Acylguanidines as bioisosteric groups in argininamide-type neuropeptide Y Y_1 and Y_2 receptor antagonists: synthesis, stability and pharmacological activity

Dissertation

zur Erlangung des Doktorgrades der Naturwissenschaften (Dr. rer. nat.)
der Naturwissenschaftlichen Fakultät IV – Chemie und Pharmazie –
der Universität Regensburg

vorgelegt von
Albert Brennauer
aus Schongau
2006

Die vorliegende Arbeit entstand in der Zeit von Juni 2000 bis September 2006 unter der Leitung von Herrn Prof. Dr. A. Buschauer am Institut für Pharmazie der Naturwissenschaftlichen Fakultät IV – Chemie und Pharmazie – der Universität Regensburg.

Das Promotionsgesuch wurde eingereicht im August 2006

Tag der mündlichen Prüfung: 11. September 2006

Prüfungsausschuss:

Prof. Dr. A. Göpferich	(Vorsitzender)
Prof. Dr. A. Buschauer	(Erstgutachter)
Prof. Dr. B. König	(Zweitgutachter)
Prof. Dr. S. Elz	(Drittprüfer)

für meine Familie

If you want to have good ideas you must have many ideas. Most of them will be wrong, and what you have to learn is which ones to throw away.

Linus Pauling, Nobel Prize 1954 (Chemistry) and 1962 (Peace)

Danksagungen

An dieser Stelle möchte ich mich bedanken bei:

Herrn Prof. Dr. A. Buschauer für die interessante Themenstellung, für seine wissenschaftlichen Anregungen, seine Förderung und für seine konstruktive Kritik bei der Durchsicht dieser Arbeit,

Herrn Prof. Dr. G. Bernhardt und Herrn Prof. Dr. S. Dove für ihre stete Unterstützung und wissenschaftlichen Ratschläge,

Frau E. Schreiber für die geduldige und zuverlässige Durchführung der pharmakologischen Testung,

Herrn Dr. Ralf Ziemek für die Ausdauer bei der Bestimmung von Bindungskonstanten und für das freundschaftliche Klima – vor allem während der Zeit des Zusammenschreibens,

Herrn M. Keller für die HPLC-Untersuchungen sowie die engagierte und anregende Zusammenarbeit an dem gemeinsamen Projekt,

Herrn M. Freund für den kollegialen und inspirierenden Austausch bei der täglichen Laborarbeit und der gemeinsamen Frustbewältigung bei der Optimierung von Synthesen,

Herrn Dr. Th. Suhs für den fachlichen Gedankenaustausch über Syntheseprobleme im Zusammenhang mit N^G-acylierten Argininen,

Frau Dr. S. Salmen, Herrn Dr. S. Braun und Herrn M. Spickenreither für die kollegiale und entspannte Atmosphäre im gemeinsamen Syntheselabor,

allen Mitarbeitern der Betriebseinheit „Zentrale Analytik“ der Fakultät für die sorgfältige und rasche Durchführung der analytischen Messungen (NMR, Massenspektrometrie, Elementaranalyse), sowie für die geduldige Beantwortung von Fragen,

allen Mitarbeitern der Elektronik-, Glasbläser- und Feinmechanikwerkstätten der Fakultät für den fachkundigen und engagierten Service,

Frau S. Heinrich, Frau M. Luginger und Herrn P. Richthammer für ihre freundliche Hilfe bei technischen und organisatorischen Problemen aller Art,

allen Mitgliedern des Lehrstuhls für ihre Kollegialität, Hilfsbereitschaft und das gute Arbeitsklima,

der Mannschaft von „Arminia Buschauer“ für unvergessliche Fußballmomente,

dem Graduiertenkolleg 760 der DFG für die wissenschaftliche Förderung,

Frau Dr. S. Salmen und Herrn Dr. A. Botzki für langjährige Freundschaft, die wertvolle Hilfe in vielen Lebenslagen und für die schöne gemeinsame Zeit am Lehrstuhl,

meinen Eltern und Schwiegereltern für ihre großartige Unterstützung und ihren Beistand in all den Jahren,

meiner Ehefrau Uta Lungwitz für ihre unerschütterliche Kraft und Liebe, und unseren Sohn Jona für die Freude, die er uns schenkt trotz der Zeit, die er seine Eltern entbehren musste.

Contents

Chapter 1: Neuropeptide Y and the Neuropeptide Y Receptor Family 1

1. Neuropeptide Y and Related Peptides 1
2. Neuropeptide Y Receptor Subtypes 3
3. References 4

Chapter 2: Structure-Activity Relationships of Non-Peptide NPY Receptor Antagonists 7

1. Introduction 7
2. First Nonspecific NPY Receptor Antagonists 10
3. Potent and Selective Non-Peptide NPY Y_1 Receptor Antagonists 13
4. Selective Non-Peptide Y_2 Receptor Antagonists 31
5. NPY Y_5 Receptor Antagonists 34
6. Conclusion 48
7. References 49

Chapter 3: Scope of the Thesis 67

Chapter 4: Overview Over the Synthetic Methods for the Preparation of NPY Y_1 and Y_2 Receptor Antagonistic Argininamides 69

1. Introduction 69
2. Retrosynthesis 71
3. Peptide Bond Formation 74
4. Protective Group Chemistry 80
5. Guanidinylation Chemistry 86
6. Arginines from Isoglutaminols 91
7. References 94

Chapter 5: On the stability of 1-(ω-aminoalkanoyl)-guanidines under alkaline conditions 99

1. Introduction 99
2. Results and Discussion 100
3. Conclusion 109
4. Experimental Section 110
5. References 117

Chapter 6: Towards the Development of NPY Y_1-Receptor Selective Tracers 121

1. Introduction 121
2. Results and Discussion 124
3. Conclusion 132
4. Experimental Section 133
5. References 176

Chapter 7: Synthesis and Y_2R Antagonistic Activity of N^{ω}-Substituted Argininamides 181

1. Introduction 181
2. Results and Discussion 182
3. Summary and Conclusion 193
4. Experimental Section 194
5. References 248

Chapter 8: Summary 251

Appendix

1. List of Abbreviations and Acronyms 255
2. Calculation of First Order Rate Constants 259
3. Spectrofluorimetric Ca^{2+} Assay 262
4. List of Publications and Abstracts 265
5. Curriculum Vitae 266

1 Neuropeptide Y and the Neuropeptide Y Receptor Family

1. Neuropeptide Y and Related Peptides

Neuropeptide Y* (NPY) is one of the most abundant neuropeptides in the central and peripheral nervous system[1]. It was first isolated from porcine brain in 1982 by Tatemoto and coworkers[2]. Together with the homologous peptides pancreatic polypeptide (PP) and peptide YY (PYY), NPY belongs to the pancreatic polypeptide family, also called neuropeptide Y peptide family. Neuropeptide Y consists of 36 amino acids and is C-terminally amidated; its sequence is highly conserved in various species[3].

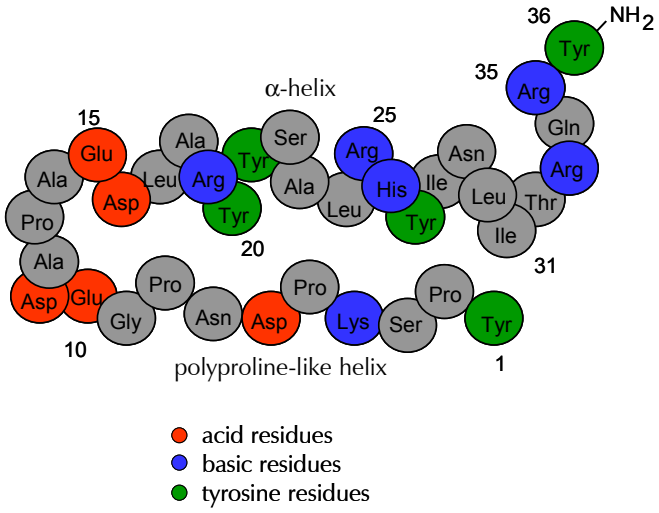


Fig. 1: Sequence of porcine NPY. The residues are arranged according to the crystal structure of the homolog aPP. In this conformation the N-terminal residues 1-8 form a polyproline-like helix, followed by a β-turn (9-13), and an α-helical region (14-31); the C-terminus (amino acids 32-36), where the residues are located that are most crucial for receptor recognition, is rather unordered and flexible. This "hairpin-like" conformation is stabilized by hydrophobic interactions between the polyproline-like and the α-helix.

* Neuropeptide Y owes his name to its tyrosine rich sequence (Y stands for tyrosine in the one-letter code).

The tertiary structure of NPY, and in particular its active conformation(s), have been the subject of numerous studies. One of the first models was based on the crystal structure of the avian pancreatic polypeptide (50 % homology)[4]. In this conformation strong hydrophobic contacts between the N-terminal polyproline-like helix and the α-helical region, formed by the C-terminus, provide an antiparallel "hairpin-like" fold that brings N- and C-terminal residues into close spatial proximity (cf. Fig. 1). This so called "pp-fold" model is in good agreement with the structure-activity relationships of shortened, discontinuous, and cyclic peptide NPY receptor ligands[5-7]. Later on, the solution structure of NPY was investigated by several groups using NMR techniques and CD-spectroscopy[8-13]. However, the pp-fold could not be confirmed as the prevalent conformation of NPY in solution. While all authors observed an α-helical section in the C-terminal half of NPY, the proposed conformations of the N-terminus were differing.

The formation of dimeric NPY aggregates, stabilized by intermolecular hydrophobic interactions of the amphiphilic α-helices ("handshake dimer") was described in the studies of Cowley et al.[8] and Monks et al.[11]. The solution structure of NPY is strongly influenced by the polarity and the pH value of the solution, the concentration of the analyte, and the temperature. At neutral pH, the water solubility of NPY is insufficient to achieve the concentrations required for 2D-NMR measurements; therefore the NMR-based structure determinations were carried out at low pH values and/or in the presence of co-solvents. Considering the results from CD-spectroscopic measurements at various concentrations and pH-values, Nordmann et al.[12] pointed out that different conformations of NPY coexist in a dynamic equilibrium, and that the monomeric pp-fold conformation is more favored under physiological conditions (low concentration, neutral pH). Furthermore, peptide-lipid interactions at the surface of the cell membrane were discussed, to support the formation of the active conformation of neuropeptide Y[14-16].

Neuropeptide Y is located in peripheral neurons of the sympathetic nervous system, where it is stored together with noradrenaline and acts as a cotransmitter (for recent reviews on the pharmacology of NPY and its receptors cf. [17, 18]). In the human central nervous system (CNS) NPY is one of the most abundant neuropeptides; high levels of NPY are found in numerous brain regions including the basal ganglia, hippocampus, amygdala, and hypothalamus. Also in central neurons neuropeptide Y is colocalized with other neurotransmitters such as noradrenaline, GABA or agouti-related peptide (AGRP).

The most prominent attribute of NPY is the strong orexigenic effect, triggered by central administration of the peptide. The important role of NPY in the regulation of appetite and food intake made neuropeptide Y receptors attractive targets for potential anti-obesity drugs. Apart from its role in the control of feeding behavior, NPY is involved in the regulation of several further physiological functions, including anxiolysis, memory retention, seizure activity, alcohol consumption, and vaso-constriction.

2. Neuropeptide Y Receptor Subtypes

The biological effects of NPY are mediated by different receptor subtypes which are all members of the large superfamily of G-protein-coupled receptors (GPCRs). In mammals five neuropeptide Y receptor subtypes have been described, denoted as Y_1, Y_2, Y_4, Y_5, and y_6[19]. All these receptors have been cloned, and belong to the rhodopsin-like (class A) GPCRs, and they are predominantly coupled to $G_{i/o}$ proteins. Table 1 gives a short overview of the main features of the mammalian NPY receptor subtypes. The individual subtypes are distinguished by characteristic selectivities for NPY analogs with altered or truncated sequences. For instance, NPY analogs, lacking the N-terminus (e.g. NPY_{2-36}, NPY_{3-36}, or NPY_{13-36}) are full Y_2R agonists, but have dramatically reduced affinities at the Y_1 receptor. Conversely, the C-terminally modified analog [Leu^{31}, Pro^{34}]NPY is a potent Y_1 receptor agonist, but has no affinity at the Y_2 receptor. NPY_{2-36} and [Leu^{31}, Pro^{34}]NPY both are active at the Y_5 receptor,

which plays an important role in the regulation of food intake. Due to its preference for PP over NPY and PYY, the Y_4 receptor sometimes was referred to as "PP preferring receptor". Unlike the human y_6 receptor the murine receptor is functionally active and has been characterized by binding profiles[20, 21].

Table 1: Overview of binding profiles, localization and physiological role of the mammalian receptor subtypes Y_1, Y_2, Y_4, Y_5, and y_6[22-24].

	Binding Profile	Localization	Physiological Role
Y_1	NPY ≈ PYY ≈ [L^{31}, P^{34}]NPY > NPY_{2-36} > NPY_{3-36} ≥ PP > NPY_{13-36}	smooth vascular muscles (postjunctionally), cerebral cortex, hypothalamus, colon, human adipocytes	regulation of blood pressure, seizures, stimulation of food intake, anxiolysis, sedation
Y_2	NPY ≥ NPY_{2-36} ≈ NPY_{3-36} ≈ NPY_{13-36} >> [L^{31}, P^{34}]NPY	hippocampus, hypothalamus, nerve ends (pre- and postjunctional), renal tubulus	presynaptic inhibition of neurotransmitter release, regulation of seizures, anxiety, pain sensitivity, food intake
Y_4	PP > PYY ≥ NPY > NPY_{2-36}	colon, intestine, prostate, CNS, coronary arteries	gastrointestinal effects
Y_5	NPY ≈ PYY ≈ NPY_{2-36} > hPP > [D-W^{32}]NPY > NPY_{13-36} > rPP	hypothalamus, hippocampus, amygdala	Stimulation of food intake
y_6	NPY ≈ PYY ≈ [L^{31}, P^{34}]NPY >> PP[21] **or** PP > [L^{31}, P^{34}]NPY > NPY ≈ PYY[20]	cardiac and skeletal muscles	pseudogene in humans (functional in mice and rabbits, absent in rats)

3. Reference List

[1] Gray, T. S.; Morley, J. E., Neuropeptide Y: anatomical distribution and possible function in mammalian nervous system. *Life Sci.* **1986**, 38, 389-401.

[2] Tatemoto, K.; Carlquist, M.; Mutt, V., Neuropeptide Y—a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. *Nature* **1982**, 296, 659-60.

[3] Larhammar, D., Evolution of neuropeptide Y, peptide YY and pancreatic polypeptide. *Regul. Pept.* **1996**, 62, 1-11.

[4] Allen, J.; Novotny, J.; Martin, J.; Heinrich, G., Molecular structure of mammalian neuropeptide Y: analysis by molecular cloning and computer-aided comparison with crystal structure of avian homologue. *Proc. Natl. Acad. Sci. U. S. A.* **1987**, 84, 2532-6.

[5] Reymond, M. T.; Delmas, L.; Koerber, S. C.; Brown, M. R.; Rivier, J. E., Truncated, branched, and/or cyclic analogues of neuropeptide Y: importance of the pancreatic peptide fold in the design of specific Y_2 receptor ligands. *J. Med. Chem.* **1992**, 35, 3653-9.

[6] Beck-Sickinger, A. G.; Jung, G., Structure-activity relationships of neuropeptide Y analogues with respect to Y_1 and Y_2 receptors. *Biopolymers* **1995**, 37, 123-42.

[7] Mörl, K.; Beck-Sickinger, A. G., Structure-Activity Relationships of Peptide-Derived Ligands at NPY Receptors. In *Handbook of Experimental Pharmacology*, Michel, M. C., Ed. Springer: Berlin · Heidelberg · New York, 2004; Vol. 162, pp 479-504.

[8] Cowley, D. J.; Hoflack, J. M.; Pelton, J. T.; Saudek, V., Structure of neuropeptide Y dimer in solution. *Eur. J. Biochem.* **1992**, 205, 1099-106.

[9] Darbon, H.; Bernassau, J. M.; Deleuze, C.; Chenu, J.; Roussel, A.; Cambillau, C., Solution conformation of human neuropeptide Y by ^{1}H nuclear magnetic resonance and restrained molecular dynamics. *Eur. J. Biochem.* **1992**, 209, 765-71.

[10] Mierke, D. F.; Durr, H.; Kessler, H.; Jung, G., Neuropeptide Y. Optimized solid-phase synthesis and conformational analysis in trifluoroethanol. *Eur. J. Biochem.* **1992**, 206, 39-48.

[11] Monks, S. A.; Karagianis, G.; Howlett, G. J.; Norton, R. S., Solution Structure of Human Neuropeptide Y. *J. Biomol. NMR* **1996**, 403, 379-90.

[12] Nordmann, A.; Blommers, M. J.; Fretz, H.; Arvinte, T.; Drake, A. F., Aspects of the molecular structure and dynamics of neuropeptide Y. *Eur. J. Biochem.* **1999**, 261, 216-26.

[13] Saudek, V.; Pelton, J. T., Sequence-specific ^{1}H NMR assignment and secondary structure of neuropeptide Y in aqueous solution. *Biochemistry* **1990**, 29, 4509-15.

[14] Bader, R.; Bettio, A.; Beck-Sickinger, A. G.; Zerbe, O., Structure and dynamics of micelle-bound neuropeptide Y: comparison with unligated NPY and implications for receptor selection. *J. Mol. Biol.* **2001**, 305, 307-29.

[15] Lerch, M.; Mayrhofer, M.; Zerbe, O., Structural similarities of micelle-bound peptide YY (PYY) and neuropeptide Y (NPY) are related to their affinity profiles at the Y receptors. *J. Mol. Biol.* **2004**, 339, 1153-68.

