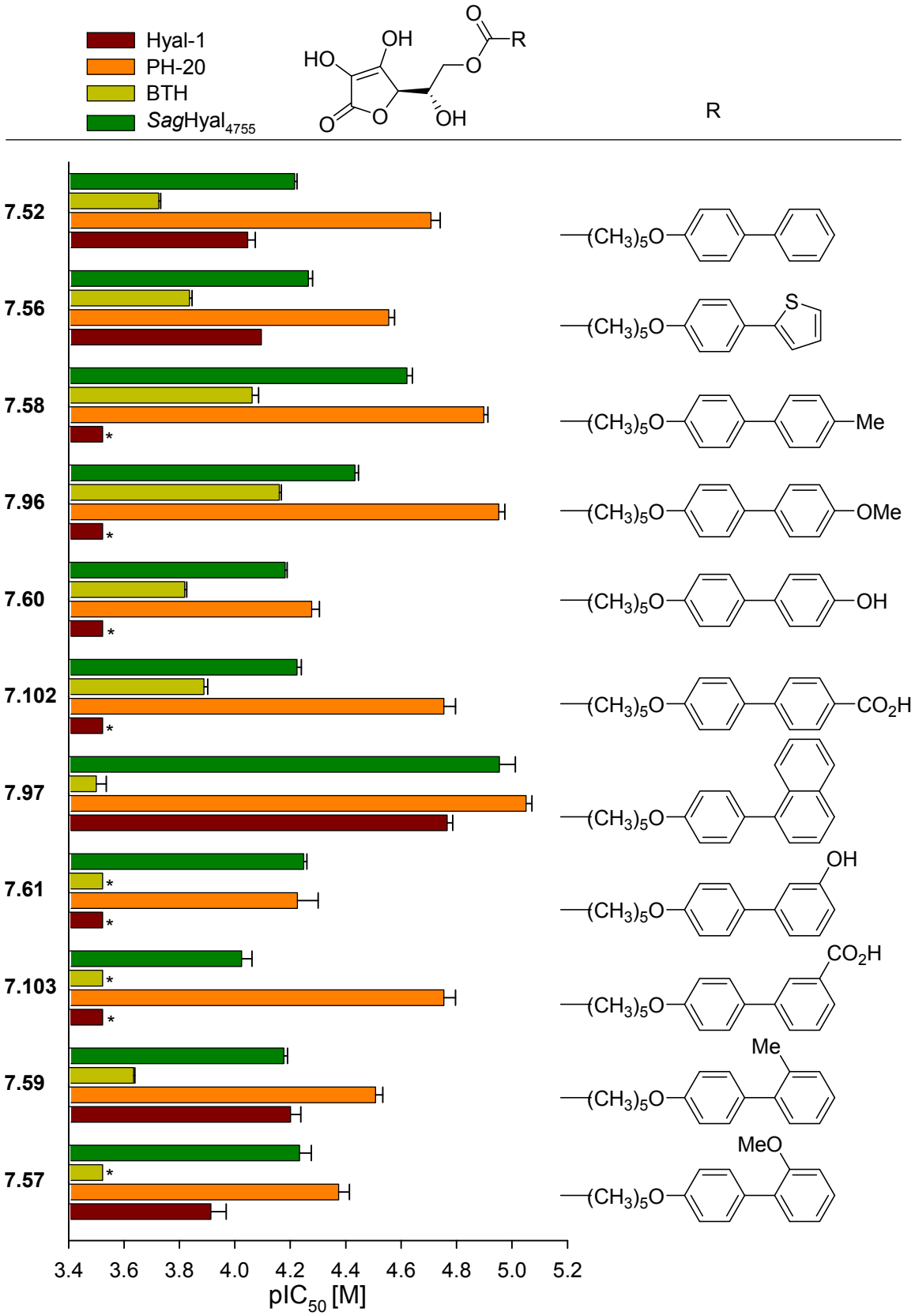


Figure 7.4. Inhibitory activity of investigated biaryl derivatives. Compounds marked with * did not show inhibitory activity on the investigated enzyme (arbitrary short bars for illustrative reasons). The bar for **7.56** Hyal-1 is based on the determined 50 % inhibition of Hyal-1 at a concentration of 80 µM. An IC_{50} value could not be determined due to the limited solubility of the compound.

The elongation of the spacer between the aromatic residue and the ascorbic acid core (compare **7.52** with **7.63**) leads to complete loss of the inhibitory activity towards Hyal-1 but to enhanced inhibition of the other three enzymes also in the case of **7.50** vs. **7.62** (see Figure 7.5: concentration-dependent activity of the hyaluronidases in the presence of **7.52** and **7.63**). Again, the increase in potency correlates with the hydrophobicity. The biphenyl derivative **7.63** is about three to nine times more potent than the corresponding phenyl derivative **7.62**. L-ascorbic acid 11-(p-biphenylyloxy)undecanoate (**7.63**) represents the most potent inhibitor of human PH-20 in this series of ascorbic acid derivatives with an IC_{50} value of 1.3 µM. Moreover, compound **7.63** is the most selective inhibitor of PH-20 *versus* Hyal-1.

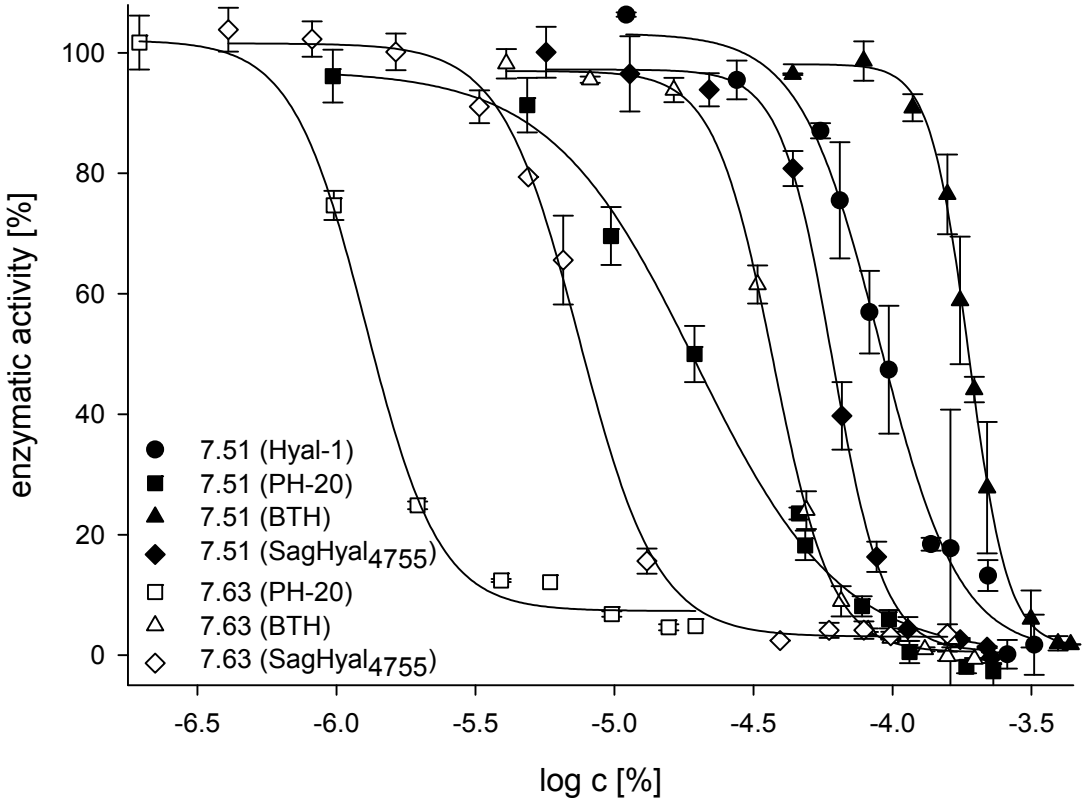


Figure 7.5. Concentration dependent activity of Hyal-1, PH-20, BTH and *Sag*$Hyal_{4755}$ in the presence of **7.52** and **7.63**.

The ω-carboxy substituted derivative **7.101** shows no inhibition of human Hyal-1, although the maximum chain length (see above) is not exceeded. Obviously, a terminal polar group (carboxy as well as *p*- and *m*-OH in **7.60** and **7.61**, respectively) is not tolerated. This finding suggests that the region of the protein surrounding the end of the aliphatic chain of the inhibitor is of strongly hydrophobic nature. By contrast, the inhibition of the human PH-20 is not affected by the additional carboxy

group in **7.101** compared to the corresponding alkanoyl derivative **7.2c**, and the activities on BTH and *Sag*Hyal$_{4755}$ are only slightly decreased. Interestingly, when an oxygen atom is introduced in the center of the aliphatic chain (**7.98** and **7.99**), the inhibition of all four hyaluronidases decreases by at least a factor of five compared to the corresponding compound lacking the additional ether bridge (**7.2e**), again indicating that a continuous hydrophobic chain is essential for high affinity to the hyaluronidases. Additionally, higher desolvation enthalpy of the ether chain may play a role. Finally, the central oxygen atom may cause a different binding mode. The significant role of the hydrophobicity is supported by the finding that an aliphatic ether group seems to be more inappropriate than an aromatic one (the oxygen contributions to log P differ by ca. 1.2 log P units in favor of the latter).

The introduction of a phenyl moiety next to the ester group (**7.87, 7.88** and **7.100**) did not significantly affect the potency of the hyaluronidase inhibitors. Comparing the IC_{50} values for the human enzymes no clear dependence on the substitution pattern of those three compounds was obvious. This is in contrast to the bovine and the bacterial hyaluronidase: the phenyl residue is also tolerated by both enzymes, but the inhibitory potency is significantly decreased when the position of the alkyl substituent at the aromatic ring is changed from para or meta to ortho.

Additional branching as in compounds **7.89-7.94** results in a considerable increase in inhibitory potency compared to the corresponding unbranched analogs **7.2b** and **7.2d**. However, only marginal activity differences between the branched derivatives were detected. Presumably, there are only weak interactions of the additional sidechain with the enzymes. SAR considerations are restricted as the branched derivatives were synthesized and investigated as diastereomers. But the separation of the stereoisomers was considered dispensable because the branched compounds are similarly hydrophobic as the unbranched derivatives (compare **7.89** and Vcpal which both have the same number of carbon atoms, i. e. in both compounds the hydrophobicity of the parent compound ascorbic acid was increased by adding 16 carbon atoms). In this series of chain-branched 6-*O*-acylated ascorbic acid derivatives the IC_{50} value of compound **7.95** on Hyal-1 is in contrast to the results for the unbranched compounds: As discussed above, inhibition significantly decreased when compounds with hydrophobic residues longer than a thirteen-membered chain were investigated. In contrast, the potency of **7.95** bearing a C-18 chain is slightly increased. Due to the highly flexible alkyl chains these compounds may easily

occupy different hydrophobic regions of the protein. Therefore, the binding mode is probably different from that of the other investigated compounds.

7.3.2 "Bivalent" ascorbic acid derivatives

Recently, the crystal structure of the hyaluronate lyase from *S. pneumoniae* in complex with ascorbic acid palmitate was elucidated (PDB code 1w3y)[19]. Interestingly, two cryoprotectant xylitol molecules were bound to *Spn*Hyal nearby the inhibitor (Figure 7.6). This position of the bound xylitol molecules suggests that the affinity of the inhibitor might be enhanced by adding matching groups at the end of the hydrophobic chain[19].

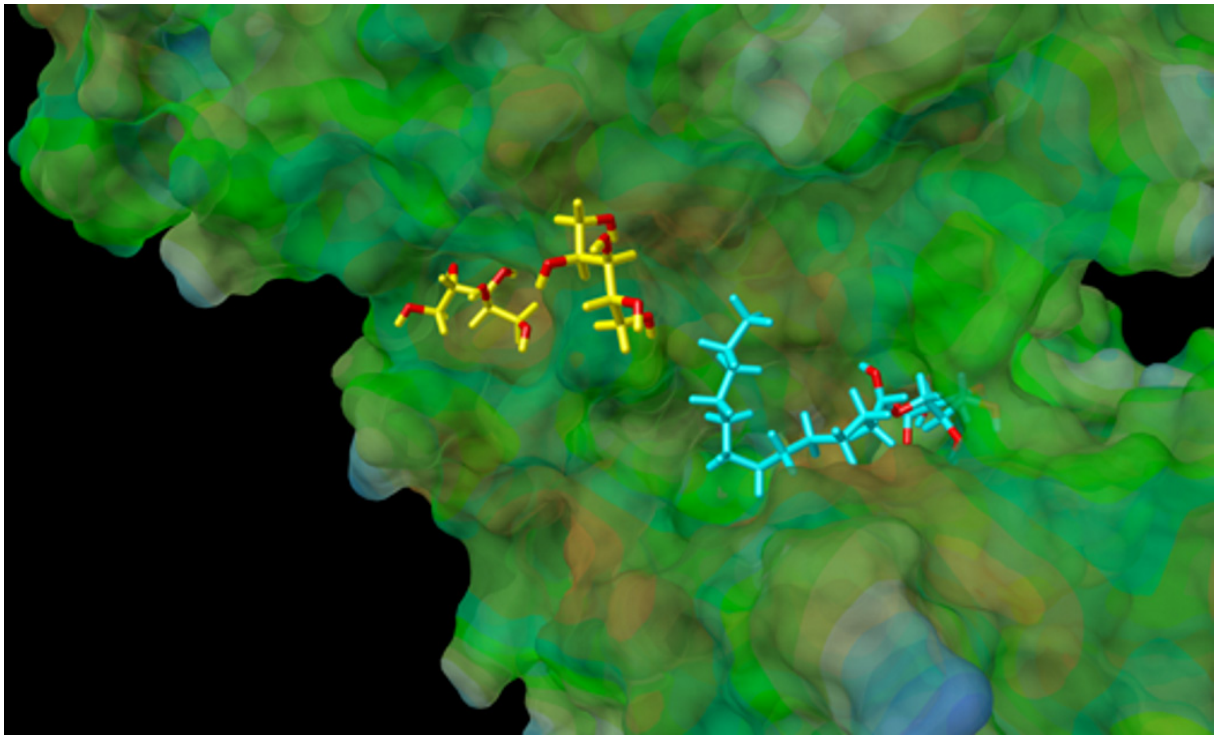
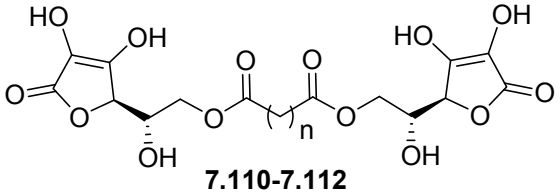


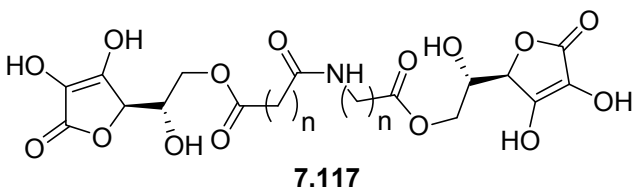
Figure 7.6. Crystal structure of *Spn*Hyal (PDB code 1w3y) in complex with ascorbic acid 6-palmitate (cyan). The protein surface is represented by a Connolly surface coloured by lipophilicity (the warmer the colour, the more lipophilic the amino acids). The two cryoprotectant xylitol molecules (yellow) were found to be bound to the enzyme nearby the end of the palmitoyl moiety.

Due to this suggestion, the "bivalent" ascorbic acid derivatives **7.110-7.112** and **7.117** were synthesized. Characteristic of these compounds is the connection of two terminal polar ascorbic acid moieties by an alkyl spacer, thus, possibly mimicking the alternate occurrence of more polar acidic regions and hydrophobic faces of hyaluronan. The IC_{50} values determined for the investigated hyaluronidases are summarized in Table 7.4.

Table 7.4. Inhibitory activity of bivalent compounds **7.110-7.112** and **7.117**.



7.110-7.112



7.117

Compound	n	Hyal-1	PH-20	BTH	*Sag*Hyal$_{4755}$
		IC$_{50}$ [µM][a]			
7.110	10	107 ± 7	7.7 ± 0.3	> 300	22 ± 2
7.111	12	101 ± 9	14 ± 3	129 ± 2	22 ± 1
7.112	14	> 150	5.0 ± 0.3	32 ± 1	7.4 ± 0.2
7.117	10	> 40	15 ± 1	> 80	55 ± 2

[a] Inhibition of enzyme expressed as IC$_{50}$ (µM), mean values ± SEM (N = 2, experiments performed in duplicate), maximal concentrations of the test compounds were selected with respect to individual terminal solubilities in the incubation mixture; IC$_{50}$ values determined at pH 5.0 (PH-20, BTH, *Sag*Hyal$_{4755}$) or 3.5 (Hyal-1) in the turbidimetric assay.

The derivatives **7.110** and **7.111** bearing the shorter spacers possess inhibitory activity towards Hyal-1 within the investigated concentration ranges, but inhibition does not change significantly when the length of the alkyl spacer is varied from 10 to 12 methylene groups. The potency decreased with a further elongation of the spacer (**7.112**). Compound **7.117** with a spacer consisting of two decamethylene chains connected by an amide bond proved to be inactive on Hyal-1. Human PH-20 was inhibited by all four bivalent compounds. Maximum inhibition was achieved with the derivatives **7.110** and **7.112**. Compound **7.111**, bearing a twelve-membered spacer, was less potent than the corresponding lower and higher homolog. Interestingly compound **7.117** is also an inhibitor of human PH-20 but inactive towards the bovine homolog BTH. Highest inhibition of BTH was found for **7.112** with an IC$_{50}$ of 32 µM. A significant loss of inhibitory activity is observed with shorter spacers. Figure 7.7 illustrates the variations in potency of bivalent ligands investigated for inhibition of Hyal-1, PH-20, BTH and *Sag*Hyal$_{4755.}$ Generally, the "bivalent" compounds did not show substantially increased inhibition of the investigated hyaluronidases compared to the "monovalent" ascorbic acid derivatives. Nevertheless, the additional ascorbic acid moiety was well tolerated and the water solubility was improved which is important for most *in vivo* studies. Due to the significant increase in potency comparing **7.111** and **7.112** it is conceivable that the inhibitory potency of Vcpal on the bacterial enzyme can be further increased by adding appropriate residues at the end of the C-16 chain. Probably another ascorbic acid moiety in this additional position is not optimal and should be replaced by other matching residues.

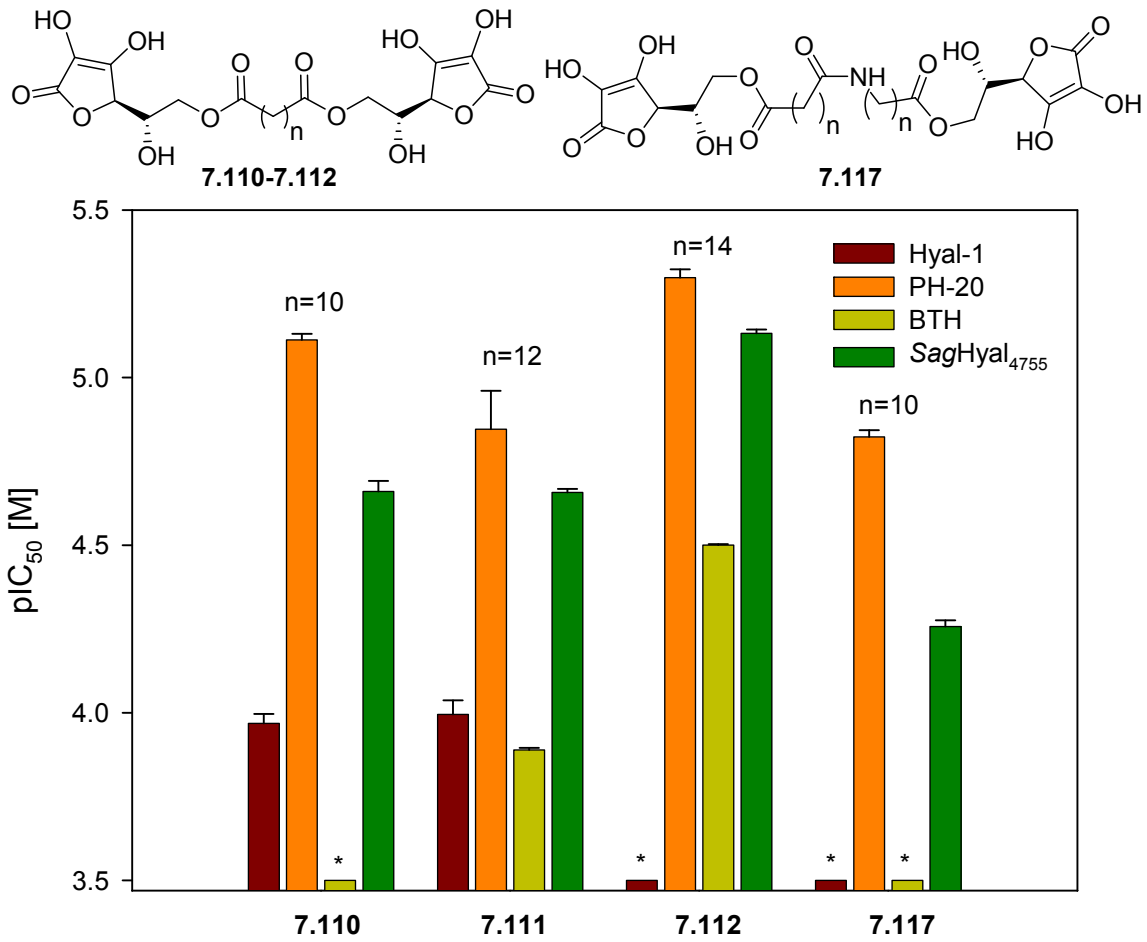
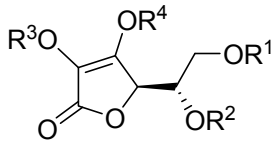


Figure 7.7. Hyaluronidase inhibitory potencies of the bivalent ligands **7.110-7.112** and **7.117**. Compounds marked with * did not show inhibition of the investigated enzyme (arbitrary low bars for illustrative reasons).

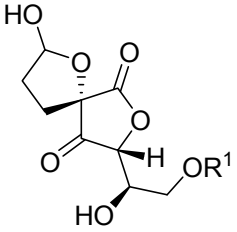
7.3.3 Mono-, di- and trisubstituted alkyl and acyl derivatives of vitamin C with increased hydrophobicity

The IC_{50} values of the acylated and alkylated ascorbic acid derivatives, of the Michael products **7.151, 7.152** and of oxidized Vcpal (**7.153**) are summarized in Table 7.6 (see Table 7.5 for the definition of residues).

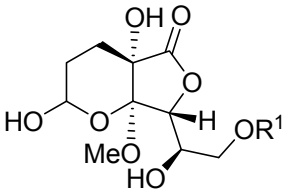
Table 7.5. Substitution patterns of investigated ascorbic acid derivatives.



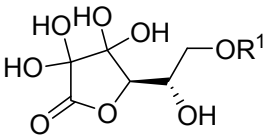
7.96a-7.100a, 7.103a, 7.106, 7.118-7.121, 7.121a, 7.127, 7.128, 7.132, 7.139, 7.140, 7.146-7.153



7.151



7.152



7.153

No	R^1	R^2	R^3	R^4
7.96a	$CO(CH_2)_5O$-*p*-C_6H_4-(*p*-OMe-C_6H_4)	$=R^1$	H	H
7.97a	$CO(CH_2)_5O$-*p*-C_6H_4-(1-naphthyl)	$=R^1$	H	H
7.98a	$CO(CH_2)_4O(CH_2)_6CH_3$	$=R^1$	H	H
7.99a	$CO(CH_2)_5O(CH_2)_5CH_3$	$=R^1$	H	H
7.100a	CO-*p*-$C_6H_4O(CH_2)_9CH_3$	$=R^1$	H	H
7.103a	$CO(CH_2)_5O$-*p*-C_6H_4-(*m*-CO_2H-C_6H_4)	$=R^1$	H	H
7.106	$CO(CH_2)_{10}CH_3$	$COCH_2CO_2H$	H	H
7.118	$CO(CH_2)_{14}CH_3$	H	$CO(CH_2)_2Ph$	H
7.119	$CO(CH_2)_{14}CH_3$	H	$CO(CH_2)_2CO_2H$	H
7.120	$CO(CH_2)_{14}CH_3$	H	$CO(CH_2)_3CO_2H$	H
7.121	$CO(CH_2)_{14}CH_3$	H	$CO(CH_2)_4CO_2H$	H
7.121a	$CO(CH_2)_{14}CH_3$	H	$CO(CH_2)_4CO_2H$	$=R^3$
7.127	$(CH_2)_{11}CH_3$	H	H	H
7.128	$(CH_2)_{17}CH_3$	H	H	H
7.132	$CO(CH_2)_{10}CH_3$	H	CH_2CO_2H	$=R^3$
7.139	H	H	$(CH_2)_{11}CH_3$	H
7.140	H	H	$(CH_2)_{15}CH_3$	H
7.146	$CO(CH_2)_{10}CH_3$	H	CH_2CO_2H	H
7.147	$CO(CH_2)_{10}O$-*p*-C_6H_4-Ph	H	CH_2CO_2H	H
7.148	$CO(CH_2)_{10}CH_3$	H	H	CH_2CO_2H
7.149	H	H	H	$(CH_2)_{11}CH_3$
7.150	H	H	H	$(CH_2)_{13}CH_3$
7.151	$CO(CH_2)_{14}CH_3$	-	-	-
7.152	$CO(CH_2)_{14}CH_3$	-	-	-
7.153	$CO(CH_2)_{14}CH_3$	-	-	-

Table 7.6. Inhibitory activity of investigated ascorbic acid derivatives.

Compound	Hyal-1	PH-20	BTH	*Sag*Hyal$_{4755}$
	IC_{50} [µM][a]			
7.96a	> 50	1.7 ± 0.2	35 ± 0.8	8.0 ± 0.6
7.97a	15 ± 2	1.9 ± 0.1	> 50	9.8 ± 1.0
7.98a	31 ± 1	15 ± 1	315 ± 5	17 ± 1
7.99a	28 ± 2	9.0 ± 0.5	300 ± 9	26 ± 3
7.100a	108 ± 6	2.2 ± 0.1	65 ± 1	15 ± 1
7.103a	> 60	1.5 ± 0.1	17 ± 1	4.7 ± 0.4
7.106	49 ± 4	4.3 ± 0.5	233 ± 7	14 ± 1
7.118	> 30	3.3 ± 0.4	> 30	4.9 ± 0.3
7.119	25 ± 3	8.6 ± 0.4	84 ± 2	1.7 ± 0.1
7.120	11 ± 1	1.5 ± 0.3	79 ± 2	2.5 ± 0.2
7.121	8.3 ± 0.4	2.0 ± 0.1	27 ± 1	2.8 ± 0.1
7.121a	8.7 ± 1.5	3.2 ± 0.2	45 ± 1	4.9 ± 0.2
7.127	62 ± 4	10 ± 1	141 ± 4	26 ± 1
7.128	> 40	2.3 ± 0.1	35 ± 1	1.6 ± 0.1
7.132	30 ± 2	3.9 ± 0.5	343 ± 14	12 ± 1
7.139	> 400	21 ± 2	564 ± 7	40 ± 2
7.140	> 50	36 ± 0.3	> 100	4.1 ± 0.2
7.146	41 ± 1	9.3 ± 0.8	297 ± 5	15 ± 0.6
7.147	> 100	1.7 ± 0.1	68 ± 2	2.4 ± 0.1
7.148	85 ± 6	6.9 ± 0.6	328 ± 15	4.8 ± 0.3
7.149	117 ± 9	4.5 ± 0.3	> 400	26 ± 1
7.150	> 100	171 ± 21	> 200	6.0 ± 0.2
7.151	39 ± 10	2.6 ± 0.2	77 ± 1	21 ± 1
7.152	> 50	33 ± 2	> 60	27 ± 1
7.153	> 50	12 ± 0.5	> 70	9.6 ± 0.4

[a] Inhibition of enzyme expressed as IC_{50} (µM), mean values ± SEM (N = 2, experiments performed in duplicate), maximal concentrations of the test compounds were selected with respect to individual terminal solubilities in the incubation mixture; IC_{50} values determined at pH 5.0 (PH-20, BTH, *Sag*Hyal$_{4755}$) or 3.5 (Hyal-1) in the turbidimetric assay.

When 5,6-di-*O*-acylated ascorbic acid derivatives **7.96a-7.100a** and **7.103a** are compared with the corresponding monoacylated derivatives **7.96-7.100** and **7.103** an increase in potency is observed in most cases. If an IC_{50} value could not be determined due to poor water-solubility, at least a percental inhibition at maximal concentrations of such compounds was obtained. More than 6-fold increase in potency is observed for **7.98a** and **7.99a** compared to the monoacylated derivatives on all enzymes except BTH. A marked increase in inhibitory activity was also observed for **7.103a** vs. **7.103**, bearing a carboxyl group in meta position of the

terminal phenyl ring(s), on human PH-20 (factor 26) and *Sag*Hyal$_{4755}$ (factor 20). In Figure 7.8 the differences in pIC$_{50}$ values determined on PH-20 are visualized for the diacylated (**7.96a-7.100a** and **7.103a**) *versus* the corresponding monoacylated ascorbic acid derivatives (**7.96a-7.100a** and **7.103a**).

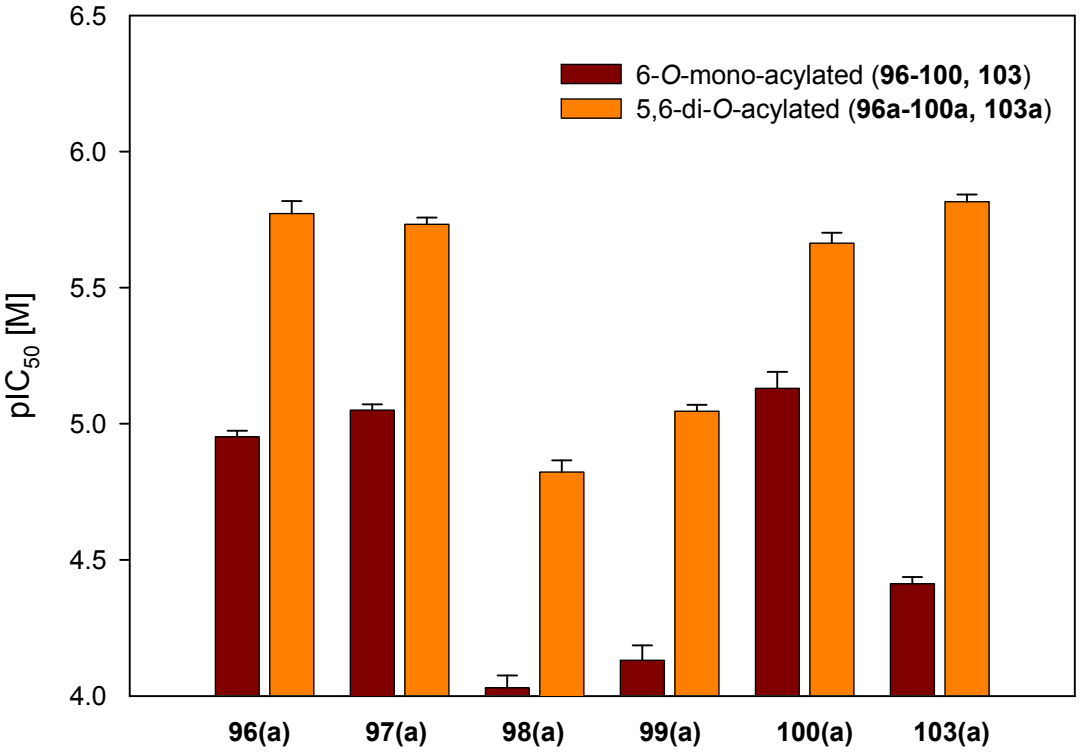


Figure 7.8. Comparison of the pIC$_{50}$ values for human PH-20 determined for monoacylated and diacylated ascorbic acid derivatives.

Whereas the monoacylated derivative **7.103** was too weakly active to determine an IC$_{50}$ value on the bovine testicular hyaluronidase, the diacylated compound **7.103a** with an IC$_{50}$ of 17 µM was among the most potent inhibitors for BTH in the ascorbic acid series. Obviously, the large additional 5-*O*-substituent contributes to the binding affinity. An exception among the 5,6-diacylated derivatives represents **7.100a**: whereas the inhibitory potency towards PH-20 is significantly higher than that of **7.100**, the activity is similar towards BTH or even weakened towards Hyal-1 and *Sag*Hyal$_{4755}$. The two rigid phenyl rings near the ascorbic acid moiety may hinder the fit compared to the other diacylated compounds where only flexible alkanoyl chains are attached to the vitamin C core of the molecules. Interestingly, the selectivity of the hyaluronidase inhibitors is affected by the diacylation (Figure 7.9): **7.100a** and

7.103a are highly selective for human PH-20 *versus* Hyal-1, whereas the PH-20 selectivity of compound **7.98a** is only weak (factor 2). The discrimination between the two PH-20 homologs, human PH-20 and BTH, remains largely unaffected by the different structural modifications, whereas the selectivity for Hyal-1 over BTH is clearly enhanced for **7.98a** and **7.99a**. By contrast, **7.96a**, **7.100a** and **7.103a** possess significant selectivity for BTH over human Hyal-1.

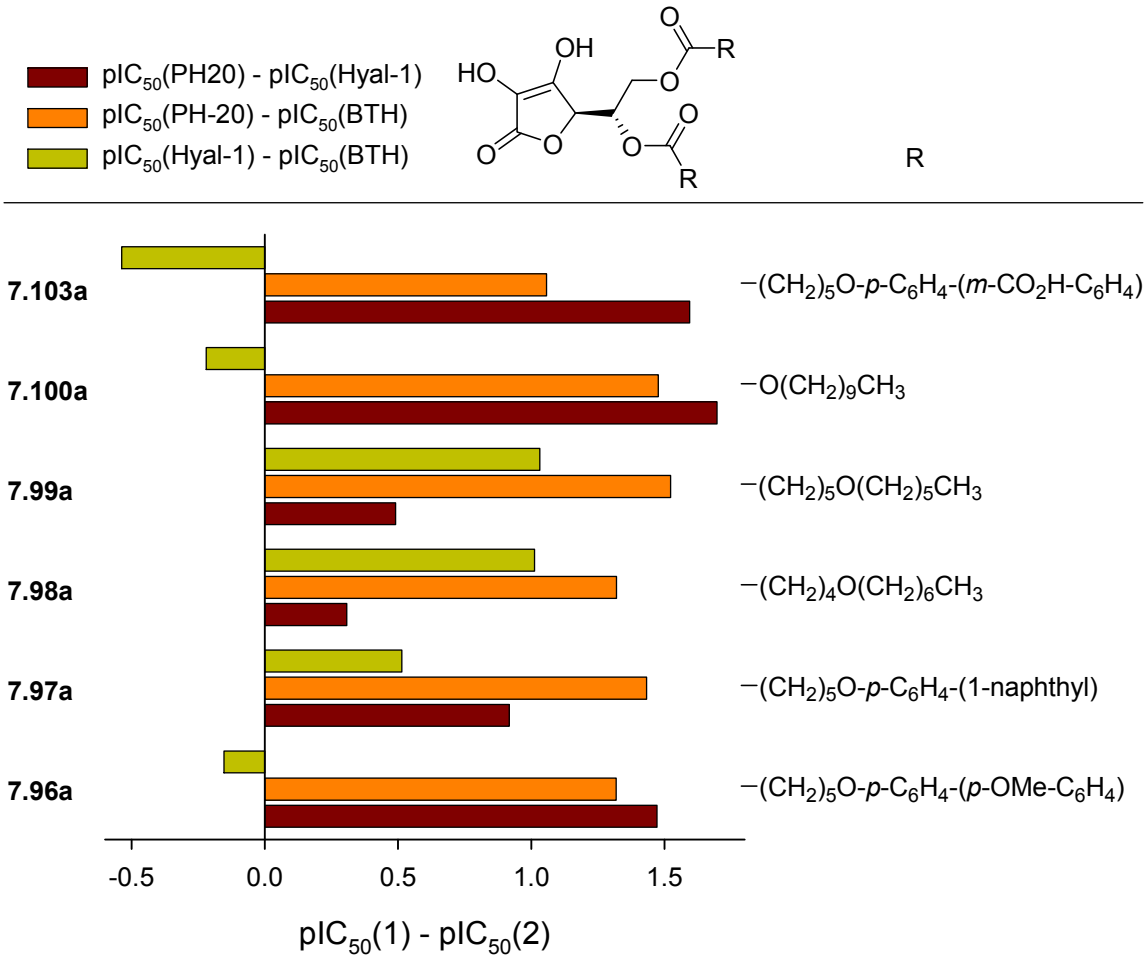


Figure 7.9. Selectivity of synthesized 5,6-di-*O*-acylated ascorbic acid derivatives for the isoforms of mammalian hyaluronidases, human Hyal-1, human PH-20 and BTH, expressed as difference of pIC_{50} values.

The additional carboxyl group in **7.106**, resulting from the acylation of *O*-5 with a malonic acid, slightly lowers the inhibitory potency towards BTH compared to the *O*-5 nonsubstituted analogue **7.2d**, whereas the inhibition of the other mammalian hyaluronidases and the bacterial enzyme is significantly increased. For the 2-*O*-acyl derivatives of ascorbic acid palmitate different SAR were observed: a carboxylic group linked to *O*-2 of Vcpal *via* different spacers (**7.119-7.121**) first decreased inhibition of BTH (**7.119, 7.120**) but the potency of **7.121** even exceeded that of Vcpal (see Figure 7.10for visualization of pIC_{50} values). Similar SAR are obtained for PH-

20, the human homologue of BTH. Whereas **7.119** is a slightly weaker inhibitor of this enzyme compared to Vcpal, the extension of the spacer to four or five methylene groups gave compounds with enhanced potency (**7.120**, **7.121**). With an IC_{50} value of 1.5 µM compound **7.120** is among the most potent inhibitors of human PH-20 known so far. An increase in inhibitory potency correlating with the length of the spacer was found for Hyal-1. The unsubstituted Vcpal was inactive at the investigated concentrations, whereas the carboxyalkanoyl substituted derivatives **7.119-7.121** were rather potent inhibitors of Hyal-1.

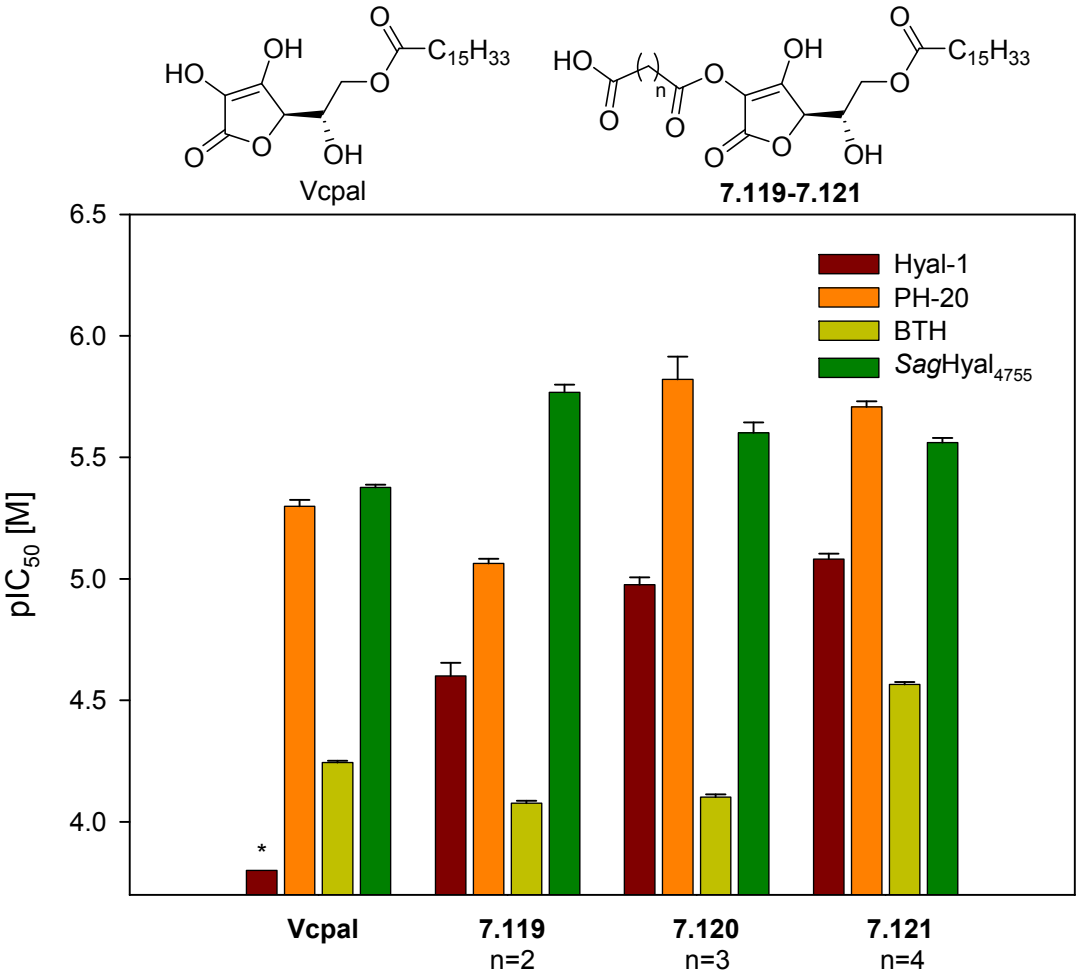


Figure 7.10. Inhibitory activity of 2-*O*-acylated ascorbic acid 6-palmitate derivatives **7.119-7.121** compared to Vcpal. Compounds marked with * did not show inhibition of the investigated enzyme (arbitrary low bars for illustrative reasons).

Regarding all enzymes except BTH, the activities of **7.120** and **7.121** do not significantly differ. Although the potency is higher than that of the reference compound Vcpal, there is no further gain in potency when a certain spacer length is exceeded. A second carboxylic acid residue introduced *via* the additional acylation of

O-3 (**7.121a**) does not increase the inhibition of the investigated hyaluronidases compared to **7.121**.

In contrast to the 2-O-carboxyalkanoyl derivatives the potency of the more hydrophobic phenylpropanoate **7.118** is not significantly different from that of the parent compound Vcpal. Generally, it seems that bulky substituents at O-2 may align with the surface of the different hyaluronidases and that a terminal polar carboxy group may contribute to the interactions.

When various 6-O-acylated vitamin C derivatives bearing additional carboxylic acid residues (**7.132** and **7.146-7.148**) are compared to the corresponding monosubstituted substances (**7.2d** and **7.63**) a tendency to increased potency is observed for Hyal-1, PH-20 and *Sag*Hyal$_{4755}$. An exception is **7.147** where the carboxymethylene group did not improve the inhibition of the two human enzymes. In the case of BTH, the additional carboxylic groups even decreased the inhibitory potency. Thus, these carboxymethylene compounds are among the vitamin C derivatives with highest selectivity for human PH-20 compared to the homologous bovine enzyme: an 88-fold higher potency in favour of the human enzyme was determined for **7.132**. Possibly, the carboxymethylene residues mimic the carboxylate moiety of glucuronic acid. The selective inhibition of the human enzyme compared to its bovine homologue suggests differences in the binding pocket of these two hyaluronidases. Significant selectivity between the two PH-20 enzymes is a surprising and important finding, as BTH has always been considered a prototypal mammalian hyaluronidase and an appropriate model for the development of inhibitors for the corresponding human enzymes.

The ether **7.127** was synthesized to get some information about the importance of the ester carbonyl group. The IC_{50} values were slightly lower than those determined for **7.2d**. This may be interpreted as a hint that the carbonyl group is not essential. However, increased flexibility of the compound could lead to a better fit to the enzymes and compensate for the lack of the carbonyl function. A prolonged chain (**7.128**) leads to a loss in activity for Hyal-1, whereas the potency on the other hyaluronidases is improved. When the location of the ether function is shifted from *O*-6 of the ascorbic acid moiety to position *O*-2 (**7.139** and **7.140**) a complete loss of the Hyal-1 inhibition is observed whereas the other hyaluronidases are still inhibited albeit with higher IC_{50} values. Interestingly, lengthening of the hydrophobic sidechain does not result in increased inhibitory activity towards the human and the bovine PH-

20, whereas the potency towards the bacterial hyaluronidase is drastically enhanced (see **7.140**). The 3-*O*-alkylated derivative **7.149** shows inhibition of Hyal-1, albeit the IC_{50} value is significantly higher than that of the corresponding 6-*O*-alkylated derivative **7.127**. Lengthening the alkyl chain by two methylene groups (**7.150**) results in a loss of the Hyal-1 inhibition. These results suggest similar binding modes of **7.149** and **7.127**. The 3-*O*-alkylated derivative **7.149** is an even more potent inhibitor of PH-20 compared to **7.127** bearing the 6-*O*-dodecyl residue, but the inhibition drastically decreases with lengthening of the chain (**7.150**). This tendency is similar to that of the 2-*O*-alkylated derivatives discussed above. For the 3-*O*-alkylated derivatives no inhibition of BTH was detectable. The IC_{50} values determined on the bacterial enzyme are comparable to those of the 6-*O*-alkylated or 6-*O*-acylated analogues. The selectivities (expressed by the difference of pIC_{50} values) of the ascorbic acid dodecyl ethers **7.127, 7.139** and **7.141** are illustrated in Figure 7.11.

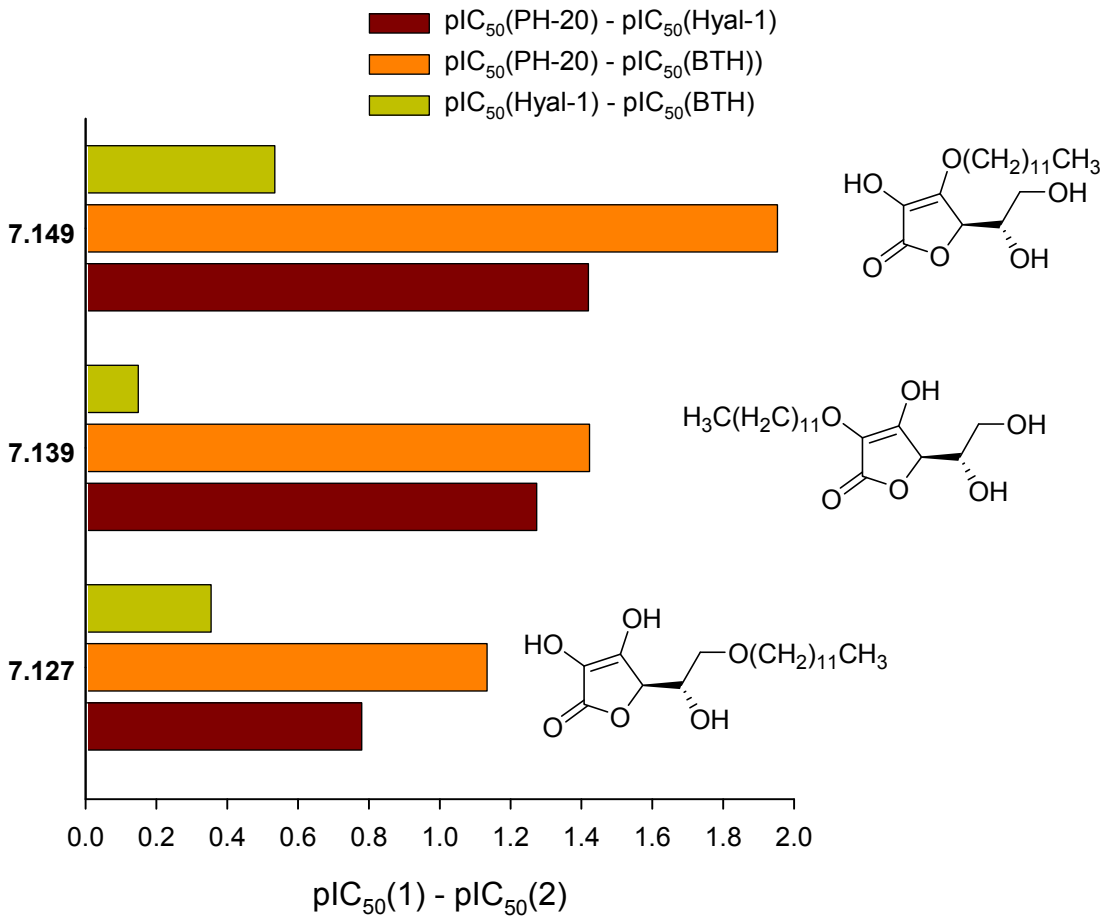


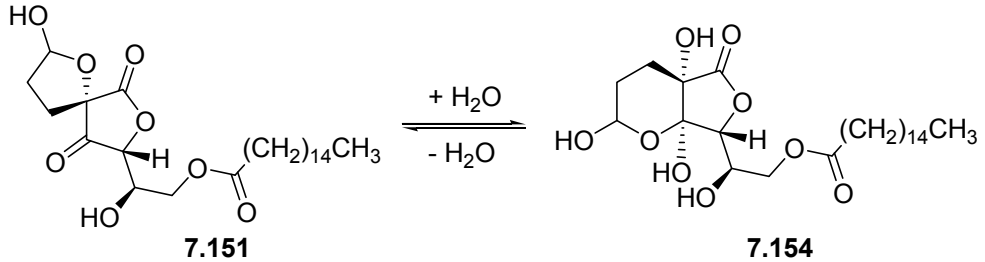
Figure 7.11. Enzyme selectivities of ascorbic acid dodecyl ethers.

Comparing the two human enzymes, the 2-*O*- and 3-*O*-alkylated derivatives **7.139** and **7.149** are most selective for PH-20 (with about the same selectivity ratio).

Considerung the two PH-20 homologs (human PH-20 vs. BTH), the selectivity of the inhibitors depending on the position of the substituent increases in the order 6-*O*-alkylated < 2-*O*-alkylated < 3-*O*-alkylated ascorbic acid. Comparing human Hyal-1 and BTH, the selectivity of the 3-*O*- and 6-*O*-alkylated derivatives for Hyal-1 is in the same range, whereas the 2-*O*-alkylated derivative **7.139** even shows weak selectivity for BTH over Hyal-1.

Regarding the inhibitory activity towards *Sag*Hyal$_{4755}$, generally, structural modifications at different positions of the ascorbic acid core lead only to minor changes in IC_{50} values, whereas changes in the overall hydrophobicity strongly affect the inhibition. This may lead to the assumption that interactions of the inhibitors with the bacterial enzyme are mainly of hydrophobic nature with major interaction sites accessible for the sidechains of the ascorbic acid derivatives, as found in the crystal structure of hyaluronate lyase from *S. pneumoniae* in complex with Vcpal[19]. This may imply slightly different binding modi of the ascorbic acid moiety depending on the position and projection of the substituents. By contrast, distinct interactions with the ascorbic acid core must be important for the inhibition of the mammalian enzymes as there are considerable changes in potency and selectivity when the region of the hydrophobic sidechain is varied.

Regarding the Michael products **7.151** and **7.152** higher inhibitory potency towards all investigated enzymes was found for **7.151** with clear differences between the IC_{50} values for Hyal-1, human PH-20 and BTH, and minor differences in activities on *Sag*Hyal$_{4755}$. Interestingly, the spiro compound **7.151** was superior to Vcpal in inhibition of the two human enzymes but was a less potent inhibitor of bovine and bacterial hyaluronidase. However, it should be stressed that interpretations of these results are ambiguous in terms of SAR due to the fact that a rearrangement of the spiro compound **7.151** was observed in aqueous solutions (Scheme 7.20)[50], i. e. it remains unclear if the increased inhibitory activity compared to **7.152** has to be attributed to **7.151** or the respective pyranoside **7.154** or to both.



7.151 **7.154**

Scheme 7.20. Equilibrium of **7.151** and **7.154** in aqueous solutions according to Witt et al.[50]

Compared to Vcpal the oxidized derivative **7.153** showed decreased inhibitory activity towards PH-20, BTH and *Sag*Hyal$_{4755}$. This may be interpreted as a hint that the flat heterocyclic ascorbic acid core is more appropriate for stacking with hydrophobic amino acids as it was found for an indole-based inhibitor of bacterial hyaluronate lyase[54] as well as for Vcpal[19]. Such a stacking, a commonly observed phenomenon also in other carbohydrate binding enzymes[55] is not optimal in the case of the oxidized compound **7.153** thus explaining the loss of potency.

7.4 *Potential binding modes of ascorbic acid derivatives at human Hyal-1*

Whereas various structural data about hyaluronate lyases are available and distinct knowledge exists about the degradation of the substrate or the binding modes of inhibitors[2, 19, 20, 54, 56-60], there is still a lack of information about mammalian hyaluronidases. In 2000, the crystal structure of bee venom hyaluronidase in complex with a hyaluronan tetrasaccharide was released[61] which provided insight into substrate binding, and first speculations about substrate degradation were made[62]. For about six years, BVH, which shares 22.9 % to 25.2 % sequence identity[62] with human hyaluronidases, was the only member of hyaluronate 4-glycanohydrolases (EC 3.2.1.35) from which extrapolations could be made. In the meantime also the structure of Ves v 2, a hyaluronidase from wasp venom has been elucidated[63]. Very recently, the crystal structure of the first human hyaluronidase was published by Chao et al.[64] It was proven that the catalytically active part (N-terminus) of the enzyme shows high homology with BVH, whereas an additional C-terminal domain in human Hyal-1 is missing in BVH. This new structural information was used to investigate potential binding modes of the developed ascorbic acid based hyaluronidase inhibitors.

The surface of Hyal-1 is shown Figure 7.12. The substrate binding site, dimensioned to bind a hyaluronan octasaccharide, is a long groove traversing the catalytic domain. The docked tetrasaccharide (taken from the BVH crystal structure PDB-code 1fcv[61]) results after cleavage at the reducing end and release of the product. There is a nearly perfect fit into the binding pocket indicating a similar binding mode as it was found for the bee venom enzyme. Hydrophobic interactions with the enzyme are

known to play a major role in substrate binding as well as in inhibition of the hyaluronidases[19, 54, 57, 62]. Thus, in a first approach hydrophobic pockets in proximity of the binding site were identified. Obviously, there are two highly lipophilic areas (see Figure 7.12): the first, marked as lipophilic area 1 is composed of residues Tyr286, Tyr202, Trp321, Tyr75, and Tyr245 and forms a narrow pocket nearby the catalytic site that surrounds the *N*-acetyl moiety of a glucosamine residue of the substrate. The second, marked as lipophilic area 2 is composed of residues Val251, Leu252, Leu213, Phe212, Tyr261, Tyr210, Tyr208, Trp141, Phe139 and Ile225 forming walls of a crevice distal from the catalytic site, but close to the hyaluronan binding groove (product moiety). These two highly lipophilic areas were considered as possible binding sites for hydrophobic residues of the inhibitors.

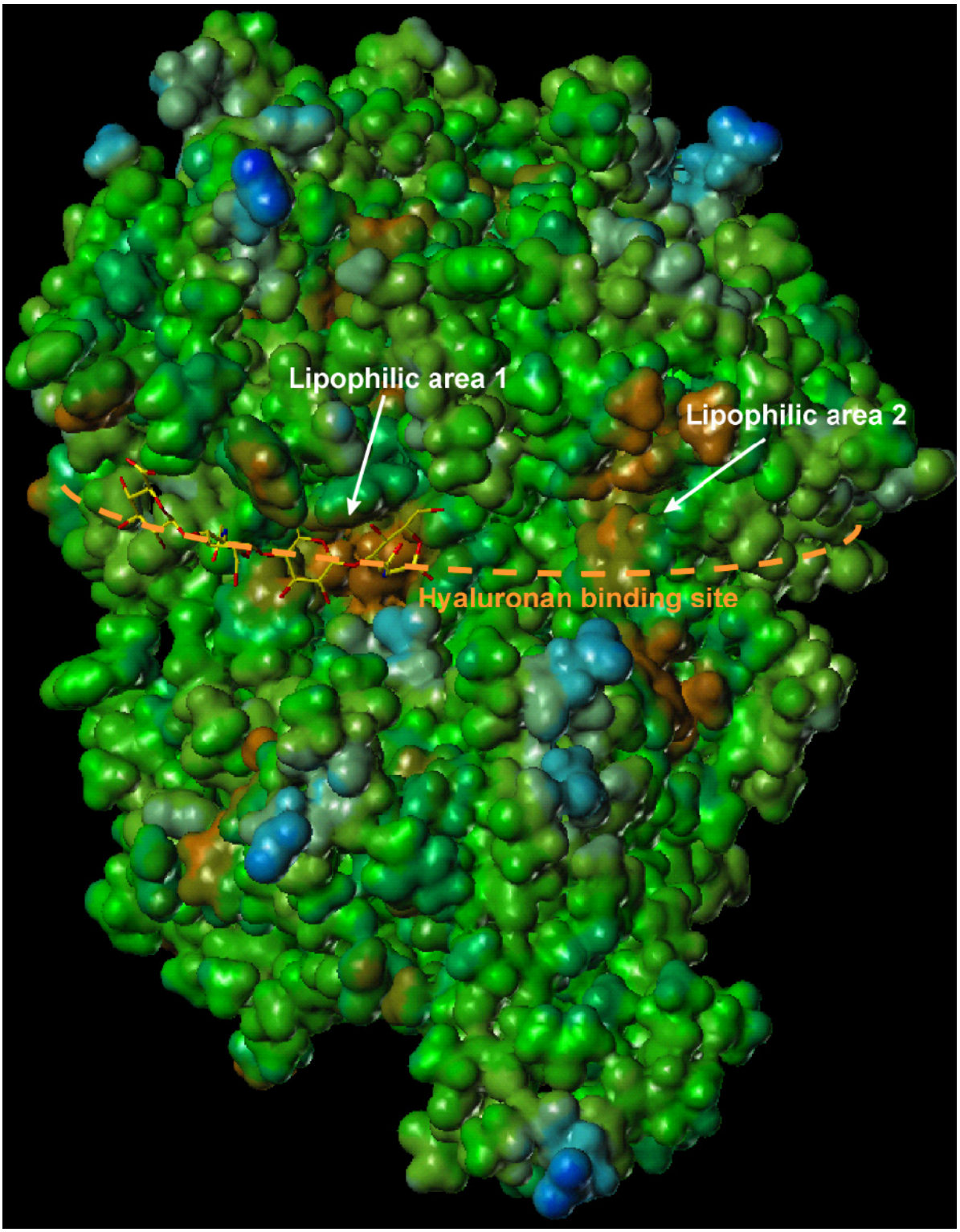


Figure 7.12. Surface of human Hyal-1 (PDB code 2pe4[64]) showing two main lipophilic areas. The protein surface is represented by a Connolly surface colored by lipophilicity (the warmer the color, the more lipophilic the amino acids). The hyaluronan tetrasaccharide (C atoms yellow) is taken from the crystal structure PDB code 1fcv[61].

The tetrasaccharide binding site of the enzyme was defined by the amino acids Trp36, Asn37, Asn39, Gln41, Trp42, Glu45, Thr65, Phe66, Arg67, Ile73, Ser74,

Tyr75, Tyr202, Ser245, Tyr247, Ile287, Trp321, Val322, Ser323, Trp324, and Glu325 and the catalytically active Asp129 and Glu131 (Figure 7.13).

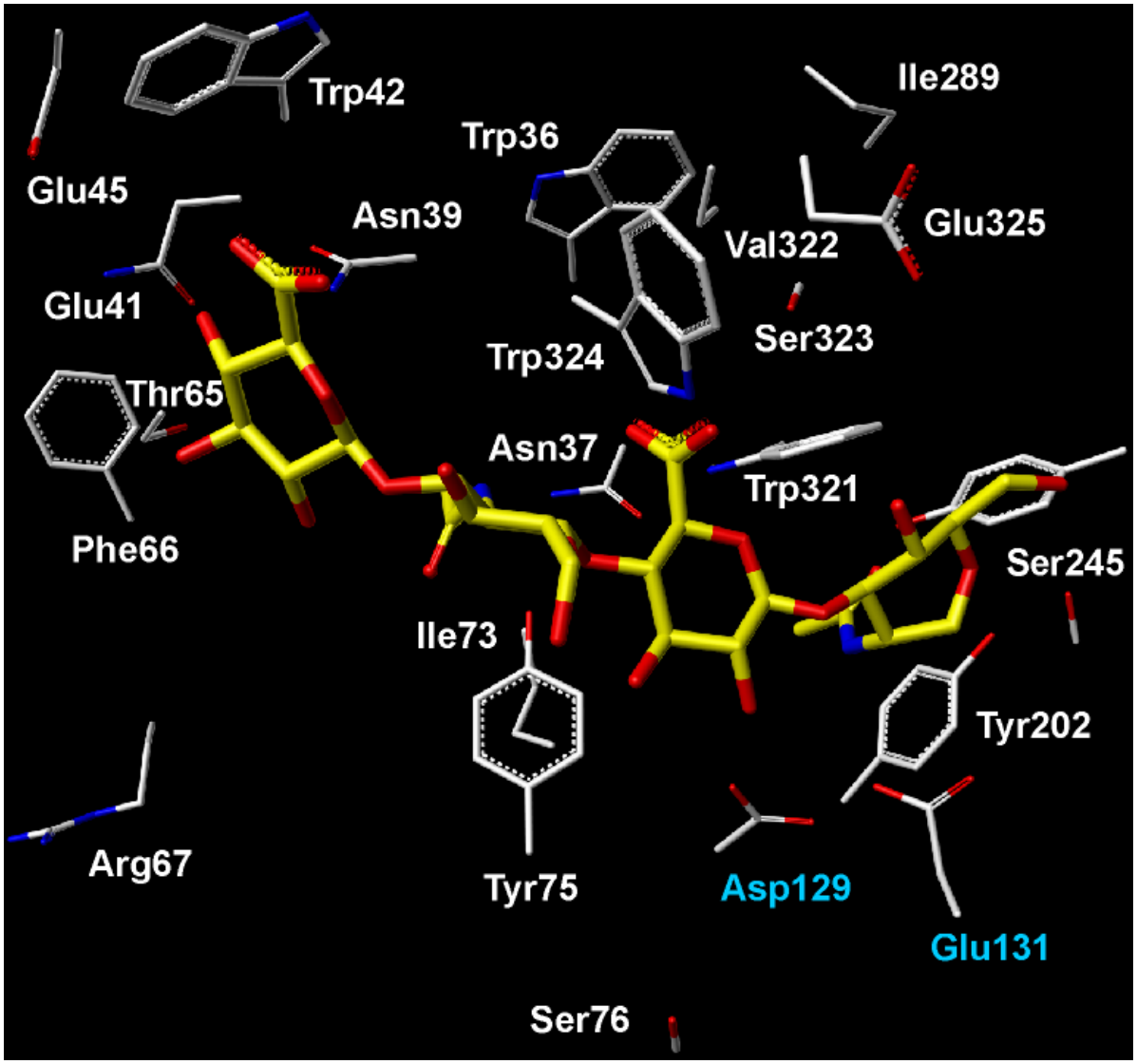


Figure 7.13. Tetrasaccharide binding site site of Hyal-1. The catalytically active Asp129 and Glu131 are marked in cyan. The hyaluronan tetramer (C atoms yellow) is taken from the crystal structure PDB code 1fcv[61].

In a first attempt 6-*O*-[6-(4-phenylphenoxy)hexanoyl]ascorbic acid (**7.52**) was chosen for docking experiments using the FlexiDock module of Sybyl 7.3. This inhibitor was selected since it is more rigid due to the biphenyl residue compared to the flexible alkyl chains present in other inhibitors. Additionally, there are several significant differences in the SAR compared to the other hyaluronidases when the biphenyl scaffold was slightly modified. As inhibition of the enzyme was determined at pH 3.5, the ascorbic acid derivative was used in the fully protonated form. Two approaches were selected for docking of **7.52**: (1) flexible ligand, fixed amino acid side chains; (2)

ligand and amino acid side chains adjustable. While the first strategy resulted in many solutions, but all with very weak protein-ligand interactions, the second approach led to multiple solutions with distinct interactions between protein and inhibitor. The resulting docking poses were analyzed by visual inspection. In most cases interactions of the biphenyl residue with hydrophobic amino acids of the lipophilic area 1 were found (Figure 7.14).

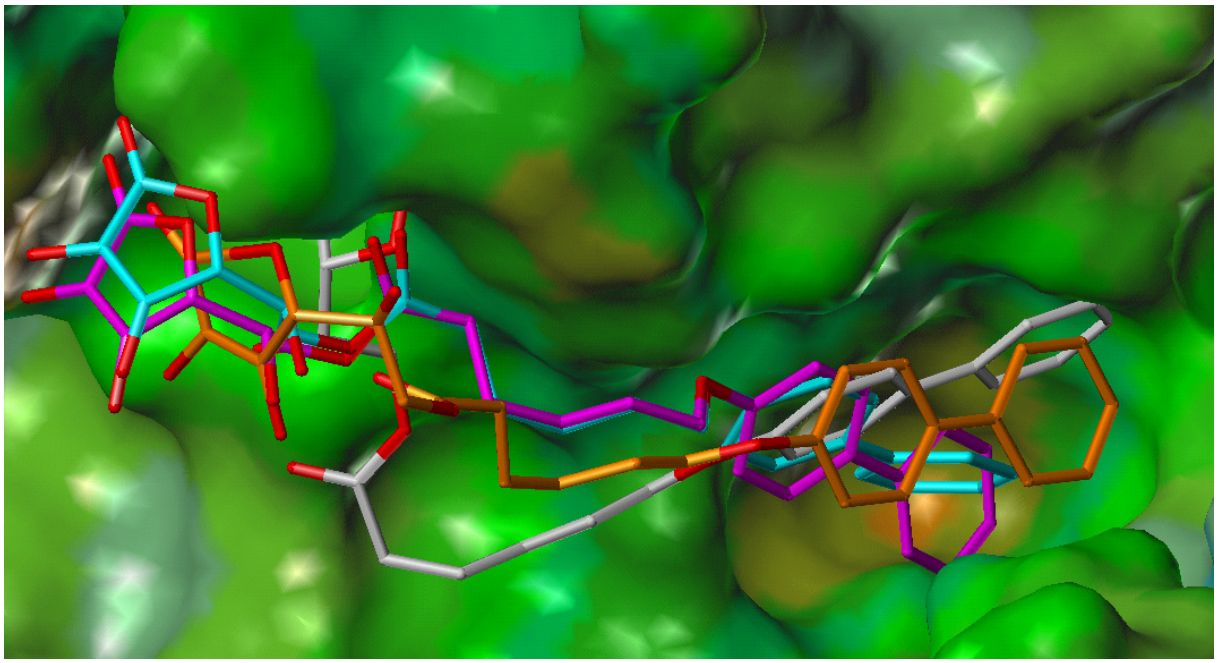


Figure 7.14. Representative binding modes of **7.52** near the lipophilic area 1 as calculated by Flexidock. The protein surface is represented by a Connolly surface colored by lipophilicity (the warmer the color, the more lipophilic the amino acids).

Most of the calculated binding modes differed especially in the binding of the biphenyl moiety to the lipophilic pocket: whereas in some poses the biphenyl moiety plugs in the narrow lipophilic pocket (binding modes colored in magenta or cyan), in others the aromatic system fits outside resulting in weaker interactions with hydrophobic residues (binding modes colored in orange and white). In all cases, the ascorbic acid moiety was found to interact with amino acids of the tetrasaccharide binding site "westwards" from lipophilic area 1. Poses with close fit of the biphenyl residue into the lipophilic pocket correspond to the results obtained for the biaryl series: para and meta substitution leads to a significant loss of Hyal-1 inhibition whereas ortho substitution is tolerated. Additionally, the overall length of the tridecanoyl substituted derivative **7.2e** is comparable to the dimensions of the biphenyl compound **7.52**. Lengthening of the alkyl chain leads to a loss of potency. Ascorbic acid derivatives bearing longer alkyl chains may also bind to the

"tetrasaccharide region" occupied by the generated docking modes, but in this case the hydrophobic chains are not able to fill the narrow pocket. No binding mode with reasonable interactions of the inhibitor with lipophilic area 2 was generated. However, binding to this second lipophilic area around the product binding site of the enzyme must also be considered, since especially disubstituted extended and flexible structures can align along both sides of the hyaluronan binding groove. Compound **7.121**, the most potent Hyal-1 inhibitor in the series, was manually docked to check if the hexadecanoyl chain may fit to the second hydrophobic pocket. Figure 7.15 displays the obtained docking mode together with a refined docking pose of **7.52** (small modifications and energy minimization of a Flexidock solution).

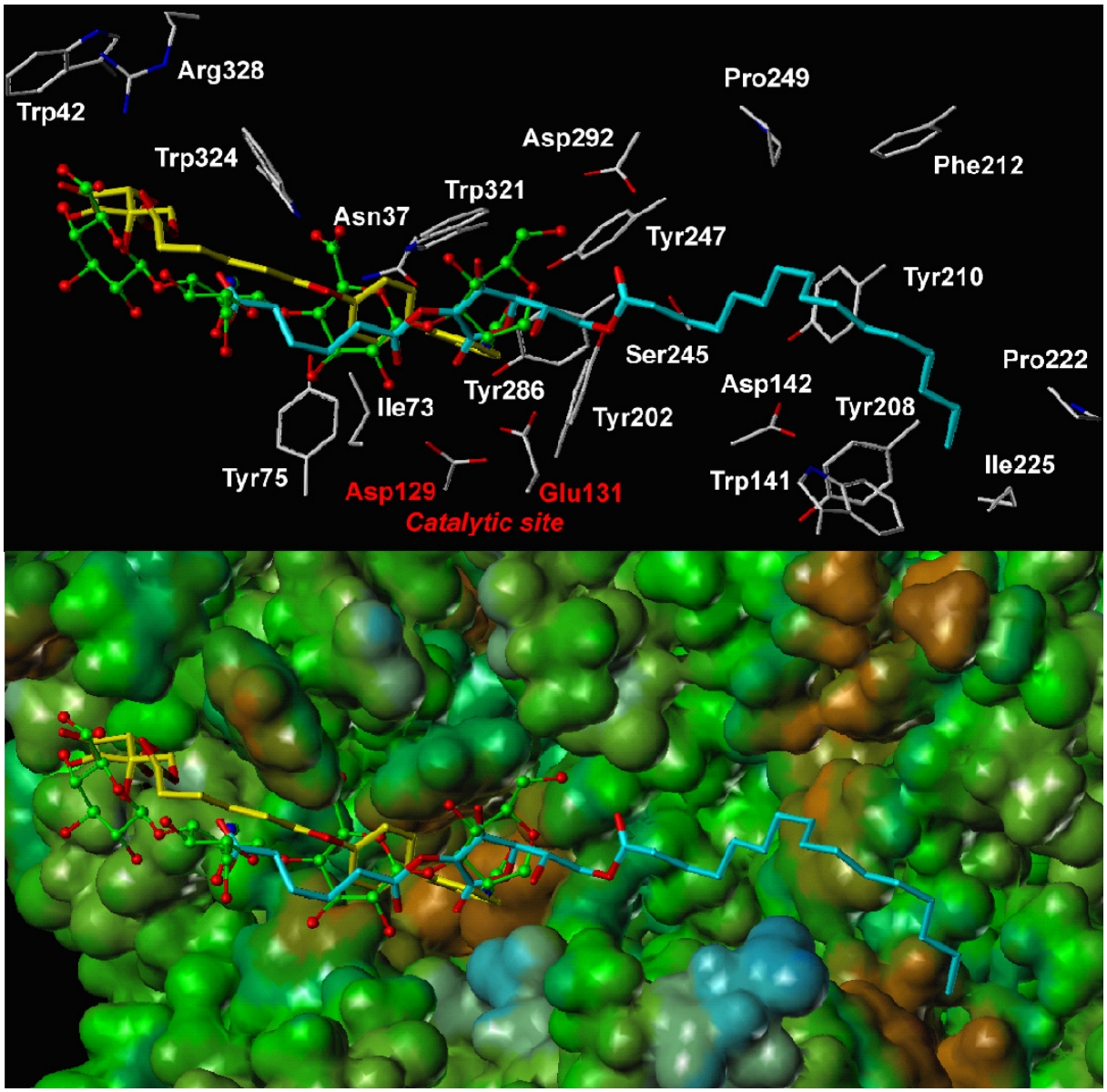


Figure 7.15. Suggested binding modes of **7.52** (C atoms yellow) and **7.121** (C atoms cyan). The hyaluronic acid tetrasaccharide (C atoms green) is taken from the complex with bee venom hyaluronidase (PDB code 1fcv)[61]. The protein surface is represented by a Connolly surface colored by lipophilicity (the warmer the color, the more lipophilic the amino acids).

The ascorbic acid core of **7.52** occupies approximately the same space as the terminal glucuronic acid moiety of the tetrasaccharide with the acidic 3-OH of vitamin C corresponding to the carboxylate of the carbohydrate. There are only weak protein-inhibitor interactions with the protein in this region indicating degrees of freedom for additional modifications. Due to the flexible link to the biphenyl residue, the ascorbic acid moiety could fit to Trp42 and Arg328. The alkyl spacer itself aligns with the indole moiety of Trp324 leading to additional hydrophobic interactions. This binding mode is in agreement with the results described in chapter 7.3.3: Various modifications of the vitamin C enediol system were tolerated and led to an increase in inhibition of Hyal-1. The position of the biphenyl residue was not modified, as this residue fills the lipophilic pocket 1 (surrounding an *N*-acetyl moiety of the substrate in the active site) and thus explains the observed SAR when the biphenyl-part is modified as described above.

Obviously, the diacylated derivative 2-*O*-(5-carboxypentanoyl)-6-*O*-hexadecanoyl-L-ascorbic acid (**7.121**) nearly fills the whole substrate groove. The flexible hexadecanoyl chain occupies the lipophilic area in the "eastern part" of the hyaluronan site (product moiety). The ascorbic acid core is allocated in the center of the active site in close proximity to the catalytic amino acids Asp129 and Glu131. The carboxypentanoyl residue may occupy the same region as the substrate tetrasaccharide by projection towards the non-reducing end. Thus, such an extended inhibitor conformation could possibly mimic the linear shape of hyaluronic acid. Again, modification of the ascorbic acid scaffold is possible because the 3-OH is solvent exposed. Therefore, this binding mode is consistent with the high inhibitory potency of the triacylated compound **7.121a**. The increased inhibitory activity of acylated ascorbic acid derivatives with additional branching in α position of the ester (**7.89-7.95**) could also be explained by this docking mode: The additional alkyl or aryl residue could occupy additional regions of this lipophilic area in the "eastern part" of the substrate binding groove.

Due to the nature of both the enzyme and the inhibitor, it is impossible to identify an explicit binding mode: The large hyaluronan binding groove of Hyal-1 (and the hyaluronidases in general) provides several potential binding sites for hydrophobic small molecule inhibitors. In addition, the flexible alkyl chains of the inhibitors are able to adopt many different conformations and thus may interact with various sites. Therefore the exemplary nature of the given binding modes must clearly be

emphasized. Nevertheless, the suggested modes could serve as a base for future work. It would be especially interesting to further investigate the binding mode of biphenyl derivatives, as the observed different selectivities for various hyaluronidases are possibly be associated with the narrow hydrophobic cleft (lipophilic area 1) in Hyal-1 if the shape of this cleft is different from other (mammalian) hyaluronidases. Thus, optimized interactions with the enzyme in this part of the molecule could lead to inhibitors with even increased selectivity. Additionally, variation of the spacer length between the biphenyl and the ascorbic acid moiety could lead to optimized interactions of the ascorbic acid with amino acids in the "western part" of the hyaluronan binding groove. Furthermore, the introduction of more rigid spacers could help to identify amino acids involved in the binding of the inhibitor.

7.5 *Summary*

Starting from ascorbic acid, which is only a weak inhibitor of human PH-20 with an IC_{50} value in the millimolar range and does dot inhibit human Hyal-1, the inhibition of the recombinantly expressed human hyaluronidases was improved by adding hydrophobic substituents in position *O*-6 of the ascorbic acid moiety as it was recently demonstrated for the bacterial hyaluronate lyase and the bovine enzyme[23, 53]. Whereas highest inhibition of PH-20 was achieved for ascorbic acid palmitate with an IC_{50} value of 5.8 µM, this compound was inactive on the human homolog Hyal-1. Highest potency on this enzyme was achieved by ascorbic acid 6-*O*-tridecanoate (**7.2e**) with an IC_{50} of 50 µM[21]. Further modification of the hydrophobic alkyl chain, e.g. introduction of various aromatic residues resulted in similarly active compounds. Again, Hyal-1 was inhibited when shorter spacers were used between the ascorbic acid core and the aromatic substituents, whereas the compounds with longer spacers were highly potent inhibitors of both PH-20 homologs and the bacterial hyaluronate lyase, e.g. 6-*O*-[11-(4-phenylphenoxy)undecanoyl]ascorbic acid (**7.63**) with IC_{50} values of 1.3 µM (human PH-20), 37 µM (BTH) and 7.5 µM (*Sag*$Hyal_{4755}$). This compound represents the most potent inhibitor of human PH-20 among the ascorbic acid derivatives. Different SAR resulted also from minor changes in the biphenyl part of 6-*O*-[6-(4-phenylphenoxy)hexanoyl]ascorbic acid (**7.52**), e.g. introduction of methyl, methoxy, hydroxy or carboxy residues, In the case of Hyal-1 major potency

differences were observed: Inhibition is lost if para- or meta-substituents are introduced, whereas hydrophobic ortho substitution is tolerated, thus indicating a lipophilic binding pocket of Hyal-1 which is possibly of different shape in the other three enzymes as the modifications of the biphenyl part caused only minor changes in inhibition of PH-20, BTH and *Sag*Hyal$_{4755}$.

Whereas the introduction of an aromatic spacer adjacent to the carbonyl group was tolerated (**7.87**, **7.88**), a drastic decrease in potency of the compounds was observed if the hydrophobic chain was disrupted by an ether oxygen (**7.98** and **7.99**). Branching of the substituent (**7.89**-**7.95**) led to inhibitors with increased potency especially for Hyal-1, but variation of the side chains resulted only in minor activity changes.

The “bivalent” compounds presented in chapter 7.3.2 are potent inhibitors of the bacterial hyaluronate lyase and the two PH-20 isoenzymes with inhibitory activities comparable to those of the “monovalent” derivatives, whereas Hyal-1 was rather weakly inhibited.

5,6-di-*O*-acylated derivatives (**7.96a**-**7.100a** and **7.103a**) are advantageous compared to the 6-O-acylated compounds (**7.96**-**7.100** and **7.103**). The greatest IC_{50} ratios (up to 26-fold) were observed for human PH-20. Additional introduction of carboxylic acid residues *via* 2-*O*-acylation of Vcpal led to potent inhibitors of human and bacterial hyaluronidases with IC_{50} values in the lower micromolar range. 2-*O*-(5-carboxypentanoyl)-6-*O*-hexadecanoyl-L-ascorbic acid (**7.121**) represents the most active inhibitor of human Hyal-1 developed in this project with an IC_{50} value of 8.3 µM und is one of the most potent inhibitors of this enzyme known to date.

Alkylation of *O*-6 (**7.127** and **7.128**) led to inhibitors which are equipotent with the corresponding acylated derivatives. Additionally, these alkyl ascorbic acids should possess increased stability in aqueous solution. When the position of the hydrophobic residue was altered from position *O*-6 to *O*-2 (**7.149** and **7.150**) or *O*-3 (**7.139** and **7.140**), drastic activity and selectivity differences between the two human enzymes Hyal-1 and PH-20 as well as between the two PH-20 homologs were observed. The inhibiton of the bacterial enzyme remained largely unaffected.

Taken together, the synthesized ascorbic acid based inhibitors are the most potent inhibitors of mammalian hyaluronidases known to date. Specific SAR were obtained for each hyaluronidase with distinct differences between the human enzymes on the one hand and the human and bovine PH-20 enzymes on the other hand. A common

selectivity of the synthesized ascorbic acid derivatives for human PH-20 compared to the bovine homolog was obvious.

The recently published[64] crystal structure of human Hyal-1 was used as model to perform docking experiments and to identify potential interaction sites explaining the results from the turbidimetric assay. As binding of the hydrophobic inhibitors to hydrophobic patches of the enzyme is most probable, two highly lipophilic areas near the catalytically active center of Hyal-1 were first considered. An automated docking (FlexiDock) was performed using 6-*O*-[6-(4-phenylphenoxy)hexanoyl]ascorbic acid (**7.52**) as ligand. The resulting docking poses suggest a close fit of the biphenyl residue into one of the identified lipohilic pockets. Manual docking of the most potent Hyal-1 inhibitor, 2-*O*-(5-carboxypentanoyl)-6-*O*-hexadecanoyl-L-ascorbic acid (**7.121**), indicates alignment of the alkyl chain with the second hydrophobic patch. The suggested binding modes are able to explain various characteristics of the SAR and thus may serve as basis for the structure-based design of further Hyal-1 inhibitors.

7.6 *Experimental Section*

7.6.1 General conditions

Chemicals were purchased from the following suppliers: Acros Organics (Geel, Belgium), IRIS Biotech GmbH (Marktredwitz, Germany), Alfa Aesar GmbH & Co. KG (Karlsruhe, Germany), Merck KGaA (Darmstadt, Germany), and Sigma-Aldrich Chemie GmbH (Munich, Germany). Deuterated solvents for NMR spectroscopy were from Deutero GmbH (Kastellaun, Germany). All solvents were of analytical grade or distilled prior to use. If moisture-free conditions were required, reactions were performed in dried glassware under inert atmosphere (argon or nitrogen); DMF (H_2O < 0.01 %) was purchased from Sigma-Aldrich Chemie GmbH. Nuclear Magnetic Resonance (^{1}H-NMR and ^{13}C-NMR) spectra were recorded on an Avance-300 NMR spectrometer from Bruker BioSpin GmbH (Rheinstetten, Germany). Tetramethylsilane was added as internal standard (chemical shift δ = 0 ppm) to all samples. Multiplicities are specified with the following abbreviations: s (singlet), d

(doublet), t (triplet), q (quartet), m (multiplet), bs (for broad singulet), as well as combinations thereof. The multiplicity of carbon atoms (^{13}C-NMR) were determined by DEPT 135 and DEPT 90 (distortionless enhancement by polarization transfer): "+" primary and tertiary carbon atom (positive DEPT 135 signal), "-" secondary carbon atom (negative DEPT 135 signal), "quat" quaternary carbon atom. Mass spectrometry analysis (MS) was performed on a Finnigan MAT 95 (PI-EIMS 70 eV, HR-MS), Finnigan SSQ 710A (CI-MS (NH_3)) and on a Finnigan ThermoQuest TSQ 7000 (ESI-MS) spectrometer. The peak-intensity in % relative to the strongest signal is indicated in parenthesis. Melting points (mp) were measured on a BÜCHI 530 using open capillaries and are uncorrected. Merck Silica Gel 60 (particle size 0.040–0.063 mm) was used for flash column chromatography. Reactions were routinely monitored by thin layer chromatography (TLC) on Merck silica gel 60 F_{254} aluminum sheets and spots were visualized with UV light at 254 nm, and/or iodine vapor or ammonium molybdate/cerium(IV) sulphate solution.

Preparative HPLC was performed with a pump model K-1800 (Knauer, Berlin, Germany), the column was Eurosphere-100 (250 x 32 mm) (Knauer), which was attached to a UV-detector model K-2000 (Knauer). UV-detection was done at 254 and 210 or 220 nm, respectively. The temperature was 25 °C and the flow rate 37 ml/min. The mobile phase was 0.1 % TFA in millipore water and MeCN. Analytical HPLC was performed on a system from Thermo Separation Products equipped with a SN 400 controller, P4000 pump, an AS3000 autosampler and a Spectra Focus UV-VIS detector. Stationary phase was a Eurosphere-100 C-18 (250 x 4.0, 5 µm) column (Knauer) thermostated at 30 °C. As mobile phase gradients of MeCN/0.02 or 0.05 % TFA/aq were used (flow rate = 0.7 ml/min). Absorbance was detected at 210 nm.

7.6.2 Chemistry

7.6.2.1 General procedure for the preparation of methyl esters 7.1a-7.1g, 14, 15 and 34

To a solution of carboxylic acid (1eq) in anhydrous methanol under nitrogen atmosphere was added chlortrimethylsilane (2.2 eq). The mixture was stirred at reflux overnight, cooled, and evaporated under reduced pressure. The residue was taken

up in diethylether and washed twice with 1 N NaOH and water. After drying over magnesium sulfate the solvent was removed under reduced pressure.

Methyl octanoate[65] (7.1a): The title compound was prepared from octanoic acid (50 mmol, 6.56 ml) and TMSCl (110 mmol, 13.9 ml) in 50 ml anhydrous methanol according to the general procedure 7.6.2.1 yielding **7.1a** as a colorless oil (7.30 g, 92 %). ^{1}H-NMR ($CDCl_3$) δ 0.87 (t, 3H, ^3J = 6.4 Hz, $CH_2C\underline{H}_3$), 1.27 (m, 8H, $(C\underline{H}_2)_4CH_3$), 1.61 (m, 2H, $COCH_2C\underline{H}_2CH_2$), 2.29 (t, 2H, ^3J = 7.5 Hz, $COC\underline{H}_2CH_2$), 3.65 (s, 3H, OCH_3). EI-MS (70 eV) *m/z (%)*: 158 (4) [$M^{+\cdot}$]. $C_9H_{18}O_2$ (158.24).

Methyl decanoate[65] (7.1b): The title compound was prepared from decanoic acid (50 mmol, 8.61 g) and TMSCl (110 mmol, 13.9 ml) in 50 ml anhydrous methanol according to the general procedure 7.6.2.1 yielding **7.1b** as a colorless oil (8.60 g, 92 %). ^{1}H-NMR ($CDCl_3$) δ 0.87 (t, 3H, ^3J = 6.7 Hz, $CH_2C\underline{H}_3$), 1.26 (m, 12H, $(C\underline{H}_2)_6CH_3$), 1.61 (m, 2H, $COCH_2C\underline{H}_2CH_2$), 2.30 (t, 2H, ^3J = 7.5 Hz, $COC\underline{H}_2CH_2$), 3.66 (s, 3H, OCH_3). EI-MS (70 eV) *m/z (%)*: 186 (5) [$M^{+\cdot}$]. $C_{11}H_{22}O_2$ (186.29).

Methyl undecanoate[66] (7.1c): The title compound was prepared from undecanoic acid (50 mmol, 9.31 g) and TMSCl (110 mmol, 13.9 ml) in 50 ml anhydrous methanol according to the general procedure 7.6.2.1 yielding **7.1c** as a colorless oil (9.29 g, 98 %). ^{1}H-NMR ($CDCl_3$) δ 0.86 (t, 3H, ^3J = 6.7 Hz, CH_3), 1.24 (m, 14H, $CH_2(C\underline{H}_2)_7CH_3$), 1.60 (m, 2H, $COCH_2C\underline{H}_2$), 2.28 (t, 2H, ^3J = 7.5 Hz, $COCH_2$), 3.65 (s, 3H, OCH_3). $C_{12}H_{14}O_2$ (190.24).

Methyl dodecanoate[67] (7.1d): The title compound was prepared from dodecanoic acid (50 mmol, 10.02 g) and TMSCl (110 mmol, 13.9 ml) in 50 ml anhydrous methanol according to the general procedure 7.6.2.1 yielding **7.1d** as a colorless oil (10.34 g, 96 %). ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.7Hz, $CH_2C\underline{H}_3$), 1.27 (m, 16H, $(C\underline{H}_2)_8CH_3$), 1.62 (m, 2H, $COCH_2C\underline{H}_2CH_2$), 2.30 (t, 2H, ^3J = 7.5 Hz, $COC\underline{H}_2CH_2$), 3.67 (s, 3H, OCH_3). EI-MS (70 eV) *m/z (%)*: 214 (10) [$M^{+\cdot}$]. $C_{13}H_{26}O_2$ (214.34).

Methyl tridecanoate[68] (7.1e): The title compound was prepared from tridecanoic acid (25 mmol, 5.36 g) and TMSCl (55 mmol, 7.0 ml) in 25 ml anhydrous methanol according to the general procedure 7.6.2.1 yielding **7.1e** as a colorless oil (5.50 g, 96 %). ^{1}H-NMR ($CDCl_3$) δ 0.87 (t, 3H, ^3J = 6.7 Hz, CH_3),1.26 (m, 18H, $CH_2(C\underline{H}_2)_9CH_3$), 1.61 (m, 2H, $COCH_2C\underline{H}_2$-), 2.29 (t, 2H, ^3J = 7.5 Hz, $COCH_2$), 3.66 (s, 3H, OCH_3). EI-MS (70 eV) *m/z (%)*: 228 (20) [$M^{+\cdot}$]. $C_{14}H_{28}O_2$ (228.37)

Methyl tetradecanoate[65] (7.1f): The title compound was prepared from tetradecanoic acid (50 mmol, 11.42 g) and TMSCl (110 mmol, 13.9 ml) in 50 ml

anhydrous methanol according to the general procedure 7.6.2.1 yielding **7.1f** as a colorless oil (11.50 g, 95 %). ^{1}H-NMR ($CDCl_3$) δ 0.87 (t, 3H, ^3J = 6.7 Hz, $CH_2C\underline{H}_3$), 1.25 (m, 20H, $(C\underline{H}_2)_{10}CH_3$), 1.61 (m, 2H, $COCH_2C\underline{H}_2CH_2$), 2.29 (t, 2H, ^3J = 7.5 Hz, $COC\underline{H}_2CH_2$), 3.66 (s, 3H, OCH_3). EI-MS (70 eV) *m/z (%)*: 242 (39) [$M^{+\cdot}$]. $C_{15}H_{30}O_2$ (242.40).

Methyl p-biphenylylacetate[69] (7.1g): The title compound was prepared from *p*-biphenylylacetic acid (25 mmol, 5.31 g) and TMSCl (55 mmol, 7.0 ml) in 25 ml anhydrous methanol according to the general procedure 7.6.2.1 yielding **7.1g** as a pale yellow oil (5.24 g, 93 %). ^{1}H-NMR ($CDCl_3$) δ 3.70 (s, 2H, CH_2), 3.74 (s, 3H, OCH_3), 7.37 (m, 3 H, Ar-H), 7.46 (m, 2H, Ar-H), 7.60 (m, 4H, Ar-H). EI-MS (70 eV) *m/z (%)*: 226 (36) [$M^{+\cdot}$]. $C_{15}H_{14}O_2$ (226.27)

7.6.2.2 General procedure for the enzymatic synthesis of 6-*O*-acylated ascorbic acids 7.2a-7.2g and 48-63

Vitamin C (1 eq), the pertinent methyl alkanoate (1.5-4 eq) and the immobilized lipase from *Candida antarctica* (Novozyme 435®) (50 mg per mmol ascorbic acid) were suspended in *tert*-amyl alcohol and submitted to rotary evaporation at 60 °C bath temperature and 200 mbar vacuum for 14-24 h. After the major part of ascorbic acid was consumed, the reaction mixture was cooled to room temperature, solids were removed by filtration, the solvent was evaporated under reduced pressure and the resulting material was taken up in EtOAc. After washing with brine and water the organic phase was dried over $MgSO_4$, the solvent was removed under reduced pressure and the crude products were recrystallized from *tert*-butyl methyl ether/hexane.

6-*O*-Octanoyl-L-ascorbic acid[70] (7.2a): The title compound was prepared from ascorbic acid (3.3 mmol, 0.58 g), **7.1a** (4 eq, 13.2 mmol, 2.09 g) and Novozyme 435® (165 mg) in 15 ml *tert*-amyl alcohol according to the general procedure 7.6.2.2 yielding **7.2a** as a white solid (0.45 g, 45 %). mp: 78-80 °C; ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H, ^3J = 6.7 Hz, CH_3), 1.24 (m, 8H, $CH_2(C\underline{H}_2)_4CH_3$), 1.53 (m, 2H, $COCH_2C\underline{H}_2$), 2.32 (t, 2H, ^3J = 7.4 Hz, $COCH_2$), 4.03 (m, 3H, CH_2O, $C\underline{H}OH$), 4.67 (d, 1H, ^3J = 1.7 Hz, H-5), 5.32 (bs, 1H, $CHO\underline{H}$), 8.42 (bs, 1H, OH), 11.12 (bs, 1H, OH). ^{13}C-NMR (DMSO-d_6) δ 13.85 (+, CH_3), 21.96 (-, CH_2), 24.30 (-, CH_2), 28.28 (-, CH_2), 28.33 (-, CH_2), 31.03 (-, CH_2), 33.30 (-, $CO\underline{C}H_2CH_2$), 64.36 (-, CH_2O), 65.39 (+, CHO), 74.92 (+, CH), 118.07 (quat, C-2), 152.13 09 (quat, C-3), 170.27 (quat,

lactone CO), 172.65 (quat, $\underline{C}O_2CH_2$). ES-MS (MeCN + TFA) *m/z (%)*: 303 (100) $[M+H]^+$. Anal. ($C_{14}H_{22}O_7$) C, H. $C_{14}H_{22}O_7$ (302.32).

6-*O*-Decanoyl-L-ascorbic acid[70] (7.2b): The title compound was prepared from ascorbic acid (3.3 mmol, 0.58 g), **7.1b** (4 eq, 13.2 mmol, 2.45 g) and Novozyme 435® (165 mg) in 15 ml *tert*-amyl alcohol according to the general procedure 7.6.2.2 yielding **7.2b** as a white solid (0.50 g, 46 %). mp: 96-97 °C (ref.[71]: 100-101 °C); ^{1}H-NMR (DMSO-d_6) δ 0.86 (t, 3H, ^3J = 6.7 Hz, CH_3), 1.24 (m, 12H, $CH_2(C\underline{H}_2)_6CH_3$), 1.53 (m, 2H, $COCH_2C\underline{H}_2$), 2.32 (t, 2H, ^3J = 7.4 Hz, $COC\underline{H}_2CH_2$), 4.03 (m, 3H, CH_2O, $C\underline{H}OH$), 4.67 (d, 1H, ^3J = 1.6 Hz, H-5), 5.30 (bs, 1H, $CHO\underline{H}$), 8.39 (bs, 1H, OH), 11.10 (bs, 1H, OH). ^{13}C-NMR (DMSO-d_6) δ 13.88 (+, CH_3), 22.01 (-, CH_2), 24.30 (-, CH_2), 28.37 (-, CH_2), 28.56 (-, CH_2), 28.63 (-, CH_2), 28.77 (-, CH_2), 31.19 (-, CH_2), 33.31 (-, $CO\underline{C}H_2CH_2$), 64.37 (-, CH_2O), 65.41 (+, CHO), 74.92 (+, CH), 118.10 (quat, C-2), 152.09 (quat, C-3), 170.25 (quat, lactone CO), 172.66 (quat, $\underline{C}O_2CH_2$). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 348 (100) $[M+NH_4]^+$. Anal. ($C_{16}H_{26}O_7$) C, H. $C_{16}H_{26}O_7$ (330.37).

6-*O*-Undecanoyl-L-ascorbic acid[70, 72] (7.2c): The title compound was prepared from ascorbic acid (3.3 mmol, 0.58 g), **7.1c** (4 eq, 13.2 mmol, 2.51 g) and Novozyme 435® (165 mg) in 15 ml *tert*-amyl alcohol according to the general procedure 7.6.2.2 yielding **7.2c** as a white solid (0.60 g, 53 %). mp: 96-98 °C; ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H, ^3J = 6.7 Hz, CH_3), 1.24 (m, 14H, $CH_2(C\underline{H}_2)_7CH_3$), 1.52 (m, 2H, $COCH_2C\underline{H}_2$), 2.32 (t, 2H, ^3J = 7.4 Hz, $COCH_2$), 4.03 (m, 3H, CH_2O, $C\underline{H}OH$), 4.68 (d, 1H, ^3J = 1.5 Hz, H-5), 5.32 (bs, 1H, $CHO\underline{H}$), 8.42 (s, 1H, OH), 11.14 (s, 1H, OH). ^{13}C-NMR (DMSO-d_6) δ 13.87 (+, CH_3), 22.02 (-, CH_2), 24.30 (-, CH_2), 28.38 (-, CH_2), 28.62 (-, CH_2), 28.82 (-, CH_2), 28.88 (-, CH_2), 31.21 (-, CH_2), 33.30 (-, $CO\underline{C}H_2CH_2$), 64.36 (-, CH_2O), 65.41 (+, CHO), 74.92 (+, CH), 118.10 (quat, C-2), 152.09 (quat, C-3), 170.25 (quat, lactone CO), 172.64 (quat, $\underline{C}O_2CH_2$). ES-MS (MeCN + TFA) *m/z (%)*: 345 (100) $[M+H]^+$. Anal. ($C_{17}H_{28}O_7$) C, H. $C_{17}H_{28}O_7$ (344.40).