[16] Thomas, L.; Scheidt, H. A.; Bettio, A.; Huster, D.; Beck-Sickinger, A. G.; Arnold, K.; Zschornig, O., Membrane interaction of neuropeptide Y detected by EPR and NMR spectroscopy. *Biochim. Biophys. Acta* **2005**, 1714, 103-13.

[17] Pedrazzini, T.; Pralong, F.; Grouzmann, E., Neuropeptide Y: the universal soldier. *Cellular and Molecular Life Sciences (CMLS)* **2003**, 60, 350-77.

[18] Michel, M. C., *Neuropeptide Y and Related Peptides*. In Handbook of Experimental Pharmacology, Springer: Berlin · Heidelberg · New York, 2004; Vol. 162.

[19] Michel, M. C.; Beck-Sickinger, A. G.; Cox, H.; Doods, H. N.; Herzog, H.; Larhammer, D.; Quirion, R.; Schwartz, T.; Westfall, T., XVI. International Union of Pharmacology Recommandations for the Nomenclature of Neuropeptide Y, Peptide YY, and Pancreatic Polypeptide Receptors. *Pharmacol. Rev.* **1998**, 50, 143-50.

[20] Gregor, P.; Millham, M. L.; Feng, Y.; DeCarr, L. B.; McCaleb, M. L.; Cornfield, L. J., Cloning and characterization of a novel receptor to pancreatic polypeptide, a member of the neuropeptide Y receptor family. *FEBS Lett.* **1996**, 381, 58-62.

[21] Weinberg, D. H.; Sirinathsinghji, D. J.; Tan, C. P.; Shiao, L. L.; Morin, N.; Rigby, M. R.; Heavens, R. H.; Rapoport, D. R.; Bayne, M. L.; Cascieri, M. A.; Strader, C. D.; Linemeyer, D. L.; MacNeil, D. J., Cloning and expression of a novel neuropeptide Y receptor. *J. Biol. Chem.* **1996**, 271, 16435-8.

[22] Schneider, E.; Mayer, M.; Ziemek, R.; Li, L.; Hutzler, C.; Bernhardt, G.; Buschauer, A., A Simple and Powerful Flow Cytometric Method for the Simultaneous Determination of Multiple Parameters at G Protein-Coupled Receptor Subtypes. *ChemBioChem* **2006**, *in press*.

[23] Schneider, E. Development of Fluorescence-Based Methods for the Determination of Ligand Affinity, Selectivity and Activity at G-Protein Coupled Receptors. Ph.D. thesis, University of Regensburg, Regensburg, **2005**.

[24] Ziemek, R. Development of binding and functional assays for the neuropeptide Y Y_2 and Y_4 receptors. Ph.D. thesis, University of Regensburg, Regensburg, **2006**, http://www.opus-bayern.de/uni-regensburg/volltexte/2006/679/.

2 Structure-Activity Relationships of Non-Peptide NPY Receptor Antagonists*

Abstract – **Reports in 1990 on some weakly to moderately active non-peptides which were not originally designed for NPY receptors, followed by the discovery of the first highly potent and selective Y_1 receptor antagonists, the (*R*)-argininamide BIBP 3226 and the benzamidine derivative SR 120819A, as well as raising hope for novel drug treatment of hypertension, obesity and metabolic diseases stimulated the search for NPY-blocking compounds. Most of the currently known non-peptidic NPY antagonists are ligands of Y_1 or Y_5 receptors, whereas only one class of Y_2 selective antagonists around the (*S*)-arginine derivative BIIE 0246 has been disclosed. Non-peptidic ligands of the Y_4 receptor are not known. In some cases the design of Y_1 antagonists followed rational strategies considering amino acids which are essential for binding to Y_1 and/or Y_2 receptors according to results of complete alanine scan of NPY. Typical Y_1 antagonists (e.g. compounds of the argininamide, benzamidine, benzimidazole, indole and aminopyridine series) have one or two basic groups which — according to the working hypothesis — could mimic Arg^{33} and/or Arg^{35} in NPY. Binding models derived for some compounds (e.g. BIBP 3226 and J-104870) based on investigations with Y_1 receptor mutants suggest key interactions between the basic group(s) and acidic residues of the Y_1 receptor protein, especially Asp^{287}. Compared to Y_1 antagonists the known Y_5 antagonists are often based on hits from screening of libraries and show a considerably higher degree of structural diversity. Nevertheless, many highly active Y_5 antagonists represent a common structural pattern suggesting at least overlapping binding sites.**

* An earlier version of this manuskript was published under the same title in *Handbook of Experimental Pharmacology*, Vol. 162, pp. 505-46, Springer Berlin Heidelberg New York, 2004. Reproduction as part of this thesis by kind permission of the publisher.

1. Introduction

Since Rudolf et al.[1] published the D-argininamide BIBP 3226 as the first highly potent and selective non-peptide Y_1 receptor antagonist, speculations about the therapeutic potential of NPY blocking agents[2, 3], e.g., as antihypertensive or anti-obesity drugs, and the discovery of additional receptor subtypes[4] tremendously stimulated the search for new drug candidates in the NPY field. Some of the early successful approaches in the design of NPY antagonists were more or less rational starting from the structure of the natural ligand NPY. Regardless of the fact that the 3D structure of NPY and its active conformation(s) at the different NPY receptors is still a matter of debate, the putative PP-fold structure of NPY[5] was used by many groups as a model to develop working hypotheses, in particular in the field of Y_1 receptor antagonists. The principle of drug design resulting in BIPB 3226 as mimic of the C-terminus of NPY appeared to be generally a promising approach, in particular, as a complete L-alanine scan has provided valuable information which residues of NPY are important for binding at Y_1 and Y_2 receptors[6]. Meanwhile, the initial optimism concerning the impact of non-peptide NPY receptor antagonists as new therapeutics in near future was dampened in some respect. This is certainly, at least in part, due to the complexity (and even redundancy) of NPY mediated physiological and pathophysiological effects resulting from the interaction of NPY with differently localized receptor subtypes and its interplay with a multitude of other neurotransmitters and hormones, e.g. in the regulation of food intake, metabolic processes, blood pressure, hormone release, modulation of emotional processing, sexual and cognitive function[3]. Although highly potent and selective substances, mainly Y_1 and Y_5 receptor antagonists, have been developed, no NPY receptor ligand was launched onto the market up to now. However, selective non-peptide NPY antagonists proved to be indispensable pharmacological tools to investigate the physiological and pathophysiologial role of NPY and the contribution of receptor subtypes, especially Y_1, Y_2, and Y_5, to complex biological responses, e.g. in feeding-related metabolic processes.

Within the last decade the number of known non-peptide NPY ligands has largely increased (for recent reviews, see lit.[7-9]). However, comparing the described compounds a strong imbalance is obvious. With respect to the possible market for anti-obesity drugs the pharmaceutical companies were focusing their research on Y_1 and Y_5 antagonists, whereas only one class of potent non-peptidic Y_2 (BIIE 0246) and no selective Y_4 receptor antagonist has been described so far. The structures of known Y_1 antagonists are less diverse than those of Y_5 antagonists, but the design strategies were rational and ligand based in some cases, leading to well explored structure-activity relationships in different series. The overall diversity of non-peptide NPY antagonists is not surprising if one considers the large spread of the putative NPY binding sites covering extracellular and transmembrane regions of the receptor protein, as indicated, for instance, by *in vitro* mutagenesis of the Y_1 receptor and modeling approaches[10-15]. Therefore, different partially or even non-overlapping antagonistic binding sites are possible which may or may not reproduce key interactions of NPY, e.g., those between crucial Arg residues and acidic amino acids of the Y_1 receptor. This is reflected by the diversity especially of the Y_5 antagonistic leads and has offered the chance of finding structurally distinct leads by high throughput screening of large and diverse compound libraries.

Compared to antagonists the structural requirements which must be fulfilled by an agonistic pharmacophore to induce (or stabilize) the active conformation of the receptor are much more stringent. Non-peptidic agonists, which could be extremely useful pharmacological tools and potential drugs as well, are not reported in the literature. In the following sections non-peptide NPY antagonists are summarized according to their Y_1, Y_2, and Y_5 selectivity and with focus on their structure-activity relationships.

2. First Nonspecific NPY Receptor Antagonists and Structurally Derived Compounds

Prior to the discovery of the first highly active non-peptide NPY receptor ligands and the application of rational approaches to design such compounds, some substances originally described to act on other targets were found to be weak or moderate NPY antagonists: D-*myo*-inositol-1,2,6-trisphosphate (α-trinositol or pp56, **1**), an isomer of the second messenger inositol-1,4,5-trisphosphate, benextramine (**2a**), an irreversible α_1-adrenergic antagonist, and BU-E-76 (He 90481, **3a**), a highly potent histamine H_2 receptor agonist (cf. Fig. 1).

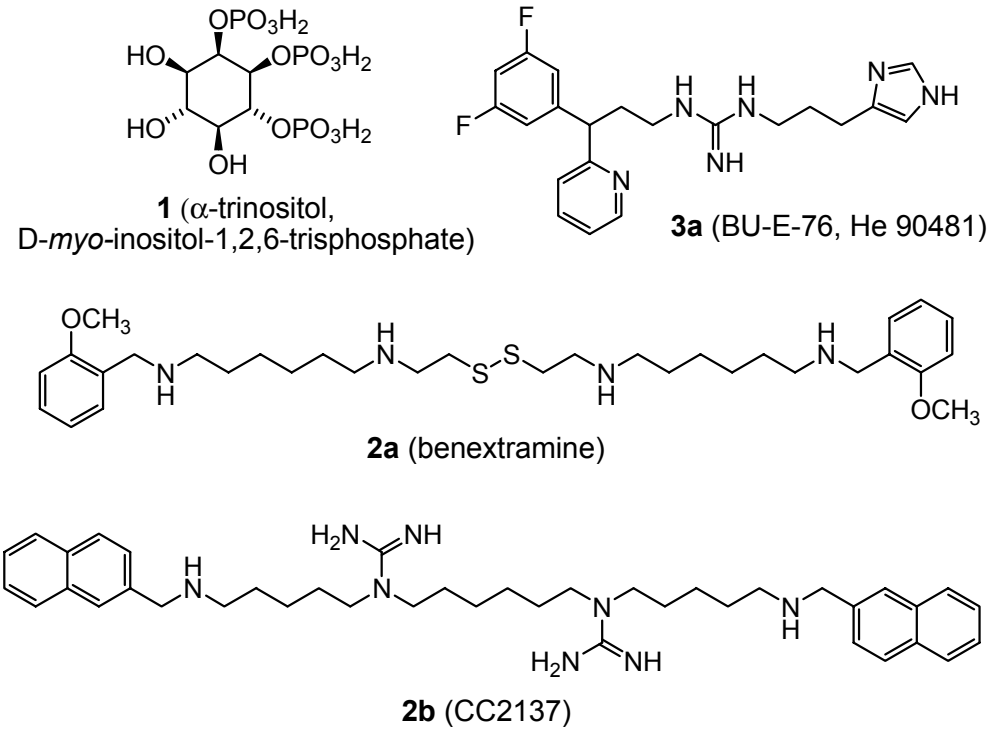


Fig. 1: First nonspecific NPY antagonists—compounds with different main pharmacological effects.

2.1. α-Trinositol

It has been reported, that α-trinositol (**1**, Fig. 1) — apart from its antiinflammatory and analgesic effects — non-competitively inhibits NPY induced vasoconstriction and pressor responses in several *in vitro* and *in vivo* assays[16]. Since the compound is

not able to displace radiolabelled NPY from Y_1 or Y_2 binding sites it is suggested that α-trinositol acts at a point in the NPY-activated signalling pathways downstreams from the receptors[17, 18]. Thus α-trinositol is not a NPY receptor ligand, but it was the first non-peptide agent described to inhibit some NPY mediated effects — amongst them the NPY induced stimulation of food intake (for a review see lit.[19]).

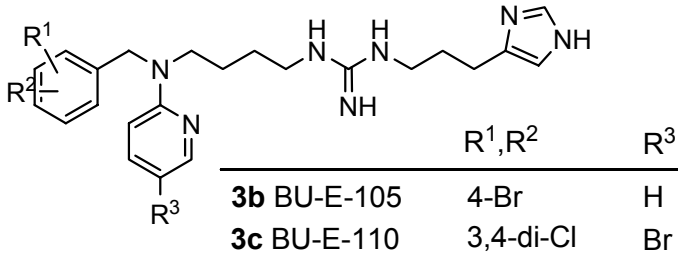
2.2. Benextramine and Related Compounds

Benextramine (**2a**, Fig. 1), an irreversible α_1-adrenoceptor antagonist[20], produces a long-lasting antagonism of NPY induced pressor effects[21] and was presented as the first non-peptide inhibiting specific binding of [^{3}H]NPY to a NPY receptor population in rat brain membranes[22]. Since the inhibition of NPY binding is irreversible, the authors suggested a covalent linkage of benextramine to a cysteine residue of the receptor protein via a thiol-disulfide exchange. Whereas functional assays indicated Y_1 selectivity[23], binding studies with cloned human NPY receptors resulted in K_i values of 2 μM at the Y_1 and the Y_4, 7.5 μM at the Y_2[24] and 5 μM at the Y_5 subtype[25]. A lead optimization approach was based on the hypothesis that the terminal benzylic moieties of benextramine possibly mimic Tyr^1 and/or Tyr^{36} of NPY. Analogs lacking a benzylic portion did not displace [^{3}H]NPY from rat brain membranes. 3-Hydroxy or 3-methoxy substituted benzyl as well as naphthyl groups are favorable[26]. Reversible antagonists were obtained when the central disulfide moiety was replaced with an ethylene bridge. Functional experiments with CC2137 (**2b**) indicated a shift towards Y_2 (*vs.* Y_1) receptor selectivity[27].

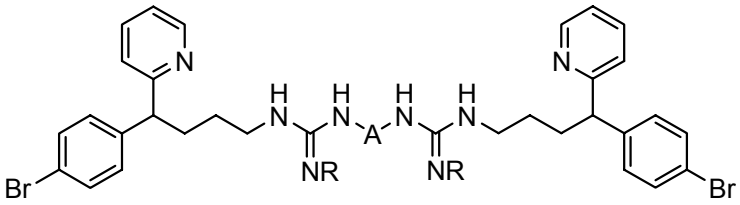
2.3. Y_1 Antagonists Related to Arpromidine

The potent histamine H_2 agonist BU-E-76 (**3a**, also named HE 90481), an analog of arpromidine[28], is a weak competitive NPY Y_1-antagonist[29, 30]. As **3a** and related substances displayed some Y_1 receptor selectivity these imidazolylpropylguanidines were considered as model compounds and investigated for inhibition of the NPY-induced Ca^{2+} mobilization in HEL cells to elaborate structure-activity relationships

and to derive a pharmacophore model. Compared to BU-E-76 the Y_1-antagonistic activity could be increased by a factor of about 100 or more by increasing lipophilicity, e.g. by introduction of two chlorine substituents[31], and/or by vicinal instead of geminal arrangement of the aromatic rings. For instance, the halogenated benzyl(2-pyridyl)aminoalkylguanidines **3b,c** achieve Y_1 antagonistic activities in the submicromolar range (**3b**: K_b 0.47 μM, **3c**: K_b 0.36 μM; calcium assay in HEL cells)[32].



	R^1,R^2	R^3
3b BU-E-105	4-Br	H
3c BU-E-110	3,4-di-Cl	Br



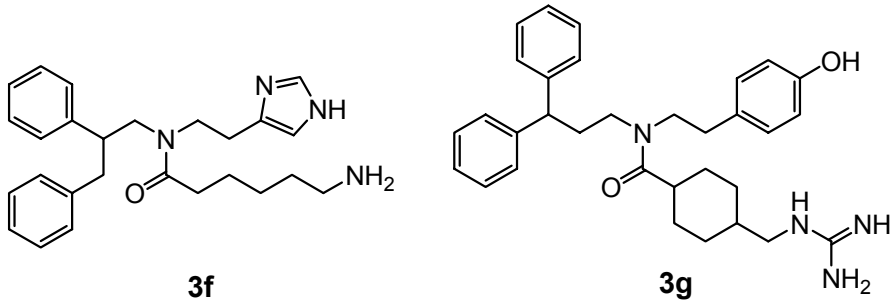
3d: A = *trans*-cyclohexane-1,4-diyl, R = H
3e: A = $(CH_2)_3$-N(CH_3)-$(CH_2)_3$, R = cyclohexyl

Fig. 2: Guanidine-type NPY antagonists derived from arpromidine.

Based on the assumption, that two basic groups, mimicking Arg^{33} and Arg^{35} in NPY, are beneficial for Y_1-receptor affinity, the imidazole ring of the arpromidine analogs, which is essential for histamine H_2 receptor agonism, was replaced by a different basic heterocycle or a second guanidino group. Active bisguanidines (e.g. **3d**)[33] with *trans*–cyclohexane-1,4-diyl spacers point to an optimal distance between the guanidine groups of about 8 Å in agreement with the $Arg^{33} - Arg^{35}$ side-chain distance postulated for the Y_1 active conformation of NPY[6, 34]. The derivative **3e** (SK 48) diplayed also Y_2 receptor binding (K_i 1 μM) and, surprisingly, a Y_2 agonist-like profile in the isolated electrically stimulated rat vas deferens (EC_{50} 2.7 μM, inhibition

of the twitch response) which could, however, not be unequivocally attributed to Y_2 receptor stimulation[35].

In another arpromidine-based series the basic center was moved to the terminus of a flexible side-chain in order to better mimic Arg^{35} of NPY[36]. Surprisingly, *N*-imidazolylethyl-*N*-diphenyl-alkanoic acid amides with a terminal amino group (e.g. **3f** with submicromolar activity) are considerably more potent than the corresponding guanidines in the functional Y_1 assay (Ca^{2+} assay, HEL cells). By contrast, when the imidazole moiety is replaced with phenol to imitate the C-terminal tyrosine of NPY, highest activity is found in combination with a guanidine (e.g. **3g**). These inverse structure-activity relationships suggest different binding modes for NPY Y_1 antagonists with one and with two basic sites.



3f **3g**

Fig. 3: *N*-Imidazolylalkyl- and *N*-(hydroxyphenyl)ethyl-*N*-diphenylalkyl-alkanoic acid amides with terminal basic functions.

3. Potent and Selective Non-Peptide NPY Y_1 Receptor Antagonists

3.1. BIBP 3226 and other (*R*)-Argininamides

3.1.1. DESIGN AND PHARMACOLOGY OF BIBP 3226

A rational mimetic strategy based on the structure of NPY led to the synthesis of the first highly active and Y_1 selective non-peptidic antagonist, BIBP 3226 (**4a**, Fig. 4) at Boehringer Ingelheim Pharma[1, 37, 38]. The complete alanine scan of NPY[6] revealed that the C-terminal tetrapeptide, in particular Arg^{35} and Tyr^{36}, is most important for

Y_1 receptor binding. Deletion of the carboxamide terminus and, surprisingly, replacing of L-arginine by its D-enantiomer proved to reproduce this pharmacophoric pattern. Lead optimization with hundreds of analogs resulted in BIBP 3226, (*R*)-N^2-(diphenylacetyl)-*N*-[(4-hydroxyphenyl)methyl]argininamide, a highly potent and selective Y_1 receptor antagonist (K_i 5.1 and 6.8 nM at human and rat Y_1 receptors, respectively[1]).

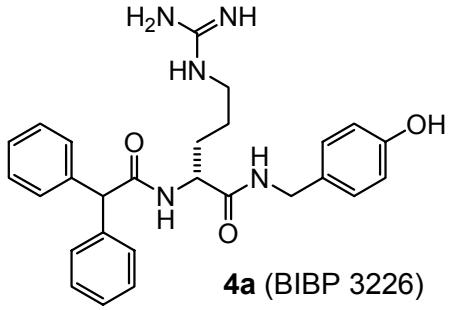


Fig. 4: Structure of the Y_1 receptor antagonist BIBP 3226 (**4a**).

BIBP 3226 was found to be active in numerous functional *in vitro* tests, e.g. on rabbit vas deferens, rat renal tissue[37], guinea-pig vena cava[39] and HEL cells[40]. Except on human cerebral arteries (pK_b 8.5)[41], *in vitro* activity (pK_b 7 – 7.6) was lower than binding affinity. The receptor selectivity was also confirmed in functional tests for NPY antagonism. For example, using rat vas deferens for Y_2 and Y_4[37, 39] and rat colon for Y_3 receptors[42] the compound was found to be inactive at concentrations ≤ 10 µM. Interestingly, BIBP 3226 also binds in a 50 – 100 nM range to human neuropeptide FF receptors and antagonizes the antiopioid effect of NPFF[43, 44], probably since the ligand fits with the C-terminus of the octapeptide NPFF, Pro^5-Gln^6-Arg^7-Phe^8-amide, like with the analogous NPY terminus.

In vivo, BIBP 3226 does not influence the basal blood pressure, but inhibits the hypertensive effect induced by administration of NPY, stimulation of the sympathetic nervous system or stress[39, 45]. Though the compound is not an appropriate drug candidate due to, e.g., lack of oral bioavailability and inability to cross the blood-brain barrier, BIBP 3226 was used as pharmacological tool in more than 100 studies to investigate Y_1 receptor mediated peripheral and central effects of NPY. Investigations of the effect of BIBP 3226 on the central regulation of feeding revealed contradictory results[46, 47]. Morgan et al.[47] and Iyengar et al.[48] reported for both, BIBP 3226 and its inactive (*S*)-enantiomer BIBP 3435, the ability to block

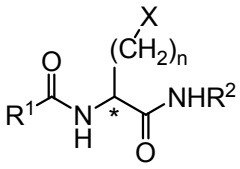
NPY induced food intake after pvn. or icv. injection, so that a Y_1 specific mechanism is questionable. However, the closely related and more potent Y_1 antagonist BIBO 3304 (**4l,** Fig. 5) does exhibit central anorexigenic effects after icv. or pvn. administration[49-52].

3.1.2. *STRUCTURE-ACTIVITY RELATIONSHIPS OF BIBP 3226 DERIVATIVES*

Some pharmacological data reflecting the structure-activity relationships of BIBP 3226 analogs are summarized in Table 1.

Table 1: NPY Y_1 receptor binding of BIBP 3226 derivatives[53].

4a-k



No.	R^1	R^2	X	n	*[a]	IC_{50} (nM)[b]
4a[c]	$CH(C_6H_5)_2$	$CH_2C_6H_4$-4-OH	NHC(=NH)NH_2	3	(*R*)-	5
4b[d]	$CH(C_6H_5)_2$	$CH_2C_6H_4$-4-OH	NHC(=NH)NH_2	3	(*S*)-	> 10000
4c	$CH_2C_6H_5$	$CH_2C_6H_4$-4-OH	NHC(=NH)NH_2	3	(*R*)-	370
4d	CH_3	$CH_2C_6H_4$-4-OH	NHC(=NH)NH_2	3	(*R*)-	> 10000
4e	9*H*-Fluoren-9-yl	$CH_2C_6H_4$-4-OH	NHC(=NH)NH_2	3	(*R*)-	72
4f	$CH(C_6H_5)_2$	$CH_2C_6H_4$-4-OH	NHC(=NH)NH_2	4	(*R*)-	220
4g	$CH(C_6H_5)_2$	$CH_2C_6H_4$-4-OH	NH_2	3	(*R*)-	> 10000
4h	$CH(C_6H_5)_2$	$CH_2C_6H_4$-4-OH	NH_2	4	(*R*)-	> 10000
4i	$CH(C_6H_5)_2$	$CH_2C_6H_5$	NHC(=NH)NH_2	3	(*R*)-	70
4j	$CH(C_6H_5)_2$	$(CH_2)_2C_6H_4$-4-OH	NHC(=NH)NH_2	3	(*R*)-	290
4k	$CH(C_6H_5)_2$	$CH_2C_6H_{10}$-4-OH	NHC(=NH)NH_2	3	(*R*)-	9000

[a] configuration of Arg
[b] receptor affinity determined by radioligand binding studies on SK-N-MC cells
[c] BIBP 3226
[d] BIBP 3435

First studies[53, 54] indicated that the fit of BIBP 3226 to the Y_1 receptor binding site is highly stereospecific and nearly optimal, hardly leaving degrees of freedom for

structural variation (but see below for N^G-substituted analogs). The (*S*)-enantiomer **4b** (BIBP 3435) is almost inactive. Moderate affinity remains if the (*R*)-arginine side chain is extended by one CH_2 group (**4f**), but, independent of the chain length, an exchange of the guanidine against an amine function results in complete loss of affinity.

With respect to better pharmacokinetic properties various basic groups such as benzamidines or aminopyridines (cf. **4n**) were incorporated as mimics of the arginine side chain, usually resulting in compounds with reduced Y_1 receptor affinity compared to that of the reference compound **4a**[40, 55, 56]. The backbone is open to modification only at the argininamide nitrogen; *N*-methylation reduces affinity by a factor of not more than five. As indicated by the weak binding of the monophenyl analog **4c**, the diphenylacetyl moiety is essential and should be sufficiently flexible since rigidization within a fluorene nucleus (**4e**) results in about 15-fold lower affinity. The *para*-OH substituent of the phenylmethyl moiety directly contributes to the high affinity of BIBP 3226. The non-hydroxylated analog **4i** is 14 times less active. However, the 4-(ureidomethyl) derivative BIBO 3304 (**4l**) has subnanomolar affinity for both the human and the rat Y_1 receptor (IC_{50} 0.38 and 0.72 nM, respectively) and is nearly inactive at Y_2, Y_4 and Y_5 receptors (IC_{50} > 1000 nM)[49]. The chain length of the amide substituent is optimal with one methylene group as in **4a**, although a 2-(4-hydroxyphenyl)ethyl residue as in **4j** should be a better mimic of the C-terminal tyrosinamide in NPY.

Additional substituents at the benzylic carbon may be tolerated as demonstrated with H409/22 (**4m**, Fig. 5) and related compounds[57-59]. The higher potency of the (*R*)-enantiomers is characteristic of the argininamide series of Y_1 antagonists (cf. BIBP 3226 (**4a**) *vs.* BIBP 3435 (**4b**); BIBO 3304 (**4l**) *vs.* its inactive enantiomer BIBO 3457). In case of the α-methylated compound, highest activity resides in the (*R,R*)-configured stereoisomer **4m**, H409/22, which was tested in man, whereas the (*S,S*)-enantiomer is inactive[59, 60]. Other examples of BIBP 3226-like Y_1 antagonists are **4n**[56] and GI264879A (**4o**)[61]. **4o** weakly binds in the micromolar range to Y_1, Y_4 and

Y_5 receptors, but reduces food intake and body weight gain in obese animals, suggesting that interaction with more than one NPY receptor and/or other mechanisms may contribute to the inhibition of NPY mediated hyperphagia[61].

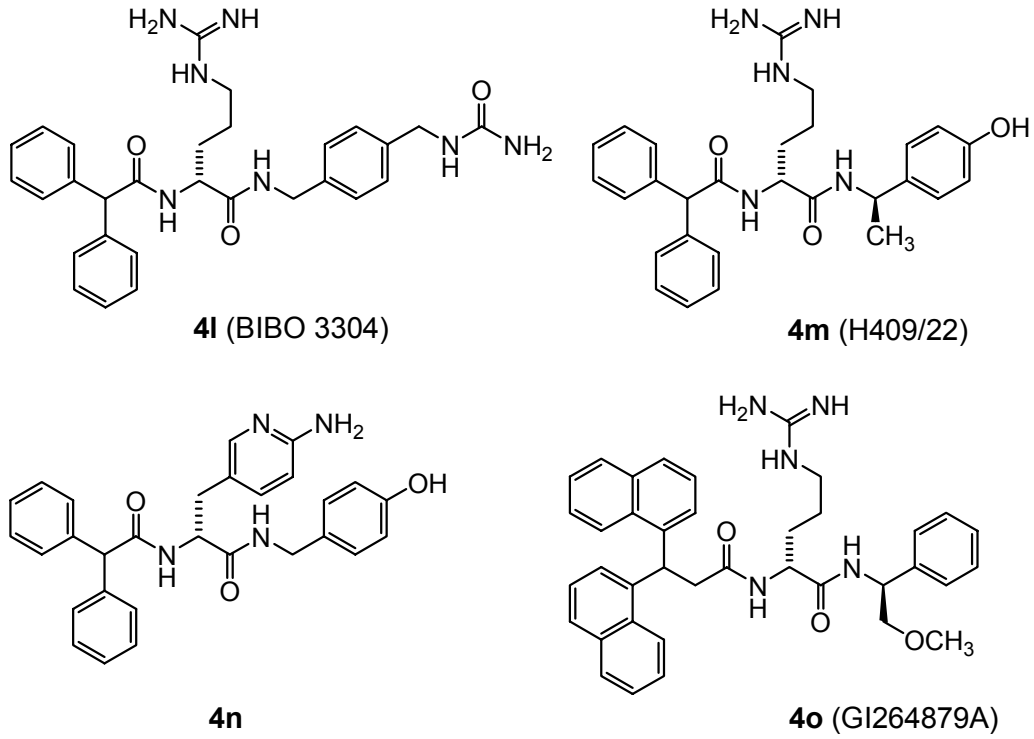


Fig. 5: NPY Y_1 antagonists from different sources based on BIBP 3226 as lead.

Further structure-activity relationships of Y_1 antagonists related to BIBP 3226 were explored by functional investigations on HEL cells (inhibition of intracellular calcium mobilization induced by 10 nM NPY)[40, 57]. Introduction of a *p*-Cl substituent at the diphenylacetyl group is tolerated and may be even favorable. The 3,3-diphenyl-propionyl homolog of BIBP 3226 (IC_{50} 510 nM compared to 17 nM for BIBP 3226) is much more active than the 2,3-diphenylpropionyl analog. Relatively open to the introduction of substituents is again the (4-hydroxyphenyl)methylamide moiety which may be incorporated into a tetrahydro-*1H*-benzo[*c*]azepine nucleus (IC_{50} 280 nM)[57]. A methylation at the hydroxybenzyl α-carbon leads to compounds with activities comparable to that of **4a**, indicating that a certain bulk is tolerated in this position. The backbone conformations of the NPY C-terminus and of BIBP 3226

should therefore be different so that the corresponding guanidino and *para*-hydroxyphenyl groups may similarly interact with the Y_1 receptor.

3.1.3. THE Y_1 RECEPTOR BINDING SITE FOR BIBP 3226: IN VITRO MUTAGENESIS RESULTS AND COMPUTER MODELS

The obvious suggestion that NPY and BIBP 3226 share an overlapping binding site at the human Y_1 receptor has been extensively investigated by *in vitro* mutagenesis and computer modeling[11, 12]. Reduced affinity of the antagonist to the respective alanine mutants indicates which residues might contribute to BIBP 3226 binding. Most of these positions, namely W163, F173, Q219, N283, F286, D287[11] and additionally Q120, F282, H306[12] are important for NPY and BIBP 3226 affinity and thus thought to form an overlapping binding region of both ligands. Positions Y211[11], Y47, W276, H298 and F302[12] seem to participate only in binding of BIBP 3226, but Y47 and H298 were demonstrated in another *in vitro* mutagenesis study[62] to interact with PYY. These experimental results have been considered in computer models of the Y_1 receptor complexed with BIBP 3226, but the proposed binding modes are rather different due to the mutants taken into account. Moreover, the homology modeling based on bacteriorhodopsin and the electron microscopy map of rhodopsin, respectively, could not represent the very recent progress resulting from the high resolution crystal structure of bovine rhodopsin[63].

Recently, a new and more reliable model of BIBP 3226 binding to the Y_1 receptor was generated[†] on the basis of an unambiguous sequence alignment of the transmembrane (TM) regions with those of bovine rhodopsin, using the crystal structure of the latter as template and taking into consideration all published results with Y_1 receptor mutants. The suggested topology of the BIBP 3226 binding site within the novel, rhodopsin-based alignment of the transmembrane and extracellular regions becomes obvious from the important residues highlighted in Fig. 6. The binding mode derived from the mutants reported by Sautel et al.[11] could be reproduced

[†] S. Dove, to be published in detail.

with the new model. All key interactions occur within a deep pocket between TMs 4 to 7. However, Y47 (TM1) and Q120 (TM3)[12] cannot approach BIBP 3226 in this mode. To include the highest possible number of responding mutants, another mode is suggested which, in principle, retains interactions of the D-argininamide and the (4-hydroxyphenyl)methyl moiety as previously proposed, but extends the diphenylacetyl site towards TMs 1 and 3 (see Fig. 6).

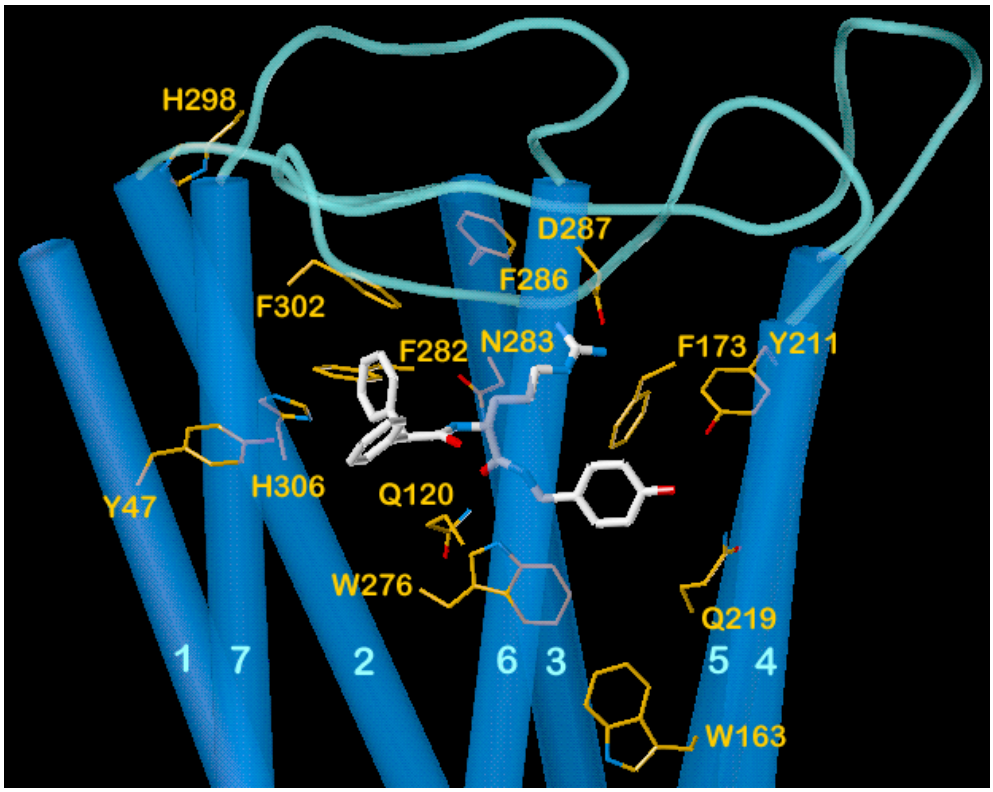


Fig. 6: Computer model of the human neuropeptide Y Y1 receptor, based on the crystal structure of bovine rhodopsin, in complex with BIBP 3226. TM regions are numbered and shown as blue cylinders. Labelled residues (C atoms: orange): weak or no binding of BIBP 3226 after mutation). The model was generated by the software package SYBYL 6.8 (Tripos Inc., St. Louis).