6-*O*-Dodecanoyl-L-ascorbic acid[70] (7.2d): The title compound was prepared from ascorbic acid (3.3 mmol, 0.58 g), **7.1d** (4 eq, 13.2 mmol, 2.83 g) and Novozyme 435® (165 mg) in 15 ml *tert*-amyl alcohol according to the general procedure 7.6.2.2 yielding **7.2d** as a white solid (0.63 g, 53 %). mp: 101-103 °C (ref.[53]: 95-97); ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H, ^3J = 6.7 Hz, $CH_2C\underline{H}_3$), 1.24 (s, 16H, $(C\underline{H}_2)_8CH_3$), 1.52 (m, 2H, $COCH_2C\underline{H}_2CH_2$), 2.32 (t, 2H, ^3J = 7.4 Hz, $COC\underline{H}_2CH_2$-), 4.01 (m, 3H, CH_2O, $C\underline{H}OH$), 4.67 (d, 1H, ^3J = 1.6 Hz, H-5), 5.33 (d, 1H, ^3J = 5.7 Hz, $CHO\underline{H}$), 8.42 (s, 1H,

OH), 11.14 (s, 1H, OH). ^{13}C-NMR (DMSO-d_6) δ 13.89 (+, CH_3), 22.04 (-, CH_2), 24.30 (-, CH_2), 28.38 (-, CH_2), 28.65 (-, CH_2), 28.83 (-, CH_2), 28.94 (-, CH_2), 31.23 (-, CH_2), 33.29 (-, CO<u>C</u>H_2CH_2), 64.36 (-, CH_2O), 65.37 (+, CHO), 74.91 (+, CH), 118.08 (quat, C-2), 152.10 (quat, C-3), 170.27 (quat, lactone CO), 172.65 (quat, <u>C</u>O_2CH_2). CI-MS (NH_3) *m/z (%)*: 376 (100) $[M+NH_4]^+$. Anal. ($C_{18}H_{30}O_7$) C, H. $C_{18}H_{30}O_7$ (358.43).

6-*O*-Tridecanoyl-L-ascorbic acid[73] (7.2e): The title compound was prepared from ascorbic acid (3.3 mmol, 0.58 g), **7.1e** (4 eq, 13.2 mmol, 3.01 g) and Novozyme 435® (165 mg) in 15 ml *tert*-amyl alcohol according to the general procedure 7.6.2.2 yielding **7.2e** as a white solid (0.79 g, 69 %). mp: 103 °C; ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H, ^3J = 6.7 Hz, CH_3), 1.24 (s, 18H, CH_2(C<u>H</u>$_2$)$_9$$CH_3$), 1.53 (m, 2H, $COCH_2$C<u>H</u>$_2$), 2.31 (t, 2H, ^3J =7.5 Hz, $COCH_2$), 4.04 (m, 3H, CH_2O, C<u>H</u>OH), 4.67 (d, 1H, ^3J = 1.7 Hz, H-5), 5.31 (d, 1H, ^3J = 5.7 Hz, CHO<u>H</u>), 8.39 (s, 1H, OH), 11.10 (s, 1H, OH). ^{13}C-NMR (DMSO-d_6) δ 13.87 (+, CH_3), 22.03 (-, CH_2), 24.30 (-, CH_2), 28.38 (-, CH_2), 28.64 (-, CH_2), 28.82 (-, CH_2), 28.93 (-, CH_2), 31.22 (-, CH_2), 33.30 (-, CO<u>C</u>H_2CH_2), 64.36 (-, CH_2O), 65.41 (+, CHO), 74.92 (+, CH), 118.10 (quat, C-2), 152.07 (quat, C-3), 170.24 (quat, lactone CO), 172.63 (quat, <u>C</u>O_2CH_2). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 371 (100) $[M-H]^-$. Anal. ($C_{19}H_{32}O_7$) C, H. $C_{19}H_{32}O_7$ (372.45).

6-*O*-Tetradecanoyl-L-ascorbic acid[74] (7.2f): The title compound was prepared from ascorbic acid (3.3 mmol, 0.58 g), **7.1f** (4 eq, 13.2 mmol, 3.20 g) and Novozyme 435® (165 mg) in 15 ml *tert*-amyl alcohol according to the general procedure 7.6.2.2 yielding **7.2f** as a white solid (0.76 g, 59 %). mp: 104-105 °C; ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H, ^3J = 6.7 Hz, CH_3), 1.24 (m, 20H, (C<u>H</u>$_2$)$_{10}$$CH_3$), 1.52 (m, 2H, $COCH_2$C<u>H</u>$_2$), 2.31 (t, 2H, ^3J = 7.4 Hz, COC<u>H</u>$_2CH_2$), 4.03 (m, 3H, CH_2O, C<u>H</u>OH), 4.67 (d, 1H, ^3J = 1.6 Hz, H-5), 5.31 (bs, 1H, CHO<u>H</u>), 8.40 (s, 1H, OH), 11.11 (s, 1H, OH). ^{13}C-NMR (DMSO-d_6) δ 13.88 (+, CH_3), 22.02 (-, CH_2), 24.30 (-, CH_2), 28.38 (-, CH_2), 28.64 (-, CH_2), 28.82 (-, CH_2), 28.95 (-, CH_2), 31.23 (-, CH_2), 33.30 (-, CO<u>C</u>H_2CH_2), 64.37 (-, CH_2O), 65.40 (+, CHO), 74.92 (+, CH), 118.10 (quat, C-2), 152.08 (quat, C-3), 170.25 (quat, lactone CO), 172.64 (quat, <u>C</u>O_2CH_2). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 404 (100) $[M+NH_4]^+$. Anal. ($C_{20}H_{34}O_7$) C, H. $C_{20}H_{34}O_7$ (386.48).

6-*O*-(*p*-Biphenylylacetyl)-L-ascorbic acid (7.2g): The title compound was prepared from ascorbic acid (3.3 mmol, 0.58 g), **7.1g** (4 eq, 13.2 mmol, 3.73 g) and Novozyme 435® (165 mg) in 15 ml *tert*-amyl alcohol according to the general procedure 7.6.2.2 yielding **7.2g** as a white solid (0.45 g, 37 %). mp: 138-141 °C; ^{1}H-NMR (DMSO-d_6) δ 3.77 (s, 2H, CH_2), 4.08 (m, 3H, CH_2O, C<u>H</u>OH) ppm 4.70 (d, 1H, ^3J=1.7 Hz, H-5), 5.37

(s, 1H, CHOH), 7.36 (m, 3H, Ar-H), 7.46 (m, 2H, Ar-H), 7.64 (m, 4H, Ar-H), 8.42 (bs, 1H, OH), 11.14 (s, 1H, OH). ^{1}H-NMR (DMSO-d_6) δ 3.77 (s, 2H, CH_2), 4.08 (m, 3H, CH_2O, CHOH) 4.70 (d, 1H, ^{3}J = 1.7 Hz, H-5), 5.37 (s, 1H, CHOH), 7.36 (m, 3H, Ar-H), 7.46 (m, 2H, Ar-H), 7.64 (m, 4H, Ar-H), 8.42 (bs, 1H, OH), 11.14 (s, 1H, OH). ^{13}C-NMR (DMSO-d_6) δ 39.62 (-, $CH_2C_6H_4$), 64.96 (-, CH_2O), 65.41 (+, CHO), 74.93 (+, CH), 118.12 (quat, C-2), 126.51 (+, Ar-C), 126.56 (+, Ar-C), 127.30 (+, Ar-C), 128.85 (+, Ar-C), 129.93 (+, Ar-C), 133.43 (quat, Ar-C), 138.67 (quat, Ar-C), 139.78 (quat, Ar-C), 152.11 (quat, C-3), 170.26 (quat, lactone CO), 170.94 (quat, CO_2CH_2). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 369 (100) $[M-H]^-$. Anal. ($C_{20}H_{18}O_7$) C, H. $C_{20}H_{18}O_7$ (370.35).

Methyl 6-bromohexanoate (7.3)[26]: A solution of ε-caprolactone (234.5 mmol, 26 ml) in 33 % hydrogen bromide in acetic acid (63 ml) was heated for 6 h at 70 °C, cooled to room temperature, treated with methanol (100 ml), and stirred overnight. Evaporation of the solvent gave a dark oil which was dissolved in EtOAc (150 ml) and washed successively with saturated sodium bicarbonate (3 x 150 ml) and saturated sodium chloride (150 ml), dried over $MgSO_4$, and evaporated to yield **7.3** as a colorless oil (41.4 g, 84 %). ^{1}H-NMR ($CDCl_3$) δ 1.45 (m, 2H, CH_2), 1.63 (m, 2H, CH_2), 1.85 (m, 2H CH_2), 2.31 (t, 2H, ^{3}J = 7.4 Hz, $COCH_2CH_2$), 3.39 (t, 2H, ^{3}J = 6.8 Hz, CH_2Br), 3.65 (s, 3H, OCH_3). EI-MS (70 eV) *m/z (%)*: 208 (20) $[M^{+\cdot}]$. Anal. ($C_7H_{13}BrO_2$) C, H. $C_7H_{13}BrO_2$ (209.08).

Methyl 6-(phenylthio)hexanoate[75] (7.4): To a solution of **7.3** (20 mmol, 4.18 g) and thiophenol (22 mmol, 2.27ml) in anhydrous THF (70 ml) was added DIEA (22 mmol, 3.77ml) and the mixture was stirred at room temperature overnight. After diluting the mixture with EtOAc (70 ml), it was washed with saturated $NaHCO_3$ and dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was submitted to flash chromatography ($CHCl_3$/PE 20/80 v/v) to obtain **7.4** as a colorless oil (2.69 g, 60 %). ^{1}H-NMR ($CDCl_3$) δ 1.46 (m, 2H, $COCH_2CH_2CH_2$), 1.65 (m, 4H, $COCH_2CH_2$, CH_2CH_2SPh), 2.30 (t, 2H, ^{3}J = 7.4 Hz, $COCH_2$), 2.91 (t, 2H, ^{3}J = 7.3 Hz, CH_2SPh), 3.66 (s, 3H, OCH_3), 7.16 (m, 1H, Ph-H), 7.29 (m, 4H, Ph-H). EI-MS (70 eV) *m/z (%)*: 238 (100) $[M^{+\cdot}]$. Anal. ($C_{13}H_{18}O_2S \cdot 0.5H_2O$) C, H. $C_{13}H_{18}O_2S$ (238,35).

Methyl 6-(benzylthio)hexanoate[76] (7.5): Solid K_2CO_3 (40 mmol, 5.53 g) was added to a solution of benzyl mercaptan (20 mmol, 2.35 ml) in 25 ml of anhydrous THF. The mixture was vigorously stirred while **7.3** (20 mmol, 4.18 g) was added dropwise. After refluxing overnight the mixture was cooled, insoluble material was filtered off and the

solution was evaporated to dryness. The residue was taken up with saturated NaCl solution, and the mixture was extracted 3 times with diethyl ether. The combined organic phases were washed with saturated $NaHCO_3$ and water, dried over $MgSO_4$ and concentrated. After flash chromatography (PE/EtOAc 90/10 v/v) the title compound was obtained as a colorless oil (3.85g, 76 %). ^{1}H-NMR ($CDCl_3$) δ 1.37 (m, 2H, $COCH_2CH_2C\underline{H}_2$), 1.58 (m, 4H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2SCH_2Ph$), 2.29 (t, 2H, ^3J = 7.5 Hz, $COCH_2$), 2.41 (t, 2H, ^3J = 7.3 Hz, $C\underline{H}_2SCH_2Ph$), 3.66 (s, 3H, OCH_3), 3.70 (s, 2H, $SC\underline{H}_2Ph$), 7.26 (m, 5H, Ph-H). CI-MS (NH_3) *m/z (%)*: 270 (100) $[M+NH_4]^+$. Anal. ($C_{14}H_{20}O_2S$) C, H. $C_{14}H_{20}O_2S$ (252.37).

7.6.2.3 General procedure for the synthesis of the ethers 6-13, 16, 33 and 36-38

Method A: To a solution of alkyl halide (1eq) and the appropriate phenol (1.5 eq) in anhydrous acetone was added K_2CO_3 (3 eq) and the mixture was refluxed overnight. After cooling insoluble material was removed by filtration and the solvent was evaporated under reduced pressure. The residue was taken up in EtOAc and washed successively with saturated K_2CO_3, water and brine. The organic phase was dried over $MgSO_4$, filtered, and evaporated. The crude product was submitted to flash chromatography.

Method B: A solution of the corresponding lactone (1eq), the appropriate alkyl halide (2 eq) and KOH (3 eq) in toluene was heated at reflux overnight. The reaction was quenched by acidification with 0.5 N HCl solution (pH value <2). The resulting mixture was then extracted four times with diethyl ether. The combined ether extracts were washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was flash chromatographed to yield the target compounds.

Method C: The corresponding phenol (1.1 eq) was dissolved in ethanol and KOH (2.2 eq) was added. The pertinent alkyl halide (1 eq) was added and the reaction mixture was refluxed 4 h under an argon atmosphere. After dilution with water and acidification with concentrated hydrochloric acid, the solvent was evaporated.

Methyl 6-phenoxyhexanoate[77] (7.6): The title compound was prepared from **7.3** (20 mmol, 4.18 g), phenol (30 mmol, 2.82 g) and K_2CO_3 (60 mmol, 8.29 g) in 150 ml anhydrous acetone according to 7.6.2.3, Method A and isolated by flash chromatography (PE/EtOAc 90/10 v/v) as a colorless oil (3.64 g, 87 %). ^{1}H-NMR ($CDCl_3$) δ 1.51 (m, 2H, $COCH_2CH_2C\underline{H}_2$), 1.76 (m, 4H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2OPh$), 2.36 (t, 2H, ^3J =7.4 Hz, $COCH_2$), 3.68 (s, 3H, OCH_3), 3.96 (t, 2H, ^3J = 6.4 Hz,

C$\underline{H_2}$OPh), 6.92 (m, 3H, Ph-H), 7.28 (m, 2H, Ph-H). CI-MS (NH_3) *m/z (%)*: 240 (20) $[M+NH_4]^+$. Anal. ($C_{13}H_{18}O_3 \cdot 0.4H_2O$) C, H. $C_{13}H_{18}O_3$ (222,28).

Methyl 6-(4-ethylphenoxy)hexanoate (7.7): The title compound was prepared from **7.3** (20 mmol, 4.18 g), *p*-ethylphenol (30 mmol, 3.67 g) and K_2CO_3 (60 mmol, 8.29 g) in 150 ml anhydrous acetone according to 7.6.2.3, Method A and was obtained after flash chromatography (PE/EtOAc 90/10 v/v) as a colorless oil (4.12 g, 87 %). ^{1}H-NMR ($CDCl_3$) δ 1.21 (t, 3H, ^3J = 7.6 Hz, $CH_2C\underline{H_3}$), 1.50 (m, 2H, $COCH_2CH_2C\underline{H_2}$), 1.74 (m, 4H, $COCH_2C\underline{H_2}$, $C\underline{H_2}CH_2OC_6H_4$), 2.35 (t, 2H, ^3J = 7.4 Hz, $COCH_2$), 2.59 (q, 2H, ^3J =7.6Hz, $C\underline{H_2}CH_3$), 3.67 (s, 3H, OCH_3), 3.93 (t, 2H, ^3J = 6.4 Hz, $C\underline{H_2}OC_6H_4$), 6.81 (m, 2H, Ar-H), 7.10 (m, 2H, Ar-H). PI-EIMS (70 eV) *m/z (%)*: 250 (50) $[M^{+\cdot}]$. Anal. ($C_{15}H_{22}O_3 \cdot 0.4H_2O$) C, H. $C_{15}H_{22}O_3$ (250.33).

Methyl 6-(*p*-biphenylyloxy)hexanoate (7.8): The title compound was prepared from **7.3** (20 mmol, 4.18g), 4-phenylphenol (30 mmol, 5.11 g) and K_2CO_3 (60 mmol, 8.29 g) in 150 ml anhydrous acetone according to 7.6.2.3, Method A. Flash chromatography (PE/EtOAc 80/20 v/v) and recrystallization from methanol yielded a white solid (3.97 g, 67 %). mp: 65-66 °C; ^{1}H-NMR ($CDCl_3$) δ 1.52 (m, 2H, $COCH_2CH_2C\underline{H_2}$), 1.77 (m, 4H, , $COCH_2C\underline{H_2}$, $C\underline{H_2}CH_2OC_6H_4$), 2.36 (t, 2H, ^3J = 7.4 Hz, $COCH_2$), 3.67 (s, 3H, OCH_3), 3.99 (t, 2H, ^3J = 6.4 Hz, $C\underline{H_2}OC_6H_4$), 6.95 (m, 2H, Ar-H), 7.29 (m, 1H, Ph-H), 7.41 (m, 2H, Ar-H), 7.53 (m, 4H, Ar-H). CI-MS (NH_3) *m/z (%)*: 316 (100) $[M+NH_4]^+$. Anal. ($C_{19}H_{22}O_3$) C, H. $C_{19}H_{22}O_3$ (298.38).

Methyl 6-[4-(benzyloxy)phenoxy]hexanoate[78] (7.9): The title compound was prepared from **7.3** (20 mmol, 4.18g), 4-benzyloxyphenol (30 mmol, 6.01 g) and K_2CO_3 (60 mmol, 8.29 g) in 150 ml anhydrous acetone according to 7.6.2.3, Method A. Flash chromatography (PE/EtOAc 90/10 v/v) yielded a white solid (4.07 g, 62 %). mp: 80-81 °C; ^{1}H-NMR ($CDCl_3$) δ 1.49 (m, 2H, $COCH_2CH_2C\underline{H_2}$), 1.72 (m, 4H, $COCH_2C\underline{H_2}$, $C\underline{H_2}CH_2OCH_2$), 2.34 (t, 2H, ^3J = 7.5 Hz, $COC\underline{H_2}CH_2$), 3.67 (s, 3H, OCH_3), 3.90 (t, 2H, ^3J = 6.4 Hz, $C\underline{H_2}OCH_2Ph$), 5.01 (s, 2H, $C\underline{H_2}Ph$), 6.81 (m, 2H, Ar-H), 6.89 (m, 2H, Ar-H), 7.36 (m, 5H, Ph-H). CI-MS (NH_3) *m/z (%)*: 346 (100) $[M+NH_4]^+$. Anal. ($C_{20}H_{24}O_4$) C, H. $C_{20}H_{24}O_4$ (328.4).

6-(Benzyloxy)hexanoic acid[79] (7.10): The title compound was prepared from ε-caprolactone (21 mmol, 2.24 ml), benzyl chloride (42 mmol, 4.83 ml) and KOH (84 mmol, 4.71 g) in 20 ml toluene according to 7.6.2.3, Method B. Flash chromatography (PE/EtOAc 80/20 v/v) gave a colorless oil (3.81 g, 82 %). ^{1}H-NMR ($CDCl_3$) δ 1.44 (m, 2H, $COCH_2CH_2C\underline{H_2}$), 1.65 (m, 4H, $COCH_2C\underline{H_2}$, $C\underline{H_2}CH_2OBn$),

2.36 (t, 2H, ^3J = 7.5 Hz, $COCH_2$), 3.48 (t, 2H, ^3J = 6.5 Hz, $C\underline{H}_2OBn$), 4.51 (s, 2H, $OC\underline{H}_2Ph$), 7.32 (m, 5H, Ph-H). EI-MS (70 eV) *m/z (%)*: 222 (4) [$M^{+\cdot}$]. $C_{13}H_{18}O_3$ (222,28).

6-(*p*-Biphenylylmethoxy)hexanoic acid (7.11): The title compound was prepared from ε-caprolactone (4 mmol, 0.44 ml), 4-(bromomethyl)biphenyl (8 mmol, 1.98 g) and KOH (16 mmol, 0.90 g) in 5 ml toluene according to 7.6.2.3, Method B and was obtained after flash chromatography (PE/EtOAc 80/20 + 0.1 % HOAc v/v) as a colorless oil (0.85 g, 71 %). ^{1}H-NMR ($CDCl_3$) δ 1.44 (m, 2H, $COCH_2CH_2C\underline{H}_2$), 1.65 (m, 4H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2OCH_2$), 2.36 (t, 2H, ^3J = 7.4 Hz, $COC\underline{H}_2CH_2$), 3.50 (t, 2H, ^3J = 6.5 Hz, $C\underline{H}_2OCH_2C_6H_4$), 4.53 (s, 2H, $OC\underline{H}_2C_6H_4$), 7.30 – 7.45 (m, 5H, Ph-H), 7.58 (m, 4H, Ar-H). CI-MS (NH_3) *m/z (%)*: 316 (100) [$M+NH_4$]$^+$. Anal. ($C_{19}H_{22}O_3 \cdot 0.2C_6H_4CH_3$) C, H. $C_{19}H_{22}O_3$ (298.38).

5-(Heptan-1-yloxy)pentanoic acid (7.12): The title compound was prepared from δ -valerolactone (42 mmol, 4.20 g), 1-iodoheptane (84 mmol, 9.5 g which was prepared from 1-bromoheptane using standard Finkelstein reaction[80, 81]) and KOH (168 mmol, 9.43 g) in 40 ml toluene according to 7.6.2.3, Method B. Flash chromatography (PE/EtOAc 80/20 + 0.1 % HOAc v/v) yielded a colorless oil (1.10 g, 12 %). ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.7 Hz, $CH_2C\underline{H}_3$), 1.29 (m, 8H, $(C\underline{H}_2)_4CH_3$),1.65 (m, 6H, $COCH_2(C\underline{H}_2)_2$, $OCH_2C\underline{H}_2$), 2.39 (t, 2H, ^3J = 7.2 Hz, $COCH_2$), 3.41 (m, 4H, $C\underline{H}_2OC\underline{H}_2$). CI-MS ($NH_3$) *m/z (%)*: 234 (100) [$M+NH_4$]$^+$. Anal. ($C_{12}H_{24}O_3 \cdot 0.1C_6H_4CH_3$) C, H. $C_{12}H_{24}O_3$ (216.32).

6-(Hexan-1-yloxy)hexanoic acid (7.13): The title compound was prepared from ε-caprolactone (21 mmol, 2.24 ml), 1-iodohexane (42 mmol, 6.2 ml) and KOH (84 mmol, 4.71 g) in 30 ml toluene according to 7.6.2.3, Method B. Flash chromatography (PE/EtOAc 80/20 + 0.1 % HOAc v/v) yielded a colorless oil (1.30 g, 29 %). ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.8 Hz, CH_3), 1.25 – 1.71 (m, 14H, $COCH_2(C\underline{H}_2)_3$, $(C\underline{H}_2)_4CH_3$), 2.36 (t, 2H, ^3J = 7.5 Hz, $COCH_2$), 3.40 (m, 4H, $C\underline{H}_2OC\underline{H}_2$). CI-MS ($NH_3$) *m/z (%)*: 215 (100) [M-H]$^-$. Anal. ($C_{12}H_{24}O_3$) calc C: 66.63, H: 11.18, found C: 65.50, H: 11.78. $C_{12}H_{24}O_3$ (216.32).

Methyl 6-(benzyloxy)hexanoate[82] (7.14): The title compound was prepared from **7.10** (15.3 mmol, 3.40 g) and TMSCl (33.7 mmol, 4.3 ml) in 25 ml anhydrous methanol according to the general procedure 7.6.2.1 yielding **7.14** as a colorless oil (2.98 g, 82 %). ^{1}H-NMR ($CDCl_3$) δ 1.41 (m, 2H, $COCH_2CH_2C\underline{H}_2$), 1.65 (m, 4H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2OBn$), 2.32 (t, 2H, ^3J = 7.5 Hz, $COCH_2$), 3.47 (t, 2H, ^3J = 6.5 Hz,

C<u>H</u>$_2$OBn), 3.66 (s, 3H, OCH$_3$), 4.50 (s, 2H, OC<u>H</u>$_2$Ph), 7.31 (m, 5H, Ph-H). EI-MS (70 eV) *m/z (%)*: 236 (1) [M$^{+\cdot}$]. Anal. ($C_{14}H_{20}O_3 \cdot 0.8H_2O$) C, H. $C_{14}H_{20}O_3$ (236,31).

Methyl 6-(*p*-biphenylylmethoxy)hexanoate (7.15): The title compound was prepared from **7.11** (4.4 mmol, 1.30 g) and TMSCl (9.7 mmol, 1.2 ml) in 20 ml anhydrous methanol according to the general procedure 7.6.2.1 yielding **7.15** as a pale yellow oil (0.87 g, 63 %). ^{1}H-NMR ($CDCl_3$) δ 1.43 (m, 2H, COCH$_2$CH$_2$C<u>H</u>$_2$-), 1.66 (m, 4H, COCH$_2$C<u>H</u>$_2$, C<u>H</u>$_2$CH$_2$OCH$_2$), 2.32 (t, 2H, ^3J = 7.5 Hz, COC<u>H</u>$_2$CH$_2$), 3.50 (t, 2H, ^3J = 6.5 Hz, C<u>H</u>$_2$OCH$_2$C$_6$H$_4$), 3.66 (s, 3H, OCH$_3$), 4.54 (s, 2H, OC<u>H</u>$_2$C$_6$H$_4$), 7.39 (m, 5H, Ar-H), 7.58 (m, 4H, Ar-H). CI-MS (NH_3) *m/z (%)*: 330 (100) [M+NH$_4$]$^+$. Anal. ($C_{20}H_{24}O_3 \cdot 0.4H_2O$) C, H. $C_{20}H_{24}O_3$ (312.4).

Methyl 6-(4-bromophenoxy)hexanoate (7.16): The title compound was prepared from **7.3** (20 mmol, 4.18g), *p*-bromophenol (30 mmol, 5.19 g) and K_2CO_3 (60 mmol, 8.29 g) in 150 ml anhydrous acetone according to 7.6.2.3, Method A. Flash chromatography (PE/EtOAc 90/10 v/v) yielded a colorless solid (4.87 g, 81 %). mp: 30-31 °C; ^{1}H-NMR ($CDCl_3$) δ 1.49 (m, 2H, COCH$_2$CH$_2$C<u>H</u>$_2$), 1.75 (m, 4H, COCH$_2$C<u>H</u>$_2$, C<u>H</u>$_2$CH$_2$OC$_6$H$_4$), 2.35 (t, 2H, ^3J = 7.4 Hz, COC<u>H</u>$_2$CH$_2$), 3.67 (s, 3H, OCH$_3$), 3.92 (t, 2H, ^3J = 6.4 Hz, C<u>H</u>$_2$OC$_6$H$_4$), 6.76 (m, 2H, Ar-H), 7.36 (m, 2H, Ar-H). EI-MS (70 eV) *m/z (%)*: 300 (15), 302 (15) [M$^{+\cdot}$]. Anal. ($C_{13}H_{17}BrO_3$) C, H. $C_{13}H_{17}BrO_3$ (301.18).

7.6.2.4 General Suzuki cross coupling conditions for the synthesis of biaryl intermediates 17-26

To a solution of **7.16** (1 eq) and the corresponding boronic acid (1.5 eq) in a mixture of 15 ml toluene and 15 ml methanol was added 2 N Na_2CO_3 (1 – 2 eq), and the resulting suspension was purged with argon for 5 min followed by addition of Pd(PPh$_3$)$_4$ (1.5 mol- %). The reaction mixture was stirred at 80 °C under argon over 48 h. After cooling the organic solvent was removed under reduced pressure and the residue was taken up in water (20 ml) and extracted tree times with EtOAc. The organic layers were combined, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography.

Methyl 6-[4-(thiophen-3-yl)phenoxy]hexanoate (7.17): The title compound was prepared from **7.16** (3 mmol, 0.90 g), thiophene-2-boronic acid (4.5 mmol, 0.58 g), 2 N Na_2CO_3 (1.5 ml) and Pd(PPh$_3$)$_4$ (0.45 mmol, 52 mg) according to general conditions 7.6.2.1. Flash chromatography (PE/EtOAc 95/5 v/v) gave a white solid

(0.60 g, 72 %). mp: 103 °C; ^{1}H-NMR ($CDCl_3$) δ 1.52 (m, 2H, $COCH_2CH_2C\underline{H}_2$), 1.76 (m, 4H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2OC_6H_4$), 2.36 (t, 2H, ^3J = 7.4 Hz, $COC\underline{H}_2CH_2$), 3.68 (s, 3H, OCH_3), 3.98 (t, 2H, ^3J = 6.4 Hz, $C\underline{H}_2OC_6H_4$), 6.91 (m, 2H, Ar-H), 7.35 (m, 3H, Ar-H), 7.51 (m, 2H, Ar-H). CI-MS (NH_3) *m/z (%)*: 322 (100) $[M+NH_4]^+$. Anal. ($C_{17}H_{20}O_3S$) C, H. $C_{17}H_{20}O_3S$ (304.40).

Methyl 6-[4-(4-methoxyphenyl)phenoxy]hexanoate (7.18): The title compound was prepared from **7.16** (3 mmol, 0.90 g), 4-methoxyphenylboronic acid which was prepared according to known procedures[33] (4.5 mmol, 0.68 g), 2 N Na_2CO_3 (1.5 ml) and $Pd(PPh_3)_4$ (0.45 mmol, 52 mg) according to general conditions 7.6.2.1. Flash chromatography (PE/EtOAc 95/5 to 80/20 v/v) yielded a white solid (0.76 g, 77 %). mp: 116-117 °C; ^{1}H-NMR ($CDCl_3$) δ 1.52 (m, 2H, $COCH_2CH_2C\underline{H}_2$), 1.77 (m, 4H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2OC_6H_4$), 2.35 (t, 2H, ^3J = 7.5 Hz, $COC\underline{H}_2CH_2$), 3.68 (s, 3H, CO_2CH_3), 3.84 (s, 3H, $C_6H_4OC\underline{H}_3$), 3.99 (t, 2H, ^3J = 6.4 Hz, $C\underline{H}_2OC_6H_4$), 6.94 (m, 4H, Ar-H), 7.47 (m, 4H, Ar-H). CI-MS (NH_3) *m/z (%)*: 346 (100) $[M+NH_4]^+$. Anal. ($C_{20}H_{24}O_4$) C, H. $C_{20}H_{24}O_4$ (328.4).

Methyl 6-[4-(2-methoxyphenyl)phenoxy]hexanoate (7.19): The title compound was prepared from **7.16** (3 mmol, 0.90 g), 2-methoxyphenylboronic acid (4.5 mmol, 0.68 g), 2 N Na_2CO_3 (1.5 ml) and $Pd(PPh_3)_4$ (0.45 mmol, 52 mg) according to general conditions 7.6.2.1. Flash chromatography (PE/EtOAc 90/10 v/v) yielded a colorless oil (0.74 g, 75 %). ^{1}H-NMR ($CDCl_3$) δ 1.52 (m, 2H, $COCH_2CH_2C\underline{H}_2$), 1.77 (m, 4H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2OC_6H_4$), 2.36 (t, 2H, ^3J = 7.4 Hz, $COC\underline{H}_2CH_2$), 3.68 (s, 3H, CO_2CH_3), 3.80 (s, 3H, $C_6H_4OC\underline{H}_3$), 3.99 (t, 2H, ^3J = 6.4 Hz, $C\underline{H}_2OC_6H_4$), 6.97 (m, 4H, Ar-H), 7.28 (m, 2H, Ar-H), 7.45 (m, 2H, Ar-H). EI-MS (70 eV) *m/z (%)*: 328 (93) $[M^{+\cdot}]$. Anal. ($C_{20}H_{24}O_4$) C, H. $C_{20}H_{24}O_4$ (328.40).

Methyl 6-[4-(4-methylphenyl)phenoxy]hexanoate (7.20): The title compound was prepared from **7.16** (3 mmol, 0.90 g), 4-methylphenylboronic acid (4.5 mmol, 0.61 g), 2 N Na_2CO_3 (1.5 ml) and $Pd(PPh_3)_4$ (0.45 mmol, 52 mg) according to general conditions 7.6.2.1. Flash chromatography (PE/EtOAc 95/5 to 80/20 v/v) yielded a white solid (0.88 g, 94 %). ^{1}H-NMR ($CDCl_3$) δ 1.52 (m, 2H, $COCH_2CH_2C\underline{H}_2$), 1.76 (m, 4H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2OC_6H_4$), 2.36 (m, 5H, $COC\underline{H}_2CH_2$, $C_6H_4C\underline{H}_3$), 3.67 (s, 3H, OCH_3), 3.99 (t, 2H, ^3J = 6.4 Hz, $C\underline{H}_2OC_6H_4$), 6.94 (m, 2H, Ar-H), 7.22 (m, 2H, Ar-H) 7.47 (m, 4H, Ar-H). CI-MS (NH_3) *m/z (%)*: 330 (100) $[M+NH_4]^+$. Anal. ($C_{20}H_{24}O_3$) C, H. $C_{20}H_{24}O_3$ (312.4).

Methyl 6-[4-(2-methylphenyl)phenoxy]hexanoate (7.21): The title compound was prepared from **7.16** (3 mmol, 0.90 g), 2-methylphenylboronic acid (4.5 mmol, 0.61 g), 2 N Na_2CO_3 (1.5 ml) and $Pd(PPh_3)_4$ (0.45 mmol, 52 mg) according to general conditions 7.6.2.1. Flash chromatography (PE/EtOAc 90/10 v/v) yielded a colorless oil (0.89 g, 95 %). ^{1}H-NMR ($CDCl_3$) δ 1.53 (m, 2H, $COCH_2CH_2C\underline{H}_2$), 1.78 (m, 4H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2OC_6H_4$), 2.28 (s, 3H, $C_6H_4C\underline{H}_3$), 2.37 (t, 2H, ^3J = 7.4 Hz, $COC\underline{H}_2CH_2$), 3.68 (s, 3H, OCH_3), 4.00 (t, 2H, ^3J = 6.4 Hz, $C\underline{H}_2OC_6H_4$), 6.93 (m, 2H, Ar-H), 7.24 (m, 7H, Ar-H). EI-MS (70 eV) *m/z (%)*: 312 (35) [$M^{+\cdot}$]. Anal. ($C_{20}H_{24}O_3 \cdot 0.1CH_2Cl_2$) C, H. $C_{20}H_{24}O_3$ (312.40).

Methyl 6-[4-(naphthalen-1-yl)phenoxy]hexanoate (7.22): The title compound was prepared from **7.16** (3 mmol, 0.90 g) naphthalene-1-boronic acid (4.5 mmol, 0.77 g), 2 N Na_2CO_3 (1.5 ml) and $Pd(PPh_3)_4$ (0.45 mmol, 52 mg) according to general conditions 7.6.2.1. Flash chromatography (PE/EtOAc 90/10 v/v) yielded a pale yellow oil (0.92 g, 88 %). ^{1}H-NMR ($CDCl_3$) δ 1.55 (m, 2H, $COCH_2CH_2C\underline{H}_2$), 1.80 (m, 4H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2OC_6H_4$), 2.38 (t, 2H, ^3J = 7.4 Hz, $COC\underline{H}_2CH_2$), 3.69 (s, 3H, OCH_3), 4.04 (t, 2H, ^3J = 6.4 Hz, $C\underline{H}_2OC_6H_4$), 7.01 (m, 2H, Ar-H), 7.45 (m, 6H, Ar-H), 7.87 (m, 3H, Ar-H). EI-MS (70 eV) *m/z (%)*: 348 (64) [$M^{+\cdot}$]. Anal. ($C_{23}H_{24}O_3$) C, H. $C_{23}H_{24}O_3$ (348.43).

Methyl 6-[4-(4-hydroxyphenyl)phenoxy]hexanoate (7.23): The title compound was prepared from **7.16** (3 mmol, 0.90 g), 4-hydroxyphenylboronic acid (4.5 mmol, 0.62 g), 2 N Na_2CO_3 (1.5 ml) and $Pd(PPh_3)_4$ (0.45 mmol, 52 mg) according to general conditions 7.6.2.1. Flash chromatography (PE/EtOAc 90/10 to 70/30 v/v) yielded a white solid (0.84 g, 89 %). mp: 113 °C; ^{1}H-NMR (DMSO-d_6) δ 1.42 (m, 2H, $COCH_2CH_2C\underline{H}_2$), 1.65 (m, 4H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2OC_6H_4$), 2.33 (t, 2H, ^3J = 7.3 Hz, $COC\underline{H}_2CH_2$), 3.59 (s, 3H, OCH_3), 3.96 (t, 2H, ^3J = 6.4 Hz, $C\underline{H}_2OC_6H_4$), 6.81 (m, 2H, Ar-H), 6.94 (m, 2H, Ar-H), 7.44 (m, 4H, Ar-H), 9.44 (s, 1H, -OH). EI-MS (70 eV) *m/z (%)*: 314 (42) [$M^{+\cdot}$]. Anal. ($C_{19}H_{22}O_4$) C, H. $C_{19}H_{22}O_4$ (314.38).

Methyl 6-[4-(3-hydroxyphenyl)phenoxy]hexanoate (7.24): The title compound was prepared from **7.16** (3 mmol, 0.90 g), 3-hydroxyphenylboronic acid (4.5 mmol, 0.62 g), 2 N Na_2CO_3 (1.5 ml) and $Pd(PPh_3)_4$ (0.45 mmol, 52 mg) according to general conditions 7.6.2.1. Flash chromatography (PE/EtOAc 90/10 v/v) yielded a white solid (0.76 g, 81 %). mp: 110-111 °C; ^{1}H-NMR ($CDCl_3$) δ 1.52 (m, 2H, $COCH_2CH_2C\underline{H}_2$), 1.76 (m, 4H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2OC_6H_4$), 2.37 (t, 2H, ^3J = 7.4 Hz, $COC\underline{H}_2CH_2$), 3.68 (s, 3H, OCH_3), 3.99 (t, 2H, ^3J = 6.4 Hz, $C\underline{H}_2OC_6H_4$), 5.15 (s, 1H, OH), 6.77 (m, 1H,

Ar-H), 6.94 (m, 2H, Ar-H), 7.02 (m, 1H, Ar-H), 7.11 (m, 1H, Ar-H), 7.26 (m, 1H, Ar-H), 7.48 (m, 2H, Ar-H). CI-MS (NH_3) *m/z (%)*: 332 (100) $[M+NH_4]^+$. Anal. ($C_{19}H_{22}O_4$) C, H. $C_{19}H_{22}O_4$ (314.38).

4-[4-(6-Methoxy-6-oxohexan-1-yloxy)phenyl]benzoic acid (7.25): The title compound was prepared from **7.16** (3 mmol, 0.90 g), 4-carboxyphenylboronic acid which was prepared according to known procedures[33] (4.5 mmol, 0.75 g), 2 N Na_2CO_3 (3 ml) and $Pd(PPh_3)_4$ (0.45 mmol, 52 mg) according to general conditions 7.6.2.1. The reaction mixture was acidified (pH <2) with 1 N HCl before extraction. Flash chromatography ($CHCl_3$/MeOH 95/5 to 90/10 v/v) yielded a white solid (0.83 g, 81 %). mp: 155-157 °C; ^{1}H-NMR (DMSO-d_6) δ 1.43 (m, 2H, $COCH_2CH_2C\underline{H}_2$), 1.66 (m, 4H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2OC_6H_4$), 2.34 (t, 2H, ^3J = 7.3 Hz, $COC\underline{H}_2CH_2$), 3.59 (s, 3H, OCH_3), 4.00 (t, 2H, ^3J = 6.4 Hz, $C\underline{H}_2OC_6H_4$), 7.03 (m, 2H, Ar-H), 7.71 (m, 4H, Ar-H), 7.98 (m, 2H, Ar-H), 12.91 (bs, 1H, CO_2H). EI-MS (70 eV) *m/z (%)*: 342 (34) $[M^{+\cdot}]$. Anal. ($C_{20}H_{22}O_5$) C, H. $C_{20}H_{22}O_5$ (342.39).

3-[4-(6-Methoxy-6-oxohexan-1-yloxy)phenyl]benzoic acid (7.26): The title compound was prepared from **7.16** (3 mmol, 0.90 g), 3-arboxyphenylboronic acid (4.5 mmol, 0.75 g), 2 N Na_2CO_3 (3 ml) and $Pd(PPh_3)_4$ (0.45 mmol, 52 mg) according to general conditions 7.6.2.1 with additional acidification of the reaction mixture to pH <2 with 1 N HCl before extraction and was obtained after flash chromatography ($CHCl_3$/MeOH 95/5 to 90/10 v/v) as a white solid (0.95 g, 92 %). ^{1}H-NMR (DMSO-d_6) δ 1.43 (m, 2H, $COCH_2CH_2C\underline{H}_2$), 1.66 (m, 4H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2OC_6H_4$), 2.34 (t, 2H, ^3J = 7.3 Hz, $COC\underline{H}_2CH_2$), 3.59 (s, 3H, OCH_3), 4.00 (t, 2H, ^3J = 6.4 Hz, $C\underline{H}_2OC_6H_4$), 7.03 (m, 2H, Ar-H), 7.58 (m, 3H, Ar-H), 7.87 (m, 2H, Ar-H), 8.13 (m, 1H, Ar-H), 12.97 (bs, 1H, CO_2H). ES-MS (MeOH + NH_4OAc) *m/z (%)*: 341 (100) $[M-H]^-$. Anal. ($C_{20}H_{22}O_5 \cdot 0.2H_2O$) C, H. $C_{20}H_{22}O_5$ (342.39).

7.6.2.5 General procedure for the preparation of the *t*butyl esters 27 and 28 using *tert*-butyl 2,2,2-trichloroacetimidate

To a solution of the appropriate carboxylic acid (1 eq) in anhydrous THF was added TBTA (2 eq, prepared according to known procedures[35]), dissolved in anhydrous cyclohexane (1ml/mmol) and BF_3Et_2O (20 µl/mmol). After stirring at room temperature overnight $NaHCO_3$ was added and the mixture was stirred for 10 min. Insoluble material was filtered off, the solvent was evaporated and the remaining crude product was subjected to flash chromatography.

***tert*-Butyl 4-[4-(6-methoxy-6-oxohexyloxy)phenyl]benzoate (7.27):** The title compound was prepared from **7.25** (2.3 mmol, 0.79 g), TBTA (4.6 mmol, 1.01 g) and BF_3Et_2O (46 µl) in anhydrous THF (10 ml) according to general procedure 7.6.2.5 and was obtained after flash chromatography (PE/EtOAc 95/5 to 90/10 v/v) as a white solid (0.58 g, 64 %). ^{1}H-NMR ($CDCl_3$) δ 1.54 (m, 2H, $COCH_2CH_2C\underline{H}_2$), 1.61 (s, 9H, $C(C\underline{H}_3)_3$), 1.78 (m, 4H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2OC_6H_4$), 2.36 (t, 2H, ^3J = 7.4 Hz, $COC\underline{H}_2CH_2$), 3.68 (s, 3H, OCH_3), 4.01 (t, 2H, ^3J = 6.4 Hz, $C\underline{H}_2OC_6H_4$), 6.97 (m, 2H, Ar-H), 7.57 (m, 4H, Ar-H), 8.02 (m, 2H, Ar-H). EI-MS (70 eV) *m/z (%)*: 398 (66) [$M^{+\cdot}$]. Anal. ($C_{24}H_{30}O_5$) C, H. $C_{24}H_{30}O_5$ (398.49).

***tert*-Butyl 3-[4-(6-methoxy-6-oxohexyloxy)phenyl]benzoate (7.28):** The title compound was prepared from **7.26** (3.4 mmol, 1.16 g), TBTA (4.6 mmol, 1.45 g) and BF_3Et_2O (68 µl) in anhydrous THF (15 ml) according to general procedure 7.6.2.5 and was obtained after flash chromatography (PE/EtOAc 90/10 to 80/20 v/v) as a white solid (0.61 g, 45 %). mp: 66 °C; ^{1}H-NMR ($CDCl_3$) δ 1.54 (m, 11H, $COCH_2CH_2C\underline{H}_2$, $C(C\underline{H}_3)_3$), 1.78 (m, 4H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2OC_6H_4$), 2.37 (t, 2H, ^3J = 7.4 Hz, $COC\underline{H}_2CH_2$), 3.68 (s, 3H, OCH_3), 4.01 (t, 2H, ^3J = 6.4 Hz, $C\underline{H}_2OC_6H_4$), 6.97 (m, 2H, Ar-H), 7.45 (m, 1H, Ar-H), 7.54 (m, 2H, Ar-H), 7.70 (m, 1H, Ar-H), 7.92 (m, 1H, Ar-H), 8.18 (m, 1H, Ar-H). EI-MS (70 eV) *m/z (%)*: 398 (42) [$M^{+\cdot}$]. Anal. ($C_{24}H_{30}O_5$) C, H. $C_{24}H_{30}O_5$ (398.49).

7.6.2.6 General procedure for the preparation of the carboxylic acids 29-32, 34a and 39-41 by cleavage of the corresponding methyl esters:

The corresponding methyl ester (1 eq) was dissolved in THF, MeOH and water. LiOH (1.5 eq) was added and the mixture was stirred overnight at room temperature. After diluting with water, acidification with 1 N HCl to pH <2 and extraction three times with EtOAc, the combined organic phases were washed with brine, dried over $MgSO_4$, filtered and evaporated under reduced pressure. The resulting free carboxylic acids were used in the next step without further purification.

6-[4-(4-Methoxyphenyl)phenoxy]hexanoic acid[83] (7.29): The title compound was prepared from **7.19** (1.6 mmol, 0.53 g), LiOH (2.4 mmol, 57 mg) THF (3 ml), MeOH (0.2 ml) and water (1ml) according to general procedure 7.6.2.6 and was obtained as a white solid (0.46 g, 91 %). mp: 151 °C; ^{1}H-NMR (DMSO-d_6) δ 1.42 (m, 2H, $COCH_2CH_2C\underline{H}_2$), 1.56 (m, 2H, $COCH_2C\underline{H}_2$), 1.72 (m, 2H, $C\underline{H}_2CH_2OC_6H_4$), 2.24 (t, 2H, ^3J = 7.2 Hz, $COC\underline{H}_2CH_2$), 3.78 (s, 3H, $C_6H_4OC\underline{H}_3$), 3.97 (t, 2H, ^3J = 6.4 Hz, ,

C$\underline{H}_2$OC$_6$H$_4$), 6.97 (m, 4H, Ar-H), 7.52 (m, 4H, Ar-H), 12.03 (s, 1H, CO_2H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 332 (100) $[M+NH_4]^+$. Anal. ($C_{19}H_{22}O_4$) C, H. $C_{19}H_{22}O_4$ (314.38).

6-[4-(Naphthalen-1-yl)phenoxy]hexanoic acid (7.30): The title compound was prepared from **7.22** (0.8 mmol, 0.28 g), LiOH (1.2 mmol, 29 mg) THF (3 ml), MeOH (0.2 ml) and water (1ml) according to general procedure 7.6.2.6 and was obtained as a white solid (0.25 g, 93 %). mp: 123 °C; ^{1}H-NMR (DMSO-d_6) δ 1.41 – 1.81 (m, 6H, COCH$_2$(C$\underline{H}_2$)$_2$), 2.26 (t, 2H, ^3J = 7.2 Hz, COC$\underline{H}_2$CH$_2$), 4.03 (t, 2H, ^3J = 6.4 Hz, C$\underline{H}_2$OC$_6$H$_4$), 7.07 (m, 2H, Ar-H), 7.37 – 7.58 (m, 6H, Ar-H), 7.91 (m, 3H, Ar-H). EI-MS (70 eV) *m/z (%)*: 334 (61) [M^+]. Anal. ($C_{22}H_{22}O_3 \cdot 0.2H_2O$) C, H. $C_{22}H_{22}O_3$ (334.41).

6-[4-(4-*tert*-Butoxycarbonylphenyl)phenoxy]hexanoic acid (7.31): The title compound was prepared from **7.27** (1.2 mmol, 0.48 g), LiOH (1.8 mmol, 43 mg) THF (3 ml), MeOH (0.2 ml) and water (1ml) according to general procedure 7.6.2.6 and was obtained as a pale yellow solid (0.40 g, 95 %). mp: 83 °C; ^{1}H-NMR ($CDCl_3$) δ 1.44 (m, 2H, COCH$_2$CH$_2$C$\underline{H}_2$), 1.57 (m, 11H, C(C$\underline{H}_3$)$_3$, COCH$_2$C$\underline{H}_2$), 1.74 (m, 2H, C$\underline{H}_2$CH$_2$OC$_6$H$_4$), 2.24 (t, 2H, ^3J = 7.2 Hz, COC$\underline{H}_2$CH$_2$), 4.01 (t, 2H, ^3J = 6.4 Hz, C$\underline{H}_2$OC$_6$H$_4$), 7.04 (m, 2H, Ar-H), 7.66 (m, 2H, Ar-H), 7.74 (m, 2H, Ar-H), 7.94 (m, 2H, Ar-H), 12.01 (s, 1H, CO_2H). EI-MS (70 eV) *m/z (%)*: 384 (6) [$M^{+\cdot}$]. Anal. ($C_{23}H_{28}O_5$) C, H. $C_{23}H_{28}O_5$ (384.47).

6-[4-(3-*tert*-Butoxycarbonylphenyl)phenoxy]hexanoic acid (7.32): The title compound was prepared from **7.28** (1.4 mmol, 0.56 g), LiOH (2.1 mmol, 57 mg) THF (3 ml), MeOH (0.2 ml) and water (1ml) according to general procedure 7.6.2.6 and was obtained as a white solid (0.52 g, 97 %). mp: 91 °C; ^{1}H-NMR (DMSO-d_6) δ 1.42 (m, 2H, COCH$_2$CH$_2$C$\underline{H}_2$), 1.57 (m, 11H, C(C$\underline{H}_3$)$_3$, COCH$_2$C$\underline{H}_2$), 1.73 (m, 2H, C$\underline{H}_2$CH$_2$OC$_6$H$_4$), 2.24 (t, 2H, ^3J = 7.2 Hz, COC$\underline{H}_2$CH$_2$), 4.00 (t, 2H, ^3J = 6.4 Hz, C$\underline{H}_2$OC$_6$H$_4$), 7.03 (m, 2H, Ar-H), 7.57 (m, 3H, Ar-H), 7.85 (m, 2H, Ar-H), 8.07 (m, 1H, Ar-H), 12.03 (bs, 1H, CO_2H). EI-MS (70 eV) *m/z (%)*: 384 (31) [$M^{+\cdot}$]. Anal. ($C_{23}H_{28}O_5$) C, H. $C_{23}H_{28}O_5$ (384.47).

Methyl 11-phenoxyundecanoate[84] (7.33): The title compound was prepared from phenol (22 mmol, 2.07 g), KOH (44 mmol, 2.47 g) and 11-bromoundecanoic acid (20 mmol, 5.30 g) in 150 ml EtOH according to general procedure 7.6.2.3, Method C. The resulting raw product was then esterified according to the general procedure for the synthesis of methyl esters using TMSCl (44 mmol, 5.6 ml) in anhydrous methanol (50 ml) and was obtained after flash chromatography (PE/EtOAc 90/10 v/v) as a

colorless semisolid substance (2.94 g, 50 %). ^{1}H-NMR ($CDCl_3$) δ 1.24 – 1.51 (m, 12H, $COCH_2CH_2(C\underline{H}_2)_6$), 1.61 (m, 2H, $COCH_2C\underline{H}_2$), 1.77 (m, 2H, $C\underline{H}_2CH_2OPh$), 2.30 (t, 2H, ^3J = 7.5 Hz, $COC\underline{H}_2CH_2$), 3.66 (s, 3H, OCH_3), 3.95 (t, 2H, ^3J = 6.6 Hz, $C\underline{H}_2OPh$), 6.90 (m, 3H, Ph-H), 7.27 (m, 2H, Ph-H). PI-EIMS (70 eV) *m/z (%)*: 292 (38) [$M^{+\cdot}$]. Anal. ($C_{18}H_{28}O_3 \cdot 0.5H_2O$) C, H. $C_{18}H_{28}O_3$ (292.41).

Methyl 11-(4-phenylphenoxy)undecanoate[85] (7.34): The title compound was prepared from 4-phenylphenol (22 mmol, 3.74 g), KOH (44 mmol, 2.47 g) and 11-bromoundecanoic acid (20 mmol, 5.30 g) in 150 ml EtOH according to Method C. The resulting raw product was then esterified according to general procedure 7.6.2.1 using TMSCl (44 mmol, 5.6 ml) in anhydrous methanol (50 ml) and was obtained after flash chromatography (PE/EtOAc 90/10 v/v) as a white solid (2.82 g, 38 %). mp: 78-80 °C; ^{1}H-NMR ($CDCl_3$) δ 1.25 - 1,49 (m, 12H, $COCH_2CH_2(C\underline{H}_2)_6$), 1.62 (m, 2H, $COCH_2C\underline{H}_2$), 1.79 (m, 2H, $C\underline{H}_2CH_2OC_6H_4$), 2.30 (t, 2H, ^3J = 7.5 Hz, $COC\underline{H}_2CH_2$), 3.66 (s, 3H, OCH_3), 3.98 (t, 2H, ^3J = 6.5 Hz, $C\underline{H}_2OC_6H_4$), 6.96 (m, 2H, Ar-H), 7.29 (m, 1H, Ph-H), 7.40 (m, 2H, Ar-H), 7.53 (m, 4H, Ar-H). EI-MS (70 eV) *m/z (%)*: 368 (8) [$M^{+\cdot}$]. Anal. ($C_{24}H_{32}O_3$) C, H. $C_{24}H_{32}O_3$ (368.51).

11-(4-Phenylphenoxy)undecanoic acid[86] (7.34a): The title compound was prepared from **7.34** (2.8 mmol, 1.03 g), LiOH (4.2 mmol, 0.10 g) THF (20 ml), MeOH (5 ml) and water (5ml) according to general procedure 7.6.2.6 and was obtained as a white solid (0.87 g, 88 %). ^{1}H-NMR (acetone-d_6) δ 1.41 (m, 14H, $COCH_2(C\underline{H}_2)_7$), 1.80 (m, 2H, $C\underline{H}_2CH_2OC_6H_4$), 2.28 (t, 2H, ^3J = 7.4 Hz, $COC\underline{H}_2CH_2$), 4.05 (t, 2H, ^3J = 6.5 Hz, $C\underline{H}_2OC_6H_4$), 7.02 (m, 2H, Ar-H), 7.29 (m, 1H, Ar-H), 7.42 (m, 2H, Ar-H), 7.59 (m, 4H, Ar-H),10.45 (bs, 1H, CO_2H). EI-MS (70 eV) *m/z (%)*: 354 (34) [$M^{+\cdot}$]. Anal. ($C_{23}H_{30}O_3 \cdot 0.5H_2O$) C, H. $C_{23}H_{30}O_3$ (354.48).

Dodecanedioic acid mono-*tert*-butyl ester (7.35)[37]: To an ice-cold solution of dodecanedioic acid (32.5 mmol, 7.48g), *tert*-butyl alcohol (32.5 mmol, 32 ml) and a catalytic amount of DMAP in anhydrous THF (40 ml), a solution of DCC (39 mmol, 8.04 g) in anhydrous THF (10 ml) was slowly added. Subsequently, the solution was warmed to room temperature and stirred overnight. The reaction mixture was filtered, concentrated under reduced pressure und subjected to flash chromatography (PE/EtOAc 80/20 to 50/50 v/v). The title compound was obtained as a colorless oil (3.02 g, 32 %). ^{1}H-NMR ($CDCl_3$) δ 1.26 (m, 12H, $COCH_2CH_2(C\underline{H}_2)_6$), 1.43 (s, 9H, $C(C\underline{H}_3)_3$), 1.58 (m, 4H, $COCH_2C\underline{H}_2$), 2.19 (t, 2H, ^3J = 7.5 Hz, $C\underline{H}_2CO_2H$), 2.33 (t, 2H,

^{3}J = 7.5 Hz, C$\underline{H}_2$CO$_2{}^t$Bu), 10.50 (bs, 1H, CO_2H). CI-MS (NH_3) *m/z (%)*: 304 (56) $[M+NH_4]^+$. $C_{16}H_{30}O_4$ (286,41).

Methyl 2-(decan-1-yloxy)benzoate[87] (7.36): The title compound was prepared from 1-bromodecane (10 mmol, 2.1 ml), methyl 2-hydroxybenzoate (15 mmol, 2.28 g) and K_2CO_3 (30 mmol, 4.15 g) in 75 ml anhydrous acetone according to general procedure 7.6.2.3, Method A and was obtained after flash chromatography (PE/EtOAc 90/10 v/v) as a colorless oil (2.50 g, 85 %). ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^{3}J = 6.7 Hz, $CH_2C\underline{H}_3$), 1.27 – 1.53 (m, 12H, $(C\underline{H}_2)_6CH_3$), 1.82 (m, 2H, $OCH_2C\underline{H}_2CH_2$), 3.88 (s, 3H, $OC\underline{H}_3$), 4.02 (t, 2H, ^{3}J = 6.5 Hz, $COC\underline{H}_2CH_2$), 6.96 (m, 2H, Ar-H), 7.42 (m, 1H, Ar-H), 7.77 (dd, 1H, ^{4}J =1.8Hz, ^{3}J = 7.9 Hz, Ar-H). EI-MS (70 eV) *m/z (%)*: 292 (4) [$M^{+\cdot}$]. Anal. ($C_{18}H_{28}O_3 \cdot 0.2Cl_2CH_2$) C, H. $C_{18}H_{28}O_3$ (292.41).

Methyl 3-(decan-1-yloxy)benzoate[88] (7.37): The title compound was prepared from 1-bromodecane (10 mmol, 2.1 ml), methyl 3-hydroxybenzoate (15 mmol, 2.28 g) and K_2CO_3 (30 mmol, 4.15 g) in 75 ml anhydrous acetone according to general procedure 7.6.2.3, Method A and was obtained after flash chromatography (PE/EtOAc 90/10 v/v) as a white solid (2.67 g, 91 %). mp: 33 °C; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^{3}J = 6.7 Hz, $CH_2C\underline{H}_3$), 1.26 – 1.50 (m, 12H, $(C\underline{H}_2)_6CH_3$), 1.79 (m, 2H, $OCH_2C\underline{H}_2CH_2$), 3.91 (s, 3H, $OC\underline{H}_3$), 3.99 (t, 2H, ^{3}J = 6.5 Hz, $COC\underline{H}_2CH_2$), 7.09 (ddd, 1H, ^{4}J = 1.0 Hz, ^{4}J = 2.6 Hz, ^{3}J = 8.3 Hz, Ar-H), 7.32 (dd, 1H, ^{3}J = 7.9 Hz, ^{3}J = 7.9 Hz, Ar-H), 7.55 (dd, 1H, ^{4}J = 1.5 Hz, ^{4}J = 2.5 Hz, 1H, Ar-H), 7.61 (m, 1H, Ar-H). EI-MS (70 eV) *m/z (%)*: 292 (13) [$M^{+\cdot}$]. Anal. ($C_{18}H_{28}O_3$) C, H. $C_{18}H_{28}O_3$ (292.41).

Methyl 4-(decan-1-yloxy)benzoate[89] (7.38): The title compound was prepared from 1-bromodecane (10 mmol, 2.1 ml), methyl 4-hydroxybenzoate (15 mmol, 2.28 g) and K_2CO_3 (30 mmol, 4.15 g) in 75 ml anhydrous acetone according to general procedure 7.6.2.3, Method A and was obtained after flash chromatography (PE/EtOAc 90/10 v/v) as a white solid (2.70 g, 92 %). mp: 47 °C (ref.[90]: 47-48 °C); ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^{3}J = 6.7 Hz, $CH_2C\underline{H}_3$), 1.27 – 1.50 (m, 12H, $(C\underline{H}_2)_6CH_3$), 1.79 (m, 2H, $OCH_2C\underline{H}_2CH_2$), 3.88 (s, 3H, $OC\underline{H}_3$), 3.99 (t, 2H, ^{3}J = 6.6 Hz, $COC\underline{H}_2CH_2$), 6.90 (m, 2H, Ar-H), 7.97 (m, 2H, Ar-H). EI-MS (70 eV) *m/z (%)*: 292 (22) [$M^{+\cdot}$]. Anal. ($C_{18}H_{28}O_3$) C, H. $C_{18}H_{28}O_3$ (292.41).

2-(Decan-1-yloxy)benzoic acid[87] (7.39): The title compound was prepared from **7.36** (4 mmol, 1.17 g), LiOH (6 mmol, 0.14 g) THF (10 ml), MeOH (2 ml) and water (5 ml) according to general procedure 7.6.2.6 and was obtained as a white semisolid substance (0.70 g, 62 %). ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 2H, ^{3}J = 6.7 Hz, $CH_2C\underline{H}_3$), 1.27

– 1.54 (m, 12H, ($C\underline{H}_2$)$_6$$CH_3$), 1.92 (m, 2H, $OCH_2C\underline{H}_2CH_2$), 4.25 (t, 2H, ^3J = 6.6Hz, $COC\underline{H}_2CH_2$), 7.05 (dd, 1H, ^4J = 0.5 Hz, ^3J = 8.4 Hz, Ar-H), 7.13 (ddd, 1H, ^4J = 1.0 Hz, ^3J = 7.8 Hz, ^3J = 7.7 Hz, Ar-H), 7.55 (ddd, 1H, ^4J = 1.9 Hz, ^3J = 7.4 Hz, ^3J = 8.4 Hz, Ar-H), 8.20 (dd, 1H, ^4J = 1.8 Hz, ^3J = 7.8 Hz, Ar-H), 10.96 (bs, 1H, CO_2H). CI-MS (NH_3) *m/z (%)*: 296 (100) $[M+NH_4]^+$. Anal. ($C_{17}H_{26}O_3$) C, H. $C_{17}H_{26}O_3$ (278.39).

3-(Decan-1-yloxy)benzoic acid[88] (7.40): The title compound was prepared from **7.37** (4 mmol, 1.17 g), LiOH (6 mmol, 0.14 g) THF (10 ml), MeOH (2 ml) and water (5 ml) according to general procedure 7.6.2.6 and was obtained as a white solid (1.06 g, 95 %). mp: 80 °C; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.7 Hz, $CH_2C\underline{H}_3$), 1.28 – 1.52 (m, 12H, ($C\underline{H}_2$)$_6$$CH_3$), 1.80 (m, 2H, $OCH_2C\underline{H}_2CH_2$), 4.01 (t, 2H, ^3J = 6.5 Hz, $COC\underline{H}_2CH_2$), 7.15 (ddd, 1H, ^4J = 1.0 Hz, ^4J = 2.6 Hz, ^3J = 8.3 Hz, Ar-H), 7.37 (dd, 1H, ^3J = 7.9 Hz, ^3J = 7.9 Hz, Ar-H), 7.62 (dd, 1H, ^4J = 1.5 Hz, ^4J = 2.5 Hz, Ar-H), 7.70 (m, 1H, Ar-H), 10.17 (bs, 1H, CO_2H). EI-MS (70 eV) *m/z (%)*: 278 (20) $[M^{+\cdot}]$. Anal. ($C_{17}H_{26}O_3$) C, H. $C_{17}H_{26}O_3$ (278.39).

4-(Decan-1-yloxy)benzoic acid[89] (7.41): The title compound was prepared from **7.38** (4 mmol, 1.17 g), LiOH (6 mmol, 0.14 g) THF (10 ml), MeOH (2 ml) and water (5 ml) according to general procedure 7.6.2.6 and was obtained as a white solid (1.03 g, 92 %). mp: 88 °C (ref.[90]: 96-97 °C); ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.7 Hz, $CH_2C\underline{H}_3$), 1.27 – 1.51 (m, 12H, ($C\underline{H}_2$)$_6$$CH_3$), 1.81 (m, 2H, $OCH_2C\underline{H}_2CH_2$), 4.02 (t, 2H, ^3J = 6.5 Hz, $COC\underline{H}_2CH_2$), 6.93 (m, 2H, Ar-H), 8.06 (m, 2H, Ar-H), 11.00 (s, 1H, CO_2H). EI-MS (70 eV) *m/z (%)*: 278 (36) $[M^{+\cdot}]$. Anal. ($C_{17}H_{26}O_3$) calcd. C: 73.34, H: 9.41, found C: 73.22, H: 10.00. $C_{17}H_{26}O_3$ (278.39).

7.6.2.7 General procedure for the synthesis of the α-branched carboxylic acids 42-47

To a solution of diethyl malonate (1 eq) in anhydrous THF was added NaH (60 % suspension, 1 eq), and the mixture was stirred for 10 min under an atmosphere of argon. The corresponding first alkyl halide (1 eq) was added followed by stirring at reflux overnight. After cooling another portion of NaH (60 % suspension, 1 eq) was added and the suspension was stirred for 1 h followed by addition of the second alkyl halide (1 eq) and stirring at reflux overnight again. After cooling, solids were removed by filtration and the solvent was evaporated. The crude product was suspended in EtOH (15 ml) and water (15 ml), KOH (10 eq) was added and the mixture was stirred at reflux overnight. The EtOH was removed under reduced pressure and the

remaining aqueous solution was diluted, washed with n-hexane, acidified with 2 N HCl to pH <2 and extracted three times with diethyl ether. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and evaporated. The crude dicarboxylic acid was decarboxylated at 170 – 200 °C in a sand bath for 5 h. The α-branched carboxylic acid was purified by flash chromatography.

2-Propyldodecanoic acid[91] (7.42): The title compound was prepared according to general procedure 7.6.2.7 by alkylating diethyl malonate (10 mmol, 1.5 ml) using NaH (60 % suspension, 10 mmol, 0.40 g) and 1-bromodecane (10 mmol, 2.1 ml) in the first step and using the same amount of NaH together with 1-iodopropane (10 mmol, 1.0 ml) in the second step. The product was obtained after flash chromatography (PE/EtOAc 90/10 v/v) as a pale yellow oil (0.88 g, 36 %). ^{1}H-NMR ($CDCl_3$) δ 0.90 (m, 6H, CH_3), 1.23 – 1.68 (m, 22H, ($C\underline{H}_2$)$_9CH_3$, ($C\underline{H}_2$)$_2CH_3$), 2.36 (tt, 1H, ^3J = 5.2 Hz, ^3J = 8.8 Hz, COCH), 11.23 (bs, CO_2H). CI-MS (NH_3) *m/z (%)*: 260 (100) $[M+NH_4]^+$. Anal. ($C_{15}H_{30}O_2$) calcd. C: 74.32, H: 12.43, found C: 74.17, H: 13.12. $C_{15}H_{30}O_2$ (242.40).

2-(Hexan-1-yl)dodecanoic acid[92] (7.43): The title compound was prepared according to general procedure 7.6.2.7 by alkylating diethyl malonate (10 mmol, 1.5 ml) using NaH (60 % suspension, 10 mmol, 0.40 g) and 1-bromodecane (10 mmol, 2.1 ml) in the first step and using the same amount of NaH together with 1-iodohexane (10 mmol, 1.5 ml) in the second step. The product was obtained after flash chromatography (PE/EtOAc 90/10 v/v) as a white foam (0.90 g, 32 %). ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 6H, ^3J =6.6Hz, $CH_2C\underline{H}_3$), 1.26 – 1.69 (m, 28H, ($C\underline{H}_2$)$_9CH_3$, ($C\underline{H}_2$)$_5CH_3$), 2.34 (tt, 1H, ^3J =5.4Hz, ^3J =8.6Hz, COCH), 11.30 (bs, 1H, CO_2H). EI-MS (70 eV) *m/z (%)*: 284 (66) [$M^{+\cdot}$]. Anal. ($C_{18}H_{36}O_2$) C, H. $C_{18}H_{36}O_2$ (284.48).

2-(Decan-1-yl)dodecanoic acid[93] (7.44): The title compound was prepared according to general procedure 7.6.2.7 by alkylating diethyl malonate (10 mmol, 1.5 ml) using NaH (60 % suspension, 10 mmol, 0.40 g) and 1-bromodecane (10 mmol, 2.1 ml) in the first step and using the same amount of NaH and 1-bromodecane in the second step. The product was obtained after flash chromatography (PE/EtOAc 90/10 v/v) as a white solid (1.33 g, 39 %). mp: 52 °C; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 6H, ^3J = 6.7 Hz, $CH_2C\underline{H}_3$), 1.27 – 1.68 (m, 36H, ($C\underline{H}_2$)$_9CH_3$), 2.34 (tt, 1H, ^3J = 5.4 Hz, ^3J = 8.6 Hz, COCH), 9.48 (s, 1H, CO_2H). CI-MS (NH_3) *m/z (%)*: 359 (100) $[M+NH_4]^+$. Anal. ($C_{22}H_{44}O_2$) C, H. $C_{22}H_{44}O_2$ (340.58).

2-(2-Phenethyl)dodecanoic acid (7.45): The title compound was prepared according to general procedure 7.6.2.7 by alkylating diethyl malonate (10 mmol, 1.5 ml) using NaH (60 % suspension, 10 mmol, 0.40 g) and 1-bromodecane (10 mmol, 2.1 ml) in the first step and using the same amount of NaH together with (2-bromoethyl)benzene (10 mmol, 1.4 ml) in the second step. The product was obtained after flash chromatography (PE/EtOAc 90/10 v/v) as a pale yellow oil (0.74 g, 24 %). ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.7 Hz, $CH_2C\underline{H}_3$), 1.62 (m, 20H, CH_2), 2.42 (m, 1H, COCH), 2.65 (m, 2H, $CH_2C\underline{H}_2Ph$), 7.23 (m, 10H, Ph-H), 11.08 (bs, 1H, CO_2H). EI-MS (70 eV) *m/z (%)*: 304 (16) [$M^{+\cdot}$]. $C_{20}H_{32}O_2$ (304.47).

2-(4-Methoxybenzyl)dodecanoic acid (7.46): The title compound was prepared according to general procedure 7.6.2.7 by alkylating diethyl malonate (10 mmol, 1.5 ml) using NaH (60 % suspension, 10 mmol, 0.40 g) and 1-bromodecane (10 mmol, 2.1 ml) in the first step and using the same amount of NaH together with 1-(chloromethyl)-4-methoxybenzene (10 mmol, 1.4 ml) in the second step. The product was obtained after flash chromatography (PE/EtOAc 90/10 v/v) as a pale yellow oil (0.53 g, 16 %). ^{1}H-NMR ($CDCl_3$) δ 0.89 (t, 3H, ^3J = 6.7 Hz, $CH_2C\underline{H}_3$), 1.25 – 1.68 (m, 16H, $(C\underline{H}_2)_8CH_3$), 2.65 (m, 2H, OCH, $CHC\underline{H}_2C_6H_4$), 2.91 (dd, 1H, ^3J = 7.7 Hz, ^2J = 13.5 Hz, $CHC\underline{H}_2C_6H_4$), 3.79 (s, 3H, OCH_3), 6.82 (m, 2H, Ar-H), 7.10 (m, 2H, Ar-H), 10.45 (bs, 1H, CO_2H). EI-MS (70 eV) *m/z (%)*: 320 (8) [$M^{+\cdot}$]. Anal. ($C_{20}H_{32}O_3 \cdot 0.01C_6H_4CH_3$) C, H. $C_{20}H_{32}O_3$ (320.47).

2-(Hexan-1-yl)octadecanoic acid[94] (7.47): The title compound was prepared according to general procedure 7.6.2.7 by alkylating diethyl malonate (10 mmol, 1.5 ml) using NaH (60 % suspension, 10 mmol, 0.40 g) and 1-bromohexadecane (10 mmol, 3.05 ml) in the first step and using the same amount of NaH together with 1-iodohexane (10 mmol, 1.5 ml) in the second step. The product was obtained after flash chromatography (PE/EtOAc 90/10 v/v) as a pale yellow oil (0.62 g, 17 %). ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 6H, ^3J = 6.6 Hz, $CH_2C\underline{H}_3$), 1.49 (m, 40H, $(C\underline{H}_2)_{15}CH_3$, $(C\underline{H}_2)_5CH_3$), 2.34 (tt, 1H, ^3J = 5.4 Hz, ^3J = 8.6Hz, COCH). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 368 (100) [M-H]$^-$. Anal. ($C_{24}H_{48}O_2 \cdot 0.3H_2O$) C, H. $C_{24}H_{48}O_2$ (368.64).

6-*O*-[6-(Phenylthio)hexanoyl]ascorbic acid (7.48): The title compound was prepared from ascorbic acid (2 mmol, 0.35 g), **7.4** (4 eq, 8 mmol, 1.79 g) and Novozyme 435® (100 mg) in 15 ml *tert*-amyl alcohol according to general procedure 7.6.2.2, yielding **7.48** as a white solid (0.40 g, 54 %). mp: 108-110 °C; ^{1}H-NMR

(DMSO-d_6) δ 1.40 (m, 2H, $COCH_2CH_2C\underline{H}_2$), 1.56 (m, 4H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2SPh$), 2.32 (t, 2H, ^3J = 7.3 Hz, $COCH_2$), 2.95 (t, 2H, ^3J = 7.2 Hz, $C\underline{H}_2SPh$), 4.02 (m, 3H, -CH_2O-, C<u>H</u>OH) ppm 4.68 (d, 1H, ^3J = 1.7 Hz, H-5), 5.31 (s, 1H, CHO<u>H</u>), 7.18 (m, 1H, Ph-H), 7.31 (m, 4H, Ph-H), 8.40 (bs, 1H, OH), 11.11 (bs, 1H, OH). ^{13}C-NMR (DMSO-d_6) δ 23.83 (-, CH_2), 27.47 (-, CH_2), 28.13 (-, CH_2), 31.67 (-, CH_2), 33.18 (-, $CO\underline{C}H_2CH_2$), 64.39 (-, CH_2O), 65.35 (+, CHO), 74.92 (+, CH), 118.08 (quat, C-2), 125.37 (+, Ph-C), 127.81 (+, Ph-C), 128.91 (+, Ph-C), 136.32 (quat, Ph-C), 152.13 (quat, C-3), 170.22 (quat, lactone CO), 172.55 (quat, $\underline{C}O_2CH_2$). ES-MS (MeCN + TFA) *m/z (%)*: 400 (100) $[M+NH_4]^+$. Anal. ($C_{18}H_{22}O_7S\cdot0.5H_2O$) C, H. $C_{18}H_{22}O_7S$ (382.43).

6-*O*-[6-(Benzylthio)hexanoyl]ascorbic acid (7.49): The title compound was prepared from ascorbic acid (3.3 mmol, 0.58 g), **7.5** (4 eq, 13.2 mmol, 3.33 g) and Novozyme 435® (165 mg) in 15 ml *tert*-amyl alcohol according to general procedure 7.6.2.2, yielding **7.49** as a white amorphous solid (0.53 g, 41 %). ^{1}H-NMR (DMSO-d_6) δ 1.31 (m, 2H, $COCH_2CH_2C\underline{H}_2$), 1.50 (m, 4H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2SBn$), 2.31 (t, 2H, ^3J = 7.4 Hz, $COCH_2$), 2.37 (t, 2H, ^3J = 7.3 Hz, $C\underline{H}_2SCH_2Ph$), 3.71 (s, 2H, $SC\underline{H}_2Ph$), 4.03 (m, 3H, CH_2O, C<u>H</u>OH), 4.68 (d, 1H, ^3J = 1.6 Hz, H-5), 5.32 (d, 1H, ^3J = 5.8 Hz, CHO<u>H</u>), 7.26 (m, 5H, Ph-H), 8.42 (s, 1H, OH), 11.13 (s, 1H, OH). ^{13}C-NMR (DMSO-d_6) δ 23.87 (-, CH_2), 27.57 (-, CH_2), 28.26 (-, CH_2), 30.36 (-, CH_2), 33.19 (-,$CO\underline{C}H_2CH_2$), 34.94 (-, $S\underline{C}H_2Ph$), 64.40 (-, CH_2O), 65.40 (+, CHO), 74.93 (+, CH), 118.09 (quat, C-2), 126.61 (+, Ph-C), 128.22 (+, Ph-C), 128.70 (+, Ph-C), 138.69 (quat, Ph-C), 152.12 (quat, C-3), 170.27 (quat, lactone CO), 172.57 (quat, $\underline{C}O_2CH_2$). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 395 (100) $[M-H]^-$. Anal. ($C_{19}H_{24}O_7S\cdot0.7H_2O$) C, H. $C_{19}H_{24}O_7S$ (396.45).

6-*O*-(6-Phenoxyhexanoyl)ascorbic acid (7.50): The title compound was prepared from ascorbic acid (3.3 mmol, 0.58 g), **7.6** (4 eq, 13.2 mmol, 2.75 g) and Novozyme 435® (165 mg) in 15 ml *tert*-amyl alcohol according to general procedure 7.6.2.2, yielding **7.50** as a white solid (0.55 g, 45 %). ^{1}H-NMR (DMSO-d_6) δ 1.43 (m, 2H, $COCH_2CH_2C\underline{H}_2$), 1.60 (m, 2H, $CH_2C\underline{H}_2CH_2$), 1.71 (m, 2H, $CH_2C\underline{H}_2CH_2$), 2.37 (t, 2H, ^3J = 7.3 Hz, $COCH_2$),4.03 (m, 5H, CH_2O, C<u>H</u>OH, $C\underline{H}_2OPh$), 4.69 (d, 1H, ^3J = 1.7 Hz, H-5), 5.32 (d, 1H, ^3J = 6.0 Hz, CHO<u>H</u>), 6.91 (m, 3H, Ph-H), 7.27 (m, 2H, Ph-H), 8.40 (s, 1H, OH), 11.12 (s, 1H, OH). ^{13}C-NMR (DMSO-d_6) δ 24.08 (-, CH_2), 25.01 (-, CH_2), 28.31 (-, CH_2), 33.27 (-, $CO\underline{C}H_2CH_2$), 64.42 (-, CH_2O), 65.43 (+, CHO), 67.02 (-, $\underline{C}H_2OPh$), 74.95 (+, CH), 114.30 (+, Ph-C), 118.12 (quat, C-2), 120.26 (+, Ph-C),

129.36 (+, Ph-C), 152.10 (quat, C-3), 158.53 (quat, Ph-C), 170.26 (quat, lactone CO), 172.61 (quat, $\underline{C}O_2CH_2$). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 365 (100) $[M-H]^-$. Anal. ($C_{18}H_{22}O_8 \cdot 0.3H_2O$) C, H. $C_{18}H_{22}O_8$ (366.36).

6-*O*-[6-(4-Ethylphenoxy)hexanoyl]ascorbic acid (7.51): The title compound was prepared from ascorbic acid (3.3 mmol, 0.58 g), **7.7** (4 eq, 13.2 mmol, 3.30 g) and Novozyme 435® (165 mg) in 15 ml *tert*-amyl alcohol according to general procedure 7.6.2.2, yielding **7.51** as a white solid (0.65 g, 50 %). mp: 109-111 °C; ^{1}H-NMR (DMSO-d_6) δ 1.13 (t, 3H, ^3J =7.6Hz, $CH_2C\underline{H}_3$), 1.41 (m, 2H, $COCH_2CH_2C\underline{H}_2$), 1.65 (m, 4H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2OC_6H_4$), 2.36 (t, 2H, ^3J = 7.3 Hz, $COCH_2$), 2.52 (q, 2H, ^3J = 7.5 Hz, $C\underline{H}_2CH_3$), 3.90 (t, 2H, ^3J = 6.4 Hz, $C\underline{H}_2OC_6H_4$), 4.03 (m, 3H, CH_2O, $C\underline{H}OH$), 4.68 (d, 1H, ^3J = 1.5 Hz, H-5), 5.33 (s, 1H, $CHO\underline{H}$), 6.81 (d, 2H, ^3J = 8.5 Hz, Ar-H), 7.09 (d, 2H, ^3J = 8.5 Hz, Ar-H), 8.42 (bs, 1H, OH), 11.11 (bs, 1H, OH). ^{13}C-NMR (DMSO-d_6) δ 15.87 (+, CH_3), 24.08 (-, CH_2), 25.02 (-, CH_2), 27.21 (-, CH_2), 28.34 (-, CH_2), 33.27 (-, $CO\underline{C}H_2CH_2$), 64.42 (-, CH_2O), 65.42 (+, CHO), 67.10 (-, $\underline{C}H_2OC_6H_4$), 74.94 (+, CH), 114.16 (+, Ph-C), 118.10 (quat, C-2), 128.51 (+, Ar-C), 135.44 (quat, Ar-C), 152.11 (quat, C-3), 156.59 (quat, Ar-C), 170.27 (quat, lactone CO), 172.61 (quat, $\underline{C}O_2CH_2$). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 393 (100) $[M-H]^-$. Anal. ($C_{20}H_{26}O_8 \cdot 0.3H_2O$) C, H. $C_{20}H_{26}O_8$ (394.42).

6-*O*-[6-(4-Phenylphenoxy)hexanoyl]ascorbic acid (7.52): The title compound was prepared from ascorbic acid (3.3 mmol, 0.58 g), **7.8** (1.5 eq, 5.0 mmol, 1.48 g) and Novozyme 435® (165 mg) in 15 ml *tert*-amyl alcohol according to general procedure 7.6.2.2, yielding **7.52** as a white solid (0.60 g, 41 %). mp: 125-126 °C; ^{1}H-NMR (DMSO-d_6) δ 1.45 (m, 2H, $COCH_2CH_2C\underline{H}_2$), 1.61 (m, 2H, $COCH_2C\underline{H}_2$), 1.74 (m, 2H, $C\underline{H}_2CH_2OC_6H_5$) 2.38 (t, 2H, ^3J = 7.3 Hz, $COCH_2$), 4.06 (m, 5H, CH_2O, $C\underline{H}OH$, $C\underline{H}_2OC_6H_4$), 4.70 (d, 1H, ^3J = 1.6 Hz, H-5), 5.33 (bs, 1H, $CHO\underline{H}$), 7.01 (m, 2H, Ar-H), 7.30 (m, 1H, Ph-H), 7.43 (m, 2H, Ar-H), 7.59 (m, 4H, Ar-H), 8.42 (s, 1H, OH), 11.14 (s, 1H, OH). ^{13}C-NMR (DMSO-d_6) δ 24.08 (-, CH_2), 25.01 (-, CH_2), 28.30 (-, CH_2), 33.28 (-, $CO\underline{C}H_2CH_2$), 64.43 (-, CH_2O), 65.42 (+, CHO), 67.27 (-, $\underline{C}H_2OC_6H_4$), 74.95 (+, CH), 114.77 (+, Ar-C), 118.11 (quat, C-2), 126.06 (+, Ar-C), 126.58 (+, Ar-C), 127.64 (+, Ar-C), 128.77 (+, Ar-C), 132.31 (quat, Ar-C), 139.76 (quat, Ar-C), 152.12 (quat, C-3), 158.20 (quat, Ar-C), 170.28 (quat, lactone CO), 172.62 (quat, $\underline{C}O_2CH_2$). ES-MS (MeCN + TFA) *m/z (%)*: 443 (100) $[M+H]^+$. Anal. ($C_{24}H_{26}O_8$) C, H. $C_{24}H_{26}O_8$ (442.46).

6-*O*-[6-(4-Benzyloxyphenoxy)hexanoyl]ascorbic acid (7.53): The title compound was prepared from ascorbic acid (3.3 mmol, 0.58 g), **7.9** (1.5 eq, 5.0 mmol, 1.63 g) and Novozyme 435® (165 mg) in 15 ml *tert*-amyl alcohol according to general procedure 7.6.2.2, yielding **7.53** as a white solid (0.51 g, 33 %). mp: 147-148 °C; ^{1}H-NMR (DMSO-d_6) δ 1.41 (m, 2H, $COCH_2CH_2C\underline{H}_2$), 1.63 (m, 4H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2OCH_2$), 2.36 (t, 2H, ^3J = 7.3 Hz, $COC\underline{H}_2CH_2$), 3.87 (t, 2H, ^3J = 6.4 Hz, $C\underline{H}_2OCH_2Ph$), 4.03 (m, 3H, -CH_2O-, C<u>H</u>OH), 4.69 (d, 1H, ^3J = 1.4 Hz, H-5), 5.02 (s, 2H, $C\underline{H}_2Ph$), 5.34 (bs, 1H, CHO<u>H</u>), 6.84 (m, 2H, Ar-H), 6.92 (m, 2H, Ar-H), 7.36 (m, 5H, Ph-H), 8.43 (s, 1H, OH), 11.14 (s, 1H, OH). ^{13}C-NMR (DMSO-d_6) δ 24.09 (-, CH_2), 25.02 (-, CH_2), 28.39 (-, CH_2), 33.27 (-, CH_2), 64.42 (-, CH_2O), 65.40 (+, CHO), 67.57 CH_2O), 69.52 (-, $\underline{C}H_2Ph$), 74.94 (+, CH), 115.15 (+, Ar-C), 115.57 (+, Ar-C), 118.09 (quat, C-2), 127.52 (+, Ar-C), 127.62 (+, Ar-C), 128.29 (+, Ar-C), 137.29 (quat, Ar-C), 152.12 (quat, C-3), 152.16 (quat, Ar-C), 152.72 (quat, Ar-C), 170.28 (quat, lactone CO), 172.62 (quat, $\underline{C}O_2CH_2$). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 471 (100) $[M-H]^-$. Anal. ($C_{25}H_{28}O_9$) C, H. $C_{25}H_{28}O_9$ (472.48).

6-*O*-(6-Benzyloxyhexanoyl)ascorbic acid (7.54): The title compound was prepared from ascorbic acid (2.5 mmol, 0.44 g), **7.14** (4 eq, 10 mmol, 2.26 g) and Novozyme 435® (125 mg) in 15 ml *tert*-amyl alcohol according to general procedure 7.6.2.2, yielding **7.54** as a colorless semisolid substance (0.35 g, 37 %). ^{1}H-NMR (DMSO-d_6) δ 1.33 (m, 2H, -$COCH_2CH_2C\underline{H}_2$-), 1.54 (m, 4H, -$COCH_2C\underline{H}_2$-, -$C\underline{H}_2CH_2OBn$), 2.33 (t, 2H, ^3J = 7.4 Hz, -$COCH_2$-), 3.41 (t, 2H, ^3J = 6.4 Hz, -$C\underline{H}_2OBn$), 4.04 (m, 3H, -CH_2O-, -C<u>H</u>OH), 4.44 (s, 2H, -$OC\underline{H}_2Ph$), 4.68 (d, 1H, ^3J = 1.6 Hz, H-5), 5.33 (s, 1H, -CHO<u>H</u>), 7.32 (m, 5H, Ph-H), 8.42 (s, 1H, -OH), 11.14 (bs, 1H, -OH). ^{13}C-NMR (DMSO-d_6) δ 24.14 (-, CH_2), 25.17(-, CH_2), 28.80 (-, CH_2), 33.28 (-, $CO\underline{C}H_2CH_2$), 64.40 (-, CH_2O), 65.40 (+, CHO), 69.35 (-, $\underline{C}H_2OCH_2Ph$), 71.71 (-, $\underline{C}H_2Ph$), 74.93 (+, CH), 118.10 (quat, C-2), 127.22 (+, Ph-C), 127.32 (+, Ph-C), 128.13 (+, Ph-C), 138.61 (quat, Ph-C), 152.11 (quat, C-3), 170.27 (quat, lactone CO), 172.63 (quat, $\underline{C}O_2CH_2$). ES-MS (MeCN + TFA) *m/z (%)*: 381 (100) $[M+H]^+$. Anal. ($C_{19}H_{24}O_8$) C, H. $C_{19}H_{24}O_8$ (380.39).

6-*O*-[6-(4-Phenylphenoxy)hexanoyl]ascorbic acid (7.55): The title compound was prepared from ascorbic acid (1.7 mmol, 0.30 g), **7.15** (1.5 eq, 2.6 mmol, 0.80 g) and Novozyme 435® (85 mg) in 15 ml *tert*-amyl alcohol according to general procedure 7.6.2.2, yielding **7.55** as a white solid (0.40 g, 51 %). mp: 104 °C; ^{1}H-NMR (DMSO-d_6) δ 1.35 (m, 2H, $COCH_2CH_2C\underline{H}_2$), 1.56 (m, 4H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2OCH_2$), 2.34 (t, 2H, ^3J = 7.3 Hz, $COC\underline{H}_2CH_2$) ppm 3.44 (t, 2H, ^3J = 6.4 Hz, $C\underline{H}_2OCH_2C_6H_4$), 4.03 (m,

3H, CH_2O, C<u>H</u>OH), 4.49 (s, 2H, OC<u>H</u>$_2C_6H_4$), 4.69 (d, 1H, ^3J = 1.6 Hz, H-5), 5.33 (bs, 1H, CHO<u>H</u>), 7.41 (m, 5H, Ar-H), 7.65 (m, 4H, Ar-H), 8.43 (s, 1H, OH), 11.14 (s, 1H, OH). ^{13}C-NMR (DMSO-d_6) δ 24.16 (-, CH_2), 25.19 (-, CH_2), 28.84 (-, CH_2), 33.28 (-, CO<u>C</u>H_2), 64.41 (-, CH_2O), 65.39 (+, CHO), 69.41 (-, CH_2O), 71.39 (-, <u>C</u>$H_2C_6H_4$), 74.94 (+, CH), 118.09 (quat, C-2), 126.47 (+, Ar-C), 126.53 (+, Ar-C), 127.30 (+, Ar-C), 127.94 (+, Ar-C), 128.85 (+, Ar-C), 137.85 (quat, Ar-C), 139.11 (quat, Ar-C), 139.84 (quat, Ar-C), 152.13 (quat, C-3), 170.29 (quat, lactone CO), 172.64 (quat, <u>C</u>O_2CH_2). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 455 (100) $[M-H]^-$. Anal. ($C_{25}H_{28}O_8$) C, H. $C_{25}H_{28}O_8$ (456.49).

6-*O*-{6-[4-(Thiophen-3-yl)phenoxy]hexanoyl}ascorbic acid (7.56): The title compound was prepared from ascorbic acid (1.7 mmol, 0.30 g), **7.17** (1.5 eq, 2.6 mmol, 0.78 g) and Novozyme 435® (85 mg) in 15 ml *tert*-amyl alcohol according to general procedure 7.6.2.2, yielding **7.56** as a white solid (0.40 g, 26 %). mp: 118-120 °C; ^{1}H-NMR (DMSO-d_6) δ 1.44 (m, 2H, $COCH_2CH_2C$<u>H</u>$_2$), 1.67 (m, 4H, $COCH_2C$<u>H</u>$_2$, C<u>H</u>$_2CH_2OC_6H_4$), 2.37 (t, 2H, ^3J = 7.3 Hz, COC<u>H</u>$_2CH_2$), 4.03 (m, 5H, CH_2O, C<u>H</u>OH, C<u>H</u>$_2OC_6H_4$), 4.70 (d, 1H, ^3J = 1.5 Hz, H-5), 5.34 (d, 1H, ^3J = 4.6 Hz, CHO<u>H</u>), 6.95 (m, 2H, Ar-H), 7.50 (m, 1H, Ar-H), 7.62 (m, 3H, Ar-H), 7.72 (m, 1H, Ar-H), 8.43 (s, 1H, OH), 11.15 (s, 1H, OH). ^{13}C-NMR (DMSO-d_6) δ 24.09 (-, CH_2), 25.01 (-, CH_2), 28.31 (-, CH_2), 33.26 (-, CO<u>C</u>H_2CH_2), 64.43 (-, CH_2O), 65.39 (+, CHO), 67.22 (-, <u>C</u>$H_2OC_6H_4$), 74.94 (+, CH), 114.62 (+, Ar-C), 118.09 (quat, C-2), 119.11 (+, Ar-C), 125.94 (+, Ar-C), 126.74 (+, Ar-C), 127.14 (+, Ar-C), 127.68 (quat, Ar-C), 141.10 (quat, Ar-C), 152.14 (quat, C-3), 157.76 (quat, Ar-C), 170.30 (quat, lactone CO), 172.63 (quat, <u>C</u>O_2CH_2). ES-MS (MeCN + TFA) *m/z (%)*: 449 (100) $[M+H]^+$. Anal. ($C_{22}H_{24}O_8S \cdot 0.3H_2O$) C, H. $C_{22}H_{24}O_8S$ (448.49).

6-*O*-{6-[4-(2-Methoxyphenyl)phenyloxy]hexanoyl}ascorbic acid (7.57): The title compound was prepared from ascorbic acid (1.6 mmol, 0.28 g), **7.19** (1.5 eq, 2.4 mmol, 0.79 g) and Novozyme 435® (80 mg) in 15 ml *tert*-amyl alcohol according to general procedure 7.6.2.2, yielding **7.57** after preparative HPLC (MeCN/0.1 % TFA (aq) 55/45 v/v), as a white solid (30 mg, 4 %). mp: 84-85 °C; ^{1}H-NMR (CD_3OD) δ 1.53 (m, 2H, $COCH_2CH_2C$<u>H</u>$_2$-), 1.76 (m, 4H, $COCH_2C$<u>H</u>$_2$, C<u>H</u>$_2CH_2OC_6H_4$), 2.42 (t, 2H, ^3J = 7.3 Hz, COC<u>H</u>$_2CH_2$), 3.76 (s, 3H, OCH_3), 3.99 (t, 2H, ^3J = 6.3 Hz, C<u>H</u>$_2OC_6H_4$), 4.07 – 4.30 (m, 3H, CH_2O, C<u>H</u>OH), 4.73 (d, 1H, ^3J = 2.0 Hz, H-5), 6.96 (m, 4H, Ar-H), 7.24 (m, 2H, Ar-H), 7.38 (m, 2H, Ar-H). ^{13}C-NMR (CD_3OD) δ 25.83 (-, CH_2), 26.82 (-, CH_2), 30.18 (-, CH_2), 34.90 (-, CO<u>C</u>H_2CH_2), 56.06 (+, OCH_3), 65.95 (-,

CH_2O), 68.14 (+, CHO), 68.85 (-, $\underline{C}H_2OC_6H_4$), 77.29 (+, CH), 112.65 (+, Ar-C), 115.01 (+, Ar-C), 120.16 (quat, C-2), 121.96 (+, Ar-C), 129.29 (+, Ar-C), 131.54 (+, Ar-C), 131.63 (+, Ar-C), 131.82 (quat, Ar-C), 132.40 (quat, Ar-C), 154.08 (quat, C-3), 157.95 (quat, Ar-C), 159.53 (quat, Ar-C), 173.22 (quat, lactone CO), 175.11 (quat, $\underline{C}O_2CH_2$). PI-EIMS (70 eV) *m/z (%)*: 472 (3) [$M^{+\cdot}$]. HRMS (PI-EIMS) calcd for $C_{26}H_{28}O_9$ [$M^{+\cdot}$], 472.1733; found, 472.1746. Anal. ($C_{25}H_{28}O_9$) C, H. $C_{25}H_{28}O_9$ (472.48).

6-*O*-{6-[4-(4-Methylphenyl)phenyloxy]hexanoyl}ascorbic acid (7.58): The title compound was prepared from ascorbic acid (1.7 mmol, 0.30 g), **7.20** (1.5 eq, 2.6 mmol, 0.81 g) and Novozyme 435® (85 mg) in 15 ml *tert*-amyl alcohol according to general procedure 7.6.2.2, yielding **7.58** as a white solid (0.39 g, 50 %). mp: 147-159 °C; ^{1}H-NMR (DMSO-d_6) δ 1.44 (m, 2H, $COCH_2CH_2C\underline{H}_2$), 1.68 (m, 4H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2OC_6H_4$), 2.32 (s, 3H, CH_3), 2.37 (t, 2H, ^3J = 7.3 Hz, $COC\underline{H}_2CH_2$), 4.04 (m, 5H, CH_2O, $C\underline{H}OH$, $C\underline{H}_2OC_6H_4$), 4.70 (d, 1H, ^3J = 1.6 Hz, H-5), 5.34 (d, 1H, ^3J = 2.8 Hz, $CHO\underline{H}$), 6.98 (m, 2H, Ar-H), 7.23 (m, 2H, Ar-H), 7.52 (m, 4H, Ar-H), 8.42 (s, 1H, OH), 11.14 (s, 1H, OH). ^{13}C-NMR (DMSO-d_6) δ 20.55 (+, CH_3), 24.08 (-, CH_2), 25.01 (-, CH_2), 28.30 (-, CH_2), 33.27 (-, $CO\underline{C}H_2CH_2$), 64.42 (-, CH_2O), 65.40 (+, CHO), 67.24 (-, $\underline{C}H_2OC_6H_4$), 74.94 (+, CH), 114.72 (+, Ar-C), 118.09 (quat, C-2), 125.88 (+, Ar-C), 127.36 (+, Ar-C), 129.36 (+, Ar-C), 132.25 (quat, Ar-C), 135.74 (quat, Ar-C), 136.89 (quat, Ar-C), 152.13 (quat, C-3), 157.95 (quat, Ar-C), 170.29 (quat, lactone CO), 172.63 (quat, $\underline{C}O_2CH_2$). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 455 (100) [M-H]$^-$. Anal. ($C_{25}H_{28}O_8$) C, H. $C_{25}H_{28}O_8$ (456.49).