With respect to the number and quality of interactions, this mode is superior to that suggested by Du et al.[12] where essentially the (4-hydroxyphenyl)methyl and diphenylacetyl sites were exchanged. Interestingly, it is never possible to include Y163 (TM4) into binding of BIBP 3226. The inability of the Y163A mutant to bind

the antagonist and NPY might be due to rearrangement of the transmembrane regions since the indole nitrogen probably forms a hydrogen bond with N81 (TM2) like the identical residues in the rhodopsin crystal structure.

The suggested key interactions are depicted in Fig. 6.

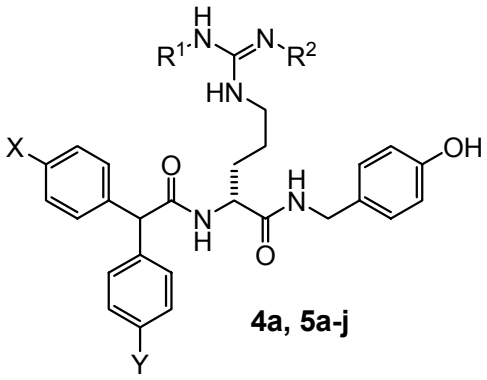
The D-argininamide backbone oxygen is hydrogen bonded to the side chain of N283 (TM6). The guanidino group interacts with the carboxylate of D287 (at the top of TM6 in the rhodopsin-based alignment). Also the suggested hydrogen bond between the amide nitrogen of Q219 (TM5) and the (4-hydroxyphenyl)methyl oxygen[11] is retained. Y211 (TM5) might form another hydrogen bond to the 4-OH group. The diphenylacetyl moiety extends, with one phenyl ring, towards Y47 (TM1) and H306 (TM7). The model suggests that a *p*-Cl substituent should be slightly favorable for interaction with Y47 as indicated by structure-activity relationships[57] (see also **5h**, Table 2). Q120 (TM3) is supposed to form an additional H-bond with the diphenylacetyl oxygen. This pattern is completed by aromatic-aromatic and π-cation interactions within a large pocket aligned by the side chains of F173 (TM4), W276 (TM6), F282 (TM6), F286 (TM6), F302 (TM7) and H306 (TM7), comprising all terminal groups of BIBP 3226.

3.1.4. N^G-SUBSTITUTED (R)-ARGININAMIDES WITH REDUCED BASICITY

Recently, the Y_1 receptor binding models of BIBP 3226 were used to suggest that appropriate N^G-substituents at the D-arginine side chain will retain or even increase antagonistic activity[64, 65]. With single alkyl or arylalkyl groups, no improvement was achieved. Radioligand binding studies on SK-N-MC cells resulted in K_i values of 2 nM (BIBP 3226), 2.6 nM (N^G-methyl), 27 nM (N^G-propyl) and 48 nM (N^G-phenylpropyl). With the intention to reduce the basicity of the guanidino group and, by this, to increase the hydrophobicity of the ligands for better blood-brain passage, electron-withdrawing substituents were introduced. Selected N^G-acylated derivatives are presented in Table 2 together with results of FURA assays on HEL cells and with binding data on Y_1, Y_2 and Y_5 receptors. Some of the compounds are

up to 20 times more active in the functional test and show more than 30 times higher Y_1 receptor affinity than BIBP 3226. Y_1 selectivity is even increased in most cases.

Table 2: Pharmacological data of N^G-acylated BIBP 3226 derivatives (Hutzler 2001).



4a, 5a-j

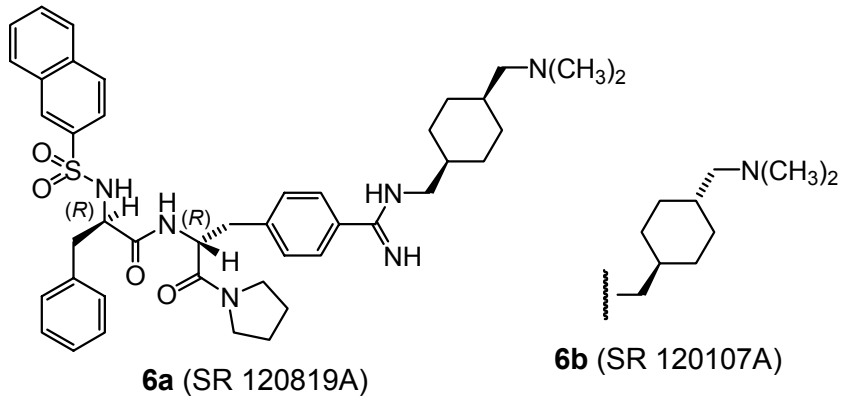
					Y_1 antagonism[a]	Binding data K_i (nM)[b]		
No.	X	Y	R^1	R^2	IC_{50} (nM)	Y_1	Y_2	Y_5
4a	H	H	H	H	14	2	8000	52300
5a	H	H	H	COMe[c]	45.4	11.9	21100	9350
5b	H	H	H	CO_2Et[c]	2.5	4.5	19100	14500
5c	H	F	H	CO_2Et	0.91	8.5	5080	12300
5d	H	H	H	CO_2CH_2Ph[c]	0.98	48.6	4200	21400
5e	H	H	H	CONHEt	1.18	0.06	19500	21300
5f	H	H	H	$CONHCH_2CO_2Et$	1.65	0.06	2480	17700
5g	H	F	H	$CONHCH_2CO_2Et$	0.86	0.31	2340	44000
5h	Cl	Cl	H	$CONHCH_2CO_2Et$	0.6	0.53	650	24100
5i	H	H	H	$CONH(CH_2)_5CO_2Et$	0.64	0.72	550	7500
5j	H	H	CO_2Et	CO_2Et	8200	-	-	-

[a] Inhibition of NPY (10 nM) stimulated Ca^{2+} mobilization in HEL cells.
[b] determined on SK-N-MC cells (Y_1), SMS-KAN cell membrane preparations (Y_2) and hY_5-transfected HEC-1B cells[66]; radioligand: [^{3}H]propionyl-NPY (1 nM).
[c] Me = CH_3; Et = C_2H_5; Ph = C_6H_5;

The basicity of the guanidino group is reduced to pK_a values of about 8, indicating that considerable amounts of the N^G-acylated argininamides are uncharged under physiological conditions. Probably, the ionic interaction of BIBP 3226 with Asp^{287} can be replaced by a charge-assisted hydrogen bond. Long N^G-substituents may interact with residues in TMs 5 and 6 and project towards the extracellular loops.The N^G-ester substituted compounds **5a-d** as such are active as Y_1 antagonists (see Table 2), but they are also prodrugs which may be enzymatically cleaved by esterases to form the unsubstituted guanidine **4a** (BIBP 3226) as demonstrated for some alkoxycarbonyl derivatives *in vitro*. The inactive diester **5j** is stepwise (via **5b**) converted to **4a**[64, 65].

3.2. Benzamidine-type Y_1 Antagonists SR 120819A and SR 120107A

The potent NPY Y_1 receptor antagonists SR120819A (**6a**), designed at Sanofi[67, 68], was published shortly after BIBP 3226 as the first orally active Y_1 antagonist. The backbone of this arylsulfonyl substituted peptide mimetic resembles that of the benzamidine-type thrombin inhibitor NAPAP. The (*R,R*)-*cis*-configured compound SR 120819A and its less active *trans*-diastereoisomer SR 120107A (**6b**)[67] are based on the C-terminus of NPY and provided with two basic centers (benzamidine and tertiary amine) presumably mimicking Arg^{33} and Arg^{35}.



6a (SR 120819A) **6b** (SR 120107A)

Fig. 7: Structure of the benzamidine derivatives SR 120819A (**6a**) and SR 120107A (**6b**).

Radioligand binding studies revealed high affinity at rat, guinea pig and human Y_1 receptors (**6a**: K_i 11-22 nM, **6b**: K_i 11-80 nM)[60, 67]. At a dosage of 5 and 10 mg/kg **6a** inhibited the rise in diastolic blood pressure induced by [Leu^{31}, Pro^{34}]NPY (5µg/kg iv) in anesthetized guinea pigs with a long duration of action of more than 4 h[68].

3.3. Indoles, Benzimidazoles and Benzothiophens

By library screening and similarity searches at Lilly Research Laboratories the trisubstituted indole **7a** (Fig. 8) was discovered as NPY Y_1 antagonistic lead with low affinity at human Y_1 receptors expressed in AV-12 cells (K_i 2.1 µM for displacement of [^{125}I]PYY)[69]. This structure was optimized in different positions, leading to some of the most potent Y_1 antagonists known so far. First attempts maintained the 1-methyl-2-(4-chlorophenoxy)methylindole scaffold. Variation of the 3-substituent resulted in markedly improved activity with a 1,4′-bipiperidine group linked by two C-atoms to C-3 (**7b**: K_i 93 nM; **7c**: K_i 26 nM). Based on the C-terminus of NPY, the introduction of an additional basic moiety at *N*-1 was suggested. Alkylpiperidine side chains with a free NH were optimal in this position. K_i values in the low nanomolar and subnanomolar range were obtained in binding studies with the compounds **7d** (K_i 1.9 nM), (*R*)-**7e** (K_i 1.4 nM), and (*S*)-**7e** (LY 357897, K_i 0.75 nM). The activity of (*S*)-**7e** in different functional assays was in a similar range: K_i 1.8 nM for reversal of NPY-induced inhibition of forskolin-stimulated cAMP, 3.2 nM for inhibition of NPY-induced Ca^{2+} mobilization in SK-N-MC cells.

The compounds proved to be highly selective for the Y_1 receptor (Y_2, Y_4, Y_5: K_i values > 10 µM). (*S*)-**7e** blocked the food consumption in mice, elicited by a submaximal (230 pmol) icv. administered dose of NPY, with an ED_{50} of 17 nmol.

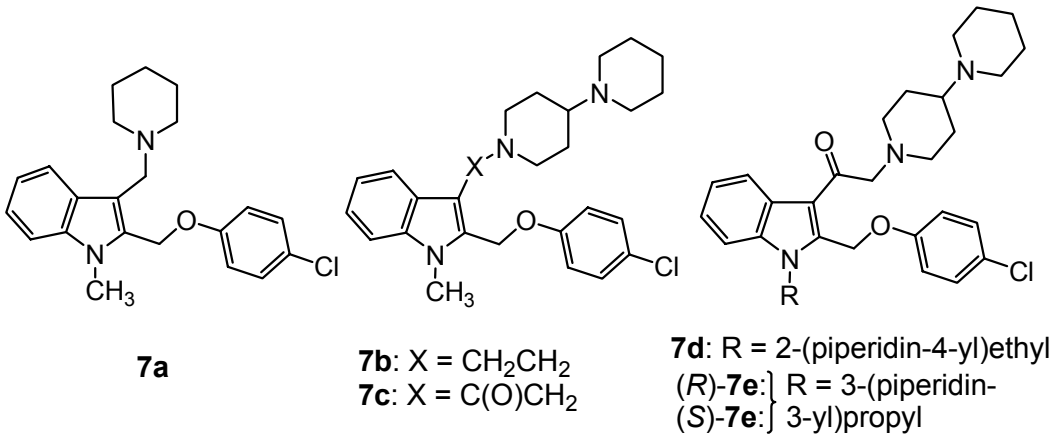
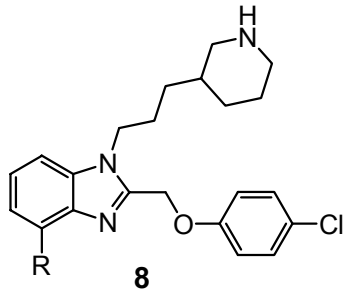


Fig. 8: Optimization of 1,3-substituted 2-[(4-chlorophenoxy)methyl]indoles.

Attempts to replace the indole moiety by other nuclei resulted in a series of 2-[(4-chlorophenoxy)methyl]benzimidazoles with an optimal 1-[3-(piperidin-3-ylpropyl)] substituent[70, 71]. Some of the analogs and their NPY Y_1 antagonistic potencies are summarized in Table 3. The parent compound **8a** was weakly active. Comparison with the most potent indoles suggested that their structure may be best matched with appropriate 4-substituents at the benzimidazole moiety. Introduction of a methyl group in **8b** produced a seven-fold increase in receptor affinity. 3-piperidinylpropoxy and 2-piperidinylethoxy substituents are more favorable. The structure-activity relationships of the piperidine isomers are not uniform and point to an optimal position of the basic nitrogen relative to the benzimidazole nucleus: 3-(piperidin-1-yl)- and 3-(piperidin-2-yl)propoxy derivatives are more active, but 3-(piperidin-3-yl)- and 3-(piperidin-4-yl)propoxy substituted compounds are less potent than their ethoxy analogs. Among the diastereomers of **8h**, highest affinity was found with the (*S,S*)-configuration. The most potent compound of Table 3, **8d**, approached the nanomolar range in NPY Y_1 receptor binding as well as in the functional data for Y_1 antagonism in SK-N-MC cells. Optimization of substituents at the piperidine nitrogen in 2-[(4-chlorophenoxy)methyl]-4-methyl-1-[3-(piperidin-4-yl)propyl]benzimidazoles **9** (Fig. 9) also resulted in very active Y_1 antagonists[70].

Table 3: *In vitro* Y_1 receptor binding (human Y_1 in AV-12 cells) and Y_1 antagonistic activity (cAMP assay in SK-N-MC cells) of selected 2-[(4-chlorophenoxy)methyl]benzimidazoles **8**[70].



8

No.	R	Y_1 binding data K_i (nM)	Y_1 antagonism K_i (nM)
4a[a]		4.6	11
8a	H	700	-
8b	CH_3	97	-
8c	[2-(piperidin-1-yl)ethyl]oxy	43	240
8d	[3-(piperidin-1-yl)propyl]oxy	1.7	2.7
8e	[2-(piperidin-2-yl)ethyl]oxy	29	-
8f	[3-(piperidin-2-yl)propyl]oxy	16	53
8g	[2-(piperidin-3-yl)ethyl]oxy	18	91
8h	[3-(piperidin-3-yl)propyl]oxy	30	77
8i	[2-(piperidin-4-yl)ethyl]oxy	7	15
8j	[3-(piperidin-4-yl)propyl]oxy	152	119
(*S,S*)-**8h**	[3-(piperidin-3-yl)propyl]oxy	6	87
(*R,R*)-**8h**	[3-(piperidin-3-yl)propyl]oxy	41	153
(*R,S*)-**8h**	[3-(piperidin-3-yl)propyl]oxy	27	137
(*S,R*)-**8h**	[3-(piperidin-3-yl)propyl]oxy	17	65

[a] BIBP 3226

The highest NPY Y_1 receptor affinity was obtained by introduction of an additional basic nitrogen separated by 3 – 4 C atoms from the piperidine-N (cf. Fig. 9; **9a**: K_i 5 nM; **9b**: K_i 6 nM). It was a reasonable extension of this work to combine such 1-substituents with the 4-[3-(piperidin-1-yl)propoxy] group present in **8d**[70]. Indeed, a

number of compounds of the common structure **10** displayed Y_1 receptor affinities in the subnanomolar range[72]. K_i values lower than 0.3 nM were found for derivatives with higher alkyl groups (cf. Fig. 9; **10a,b**). Phenylalkyl or phenylalkenyl substitution (**10c,d**) or the attachment of a moiety with a polar group as in **10e,f** led to even higher affinities (K_i 0.1 – 0.2 nM). The derivative with a *p*-iodophenylethyl substituent R (**10g**) is among the most potent non-peptide NPY Y_1 receptor ligands known so far (K_i 0.05 nM).

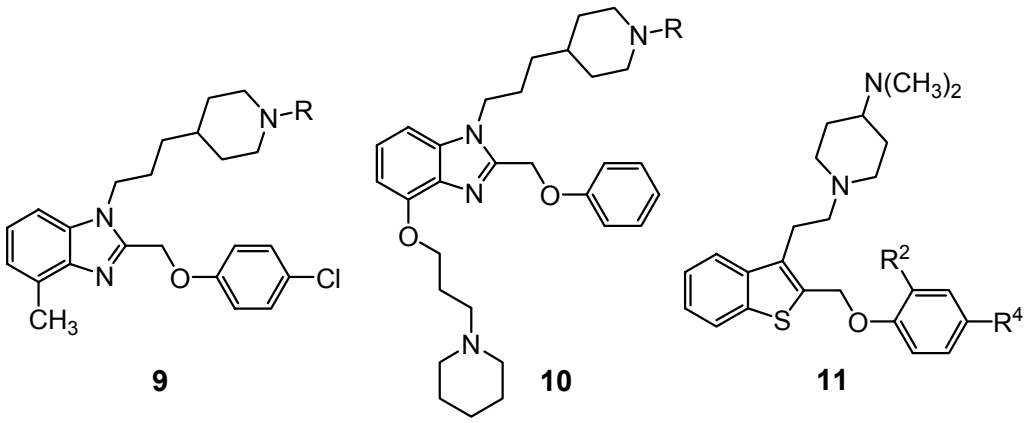


Fig. 9: General structures of highly active NPY Y_1 antagonistic benzimidazole (**9**, **10**) and benzothiophene (**11**) derivatives. Example structures: **9a**: R = 3-(piperidin-1-yl)propyl; **9b**: R = 4-(piperidin-1-yl)butyl; **10a**: R = isobutyl; **10b**: R = cyclohexylmethyl; **10c**: R = 2-phenylethyl; **10d**: R = 3-phenylprop-2-en-1-yl; **10e**: R = 3-(piperidin-1-yl)propyl; **10f**: R = 4-oxo-4-phenylbutyl; **10g**: R = 2-(4-iodophenyl)ethyl; **11a**: R^2 = CH_2OH, R^4 = Br; **11b**: R^2 = CH_2OCH_3, R^4 = Br; **11c**: R^2 = CN, R^4 = Br.

With benzothiophene derivatives a third nucleus was used at Lilly as scaffold for the design of NPY Y_1 antagonists[73]. Optimization of both side chains in 2- and 3-position resulted in the common structure **11** (Fig. 9). A 4-chlorophenoxymethyl group as present in the indole and benzimidazole series does only lead to moderate affinity (K_i 310 nM). However, the potency may be significantly increased by appropriate multiple substitution at the phenyl ring. The most active Y_1 antagonists in this series (K_i values: 11 – 15 nM) are those with polar groups in ortho position (R^2 = CH_2OH, CH_2OMe, CN) and an additional *para*-Br substituent (**11a-c**).

3.4. Y_1 Antagonists Based on other Common Structures

3.4.1. 6-ARYLSULFONYL-5-NITROQUINOLINES

Arylsulfonyl compounds from Parke-Davis/Warner-Lambert with a nitroquinoline nucleus are only weakly basic and do not obviously overlap with NPY[74]. The 8-amino-5-nitroquinoline and the phenylsulfonyl moieties in the general structure **12** (Fig. 10) are essential for Y_1 antagonistic activity. Whereas the parent compound PD 9262 (**12a**) is not very potent (K_i 282 nM for displacement of [^{125}I]PYY from SK-N-MC membranes), *ortho*-alkyl or halogen substituents at the phenyl ring enhance affinity up to a K_i value of 48 nM for the *ortho*-isopropyl derivative PD 160170 (**12b**).

3.4.2. PHENYLPIPERAZINES

Neurogen and Pfizer have patented 1-(1-phenylcyclohexyl)-4-phenyl-piperazines with amide, amine and ether substituents R (**13**, Fig. 10) as novel class of NPY Y_1 specific ligands[75, 76]. No biological data were given for the new amides (e.g., **13a**). An ether derivative (**13b**) displaced [^{125}I]PYY with an IC_{50} value of 30 nM.

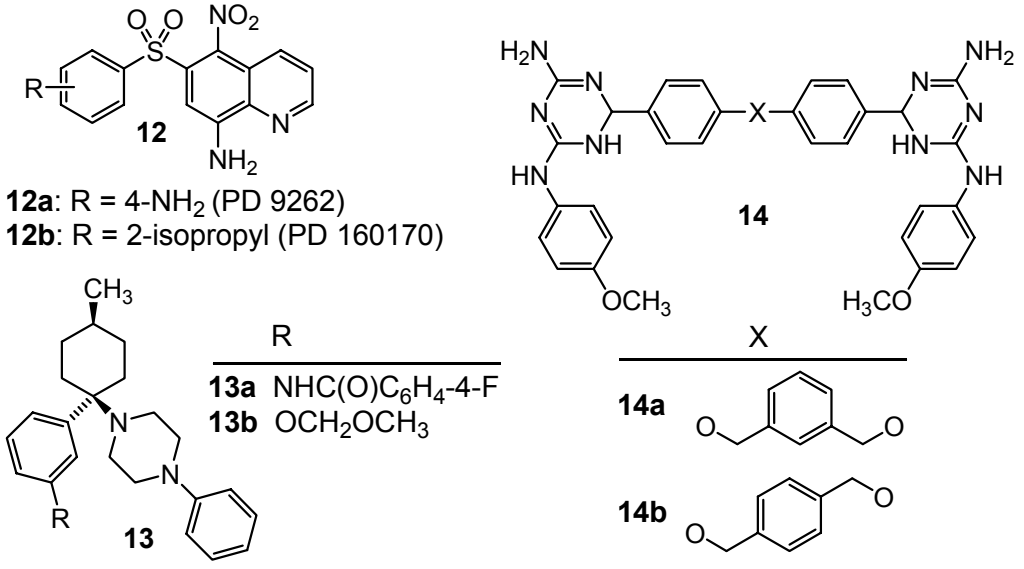


Fig. 10: 6-Arylsulfonyl-5-nitroquinolines **12**, phenylpiperazines **13** and bis[diamino(phenyl)triazines] **14**.

3.4.3. BIS[DIAMINO(PHENYL)TRIAZINES]

At Alanex different structures were identified as non-peptide NPY Y_1 receptor antagonists by pharmacophore-based approaches and by screening of combinatorial libraries[77]. Examples of discovered compounds are symmetric bis[diamino(phenyl)-triazines] **14** with a disubstituted central benzene ring as spacer (Fig. 10). The K_i values for Y_1 receptor binding are 117 nM (*meta* derivative AXC01829) and 150 nM (*para* analog AXC011018). A certain similarity of the compounds to benextramine (**2a**) and analogs is obvious.

3.4.4. BENZAZEPINES AND BENZODIAZEPINES

Hybrid compounds combining a CCK-B receptor antagonistic benzodiazepine and a histamine H_2 receptor blocking roxatidine-like moiety were synthesized at Shionogi and Co.[78], e.g. the derivative **15** (Fig. 11), which was about equiactive with BIBP 3226 as NPY Y_1 antagonist (K_i 6.4 nM in radioligand binding studies, IC_{50} 95 and 320 nM in functional Ca^{2+} and cAMP assays in SK-N-MC cells). No binding to Y_2 and Y_5 receptors was observed up to concentrations of 1 µM. The hybrid molecule maintains the CCK-B and histamine H_2 antagonistic potency of the components, which were, however, both inactive at NPY Y_1 receptors. Other series of Y_1 antagonists from Shionogi and Co. are based on a 1,3-disubstituted benzazepine nucleus[79, 80]. The common structure **16** was optimized at both positions. Generally, derivatives with urea moieties (R^1 = NH-alkyl) are about ten times more potent than the corresponding carbamates (R^1 = O-alkyl). Maximal Y_1 receptor binding affinity (K_i 2.9 nM) was observed for the 3-guanidino derivative (X = NH) with an isopropylamino and a 4-hydroxyphenyl group as R^1 and R^2, respectively, whereas the 3-ureido analog (X = O) was much less potent (K_i 82 nM). Further optimization of R^2 in a 3-ureido series (R^1 = NH-isopropyl) also led to compounds with K_i values lower than 10 nM (R^2 = 6-benzofuryl, 6-benzothienyl, 6-benzothiazolyl, 2-F-phenyl, 2,4-di-F-phenyl)[79]. The 6-benzothiazolyl derivative (K_i 5.1 nM) was functionally

characterized as an Y_1 antagonist and did not show any effects on Y_2, Y_4 and Y_5 receptors.

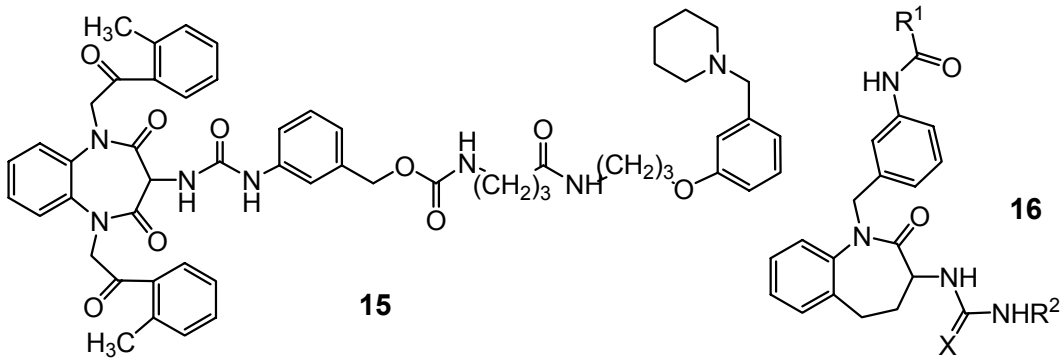


Fig. 11: Benzodiazepine **15** and general structure of benzazepines **16** (X = O; R^1 = NH-alkyl, O-alkyl; R^2 = aryl, heteroaryl).

3.4.5. MORPHOLINOPYRIDINES J-104870 AND J-115814

Two highly potent NPY Y_1 receptor antagonists, the morpholinopyridines J-104870 (**17a**) and J-115814 (**17b**, Fig. 12) were disclosed by Banyu[81, 82]. J-104870 displaced [^{125}I]PYY binding to cloned human and rat Y_1 receptors with K_i values of 0.29 and 0.54 nM, respectively, and inhibited the NPY-induced intracellular calcium mobilization (IC_{50} 3.2 nM). K_i values determined for the binding at other NPY receptors were greater than 5 µM. Anorexigenic effects on NPY-mediated feeding of rats were demonstrated by both intracerebroventricular and oral administration of the compound[82]. J-115814 (K_i 1.4 – 1.8 nM) was nearly as potent as J-104870. Feeding induced by icv. NPY was unaffected by ip. injected J-115814 in Y_1(-/-) mice, but suppressed in wildtype and Y_5(-/-) mice[81]. Together these findings suggest the contribution of Y_1 receptors in the regulation of food intake. *In vitro* mutagenesis studies on the human Y_1 receptor[62] resulted in reduced affinity of J-104870 at alanine mutants of amino acids Trp^{163}, Phe^{173}, Asn^{283}, Asp^{287} and Leu^{303}, indicating that the compound recognizes a pocket formed by TMs 4, 5 and 6 which only

partially overlaps with the binding site of other antagonists like BIBP 3226 or the peptide 1229U91.

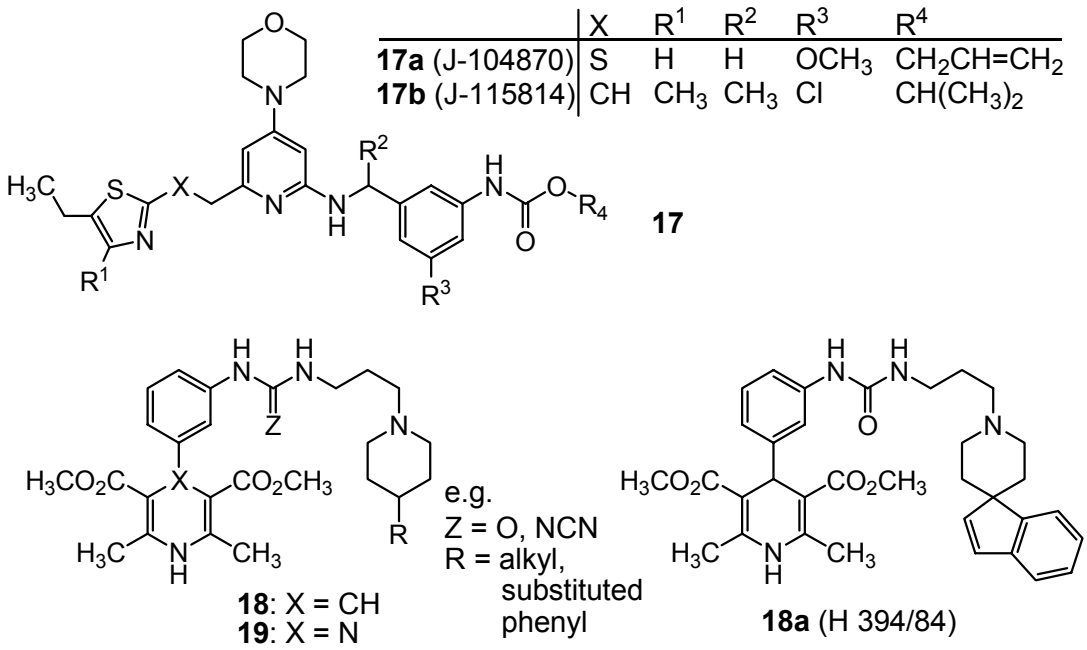


Fig. 12: Morpholinopyridines **17**, dihydropyridines **18** and dihydropyrazines **19**

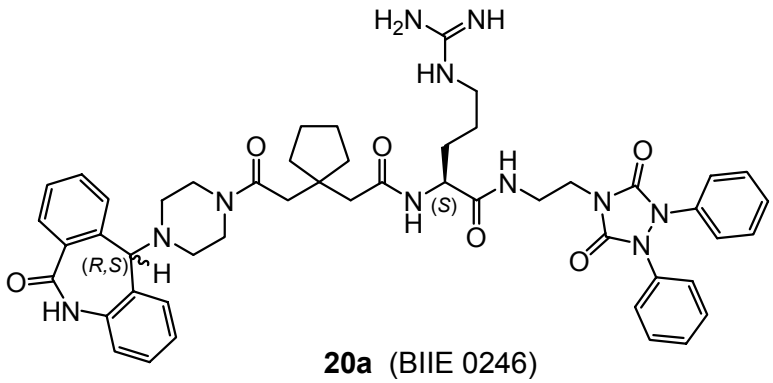
3.4.6. *DIHYDROPYRIDINES AND DIHYDROPYRAZINES*

Recently, dihydropyridine (**18**) and dihydropyrazine derivatives (**19**, Fig. 12) from Bristol-Myers Squibb[83-85] were described as NPY Y_1 antagonists. Generally the dihydropyridines **18** were up to about 100 times more potent in displacing [^{125}I]PYY from human Y_1 receptors than the corresponding dihydropyrazine analogs **19**. Highly active compounds **18** (K_i 2-5 nM) are urea derivatives (Z = O) with 2-methoxy-, 3-methoxy- or 3-hydroxy-substituted phenyl rings as R. Replacement of the urea with a cyanoguanidine group (Z = NCN) results in a further increase in activity (e.g. with R = *tert*-butyl: K_i < 1 nM). In comparison to the urea analogs, members of the cyanoguanidine series show improved permeability properties in Caco-2 cells[86]. For the derivative BMS-193885 (Z = O, R = 2-methoxyphenyl) full

functional Y_1 antagonism (K_b 4.5 nM) was observed in a cAMP assay using human Y_1 receptor expressing CHO cells. The compounds are Y_1 selective and specific in spite of the presence of α_1 adrenoceptor and calcium channel blocking pharmacophores. H 394/84 (**18a**) antagonized vascular responses to exogenous and endogenous, neuronally released NPY with similar potency already at plasma levels of 29 nM with a long duration of action *in vivo*[60, 87].

4. Selective Non-Peptide Y_2 Receptor Antagonists

For a long time Y_2 receptor blocking agents were eagerly awaited as pharmacological tools. First approaches to the design of NPY Y_2 receptor antagonists, described by Grouzman et al.[88], were based on the structure of the C-terminal tetrapeptide in NPY. A template-assembled synthetic protein (TASP), T_4-[NPY(33-36)]$_4$, consisting of four NPY(33-36) residues bound via spacer groups to a cyclic template (T_4) was reported to display Y_2 receptor binding in the submicromolar range[88]. However, it was not before 1999 that a non-peptide ligand, the L-arginine derivative BIIE 0246 (**20a**, Fig. 13), was disclosed (Boehringer Ingelheim Pharma)[54]. BIIE 0246 proved to be a highly potent and selective Y_2 receptor antagonist in binding experiments as well as in functional pharmacological studies[54, 89].



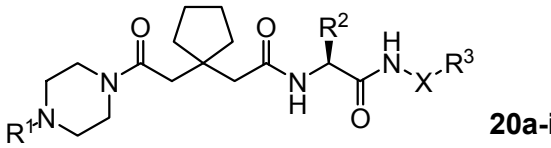
20a (BIIE 0246)

Fig. 13: Structure of the non-peptide Y_2 receptor antagonist BIIE 0246 (**20a**).

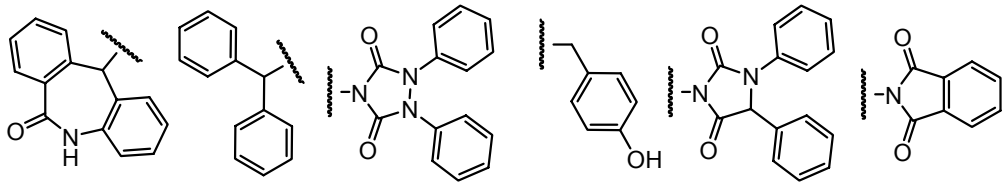
Radioligand binding studies on Y_2 receptors (SMS-KAN cells) revealed an IC_{50} value of 3.3 nM, whereas no displacement of radiolabelled NPY was observed in Y_1, Y_4

and Y_5 receptor assays[54]. Competitive Y_2 antagonism of **20a** was demonstrated, for instance, in pharmacological investigations on the isolated electrically stimulated rat *vas deferens*. The compound was used in numerous studies to investigate the contribution of NPY Y_2 receptors to complex physiological effects of NPY, e.g., in tissues with heterogenous receptor populations[89-94].

Table 4: Structure-activity relationships of BIIE 0246 related argininamides[95-97].



20a-i



i ii iii iv v vi

No.	R^1	R^2	X	R^3	IC_{50} (nM)[a]
20a[b]	i	$(CH_2)_3NHC(=NH)NH_2$	$(CH_2)_2$	iii	7.5
20b	i	$(CH_2)_3NHC(=NH)NH_2$	(*S*)-$CH(CONH_2)$	iv	36
20c	i	4-C_6H_4-$C(=NH)NH_2$	(*S*)-$CH(CONH_2)$	iv	1 000
20d	ii	$(CH_2)_3NHC(=NH)NH_2$	(*S*)-$CH(CONH_2)$	iv	40
20e	ii	$(CH_2)_3NHC(=NH)NH_2$	$(CH_2)_2$	iii	32
20f	ii	$(CH_2)_3NHC(=NH)NH_2$	$(CH_2)_2$	v	220
20g	ii	$(CH_2)_3NHC(=NH)NH_2$	$(CH_2)_2$	vi	340
20h	ii	$(CH_2)_3NHC(=NH)NH_2$	$(CH_2)_4$	iii	68

[a] displacement of ^{125}I labeled NPY from rabbit kidney preparations

[b] BIIE 0246

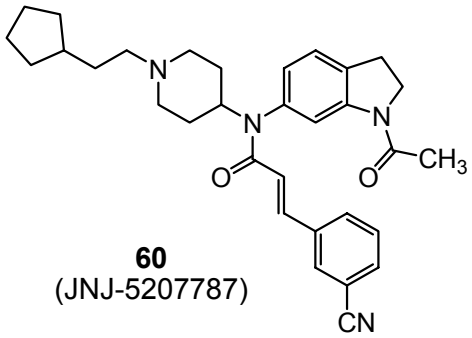
Similar to the discovery of the Y_1-selective antagonist BIBP 3226 (**4a**, see section 3.1), BIIE 0246 was synthesized as a member of an extensive set of related peptidomimetics which were designed as putative mimics of the C-terminus in NPY.