6-*O*-{6-[4-(2-Methylphenyl)phenyloxy]hexanoyl}ascorbic acid (7.59): The title compound was prepared from ascorbic acid (1.5 mmol, 0.26 g), **7.21** (1.5 eq, 2.3 mmol, 0.71 g) and Novozyme 435® (75 mg) in 15 ml *tert*-amyl alcohol according to general procedure 7.6.2.2, yielding **7.59** as a white solid (0.30 g, 44 %). mp: 97-99 °C; ^{1}H-NMR (DMSO-d_6) δ 1.45 (m, 2H, $COCH_2CH_2C\underline{H}_2$), 1.68 (m, 4H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2OC_6H_4$), 2.22 (s, 3H, $C_6H_4C\underline{H}_3$), 2.38 (t, 2H, ^3J = 7.3 Hz, $COC\underline{H}_2CH_2$), 4.03 (m, 5H, CH_2O, $C\underline{H}OH$, $C\underline{H}_2OC_6H_4$-), 4.70 (d, 1H, ^3J = 1.5 Hz, H-5), 5.35 (bs, 1H, $CHO\underline{H}$), 6.97 (m, 2H, Ar-H), 7.21 (m, 6H, Ar-H), 8.43 (s, 1H, OH), 11.15 (s, 1H, OH). ^{13}C-NMR (DMSO-d_6) δ 20.21 (+, CH_3), 24.09 (-, CH_2), 25.04 (-, CH_2), 28.35 (-, CH_2), 33.28 (-, $\underline{C}H_2OC_6H_4$), 64.43 (-, CH_2O), 65.41 (+, CHO), 67.18 (-, $\underline{C}H_2OC_6H_4$), 74.94 (+, CH), 114.01 (+, Ar-C), 118.09 (quat, C-2), 125.81 (+, Ar-C), 126.82 (+, Ar-C), 129.46 (+, Ar-C), 129.96(+, Ar-C), 130.20 (+, Ar-C), 133.28 (quat, Ar-C), 134.64 (quat, Ar-C), 140.88 (quat, Ar-C), 152.13 (quat, C-3), 157.53 (quat, Ar-

C), 170.29 (quat, lactone CO), 172.63 (quat, $\underline{C}O_2CH_2$). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 455 (100) $[M-H]^-$. Anal. ($C_{25}H_{28}O_8$) C, H. $C_{25}H_{28}O_8$ (456.49).

6-*O*-{6-[4-(4-Hydroxyphenyl)phenyloxy]hexanoyl}ascorbic acid (7.60): The title compound was prepared from ascorbic acid (1.6 mmol, 0.28 g), **7.22** (1.5 eq, 2.4 mmol, 0.75 g) and Novozyme 435® (80 mg) in 15 ml *tert*-amyl alcohol according to general procedure 7.6.2.2, yielding **7.60** as a white solid (0.40 g, 55 %). mp: 158-161 °C; ^{1}H-NMR (DMSO-d_6) δ 1.44 (m, 2H, $COCH_2CH_2C\underline{H}_2$), 1.67 (m, 4H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2OC_6H_4$), 2.37 (t, 2H, ^3J = 7.3 Hz, $COC\underline{H}_2CH_2$), 4.03 (m, 5H, CH_2O, $C\underline{H}OH$, $C\underline{H}_2OC_6H_4$), 4.70 (d, 1H, ^3J = 1.5 Hz, H-5), 5.35 (bs, 1H, $CHO\underline{H}$), 6.81 (m, 2H, Ar-H), 6.95 (m, 2H, Ar-H), 7.44 (m, 4H, Ar-H), 8.44 (bs, 1H, OH), 9.45 (s, 1H, $C_6H_4O\underline{H}$), 11.15 (s, 1H, -OH). ^{13}C-NMR (DMSO-d_6) δ 24.08 (-, CH_2), 25.01 (-, CH_2), 28.32 (-, CH_2), 33.27 (-, $CO\underline{C}H_2CH_2$), 64.42 (-, CH_2O), 65.40 (+, CHO), 67.20 (-, $\underline{C}H_2OC_6H_4$), 74.94 (+, CH), 114.64 (+, Ar-C), 115.53 (+, Ar-C), 118.09 (quat, C-2), 126.89 (+, Ar-C), 127.10 (+, Ar-C), 130.65 (quat, Ar-C), 132.52 (quat, Ar-C), 152.13 (quat, C-3), 156.38 (quat, Ar-C), 157.39 (quat, Ar-C), 170.29 (quat, lactone CO), 172.63 (quat, $\underline{C}O_2CH_2$). ES-MS (MeCN + TFA) *m/z (%)*: 459 (100) $[M+H]^+$. Anal. ($C_{24}H_{26}O_9$) C, H. $C_{24}H_{26}O_9$ (458.46).

6-*O*-{6-[4-(3-Hydroxyphenyl)phenyloxy]hexanoyl}ascorbic acid (7.61): The title compound was prepared from ascorbic acid (1.5 mmol, 0.26 g), **7.23** (1.5 eq, 2.3 mmol, 0.71 g) and Novozyme 435® (75 mg) in 15 ml *tert*-amyl alcohol according to general procedure 7.6.2.2, yielding **7.61** as a white solid (0.30 g, 44 %). mp: 143-144 °C; ^{1}H-NMR (DMSO-d_6) δ 1.45 (m, 2H, $COCH_2CH_2C\underline{H}_2$), 1.68 (m, 4H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2OC_6H_4$), 2.37 (t, 2H, ^3J = 7.3 Hz, $COC\underline{H}_2CH_2$), 4.04 (m, 5H, CH_2O, $C\underline{H}OH$, $C\underline{H}_2OC_6H_4$), 4.70 (d, 1H, ^3J = 1.6 Hz, H-5), 5.33 (bs, 1H, $CHO\underline{H}$), 6.70 (m, 1H, Ar-H), 6.98 (m, 4H, Ar-H), 7.21 (m, 1H, Ar-H), 7.50 (m, 2H, Ar-H), 8.42 (s, 1H, OH), 9.45 (s, 1H, $C_6H_4O\underline{H}$), 11.13 (s, 1H, OH). ^{13}C-NMR (DMSO-d_6) δ 24.08 (-, CH_2), 25.01 (-, CH_2), 26.76 (-, CH_2), 28.30 (-, CH_2), 33.28 $CO\underline{C}H_2CH_2$), 64.43 (-, CH_2O), 65.42 (+, CHO), 67.26 (-, $\underline{C}H_2OC_6H_4$), 74.95 (+, CH), 112.88 (+, Ar-C), 113.60 (+, Ar-C), 114.69 (+, Ar-C), 116.87 (+, Ar-C), 118.11 (quat, C-2), 127.52 (+, Ar-C), 129.73 (+, Ar-C), 132.46 (quat, Ar-C), 141.20 (quat, Ar-C), 152.12 (quat, C-3), 157.66 (quat, Ar-C), 158.14 (quat, Ar-C), 170.28 (quat, lactone CO), 172.62 (quat, $\underline{C}O_2CH_2$). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 457 (100) $[M-H]^-$. Anal. ($C_{24}H_{26}O_9$) C, H. $C_{24}H_{26}O_9$ (458.46).

6-*O*-(11-Phenoxyundecanoyl)ascorbic acid (7.62): The title compound was prepared from ascorbic acid (3.3 mmol, 0.58 g), **7.33** (1.5 eq, 5.0 mmol, 1.45 g) and Novozyme 435® (165 mg) in 15 ml *tert*-amyl alcohol according to general procedure 7.6.2.2, yielding **7.62** as a white solid (0.69 g, 50 %). mp: 100-103 °C; ^{1}H-NMR (DMSO-d_6) δ 1.19 – 1.58 (m, 14H, $COCH_2(CH_2)_7$), 1.69 (m, 2H, CH_2CH_2OPh), 2.32 (t, 2H, ^{3}J = 7.4Hz, $COCH_2CH_2$), 3.91 – 4.11 (m, 5H, CH_2O, CHOH, CH_2OPh), 4.68 (d, 1H, ^{3}J = 1.6 Hz, H-5), 5.32 (d, 1H, ^{3}J = 6.4 Hz, CHOH), 6.90 (m, 3H, Ph-H), 7.27 (m, 2H, Ph-H), 8.42 (s, 1H, OH), 11.13 (s, 1H, OH). ^{13}C-NMR (DMSO-d_6) δ 24.30 (-, CH_2), 25.45 (-, CH_2), 28.38 (-, CH_2), 28.62 (-, CH_2), 28.68 (-, CH_2), 28.77 (-, CH_2), 28.87 (-, CH_2), 33.30 (-, $COCH_2CH_2$), 64.37 (-, CH_2O), 65.39 (+, CHO), 67.12 (-, $CH_2OC_6H_4$), 74.92 (+, CH), 114.27 (+, Ph-C), 118.09 (quat, C-2), 120.22 (+, Ph-C), 129.35 (+, Ph-C), 152.10 (quat, C-3), 158.55 (quat, Ph-C), 170.27 (quat, lactone CO), 172.66 (quat, CO_2CH_2). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 437 (100) $[M+H]^+$. Anal. ($C_{23}H_{32}O_8$) C, H. $C_{23}H_{32}O_8$ (436,50).

6-*O*-[11-(4-Phenylphenoxy)undecanoyl]ascorbic acid (7.63): The title compound was prepared from ascorbic acid (3.0 mmol, 0.53 g), **7.34** (1.5 eq, 4.5 mmol, 1.66 g) and Novozyme 435® (150 mg) in 15 ml *tert*-amyl alcohol according to general procedure 7.6.2.2, yielding **7.63** as a white solid (0.40 g, 26 %). mp: 148-149 °C; ^{1}H-NMR (DMSO-d_6) δ 1.26 – 1.55 (m, 14H, $COCH_2(CH_2)_7$), 1.72 (m, 2H, $CH_2CH_2OC_6H_4$), 2.32 (t, 2H, ^{3}J = 7.4 Hz, $COCH_2CH_2$), 4.03 (m, 5H, CH_2O, CHOH, $CH_2OC_6H_4$), 4.68 (d, 1H, ^{3}J = 1.6 Hz, H-5), 5.33 (bs, 1H, CHOH), 7.00 (m, 2H, Ar-H), 7.30 (m, 1H, Ph-H), 7.42 (m, 2H, Ar-H), 7.59 (m, 4H, Ar-H), 8.43 (s, 1H, OH), 11.14 (s, 1H, OH). ^{13}C-NMR (DMSO-d_6) δ 24.31 (-, CH_2), 25.45 (-, CH_2), 28.39 (-, CH_2), 28.63 (-, CH_2), 28.70 (-, CH_2), 28.78 (-, CH_2), 28.89 (-, CH_2), 33.30 (-, $COCH_2CH_2$), 64.37 (-, CH_2O), 65.39 (+, CHO), 67.38 (-, $CH_2OC_6H_4$), 74.93 (+, CH), 114.75 (+, Ar-C), 118.08 (quat, C-2), 126.04 (+, Ar-C), 126.57 (+, Ar-C), 127.63 (+, Ar-C), 128.76 (+, Ar-C), 132.26 (quat, Ar-C), 139.76 (quat, Ar-C), 152.11 (quat, C-3), 158.23 (quat, Ar-C), 170.28 (quat, lactone CO), 172.67 (quat, CO_2CH_2). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 513 (30) $[M+H]^+$. Anal. ($C_{29}H_{36}O_8$) C, H. $C_{29}H_{36}O_8$ (512,59).

5,6-*O*-Isopropylidene-L-ascorbic acid (7.64)[39]: To L-ascorbic acid (100 g, 568 mmol), suspended in absolute acetone (1 l), was added acetyl chloride (8.0 ml, 113 mmol) under vigorous stirring. After stirring overnight at room temperature, the solid was collected by filtration, washed with EtOAc and dried over potassium carbonate to yield the title compound as white solid (89,85 g, 73 %). mp: 193-194 °C

(ref.[95]: 201-203 °C); ^{1}H-NMR (DMSO-d_6) δ 1.26 (s, 6H, C(CH_3)$_2$), 3.88 (dd, 1H, ^3J = 6.3 Hz, ^2J = 8.4 Hz, CH_2O), 4.10 (dd, 1H, ^3J = 7.1 Hz, ^2J = 8.3 Hz, CH_2O), 4.26 (ddd, 1H, ^3J = 7.1 Hz, ^3J = 5.6 Hz, ^3J = 2.5 Hz, CHO), 4.71 (d, 1H, ^3J = 2.9 Hz, H-5), 8.48 (bs, 1H, OH), 11.30 (s, 1H, OH). $C_9H_{12}O_6$ (216.19).

7.6.2.8 General procedure for the synthesis of 2,3-di-*O*-alkylated 5,6-*O*-isopropylidene-L-ascorbic acid derivatives 65 and 129

A solution of 5,6-*O*-isopropylidene-L-ascorbic acid (**7.64**) (1 eq), potassium carbonate (1.1 eq) and the pertinent alkyl halide (2.2 eq) in anhydrous DMF was heated under stirring at 40 °C for 4 h. The solvent was removed under reduced pressure, the oily residue was taken up in water and extracted tree times with dichloromethane. The combined organic phases were dried over magnesium sulfate and evaporated *in vacuo*. The remaining raw product was recrystallized or subjected to flash chromatography.

2,3-Di-*O*-benzyl-5,6-*O*-isopropylidene-L-ascorbic acid[96] (7.65): The title compound was prepared from **7.64** (100 mmol, 21.60 g), potassium carbonate (110 mmol, 15.20 g) and benzylbromide (220 mmol, 37.6 g) in anhydrous DMF (300 ml) according to general procedure 7.6.2.8. Recrystallization from methanol yielded a white solid (29.10 g, 73 %). mp: 116-118 °C (ref.[40]: 123-125 °C); ^{1}H-NMR (DMSO-d_6): δ 1.28 (s, 3H, C(CH_3)$_2$), 1.29 (s, 3H, C(CH_3)$_2$), 3.87 (dd, 1H, ^2J = 8.5 Hz, ^3J = 5.9 Hz, CH_2O), 4.08 (dd, 1H, ^2J = 8.5 Hz, ^3J = 7.1 Hz, CH_2O), 4.27 (ddd, 1H, ^3J = 7.1 Hz, ^3J = 5.9 Hz, ^3J = 2.5 Hz, CHO), 4.94 (d, 1H, ^2J = 11.2 Hz, C$\underline{H}_2$Ph), 4.95 (d, 1H, ^3J = 2.5 Hz, H-5), 5.01 (d, 1H, ^2J =11.2 Hz, C$\underline{H}_2$Ph), 5.16 (d, 1H, ^2J = 11.8 Hz, C$\underline{H}_2$Ph), 5.25 (d, 1H, ^2J = 11.8 Hz, C$\underline{H}_2$Ph), 7.27-7.43 (m, 10H, Ph-H). CI-MS (NH_3) *m/z (%)*: 414 (100) $[M+NH_4]^+$. $C_{23}H_{24}O_6$ (396.43).

2,3-Di-*O*-benzyl-L-ascorbic acid[96] (7.66): A solution of **7.65** (50 mmol, 19.8 g), methanol (200 ml) and aqueous acetic acid (50 %, 500 ml) was stirred at 90 °C for 5 h. The solvent was evaporated and the oily yellow residue was dissolved in ethyl acetate (400 ml). After washing three times with a saturated solution of sodium carbonate, the organic layer was washed with water, dried over magnesium sulfate and evaporated under reduced pressure to obtain the title compound as a colorless oil which solidified over time (15.1 g, 85 %). mp: 65 °C (ref.[40]: 67-69 °C); ^{1}H-NMR (DMSO-d_6) δ 3.36-3.51 (m, 2H, C$\underline{H}_2$OH), 3.66-3.73 (m, 1H, C$\underline{H}$OH), 4.88 (dd, 1H, ^3J = 6.1 Hz, ^3J = 4.8 Hz, $CH_2O\underline{H}$), 4.90 (d, 1H, ^3J = 1.4 Hz, H-5), 4.94 (d, 1H, ^2J = 11.3

Hz, C$\underline{H}_2$Ph), 4.98 (d, 1H, ^2J = 11.3 Hz, C$\underline{H}_2$Ph), 5.15 (d, 1H, ^3J = 6.3 Hz, CHO$\underline{H}$), 5.20 (d, 1H, ^2J = 11.5 Hz, C$\underline{H}_2$Ph), 5.26 (d, 1H, ^2J = 11.5 Hz, C$\underline{H}_2$Ph), 7.29-7.45 (m, 10H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 374 (100) $[M+NH_4]^+$. $C_{20}H_{20}O_6$ (356.37).

7.6.2.9 General procedure for the synthesis of 6-*O*-acylated 2,3-di-*O*-protected ascorbic acids 67-83, 77a, 78a, 80a-83a, 104, 107-109, 114, 131 and 143-145

To a solution of the corresponding 2,3-di-*O*-protected ascorbic acid (1 eq), the pertinent carboxylic acid (1 eq) and DMAP (1.2 eq) in anhydrous DMF was added EDAC (1.1 eq) portion wise under an atmosphere of argon at 0 °C. The mixture was stirred overnight at room temperature and the solvent was removed under reduced pressure. The remaining raw product was taken up in water and extracted tree times with EtOAc. The combined organic layers were washed with 1 N HCl and brine, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The acylated products were purified by flash chromatography.

2,3-Di-*O*-benzyl-6-*O*-(12-*tert*-butoxy-12-oxododecanoyl)-L-ascorbic acid (7.67): The title compound was prepared from **7.66** (4.0 mmol, 1.42 g), **7.35** (4.0 mmol, 1.14 g), DMAP (4.4 mmol, 0.54 g) and EDAC (4.2 mmol, 0.80 g) in anhydrous DMF (15 ml) according to general procedure 7.6.2.9. Compound **7.67** was obtained after flash chromatography (PE/EtOAc 90/10 to 80/20 v/v) as a pale yellow oil (0.63 g, 25 %). ^{1}H-NMR ($CDCl_3$) δ 1.22 (m, 12H, $COCH_2CH_2(C\underline{H}_2)_6$), 1.39 (s, 9H, $C(C\underline{H}_3)_3$), 1.53 (m, 4H, $COCH_2C\underline{H}_2$), 2.15 (t, 2H, ^3J = 7.5 Hz, $COC\underline{H}_2$), 2.28 (t, 2H, ^3J = 7.6 Hz, $COC\underline{H}_2$), 4.01 (ddd, 1H, ^3J = 1.9 Hz, ^3J = 5.1 Hz, ^3J = 6.7 Hz, C$\underline{H}$OH), 4.15 (dd, 1H, ^3J = 5.0 Hz, ^2J = 11.6 Hz, CH_2O), 4.27 (dd, 1H, ^3J = 6.8 Hz, ^2J = 11.6 Hz, CH_2O), 4.62 (d, 1H, ^3J = 2.1 Hz, H-5), 5.04 (d, 2H, ^2J = 11.5 Hz, C$\underline{H}_2$Ph), 5.08 (d, 1H, ^2J = 11.6 Hz, C$\underline{H}_2$Ph), 5.17 (d, 1H, ^2J = 11.7 Hz, C$\underline{H}_2$Ph), 7.17 (m, 2H, Ph-H), 7.32 (m, 8H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 642 (100) $[M+NH_4]^+$. $C_{36}H_{48}O_9$ (624.76).

2,3-Di-*O*-benzyl-6-*O*-[2-(decan-1-yloxy)benzoyl]-L-ascorbic acid (7.68): The title compound was prepared from **7.66** (2.0 mmol, 0.71 g), **7.39** (2.0 mmol, 0.56 g), DMAP (2.4 mmol, 0.29 g) and EDAC (2.2 mmol, 0.42 g) in anhydrous DMF (10 ml) according to general procedure 7.6.2.9. Compound **7.68** was obtained after flash chromatography (PE/EtOAc 90/10 to 70/30 v/v) as colorless oil (0.43 g, 35 %). ^{1}H-NMR ($CDCl_3$) δ 0.87 (t, 3H, ^3J = 6.7 Hz, $CH_2C\underline{H}_3$), 1.31 (m, 12H, $(C\underline{H}_2)_6CH_3$), 1.78 (m,

2H, $OCH_2C\underline{H}_2CH_2$), 4.00 (t, 1H, ^3J = 6.8 Hz, $COC\underline{H}_2CH_2$), 4.20 (m, 1H, C<u>H</u>OH), 4.42 (dd, 1H, ^3J = 5.4 Hz, ^2J = 11.4 Hz, CH_2O), 4.52 (dd, 1H, ^3J = 6.8 Hz, ^2J = 11.4 Hz, CH_2O), 4.79 (d, 1H, ^3J = 2.1 Hz, H-5), 5.10 (s, 2H, $C\underline{H}_2Ph$), 5.13 (d, 1H, ^2J = 11.9 Hz, $C\underline{H}_2Ph$), 5.22 (d, 1H, ^2J = 11.7 Hz, $C\underline{H}_2Ph$), 6.95 (m, 2H, Ar-H) 7.20 (m, 2H, Ar-H), 7.39 (m, 9H, Ar-H), 7.79 (dd, 1H, ^4J =1.8Hz, ^3J =8.0Hz, Ar-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 617 (100) $[M+H]^+$. Anal. ($C_{37}H_{44}O_8$) C, H. $C_{37}H_{44}O_8$ (616.74).

2,3-Di-*O*-benzyl-6-*O*-[3-(decan-1-yl)benzoyl]-L-ascorbic acid (7.69): The title compound was prepared from **7.66** (2.0 mmol, 0.71 g), **7.40** (2.0 mmol, 0.56 g), DMAP (2.4 mmol, 0.29 g) and EDAC (2.2 mmol, 0.42 g) in anhydrous DMF (10 ml) according to to general procedure 7.6.2.9. The title compound was obtained after flash chromatography (PE/EtOAc 90/10 to 70/30 v/v) as a colorless oil (0.39 g, 32 %). ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.7 Hz, $CH_2C\underline{H}_3$), 1.26 – 1.50 (m, 12H, $(C\underline{H}_2)_6CH_3$), 1.78 (m, 2H, $OCH_2C\underline{H}_2CH_2$), 3.98 (t, 2H, ^3J = 6.5 Hz, $COC\underline{H}_2CH_2$), 4.21 (m, 1H, C<u>H</u>OH), 4.45 (dd, 1H, ^3J = 5.2 Hz, ^2J = 11.6 Hz, CH_2O), 4.55 (dd, 1H, ^3J = 6.7 Hz, ^2J = 11.6 Hz, CH_2O), 4.76 (d, 1H, ^3J = 2.1 Hz, H-5), 5.11 (s, 2H, $C\underline{H}_2Ph$), 5.13 (d, 1H, ^2J = 12.7 Hz, $C\underline{H}_2Ph$), 5.22 (d, 1H, ^2J = 11.7 Hz, $C\underline{H}_2Ph$), 7.21 (m, 1H, Ar-H), 7.35 (m, 9H, Ar-H), 7.53 (dd, 1H, ^4J = 1.5 Hz, ^4J = 2.4 Hz, Ar-H), 7.60 (m, 1H, Ar-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 634 (100) $[M+NH_4]^+$. Anal. ($C_{37}H_{44}O_8$) C, H. $C_{37}H_{44}O_8$ (616.74).

2,3-Di-*O*-benzyl-6-*O*-[2-(hexan-1-yl)decanoyl]-L-ascorbic acid (7.70): The title compound was prepared from **7.66** (2.0 mmol, 0.71 g), commercially available 2-hexyldecanoic acid (2.0 mmol, 0.59 ml), DMAP (2.4 mmol, 0.29 g) and EDAC (2.2 mmol, 0.42 g) in anhydrous DMF (10 ml) according to general procedure 7.6.2.9. Compound **7.70** was obtained after flash chromatography (PE/EtOAc 90/10 v/v) as a white solid (0.6 g, 50 %). mp: 43 °C; ^{1}H-NMR ($CDCl_3$) δ 0.87 (m, 6H, $CH_2C\underline{H}_3$), 1.23 (m, 20H, $(C\underline{H}_2)_4CH_3$, $(C\underline{H}_2)_6CH_3$), 1.51 (m, 4H, $COCHC\underline{H}_2$), 2.35 (tt, 1H, ^3J = 5.5 Hz, ^3J = 8.7 Hz, COCH), 4.02 – 4.36 (m, 3H, CH_2O, C<u>H</u>OH), 4.66 (d, 1H, ^3J = 2.0 Hz, H-5), 5.10 (s, 2H, $C\underline{H}_2Ph$), 5.14 (d, 1H, ^2J = 11.7 Hz, $C\underline{H}_2Ph$), 5.22 (d, 1H, ^3J = 11.7 Hz, $C\underline{H}_2Ph$), 7.22 (m, 2H, Ph-H), 7.36 (m, 8H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 613 (100) $[M+NH_4]^+$. Anal. ($C_{36}H_{50}O_7$) C, H. $C_{36}H_{50}O_7$ (594.78).

2,3-Di-*O*-benzyl-6-*O*-[2-(propan-1-yl)dodecanoyl]-L-ascorbic acid (7.71): The title compound was prepared from **7.66** (2.0 mmol, 0.71 g), **7.42** (2.0 mmol, 0.49 mg), DMAP (2.4 mmol, 0.29 g) and EDAC (2.2 mmol, 0.42 g) in anhydrous DMF (10 ml) according to general procedure 7.6.2.9. After flash chromatography (PE/EtOAc 80/20

v/v) compound **7.71** was obtained as a colorless oil (0.49 g, 42 %). ^{1}H-NMR ($CDCl_3$) δ 0.89 (m, 6H, $CH_2C\underline{H}_3$), 1.18 – 1.68 (m, 22H, $(C\underline{H}_2)_9CH_3$, $(C\underline{H}_2)_2CH_3$), 2.37 (m, 1H, COCH), 4.05 (ddd, 1H, ^3J = 1.6 Hz, ^3J = 4.8 Hz, ^3J = 6.6 Hz, C<u>H</u>OH), 4.17 (ddd, 1H, ^3J = 2.9 Hz, ^3J = 5.1 Hz, ^2J = 11.6 Hz, CH_2O), 4.32 (ddd, 1H, ^3J = 3.4 Hz, ^3J = 6.9 Hz, ^2J = 11.4 Hz, CH_2O), 4.65 (d, 1H, ^3J = 2.1 Hz, H-5), 5.07 – 5.25 (m, 4H, $C\underline{H}_2$Ph), 7.22 (m, 2H, Ph-H), 7.36 (m, 8H, Ph-H).ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 598 (100) $[M+NH_4]^+$. Anal. ($C_{35}H_{48}O_7$) C, H. $C_{35}H_{48}O_7$ (580.75).

2,3-Di-*O*-benzyl-6-*O*-[2-(hexan-1-yl)dodecanoyl]-L-ascorbic acid (7.72): The title compound was prepared from **7.66** (2.0 mmol, 0.71 g), **7.43** (2.0 mmol, 0.49 mg), DMAP (2.4 mmol, 0.29 g) and EDAC (2.2 mmol, 0.42 g) in anhydrous DMF (10 ml) according to general procedure 7.6.2.9. Flash chromatography (PE/EtOAc 80/20 v/v) yielded compound **7.72** as a colorless oil (0.52 g, 42 %). ^{1}H-NMR ($CDCl_3$) δ 0.86 (m, 6H, $CH_2C\underline{H}_3$), 1.24 – 1.65 (m, 28H, $(C\underline{H}_2)_9CH_3$, $(C\underline{H}_2)_5CH_3$), 2.35 (m, 1H, COCH), 4.06 (ddd, 1H, ^3J = 1.9 Hz, ^3J = 4.5 Hz, ^3J = 7.1 Hz, C<u>H</u>OH), 4.18 (ddd, 1H, ^3J = 1.3 Hz, ^3J = 5.3 Hz, ^2J = 11.5 Hz, CH_2O), 4.33 (ddd, 1H, ^3J = 1.5 Hz, ^3J = 6.9 Hz, ^2J = 11.5 Hz, CH_2O), 4.66 (d, 1H, ^3J = 2.1 Hz, H-5), 5.10 (s, 2H, $C\underline{H}_2$Ph), 5.14 (d, 1H, ^2J = 11.7 Hz, $C\underline{H}_2$Ph), 5.22 (d, 1H, ^2J = 11.7 Hz, $C\underline{H}_2$Ph), 7.21 (m, 2H, Ph-H), 7.36 (m, 8H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 640 (100) $[M+NH_4]^+$. Anal. ($C_{38}H_{54}O_7$) C, H. $C_{38}H_{54}O_7$ (622.83).

2,3-Di-*O*-benzyl-6-*O*-[2-(decan-1-yl)dodecanoyl]-L-ascorbic acid (7.73): The title compound was prepared from **7.66** (2.0 mmol, 0.71 g), **7.44** (2.0 mmol, 0.68 mg), DMAP (2.4 mmol, 0.29 g) and EDAC (2.2 mmol, 0.42 g) in anhydrous DMF (10 ml) according to general procedure 7.6.2.9. Flash chromatography (PE/EtOAc 90/10 v/v) gave compound **7.73** as a pale yellow oil (0.45 g, 33 %). ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 6H, ^3J = 6.7 Hz, $CH_2C\underline{H}_3$), 1.24 – 1.65 (m, 36H, $(C\underline{H}_2)_9CH_3$), 2.35 (m, 1H, COCH), 4.06 (ddd, 1H, ^3J = 2.0 Hz, ^3J = 5.3 Hz, ^3J = 7.1 Hz, C<u>H</u>OH), 4.18 (dd, 1H, ^3J = 5.3 Hz, ^2J = 11.5 Hz, CH_2O), 4.32 (dd, 1H, ^3J = 7.0 Hz, ^2J = 11.5 Hz, CH_2O), 4.66 (d, 1H, ^3J = 2.0 Hz, H-5), 5.10 (s, 2H, $C\underline{H}_2$Ph), 5.14 (d, 1H, ^2J = 11.7 Hz, $C\underline{H}_2$Ph), 5.22 (d, 1H, ^2J = 11.7 Hz, $C\underline{H}_2$Ph), 7.22 (m, 2H, Ar-H), 7.36 (m, 8H, Ar-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 697 (100) $[M+NH_4]^+$. $C_{42}H_{62}O_7$ (678.94).

2,3-Di-*O*-benzyl-6-*O*-[2-(2-phenylethyl)dodecanoyl]-L-ascorbic acid (7.74): The title compound was prepared from **7.66** (2.0 mmol, 0.71 g), **7.45** (2.0 mmol, 0.61 mg), DMAP (2.4 mmol, 0.29 g) and EDAC (2.2 mmol, 0.42 g) in anhydrous DMF (10 ml) according to general procedure 7.6.2.9. Flash chromatography (PE/EtOAc 90/10 to

80/20 v/v) yielded **7.74** as a colorless oil (0.30 g, 23 %). ^{1}H-NMR ($CDCl_3$) δ 0.87 (t, 3H, ^3J = 6.7 Hz, $CH_2C\underline{H}_3$), 1.23 – 2.02 (m, 20H, CH_2), 2.40 (m, 1H, COCH), 2.58 (m, 2H, $CH_2C\underline{H}_2Ph$), 4.02 – 4.36 (m, CH_2O, C<u>H</u>OH), 4.65 (m, 1H, H-5), 5.09 (d, 1H, ^2J = 11.4 Hz, OCH_2Ph), 5.13 (d, 1H, ^2J = 11.5 Hz, OCH_2Ph), 5.15 (d, 1H, ^2J = 11.7 Hz, OCH_2Ph), 5.22 (d, 1H, ^2J = 11.7 Hz, OCH_2Ph), 7.26 (m, 15H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 661 (100) $[M+NH_4]^+$. Anal. ($C_{40}H_{50}O_7$) C, H. $C_{40}H_{50}O_7$ (642.82).

2,3-Di-*O*-benzyl-6-*O*-[2-(4-methoxybenzyl)dodecanoyl]-L-ascorbic acid (7.75): The title compound was prepared from **7.66** (1.0 mmol, 0.36 g), **7.46** (1.0 mmol, 0.32 mg), DMAP (1.2 mmol, 0.15 g) and EDAC (1.1 mmol, 0.21 g) in anhydrous DMF (5 ml) according to general procedure 7.6.2.9. Flash chromatography (PE/EtOAc 85/15 to 75/35 v/v) yielded **7.75** as a colorless oil (0.26 g, 39 %). ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.7 Hz, $CH_2C\underline{H}_3$), 1.20 – 1.69 (m, 18H, $(C\underline{H}_2)_9CH_3$), 2.73 (m, 3H, $COC\underline{H}C\underline{H}_2C_6H_4$), 3.73 – 4.68 (m, 7H, OCH_3, CH_2O, C<u>H</u>OH, H-5), 5.16 (m, 4H, $C\underline{H}_2Ph$), 6.77 (m, 2H, Ar-H), 7.05 (m, 2H, Ar-H), 7.22 (m, 2H, Ph-H), 7.36 (m, 8H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 676 (100) $[M+NH_4]^+$. Anal. ($C_{40}H_{50}O_8 \cdot 0.3H_2O$) C, H. $C_{40}H_{50}O_8$ (658.82).

2,3-Di-*O*-benzyl-6-*O*-[2-(hexan-1-yl)octadecanoyl]-L-ascorbic acid (7.76): The title compound was prepared from **7.66** (1.5 mmol, 0.54 g), **7.47** (1.5 mmol, 0.55 mg), DMAP (1.8 mmol, 0.22 g) and EDAC (1.65 mmol, 0.32 g) in anhydrous DMF (8 ml) according to general procedure 7.6.2.9. Compound **7.76** was obtained after flash chromatography (PE/EtOAc 90/10 to 80/20 v/v) as a white solid (0.29 g, 27 %). mp: 53-54 °C; ^{1}H-NMR ($CDCl_3$) δ 0.87 (m, 6H, $CH_2C\underline{H}_3$), 1.24 – 1.65 (m, 40H, $(C\underline{H}_2)_{15}CH_3$, $(C\underline{H}_2)_5CH_3$), 2.35 (tt, 1H, ^3J = 5.5 Hz, ^3J = 8.6 Hz, COCH), 4.05 (ddd, 1H, ^3J = 2.0 Hz, ^3J = 5.3 Hz, ^3J = 7.1 Hz, C<u>H</u>OH), 4.18 (ddd, 1H, ^3J = 1.4 Hz, ^3J = 5.3 Hz, ^2J = 11.5 Hz, CH_2O), 4.32 (ddd, 1H, ^3J = 1.6 Hz, ^3J = 7.0 Hz, ^2J = 11.5 Hz, CH_2O), 4.66 (d, 1H, ^3J = 2.1 Hz, H-5), 5.10 (s, 2H, $C\underline{H}_2Ph$), 5.15 (d, 1H, ^2J = 11.7 Hz, $C\underline{H}_2Ph$), 5.23 (d, 1H, ^2J = 11.7 Hz, $C\underline{H}_2Ph$), 7.22 (m, 2H, Ph-H), 7.36 (m, 8H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 725 (100) $[M+NH_4]^+$. Anal. ($C_{44}H_{66}O_7$) C, H. $C_{44}H_{66}O_7$ (706.99).

2,3-Di-*O*-benzyl-6-*O*-{6-[4-(4-methoxyphenyl)phenoxy]hexanoyl}-L-ascorbic acid (7.77): The title compound was prepared from **7.66** (2.0 mmol, 0.71 g), **7.29** (2.0 mmol, 0.63 g), DMAP (2.4 mmol, 0.29 g) and EDAC (2.2 mmol, 0.42 g) in anhydrous DMF (15 ml) according to general procedure 7.6.2.9. Compound **7.77** was obtained

after flash chromatography (PE/EtOAc 80/20 to 60/40 v/v) as a white solid (0.39 g, 30 %). ^{1}H-NMR ($CDCl_3$) δ 1.51 (m, 2H, $COCH_2CH_2C\underline{H}_2$), 1.76 (m, 4H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2OCH_2$), 2.38 (t, 2H, ^3J = 7.4 Hz, $COC\underline{H}_2CH_2$), 3.83 (s, 3H, OCH_3), 3.98 (t, 2H, ^3J = 6.3 Hz, $C\underline{H}_2OC_6H_4$), 4.14 (m, 2H, CH_2O, C<u>H</u>OH), 4.33 (dd, 1H, ^3J = 6.7 Hz, ^2J = 11.6 Hz, CH_2O), 4.67 (d, 1H, ^3J = 2.0 Hz, H-5), 5.09 (s, 2H, $C\underline{H}_2Ph$), 5.09 (d, 1H, ^2J = 12.8 Hz, $C\underline{H}_2Ph$), 5.20 (d, 1H, ^2J = 11.7 Hz, $C\underline{H}_2Ph$), 6.94 (m, 4H, Ar-H), 7.21 (m, 2H, Ar-H), 7.35 (m, 8H, Ar-H) 7.46 (m, 4H, Ar-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 670 (100) $[M+NH_4]^+$. Anal. ($C_{39}H_{40}O_9$) C, H. $C_{39}H_{40}O_9$ (652.73).

2,3-Di-*O*-benzyl-5,6-*O*-bis{6-[4-(4-methoxyphenyl)phenoxy]hexanoyl}-L-ascorbic acid (7.77a): Compound **7.77a** was obtained as a byproduct in the synthesis of **7.77** as a colorless oil (0.31 g, 16 %). ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 6H, ^3J = 6.7 Hz, $CH_2C\underline{H}_3$), 1.27 – 1.49 (m, 24H, $(C\underline{H}_2)_6CH_3$), 1.78 (m, 4H, $OCH_2C\underline{H}_2CH_2$), 3.99 (m, 4H, $COC\underline{H}_2CH_2$), 4.62 (m, 2H, CH_2O), 4.82 (d, 1H, ^2J = 11.2 Hz, $C\underline{H}_2Ph$), 4.99 (m, 2H, H-5, $C\underline{H}_2Ph$), 5.08 (d, 1H, ^2J = 11.1 Hz, $C\underline{H}_2Ph$), 5.14 (d, 1H, ^2J = 11.4 Hz, $C\underline{H}_2Ph$), 5.77 (ddd, 1H, ^3J = 1.8 Hz, ^3J = 6.4 Hz, ^3J = 6.0 Hz, CHO), 6.88 (m, 4H, Ar-H), 7.24 (m, 10H, Ar-H), 7.93 (m, 4H, Ar-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 895 (100) $[M+NH_4]^+$. $C_{54}H_{68}O_{10}$ (877.11).

2,3-Di-*O*-benzyl-6-*O*-{6-[4-(4-naphthalen-1-yl)phenoxy]hexanoyl}-L-ascorbic acid (7.78): The title compound was prepared from **7.66** (0.67 mmol, 0.24 g), **7.30** (0.67 mmol, 0.22 g), DMAP (0.80 mmol, 0.10 g) and EDAC (0.73 mmol, 0.14 g) in anhydrous DMF (5 ml) according to general procedure 7.6.2.9. Compound **7.78** was obtained after flash chromatography (PE/EtOAc 80/20 to 60/40 v/v) as a pale yellow oil (0.16 g, 35 %). ^{1}H-NMR ($CDCl_3$) δ 1.55 (m, 2H, $COCH_2CH_2C\underline{H}_2$), 1.80 (m, 4H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2OC_6H_4$), 2.41 (t, 2H, ^3J = 7.4 Hz, $COC\underline{H}_2CH_2$), 4.04 (m, 3H, $C\underline{H}_2OC_6H_4$, C<u>H</u>OH), 4.23 (dd, 1H, ^3J = 4.9 Hz, ^2J = 11.6 Hz, CH_2O), 4.35 (dd, 1H, ^3J = 6.8 Hz, ^2J = 11.6 Hz, CH_2O), 4.68 (d, 1H, ^3J = 2.1 Hz, H-5), 5.12 (m, 3H, $C\underline{H}_2Ph$), 5.22 (d, 1H, ^2J = 11.6 Hz, $C\underline{H}_2Ph$), 7.01 (m, 2H, Ar-H), 7.22 (m, 2H, Ar-H), 7.32 – 7.53 (m, 14H, Ar-H), 7.88 (m, 3H, Ar-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 690 (100) $[M+NH_4]^+$. Anal. ($C_{42}H_{40}O_8 \cdot 0.2H_2O$) C, H. $C_{42}H_{40}O_8$ (672.76).

2,3-Di-*O*-benzyl-5,6-*O*-bis{6-[4-(4-napthalen-1-yl)phenoxy]hexanoyl}-L-ascorbic acid (7.78a): Compound **7.78a** was obtained as byproduct in the synthesis of **7.78** as a pale yellow oil (0.11 g, 17 %). ^{1}H-NMR ($CDCl_3$) δ 1.49 – 2.40 (m, 16H), 4.02 (m, 4H), 4.28 (dd, 1H, ^3J = 7.0 Hz, ^2J = 11.6 Hz, CH_2O), 4.39 (dd, 1H, ^3J = 5.5 Hz, ^2J = 11.6 Hz, CH_2O), 4.83 (d, 1H, ^3J = 2.1 Hz, H-5), 5.14 (m, 4H, $C\underline{H}_2Ph$), 5.42 (ddd, 1H,

^3J = 2.1 Hz, ^3J = 5.6 Hz, ^3J = 7.5 Hz, CHO), 6.99 (m, 4H, Ar-H), 7.24 (m, 2H, Ar-H), 7.44 (m, 22H, Ar-H), 7.88 (m, 4H, Ar-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 1007 (100) $[M+NH_4]^+$. Anal. ($C_{64}H_{60}O_{10}$) calc C:77.81, H:6.11 found C:77.29 H:6.67. $C_{64}H_{60}O_{10}$ (989.16).

2,3-Di-*O*-benzyl-6-*O*-{6-[4-(4-*tert*-butoxycarbonylphenyl)phenoxy]hexanoyl}-L-ascorbic acid (7.79): The title compound was prepared from **7.66** (0.94 mmol, 0.33 g), **7.31** (0.94 mmol, 0.36 g), DMAP (1.12 mmol, 0.14 g) and EDAC (1.03 mmol, 0.20 g) in anhydrous DMF (5 ml) according to general procedure 7.6.2.9. Compound **7.79** was obtained after flash chromatography (PE/EtOAc 80/20 to 70/30 v/v) as a white solid (0.21 g, 31 %). mp: 81-83 °C; ^{1}H-NMR ($CDCl_3$) δ 1.55 (m, 11H, $COCH_2CH_2C\underline{H}_2$, $C(C\underline{H}_3)_3$), 1.77 (m, 4H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2OC_6H_4$), 2.39 (t, 2H, ^3J = 7.4 Hz, $COC\underline{H}_2CH_2$), 4.00 (t, 2H, ^3J = 6.3 Hz, $C\underline{H}_2OC_6H_4$), 4.06 (ddd, 1H, ^3J = 2.0 Hz, ^3J = 5.0 Hz, ^3J = 6.8 Hz, C<u>H</u>OH), 4.21 (dd, 1H, ^3J = 5.0 Hz, ^2J = 11.6 Hz, CH_2O), 4.34 (dd, 1H, ^3J = 6.7 Hz, ^2J = 11.6 Hz, CH_2O), 4.67 (d, 1H, ^3J = 2.1 Hz, H-5), 5.10 (s, 2H, $C\underline{H}_2Ph$), 5.12 (d, 1H, ^2J = 11.9 Hz, $C\underline{H}_2Ph$), 5.20 (d, 1H, ^2J = 11.7 Hz, $C\underline{H}_2Ph$), 6.97 (m, 2H, Ar-H), 7.21 (m, 2H, Ar-H), 7.36 (m, 8H, Ar-H), 7.56 (m, 4H, Ar-H), 8.02 (m, 2H, Ar-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 740 (100) $[M+NH_4]^+$. Anal. ($C_{43}H_{46}O_{10}$) C, H. $C_{43}H_{46}O_{10}$ (722.82).

2,3-Di-*O*-benzyl-6-*O*-{6-[4-(3-*tert*-butoxycarbonylphenyl)phenoxy]hexanoyl}-L-ascorbic acid (7.80): The title compound was prepared from **7.66** (1.20 mmol, 0.43 g), **7.32** (1.20 mmol, 0.46 g), DMAP (1.44 mmol, 0.18 g) and EDAC (1.32 mmol, 0.25 g) in anhydrous DMF (5 ml) according to general procedure 7.6.2.9. Compound **7.80** was obtained after flash chromatography (PE/EtOAc 80/20 to 70/30 v/v) as a colorless oil (0.27 g, 31 %). ^{1}H-NMR ($CDCl_3$) δ 1.53 (m, 2H, $COCH_2CH_2C\underline{H}_2$), 1.61 (s, 9H, $C(C\underline{H}_3)_3$), 1.77 (m, 4H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2OC_6H_4$), 2.39 (t, 2H, ^3J = 7.4 Hz, $COC\underline{H}_2CH_2$), 4.00 (t, 2H, ^3J = 6.3 Hz, $C\underline{H}_2OC_6H_4$), 4.07 (ddd, 1H, ^3J = 2.0 Hz, ^3J = 5.0 Hz, ^3J = 6.8 Hz, C<u>H</u>OH), 4.21 (dd, 1H, ^3J = 4.9 Hz, ^2J = 11.6 Hz, CH_2O), 4.34 (dd, 1H, ^3J = 6.7 Hz, ^2J = 11.6 Hz, CH_2O), 4.67 (d, 1H, ^3J = 2.1 Hz, H-5), 5.10 (s, 2H, $C\underline{H}_2Ph$), 5.12 (d, 1H, ^2J = 12.0 Hz, $C\underline{H}_2Ph$), 5.21 (d, 1H, ^2J = 11.7 Hz, $C\underline{H}_2Ph$) 6.96 (m, 2H, Ar-H), 7.21 (m, 2H, Ar-H), 7.43 (m, 11H, Ar-H), 7.70 (m, 1H, Ar-H), 7.92 (m, 1H, Ar-H), 8.17 (m, 1H, Ar-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 740 (100) $[M+NH_4]^+$. Anal. ($C_{43}H_{46}O_{10}$) C, H. $C_{43}H_{46}O_{10}$ (722.82).

2,3-Di-*O*-benzyl-5,6-*O*-bis{6-[4-(3-*tert*-butoxycarbonylphenyl)phenoxy]-hexanoyl}-L-ascorbic acid (7.80a): Compound **7.80a** was obtained as a byproduct

in the synthesis of **7.80** as a pale yellow oil (0.23 g, 18 %). ^{1}H-NMR ($CDCl_3$) δ 1.45 – 1.85 (m, 30H, $C(C\underline{H}_3)_3$, $COCH_2(C\underline{H}_2)_3$), 2.29 (m, 4H, $COC\underline{H}_2CH_2$), 3.97 (m, 4H, $C\underline{H}_2OC_6H_4$), 4.26 (dd, 1H, ^3J = 7.0 Hz, ^2J = 11.5 Hz, CH_2O), 4.36 (dd, 1H, ^3J = 5.6 Hz, ^3J = 11.5 Hz, CH_2O), 4.81 (d, 1H, ^3J = 2.1 Hz, H-5), 5.05 – 5.18 (m, 4H, $C\underline{H}_2Ph$), 5.40 (ddd, 1H, ^3J = 2.1 Hz, ^3J = 5.6 Hz, ^3J = 7.4 Hz, $C\underline{H}O$), 6.94 (m, 4H, Ar-H), 7.22 (m, 2H, Ar-H), 7.32 – 7.54 (m, 14H, Ar-H), 7.69 (m, 2H, Ar-H), 7.91 (m, 2H, Ar-H), 8.17 (m, 2H, Ar-H). ES-MS (MeOH + NH_4OAc) *m/z (%)*: 909 (100) $[M+NH_4]^+$. Anal. ($C_{66}H_{72}O_{14}\cdot 0.2H_2O$) C, H. $C_{66}H_{72}O_{14}$ (1089.27).

2,3-Di-*O*-benzyl-6-*O*-[6-(hexan-1-yloxy)hexanoyl]-L-ascorbic acid (7.81): The title compound was prepared from **7.66** (1.5 mmol, 0.53 g), **7.13** (1.5 mmol, 0.32 g), DMAP (1.8 mmol, 0.22 g) and EDAC (1.68 mmol, 0.32 g) in anhydrous DMF (10 ml) according to general procedure 7.6.2.9. Flash chromatography (PE/EtOAc 80/20 to 70/30 v/v) yielded compound **7.81** as a colorless oil (0.21 g, 25 %). ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.8 Hz, $CH_2C\underline{H}_3$), 1.32 (m, 8H, $(C\underline{H}_2)_3CH_3$, $COCH_2CH_2C\underline{H}_2$), 1.60 (m, 6H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2OCH_2C\underline{H}_2$), 2.34 (t, 2H, ^3J = 7.5 Hz, $COC\underline{H}_2CH_2$), 3.38 (m, 4H, $C\underline{H}_2OC\underline{H}_2$), 4.05 (ddd, 1H, ^3J = 2.0 Hz, ^3J = 5.0 Hz, ^3J = 6.8 Hz, $C\underline{H}OH$), 4.20 (dd, 1H, ^3J = 4.9 Hz, ^2J = 11.6 Hz, CH_2O), 4.31 (dd, 1H, ^3J = 6.9 Hz, ^2J = 11.6 Hz, CH_2O), 4.66 (d, 1H, ^3J = 2.1 Hz, H-5), 5.11 (s, 2H, $C\underline{H}_2Ph$), 5.13 (d, 1H, ^2J = 11.7 Hz, $C\underline{H}_2Ph$), 5.21 (d, 1H, ^2J = 11.7 Hz, $C\underline{H}_2Ph$), 7.21 (m, 2H, Ph-H), 7.36 (m, 8H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 555 (100) $[M+H]^+$. Anal. ($C_{32}H_{42}O_8$) C, H. $C_{32}H_{42}O_8$ (554.67).

2,3-Di-*O*-benzyl-5,6-*O*-bis[6-(hexan-1-yloxy)hexanoyl]-L-ascorbic acid (7.81a): The title compound was obtained as byproduct in the synthesis of **7.81** as a colorless oil (0.17 g, 15 %). ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 6H, ^3J = 6.7 Hz, $CH_2C\underline{H}_3$), 1.32 (m, 16H, $(C\underline{H}_2)_3CH_3$, $COCH_2CH_2C\underline{H}_2$), 1.57 (m, 12H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2OCH_2C\underline{H}_2$), 2.24 (m, 4H, $COC\underline{H}_2CH_2$), 3.37 (m, 8H, $C\underline{H}_2OC\underline{H}_2$), 4.22 (dd, 1H, ^3J = 7.1 Hz, ^2J = 11.6 Hz, CH_2O), 4.32 (dd, 1H, ^3J = 5.7 Hz, ^2J = 11.5 Hz, CH_2O), 4.80 (d, 1H, ^3J = 2.1 Hz, H-5), 5.07 – 5.18 (m, 4H, $C\underline{H}_2Ph$), 5.36 (ddd, 1H, ^3J = 2.1 Hz, ^3J = 5.7 Hz, ^3J = 7.1 Hz, $C\underline{H}O$), 7.23 (m, 2H, Ph-H), 7.36 (m, 8H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 771 (100) $[M+NH_4]^+$. $C_{44}H_{64}O_{10}$ (752.97).

2,3-Di-*O*-benzyl-6-*O*-[5-(heptan-1-yloxy)pentanoyl]-L-ascorbic acid (7.82): The title compound was prepared from **7.66** (1.5 mmol, 0.53 g), **7.14** (1.5 mmol, 0.32 g), DMAP (1.8 mmol, 0.22 g) and EDAC (1.68 mmol, 0.32 g) in anhydrous DMF (10 ml) according to general procedure 7.6.2.9. Flash chromatography (PE/EtOAc 80/20 to

70/30 v/v) yielded compound **7.82** as a colorless oil (0.22 g, 26 %). ^{1}H-NMR ($CDCl_3$) δ 0.87 (t, 3H, ^3J = 6.7 Hz, $CH_2C\underline{H}_3$), 1.27 (m, 8H, $(C\underline{H}_2)_4CH_3$), 1.61 (m, 4H, $C\underline{H}_2CH_2OCH_2C\underline{H}_2$), 2.37 (t, 2H, ^3J = 7.3 Hz, $COC\underline{H}_2CH_2$), 3.39 (m, 4H, $C\underline{H}_2OC\underline{H}_2$), 4.05 (ddd, 1H, ^3J = 1.9 Hz, ^3J = 4.9 Hz, ^3J = 6.8 Hz, C<u>H</u>OH), 4.20 (dd, 1H, ^3J =4.9Hz, ^2J =11.6Hz, CH_2O), 4.32 (dd, 1H, ^3J = 6.9 Hz, ^2J = 11.6 Hz, CH_2O), 4.67 (d, 1H, ^3J = 2.1 Hz, H-5), 5.10 (s, 2H, $C\underline{H}_2Ph$), 5.12 (d, 1H, ^2J = 11.7 Hz, $C\underline{H}_2Ph$), 5.21 (d, 1H, ^2J = 11.7 Hz, $C\underline{H}_2Ph$), 7.21 (m, 2H, Ph-H), 7.36 (m, 8H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 572 (100) $[M+NH_4]^+$. Anal. ($C_{32}H_{42}O_8$) C, H. $C_{32}H_{42}O_8$ (554.67).

2,3-Di-*O*-benzyl-5,6-*O*-bis[5-(heptan-1-yloxy)pentanoyl]-L-ascorbic acid (7.82a): The title compound was obtained as byproduct in the synthesis of **7.82** as a colorless oil (0.16 g, 12 %). ^{1}H-NMR ($CDCl_3$) δ 0.87 (t, 6H, ^3J = 6.2 Hz, $CH_2C\underline{H}_3$), 1.28 (m, 16H, $(C\underline{H}_2)_4CH_3$), 1.60 (m, 8H, $C\underline{H}_2CH_2OCH_2C\underline{H}_2$), 2.28 (m, 4H, $COC\underline{H}_2CH_2$), 3.38 (m, 8H, $C\underline{H}_2OC\underline{H}_2$), 4.22 (dd, 1H, ^3J = 7.0 Hz, ^2J = 11.5 Hz, CH_2O), 4.33 (dd, 1H, ^3J = 5.7 Hz, ^2J = 11.5 Hz, CH_2O), 4.80 (d, 1H, ^3J = 2.1 Hz, H-5), 5.07 – 5.22 (m, 4H, $C\underline{H}_2Ph$), 5.36 (ddd, 1H, ^3J = 2.1 Hz, ^3J = 5.9 Hz, ^3J = 7.7 Hz, CHO), 7.23 (m, 4H, Ph-H), 7.36 (m, 8H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 771 (100) $[M+NH_4]^+$. Anal. ($C_{44}H_{64}O_{10}$) C, H. $C_{44}H_{64}O_{10}$ (752.97).

2,3-Di-*O*-benzyl-6-*O*-[4-(decan-1-yloxy)benzoyl]-L-ascorbic acid (7.83): The title compound was prepared from **7.66** (2.0 mmol, 0.71 g), **7.41** (2.0 mmol, 0.56 g), DMAP (2.4 mmol, 0.29 g) and EDAC (2.2 mmol, 0.42 g) in anhydrous DMF (10 ml) according to general procedure 7.6.2.9. Compound **7.83** was obtained after flash chromatography (PE/EtOAc 90/10 to 70/30 v/v) as a white solid (0.34 g, 28 %). mp: 78 °C; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.7 Hz, $CH_2C\underline{H}_3$), 1.26 – 1.50 (m, 12H, $(C\underline{H}_2)_6CH_3$), 1.79 (m, 2H, $OCH_2C\underline{H}_2CH_2$), 3.99 (t, 2H, ^3J = 6.6 Hz, $COC\underline{H}_2CH_2$), 4.20 (m, 1H, C<u>H</u>OH), 4.42 (dd, 1H, ^3J = 5.2 Hz, ^2J = 11.6 Hz, $C\underline{H}_2O$), 4.53 (dd, 1H, ^3J = 6.7 Hz, ^2J = 11.6 Hz, $C\underline{H}_2O$), 4.75 (d, 1H, ^3J = 2.1 Hz, H-5), 5.11 (s, 2H, $C\underline{H}_2Ph$), 5.13 (d, 1H, ^2J = 12.3 Hz, $C\underline{H}_2Ph$), 5.22 (d, 1H, ^2J = 11.7 Hz, $C\underline{H}_2Ph$), 6.89 (m, 2H, Ar-H), 7.21 (m, 2H, Ar-H), 7.35 (m, 8H, Ar-H), 7.96 (m, 2H, Ar-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 618 (100) $[M+H]^+$. Anal. ($C_{37}H_{44}O_8$) C, H. $C_{37}H_{44}O_8$ (616.74).

2,3-Di-*O*-benzyl-5,6-*O*-bis[4-(decan-1-yloxy)benzoyl]-L-ascorbic acid (7.83a): The title compound was obtained as byproduct in the synthesis of **7.83** as a colorless oil (0.18 g, 10 %). ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 6H, ^3J = 6.7 Hz, $CH_2C\underline{H}_3$), 1.27 – 1.49 (m, 24H, $(C\underline{H}_2)_6CH_3$), 1.78 (m, 4H, $OCH_2C\underline{H}_2CH_2$), 3.99 (m, 4H, $COC\underline{H}_2CH_2$), 4.62 (m, 2H, CH_2O), 4.82 (d, 1H, ^2J = 11.2 Hz, $C\underline{H}_2Ph$), 4.99 (m, 2H, H-5, $C\underline{H}_2Ph$), 5.08 (d,

1H, ^{2}J = 11.1 Hz, C$\underline{H}_2$Ph), 5.14 (d, 1H, ^{2}J = 11.4 Hz, C$\underline{H}_2$Ph), 5.77 (ddd, 1H, ^{3}J = 1.8 Hz, ^{3}J = 6.4 Hz, ^{3}J = 6.0 Hz, CHO), 6.88 (m, 4H, Ar-H), 7.24 (m, 10H, Ar-H), 7.93 (m, 4H, Ar-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 895 (100) $[M+NH_4]^+$. $C_{54}H_{68}O_{10}$ (877.11).

7.6.2.10 General procedure for the synthesis of carboxylic acids 84-86, 86a and 115 *via* cleavage of the corresponding *tert*-butyl esters

The pertinent *tert*-butyl ester was dissolved in CH_2Cl_2/TFA (80/20 v/v) at 0 °C. After stirring for 6 h at room temperature the solvent was evaporated to obtain the free carboxylic acid derivative.

2,3-Di-*O*-benzyl-6-*O*-(11-carboxyundecanoyl)-L-ascorbic acid (7.84): The title compound was prepared from **7.67** (0.85mmol, 0.52 g), in CH_2Cl_2/TFA (80/20 v/v, 8 ml) according to general procedure 7.6.2.10. Compound **7.84** was obtained as a yellow oil (0.48 g, 100 %). ^{1}H-NMR ($CDCl_3$) δ 1.22 (m, 12H, $COCH_2CH_2(C\underline{H}_2)_6$), 1.57 (m, 4H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2CO_2H$), 2.30 (m, 4H, $COC\underline{H}_2CH_2$, $C\underline{H}_2CO_2H$), 4.02 (ddd, 1H, ^{3}J = 2.0 Hz, ^{3}J = 4.9 Hz, ^{3}J = 6.8 Hz, C$\underline{H}$OH), 4.15 (dd, 1H, ^{3}J =4.9Hz, ^{2}J = 11.6 Hz, CH_2O), 4.27 (dd, 1H, ^{3}J = 6.9 Hz, ^{2}J = 11.6 Hz, CH_2O), 4.63 (d, 1H, ^{3}J = 2.0 Hz, H-5), 5.02 – 5.19 (m, 4H, C$\underline{H}_2$Ph), 7.09 – 7.26 (m, 10H, Ph-H). $C_{32}H_{40}O_9$ (568.65).

2,3-Di-*O*-benzyl-6-*O*-{6-[4-(4-carboxyphenyl)phenoxy]hexanoyl}-L-ascorbic acid (7.85): The title compound was prepared from **7.79,** (0.24 mmol, 0.18 g), in CH_2Cl_2/TFA (80/20 v/v, 4 ml) according to general procedure 7.6.2.10. Compound **7.85** was obtained as a white solid (0.16 g, 100 %). mp: 132-135 °C; ^{1}H-NMR (DMSO-d_6) δ 1.43 (m, 2H, $COCH_2CH_2C\underline{H}_2$), 1.66 (m, 4H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2OC_6H_4$), 2.37 (t, 2H, ^{3}J = 7.3 Hz, $COC\underline{H}_2CH_2$), 3.92 – 4.12 (m, 6H, $C\underline{H}_2OC_6H_4$, CH_2O, C$\underline{H}$OH), 4.94 (m, 3H, H-5, C$\underline{H}_2$Ph), 5.19 (d, 1H, ^{3}J = 11.8 Hz, C$\underline{H}_2$Ph), 5.24 (d, 1H, ^{2}J = 11.8 Hz, C$\underline{H}_2$Ph), 7.03 (m, 2H, Ar-H), 7.36 (m, 10H, Ar-H), 7.67 (m, 2H, Ar-H), 7.74 (m, 2H, Ar-H), 7.98 (m, 2H, Ar-H), 12.85 (bs, 1H, CO_2H). ES-MS (MeOH + NH_4OAc) *m/z (%)*: 684 (100) $[M+NH_4]^+$. Anal. ($C_{39}H_{38}O_{10}\cdot 0.3H_2O$) C, H. $C_{39}H_{38}O_{10}$ (666,71).

2,3-Di-*O*-benzyl-6-*O*-{6-[4-(3-carboxyphenyl)phenoxy]hexanoyl}-L-ascorbic acid (7.86): The title compound was prepared from **7.80** (0.37 mmol, 0.27 g), in CH_2Cl_2/TFA (80/20 v/v, 4 ml) according to general procedure 7.6.2.10. Compound **7.86** was obtained as a pale yellow solid (0.24 g, 100 %). mp: 77-80 °C; ^{1}H-NMR (acetone-d_6) δ 1.53 (m, 2H, $COCH_2CH_2C\underline{H}_2$), 1.76 (m, 4H, $COCH_2C\underline{H}_2$,

$C\underline{H}_2CH_2OC_6H_4$), 2.40 (t, 2H, ^3J = 7.3 Hz, $COC\underline{H}_2CH_2$), 4.05 (t, 2H, ^3J =6.4Hz, $C\underline{H}_2OC_6H_4$), 4.13 – 4.32 (m, 3H, CH_2O, C<u>H</u>OH), 4.88 (d, 1H, ^3J = 1.4 Hz, H-5), 5.05 (s, 2H, $C\underline{H}_2Ph$), 5.25 (d, 1H, ^2J = 11.8 Hz, $C\underline{H}_2Ph$), 5.31 (d, 1H, ^3J = 11.8 Hz, $C\underline{H}_2Ph$), 7.05 (m, 2H, Ar-H), 7.40 (m, 10H, Ar-H), 7.60 (m, 3H, Ar-H), 7.87 (m, 1H, Ar-H), 7.98 (m, 1H, Ar-H), 8.26 (m, 1H, Ar-H). ES-MS (MeOH + NH_4OAc) *m/z (%)*: 684 (100) $[M-H]^-$. Anal. ($C_{39}H_{38}O_{10}\cdot H_2O$) C, H. $C_{39}H_{38}O_{10}$ (666.71).

2,3-Di-*O*-benzyl-5,6-*O*-bis{6-[4-(3-carboxyphenyl)phenoxy]hexanoyl}-L-ascorbic acid (7.86a): The title compound was prepared from **7.80a** (0.21 mmol, 0.23 g), in CH_2Cl_2/TFA (80/20 v/v, 4 ml) according to general procedure 7.6.2.10. Compound **7.86a** was obtained as a white solid (0.20 g, 100 %). mp: 117-119 °C; ^{1}H-NMR (acetone-d_6) δ 1.51 (m, 4H, $COCH_2CH_2C\underline{H}_2$), 1.72 (m, 8H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2OC_6H_4$), 2.36 (m, 4H, $COC\underline{H}_2CH_2$), 4.01 (m, 4H, $C\underline{H}_2OC_6H_4$), 4.31 (dd, 1H, ^3J = 7.8 Hz, ^2J = 11.6 Hz, CH_2O), 4.44 (dd, 1H, ^3J = 4.7 Hz, ^2J = 11.6 Hz, CH_2O), 5.11 (m, 3H, $C\underline{H}_2Ph$, H-5), 5.25 (d, 1H, ^2J = 11.6 Hz, $C\underline{H}_2Ph$), 5.32 (d, 1H, ^2J = 11.7 Hz, $C\underline{H}_2Ph$), 5.49 (ddd, 1H, ^3J = 1.9 Hz, ^3J = 4.7 Hz, ^3J = 7.6 Hz, CHO), 7.02 (m, 4H, Ar-H), 7.39 (m, 10H, Ar-H), 7.60 (m, 6H, Ar-H), 7.85 (m, 2H, Ar-H), 7.98 (m, 2H, Ar-H), 8.25 (m, 2H, Ar-H). ES-MS (MeOH + NH_4OAc) *m/z (%)*: 976 (100) $[M-H]^-$. Anal. ($C_{58}H_{56}O_{14}$) C, H. $C_{58}H_{56}O_{14}$ (977.06).

7.6.2.11 General procedure for the synthesis of deprotected ascorbic acid derivatives 87-103, 96a-100a, 103a, 106, 110-112, 117, 127, 128, 132, 139, 140 and 146-148 *via* hydrogenolytic removal of benzyl groups

The pertinent benzyl ether or benzyl ester was dissolved in EtOH and EtOAc and a catalytic amount of palladium on activated charcoal (10 % Pd) was added. Hydrogenolysis was performed in an autoclave under a pressure of 3 bar at room temperature for 24 h. Insoluble material was filtered off, and the solvent was evaporated to yield the target compound.

6-*O*-[2-(Decan-1-yloxy)benzoyl]-L-ascorbic acid (7.87): The title compound was prepared from **7.68** (0.54 mmol, 0.33 g), 10 % Pd/C (30 mg) in EtOH (5 ml) and EtOAc (5 ml) according to general procedure 7.6.2.11 and was obtained as a white solid (0.23 g, 98 %). mp: 53-54 °C; ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H, ^3J = 6.7Hz, $CH_2C\underline{H}_3$), 1.25 – 1.45 (m, 12H, $(C\underline{H}_2)_6CH_3$),1.69 (m, 2H, $OCH_2C\underline{H}_2CH_2$), 4.05 (m, 3H, $COC\underline{H}_2CH_2$, C<u>H</u>OH), 4.20 (dd, 1H, ^3J = 6.9 Hz, ^2J = 10.7 Hz, CH_2O), 4.30 (dd, 1H, ^3J = 6.7 Hz, ^2J = 10.7 Hz, CH_2O), 4.79 (d, 1H, ^3J = 1.5 Hz, H-5), 5.40 (d, 1H, ^3J = 6.2

Hz, CHOH), 7.00 (dd, 1H, ^{3}J = 7.5 Hz, ^{3}J = 7.5 Hz, Ar-H), 7.13 (d, 1H, ^{3}J = 8.3 Hz, Ar-H), 7.52 (ddd, 1H, ^{4}J = 1.8 Hz, ^{3}J = 7.5 Hz, ^{3}J = 9.0 Hz, Ar-H), 7.71 (dd, 1H, ^{4}J = 1.7 Hz, ^{3}J = 7.7 Hz, Ar-H), 8.41 (s, 1H, OH), 11.12 (s, 1H, OH). ^{13}C-NMR (DMSO-d_6) δ 13.88 (+, CH_3), 22.02 (-, CH_2), 25.36 (-, CH_2), 28.51 (-, CH_2), 28.62 (-, CH_2), 28.88 (-, CH_2), 28.95 (-, CH_2), 31.24 (-, CH_2), 64.59 (+, CHO), 65.35 (+, CHO), 68.24 (-, CH_2O), 74.86 (+, CH), 113.37 (+, Ar-C), 118.16 (quat, C-2), 119.86 (+, Ar-C), 130.93 (+, Ar-C), 133.54 (+, Ar-C), 151.98 (quat, C-3), 157.71 (quat, Ar-H), 165.41 (lactone CO), 170.25 (quat, $\underline{C}O_2C_6H_4$). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 435 (100) $[M-H]^-$. Anal. ($C_{23}H_{32}O_8 \cdot 0.2H_2O$) C, H. $C_{23}H_{32}O_8$ (436.50).

6-*O*-[3-(Decan-1-yloxy)benzoyl]-L-ascorbic acid (7.88): The title compound was prepared from **7.69** (0.50 mmol, 0.31 g), 10 % Pd/C (30 mg) in EtOH (5 ml) and EtOAc (5 ml) according to general procedure 7.6.2.11 and was obtained as a white solid (0.21 g, 96 %). mp: 93-95 °C; ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H, ^{3}J = 6.7 Hz, $CH_2C\underline{H}_3$), 1.25 – 1.45 (m, 12H, $(C\underline{H}_2)_6CH_3$), 1.72 (m, 2H, $OCH_2C\underline{H}_2CH_2$), 4.02 (t, 2H, ^{3}J = 6.4 Hz, $COC\underline{H}_2CH_2$), 4.13 (m, 1H, C<u>H</u>OH), 4.32 (m, 2H, CH_2O), 4.81 (d, 1H, ^{3}J = 1.7Hz, H-5), 5.46 (bs, 1H, CHO<u>H</u>), 7.22 (ddd, 1H, ^{4}J = 0.7 Hz, ^{4}J = 2.5 Hz, ^{3}J = 8.2 Hz, Ar-H), 7.45 (m, 2H, Ar-H), 7.59 (m, 1H, Ar-H), 8.41 (bs, 1H, OH), 11.10 (bs, 1H, OH). ^{13}C-NMR (DMSO-d_6) δ 13.87 (+, CH_3), 22.01 (-, CH_2), 25.39 (-, CH_2), 28.51 (-, CH_2), 28.60 (-, CH_2), 28.67 (-, CH_2), 28.86 (-, CH_2), 28.90 (-, CH_2), 31.21 (-, CH_2), 65.56 (+, CHO), 67.66 (-, CH_2O), 75.12 (+, CH), 114.67 (+, Ar-C), 118.17 (quat, C-2), 119.60 (+, Ar-C), 121.41 (+, Ar-C), 129.78 (+, Ar-C), 130.81 (quat, Ar-C), 152.08 (quat, C-3), 158.64 (quat, Ar-C), 165.32 (lactone CO), 170.26 (quat, $\underline{C}O_2C_6H_4$). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 435 (100) $[M-H]^-$. Anal. ($C_{23}H_{32}O_8 \cdot 0.4H_2O$) C, H. $C_{23}H_{32}O_8$ (436.50).

6-*O*-[2-(Hexan-1-yl)decanoyl]-L-ascorbic acid (7.89): The title compound was prepared from **7.70** (0.84 mmol, 0.50 g), 10 % Pd/C (50 mg) in EtOH (10 ml) and EtOAc (10 ml) according to general procedure 7.6.2.11 and was obtained as a white solid (0.33 g, 95 %). mp: 73 °C; ^{1}H-NMR (DMSO-d_6) δ 0.85 (m, 6H, CH_2CH_3), 1.22 (m, 10H, $(C\underline{H}_2)_4CH_3$, $(C\underline{H}_2)_6CH_3$), 1.46 (m, 4H, $COCH(C\underline{H}_2\text{-})C\underline{H}_2$), 2.33 (m, 1H, COCH), 4.01 (m, 3H, CH_2O, C<u>H</u>OH), 4.63 (d, 1H, ^{3}J = 1.6 Hz, H-5), 5.33 (s, 1H, CHO<u>H</u>) 8.42 (s, 1H, OH), 11.15 (s, 1H, OH). ^{13}C-NMR (DMSO-d_6) δ 13.85 (+, CH_3), 13.89 (+, CH_3), 21.94 (-, CH_2), 22.01 (-, CH_2), 26.66 (-, CH_2), 28.55 (-, CH_2), 28.74 (-, CH_2), 28.84 (-, CH_2), 31.18 (-, CH_2), 31.74 (-, CH_2), 31.01 (-, CH_2), 31.79 (-, CH_2), 44.71 (+, CO<u>C</u>H), 64.14 (-, CH_2O), 65.42 (+, CHO), 74.90 (+, CH), 118.10 (quat, C-

2), 151.99 (quat, C-3), 170.23 (quat, lactone CO), 175.15 (quat, $\underline{C}O_2CH$). CI-MS (NH_3) *m/z (%)*: 432 (100) $[M+NH_4]^+$. Anal. ($C_{22}H_{38}O_7 \cdot 0.4H_2O$) C, H. $C_{22}H_{38}O_7$ (414.53).

6-*O*-[2-(Propan-1-yl)dodecanoyl]-L-ascorbic acid (7.90): The title compound was prepared from **7.71** (0.67 mmol, 0.39 g), 10 % Pd/C (30 mg) in EtOH (3 ml) and EtOAc (3 ml) according to general procedure 7.6.2.11. Purification by preparative HPLC (0 min: MeCN/0.1 % TFA (aq) 80/20, 15 min: 90/10 v/v) yielded **7.90** as a white semisolid substance (0.16 g, 60 %). ^{1}H-NMR (CD_3OD) δ 0.85 (m, 6H, $CH_2C\underline{H}_3$), 1.23 - 1.59 (m, 22H, $CH(C\underline{H}_2)_2CH_3$, $CH(C\underline{H}_2)_8CH_3$), 2.38 (m, 1H, $COC\underline{H}$), 4.13 (m, 3H, CH_2O, $C\underline{H}OH$), 4.67 (d, 1H, ^3J = 2.1 Hz, H-5). ^{13}C-NMR (CD_3OD) δ 14.41 (+, CH_3), 14.54 (+, CH_3), 21.75 (-, CH_2) ,23.83 (-, CH_2), 26.58 (-, CH_2), 28.61 (-, CH_2), 30.55 (-, CH_2), 30.67 (-, CH_2), 30.73 (-, CH_2), 30.81 (-, CH_2), 33.16 (-, CH_2), 33.72 (-, CH_2), 35.94 (-, CH_2), 46.81 (+, $CO\underline{C}H$), 65.65 (-, CH_2O), 68.13 (+, CHO), 77.21 (+, CH), 120.17 (quat, C-2), 153.96 (quat, C-3), 173.17, (lactone CO), 177.86 (quat, $\underline{C}O_2CH$). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 418 (100) $[M+NH_4]^+$. HRMS (PI-EIMS) calcd for $C_{21}H_{36}O_7$ $[M^{+\cdot}]$, 400.2461; found, 400.2456. Anal. ($C_{21}H_{36}O_7$) calc C: 62.98, H: 9.06, found C: 62.41, H: 9.61. $C_{21}H_{36}O_7$ (400.51).

6-*O*-[2-(Hexan-1-yl)dodecanoyl]-L-ascorbic acid (7.91): The title compound was prepared from **7.72** (0.69 mmol, 0.43 g), 10 % Pd/C (30 mg) in EtOH (3 ml) and EtOAc (3 ml) according to general procedure 7.6.2.11. Purification by preparative HPLC (0 min: MeCN/0.1 % TFA (aq) 85/15, 15 min: 95/5 v/v) yielded **7.91** as a white semisolid (0.17 g, 56 %). ^{1}H-NMR (CD_3OD) δ 0.89 (t, 6H, ^3J = 6.3 Hz, $CH_2C\underline{H}_3$), 1.28 – 1.67 (m, 28H, $CH(C\underline{H}_2)_5CH_3$, $CH(C\underline{H}_2)_9CH_3$), 2.41 (m, 1H, $COC\underline{H}$), 4.08 (ddd, 1H, ^3J = 2.0 Hz, ^3J = 6.6 Hz, ^3J = 6.7 Hz, $C\underline{H}OH$), 4.21 (m, 2H, CH_2O), 4.71 (d, 1H, ^3J = 2.1 Hz, H-5). ^{13}C-NMR (CD_3OD) δ 14.49 (+, CH_3), 14.54 (+, CH_3), 23.74 (-, CH_2), 23.83 (-, CH_2), 28.58 (-, CH_2), 30.41 (-, CH_2), 30.55 (-, CH_2), 30.65 (-, CH_2), 30.70 (-, CH_2), 30.80 (-, CH_2), 32.93 (-, CH_2), 33.16 (-, CH_2), 33.73 (-, CH_2), 46.99 (+, $CO\underline{C}H$), 65.56 (-, CH_2O), 68.08 (+, CHO), 77.16 (+, CH), 120.16 (quat, C-2), 153.97 (quat, C-3), 173.17 (lactone CO), 177.86 (quat, $\underline{C}O_2CH$). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 441 (100) $[M-H]^-$. HRMS (PI-EIMS) calcd for $C_{24}H_{42}O_7$ $[M^{+\cdot}]$, 442.2931; found, 442.2929. Anal. ($C_{24}H_{42}O_7 \cdot 0.7H_2O$) C, H. $C_{24}H_{42}O_7$ (442.59).

6-*O*-[2-(Decan-1-yl)dodecanoyl]-L-ascorbic acid (7.92): The title compound was prepared from **7.73** (0.62 mmol, 0.42 g), 10 % Pd/C (30 mg) in EtOH (5 ml) and EtOAc (5 ml) according to general procedure 7.6.2.11and was recrystallized from

IPE/*n*-hexane to give a white solid (0.20 g, 64 %). ^{1}H-NMR (CD_3OD) δ 0.89 (t, 3H, ^{3}J = 6.6 Hz, $CH_2C\underline{H}_3$),1.28 – 1.67 (m, 36H, $(C\underline{H}_2)_9CH_3$), 2.41 (m, 1H, COCH), 4.08 (ddd, 1H, ^{3}J = 1.8 Hz, ^{3}J = 6.6 Hz, ^{3}J = 6.6 Hz, C<u>H</u>OH), 4.21 (m, 2H, CH_2O), 4.71 (d, 1H, ^{3}J = 2.0 Hz, H-5). ^{13}C-NMR (CD_3OD) δ 14.57 (+, CH_3), 23.84 (-, CH_2), 28.58 (-, CH_2), 30.57 (-, CH_2), 30.67 (-, CH_2), 30.71 (-, CH_2), 30.82 (-, CH_2), 33.17 (-, CH_2), 33.66 (-, CH_2), 33.70 (-, CH_2), 46.95 (+, CO<u>C</u>H), 65.57 (-, CH_2O), 68.09 (+, CHO), 77.17 (+, CH), 120.16 (quat, C-2), 154.00 (quat, C-3), 173.19 (lactone CO), 177.84 (quat, <u>C</u>O_2CH). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 617 (100) $[M+NH_4]^+$. Anal. ($C_{28}H_{50}O_7$) C, H. $C_{28}H_{50}O_7$ (498.69).

6-*O*-[2-(2-Phenylethyl)dodecanoyl]-L-ascorbic acid (7.93): The title compound was prepared from **7.74** (0.35 mmol, 0.23 g), 10 % Pd/C (30 mg) in EtOH (3 ml) and EtOAc (3 ml) according to general procedure 7.6.2.11. Purification by preparative HPLC (0 min: MeCN/0.1 % TFA (aq) 80/20, 15 min: 90/10 v/v) yielded **7.93** as a white semisolid substance (0.12 g, 75 %). ^{1}H-NMR (CD_3OD) δ 0.89 (t, 3H, ^{3}J = 6.6 Hz, $CH_2C\underline{H}_3$), 1.27 – 2.00 (m, 20H, CH_2), 2.43 (m, 1H, COCH), 2.59 (m, 2H, $CH_2C\underline{H}_2Ph$), 4.22 (m, 3H, -CH_2O-, C<u>H</u>OH), 4.72 (m, 1H, H-5), 7.19 (m, 5H, Ph-H). ^{13}C-NMR (CD_3OD) δ 14.55 (+, CH_3), 23.83 (-, CH_2), 28.47 (-, CH_2), 30.55 (-, CH_2), 30.64 (-, CH_2), 30.80 (-, CH_2), 33.16 (-, CH_2), 33.66 (-, CH_2), 34.73 (-, CH_2), 35.47 (-, CH_2), 46.35 (+, CO<u>C</u>H), 65.75 (-, CH_2O), 68.19 (+, CHO), 77.25, (+, CH), 120.20 (quat, C-2), 127.02 (+, Ar-H),129.46 (+, 2 Ar-H),129.57 (+, 2 Ar-H),143.01 (quat, Ar-H), 153.97 (quat, C-3),173.18 (lactone CO), 177.58 (quat, <u>C</u>O_2CH). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 462 (100) $[M-H]^-$. HRMS (PI-LSIMS) calcd for $C_{26}H_{39}O_7$ $[M+H]^+$, 463.2696; found, 463.2709. Anal. ($C_{26}H_{38}O_7 \cdot 0.3H_2O$) C, H. $C_{26}H_{38}O_7$ (462.58).

6-*O*-[2-(4-Methoxybenzyl)dodecanoyl]-L-ascorbic acid (7.94): The title compound was prepared from **7.75** (0.29 mmol, 0.19 g), 10 % Pd/C (30 mg) in EtOH (3 ml) and EtOAc (3 ml) according to general procedure 7.6.2.11 and was obtained after preparative HPLC (MeCN/0.1 % TFA (aq) 65/35 v/v) as a white semisolid (80 mg, 57 %). mp: 85-87 °C; ^{1}H-NMR (CD_3OD) δ 0.89 (t, 3H, ^{3}J = 6.6 Hz, $CH_2C\underline{H}_3$), 1.21 – 1.70 (m, 18H, $(C\underline{H}_2)_2CH_3$), 2.74 (m, 3H, $COC\underline{H}C\underline{H}_2C_6H_4$), 3.72 – 4.38 (m, 7H, OCH_3, CH_2O, C<u>H</u>OH, H-5), 6.80 (m, 2H, Ar-H), 7.07 (m, 2H, Ar-H). ^{13}C-NMR (CD_3OD) δ 14.47 (+, CH_3), 23.76 (-, CH_2), 28.49 (-, CH_2), 30.48 (-, CH_2), 30.54 (-, CH_2), 30.71 (-, CH_2), 33.10 (-, CH_2), 33.35 (-, CH_2), 33.49 (-, CH_2), 38.90 (-, CH_2), 39.16 (-, CH_2), 49.36 (+, CO<u>C</u>H), 49.61 (+, CO<u>C</u>H), 55.64 (+, OCH_3), 55.67 (+,OCH_3), 65.07 (-,

CH_2O), 65.30 (-, CH_2O), 67.65 (+, CHO), 76.57 (+, CH), 76.87 (+, CH), 114.86 (+, Ar-H), 114.92 (+, Ar-H), 120.06 (quat, C-2), 130.88 (+, Ar-H), 132.57 (quat, Ar-H), 154.04 (quat, C-3), 154.21 (quat, Ar-H), 159.81 (quat, Ar-H), 173.19 (lactone CO), 177.12 (quat, $\underline{C}O_2CH$), 177.19 (quat, $\underline{C}O_2CH$). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 477 (100) $[M-H]^-$. HRMS (PI-LSIMS) calcd for $C_{26}H_{39}O_8$ $[M+H]^+$, 479.2645; found, 479.2636. Anal. ($C_{26}H_{38}O_8 \cdot 0.3H_2O$) C, H. $C_{26}H_{38}O_8$ (478.58).

6-*O*-[2-(Hexan-1-yl)octadecanoyl]-L-ascorbic acid (7.95): The title compound was prepared from **7.76** (0.31 mmol, 0.22 g), 10 % Pd/C (30 mg) in EtOH (3 ml) and EtOAc (3 ml) according to general procedure 7.6.2.11. Purification by preparative HPLC (MeCN/0.1 % TFA (aq) 98/2 v/v) yielded compound **7.95** as a white semisolid substance (0.10 g, 61 %). ^{1}H-NMR (CD_3OD) δ 0.89 (t, 1H, ^3J = 5.8 Hz, $CH_2C\underline{H}_3$), 1.28 – 1.67 (m, 40H, $(C\underline{H}_2)_{15}CH_3$, $(C\underline{H}_2)_5CH_3$), 2.41 (m, 1H, COCH), 4.14 (m, 3H, CH_2O, $C\underline{H}OH$), 4.71 (d, 1H, ^3J = 1.9 Hz, H-5). ^{13}C-NMR (CD_3OD) δ 14.51 (+, CH_3), 14.56 (+, CH_3), 23.75 (-, CH_2), 23.84 (-, CH_2), 28.58 (-, CH_2), 30.43 (-, CH_2), 30.59 (-, CH_2), 30.65 (-, CH_2), 30.71 (-, CH_2), 30.78 (-, CH_2), 30.88 (-, CH_2), 32.94 (-, CH_2), 33.18 (-, CH_2), 33.72 (-, CH_2), 46.99 (+, $CO\underline{C}H$), 65.57 (-, CH_2O), 68.09 (+, CHO), 77.17 (+, CH), 120.16 (quat, C-2), 153.99 (quat, C-3), 173.18 (lactone CO), 177.84 (quat, $\underline{C}O_2CH$). HRMS (PI-EIMS) calcd for $C_{30}H_{52}O_6$ $[M^{+\cdot}-H_2O]$, 508.3764; found, 508.3789. $C_{30}H_{54}O_7$ (526.75).

6-*O*-{6-[4-(4-Methoxyphenyl)phenoxy]hexanoyl}-L-ascorbic acid (7.96): The title compound was prepared from **7.57** (0.49 mmol, 0.32 g), 10 % Pd/C (20 ml) in EtOH (5 ml) and EtOAc (5 ml) according to general procedure 7.6.2.11. Compound **7.96** was obtained as a white solid (0.22 g, 95 %). mp: 148-150 °C; ^{1}H-NMR (DMSO-d_6) δ 1.44 (m, 2H, $COCH_2CH_2C\underline{H}_2$), 1.68 (m, 4H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2OC_6H_4$), 2.38 (t, 2H, ^3J = 7.3 Hz, $COC\underline{H}_2CH_2$), 3.78 (s, 3H, OCH_3), 4.04 (m, 5H, CH_2O, $C\underline{H}OH$, $C\underline{H}_2OC_6H_4$), 4.70 (d, 1H, ^3J = 1.6 Hz, H-5), 5.34 (bs, 1H, $CHO\underline{H}$), 6.98 (m, 4H, Ar-H), 7.52 (m, 4H, Ar-H), 8.43 (s, 1H, OH) 11.14 (s, 1H, OH). ^{13}C-NMR (DMSO-d_6) δ 24.08 (-, CH_2), 25.01 (-, CH_2), 28.32 (-, CH_2), 33.28 (-, $CO\underline{C}H_2CH_2$), 55.04 (+, OCH_3), 64.43 (-, CH_2O), 65.42 (+, CHO), 67.24 (-, $\underline{C}H_2OC_6H_4$), 74.95 (+, CH), 114.18 (+, Ar-C), 114.71 (+, Ar-C), 118.11 (quat, C-2), 127.11 (+, Ar-C), 132.10 (quat, Ar-C), 132.25 (quat, Ar-C), 152.11 (quat, C-3), 157.65 (quat, Ar-C), 158.23 (quat, Ar-C), 170.27 (quat, lactone CO), 172.62 (quat, $\underline{C}O_2CH_2$). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 490 (100) $[M+NH_4]^+$. Anal. ($C_{25}H_{28}O_9$) C, H. $C_{25}H_{28}O_9$ (472.48).

5-*O*-,6-*O*-Bis{6-[4-(4-methoxyphenyl)phenoxy]hexanoyl}-L-ascorbic acid (7.96a): The title compound was prepared from **7.77a** (0.28 mmol, 0.26 g), 10 % Pd/C (20 mg) in EtOH (5 ml) and EtOAc (5 ml) according to general procedure 7.6.2.11. Compound **7.96a** was obtained as a white solid (0.18 g, 84 %). mp: 119-120 °C; ^{1}H-NMR (DMSO-d_6) δ 1.34 – 1.72 (m, 12H, $COCH_2(C\underline{H}_2)_3$), 2.29 (m, 4H, $COC\underline{H}_2CH_2$), 3.77 (s, 6H, OCH_3), 3.93 (t, 4H, ^{3}J = 6.3 Hz, $C\underline{H}_2OC_6H_4$), 4.19 (dd, 1H, ^{3}J = 8.5 Hz, ^{2}J = 11.6 Hz, CH_2O), 4.40 (dd, 1H, ^{3}J = 4.0 Hz, ^{2}J = 11.7 Hz, CH_2O), 5.02 (d, 1H, ^{3}J = 2.1 Hz, H-5), 5.42 (ddd, 1H, ^{3}J = 2.3 Hz, ^{3}J = 3.9 Hz, ^{3}J = 8.3 Hz, C<u>H</u>O), 6.95 (m, 8H, Ar-H), 7.50 (m, 8H, Ar-H), 8.68 (bs, 1H, OH), 11.39 (bs, 1H, OH). ^{13}C-NMR (DMSO-d_6) δ 24.00 (-, CH_2), 24.27 (-, CH_2), 24.82 (-, CH_2), 24.92 (-, CH_2), 28.29 (-, CH_2), 33.18 (-, $CO\underline{C}H_2CH_2$), 33.28 (-, $CO\underline{C}H_2CH_2$), 55.01 (+, OCH_3), 62.19 (-, CH_2O), 67.09 (-, $\underline{C}H_2OC_6H_4$), 67.19 (-, $\underline{C}H_2OC_6H_4$), 73.25 (+, CHO), 114.16 (+, Ar-C), 114.66 (+, Ar-C), 118.31 (quat, C-2), 127.07 (+, Ar-C), 132.05 (quat, Ar-C), 132.20 (quat, Ar-C), 150.68 (quat, C-3), 157.63 (quat, Ar-C), 158.22 (quat, Ar-C), 169.54 (quat, lactone CO), 171.55 (quat, $\underline{C}O_2CH_2$), 172.38 (quat, $\underline{C}O_2CH_2$). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 767 (100) $[M-H]^-$. Anal. ($C_{44}H_{48}O_{12}$) C, H. $C_{44}H_{48}O_{12}$ (768.84).

6-*O*-{6-[4-(Naphthalen-1-yl)phenoxy]hexanoyl}-L-ascorbic acid (7.97): The title compound was prepared from **7.78** (0.21 mmol, 0.14 g), 10 % Pd/C (20 mg) in EtOH (3 ml) and EtOAc (3 ml) according to general procedure 7.6.2.11. Compound **7.97** was obtained as a white foam (0.10 g, 97 %). mp: 80-81 °C; ^{1}H-NMR (CD_3OD+ $CDCl_3$) δ 1.46 – 1.87 (m, 6H, $COCH_2(CH_2)_3$), 2.42 (t, 2H, ^{3}J = 7.3 Hz, $COC\underline{H}_2CH_2$), 4.00 – 4.31 (m, 5H, $C\underline{H}_2OC_6H_4$, CH_2O, C<u>H</u>OH), 4.70 (d, 1H, ^{3}J = 2.2 Hz, H-5), 6.99 (m, 2H, Ar-H), 7.31 – 7.49 (m, 6H, Ar-H), 7.82 (m, 3H, Ar-H). ^{13}C-NMR (CD_3OD+ $CDCl_3$) δ 25.85 (-, CH_2), 26.88 (-, CH_2), 30.20 (-, CH_2), 35.03 (-, $CO\underline{C}H_2CH_2$), 65.96 (-, CH_2O), 68.26 (+, CHO), 68.98 (-, $\underline{C}H_2OC_6H_4$), 77.42 (+, CH), 115.51 (+, Ar-C), 126.52 (+, Ar-C), 126.81 (+, Ar-C), 127.01 (+, Ar-C), 127.08 (+, Ar-C), 127.98 (+, Ar-C), 128.43 (+, Ar-C), 129.41 (+, Ar-C), 132.20 (+, Ar-C), 133.19 (quat, Ar-C), 134.38 (quat, Ar-C), 135.41 (quat, Ar-C), 141.34 (quat, Ar-C), 155.06 (quat, C-3), 159.91 (quat, Ar-C), 173.47 (quat, lactone CO), 175.19 (quat, $\underline{C}O_2CH_2$). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 493 (100) $[M+NH_4]^+$. Anal. ($C_{28}H_{28}O_8 \cdot 1.3H_2O$) C, H. $C_{28}H_{28}O_8$ (492.52).

5,6-*O*-Bis{6-[4-(naphthalen-1-yl)phenoxy]hexanoyl}-L-ascorbic acid (7.97a): The title compound was prepared from **7.78a** (0.10 mmol, 0.10 g), 10 % Pd/C (20 mg) in

EtOH (3 ml) and EtOAc (3 ml) according to general procedure 7.6.2.11 and was obtained as a white foam (0.07 g, 87 %). mp: 63-65 °C; ^{1}H-NMR ($CDCl_3$) δ 1.46 – 1.87 (m, 12H, $COCH_2(CH_2)_3$), 2.39 (m, 4H, $COC\underline{H}_2CH_2$), 4.00 (m, 4H, $C\underline{H}_2OC_6H_4$), 4.34 (dd, 1H, ^{3}J = 6.8 Hz, ^{2}J = 11.4 Hz, CH_2O), 4.49 (dd, 1H, ^{3}J = 4.0 Hz, ^{2}J = 11.5 Hz, CH_2O), 4.94 (d, 1H, ^{3}J = 3.1 Hz, H-5), 5.50 (m, 1H, CHO), 6.99 (m, 4H, Ar-H), 7.41 (m, 12H, Ar-H), 7.87 (m, 6H, Ar-H). ^{13}C-NMR ($CDCl_3$) δ 24.55 (-, CH_2), 24.79 (-, CH_2), 25.51 (-, CH_2), 25.69 (-, CH_2), 28.91 (-, CH_2), 29.01 (-, CH_2), 29.73 (-, CH_2), 33.93 (-, CH_2), 62.29 (-, CH_2O), 67.70 (-, $\underline{C}H_2OC_6H_4$), 68.15 (-, $\underline{C}H_2OC_6H_4$), 68.35 (+, CHO), 74.84 (+, CH), 114.28 (+, Ar-C), 114.46 (+, Ar-C), 119.07 (quat, C-2), 125.41 (+, Ar-C), 125.70 (+, Ar-C), 125.92 (+, Ar-C), 126.02 (+, Ar-C), 126.07 (+, Ar-C), 126.91 (+, Ar-C), 127.31 (+, Ar-C), 127.37 (+, Ar-C), 128.27 (+, Ar-C), 131.11 (+, Ar-C), 131.16 (+, Ar-C), 131.78 (quat, Ar-C), 131.82 (quat, Ar-C), 133.04 (quat, Ar-C), 133.37 (quat, Ar-C), 133.85 (quat, Ar-C), 139.81 (quat, Ar-C), 139.93 (quat, Ar-C), 150.75 (quat, C-3), 158.11 (quat, Ar-C), 158.36 (quat, Ar-C), 171.28 (quat, lactone CO), 172.75 (quat, $\underline{C}O_2CH_2$), 173.46 (quat, $\underline{C}O_2CH_2$). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 810 (60) $[M+NH_4]^+$. Anal. ($C_{50}H_{48}O_{10}$·1.5EtOH) C, H. $C_{50}H_{48}O_{10}$ (808.91).

6-*O*-[5-(Heptan-1-yloxy)pentanoyl]-L-ascorbic acid (7.98): The title compound was prepared from **7.81** (0.34 mmol, 0.19 g), 10 % Pd/C (20 mg) in EtOH (3 ml) and EtOAc (3 ml) according to general procedure 7.6.2.11 and was obtained as a colorless oil (0.13 g, quantitative). ^{1}H-NMR (CD_3OD) δ 0.90 (t, 3H, ^{3}J = 6.0 Hz, $CH_2C\underline{H}_3$), 1.32 (m, 8H, $(C\underline{H}_2)_4CH_3$), 1.62 (m, 4H, $C\underline{H}_2CH_2OCH_2C\underline{H}_2$), 2.41 (t, 2H, ^{3}J = 7.2 Hz, $COC\underline{H}_2CH_2$), 3.42 (m, 4H, $C\underline{H}_2OC\underline{H}_2$) ppm 4.06 – 4.29 (m, 3H, CH_2O, $C\underline{H}OH$), 4.71 (d, 1H, ^{3}J = 2.1 Hz, H-5). ^{13}C-NMR (CD_3OD) δ 14.51 (+, CH_3), 22.88 (-, CH_2), 23.77 (-, CH_2), 27.33 (-, CH_2), 30.14 (-, CH_2), 30.36 (-, CH_2), 30.85 (-, CH_2), 33.10 (-, CH_2), 34.65 (-, $CO\underline{C}H_2CH_2$), 65.92 (-, CH_2O), 68.15 (+, CHO), 71.45 (-, CH_2O), 72.04 (-, CH_2O), 77.39 (+, CH), 119.89 (quat, C-2), 155.20 (quat, C-3), 173.49 (quat, lactone CO), 174.99 (quat, $\underline{C}O_2CH_2$). ES-MS (MeCN + TFA) *m/z (%)*: 375 (100) $[M+H]^+$. Anal. ($C_{18}H_{30}O_8$) C, H. $C_{18}H_{30}O_8$ (374.43).