For some of these BIIE 0246 analogs Y_2 receptor binding data are given in the patent literature[95-97] (examples cf. Table 4). The 5,11-dihydrodibenzo[*b*,*e*]azepin-6-one group, though obviously representing the best suited substructure, can be replaced by an *α*-diphenylmethyl residue resulting in a relatively small decrease in affinity at the Y_2 receptor by a factor of 4-5 (**20e**)[95]. By contrast, exchange of the L-Arg side-chain, which is presumably mimicking Arg^{35} of NPY, by an isosteric *p*-benzamidino group is not tolerated (**20c**). Another interesting observation is that the replacement of the 4-(2-aminoethyl)-1,2-diphenyl-1,2,4-triazolidine-3,5-dione group by a L-tyrosinamide residue (**20b**) does not strongly alter affinity. This may be interpreted that both substructures are bioisosters mimicking the C-terminus in NPY. In contrast to BIIE 0246, other compounds of this series, e.g. **20d**, were found to produce an elevation of blood pressure in anesthetized rats which was attributed to different qualities of action (agonism/antagonism) at NPY receptors[96]. Variations of the 4-(2-aminoethyl)-1,2-diphenyl-1,2,4-triazolidine-3,5-dione motif (cf. **20f** – **20h**) led to diminished Y_2 affinity.

Interestingly, **20a** (BIIE 0246) does not bind to avian NPY Y_2 receptors[98]. Reciprocal mutagenesis between human (hY_2) and chicken Y_2 receptor (chY_2) revealed that three amino acids in hY_2 are especially important for BIIE 0246 binding: Gln^{135} in transmembrane domain 3 (TM3), Leu^{227} in TM5, and Leu^{284} in TM6[99] (Berglund et al. 2002). Mutagenesis of hY_2 to the corresponding amino acids in chY_2 (Q135H, L227Q, L284F) resulted in low affinity of BIIE 0246. Inversely, the introduction of the three human residues into chY_2 reproduced the high affinity to hY_2[99]. These results are first clues to models of the Y_2 receptor binding site of BIIE 0246.

Recently, a novel non-peptide, low molecular weight Y_2 receptor selective ligand with moderate antagonistic activity has been developed at Johnson & Johnson. JNJ-5207787 (**60**, Fig. 14) was the result of a SAR-driven optimization of a *N*-(indolin-6-yl)-*N*-(piperidin-4-yl)acrylamide lead structure, identified by high-throughput screening[100]. **60** inhibited the PYY (300 nM)-stimulated binding of [^{35}S]GTPγS to membranes of KAN-Ts cells, endogenously expressing hY_2 receptors, with a pIC_{50}

value of 7.20. For the displacement of [^{125}I]PYY (80 pM) from KAN-Ts cell membranes a pIC_{50} value of 7.00 was reported. After intraperitoneal administration in rats, JNJ-5207787 (**60**) penetrated into the brain. Moreover, **60** was used for *in vitro* autoradiography experiments in rat brain tissue sections[101].



60
(JNJ-5207787)

Fig. 14: Structure of small molecule Y_2 antagonist JNJ-5207787 (**60**).

5. NPY Y_5 Receptor Antagonists

The cloning of Y_5 receptors[102] and the discovery of first low molecular weight Y_5-blocking compounds stimulated an intensive and successful search for potent and selective non-peptide ligands. The interest in such compounds was not surprising as the Y_5 receptor — previously referred to as "Y_1-like" or the putative "feeding receptor" — has been considered as a key target for the control of body weight. Meanwhile various Y_1 and Y_5 receptor antagonists have been investigated as potential anti-obesity agents. The results do not allow to conclude that the orexigenic effect of NPY depends on a single receptor subtype[103], and there is some doubt concerning a significant contribution of the Y_5 receptor[104]. Investigations of the NPY receptor mediated feeding response in Y_1 and Y_5 receptor knockout mice indicated a dominant role of Y_1 receptors[105]. Nevertheless, the following selection of compounds reflects the enormous efforts of pharmaceutical companies in the Y_5 field: all non-peptide Y_5 receptor ligands mentioned in this section have been disclosed in patents over the last few years. The novel structures were summarized

in detailed reviews by A. Ling[106], M. Hammond[8], and S. L. Dax[107]. Published pharmacological data of these compounds are mostly restricted to IC_{50} values for the displacement of radioligands, and information on structure-activity relationships is limited to a few series.

Although the diversity of the known Y_5 antagonists is very large, many of the highly active structures seem to represent a common pattern which may be roughly characterized as "barbell-shaped": a non-bulky central group containing heteroatoms connects two larger terminal moieties consisting of polar or hydrophobic, aromatic or alicyclic, hetero- and/or polycyclic rings. Although this very general and simplified pattern does not necessarily suggest common ligand-receptor interactions, overlapping binding sites with a similar orientation of the structures may be assumed. The superposition of electrostatic and hydrophobic potentials would further elucidate this point.

5.1. Arylsulfonamide-type and Related NPY Y_5 Receptor Antagonists

The first non-peptide Y_5 receptor antagonists with binding affinities in the micromolar range, e.g. diarylalkanediamines such as JCF 104 (**21a**) and JCF 105 (**21b**, Fig. 15), were disclosed in patents by Synaptic Pharma and Eli Lilly & Co.[108, 109]. Moreover, diarylalkanediamines structurally similar to benextramine, which has some affinity to the hY_5 receptor (K_i = 5 μM)[25], were prepared, e.g. **21c** (Y_5: K_i = 1.7 μM)[25], and optimized in subsequent work. Y_5 receptor affinity and selectivity could be considerably improved by replacement of one amine center with a (non-basic) arylsulfonamide group and optimization of the connecting chain. A *trans*-cyclohexane-1,4-diyldimethyl spacer was found to be the most favorable linker between the two nitrogen centers[25]. Examples of the resulting arylsulfonamides are **22a** (JCF 109; hY_5: K_i = 10 nM, radioligand: [^{125}I]PYY)[103] and **22b** (hY_5: K_i = 11 nM, radioligand: [^{125}I]PYY)[110].

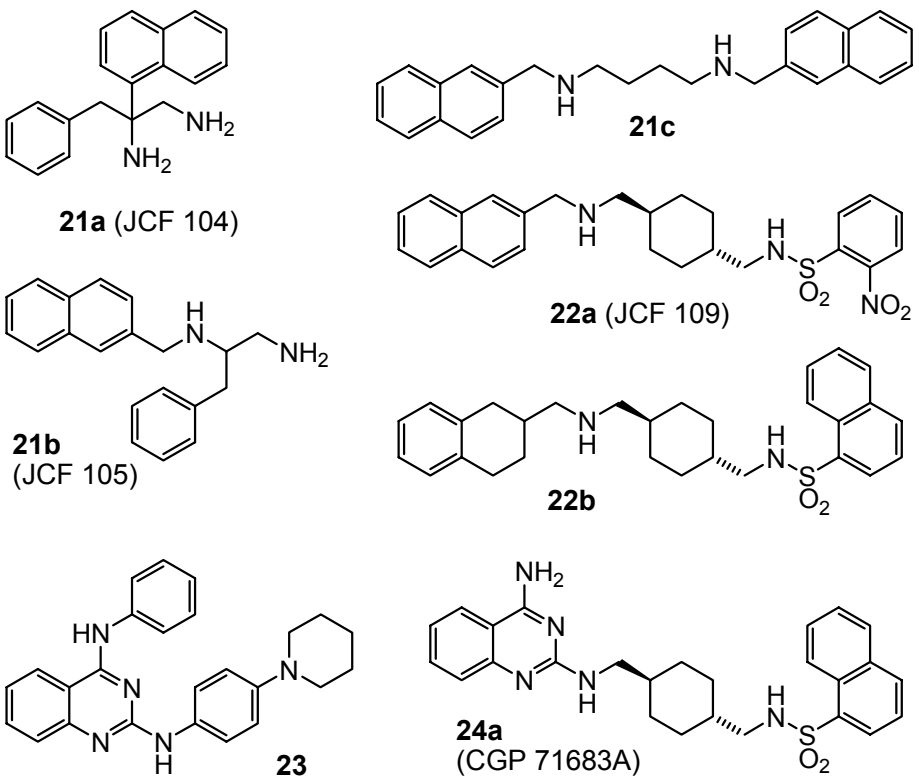
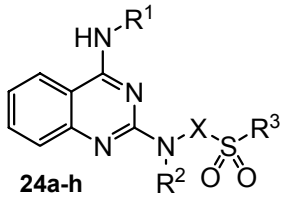


Fig. 15: Examples of diarylalkanediamine-, diaminoquinazoline- and arylsulfonamide-type Y_5 antagonists.

Based on homology modeling and ligand binding data obtained from studies with a set of receptor mutants, a ligand-receptor interaction model was generated[25]. According to this model a hydrogen bond is possible between the sulfonamide NH and His^{398} in TM6, which is absent in all other NPY receptor subtypes. Furthermore, the model suggests a salt bridge between the basic amino function of the ligand and Glu^{211} as well as hydrophobic interactions of the terminal aromatic rings with the receptor protein.

The 2,4-diaminoquinazoline motif was the scaffold of some moderate Y_5 ligands with slight Y_1/Y_5 selectivity[111]. Structural modifications led to compounds with nanomolar affinity for Y_5 receptors (e.g. **23**, hY_5: K_i 10 nM, radioligand: [^{125}I][Pro^{34}]hPYY)[112].

Table 5: Selected structures and binding data of substituted 2,4-diaminoquinazolines with Y_5 affinity[113].



24a-h

No.	R^1	R^2	X	R^3	IC_{50} (nM)[a]
24a[b]	H	H	CH_2-*t*-chx[c]-CH_2NH	1-naphthyl	2.9
24b	H	CH_3	CH_2-*t*-chx[c]-$CH_2N(CH_3)$	1-naphthyl	710
24c	H	H	CH_2-*p*-C_6H_4-CH_2NH	1-naphthyl	290
24d	phenyl	H	*p*-C_6H_4-CH_2	C_2H_5	0.6
24e	phenyl	H	*p*-C_6H_4-CH_2	$N(CH_3)_2$	0.9
24f	phenyl	H	*t*-chx[c]-CH_2NH	CH_3	2
24g	H	H	*t*-chx[c]-CH_2NH	4-methylphenyl	4
24h	H	H	$(CH_2)_6$	1-naphthyl	28

[a] binding affinities to human NPY Y_5 receptors stably expressed in LM(tk$^-$) cells.
[b] CGP 71683A
[c] *trans*-cyclohexane-1,4-diyl

A crucial step towards further increase in Y_5 receptor affinity and selectivity was the combination of the diaminoquinazoline moiety with *N*-cyclohexylmethyl-aryl-sulfonamide substructures like those present in compounds **22**. This approach resulted in the synthesis of the Y_5 receptor antagonist CGP 71683A (**24a**, Fig. 15) and analogs at Novartis Pharma[112, 114-116]. Substitution patterns and Y_5 receptor binding data of some diaminoquinazolines are exemplarily given in Table 5.

JCF 104 (**21a**), JCF 109 (**22a**) and CGP 71683A (**24a**, Fig. 15 and Table 5) were among the first Y_5 antagonistic tools available for the exploration of the occurrence and the pharmacological role of Y_5 receptors — especially their influence on feeding [50, 52, 103, 117-122]. CGP 71683A binds to Y_5 receptors with >1000-fold higher affinity than to the Y_1, Y_2 and Y_4 subtypes. It is probably the most intensively studied Y_5

antagonist so far. In fact, CGP 71683A (**24a**) is able to reduce food intake in animals. However, application of the substance *in vivo* is limited due to unfavorable properties (poor solubility, induction of local inflammatory changes) and significant affinity to other neurotransmitter receptors (e.g. muscarinic acetylcholine receptors) and to the serotonin transporter (5-HT reuptake) which may interfere with the regulation of food consumption[123]. Reports on the peripheral effects of CGP 71683A and other Y_5 antagonists were contradictory. Inhibition of PP-induced relaxation of rabbit ileum preparations was originally ascribed to Y_5 receptors[124], but further studies suggest that other NPY receptor subtypes (Y_4) are involved in this biological response[118].

The combination of the aforementioned structural motifs proved to be very successful. Neither a sulfonamide moiety nor a cyclic hydrocarbon or a quinazoline ring is essential for Y_5 receptor affinity. Consequently, the substructures of the Y_5 antagonists **22** and **24** were used as scaffolds by many groups to synthesize new potent and selective non-peptide Y_5 antagonists. In the following, sulfonamides related to **22** and **24** are subdivided into two chemical classes:

a. analogs of **22**, i. e., compounds having a (partially hydrogenated) hydrocarbon system, e.g., tetraline or a homolog,
b. analogs of **24a** (CGP 71683), i. e. compounds with other heterocyclic rings in place of the quinazoline.

Similar structures with other groups in place of the sulfonamide are included as analogs in section 5.1, whereas the majority of structurally diverse heterocyclic compounds is summarized in section 5.2.

5.1.1. SULFONAMIDES WITH TETRALINE OR HOMOLOGOUS CYCLIC HYDROCARBON MOIETIES

Recently, Itani et al.[125] described the synthesis of compounds in which, for instance, the tetrahydronaphthalene moiety of **22b** was expanded to a benzo[a]cycloheptene one. Additional exchange of the cyclohexane-1,4-diyl group against a piperidine containing central spacer results in **25a** (FR 226928, Fig. 16) as the most potent

compound (Y_5 IC_{50} 16 nM). Further optimization led to the structures **25b** and the non-sulfonamide **25c** with subnanomolar affinities[126].

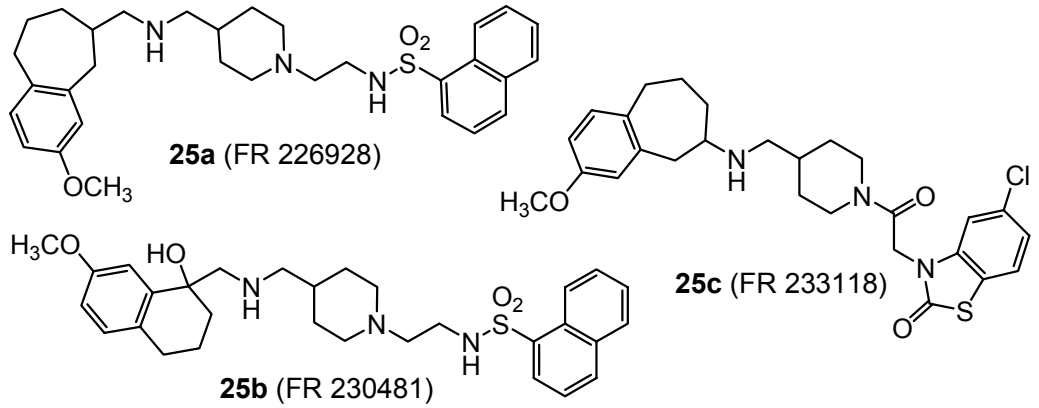


Fig. 16: Sulfonamide-type and related Y_5 antagonists with piperidine-containing spacer group.

NPY Y_5 antagonists with a common 2-aminotetraline motif were disclosed by Ortho-McNeil/R. W. Johnson in patent applications[127-129].

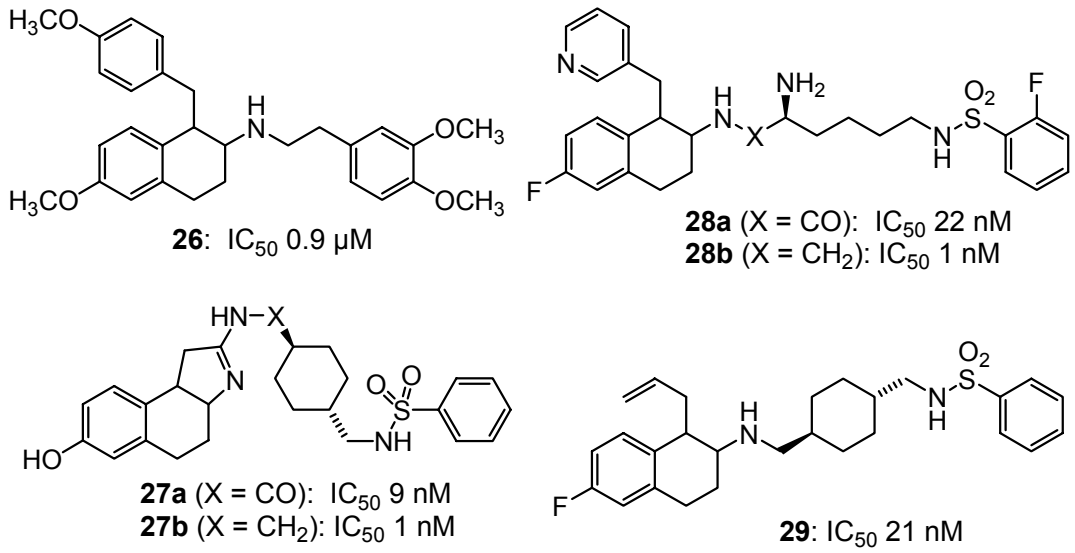


Fig. 17: Structures of *cis*-configured 1-substituted 2-aminotetralines and hY_5 receptor binding data (IC_{50} values; displacement of [^{125}I]PYY (80 pM), HEK 293 cells) of some Y_5 antagonists with aminotetraline portion[131, 132].

The structure-activity relationships of some α-substituted *N*-(sulfonamido)alkyl-β-aminotetralines have been the subject of subsequent journal papers[130-132]. Some representative structures (**26**-**29**) are depicted in Fig. 17.

The substituted *trans*-cyclohexane-1,4-diyl scaffold is present in many of the derivatives, though there are equipotent Y_5 antagonists with flexible alkyl chains such as *n*-pentyl instead of cyclohexylmethyl as central spacer. Moreover, analogs of, e.g., **28** with a carboxylic acid amide in place of the sulfonamide group were also found to have high Y_5 receptor affinity[107, 129]. Though not exactly matching the features of compounds **22a,b** or **24a** a large series of sulfonamides and sulfinamides covered in a patent application by Shionogi[133] may be subsumed in this group of Y_5 antagonists due to their structural design. Highly potent representative examples are **30a** and **30b** (Fig. 18) with IC_{50} values of 0.3 and 0.17 nM for binding affinity (Y_5 receptor expressing CHO cells) and 8.4 and 2.6 nM for antagonistic activity in the cAMP assay, respectively[133].

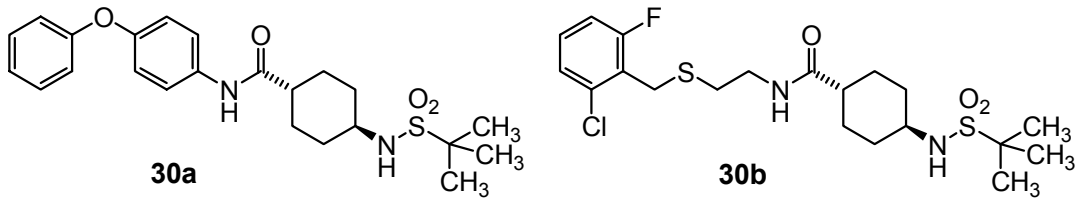


Fig. 18: 4-(Sulfonylamino)cyclohexanecarboxylic acid amides.

5.1.2. HETEROCYCLIC ANALOGS OF CGP 71863

Numerous heterocyclic analogs of **24a** (CGP 71683A), for instance, compounds with an aminothiazole or aminotriazine group or tricyclic ring systems[134-137] in place of the aminoquinazoline moiety, were disclosed as Y_5 antagonists with high affinity and selectivity for the human Y_5 receptor. Examples of such compounds (**31-35**) with Y_5 receptor binding data are given in Fig. 19. For some examples, e.g. **31**, **32** and **35**, hY_5 selectivity (vs. hY_1, hY_2, and hY_4 receptors) was demonstrated and the Y_5 antagonistic activity was confirmed in a cAMP assay. Tricyclic thiazole **61** and related compounds exhibit an attractive pharmacokinetic profile as selective, orally and centrally available NPY Y_5 receptor antagonists. Though **61** inhibited Y_5 agonist mediated feeding after oral administration, it did influence free- or fasting-induced feeding in rats[138]. Galiano et al.[139] described benzo[*b*]thiophene hydrazide

derivatives with an arylsulfonamidomethylcyclohexyl substructure as selective Y_5 receptor antagonists (e.g. **62**, IC_{50} 7.7 nM).

Aminotriazoles such as compound **36** were claimed as Y_5 antagonists in a patent application by Adir[140]. Substance **36** was reported to displace radiolabeled [^{125}I]PYY from Y_5 receptors with an IC_{50} value of 7 nM (assay not specified) and to lower food consumption and body weight in *ob/ob* mice (5 mg/kg ip bid for 3 days)[140].

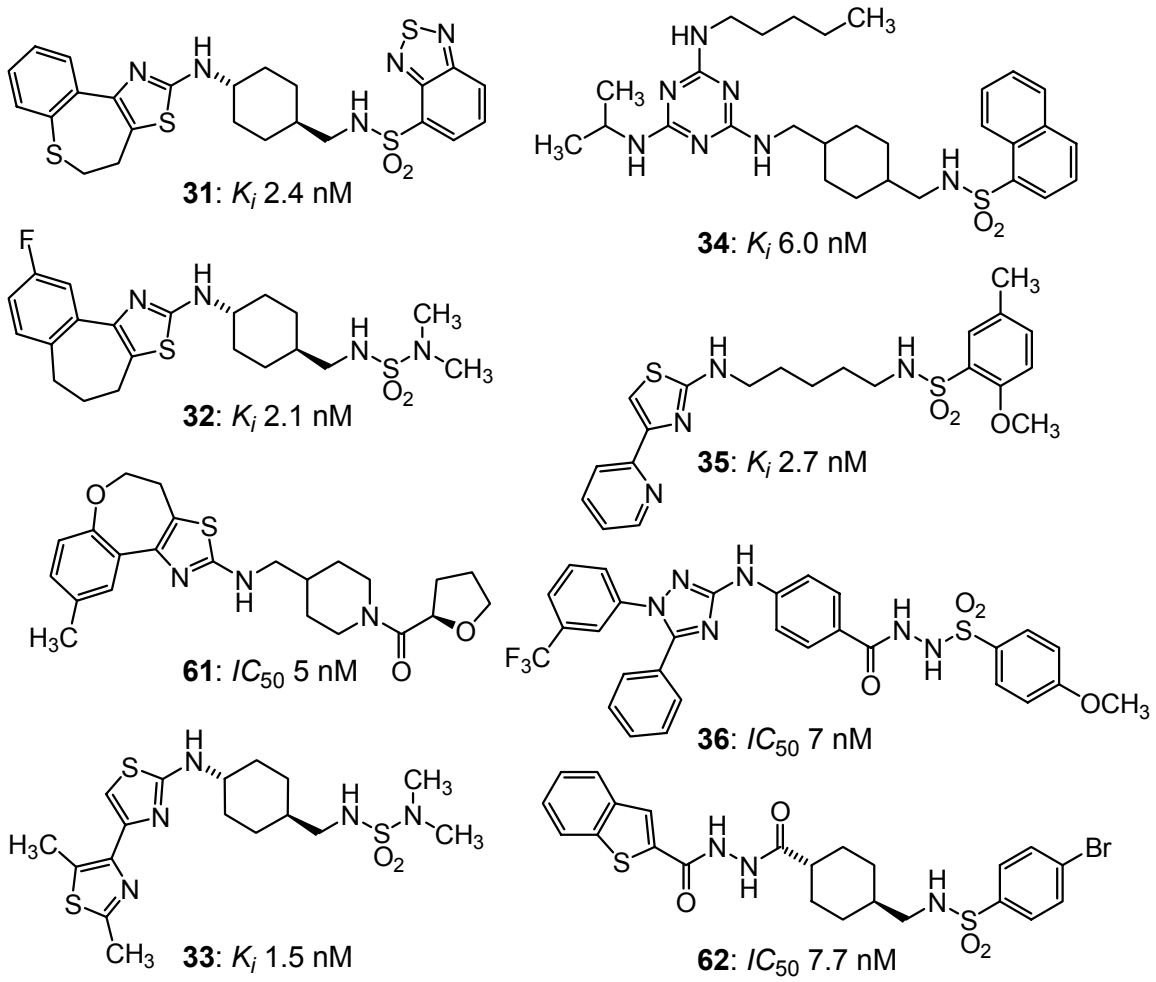


Fig. 19: Heterocyclic analogs of CGP 71683A. Binding constants (hY_5) for compounds **31-36** determined in radioligand binding studies on cell membrane preparations[134-136].

5.2. Various Heterocyclic NPY Y_5 Receptor Antagonists

5.2.1. AZOLES, PYRIDINES AND DIAZINES

Nitrogen-containing heterocycles are recurring structural elements in many non-peptide Y_5 ligands. For instance, several series of aminopyrazoles (e.g., **37a** – **37d**, Fig. 20) were presented by Banyu Pharm[141-145]. Compounds **37a** (JCF 114) and **37c** are reported to have Y_5 receptor affinities in the low nanomolar range (IC_{50} values: **37a**: 8.3 nM; **37c**: 2.5 nM). Very recently, the (–)-enantiomer of **37d** was described as an orally available and brain-penetrating Y_5 antagonist[146]. The compound displaces [^{125}I]PYY from human recombinant Y_5 receptors in LM(tk$^-$) cells with a K_i value of 3.5 nM. Though **37d** can be detected in the brain of SD rats after oral administration, its ability to suppress bPP-induced food-uptake is only moderate[146].

Among some pyrazole-3-carboxamides claimed by Ortho McNeil compound **38** (IC_{50} 80 nM) was reported to produce a 39 % reduction in food intake in fasted rats within the first 6 h after resumption of feeding, relative to control rats[147, 148].

Imidazole derivatives (cf. examples **39a** and **39b**, Fig. 20) were described as Y_5 antagonists by Neurogen and Pfizer[149-151]. Replacement of the 2-aryl ring of 2,4-diarylimidazole derivatives, related to **39b**, with a saturated, 6-membered ring produced compounds with only modest loss of activity at the Y_5 receptor[152]. Introduction of hydrophilic functionalities in 4-position of the cyclohexyl ring led to potent Y_5 antagonists with improved pharmacokinetic properties (e.g. **39c**, K_i 2.8 nM). In contrast to the parent 2,4-diarylimidazoles, **39c** shows almost no affinity for the hERG potassium channel[152]. A large collection of 5,5-diaryl-substituted imidazolones of general formula **40** was disclosed by Bristol-Myers Squibb without specified pharmacological data[153-155].

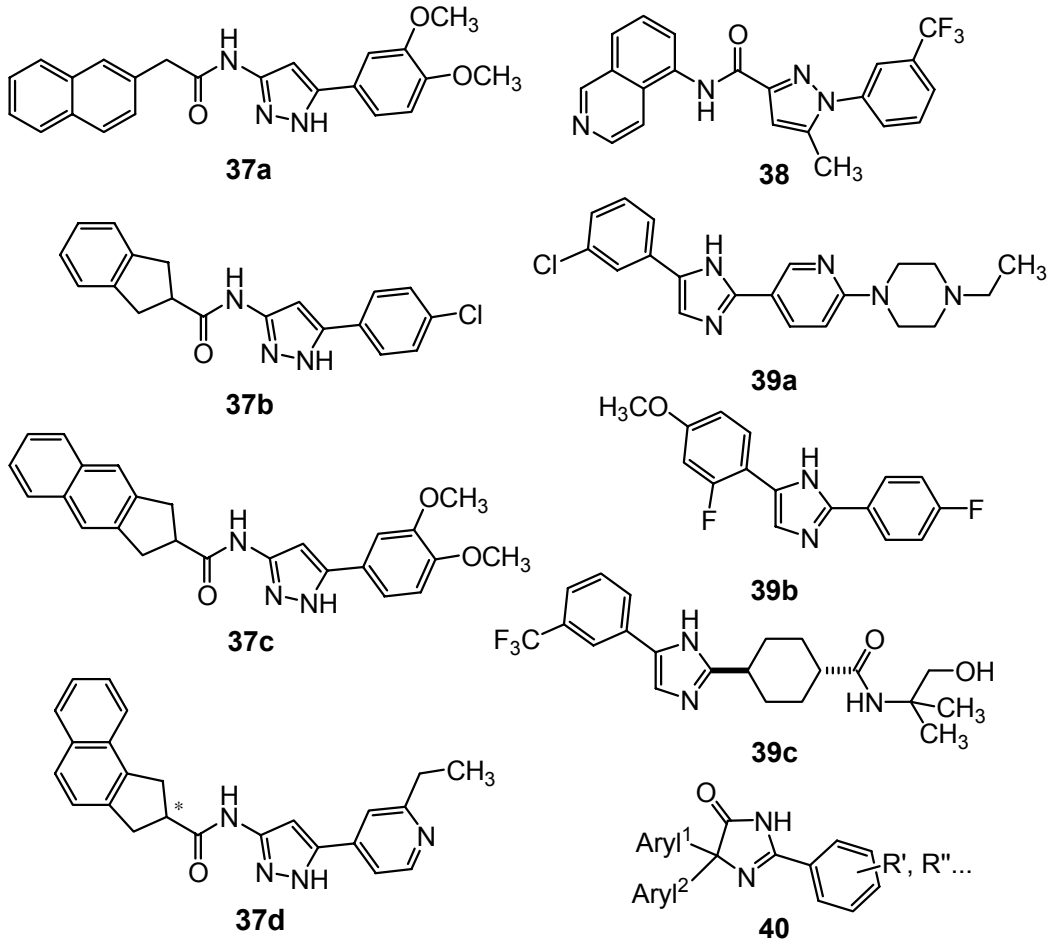


Fig. 20: Pyrazoles and imidazoles described as Y_5 antagonists.

Phenylacetamide derivatives (e.g. **45**) are the subject of a further patent application by Pfizer[162], and a series of benzimidazole-based Y_5 antagonists has been reported by the GlaxoSmithKline group[163-168]. The latter series covers orally available compounds such as **46** which penetrate into the CNS and reduce food intake in fasted rats (**46**: Y_5 IC_{50} 7.5 nM). Diarylguanidines (e.g. **47**: Y_5 IC_{50} 6 nM) are reported to be active in Zucker rats[169]. 4-Aminopyridines such as **48** from Banyu are described as Y_5 antagonists with affinity in the nanomolar range (**48**: IC_{50} 4.1 nM)[170].

Screening of an in-house library at Fujisawa Pharm. led to the discovery of benzothiazolone derivatives (e.g. **49** FR236478) with high Y_5 affinity but poor

bioavailability[171] and of tetrahydrodiazabenzazulenes like **50a** and **50b** which combine nanomolar affinity to the Y_5 receptor with oral absorption and penetration into brain[172].

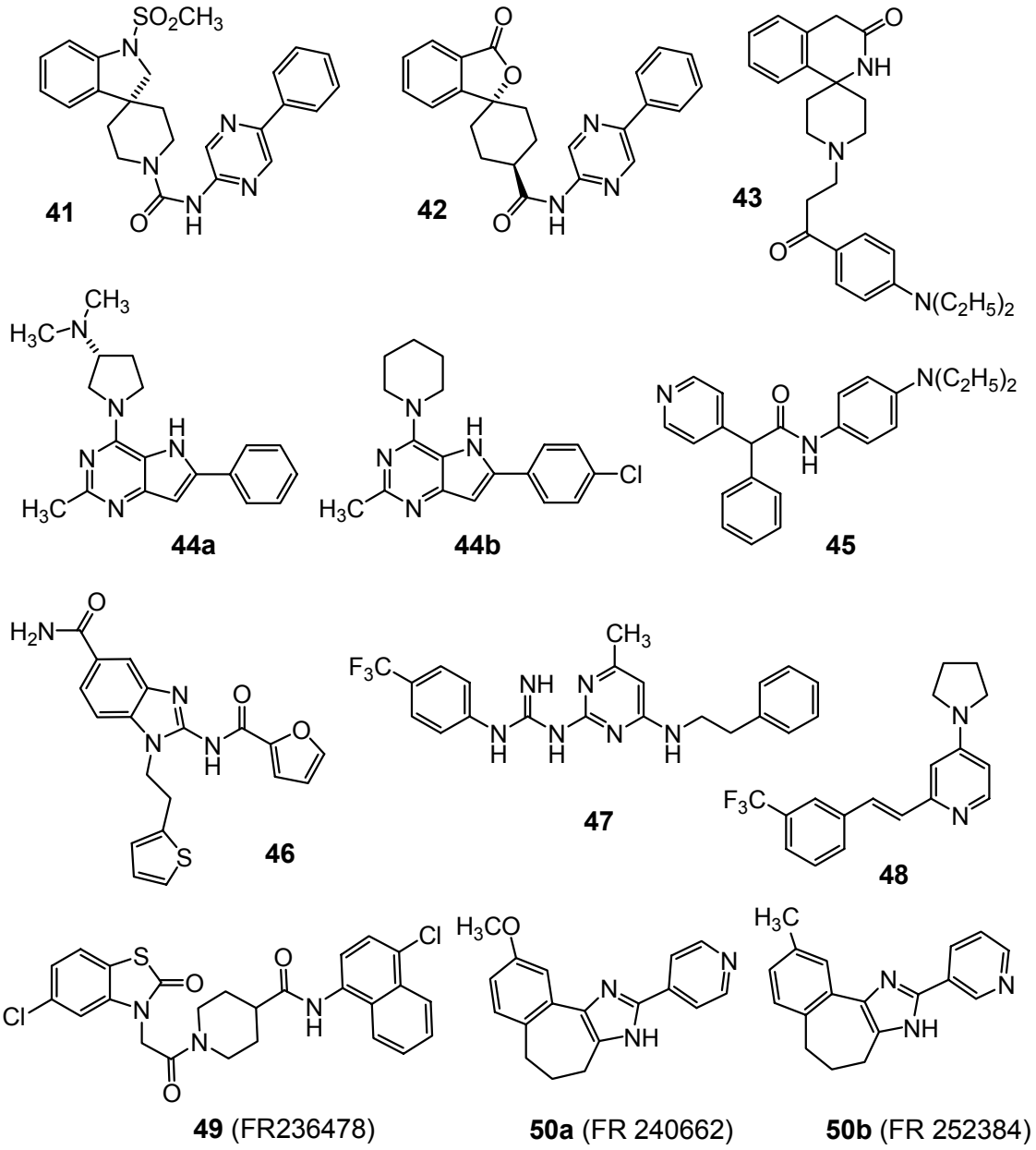


Fig. 21: Spiro compounds, annelated azoles and pyridines described as Y_5 antagonists.