5,6-*O*-Bis[5-(heptan-1-yloxy)pentanoyl]-L-ascorbic acid (7.98a): The title compound was prepared from **7.81a** (0.25 mmol, 0.19 g), 10 % Pd/C (20 mg) in EtOH (3 ml) and EtOAc (3 ml) according to general procedure 7.6.2.11 and was obtained after preparative HPLC (0 min: MeCN/0.1 % TFA (aq) 80/20, 30 min: 95/5 v/v) as a colorless oil (0.5 g, 35 %). ^{1}H-NMR ($CDCl_3$) δ 0.87 (t, 6H, ^{3}J = 6.7 Hz,

$CH_2C\underline{H}_3$), 1.28 (m, 16H, $(C\underline{H}_2)_4CH_3$), 1.62 (m, 8H, $C\underline{H}_2CH_2OCH_2C\underline{H}_2$), 2.35 (m, 4H, $COC\underline{H}_2CH_2$), 3.40 (m, 8H, $C\underline{H}_2OC\underline{H}_2$), 4.26 (dd, 1H, ^3J = 6.9 Hz, ^2J = 11.7 Hz, CH_2O), 4.42 (dd, 1H, ^3J = 5.1 Hz, ^2J = 11.7 Hz, CH_2O), 4.89 (d, 1H, ^3J = 2.7 Hz, H-5), 5.42 (m, 1H, CHO). ^{13}C-NMR (CD_3OD) δ 14.54 (+, CH_3), 22.80 (-, CH_2), 22.97 (-, CH_2), 23.78 (-, CH_2), 27.35 (-, CH_2), 29.99 (-, CH_2), 30.14 (-, CH_2), 30.38 (-, CH_2), 30.88 (-, CH_2), 33.11 (-, CH_2), 34.60 (-, $CO\underline{C}H_2CH_2$), 34.64 (-, $CO\underline{C}H_2CH_2$), 63.79 (-, CH_2O), 69.16 (+, CHO), 71.37 (-, CH_2O), 71.43 (-, CH_2O), 72.06 (-, CH_2O), 75.67 (+, CH), 120.37 (quat, C-2), 152.59 (quat, C-3), 172.37 (quat, lactone CO), 173.72 (quat, $\underline{C}O_2CH_2$), 174.66 (quat, $\underline{C}O_2CH_2$). ES-MS (MeCN + TFA) *m/z (%)*: 574 (100) $[M+H]^+$. HRMS (EI-PIMS) calcd for $C_{30}H_{52}O_{10}$ [$M^{+\cdot}$], 572.3560; found, 572.3551. Anal. ($C_{30}H_{52}O_{10}$) C, H. $C_{30}H_{52}O_{10}$ (572.73).

6-*O*-[6-(Hexan-1-yloxy)hexanoyl]-L-ascorbic acid (7.99): The title compound was prepared from **7.82** (0.36 mmol, 0.20 g), 10 % Pd/C (20 mg) in EtOH (3 ml) and EtOAc (3 ml) according to general procedure 7.6.2.11 and was obtained as a pale yellow oil (0.13 g, 96 %). ^{1}H-NMR (CD_3OD) δ 0.90 (t, 3H, ^3J = 6.8 Hz, $CH_2C\underline{H}_3$), 1.34 (m, 8H, $(C\underline{H}_2)_3CH_3$, $COCH_2CH_2C\underline{H}_2$), 1.60 (m, 6H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2OCH_2C\underline{H}_2$), 2.38 (t, 2H, ^3J = 7.4 Hz, $COC\underline{H}_2CH_2$), 3.42 (m, 4H, $C\underline{H}_2OC\underline{H}_2$), 4.09 (ddd, 1H, ^3J = 2.0 Hz, ^3J = 5.7 Hz, ^3J = 7.4 Hz, C<u>H</u>OH), 4.22 (m, 2H, CH_2O-), 4.71 (d, 1H, ^3J = 2.1 Hz, H-5). ^{13}C-NMR (CD_3OD) δ 14.47 (+, CH_3), 23.79 (-, CH_2), 25.86 (-, CH_2), 26.89 (-, CH_2), 27.04 (-, CH_2), 30.46 (-, CH_2), 30.80 (-, CH_2), 32.93 (-, CH_2), 34.90 (-, $CO\underline{C}H_2CH_2$), 65.93 (-, CH_2O), 68.15 (+, CHO), 71.73 (-, CH_2O), 72.05 (-, CH_2O), 77.38 (+, CH), 119.92 (quat, C-2), 155.07 (quat, C-3), 173.45 (quat, lactone CO), 175.08 (quat, $\underline{C}O_2CH_2$). ES-MS (MeCN + TFA) *m/z (%)*: 375 (100) $[M+H]^+$. Anal. ($C_{18}H_{30}O_8$) C, H. $C_{18}H_{30}O_8$ (374.43).

5,6-*O*-Bis[6-(hexan-1-yloxy)hexanoyl]-L-ascorbic acid (7.99a): The title compound was prepared from **7.82a** (0.21 mmol, 0.16 g), 10 % Pd/C (20 mg) in EtOH (3 ml) and EtOAc (3 ml) according to general procedure 7.6.2.11 and was obtained as a pale yellow oil (0.11 g, 90 %). ^{1}H-NMR (CD_3OD + $CDCl_3$) δ 0.89 (t, 6H, ^3J = 6.8 Hz, $CH_2C\underline{H}_3$), 1.33 (m, 16H, $(C\underline{H}_2)_3CH_3$, $COCH_2CH_2C\underline{H}_2$), 1.58 (m, 12H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2OCH_2C\underline{H}_2$), 2.31 (m, 4H, $COC\underline{H}_2CH_2$), 3.41 (m, 8H, $C\underline{H}_2OC\underline{H}_2$), 4.26 (dd, 1H, ^3J = 7.6 Hz, ^2J = 11.7 Hz, CH_2O), 4.43 (dd, 1H, ^3J = 4.5 Hz, ^2J = 11.7 Hz, CH_2O), 4.86 (d, 1H, ^3J = 2.6 Hz, H-5), 5.44 (ddd, 1H, ^3J = 2.6 Hz, ^3J = 4.5 Hz, ^3J = 7.3 Hz, C<u>H</u>O). ^{13}C-NMR (CD_3OD + $CDCl_3$) δ 14.75 (+, CH_3), 23.86 (-, CH_2), 25.84 (-, CH_2), 26.06 (-, CH_2), 26.81 (-, CH_2), 26.91 (-, CH_2), 27.09 (-, CH_2), 30.44 (-, CH_2),

30.52 (-, CH_2), 30.85 (-, CH_2), 32.99 (-, CH_2), 35.01(-, CO<u>C</u>H_2CH_2), 35.08 (-, CO<u>C</u>H_2CH_2), 63.83 (-, CH_2O), 69.28 (+, CHO), 71.83 (-, CH_2O), 72.22 (-, CH_2O), 75.81 (+, CH), 120.15 (quat, C-2), 153.61 (quat, C-3), 172.71 (quat, lactone CO), 173.93 (quat, <u>C</u>O_2CH_2), 174.83 (quat, <u>C</u>O_2CH_2). ES-MS (MeCN + TFA) *m/z (%)*: 573 (100) $[M+H]^+$. Anal. ($C_{30}H_{52}O_{10}$·0.4H_2O) C, H. $C_{30}H_{52}O_{10}$ (572.73).

6-*O*-[4-(Decan-1-yloxy)benzoyl]-L-ascorbic acid (7.100): The title compound was prepared from **7.83** (0.42 mmol, 0.26 g), 10 % Pd/C (30 mg) in EtOH (5 ml) and EtOAc (5 ml) according to general procedure 7.6.2.11 and was obtained as a white semisolid substance (0.18 g, 98 %). mp: 118-120 °C; ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H, ^3J = 6.7 Hz, CH_2C<u>H_3</u>), 1.34 (m, 12H, (C<u>H_2</u>)$_6CH_3$), 1.72 (m, 2H, OCH_2C<u>H_2</u>CH_2), 4.04 (t, 2H, ^3J = 6.5 Hz, COC<u>H_2</u>CH_2), 4.19 (m, 3H, C<u>H</u>OH, C<u>H_2</u>O), 4.80 (d, 1H, ^3J = 1.6 Hz, H-5), 5.43 (d, 1H, ^3J = 4.7 Hz, CHO<u>H</u>), 7.03 (m, 2H, Ar-H), 7.95 (m, 2H, Ar-H), 8.41 (s, 1H, OH), 11.11 (s, 1H, OH). ^{13}C-NMR (DMSO-d_6) δ 13.85 (+, CH_3), 22.00 (-, CH_2), 25.34 (-, CH_2), 28.42 (-, CH_2), 28.59 (-, CH_2), 28.64 (-, CH_2), 28.85 (-, CH_2), 28.89 (-, CH_2), 31.20 (-, CH_2), 65.00 (-, CH_2O), 65.64 (+, CHO), 67.79 (-, CH_2O), 75.09 (+, CH), 114.29 (+, Ar-C), 118.18 (quat, C-2), 121.51 (quat, Ar-C), 131.38 (+, Ar-C), 152.08 (quat, C-3), 162.62 (quat, Ar-C), 165.13 (lactone CO), 170.26 (quat, <u>C</u>$O_2C_6H_4$). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 499 (100) $[M+H]^+$. Anal. ($C_{23}H_{32}O_8$) C, H. $C_{23}H_{32}O_8$ (436.50).

5,6-*O*-Bis[4-(decan-1-yloxy)benzoyl]-L-ascorbic acid (7.100a): The title compound was prepared from **7.83a** (0.15 mmol, 0.13 g), 10 % Pd/C (30 mg) in EtOH (5 ml) and EtOAc (5 ml) according to general procedure 7.6.2.11 and was obtained as a white semisolid substance (0.10 g, 97 %). ^{1}H-NMR (DMSO-d_6) δ 0.84 (t, 6H, ^3J = 6.6 Hz, CH_2C<u>H_3</u>), 1.23 – 1.40 (m, 24H, (C<u>H_2</u>)$_6CH_3$), 1.70 (m, 4H, OCH_2C<u>H_2</u>CH_2), 4.01 (t, 4H, ^3J = 6.0 Hz, COC<u>H_2</u>CH_2), 4.52 (dd, 1H, ^3J = 8.3 Hz, ^2J = 11.6 Hz, CH_2O), 4.67 (dd, 1H, ^3J = 4.1 Hz, ^2J = 11.6 Hz, CH_2O), 5.21 (d, 1H, ^3J = 2.0 Hz, H-5), 5.73 (ddd, 1H, ^3J = 2.1 Hz, ^3J = 4.2 Hz, ^3J = 8.1 Hz, CHO), 6.99 (m, 4H, Ar-H), 7.80 (m, 4H, Ar-H), 8.61 (bs, 1H, OH), 11.40 (bs, 1H, OH). ^{13}C-NMR (DMSO-d_6) δ 13.84 (+, CH_3), 22.00 (-, CH_2), 25.32 (-, CH_2), 28.38 (-, CH_2), 28.59 (-, CH_2), 28.85 (-, CH_2), 28.89 (-, CH_2), 31.20 (-, CH_2), 62.82 (-, CH_2O), 67.77 (-, CH_2O), 67.81 (+, CHO), 73.49 (+, CH), 114.32 (+, Ar-C), 114.46 (+, Ar-C), 118.31 (quat, C-2), 120.77 (quat, Ar-C), 121.06 (quat, Ar-C), 131.25 (+, Ar-C), 150.82 (quat, C-3), 162.69 (quat, Ar-C), 162.84 (quat, Ar-C), 164.11 (quat, CO), 164.87 (quat, CO), 169.61 (quat, CO). ES-MS

(DCM/MeOH + NH_4OAc) *m/z (%)*: 696 (100) $[M-H]^-$. Anal. ($C_{40}H_{56}O_{10}\cdot 0.5H_2O$) C, H. $C_{40}H_{56}O_{10}$ (696.87).

6-*O*-(11-Carboxyundecanoyl)-L-ascorbic acid (7.101): The title compound was prepared from **7.84** (0.23 mmol, 0.13 g), 10 % Pd/C (20 mg) in EtOH (3 ml) and EtOAc (3 ml) according to general procedure 7.6.2.11 and was obtained as a white solid (0.08 g, 89 %). mp: 114-115 °C; ^{1}H-NMR (CD_3OD) δ 1.31 (m, 12H, $COCH_2CH_2(C\underline{H}_2)_6$), 1.60 (m, 4H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2CO_2H$), 2.32 (m, 4H, $COC\underline{H}_2CH_2$, $C\underline{H}_2CO_2H$), 4.06 – 4.28 (m, 3H, CH_2O, C<u>H</u>OH), 4.73 (d, 1H, ^3J = 2.3 Hz, H-5). ^{13}C-NMR (CD_3OD) δ 26.05 (-, CH_2), 26.17 (-, CH_2), 30.24 (-, CH_2), 30.30 (-, CH_2), 30.45 (-, CH_2), 30.48 (-, CH_2), 30.60 (-, CH_2), 34.95 (-, $\underline{C}H_2CO_2$), 35.02 (-, $\underline{C}H_2CO_2$), 65.88 (-, CH_2O), 68.12 (+, CHO), 77.26 (+, CH), 120.15 (quat, C-2), 154.05 (quat, C-3), 173.21 (quat, lactone CO), 175.22 (quat, $\underline{C}O_2CH_2$), 177.80 (quat, $\underline{C}O_2H$). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 385 (100) $[M-H]^-$. Anal. ($C_{18}H_{28}O_9$) C, H. $C_{18}H_{28}O_9$ (388.41).

6-*O*-{6-[4-(4-Carboxyphenyl)phenoxy]hexanoyl}-L-ascorbic acid (7.102): The title compound was prepared from **7.85** (0.21 mmol, 0.14 g), 10 % Pd/C (20 mg) in EtOH (10 ml) and EtOAc (5 ml) according to general procedure 7.6.2.11 and was obtained as a white solid (0.10 g, 98 %). mp: 149-151 °C; ^{1}H-NMR (DMSO-d_6) δ 1.65 (m, 6H, $COCH_2(C\underline{H}_2)_6$), 2.37 (t, 2H, ^3J = 7.0 Hz, $COC\underline{H}_2CH_2$), 4.04 (m, 5H, $C\underline{H}_2OC_6H_4$, CH_2O, C<u>H</u>OH), 4.69 (d, 1H, ^3J = 1.5 Hz, H-5), 5.34 (bs, 1H, CHO<u>H</u>), 7.04 (m, 2H, Ar-H), 7.71 (m, 4H, Ar-H), 7.98 (m, 2H, Ar-H), 8.49 (bs, 1H, OH), 11.18 (bs, 1H, OH), 12.82 (bs, 1H, CO_2H). ^{13}C-NMR (DMSO-d_6) δ 24.07 (-, CH_2), 24.98 (-, CH_2), 28.26 (-, CH_2), 33.27 (-, $CO\underline{C}H_2CH_2$), 64.42 (-, CH_2O), 65.41 (+, CHO), 67.33 (-, $\underline{C}H_2OC_6H_4$), 74.94 (+, CH), 114.90 (+, Ar-C), 118.09 (quat, C-2), 126.00 (+, Ar-C), 128.03 (+, Ar-C), 128.68 (quat, Ar-C), 129.85 (+, Ar-C), 130.98 (quat, Ar-C), 143.88 (quat, Ar-C), 152.08 (quat, C-3), 158.87 (quat, Ar-C), 167.10 (quat, CO_2H), 170.26 (quat, lactone CO), 172.62 (quat, $\underline{C}O_2CH_2$). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 485 (100) $[M-H]^-$. Anal. ($C_{25}H_{26}O_{10}\cdot 0.3H_2O$) C, H. $C_{25}H_{26}O_{10}$ (486.47).

6-*O*-{6-[4-(3-Carboxyphenyl)phenoxy]hexanoyl}-L-ascorbic acid (7.103): The title compound was prepared from **7.86** (0.33 mmol, 0.22 g), 10 % Pd/C (20 mg) in EtOH (3 ml) and EtOAc (3 ml) according to general procedure 7.6.2.11 and was obtained as a white solid (0.16 g, 99 %). mp: 145-148 °C; ^{1}H-NMR (DMSO-d_6) δ 1.45 (m, 2H, $COCH_2CH_2C\underline{H}_2$), 1.68 (m, 4H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2OC_6H_4$), 2.38 (t, 2H, ^3J = 7.2 Hz, $COC\underline{H}_2CH_2$), 4.04 (m, 5H, $C\underline{H}_2OC_6H_4$, CH_2O, C<u>H</u>OH), 4.70 (d, 1H, ^3J = 1.4 Hz, H-5),

5.32 (bs, 1H, CHOH), 7.03 (m, 2H, Ar-H), 7.58 (m, 3H, Ar-H), 7.87 (m, 2H, Ar-H), 8.13 (m, 1H, Ar-H), 8.42 (bs, 1H, OH), 11.15 (s, 1H, OH), 13.00 (bs, 1H, CO_2H). ^{13}C-NMR (DMSO-*d*$_6$) δ 24.08 (-, CH_2), 25.00 (-, CH_2), 28.29 (-, CH_2), 33.27 (-, $COCH_2CH_2$), 64.43 (-, CH_2O), 65.41 (+, CHO), 67.31 (-, $CH_2OC_6H_4$), 74.94 (+, CH), 114.91 (+, Ar-C), 118.10 (quat, C-2), 126.64 (+, Ar-C), 127.42 (+, Ar-C), 127.80 (+, Ar-C), 129.15 (+, Ar-C), 130.46 (+, Ar-C), 131.29 (quat, Ar-C), 131.32 (quat, Ar-C), 140.08 (quat, Ar-C), 152.11 (quat, C-3), 158.53 (quat, Ar-C), 167.21 (quat, CO_2H), 170.28 (quat, lactone CO), 172.62 (quat, CO_2CH_2). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 485 (100) $[M-H]^-$. Anal. ($C_{25}H_{26}O_{10}$) C, H. $C_{25}H_{26}O_{10}$ (486.47).

5,6-*O*-Bis{6-[4-(3-carboxyphenyl)phenoxy]hexanoyl}-L-ascorbic acid (7.103a): The title compound was prepared from **7.86a** (0.14 mmol, 0.14 g), 10 % Pd/C (20 mg) in EtOH (10 ml) and EtOAc (5 ml) according to general procedure 7.6.2.11 and was obtained as a white solid (0.11 g, 88 %). 1H-NMR (DMSO-*d*$_6$) δ 1.35 – 1.74 (m, 12H, $COCH_2(CH_2)_3$), 2.30 (m, 4H, $COCH_2CH_2$), 3.96 (t, 4H, ^3J = 6.1 Hz, $CH_2OC_6H_4$), 4.19 (dd, 1H, ^3J = 8.4 Hz, ^2J = 11.6 Hz, CH_2O), 4.40 (dd, 1H, ^3J = 4.0 Hz, ^2J = 11.7 Hz, CH_2O), 5.02 (d, 1H, ^3J = 2.1 Hz, H-5), 5.42 (ddd, 1H, ^3J = 2.3 Hz, ^3J = 3.9 Hz, ^3J = 8.2 Hz, CHO), 7.00 (m, 4H, Ar-H), 7.57 (m, 6H, Ar-H), 7.86 (m, 4H, Ar-H), 8.12 (m, 2H, Ar-H), 8.69 (bs, 1H, OH), 11.39 (bs, 1H, OH), 13.00 (bs, 2H, CO_2H). Anal. ($C_{44}H_{44}O_{14}\cdot 1.4H_2O$) C, H. $C_{44}H_{44}O_{14}$ (796.81).

2,3-Di-*O*-benzyl-6-O-dodecanoyl-L-ascorbic acid (7.104): The title compound was prepared from **7.66** (8.0 mmol, 4.29 g), lauric acid (8.0 mmol, 1.60 g), DMAP (9.6 mmol, 1.17 g) and EDAC (8.8 mmol, 1.68 g) in anhydrous DMF (20 ml) according to general procedure 7.6.2.9 and was obtained after flash chromatography (PE/EtOAc 90/10) as a white solid (1.80 g, 43 %). mp: 42 °C (ref.[53]: 49-50 °C); ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 2H, ^3J = 6.7 Hz, CH_2CH_3), 1.25 (s, 16H, $(CH_2)_8CH_3$), 1.61 (m, 2H, $COCH_2CH_2CH_2$), 2.33 (t, 2H, ^3J = 7.6 Hz, $COCH_2CH_2$-), 4.06 (ddd, 1H, ^3J = 1.8 Hz, ^3J = 5.1 Hz, ^3J = 6.7 Hz, CHOH), 4.20 (dd, 1H, ^3J = 5.1 Hz, ^2J = 11.6 Hz, CH_2O), 4.32 (dd, 1H, ^3J = 6.8 Hz, ^2J = 11.6 Hz, CH_2O), 4.67 (d, 1H, ^3J = 2.0 Hz, H-5), 5.10 (s, 2H, CH_2Ph), 5.14 (d, 1H, ^2J = 11.9 Hz, CH_2Ph), 5.21 (d, 1H, ^2J = 11.7 Hz, CH_2Ph), 7.22 (m, 2H, Ph-H), 7.36 (m, 8H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 556 (100) $[M+NH_4]^+$. Anal. ($C_{32}H_{42}O_7$) C, H. $C_{32}H_{42}O_7$ (538.67).

Benzyl 3-{(1*S*)-1-[(5*R*)-3,4-bis(benzyloxy)-2-oxo-2,5-dihydrofuran-5-yl]-2-(dodecanoyloxy)ethyloxy}-3-oxopropanoate (7.105): The title compound was prepared from **7.104** (1.0 mmol, 0.54 g), **6.33** (1.0 mmol, 0.194 g), DMAP (1.2 mmol,

0.15 g) and EDAC (1.1 mmol, 0.11 g) in anhydrous DMF (10 ml) by analogy with general procedure 7.6.2.9 and was obtained after flash chromatography (PE/EtOAc 90/10 v/v) as a colorless oil (0.65 g, 91 %). ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J =6.7Hz, $CH_2C\underline{H}_3$), 1.25 (m, 16H, $(C\underline{H}_2)_8CH_3$), 1.57 (m, 2H, $COCH_2C\underline{H}_2CH_2$), 2.26 (t, 2H, ^3J = 7.7 Hz, $-COC\underline{H}_2CH_2$), 3.34 (s, 2H, $COCH_2CO_2$), 4.24 (dd, 1H, ^3J = 7.2 Hz, ^2J = 11.6 Hz, CH_2O), 4.34 (dd, 1H, ^3J = 5.6 Hz, ^2J =11.6Hz, CH_2O), 4.80 (d, 1H, ^3J = 2.0 Hz, H-5), 5.17 (m, 6H, $C\underline{H}_2Ph$), 5.44 (ddd, 1H, ^3J = 2.0 Hz, ^3J = 5.6 Hz, ^3J = 7.4 Hz, CHO), 7.32 (m, 15H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 732 (100) $[M+NH_4]^+$. Anal. ($C_{42}H_{50}O_{10}\cdot 1.5H_2O$) C, H. $C_{42}H_{50}O_{10}$ (714.84).

3-{(1*S*)-1-[(5*R*)-3,4-Dihydroxy-2-oxo-2,5-dihydrofuran-5-yl]-2-(dodecanoyloxy)-ethyloxy}-3-oxopropanoic acid (7.106): The title compound was prepared from **7.105** (0.74 mmol, 0.53 g), 10 % Pd/C (30 mg) in EtOH (3 ml) and EtOAc (3 ml) according to general procedure 7.6.2.11 and was obtained after preparative HPLC (MeCN/0.1 % TFA (aq) 70/30 v/v) as a white semisolid substance (0.20 g, 61 %). ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H, ^3J = 6.7 Hz, CH_3), 1.24 (m, 16H, $(C\underline{H}_2)_8CH_3$), 1.50 (m, 2H, $COCH_2C\underline{H}_2$), 2.28 (t, 2H, ^3J = 7.4 Hz, $COC\underline{H}_2CH_2$), 3.31 (s, 2H $COC\underline{H}_2CO_2H$), 4.19 (dd, 1H, ^3J = 8.1 Hz, ^2J = 11.7 Hz, CH_2O), 4.32 (dd, 1H, ^3J = 4.5 Hz, ^2J = 11.7 Hz, CH_2O), 5.00 (d, 1H, ^3J = 2.5 Hz, H-5), 5.39 (ddd, 1H, ^3J = 2.5 Hz, ^3J = 4.4 Hz, ^3J = 7.2 Hz, $C\underline{H}O$), 8.61 (s, 1H, OH), 11.54 (s, 1H, OH), 12.72 (s, 1H, CO_2H). ^{13}C-NMR (DMSO-d_6) δ 13.88 (+, CH_3), 22.02 (-, CH_2), 24.11 (-, CH_2), 28.35 (-, CH_2), 28.61 (-, CH_2), 28.63 (-, CH_2), 28.81 (-, CH_2), 28.93 (-, CH_2), 31.22 (-, CH_2), 33.12 (-, $CO\underline{C}H_2CH_2$), 40.99 (-, $CO\underline{C}H_2CO_2$), 61.62 (-, CH_2O), 68.08 (+, CHO), 72.91 (+, CH), 118.28 (quat, C-2), 150.67 (quat, C-3), 165.72 (quat, $\underline{C}OCH_2CO_2H$), 167.22 (quat, $COCH_2\underline{C}O_2H$), 169.40 (quat, lactone CO), 172.46 (quat, $\underline{C}O_2CH_2$). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 443 (100) $[M-H]^-$. HRMS (PI-LSIMS) calcd for $C_{21}H_{33}O_{10}$ $[M+H]^+$, 445.2074; found, 445.2082. Anal. ($C_{21}H_{32}O_{10}\cdot 1.2H_2O$) C, H. $C_{21}H_{32}O_{10}$ (444.47).

Bis{(2*S*)-2-[(5*R*)-3,4-bis(benzyloxy)-2-oxo-2,5-dihydrofuran-5-yl]-2-hydroxyethyl} dodecanedioate (7.107): The title compound was prepared by analogy with general method 7.6.2.9 using **7.66** (2.0 mmol, 0.71 g), dodecanedioic acid (1.0 mmol, 0.23 g), DMAP (2.6 mmol, 0.32 g) and EDAC (2.4 mmol, 0.46 g) in anhydrous DMF (10 ml). Compound **7.107** was obtained after flash chromatography (PE/EtOAc 80/20 v/v) as a pale yellow oil (0.13 g, 14 %). ^{1}H-NMR ($CDCl_3$) δ 1.26 (m, 12H, $COCH_2CH_2(C\underline{H}_2)_6$), 1.57 (m, 4H, $COCH_2C\underline{H}_2$), 2.32 (t, 4H, ^3J = 7.5 Hz, $COC\underline{H}_2CH_2$), 4.06 (m, 2H,

C<u>H</u>OH), 4.20 (dd, 2H, ^{3}J = 5.2 Hz, ^{2}J = 11.5 Hz, CH_2O), 4.32 (dd, 2H, ^{3}J = 6.8 Hz, ^{2}J = 11.5 Hz, CH_2O), 4.68 (d, 2H, ^{3}J = 2.0 Hz, H-5), 5.04 – 5.23 (m, 8H, C<u>H</u>$_2$Ph), 7.22 (m, 4H, Ph-H), 7.36 (m, 16H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 925 (100) $[M+NH_4]^+$. Anal. ($C_{52}H_{58}O_{14}\cdot 1.2H_2O$) C, H. $C_{52}H_{58}O_{14}$ (907.01).

Bis{(2*S*)-2-[(5*R*)-3,4-bis(benzyloxy)-2-oxo-2,5-dihydrofuran-5-yl]-2-hydroxyethyl} tetradecanedioate (7.108): The title compound was prepared by analogy with general method 7.6.2.9 using **7.66** (2.0 mmol, 0.71 g), tetradecanedioic acid (1.0 mmol, 0.26 g), DMAP (2.6 mmol, 0.32 g) and EDAC (2.4 mmol, 0.46 g) in anhydrous DMF (10 ml). Compound **7.108** was obtained after flash chromatography (PE/EtOAc 70/30 v/v) as a colorless oil (0.3 g, 16 %). ^{1}H-NMR ($CDCl_3$) δ 1.25 (m, 16H, $COCH_2CH_2(C\underline{H}_2)_8$), 1.60 (m, 4H, $COCH_2C\underline{H}_2$), 2.32 (t, 4H, ^{3}J = 7.6 Hz, $COC\underline{H}_2CH_2$), 4.14 (m, 4H, CH_2O, C<u>H</u>OH), 4.33 (dd, 2H, ^{3}J = 6.8 Hz, ^{2}J = 11.5 Hz, CH_2O), 4.68 (d, 2H, ^{3}J = 2.0 Hz, H-5), 5.08 – 5.23 (m, 8H, C<u>H</u>$_2$Ph), 7.22 (m, 4H, Ph-H), 7.36 (m, 16H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 952 (100) $[M+NH_4]^+$. $C_{54}H_{62}O_{14}$ (935.06).

Bis{(2*S*)-2-[(5*R*)-3,4-bis(benzyloxy)-2-oxo-2,5-dihydrofuran-5-yl]-2-hydroxyethyl} hexadecanedioate (7.109): The title compound was prepared by analogy with general method 7.6.2.9 using **7.66** (2.0 mmol, 0.71 g), hexadecanedioic acid (1.0 mmol, 0.29 g), DMAP (2.6 mmol, 0.32 g) and EDAC (2.4 mmol, 0.46 g) in anhydrous DMF (10 ml). Compound **7.109** was obtained after flash chromatography (PE/EtOAc 95/5 to 60/40 v/v) as a colorless oil (0.12 g, 12 %). ^{1}H-NMR ($CDCl_3$) δ 1.26 (m, 20H, $COCH_2CH_2(C\underline{H}_2)_{10}$), 1.60 (m, 4H, $COCH_2C\underline{H}_2$), 2.32 (t, 4H, ^{3}J = 7.6 Hz, $COC\underline{H}_2CH_2$), 4.05 – 4.35 (m, 6H, CH_2O, C<u>H</u>OH), 4.68 (d, 2H, ^{3}J = 2.0 Hz, H-5), 5.09 – 5.32 (m, 8H, C<u>H</u>$_2$Ph), 7.21 (m, 4H, Ph-H), 7.36 (m, 16H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 981 (100) $[M+NH_4]^+$. $C_{56}H_{66}O_{14}$ (963.11).

Bis{(2*S*)-2-[(5*R*)-3,4-dihydroxy-2-oxo-2,5-dihydrofuran-5-yl]-2-hydroxyethyl} dodecanedioate (7.110): The title compound was prepared from **7.107** (0.14 mmol, 0.13 g), 10 % Pd/C (30 mg) in EtOH (4 ml) and EtOAc (4 ml) according to general procedure 7.6.2.11 and was obtained as a pale yellow oil (65 mg, 85 %). ^{1}H-NMR (CD_3OD + $CDCl_3$) δ 1.21 (m, 12H, $COCH_2CH_2(C\underline{H}_2)_6$), 1.53 (m, 4H, $COCH_2C\underline{H}_2$), 2.28 (t, 4H, ^{3}J = 7.5 Hz, $COC\underline{H}_2CH_2$), 4.02 (ddd, 1H, ^{3}J = 2.2 Hz, ^{3}J = 6.3 Hz, ^{3}J = 6.4 Hz, C<u>H</u>OH), 4.12 (dd, 1H, ^{3}J = 5.9 Hz, ^{2}J = 11.1 Hz, CH_2O), 4.21 (dd, 1H, ^{3}J = 6.7 Hz, ^{2}J = 11.2 Hz, CH_2O), 4.63 (d, 1H, ^{3}J = 2.2 Hz, H-5). ^{13}C-NMR (CD_3OD + $CDCl_3$) δ 26.21 (-, CH_2), 30.42 (-, CH_2), 30.56 (-, CH_2), 30.73 (-, CH_2), 31.04 (-, CH_2), 35.44

(-, CH_2), 35.55 (-, CH_2), 65.83 (-, CH_2O), 68.51 (+, CHO), 120.22 (quat, C-2), 153.86 (quat, C-3), 173.20 (quat, lactone CO), 175.58 (quat, $\underline{C}O_2CH_2$). ES-MS (MeCN + TFA) *m/z (%)*: 547 (5) $[M+H]^+$. Anal. ($C_{24}H_{34}O_{14}\cdot 3H_2O$) C, H. $C_{24}H_{34}O_{14}$ (546.52).

Bis{(2*S*)-2-[(5*R*)-3,4-dihydroxy-2-oxo-2,5-dihydrofuran-5-yl]-2-hydroxyethyl} tetradecanedioate (7.111): The title compound was prepared from **7.108** (0.32 mmol, 0.30 g), 10 % Pd/C (30 mg) in EtOH (4 ml) and EtOAc (4 ml) according to general procedure 7.6.2.11 and was obtained as a white solid (0.17 g, 95 %). ^{1}H-NMR (DMSO-d_6) δ 1.23 (m, 16H, $COCH_2CH_2(C\underline{H}_2)_8$), 1.52 (m, 4H, $COCH_2C\underline{H}_2$), 2.32 (t, 4H, ^3J = 7.4 Hz, $COC\underline{H}_2CH_2$), 4.01 (m, 6H, CH_2O, C<u>H</u>OH), 4.68 (d, 2H, ^3J = 1.5 Hz, H-5), 5.33 (bs, 2H, CHO<u>H</u>), 8.43 (s, 2H, OH), 11.14 (s, 2H, OH). ^{13}C-NMR (DMSO-d_6) δ 24.31 (-, CH_2), 28.39 (-, CH_2), 28.66 (-, CH_2), 28.84 (-, CH_2), 28.93 (-, CH_2), 33.29 (-, $CO\underline{C}H_2$), 64.37 (-, CH_2O), 65.37 (+, CHO), 74.91 (+, CH), 118.07 (quat, C-2), 152.07 (quat, C-3), 170.26 (quat, lactone CO), 172.67 (quat, $\underline{C}O_2CH_2$). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 573 (100) $[M-H]^-$. Anal. ($C_{26}H_{38}O_{14}\cdot H_2O$) C, H. $C_{26}H_{38}O_{14}$ (574.57).

Bis{(2*S*)-2-[(5*R*)-3,4-dihydroxy-2-oxo-2,5-dihydrofuran-5-yl]-2-hydroxyethyl} hexadecanedioate (7.112): The title compound was prepared from **7.109** (0.11 mmol, 0.11 g), 10 % Pd/C (30 mg) in EtOH (4 ml) and EtOAc (4 ml) according to general procedure 7.6.2.11 as a white solid (65 mg, 98 %). ^{1}H-NMR (CD_3OD + $CDCl_3$) δ 1.27 (m, 20H, $COCH_2CH_2(C\underline{H}_2)_{10}$), 1.60 (m, 4H, $COCH_2C\underline{H}_2$), 2.36 (t, 4H, ^3J = 7.4 Hz, $COC\underline{H}_2CH_2$), 4.08 (ddd, 2H, ^3J = 2.2 Hz, ^3J = 5.8 Hz, ^3J = 7.1 Hz, C<u>H</u>OH), 4.18 (dd, 2H, ^3J = 5.8 Hz, ^3J = 11.1 Hz, CH_2O), 4.26 (dd, 2H, ^3J = 7.1 Hz, ^3J = 11.1 Hz, CH_2O), 4.71 (d, 1H, ^3J = 2.2 Hz, H-5). ^{13}C-NMR (CD_3OD + $CDCl_3$) δ 26.12 (-, CH_2), 30.34 (-, CH_2), 30.53 (-, CH_2), 30.72 (-, CH_2), 30.87 (-, CH_2), 35.14 (-, $CO\underline{C}H_2CH_2$), 65.88 (-, CH_2O), 68.21 (+, CHO), 77.30 (+, CH), 120.20 (quat, C-2), 154.04 (quat, C-3), 173.24 (quat, lactone CO), 175.37 (quat, $\underline{C}O_2CH_2$). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 620 (100) $[M+NH_4]^+$. Anal. ($C_{28}H_{42}O_{14}\cdot 2.5H_2O$) C, H. $C_{28}H_{42}O_{14}$ (602.62).

11-(*tert*-Butoxycarbonylamino)undecanoic acid[41] (7.113): To a solution of 11-aminoundecanoic acid (24.8 mmol, 5.0 g) in THF (130 ml) and water (130 ml) was added NaOH (54,5 mmol, 2.2 g) and the mixture was stirred for 10 min at room temperature. Di-*tert*-butyl dicarbonate (27.3 mmol, 5.3 g) was added and stirring was continued overnight. After evaporation of the solvent the residue was taken up in $CHCl_3$ (150 ml) and washed three times with 1 N HCl. The organic layer was dried

over $MgSO_4$, filtered and evaporated under reduced pressure. The raw product was recrystallized from *n*-hexane to yield the title compound as a white solid (6.44 g, 90 %). mp: 64 °C (ref.[41]: 68 °C); ^{1}H-NMR ($CDCl_3$) δ 1.27 (s, 12H, $COCH_2CH_2(C\underline{H}_2)_6$), 1.44 (m, 11H, $C(C\underline{H}_3)_3$, $NHCH_2C\underline{H}_2$), 1.62 (m, 2H, $COCH_2C\underline{H}_2$), 2.34 (t, 2H, ^3J = 7.5 Hz, $COC\underline{H}_2$), 3.09 (t, 2H, ^3J = 6.1 Hz, $NHCH_2$), 4.53 (bs, 1H, CONH), 9.57 (s, 1H, CO_2H). $C_{16}H_{31}NO_4$ (301.42).

(2*S*)-2-[(5*R*)-3,4-Bis(benzyloxy)-2-oxo-2,5-dihydrofuran-5-yl]-2-hydroxyethyl 11-(*tert*-butoxycarbonylamino)undecanoate (7.114): The title compound was prepared from **7.66** (4.0 mmol, 1.42 g), **7.113** (4.0 mmol, 1.21 g), DMAP (4.8 mmol, 0.59 g) and EDAC (4.4 mmol, 0.84 g) in anhydrous DMF (15 ml) according to general procedure 7.6.2.9 and was obtained after flash chromatography (PE/EtOAc 90/10 to 80/20 v/v) as a pale yellow oil (0.79 g, 31 %). ^{1}H-NMR ($CDCl_3$) δ 1.26 (m, 12H, $COCH_2CH_2(C\underline{H}_2)_6$), 1.44 (s, 9H, $C(CH_3)_3$), 1.60 (m, 4H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2NH$), 2.32 (t, 2H, ^3J = 7.5 Hz, $COC\underline{H}_2CH_2$), 3.09 (m, 2H, $C\underline{H}_2NH$), 4.05 (ddd, 1H, ^3J =2.0Hz, ^3J =5.0Hz, ^3J =6.9Hz, C<u>H</u>OH), 4.19 (dd, 1H, ^3J = 5.0 Hz, ^2J = 11.6 Hz, CH_2O), 4.32 (dd, 1H, ^3J = 6.8 Hz, ^2J = 11.6 Hz, CH_2O), 4.67 (d, 1H, ^3J = 2.1 Hz, H-5), 5.10 (s, 2H, $C\underline{H}_2Ph$), 5.12 (d, 1H, ^2J = 10.9 Hz, $C\underline{H}_2Ph$), 5.21 (d, 1H, ^2J = 11.7 Hz, $C\underline{H}_2Ph$), 7.21 (m, 2H, Ph-H), 7.36 (m, 8H, Ph-H). ES-MS (MeCN + TFA) *m/z (%)*: 640 (100) $[M+H]^+$. $C_{36}H_{49}NO_9$ (639.78).

(2*S*)-2-[(5*R*)-3,4-Bis(benzyloxy)-2-oxo-2,5-dihydrofuran-5-yl]-2-hydroxyethyl 11-aminoundecanoate trifluoroacetic acid salt (7.115): The title compound was prepared from **7.114** (1.16 mmol, 0.73 g), in CH_2Cl_2/TFA (80/20 v/v, 10 ml) according to general procedure 7.6.2.10 and was obtained as pale yellow oil (0.76 g, 100 %). ^{1}H-NMR ($CDCl_3$) δ 1.25 (s, 12H, $COCH_2CH_2(C\underline{H}_2)_6$), 1.60 (m, 4H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2NH_2$), 2.32 (t, 2H, ^3J = 7.3 Hz, $COC\underline{H}_2CH_2$), 2.99 (m, 2H, $C\underline{H}_2NH_2$), 4.19 (m, 3H, CH_2O, C<u>H</u>OH), 4.68 (d, 1H, ^3J = 1.9 Hz, H-5), 5.04 (s, 2H, $C\underline{H}_2Ph$), 5.13 (d, 1H, ^2J = 11.5 Hz, $C\underline{H}_2Ph$), 5.20 (d, 1H, ^2J = 11.6 Hz, $C\underline{H}_2Ph$), 7.20 (m, 2H, Ph-H), 7.35 (m, 8H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 540 (100) $[M+H]^+$. $C_{31}H_{41}NO_7$ CF_3COOH (653.68).

Bis{(2*S*)-2-[(5*R*)-3,4-bis(benzyloxy)-2-oxo-2,5-dihydrofuran-5-yl]-2-hydroxyethyl} 13-oxo-12-azatricosanedioate (7.116): A solution of **7.115** (0.21 mmol, 0.14 g), **7.84** (0.21 mmol, 0.12 g), DIEA (0.46 mmol, 79 µl), HOBt (0.25 mmol, 39 mg) and EDAC (0.25 mmol, 48 mg) in DMF (10 ml) was stirred at room temperature overnight. After removal of the solvent under reduced pressure the remaining residue was

subjected to flash chromatography to yield the title compound as a colorless oil (0.10 g, 43 %). ^{1}H-NMR ($CDCl_3$), δ 1.23 (m, 26H, $COCH_2CH_2(C\underline{H}_2)_6$, $NHCH_2(C\underline{H}_2)_7$), 1.51 (m, 6H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2NH$), 2.15 (t, 2H, ^3J = 7.6 Hz, $C\underline{H}_2CONH$), 2.31 (t, 4H, ^3J = 7.5 Hz, $COC\underline{H}_2CH_2$), 3.20 (m, 2H, $NHC\underline{H}_2$), 4.08 (m, 2H, $C\underline{H}OH$), 4.20 (dd, 2H, ^3J = 5.4 Hz, ^2J = 11.5 Hz, CH_2O), 4.32 (dd, 2H, ^3J = 6.8 Hz, ^2J = 11.5 Hz, CH_2O), 4.69 (d, 2H, ^3J = 2.0 Hz, H-5), 5.08 (s, 4H, $C\underline{H}_2Ph$), 5.11 (d, 2H, ^2J = 11.8 Hz, $C\underline{H}_2Ph$), 5.21 (d, 2H, ^2J = 11.7 Hz, $C\underline{H}_2Ph$) 5.81 (t, 1H, ^3J = 5.5 Hz, CONH-), 7.21 (m, 4H, Ar-H), 7.38 (m, 16H, Ar-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 1091 (100) $[M+H]^+$. $C_{63}H_{79}NO_{15}$ (1090.30).

Bis{(2*S*)-2-[(5*R*)-3,4-dihydroxy-2-oxo-2,5-dihydrofuran-5-yl]-2-hydroxyethyl} 13-oxo-12-azatricosanedioate (7.117): The title compound was prepared from **7.116** (0.08 mmol, 90 mg), 10 % Pd/C (20 mg) in EtOH (3 ml) and EtOAc (3 ml) according to general procedure 7.6.2.11 and was obtained as a white solid (60 mg, 100 %). ^{1}H-NMR (CD_3OD) δ 1.24 (m, 26H, $COCH_2CH_2(C\underline{H}_2)_6$, $NHCH_2(C\underline{H}_2)_7$), 1.49 (m, 6H, $COCH_2C\underline{H}_2$, $C\underline{H}_2CH_2NH$), 2.10 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2CONH$), 2.30 (t, 4H, ^3J = 7.4 Hz, $COC\underline{H}_2CH_2$), 3.24 (m, 2H, $NHC\underline{H}_2$), 4.09 (m, 6H, $C\underline{H}OH$, CH_2O), 4.67 (d, 2H, ^3J = 1.6 Hz, H-5). ES-MS (MeCN + TFA) *m/z (%)*: 729 (5) $[M-H]^-$. $C_{35}H_{55}NO_{15}$ (729.81).

7.6.2.12 General procedure for the synthesis of 2-*O*-acylated derivatives of 6-*O*-palmitoylascorbic acid 118-121

To a solution of 6-*O*-palmitoylascorbic acid (1 eq) and a catalytic amount of DMAP in a mixture of anhydrous THF and anhydrous CH_2Cl_2 was added a solution of the pertinent anhydride (1 eq) dissolved in anhydrous THF dropwise at 0 °C under an atmosphere of argon. After stirring at room temperature overnight the mixture was quenched with 1 N HCl and extracted three times with EtOAc. The combined organic phases were washed with brine and water, dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The products were purified by preparative HPLC.

6-*O*-Hexadecanoyl-2-*O*-(3-phenylpropanoyl)-L-ascorbic acid (7.118): The title compound was prepared by analogy with general procedure 7.6.2.12 using 6-*O*-palmitoylascorbic acid (0.5 mmol, 0.21 g), a catalytic amount of DMAP and 3-phenylpropanoyl chloride (0.5 mmol, 74 µl) together with NEt_3 (0.5 mmol, 69 µl) instead of the anhydride in anhydrous CH_2Cl_2 (30 ml) and anhydrous THF (10 ml). Compound **7.118** was obtained after preparative HPLC (0 min: MeCN/0.1 % TFA

(aq) 90/10, 20 min: 95/5 v/v) as a white semisolid substance (0.10 g, 37 %). mp: 95 °C; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^{3}J = 6.7 Hz, $CH_2C\underline{H}_3$), 1.25 (m, 24H, $(C\underline{H}_2)_{12}CH_3$), 1.63 (m, 2H, $COCH_2C\underline{H}_2$), 2.36 (t, 2H, ^{3}J = 7.6Hz, $COCH_2$), 2.98 (m, 4H, $CO(C\underline{H}_2)_2Ph$), 4.23 (m, 2H, -C<u>H</u>OH, CH_2O), 4.39 (dd, 1H, ^{3}J = 5.9 Hz, ^{2}J = 11.0 Hz, CH_2O), 4.82 (d, 1H, ^{3}J = 2.0 Hz, H-5), 7.27 (m, 5H, Ph-H). ^{13}C-NMR ($CDCl_3$) δ 14.19 (+, CH_3), 22.75 (-, CH_2), 24.88 (-, CH_2), 29.16 (-, CH_2), 29.30 (-, CH_2), 29.42 (-, CH_2), 29.50 (-, CH_2), 29.66 (-, CH_2), 29.71 (-, CH_2), 29.74 (-, 4 CH_2), 30.46 (-, CH_2), 31.98 (-, CH_2), 34.12 (-, CH_2), 35.39 (-, CH_2), 64.65 (-, CH_2O), 68.13 (+, CHO), 75.38 (+, CH), 115.24 (quat, C-2), 126.79 (+, Ph-C), 128.31 (+, 2 Ph-C), 128.78 (+, 2 Ph-C), 139.12 (quat, Ph-C), 155.71 (quat, C-3), 166.27 (quat, lactone CO), 173.06 (quat, $CH_2\underline{C}O_2$), 174.09 (quat, $CH_2\underline{C}O_2$). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 564 (100) $[M+NH_4]^+$. HRMS (PI-EIMS) calcd for $C_{31}H_{46}O_8$ $[M^{+\cdot}]$, 546.3193; found, 546.3190. Anal. ($C_{31}H_{46}O_8$) C, H. $C_{31}H_{46}O_8$ (546.69).

2-*O*-(3-Carboxypropanoyl)-6-*O*-hexadecanoyl-L-ascorbic acid (7.119): The title compound was prepared according to general method 7.6.2.12 using 6-*O*-palmitoylascorbic acid (0.5 mmol, 0.21 g), a catalytic amount of DMAP and succinic anhydride (0.5 mmol, 50 mg) in anhydrous CH_2Cl_2 (30 ml) and anhydrous THF (10 ml). Compound **7.119** was obtained after preparative HPLC (0 min: MeCN/0.1 % TFA (aq) 80/20, 20 min: 95/5 v/v) as a white solid (0.15 g, 58 %). mp: 117-119 °C; ^{1}H-NMR (CD_3OD) δ 0.89 (t, 3H, ^{3}J = 6.1 Hz CH_2CH_3), 1.28 (m, 24H, $(C\underline{H}_2)_{12}CH_3$), 1.64 (m, 2H, $COCH_2C\underline{H}_2$), 2.37 (t, 2H, ^{3}J = 7.4 Hz, $COCH_2$), 2.67 (t, 2H, ^{3}J = 6.7 Hz, $COCH_2$), 2.85 (t, 2H, ^{3}J = 6.7 Hz, $COCH_2$), 4.19 (m, 3H, CH_2O, C<u>H</u>OH), 4.92 (d, 1 H, under solvent peak, H-5). ^{13}C-NMR (CD_3OD) δ 14.53 (+, CH_3), 23.83 (-, CH_2), 26.06 (-, CH_2), 29.34 (-, CH_2), 29.48 (-, CH_2), 29.56 (-, CH_2), 30.27 (-, CH_2), 30.50 (-, CH_2), 30.57 (-, CH_2), 30.69 (-, CH_2), 30.81 (-, CH_2), 30.85 (-, CH_2), 33.16 (-, CH_2), 34.92 (-, CH_2), 65.58 (-, CH_2O), 67.99 (+, CHO), 77.99 (+, CH), 114.50 (quat, C-2), 165.07 (quat, C-3), 170.73 (quat, lactone CO), 171.36 (quat, $CH_2\underline{C}O_2$), 175.15 (quat, $CH_2\underline{C}O_2$), 175.84 (quat, $\underline{C}O_2H$). ES-MS (MeOH + NH_4OAc) *m/z (%)*: 532 (100) $[M+NH_4]^+$. HRMS (PI-LSIMS) calcd for $C_{26}H_{43}O_{10}$ $[M+H]^+$, 515.2856; found, 515.2854. Anal. ($C_{26}H_{42}O_{10}\cdot 0.8H_2O$) C, H. $C_{26}H_{42}O_{10}$ (514.61).

2-*O*-(4-Carboxybutanoyl)-6-*O*-hexadecanoyl-L-ascorbic acid (7.120): The title compound was prepared according to general method 7.6.2.12 using 6-*O*-palmitoylascorbic acid (0.5 mmol, 0.21 g), a catalytic amount of DMAP and glutaric anhydride (0.5 mmol, 57 mg) in anhydrous CH_2Cl_2 (30 ml) and anhydrous THF (10

ml). Compound **7.120** was obtained after preparative HPLC (0 min: MeCN/0.1 % TFA (aq) 85/15, 15 min: 95/5 v/v) as a white semisolid substance (0.2 g, 76 %). mp: 116-119 °C; ^{1}H-NMR (CD_3OD) δ 0.90 (t, 3H, ^{3}J = 6.6 Hz, $CH_2C\underline{H}_3$), 1.28 (m, 24H, $(C\underline{H}_2)_{12}CH_3$), 1.63 (m, 2H, $COCH_2C\underline{H}_2$), 1.96 (m, 2H, $COCH_2C\underline{H}_2CH_2CO_2H$), 2.40 (m, 4H, $COC\underline{H}_2CH_2C\underline{H}_2CO_2H$), 2.64 (t, 2H, ^{3}J = 7.3 Hz, $COCH_2$), 4.22 (m, 3H, CH_2O, $C\underline{H}OH$), 4.93 (d, 1H, ^{3}J = 1.6 Hz, H-5). ^{13}C-NMR (CD_3OD) δ 14.54 (+, CH_3), 21.05 (-, CH_2), 23.83 (-, CH_2), 26.06 (-, CH_2), 30.28 (-, CH_2), 30.51 (-, CH_2), 30.57 (-, CH_2), 30.70 (-, CH_2), 30.82 (-, CH_2), 30.86 (-, CH_2), 33.17 (-, CH_2), 33.20 (-, CH_2), 33.64 (-, CH_2), 34.92 (-, CH_2), 65.57 (-, CH_2O), 67.99 (+, CHO), 77.99 (+, CH), 114.53 (quat, C-2), 164.98 (quat, C-3), 170.83 (quat, lactone CO), 171.75 (quat, $CH_2\underline{C}O_2$), 175.14 (quat, $CH_2\underline{C}O_2$), 176.76 (quat, $\underline{C}O_2H$). ES-MS (MeCN + TFA) *m/z (%)*: 546 (100) $[M+H]^+$. Anal. ($C_{27}H_{44}O_{10}$) C, H. $C_{27}H_{44}O_{10}$ (528.63).

2-*O*-(5-Carboxypentanoyl)-6-*O*-hexadecanoyl-L-ascorbic acid (7.121): The title compound was prepared according to general method 7.6.2.12 using 6-*O*-palmitoylascorbic acid (0.5 mmol, 0.21 g), a catalytic amount of DMAP and oxepane-2,7-dione (0.5 mmol, 64 mg) which was prepared according to known procedures[45] in anhydrous CH_2Cl_2 (30 ml) and anhydrous THF (10 ml). Compound **7.121** was obtained after preparative HPLC (0 min: MeCN/0.1 % TFA (aq) 70/30, 30 min: 90/10 v/v) as a white semisolid substance (0.08 g, 29 %). mp: 102-104 °C; ^{1}H-NMR (CD_3OD) δ 0.89 (t, 3H, ^{3}J = 6.9 Hz, $CH_2C\underline{H}_3$), 1.29 (m, 24H, $(C\underline{H}_2)_{12}CH_3$), 1.67 (m, 4H, $(C\underline{H}_2)_2CH_2CO_2H$), 2.35 (m, 4H, $COCH_2$, $C\underline{H}_2CO_2H$), 2.58 (t, 2H, ^{3}J = 6.7 Hz, $COCH_2$), 4.19 (m, 3H, $C\underline{H}OH$, CH_2O), 4.91 (d, 1H, ^{3}J = 1.9 Hz, H-5). ^{13}C-NMR (CD_3OD) δ 14.41 (+, CH_3), 23.71 (-, CH_2), 25.09 (-, CH_2), 25.31 (-, CH_2), 25.96 (-, CH_2), 30.17 (-, CH_2), 30.39 (-, CH_2), 30.45 (-, CH_2), 30.58 (-, CH_2), 30.70 (-, CH_2), 30.74 (-, CH_2), 33.05 (-, CH_2), 33.68 (-, CH_2), 34.47 (-, CH_2), 34.85 (-, CH_2), 65.50 (-, CH_2O), 67.96 (+, CHO), 77.92 (+, CH), 114.52 (quat, C-2), 164.93 (quat, C-3), 170.77 (quat, lactone CO), 171.91 (quat, $CH_2\underline{C}O_2$), 175.09 (quat, $CH_2\underline{C}O_2$), 177.17 (quat, $\underline{C}O_2H$). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 541 (100) $[M-H]^-$. Anal. ($C_{28}H_{46}O_{10}$) C, H. $C_{28}H_{46}O_{10}$ (542.66).

2,3-*O*-Bis(5-carboxypentanoyl)-6-*O*-hexadecanoyl-L-ascorbic acid (7.121a): The title compound was obtained as byproduct in the synthesis of **7.121** as a white solid (40 mg, 12 %). mp: 80-81 °C; ^{1}H-NMR (CD_3OD) δ 0.90 (t, 3H, ^{3}J = 6.7 Hz, $CH_2C\underline{H}_3$), 1.30 (m, 24H, $(C\underline{H}_2)_{12}CH_3$), 1.66 (m, 10H, $COCH_2CH_2$, $COCH_2(C\underline{H}_2)_2CH_2CO_2H$), 2.35 (m, 6H, $COCH_2$, $C\underline{H}_2CO_2H$), 2.61 (m, 4H, $COCH_2$), 4.21 (m, 3H, $C\underline{H}OH$, CH_2O), 5.31

(d, 1H, ^{3}J = 1.5 Hz, H-5). ^{13}C-NMR (CD_3OD) δ 14.51 (+, CH_3), 23.80 (-, CH_2), 24.97 (-, CH_2), 25.18 (-, CH_2), 25.20 (-, CH_2), 25.29 (-, CH_2), 26.00 (-, CH_2), 30.25 (-, CH_2), 30.47 (-, CH_2), 30.54 (-, CH_2), 30.67 (-, CH_2), 30.80 (-, CH_2), 30.85 (-, CH_2), 33.14 (-, CH_2), 33.79 (-, CH_2), 34.05 (-, CH_2), 34.42 (-, CH_2), 34.49 (-, CH_2), 34.89 (-, CH_2), 65.36 (-, CH_2O), 67.50 (+, CHO), 78.08 (+, CH), 123.08 (quat, C-2), 153.14 (quat, C-3), 168.03 (quat, CO), 168.89 (quat, CO), 170.39 (quat, $CH_2\underline{C}O_2$), 175.06 (quat, $CH_2\underline{C}O_2$), 177.07 (quat, $\underline{C}O_2H$), 177.13 (quat, $\underline{C}O_2H$). HRMS (PI-LSIMS) calcd. for $C_{34}H_{55}O_{13}$ $[M+H]^+$, 671.3543; found, 671.3656. Anal. ($C_{34}H_{54}O_{13}\cdot 1.5H_2O$) C, H. $C_{34}H_{54}O_{13}$ (670.78).

2,3-*O*-Dibenzyl-6-*O*-(4-methylphenylsulfonyl)-L-ascorbic acid[40] (7.122): To a solution of **7.66** (42 mmol, 15.1 g) in pyridine (40 ml) and CH_2Cl_2 (130 ml) was added a solution of *p*-toluenesulfonyl chloride (46.2 mmol, 8.81 g) in CH_2Cl_2 (80 ml) at 0 °C dropwise over a period of 2 h. After stirring overnight CH_2Cl_2 (340 ml) was added and the solution was washed twice with water, dried over $MgSO_4$, filtered and evaporated under reduced pressure. Recrystallization of the crude product yielded the title compound as colorless crystals (13.3 g, 62 %). mp: 122-123 °C (ref.[40]: 133-134 °C); ^{1}H-NMR ($CDCl_3$) δ 2.44 (s, 3H, CH_3) 4.12 (m, 3H, CH_2O, C$\underline{H}$OH), 4.65 (s, 1H, H-5), 5.06 (s, 2H, C$\underline{H}_2$Ph), 5.11 (d, 1H, ^{2}J = 11.6 Hz, C$\underline{H}_2$Ph), 5.19 (d, 1H, ^{2}J = 11.6 Hz, C$\underline{H}_2$Ph), 7.21 (m, 2H, Ph-H), 7.34 (m, 10H, Ar-H), 7.77 (m, 2H, Ar-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 528 (100) $[M+NH_4]^+$. $C_{27}H_{26}O_8S$ (510.56).

(5*R*)-3,4-Bis(benzyloxy)-5-[(1*R*)-2-bromo-1-hydroxyethyl]-2,5-dihydrofuran-2-one[40] (7.123): A mixture of **7.122** (25 mmol, 12.8 g), NaBr (30 mmol, 3.1 g) in anhydrous acetone (300 ml) was stirred at 120 °C in an autoclave for 7 h. After cooling to room temperature solids were removed by filtration and the solvent was evaporated to yield the title compound as a brown oil (10.3 g, 98 %) which was used without further purification in the next step. CI-MS (NH_3) *m/z (%)*: 436 (100) $[M+NH_4]^+$. $C_{20}H_{19}BrO_5$ (419.27).

(5*R*)-3,4-Bis(benzyloxy)-5-[(2*S*)-oxiran-2-]-2,5-dihydrofuran-2-one[40] (7.124): A mixture of **7.123** (20 mmol, 8.4 g), KOH (25 mmol, 1.4 g) and $N(Bu)_4I$ (100 mg) in CH_2Cl_2 (200 ml) and water (30 ml) was vigorously stirred for 1 h. After separation of the water from the organic layer, the organic phase was washed twice with brine and once with water, dried over K_2CO_3, filtered and the solvent was removed under reduced pressure. The raw product was submitted to flash chromatography to yield the title compound as white solid (5.3 g, 78 %). mp: 48-49 °C (ref.[40]: 86-87 °C); ^{1}H-

NMR ($CDCl_3$) δ 2.84 (m, 2H, CHC<u>H</u>$_2$O), 3.12 (ddd, 1H, ^3J = 2.7 Hz, ^3J = 4.0 Hz, ^3J = 4.0 Hz, CHO), 4.56 (d, 1H, ^3J = 3.9 Hz, H-5), 5.06 – 5.23 (m, 4H, C<u>H</u>$_2$Ph), 7.20 (m, 2H, Ph-H), 7.37 (m, 8H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 356 (100) $[M+NH_4]^+$. $C_{20}H_{18}O_5$ (338.35).

7.6.2.13 General procedure for the synthesis of 6-*O*-alkylated ascorbic acids 125 and 126

A solution of **7.124** (1 eq), the pertinent alcohol (1.5 eq) and BF_3Et_2O (0.13 eq) in anhydrous CH_2Cl_2 was stirred at room temperature for 48 h. After dilution with CH_2Cl_2 the solution was washed with saturated $NaHCO_3$ and water, dried over $MgSO_4$, filtered and the solvent was evaporated. The raw product was subjected to flash chromatography.

2,3-Di-*O*-benzyl-6-*O*-(dodecan-1-yl)-L-ascorbic acid (7.125): The title compound was prepared according to general procedure 7.6.2.13 using **7.124** (1 mmol, 0.34 g), 1-dodecanol (1.5 mmol, 0.28 g) and BF_3Et_2O (0.13 mmol, 16 µl) in CH_2Cl_2 (15 ml). After flash chromatography ($CHCl_3$/MeOH 98/2 v/v) compound **7.125** was obtained as a colorless oil (0.28 g, 53 %). ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.7 Hz, CH_3), 1.25 (m, 18H, (C<u>H</u>$_2$)$_9$CH$_3$), 1.54 (m, 2H, OCH$_2$C<u>H</u>$_2$CH$_2$), 3.44 (t, 1H, ^3J = 6.7 Hz, OC<u>H</u>$_2$CH$_2$), 3.54 (m, 2H, C<u>H</u>$_2$OCH$_2$CH$_2$), 4.02 (m, 1H, C<u>H</u>OH), 4.72 (d, 1H, ^3J = 2.0 Hz, H-5), 5.09 (s, 2H, C<u>H</u>$_2$Ph), 5.13 (d, 1H, ^2J = 11.7 Hz, C<u>H</u>$_2$Ph), 5.21 (d, 1H, ^2J = 11.7 Hz, C<u>H</u>$_2$Ph), 7.22 (m, 2H, Ph-H), 7.36 (m, 10H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 542 (100) $[M+NH_4]^+$. $C_{32}H_{44}O_6$ (524.69).

2,3-Di-*O*-benzyl-6-*O*-(octandecan-1-yl)-L-ascorbic acid (7.126): The title compound was prepaded according to general procedure 7.6.2.13 using **7.124** (1 mmol, 0.34 g), 1-octadecanol (1.5 mmol, 0.41 g) and BF_3Et_2O (0.13 mmol, 16 µl) in CH_2Cl_2 (15 ml). After flash chromatography ($CHCl_3$/MeOH 98/2 v/v) compound **7.126** was obtained as a colorless oil (0.38 g, 53 %). ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.7 Hz, CH_3), 1.25 (m, 30H, (C<u>H</u>$_2$)$_{15}$CH$_3$), 1.54 (m, 2H, OCH$_2$C<u>H</u>$_2$CH$_2$), 3.44 (t, 2H, ^3J = 6.7 Hz, OC<u>H</u>$_2$CH$_2$), 3.58 (m, 2H, C<u>H</u>$_2$OCH$_2$CH$_2$), 4.02 (ddd, 1H, ^3J = 2.1 Hz, ^3J = 6.4 Hz, ^3J = 6.4 Hz, C<u>H</u>OH), 4.72 (d, 1H, ^3J = 2.1 Hz, H-5), 5.10 (s, 2H, C<u>H</u>$_2$Ph), 5.14 (d, 1H, ^2J = 11.8 Hz, C<u>H</u>$_2$Ph), 5.21 (d, 1H, ^2J = 11.7 Hz, C<u>H</u>$_2$Ph), 7.22 (m, 2H, Ph-H), 7.36 (m, 8H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 626 (100) $[M+NH_4]^+$. $C_{38}H_{56}O_6$ (608.85).

6-*O*-(Dodecan-1-yl)-L-ascorbic acid (7.127): The title compound was prepared from **7.125** (0.42 mmol, 0.22 g), 10 % Pd/C (30 mg) in EtOH (5 ml) and EtOAc (5 ml) according to general procedure 7.6.2.11. After recystallization from TBME/*n*-hexane compound **7.127** was obtained as a white solid (0.10 g, 69 %). mp: 105-106 °C; ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H, ^3J = 6.6 Hz, CH_3), 1.24 (m, 18H, $(C\underline{H}_2)_9CH_3$), 1.49 (m, 2H, $OCH_2C\underline{H}_2CH_2$), 3.40 (m, 4H, $C\underline{H}_2OC\underline{H}_2$), 3.87 (m, 1H, C<u>H</u>OH), 4.64 (d, 1H, ^3J = 1.3 Hz, H-5), 5.03 (s, 1H, CHO<u>H</u>), 8.35 (s, 1H, OH), 11.03 (s, 1H, OH). ^{13}C-NMR (DMSO-d_6) δ 13.90 (+, CH_3), 22.04 (-, CH_2), 25.54 (-, CH_2), 28.65 (-, CH_2), 28.81 (-, CH_2), 28.95 (-, CH_2), 29.07 (-, CH_2), 31.23 (-, CH_2), 65.97 (+, CHO), 70.46 (-, OCH_2), 70.84 (-, OCH_2), 74.90 (+, CH), 117.93 (quat, C-2), 152.47 (quat, C-3), 170.45 (quat, lactone CO). CI-MS (NH_3) *m/z (%)*: 362 (100) $[M+NH_4]^+$. Anal. ($C_{18}H_{32}O_6$) C, H. $C_{18}H_{32}O_6$ (344.44).

6-*O*-(Octandecan-1-yl)-L-ascorbic acid (7.128): The title compound was prepared from **7.126** (0.57 mmol, 0.35 g), 10 % Pd/C (30 mg) in EtOH (5 ml) and EtOAc (5 ml) according to general procedure 7.6.2.11. After recystallization from IPE compound **7.128** was obtained as a white solid (0.20 g, 81 %). mp: 104-106 °C; ^{1}H-NMR (CD_3OD + $CDCl_3$), 0.89 (t, 3H, ^3J = 6.7 Hz, CH_3), 1.28 (s, 30H, $(C\underline{H}_2)_{15}CH_3$), 1.58 (m, 2H, $OCH_2C\underline{H}_2CH_2$), 3.52 (m, 4H, $C\underline{H}_2OC\underline{H}_2$), 4.01 (ddd, 1H, ^3J = 1.8 Hz, ^3J = 6.8 Hz, ^3J = 6.8 Hz, C<u>H</u>OH), 4.75 (d, 1H, ^3J = 1.9 Hz, H-5). ^{13}C-NMR (DMSO-d_6) δ 13.87 (+, CH_3), 22.03 (-, CH_2), 25.55 (-, CH_2), 28.64 (-, CH_2), 28.82 (-, CH_2), 28.96 (-, CH_2), 29.08 (-, CH_2), 31.22 (-, CH_2), 66.00 (+, CHO), 70.48 (-, OCH_2), 70.86 (-, OCH_2), 74.89 (+, CH), 117.95 (quat, C-2), 152.42 (quat, C-3), 170.41 (quat, lactone CO). ES-MS (MeCN + TFA) *m/z (%)*: 429 (100) $[M+H]^+$. Anal. ($C_{24}H_{44}O_6$) C, H. $C_{24}H_{44}O_6$ (428.60).

2,3-*O*-Bis(benzyloxycarbonylmethyl)-5-*O*-,6-*O*-isopropylidene-L-ascorbic acid (7.129): The title compound was prepared from **7.64** (25.0 mmol, 4.40 g), potassium carbonate (27.5 mmol, 3.80 g) and benzyl bromoacetate (55.0 mmol, 12.60 g) in anhydrous DMF (75 ml) according to general 7.6.2.8. Flash chromatography (PE/EtOAc 70/30 v/v) yielded compound **7.129** as a white solid (10.9 g, 85 %). mp: 64 °C; ^{1}H-NMR ($CDCl_3$) δ 1.35 (s, 3H, $C(CH_3)_2$), 1.38 (s, 3H, $C(CH_3)_2$), 4.03 (dd, 1H, ^3J = 6.4 Hz, ^2J = 8.7 Hz, CH_2O), 4.13 (dd, 1H, ^3J = 6.7 Hz, ^2J = 8.6 Hz, CH_2O), 4.26 (ddd, 1H, ^3J = 4.2 Hz, ^3J = 6.5 Hz, ^3J = 6.5 Hz, CHO), 4.62 (d, 1H, ^3J = 4.2 Hz, H-5), 4.71 (d, 1H, ^2J = 16.8 Hz, OCH_2CO_2), 4.80 (d, 1H, ^2J = 16.8 Hz, OCH_2CO_2), 5.10 - 8.28 (m, 6H, OCH_2CO_2, $C\underline{H}_2Ph$), 7.34 (m, 10H, Ph-H). ES-MS (DCM/MeOH +

NH_4OAc) *m/z (%)*: 530 (100) $[M+NH_4]^+$. Anal. ($C_{27}H_{28}O_{10}\cdot 0.1CH_2Cl_2$) C, H. $C_{27}H_{28}O_{10}$ (512.51).

7.6.2.14 General procedure for the synthesis of 5,6-*O*-unprotected ascorbic acid derivatives 130 and 139-142 by acid catalyzed acetal cleavage

The pertinent acetal was stirred in a mixture of methanol, THF and 2 N HCl for 6 h. After evaporating the solvent the remaining residue was taken up with water and extracted tree times with EtOAc. The combined organic layers were washed with water, dried over $MgSO_4$, filtered and evaporated under reduced pressure. The raw product was purified, if necessary, using flash chromatography

2,3-*O*-Bis(benzyloxycarbonylmethyl)-L-ascorbic acid (7.130): The title compound was prepared according to general procedure 7.6.2.14 using **7.129** (2.54 mmol, 1.30 g) in MeOH (4 ml), THF (4 ml) and 2 N HCl (2,5 ml) and was obtained after flash chromatography (PE/EtOAc 2/1 v/v) as a colorless oil (0.81 g, 68 %). ^{1}H-NMR ($CDCl_3$) δ 2.45 (dd, 1H, ^3J = 3.9 Hz, ^3J = 7.9 Hz, $CH_2O\underline{H}$), 3.68 (d, 1H, ^3J = 4.7 Hz, $CHO\underline{H}$), 3.75 (m, 1H), 3.86 (ddd, 1H, ^3J = 3.3 Hz, ^3J = 6.9 Hz, ^2J = 10.4 Hz, $C\underline{H}_2OH$), 4.11 (ddd, 1H, ^3J = 5.0 Hz, ^3J = 6.8 Hz, ^2J = 6.8 Hz, $C\underline{H}_2OH$), 4.63 (d, 1H, ^2J = 16.9 Hz, $OC\underline{H}_2$), 4.76 (d, 1H, ^3J = 2.0 Hz, H-5), 4.85 (d, 2H, ^2J = 16.8 Hz, $OC\underline{H}_2$), 5.14 (s, 2H, $OC\underline{H}_2$), 5.20 (s, 2H, $OC\underline{H}_2$), 5.64 (d, 1H, ^2J = 16.6 Hz, $OC\underline{H}_2$), 7.34 (m, 10H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 490 (100) $[M+NH_4]^+$. $C_{24}H_{24}O_{10}$ (472.44).

2,3-*O*-Bis(benzyloxycarbonylmethyl)-6-*O*-dodecanoyl-L-ascorbic acid (7.131): The title compound was prepared from **7.130** (1.0 mmol, 0.47 g), lauric acid (1.0 mmol, 0.20 g), DMAP (1.2 mmol, 0.15 g) and EDAC (1.1 mmol, 0.21 g) in anhydrous DMF (10 ml) according to general procedure 7.6.2.9 and was obtained after flash chromatography (PE/EtOAc 80/20 to 60/40 v/v) as a white solid (0.20 g, 31 %). mp: 44 °C; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.6 Hz, $CH_2C\underline{H}_3$) 1.26 (m, 16H, $(C\underline{H}_2)_4CH_3$), 1.63 (m, 2H, $COCH_2C\underline{H}_2CH_2$), 2.34 (t, 2H, ^3J = 7.5 Hz, $COC\underline{H}_2CH_2$), 4.30 (m, 3H, CH_2O, $C\underline{H}OH$), 4.62 (d, 1H, ^2J = 16.9 Hz, OCH_2CO_2), 4.76 (d, 1H, ^3J = 1.3 Hz, H-5), 4.81 (d, 1H, ^2J = 16.7 Hz, OCH_2CO_2), 4.87 (d, 1H, ^2J = 17.0 Hz, OCH_2CO_2), 5.14 (s, 2H, CH_2Ph), 5.20 (s, 2H, CH_2Ph) ppm 5.69 (d, 1H, ^2J = 16.7 Hz, OCH_2CO_2), 7.34 (m, 10H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 672 (100) $[M+NH_4]^+$. Anal. ($C_{36}H_{46}O_{11}$) C, H. $C_{36}H_{46}O_{11}$ (654.74).

2,3-*O*-Bis(carboxymethyl)-6-*O*-dodecanoyl-L-ascorbic acid (7.132): The title compound was prepared from **7.131** (0.18 mmol, 0.12 g), 10 % Pd/C (30 mg) in

EtOH (3 ml) and EtOAc (3 ml) according to general procedure 7.6.2.11 and was obtained as a white solid (80 mg, 94 %). mp: 89-90 °C; ^{1}H-NMR (CD_3OD) δ 0.89 (t, 3H, ^{3}J = 6.7 Hz, $CH_2C\underline{H}_3$), 1.29 (s, 16H, $(C\underline{H}_2)_4CH_3$), 1.62 (m, 2H, $COCH_2C\underline{H}_2CH_2$), 2.37 (t, 2H, ^{3}J = 7.4 Hz, $COC\underline{H}_2CH_2$), 4.21 (m, 3H, CH_2O, $C\underline{H}OH$), 4.64 (d, 1H, ^{2}J = 16.5 Hz, $OC\underline{H}_2CO_2H$), 4.71 (d, 1H, ^{2}J = 16.5 Hz, $OC\underline{H}_2CO_2H$), 4.88 (d, 1H, ^{3}J = 1.7 Hz, H-5), 5.20 (d, 1H, ^{2}J = 16.5 Hz, $OC\underline{H}_2CO_2H$), 5.29 (d, 1H, ^{2}J = 16.5 Hz, $OC\underline{H}_2CO_2H$). ^{13}C-NMR (CD_3OD) δ 14.53 (+, CH_3), 23.82 (-, CH_2), 26.05 (-, CH_2), 30.26 (-, CH_2), 30.48 (-, CH_2), 30.55 (-, CH_2), 30.68 (-, CH_2), 30.81 (-, CH_2), 33.15 (-, CH_2), 34.93 (-, $CO\underline{C}H_2CH_2$), 65.35 (-, CH_2), 67.91 (+, CHO), 68.16 (-, CH_2), 68.86 (-, CH_2), 77.21 (+, CH), 122.92 (quat, C-2), 157.19 (quat, C-3), 170.53 (quat, lactone CO), 172.33 (quat, $\underline{C}O_2CH_2$), 175.14 (quat, CO_2H). ES-MS ($MeCN/H_2O$/MeOH + NH_4OAc) *m/z (%)*: 492 (100) $[M+NH_4]^+$. Anal. ($C_{22}H_{34}O_{11}$) C, H. $C_{22}H_{34}O_{11}$ (474.50).

7.6.2.15 General procedure for the synthesis of 3-*O*-mono-alkylated 5,6-*O*-isopropylidene-L-ascorbic acid derivatives 133 and 134

K_2CO_3 (1 eq) was added to a solution of **7.64** (1 eq) in THF/DMF. After stirring for 10 min at room temperature an alkyl halide was added dropwise. Stirring was continued for 3 h and then the reaction was quenched by addition of water. The mixture was neutralized with 1 N HCl and extracted twice with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, concentrated and subjected to flash chromatography.

3-*O*-Benzyl-5,6-*O*-isopropylidene-L-ascorbic acid[39] (7.133): The title compound was prepared according to general procedure 7.6.2.15 using **7.64** (50 mmol, 10.81 g), K_2CO_3 (50 mmol, 6.91 g) and benzyl bromide (50 mmol, 5.94 ml) in anhydrous THF (30 ml) and anhydrous DMF (10 ml). After flash chromatography (PE/EtOAc 80/20 v/v) and recrystallization from IPE the product was obtained as a white solid (7.0 g, 46 %). mp: 89-91 °C (ref.[39]: 105-106 °C); ^{1}H-NMR ($CDCl_3$) δ 1.36 (s, 3H, $C(CH_3)_2$), 1.39 (s, 3H, $C(CH_3)_2$), 4.02 (dd, 1H, ^{3}J = 6.8 Hz, ^{2}J = 8.6 Hz, CH_2O), 4.10 (dd, 1H, ^{3}J =6.7Hz, ^{2}J =8.6Hz, CH_2O), 4.26 (ddd, 1H, ^{3}J = 3.7 Hz, ^{3}J = 6.7 Hz, ^{3}J = 6.7 Hz, CHO), 4.57 (d, 1H, ^{3}J = 3.7 Hz, H-5), 5.49 (d, 1H, ^{2}J = 11.8 Hz, $C\underline{H}_2Ph$), 5.53 (d, 1H, ^{2}J = 11.8 Hz, $C\underline{H}_2Ph$), 7.39 (m, 5H, Ph-H). CI-MS (NH_3) *m/z (%)*: 324 (100) $[M+NH_4]^+$. $C_{16}H_{18}O_6$ (306.31).

3-*O*-(Benzyloxycarbonylethyl)-5,6-*O*-isopropylidene-L-ascorbic acid (7.134): The title compound was prepared according to general procedure 7.6.2.15 using **7.64**

(15 mmol, 3.24 g), K_2CO_3 (15 mmol, 2.07 g) and benzyl bromoacetate (15 mmol, 3.44 ml) in anhydrous THF (10 ml) and anhydrous DMF (3 ml). Flash chromatography (PE/EtOAc 70/30 to 60/40 v/v) yielded a colorless oil (1.4 g, 26 %). ^{1}H-NMR ($CDCl_3$) δ 1.36 (s, 3H, $C(CH_3)_2$), 1.39 (s, 3H, $C(CH_3)_2$), 4.10 (m, 2H, CH_2O), 4.27 (ddd, 1H, ^3J = 4.2 Hz, ^3J = 6.6 Hz, ^3J = 6.6 Hz, CHO), 4.67 (d, 1H, ^3J = 4.2 Hz, H-5), 4.95 (d, 1H, ^2J = 16.4 Hz, OCH_2CO_2), 5.06 (d, 1H, ^2J = 16.4 Hz, OCH_2CO_2), 5.24 (s, 2H, $C\underline{H}_2Ph$), 5.95 (bs, 1H, OH), 7.36 (m, 5H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 382 (100) $[M+NH_4]^+$. Anal. ($C_{18}H_{20}O_8 \cdot 0.9CH_2Cl_2$) C, H. $C_{18}H_{20}O_8$ (364.35).

7.6.2.16 General procedure for the synthesis of 2-*O*-alkylated 3-*O*-alkyl,5,6-*O*-isopropylidene-L-ascorbic acid derivatives 135-138

K_2CO_3 (1.1 eq) and the pertinent alkyl halide (1.1 eq) were added to a solution of the corresponding ascorbic acid derivative (1 eq) in anhydrous THF/DMSO. After stirring for 3 h at 50 °C the reaction was quenched by addition of water. The mixture was neutralized with 1 N HCl and extracted three times with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and subjected to flash chromatography.

3-*O*-Benzyl-2-*O*-(dodecan-1-yl)-5-*O*-,6-*O*-isopropylidene-L-ascorbic acid[97] (7.135): The title compound was prepared according to general procedure 7.6.2.16 using **7.133** (2 mmol, 0.61 g), K_2CO_3 (2.2 mmol, 0.30 g) and 1-bromododecane (2.2 mmol, 0.53 ml) in anhydrous THF (2 ml) and DMSO (2 ml). After flash chromatography (PE/EtOAc 80/20 v/v) the product was obtained as a colorless oil (0.55 g, 58 %). ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.7 Hz, $CH_2C\underline{H}_3$), 1.26 (m, 18H, $CH_2(C\underline{H}_2)_9CH_3$), 1.36 (s, 3H, $C(CH_3)_2$), 1.39 (s, 3H, $C(CH_3)_2$), 1.65 (m, 2H, $COCH_2C\underline{H}_2$), 4.06 (m, 4H, CH_2O, $OC\underline{H}_2CH_2$), 4.29 (ddd, 1H, ^3J = 3.2 Hz, ^3J = 6.7 Hz, ^3J = 6.7 Hz, CHO), 4.54 (d, 1H, ^3J = 3.2 Hz, H-5), 5.48 (s, 2H, $C\underline{H}_2Ph$), 7.38 (m, 5H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 492 (100) $[M+NH_4]^+$. $C_{28}H_{42}O_6$ (474.63).

3-*O*-Benzyl-2-*O*-(hexadecan-1-yl)-5,6-*O*-isopropylidene-L-ascorbic acid[98] (7.136): The title compound was prepared according to general procedure 7.6.2.16 using **7.133** (2 mmol, 0.61 g), K_2CO_3 (2.2 mmol, 0.30 g) and 1-bromohexadecane (2.2 mmol, 0.67 ml) in anhydrous THF (2 ml) and DMSO (2 ml). After flash chromatography (PE/EtOAc 90/10 to 80/20 v/v) the product was obtained as a pale

yellow oil (0.43 g, 41 %). ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.7 Hz, $CH_2C\underline{H}_3$), 1.32 (m, 32H, $CH_2(C\underline{H}_2)_{13}CH_3$), 1.65 (m, 2H, $OCH_2C\underline{H}_2$), 4.05 (m, 4H, $OC\underline{H}_2CH_2$, CH_2O), 4.29 (ddd, 1H, ^3J = 3.2 Hz, ^3J = 6.7 Hz, ^3J = 6.7 Hz, CHO), 4.54 (d, 1H, ^3J = 3.2 Hz, H-5), 5.48 (s, 2H, CH_2Ph), 7.38 (m, 5H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 548 (100) $[M+NH_4]^+$. Anal. ($C_{32}H_{50}O_6$) C, H. $C_{32}H_{50}O_6$ (530.74).

2-*O*-(Benzyloxycarbonylmethyl)-3-*O*-benzyl-5,6-*O*-isopropylidene-L-ascorbic acid (7.137): The title compound was prepared according to general procedure 7.6.2.16 using **7.133** (3 mmol, 0.92 g), K_2CO_3 (3.3 mmol, 0.46 g) and benzyl bromoacetate (3.3 mmol, 0.76 g) in anhydrous THF (3 ml) and DMSO (3 ml). After flash chromatography (PE/EtOAc 80/20 to 70/30 v/v) the product was obtained as a pale yellow oil (1.20 g, 88 %). ^{1}H-NMR ($CDCl_3$) δ 1.35 (s, 3H, $C(CH_3)_2$), 1.37 (s, 3H, $C(CH_3)_2$), 4.01 (dd, 1H, ^3J = 6.7 Hz, ^2J = 8.6 Hz, CH_2O), 4.09 (dd, 1H, ^3J = 6.8 Hz, ^2J = 8.3 Hz, CH_2O), 4.28 (ddd, 1H, ^3J = 3.4 Hz, ^3J = 6.7 Hz, ^3J = 6.7 Hz, CHO), 4.54 (d, 1H, ^3J = 3.4 Hz, H-5), 4.72 (d, 1H, ^2J = 16.6 Hz, OCH_2CO_2), 4.86 (d, 1H, ^2J = 16.6 Hz, OCH_2CO_2), 5.18 (d, 1H, ^2J = 12.1 Hz, $OC\underline{H}_2Ph$), 5.23 (d, 1H, ^2J = 12.1 Hz, $OC\underline{H}_2Ph$), 5.58 (d, 1H, ^2J = 11.9 Hz, $OC\underline{H}_2Ph$), 5.66 (d, 1H, ^2J = 11.9 Hz, $OC\underline{H}_2Ph$), 7.35 (m, 10H, Ph-H). CI-MS (NH_3) *m/z (%)*: 472 (100) $[M+NH_4]^+$. Anal. ($C_{25}H_{26}O_8 \cdot 0.3CH_2Cl_2$) C, H. $C_{25}H_{26}O_8$ (454.47).

2-*O*-Benzyl-3-*O*-(benzyloxycarbonylmethyl)-5,6-*O*-isopropylidene-L-ascorbic acid (7.138): The title compound was prepared according to to general procedure 7.6.2.16 using **7.134** (2.23 mmol, 0.82 g), K_2CO_3 (2.46 mmol, 0.34 g) and benzyl bromide (2.45 mmol, 0.29 ml) in anhydrous THF (3 ml) and DMSO (3 ml). After flash chromatography (PE/EtOAc 70/30 v/v) the product was obtained as a colorless oil (0.83 g, 82 %). ^{1}H-NMR ($CDCl_3$) δ 1.36 (s, 3H, $C(CH_3)_2$), 1.41 (s, 3H, $C(CH_3)_2$), 4.07 (m, 2H, CH_2O), 4.25 (ddd, 1H, ^3J = 3.7 Hz, ^3J = 6.6 Hz, ^3J = 6.6 Hz, CHO), 4.70 (m, 3H, H-5, OCH_2CO_2), 5.08 (m, 4H, $C\underline{H}_2Ph$), 7.32 (m, 10H, Ar-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 472 (100) $[M+NH_4]^+$. Anal. ($C_{25}H_{26}O_8 \cdot 0.3H_2O$) C, H. $C_{25}H_{26}O_8$ (454.47).

2-*O*-(Dodecan-1-yl)-L-ascorbic acid[95] (7.139): The title compound was prepared according to general procedure 7.6.2.14 using **7.135** (0.83 mmol, 0.40 g) in MeOH (3 ml), THF (1 ml) and 2 N HCl (1 ml). The obtained colorless oil was hydrogenated according to general procedure 7.6.2.11 in EtOH (3 ml) and EtOAc (3 ml) using 10 % Pd/C (50 mg). Recrystallization from EtOAc/IPE yielded a white solid (0.20 g, 70 %). mp: 117-119 °C (ref.[98]: 127-128 °C); ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H, ^3J = 6.5 Hz,

$CH_2C\underline{H}_3$), 1.24 (m, 18H, $CH_2(C\underline{H}_2)_9CH_3$), 1.57 (m, 2H, $COCH_2C\underline{H}_2$), 3.42 (m, 2H, CH_2O), 3.83 (m, 3H, $OC\underline{H}_2CH_2$, C<u>H</u>OH), 4.76 (d, 1H, ^3J = 1.0 Hz, H-5), 4.93 (s, 1H, OH), 11.58 (s, 1H, OH). ^{13}C-NMR (DMSO-d_6) δ 13.90 (+, CH_3), 22.04 (-, CH_2), 25.10 (-, CH_2), 28.66 (-, CH_2), 28.74 (-, CH_2), 28.96 (-, CH_2), 31.24 (-, CH_2), 61.74 (-, CH_2OH), 68.27 (+, CHOH), 70.93 (-, OCH_2), 74.45 (+, CH), 119.79 (quat, C-2), 159.01 (quat, C-3), 169.65 (quat, $\underline{C}O_2CH_2$). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 362 (100) $[M+NH_4]^+$. $C_{18}H_{32}O_6$ (344.44).

2-*O*-(Hexadecan-1-yl)-L-ascorbic acid[97] (7.140): The title compound was prepared according to general procedure 7.6.2.14 using **7.136** (0.64 mmol, 0.34 g) in MeOH (3 ml), THF (1 ml) and 2 N HCl (1 ml). The obtained pale yellow oil was hydrogenated according to general procedure 7.6.2.11 in EtOH (3 ml) and EtOAc (3 ml) using 10 % Pd/C (50 mg). Recrystallization from EtOAc/IPE yielded a white solid (0.13 g, 51 %). mp: 118-120 °C (ref.[98]: 128-129 °C); ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H, ^3J = 6.4 Hz, $CH_2C\underline{H}_3$), 1.24 (m, 26H, $CH_2(C\underline{H}_2)_{13}CH_3$), 1.57 (m, 2H, $COCH_2C\underline{H}_2$), 3.43 (dd, 2H, ^3J = 6.8 Hz, ^2J = 12.6 Hz, CH_2O), 3.83 (m, 3H, $OC\underline{H}_2CH_2$, C<u>H</u>OH), 4.75 (d, 1H, ^3J = 0.8 Hz, H-5), 4.93 (bs, 1H, OH), 11.51 (bs, 1H, OH). ^{13}C-NMR (DMSO-d_6) δ 13.89 (+, CH_3), 22.03 (-, CH_2), 25.11 (-, CH_2), 28.65 (-, CH_2), 28.76 (-, CH_2), 28.99 (-, CH_2), 31.23 (-, CH_2), 61.73 (-, CH_2OH), 68.28 (+, CHOH), 70.92 (-, OCH_2), 74.48 (+, CH), 119.74 (quat, C-2), 159.14 (quat, C-3), 169.67 (quat, $\underline{C}O_2CH_2$). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 401 (100) $[M+NH_4]^+$. Anal. ($C_{22}H_{40}O_6$) C, H. $C_{22}H_{40}O_6$ (400.55).

2-*O*-(Benzyloxycarbonylmethyl)-3-*O*-benzylascorbic acid (7.141): The title compound was prepared according to general procedure 7.6.2.14 using **7.137** (2.3 mmol, 1.1 g) in MeOH (3 ml), THF (3 ml) and 2 N HCl (2 ml). Flash chromatography (PE/EtOAc 3/1 v/v) yielded a white solid (0.64 g, 67 %). mp: 93 °C; ^{1}H-NMR (DMSO-d_6) δ 3.43 (m, 2H, CH_2O), 3.72 (ddd, 1H, ^3J = 1.0 Hz, ^3J = 7.5 Hz, ^3J = 7.3 Hz, C<u>H</u>OH), 4.71 (d, 1H, ^2J = 16.2 Hz, OCH_2CO_2), 4.77 (d, 1H, ^2J = 16.2Hz, OCH_2CO_2), 4.89 (m, 2H, H-5, OH), 5.17 (m, 3H, $OC\underline{H}_2$Ph, OH), 5.50 (d, 1H, ^2J = 11.8 Hz, $OC\underline{H}_2$Ph), 5.61 (d, 1H, ^2J = 11.8 Hz, $OC\underline{H}_2$Ph), 7.38 (m, 10H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 415 (100) $[M+H]^+$. Anal. ($C_{22}H_{22}O_8$) C, H. $C_{22}H_{22}O_8$ (414.41).

2-*O*-Benzyl-3-*O*-(benzyloxycarbonylmethyl)ascorbic acid (7.142): The title compound was prepared according to general procedure 7.6.2.14 using **7.138** (1.67 mmol, 0.76 g) in MeOH (3 ml), THF (3 ml) and 2 N HCl (2 ml). Flash chromatography (PE/EtOAc 2/1 v/v) yielded a white solid (0.50 g, 72 %). mp: 77 °C; ^{1}H-NMR ($CDCl_3$)

δ 2.33 (bs, 1H, $CH_2O\underline{H}$), 3.51 (d, 1H, ^3J = 3.8 Hz, $CHO\underline{H}$), 3.90 (m, 3H, $C\underline{H}_2OH$, $C\underline{H}OH$), 4.61 (d, 1H, ^2J = 16.5 Hz, $OC\underline{H}_2CO_2$), 4.75 (d, 1H, ^3J = 2.0 Hz, H-5), 5.09 (m, 5H, $OC\underline{H}_2CO_2$, $C\underline{H}_2Ph$), 7.31 (m, 10H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 432 (100) $[M+NH_4]^+$. Anal. ($C_{22}H_{22}O_8$) C, H. $C_{22}H_{22}O_8$ (414.41).

2-*O*-(2-Benzyloxycarbonylmethyl)-3-*O*-benzyl-6-*O*-dodecanoylascorbic acid (7.143): The title compound was prepared from **7.141** (1.0 mmol, 0.41 g), lauric acid (1.0 mmol, 0.20 g), DMAP (1.2 mmol, 0.15 g) and EDAC (1.1 mmol, 0.21 g) in anhydrous DMF (6 ml) according to general procedure 7.6.2.9 and was obtained after flash chromatography (PE/EtOAc 80/20 v/v) as a colorless oil (0.21 g, 35 %). ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.6Hz, $CH_2C\underline{H}_3$), 1.26 (m, 16H, $(C\underline{H}_2)_8CH_3$), 1.62 (m, 2H, $COCH_2C\underline{H}_2CH_2$), 2.33 (t, 2H, ^3J = 7.6Hz, $COC\underline{H}_2CH_2$), 4.09 – 4.36 (m, 3H, CH_2O-, $C\underline{H}OH$), 4.69 (d, 1H, ^3J = 2.0 Hz, H-5), 4.77 (d, 1H, ^2J = 16.8 Hz, $OC\underline{H}_2CO_2$), 4.83 (d, 1H, ^2J = 16.8 Hz, $OC\underline{H}_2CO_2$), 5.20 (s, 2H, $C\underline{H}_2Ph$), 5.60 (d, 1H, ^2J = 11.8 Hz, $C\underline{H}_2Ph$), 5.66 (d, 1H, ^2J = 11.8 Hz, $C\underline{H}_2Ph$), 7.36 (m, 10H, Ph-H). CI-MS (NH_3) *m/z (%)*: 614 (100) $[M+NH_4]^+$. Anal. ($C_{34}H_{44}O_9$) C, H. $C_{34}H_{44}O_9$ (596.71).

2-*O*-(Benzyloxycarbonylmethyl)-3-*O*-benzyl-6-*O*-[11-(4-phenylphenoxy)-undecanoyl]ascorbic acid (7.144): The title compound was prepared from **7.142** (1.5 mmol, 0.62 g), **7.34a** (1.5 mmol, 0.53 g), DMAP (1.8 mmol, 0.22 g) and EDAC (1.65 mmol, 0.32 g) in anhydrous DMF (10 ml) according to general procedure 7.6.2.9 and was obtained after flash chromatography (PE/EtOAc 80/20 to 70/30 v/v) as a colorless oil (0.27 g, 24 %). mp: 115 °C; ^{1}H-NMR ($CDCl_3$) δ 1.26 – 1.84 (m, 16H, $COCH_2(C\underline{H}_2)_8$), 2.33 (t, 2H, ^3J = 7.5Hz, $COC\underline{H}_2CH_2$), 3.99 (t, 2H, ^3J = 6.6 Hz, $C\underline{H}_2OC_6H_4$), 4.11 (m, 1H, $C\underline{H}OH$), 4.19 (dd, 1H, ^3J = 4.9 Hz, ^2J = 11.6 Hz, CH_2O), 4.33 (dd, 1H, ^3J = 6.7 Hz, ^2J = 11.5 Hz, CH_2O), 4.69 (d, 1H, ^3J = 2.0 Hz, H-5), 4.81 (s, 2H, OCH_2CO_2), 5.20 (s, 2H, $C\underline{H}_2Ph$), 5.61 (d, 1H, ^2J = 11.8 Hz, $C\underline{H}_2Ph$), 5.66 (d, 1H, ^2J = 11.8 Hz, $C\underline{H}_2Ph$), 6.97 (m, 2H, Ar-H), 7.35 (m, 13H, Ar-H), 7.53 (m, 4H, Ar-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 769 (100) $[M+NH_4]^+$. $C_{45}H_{50}O_{10}$ (750.87).

2-*O*-Benzyl-3-*O*-(benzyloxycarbonylmethyl)-6-*O*-dodecanoylascorbic acid (7.145): The title compound was prepared from **7.142** (1.0 mmol, 0.41 g), lauric acid (1.0 mmol, 0.20 g), DMAP (1.2 mmol, 0.15 g) and EDAC (1.1 mmol, 0.21 g) in anhydrous DMF (10 ml) according to general procedure 7.6.2.9 and was obtained after flash chromatography (PE/EtOAc 80/20 v/v) as a white solid (0.21 g, 35 %). mp: 55-56 °C; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.6 Hz, $CH_2C\underline{H}_3$), 1.26 (s, 16H,

($C\underline{H}_2)_4CH_3$), 1.63 (m, 2H, $COCH_2C\underline{H}_2CH_2$), 2.34 (t, 2H, ^3J = 7.6 Hz, $CO\underline{C}H_2CH_2$), 4.22 (m, 2H, CH_2O, C<u>H</u>OH), 4.36 (dd, 1H, ^3J =6.8 Hz, ^2J =11.1 Hz, CH_2O), 4.59 (d, 1H, ^2J = 16.5 Hz, $OC\underline{H}_2CO_2$), 4.75 (d, 1H, ^3J = 1.6 Hz, H-5), 5.12 (m, 5H, $OC\underline{H}_2CO_2$, CH_2Ph), 7.32 (m, 10H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 614 (100) $[M+NH_4]^+$. Anal. ($C_{34}H_{44}O_9$) C, H. $C_{34}H_{44}O_9$ (596.71).

2-*O*-(Carboxymethyl)-6-O-dodecanoyl-L-ascorbic acid (7.146): The title compound was prepared from **7.143** (0.30 mmol, 0.18 g), 10 % Pd/C (30 mg) in EtOH (3 ml) and EtOAc (3 ml) according to general procedure 7.6.2.11. Recrystallization from IPE/*n*-hexane yielded a white solid (0.90 g, 50 %). mp: 109-111 °C; ^{1}H-NMR (CD_3OD) δ 0.89 (t, 3H, ^3J = 6.6 Hz, $CH_2C\underline{H}_3$), 1.29 (s, 16H, $(C\underline{H}_2)_8CH_3$), 1.62 (m, 2H, $COCH_2C\underline{H}_2CH_2$), 2.37 (t, 2H, ^3J = 7.4 Hz, $CO\underline{C}H_2CH_2$), 4.18 (m, 3H, CH_2O-, C<u>H</u>OH), 4.57 (d, 1H, ^2J =15.0 Hz, $C\underline{H}_2CO_2$), 4.63 (d, 1H, ^2J = 16.4 Hz, $C\underline{H}_2CO_2$), 4.79 (d, 1H, ^3J = 1.8Hz, H-5). ^{13}C-NMR (CD_3OD) δ 14.56 (+, CH_3), 23.83 (-, CH_2), 26.06 (-, CH_2), 30.27 (-, CH_2), 30.50 (-, CH_2), 30.56 (-, CH_2), 30.69 (-, CH_2), 30.82 (-, CH_2), 33.16 (-, CH_2), 34.95 (-, $CO\underline{C}H_2CH_2$), 65.65 (-, CH_2O), 68.01 (+, CHO), 68.72 (-, CH_2O), 77.30 (+, CH), 121.77 (quat, C-2), 161.08 (quat, C-3), 171.73 (quat, lactone CO), 173.86 (quat, $CH_2\underline{C}O_2$) 175.18 (quat, $CH_2\underline{C}O_2$). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 434 (100) $[M+NH_4]^+$. Anal. ($C_{20}H_{32}O_9$) C, H. $C_{20}H_{32}O_9$ (416.46).

2-*O*-(Carboxymethyl)-6-*O*-[11-(4-phenylphenoxy)undecanoyl]-L-ascorbic acid (7.147): The title compound was prepared from **7.144** (0.33 mmol, 0.25 g), 10 % Pd/C (30 mg) in EtOH (3 ml) and EtOAc (3 ml) according to general procedure 7.6.2.11. Compound **7.147** and was obtained as a white solid (0.17 g, 89 %). mp: 109-112 °C; ^{1}H-NMR (CD_3OD + $CDCl_3$) δ 1.27 – 1.83 (m, 16H, $COCH_2(C\underline{H}_2)_8$), 2.35 (t, 2H, ^3J = 7.4 Hz, $CO\underline{C}H_2CH_2$), 3.99 (t, 2H, ^3J = 6.4 Hz, $C\underline{H}_2OC_6H_4$), 4.17 (m, 3H, C<u>H</u>OH, CH_2O), 4.52 (d, 1H, ^2J = 16.3 Hz, $OC\underline{H}_2CO_2H$), 4.59 (d, 1H, ^2J = 16.4 Hz, $OC\underline{H}_2CO_2H$), 4.76 (d, 1H, ^3J = 1.9 Hz, H-5). ^{13}C-NMR (CD_3OD + CD_3OD) δ 26.09 (-, CH_2), 27.28 (-, CH_2), 30.30 (-, CH_2), 30.48 (-, CH_2), 30.54 (-, CH_2), 30.60 (-, CH_2), 30.62 (-, CH_2), 30.74 (-, CH_2), 35.08 (-, $CO\underline{C}H_2CH_2$), 65.66 (-, CH_2O), 68.05 (+, CHO), 69.21 (-, CH_2O), 69.41 (-, CH_2O), 77.33 (+, CH), 115.98 (+, Ar-H), 121.95 (quat, C-2), 127.67 (+, Ar-H), 127.71 (+, Ar-H), 129.11 (+, Ar-H), 129.85 (+, Ar-H), 134.90 (quat, Ar-C), 142.25 (quat, Ar-C), 160.17 (quat, Ar-C), 161.18 (quat, C-3), 171.98 (quat, $CH_2\underline{C}O_2$), 175.26 (quat, $CH_2\underline{C}O_2$). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 588 (100) $[M+NH_4]^+$. Anal. ($C_{31}H_{38}O_{10}$) C, H. $C_{31}H_{38}O_{10}$ (570.63).

3-*O*-(Carboxymethyl)-6-*O*-dodecanoyl-L-ascorbic acid (7.148): The title compound was prepared from **7.145** (0.23 mmol, 0.14 g), 10 % Pd/C (30 mg) in EtOH (3 ml) and EtOAc (3 ml) according to general procedure 7.6.2.11. Preparative HPLC (MeCN/0.1 % TFA (aq) 70/30 v/v) yielded **7.148** as a white semisolid substance (60 mg, 63 %). ^{1}H-NMR (CD_3OD) δ 0.89 (t, 3H, ^{3}J = 6.7 Hz, $CH_2C\underline{H}_3$), 1.29 (m, 16H, $(C\underline{H}_2)_4CH_3$), 1.62 (m, 2H, $COCH_2C\underline{H}_2CH_2$), 2.37 (t, 2H, ^{3}J = 7.4 Hz, $COC\underline{H}_2CH_2$), 4.20 (m, 3H, CH_2O, C<u>H</u>OH), 4.82 (d, 1H, ^{3}J = 1.6 Hz, H-5), 4.98 (d, 1H, ^{2}J = 16.5 Hz, $OC\underline{H}_2CO_2$), 5.07 (d, 1H, ^{2}J = 16.5 Hz, $OC\underline{H}_2CO_2$). ^{13}C-NMR (CD_3OD) δ 14.52 (+, CH_3), 23.82 (-, CH_2), 26.06 (-, CH_2), 30.25 (-, CH_2), 30.48 (-, CH_2), 30.55 (-, CH_2), 30.68 (-, CH_2), 30.81 (-, CH_2), 33.15 (-, CH_2), 34.95 (-, $CO\underline{C}H_2CH_2$), 65.53 (-, CH_2), 67.98 (-, CH_2), 68.23 (+, CHO), 77.13 (+, CH), 122.41 (quat, C-2), 149.73 (quat, C-3), 172.03 (quat, $\underline{C}O_2CH_2$), 175.20 (quat, CO_2H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 434 (100) $[M+NH_4]^+$. Anal. ($C_{20}H_{32}O_9$) C, H. $C_{20}H_{32}O_9$ (416.46).

7.6.2.17 General procedure for the synthesis of 3-*O*-alkylated ascorbic acid derivatives 149 and 150 using Mitsunobu conditions

To a solution of PPh_3 (1.14 eq) in anhydrous THF at -78°C was added DIAD (1.12 eq) under an atmosphere of argon. After a white solid was formed the mixture was stirred for 20 min before a solution of L-ascorbic acid (1 eq) in anhydrous DMF was added. The cooling bath was removed and after stirring for 5 min the pertinent alcohol (1.25 eq) dissolved in anhydrous THF was added and stirring was continued at room temperature overnight. After the solvent was evaporated, the remaining residue was subjected to flash chromatography.

3-*O*-(Dodecan-1-yl)-L-ascorbic acid[99] (7.149): The title compound was prepared according to general procedure 7.6.2.17 from PPh_3 (2.25 mmol, 0.59 g) in anhydrous THF (10 ml), DIAD (2.22 mmol, 0.44 ml), L-ascorbic acid (1.98 mmol, 0.35 g) in anhydrous DMF (12 ml) and 1-dodecanol (2.49 mmol, 0.46 g) in anhydrous THF (2 ml). Compound **7.149** was obtained after flash chromatography ($CHCl_3$/MeOH 97/3 v/v) as a pale yellow solid (0.18 g, 26 %). mp: 87 °C (ref.[99]: 90-92 °C); ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H, ^{3}J = 6.7 Hz, $CH_2C\underline{H}_3$) , 1.24 (m, 18H, $(C\underline{H}_2)_9CH_3$), 1.64 (m, 2H, $OCH_2C\underline{H}_2$), 3.41 (m, 2H, $C\underline{H}_2OH$), 3.63 (m, 1H, C<u>H</u>OH), 4.36 (m, 2H, $OC\underline{H}_2CH_2$), 4.73 (d, 1H, ^{3}J = 0.9 Hz, H-5), 4.84 (dd, 1H, ^{3}J = 5.3 Hz, ^{3}J = 6.0 Hz, OH), 4.93 (d, 1H, ^{3}J = 6.3 Hz, OH), 8.67 (s, 1H, OH). ^{13}C-NMR (DMSO-d_6) δ 13.88 (+, CH_3), 22.03 (-, CH_2), 24.93 (-, CH_2), 28.65 (-, CH_2), 28.90 (-, CH_2), 28.95 (-, CH_2), 29.00 (-, CH_2),

29.13 (-, CH_2), 31.23 (-, CH_2), 61.71 (-, CH_2O), 68.53 (+, CHO), 70.58 (-, CH_2O), 74.35 (+, CH), 118.80 (quat, C-2), 150.64 (quat, C-3), 170.55 (quat, lactone CO). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 362 (100) $[M+NH_4]^+$. $C_{18}H_{32}O_6$ (344.44).

3-*O*-(Tetradecan-1-yl)-L-ascorbic acid[99] (7.150): The title compound was prepared according to general procedure 7.6.2.17 using PPh_3 (2.25 mmol, 0.59 g) in anhydrous THF (10 ml), DIAD (2.22 mmol, 0.44 ml), L-ascorbic acid (1.98 mmol, 0.35 g) in anhydrous DMF (12 ml) and 1-dodecanol (2.49 mmol, 0.53 g) in anhydrous THF (10 ml). Compound **7.150** was obtained after flash chromatography ($CHCl_3$/MeOH 97/3 v/v) as a white solid (0.51 g, 26 %). mp: 85-86 °C (ref.[99]: 86-88 °C); ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H, ^3J = 6.7 Hz, $CH_2C\underline{H}_3$), 1.24 (m, 22H, $(C\underline{H}_2)_{11}CH_3$), 1.64 (m, 2H, $OCH_2C\underline{H}_2$), 3.42 (m, 2H, $C\underline{H}_2OH$), 3.64 (m, 1H, C<u>H</u>OH), 4.35 (m, 2H, $OC\underline{H}_2CH_2$), 4.73 (d, 1H, ^3J = 1.1 Hz, H-5), 4.84 (dd, 1H, ^3J = 5.6 Hz, ^3J = 5.6 Hz, OH), 4.93 (d, 1H, ^3J = 6.3 Hz, OH), 8.67 (s, 1H, OH). ^{13}C-NMR (DMSO-d_6) δ 13.87 (+, CH_3), 22.02 (-, CH_2), 24.93 (-, CH_2), 28.64 (-, CH_2), 26.67 (-, CH_2), 28.91 (-, CH_2), 28.94 (-, CH_2), 28.99 (-, CH_2), 29.14 (-, CH_2), 31.23 (-, CH_2), 61.72 (-, CH_2O), 68.55 (+, CHO), 70.58 (-, CH_2O), 74.36 (+, CH), 118.82 (quat, C-2), 150.64 (quat, C-3), 170.54 (quat, lactone CO). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 390 (100) $[M+NH_4]^+$. $C_{20}H_{36}O_6$ (372.50).

7.6.3 Molecular modeling

All calculations were done using Sybyl 7.3 (Tripos Inc., St. Louis, USA). The crystal structure of Hyal-1 (PDB code 2pe4[64]) was prepared using the Biopolymer structure preparation tool. Hydrogens and Amber FF99 charges were added and the additional carbohydrates found in the crystal structure were deleted. The structure was minimized first with the steepest descent and then with the Powell method using the Amber FF99 force field. The dielectric constant was set to 4, one four scaling to 1, NB cutoff to 12 Å. The backbone of the protein was initially fixed and freed after the first 100 cycles. The termination criterion was set to a gradient norm of 0.1 kcal/mol*Å. All other variables were set to standard values.

The inhibitors used for docking were generated with the sketch module of Sybyl and energetically minimized using the Tripos Force Field with Gasteiger-Hückel charges and a termination gradient norm of 0.05 kcal/mol*Å. The docking calculations with compound **7.51** were performed with the FlexiDock module of Sybyl. The binding

pocket was either defined by a sphere of 5 Å surrounding the set of amino acids of the tetrasaccharide site (Trp36, Asn37, Asn39, Gln41, Trp42, Glu45, Thr65, Phe66, Arg67, Ile73, Ser74, Tyr75, Asp129, Glu131, Tyr202, Ser245, Tyr247, Ile287, Trp321, Val322, Ser323, Trp324, and Glu325) or a sphere of 15 Å surrounding Trp321 and Tyr202 in the active site of Hyal-1. Freely rotatable bonds were: in the first approach all bonds of the ligand, in a second approach additionally all bonds of the binding site. The ligand was pre-positioned in the binding pocket using various starting positions in the different FlexiDock runs. The total number of generations was set according to the suggestions made in the Tripos Bookshelf (at least 500 iterations per rotatable bond). Compound **7.121** was manually docked to check if the hexadecanoyl chain may fit to the second hydrophobic pocket. Visualizations of protein surfaces were made using the Molcad tool.

The final docking poses presented for **7.51** and **7.121** were a result of an iterative minimization process: After docking, the complex was minimized (steepest descent, then Powell) in the Amber99 force field fixing the ligands as aggregates. In a second step, the inhibitor together with a sphere of the enzyme 6 Å around the ligand was minimized (Tripos force field, Powell method) using the Minimize Subset option of Sybyl 7.3. The two steps were repeated in succession until both approached a termination gradient norm of 0.05 kcal/mol*Å.

7.7 *References*

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Chapter 8
Indolylalkanoic acid derivatives as potent inhibitors of human PH-20 and bacterial hyaluronidase *Sag*Hyal$_{4755}$

8.1 *Introduction*

Recently, a structure-based strategy for the design of inhibitors of the bacterial hyaluronate lyase *Sag*Hyal$_{4755}$ was successfully pursued in our workgroup[1, 2]. Based on two crystal structures from homologous bacterial enzymes (PDB code 1c82 and 1egu)[3, 4] a *de novo* approach using the program LUDI[5, 6] resulted in the identification of indole-2-carboxylic acid as a lead structure for the development of hyaluronidase inhibitors. Further modifications of the indole scaffold led to the identification of indole-based inhibitors of bacterial hyaluronidases with IC_{50} values in the lower micromolar range. Moreover, the crystal structure of bacterial hyaluronidase from *Streptococcus pneumoniae* in complex with 1-decyl-2-(4-sulfamoyloxyphenyl)-1*H*-indol-6-yl sulfamate (Figure 8.1) was elucidated (PDB code 2brp)[7].

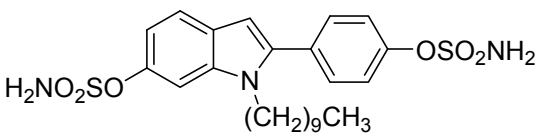


Figure 8.1. Structure of 1-decyl-2-(4-sulfamoyloxyphenyl)-1*H*-indol-6-yl sulfamate.

The indole moiety of the co-crystallized compound was bound to the active site explaining the inhibitory activity of the indole derivatives. Figure 8.2 shows the binding mode of the indole-type inhibitor within the bacterial enzyme.

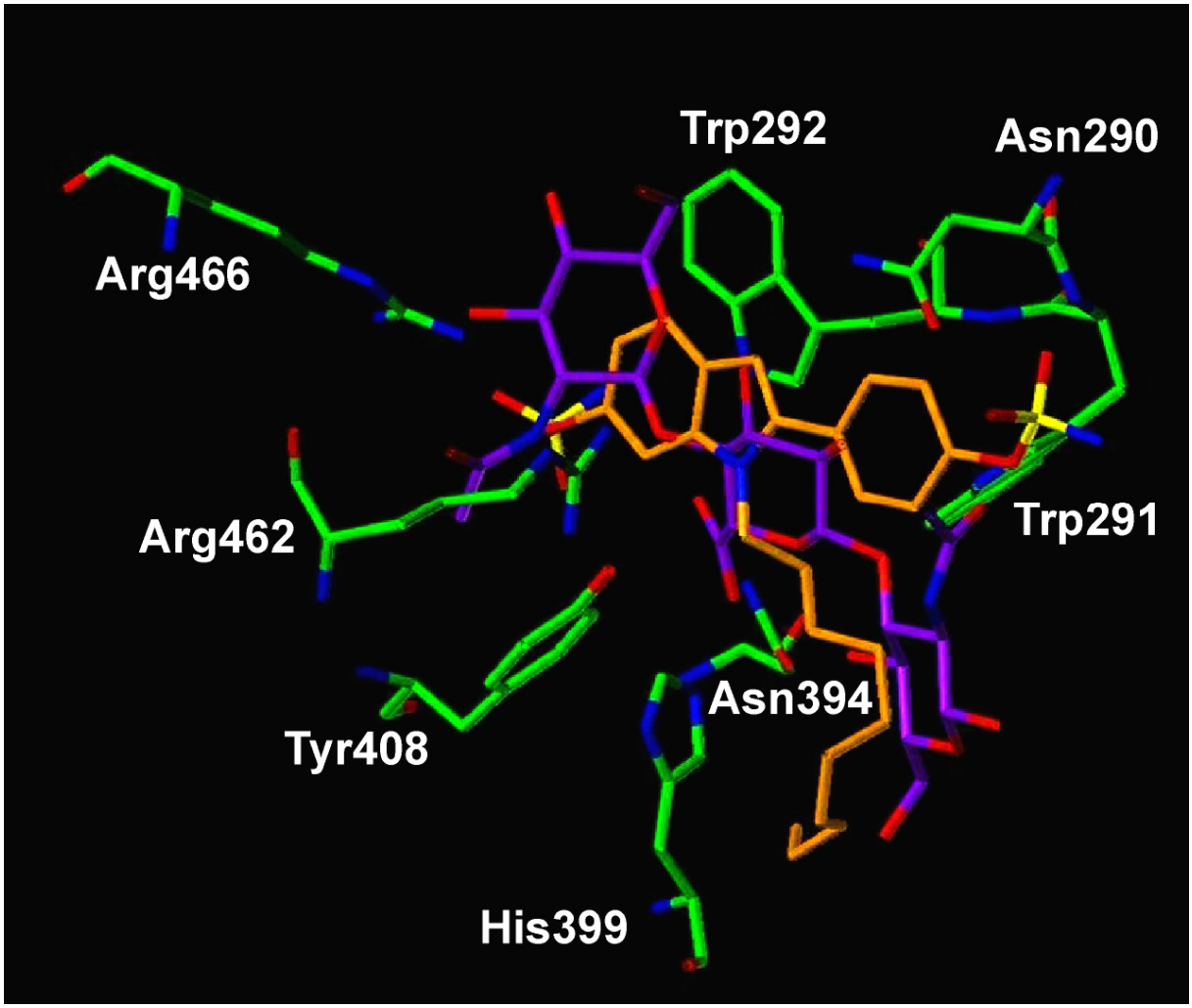


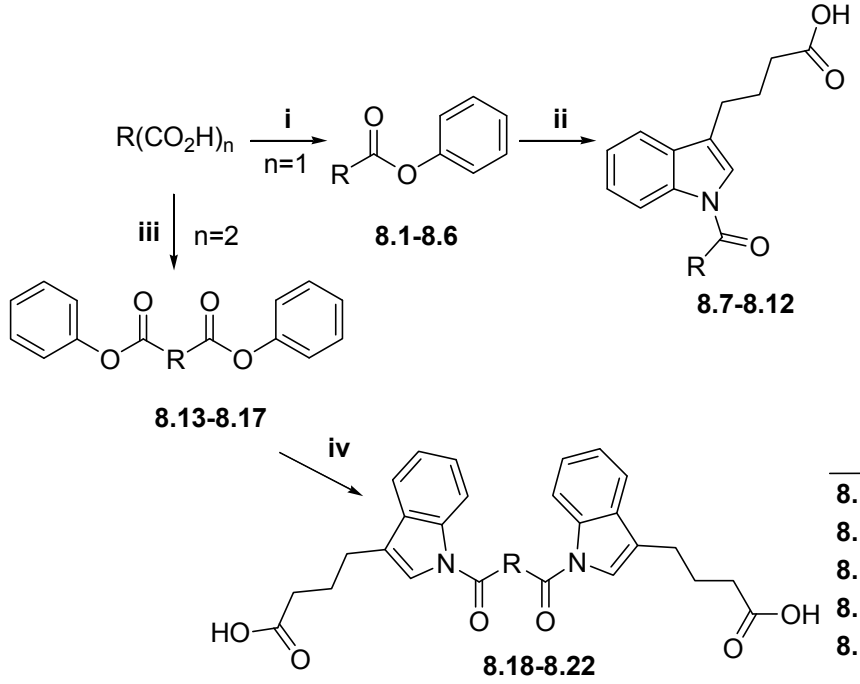
Figure 8.2. Comparison of binding modes of hyl*Spn* inhibitor sulfamic acid 1-decyl-2-(4-sulfamoyloxyphenyl)-1*H*-indol-6-yl ester (carbon atoms orange) and a HA hexasaccharide (carbon atoms purple, taken from the X-ray structure PDB code 1loh[8], for clarity only a trisaccharide moiety is shown). Modified from Botzki, 2004[9].

Interestingly, the indole derivatives synthesized by Salmen[2] and Braun[1] did not or only very weakly inhibit the bovine testicular hyaluronidase. Due to the fact that indomethacin, a non-steroidal anti-inflammatory drug, is described as a hyaluronidase inhibitor[10] and also does inhibit the recombinantly expressed human hyaluronidases Hyal-1 and PH-20 in the used turbidimetric assay (see chapter 5), a small collection of commercially available indole derivatives was tested for inhibition of Hyal-1 and PH-20, namely indole, indole-2-carboxylic acid, indole-3-carboxylic acid, indole-3-acetic acid, indole-3-propionic acid, indole-3-butanoic acid and indole-

5-carboxylic acid (data not shown). Highest potency among these small molecules was found for indole-3-butanoic acid with an IC_{50} value in the lower millimolar range for human PH-20 (Table 8.3, chapter 8.3.2). This result is in agreement with previous studies where indole-3-butanoic acid was identified as the most potent inhibitor of the bacterial enzyme in this series of compounds[2]. Due to these promising results additional indole derivatives were synthesized. The synthesis and structure-activity relationships of substituted indole derivatives are reported in this chapter.

8.2 *Chemistry*

The *N*-acylated indole-3-butanoic acid derivatives **8.7-8.12** and **8.18-8.22** were synthesized according to Scheme 8.1 using phenol esters as acylation reagents[11] which were prepared from the pertinent carboxylic acids and phenol in the presence of standard coupling reagents[12]. The synthesis of **8.10-8.12** and **8.18** as well as the preparation of the required phenol esters (**8.4-8.6** and **8.13**) was already described elsewhere[13].

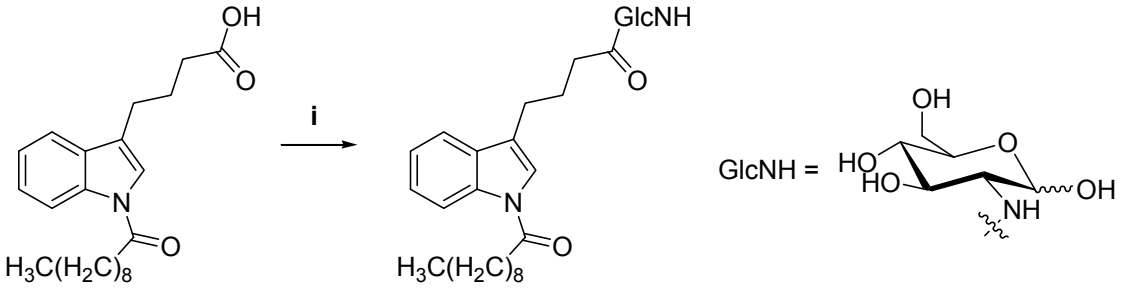


	R
8.1, 8.7	$(CH_2)_2Ph$
8.2, 8.8	$(CH_2)_8CH_3$
8.3, 8.9	*p*-Cl-C_6H_4
8.4, 8.10	*p*-MeO-C_6H_4
8.5, 8.11	*p*-C_6H_4-Ph
8.6, 8.12	CH_2-*p*-MeO-C_6H_4

	R
8.13, 8.18	-*p*-C_6H_4-
8.14, 8.19	-CH_2-*p*-$C_6H_4CH_2$-
8.15, 8.20	-$(CH_2)_6$-
8.16, 8.21	-$(CH_2)_{10}$-
8.17, 8.22	-$(CH_2)_{14}$-

Scheme 8.1. Synthesis of *N*-acylated indole-3-butanoic acid derivatives **8.7-8.12** and **8.18-8.22**. Reagents and conditions: (i) DCC (1.1 eq), phenol (1.2 eq), DMAP (cat.), CH_2Cl_2 / THF, RT overnight; (ii) indole-3-butanoic acid (1.02 eq), NaH (3 eq), DMF, -41 to -20 °C, 30 min; (iii) DCC (2.2 eq), phenol (2.4 eq), DMAP (cat.), CH_2Cl_2 / THF, RT overnight; (iv) indole-3-butanoic acid (2.04 eq), NaH (6 eq), DMF, -41 to -20 °C, 30 min.

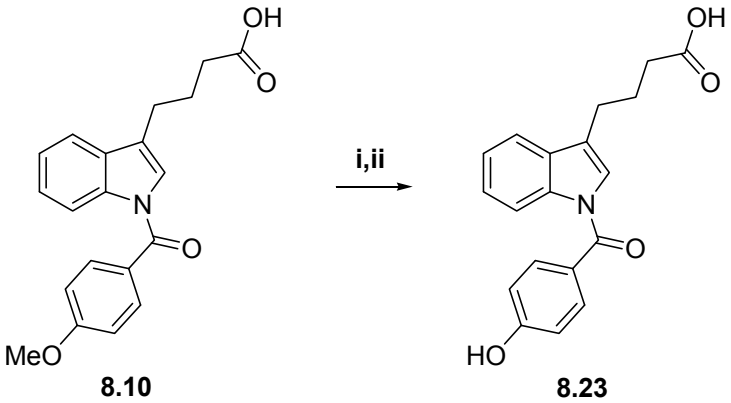
The glycosylamide **8.8a** was synthesized from **8.8** and D-glucosamine in the presence of EDAC according to Scheme 8.2.



8.8 **8.8a**

Scheme 8.2. Synthesis of **8.8a**. Reagents and conditions: (i) Glucosamine hydrochloride (2 eq), HOBt (1.2 eq), EDAC (1.2 eq), NEt_3 (3.2 eq), DMF / H_2O, RT overnight.

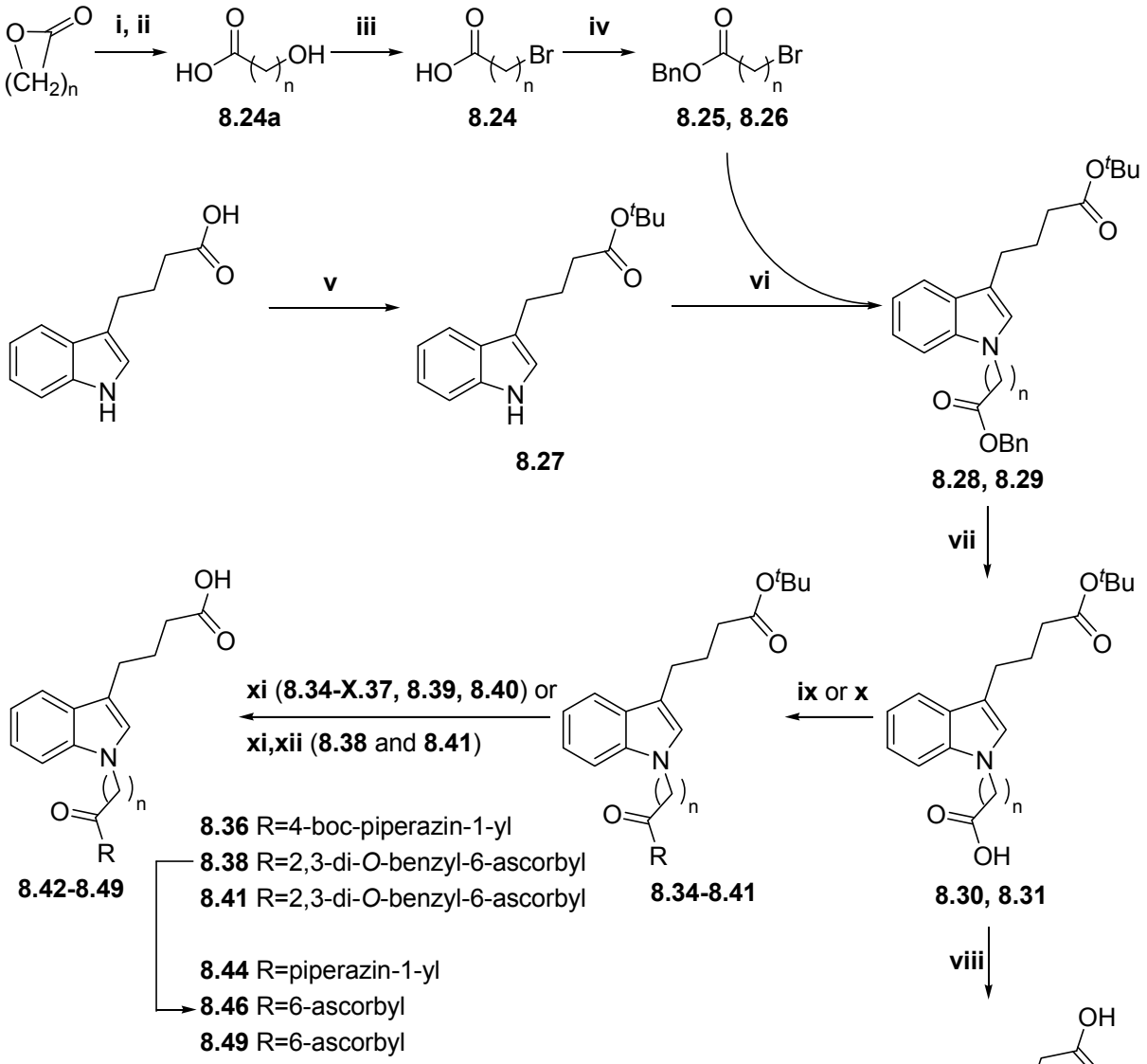
The methyl ether in **8.10** was cleaved (Scheme 8.3) to yield the corresponding hydroxyl derivative **8.23** as described[13].



8.10 **8.23**

Scheme 8.3. Synthesis of **8.23** by cleavage of the methyl ether in **8.23**. Reagents and conditions: (i) BBr_3SMe_3, reflux, 24 h; (ii) H_2O, RT, 20 min.

Alkyl spacer containing derivatives **8.32, 8.33** and **8.42-8.49** were synthesized according to Scheme 8.4. ω-Bromo alkanoic acids were used to alkylate the carboxy-protected indole. Hence 11-bromoundecanoic acid was esterified with benzyl alcohol in the presence of DCC and DMAP and used to *N*-alkylate *tert*-butyl indole-3-butanoate (**8.27**) which was prepared from the pertinent indolylalkanoic acid[14]. 16-Bromohexadecanoic acid (**8.24**) was prepared from hexadecano-16-lactone by saponification to 16-hydroxyhexadecanoic acid (**8.24a**) and subsequent halogenation according to known procedures[15] and then treated as in the case of the shorter bromoalkanoic acid. After hydrogenolytic cleavage of the benzyl ester, amines or alcohols in the presence of standard coupling reagents were used to form amides or esters of **8.30** and **8.31**. In a last step the *tert*-butyl ester was cleaved using TFA to yield the pertinent *N*-alkylated indole-3-butanoic acid derivatives.

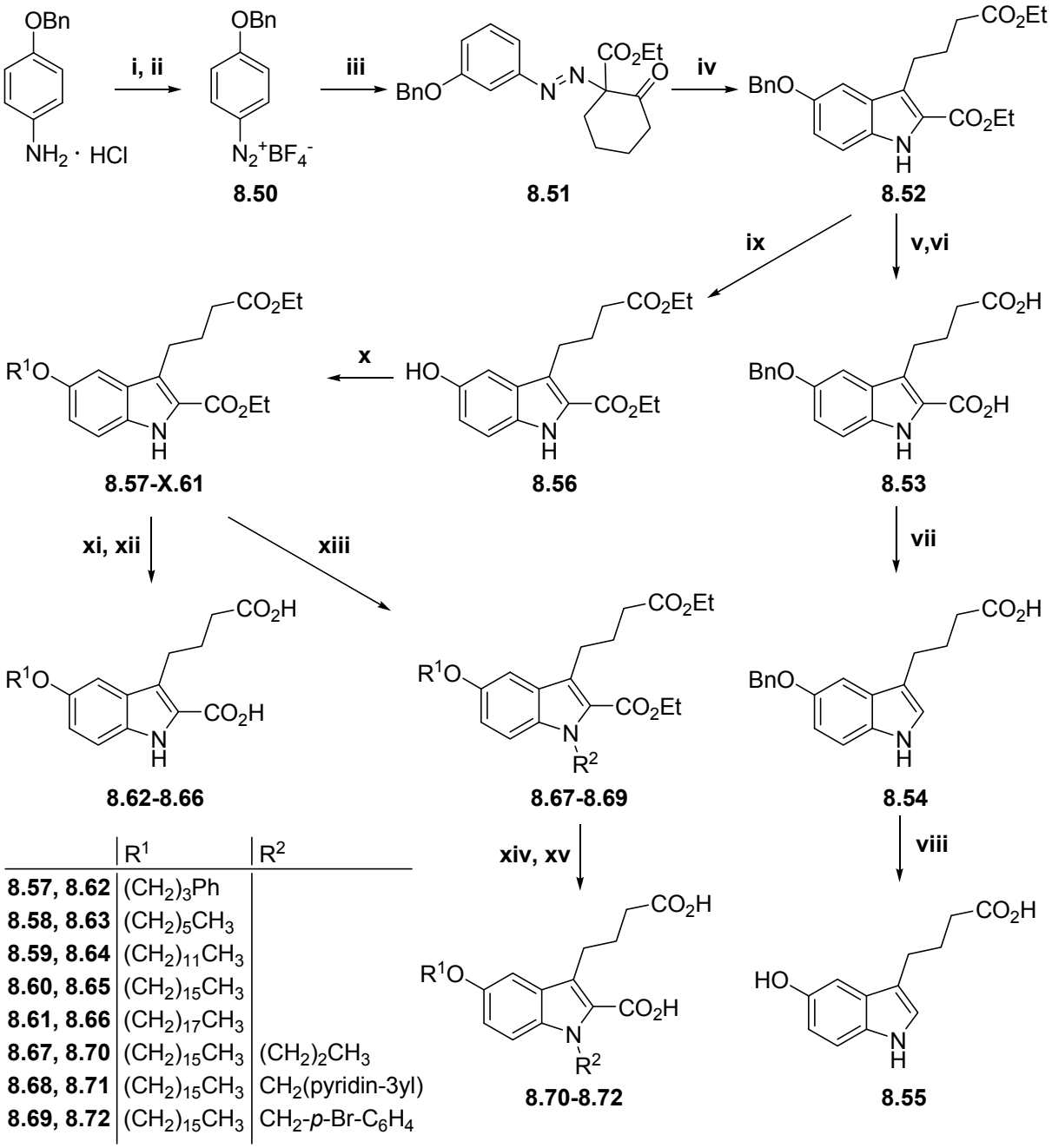


	n	R
8.23, 8.24, 8.26, 8.29, 8.31, 8.33	15	
8.25, 8.28, 8.30, 8.32	10	
8.34, 8.42	10	NH(thiazole-2-yl)
8.35, 8.43	10	4-phenylpiperazin-1-yl
8.36	10	4-boc-piperazin-1-yl
8.37, 8.45	10	NH-*3*-OH-C_6H_4
8.38	10	2,3-di-*O*-benzyl-6-ascorbyl
8.39, 8.47	15	4-phenylpiperazin-1-yl
8.40, 8.48	15	NH-*m*-OH-C_6H_4
8.41	15	2,3-di-*O*-benzyl-6-ascorbyl
8.44	10	piperazin-1-yl
8.46	10	6-ascorbyl
8.49	15	6-ascorbyl

Scheme 8.4. Synthesis of the *N*-alkylated indole-3-butanoic acid derivatives **8.42-8.49** and the corresponding intermediates. Reagents and conditions: (i) 50 % NaOH, NBu_4HSO_4 (0.01 eq), toluene, 80-90 °C overnight; (ii) HCl (conc.); (iii) HBr/HOAc, H_2SO_4 (conc.), RT overnight, 6 h reflux; (iv) BnOH (1.1 eq), DCC (1.1 eq), DMAP (0.1 eq), CH_2Cl_2, RT overnight; (v) TBTA (2 eq), BF_3Et_2O (cat), THF, RT overnight; (vi) NaH (1.2 eq), $BnO_2C(CH_2)_nBr$ (1.2 eq), DMF 60-80 °C; (vii) 10 % Pd/C (cat.), H_2 (1

atm), EtOH/EtOAc, RT, 30 min; (viii) TFA, CH_2Cl_2, RT, 6 h; (ix) NHR (2 eq), HOBt (1.2 eq), DIEA (1.2 eq), EDAC (1.2 eq), DMF, RT overnight; (x) **7.66** (1 eq), EDAC (1.1 eq), DMAP (1.1 eq), DMF, RT overnight; (xi) TFA, CH_2Cl_2, RT, 6 h; (xii) 10 % Pd/C (cat.), H_2, 4 bar, EtOH/EtOAc, RT overnight.

To be able to introduce various residues at position C-5 of the indole ring, building block **8.52** was prepared from 4-benzyloxyaniline and ethyl 2-oxocyclohexane-carboxylate using the Japp-Klingemann variant[16] of the Fischer indole synthesis (Scheme 8.5)[17]. **8.52** was deprotected and decarboxylated to obtain **8.53-8.55**, or the benzyl ether of **8.52** was cleaved followed by alkylation of the 5-OH under Mitsunobu conditions (compounds **8.57-8.61**)[18]. Subsequent saponification of the ester groups led to **8.62-8.66** or *N*-alkylation of **8.60** was performed before ester cleavage to obtain **8.70-8.72**.

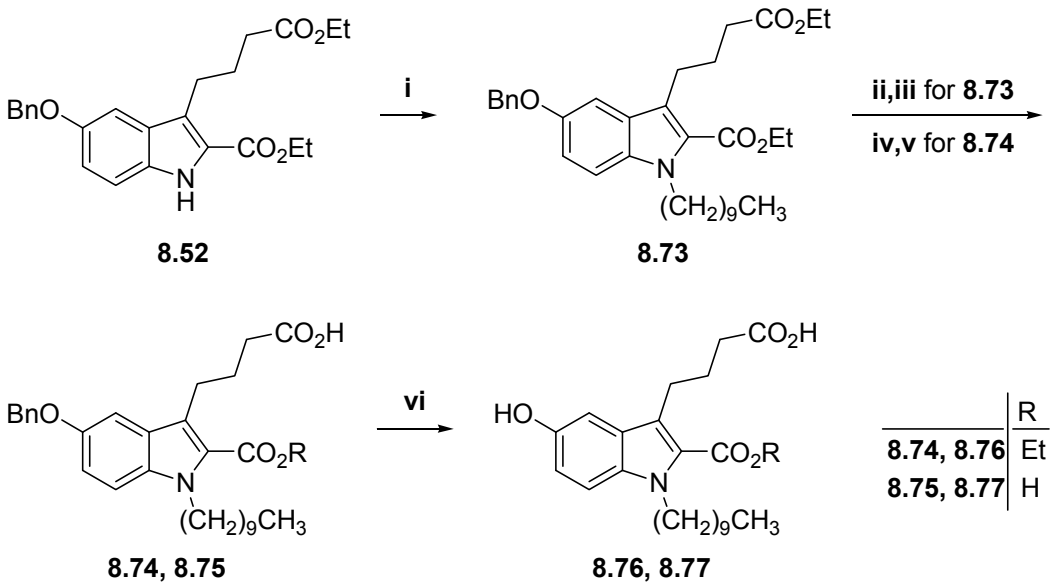


	R^1	R^2
8.57, 8.62	$(CH_2)_3Ph$	
8.58, 8.63	$(CH_2)_5CH_3$	
8.59, 8.64	$(CH_2)_{11}CH_3$	
8.60, 8.65	$(CH_2)_{15}CH_3$	
8.61, 8.66	$(CH_2)_{17}CH_3$	
8.67, 8.70	$(CH_2)_{15}CH_3$	$(CH_2)_2CH_3$
8.68, 8.71	$(CH_2)_{15}CH_3$	CH_2(pyridin-3yl)
8.69, 8.72	$(CH_2)_{15}CH_3$	CH_2-*p*-Br-C_6H_4

Scheme 8.5. Synthesis of *N*-alkylated indolylalkanoic acids derivatives **8.53-8.55**, **8.62-8.66**, **8.70-8.72** and the pertinent intermediates. Reagents and conditions: (i) $NaNO_2$ (1.05 eq), HCl (conc.) / H_2O, 0 °C, (ii) $NaBF_4$ (1 eq), RT, 20 min; (iii) NaH (1.14 eq), Ethyl 2-oxocyclohexanecarboxylate (1.02 eq), THF, reflux, 30 min, then RT, 1 h; (iv) H_2SO_4, EtOH, reflux overnight; (v) KOH (3 eq), EtOH, reflux 30 min; (vi) 2 N HCl; (vii) 200-230 °C; (viii) 10 % Pd/C (cat.), H_2 (1 atm), EtOH, RT, 30 min; (ix) 10 % Pd/C (cat.), H_2 (1 atm), EtOH/EtOAc, RT, 30 min; (x) R^1OH (1 eq), DIAD (1 eq), PPh_3 (1 eq), THF, RT overnight; (xi) LiOH (3 eq), THF/MeOH/H_2O, RT overnight; (xii) 2 N HCl; (xiii) NaH (1.2 eq), R^2Hal (1.2 eq), DMF, 40-60 °C, 3 h; (xiv) LiOH (3 eq), THF/MeOH/H_2O, RT overnight; (xv) 2 N HCl.

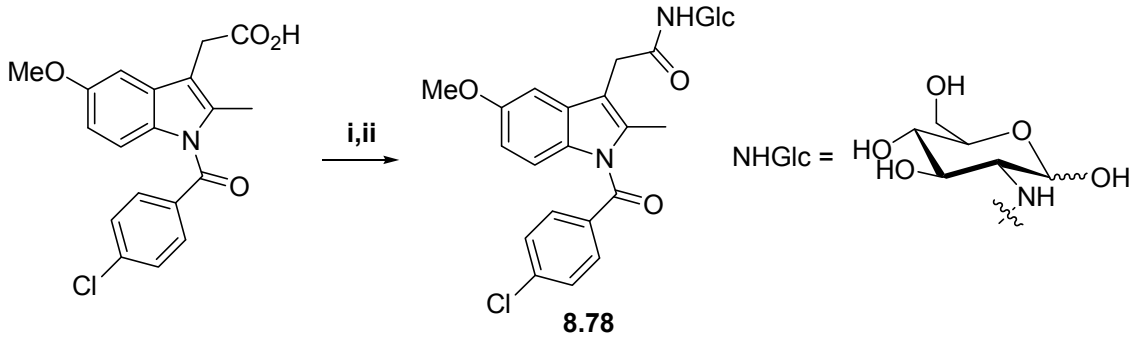
N-Alkylation of **8.52** yielded the diester **8.73** which then was saponified to give the carboxylic acid (Scheme 8.6). When a slight excess of LiOH was used at room temperature the monoester **8.74** was obtained whereas saponification with excess

NaOH at reflux resulted in complete conversion to **8.75**. Both *N*-alkylindoles were subjected to hydrogenolysis to obtain **8.76** and **8.77**.



Scheme 8.6. Synthesis of **8.73-8.77**. Reagents and conditions: (i) NaH (1.2 eq), Br$(CH_2)_9CH_3$ (1.2 eq), DMF, 40-60 °C, 3 h; (ii) LiOH (3 eq), THF/H_2O, RT overnight; (iii) 2 N HCl; (iv) NaOH (10 eq), reflux, 30 min; (v) 2 N HCl; (vi) 10 % Pd/C (cat.), H_2 (1 atm), EtOH/EtOAc, RT, 30 min.

The glycosylamide of indomethacin (**8.78**) was synthesized from indomethacin and D-glucosamine according to known procedures[19, 20] (Scheme 8.7).



Scheme 8.7. Synthesis of **8.78**. Reagents and conditions: (i) $SOCl_2$ (1.05 eq), DMF (1.05 eq), CH_2Cl_2, RT; (ii) Glucosamine hydrochloride (1 eq), NEt_3 (2 eq), MeOH / CH_2Cl_2, RT, 20 min.

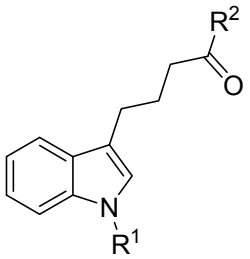
8.3 *Inhibition of hyaluronidases: results and discussion*

All synthesized indole derivatives were investigated for inhibition of recombinant human hyaluronidases PH-20 and Hyal-1, the bovine testicular enzyme BTH (Neopermease®) and the bacterial hyaluronan lyase *Sag*Hyal$_{4755}$ in a modified turbidimetric assay according to the method of Di Ferrante[21] as described in chapter 3.

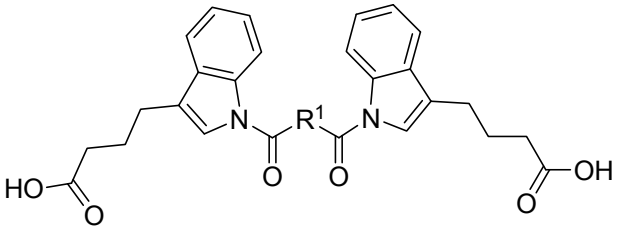
8.3.1 *N*-Substituted indole-3-butanoic acid derivatives

The IC_{50} values of the investigated indole-3-butanoic acid derivatives are summarized in Table 8.1.

Table 8.1. Inhibitory activity of *N*-substituted indole-3-butanoic acid derivatives, determined on the hyaluronidases Hyal-1, PH-20, BTH and *Sag*Hyal$_{4755}$.



8.7-8.12, 8.23, 8.32, 8.33, 8.42-8.49



8.18-8.22

No	R^1	R^2	Hyal-1	PH-20	BTH	*Sag*Hyal$_{4755}$
			IC_{50} [µM][a]			
8.7	$CO(CH_2)_2Ph$	OH	> 500	83 ± 6	> 500	72 ± 4
8.8	$CO(CH_2)_8CH_3$	OH	> 100	36 ± 6	> 100	> 100
8.8a	$CO(CH_2)_8CH_3$	NHGlc[b]	> 150	≥ 200	> 200	> 200
8.9	CO-*p*-Cl-C_6H_4	OH	> 300	41 ± 6	> 1000	125 ± 5
8.10	CO-*p*-OMe-C_6H_4	OH	> 400	105 ± 7	> 400	> 400
8.11	CO-*p*-C_6H_4-Ph	OH	> 100	9.1 ± 0.7	> 100	≥ 60
8.12	$COCH_2$-*p*-OMe-C_6H_4	OH	> 250	113 ± 7	>250	> 250
8.23	$COCH_2$- *p*-OH-C_6H_4	OH	575 ± 25	89 ± 12	1670 ± 20	490 ± 30
8.18	-*p*-C_6H_4-	-	> 100	6.7 ± 0.6	> 100	> 100
8.19	-CH_2-*p*-$C_6H_4CH_2$-	-	>100	16 ± 2	>100	17 ± 3
8.20	-$(CH_2)_6$-	-	> 70	23 ± 2	> 70	> 70
8.21	-$(CH_2)_{10}$-	-	> 50	8.3 ± 3.2	> 50	18 ± 1
8.22	-$(CH_2)_{14}$-	-	> 30	4.4 ± 0.3	> 30	13 ± 1
8.32	$(CH_2)_{10}CO_2H$	OH	> 150	6.9 ± 0.7	> 150	12 ± 1
8.33	$(CH_2)_{15}CO_2H$	OH	> 100	3.0 ± 0.5	> 100	16.5 ± 0.4
8.42	$(CH_2)_{10}CONH$(thiazole-2-yl)	OH	> 100	6.9 ± 0.7	> 100	> 100
8.43	$(CH_2)_{10}CO$(4-phenylpiperazin-1-yl)	OH	> 50	20 ± 2	> 50	> 50
8.44	$(CH_2)_{10}CO$(piperazin-1-yl)	OH	> 100	> 100	> 100	61 ± 1
8.45	$(CH_2)_{10}CONH$-3-OH-C_6H_4	OH	> 100	20 ± 3	> 100	7.4 ± 3
8.46	$(CH_2)_{10}CO$(6-ascorbyl)	OH	29 ± 1	17 ± 1	284 ± 10	7.7 ± 0.5
8.47	$(CH_2)_{15}CO$(4-phenylpiperazin-1-yl)	OH	> 30	> 30	> 30	> 30

Table 8.1 (continued)

8.48	$(CH_2)_{15}CONH$-3-OH-C_6H_4	OH	> 30	3.9 ± 0.8	> 30	> 30
8.49	$(CH_2)_{15}CO$(6-ascorbyl)	OH	35 ± 3	3.1 ± 0.5	171 ± 7	8.1 ± 0.3

[a] inhibition of enzyme expressed as IC_{50} (µM), mean values ± SEM (N = 2, experiments performed in duplicate), maximal concentrations of the test compounds were selected with respect to individual terminal solubilities in the incubation mixture; IC_{50} values determined at pH 5.0 (PH-20, BTH, *Sag*Hyal$_{4755}$) or 3.5 (Hyal-1) in the turbidimetric assay; [b]Glc: 2-Deoxy-D-glucopyranose-2-yl.

Compared to indole-3-butanoic acid (see Table 8.3, chapter 8.3.2 for IC_{50} values) a dramatic increase in the inhibition of PH-20 and the bacterial enzyme is observed when hydrophobic residues are introduced by *N*-acylation of the indole moiety (**8.7-8.12**), e.g. potency increases by over 150-fold to an IC_{50} of 9.1 µM for the biphenylyl derivative **8.11** determined for the human PH-20. Figure 8.3 illustrates the inhibitory potencies of **8.7-8.12** and **8.23** on human PH-20. In some cases IC_{50} values could not be calculated for the inhibition of the bacterial enzyme due to poor solubilities of the compounds. Nevertheless, a certain percentage of inhibition was observed in all cases at micromolar concentrations (data not shown). Some noticeable differences in the SAR are observed when bacterial hyaluronate lyase and the human hyaluronidase PH-20 are compared: whereas inhibition of the human hyaluronidase is significantly increased when the phenylpropanoyl residue of **8.7** is replaced by a decanoyl chain (**8.8**), inhibition of *Sag*Hyal$_{4755}$ is decreased. This is in agreement with earlier results, where phenylpropanoyl substitution of the structurally related benzoxazole-2-thione scaffold led to increased inhibition of the bacterial hyaluronidase, probably by an energetically favored interaction of the phenyl residue with a hydrophobic patch which is assumed to interact with the hydrophobic sugar ring of *N*-acetylglucosamine of the substrate hyaluronan[1, 7]. This result could indicate similar binding modes of the presented indole-3-butanoic acids and the earlier described benzoxazole-2-thiones when the bacterial enzyme is regarded. An additional carbohydrate residue (**8.8a**) which replaces the carboxyl moiety in **8**, leads to complete loss of inhibitory activity. When **8.7** is compared with **8.9** bearing a *para*-chlorophenyl residue, again differences in SAR of both enzymes are obvious: inhibition of PH-20 is increased, whereas inhibition of the bacterial enzyme is significantly decreased. Hyal-1 and BTH are not inhibited by **8.7-8.12** and **8.8a**.

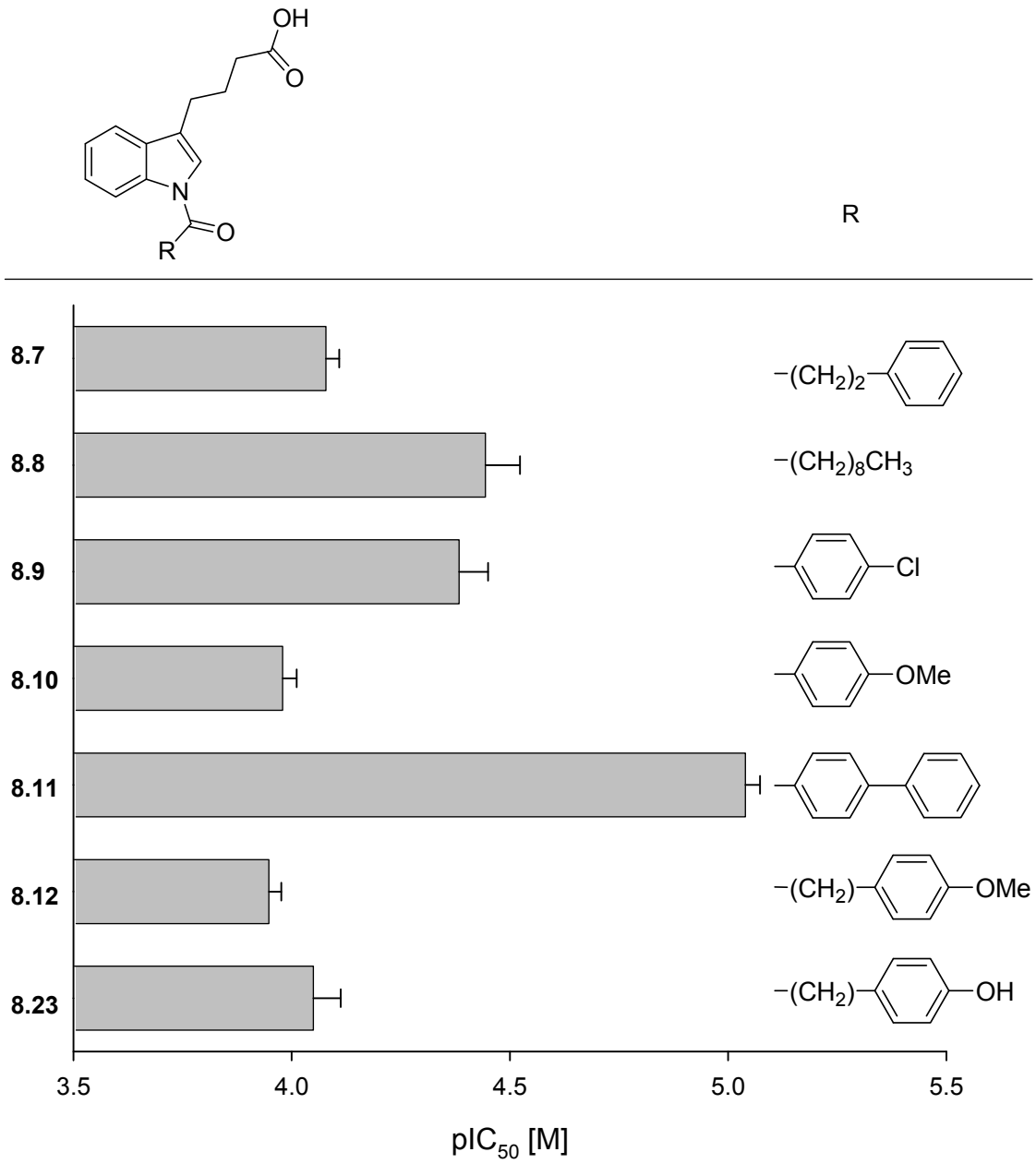


Figure 8.3. PH-20 inhibitory potencies of *N*-acylated indole-3-butanoic acid derivatives **8.7-8.12** and **8.23**.

An additional methylene group between methoxyphenyl and indole residue (compare **8.10** with **8.12**) does not lead to significant changes of the IC_{50} values. When the hydroxy derivative **8.23** is compared with the methoxy substituted compound **8.12** an increase in potency is obvious. Interestingly, **8.23** also inhibits human Hyal-1 and bovine PH-20 suggesting a contribution of the OH-group to binding by forming a hydrogen bond. Possibly the interaction site of the indole moiety at both human enzymes is different from that of the ascorbic acid derivatives described in chapter 7, because no increase in potency is achieved by adding hydrophobic sidechains (compound **8.8**, also see Table 8.3, compounds **8.63-8.66**).

Interesting results were obtained for the "bivalent" derivatives **8.18-8.22** (see Figure 8.4 for visualization of the potencies).

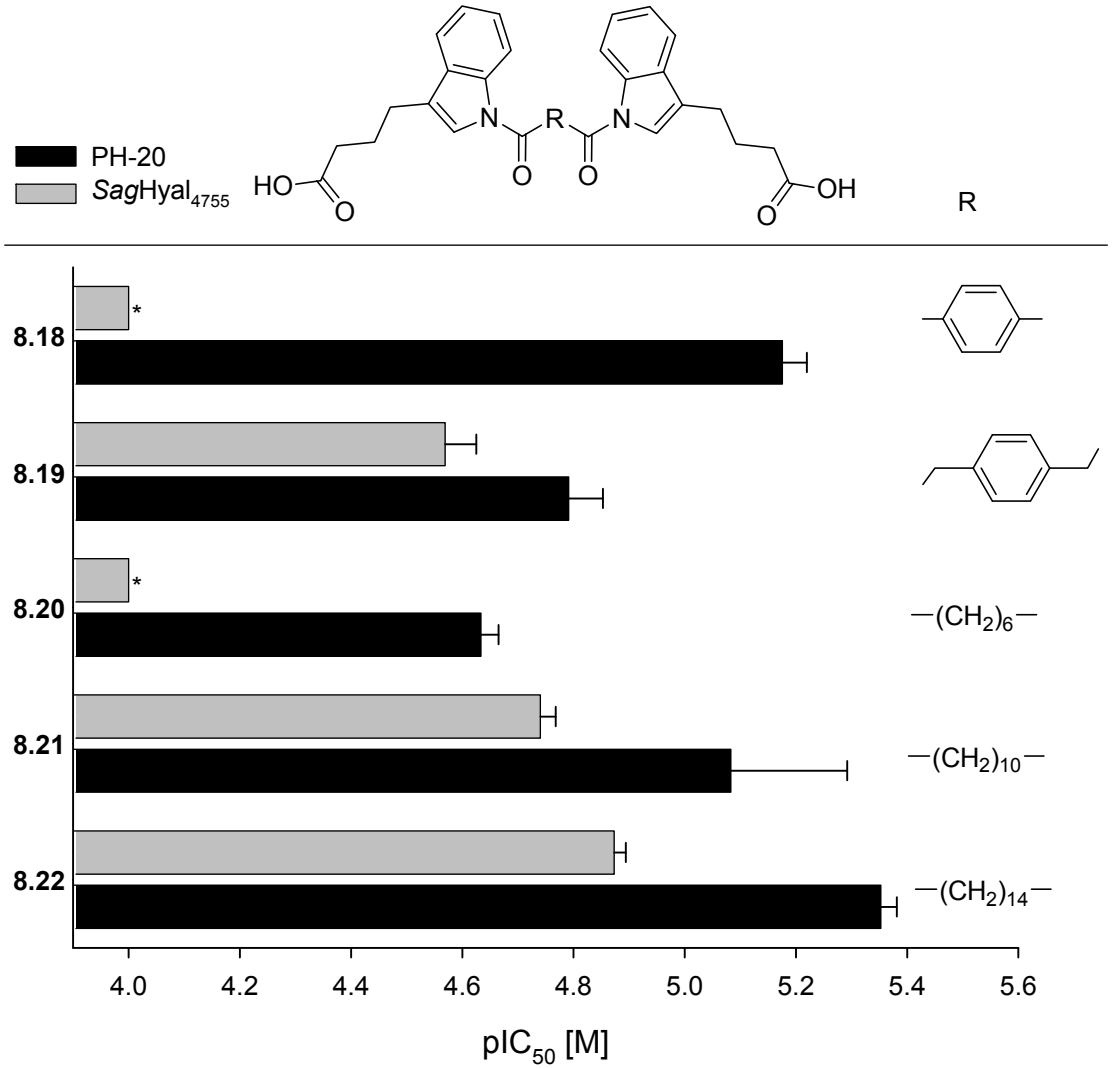


Figure 8.4. Inhibitory activities of bivalent indole-3-butanoic acid derivatives on human PH-20 and *Sag*Hyal$_{4755}$. Compounds marked with * did not show inhibitory activity on the investigated enzyme (arbitrary short bars for illustrative reasons).

Again, Hyal-1 and BTH are not inhibited while human PH-20 and bacterial hyaluronidases show a concentration dependent decrease in activity in the presence of those compounds. Compound **8.18** consisting of two indole-3-butanoic acid moieties connected *via* terephthalic acid possesses remarkable selectivity for the human PH-20 compared to the bacterial enzyme. Exceedingly strong inhibition of human PH-20 with an IC_{50} value of 6.7 µM was found for this derivative, whereas *Sag*Hyal$_{4755}$ was only slightly inhibited up to a concentration of 100 µM (data not shown); an exact IC_{50} value could not be determined due to insufficient terminal solubility. Possibly this relatively rigid molecule does not fit into the narrow crevice

which is present in the active site of the bacterial enzyme. A wider cleft as probably present in human PH-20[22] could accept such a rigid molecule more easily, and thus explain the higher inhibitory potency. The elongated aromatic spacer of **8.19** leads to a two-fold decrease in inhibition of PH-20, whereas the inhibition of *Sag*Hyal$_{4755}$ is drastically increased. Therefore, these two molecules are indicative of the different SAR for the human hyaluronidase PH-20 and the bacterial hyaluronate lyase from *Streptococcus agalactiae*, suggesting interactions with different amino acids in the binding site of those proteins. When the derivatives **8.20-8.22** which contain alkyl spacers are compared, an increase in potency which correlates with the length of the aliphatic chain is obvious. By adding four methylene groups to the alkyl spacer (compare **8.20** with **8.21** and **8.21** with **8.22**) the IC_{50} value for the human PH-20 decreases approximately by a factor of two. Regarding the bacterial enzyme, the IC_{50} also increases (compare **8.20** and **8.21**) but by adding additional four C-atoms the gain in inhibitory activity is only marginal (**8.22**). The bivalent ligands were inactive on both human Hyal-1 and BTH within the investigated concentration ranges.

A striking increase in potency is achieved by adding a terminal carboxyl group (**8.32** and **8.33**). The enzymatic activity of PH-20 and the bacterial hyaluronidase in the presence of **8.8**, **8.32** and **8.33** is depicted in Figure 8.5. Compound **8.32** is about 12-fold more potent as an inhibitor of human PH-20 compared to the analog lacking the additional carboxyl group (**8.8**). An at least 8-fold increase in potency is observed for the bacterial enzyme, too. Lengthening the connecting chain between the indole and carboxyl group by five carbon atoms leads to increased inhibition of PH-20, whereas a slight decreased inhibition was observed for the hyaluronate lyase. Regarding the synthesized esters or amides **8.42-8.49** again distinct differences between PH-20 and *Sag*Hyal$_{4755}$ are observed: whereas an additional 2-thiazolyl residue does not significantly affect the potency at the human enzyme (compare **8.42** and **8.32**), the inhibition of the bacterial enzyme is lost. Introduction of a basic piperazine ring as in **8.43** and **8.44** leads to lower inhibitory potency compared to the carboxyl derivative **8.32** in both cases, however, on PH-20 the piperazinyl derivative **8.44** was less potent than the derivative with an additional *N*-phenyl residue (**8.43**), and in the case of *Sag*Hyal$_{4755}$ the SAR is inverted. The introduction of structural motives bearing hydroxyl subtituents (**8.45** and **8.46**) leads to decreased inhibition of PH 20 (compared to **8.32**), whereas the inhibition of the bacterial enzyme is increased. Elongation of the alkyl spacer (compare **8.47-8.49** with **8.44-8.46**) does not result in

increased potency in the case of the piperazine containing compound, but leads to distinct increase in potency against PH-20 when hydroxyl substituted derivatives **8.48** and **8.49** are regarded.

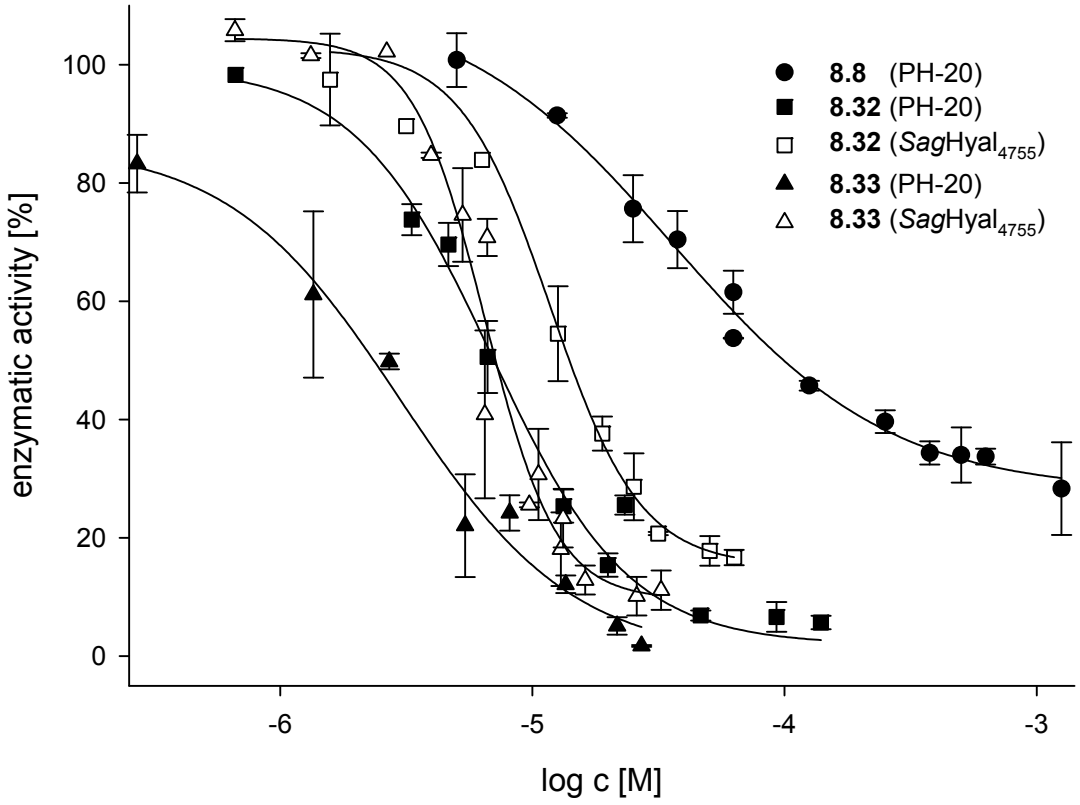


Figure 8.5. Enzymatic activity of human PH-20 (black symbols) and *Sag*Hyal$_{4755}$ (open symbols) in the presence of **8.8**, **8.32** and **3.33**. The different shape of the inhibition curve in the presence of high concentrations of **8.8** most probably results from the limited water solubility of this compound.

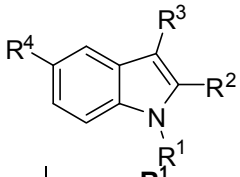
In contrast, the inhibition of the bacterial enzyme was reduced for the compounds with longer chains, **8.48** and **8.49**. Nevertheless, the compounds bearing hydroxyl moieties are potent inhibitors of bacterial hyaluronidase and PH-20. Under the assumption that the hydrophobic chain covers the same space of the protein as the substrate hyaluronan, the additional OH-groups may confer additional binding by mimicking functional groups of hyaluronic acid. Interestingly **8.46** and **8.49** also inhibit human Hyal-1 and the bovine hyaluronidase with IC_{50} values of 29 µM and 284 µM (**8.46**) and 35 µM and 171 µM (**8.49**), respectively. Certainly, the additional ascorbic acid residue accounts for the binding and inhibition of these enzymes, as no effect of other investigated alkyl-indoles lacking the vitamin C moiety was noticed. This may be attributed to a change in the binding mode of the compounds when the bacterial enzyme is regarded. X-ray analyses of hyaluronate lyase in complex with inhibitors

having either an indole or a vitamin C moiety support the working hypothesis that the indole occupies the same binding site of *Sag*Hyal$_{4755}$ as the ascorbic acid portion[7, 23]. Whereas the IC_{50} values of **8.46** and **8.49** are similar for the bacterial enzyme, an increase in potency towards human PH-20 is noticed for **8.49**, the compound bearing the longer alkyl spacer at the indole nitrogen, compared to **8.46**.

8.3.2 *N*-Acylated and *N*-alkylated indole-3-alkanoic acid derivatives with increased lipophilicity

The IC_{50} values of the investigated indoles, determined on Hyal-1, PH-20, BTH and *Sag*Hyal$_{4755}$, are summarized in Table 8.3, the substitution patterns are given in Table 8.2.

Table 8.2. Structures of investigated indole derivatives.



8.53-8.55, 8.62-8.66, 8.70-8.72, 8.74-8.78

Compound	R^1	R^2	R^3	R^4
Indole-3-butanoic acid	H	H	$(CH_2)_3CO_2H$	H
Indomethacin	CO-*p*-Cl-C_6H_4	Me	CH_2CO_2H	OMe
8.53	H	CO_2H	$(CH_2)_3CO_2H$	OCH_2Ph
8.54	H	H	$(CH_2)_3CO_2H$	OCH_2Ph
8.55	H	H	$(CH_2)_3CO_2H$	OH
8.62	H	CO_2H	$(CH_2)_3CO_2H$	$O(CH_2)_3Ph$
8.63	H	CO_2H	$(CH_2)_3CO_2H$	$O(CH_2)_5CH_3$
8.64	H	CO_2H	$(CH_2)_3CO_2H$	$O(CH_2)_{11}CH_3$
8.65	H	CO_2H	$(CH_2)_3CO_2H$	$O(CH_2)_{15}CH_3$
8.66	H	CO_2H	$(CH_2)_3CO_2H$	$O(CH_2)_{17}CH_3$
8.70	$(CH_2)_2CH_3$	CO_2H	$(CH_2)_3CO_2H$	$O(CH_2)_{15}CH_3$
8.71	CH_2-pyridin-3-yl	CO_2H	$(CH_2)_3CO_2H$	$O(CH_2)_{15}CH_3$
8.72	CH_2-*p*-Br-C_6H_4	CO_2H	$(CH_2)_3CO_2H$	$O(CH_2)_{15}CH_3$
8.74	$(CH_2)_9CH_3$	CO_2Et	$(CH_2)_3CO_2H$	OCH_2Ph
8.75	$(CH_2)_9CH_3$	CO_2H	$(CH_2)_3CO_2H$	OCH_2Ph
8.76	$(CH_2)_9CH_3$	CO_2Et	$(CH_2)_3CO_2H$	OH
8.77	$(CH_2)_9CH_3$	CO_2H	$(CH_2)_3CO_2H$	OH
8.78	CO-*p*-Cl-C_6H_4	Me	$CH_2CONHGlc^*$	OMe

*Glc: 2-Deoxy-D-glucopyranose-2-yl

Table 8.3. Inhibitory activity of indole derivatives on Hyal-1, PH-20, BTH and *Sag*Hyal$_{4755}$.

Compound	Hyal-1	PH-20	BTH	*Sag*Hyal$_{4755}$
	IC_{50} [µM][a]			
Indole-3-butanoic acid	> 5000	1425 ± 170	> 5000[b]	2200[b]
Indomethacin	390 ± 30	82 ± 9	540[c]	350[b]
8.53	≥ 600	141 ± 16	> 1200	164 ± 1
8.54	> 350	84 ± 8	> 350	318 ± 9
8.55	> 1000	1082 ± 57	> 1000	n.d.
8.62	> 600	35 ± 2	> 600	53 ± 2
8.63	> 700	60 ± 5	> 900	44 ± 3
8.64	> 80	3.6 ± 0.4	> 90	3.0 ± 0.1
8.65	> 40	1.3 ± 0.1	> 50	2.4 ± 0.1
8.66	> 30	5.0 ± 0.9	> 40	6.3 ± 0.2
8.70	> 30	> 30	> 30	> 50
8.71	> 50	2.3 ± 0.7	> 50	15 ± 1
8.72	> 50	11 ± 2	> 50	108 ± 10
8.74	> 40	11 ± 2	> 40	> 40
8.75	> 100	4.7 ± 0.3	> 130	20 ± 2
8.76	> 70	7.5 ± 0.2	> 70	7.5 ± 0.6
8.77	> 100	4.6 ± 0.2	> 150	9.3 ± 0.3
8.78	> 1000	604 ± 18	> 1000	189 ± 33

[a] inhibition of enzyme expressed as IC_{50} (µM), mean values ± SEM (N = 2, experiments performed in duplicate), maximal concentrations of the test compounds were selected with respect to individual terminal solubilities in the incubation mixture; IC_{50} values determined at pH 5.0 (PH-20, BTH, *Sag*Hyal$_{4755}$) or 3.5 (Hyal-1) in the turbidimetric assay; [b]as determined by Salmen[2]; [c]as determined by Salmen[2] at pH 3.6.

Most of the synthesized indole derivatives described in this chapter were inactive at the tested concentrations on human Hyal-1 and BTH, but turned out to be inhibitors of human PH-20 and the bacterial enzyme *Sag*Hyal$_{4755}$. Therefore, these indole based inhibitors must be clearly differentiated from the ascorbic acid derivatives described in chapter 7. Whereas the selectivity of the ascorbic acid type inhibitors is generally low, the indole derivatives show distinct selectivity for the bacterial enzyme and the human PH-20. Surprisingly, the bovine enzyme and the human Hyal-1 remained unaffected or were only very weakly inhibited in the presence of the indole derivatives, and it was not possible to determine IC_{50} values.

Indole-3-butanoic acid which was previously identified as an inhibitor of *Sag*Hyal$_{4755}$[2] turned out to be also an inhibitor of the recombinantly expressed human PH-20 with comparable inhibitory potency in the millimolar range. 5-Hydroxyindole-3-butanoic acid (**8.55**) was only slightly more potent than indole-3-butanoic acid PH-20, thus, there appears to be no relevant interaction of the additional 5-hydroxy group with the

enzyme. By contrast, an additional benzyloxy residue (**8.54**) leads to a drastic increase in potency, especially, when human PH-20 is regarded: **8.54** (IC_{50} 84 µM) is 17 times and 12 times more potent than indole-3-butanoic acid and **8.55**, repectively. An additional carboxyl residue (**8.53**) has only minor influence on the IC_{50} values: the inhibitory activities of **8.53** and **8.54** against PH-20 and *Sag*Hyal$_{4755}$ are in the same concentration ranges. However, the solubility of the compound in aqueous medium is considerably improved. Therefore, compound **8.53** was considered a promising lead for further structural variations in 5-position. The pIC_{50} values of such indole derivatives characterized by two carboxyl groups and different hydrophobic substituent in 5-position are visualized in Figure 8.6.

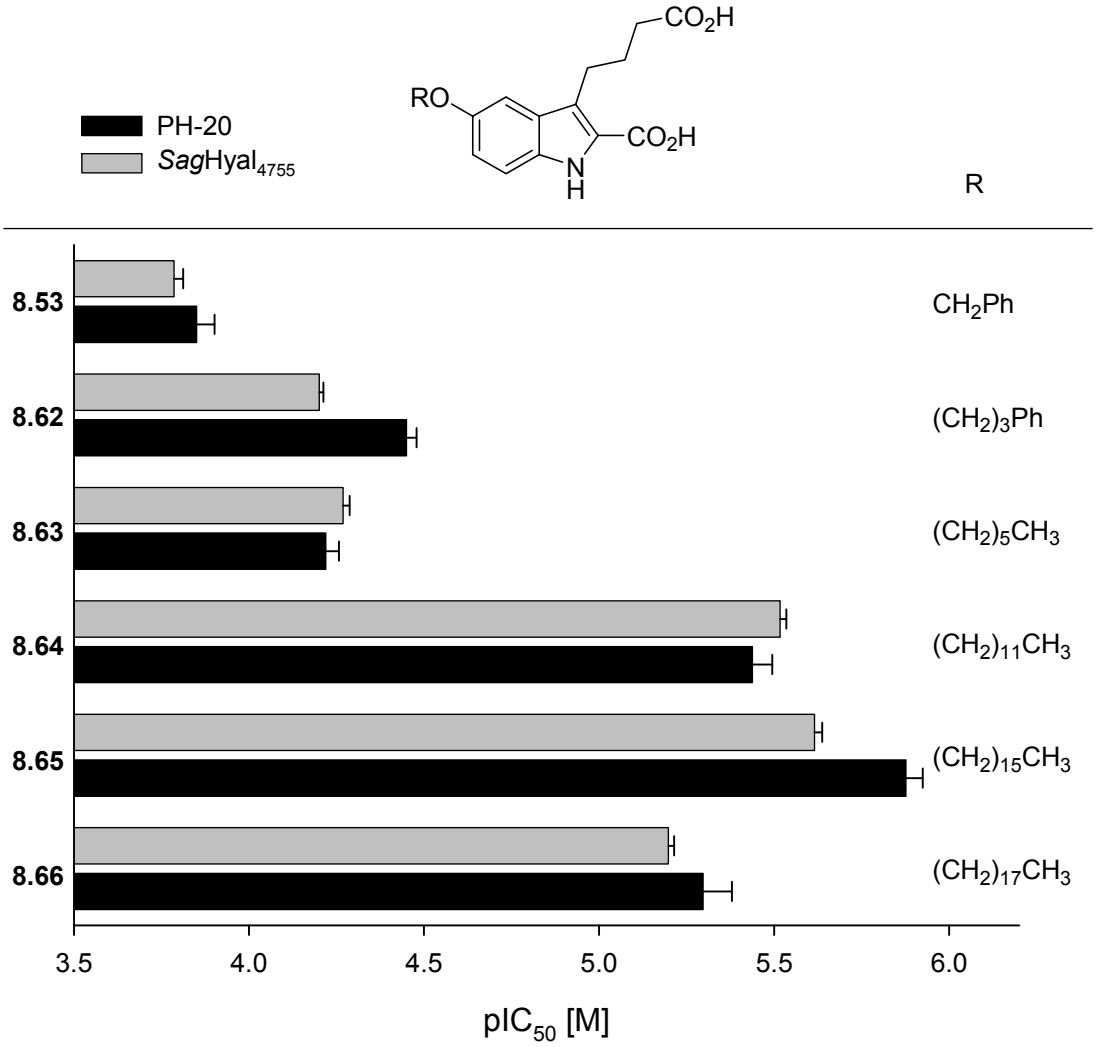


Figure 8.6. Inhibitory activity of dicarboxylic acids bearing hydrophobic substituents in position 5 of the indole scaffold, investigated on human PH-20 and the bacterial hyaluronate lyase.

The extension of the chainlength of hydrophobic substituents in position 5 of the indole scaffold (**8.62-8.66**) resulted in increased potency compared to **8.53**. The contribution of the hydrophobic residue to the affinity of the compounds is most

obvious from the drastic gain in potency found after extension of the alkyl chain from 6 (**8.63**) to 12 carbon atoms (**8.64**): IC_{50} values of 3.6 µM and 3.0 µM were determined for human PH-20 and *Sag*Hyal$_{4755}$ respectively. A further increase in chainlength to 16 carbon atoms leads only to a weak increase in potency, and extension to a 18-membered carbon chain (**8.66**) even leads to a decrease in inhibitory potency on both enzymes, indicating that there is an optimum size of the hydrophobic group around 16 carbon atoms. Thus, in this series of compounds the hexadecyloxy substituted indole **8.65** with IC_{50} values of 1.3 µM and 2.4 µM is the most potent inhibitor of human PH-20 and the bacterial enzyme, respectively. The compound is characterized by high selectivity for PH-20 and *Sag*Hyal$_{4755}$ compared to human Hyal-1 and BTH.

The introduction of additional residues at the indole nitrogen (**8.70-8.72**) does not lead to increased potency. This may suggest a possible role of the indole-nitrogen as H-donor, however, it should be stressed that N-substituents are generally tolerated. Interestingly, for compound **8.71** bearing a pyridyl-substituent an IC_{50} value of 2.3 µM was determined on human PH-20, i. e. the potency of this hyaluronidase inhibitor is in the same range as that of the *N*-unsubstituted analog **8.65**. It is tempting to speculate that the weakly basic pyridine nitrogen is involved in the binding mode because the the two *N*-substituted derivatives **8.70** and **8.72** bearing a propyl- or para-bromobenzyl-moiety are much less potent. Therefore, functionalized *N*-substituents might be useful with respect to further structural optimization.

The indole derivatives **8.74-8.77** contain an *N*-decyl chain. The potency of the dicarboxylic acids **8.75** and **8.77** is clearly enhanced when compared to the indole-3-butanoic acid derivative **8.8** which is bearing a decanoyl sidechain and is lacking the 2-carboxylic acid and the 5-benzyloxy residue. The ethyl esters **8.74** and **8.76**, are slightly superior to their corresponding carboxylic acids **8.75** and **8.77**, when investigated for inhibition of PH-20. On the bacterial enzyme **8.76** and **8.77** are in the same range of potency, whereas the inhibitory activity of **8.75** is lost upon esterification of 2-carboxyl group (compare **8.75** and **8.74**). The only structural difference between compounds **8.74/8.75** and **8.76/8.77** is the substitution in position 5 of the indole ring. Therefore, it cannot be ruled out that the additional hydroxyl or benzyloxy moiety causes changes in the binding mode. Concerning the bacterial hyaluronidase, the significantly lower IC_{50} values of **8.76** and **8.77** may be explained by additional hydrogen bonds of the free hydroxyl group with amino acids in the

binding site. In contrast to the observation made for the bacterial hyaluronate lyase, no major changes in potency on human PH-20 are noticed when benzyloxy and the hydroxy derivatives are compared (IC_{50} values: 4.7 µM determined for **8.75** and 4.6 µM for **8.77**). Thus, the hydroxyl or the benzyloxy group in 5-position are presumably not essentially involved in binding to the human enzyme. This is in clear contrast with the results obtained for the small indole derivatives **8.53-8.55** and indole-3-butanoic acid as discussed above, where the additional benzyl residue causes a clear increase in potency. This fact is probably the result of a change in binding mode again. It is conceivable that the benzyloxy residues of **8.53** and **8.54** occupy the same hydrophobic area of the enzymes as the decyl chains of **8.74-8.77,** resulting in a different orientation of the indole scaffold relative to the enzyme.

Indomethacin, a known hyaluronidase inhibitor[10] inhibits both human enzymes, Hyal-1 and PH-20, with IC_{50} values of 390 µM and 82 µM, respectively. The inhibition of Hyal-1 and BTH is lost and the activity against human PH-20 is drastically weakened when a carbohydrate scaffold is introduced (**8.78**) to replace the carboxylate in indomethacin. Interestingly, this modification yields an inhibitor of *Sag*Hyal$_{4755}$ which is more potent than the parent compound, indomethacin. These observations again underline the differences in SAR between mammalian hyaluronidases and bacterial hyaluronate lyases.

8.4 *Summary*

Starting from the lead compound, indole-3-butanoic acid, the hydrophobicity of the derivatives was increased by *N*-acylation resulting in an increase in inhibition of human PH-20 and the bacterial hyaluronate lyase *Sag*Hyal$_{4755}$. Among the synthesized derivatives, the compound bearing a *p*-biphenylylcarbonyl residue (**8.11**) is a surprisingly potent inhibitor of PH-20 with an IC_{50} value of 9.1 µM, whereas *Sag*Hyal$_{4755}$ is only weakly inhibited. As most of the indoles described in this chapter are inactive on human Hyal-1 and bovine testicular hyaluronidase, *N*-(*p*-hydroxybenzoyl)indole-3-butanoic acid (**8.23**) represents an exception: these two enzymes are weakly inhibited by **8.23**. Strong inhibition of both human PH-20 and the bacterial enzyme is achieved by “bivalent” ligands which are composed of two indole-3-butanoic acids connected *via* hydrophobic aromatic or aliphatic spacers attached to

the indole-N. Additionally, the derivative containing a phenyl spacer between the two indole scaffolds (**8.18**) was found to possess increased selectivity for PH-20 *versus* *Sag*Hyal$_{4755}$. Whereas additional carboxyl groups at the end of hydrophobic undecanoyl or hexadecanoyl spacers are very well tolerated by PH-20 and *Sag*Hyal$_{4755}$ and can even enhance the inhibitory activity, additional basic piperazinyl moieties drastically decrease potency. When ascorbic acid moieties are attached at the end of the hydrophobic chain, inhibition of Hyal-1 and BTH is observed. Possibly, the vitamin C scaffold acts as an anchor allowing a switch in the binding mode. The ascorbic acid moiety might bind to regions of the enzymes which are different from the indole binding site(s).

Interestingly, the introduction of hydrophobic motives in position 5 of the indole scaffold also led to drastic increase in inhibitory activity against human PH-20 and the bacterial hyaluronat lyase. A second carboxyl residue, introduced in position 2 of indole-3-butanoic acid, retains the potency and considerably improves the solubility in water. Lengthening the aliphatic 5-alkyloxy chain results in increased inhibition of the two enzymes: IC_{50} values of 1.3 µM and 2.4 µM were determined for the 5-hexadecyloxy derivative **8.65** on human PH-20 and *Sag*Hyal$_{4755}$, respectively. A further extension of the chainlength leads to a significant decrease in potency. This may be interpreted a hint to the dimension of the hydrophobic patch in the binding site of those two enzymes. Additional substitution of the indole nitrogen in 5-alkyloxy-indoledicarboxylic acids does not increase the inhibitory effect. Differences in the SAR between human PH-20 and the bacterial enzyme are obvious, especially when minor modifications in position 2 and 5 of the indole moiety (compounds **8.74-8.77**) are considered.

The presented indole-3-butanoic acid derivatives presented in this chapter must be clearly differentiated from the derivatives of glucurono-3,6-lactone (chapter 6) and ascorbic acid (chapter 7) in terms of selectivity. Whereas those compounds possess more or less pronounced inhibitory activity against all four investigated enzymes, the synthesized indole derivatives show distinct selectivity for the human PH-20 and the bacterial hyaluronate lyase, whereas human Hyal-1 and bovine testicular hyaluronidase are generally not inhibited in the investigated concentration ranges. Thus, the synthesized derivatives are the most potent indole-based inhibitors of *Sag*Hyal$_{4755}$ and human PH-20 and they are characterized by distinct selectivity for PH-20 compared to the other investigated mammalian hyaluronidases. This

selectivity is proven by testing at equiactive enzyme concentrations as described for selected compounds in chapter 10.3.5. BTH, the prototype of class I hyaluronidases (E.C. 3.2.1.35), has been broadly accepted as a model enzyme for the design of inhibitors for human hyaluronidases. This working hypothesis is challenged by striking results of this thesis: recombinant human PH-20 is strongly inhibited by some indoles and the bovine homolog is not. Moreover, the phenomenon that in terms of SAR the human PH-20 is more similar to the bacterial hyaluronate lyase than to its human homolog Hyal-1 is highly surprising, as there are distinct differences between PH-20 and *Sag*Hyal$_{4755}$ in terms of structure and mechanism of action. Both enzymes are representatives of different classes of hyaluronidases: whereas the bacterial enzyme utilizes a β-elimination mechanism, the human PH-20 is a hydrolase. Thus, a comparable behavior of human hyaluronidases Hyal-1 and PH-20 was expected rather than similarities between PH-20 and *Sag*Hyal$_{4755}$.

8.5 *Experimental Section*

8.5.1 General conditions

Chemicals were purchased from the following suppliers: Acros Organics (Geel, Belgium), IRIS Biotech GmbH (Marktredwitz, Germany), Alfa Aesar GmbH & Co. KG (Karlsruhe, Germany), Merck KGaA (Darmstadt, Germany), and Sigma-Aldrich Chemie GmbH (Munich, Germany). Deuterated solvents for NMR spectroscopy were from Deutero GmbH (Kastellaun, Germany). All solvents were of analytical grade or distilled prior to use. If moisture-free conditions were required, reactions were performed in dried glassware under inert atmosphere (argon or nitrogen); DMF (H_2O < 0.01 %) was purchased from Sigma-Aldrich Chemie GmbH. Nuclear Magnetic Resonance (^{1}H-NMR and ^{13}C-NMR) spectra were recorded on an Avance-300 NMR spectrometer from Bruker BioSpin GmbH (Rheinstetten, Germany). Tetramethyl-silane was added as internal standard (chemical shift δ = 0 ppm) to all samples. Multiplicities are specified with the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), bs (for broad singulet), as well as combinations thereof. The multiplicity of carbon atoms (^{13}C-NMR) were determined by DEPT 135

and DEPT 90 (distortionless enhancement by polarization transfer): "+" primary and tertiary carbon atom (positive DEPT 135 signal), "-" secondary carbon atom (negative DEPT 135 signal), "quat" quaternary carbon atom. Mass spectrometry analysis (MS) was performed on a Finnigan MAT 95 (PI-EIMS 70 eV, HR-MS), Finnigan SSQ 710A (CI-MS (NH_3)) and on a Finnigan ThermoQuest TSQ 7000 (ESI-MS) spectrometer. The peak-intensity in parenthesis is indicated relatively to the strongest signal in %. Melting points (mp) were measured on a BÜCHI 530 using open capillaries and are uncorrected. Merck Silica Gel 60 (particle size 0.040–0.063 mm) was used for flash column chromatography. Reactions were routinely monitored by thin layer chromatography (TLC) on Merck silica gel 60 F_{254} aluminum sheets and spots were visualized with UV light at 254 nm, and/or iodine vapor or ammonium molybdate/cerium(IV) sulfate solution.

8.5.2 Chemistry

8.5.2.1 General procedure for the synthesis of phenyl esters 8.1-8.3, 8.14-8.17

To a stirred solution of the pertinent carboxylic acid (1 eq) in CH_2Cl_2/THF was added DMAP (0.2 eq / carboxylic acid moiety) and DCC (1.1 eq / carboxylic acid moiety). A solution of phenol (1.2 eq / carboxylic acid moiety) in CH_2Cl_2 was added dropwise and the mixture was stirred at room temperature overnight. After removal of the solvent under reduced pressure, the residue was taken up in EtOAc, filtered and evaporated again. The raw product was submitted to flash chromatography.

Phenyl 3-phenylpropanoate[24] (8.1): The title compound was prepared according to general procedure 8.5.2.1 using hydrocinnamic acid (3 mmol, 0.45 g), DMAP (0.6 mmol, 73 mg), DCC (3.3 mmol, 0.68 g) and phenol (3.6 mmol, 0.34 g) in anhydrous CH_2Cl_2 (10 ml) and was obtained after flash chromatography (PE/$CHCl_3$ 2/1 to 1/1 v/v) as colorless oil (0.64 g, 92 %). ^{1}H-NMR ($CDCl_3$) δ 2.88 (m, 2H, C$\underline{H_2}$Ph), 3.08 (t, 2H, ^3J = 7.6 Hz, $COCH_2$), 7.01 (m, 2H, Ph-H), 7.18 – 7.39 (m, 8H, Ph-H). EI-MS (70 eV) *m/z (%)*: 226 (24) [$M^{+\cdot}$]. $C_{15}H_{14}O_2$ (226.27).

Phenyl decanoate[25] (8.2): The title compound was prepared according to general procedure 8.5.2.1 using decanoic acid (3 mmol, 0.52 g), DMAP (0.6 mmol, 73 mg), DCC (3.3 mmol, 0.68 g) and phenol (3.6 mmol, 0.34 g) in anhydrous CH_2Cl_2 (10 ml) and was obtained after flash chromatography (PE/$CHCl_3$ 2/1 v/v) as colorless oil

(0.71 g, 96 %). ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.7 Hz, CH_3), 1.36 (m, 12H, ($C\underline{H}_2$)$_6CH_3$), 1.75 (m, 2H, $COCH_2C\underline{H}_2$), 2.54 (t, 2H, ^3J = 8.0 Hz, $COCH_2$), 7.07 (m, 2H, Ph-H), 7.21 (m, 1H, Ph-H), 7.36 (m, 2H, Ph-H). EI-MS (70 eV) *m/z (%)*: 248 (28) [$M^{+\cdot}$]. $C_{16}H_{24}O_2$ (248.36).

Phenyl 4-chlorobenzoate[26] (8.3): The title compound was prepared according to general procedure 8.5.2.1 using 4-chlorobenzoic acid (3 mmol, 0.47 g), DMAP (0.6 mmol, 73 mg), DCC (3.3 mmol, 0.68 g) and phenol (3.6 mmol, 0.34 g) in anhydrous CH_2Cl_2 (10 ml) and was obtained after flash chromatography (PE/$CHCl_3$ 1/1 v/v) as a white solid (0.64 g, 92 %). mp: 95 °C (ref.[26]: 103-104 °C); ^{1}H-NMR ($CDCl_3$) δ 7.25 (m, 3H, Ar-H), 7.47 (m, 4H, Ar-H), 8.15 (m, 2H, Ar-H). $C_{13}H_9ClO_2$ (232.66).

8.5.2.2 General procedure for the synthesis of *N*-acylated indole-3-butanoic acids 8.7-8.9 and 8.19-8.22

A solution of indole-3-butanoic acid (1.02 eq) in anhydrous DMF (3 ml) was added to a suspension of NaH (60 % suspension in mineral oil, 3.2 eq) in anhydrous DMF (4 ml) under an atmosphere of argon at -41 °C. The cooling bath was removed and the mixture was stirred at 0 °C for 15 min. After cooling to -20 °C a solution of the pertinent phenol ester (1 eq) in anhydrous DMF (3 ml) was added dropwise and the mixture was stirred for additional 30 min. The reaction mixture was quenched by adding icecold 1 N HCl. The organic compounds were extracted three times with EtOAc. After washing the combined organic layers with water and brine, the organic phase was dried over $MgSO_4$, filtered and evaporated to dryness. Flash chromatography or recrystallization yielded the target compounds.

1-(3-Phenylpropanoyl)-1*H*-indole-3-butanoic acid (8.7): The title compound was prepared according to general procedure 8.5.2.2 using indole-3-butanoic acid (1.02 mmol, 0.21 g), NaH (60 % suspension in mineral oil, 3.3 mmol, 0.12 g) and **8.1** (1 mmol, 0.23 g) and was obtained after flash chromatography ($CHCl_3$/MeOH 93/7 v/v) as a pale yellow solid (0.21 g, 63 %). mp: 128-129 °C; ^{1}H-NMR (DMSO-d_6) δ 1.90 (m, 2H, $C\underline{H}_2CH_2CO_2H$), 2.31 (t, 2H, ^3J = 7.3 Hz, $C\underline{H}_2CO_2H$), 2.67 (t, 2H, ^3J = 7.5 Hz, $C\underline{H}_2(CH_2)_2CO_2H$), 3.02 (t, 2H, ^3J = 7.6 Hz, $COCH_2$), 3.36 (t, 2H, ^3J = 7.6 Hz, $C\underline{H}_2Ph$), 7.25 (m, 8H, Ar-H), 7.59 (m, 1H, Ar-H), 7.72 (s, 1H, H-2), 8.35 (m, 1H, Ar-H), 12.08 (m, 1H, CO_2H). ^{13}C-NMR (DMSO-d_6) δ 23.61 (-, CH_2), 24.03 (-, CH_2), 29.76 (-, CH_2), 33.25 (-, CH_2), 36.22 (-, CH_2), 115.93 (+, indole-C), 118.89 (+, indole-C), 121.00 (quat, indole-C), 122.85 (+, indole-C), 123.07 (+, indole-C), 124.60 (+, indole-C), 125.93 (+, Ph-C), 128.20 (+, Ph-C), 128.41 (+, Ph-C), 130.20 (quat, C-8), 135.23

(quat, C-7), 140.62 (quat, Ph-C), 170.92 (quat, NCO), 174.27 (quat, CO_2H). CI-MS (NH_3) *m/z (%)*: 353 (100) $[M+NH_4]^+$. Anal. ($C_{21}H_{21}NO_3$) C, H, N. $C_{21}H_{21}NO_3$ (335.40).

1-Decanoyl-1*H*-indole-3-butanoic acid (8.8): The title compound was prepared according to general procedure 8.5.2.2 using indole-3-butanoic acid (1.02 mmol, 0.21 g), NaH (60 % suspension in mineral oil, 3.3 mmol, 0.12 g) and **8.2** (1 mmol, 0.25 g) and was obtained after flash chromatography ($CHCl_3$/MeOH 93/7 v/v) and recrystallization from EtOAc/hexane as a white solid (0.19 g, 53 %). mp: 94 °C; ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H, ^3J = 6.7 Hz, CH_3), 1.30 (m, 12H, $(C\underline{H}_2)_6CH_3$), 1.67 (m, 2H, $COCH_2C\underline{H}_2$), 1.91 (m, 2H, $C\underline{H}_2CH_2CO_2H$), 2.32 (t, 2H, ^3J = 7.3 Hz, $C\underline{H}_2CO_2H$), 2.69 (t, 2H, ^3J = 7.5 Hz, $C\underline{H}_2(CH_2)_2CO_2H$), 2.98 (t, 2H, ^3J = 7.3 Hz, $COCH_2$), 7.29 (m, 2H, H-5, indole-H), 7.59 (dd, 1H, ^4J = 1.4 Hz, ^3J = 6.9 Hz, indole-H), 7.68 (s, 1H, H-2), 8.34 (dd, 1H, ^4J = 1.0 Hz, ^3J = 7.3 Hz, indole-H), 12.09 (bs, 1H, CO_2H). ^{13}C-NMR (DMSO-d_6) δ 13.87 (+, CH_3), 22.03 (-, CH_2), 23.63 (+, CH_2), 24.11 (+, CH_2), 28.42 (+, CH_2), 28.60 (+, CH_2), 28.76 (+, CH_2), 28.85 (+, CH_2), 31.21 (+, CH_2), 33.26 (+, CH_2), 34.75 (+, CH_2), 115.93 (+, indole-C), 118.85 (+, indole-C), 120.91 (quat, C-3), 122.81 (+, indole-C), 122.99 (+, indole-C), 124.56 (+, indole-C), 130.17 (quat, C-8), 135.22 (quat, C-7), 171.67 (quat, NCO), 174.26 (quat, CO_2H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 358 (100) $[M+H]^+$. Anal. ($C_{22}H_{31}NO_3$) C: 73.91, H: 8.74, N: 3.92, found C: 73.37, H: 9.30, N: 3.79. $C_{22}H_{31}NO_3$ (357.49).

***N*-[2-Deoxy-D-glucopyranose-2-yl]-1-decanoyl-1*H*-indole-3-butanoic acid amide (8.8a):** To a solution of **8.8** (0.20 mmol 71 mg), HOBt (0.24 mmol, 32 mg), NEt_3 (0.24 mmol, 33 µl) and EDAC (0.24 mmol, 46 mg) in DMF (2 ml) was added a solution of D-glucosamine hydrochloride (0.40 mmol, 86 mg) and NEt_3 (0.40 mmol, 55 µl) in DMF (1 ml) and water (0.5 ml). After stirring overnight the solvent was removed under reduced pressure and the remaining raw product was subjected to flash chromatography ($CHCl_3$/MeOH 95/5 to 85/15 v/v) to yield the title compound as white solid (85 mg, 82 %). mp: 157 °C; ^{1}H-NMR (CD_3OD) δ 0.89 (t, 3H, ^3J = 6.2 Hz, CH_3), 1.36 (m, 12H, $(C\underline{H}_2)_6CH_3$), 1.78 (m, 2H, $COCH_2C\underline{H}_2$), 2.03 (m, 2H, $C\underline{H}_2CH_2CO_2H$), 2.34 (t, 2H, ^3J = 7.2 Hz, $C\underline{H}_2CO_2H$), 2.75 (t, 2H, ^3J = 7.2 Hz, $C\underline{H}_2(CH_2)_2CO_2H$), 2.96 (t, 2H, ^3J = 7.3 Hz, $COCH_2$), 3.35 – 3.91 (m, 6H, $C\underline{H}_2OH$, H-2, H-3, H-4, H-5), 5.14 (d, 1H, ^3J = 3.4 Hz, H-1), 7.26 (m, 2H, indole-H), 7.55 (m, 2H, indole-H), 8.37 (d, 1H, ^3J = 7.8 Hz, indole-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 519 (100) $[M+H]^+$. Anal. ($C_{28}H_{42}N_2O_7$) C, H, N. $C_{28}H_{42}N_2O_7$ (518.64).

1-(4-Chlorobenzoyl)-1*H*-indole-3-butanoic acid (8.9)[27]: The title compound was prepared according to general procedure 8.5.2.2 using indole-3-butanoic acid (1.02 mmol, 0.21 g), NaH (60 % suspension in mineral oil, 3.3 mmol, 0.12 g) and **8.3** (1 mmol, 0.23 g) and was obtained after flash chromatography ($CHCl_3$/MeOH 93/7 v/v) and recrystallization from EtOAc/hexane as a white solid (0.20 g, 59 %). mp: 144 °C; ^{1}H-NMR (DMSO-d_6) δ 1.84 (m, 2H, C$\underline{H}_2$CH$_2$CO$_2$H), 2.27 (t, 2H, ^3J = 7.3 Hz, C$\underline{H}_2$CO$_2$H), 2.67 (t, 2H, ^3J = 7.5 Hz, C$\underline{H}_2$(CH$_2$)$_2$CO$_2$H), 7.19 (s, 1H, H-2), 7.37 (m, 2H, Ar-H), 7.67 (m, 3H, Ar-H), 7.78 (m, 2H, Ar-H), 8.27 (dd, 1H, ^3J = 1.2 Hz, ^3J = 7.1 Hz, indole-H), 12.08 (s, 1H, CO$_2$H). ^{13}C-NMR (DMSO-d_6) δ 23.41 (-, CH$_2$), 24.03 (-, CH$_2$), 33.11 (-, CH$_2$), 115.86 (+, indole-C), 119.18 (+, indole-C), 121.43 (quat, C-3), 123.69 (+, indole-C), 124.36 (+, indole-C), 124.76 (+, indole-C), 128.71 (+, Ar-C), 130.61 (quat, C-8), 130.75 (+, Ar-C), 132.98 (quat, Ar-C), 135.62 (quat, C-7), 136.53 (quat, Ar-C), 166.83 (quat, NCO), 174.22 (quat, CO$_2$H). Anal. ($C_{19}H_{16}ClNO_3$) C, H, N. $C_{19}H_{16}ClNO_3$ (341.79).

Diphenyl 2,2'-(1,4-phenylene)diacetate[28] (8.14): The title compound was prepared according to general procedure 8.5.2.1 using 1,4-phenylenediacetic acid (5 mmol, 0.97 g), DMAP (2 mmol, 0.24 g), DCC (11 mmol, 2.27 g) and phenol (12 mmol, 1.13 g) in anhydrous CH_2Cl_2 (20 ml) and was obtained after flash chromatography (PE/$CHCl_3$ 1/2 v/v) as a white solid (1.47 g, 85 %). mp: 96-97 °C; ^{1}H-NMR ($CDCl_3$) δ 3.86 (s, 4H, CH$_2$), 7.06 (m, 4H, Ar-H), 7.21 (m, 2H, Ar-H), 7.35 (m, 8H, Ar-H). EI-MS (70 eV) *m/z (%)*: 346 (4) [$M^{+\cdot}$]. Anal. ($C_{22}H_{18}O_4$) C, H. $C_{22}H_{18}O_4$ (346.38).

Diphenyl octanedioate[29] (8.15): The title compound was prepared according to general procedure 8.5.2.1 using octanedioic acid (5 mmol, 0.97 g), DMAP (2 mmol, 0.24 g), DCC (11 mmol, 2.27 g) and phenol (12 mmol, 1.13 g) in anhydrous CH_2Cl_2 (20 ml) and was obtained after flash chromatography (PE/$CHCl_3$ 1/2 v/v) as a white solid (1.30 g, 80 %). mp: 68 °C (ref.[30]: 70-71 °C); ^{1}H-NMR ($CDCl_3$) δ 1.49 (m, 4H, COCH$_2$CH$_2$(C$\underline{H}_2$)$_2$), 1.79 (m, 4H, COCH$_2$C$\underline{H}_2$), 2.57 (t, 4H, ^3J = 7.4 Hz COCH$_2$), 7.07 (m, 4H, Ph-H), 7.22 (m, 2H, Ph-H), 7.37 (m, 4H, Ph-H). EI-MS (70 eV) *m/z (%)*: 326 (3) [$M^{+\cdot}$]. Anal. ($C_{20}H_{22}O_4$) C, H. $C_{20}H_{22}O_4$ (326.39).

Diphenyl dodecanedioate[31] (8.16): The title compound was prepared according to general procedure 8.5.2.1 using dodecanedioic acid (5 mmol, 1.15 g), DMAP (2 mmol, 0.24 g), DCC (11 mmol, 2.27 g) and phenol (12 mmol, 1.13 g) in anhydrous CH_2Cl_2 (20 ml) and was obtained after flash chromatography (PE/$CHCl_3$ 1/2 v/v) as a white solid (1.80 g, 94 %). mp: 64 °C (ref.[30]: 70-71 °C); ^{1}H-NMR ($CDCl_3$) δ 1.34 (m,

12H, $COCH_2CH_2(C\underline{H}_2)_6$), 1.75 (m, 4H, $COCH_2C\underline{H}_2$), 2.55 (t, 4H, ^3J = 7.5 Hz, $COCH_2$), 7.07 (m, 4H, Ph-H), 7.21 (m, 2H, Ph-H), 7.36 (m, 4H, Ph-H). EI-MS (70 eV) *m/z (%)*: 382 (10) [$M^{+\cdot}$]. Anal. ($C_{24}H_{30}O_4$) C, H. $C_{24}H_{30}O_4$ (382.49).

Diphenyl hexadecanedioate[32] (8.17): The title compound was prepared according to general procedure 8.5.2.1 using hexadecanedioic acid (5 mmol, 1.43 g), DMAP (2 mmol, 0.24 g), DCC (11 mmol, 2.27 g) and phenol (12 mmol, 1.13 g) in anhydrous CH_2Cl_2 (20 ml) and was obtained after flash chromatography (PE/$CHCl_3$ 1/2 v/v) as a white solid (1.91 g, 87 %). mp: 74 °C (ref.[32]: 86-87 °C); ^{1}H-NMR ($CDCl_3$) δ 1.34 (m, 20H, $COCH_2CH_2(C\underline{H}_2)_{10}$), 1.75 (m, 2H, $COCH_2C\underline{H}_2$), 2.55 (t, 4H, ^3J = 7.5 Hz, $COCH_2$), 7.07 (m, 4H, Ph-H), 7.21 (m, 2H, Ph-H), 7.37 (m, 4H, Ph-H). EI-MS (70 eV) *m/z (%)*: 345 (100) [M-$C_6H_5O^{+\cdot}$]. Anal. ($C_{28}H_{38}O_4$) C, H. $C_{28}H_{38}O_4$ (438.60).

1,1'-(*p*-Phenylendiacetyl)diindol-3,3'-dibutanoic acid (8.19): The title compound was prepared according to general procedure 8.5.2.2 using indole-3-butanoic acid (1.02 mmol, 0.21 g), NaH (60 % suspension in mineral oil, 3.3 mmol, 0.12 g) and **8.14** (0.5 mmol, 0.17 g). After quenching the reaction with 1 N HCl, the resulting white solid was collected by filtration and recrystallized from methanol to yield **8.19** as a white solid (0.15 g, 53 %). ^{1}H-NMR (DMSO-d_6) δ 1.92 (m, 4H, $C\underline{H}_2CH_2CO_2H$), 2.34 (t, 4H, ^3J = 7.3 Hz, $C\underline{H}_2CO_2H$), 2.71 (t, 4H, ^3J = 7.4 Hz, $C\underline{H}_2(CH_2)_2CO_2H$), 4.39 (s, 4H, $C\underline{H}_2C_6H_4$), 7.30 (m, 8H, indole-H, Ph-H), 7.61 (dd, 2H, ^4J = 1.2 Hz, ^3J = 6.6 Hz, indole-H), 7.85 (s, 2H, H-2), 8.33 (dd, 2H, ^4J = 1.2 Hz, ^3J = 7.4 Hz, indole-H), 12.11 (s, 2H, CO_2H). ^{13}C-NMR (DMSO-d_6) δ 23.63 (-, CH_2), 24.09 (-, CH_2), 33.25 (-, CH_2), 41.18 (-, $\underline{C}H_2C_6H_4$), 115.98 (+, indole-C), 118.94 (+, indole-C), 121.26 (quat, indole-C), 123.03 (+, indole-C), 123.23 (+, indole-C), 124.70 (+, indole-C), 129.61 (+, Ar-C), 130.29 (quat, C-8), 132.91 (quat, Ar-H), 135.40 (quat, C-7), 169.80 (quat, NCO), 174.26 (quat, CO_2H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 563 (100) [M-H]$^-$. Anal. ($C_{34}H_{32}N_2O_6 \cdot 0.5H_2O$) C, H, N. $C_{34}H_{32}N_2O_6$ (564.63).

1,1'-Octanedioyldiindol-3,3'-dibutanoic acid (8.20): The title compound was prepared according to general procedure 8.5.2.2 using indole-3-butanoic acid (1.02 mmol, 0.21 g), NaH (60 % suspension in mineral oil, 3.3 mmol, 0.12 g) and **8.15** (0.5 mmol, 0.16 g). After quenching the reaction with 1 N HCl, the resulting white solid was collected by filtration and recrystallized from methanol to yield **8.20** as a white solid (0.18 g, 66 %). ^{1}H-NMR (DMSO-d_6) δ 1.45 (m, 4H, $COCH_2CH_2(C\underline{H}_2)_2$), 1.71 (m, 4H, $COCH_2C\underline{H}_2$), 1.91 (m, 4H, $C\underline{H}_2CH_2CO_2H$), 2.32 (t, 4H, ^3J = 7.3 Hz, $C\underline{H}_2CO_2H$), 2.69 (t, 4H, ^3J = 7.5 Hz, $C\underline{H}_2(CH_2)_2CO_2H$), 3.01 (t, 4H, ^3J = 7.2 Hz, $COCH_2$), 7.29 (m,

4H, H-5, indole-H), 7.60 (m, 2H, indole-H), 7.69 (s, 2H, H-2), 8.35 (m, 2H, indole-H), 12.09 (s, 2H, CO_2H). ^{13}C-NMR (DMSO-d_6) δ 23.63 (-, CH_2), 23.94 (-, CH_2), 24.11 (-, CH_2), 28.19 (-, CH_2), 33.26 (-, CH_2), 34.70 (-, CH_2), 115.95 (+, indole-C), 118.87 (+, indole-C), 120.94 (quat, indole-C), 122.78 (+, indole-C), 123.00 (+, indole-C), 124.57 (+, indole-C), 130.18 (quat, C-8), 135.23 (quat, C-7), 171.64 (quat, NCO), 174.26 (quat, CO_2H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 546 (100) $[M+H]^+$. Anal. ($C_{32}H_{36}N_2O_6 \cdot 0.25H_2O$) C, H, N. $C_{32}H_{36}N_2O_6$ (544.64).

1,1'-Dodecanedioyldiindol-3,3'-dibutanoic acid (8.21): The title compound was prepared according to general procedure 8.5.2.2 using indole-3-butanoic acid (1.02 mmol, 0.21 g), NaH (60 % suspension in mineral oil, 3.3 mmol, 0.12 g) and **8.16** (0.5 mmol, 0.19 g). After quenching the reaction with 1 N HCl, the resulting white solid was collected by filtration and recrystallized from methanol to yield **8.21** as a white solid (0.15 g, 50 %). ^{1}H-NMR (DMSO-d_6) δ 1.32 (m, 12H, $COCH_2CH_2(C\underline{H}_2)_6$), 1.67 (m, 4H, $COCH_2C\underline{H}_2$), 1.90 (m, 4H, $C\underline{H}_2CH_2CO_2H$), 2.32 (t, 4H, ^3J = 6.8 Hz, $C\underline{H}_2CO_2H$), 2.68 (t, 4H, ^3J = 6.8 Hz, $C\underline{H}_2(CH_2)_2CO_2H$), 2.98 (t, 4H, ^3J = 6.4 Hz, $COCH_2$), 7.28 (m, 4H, H-5, indole-H), 7.59 (d, 2H, ^3J = 7.0 Hz, indole-H), 7.68 (s, 2H, H-2), 8.34 (d, 2H, ^3J = 7.6 Hz, indole-H), 12.10 (s, 2H, CO_2H). ^{13}C-NMR (DMSO-d_6) δ 23.62 (-, CH_2), 24.10 (-, CH_2), 28.43 (-, CH_2), 28.76 (-, CH_2), 28.85 (-, CH_2), 33.25 (-, CH_2), 34.75 (-, CH_2), 115.93 (+, indole-C), 118.85 (+, indole-C), 120.90 (quat, indole-C), 122.80 (+, indole-C), 122.99 (+, indole-C), 124.56 (+, indole-C), 130.16 (quat, C-8), 135.21 (quat, C-7), 171.67 (quat, NCO), 174.27 (quat, CO_2H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 618 (100) $[M+NH_4]^+$. Anal. ($C_{36}H_{44}N_2O_6 \cdot 0.5H_2O$) C, H, N. $C_{36}H_{44}N_2O_6$ (600.74).

1,1'-Hexadecanedioyldiindol-3,3'-dibutanoic acid (8.22): The title compound was prepared according to general procedure 8.5.2.2 using indole-3-butanoic acid (1.02 mmol, 0.21 g), NaH (60 % suspension in mineral oil, 3.3 mmol, 0.12 g) and **8.17** (0.5 mmol, 0.22 g). The obtained raw product was recrystallized from methanol to yield **8.22** as a white solid (0.10 g, 30 %). mp: 156-158 °C; ^{1}H-NMR (DMSO-d_6) δ 1.28 (m, 20H, $COCH_2CH_2(C\underline{H}_2)_{10}$), 1.68 (m, 4H, $COCH_2C\underline{H}_2$), 1.91 (m, 4H, $C\underline{H}_2CH_2CO_2H$), 2.32 (t, 4H, ^3J = 7.3 Hz, $C\underline{H}_2CO_2H$), 2.69 (t, 4H, ^3J = 7.5 Hz, $C\underline{H}_2(CH_2)_2CO_2H$), 2.98 (t, 4H, ^3J = 7.3 Hz, $COCH_2$), 7.28 (m, 4H, H-5, indole-H), 7.59 (dd, 2H, ^4J = 1.1 Hz, ^3J = 6.8 Hz, indole-H), 7.68 (s, 2H, H-2), 8.34 (dd, 2H, ^4J = 1.0 Hz, ^3J = 7.4 Hz, indole-H), 12.03 (s, 2H, CO_2H). ^{13}C-NMR (DMSO-d_6) δ 23.59 (-, CH_2), 24.08 (-, CH_2), 28.36 (-, CH_2), 28.68 (-, CH_2), 28.82 (-, CH_2), 28.86 (-, CH_2), 28.91 (-, CH_2), 33.23 (-, CH_2),

34.72 (-, CH_2), 115.91 (+, indole-C), 118.83 (+, indole-C), 120.89 (quat, indole-C), 122.79 (+, indole-C), 122.97 (+, indole-C), 124.53 (+, indole-C), 130.16 (quat, C-8), 135.21 (quat, C-7), 171.66 (quat, NCO), 174.22 (quat, CO_2H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 656 (100) $[M-H]^-$. Anal. ($C_{40}H_{52}N_2O_6 \cdot 0.25H_2O$) C, H, N. $C_{40}H_{52}N_2O_6$ (656.85).

16-Hydroxyhexadecanoic acid (8.24a)[15]: A mixture of hexadecano-16-lactone (30 mmol, 7.63 g) and NBu_4HSO_4 (0.3 mmol, 0.10 g) in toluene (30 ml) and 50 % aqueous NaOH (25 ml) was stirred at 80-90 °C overnight. After cooing the resulting solid was isolated by filtration, washed with ether and dissolved in water. Acidification with concentrated HCl to pH <2 led to precipitation of the product which was isolated by filtration, washed with water and dried under reduced pressure to yield the title compound as white solid (7.76 g, 95 %). mp: 89-90 °C (Lit.[15]: 94-95 °C); ^{1}H-NMR (DMSO-d_6) δ 1.24 (s, 22H, $COCH_2CH_2(C\underline{H}_2)_{11}$), 1.43 (m, 4H, $COCH_2CH_2$, $C\underline{H}_2CH_2OH$), 2.18 (t, 2H, ^3J = 7.3 Hz, $COCH_2$), 3.37 (m, 2H, ^3J = 5.6 Hz, $C\underline{H}_2OH$), 4.32 (t, 2H, ^3J = 5.0 Hz, $CH_2O\underline{H}$), 11.96 (s, 1H, CO_2H). CI-MS (NH_3) *m/z (%)*: 271 (100) $[M-H]^-$. $C_{16}H_{32}O_3$ (272.42).

16-Bromohexadecanoic acid (8.24)[15]: A mixture of **8.24a** (26.8 mmol, 7.29 g) in 42 ml HBr (33 % solution in acetic acid) and concentrated H_2SO_4 (13 ml) was stirred overnight under an atmosphere of nitrogen. Subsequently the solution was stirred at reflux for 6 h. The reaction was quenched by adding icewater and the resulting solid was sucked off, washed with water and dried under reduced pressure to yield the title compound as white solid (8.57 g, 95 %). mp: 64-66 °C (Lit.[15]: 67-69 °C); ^{1}H-NMR ($CDCl_3$) δ 1.30 (m, 22H, $COCH_2CH_2(C\underline{H}_2)_{11}$), 1.63 (m, 2H, $COCH_2CH_2$), 1.85 (m, 2H, $C\underline{H}_2CH_2OH$), 2.35 (t, 2H, ^3J = 7.5 Hz, $COCH_2$), 3.41 (t, 2H, ^3J = 6.9 Hz, $C\underline{H}_2OH$). EI-MS (70 eV) *m/z (%)*: 334 (13) $[M^{+\cdot}]$. $C_{16}H_{31}BrO_2$ (335.32).

8.5.2.3 General procedure for the synthesis of benzyl esters 8.25 and 8.26

A mixture of the pertinent carboxylic acid (1 eq), benzyl alcohol (1.1 eq), DMAP (0.1 eq) and DCC (1.1 eq) in anhydrous CH_2Cl_2 was stirred overnight at room temperature under an atmosphere of argon. After filtration the solvent was removed under reduced pressure and the resulting raw product was subjected to flash chromatography.

Benzyl 11-bromoundecanoate[33] (8.25): The title compound was prepared according to general procedure 8.5.2.3 using 11-bromoundecanoic acid (10 mmol, 2.56 g), DMAP (1 mmol, 0.12 g), DCC (11 mmol, 2.27 g) and benzyl alcohol (11

mmol, 1.13 ml) in anhydrous CH_2Cl_2 (40 ml) and was obtained after flash chromatography (PE/EtOAc 90/10 v/v) as a colorless oil (3.27 g, 92 %). ^{1}H-NMR ($CDCl_3$) δ 1.29 – 1.89 (m, 16H, $COCH_2(C\underline{H}_2)_8$), 2.35 (t, 2H, ^3J = 7.5 Hz, $COCH_2$), 3.39 (t, 2H, ^3J = 6.9 Hz, CH_2Br), 5.11 (s, 2H, $C\underline{H}_2Ph$), 7.35 (m, 5H, Ph-H). CI-MS (NH_3) *m/z (%)*: 355 (100) $[M+H]^+$. $C_{18}H_{27}BrO_2$ (355.31).

Benzyl 16-bromohexadecanoate (8.26): The title compound was prepared according to general procedure 8.5.2.3 using **8.24** (15 mmol, 5.03 g), DMAP (1.5 mmol, 0.18 g), DCC (16.5 mmol, 3.40 g) and benzyl alcohol (16.5 mmol, 1.70 ml) in anhydrous CH_2Cl_2 (50 ml) and was obtained after flash chromatography (PE/EtOAc 95/5 to 90/10 v/v) as a pale yellow solid (5.34 g, 84 %). ^{1}H-NMR ($CDCl_3$) δ 1.35 (m, 22H, $COCH_2CH_2(C\underline{H}_2)_{11}$), 1.64 (m, 2H, $COCH_2CH_2$), 1.85 (m, 2H, $C\underline{H}_2CH_2OH$), 2.35 (t, 2H, ^3J = 7.5 Hz, $COCH_2$), 3.41 (t, 2H, ^3J = 6.9 Hz, $C\underline{H}_2OH$), 5.12 (s, 2H, $C\underline{H}_2Ph$), 7.36 (m, 5H, Ph-H). EI-MS (70 eV) *m/z (%)*: 424 (2) $[M^{+\cdot}]$. $C_{23}H_{37}BrO_2$ (425.44).

***tert*-Butyl 1*H*-indole-3-butanoate[34] (8.27):**To a solution of indole-3-butanoic acid (10 mmol) in anhydrous THF (10 ml) was added TBTA (20 mmol, 4.38 g) dissolved in anhydrous cyclohexane (20 ml) and BF_3Et_2O (0.2 ml). After stirring at room temperature overnight $NaHCO_3$ was added and the mixture was stirred for 10 min. Solids were removed by filtration, the solvent was evaporated and the remaining crude product was subjected to flash chromatography (PE/EtOAc 90/10 v/v) to yield the title compound as a white solid (1.53 g, 59 %). mp: 62-64 °C (ref.[34]: 69-61 °C); ^{1}H-NMR ($CDCl_3$) δ 1.45 (s, 9H, $C(CH_3)_3$), 2.00 (m, 2H, $C\underline{H}_2CH_2CO_2$), 2.30 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2CO_2$), 2.79 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2(CH_2)_2CO_2$), 6.97 (s, 1H, H-2), 7.15 (m, 2H, indole-H), 7.34 (m, 1H, indole-H), 7.61 (d, 1H, ^3J = 8.0 Hz, indole-H), 8.00 (s, 1H). PI-EIMS (70 eV) *m/z (%)*: 259 (47) $[M^{+\cdot}]$. $C_{16}H_{21}NO_2$ (259.34).

8.5.2.4 General procedure for the synthesis of *N*-alkylated indoles 8.28, 8.29, 8.67-8.69 and 8.73

To a solution of indole derivative (1 eq) in anhydrous DMF was added NaH (60 % suspension in mineral oil, 1.2 eq) under an atmosphere of argon. After stirring at room temperature for 30 min the pertinent alkyl halide (1.2 eq) was added and stirring was continued for 3 h at 40-60 °C. The reaction was quenched by adding water and the mixture was extracted tree times with EtOAc. The combined organic phases were washed with brine, dried over $MgSO_4$, filtered and evaporated to dryness. The crude product was subjected to flash chromatography to yield the target compound.

Benzyl 11-[3-(4-*tert*-butoxy-4-oxobutyl)-1*H*-indol-1-yl]undecanoate (8.28): The title compound was prepared according to general method 8.5.2.4 using **8.27** (1 mmol, 0.26 g), NaH (1.2 mmol, 48 mg) and **8.25** (1.2 mmol, 0.43 g) in anhydrous DMF (5 ml) and was obtained after flash chromatography (PE/EtOAc 95/5 to 90/10 v/v) as a colorless oil (0.31 g, 58 %). ^{1}H-NMR ($CDCl_3$) δ 1.26 (m, 12H, $NCH_2CH_2(C\underline{H}_2)_6$), 1.44 (s, 9H, $C(CH_3)_3$), 1.63 (m, 2H, $C\underline{H}_2CH_2CO$), 1.79 (m, 2H, $NCH_2C\underline{H}_2$), 1.98 (m, 2H, $C\underline{H}_2CH_2CO_2$), 2.28 (t, 2H, ^3J = 7.5 Hz, $C\underline{H}_2CO_2$), 2.34 (t, 2H, ^3J = 7.5 Hz, $C\underline{H}_2CO_2$), 2.77 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2(CH_2)_2CO_2$), 4.04 (t, 2H, ^3J = 7.2 Hz, NCH_2), 5.11 (s, 2H, $C\underline{H}_2Ph$), 6.88 (s, 1H, H-2), 7.07 (ddd, 1H, ^4J = 1.0 Hz, ^3J = 7.1 Hz, ^3J = 7.9 Hz, indole-H), 7.18 (ddd, 1H, ^4J = 1.1 Hz, ^3J = 6.9 Hz, ^3J = 8.2 Hz, indole-H), 7.31 (m, 6H, Ph-H, indole-H), 7.58 (m, 1H, indole-H). CI-MS (NH_3) *m/z (%)*: 534 (32) $[M+H]^+$. $C_{34}H_{47}NO_4$ (533.74).

Benzyl 16-[3-(4-*tert*-butoxy-4-oxobutyl)-1*H*-indol-1-yl]hexadecanoate (8.29): The title compound was prepared according to general method 8.5.2.4 using **8.27** (10 mmol, 2.59 g), NaH (1.2 mmol, 0.48 g) and **8.26** (12 mmol, 5.11 g) in anhydrous DMF (25 ml) and was obtained after flash chromatography (PE/EtOAc 95/5 to 90/10 v/v) as yellow oil (2.06 g, 34 %). ^{1}H-NMR ($CDCl_3$) δ 1.28 (m, 22H, $NCH_2CH_2(C\underline{H}_2)_{11}$), 1.46 (s, 9H, $C(CH_3)_3$), 1.65 (m, 2H, $C\underline{H}_2CH_2CO$), 1.81 (m, 2H, $NCH_2C\underline{H}_2$), 2.00 (m, 2H, $C\underline{H}_2CH_2CO_2$), 2.30 (t, 2H, ^3J = 7.5 Hz, $C\underline{H}_2CO_2$), 2.36 (t, 2H, ^3J = 7.5 Hz, $C\underline{H}_2CO_2$), 2.79 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2(CH_2)_2CO_2$), 4.06 (t, 2H, ^3J = 7.2 Hz, NCH_2), 5.13 (s, 2H, $C\underline{H}_2Ph$), 6.90 (s, 1H, H-2), 7.09 (ddd, 1H, ^4J = 1.0 Hz, ^3J = 7.0 Hz, ^3J = 7.9 Hz, indole-H), 7.20 (ddd, 1H, ^4J = 1.1 Hz, ^3J = 7.0 Hz, ^3J = 8.2 Hz, indole-H), 7.34 (m, 6H, indole-H, Ph-H), 7.60 (m, 1H, indole-H). CI-MS (NH_3) *m/z (%)*: 604 (100) $[M+H]^+$. $C_{39}H_{57}NO_4$ (603.87).

8.5.2.5 General procedure for the synthesis of alcohols or carboxylic acids 8.30, 8.46, 8.49, 8.55, 8.56, 8.76 and 8.77 *via* cleavage of benzyl ether/ester precursors:

The pertinent benzyl ester/ether was dissolved in EtOH and EtOAc and a catalytic amount of palladium on activated charcoal (10 % Pd) was added. A slow stream of hydrogen was then bubbled through the suspension for 30 min. Insoluble material was filtered off, and the solvent was evaporated to yield the target compound.

11-[3-(4-*tert*-Butoxy-4-oxobutyl)-1*H*-indol-1-yl]undecanoic acid (8.30): The title compound was prepared according to general procedure 8.5.2.5 using **8.27** (0.54 mmol, 0.29 g) and 10 % Pd/C (50 mg) in EtOH (2 ml) and EtOAc (5 ml) and was

obtained as a colorless oil (0.23 g, 96 %). ^{1}H-NMR ($CDCl_3$) δ 1.27 (m, 12H, $NCH_2CH_2(C\underline{H}_2)_6$), 1.45 (s, 9H, $C(CH_3)_3$), 1.61 (m, 2H, $C\underline{H}_2CH_2CO_2H$), 1.80 (m, 2H, $NCH_2C\underline{H}_2$), 1.98 (m, 2H, $C\underline{H}_2CH_2CO_2$), 2.31 (m, 4H, $C\underline{H}_2CO_2$, $C\underline{H}_2CO_2H$), 2.77 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2(CH_2)_2CO_2$), 4.04 (t, 2H, ^3J = 7.1 Hz, NCH_2), 6.88 (s, 1H, H-2), 7.08 (ddd, 1H, ^4J = 1.0 Hz, ^3J = 7.1 Hz, ^3J = 7.9 Hz, indole-H), 7.18 (ddd, 1H, ^2J = 1.1 Hz, ^3J = 6.9 Hz, ^3J = 8.2 Hz, indole-H), 7.29 (m, 1H, indole-H), 7.59 (m, 1H, indole-H). EI-MS (70 eV) *m/z (%)*: 443 (27) [$M^{+\cdot}$]. $C_{27}H_{41}NO_4$ (443.62).

16-[3-(4-*tert*-Butoxy-4-oxobutyl)-1*H*-indol-1-yl]hexadecanoic acid (8.31): The title compound was prepared according to the general procedure for cleavage of benzyl esters using **8.28** (3.20 mmol, 1.93 g) and 10 % Pd/C (0.30 g) in EtOH (15 ml) and EtOAc (15 ml) and was obtained as a pale yellow oil (1.64 g, 99 %). ^{1}H-NMR ($CDCl_3$) δ 1.29 (m, 22H, $NCH_2CH_2(C\underline{H}_2)_{11}$), 1.46 (s, 9H, $C(CH_3)_3$), 1.64 (m, 2H, $C\underline{H}_2CH_2CO_2H$), 1.81 (m, 2H, $NCH_2C\underline{H}_2$), 2.00 (m, 2H, $C\underline{H}_2CH_2CO_2$), 2.32 (m, 4H, $C\underline{H}_2CO_2$, $C\underline{H}_2CO_2H$), 2.79 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2(CH_2)_2CO_2$), 4.05 (t, 2H, ^3J = 7.2 Hz, NCH_2), 6.89 (s, 1H, H-2), 7.09 (ddd, 1H, ^4J = 1.0 Hz, ^3J = 7.1 Hz, ^3J = 7.9 Hz, indole-H), 7.20 (ddd, 1H, ^3J = 1.1 Hz, ^3J = 7.0 Hz, ^3J = 8.2 Hz, indole-H), 7.31 (m, 1H, indole-H), 7.60 (m, 1H, indole-H), 9.95 (bs, 1H, CO_2H). EI-MS (70 eV) *m/z (%)*: 514 (78) [$M^{+\cdot}$]. $C_{32}H_{51}NO_4$ (513.75).

8.5.2.6 General procedure for the synthesis of carboxylic acids 8.32, 8.33, 8.42-8.45, 8.47 and 8.48 *via* cleavage of *tert*-butyl ester precursors:

The pertinent *tert*-butyl ester was dissolved in CH_2Cl_2/TFA (80/20 v/v) at 0 °C. After stirring for 6 h at room temperature the solvent was evaporated to yield the free carboxylic acid derivative.

11-[3-(3-Carboxypropyl)-1*H*-indol-1-yl]undecanoic acid (8.32): The title compound was prepared according to general procedure 8.5.2.6 using **8.30** (0.5 mmol, 0.22 g) in 4 ml of CH_2Cl_2/TFA (80/20 v/v) and was obtained after flash chromatography (PE/EtOAc 1/1 + 0.1 % HOAc v/v) as a white solid (0.16 g, 83 %). ^{1}H-NMR (CD_3OD) δ 1.25 (s, 12H, $NCH_2CH_2(C\underline{H}_2)_6$), 1.56 (m, 2H, $C\underline{H}_2CH_2COH$), 1.78 (m, 2H, $NCH_2C\underline{H}_2$), 1.97 (m, 2H, $C\underline{H}_2CH_2CO_2$), 2.25 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2CO_2H$), 2.33 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2CO_2H$), 2.77 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2(CH_2)_2CO_2H$), 4.09 (t, 2H, ^3J = 6.9 Hz, NCH_2), 6.99 (m, 2H, H-2, indole-H), 7.11 (ddd, 1H, ^4J = 1.1 Hz, ^3J = 7.0 Hz, ^3J = 7.9 Hz, indole-H), 7.30 (d, 1H, ^3J = 8.2 Hz, indole-H), 7.52 (m, 1H, indole-H). ^{13}C-NMR (CD_3OD) δ 25.47 (-, CH_2), 26.15 (-, CH_2), 27.03 (-, CH_2), 27.99 (-, CH_2), 30.28 (-, CH_2), 30.34 (-, CH_2), 30.41 (-, CH_2), 30.50 (-, CH_2), 30.60 (-, CH_2),

31.43 (-, CH_2), 34.57 (-, CH_2), 35.03 (-, CH_2), 46.87 (-, NCH_2), 110.42 (+, indole-C), 115.08 (quat, C-3), 119.45 (+, indole-C), 119.84 (+, indole-C), 122.26 (+, indole-C), 126.66 (+, indole-C), 129.40 (quat, C-9), 138.00 (quat, C-8), 177.79 (quat, CO_2H). EI-MS (70 eV) *m/z (%)*: 387 (15) [M^+]. Anal. ($C_{23}H_{33}NO_4$) C, H, N. $C_{23}H_{33}NO_4$ (387.51).

16-[3-(3-Carboxypropyl)-1*H*-indol-1-yl]hexadecanoic acid (8.33): The title compound was prepared according to general procedure 8.5.2.5 esters using **8.31** (0.33 mmol, 0.20 g) in 4 ml of CH_2Cl_2/TFA (80/20 v/v) and was obtained after flash chromatography (PE/EtOAc 60/40 + 0.1 % HOAc v/v) as a white solid (0.13 g, 86 %). mp: 85-86 °C; ^{1}H-NMR (CD_3OD+$CDCl_3$) δ 1.27 (m, 22H, $NCH_2CH_2(C\underline{H}_2)_{11}$), 1.58 (m, 2H $C\underline{H}_2CH_2CO_2H$), 1.78 (m, 2H, $NCH_2C\underline{H}_2$), 1.98 (m, 2H, $C\underline{H}_2CH_2CO_2H$), 2.26 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2CO_2H$), 2.33 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2CO_2$), 2.77 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2(CH_2)_2CO_2H$), 4.07 (t, 2H, ^3J = 7.0 Hz, NCH_2), 6.94 (s, 1H, H-2), 6.99 (ddd, 1H, ^4J = 0.9 Hz, ^3J = 7.1 Hz, ^3J = 7.9 Hz, indole-H), 7.11 (ddd, 1H, ^4J = 1.1 Hz, ^3J = 7.0 Hz, ^3J = 8.2 Hz, indole-H), 7.29 (d, 1H, ^3J = 8.2 Hz, indole-H), 7.52 (m, 1H, indole-H). ^{13}C-NMR (CD_3OD+$CDCl_3$) δ 25.54 (-, CH_2), 26.19 (-, CH_2), 26.98 (-, CH_2), 28.07 (-, CH_2), 30.34 (-, CH_2), 30.40 (-, CH_2), 30.51 (-, CH_2), 30.68 (-, CH_2), 30.80 (-, CH_2), 31.47 (-, CH_2), 34.71 (-, CH_2), 35.16 (-, CH_2), 47.02 (-, NCH_2), 110.46 (+, indole-C), 115.13 (quat, C-3), 119.52 (+, indole-C), 119.94 (+, indole-C), 122.33 (+, indole-C), 126.65 (+, indole-C), 129.37 (quat, C-9), 137.94 (quat, C-8), 177.90 (quat, CO_2H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 458 (100) [M+H]$^+$. $C_{28}H_{43}NO_4$ (457.65).

8.5.2.7 General procedure for the synthesis of amides 8.34-8.37, 8.39 and 8.40

A mixture of the pertinent carboxylic acid (1 eq), HOBt (1.2 eq), DIEA (1.2 eq), EDAC (1.2 eq) and the pertinent amine (2 eq) in DMF was stirred at room temperature overnight. After removal of the solvent the remaining residue was subjected to flash chromatography to yield the target compound.

***tert*-Butyl {1-[11-oxo-11-(thiazol-2-ylamino)undecan-1-yl]-1*H*-indole-3-butanoate (8.34):** The title compound was prepared according to general procedure 8.5.2.7 using **8.30** (0.5 mmol, 0.22 g), HOBt (0.6 mmol, 81 mg), DIEA (0.6 mmol, 0.10 ml), EDAC (0.6 mmol, 115 mg) and 2-aminothiazole (1 mmol, 0.10 g) in DMF (5 ml) and was obtained after flash chromatography ($CHCl_3$/MeOH 98/2 v/v) as a pale yellow oil (0.20 g, 76 %). ^{1}H-NMR ($CDCl_3$) δ 1.32 (m, 12H, $NCH_2CH_2(C\underline{H}_2)_6$), 1.44 (s, 9H, $C(CH_3)_3$), 1.77 (m, 2H, $NCH_2C\underline{H}_2$), 1.98 (m, 2H, $C\underline{H}_2CH_2CO_2$), 2.29 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2CO_2$), 2.54 (t, 2H, ^3J = 7.5 Hz, $C\underline{H}_2CONH$), 2.77 (t, 2H, ^3J =7.4Hz,

C$\underline{H}_2$(CH$_2$)$_2$CO$_2$), 4.04 (t, 2H, ^3J = 7.1 Hz, NCH$_2$), 6.88 (s, 1H, H-2), 6.98 (d, 1H, ^3J = 2.9 Hz, thiazole-H), 7.07 (ddd, 1H, ^4J = 1.0 Hz, ^3J = 7.0 Hz, ^3J = 7.9 Hz, indole-H), 7.18 (ddd, 1H, ^4J = 1.0 Hz, ^3J = 6.9 Hz, ^3J = 7.9 Hz, indole-H), 7.29 (m, 1H, indole-H), 7.42 (d, 1H, ^3J = 2.9 Hz, thiazole-H), 7.59 (m, 1H, indole-H), 8.03 (s, 1H). CI-MS (NH$_3$) *m/z (%)*: 526 (100) [M+H]$^+$. Anal. (C$_{30}$H$_{43}$N$_3$O$_3$S·0.4H$_2$O) C, H, N. C$_{30}$H$_{43}$N$_3$O$_3$S (525.75).

***tert*-Butyl 1-[11-oxo-11-(4-phenylpiperazin-1-yl)undecan-1-yl]-1*H*-indole-3-butanoate (8.35):** The title compound was prepared according to general procedure 8.5.2.7 using **8.30** (0.5 mmol, 0.22 g), HOBt (0.6 mmol, 81 mg), DIEA (0.6 mmol, 0.10 ml), EDAC (0.6 mmol, 115 mg) and *N*-phenylpiperazine (1 mmol, 0.15 ml) in DMF (5 ml) and was obtained after flash chromatography (PE/EE 60/40 + 0.1 % NEt$_3$ v/v) as a colorless oil (0.17 g, 98 %). ^{1}H-NMR (CDCl$_3$) δ 1.29 (m, 12H, NCH$_2$CH$_2$(C$\underline{H}_2$)$_6$), 1.45 (s, 9H, C(CH$_3$)$_3$), 1.64 (m, 2H, C$\underline{H}_2$CH$_2$CO), 1.80 (m, 2H, NCH$_2$C$\underline{H}_2$), 1.99 (m, 2H, C$\underline{H}_2$CH$_2$CO$_2$), 2.29 (t, 2H, ^3J = 7.5 Hz, C$\underline{H}_2$CO$_2$), 2.36 (t, 2H, ^3J = 7.7 Hz, C$\underline{H}_2$CON), 2.78 (t, 2H, ^3J = 7.4 Hz, C$\underline{H}_2$(CH$_2$)$_2$CO$_2$), 3.16 (m, 4H, piperazine-H), 3.63 (m, 2H, piperazine-H), 3.78 (m, 2H, piperazine-H), 4.05 (t, 2H, ^3J = 7.1 Hz, NCH$_2$), 6.89 (s, 1H, H-2), 6.93 (m, 4H, Ar-H), 7.08 (ddd, 1H, ^4J = 0.9 Hz, ^3J = 7.1 Hz, ^3J = 7.8 Hz, indole-H), 7.19 (ddd, 1H, ^4J = 1.1 Hz, ^3J = 7.0 Hz, ^3J = 8.2 Hz, indole-H), 7.29 (m, 2H, Ar-H), 7.60 (m, 1H, indole-H). ES-MS (EtOAc/MeOH + NH$_4$OAc) *m/z (%)*: 589 (100) [M+H]$^+$. C$_{37}$H$_{53}$N$_3$O$_3$ (587.84).

***tert*-Butyl 4-{11-[3-(4-*tert*-butoxy-4-oxobutyl)-1*H*-indol-1-yl]undecanoyl}-piperazine-1-carboxylate (8.36):** The title compound was prepared according to general procedure 8.5.2.7 using **8.30** (0.5 mmol, 0.22 g), HOBt (0.6 mmol, 81 mg), DIEA (0.6 mmol, 0.10 ml), EDAC (0.6 mmol, 115 mg) and *N*-boc-piperazine (1 mmol, 0.19 g) in DMF (5 ml) and was obtained after flash chromatography (PE/EE 60/40 + 0.1 % NEt$_3$ v/v) as a colorless oil (0.26 g, 85 %). ^{1}H-NMR (CDCl$_3$) δ 1.27 (m, 12H, NCH$_2$CH$_2$(C$\underline{H}_2$)$_6$), 1.45 (s, 9H, C(CH$_3$)$_3$), 1.47 (s, 9H, C(CH$_3$)$_3$), 1.61 (m, 2H, C$\underline{H}_2$CH$_2$CO), 1.79 (m, 2H, NCH$_2$C$\underline{H}_2$), 1.98 (m, 2H, C$\underline{H}_2$CH$_2$CO$_2$), 2.30 (m, 4H, C$\underline{H}_2$CO$_2$, C$\underline{H}_2$CON), 2.77 (t, 2H, ^3J = 7.4 Hz, C$\underline{H}_2$(CH$_2$)$_2$CO$_2$), 3.43 (m, 6H, piperazine-H), 3.58 (m, 2H, piperazine-H), 4.04 (t, 2H, ^3J = 7.1 Hz, NCH$_2$), 6.88 (s, 1H, H-2), 7.08 (ddd, 1H, ^2J = 1.0 Hz, ^3J = 7.0 Hz, ^3J = 7.9 Hz, indole-H), 7.18 (ddd, 1H, ^2J = 1.0 Hz, ^3J = 6.9 Hz, ^3J = 8.1 Hz, indole-H), 7.29 (m, 1H, indole-H), 7.59 (m, 1H, indole-H). ES-MS (DCM/MeOH + NH$_4$OAc) *m/z (%)*: 613 (100) [M+H]$^+$. C$_{36}$H$_{57}$N$_3$O$_5$ (611.85).

***tert*-Butyl 1-[11-(3-hydroxyphenylamino)-11-oxoundecan-1-yl]-1*H*-indole-3-butanoate (8.37):** The title compound was prepared according to general procedure 8.5.2.7 using **8.30** (0.5 mmol, 0.22 g), HOBt (0.6 mmol, 81 mg), DIEA (0.6 mmol, 0.10 ml), EDAC (0.6 mmol, 115 mg) and 3-hydroxyphenol (1 mmol, 0.11 g) in DMF (5 ml) and was obtained after flash chromatography (PE/EE 60/40 + 0.1 % NEt_3 v/v) as a colorless oil (0.22 g, 82 %). ^{1}H-NMR ($CDCl_3$) δ 1.29 (m, 12H, $NCH_2CH_2(C\underline{H}_2)_6$), 1.45 (s, 9H, $C(CH_3)_3$), 1.72 (m, 4H, $C\underline{H}_2CH_2CO$, $NCH_2C\underline{H}_2$), 1.98 (m, 2H, $C\underline{H}_2CH_2CO_2$), 2.29 (t, 2H, ^3J = 7.5 Hz, CH_2CO), 2.36 (t, 2H, ^3J = 7.6 Hz, CH_2CO), 2.77 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2(CH_2)_2CO_2$), 4.03 (t, 2H, ^3J = 7.1 Hz, NCH_2), 6.53 (dd, 1H, ^4J = 0.9 Hz, ^3J = 7.9 Hz, Ph-H), 6.64 (dd, 1H, ^4J = 1.9 Hz, ^3J = 8.1 Hz, Ph-H), 6.87 (s, 1H, H-2), 7.13 (m, 3H, indole-H, Ph-H), 7.29 (d, 1H, ^3J = 8.2 Hz, indole-H), 7.38 (s, 1H, Ph-H), 7.58 (d, 1H, ^3J = 7.8 Hz, indole-H), 7.93 (t, 1H, ^3J = 2.0 Hz, CONH), 8.19 (bs, 1H, OH). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 552 (100) $[M+NH_4]^+$. $C_{33}H_{46}N_2O_4$ (534.73).

8.5.2.8 General procedure for the synthesis of esters 8.38 and 8.41

To a solution of the corresponding carboxylic acid (1eq), the pertinent alcohol (1eq) and DMAP (1.2 eq) in anhydrous DMF was added EDAC (1.1 eq) portionwise under an atmosphere of argon at 0 °C. The mixture was stirred overnight at room temperature and the solvent was removed under reduced pressure. The remaining raw product was taken up in water and extracted tree times with EtOAc. The combined organic layers were washed with 1 N HCl and brine, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The product was obtained after flash chromatography.

(*S*)-2-[(*R*)-3,4-Bis(benzyloxy)-2-oxo-2,5-dihydrofuran-5-yl]-2-hydroxyethyl 11-[3-(4-*tert*-butoxy-4-oxobutyl)-1*H*-indol-1-yl]undecanoate (8.38): The title compound was prepared according to general procedure 8.5.2.8 using **8.30** (0.5 mmol, 0.22 g), **7.66** (0.5 mmol, 0.18 g), DMAP (0.6 mmol, 73 mg) and EDAC (0.55 mmol, 105 mg) in anhydrous DMF (5 ml) and was obtained after flash chromatography (PE/EE 90/10 v/v) as a pale yellow oil (0.13 g, 33 %). ^{1}H-NMR ($CDCl_3$) δ 1.26 (m, 12H, $NCH_2CH_2(C\underline{H}_2)_6$), 1.45 (s, 9H, $C(CH_3)_3$), 1.60 (m, 2H, $C\underline{H}_2CH_2CO$), 1.80 (m, 2H, $NCH_2C\underline{H}_2$), 1.98 (m, 2H, $C\underline{H}_2CH_2CO_2$), 2.30 (m, 4H, $C\underline{H}_2CO_2$, $C\underline{H}_2CO_2$), 2.78 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2(CH_2)_2CO_2$), 4.05 (m, 3H, NCH_2, $C\underline{H}OH$), 4.19 (dd, 1H, ^3J = 5.0 Hz, ^2J = 11.6 Hz, CH_2O), 4.32 (dd, 1H, ^3J = 6.8 Hz, ^2J = 11.6 Hz, CH_2O), 4.67 (d, 1H, ^3J = 2.0 Hz, ascorbic acid H-5), 5.11 (s, 2H, $C\underline{H}_2Ph$), 5.12 (d, 1H, ^2J = 10.6 Hz, $C\underline{H}_2Ph$),

5.21 (d, 1H, ^{2}J = 11.7 Hz, C$\underline{H}_2$Ph), 6.88 (s, 1H, indole H-2), 7.08 (ddd, 1H, ^{4}J = 0.9 Hz, ^{3}J = 7.0 Hz, ^{3}J = 7.8 Hz, indole-H), 7.16 – 7.40 (m, 12H, indole-H, Ph-H), 7.59 (d, 1H, ^{3}J = 7.9 Hz, indole-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 800 (100) $[M+NH_4]^+$. $C_{47}H_{59}NO_9$ (781.97).

***tert*-Butyl 1-[16-oxo-16-(4-phenylpiperazin-1-yl)hexadecan-1-yl]-1*H*-indole-3-butanoate (8.39):** The title compound was prepared according to general procedure 8.5.2.7 using **8.31** (0.4 mmol, 0.24 g), HOBt (0.48 mmol, 65 mg), DIEA (0.48 mmol, 82 µl), EDAC (0.48 mmol, 92 mg) and *N*-phenylpiperazine (0.8 mmol, 0.12 ml) in DMF (5 ml) and was obtained after flash chromatography (PE/EE 80/20 + 0.1 % NEt_3 v/v) as a colorless oil (0.14 g, 53 %). ^{1}H-NMR ($CDCl_3$) δ 1.29 (m, 22H, $NCH_2CH_2(C\underline{H}_2)_{11}$), 1.45 (s, 9H, $C(CH_3)_3$), 1.65 (m, 2H, $C\underline{H}_2CH_2CO$), 1.80 (m, 2H, $NCH_2C\underline{H}_2$), 1.99 (m, 2H, $C\underline{H}_2CH_2CO_2$), 2.29 (t, 2H, ^{3}J = 7.4 Hz, $C\underline{H}_2CO_2$), 2.36 (t, 2H, ^{3}J = 7.7 Hz, $C\underline{H}_2CON$), 2.78 (t, 2H, ^{3}J = 7.4 Hz, $C\underline{H}_2(CH_2)_2CO_2$), 3.16 (m, 4H, piperazine-H), 3.63 (m, 2H, piperazine-H), 3.78 (m, 2H, piperazine-H), 4.05 (t, 2H, ^{3}J = 7.2 Hz, NCH_2), 6.89 (s, 1H, H-2), 6.93 (m, 4H, Ar-H), 7.08 (ddd, 1H, ^{2}J = 1.0 Hz, ^{3}J = 7.0 Hz, ^{3}J = 7.9 Hz, indole-H), 7.19 (ddd, 1H, ^{2}J = 1.1 Hz, ^{3}J = 7.0 Hz, ^{3}J = 8.2 Hz, indole-H), 7.30 (m, 2H, Ar-H), 7.59 (m, 1H, indole-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 658 (100) $[M+H]^+$. $C_{42}H_{63}N_3O_3$ (657.97).

***tert*-Butyl 1-[16-(3-hydroxyphenylamino)-16-oxo(hexadecan-1yl)]-1*H*-indole-3-butanoate (8.40):** The title compound was prepared according to general procedure 8.5.2.7 using **8.31** (0.4 mmol, 0.24 g), HOBt (0.48 mmol, 65 mg), DIEA (0.48 mmol, 82 µl), EDAC (0.48 mmol, 92 mg) and 3-aminophenol (0.8 mmol, 87 mg) in DMF (5 ml) and was obtained after flash chromatography (PE/EE 80/20 v/v) as a white semisolid substance (0.21 g, 87 %). ^{1}H-NMR ($CDCl_3$) δ 1.26 (m, 22H, $NCH_2CH_2(C\underline{H}_2)_{11}$), 1.45 (s, 9H, $C(CH_3)_3$), 1.74 (m, 4H, $C\underline{H}_2CH_2CO$, $NCH_2C\underline{H}_2$), 1.98 (m, 2H, $C\underline{H}_2CH_2CO_2$), 2.29 (t, 2H, ^{3}J = 7.5 Hz, $C\underline{H}_2CO_2$), 2.37 (t, 2H, ^{3}J = 7.6 Hz, $C\underline{H}_2CON$), 2.77 (t, 2H, ^{3}J = 7.4 Hz, $C\underline{H}_2(CH_2)_2CO_2$), 4.04 (t, 2H, ^{3}J = 7.2 Hz, NCH_2), 6.55 (m, 1H, Ar-H), 6.64 (m, 1H, Ar-H), 6.88 (s, 1H, H-2), 7.05 – 7.31 (m, 4H, indole-H, Ar-H), 7.59 (m, 1H, indole-H), 7.88 (m, 1H, Ar-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 604 (100) $[M-H]^-$. Anal. ($C_{38}H_{56}N_2O_4 \cdot 0.4H_2O$) C, H, N. $C_{38}H_{56}N_2O_4$ (604.86).

(*S*)-2-[(*R*)-3,4-Bis(benzyloxy)-2-oxo-2,5-dihydrofuran-5-yl]-2-hydroxyethyl 16-[3-(4-*tert*-butoxy-4-oxobutyl)-1*H*-indol-1-yl]hexadecanoate (8.41): The title compound was prepared according to general procedure 8.5.2.8 using **8.31** (0.5

mmol, 0.22 g), **7.66** (0.5 mmol, 0.18 g), DMAP (0.6 mmol, 73 mg) and EDAC (0.55 mmol, 105 mg) in anhydrous DMF (5 ml) and was obtained after flash chromatography (PE/EE 90/10 v/v) as a pale yellow oil (90 mg, 21 %). ^{1}H-NMR ($CDCl_3$) δ 1.26 (m, 22H, $NCH_2CH_2(C\underline{H}_2)_{11}$), 1.45 (s, 9H, $C(CH_3)_3$), 1.61 (m, 2H $C\underline{H}_2CH_2CO$), 1.80 (m, 2H, $NCH_2C\underline{H}_2$), 1.98 (m, 2H, $C\underline{H}_2CH_2CO_2$), 2.31 (m, 4H, $C\underline{H}_2CO_2$, $C\underline{H}_2CO_2$), 2.78 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2(CH_2)_2CO_2$), 4.05 (m, 3H, NCH_2, C<u>H</u>OH), 4.19 (dd, 1H, ^3J = 5.0 Hz, ^2J = 11.6 Hz, CH_2O), 4.32 (dd, 1H, ^3J = 6.8 Hz, ^2J = 11.6 Hz, CH_2O), 4.67 (d, 1H, ^3J = 2.1 Hz, ascorbic acid H-5), 5.11 (s, 2H, $C\underline{H}_2Ph$), 5.13 (d, 1H, ^2J = 10.7 Hz, $C\underline{H}_2Ph$), 5.21 (d, 1H, ^2J = 11.7 Hz, $C\underline{H}_2Ph$), 6.89 (s, 1H, indole H-2), 7.08 (ddd, 1H, ^3J = 1.0 Hz, ^3J = 7.0 Hz, ^3J = 7.9 Hz, indole-H), 7.16 – 7.40 (m, 12H, indole-H, Ph-H), 7.59 (m, 1H, indole-H). $C_{52}H_{69}NO_9$ (852.11).

1-[11-Oxo-11-(thiazol-2-ylamino)undecan-1yl]-1*H*-indole-3-butanoic acid (8.42): The title compound was prepared according to general procedure 8.5.2.6 using **8.34** (0.36 mmol, 0.19 g) in 4 ml of CH_2Cl_2/TFA (80/20 v/v) and was obtained as a colorless oil (0.17 g, 100 %). ^{1}H-NMR (DMSO-*d*$_6$) δ 1.21 (m, 12H, $NCH_2CH_2(C\underline{H}_2)_6$), 1.50 – 1.90 (m, 6H, $NCH_2C\underline{H}_2$, $C\underline{H}_2CH_2CON$, $C\underline{H}_2CH_2CO_2H$), 2.25 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2CO_2H$), 2.40 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2CONH$), 2.68 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2(CH_2)_2CO_2H$), 4.08 (t, 2H, ^3J = 6.9 Hz, NCH_2), 6.95 – 7.28 (m, 4H, indole-H, thiazole-H), 7.38 (d, 1H, ^3J = 8.2 Hz, indole-H), 7.44 (d, 1H, ^3J = 3.6 Hz, thiazole-H), 7.50 (m, 1H, indole-H), 12.01 (bs, 2H, CO_2H, CONH). ES-MS (MeOH + NH_4OAc) *m/z (%)*: 470 (100) $[M+H]^+$. $C_{26}H_{35}N_3O_3S$ (469.64).

1-[11-Oxo-11-(4-phenylpiperazin-1-yl)undecan-1yl]-1*H*-indole-3-butanoic acid ammonium salt (8.43): The title compound was prepared according to general procedure 8.5.2.6 using **8.35** (0.29 mmol, 0.17 g) in 4 ml of CH_2Cl_2/TFA (80/20 v/v) and was obtained after flash chromatography ($CHCl_3$/MeOH 80/20 + NH_3 v/v) as a colorless oil (0.12 g, 75 %). ^{1}H-NMR (CD_3OD) δ 1.28 (m, 12H, $NCH_2CH_2(C\underline{H}_2)_6$), 1.57 (m, 2H, $C\underline{H}_2CH_2CO$), 1.76 (m, 2H, $NCH_2C\underline{H}_2$), 1.97 (m, 2H, $C\underline{H}_2CH_2CO_2H$), 2.32 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2CO_2H$), 2.39 (t, 2H, ^3J = 7.6 Hz, $C\underline{H}_2CON$), 2.77 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2(CH_2)_2CO_2H$), 3.10 (m, 4H, piperazine-H), 3.67 (m, 4H, piperazine-H), 4.07 (t, 2H, ^3J = 6.9 Hz, NCH_2), 6.85 (m, 1H, indole-H), 6.97 (m, 4H, Ar-H), 7.11 (ddd, 1H, ^2J = 1.0 Hz, ^3J = 7.0 Hz, ^3J = 8.2 Hz, indole-H), 7.25 (m, 3H, Ar-H), 7.52 (m, 1H, indole-H). ^{13}C-NMR (CD_3OD) δ 25.53 (-, CH_2), 26.66 (-, CH_2), 27.11 (-, CH_2), 27.99 (-, CH_2), 30.33 (-, CH_2), 30.43 (-, CH_2), 30.48 (-, CH_2), 30.56 (-, CH_2), 31.42 (-, CH_2), 34.09 (-, CH_2), 34.80 (-, CH_2), 42.87 (-, piperazine-C), 46.88 (-, NCH_2), 46.98 (-, piperazine-C),

50.73 (-, piperazine-C), 51.25 (-, piperazine-C), 110.44 (+, indole-C), 115.13 (quat, C-3), 117.98 (+, Ph-C), 119.46 (+, indole-C), 119.87 (+, indole-C), 121.63 (+, Ph-C), 122.27 (+, indole-C), 126.67 (+, indole-C), 129.42 (quat, C-9), 130.20 (+, Ph-C), 138.00 (quat, C-8), 152.59 (quat, Ph-C), 174.27 (quat, $CH_2\underline{C}ON$), 177.97 (quat, CO_2H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 532 (100) $[M+H]^+$. $C_{33}H_{48}N_4O_3$ (548.76).

1-[11-Oxo-11-(piperazin-1-yl)undecan-1-yl]-1*H*-indole-3-butanoic acid ammonium salt (8.44): The title compound was prepared according to general procedure 8.5.2.6 using **8.36** (0.40 mmol, 0.24 g) in 4 ml of CH_2Cl_2/TFA (80/20 v/v) and was obtained after flash chromatography ($CHCl_3$/MeOH 80/20 + NH_3 v/v) as a pale yellow oil (0.20 g, 95 %). ^{1}H-NMR (CD_3OD) δ 1.27 (m, 12H, $NCH_2CH_2(C\underline{H}_2)_6$), 1.55 (m, 2H, $C\underline{H}_2CH_2CO$), 1.78 (m, 2H, $NCH_2C\underline{H}_2$), 1.97 (m, 2H, $C\underline{H}_2CH_2CO_2H$), 2.30 (t, 2H, ^3J = 7.5 Hz, $C\underline{H}_2CO_2H$), 2.38 (t, 2H, ^3J = 7.5 Hz, $C\underline{H}_2CON$), 2.77 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2(CH_2)_2CO_2H$), 3.12 (m, 4H, piperazine-H), 3.73 (m, 4H, piperazine-H), 4.09 (t, 2H, ^3J = 6.9 Hz, NCH_2), 6.98 (m, 2H, indole-H), 7.10 (ddd, 1H, ^2J = 1.0 Hz, ^3J = 6.9 Hz, ^3J = 8.2 Hz, indole-H), 7.30 (m, 1H, indole-H), 7.52 (m, 1H, indole-H). ^{13}C-NMR (CD_3OD) δ 25.68 (-, CH_2), 26.27 (-, CH_2), 27.61 (-, CH_2), 27.91 (-, CH_2), 30.26 (-, CH_2), 30.30 (-, CH_2), 30.41 (-, CH_2), 30.48 (-, CH_2), 31.36 (-, CH_2), 33.69 (-, CH_2), 36.17 (-, CH_2), 40.11 (-, piperazine-C), 44.26 (-, piperazine-C), 44.68 (-, piperazine-C), 44.89 (-, piperazine-C), 46.82 (-, NCH_2), 110.38 (+, indole-C), 115.33 (quat, C-3), 119.38 (+, indole-C), 119.86 (+, indole-C), 122.19 (+, indole-C), 126.60 (+, indole-C), 129.43 (quat, C-9), 137.97 (quat, C-8), 174.37 (quat, $CH_2\underline{C}ON$), 179.70 (quat, CO_2H). ES-MS (DCM/MeCN + TFA) *m/z (%)*: 456 (100) $[M+H]^+$. Anal. ($C_{27}H_{44}N_4O_3$) C: 70.87, H: 9.39, N: 6.87, found C: 70.43, H: 8.83, N: 6.36. $C_{27}H_{44}N_4O_3$ (472.66).

1-[11-(3-Hydroxyphenylamino)-11-oxoundecan-1yl]-1*H*-indole-3-butanoic acid (8.45): The title compound was prepared according to general procedure 8.5.2.6 using **8.37** (0.36 mmol, 0.19 g) in 4 ml of CH_2Cl_2/TFA (80/20 v/v) and was obtained after flash chromatography ($CHCl_3$/MeOH 80/20 + NH_3 v/v) as a brown solid (0.14 g, 81 %). mp: 65-66 °C; ^{1}H-NMR (CD_3OD) δ 1.25 (m, 12H, $NCH_2CH_2(C\underline{H}_2)_6$), 1.64 (m, 2H, $C\underline{H}_2CH_2CO$), 1.76 (m, 2H, $NCH_2C\underline{H}_2$), 1.97 (m, 2H, $C\underline{H}_2CH_2CO_2H$), 2.31 (m, 4H, $C\underline{H}_2CO_2H$, $C\underline{H}_2CON$), 2.77 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2(CH_2)_2CO_2H$), 4.07 (t, 2H, ^3J = 6.9 Hz, NCH_2), 6.51 (m, 1H, Ar-H), 6.90 – 7.23 (m, 6H, Ar-H), 7.29 (m, 1H, indole-H), 7.51 (m, 1H, indole-H). ^{13}C-NMR (CD_3OD) δ 25.47 (-, CH_2), 27.02 (-, CH_2), 27.99 (-, CH_2), 30.34 (-, CH_2), 30.43 (-, CH_2), 30.59 (-, CH_2), 31.43 (-, CH_2), 30.48 (-, CH_2),

34.56 (-, CH_2), 38.13 (-, CH_2), 46.87 (-, NCH_2), 108.46 (+, Ph-C), 110.43 (+, indole-C), 112.13 (+, Ph-C), 112.39 (+, Ph-C), 115.06 (quat, C-3), 119.45 (+, indole-C), 119.84 (+, indole-C), 122.26 (+, indole-C), 126.67 (+, indole-C), 129.39 (quat, C-9), 130.53 (+, Ph-C), 138.00 (quat, C-8), 141.06 (quat, Ph-C), 158.94 (quat, Ph-C), 174.77 (quat, $CH_2\underline{C}ON$), 177.80 (quat, CO_2H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 496 (100) $[M+NH_4]^+$. $C_{29}H_{38}N_2O_4$ (478.62).

1-{11-[(*S*)-2-((*R*)-3,4-Dihydroxy-2-oxo-2,5-dihydrofuran-5-yl)-2-hydroxyethoxy]-11-oxoundecan-1-yl}-1*H*-indole-3-butanoic acid (8.46): The title compound was prepared from **8.38** (0.15 mmol, 0.12 g) by removing the *tert*-butyl moiety according to general method 8.5.2.6 in 4 ml of CH_2Cl_2/TFA (80/20 v/v) and subsequent hydrogenolysis according to general procedure 8.5.2.5 using 10 % Pd/C (20 mg) in EtOH (2 ml) and EtOAc (2 ml) to yield **8.46** as a pale yellow semisolid substance (60 mg, 73 %). ^{1}H-NMR (acetone-d_6) δ 1.29 (m, 12H, $NCH_2CH_2(C\underline{H}_2)_6$), 1.58 (m, 2H, $C\underline{H}_2CH_2CO$), 1.79 (m, 2H, $NCH_2C\underline{H}_2$), 1.98 (m, 2H, $C\underline{H}_2CH_2CO_2H$), 2.35 (m, 4H, $C\underline{H}_2CO_2$, $C\underline{H}_2CO_2H$), 2.80 (t, 2H, ^3J = 7.5 Hz, $C\underline{H}_2(CH_2)_2CO_2H$), 4.22 (m, 5H, NCH_2, $C\underline{H}OH$, CH_2O), 4.78 (d, 1H, ^3J = 1.5 Hz, ascorbic acid H-5), 7.00 (ddd, 1H, ^2J = 0.8 Hz, ^3J = 7.1 Hz, ^3J = 7.9 Hz, indole-H), 7.12 (m, 2H, indole-H), 7.38 (m, 1H, indole-H), 7.57 (m, 1H, indole-H). ^{13}C-NMR (acetone-d_6) δ 25.13 (-, CH_2), 25.64 (-, CH_2), 26.22 (-, CH_2), 26.59 (-, CH_2), 27.64 (-, CH_2), 29.77 (-, CH_2), 29.98 (-, CH_2), 30.22 (-, CH_2), 31.12 (-, CH_2), 33.87 (-, CH_2), 34.52 (-, CH_2), 46.52 (-, CH_2), 65.42 (-, CH_2), 67.66 (+, CHOH), 68.12 (-, CH_2), 76.14 (+, CH), 110.35 (+, indole-C), 114.77 (quat, indole C-3), 119.16 (+, indole-C), 119.68 (+, indole-C), 120.10 (quat, ascorbic acid C-3), 121.94 (+, indole-C), 126.50 (+, indole-C), 129.02 (quat, indole C-9), 137.51 (quat, indole C-8), 151.05 (quat, ascorbic acid C-4), 170.39 (quat, lactone CO), 173.64 (quat, $\underline{C}O_2CH_2$), 174.79 (quat, CO_2H). ES-MS (MeCN + TFA) *m/z (%)*: 546 (100) $[M+H]^+$. $C_{29}H_{39}NO_9$ (545.62).

1-[16-Oxo-16-(4-phenylpiperazin-1-yl)hexadecan-1-yl)]-1*H*-indole-3-butanoic acid trifluoroacetic acid salt (8.47): The title compound was prepared according to general procedure 8.5.2.6 using **8.39** (0.20 mmol, 0.13 g) in 4 ml of CH_2Cl_2/TFA (80/20 v/v) and was obtained after flash chromatography ($CHCl_3$/MeOH 80/20 + NH_3 v/v) as a yellow oil (0.14 g, quantitative). ^{1}H-NMR (CD_3OD) δ 1.29 (m, 1H, $NCH_2CH_2(C\underline{H}_2)_{11}$), 1.61 (m, 2H, $C\underline{H}_2CH_2CO$), 1.77 (m, 2H, $NCH_2C\underline{H}_2$), 1.97 (m, 2H, $C\underline{H}_2CH_2CO_2H$), 2.32 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2CO_2H$), 2.44 (t, 2H, ^3J = 7.6 Hz, $C\underline{H}_2CON$), 2.77 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2(CH_2)_2CO_2H$), 3.41 (m, 4H, piperazine-H),

3.85 (m, 4H, piperazine-H), 4.08 (t, 2H, ^{3}J = 6.9 Hz, NCH_2), 6.99 (m, 2H, H-2, Ar-H), 7.11 (m, 1H, indole-H), 7.22 (m, 1H, indole-H), 7.31 (m, 3H, Ar-H), 7.46 (m, 3H, Ar-H). ^{13}C-NMR (CD_3OD) δ 25.46 (-, CH_2), 26.44 (-, CH_2), 26.99 (-, CH_2), 27.95 (-, CH_2), 30.31 (-, CH_2), 30.46 (-, CH_2), 30.57 (-, CH_2), 30.61 (-, CH_2), 30.75 (-, CH_2), 31.40 (-, CH_2), 33.89 (-, CH_2), 34.55 (-, CH_2), 41.59 (-, piperazine-C), 45.63 (-, piperazine-C), 46.85 (-, NCH_2), 53.35 (-, piperazine-C), 53.66 (-, piperazine-C), 110.41 (+, indole-C), 115.08 (quat, C-3), 119.45 (+, indole-C), 119.84 (+, indole-C), 119.98 (+, Ph-C), 122.25 (+, indole-C), 126.44 (+, Ph-C), 126.64 (+, indole-C), 129.39 (quat, C-9), 130.99 (+, Ph-C), 137.98 (quat, C-8), 147.95 (quat, Ph-C), 174.27 (quat, $CH_2\underline{C}ON$), 177.72 (quat, CO_2H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 602 (100) $[M+H]^+$. $C_{40}H_{56}F_3N_3O_5$ (715.88).

1-[16-(3-Hydroxyphenylamino)-16-oxohexadecan-1-yl]-1*H*-indole-3-butanoic acid (8.48): The title compound was prepared according to general procedure 8.5.2.6 using **8.40** (0.31 mmol, 0.19 g) in 4 ml of CH_2Cl_2/TFA (80/20 v/v) and was obtained after flash chromatography ($CHCl_3$/MeOH 80/20 + NH_3 v/v) as a white semisolid (0.17 g, quantitative). ^{1}H-NMR (CD_3OD) δ 1.29 (m, 22H, $NCH_2CH_2(C\underline{H}_2)_{11}$), 1.72 (m, 4H, $C\underline{H}_2CH_2CO$, $NCH_2C\underline{H}_2$), 1.97 (m, 2H, $C\underline{H}_2CH_2CO_2H$), 2.32 (m, 4H, $C\underline{H}_2CO_2H$, $C\underline{H}_2CON$), 2.77 (t, 2H, ^{3}J = 7.4 Hz, $C\underline{H}_2(CH_2)_2CO_2H$), 4.07 (t, 2H, ^{3}J = 6.9 Hz, NCH_2), 6.51 (m, 1H, Ar-H), 7.11 (m, 7H), 7.51 (d, 1H, ^{3}J = 7.6 Hz, Ar-H). ^{13}C-NMR (CD_3OD) δ 24.36 (-, CH_2), 25.92 (-, CH_2), 26.88 (-, CH_2), 29.29 (-, CH_2), 29.42 (-, CH_2), 29.56 (-, CH_2), 29.66 (-, CH_2), 30.32 (-, CH_2), 33.45 (-, CH_2), 37.02 (-, CH_2), 45.76 (-, NCH_2), 107.36 (+, Ph-C), 109.31 (+, indole-C), 111.02 (+, Ph-C), 111.29 (+, Ph-C), 113.96 (quat, C-3), 118.34 (+, indole-C), 118.73 (+, indole-C), 121.15 (+, indole-C), 125.27 (+, indole-C), 125.53 (quat, C-9), 129.41 (+, Ph-C), 136.89 (quat, C-8), 139.95 (quat, Ph-C), 157.84 (quat, Ph-C), 173.65 (quat, $CH_2\underline{C}ON$), 176.67 (quat, CO_2H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 549 (100) $[M+H]^+$. $C_{34}H_{48}N_2O_4$ (548.76).

1-{16-[(*S*)-2-((*R*)-3,4-Dihydroxy-2-oxo-2,5-dihydrofuran-5-yl)-2-hydroxyethoxy]-16-oxohexadecan-1-yl}-1*H*-indole-3-butanoic acid (8.49): The title compound was prepared from **8.41** (0.09 mmol, 80 mg) by removing the *tert*-butyl moiety according to general method 8.5.2.6 in 4 ml of CH_2Cl_2/TFA (80/20 v/v) and subsequent hydrogenolysis according to general procedure 8.5.2.5 using 10 % Pd/C (20 mg) in EtOH (2 ml) and EtOAc (2 ml) to yield **8.49** as a pale yellow semisolid substance (50 mg, 86 %). ^{1}H-NMR (CD_3OD+$CDCl_3$) δ 1.28 (m, 22H, $NCH_2CH_2(C\underline{H}_2)_6$), 1.61 (m, 2H,

C$\underline{H}_2$CH$_2$CO), 1.78 (m, 2H, NCH$_2$C$\underline{H}_2$), 1.98 (m, 2H, C$\underline{H}_2$CH$_2$CO$_2$H), 2.31 (m, 4H, C$\underline{H}_2$CO$_2$, C$\underline{H}_2$CO$_2$H), 2.77 (t, 2H, ^3J = 7.3 Hz, C$\underline{H}_2$(CH$_2$)$_2$CO$_2$H), 4.03 – 4.29 (m, 5H, NCH$_2$, C$\underline{H}$OH, CH$_2$O), 4.68 (d, 1H, ^3J = 2.1 Hz, ascorbic acid H-5), 6.97 (m, 2H, indole-H), 7.12 (ddd, 1H, ^4J = 1.1 Hz, ^3J = 5.0 Hz, ^3J = 8.3 Hz, indole-H), 7.29 (m, 1H, indole-H), 7.52 (m, 1H, indole-H). ES-MS (DCM/MeOH + NH$_4$OAc) *m/z (%)*: 614 (100) [M-H]$^-$. $C_{34}H_{49}NO_9$ (615.75).

4-Benzyloxybenzenediazonium tetrafluoroborate[35] (8.50): To a suspension of 4-benzyloxyaniline hydrochloride (33.0 mmol, 7.78) in water (24 ml) and concentrated HCl (24 ml) was added dropwise a solution of sodium nitrite (34.65 mmol, 2.39 g) in water (10 ml) at 0 °C. After stirring for 10 min a solution of $NaBF_4$ (33 mmol, 3.62 g) in water (8 ml) was added and the cooling bath was removed. Stirring was continued for additional 20 min at room temperature. The resulting grey solid was isolated by filtration and washed tree times with water, methanol and ether. After drying under reduced pressure the title compound was obtained as a pale grey solid which was used without further purification in the next step (7.90 g, 80 %). mp: 136 °C (ref.[35]: 137 °C); $C_{13}H_{11}BF_4N_2O$ (298.04).

Ethyl 1-(4-benzyloxyphenyldiazenyl)-2-oxocyclohexanecarboxylate (8.51): To a suspension of NaH (60 % suspension in mineral oil, 20.8 mmol, 0.83g) in anhydrous THF (25 ml) was added dropwise a solution of ethyl 2-oxocyclohexanecarboxylate (18.3 mmol, 2.91 ml) and the mixture was refluxed for 30 min. After cooling to -5 °C, **8.50** (18.0 mmol, 5.36) was added in portions and the resulting dark red solution was stirred for 1 h at room temperature. The mixture was then poured into water (200 ml) and extracted with ether (3 x 100 ml). The combined organic layers were washed with water (100 ml) dried over $MgSO_4$, filtered and evaporated to afford the title compound as dark red oil which was used without further purification in the next step (6.5 g, 95 %). $C_{22}H_{24}N_2O_4$ (380.44).

Ethyl 5-benzyloxy-2-ethoxycarbonyl-1*H*-indole-3-butanoate (8.52): A solution of **8.51** (21.6 mmol, 8.2 g) and concentrated H_2SO_4 (2 ml) in anhydrous EtOH (50 ml) was refluxed overnight. After cooling the solution was diluted with water (250 ml) and extracted tree times with $CHCl_3$. The combined organic phases were washed with water, dried over $MgSO_4$, filtered and evaporated to dryness. The raw product was subjected to flash chromatography (PE/EtOAc 80/20 v/v) to obtain the title compound as pale brown solid (3.8 g, 42 %). ^{1}H-NMR ($CDCl_3$) δ 1.24 (t, 3H, ^3J = 7.1 Hz, CH$_2$C$\underline{H}_3$), 1.41 (t, 3H, ^3J = 7.1 Hz, CH$_2$C$\underline{H}_3$), 1.96 (m, 2H, C$\underline{H}_2$CH$_2$CO$_2$), 2.33 (t, 1H,

^{3}J = 7.5 Hz, C$\underline{H}_2$CO$_2$), 3.06 (t, 2H, ^{3}J = 7.4 Hz, C$\underline{H}_2$(CH$_2$)$_2$CO$_2$), 4.11 (q, 2H, ^{3}J = 7.1 Hz, C$\underline{H}_2$CH$_3$), 4.38 (q, 2H, ^{3}J = 7.1 Hz, C$\underline{H}_2$CH$_3$), 5.11 (s, 2H, C$\underline{H}_2$Ph), 7.07 (dd, 1H, ^{4}J = 2.4 Hz, ^{3}J = 9.0 Hz, H-6), 7.14 (d, 1H, ^{4}J = 2.2 Hz, H-4), 7.22 (d, 1H, ^{3}J = 9.0 Hz, H-7), 7.37 (m, 3H, Ph-H), 7.49 (m, 2H, Ph-H), 8.02 (s, 1H, NH). CI-MS (NH$_3$) *m/z (%)*: 427 (53) [M+NH$_4$]$^+$. Anal. (C$_{24}$H$_{27}$NO$_5$·0.75H$_2$O) C, H, N. C$_{24}$H$_{27}$NO$_5$ (409.47).

5-Benzyloxy-2-carboxy-1*H*-indole-3-butanoic acid[36] (8.53): A solution of **8.52** (13.1 mmol, 5.37 g) and KOH (39.3 mmol, 2.21 g) in ethanol (50 ml) was refluxed for 30 min. After cooling, the diacid which precipitated was isolated by filtration and resuspended in 2 N HCl (30 ml). The mixture was stirred for 2 h, solids were isolated by filtration, washed with water and dried under reduced pressure to obtain the title compound as a pale brown solid (2.3 g, 49 %). mp: 185-187 °C (ref.[36]: 185 °C); ^{1}H-NMR (DMSO-d_6) δ 1.83 (m, 2H, C$\underline{H}_2$CH$_2$CO$_2$H), 2.22 (t, 2H, ^{3}J = 7.4 Hz, C$\underline{H}_2$CO$_2$H), 3.04 (t, 2H, ^{3}J = 7.1 Hz, C$\underline{H}_2$(CH$_2$)$_2$CO$_2$H), 5.11 (s, 2H, C$\underline{H}_2$Ph), 6.97 (dd, 1H, ^{4}J = 2.3 Hz, ^{3}J = 8.9 Hz, H-6), 7.23 (d, 1H, ^{3}J = 2.0 Hz, H-4), 7.35 (m, 4H, H-7, Ph-H), 7.50 (m, 2H, Ph-H), 11.28 (s, 1H, NH), 12.45 (s, 2H, CO$_2$H). ^{13}C-NMR (DMSO-d_6) δ 23.25 (-, CH$_2$), 33.24 (-, CH$_2$), 25.80 (-, $\underline{C}$H$_2$CO$_2$H), 69.62 (-, CH$_2$Ph), 102.07 (+, C-4), 113.21 (+, indole-C), 116.24 (+, indole-C), 121.33 (quat, C-3), 124.54 (quat, C-2), 127.52 (quat, indole-C), 127.62 (+, Ph-C), 127.78 (+, Ph-C), 128.26 (+, Ph-C), 131.39 (quat, indole-C), 137.40 (quat, Ph-C), 152.30 (quat, C-5), 163.26 (quat, CO$_2$H), 174.49 (quat, CO$_2$H). ES-MS (MeOH + NH$_4$OAc) *m/z (%)*: 371 (100) [M+NH$_4$]$^+$. Anal. (C$_{20}$H$_{19}$NO$_5$·0.2H$_2$O) C, H, N. C$_{20}$H$_{19}$NO$_5$ (353.37).

5-Benzyloxy-1*H*-indole-3-butanoic acid[36] (8.54): 8.53 (2.83 mmol, 1.00 g) was heated for 2 h at 200-230 °C. After cooling, the raw product was subjected to flash chromatography (CHCl$_3$/MeOH 98/2 v/v) to obtain the title compound as brown solid (0,79 g, 90 %). mp: 100 °C (ref.[36]: 86-95 °C); ^{1}H-NMR (DMSO-d_6) δ 1.85 (m, 2H, C$\underline{H}_2$CH$_2$CO$_2$H), 2.27 (t, 2H, ^{3}J = 7.3 Hz, C$\underline{H}_2$CO$_2$H), 2.66 (t, 2H, ^{3}J = 7.4 Hz, C$\underline{H}_2$(CH$_2$)$_2$CO$_2$H), 5.09 (s, 2H, C$\underline{H}_2$Ph), 6.79 (dd, 1H, ^{4}J = 2.4 Hz, ^{3}J = 8.7 Hz, H-6), 7.07 (d, 1H, ^{4}J = 2.1 Hz, H-4), 7.11 (d, 1H, ^{3}J = 2.3 Hz, H-2), 7.32 (m, 4H, H-7, Ph-H), 7.48 (m, 2H, Ph-H), 10.63 (d, 1H, ^{3}J = 1.5 Hz, NH), 12.04 (s, 1H, CO$_2$H). ^{13}C-NMR (DMSO-d_6) δ 24.01 (-, $\underline{C}$H$_2$CH$_2$CO$_2$H), 25.23 (-, $\underline{C}$H$_2$(CH$_2$)$_2$CO$_2$H), 33.33 (-, $\underline{C}$H$_2$CO$_2$H), 69.75 (-, CH$_2$Ph), 101.76 (+, C-4), 111.49 (+, indole-C), 111.87 (+, indole-C), 113.62 (quat, C-3), 123.01 (+, C-2), 127.37 (quat, indole-C), 127.50 (+, Ph-C), 127.61 (+, Ph-C), 128.23 (+, Ph-C), 131.53 (quat, indole-C), 137.76 (quat, Ph-C),

151.80 (quat, C-5), 174.48 (quat, CO_2H). CI-MS (NH_3) *m/z (%)*: 327 (100) $[M+NH_4]^+$. $C_{19}H_{19}NO_3$ (309.36).

5-Hydroxy-1*H*-indole-3-butanoic acid (8.55): The title compound was prepared according to general procedure 8.5.2.5 using **8.54** (0.32 mmol, 0.10 g) and 10 % Pd/C (50 mg) in EtOH (5 ml) and was obtained as a pale brown solid (0.23 g, 96 %). mp: 143 °C; ^{1}H-NMR (CD_3OD) δ 1.96 (m, 2H, C$\underline{H}_2$CH_2CO_2H), 2.33 (t, 2H, ^3J = 7.4 Hz, C$\underline{H}_2$CO_2H), 2.71 (t, 2H, ^3J = 7.4 Hz, C$\underline{H}_2$(CH_2)$_2$CO_2H), 6.65 (dd, 1H, ^4J = 2.3 Hz, ^3J = 8.6 Hz, H-6), 6.90 (d, 1H, ^3J = 2.3 Hz, H-4), 6.95 (s, 1H, H-2), 7.14 (d, 1H, ^3J = 8.5 Hz, H-7). EI-MS (70 eV) *m/z (%)*: 219 (29) $[M^{+\cdot}]$. $C_{12}H_{13}NO_3$ (219.24).

Ethyl 3-(4-ethoxy-4-oxobutyl)-5-hydroxy-1*H*-indole-2-carboxylate[37] (8.56): The title compound was prepared according to general procedure 8.5.2.5 using **8.52** (1.0 mmol, 0.41 g) and 10 % Pd/C (50 mg) in EtOH (10 ml) and was obtained as a pale yellow solid (0.30 g, 95 %). mp: 125-127 °C; ^{1}H-NMR (DMSO-d_6) δ 1.15 (t, 3H, ^3J = 7.1Hz, CH_2C$\underline{H}_3$), 1.33 (t, 3H, ^3J = 7.1 Hz, CH_2C$\underline{H}_3$), 1.83 (m, 2H, C$\underline{H}_2$CH_2CO_2), 2.27 (t, 2H, ^3J = 7.4 Hz, C$\underline{H}_2$CO_2), 2.97 (t, 2H, ^3J = 7.3 Hz, C$\underline{H}_2$(CH_2)$_2$CO_2), 4.02 (q, 2H, ^3J = 7.1 Hz, C$\underline{H}_2$CH_3), 4.31 (q, 2H, ^3J = 7.1 Hz, C$\underline{H}_2$CH_3), 6.81 (dd, 1H, ^4J = 2.3 Hz, ^3J = 8.8 Hz, H-6), 6.89 (d, 1H, ^4J = 2.2 Hz, H-4), 7.23 (d, 1H, ^3J = 8.8 Hz, H-7), 8.92 (s, 1H, NH), 11.23 (s, 1H, OH). CI-MS (NH_3) *m/z (%)*: 320 (100) $[M+H]^+$. $C_{17}H_{21}NO_5$ (319.35). CD_3OD

8.5.2.9 General procedure for the synthesis of 5-*O*-alkylated indoles 8.57-8.61 using Mitsunobu conditions

To a solution of **8.56** (1 eq) in anhydrous THF was added the pertinent alcohol (1 eq), PPh_3 (1 eq) and DIAD (1 eq) at 0 °C under an atmosphere of argon. After stirring for 10 min the cooling bath was removed and stirring was continued at room temperature overnight. The mixture was poured into water (20 ml) and extracted three times with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and evaporated to dryness. The 5-*O*-alkylated indoles were then obtained after flash chromatography.

Ethyl 3-(4-ethoxy-4-oxobutyl)-5-(3-phenylpropoxy)-1*H*-indole-2-carboxylate (8.57): The title compound was prepared according to general procedure 8.5.2.9 from **8.56** (1.0 mmol, 0.32 g), 3-phenylpropan-1-ol (1.0 mmol, 0,14 g), PPh_3 (1.0 mmol, 0.26 g) and DIAD (1.0 mmol, 0.20 ml) in anhydrous THF (10 ml) and was obtained after flash chromatography (PE/EtOAc 80/20 v/v) as a pale yellow semisolid (0.27 g, 62 %). ^{1}H-NMR (DMSO-d_6) δ 1.14 (t, 3H, ^3J = 7.1 Hz, CH_2C$\underline{H}_3$), 1.34 (t, 3H,

^3J = 7.1 Hz, $CH_2C\underline{H}_3$), 1.84 (m, 2H, $C\underline{H}_2CH_2CO_2$), 2.03 (m, 2H, $OCH_2C\underline{H}_2$), 2.28 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2CO_2$), 2.77 (m, 2H, $C\underline{H}_2Ph$), 3.02 (t, 2H, ^3J = 7.2 Hz, $C\underline{H}_2(CH_2)_2CO_2$), 4.01 (m, 4H, $C\underline{H}_2CH_3$, OCH_2), 4.32 (q, 2H, ^3J = 7.1 Hz, $C\underline{H}_2CH_3$), 6.94 (dd, 1H, ^4J = 2.3 Hz, ^3J = 8.9 Hz, H-6), 7.08 (d, 1H, ^3J = 2.1 Hz, H-4), 7.25 (m, 6H, H-7, Ph-H), 11.38 (s, 1H, NH). CI-MS (NH_3) *m/z (%)*: 455 (100) $[M+NH_4]^+$. Anal. ($C_{26}H_{31}NO_5 \cdot 0.4H_2O$) C, H, N. $C_{26}H_{31}NO_5$ (437.53).

Ethyl 3-(4-ethoxy-4-oxobutyl)-5-hexyloxy-1*H*-indole-2-carboxylate (8.58): The title compound was prepared according to general procedure 8.5.2.9 from **8.56** (1.0 mmol, 0.32 g), hexan-1-ol (1.0 mmol, 0,13 ml), PPh_3 (1.0 mmol, 0.26 g) and DIAD (1.0 mmol, 0.20 ml) in anhydrous THF (10 ml) and was obtained after flash chromatography (PE/EtOAc 90/10 to 80/20 v/v) as pale yellow semisolid substance (0.21 g, 52 %). ^{1}H-NMR (DMSO-d_6) δ 0.88 (t, 3H, ^3J = 7.0 Hz, $(CH_2)_5C\underline{H}_3$), 1.15 (t, 3H, ^3J = 7.1 Hz, $CH_2C\underline{H}_3$), 1.37 (m, 9H, $CH_2C\underline{H}_3$, $(C\underline{H}_2)_3CH_3$), 1.72 (m, 2H, $OCH_2C\underline{H}_2$), 1.85 (m, 2H, $C\underline{H}_2CH_2CO_2$), 2.29 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2CO_2$), 3.02 (t, 2H, ^3J = 7.2 Hz, $C\underline{H}_2(CH_2)_2CO_2$), 3.99 (m, 4H, $C\underline{H}_2CH_3$, OCH_2), 4.32 (q, 2H, ^3J = 7.1 Hz, $C\underline{H}_2CH_3$), 6.91 (dd, 1H, ^4J = 2.3 Hz, ^3J = 8.9 Hz, H-6), 7.08 (d, 1H, ^4J = 2.2 Hz, H-4), 7.30 (d, 1H, ^3J = 8.9 Hz, H-7), 11.36 (s, 1H, NH). CI-MS (NH_3) *m/z (%)*: 421 (100) $[M+NH_4]^+$. Anal. ($C_{23}H_{33}NO_5$) C, H, N. $C_{23}H_{33}NO_5$ (403.51).

Ethyl 3-(4-ethoxy-4-oxobutyl)-5-(dodecan-1-yl)-oxy-1*H*-indole-2-carboxylate (8.59): The title compound was prepared according to general procedure 8.5.2.9 from **8.56** (1.0 mmol, 0.32 g), dodecan-1-ol (1.0 mmol, 0,19 g), PPh_3 (1.0 mmol, 0.26 g) and DIAD (1.0 mmol, 0.20 ml) in anhydrous THF (10 ml) and was obtained after flash chromatography (PE/EtOAc 90/10 to 80/20 v/v) as a pale yellow solid (0.30 g, 62 %). ^{1}H-NMR (DMSO-d_6) δ 0.84 (t, 6H, ^3J = 6.5 Hz, $(CH_2)_{11}C\underline{H}_3$), 1.12 – 1.45 (m, 24H, $(C\underline{H}_2)_9CH_3$, $CH_2C\underline{H}_3$), 1.71 (m, 2H, $OCH_2C\underline{H}_2$), 1.85 (m, 2H, $C\underline{H}_2CH_2CO_2$), 2.29 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2CO_2$), 3.02 (t, 2H, ^3J = 7.2 Hz, $C\underline{H}_2(CH_2)_2CO_2$), 3.99 (m, 4H, $C\underline{H}_2CH_3$, OCH_2), 4.32 (q, 2H, ^3J = 7.2 Hz, $C\underline{H}_2CH_3$), 6.90 (dd, 1H, ^4J = 2.3 Hz, ^3J = 8.9 Hz, H-6), 7.07 (d, 1H, ^4J = 2.1 Hz, H-4), 7.30 (d, 1H, ^3J = 8.9 Hz, H-7), 11.36 (s, 1H, NH). CI-MS (NH_3) *m/z (%)*: 505 (100) $[M+NH_4]^+$. $C_{29}H_{45}NO_5$ (487.67).

Ethyl 3-(4-ethoxy-4-oxobutyl)-5-(hexadecan-1-yl)-oxy-1*H*-indole-2-carboxylate (8.60): The title compound was prepared according to general procedure 8.5.2.9 from **8.56** (1.0 mmol, 0.32 g), hexadecan-1-ol (1.0 mmol, 0,24 g), PPh_3 (1.0 mmol, 0.26 g) and DIAD (1.0 mmol, 0.20 ml) in anhydrous THF (10 ml) and was obtained after flash chromatography (PE/EtOAc 95/5 to 80/20 v/v) as a white solid (0.36 g, 64

%). mp: 50 °C; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.7 Hz, $(CH_2)_{15}C\underline{H}_3$), 1.21 – 1.60 (m, 32H, $(C\underline{H}_2)_{13}CH_3$, $CH_2C\underline{H}_3$), 1.81 (m, 2H, $OCH_2C\underline{H}_2$), 2.02 (m, 2H, $C\underline{H}_2CH_2CO_2$), 2.36 (t, 2H, ^3J = 7.6 Hz, $C\underline{H}_2CO_2$), 3.12 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2(CH_2)_2CO_2$), 4.00 (t, 2H, ^3J = 6.6 Hz, OCH_2), 4.10 (q, 2H, ^3J = 7.1 Hz, $C\underline{H}_2CH_3$), 4.40 (q, 2H, ^3J = 7.1 Hz, $C\underline{H}_2CH_3$), 6.99 (dd, 1H, ^4J = 2.4 Hz, ^3J = 8.8 Hz, H-6), 7.05 (d, 1H, ^4J = 2.2 Hz, H-4), 7.26 (d, 1H, ^3J = 8.8 Hz, H-7), 8.66 (s, 1H, NH). CI-MS (NH_3) *m/z (%)*: 561 (100) $[M+NH_4]^+$. $C_{33}H_{53}NO_5$ (543.78).

Ethyl 3-(4-ethoxy-4-oxobutyl)-5-(octadecan-1-yl)-oxy-1*H*-indole-2-carboxylate (8.61): The title compound was prepared according to general procedure 8.5.2.9 from **8.56** (1.0 mmol, 0.32 g), octadecan-1-ol (1.0 mmol, 0,27 g), PPh_3 (1.0 mmol, 0.26 g) and DIAD (1.0 mmol, 0.20 ml) in anhydrous THF (10 ml) and was obtained after flash chromatography (PE/EtOAc 95/5 to 80/20 v/v) as pale yellow solid (0.35 g, 61 %). mp: 52 °C; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.7 Hz, $(CH_2)_{17}C\underline{H}_3$), 1.18 – 1.57 (m, 36H, $(C\underline{H}_2)_{15}CH_3$, $CH_2C\underline{H}_3$), 1.81 (m, 2H, $OCH_2C\underline{H}_2$), 2.02 (m, 2H, $C\underline{H}_2CH_2CO_2$), 2.36 (t, 2H, ^3J = 7.6 Hz, $C\underline{H}_2CO_2$), 3.12 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2(CH_2)_2CO_2$), 4.00 (t, 2H, ^3J = 6.6 Hz, OCH_2), 4.11 (q, 2H, ^3J = 7.1 Hz, $C\underline{H}_2CH_3$), 4.40 (q, 2H, ^3J = 7.1 Hz, $C\underline{H}_2CH_3$), 6.99 (dd, 1H, ^4J = 2.4 Hz, ^3J = 8.8 Hz, H-6), 7.04 (d, 1H, ^4J = 2.3 Hz, H-4), 7.26 (d, 1H, ^3J = 8.8 Hz, H-7), 8.64 (s, 1H, NH). CI-MS (NH_3) *m/z (%)*: 589 (100) $[M+NH_4]^+$. $C_{35}H_{57}NO_5$ (571.83).

8.5.2.10 General method for the synthesis of carboxylic acids 8.62-8.66, 8.70-8.72 and 8.74 *via* saponification of ethyl ester precursors

To a solution of the pertinent ethyl ester (1 eq) in THF/MeOH/H_2O was added LiOH (1.5 eq) and the mixture was stirred at room temperature overnight. After dilution with water and acidification with 2 N HCl to pH <2 the mixture was extracted three times with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and evaporated to dryness to obtain the pertinent carboxylic acids.

2-Carboxy-5-(3-phenylpropoxy)-1*H*-indole-3-butanoic acid (62): The title compound was prepared according to general procedure 8.5.2.10 using **8.57** (0.75 mmol, 0.33 g) and LiOH (2.26 mmol, 54 mg) in THF (5 ml), MeOH (1 ml) and H_2O (2 ml) and was obtained after recrystallization from EtOAc/PE as a white solid (0.20 g, 70 %). mp: 170-171 °C; ^{1}H-NMR (DMSO-d_6) δ 1.81 (m, 2H, $C\underline{H}_2CH_2CO_2H$), 2.03 (m, 2H, $OCH_2C\underline{H}_2$), 2.21 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2CO_2H$), 2.77 (m, 2H, $C\underline{H}_2Ph$), 3.01 (t, 2H, ^3J = 7.2 Hz, $C\underline{H}_2(CH_2)_2CO_2H$), 3.99 (t, 2H, ^3J = 6.3 Hz, OCH_2), 6.92 (dd, 1H, ^4J = 2.3 Hz, ^3J = 8.9 Hz, H-6), 7.09 (d, 1H, ^4J = 2.2 Hz, H-4), 7.23 (m, 6H, H-7, Ph-H), 11.27

(s, 1H, NH), 12.41 (bs, 1H, CO_2H). ^{13}C-NMR (DMSO-d_6) δ 23.24 (-, CH_2), 25.78 (-, CH_2), 30.57 (-, CH_2), 31.56 (-, CH_2), 33.21 (-, CH_2), 67.02 (-, OCH_2), 101.58 (+, C-4), 113.19 (+, indole-C), 116.22 (+, indole-C), 121.45 (quat, C-3), 124.23 (quat, C-2), 125.71 (+, Ph-C), 127.52 (quat, indole-C), 128.24 (+, Ph-C), 128.27 (+, Ph-C), 131.37 (quat, indole-C), 141.49 (quat, Ph-C), 152.60 (quat, C-5), 163.16 (quat, CO_2H), 174.42 (quat, CO_2H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 380 (100) $[M-H]^-$. Anal. ($C_{22}H_{23}NO_5 \cdot 0.2H_2O$) C, H, N. $C_{22}H_{23}NO_5$ (381.42).

2-Carboxy-5-(hexan-1-yloxy)-1*H*-indole-3-butanoic acid (8.63): The title compound was prepared according to general procedure 8.5.2.10 using **8.58** (0.40 mmol, 0.16 g) and LiOH (1.20 mmol, 29 mg) in THF (5 ml), MeOH (1 ml) and H_2O (2 ml) and was obtained after recrystallization from EtOAc/PE as a white solid (90 mg, 65 %). mp: 154 °C; ^{1}H-NMR (DMSO-d_6) δ 0.88 (t, 3H, ^3J = 7.0 Hz, $CH_2C\underline{H}_3$), 1.37 (m, 6H, $(C\underline{H}_2)_3CH_3$), 1.77 (m, 4H, $OCH_2C\underline{H}_2$), 2.21 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2CH_2CO_2H$), 3.01 (t, 2H, ^3J = 7.2 Hz, $C\underline{H}_2(CH_2)_2CO_2H$), 3.96 (t, 2H, ^3J = 6.4 Hz, OCH_2), 6.88 (dd, 2H, ^4J = 2.3 Hz, ^3J = 8.9 Hz, H-6), 7.08 (d, 1H, ^4J = 2.2 Hz, H-4), 7.27 (d, 1H, ^3J = 8.9 Hz, H-7), 11.25 (s, 1H, NH), 12.39 (bs, 2H, $CO_2\underline{H}$). ^{13}C-NMR (DMSO-d_6) δ 13.84 (+, CH_3), 22.02 (-, CH_2), 23.22 (-, CH_2), 25.25 (-, CH_2), 25.77 (-, CH_2), 28.79 (-, CH_2), 31.01 (-, CH_2), 33.19 (-, CH_2), 67.69 (-, CH_2O), 101.36 (+, C-4), 113.15 (+, indole-C), 116.21 (+, indole-C), 121.45 (quat, C-3), 124.17 (quat, C-2), 127.53 (quat, indole-C), 131.30 (quat, indole-C), 152.69 (quat, C-5), 163.16 (quat, CO_2H), 174.42 (quat, CO_2H). CI-MS (NH_3) *m/z (%)*: 346 (100) $[M-H]^-$. Anal. ($C_{19}H_{25}NO_5$) C, H, N. $C_{19}H_{25}NO_5$ (347.41).

2-Carboxy-5-(dodecan-1-yloxy)-1*H*-indole-3-butanoic acid (8.64): The title compound was prepared according to general procedure 8.5.2.10 using **8.59** (0.45 mmol, 0.22 g) and LiOH (1.35 mmol, 32 mg) in THF (5 ml), MeOH (0,5 ml) and H_2O (1 ml) and was obtained after recrystallization from EtOAc/PE as a pale yellow solid (130 mg, 67 %). mp: 145 °C; ^{1}H-NMR (DMSO-d_6) δ 0.85 (t, 3H, ^3J = 6.7 Hz, $(CH_2)_{11}C\underline{H}_3$), 1.31 (m, 18H, $(C\underline{H}_2)_9CH_3$), 1.71 (m, 2H, $OCH_2C\underline{H}_2$), 1.81 (m, 2H, $C\underline{H}_2CH_2CO_2H$), 2.21 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2CO_2H$), 3.01 (t, 2H, ^3J = 7.2 Hz, $C\underline{H}_2(CH_2)_2CO_2H$), 3.95 (t, 2H, ^3J = 6.4 Hz, OCH_2), 6.88 (dd, 1H, ^4J = 2.3 Hz, ^3J = 8.9 Hz, H-6), 7.07 (d, 1H, ^4J = 2.2 Hz, H-4), 7.27 (d, 1H, ^3J = 8.9 Hz, H-7), 11.25 (s, 1H, NH), 12.39 (s, 2H, CO_2H). ^{13}C-NMR (DMSO-d_6) δ 13.87 (+, CH_3), 22.02 (-, CH_2), 23.22 (-, CH_2), 25.56 (-, CH_2), 25.77 (-, CH_2), 28.64 (-, CH_2), 28.77 (-, CH_2), 28.81 (-, CH_2), 28.94 (-, CH_2), 31.22 (-, CH_2), 33.19 (-, CH_2), 67.66 (-, OCH_2), 101.33 (+, C-4),

113.13 (+, indole-C), 116.19 (+, indole-C), 121.42 (quat, C-3), 124.18 (quat, C-2), 127.52 (quat, indole-C), 131.29 (quat, indole-C), 152.68 (quat, C-5), 163.17 (quat, CO_2H), 174.41 (quat, CO_2H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 430 (100) $[M-H]^-$. Anal. ($C_{25}H_{37}NO_5$) C, H, N. $C_{25}H_{37}NO_5$ (431.56).

2-Carboxy-5-(hexadecan-1-yloxy)-1*H*-indole-3-butanoic acid (8.65): The title compound was prepared according to general procedure 8.5.2.10 using **8.60** (0.53 mmol, 0.29 g) and LiOH (1.60 mmol, 38 mg) in THF (4 ml), MeOH (1 ml) and H_2O (2 ml) and was obtained after recrystallization from EtOAc/PE as a white solid (0.15 g, 58 %). mp: 143 °C; ^{1}H-NMR (DMSO-d_6) δ 0.84 (t, 3H, ^3J = 6.7 Hz, $(CH_2)_{15}C\underline{H}_3$), 1.35 (m, 26H, $(C\underline{H}_2)_{13}CH_3$), 1.71 (m, 2H, $OCH_2C\underline{H}_2$), 1.81 (m, 2H, $C\underline{H}_2CH_2CO_2H$), 2.21 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2CO_2H$), 3.01 (t, 2H, ^3J = 7.1 Hz, $C\underline{H}_2(CH_2)_2CO_2H$), 3.95 (t, 2H, ^3J = 6.4 Hz, OCH_2), 6.87 (dd, 1H, ^4J = 2.3 Hz, ^3J = 8.9 Hz, H-6), 7.07 (d, 1H, ^4J = 2.2 Hz, H-4), 7.27 (d, 1H, ^3J = 8.9 Hz, H-7), 11.24 (s, 1H, NH), 12.38 (s, 2H, CO_2H). ^{13}C-NMR (DMSO-d_6) δ 13.86 (+, CH_3), 22.02 (-, CH_2), 23.24 (-, CH_2), 25.56 (-, CH_2), 25.77 (-, CH_2), 28.63 (-, CH_2), 28.77 (-, CH_2), 28.82 (-, CH_2), 28.96 (-, CH_2), 31.22 (-, CH_2), 33.20 (-, CH_2), 67.67 (-, OCH_2), 101.33 (+, C-4), 113.12 (+, indole-C), 116.17 (+, indole-C), 121.41 (quat, C-3), 124.18 (quat, C-2), 127.52 (quat, indole-C), 131.31 (quat, indole-C), 152.68 (quat, C-5), 163.16 (quat, CO_2H), 174.39 (quat, CO_2H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 486 (100) $[M-H]^-$. Anal. ($C_{29}H_{45}NO_5$) C, H, N. $C_{29}H_{45}NO_5$ (487.67).

2-Carboxy-5-(octadecan-1-yloxy)-1*H*-indole-3-butanoic acid (8.66): The title compound was prepared according general procedure 8.5.2.10 using **8.61** (0.48 mmol, 0.28 g) and LiOH (1.44 mmol, 34 mg) in THF (4 ml), MeOH (1 ml) and H_2O (2 ml) and was obtained after recrystallization from EtOAc/PE as a white solid (0.15 g, 61 %). mp: 138-139 °C; ^{1}H-NMR (DMSO-d_6) δ 0.84 (t, 3H, ^3J = 6.6 Hz, $(CH_2)_{18}C\underline{H}_3$), 1.31 (m, 30H, $(C\underline{H}_2)_{15}CH_3$), 1.77 (m, 4H, $C\underline{H}_2CH_2CO_2H$, $OCH_2C\underline{H}_2$), 2.21 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2CO_2H$), 3.01 (t, 2H, ^3J = 7.1 Hz, $C\underline{H}_2(CH_2)_2CO_2H$), 3.95 (t, 2H, ^3J = 6.3 Hz, OCH_2), 6.87 (dd, 1H, ^4J = 2.2 Hz, ^3J = 8.9 Hz, H-6), 7.07 (d, 1H, ^4J = 2.0 Hz, H-4), 7.27 (d, 1H, ^3J = 8.9 Hz, H-7), 11.24 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ 13.86 (+, CH_3), 22.03 (-, CH_2), 23.23 (-, CH_2), 25.57 (-, CH_2), 25.76 (-, CH_2), 28.65 (-, CH_2), 28.78 (-, CH_2), 28.83 (-, CH_2), 28.96 (-, CH_2), 31.23 (-, CH_2), 33.18 (-, CH_2), 67.63 (-, OCH_2), 101.27 (+, C-4), 113.12 (+, indole-C), 116.16 (+, indole-C), 121.41 (quat, C-3), 124.15 (quat, C-2), 127.51 (quat, indole-C), 131.29 (quat, indole-C), 152.67 (quat,

C-5), 163.16 (quat, CO_2H), 174.41 (quat, CO_2H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 514 (100) $[M-H]^-$. Anal. ($C_{31}H_{49}NO_5$) C, H, N. $C_{31}H_{49}NO_5$ (515.72).

Ethyl 2-ethoxycarbonyl-5-(hexadecan-1-yloxy)-1-propyl-1*H*-indole-3-butanoate (8.67): The title compound was prepared according to general procedure 8.5.2.4 using **8.60** (0.5 mmol, 0.27 g), NaH (0.6 mmol, 24 mg) and 1-iodopropane (0.6 mmol, 68 µl) in anhydrous DMF (3 ml) and was obtained after flash chromatography (PE/EtOAc 95/5 v/v) as a pale yellow semisolid substance (0.15 g, 51 %). ^{1}H-NMR ($CDCl_3$) δ 0.89 (m, 6H, $CH_2C\underline{H}_3$), 1.19 – 1.54 (m, 30H, $(C\underline{H}_2)_{12}CH_3$, $CH_2C\underline{H}_3$), 1.79 (m, 4H, $OCH_2C\underline{H}_2$, $NCH_2C\underline{H}_2$), 1.99 (m, 2H, $C\underline{H}_2CH_2CO_2$), 2.36 (t, 2H, ^3J = 7.6 Hz, $C\underline{H}_2CO_2$), 3.08 (t, 2H, ^3J = 7.5 Hz, $C\underline{H}_2(CH_2)_2CO_2$), 4.00 (t, 2H, ^3J = 6.6 Hz, OCH_2), 4.12 (q, 2H, ^3J = 7.1 Hz, $C\underline{H}_2CH_3$), 4.39 (m, 4H, $C\underline{H}_2CH_3$, NCH_2), 7.03 (m, 2H, H-4, H-6), 7.25 (d, 1H, ^3J = 8.7 Hz, H-7). EI-MS (70 eV) *m/z (%)*: 585 (100) $[M^{+\cdot}]$. Anal. ($C_{36}H_{59}NO_5 \cdot 0.2H_2O$) C, H, N. $C_{36}H_{59}NO_5$. (585.86).

Ethyl 2-ethoxycarbonyl-5-(hexadecan-1-yloxy)-1-(pyridin-3-ylmethyl)-1*H*-indole-3-butanoate (8.68): The title compound was prepared according to general procedure 8.5.2.4 using **8.60** (0.5 mmol, 0.27 g), NaH (1.2 mmol, 48 mg) and 3-chloromethylpyridinium chloride (0.6 mmol, 0,10 g) in anhydrous DMF (3 ml) and was obtained after flash chromatography (PE/EtOAc 80/20 v/v) as a pale yellow semisolid (0.16 g, 50 %). ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.7 Hz, $(CH_2)_{15}C\underline{H}_3$), 1.31 (m, 38H), 1.81 (m, 2H, $OCH_2C\underline{H}_2$), 2.01 (m, 2H, $C\underline{H}_2CH_2CO_2$), 2.38 (t, 2H, ^3J = 7.5 Hz, $C\underline{H}_2CO_2$), 3.12 (t, 2H, ^3J = 7.5 Hz, $C\underline{H}_2(CH_2)_2CO_2$), 4.01 (t, 2H, ^3J = 6.5 Hz, OCH_2), 4.12 (q, 2H, ^3J = 7.1 Hz, $C\underline{H}_2CH_3$), 4.33 (q, 2H, ^3J = 7.1 Hz, $C\underline{H}_2CH_3$), 5.75 (s, 2H, NCH_2), 7.00 (dd, 1H, ^4J = 2.4 Hz, ^3J = 9.0 Hz, H-6), 7.09 (d, 1H, ^4J = 2.2 Hz, H-4), 7.19 (m, 2H, H-7, pyridine-H), 7.32 (m, 1H, pyridine-H), 8.45 (m, 2H, pyridine-H). CI-MS (NH_3) *m/z (%)*: 635 (100) $[M+H]^+$. Anal. ($C_{39}H_{58}N_2O_5 \cdot 0.2H_2O$) C, H, N. $C_{39}H_{58}N_2O_5$ (634.89).

Ethyl 2-ethoxycarbonyl-1-(4-bromobenzyl)-5-(hexadecane-1-yloxy)-1*H*-indole-3-butanoate (8.69): The title compound was prepared according to general procedure 8.5.2.4 using **8.60** (0.5 mmol, 0.27 g), NaH (0.6 mmol, 24 mg) and 4-bromobenzyl bromide (0.6 mmol, 0,15 g) in anhydrous DMF (3 ml) and was obtained after flash chromatography (PE/EtOAc 90/10 v/v) as a white solid (0.23 g, 64 %). mp: 53 °C; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.7 Hz, $CH_2C\underline{H}_3$), 1.04 – 1.53 (m, 30H, $(C\underline{H}_2)_{12}CH_3$, $CH_2C\underline{H}_3$), 1.81 (m, 2H, $OCH_2C\underline{H}_2$), 2.01 (m, 2H, $C\underline{H}_2CH_2CO_2$), 2.37 (t, 2H, ^3J = 7.5 Hz, $C\underline{H}_2CO_2$), 3.11 (t, 2H, ^3J = 7.5 Hz, $C\underline{H}_2(CH_2)_2CO_2$), 4.00 (t, 2H, ^3J =

6.5 Hz, OCH_2), 4.12 (q, 2H, ^3J = 7.1 Hz, $C\underline{H}_2CH_3$), 4.32 (q, 2H, ^3J = 7.1 Hz, $C\underline{H}_2CH_3$), 5.66 (s, 2H, $C\underline{H}_2C_6H_4$), 6.86 (m, 2H, Ar-H), 6.99 (dd, 1H, ^4J =2.4Hz, ^3J =9.0Hz, H-6), 7.08 (d, 1H, ^4J = 2.2 Hz, H-4), 7.15 (d, 1H, ^3J = 9.0 Hz, H-7), 7.35 (m, 2H, Ar-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 772 (100) $[M+CH_3COO]^-$. $C_{40}H_{58}BrNO_5$ (712.80).

2-Carboxy-5-(hexadecan-1-yloxy)-1-propyl-1*H*-indole-3-butanoic acid (8.70): The title compound was prepared according to general procedure 8.5.2.10 using **8.67** (0.18 mmol, 0.11 g) and LiOH (0.54 mmol, 13 mg) in THF (3 ml), MeOH (0.5 ml) and H_2O (1 ml) and was obtained after recrystallization from EtOAc/PE as a white solid (50 mg, 52 %). mp: 61 °C; ^{1}H-NMR ($CDCl_3$) δ 0.89 (m, 6H, $C\underline{H}_3$), 1.35 (m, 26H, $(C\underline{H}_2)_{13}CH_3$), 1.78 (m, 4H, CH_2), 2.01 (m, 2H, CH_2), 2.43 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2CO_2H$), 3.10 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2(CH_2)_2CO_2H$), 4.00 (t, 2H, ^3J = 6.5 Hz, OCH_2), 4.38 (m, 2H, NCH_2), 7.03 (m, 2H, indole-H), 7.26 (m, 1H, indole-H). ^{13}C-NMR ($CDCl_3$) δ 11.38 (+, CH_3), 14.34 (+, CH_3), 22.71 (-, CH_2), 24.01 (-, CH_2), 24.70 (-, CH_2), 25.78 (-, CH_2), 26.18 (-, CH_2), 29.38 (-, CH_2), 29.50 (-, CH_2), 29.65 (-, CH_2), 29.72 (-, CH_2), 31.94 (-, CH_2), 33.56 (-, CH_2), 60.39 (-, NCH_2), 68.68 (-, OCH_2), 101.73 (+, indole-C), 111.31 (+, indole-C), 117.15 (+, indole-C), 123.27 (quat, indole-C), 124.54 (quat, indole-C), 126.77 (quat, indole-C), 133.78 (quat, indole-C), 153.71 (quat, C-5), 162.56 (quat, CO_2H), 179.46 (quat, CO_2H). ES-MS (MeOH + NH_4OAc) *m/z (%)*: 547 (100) $[M+NH_4]^+$. Anal. ($C_{32}H_{51}NO_5$) C, H, N. $C_{32}H_{51}NO_5$ (529.75).

2-Carboxy-5-(hexadecan-1-yloxy)-1-(pyridin-3-ylmethyl)-1*H*-indole-3-butanoic acid hydrochloride (8.71): The title compound was prepared according to general procedure 8.5.2.10 using **8.68** (0.22 mmol, 0.14 g) and LiOH (0.55 mmol, 16 mg) in THF (4 ml), MeOH (0.5 ml) and H_2O (1 ml) and was obtained after recrystallization from EtOAc/PE as a white solid (44 mg, 32 %). mp: 114-116 °C; ^{1}H-NMR ($CDCl_3$+CD_3OD) δ 0.74 (t, 3H, ^3J = 6.0 Hz), 1.09 – 1.40 (m, 26H), 1.68 (m, 2H, $OCH_2C\underline{H}_2$), 1.88 (m, 2H, $C\underline{H}_2CH_2CO_2H$), 2.25 (t, 2H, ^3J = 7.3 Hz, $C\underline{H}_2CO_2H$), 3.03 (m, 2H, $C\underline{H}_2(CH_2)_2CO_2H$), 3.90 (t, 2H, ^3J = 6.4 Hz, OCH_2), 5.69 (d, 2H, ^2J = 11.2 Hz, CH_2Ph), 6.89 (m, 1H, H-6), 7.01 (m, 1H, H-4), 7.13 (m, 1H, H-7), 7.34 (m, 1H, Ar-H), 7.49 (m, 1H, Ar-H), 8.29 (m, 2H, Ar-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 577 (100) $[M-H]^-$. Anal. ($C_{35}H_{51}ClN_2O_5$) C: 68.33, H: 8.36, N: 4.55, found C: 70.94, H: 8.51, N: 4.32. $C_{35}H_{51}ClN_2O_5$ (615.24).

1-(4-Bromobenzyl)-2-carboxy-5-(hexadecan-1yloxy)-1*H*-indole-3-butanoic acid (8.72): The title compound was prepared according to general procedure 8.5.2.10

using **8.69** (0.24 mmol, 0.17 g) and LiOH (0.72 mmol, 17 mg) in THF (4 ml), MeOH (0.5 ml) and H_2O (1 ml) and was obtained after recrystallization from EtOAc/PE as a white solid (64 mg, 41 %). mp: 153-155 °C; ^{1}H-NMR ($CDCl_3$+CD_3OD) δ 0.74 (t, 3H, ^3J = 5.7 Hz, $CH_2C\underline{H}_3$), 1.07 – 1.39 (m, 26H, $(C\underline{H}_2)_{13}CH_3$), 1.67 (m, 2H, $OCH_2C\underline{H}_2$), 1.88 (m, 2H, $C\underline{H}_2CH_2CO_2H$), 2.24 (t, 2H, ^3J = 7.5 Hz, $C\underline{H}_2CO_2H$), 3.03 (t, 2H, ^3J = 7.3 Hz, $C\underline{H}_2(CH_2)_2CO_2H$), 3.88 (t, 2H, ^3J = 6.4 Hz, OCH_2), 5.56 (s, 2H, $C\underline{H}_2C_6H_4$), 6.74 (m, 2H, Ar-H), 6.84 (dd, 1H, ^4J = 2.2 Hz, ^3J = 9.0 Hz, H-6), 6.97 (d, 1H, ^4J = 1.9 Hz, H-4), 7.02 (d, 1H, ^3J = 9.0 Hz, H-7), 7.20 (m, 2H, Ar-H). ^{13}C-NMR ($CDCl_3$+CD_3OD) δ 17.84 (+, CH_3), 26.52 (-, CH_2), 28.45 (-, CH_2), 29.87 (-, CH_2), 29.99 (-, CH_2), 33.21 (-, CH_2), 33.32 (-, CH_2), 33.47 (-, CH_2), 33.50 (-, CH_2), 33.53 (-, CH_2), 35.78 (-, CH_2), 37.58 (-, CH_2), 51.54 (-, $\underline{C}H_2C_6H_4$), 72.74 (-, OCH_2), 106.22 (+, indole-C), 115.30 (+, indole-C), 121.33 (+, indole-C), 124.49 (quat, C-2), 128.76 (quat, indole-C), 129.01 (quat, indole-C), 131.09 (+, Ar-C), 131.83 (+, Ar-C), 135.37 (quat, Ar-C), 138.11 (quat, Ar-C), 141.90 (quat, Ar-C), 157.80 (quat, C-5), 168.17 (quat, CO_2H), 180.42 (quat, CO_2H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 656 (100) $[M-H]^-$. Anal. ($C_{36}H_{50}BrNO_5$) C, H, N. $C_{36}H_{50}BrNO_5$ (656.69).

Ethyl 5-benzyloxy-1-(decan-1-yl)-2-ethoxycarbonyl-1*H*-indole-3-butanoate (8.73): The title compound was prepared according to general procedure 8.5.2.4 using **8.52** (1.5 mmol, 0.61 g), NaH (1.8 mmol, 72 mg) and 1-bromodecane (1.8 mmol, 0.38 ml) in anhydrous DMF (8 ml) and was obtained after flash chromatography (PE/EtOAc 90/10 v/v) as a pale yellow oil (0.55 g, 67 %). ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.7 Hz, $CH_2C\underline{H}_3$), 1.27 (m, 20H, $(C\underline{H}_2)_7CH_3$, $CH_2C\underline{H}_3$), 1.74 (m, 2H, $NCH_2C\underline{H}_2$), 1.98 (m, 2H, $C\underline{H}_2CH_2CO_2$), 2.34 (t, 2H, ^3J = 7.6 Hz, $C\underline{H}_2CO_2$), 3.07 (t, 2H, ^3J = 7.4 Hz, $C\underline{H}_2(CH_2)_2CO_2$), 4.12 (q, 2H, ^3J = 7.1 Hz, $C\underline{H}_2CH_3$), 4.40 (q, 2H, ^3J = 7.1 Hz, $C\underline{H}_2CH_3$), 4.44 (t, 2H, ^3J = 7.5 Hz, NCH_2), 5.12 (s, 2H, $C\underline{H}_2Ph$), 7.09 (dd, 1H, ^3J = 2.4 Hz, ^3J = 9.0 Hz, indole-H), 7.15 (d, 1H, ^3J = 2.2 Hz, indole-H), 7.30 – 7.43 (m, 4H, indole-H, Ph-H), 7.49 (m, 2H, Ph-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 551 (100) $[M+H]^+$. Anal. ($C_{34}H_{47}NO_5$) C, H, N. $C_{34}H_{47}NO_5$ (549.74).

5-Benzyloxy-1-(decan-1-yl)-2-ethoxycarbonyl-1*H*-indole-3-butanoic acid (8.74): The title compound was prepared according to general procedure 8.5.2.10 using **8.73** (0.89 mmol, 0.49 g) and LiOH (2.67 mmol, 64 mg) in THF (8 ml), and H_2O (2 ml) and was obtained as a pale yellow solid (0.43 g, 93 %). ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.7 Hz, $CH_2C\underline{H}_3$), 1.27 (m, 14H, $(C\underline{H}_2)_7CH_3$), 1.42 (t, 3H, ^3J = 7.1 Hz, $CH_2C\underline{H}_3$), 1.74 (m, 2H, $NCH_2C\underline{H}_2$), 1.98 (m, 2H, $C\underline{H}_2CH_2CO_2H$), 2.39 (t, 2H, ^3J = 7.3 Hz,

C$\underline{H}_2$CO$_2$H), 3.09 (t, 2H, ^3J = 7.4 Hz, C$\underline{H}_2$(CH$_2$)$_2$CO$_2$H), 4.39 (q, 2H, ^3J = 7.2 Hz, C$\underline{H}_2$CH$_3$), 4.44 (m, 2H, ^3J = 7.6 Hz, NCH$_2$), 5.10 (s, 2H, C$\underline{H}_2$Ph), 7.10 (dd, 1H, ^4J = 2.4 Hz, ^3J = 9.0 Hz, indole-H), 7.14 (d, 1H, ^4J = 2.2 Hz, indole-H), 7.25 – 7.40 (m, 4H, indole-H, Ph-H), 7.48 (m, 2H, Ph-H), 10.87 (bs, 1H, CO$_2$H). ^{13}C-NMR (CDCl$_3$) δ 14.13 (+, CH$_3$), 14.35 (+, CH$_3$), 22.69 (-, CH$_2$), 24.68 (-, CH$_2$), 25.73 (-, CH$_2$), 27.05 (-, CH$_2$), 29.31 (-, CH$_2$), 29.41 (-, CH$_2$), 29.58 (-, CH$_2$), 30.81 (-, CH$_2$), 31.89 (-, CH$_2$), 33.52 (-, CH$_2$), 60.43 (-, O$\underline{C}$H$_2$CH$_3$), 70.73 (-, $\underline{C}$H$_2$Ph), 102.37 (+, C-4), 111.39 (+, indole-C), 117.27 (+, indole-C), 123.34 (quat, C-3), 124.60 (quat, C-2), 126.76 (quat, indole-C), 127.69 (+, Ph-C), 127.87 (+, Ph-C), 128.53 (+, Ph-C), 133.83 (quat, indole-C), 137.37 (quat, Ph-C), 153.35 (quat, C-5), 162.51 (quat, $\underline{C}$O$_2$Et), 179.64 (quat, CO$_2$H). ES-MS (DCM/MeOH + NH$_4$OAc) *m/z (%)*: 522 (100) [M+H]$^+$. Anal. (C$_{32}$H$_{43}$NO$_5$) C, H, N. C$_{32}$H$_{43}$NO$_5$ (521.69).

5-Benzyloxy-3-(3-carboxypropyl)-1-(decan-1-yl)-1*H*-indole-2-carboxylic acid (8.75): A solution of **8.73** (1 mmol, 0.55 g) and KOH (10 mmol, 0.56 g) in ethanol (10 ml) was refluxed for 45 min. After cooling, the diacid which precipitated was isolated by filtration and resuspended in 2 N HCl (10 ml). The mixture was stirred for 2 hours, and then extracted tree times with EtOAc. The combined organic layers were washed with brine, dried over MgSO$_4$, filtered and the solvent was evaporated to give the title compound as a white solid (0.37 g, 75 %). ^{1}H-NMR (CDCl$_3$) δ 0.86 (t, 3H, ^3J = 6.7 Hz, CH$_2$C$\underline{H}_3$), 1.28 (m, 14H, (C$\underline{H}_2$)$_7$CH$_3$), 1.77 (m, 2H, NCH$_2$C$\underline{H}_2$), 2.04 (m, 2H, C$\underline{H}_2$CH$_2$CO$_2$H), 2.39 (t, 2H, ^3J = 7.4 Hz, C$\underline{H}_2$CO$_2$H), 3.29 (t, 2H, ^3J = 7.1 Hz, C$\underline{H}_2$(CH$_2$)$_2$CO$_2$H), 4.47 (t, 2H, ^3J = 7.4 Hz, NCH$_2$), 5.13 (s, 2H, C$\underline{H}_2$Ph), 7.15 (m, 2H, indole-H), 7.25 – 7.51 (m, 6H, indole-H, Ph-H), 12.59 (s, 2H, CO$_2$H). ES-MS (DCM/MeOH + NH$_4$OAc) *m/z (%)*: 494 (100) [M+H]$^+$. Anal. (C$_{30}$H$_{39}$NO$_5$) C, H, N. C$_{30}$H$_{39}$NO$_5$ (493.63).

1-(Decan-1-yl)-2-ethoxycarbonyl-5-hydroxy-1*H*-indole-3-butanoic acid (8.76): The title compound was prepared according to general procedure 8.5.2.5 using **8.74** (0.38 mmol, 0.20 g) and 10 % Pd/C (50 mg) in EtOH (3 ml) and EtOAc (3 ml) and was obtained as a pale grey solid (0.30 g, 95 %). ^{1}H-NMR (CDCl$_3$) δ 0.87 (t, 3H, ^3J = 6.6 Hz, CH$_2$C$\underline{H}_3$), 1.25 (m, 14H, (C$\underline{H}_2$)$_7$CH$_3$), 1.40 (t, 3H, ^3J = 8.3 Hz, CH$_2$C$\underline{H}_3$), 1.71 (m, 2H, NCH$_2$C$\underline{H}_2$), 1.96 (m, 2H, C$\underline{H}_2$CH$_2$CO$_2$H), 2.39 (t, 2H, ^3J = 6.9 Hz, C$\underline{H}_2$CO$_2$H), 3.04 (t, 2H, ^3J = 7.4 Hz, C$\underline{H}_2$(CH$_2$)$_2$CO$_2$H), 4.38 (m, 4H, C$\underline{H}_2$CH$_3$, NCH$_2$), 6.96 (dd, 1H, ^4J = 2.2 Hz, ^3J = 8.9 Hz, H-6), 7.08 (d, 1H, ^4J = 2.0 Hz, H-4), 7.20 (d, 1H, ^3J = 8.9

Hz, H-7). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 432 (100) $[M+H]^+$. Anal. ($C_{25}H_{37}NO_5$) C, H, N. $C_{25}H_{37}NO_5$ (431.56).

2-Carboxy-1-(decan-1-yl)-5-hydroxy-1*H*-indole-3-butanoic acid (77): The title compound was prepared according to general procedure 8.5.2.5 using **8.75** (0.13 mmol, 64 mg) and 10 % Pd/C (20 mg) in EtOH (2 ml) and EtOAc (2 ml) and was obtained as a grey semisolid (50 mg, 95 %). ^{1}H-NMR ($CDCl_3$+ CD_3OD) δ 0.84 (t, 3H, ^3J = 6.7 Hz, $CH_2C\underline{H}_3$), 1.23 (m, 14H, $(C\underline{H}_2)_7CH_3$), 1.69 (m, 2H, $NCH_2C\underline{H}_2$), 1.94 (m, 2H, $C\underline{H}_2CH_2CO_2H$), 2.31 (t, 2H, ^3J = 7.5 Hz, $C\underline{H}_2CO_2H$), 3.06 (t, 2H, ^3J = 7.5 Hz, $C\underline{H}_2(CH_2)_2CO_2H$), 4.42 (t, 2H, ^3J = 7.3 Hz, NCH_2), 6.90 (m, 1H, H-6), 7.01 (d, 1H, ^4J = 2.3 Hz, H-4), 7.19 (dd, 1H, ^4J = 2.6 Hz, ^3J = 8.9 Hz, H-7). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 404 (100) $[M+H]^+$. $C_{23}H_{33}NO_5$ (403.51).

***N*-[2-Deoxy-D-glucopyranose-2-yl]-1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indole-3-acetamide (8.78)[20]**: To a solution of $SOCl_2$ (1.05 mmol, 76 µl) in anhydrous CH_2Cl_2 (5 ml) was added a solution of DMF (1.05 mmol, 77 76 µl) in anhydrous CH_2Cl_2 (1 ml) and the mixture was stirred for 10 min at room temperature. The solution then was added to a suspension of indomethacin (1 mmol, 0.36 g) in anhydrous CH_2Cl_2 (2 ml). After evaporation of the solvent, the oily residue was treated with ether to get a pale yellow solid which was dried under reduced pressure. The crude acid chloride was then dissolved in anhydrous CH_2Cl_2 (2 ml) and was added dropwise to a solution of D-glucosamine hydrochloride (1 mmol, 0.22 g) and NEt_3 (2 mmol, 0.28 ml) in methanol (10 ml) at 0 °C. After 10 min the resulting solid was isolated by filtration and dried under reduced pressure to obtain the title compound as pale yellow solid (0.32 g, 61 %). ^{1}H-NMR (DMSO-d_6) δ 2.22 (s, 3H, CH_3), 3.06 (m, 2H, H-3, H-6), 3.48 (m, 1H, H-6), 3.59 (m, 6H, CH_2CO, H-2, H-4, H-5, H-6), 3.76 (s, 3H, OCH_3), 4.44 (t, 1H, ^3J = 5.8 Hz, 6-OH), 4.66 (d, 1H, ^3J = 5.3 Hz, 4-OH), 4.94 (m, 2H, H-1, 3-OH), 6.47 (d, 1H, ^3J = 4.2 Hz, 1-OH), 6.68 (dd, 1H, ^4J = 2.5 Hz, ^3J = 9.0 Hz, Ar-H), 6.93 (d, 1H, ^3J = 8.9 Hz, Ar-H), 7.17 (d, 1H, ^4J = 2.4 Hz, Ar-H), 7.66 (m, 4H, Ar-H), 7.99 (d, 1H, ^3J = 7.4 Hz, NH). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 519 (100) $[M+H]^+$. $C_{25}H_{27}ClN_2O_8$ (518.94).

8.6 *References*

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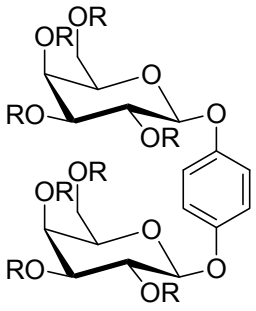
Chapter 9
Miscellaneous compounds as potential hyaluronidase inhibitors

9.1 *Introduction*

Previous studies led to the identification of sulfated carbohydrates as inhibitors of bacterial and bovine hyaluronidase[1, 2]. The sulfated hydroquinone digalactoside **9.1** (Figure 9.1) was among inhibitors with potencies in the lower micromolar range on BTH, BVH and *Sag*Hyal$_{4755}$. A major disadvantage of such sulfated carbohydrates is the lack of information on the exact structure. Mixtures of sulfated compounds were used with a mean degree of sulfation (DS) of 0.81. For SAR considerations information about the position of the sulfate groups is essential. As a starting point for the synthesis of sulfated and non-sulfated carbohydrates as potential hyaluronidase inhibitors, the synthesis and inhibitory activity of a non-sulfated carbohydrate connected *via* a hydroquinone linker is described in this chapter.



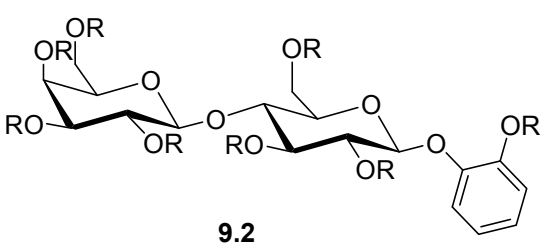
9.1

R = H or SO_3Na
DS = 0.81

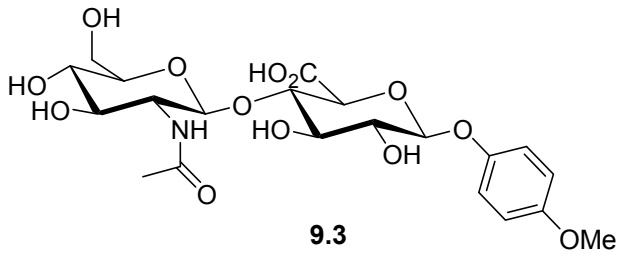
	IC_{50} [µM]
BTH	40 ± 5
BVH	25 ± 2.5
*Sag*Hyal$_{4755}$	630 ± 70

Figure 9.1. Structure and inhibitory activity of **9.1** as determined by Salmen[1, 2] using the colorimetric Morgan Elson assay. IC_{50} values determined at pH 3.6 (BTH and BVH) or 5.0 (*Sag*Hyal$_{4755}$).

Salmen also demonstrated the inhibitory activity of a sulfated disaccharide (**9.2**) bearing a phenyl residue at the reducing end (Figure 9.2). Following a substrate analog approach, the synthesis of a hyaluronan disaccharide fragment bearing a hydrophobic methoxyphenyl substituent (**9.3**) was proposed and is presented here.



9.2



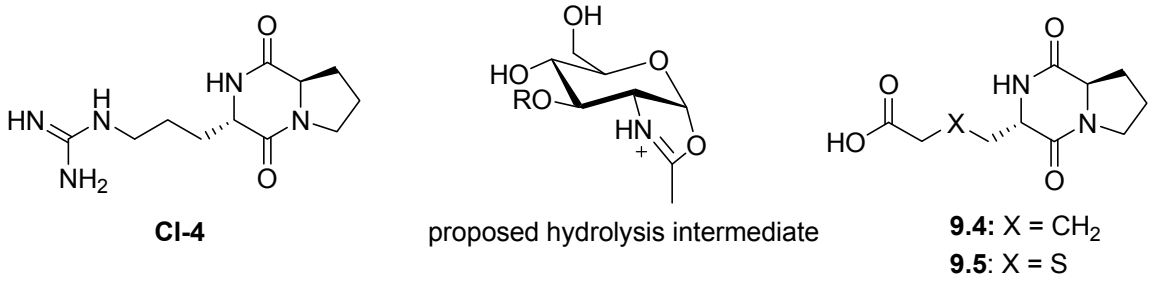
9.3

R = H or SO_3Na
DS = 0.67

	IC_{50} [µM]
BTH	25 ± 3
*Sag*Hyal$_{4755}$	350 ± 15

Figure 9.2. Structure and inhibitory activity of **9.2** as determined by Salmen[1, 2] and structure of **9.3**. IC_{50} values determined at pH 3.6 (BTH and BVH) or 5.0 (*Sag*Hyal$_{4755}$) in the Morgan Elson Assay.

Based on theoretical considerations Botzki suggested diketopiperazines as potential inhibitors of mammalian hyaluronidases[3]. Diketopiperazines were recently investigated as inhibitors of chitinase[4, 5] which belong to a class of enzymes related to the hyaluronidases[6]. Thus, compounds like **CI-4** are suggested to mimic a hydrolysis intermediate (Figure 9.3). Based on the superposition of **CI-4** and a hyaluronic acid disaccharide fragment using a model of BTH[3], **9.4** was suggested as an inhibitor of mammalian hyaluronidases. The synthesis and inhibitory activity of the thio analog **9.5**, which was chosen for the synthesis due to economic reasons, is presented in this chapter.



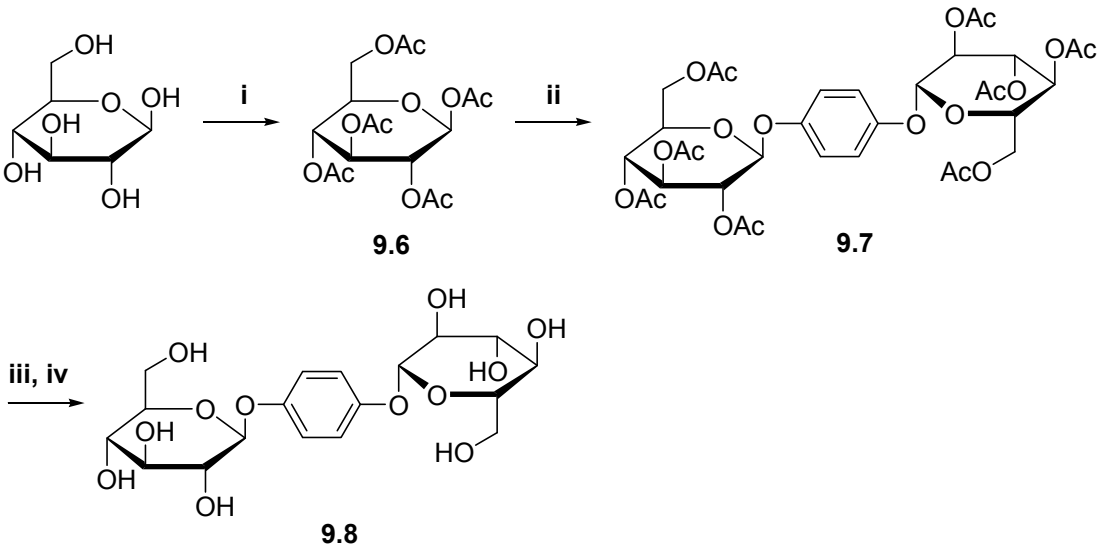
CI-4 proposed hydrolysis intermediate **9.4:** X = CH_2 **9.5**: X = S

Figure 9.3. Structures of the chitinase inhibitors **CI-4**, the proposed hydrolysis intermediate of *N*-acetylglucosamine residue, the derived potential BTH inhibitor **9.4** and the synthesized analog **9.5**.

Furthermore, the investigation of several additional compounds for inhibition of hyaluronidases is presented in this chapter. Due to structural similarities with the synthesized ascorbic acid derivatives described in chapter 8, melophlins, a class of *N*-methyl-3-acyltetramic acids that possess biological effects such as general cytotoxic, antiproliferative, antifungal, antibacterial, and antiviral activity[7], were investigated for hyaluronidase inhibition. Additionally, a selection of alkylphosphocholines which were previously broadly studied due to their antitumor activities[8-12] were included in our screening for hyaluronidase inhibitors as those substances contain long hydrophobic structural elements, a common motive of several hyaluronidase inhibitors presented in chapters 6-8.

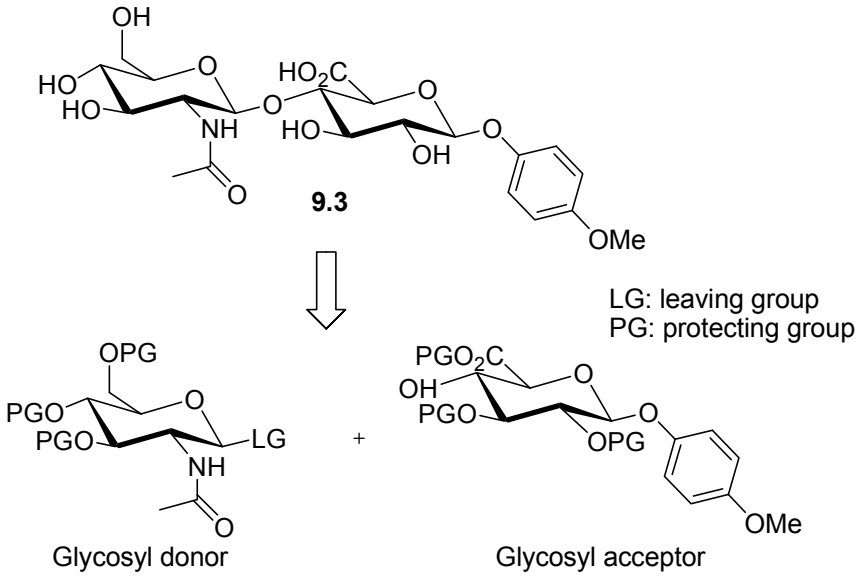
9.2 *Synthesis*

The glucopyranoside **9.8** was synthesized according to Scheme 9.1 starting from glucose, which was peracetylated[13] und subsequently treated with hydroquinone in the presence of TMSTf[14] to obtain the fully acetylated glycoside **9.7** which was deprotected using catalytic amounts of NaOMe[15].

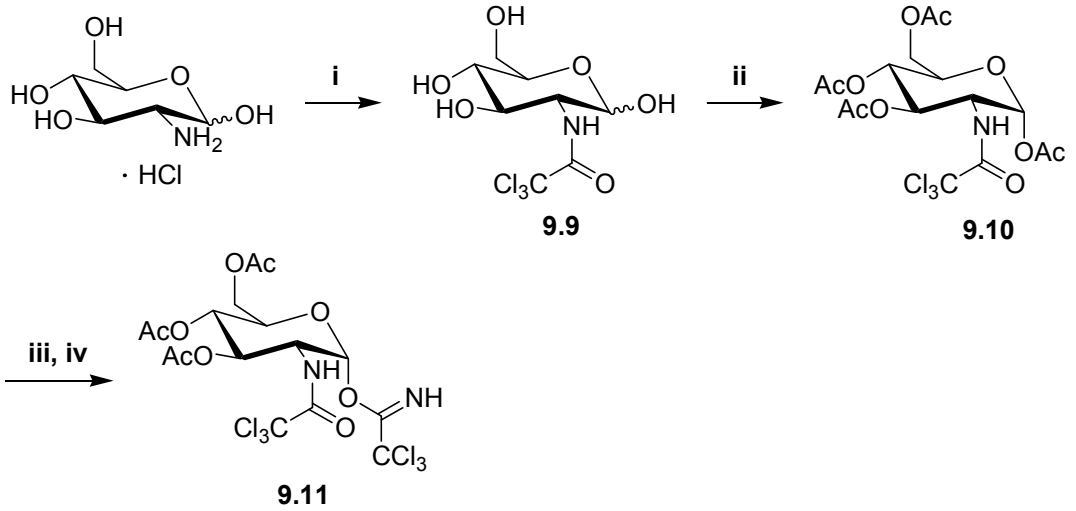


Scheme 9.1. Synthesis of **9.8**.

For the synthesis of **9.3**, which contains a hyaluronic acid disaccharide, two building blocks had to be prepared, the glycosyldonor and –acceptor (Scheme 9.2). TMSTf catalyzed reaction of a trichloroacetimidate donor was chosen for the formation of the glycosidic bond to obtain high β-selectivity. As the neighboring *N*-acetyl group of the glycosyl donor is incompatible with that method[16], the N-acetyl moiety had to be introduced after the glycosylation reaction. The preparation of the glycosyl donor **9.12** according to Blattner et al.[17] is depicted in Scheme 9.3.



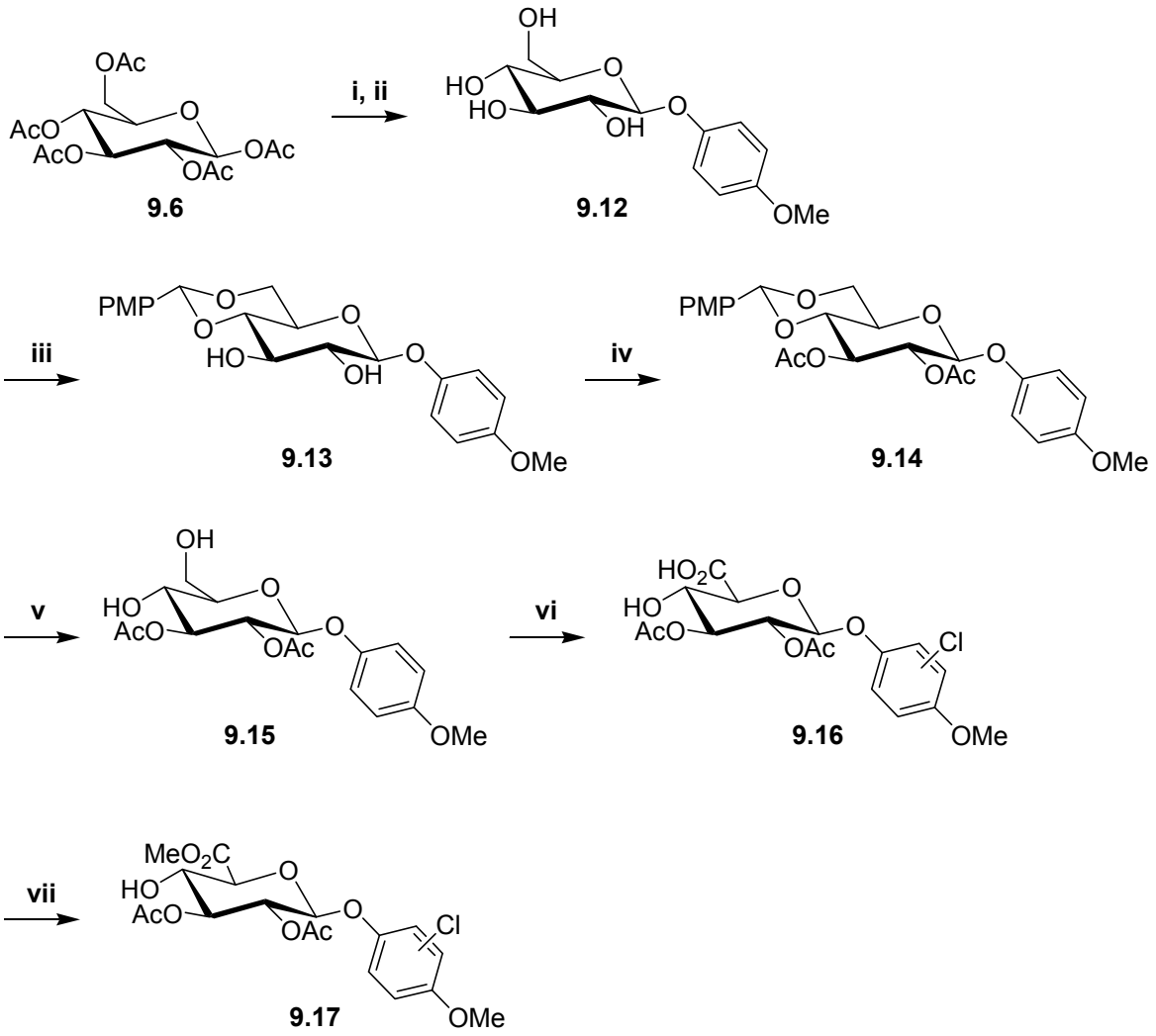
Scheme 9.2. Retrosynthetic approach for the synthesis of disaccharide **9.3**.



Scheme 9.3. Preparation of glycosyl donor **9.11**. Reagents and conditions: (i) $NaHCO_3$ (3 eq), Cl_3COCl (1.5 eq), H_2O, RT, 1 h; (ii) Ac_2O, pyridine, RT overnight; (iii) Hydrazine acetate (1.5 eq), DMF, RT, 20 min; (iv) CCl_3CN (10 eq), DBU (0.25 eq), RT, 30 min.

In the first step the nitrogen of D-glucosamine was acylated using trichloroacetyl chloride followed by acylation of the hydroxyl residues to form **9.10**. Afterwards, selective cleavage of the anomeric acetyl group was afforded using hydrazine acetate. The crude product was used in the next step without further purification to obtain the acetimidate **9.11** *via* reaction of the carbohydrate with trichloroacetonitrile in presence of DBU.

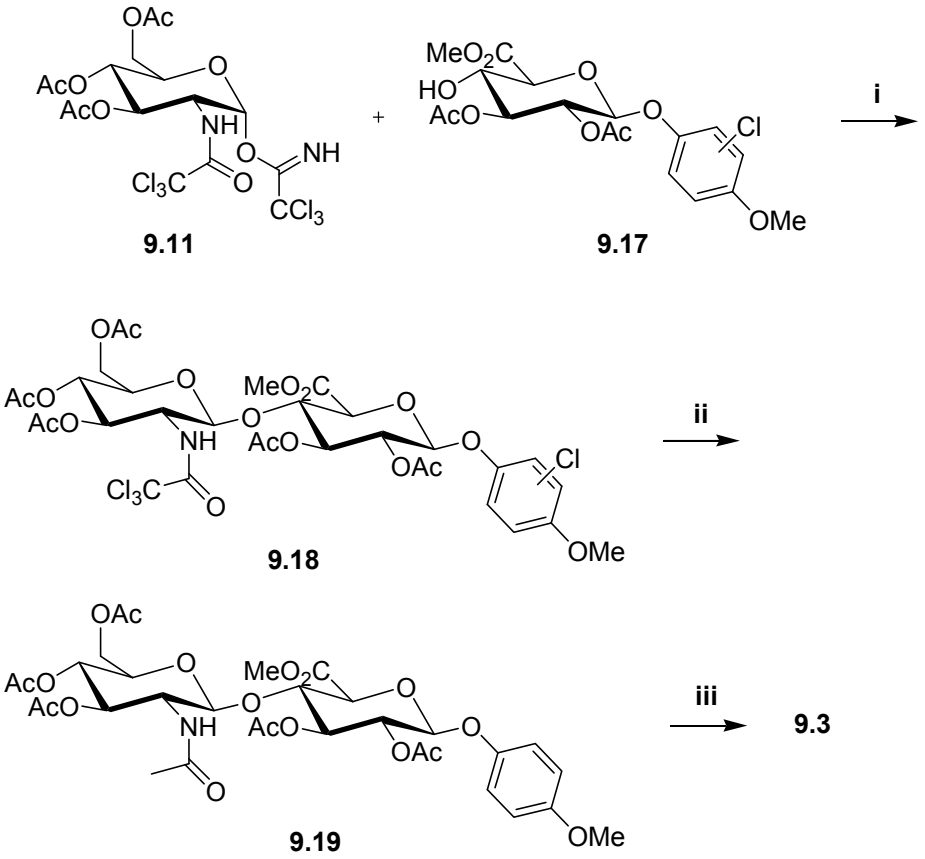
The glycosyl donor **9.17** was synthesized by analogy with the method described by Rye and Withers[18] with minor modifications (Scheme 9.4). After alkylation of **9.6** using *p*-methoxyphenol and catalytic amounts of trifluorosulfonic acid[19] and subsequent cleavage of acetyl groups to form **9.12**, anisaldehyde dimethyl acetal in the presence of *p*-toluenesulfonic acid was used to protect the 4-OH and 6-OH of the carbohydrate scaffold (**9.13**)[18]. Acetylation of the free 2-OH and 3-OH followed by removal of the acetal under acidic conditions[20] yielded **9.15** which was oxidized using NaOCl and catalytic amounts of TEMPO under phase transfer conditions[18] to get the carboxylic acid **9.16**. Although no side reactions occurred when this reaction was performed on a small scale, the aromatic core was variously halogenated when the reaction was performed on a larger scale. As the various halogenated products could not be separated and a dehalogenation reaction was already planned in a later step to prepare the *N*-acetyl moiety, the reactions were carried on using the partly purified mixture of halogenated derivatives.



Scheme 9.4. Synthesis of the glycoside acceptor **9.17**. Reagents and conditions: (i) *p*-Methoxyphenol (2 eq), CF_3SO_3H (0.16 eq), CH_2Cl_2, 0 °C, 6 h; (ii) NaOMe (cat.), MeOH, RT overnight; (iii) *p*-Anisaldehyde dimethyl acetal (1.5 eq), TsOH (0.02 eq), DMF, 50-60 °C, 20 mbar, 4.5 h; (iv) Ac_2O, pyridine, RT, 1.5 h; (v) HOAc (aq), 70 °C overnight; (vi) TEMPO (cat.) NaOCl, TBAB (cat.), EtOAc, sat. $NaHCO_3$, sat NaCl, NaBr, O °C, 1h; (vii) Acidic ion exchanger, MeOH, RT overnight.

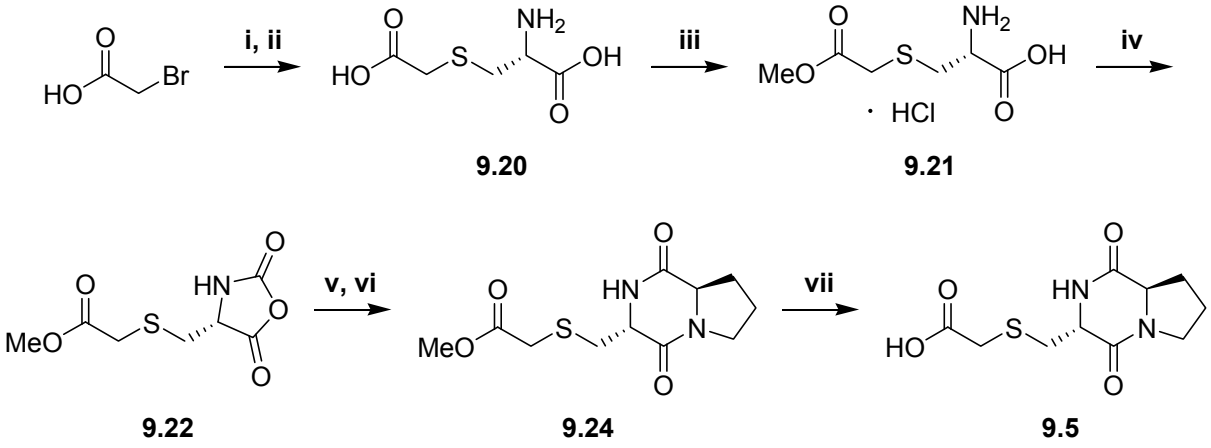
To protect the carboxylic acid residue, a methyl ester was introduced utilizing an acidic ion exchanger in methanol[18] to obtain the glycosyl acceptor **9.17**.

The two building blocks **9.11** and **9.17** were finally coupled to form the protected disaccharide **9.17** in the presence of catalytic amounts of TMSTf (Scheme 9.5). Hydrogenolytic dehalogenation of **9.18** was successfully performed using palladium on activated carbon in the presence of sodium acetate[21]. Finally, compound **9.3** was obtained after removal of the protecting groups under aqueous basic conditions.



Scheme 9.5. Synthesis of **9.3** starting from the building blocks **9.11** and **9.17**. Reagents and conditions: (i) TMSTf (0.2 eq), molsieves, 1,2-dichloroethane, 0 °C, 4.5 h; (ii) 10 % Pd/C (cat.), NaOAc (4 eq), H_2 (6 atm), THF, RT, 72 h; (iii) 3 N NaOH (30 eq), MeOH / H_2O, RT, 6 h, then HOAc.

The synthesis of the diketopiperazine **9.5** is depicted in Scheme 9.6. Starting from bromoacetic acid, L-cysteine was used for the alkylation[22] to obtain **9.20** which was selectively esterified using TMSCl in methanol[23]. After treating with triphosgene[24] the obtained *N*-carboxyanhydride **9.22** was allowed to react with D-proline methyl ester (**9.23**)[25] according to the synthetic procedure described by Schöllkopf[26]. Basic hydrolysis of the methyl ester **9.24** yielded too much side products, therefore **9.5** was prepared by enzymatic cleavage of the ester moiety using porcine liver esterase (EC 3.1.1.1) in buffered solution[27].



Scheme 9.6. Syntesis of diketopiperazine **9.5**. Reagents and conditions: (i) L-Cysteine (1.2 eq), H_2O, 7 N NaOH, 1 h, RT; (ii) HOAc; (iii) TMSCl (1.8 eq), MeOH, 6 min, RT; (iv) Triphosgene (0.33 eq), THF, 50 °C, 3 h; (v) Proline methyl ester hydrochloride (**9.23**, 1 eq), NEt_3 (2.25 eq), CH_2Cl_2, -78 °C, 30 min; (vi) Toluene, reflux; (vii) Porcine liver esterase (EC 3.1.1.1), acetone / phosphate buffer (pH 8.0), RT overnight.

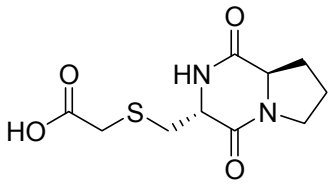
9.3 *Inhibition of hyaluronidases: results and discussion*

9.3.1 Compounds with carbohydrate or peptidic scaffolds

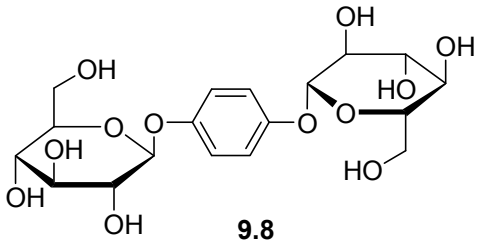
All synthesized L-ascorbic acid derivatives were investigated for inhibition of recombinant human hyaluronidases PH-20 and Hyal-1, the bovine testicular enzyme BTH (Neopermease®) and the bacterial hyaluronan lyase *Sag*Hyal$_{4755}$ in a modified turbidimetric assay based on the method of Di Ferrante[28] as described in chapter 3.
The IC_{50} values of the synthesized compounds are summarized in Table 9.1. Significant inhibition was only found for **9.8** when investigated on human PH-20 and the bacterial hyaluronate lyase with IC_{50} values of 336 µM and 1608 µM, respectively (see Figure 9.4 for the dose dependent inhibition). Compared to the sulfated glycoside **9.1** which inhibits BTH and *Sag*Hyal$_{4755}$ with IC_{50} values in the lower micromolar range (see chapter 9.1), there is an enormous decrease in potency. Similarly, the hyaluronic acid fragment **9.3** turned out to be inactive, whereas the IC_{50} values determined for the sulfated disaccharide **9.2** are in the micromolar range. These results again underline the importance of the strongly acidic hydrogensulfate groups for the inhibition of the hyaluronidases. In chapter 6-8 it was demonstrated for different classes of compounds that the hydrophobic aryl or alkyl motive which is generally required to achieve reasonable inhibitory potency should have at least the

dimension of an octyl residue. Probably, the hydrophobicity of the synthesized disaccharide **9.3** bearing a methoxyphenyl residue is too low. Possibly, inhibition could also be achieved by extension of the disaccharide to a tetra- or even hexasaccharide to improve the binding of the compound to the hyaluronidases. Nevertheless, the synthetic effort to obtain such oligosaccharides is very high and not appropriate to produce substance libraries for inhibitor screening. Therefore, this carbohydrate based approach was discontinued.

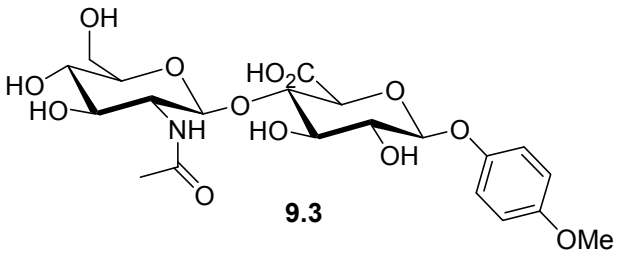
Table 9.1. Inhibitory activity of compounds **9.3**, **9.5** and **9.8**.



9.5



9.8



9.3

Compound	Hyal-1	PH-20	BTH	*Sag*Hyal$_{4755}$
	IC$_{50}$ [µM][a]			
9.3	> 3000	> 3000	> 3000	> 3000
9.5	> 3000	> 3000	> 3000	> 3000
9.8	> 6000	336 ± 75	> 6000	1608 ± 60

[a] Inhibition of enzyme expressed as IC$_{50}$ (µM), mean values ± SEM (N = 2, experiments performed in duplicate); IC$_{50}$ values determined at pH 5.0 (PH-20, BTH, *Sag*Hyal$_{4755}$) or 3.5 (Hyal-1) in the turbidimetric assay.

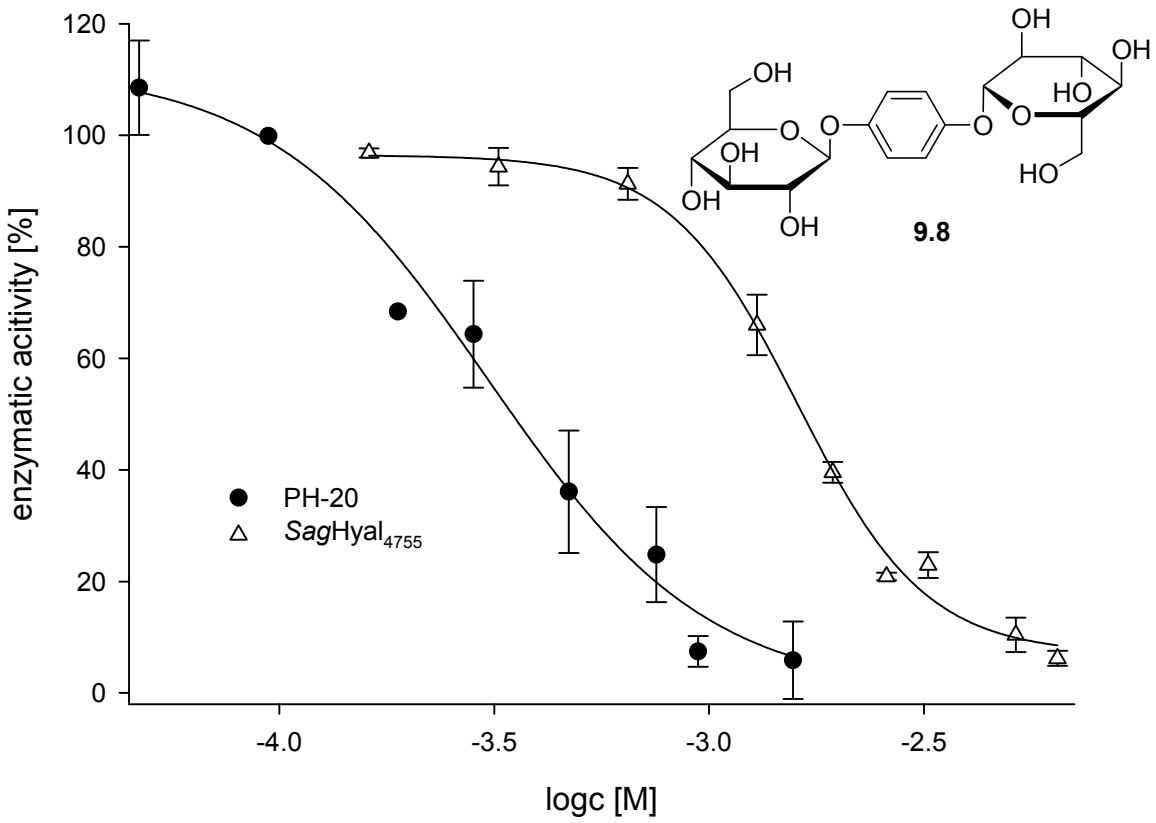


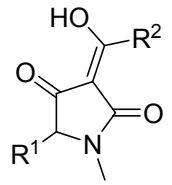
Figure 9.4. Activity of human PH-20 and *Sag*Hyal$_{4755}$ in the presence of **9.8**.

In contrast to previous suggestions based on theoretical considerations the synthesized diketopiperazine **9.5** does not inhibit the hyaluronidases in the turbidimetric assay. It remains unclear, if structural differences between hyaluronidases and chitinases or shortcomings of the BTH model account for that negative result. As a consequence the peptide based approach was discontinued.

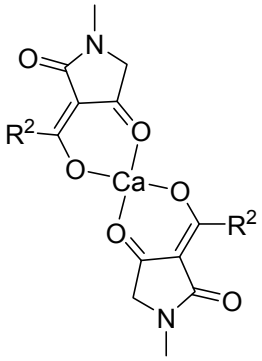
9.3.2 Melophlins and related structures

The melophlins and related compounds were kindly provided by Prof. Dr. R. Schobert (Institute of Organic Chemistry, University of Bayreuth). The IC_{50} values determined in the turbidimetric assay (chapter 3) are summarized in Table 9.3, substitution patterns are given in Table 9.2.

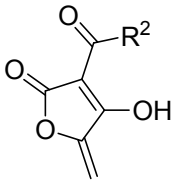
Table 9.2. Substitution patterns of investigated melophlin and agglomerin derivatives.



Melophlin
A, B, C, G, P, Q



$Ca(Mel\text{-}A)_2$



Agglomerin B

Compound	R^1	R^2
Melophlin A	H	$(CH_2)_{14}CH_3$
Melophlin B	*(S)*-CH_3	$(CH_2)_2CHMe(CH_2)_7CH_3$
Melophlin C	*(S,R)*-CH_3	$(CH_2)_3CHMe(CH_2)_6CH_3$
Melophlin G	H	$(CH_2)_{12}CH_3$
Melophlin P	*(S,R)*-CH_3	$(CH_2)_{14}CH_3$
Melophlin Q	*(S,R)*-CH_3	$(CH_2)_{11}CH(CH_3)_2$
Melophlin R	*(S,R)*-CH_3	$(CH_2)_{10}CH(CH_3)(CH_2CH_3)$
$Ca(Mel\text{-}A)_2$	-	$(CH_2)_{14}CH_3$
Agglomerin B	-	$(CH_2)_3CH{=}CH(CH_2)_5CH_3$

Table 9.3. Inhibitory activity of melophlin and agglomerin derivatives on hyaluronidases.

Compound	Hyal-1	PH-20	BTH	*Sag*Hyal$_{4755}$
	IC_{50} [µM][a]			
Melophlin A	> 100	2.6 ± 0.2	> 100	6.6 ± 3
Melophlin B	> 200	15 ± 1	> 200	75 ± 7
Melophlin C	> 200	12 ± 1	> 200	33 ± 2
Melophlin G	> 200	6.9 ± 0.2	> 200	14 ± 1
Melophlin P	> 100	2.3 ± 0.1	> 100	9.0 ± 1.0
Melophlin Q	> 200	177 ± 17	> 250	> 250
Melophlin R	> 200	8.2 ± 0.4	> 200	18 ± 1
$Ca(Mel\text{-}A)_2$	> 100	1.9 ± 0.1	> 100	5.0 ± 0.2
Agglomerin B	> 100	> 50	> 100	> 100

[a] inhibition of enzyme expressed as IC_{50} (µM), mean values ± SEM (N = 2, experiments performed in duplicate); IC_{50} values determined at pH 5.0 (PH-20, BTH, *Sag*Hyal$_{4755}$) or 3.5 (Hyal-1) in the turbidimetric assay.

Obviously, none of the investigated compounds inhibits human Hyal-1 and BTH, whereas several compounds show inhibition of human PH-20 and the bacterial hyaluronate lyase. Strongest inhibition of PH-20 was found for monomeric, pentadecyl substituted melophlins A and P with IC_{50} values around 2 µM. The additional methyl group in melophlin P does not lead to noteworthy changes in

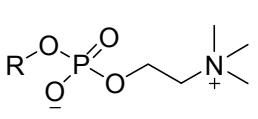
potency. The dimeric calcium complex Ca(Mel-A)$_2$ possesses even increased potency, but the stability of this complex under the aqueous assay conditions is questionable. Thus, by dissociation, the lower IC_{50} value could possibly be the result of an increased concentration of free monomer in solution. Similar results were obtained for *Sag*Hyal$_{4755}$, albeit IC_{50} values are higher. A drastic drop in potency is observed for melophlin Q when compared to melophlin P: a comparable overall length but an additional terminal branching leads to 77 times lower potency on PH-20 and a complete loss of activity against *Sag*Hyal$_{4755}$. However, the inhibitory activity against both enzymes is regained, when the branching is moved away from the chain terminus (melophlin R). Compounds with shorter alkyl chains are significantly less potent hyaluronidase inhibitors (compare melophlins A and G). An additional methyl residue situated at different positions of the alkyl chain does contribute to increased potency (melophlins B and C), but it should be considered that the alkyl residues in these derivatives are shorter than that in melophlin P, and do not fulfill the structural requirements to give potent inhibitors as shown in chapters 6-8. The different position of the methyl residue causes only minor changes, albeit melophlin C is more potent than melophlin B, especially when the bacterial enzyme is regarded. Interestingly, none of the four investigated hyaluronidases is inhibited by agglomerin B. This cannot be explained on the basis of available data, but might be associated with the differences in the heterocyclic part of the molecule, or depend on the double bond.

9.3.3 Alkylphosphocholines

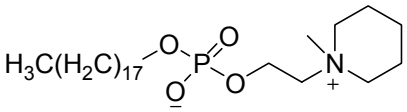
A selection of alkylphosphocholines (Table 9.4) was investigated for hyaluronidase inhibition in the turbidimetric assay (chapter 3). The obtained IC_{50} values are presented in Table 9.4. No inhibition of Hyal-1 and BTH was observed for the phosphocholines in the investigated concentration range, whereas PH-20 and the bacterial hyaluronate lyase were inhibited in a concentration-dependent manner and with similar SAR: Comparing miltefosine (**9.25**) with the derivative bearing the longer octadecyl sidechain (**9.26**) no significant change in potencies is obvious when tested upon PH-20 and *Sag*Hyal$_{4755}$ inhibition. This result is in agreement with observations made for indole-type hyaluronidase inhibitors (chapter 8) where no further increase in inhibition occurs if a certain length of the hydrophobic motive is exceeded.

Replacement of the trimethylammonium residue with an *N*-methyl-piperidinium moiety results in a significant increase in potency.

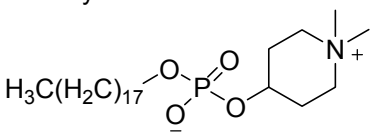
Table 9.4. Structures and inhibitory activity of alkylphosphocholines on hyaluronidases.



9.25: R=$(CH_2)_{15}CH_3$
9.26: R=$(CH_2)_{17}CH_3$



9.27



9.28

Compound	Hyal-1	PH-20	BTH	*Sag*Hyal$_{4755}$
	IC_{50} [µM][a]			
9.25	> 200	6.8 ± 0.8	> 200	1.6 ± 0.3
9.26	> 200	6.5 ± 0.5	> 200	1.9 ± 0.1
9.27	> 200	4.0 ± 0.3	> 200	0.88 ± 0.1
9.28	> 200	7.5 ± 1.1	> 200	1.5 ± 0.2

[a] inhibition of enzyme expressed as IC_{50} (µM), mean values ± SEM (N = 2, experiments performed in duplicate); IC_{50} values determined at pH 5.0 (PH-20, BTH, *Sag*Hyal$_{4755}$) or 3.5 (Hyal-1) in the turbidimetric assay.

For compound **9.27** IC_{50} values of 4.0 µM and 0.88 µM were determined for human PH-20 and *Sag*Hyal$_{4755}$, thus this derivative represents the most potent inhibitor of the bacterial enzyme in this work. Perifosine (**9.28**) is a weaker hyaluronidase inhibitor than **9.27**, the IC_{50} value is comparable to that of **9.26** which has the same octadecyl chain but different substitution in the charged ammonium part of the molecule. The phosphocholines have a significant preference for the bacterial enzyme over the human PH-20. This is in contrast to the melophlines which turned out to slightly prefer the human enzyme.

9.4 *Summary*

In this chapter several approaches for the design and synthesis of hyaluronidase inhibitors as well as the investigation of compounds provided by other groups are described. In a first effort carbohydrate based molecules were synthesized by analogy with the sulfated inhibitors described by Salmen[2]. Unfortunately, the nonsulfated derivatives did not or only very weakly inhibit the investigated mammalian and bacterial hyaluronidases, thus confirming the conclusion that the sulfate groups of the previously published compounds are key to high affinity. A second approach based on a homology model of bovine testicular hyaluronidsase led

to the proposal of a peptidic transition state mimic as potential inhibitor of mammalian hyaluronidase[3]. As the synthesized diketopiperazine did not meet the predictions in terms of hyaluronidase inhibitory activity this approach was discontinued.

Some aza-analogs of ascorbic acid, so-called melophlins, which are structurally related to the synthesized vitamin C based inhibitors, were investigated for hyaluronidase inhibition. These compounds proved to be rather potent inhibitors of human PH-20 and bacterial hyaluronate lyase, whereas human Hyal-1 and BTH were not affected. Thus, despite structural similarity with vitamin C derivatives these compounds must be clearly separated from the ascorbic acid derivatives described in chapter 7, but may rather be compared with the PH-20 and $SagHyal_{4755}$ inhibitors presented in the indole chapter (chapter 8). Finally, a set of alkylphosphocholines was investigated. Among them, **9.27** was found to be the most potent inhibitor of bacterial hyaluronate lyase identified in this work with an IC_{50} value of 0.88 µM. Interestingly, no inhibition of Hyal-1 and the bovine enzyme was observed, but in contrast to the melophlins which possess slight selectivity for human PH-20 *versus* $SagHyal_{4755}$, the alkylphosphocholines have significant preference for the bacterial over the human enzyme.

9.5 *Experimental Section*

9.5.1 General conditions

Chemicals were purchased from the following suppliers: Acros Organics (Geel, Belgium), IRIS Biotech GmbH (Marktredwitz, Germany), Alfa Aesar GmbH & Co. KG (Karlsruhe, Germany), Merck KGaA (Darmstadt, Germany), and Sigma-Aldrich Chemie GmbH (Munich, Germany). Esterase (EC 3.1.1.1) from porcine liver with an activity of 20 units/mg was purchased from Sigma-Aldrich Chemie GmbH (Munich, Germany). The investigated alkylphosphocholines were kindly provided by Asta Medica AG (Frankfurt, Germany). Deuterated solvents for NMR spectroscopy were from Deutero GmbH (Kastellaun, Germany). All solvents were of analytical grade or distilled prior to use. If moisture-free conditions were required, reactions were performed in dried glassware under inert atmosphere (argon or nitrogen); DMF (H_2O < 0.01 %) was purchased from Sigma-Aldrich Chemie GmbH. Nuclear Magnetic

Resonance (^{1}H-NMR and ^{13}C-NMR) spectra were recorded on an Avance-300 NMR spectrometer from Bruker BioSpin GmbH (Rheinstetten, Germany). Tetramethylsilane was added as internal standard (chemical shift δ = 0 ppm) to all samples. Multiplicities are specified with the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), bs (for broad singulet), as well as combinations thereof. The multiplicity of carbon atoms (^{13}C-NMR) were determined by DEPT 135 and DEPT 90 (distortionless enhancement by polarization transfer): "+" primary and tertiary carbon atom (positive DEPT 135 signal), "-" secondary carbon atom (negative DEPT 135 signal), "quat" quaternary carbon atom. Mass spectrometry analysis (MS) was performed on a Finnigan MAT 95 (PI-EIMS 70 eV, HR-MS), Finnigan SSQ 710A (CI-MS (NH_3)) and on a Finnigan ThermoQuest TSQ 7000 (ESI-MS) spectrometer. The peak-intensity in parenthesis is indicated relatively to the strongest signal in %. Melting points (mp) were measured on a BÜCHI 530 using open capillaries and are uncorrected. Merck Silica Gel 60 (particle size 0.040–0.063 mm) was used for flash column chromatography. Reactions were routinely monitored by thin layer chromatography (TLC) on Merck silica gel 60 F_{254} aluminum sheets and spots were visualized with UV light at 254 nm, and/or iodine vapor or ammonium molybdate/cerium(IV) sulfate solution.

9.5.2 Chemistry

β-D-Glucose pentaacetate (9.6)[13]: To a mixture of anhydrous pyridine (50 ml) and acetic acid anhydride (0.5 mol, 47.0 ml) was added β-D-glucose (50 mmol, 11.0 g) at 0 °C and the reaction was stirred overnight at room temperature. After evaporation of the solvent and recrystallization of the crude material, the title compound was obtained as a white solid (16.3 g, 84 %). EI-MS (70 eV) *m/z (%)*: 390 (1) [$M^{+\cdot}$]. $C_{16}H_{22}O_{11}$ (390.34).

1,4-Phenylene-bis(2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside) (9.7): To a solution of **9.6** (4.0 mmol, 1.56 g) in anhydrous CH_2Cl_2 (5 ml) was subsequently added freshly activated molecular sieves and TMSTf (4.0 mmol, 0.77 ml). After stirring for 30 min at room temperature, hydroquinone (2.0 mmol, 0.22 g) was added and stirring was continued overnight. Solids were removed by filtration and after dilution with CH_2Cl_2 and addition of saturated $NaHCO_3$, the mixture was extracted

three times with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered and evaporated. The title compound was obtained after flash chromatography (PE/EtOAc 90/10 v/v) as a white foam (0.23 g, 15 %). ^{1}H-NMR ($CDCl_3$) δ 2.04 – 2.09 (m, 24H, CH_3), 4.17 (m, 4H, CH_2O), 4.97 – 5.72 (m, 10H, CH), 6.97 (m, 4H, Ar-H). PI-EIMS (70 eV) *m/z (%)*: 770 (20) [$M^{+\cdot}$]. $C_{34}H_{42}O_{20}$ (770.69).

1,4-Phenylene-bis(β-D-glucopyranoside) (9.8): To a suspension of **9.7** (0.29 mmol, 0.22 g) in anhydrous methanol (5 ml) was added 1 N methanolic NaOMe (20 µl) and the mixture was stirred at room temperature overnight. The precipitated product was dissolved by adding water and the mixture was neutralized with an acidic ionic exchanger (Amberlite® CG-120, loaded prior to use). After filtration and evaporation to dryness, the title compound was obtained as a pale brown solid (0.12 g, 95 %). ^{1}H-NMR (DMSO-d_6) δ 3.11 – 3.79 (m, 12H, CH, CH_2), 4.50 – 5.31 (m, 10H, OH, H-1), 6.99 (m, 4H, Ar-H). ES-MS (H_2O/MeOH + NH_4OAc) *m/z (%)*: 452 (100) [$M+NH_4$]$^+$. $C_{18}H_{26}O_{12}$ (434.39).

2-Deoxy-2-trichloroacetamido-D-glucopyranose (9.9)[17]: To a vigorously stirred solution of D-glucosamine hydrochloride (0.1 mol, 21.6 g) and $NaHCO_3$ (0.3 mol, 25.2 g) in water (200 ml) was added trichloroacetyl chloride (0.15 mol, 16.8 ml) dropwise over a period of 1 h at room temperature. After stirring of the solution for an additional hour, the mixture was neutralized by addition of 1 N HCl and the solvent was evaporated. The remaining residue was taken up in methanol (200 ml) und stirred for 2 h. The precipitated salt was removed by filtration, and after evaporation of the solvent the crude material was recrystallized from cold water to obtain the title compound as a white solid (19.1 g, 59 %). ^{1}H-NMR (DMSO-d_6) δ 2.09 (s, 1H, 1-OH), 3.49 (m, 6H, CH, OH), 4.65 (d, 1H, ^3J = 6.7 Hz, H-1*β*), 5.05 (s, 1H, H-1*α*), 8.34 (s, 1H, NH*α*), 8.66 (s, 1H, NH*β*). mp: 160-163 °C (Lit: 163-165 °C[17]); ES-MS (MeOH + NH_4OAc) *m/z (%)*: 322 (100) [M-H]$^-$. $C_8H_{12}Cl_3NO_6$ (324.54).

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-trichloroacetamido-α-D-glucopyranose (9.10)[17]: To a solution of **9.9** (6.2 mmol, 2.0 g) in anhydrous pyridine (20 ml) was added acetic anhydride (10 ml) and the mixture was stirred at room temperature overnight. After addition of toluene (40 ml), the mixture was filtrated and the solvent was evaporated under reduced pressure. Recrystallization from EtOAc/Hexane yielded the title compound as a pale yellow solid (2.0 g, 65 %). mp: 153-155 °C (ref. [17]: 156-157 °C); ^{1}H-NMR ($CDCl_3$) δ 2.07 (s, 6H, CH_3), 2.11 (s, 3H, CH_3), 2.20 (s, 3H, CH_3), 4.02 – 4.11 (m, 2H, H-5, H-6b), 4.28 – 4.39 (m, 2H, H-6a, H-2), 5.26 (t, 1H, ^3J =

9.8 Hz, H-4), 5.35 (dd, 1H, ^3J = 10.8 Hz, ^3J = 9.6 Hz, H-3), 6.31 (d, 1H, ^3J = 3.7 Hz, H-1), 6.83 (d, 1H, ^3J = 8.3 Hz, NH). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 509 (100) $[M+NH_4]^+$. $C_{16}H_{20}Cl_3NO_{10}$ (492.69).

3,4,6-Tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-α-D-glucopyranosyl trichloroacetimidate (9.11)[17]: To a solution of **9.10** (4.0 mmol, 1.96 g) in anhydrous DMF (20 ml) was added hydrazine acetate (6 mmol, 0.55 g) and the mixture was stirred for 20 min at room temperature. After addition of EtOAc (80 ml), the mixture was washed subsequently with water, saturated $NaHCO_3$ and water again. The organic phase was dried over $MgSO_4$, filtered and evaporated to dryness. The remaining residue was dissolved in anhydrous CH_2Cl_2 (12 ml) and trichloroacetonitrile (30 mmol, 4.0 ml) and DBU (1 mmol, 0.15 ml) were added. After stirring for 30 min at room temperature, the solvent was distilled off and the crude product was purified by flash chromatography (PE/EtOAc 2/1 v/v + 0.1 % NEt_3) to obtain the title compound as a white solid (1.5 g, 42 %). mp: 150-153 °C (ref.[17]: 160-161 °C); ^{1}H-NMR ($CDCl_3$) δ 2.07 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 4.15 (m, 2H, H-6b, H-5), 4.30 (dd, 1H, ^3J = 4.1 Hz, ^3J = 12.5 Hz, H-6a), 4.46 (ddd, 1H, ^3J = 3.6 Hz, ^3J = 8.5 Hz, ^3J = 10.7 Hz, H-2), 5.31 (t, 1H, ^3J = 9.9 Hz, H-4), 5.45 (dd, 1H, ^3J = 9.8 Hz, ^3J = 10.6 Hz, H-3), 6.50 (d, 1H, ^3J = 3.6 Hz, H-1), 7.01 (d, 1H, ^3J = 8.5 Hz, NH), 8.85 (s, 1H, C=NH). $C_{16}H_{18}Cl_6N_2O_9$ (595.04).

***p*-Methoxyphenyl-β-D-glucopyranoside (9.12)[29]:** To a solution of **9.6** (46.3 mmol, 18.1 g) and *p*-methoxyphenol (92.6 mmol, 11.5 g) in anhydrous CH_2Cl_2 (100 ml) was added CF_3SO_3H (7.4 mmol, 0.65 ml) dropwise at 0 °C. After the solution was stirred for 6 h at room temperature, CH_2Cl_2 (100 ml) was added and the mixture was washed twice with 2 N NaOH and water. The organic phase was dried over $MgSO_4$, filtered and evaporated to give a colorless oil which was dissolved in anhydrous methanol (100 ml). After addition of a catalytic amount of NaOMe (50 mg) and stirring overnight at room temperature, the solution was neutralized with an acidic ion exchanger (Amberlite® CG-120, loaded prior to use), filtered and evaporated to dryness. The title compound was obtained after recrystallization of the crude material from ethanol as a white solid (8.6 g, 64 %). mp: 155-156 °C (ref.[30]: 160 °C); ^{1}H-NMR (DMSO-d_6) δ 3.09 – 3.68 (m, 6H, CH, CH_2), 3.70 (s, 3H, OCH_3), 4.56 (t, 1H, ^3J = 5.7 Hz, OH), 4.70 (d, 1H, ^3J = 7.3 Hz, OH), 5.00 (d, 1H, ^3J = 5.1 Hz, OH), 5.06 (d, 1H, ^3J = 4.1 Hz, OH), 5.27 (d, 1H, ^3J = 4.7 Hz, H-1), 6.85 (m, 2H, Ar-H), 6.98 (m, 2H, Ar-H). ES-MS (H_2O/MeOH + NH_4OAc) *m/z (%)*: 304 (100) $[M+NH_4]^+$. $C_{13}H_{18}O_7$ (286.28).

***p*-Methoxyphenyl-4,6-*O*-(4-methoxybenzylidene)-β-D-glucopyranoside (9.13):** **9.12** (36.3 mmol, 10.3 g), 4-methoxybenzaldehyde dimethyl acetal (54.4 mmol, 9.3 ml) and *p*-toluenesulfonic acid monohydrate (0.7 mmol, 0.13 g) were suspended in DMF (50 ml) and submitted to rotary evaporation at 50-60 °C bath temperature and 20 mbar vacuum for 4.5 h. The temperature was raised to 70 °C and the mixture was concentrated until a solid began to form. The residue was poured into a mixture of saturated $NaHCO_3$ (50 ml), diethyl ether (50 ml) and ice. The resulting white solid was sucked off, washed with water and diethyl ether und was dried over P_2O_5 to obtain the title compound as a white solid which was used without further purification in the next step (13.20 g, 90 %). $C_{21}H_{24}O_8$ (404.41).

***p*-Methoxyphenyl-2,3-di-*O*-acetyl-4,6-*O*-(4-methoxybenzylidene)-β-D-glucopyranoside (9.14):** The crude **9.13** (13.2 g, 32.6 mmol) was dissolved in anhydrous pyridine (25 ml) and acetic acid anhydride (32 ml) and was stirred for 1.5 h at room temperature. After dilution with CH_2Cl_2 (100 ml), the mixture was subsequently washed with water, 1 N HCl, saturated $NaHCO_3$ and water. The organic layer was dried over $MgSO_4$, filtered and evaporated to dryness. The target compound was obtained after recrystallization of the crude product as a white solid (12.7 g, 80 %). ^{1}H-NMR (DMSO-*d*$_6$) δ 2.01 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 3.71 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 3.79 (m, 1H, CH), 3.89 (m, 2H, CH), 4.27 (m, 1H, CH), 5.04 (dd, 1H, ^3J = 7.9 Hz, ^3J = 9.4 Hz, CH), 5.40 (m, 2H, CH), 5.59 (s, 1H, CH), 6.91 (m, 8H, Ar-H), 7.30 (m, 2H, Ar-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 489 (100) $[M+H_4]^+$. $C_{25}H_{28}O_{10}$ (488.48).

***p*-Methoxyphenyl-2,3-di-*O*-acetyl-β-D-glucopyranoside (9.15):** A solution of **9.14** (20 mmol, 9.8 g) in 80 % aqueous HOAc (300 ml) was stirred at 70 °C overnight. After removal of the solvent at reduced pressure, the raw product was subjected to flash chromatography (PE/EtOAc 30/70 v/v) to obtain the target compound as a white solid (5.6 g, 75 %). ^{1}H-NMR (CD_3OD) δ 1.99 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 3.58 (ddd, 1H, ^3J = 2.1 Hz, ^3J = 5.0 Hz, ^2J = 10.0Hz, H-6a), 3.68 (m, 2H, H-6b, CH), 3.69 (s, 3H, OCH_3), 3.83 (dd, 1H, ^3J = 2.1 Hz, ^3J = 12.5 Hz, CH), 4.95 (dd, 1H, ^3J = 8.0 Hz, ^3J = 9.6 Hz, CH), 5.10 (m, 1H, CH), 5.19 (d, 1H, ^3J = 7.9 Hz, CH), 6.85 (m, 2H, Ar-H), 6.95 (m, 2H, Ar-H). ES-MS (MeOH + NH_4OAc) *m/z (%)*: 388 (100) $[M+NH_4]^+$. $C_{17}H_{22}O_9$ (370.35).

***p*-Methoxyphenyl-2,3-di-*O*-acetyl-β-D-glucopyranosiduronic acid (9.16):** To a solution of **9.15** (14 mmol, 5.2 g), TEMPO (0.35 mmol, 55 mg), TBAB (1 mmol, 0.34

g) and NaBr (1.7 mmol, 0.17 g) in EtOAc (60 ml) and saturated $NaHCO_3$ (40 ml) was added a solution of 12 % NaOCl (52 mmol, 32 ml), saturated $NaHCO_3$ (30 ml) and saturated NaCl (60 ml) dropwise at 0 °C. After stirring for 1 h at 0 °C, 6 N HCl was added to adjust the pH value <2, and the mixture was extracted three times with EtOAc. The product was obtained after partial purification using flash chromatography (CH_2Cl_2/acetone 80/20 v/v + 0.1 % HOAc) together with halogenated byproducts as a pale yellow foam (4.39 g, 82 %) and was used in the next step without further purification. ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 383 (100) $[M-H]^-$, 417 (85) $[M+Cl-H]^-$, 461 (40) $[M+2Cl-H]^-$. $C_{17}H_{20}O_{10}$ (384.33).

Methyl *p*-methoxyphenyl-2,3-di-*O*-acetyl-β-D-glucopyranosiduronate (9.17): A solution of **9.16** (3.9 mmol, 1.5 g) and Amberlite® CG-120 (1 g, loaded prior to use) in absolute methanol (50 ml) was stirred at room temperature overnight. After filtration and evaporation of the solvent, the crude product was partly purified using flash chromatography (PE/EtOAc 40/60 v/v) to obtain the title compound with halogenated impurities as a colorless amorphous substance (0.98 g, 63 %). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 416 (20) $[M+NH_4]^+$, 450 (100) $[M+Cl+NH_4]^+$, 494 (35) $[M+2Cl+NH_4]^+$. $C_{18}H_{22}O_{10}$ (398.36).

Methyl *p*-methoxyphenyl-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-acetyl-β-D-glucopyranosiduronate (9.18): A solution of **9.11** (0.65 mmol, 0.39 g) and **9.17** (0.5 mmol, 0.20 g) in anhydrous 1,2-dichloroethane was stirred with molecular sieves under an atmosphere of argon for 1.h at room temperature. After cooling to 0 °C, TMSTf (0.1 mmol, 20 µl) was added and stirring was continued for additional 3 h. The mixture was quenched by adding NEt_3 and solids were removed by filtration. After evaporation of the solvent the crude mixture was subjected to flash chromatography (PE/EtOAc 70/30 v/v) to obtain the title compound with halogenated impurities as a white solid (0.21 g, 51 %). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 849 (20) $[M+NH_4]^+$, 883 (100) $[M+Cl+NH_4]^+$, 927 (45) $[M+2Cl+NH_4]^+$. $C_{32}H_{38}Cl_3NO_{18}$ (831.00).

Methyl (*p*-methoxyphenyl-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-acetyl-β-D-glucopyranosid)uronate (9.19): A mixture of **9.18** (0.59 mmol, 0.49 g), sodium acetate (2.36 mmol, 0.19 g), palladium on activated charcoal (10 % Pd, 0.49 g) in THF (20 ml) was hydrogenated in an autoclave under a pressure of 6 bar at room temperature for 72 h. Insoluble material was filtered off, and the remaining solution was diluted with EtOAc. After washing

successively with 1 N NaOH, water and brine, the organic layer was dried over $MgSO_4$, filtered and concentrated to dryness. The title compound was obtained after flash chromatography (EtOAc) as a white solid (0.25 g, 58 %). ^{1}H-NMR ($CDCl_3$) δ 1.89 (s, 3H, CH_3), 1.99 (s, 3H, CH_3), 1.99 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 3.66 – 4.39 (m, 11H, CH, CH_2, CO_2CH_3, OCH_3), 4.73 (d, 1H, ^3J = 8.4 Hz, CH), 5.02 (m, 2H, CH), 5.20 (m, 3H, CH), 5.70 (d, 1H, ^3J = 8.7 Hz, CH), 6.80 (m, 2H, Ar-H), 6.91 (m, 2H, Ar-H). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 728 (100) $[M+H]^+$. $C_{32}H_{41}NO_{18}$ (727.66).

***p*-Methoxyphenyl-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-β-D-glucopyranosiduronic acid (9.3):** To a suspension of **9.19** (0.32 mmol, 0.22 g) in methanol (5 ml) and water (1 ml) was slowly added 3 N NaOH (9.6 mmol, 3.2 ml) dropwise. After stirring for 6 h, HOAc was added and the solvent was evaporated. Flash chromatography (EtOAc/MeOH/H_2O 10/6/2 v/v/v + 0.5 % HOAc) yielded the target compound with salt impurities which were removed by passing an ion exchanger (Bio Rad, AG 50W-X8, loaded with NH_4^+, mobile phase: H_2O). After drying under reduced pressure the target compound was obtained as a white hygroscopic solid (50 mg, 31 %). ^{1}H-NMR (600 MHz, DMSO-d_6) δ 1.89 (s, 3H, CH_3), 3.27 (m, 8H, CH), 3.58 (d, 1H, ^3J = 9.4 Hz, CH), 3.69 (m, 1H, CH), 3.70 (s, 3H, OCH_3), 4.42 (d, 1H, ^3J = 8.4 Hz, CH), 4.75 (d, 1H, ^3J = 7.6 Hz, CH), 6.84 (m, 2H, Ar-H), 6.95 (m, 2H, Ar-H), 8.53 (d, 1H, ^3J = 5.9 Hz, NH). ES-MS (DCM/MeOH + NH_4OAc) *m/z (%)*: 504 (100) $[M+H]^+$. $C_{21}H_{29}NO_{13}$ (503.45).

***S*-Carboxymethyl-L-cysteine (9.20)[22]:** To a solution of bromoacetic acid (56 mmol, 7.8 g) and L-cysteine hydrochloride monohydrate (67 mmol, 11.8 g) in water (60 ml) was added 7 N NaOH (0.34 mol, 49 ml) at 0 °C over a period of 30 min. After stirring for additional 60 min, acetic acid (200 ml) was added and the mixture was kept in the refrigerator overnight. The precipitated product was sucked off, recrystallized from water and dried under reduced pressure to obtain a white solid (5.6 g, 52 %). mp: 187-189 °C (ref.[22]: 191 °C). ES-MS (H_2O/MeOH + NH_4OAc) *m/z (%)*: 180 (100) $[M+H]^+$. $C_5H_9NO_4S$ (179.19).

***S*-Methoxycarbonylmethyl-L-cysteine hydrochloride (9.21)[23]:** To a suspension of **9.20** (8.0 mmol, 1.55 g) in anhydrous methanol (50 ml) was added TMSCl (17.6 mmol, 2.2 ml) dropwise. After stirring for 6 min at room temperature, the solution was concentrated and poured into a mixture of EtOAc (100 ml) and Et_2O (100 ml). Crystals obtained after storage in the refrigerator overnight and were collected by

filtration to yield the title compound as a white solid (1.7 g, 93 %). ^{1}H-NMR (D_2O) δ 3.05 (dd, 1H, ^3J = 7.8 Hz, ^2J = 15.1 Hz, SC$\underline{H}_2$CH), 3.20 (dd, 1H, ^3J = 4.5 Hz, ^2J = 15.2 Hz, SC$\underline{H}_2$CH), 3.41 (s, 2H, SCH_2CO), 3.66 (s, 3H, OCH_3), 4.16 (dd, 1H, ^3J = 4.4 Hz, ^3J = 7.8 Hz, CH). $C_6H_{12}ClNO_4S$ (229.68).

Methyl (*R*)-2-(2,5-dioxooxazolidin-4-ylmethylthio)acetate (9.22): To a suspension of **9.21** (3.0 mmol, 0.66 g) in anhydrous THF (10 ml) was added triphosgene (1.0 mmol, 0.30 g) at 50 °C under an atmosphere of argon. After stirring for 3 h at 50 °C, the solution was purred into hexane (50 ml) and stored in the refrigerator overnight. The crystals were sucked off to yield the target compound as a white solid (0.57 g, 87 %). ^{1}H-NMR ($CDCl_3$) δ 2.98 (dd, 1H, ^3J = 7.7 Hz, ^2J = 14.8 Hz, SC$\underline{H}_2$CH), 3.27 (dd, 1H, ^3J = 3.6 Hz, ^2J = 14.7 Hz, SC$\underline{H}_2$CH), 3.36 (s, 2H, SC$\underline{H}_2$CH), 3.78 (s, 3H, OCH_3), 4.59 (ddd, 1H, ^3J = 0.9 Hz, ^3J = 3.6 Hz, ^3J = 7.7 Hz, CH), 7.05 (bs, 1H, NH). $C_7H_9NO_5S$ (219.22).

D-Proline methyl ester hydrochloride (9.23)[31]: To a suspension of D-proline (20 mmol, 2.3 g) in anhydrous methanol (20 ml) was added thionyl chloride (22 mmol, 1.6 ml) at 0 °C and the mixture was stirred for 2 h at room temperature. After stirring for additional 1.5 h at reflux, volatiles were removed to obtain the title compound as a very hygroscopic semisolid substance (3.3 g, 100 %). ^{1}H-NMR (D_2O) δ 1.92 – 2.41 (m, 4H, CH_2), 3.33 (m, 2H, C$\underline{H}_2$NH), 3.75 (s, 3H, CO_2CH_3), 4.40 (dd, 1H, ^3J = 7.4 Hz, ^3J = 8.5 Hz, CH). EI-MS (70 eV) *m/z (%)*: 129 (4) [$M^{+\cdot}$]. $C_6H_{12}ClNO_2$ (165.62).

Methyl 2-[((3R,8aR)-1,4-dioxooctahydropyrrolo[1,2-a]pyrazin-3-yl)methylthio]-acetate (9.24): To a solution of **9.21** (1.5 mmol, 0.33 g) in anhydrous THF (5 ml) was added a solution of **9.23** (1.5 mmol, 0.25 g) and NEt_3 (3.38 mmol, 0.47 ml) in anhydrous CH_2Cl_2 (10 ml) dropwise at -70 °C under an atmosphere of argon. After stirring for 3 h at -70 °C, the cooling bath was removed and stirring was continued for additional 30 min at room temperature. The mixture was filtered, the solvent was evaporated and the crude residue was dissolved in anhydrous toluene (20 ml) and stirred at reflux overnight. After removal of the solvent under reduced pressure, the raw product was subjected to flash chromatography ($CHCl_3$/MeOH 97/3 v/v) to obtain the title compound as a pale yellow oil (0.35 g, 68 %). ^{1}H-NMR (DMSO-d_6) δ 1.69 – 2.63 (m, 4H, CH_2), 2.93 (dd, 1H, ^3J = 4.9 Hz, ^2J = 13.8 Hz, SC$\underline{H}_2$CH), 3.02 (dd, 1H, ^3J = 6.7 Hz, ^2J = 13.8 Hz, SC$\underline{H}_2$CH), 3.40 (m, 4H, SCH_2, NCH_2), 3.65 (s, 3H, CO_2CH_3), 3.93 (ddd, 1H, ^3J = 4.5 Hz, ^3J = 4.5 Hz, ^3J = 6.5 Hz, CH), 4.24 (dd, 1H, ^3J = 6.9 Hz, ^3J

= 9.3 Hz, CH), 8.29 (d, 1H, ^3J = 3.8 Hz, NH). EI-MS (70 eV) *m/z (%)*: 272 (21) [$M^{+\cdot}$]. $C_{11}H_{16}N_2O_4S$ (272.32).

Sodium 2-[((3R,8aR)-1,4-dioxooctahydropyrrolo[1,2-a]pyrazin-3-yl)methylthio]-acetate (9.5): To a solution of **9.23** (0.59 mmol, 0.16 g) in acetone (1ml) and 0.1 N phosphate buffer (13 ml, pH 8.0) was added porcine liver esterase (30 mg) and the mixture was stirred at room temperature overnight. The mixture was concentrated under reduced pressure and the remaining suspension was subjected to flash chromatography ($CHCl_3$/MeOH 80/20) to obtain the title compound as a white solid (0.10 g, 66 %). ^{1}H-NMR (DMSO-d_6) δ 1.67 – 2.21 (m, 4H, CH_2), 2.90 (d, 2H, ^3J = 6.2 Hz, SCH_2CH), 3.07 (s, 2H, SCH_2), 3.38 (m, 2H, NCH_2), 3.88 (m, 1H, CH), 4.26 (dd, 1H, ^3J = 6.9 Hz, ^3J = 9.1 Hz, H), 8.56 (d, 1H, ^3J = 3.6 Hz, NH). ES-MS (H_2O/MeOH + NH_4OAc) *m/z (%)*: 257 (100) [M-H]$^-$. $C_{10}H_{13}N_2NaO_4S$ (280.28).

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Chapter 10
Biopharmaceutical and toxicological investigations on representative hyaluronidase inhibitors

10.1 *Introduction*

Many hyaluronidase inhibitors synthesized in this work comprise on one hand a large lipophilic fragment (alkyl, aryl residues) and on the other hand a polar, acidic motive (e.g. polyhydroxy compounds such as ascorbic acid and carbohydrate(-like) structures, indole carboxylic acids). Thereby, these molecules mimic respective amphiphilic repetitive motives of the substrate hyaluronan. Due to the aforementioned structural features these hyaluronidase inhibitors possess detergent-like properties which may compromise their hyaluronidase inhibitory activity *in vitro* and *in vivo.* Therefore, selected hyaluronidase inhibitors were investigated with respect to micelle formation and the induction of hemolysis, both characteristic for detergent-like molecules. Due to observed inconsistencies, when the compounds were investigated for hyaluronidase inhibition on crude hyaluronidase preparations, protein binding of the compounds was studied, too. Furthermore, selectivity of representative inhibitors from various compound classes against different mammalian hyaluronidases was determined using equiactive enzyme concentrations. In addition, the toxicity of selected substances was investigated in a standard chemosensitivity assay.

10.2 *Materials and methods*

10.2.1 Determination of critical micelle concentrations (CMCs)

The determination of CMC values based upon measurement of the surface tension was performed according to literature procedures[1-3].
Stock solutions of the investigated hyaluronidase inhibitors were prepared in DMSO. For CMC determination solutions were prepared, which contained 0.6 ml of each inhibitor solution, 6.2 ml of McIlvaine's buffer (solution A: 0.2 M Na_2HPO_4, 0.1 M NaCl, solution B: 0.1 M citric acid, 0.1 M NaCl; solution A and B were mixed in the appropriate proportions to reach pH 5.0), 3.62 ml of BSA solution (0.2 mg/ml in water), 2.56 ml of water and 1.62 ml of HA solution (2 mg/ml in water). The surface tension of the resulting samples was determined using a ring tensiometer (Lecompte du Noüy) K8600 equipped with a platinum-iridium ring from Kruss, Optisch-mechanische Werkstätten (Hamburg, Germany). Measurements were performed in duplicate.

10.2.2 Determination of hemolytic properties using human erythrocytes

The determination of hemolytic properties of the compounds was performed as previously described[4, 5].

10.2.2.1 Isolation and purification of erythrocytes

Isotonic NaCl solution (4 ml) was added to fresh human heparinized blood (8 ml) and the suspension was centrifuged at 4 °C (2000 g, 10 min). After removal of the supernatant plasma and the thin colorless leukocyte-layer using a Pasteur pipette, the erythrocytes were re-suspended in isotonic NaCl solution (8 ml) and centrifuged again (2000 g, 10 min, 4 °C). After removal of the supernatant, the washing

procedure was repeated twice. Finally, the supernatant was discarded and the erythrocytes were stored on ice before use on the same day.

10.2.2.2 Determination of hemolysis

Stock solutions (20 mM) of inhibitor dissolved in DMSO were prepared and diluted to concentrations of 0.2, 2 and 10 mM with DMSO.
To a suspension of freshly prepared erythrocytes (50 µl) and isotonic NaCl solution (0.945 µl) appropriate volumes of the solutions of the inhibitors were added to an Eppendorf reaction vessel to obtain the final concentrations of the test compounds (1, 10, 50 and 100 µM). To achieve 100 % hemolysis, a solution of digitonin in DMSO (2 %, w/v) was used as reference. For the 0 % value DMSO without any inhibitor was added. After careful mixing, the reaction vessels were incubated for 1 h at 37 °C and vortexed periodically every 15 min. The suspensions were centrifuged at 4 °C (2000 g, 10 min), and 50 µl of the supernatant were transferred to acryl cuvettes and diluted with water (950 µl). Absorbance of the samples was measured at 585 nm. The hemolytic activity (percentage) was calculated according to the following equation:

$$\% \text{ Hemolysis} = 100 \cdot (A - A_{0\%}) / A_{100\%}$$

whereas A is the measured absorbance of the sample, $A_{0\%}$ is the absorbance of the references with DMSO only and $A_{100\%}$ is the absorption of the sample with digitonin.

10.2.3 Chemosensitivity assay

The assay was performed as previously described by Bernhardt et al.[6]: Tumor cell suspensions (100 µl/well) were seeded into 96-well flat-bottomed microtitration plates (Greiner, Frickenhausen, Germany) at a density of ca 15 cells/microscopic field (magnification 320x). After 2 days of incubation, the culture medium was removed by suction and replaced by fresh medium (200 µl/well) containing varying hyaluronidase inhibitor concentrations. On every plate 16 wells served as controls and 16 wells were used per compound concentration. After various periods of incubation the cells were fixed with glutaraldehyde (Merck, Darmstadt, Germany) and stored at 4 °C. At the end of the experiment all plates were stained with crystal violet (Serva, Heidelberg, Germany) simultaneously. Absorbance was measured at 578 nm using a

Biotek 309 Autoreader (Tecnomara, Fernwald, Germany). Growth curves were constructed using SigmaPlot analysis software (Systat Software GmBH, Erkrath, Germany).

10.2.4 Determination of hyaluronidase activitiy

10.2.4.1 Morgan-Elson assay

The Morgan-Elson assay was carried out as described in chapter 3.4.2 at pH 3.5 using murine serum (50 µl) instead of enzyme solution and an incubation period of 48 h. For experiments with BTH in the presence of murine serum, 50 µl of a solution of Neopermease® (400 IE/ml* in BSA (0.2 mg/ml)) were used, and 50 µl of water were replaced with murine serum. In this case the samples were incubated for 1 h. 50 µl of Neopermease® (400 IE/ml* in BSA (0.2 mg/ml)) and an incubation period of 1 h were also chosen to perform the assay in the presence of various concentrations of (heat-denatured) BSA. In the latter case the standard BSA solution (0.2 mg/ml) was replaced by a solution containing 40 mg/ml of BSA.

10.2.4.2 Turbidimetric assay using equiactive hyaluronidase concentrations

To determine the selectivity of hyaluronidase inhibitors the turbidimetric assay was performed as described in chapter 3.4.3 using equiactive enzyme concentrations: A final enzymatic activity of 0.01 mU of each enzyme and incubation times of 4.5 h were used in the assay.

10.2.5 Investigations on serum protein binding using HPLC

A mixture of 240 µl of phosphate buffer (pH 5.0 as described in chapter 3), 200 µl of water, 60 µl of human plasma, 20 µl of DMSO and 20 µl of inhibitor (0.93 mg **7.52** dissolved in 400 µl DMSO) were incubated for 30 min at 37 °C. 400 µl of the incubation mixture were filtered using Microcon centrifugal filter devices (Microcon

* according to the declaration of the supplier

YM-10, 10000 MWCO from Micon® Bioseparations, Millipore, Eschborn, Germany). After approximately half of the solution was filtered (13000 rpm, 13000 g), 100 µl samples of the filtrate, the supernatant and the unfiltered sample were taken and diluted with ice-cold acetonitrile. To complete deproteinisation, the solutions were stored on ice for 30 min and centrifuged again (13000 rpm, 13000 g, 4°C, 15 min). 200 µl of the supernatants were diluted with aqueous TFA (0.1 %). The solutions were used for HPLC analysis immediately. As a reference, the same procedure was repeated without human serum (replaced by water).

Analytical HPLC was performed on a Kontron system consisting of an HPLC Pump 430, a gradient mixer M491, an autosampler 460, and a 430 UV/VIS detector (Kontron, Neufahrn, Germany). The stationary phase was a Eurosphere-100 C-18 (250 x 4.0, 5 µm) column (Knauer) thermostatted at 30 °C with a Shimadzu CTO-2A column oven. 50 µl of each sample were injected. As mobile phase a mixture (65/45 (v/v)) of MeCN/TFAaq (0.1 %) was used at a flow rate of 0.7 ml/min. Absorbance was detected at 210 nm.

10.3 *Results and discussion*

10.3.1 Determination of the CMCs of selected compounds

Vcpal is known to form micelles in aqueous solutions. CMC values of 120-260 µM were determined at pH 7.00[7]. Due to the fact that micelle formation depends on various conditions like temperature, pH-value, ionic strength or concentration of co-solutes, CMC values vary over o broad range. It is known that hyaluronan can influence surfactant properties in aqueous media[8]. To investigate, if the synthesized hyaluronidase inhibitors form micelles under the conditions of the enzymatic assays performed in this work, the concentration dependent surface tension of solutions containing selected hyaluronidase inhibitors, namely the ascorbic acid derivatives Vcpal and **7.112** and the indole-3-butyric acid derivative **8.32** (see Figure 10.1 for structures) was determined using a ring tensiometer.

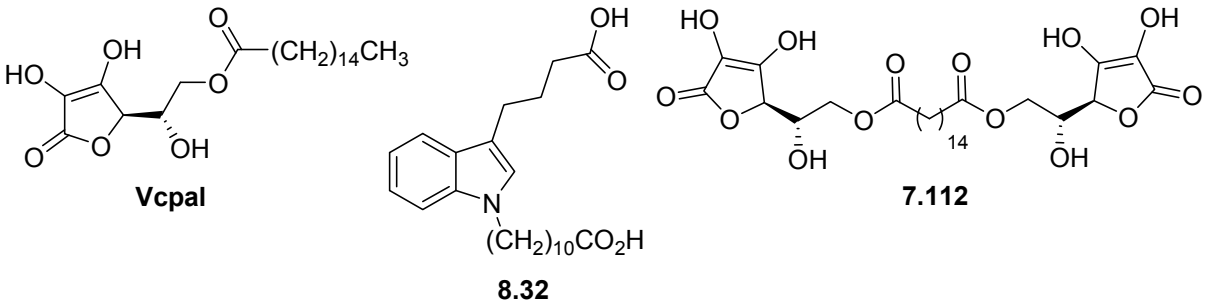


Figure 10.1. Chemical structures of investigated hyaluronidase inhibitors.

Solutions were prepared by analogy with the incubation mixture of the turbidimetric hyaluronidase activity assay. Figure 10.2 shows the surface tension in the presence of varying concentrations of the investigated compounds under assay conditions.

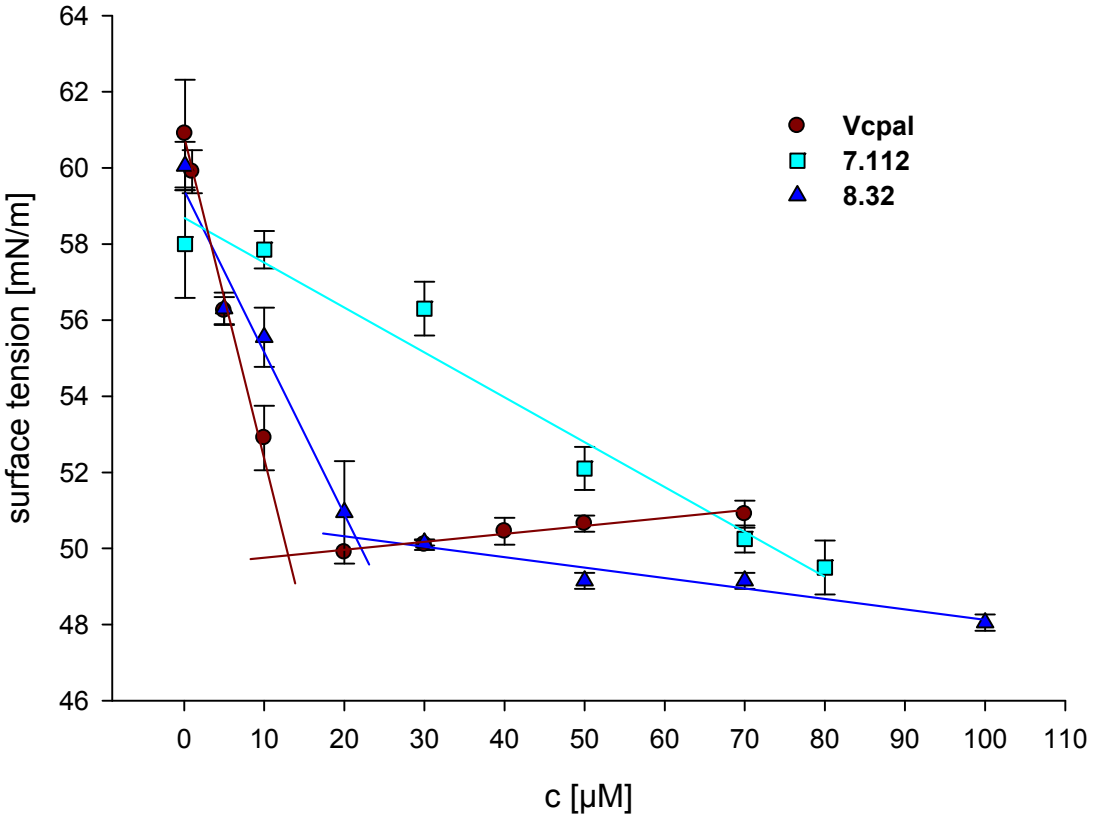


Figure 10.2. Suface tension in the presence of varying concentrations of selected hyaluronidase inhibitors. CMC values were determined by analogy of the conditions of the turbidimetric hyaluronidase assay at pH 5.0, 20 °C.

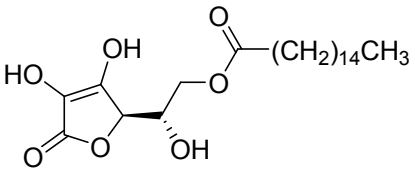
In the case of Vcpal it is obvious from Figure 10.2 that an increase in the concentration of the compound (0.1 µM-20 µM) initially leads to a steep decrease in surface tension. This behavior is typical for surfactants, and a critical micelle concentration of 13 µM can be calculated from the two linear regression graphs (0.1 µM -10 µM and 20 µM-70 µM). The determined CMC value is much lower compared

to literature values which probably results from different conditions such as pH value, ionic strength and compounds like BSA and HA which are also present in the incubation mixture.

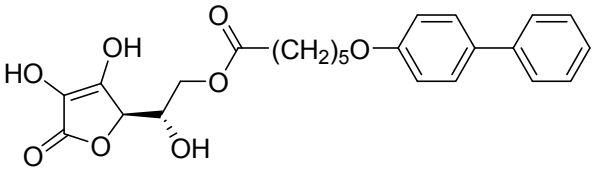
The indole-3-butyric acid derivative (**8.32**) is also surface-active and forms micelles with a CMC of 22 µM, although this compound bears a carboxylic acid at the end of the lipophilic alkyl chain. Thus, micelle formation or better the formation of bilayers, ellipsoids, cylinders or other aggregates cannot be prevented by the simple introduction of polar residues at the end of the alkyl chain. As long as there are large lipophilic fragments in the inhibitor, aggregation will always be a problem. In contrast to Vcpal, no CMC was measured for the "bivalent" ascorbic acid **7.112** up to a concentration of 80 µM. Therefore, this compound may be a candidate for further investigations due to its improved physicochemical properties compared to the other hyaluronidase inhibitors with a highly lipophilic residue facilitating micelle formation. There are also consequences for the determination of the hyaluronidase inhibitory potency of the compounds: as soon as micelles begin to form, the concentration of "inhibitor monomers" decreases in solution, which affects the determined IC_{50} values. It is known that micelle formation often leads to a flattening of the SAR. This may also be the case in the SAR presented in this work, especially when only weak inhibition of the enzymes is observed as it is often the case with bovine testicular hyaluronidase.

10.3.2 Hemolytic properties of selected hyaluronidase inhibitors

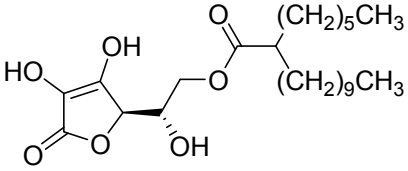
Ionic and non-ionic detergents often induce hemolysis[9], that means the abnormal breakdown of red blood cells, which leads to the release of hemoglobin into the surrounding fluid. As proven in chapter 10.3.1 at least some hyaluronidase inhibitors show surface activity like formation of micelles, thus the hemolytic properties were investigated as a prerequisite for *in vivo* investigations. Figure 10.4 shows the percentage of hemolysis compared to the reference compound digitonin, which is known to be strongly hemolytic[10] (see Figure 10.3 for structures of investigated hyaluronidase inhibitors). The detergent sodium dodecyl sulfate (SDS) was also investigated for comparative reasons.



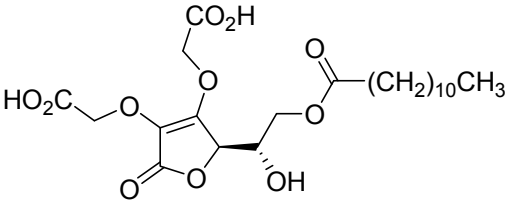
Vcpal



7.52



7.91



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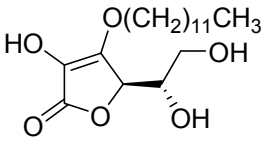
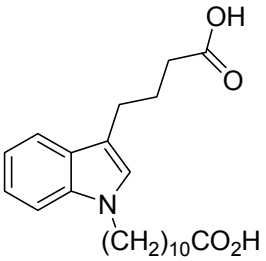


7.149



8.32

Figure 10.3. Structures of investigated hyaluronidase inhibitors.

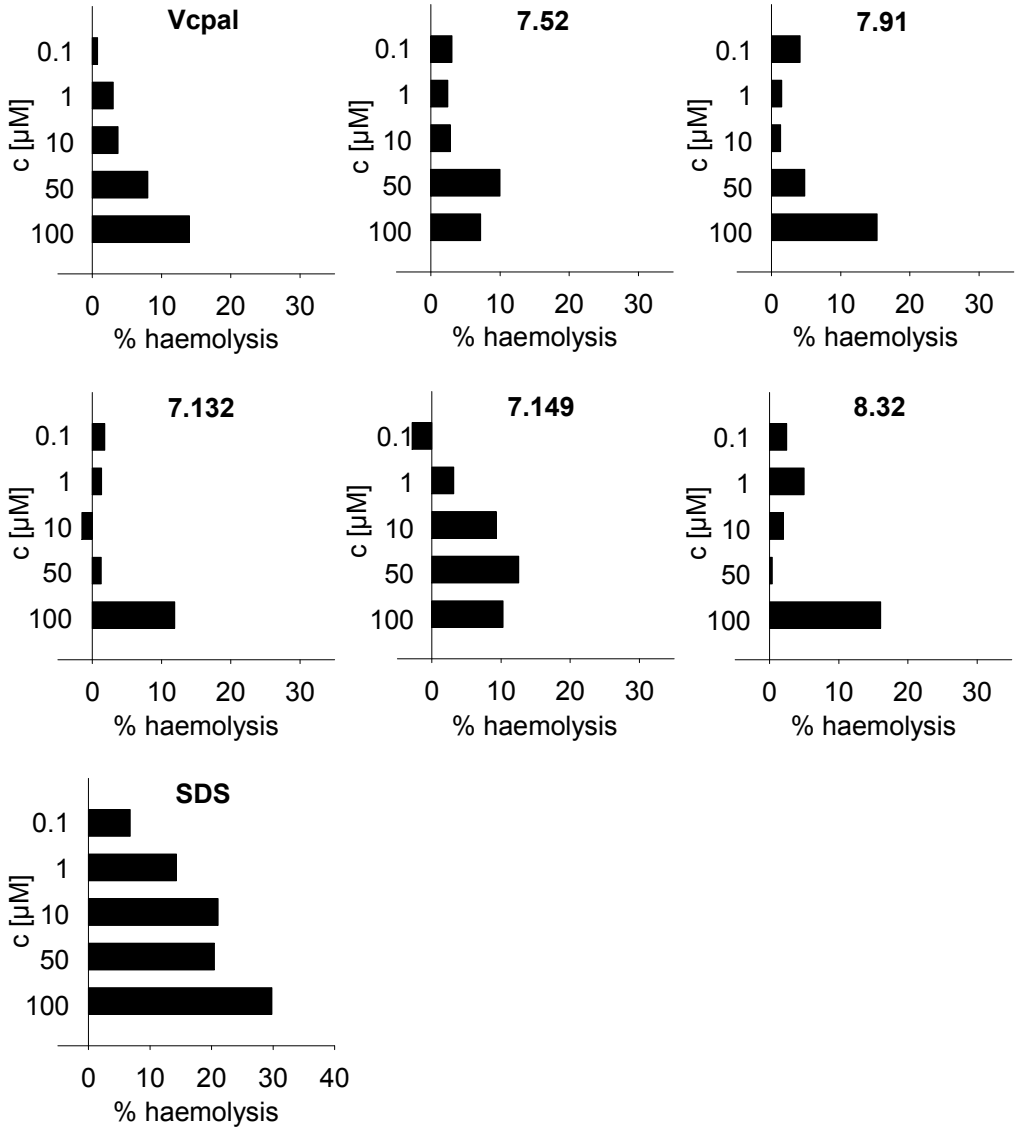
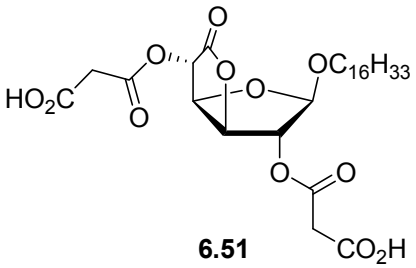


Figure 10.4. Hemolysis induced by hyaluronidase inhibitors and SDS in % values at various concentrations compared to digitonin.

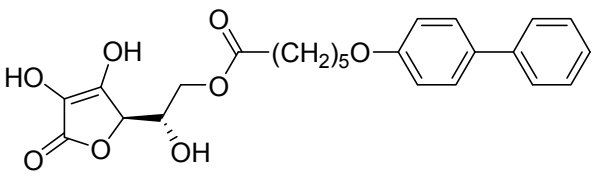
All investigated hyaluronidase inhibitors induced weak hemolysis at the highest concentrations tested (100 µM). Nevertheless, compared to SDS the hemolytic effect was significantly lower at every concentration. At concentrations below 50 µM the hemolytic effect of the inhibitors is marginal. Negligible hemolysis was also observed for **7.52**, a compound where the alkyl chain present in all the other inhibitors is replaced by a biphenyl containing residue. On the contrary, distinct hemolysis was observed for **7.149,** where additional carboxy substituents were introduced. The indole derivative **8.32** possesses no significant hemolytic activity below 100 µM. Taken together, with respect to *in vivo* experiments, inhibitor concentrations higher than 50-100 µM should be avoided.

10.3.3 Cytotoxicity of representative hyaluronidase inhibitors

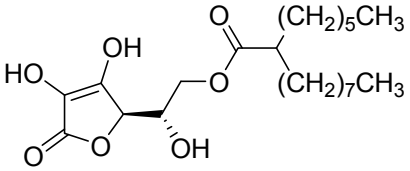
As a prerequisite for application as pharmacological tools *in vivo*, several hyaluronidase inhibitors were tested with respect to cytotoxicity in the crystal violet based chemosensitivity assay[6]. The human glioblastoma cell variant U-373 MG and the colon carcinoma cells HT-29 were used for these investigations. Paclitaxel was taken as reference compound. Structures of investigated compounds are shown in Figure 10.5.



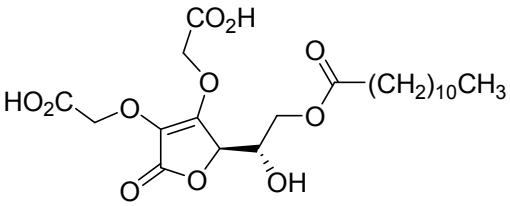
6.51



7.52



7.89



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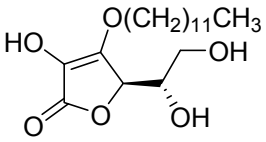

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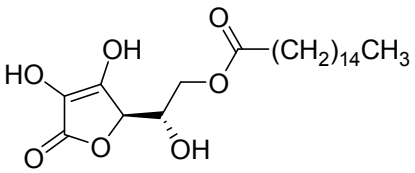
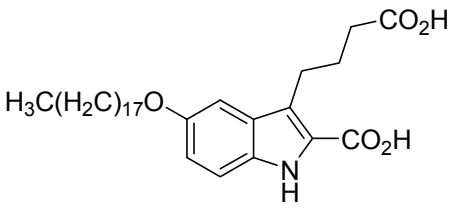
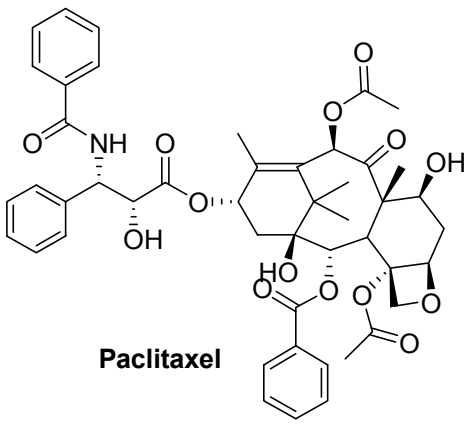


Vcpal



8.65



Paclitaxel

Figure 10.5. Chemical structures of the compounds investigated in the cytotoxicity assay.

Exemplary data is depicted in Figure 10.6.

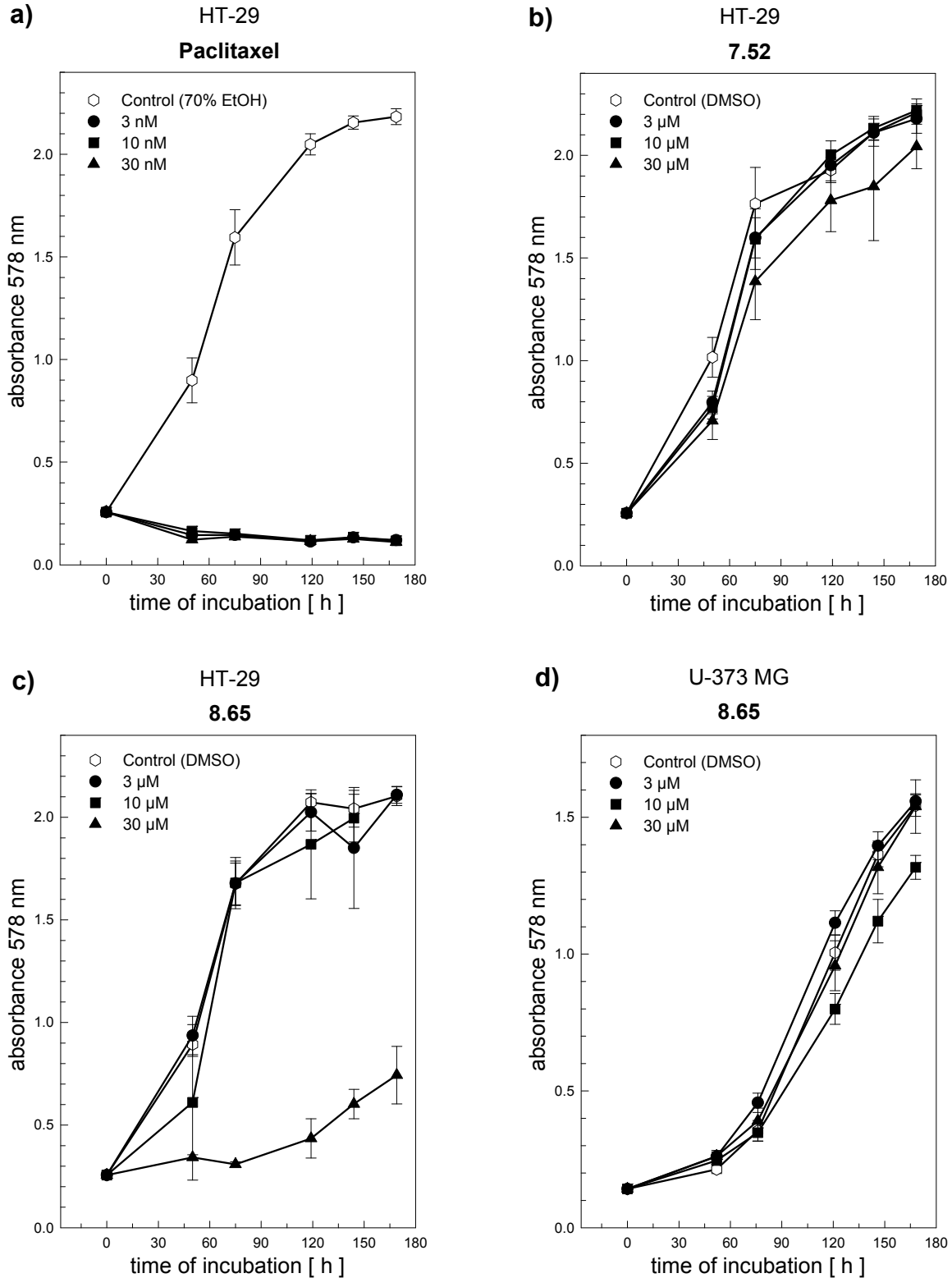


Figure 10.6. Incubation of a) HT-29 cells with various concentrations of paclitaxel b) HT-29 cells with various concentrations of **7.52** c) HT-29 cells with various concentrations of **8.65** and d) U-272 MG cells with various concentrations of **8.65**.

The reference compound paclitaxel clearly caused a cytostatic effect at the investigated concentrations (Figure 10.6a), whereas cell proliferation was not affected by all but one of the investigated hyaluronidase inhibitors up to a concentration of 30 µM (see Figure 10.6b for the representative ascorbic acid derivative **7.52**). The only exception was the indole based inhibitor **8.65** when tested against HT-29 cells (Figure10.6c). At the highest concentration of 30 µM cell growth was significantly reduced. Due to the fact that the compound was barely soluble in the cell suspension at this concentration and precipitation of the compound was observed after adding the stock solution to the cell medium, this observation might be the result of mechanical processes rather than of toxic properties of the compound. As shown in Figure 10.6d, the U-373 MG cells were not affected by the highest concentration of **8.65**.

10.3.4 Effect of murine serum and BSA on the inhibitory activity of representative compounds

During investigations with mouse serum using the colorimetric Morgan Elson assay (see chapter 3), we encountered problems when serum was taken as a source of hyaluronidase (see Figur 10.7 for the results of the Morgan Elson assay). None of the previously identified inhibitors was able to inhibit the murine hyaluronidase at concentration of 1 mM (Figure 10.7a), although mouse Hyal-1, which is present in the serum, is closely related to the human enzyme (the degree of homology between the pairs of orthologs of human and mouse is much higher than that between human paralogs[11]). To verify if the compounds are active in presence of mouse serum, we added bovine testicular hyaluronidase, which is definitely inhibited by these compounds (see chapter 6-8): No inhibitory activity was found for **7.127**, although BTH was clearly inhibited without serum (Figur 10.7b). The activity of BTH in presence of murine serum was proven previously[12]. In a next step we replaced the mouse serum by a physiological concentration of BSA (solutions of 40 mg/ml were used for the incubation mixture instead of the 0.2 mg/ml BSA solution, which was routinely used in this work). Again, no inhibition of the hyaluronidase was observed. Boiling of the BSA-solution for 5 min prior to use led to a significant increase in the inhibition of BTH by **7.127** and **6.42**, an indication for specific binding of the

compounds to native BSA. These results suggest that the hyaluronidase inhibitors possess high affinity to serum albumin.

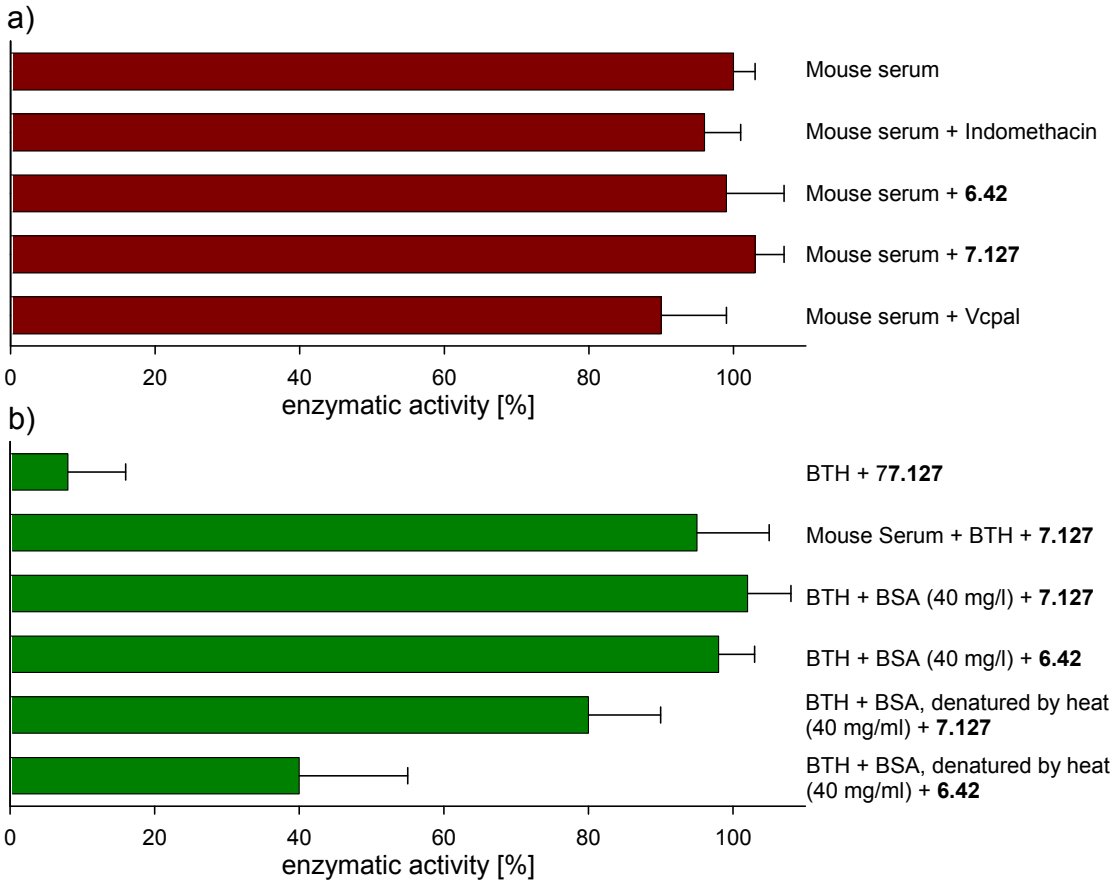


Figure 10.7. Effect of mouse serum and BSA on the inhibitory activity of selected compounds. Enzymatic activity determined at pH 3.5 using the Morgan Elson assay under different assay conditions: a) in the presence of murine serum (incubation time: 48 h), b) in the presence of BTH (incubation time: 1h). Final inhibitor concentration in the assay was 1 mM.

Extensive investigations on the binding properties of low molecular weight compounds to serum albumin led to the identification of two main high affinity binding sites. The existence of several additional binding sites has been proposed[13-18]. Especially anionic and lipophilic drugs are known to show high binding to serum albumin[15]. As the investigated hyaluronidase inhibitors possess both, anionic and lipophilic motives, the protein binding was further investigated.

Thus, ascorbic acid 6-palmitate was tested in the turbidimetric assay (see chapter 3) with respect to the inhibition of the bacterial hyaluronate lyase in the presence of various concentrations of BSA. The results are summarized in Table 10.1.

Table 10.1. IC_{50} values of Vcpal against *Hyl*B_{4755} at pH 5.0 from the turbidimetric assay in the presence of varying concentrations of BSA in the incubation mixture.

c(BSA) [µM]*	IC_{50} [µM]	Δc(BSA) [µM]	ΔIC_{50} [µM]	ΔIC_{50} / Δc(BSA)
0	3.6			
		0.3	1.6	5.3
0.3	5.2			
		0.3	1.5	5.0
0.6	6.7			
		0.8	3.5	4.4
1.4	10.2			
		1.2	7.2	6.0
2.6	17.4			
		2.4	2.5	1.0
5.0	19.9			
		11.8	47.9	4.1
16.8	67.8			
		165	666	4.0
182	734			

*a molar weight of 66.0 kDa was used to calculate the BSA concentrations.

Obviously an increase in protein concentration leads to an increase in the IC_{50} values from 3.6 µM in the absence of BSA to 734 µM determined at a BSA concentration of 182 µM. In the last column the difference of the IC_{50} values divided by the differences in BSA-concentration were calculated. This quotient is approximately 5 at lower BSA concentrations, i.e. 5 molecules of the inhibitor are bound to one molecule of BSA. At concentrations around 20 µM the number of molecules bound to BSA drops to one. Interestingly, this is exactly the concentration range where micelle formation occurs (see chapter 10.3.1). Thus, *via* micelle formation the concentration of free monomer is lowered in solution and a smaller number of inhibitor monomers are bound to BSA. An increase in the BSA concentration to 16.8 µM leads to an increase in the number of molecules bound to BSA. The protein concentration (c(BSA)=182 µM = 12 mg/ml) in last row of Table 10.1 amounts approx. to the serum albumin concentration in human blood (c(HSA) ≥ 30 mg/ml).

To investigate, if, beside Vcpal, other inhibitors also bind to BSA, the IC_{50} values of selected compounds were determined (see Figure 10.8 for chemical structures of investigated compounds) in the presence of elevated BSA concentrations. The IC_{50} values determined at different BSA concentrations are summarized in Table 10.2.

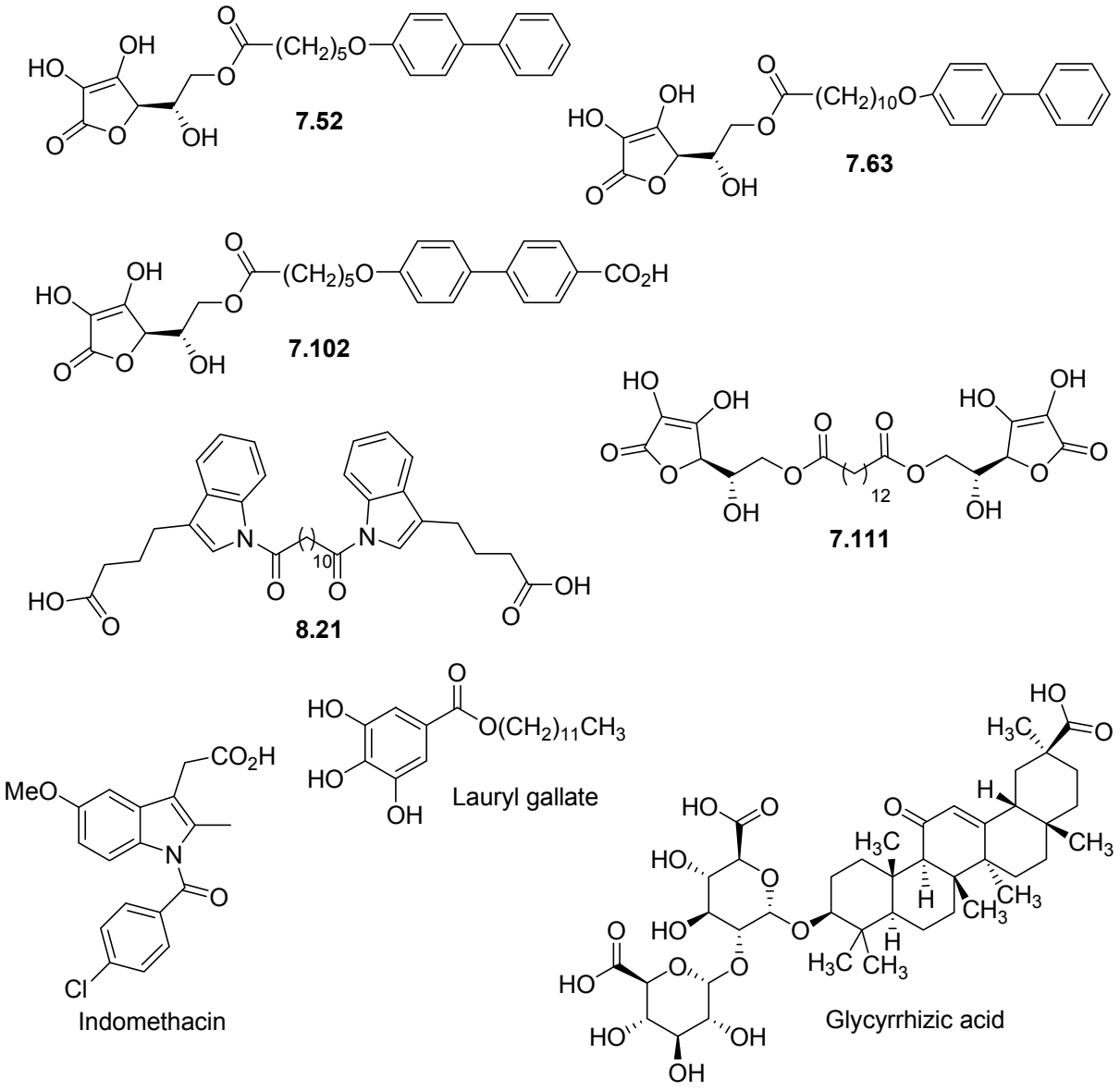


Figure 10.8. Investigated hyaluronidase inhibitors.

Table 10.2. IC_{50} values of selected inhibitors in the presence of 0.3 µM BSA and 16.8 µM BSA.

Compound	IC_{50} [µM][a] c(BSA)=0.3 µM	IC_{50} [µM][a] c(BSA)=16.8 µM	ΔIC_{50} / Δc(BSA)
7.52	61	127	4.0
7.63	7.5	225	13.2
7.102	60	137	4.7
7.111	22	138	7.0
8.21	18	73	3.3
Glycyrrhizic acid	46	129	5.0
Lauryl gallate	13	155	8.6
Indomethacin	350[b]	590	14.5

[a]SEM values < 5 %.[b]determined by Salmen.

In every case, a significant drop of the IC_{50} value was observed when a high concentration of BSA was used in the turbidimetric assay. Around five molecules of each inhibitor were calculated to be bound per molecule of BSA as in the case of Vcpal. An exceptional loss of potency was observed for the ascorbic acid derivative **7.63** bearing an elongated alkyl spacer, when compared to **7.52**. The lowest protein binding was found for the bivalent indole moiety **8.21** with around three molecules bound per molecule of serum albumin. Glycyrrhizic acid, which is known to be among the most potent inhibitors for human hyaluronidases also seems to bind to BSA with a ratio of 5.0. By contrast, the decrease in the inhibitory activity of lauryl gallate is more pronounced: The IC_{50} value drops to 155 µM in the presence of higher protein concentration, which corresponds to 8.6 molecules bound per molecule of BSA. For indomethacin an exceedingly high number of molecules bound to BSA was determined. The value of 14.5 determined *via* the turbidimetric assay is in good agreement with previous investigations, where 15 molecules of indomethacin were found to bind so serum albumin by different techniques[13]. Generally, the obtained results are indications for a specific binding to serum albumin rather than unspecific binding.

To further investigate the protein binding, an HPLC based approach was carried out: a mixture containing phosphate buffer, human plasma (which contains human serum albumin as major protein component) and inhibitor (**7.52**) was incubated at 37 °C and then filtered using a cutoff of 10 kDa to remove serum albumin. The concentration of serum proteins was approximately 100 µM in the incubation mixture; the inhibitor concentration was 200 µM. As a control experiment, the same procedure was carried out with solutions containing water instead of plasma. After preparation for analytical HPLC, samples before filtration, and samples from the filtrate as well from the supernatant were analyzed.

In the control experiment without serum, the inhibitor was able to pass the membrane. The obtained HPLC-traces for the plasma samples are depicted in Figure 10.9.

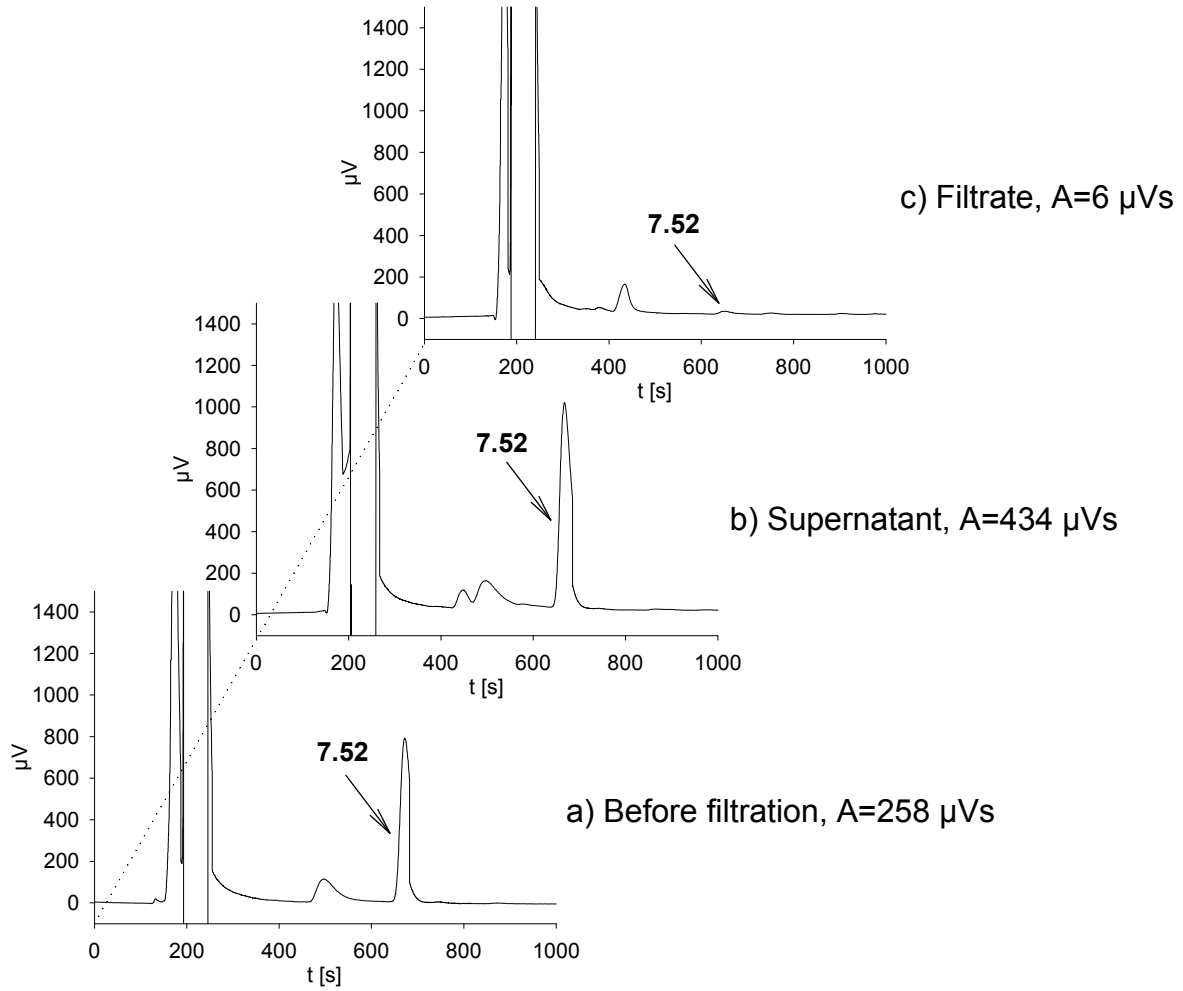


Figure 10.9. HPLC traces of samples containing **7.51** in presence of human plasma taken a) before filtration, b) from the supernatant and c) from the filtrate. The area under the curve (A) is given for the inhibitor peak.

As shown in Figure 10.9 a higher concentration of **7.51** was found in the supernatant when compared to the sample without filtration. In the filtrate only a very small amount of the inhibitor was detectable, indicating that the inhibitor was nearly completely bound to serum proteins and therefore did not pass the membrane in the filtration step. Due to the fact that the inhibitor *versus* protein ratio in this experiment was 2:1 it can be concluded that at least two inhibitor molecules are bound to one molecule of protein. These results are in agreement with the data obtained before.

Taken together, both experiments show that the synthesized inhibitors share the fate of many other drugs and bind to serum proteins. This protein binding will be a general problem of hyaluronidase inhibitors as long as lipophilic fatty acid-like moieties on the one hand and negatively charged groups on the other hand are required to achieve inhibition of the enzymes, as these characteristics also promote

the protein binding capabilities. A literature example is the NSAID indomethacin, which is known to inhibit hyaluronidases *in vivo*: The protein binding of indomethacin in human plasma was calculated to be 90% and the number of binding sites on albumin was determined to be around 15[13]. Due to the high potency of many drugs (IC_{50} values in the nanomolar range), protein binding may not be a serious problem as the amount of unbound drug is still sufficient to generate the desired effect. However, in the case of the investigated hyaluronidase inhibitors, the IC_{50} values are in the (lower) micromolar range and therefore probably concentrations in the millimolar range would be necessary to achieve inhibition in presence of physiological amounts of plasma proteins. Surely, these results must be considered, when the inhibitors are used in *in vivo* experiments.

10.3.5 Determination of the selectivity of representative hyaluronidase inhibitors under “equiactive conditions”

Routinely, slightly different amounts of hyaluronidase were used in the turbidimetric assay to determine the inhibitory activities of investigated compounds: whereas comparable amounts of Hyal-1 and BTH were used in this thesis, a lower PH-20 concentration was used in the assays due to economic reasons. The incubation times were adjusted to obtain comparable substrate degradation. In chapters 6-9 a distinct selectivity of the inhibitors for human PH-20 *versus* the bovine homolog was derived from the IC_{50} values. As IC_{50} values are dependent on the assay conditions, this could possibly be the result of the different enzyme concentrations used in the turbidimetric assay. Due to that reason selected compounds were investigated using the turbidimetric assay under “equiactive conditions”, that means the concentrations of Hyal-1 and BTH were reduced to adjust the enzymatic activities to that of PH-20 used in routine. Furthermore, incubation periods were adjusted to exclude changes in the IC_{50} values which could result from degradation of the inhibitors due to limited stability in aqueous solution. The results are summarized in Table 10.3. IC_{50} values obtained using the standard assay conditions as in chapters 5-9 are added for comparative reasons.

Table 10.3. Inhibitory activities (selectivities) of representative compounds determined under standard assay conditions used in chapters 5-9 compared to the values determined under "equiactive conditions".

Compound	Hyal-1	PH-20	BTH	Hyal-1	PH-20	BTH
	IC_{50} [µM][a] (as determined in chapters 6-9)			IC_{50} [µM][a] (as determined under equiactive conditions)		
6.6	> 30	> 30	> 30	> 30	> 30	> 30
6.51	33 ± 1	2.3 ± 0.1	49 ± 1	10 ± 2	2.3 ± 0.1	42 ± 3
6.55	14 ± 2	1.4 ± 0.1	31 ± 1	n.d.	1.4 ± 0.1	26 ± 1
7.121	8.3 ± 0.4	2.0 ± 0.1	27 ± 1	8.8 ± 0.7	2.0 ± 0.1	23 ± 1
7.127	62 ± 4	10 ± 1	141 ± 4	40 ± 2	10 ± 1	164 ± 10
7.149	117 ± 9	4.5 ± 0.3	> 400	n.d.	4.5 ± 0.3	> 400
8.18	> 100	6.7 ± 0.6	> 100	> 100	6.7 ± 0.6	> 100
8.19	> 100	16 ± 2	> 100	n.d.	16 ± 2	≥ 100
8.64	> 80	3.6 ± 0.4	> 90	> 100	3.6 ± 0.4	> 100
Glycyrrhizin	26 ± 1	17 ± 1	737 ± 30	n.d.	17 ± 1	587 ± 11
Melophlin P	> 100	2.3 ± 0.1	> 100	> 100	2.3 ± 0.1	> 100

[a] inhibition of enzyme expressed as IC_{50} (µM), mean values ± SEM; IC_{50} values determined at pH 5.0 (PH-20, BTH) or 3.5 (Hyal-1) in the turbidimetric assay.

The trends in selectivity are comparable with those described in the previous chapters. All compounds, except **6.6,** for which no inhibition of the investigated enzymes was obtained, possess distinct selectivity for the human PH-20 *versus* the bovine homolog. IC_{50} values determined under "equiactive conditions" for Hyal-1 and BTH are significantly lower for the glucurono-6,3-lactones **6.51** and **6.55**. This was expected due to the reduced amount of Hyal-1 and BTH in the assay compared to the standard conditions. The enzyme selectivity of compound **6.51** and **6.55** for PH-20 *versus* the human isoenzyme drops marginally in both cases. When the ascorbic acid derivative **7.127** is regarded, even a decrease in inhibition at equiactive concentrations is observed for BTH. This surely is a result of the longer period of incubation: whereas the ester moiety of acylated vitamin C is stable under aqueous conditions for hours to days, and the alkyl ether in **7.127** should be stable in slightly acidic aqueous solution, the ascorbic acid core is known to be slowly degraded under these conditions[19, 20]. This effect is much more pronounced in neutral to basic solutions where the 3-OH of ascorbic acid is deprotonated compared to acidic conditions around pH 3.0, where this hydroxyl is protonated[20]. This pH-dependency of stability explains the results obtained at equiactive enzyme concentrations: the decrease in inhibitory potency against BTH (tested at pH 5.0) is significantly higher

compared to Hyal-1 (tested at pH 3.5). In the case of **7.121** lower IC_{50} values were determined, indicating that the degradation of the acylated ascorbic acid plays only a minor role. This is in agreement with investigations of 2-*O*-modified ascorbic acids which are known to possess increased stability[19]. Thus, the selectivity of **7.121** for the human PH-20 drops slightly, but the IC_{50} value for inhibition of BTH is still more than an order of magnitude higher when compared to the human homolog. The selectivity of the 3-*O*-alkylated ascorbic acid **7.149** for human PH-20 *versus* the bovine enzyme was proven, too. When the results for the investigated indoles (**8.18, 8.19** and **8.64**) are regarded, still no significant inhibition of Hyal-1 and BTH is observable in the investigated concentration ranges with the exception of **8.19,** where the enzymatic activity of BTH was reduced to 60 % in presence of 100 µM of this "bivalent" compound (data not shown). Thus, the remarkable selectivity of those compounds for human PH-20 compared to the other mammalian hyaluronidases is confirmed. The same observations were made for the melophlin derivative tested under "equiactive conditions". Glycyrrhizin follows the trend found for the glucurono-6,3-lactones, that is the inhibitory potency against BTH increased under "equiactive conditions". Nevertheless, this compound is still 35 times more potent when the human PH-20 is investigated under those assay conditions.

10.4 *Summary and conclusions*

In a fist attempt, selected inhibitors were characterized according to their surfactant properties. By measuring the surface tension, it was proven that ascorbic acid palmitate probably forms micelles under the conditions applied in the enzymatic assay used in this work. A CMC of 13 µM was determined. Thus, it cannot be ruled out that at least in some of the cases of lower inhibitory activity, the IC_{50} values are influenced by micelle formation, a phenomenon which reduces the concentration of free inhibitor monomer in solution. Considering these circumstances, the "bivalent" inhibitors are clearly advantageous, as no CMC was found for **7.112**, a representative compound of this class of inhibitors.

In preparation for *in vivo* experiments using the synthesized inhibitors, the hemolytic properties and the toxicity of selected compounds were determined: although weak hemolytic effects were induced by the investigated inhibitors at higher

concentrations, which are certainly due to the lipophilic alkyl chains, the compounds were generally inconspicuous at concentrations below 50-100 µM. In the chemosensitivity assay using U-373 MG and HT-29 cells the investigated inhibitors did not show any cytotoxic effects. Only one indole-based inhibitor significantly reduced cell growth at the highest concentration tested, but this effect is supposed to result from physical processes due to the limited solubility of this compound rather than from chemical reasons. Thus, the presented inhibitors should be applicable *in vivo*.

The investigation of protein binding revealed that the synthesized hyaluronidase inhibitiors as well as reference compounds known from the literature tend to bind to plasma proteins and thus share the fate of well known drugs with high affinity to various proteins. It was shown that in most cases roughly five molecules bind to one molecule serum albumin. This is not an exceptionally high value when it is compared to 15 molecules of the NSAID (and hyaluronidase inhibitor) indomethacin binding to one molecule of albumin[13]. Additionally, these results suggest a distinct interaction between protein and the hyaluronidase inhibitor rather than unspecific binding as supposed for strong detergents. These findings must be considered, when the inhibitors are used in the presence of high amounts of protein especially *in vivo*.

In the last part of this chapter, investigations on the selectivity of enzyme inhibition were performed. Testing at equiactive enzyme concentrations revealed selectivity of indole based inhibitors, melophlins, glucurono-6,3-lactone and ascorbic acid based inhibitors for human PH-20 over the bovine homolog.

10.5 *References*

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Chapter 11
Summary

There is a need for hyaluronidase inhibitors as pharmacological tools to study the (patho)physiological role of these enzymes. As such compounds could also be useful as drugs, e.g. in the treatment of (bacterial) infections, arthroses and cancer or as contraceptives, the goal of this thesis was the synthesis, identification and structural optimization of low molecular weight inhibitors.

The synthesized compounds were tested in a turbidimetric assay for inhibition of the recombinantly expressed human hyaluronidases Hyal-1 and PH-20, bovine testicular hyaluronidase (BTH) and a bacterial hyaluronate lyase (*Sag*Hyal$_{4755}$).

In a first attempt derivatives of the glucurono-6,3-lactone were synthesized and investigated for their inhibitory activity. Whereas 1-*O*-alkyl derivatives were inactive or only weakly active on human Hyal-1 and BTH, these compounds exhibited pronounced inhibition of human PH-20 and *Sag*Hyal$_{4755}$. The inhibitory potency against all investigated hyaluronidases was increased by adding polar substituents (IC_{50} values in the lower micromolar range). Furthermore, 1-*O*-alkyl-β-D-gluco-furanosiduronic acids and -uronamides were prepared which proved to be preferably inhibitors of PH-20 and *Sag*Hyal$_{4755}$.

In a second attempt various ascorbic acid derivatives with increased hydrophobicity were prepared, since the inhibitors ascorbic acid and its 6-*O*-palmitoyl derivative were proven to bind to the active center of a bacterial hyaluronate lyase by X-ray crystallography. A clear correlation between chain length of the 6-alkanoyl residue

and inhibitory potency was found. Whereas BTH, PH-20 and *Sag*Hyal$_{4755}$ were most potently inhibited by ascorbic acid 6-*O*-palmitate, strongest inhibition of Hyal-1 was achieved with the 6-*O*-tridecanoate. The differences in inhibitory activity became even more prominent when compounds bearing biaryl residues were investigated: rather small modifications of the terminal phenyl residue led to different SAR for all investigated hyaluronidases. Among the ascorbic acid derivatives 6-*O*-[11-(4-phenylphenoxy)undecanoyl]ascorbic acid was identified as one of the most potent low molecular weight inhibitors of human PH-20 known so far with an IC_{50} value of 1.3 µM. “Bivalent” inhibitors, characterized by two vitamin C moieties connected by alkanedioyl spacer groups, proved to be roughly equipotent with the corresponding “monovalent” ligands. By contrast, 5,6-di-*O*-acylation of ascorbic acid led to significantly enhanced inhibition compared to monoacyl derivatives. The differences in IC_{50} values due to diacylation were most evident for human PH-20 where up to 26-fold higher potency was obtained. Introduction of carboxylic acid residues led to potent inhibitors of the hyaluronidases, e.g. 2-*O*-(5-carboxypentanoyl)-6-*O*-hexadecanoyl-L-ascorbic acid (IC_{50} values: 8.3 µM (Hyal-1), 2.0 µM (PH-20), 27 µM (BTH), 2.8 µM (*Sag*Hyal$_{4755}$)) represents the most potent inhibitor of human Hyal-1 developed in this work and thus is one of the most potent inhibitors of Hyal-1 known to date. Additionally, 2-, 3- and 6-*O*-alkylated ascorbic acid derivatives were prepared with respect to higher stability under physiological conditions compared to carboxylic esters. Strikingly, distinct differences in the SAR for the mammalian and bacterial hyaluronidases are obvious in this series again.

The recently published crystal structure of human Hyal-1 was used as a 3-D model to perform an automated (FlexiDock) as well as a manual docking using potent ascorbic acid based inhibitors as ligands. The suggested binding modes are appropriate to explain several characteristics of the SAR and thus could provide the basis for further structure-based development of Hyal-1 inhibitors.

In a third synthetic approach indole based inhibitors were synthesized. Especially “bivalent” indole-3-butanoic acids, derivatives containing large hydrophobic fragments and manifold substituted indoles proved to be potent inhibitors of human PH-20 and bacterial hyaluronidase with selectivity over human Hyal-1 and BTH (e.g. 2-carboxy-5-(hexadecan-1-yl)oxy-1*H*-indole-3-butanoic acid, IC_{50} values: 1.3 µM

(PH-20) and 3.4 µM (*Sag*Hyal$_{4755}$)). These compounds are characterized by distinct selectivity for human PH-20 compared to the other investigated mammalian hyaluronidases. Previously, BTH has been broadly accepted as a model enzyme for human hyaluronidases. This working hypothesis is challenged by the results of this thesis as human PH-20 is strongly inhibited by some indoles and the bovine homolog is not.

Whereas additional approaches to develop carbohydrate- or peptide-based inhibitors failed, rather potent inhibitors of human PH-20 and *Sag*Hyal$_{4755}$ were identified among small series of melophlins and alkylphosphocholines.
With respect to planned *in vivo* investigations selected hyaluronidase inhibitors were investigated in more detail. Whereas cytotoxicity was not detected and micelle formation and hemolytic activity, respectively, became prominent at irrelevantly high concentrations the detected plasma protein binding must be considered in further investigations.

In summary, the compounds described in this thesis are among the most potent low molecular weight inhibitors of human and bacterial hyaluronidases known to date. Structure-activity relationships were studied in detail using purified human hyaluronidases for the first time. The SAR, which are surprisingly unique for the investigated hyaluronidases, in combination with molecular modeling and structural information could provide the basis for the rational design of more potent and selective inhibitors.

Chapter 12
Appendix

12.1 *Appendix 1: Elemental analysis data*

No	Formula	Calculated			Found		
		C	H	N	C	H	N
6.2	$C_{15}H_{18}O_6 \cdot 0.8H_2O$	58.36	6.40		58.12	6.16	
6.3	$C_{14}H_{24}O_6$	58.32	8.39		58.14	8.39	
6.4	$C_{14}H_{25}NaO_7 \cdot 0.6H_2O$	49.58	7.79		49.44	7.99	
6.6	$C_{22}H_{40}O_6$	65.97	10.07		65.97	10.25	
6.9	$C_{18}H_{33}NaO_7 \cdot 0.6H_2O$	54.70	8.72		54.52	8.66	
6.12	$C_{18}H_{35}NO_6$	59.81	9.76	3.87	59.53	9.34	3.45
6.13	$C_{29}H_{49}NO_6$	68.61	9.73	2.76	68.45	9.83	2.46
6.14	$C_{30}H_{51}NO_6$	69.06	9.86	2.68	68.94	9.95	2.24
6.15	$C_{26}H_{51}NO_6$	65.93	10.85	2.96	65.79	10.85	2.60
6.16	$C_{32}H_{63}NO_6$	68.9	11.38	2.51	68.55	11.53	2.14
6.17	$C_{24}H_{47}NO_7$	62.44	10.26	3.03	62.24	10.35	2.55
6.20	$C_{26}H_{49}NO_8$	62.00	9.81	2.78	61.92	9.92	2.44
6.21	$C_{27}H_{51}NO_8$	62.64	9.93	2.71	62.76	10.17	2.44
6.22	$C_{31}H_{57}NO_8$	65.12	10.05	2.45	64.91	10.41	2.09
6.23	$C_{25}H_{47}NO_8 \cdot 0.2H_2O$	60.88	9.69	2.84	60.59	9.77	2.56
6.25	$C_{30}H_{55}NO_8 \cdot 0.8H_2O$	62.97	9.97	2.45	62.72	9.72	2.07
6.27	$C_{27}H_{48}O_7$	66.91	9.98		66.77	10.18	
6.29	$C_{25}H_{41}NO_7$	64.22	8.84	3.00	64.07	9.19	2.61
6.30	$C_{23}H_{39}F_3O_8S$	51.87	7.38		51.81	7.32	
6.32	$C_{29}H_{46}O_6$	70.99	9.45		70.79	9.56	
6.36	$C_{30}H_{46}O_8$	67.39	8.67		67.13	8.41	
6.41	$C_{21}H_{34}O_9 \cdot 0.3H_2O$	57.86	8.00		57.99	8.23	

Elemental analysis data (continued)

6.42	$C_{25}H_{42}O_9$	61.71	8.70		61.71	8.66	
6.43	$C_{26}H_{44}O_9$	62.38	8.86		62.67	8.60	
6.47	$C_{25}H_{42}O_9$	61.71	8.70		62.03	8.76	
6.48	$C_{26}H_{44}O_9$	62.38	8.86		62.44	8.91	
6.51	$C_{28}H_{44}O_{12}\cdot 1.8H_2O$	55.58	7.93		55.58	7.71	
6.52	$C_{30}H_{48}O_{12}\cdot H_2O$	58.24	8.15		58.01	8.09	
7.2a	$C_{14}H_{22}O_7$	55.62	7.33		55.50	7.54	
7.2b	$C_{16}H_{26}O_7$	58.17	7.93		57.80	8.18	
7.2c	$C_{17}H_{28}O_7$	59.29	8.19		58.91	8.32	
7.2d	$C_{18}H_{30}O_7$	60.32	8.44		60.30	8.79	
7.2e	$C_{19}H_{32}O_7$	61.27	8.66		61.14	9.00	
7.2f	$C_{20}H_{34}O_7$	62.15	8.87		61.90	9.06	
7.2g	$C_{20}H_{18}O_7$	65.86	4.90		64.73	5.17	
7.3	$C_7H_{13}BrO_2$	40.21	6.27		40.00	6.05	
7.4	$C_{13}H_{18}O_2S\cdot 0.5H_2O$	63.12	7.74		63.48	7.63	
7.5	$C_{14}H_{20}O_2S$	66.63	7.99		66.44	8.22	
7.6	$C_{13}H_{18}O_3\cdot 0.4H_2O$	68.04	8.26		67.91	8.28	
7.7	$C_{15}H_{22}O_3\cdot 0.4H_2O$	69.95	8.92		69.95	8.74	
7.8	$C_{19}H_{22}O_3$	76.13	7.09		76.24	7.35	
7.9	$C_{20}H_{24}O_4$	73.15	7.37		73.11	7.49	
7.11	$C_{19}H_{22}O_3\cdot 0.2C_6H_4CH_3$	77.54	7.53		77.20	7.43	
7.12	$C_{12}H_{24}O_3\cdot 0.1C_6H_4CH_3$	67.63	11.08		67.54	11.46	
7.13	$C_{12}H_{24}O_3$	66.63	11.18		65.50	11.78	
7.14	$C_{14}H_{20}O_3\cdot 0.8H_2O$	67.07	8.68		67.19	8.57	
7.15	$C_{20}H_{24}O_3\cdot 0.4H_2O$	75.16	7.82		74.98	7.51	
7.16	$C_{13}H_{17}BrO_3$	51.84	5.69		51.98	5.67	
7.17	$C_{17}H_{20}O_3S$	67.08	6.62		67.01	6.72	
7.18	$C_{20}H_{24}O_4$	73.15	7.37		73.34	7.56	
7.19	$C_{20}H_{24}O_4$	79.28	6.94		78.92	6.87	
7.20	$C_{20}H_{24}O_3$	76.86	7.74		76.62	7.59	
7.21	$C_{20}H_{24}O_3\cdot 0.1CH_2Cl_2$	75.23	7.60		75.41	7.36	
7.22	$C_{23}H_{24}O_3$	79.28	6.94		78.92	6.87	
7.23	$C_{19}H_{22}O_4$	72.59	7.05		72.51	6.89	
7.24	$C_{19}H_{22}O_4$	72.59	7.05		72.77	7.16	
7.25	$C_{20}H_{22}O_5$	70.16	6.48		69.88	6.26	
7.26	$C_{20}H_{22}O_5\cdot 0.2H_2O$	69.43	5.53		69.55	6.30	
7.27	$C_{24}H_{30}O_5$	72.34	7.59		72.26	7.96	
7.28	$C_{24}H_{30}O_5$	72.34	7.59		72.24	7.46	
7.29	$C_{19}H_{22}O_4$	72.59	7.05		72.39	6.95	
7.30	$C_{22}H_{22}O_3\cdot 0.2H_2O$	78.17	6.68		78.37	7.06	

Elemental analysis (continued)

7.31	$C_{23}H_{28}O_5$	71.85	7.34		71.88	7.47	
7.32	$C_{23}H_{28}O_5$	71.85	7.34		71.54	7.47	
7.33	$C_{18}H_{28}O_3 \cdot 0.5H_2O$	71.72	9.70		71.68	9.64	
7.34	$C_{24}H_{32}O_3$	78.22	8.75		77.82	8.65	
7.34a	$C_{23}H_{30}O_3 \cdot 0.5H_2O$	76.00	8.60		76.16	8.94	
7.36	$C_{18}H_{28}O_3 \cdot 0.2Cl_2CH_2$	70.65	9.25		70.45	8.90	
7.37	$C_{18}H_{28}O_3$	73.93	9.65		73.88	9.59	
7.38	$C_{18}H_{28}O_3$	73.93	9.65		73.92	9.78	
7.39	$C_{17}H_{26}O_3$	73.34	9.41		73.13	9.79	
7.40	$C_{17}H_{26}O_3$	73.34	9.41		73.24	9.48	
7.41	$C_{17}H_{26}O_3$	73.34	9.41		73.22	10.00	
7.42	$C_{15}H_{30}O_2$	74.32	12.47		74.17	13.12	
7.43	$C_{18}H_{36}O_2$	62.88	5.72		62.81	6.04	
7.44	$C_{22}H_{44}O_2$	77.58	13.02		77.33	13.33	
7.46	$C_{20}H_{32}O_3 \cdot 0.01C_6H_4CH_3$	75.00	10.06		74.69	10.09	
7.47	$C_{24}H_{48}O_2 \cdot 0.3H_2O$	77.07	13.10		77.09	13.16	
7.48	$C_{18}H_{22}O_7S \cdot 0.5H_2O$	53.99	6.04		54.32	5.83	
7.49	$C_{19}H_{24}O_7S \cdot 0.7H_2O$	55.79	6.26		55.86	6.59	
7.50	$C_{18}H_{22}O_8 \cdot 0.3H_2O$	58.15	6.13		58.06	6.14	
7.51	$C_{20}H_{26}O_8 \cdot 0.3H_2O$	60.08	6.71		59.85	6.38	
7.52	$C_{24}H_{26}O_8$	60.32	8.44		60.30	8.79	
7.53	$C_{25}H_{28}O_9$	63.55	5.97		63.88	6.05	
7.54	$C_{19}H_{24}O_8$	59.99	6.36		59.65	6.68	
7.55	$C_{25}H_{28}O_8$	65.78	6.18		65.90	6.27	
7.56	$C_{22}H_{24}O_8S \cdot 0.3H_2O$	58.22	5.46		57.95	5.57	
7.57	$C_{25}H_{28}O_9$	63.55	5.97		63.84	5.91	
7.58	$C_{25}H_{28}O_8$	65.78	6.18		60.97	5.89	
7.59	$C_{25}H_{28}O_8$	65.78	6.18		65.96	6.56	
7.60	$C_{24}H_{26}O_9$	62.88	5.72		62.81	6.04	
7.61	$C_{24}H_{26}O_9$	62.88	5.72		62.91	5.93	
7.62	$C_{23}H_{32}O_8$	63.29	7.39		63.24	7.38	
7.63	$C_{29}H_{36}O_8$	67.95	7.18		68.17	7.08	
7.68	$C_{37}H_{44}O_8$	72.06	70.19		72.12	7.38	
7.69	$C_{37}H_{44}O_8$	72.06	7.19		71.83	6.99	
7.70	$C_{36}H_{50}O_7$	72.70	8.47		72.48	8.64	
7.71	$C_{35}H_{48}O_7$	72.38	8.33		72.58	8.15	
7.72	$C_{38}H_{54}O_7$	73.28	8.74		73.31	8.78	
7.74	$C_{40}H_{50}O_7$	74.74	7.84		74.98	7.66	
7.75	$C_{40}H_{50}O_8 \cdot 0.3H_2O$	72.33	7.68		72.35	7.86	
7.76	$C_{44}H_{66}O_7$	74.75	9.41		74.12	9.49	

Elemental analysis (continued)

7.77	$C_{39}H_{40}O_9$	71.76	6.18		71.76	6.24	
7.78	$C_{42}H_{40}O_8 \cdot 0.2H_2O$	74.58	6.02		74.45	6.39	
7.78a	$C_{64}H_{60}O_{10}$	77.71	6.11		77.26	6.67	
7.79	$C_{43}H_{46}O_{10}$	71.45	6.41		71.57	6.63	
7.80	$C_{43}H_{46}O_{10}$	71.45	6.41		71.23	6.43	
7.80a	$C_{66}H_{72}O_{14} \cdot 0.2H_2O$	72.53	6.68		72.31	6.87	
7.81	$C_{32}H_{42}O_8$	62.29	7.63		69.11	7.83	
7.82	$C_{32}H_{42}O_8$	62.29	7.63		69.01	7.68	
7.82a	$C_{44}H_{64}O_{10}$	70.18	8.57		70.39	8.86	
7.83	$C_{37}H_{44}O_8$	72.16	7.19		72.18	7.40	
7.85	$C_{39}H_{38}O_{10} \cdot 0.3H_2O$	69.63	5.79		69.64	5.85	
7.86	$C_{39}H_{38}O_{10} \cdot H_2O$	68.41	5.89		68.78	5.91	
7.86a	$C_{58}H_{56}O_{14}$	71.30	5.78		71.19	5.89	
7.87	$C_{23}H_{32}O_8 \cdot 0.2H_2O$	62.77	7.42		62.87	7.77	
7.88	$C_{23}H_{32}O_8 \cdot 0.4H_2O$	62.26	7.45		62.41	7.05	
7.89	$C_{22}H_{38}O_7 \cdot 0.4H_2O$	62.65	9.27		62.66	9.04	
7.90	$C_{21}H_{36}O_7$	62.98	9.06		62.41	9.61	
7.91	$C_{24}H_{42}O_7 \cdot 0.7H_2O$	63.33	9.61		63.32	9.30	
7.92	$C_{28}H_{50}O_7$	67.44	10.11		67.09	9.98	
7.93	$C_{26}H_{38}O_7 \cdot 0.3H_2O$	66.73	8.31		66.88	8.57	
7.94	$C_{26}H_{38}O_8 \cdot 0.3H_2O$	64.52	8.04		64.58	7.94	
7.96	$C_{25}H_{28}O_9$	63.55	5.97		63.86	6.07	
7.96a	$C_{44}H_{48}O_{12}$	68.74	6.29		68.43	5.91	
7.97	$C_{28}H_{28}O_8 \cdot 1.3H_2O$	65.18	5.98		65.13	5.68	
7.97a	$C_{50}H_{48}O_{10} \cdot 1.5EtOH$	72.5	6.54		72.45	6.56	
7.98	$C_{18}H_{30}O_8$	57.74	8.08		57.75	7.89	
7.98a	$C_{30}H_{52}O_{10}$	62.15	9.15		62.53	8.80	
7.99	$C_{18}H_{30}O_8$	57.74	8.08		57.95	8.44	
7.99a	$C_{30}H_{52}O_{10} \cdot 0.4H_2O$	62.13	9.18		61.96	9.44	
7.100	$C_{23}H_{32}O_8$	63.29	7.39		63.55	7.34	
7.100a	$C_{40}H_{56}O_{10} \cdot 0.5H_2O$	68.08	8.14		68.06	8.29	
7.101	$C_{18}H_{28}O_9$	55.66	7.27		55.55	7.60	
7.102	$C_{25}H_{26}O_{10} \cdot 0.3H_2O$	62.05	5.45		60.40	5.06	
7.103	$C_{25}H_{26}O_{10}$	61.72	5.39		61.54	5.30	
7.103a	$C_{44}H_{44}O_{14} \cdot 1.4H_2O$	64.29	5.74		64.19	5.58	
7.104	$C_{32}H_{42}O_7$	71.35	7.86		71.36	7.90	
7.105	$C_{42}H_{50}O_{10} \cdot 1.5H_2O$	68.00	7.20		67.93	6.84	
7.106	$C_{21}H_{32}O_{10} \cdot 1.2H_2O$	54.11	7.44		54.17	7.83	
7.107	$C_{52}H_{58}O_{14} \cdot 1.2H_2O$	67.26	6.56		67.10	6.44	
7.110	$C_{24}H_{34}O_{14} \cdot 3H_2O$	48.00	6.71		48.05	6.41	

Elemental analysis (continued)

7.111	$C_{26}H_{38}O_{14}\cdot H_2O$	52.70	8.80		52.58	7.10	
7.112	$C_{28}H_{42}O_{14}\cdot 2.5H_2O$	51.93	7.31		51.83	7.30	
7.118	$C_{31}H_{46}O_8$	68.11	8.48		68.06	8.78	
7.118	$C_{26}H_{42}O_{10}\cdot 0.8H_2O$	59.03	8.31		58.76	7.92	
7.120	$C_{27}H_{44}O_{10}$	61.34	8.39		61.01	8.40	
7.121	$C_{28}H_{46}O_{10}$	61.97	8.54		61.73	8.64	
7.121a	$C_{34}H_{54}O_{13}\cdot 1.5H_2O$	58.52	8.23		58.62	8.35	
7.127	$C_{18}H_{32}O_6$	62.77	9.36		62.84	9.56	
7.128	$C_{24}H_{44}O_6$	67.26	10.35		67.31	10.21	
7.129	$C_{27}H_{28}O_{10}\cdot 0.1CH_2Cl_2$	62.47	5.45		62.74	5.32	
7.131	$C_{36}H_{46}O_{11}$	66.04	7.08		66.42	7.42	
7.132	$C_{22}H_{34}O_{11}$	55.69	7.22		55.52	7.53	
7.134	$C_{18}H_{20}O_8\cdot 0.9CH_2Cl_2$	51.50	4.98		51.19	5.17	
7.136	$C_{32}H_{50}O_6$	72.42	9.50		72.28	9.83	
7.137	$C_{25}H_{26}O_8\cdot 0.3CH_2Cl_2$	63.31	5.59		62.91	5.43	
7.138	$C_{25}H_{26}O_8\cdot 0.3H_2O$	65.29	5.83		65.20	5.74	
7.140	$C_{22}H_{40}O_6$	65.97	10.07		65.92	10.19	
7.141	$C_{22}H_{22}O_8$	63.76	5.35		63.77	5.48	
7.142	$C_{22}H_{22}O_8$	63.76	5.35		63.67	5.59	
7.143	$C_{34}H_{44}O_9$	68.44	7.43		77.37	7.49	
7.145	$C_{34}H_{44}O_9$	68.44	7.43		68.38	7.57	
7.146	$C_{20}H_{32}O_9$	57.68	7.74		57.74	8.03	
7.147	$C_{31}H_{38}O_{10}$	65.25	6.71		64.91	6.24	
7.148	$C_{20}H_{32}O_9$	57.68	7.74		57.65	8.03	
7.150	$C_{20}H_{36}O_6\cdot 0.3H_2O$	63.57	9.56		63.52	9.66	
8.7	$C_{21}H_{21}NO_3$	75.20	6.31	4.18	74.80	6.36	3.92
8.8	$C_{22}H_{31}NO_3$	73.91	8.74	3.92	73.37	9.30	3.79
8.8a	$C_{28}H_{42}N_2O_7$	64.84	8.16	5.40	63.15	8.54	5.08
8.9	$C_{19}H_{16}ClNO_3$	66.77	4.72	4.10	66.60	4.71	4.06
8.14	$C_{22}H_{18}O_4$	76.29	5.24		76.42	5.33	
8.15	$C_{20}H_{22}O_4$	73.60	6.79		73.73	7.01	
8.16	$C_{24}H_{30}O_4$	72.36	7.91		75.21	7.83	
8.17	$C_{28}H_{38}O_4$	76.68	8.73		76.48	8.54	
8.19	$C_{34}H_{32}N_2O_6\cdot 0.5H_2O$	71.19	5.80	4.88	71.31	5.63	4.83
8.20	$C_{32}H_{36}N_2O_6\cdot 0.25H_2O$	69.99	6.17	5.10	69.86	6.77	5.04
8.21	$C_{36}H_{44}N_2O_6\cdot 0.5H_2O$	70.91	7.44	4.59	70.81	7.23	4.33
8.22	$C_{40}H_{52}N_2O_6\cdot 0.25H_2O$	72.64	8.00	4.24	72.37	8.18	4.03
8.32	$C_{23}H_{33}NO_4\cdot 0.1H_2O$	70.96	8.60	3.60	70.83	8.88	3.36
8.34	$C_{30}H_{43}N_3O_3S\cdot 0.4H_2O$	67.61	8.28	7.88	67.65	8.37	8.01
8.40	$C_{38}H_{56}N_2O_4\cdot 0.4H_2O$	74.57	9.35	4.58	74.85	9.73	4.50

Elemental analysis (continued)

8.44	$C_{27}H_{44}N_4O_3$	70.87	9.39	6.87	70.43	9.83	6.36
8.52	$C_{24}H_{27}NO_5 \cdot 0.75H_2O$	68.15	6.79	3.31	68.29	7.02	3.51
8.53	$C_{20}H_{19}NO_5 \cdot 0.2H_2O$	67.29	5.28	3.92	67.00	5.46	3.82
8.57	$C_{26}H_{31}NO_5 \cdot 0.4H_2O$	70.22	7.21	3.15	70.23	7.44	3.41
8.58	$C_{23}H_{33}NO_5$	68.46	8.24	3.47	68.74	8.19	3.30
8.62	$C_{22}H_{23}NO_5 \cdot 0.2H_2O$	68.63	6.13	3.64	68.50	6.09	3.33
8.63	$C_{19}H_{25}NO_5$	65.69	7.25	4.03	65.81	7.53	3.96
8.64	$C_{25}H_{37}NO_5$	69.58	8.64	3.25	69.47	8.48	3.01
8.65	$C_{29}H_{45}NO_5$	71.42	9.30	2.87	71.37	9.37	2.56
8.66	$C_{31}H_{49}NO_5$	71.69	9.59	2.70	71.68	9.40	2.54
8.67	$C_{36}H_{59}NO_5 \cdot 0.2H_2O$	73.35	10.16	2.38	73.32	10.37	2.44
8.68	$C_{39}H_{58}N_2O_5 \cdot 0.2H_2O$	73.36	9.22	4.39	73.12	9.32	4.32
8.70	$C_{32}H_{51}NO_5$	72.55	9.70	2.64	72.61	9.78	2.32
8.71	$C_{35}H_{50}N_2O_5 \cdot HCl$	68.33	8.36	4.55	70.94	8.51	4.32
8.72	$C_{36}H_{50}BrNO_5$	65.84	7.67	2.13	65.80	7.64	1.94
8.73	$C_{34}H_{47}NO_5$	74.28	8.62	2.55	74.25	8.69	2.31
8.74	$C_{32}H_{43}NO_5$	73.67	8.31	2.68	73.47	8.20	2.47
8.75	$C_{30}H_{39}NO_5$	72.99	7.96	2.84	73.16	8.09	2.62
8.76	$C_{25}H_{37}NO_5$	69.58	8.64	3.25	69.23	8.44	2.97

12.2 *Appendix 2: Abbreviations*

Ac	Acetyl
Bn	Benzyl
BSA	Bovine serum albumin
BSE	Bovine spongiform encephalopathy
BTH	Bovine testicular hyaluronidase
BVH	Bee venom hyaluronidase
CDI	*N*,*N*'-Carbonyldiimidazole
CAZY	Carbohydrate active enzyme database
CD44	Cluster determinant 44
CMC	Critical micelle concentration
Cmpd	Compound
CTAB	Cetyltrimethylammonium bromide
cy	Cyclohexyl
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene

DCC	*N*,*N*′-Dicyclohexylcarbodiimide
DEPT	Distortionless enhancement by polarization transfer
DIAD	Diisopropyl azodicarboxylate
DIEA	*N*,*N*-Diisopropylethylamine
DMAP	4-(Dimethylamino)pyridine
DMF	*N*,*N*-Dimethylformamide
DSCG	Disodium cromoglycate
DMSO	Dimethylsulfoxide
ECM	Extracellular matrix
EDAC	*N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride
ENTV	Enzootic nasal tumor virus
EtOAc	Ethyl acetate
GAG	Glucosaminoglycane
GlcNAc	*N*-Acetylglucosamine
GlcUA	Glucuronic acid
GPI	Glycosylphosphatidylinositol
h	Hours
HA	Hyaluronic acid
HARE	Hyaluronan receptor for endocytosis
HAS	Hyaluronan synthase
HOAc	Acetic acid
HOBT	1-Hydroxybenzotriazole
HPLC	High-pressure liquid chromatography
HR-MS	High resolution mass spectroscopy
HSA	Human serum albumin
hylSpn	*S. pneumoniae* hyaluronidase
IC_{50}	concentration of an inhibitor required to give 50% inhibition of enzyme activity
IPE	Diisopropylether
JSRV	Jaagsiekte sheep retrovirus
LUCA-1	Lung cancer gene 1
LYVE-1	Lymphatic vessel endothelial HA receptor
LEC	Liver endothelial cell clearance

mp	Melting point
MS	Mass spectrometry
Mw	Molecular weight
n.d.	Not determined
NMR	Nuclear magnetic resonance
NSAID	Non-steroidal anti-inflammatory drug
OD	Optical density
pH	Negative logarithm of the hydrogen ion concentration
ppm	Parts per million
RT	Room temperature
*Sag*Hyal$_{3502}$	Hyaluronate lyase of *S. agalactiae* strain 3502
*Sag*Hyal$_{4755}$	Hyaluronate lyase of *S. agalactiae* strain 4755
SAR	Structure-activity relationships
SEM	Standard error of the mean
SPAM1	Sperm adhesion molecule
TBAB	Tetrabutylammonium bromide
TBME	*tert*-Butyl methyl ether
TBTA	*tert*-Butyl 2,2,2-trichloroacetimidate
TEMPO	2,2,6,6-Tetramethylpiperidine 1-oxyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TIM	Triose phosphate isomerase
TLC	Thin layer chromatography
TLR-4	Toll like receptor 4
TMSCl	Chlortrimethylsilane
PE	Petrol ether
PG	Protecting group
RHAMM	Receptor for hyaluronate-mediated motility

12.3 *Appendix 3: List of publications and poster presentations*

Hofinger E.S.A., Spickenreither M., Oschmann J., Bernhardt G., Rudolph R., Buschauer A., Recombinant human hyaluronidase Hyal-1: insect cells versus E. coli as expression system and identification of low molecular weight inhibitors. *Glycobiology* **2007**, 17 (4), 444-453.

Spickenreither M., Braun S., Bernhardt G., Dove S., Buschauer A., Novel 6-*O*-acylated vitamin C derivatives as hyaluronidase inhibitors with selectivity for bacterial lyases. *Bioorg. Med. Chem. Lett*. **2006**, 16 (20), 5313-5316.

Spickenreither M., Hofinger E., Bernhardt G., Dove S., Buschauer A., Lipophilic vitamin C derivatives are potent inhibitors of bacterial and human hyaluronidases. (2007) Annual Meeting of the German Pharmaceutical Society (DPhG), Universität Erlangen-Nürnberg, Erlangen, Germany. Abstract no. A17

Spickenreither M., Hofinger E.S.A., Bernhardt G., Dove S., Buschauer A., Ascorbic acid derivatives as potent inhibitors of bacterial and human hyaluronidases. (2007) Gordon Research Conference on Medicinal Chemistry, New London, USA.

Spickenreither M., Hofinger E., Bernhardt G., Dove S., Buschauer A., Ascorbic acid derivatives with increased lipophilicity as potent inhibitors of bacterial and human hyaluronidases. (2007) Annual Meeting “Frontiers in Medicinal Chemistry”, Berlin, Germany. Abstract no. HMC14

Spickenreither M., Hofinger E., Bernhardt G., Dove S., Buschauer A., Acylated and alkylated vitamin C derivatives - potent inhibitors of bacterial and mammalian hyaluronidases. (2006) 3rd Summer School Medicinal Chemistry, University of Regensburg, Germany. Abstract no. 67

Spickenreither M., Hofinger E., Bernhardt G., Dove S., Buschauer A., L-ascorbic acid derivatives as inhibitors of bacterial and mammalian hyaluronidases. (2006) XIXth International Symposium on Medicinal Chemistry, Istanbul, Turkey.
Abstract published in: *Drugs of the Future* **31** (Suppl. A), 125.

Spickenreither M., Bernhardt G., Dove S., Buschauer A., Alkyl-D-glucurono-6,3-lactones and derivatives: potent inhibitors of hyaluronidases. (2005) Annual Meeting of the German Pharmaceutical Society (DPhG), Johannes Gutenberg University of Mainz, Germany. Abstract no. C109

Spickenreither M., Braun S., Botzki A., Jedrzejas M., Bernhardt G., Dove S., von Angerer E., Buschauer A., Design, synthesis and structure-activity relationships of acylated indoles and benzoxazoles as inhibitors of bacterial hyaluronidase (2005) 6th Carbohydrate Bioengineering Meeting, Barcelona, Spain.
Abstract published in: *Book of abstracts*, P87 (ISBN: 84-609-4444-1)

Spickenreither M., Braun S., Botzki A., Jedrzejas M., Bernhardt G., Dove S., von Angerer E., Buschauer A., (2005) Acylated indoles and benzoxazoles as potential hyaluronidase inhibitors: Design, synthesis and structure-activity relationships, Annual Meeting “Frontiers in Medicinal Chemistry”, Leipzig, Germany. Abstract no. PSA02

Erklärung

Ich erkläre hiermit an Eides statt, dass ich die vorliegende Arbeit ohne unzulässige Hilfe Dritter und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe; die aus anderen Quellen direkt oder indirekt übernommenen Daten und Konzepte sind unter Angabe des Literaturzitats gekennzeichnet.

Regensburg, im November 2007

(Martin Spickenreither)