The spiroindoline **41** (Fig. 21) from Merck and Banyu suppressed bPP-induced food intake in rats at an oral dosage of 3 mg/kg[156]. Related spirolactones were disclosed

by Banyu; **42** inhibited binding of [^{125}I]PP with an IC_{50} value of 0.48 nM[157]. Spiroisoquinolines such as **43** from Bristol-Myers Squibb belong to the same type of scaffold[158]. Pfizer introduced some 4-aminopyrrole[3,2-d]pyrimidines (e.g. **44a**) as Y_5 antagonists[159]. Similar bicyclic pyridine and pyrimidine derivatives (e.g. **44b**, furano- and thienopyrimidines) were reported by Amgen[160]. Within a series of pyrrolo[3,2-d]pyrimidines, structure-activity relationships of Y_5 receptor binding were analyzed and a pharmacophore model was derived[161]. Potent Y_5 antagonists of this series have IC_{50} values in the subnanomolar range (e.g. **44b**, Y_5: IC_{50} <0.1 nM).

5.2.2. *CARBAZOLES, FLUORENONES AND PHENYLUREAS*

AstraZeneca disclosed carbazole derivatives (Fig. 22) as NPY Y_5 antagonists[173, 174]. Recently, the discovery of these compounds and the optimization of their pharmacokinetic and toxicological properties were described[175]. **51a** is reported to bind with high affinity and selectivity to the human Y_5 receptor (IC_{50},Y_5: 2 nM; Y_1, Y_2, and Y_4: > 10 μM). But poor phamacokinetic properties (half-life ~15 min, oral bioavailability ~1 %) and potential mutagenicity and carcinogenicity disqualify the compound for the use *in vivo*. Replacement of the pyridylpropionamide sidechain by a morpholinocarboxamide, i. e. an urea group, as in **51b** greatly improved the oral bioavailability and increased the half-life to 3 h[175]. Moreover, compound **51b** is able to penetrate into the CNS. However, further modifications were necessary to suppress the mutagenic potential. In this context an isopropyl substituent at the carbazole-nitrogen turned out to be superior to ethyl, and further improvement was achieved by an additional methyl group *ortho* to the aniline function. The resulting optimized compound **51c** is reported to combine high Y_5 receptor affinity (IC_{50} 3 nM) with high selectivity, good bioavailability and central activity[175]. **51c** (3 mg/kg, dosed orally) is able to completely block food uptake provoked by injection of Y_5 selective agonists into the third ventricle of the brain of rats. By contrast, the

compound has no or only little effect on fasting-induced feeding or on NPY-induced-feeding.

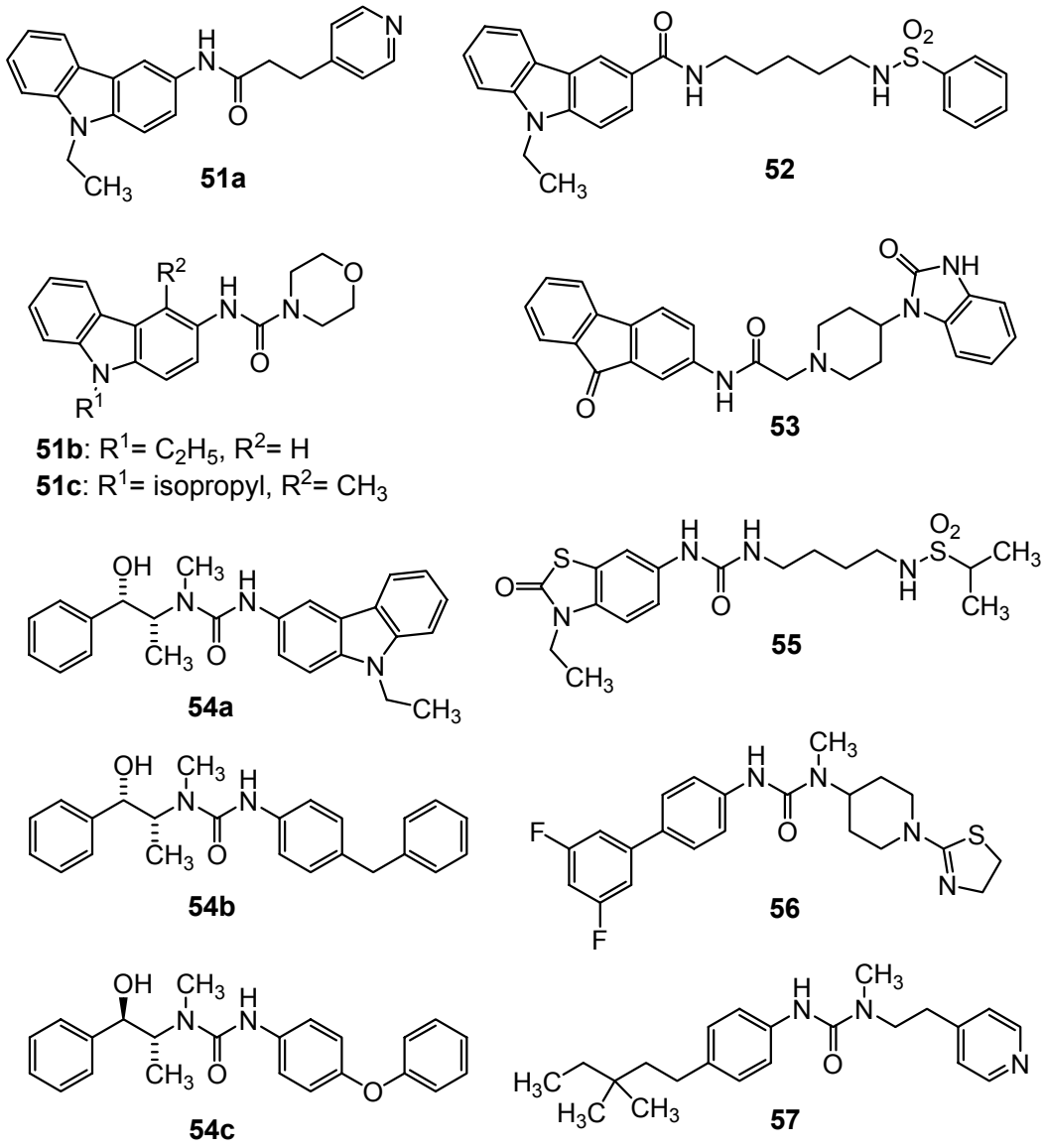


Fig. 22: Carbazole, fluorenone and phenylurea derivatives described as Y_5 antagonists.

Carbazole derivatives with inverted amide function were also described: the carbazole carboxamide **52** (Meiji Seika Kaisha Ltd.) was reported to completely displace radiolabeled NPY from membranes prepared from insect cells in which the human Y_5 receptor was expressed at a concentration of 10 μM (no detailed data included)[176].

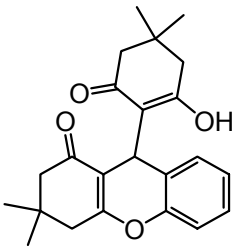
Within a series of Y_5 selective amides some fluorenone derivatives resembling the aforementioned carbazoles were presented by Bayer[177]. Compound **53** displaced ^{125}I-labeled pPP from human NPY Y_5 receptors with an IC_{50} value of 0.47 nM.

At Amgen, some trisubstituted phenyl urea derivatives with subnanomolar affinity were designed[178]. The starting point was structure **54c**, which was identified as hit by random screening. Lead optimization resulted in highly potent Y_5 antagonists, e.g. **54b** (IC_{50} < 0.1 nM) and **54a**, a carbazolylurea which resembles **51a-c**.

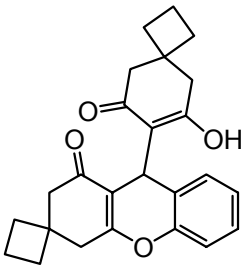
Similar approaches combined a central urea group with many other substituents to obtain Y_5 antagonists such as the ureidobenzothiazolone **55** (Meiji Seika Kaisha Ltd.)[179] or the ureas **56** and **57** which belong to a large series of NPY Y_5 receptor antagonists recently claimed by Schering-Plough[180-184]. For instance **57** was found to have a K_i value of 0.4 nM (Y_5), and **56** inhibited [D-Trp^{34}]NPY-stimulated food intake in a dose-dependent manner (ID_{50} 0.5 mg/kg)[184].

5.2.3. TETRAHYDROXANTHENE-1-ONES

The tetrahydro-xanthene-1-one derivatives **58** (L-152,804) and **59**[185] were introduced by Banyu as new orally active and selective neuropeptide Y Y_5 antagonists[186]. Compared to all the other potent NPY antagonists the structures look rather exotic as nitrogen atoms are completely lacking. No information on the binding mode of L-152,804 (**58**) and the molecular ligand-receptor interactions is published so far. Nevertheless, IC_{50} values of 52 and 14 nM for the inhibition of [^{125}I]PYY binding to a NPY Y_5 receptor membrane preparation are reported for the compounds **58** and **59**, respectively[185], and a significant reduction of bPP-induced food consumption was found both after intracerebroventricular (30 μg) and oral (10 mg/kg) administration of L-152,804 (**58**) in Sprague-Dawley rats. Interestingly, enhanced feeding, evoked by NPY, could not be influenced by application of L-152,804.



58 (L-152,804)



59

Fig. 23: Tetrahydroxanthene-1-one derivatives with Y_5 antagonistic activity

6. Conclusion

Since the discovery of the first non-peptide NPY antagonists the number of known highly potent and selective small molecular ligands and the structural diversity has considerably increased, mainly due to strategies based on screening of in-house libraries followed by rational lead optimization approaches based on pharmacophore hypotheses. As for other G-protein-coupled receptors, structure-based (receptor-based) design methods are not yet generally applicable. However, site-directed mutagenesis of NPY receptors and binding studies supported by molecular modeling have led to insight into ligand-receptor interactions on the molecular level and to the construction of receptor models complexed with ligands. The potential market for Y_1 and/or Y_5 antagonists as anti-obesity drugs has been the major stimulus of industrial NPY research. Considering the effort which has been spent on the discovery of neuropeptide Y Y_5 receptor ligands by various research groups in the last few years, some disillusion arises since no agent has entered human clinical trial, although many compounds with good bioavailability and reducing effects on food consumption in animal experiments have been identified. Unfortunately, there is increasing evidence that blocking a single receptor, in particular the Y_5 receptor[104, 105], is not suitable for long term body weight control as there are many redundant mechanisms in the regulation of food uptake and energy expenditure[187, 188]. The role of NPY in feeding is by far not the only interesting

therapeutic implication. Selective antagonists and agonists for all known NPY receptors are required as pharmacological tools to study the contribution of NPY and NPY receptor subtypes to different central and peripheral effects. Obviously, for NPY receptor antagonists (and agonists) as drug candidates the most interesting targets are located in the central nervous system, i. e., optimization of ADME parameters affecting pharmacokinetics, in particular penetration across the blood-brain barrier, is the bottle neck rather than further increasing binding affinity, provided that sufficiently potent and selective ligands for the respective NPY receptor subtypes are already available.

7. Reference list

[1] Rudolf, K.; Eberlein, W.; Wieland, H. A.; Engel, W.; Willim, K. D.; Entzeroth, M.; Wienen, W.; Beck-Sickinger, A. G.; Doods, H., The first highly potent and selective non-peptide NPY Y_1 receptor antagonist: BIBP 3226. *Eur. J. Pharm.* **1994,** 271, R11-3.

[2] Grundemar, L.; Bloom, S. R., *Neuropeptide Y and drug development*. Academic Press: London, San Diego, 1997; p 207.

[3] Silva, A. P.; Cavadas, C.; Grouzmann, E., Neuropeptide Y and its receptors as potential therapeutic drug targets. *Clin. Chim. Acta* **2002,** 326, 3-25.

[4] Michel, M. C.; Beck-Sickinger, A. G.; Cox, H.; Doods, H. N.; Herzog, H.; Larhammer, D.; Quirion, R.; Schwartz, T.; Westfall, T., XVI. International Union of Pharmacology Recommandations for the Nomenclature of Neuropeptide Y, Peptide YY, and Pancreatic Polypeptide Receptors. *Pharmacol. Rev.* **1998,** 50, 143-50.

[5] Allen, J.; Novotny, J.; Martin, J.; Heinrich, G., Molecular structure of mammalian neuropeptide Y: analysis by molecular cloning and computer-aided comparison with crystal structure of avian homologue. *Proc. Natl. Acad. Sci. U. S. A.* **1987,** 84, 2532-6.

[6] Beck-Sickinger, A. G.; Wieland, H. A.; Wittneben, H.; Willim, K. D.; Rudolf, K.; Jung, G., Complete L-alanine scan of neuropeptide Y reveals ligands binding to Y_1 and Y_2 receptors with distinguished conformations. *Eur. J. Biochem.* **1994,** 225, 947-58.

[7] Carpino, P. A., Patent focus on new anti-obesity agents: September 1999 - February 2000. *Expert Opin. Ther. Pat.* **2000,** 10, 819-31.

[8] Hammond, M., Neuropeptide Y receptor antagonists. *IDrugs* **2001,** 4, 920-7.

[9] Zimanyi, I. A.; Poindexter, G. S., NPY-ergic agents for the treatment of obesity. *Drug Dev. Res.* **2000,** 51, 94-111.

[10] Walker, P.; Munoz, M.; Martinez, R.; Peitsch, M. C., Acidic residues in extracellular loops of the human Y_1 neuropeptide Y receptor are essential for ligand binding. *J. Biol. Chem.* **1994,** 269, 2863-9.

[11] Sautel, M.; Rudolf, K.; Wittneben, H.; Herzog, H.; Martinez, R.; Munoz, M.; Eberlein, W.; Engel, W.; Walker, P.; Beck-Sickinger, A. G., Neuropeptide Y and the nonpeptide antagonist BIBP 3226 share an overlapping binding site at the human Y_1 receptor. *Mol. Pharmacol.* **1996,** 50, 285-92.

[12] Du, P.; Salon, J. A.; Tamm, J. A.; Hou, C.; Cui, W.; Walker, M. W.; Adham, N.; Dhanoa, D. S.; Islam, I.; et al., Modeling the G-protein-coupled neuropeptide Y Y_1 receptor agonist and antagonist binding sites. *Protein Eng.* **1997,** 10, 109-17.

[13] Robin-Jagerschmidt, C.; Sylte, I.; Bihoreau, C.; Hendricksen, L.; Calvet, A.; Dahl, S. G.; Benicourt, C., The ligand binding site of NPY at the rat Y_1 receptor investigated by site-directed mutagenesis and molecular modeling. *Mol. Cell. Endocrinol.* **1998,** 139, 187-98.

[14] Sylte, I.; Andrianjara, C. R.; Calvet, A.; Pascal, Y.; Dahl, S. G., Molecular dynamics of NPY Y_1 receptor activation. *Bioorg. Med. Chem.* **1999,** 7, 2737-48.

[15] Sylte, I.; Robin-Jagerschmidt, C.; Bihoreau, C.; Hendricksen, L.; Calvet, A.; Benicourt, C.; Dahl, S. G., Molecular modeling of the NPY binding site on the Y_1 receptor. *J. Mol. Model.* **1998,** 4, 221-33.

[16] Edvinsson, L.; Adamsson, M.; Jansen, I., Neuropeptide Y antagonistic properties of D-myo-inositol-1.2.6-trisphosphate in guinea pig basilar arteries. *Neuropeptides* **1990,** 17, 99-105.

[17] Feth, F.; Erdbrugger, W.; Rascher, W.; Michel, M. C., Is PP56 (D-myo-inositol-1,2,6-trisphosphate) an antagonist at neuropeptide Y receptors? *Life Sci.* **1993,** 52, 1835-44.

[18] Heilig, M.; Edvinsson, L.; Wahlestedt, C., Effects of intracerebroventricular D-myo-inositol-1,2,6-trisphosphate (PP56), a proposed neuropeptide Y (NPY) antagonist, on locomotor activity, food intake, central effects of NPY and NPY-receptor binding. *Eur. J. Pharmacol.* **1991,** 209, 27-32.

[19] Bell, D.; McDermott, B. J., D-myo inositol 1,2,6, trisphosphate (α-trinositol, pp56): selective antagonist at neuropeptide Y (NPY) Y-receptors or selective inhibitor of phosphatidylinositol cell signaling? *Gen. Pharmacol.* **1998,** 31, 689-96.

[20] Benfey, B. G., Two long acting α-adrenoceptor blocking drugs: benextramine and phenoxybenzamide. *Trends Pharmacol. Sci.* **1982,** 8, 231.

[21] Doughty, M. B.; Chu, S. S.; Miller, D. W.; Li, K.; Tessel, R. E., Benextramine: a long-lasting neuropeptide Y receptor antagonist. *Eur. J. Pharmacol.* **1990,** 185, 113-14.

[22] Doughty, M. B.; Chu, S. S.; Misse, G. A.; Tessel, R., Neuropeptide Y (NPY) functional group mimetics: design, synthesis, and characterization as NPY receptor antagonists. *Bioorg. Med. Chem. Lett.* **1992,** 2, 1497-502.

[23] Palea, S.; Corsi, M.; Rimland, J. M.; Trist, D. G., Discrimination by benextramine between the NPY Y_1 receptor subtypes present in rabbit isolated vas deferens and saphenous vein. *Br. J. Pharmacol.* **1995,** 115, 3-10.

[24] Wright, J., Design and discovery of neuropeptide Y_1 receptor antagonists. *Drug Discovery Today* **1997,** 2, 19-24.

[25] Islam, I.; Dhanoa, D.; Finn, J.; Du, P.; Walker, M. W.; Salon, J. A.; Zhang, J.; Gluchowski, C., Discovery of potent and selective small molecule NPY Y_5 receptor antagonists. *Bioorg. Med. Chem. Lett.* **2002,** 12, 1767-9.

[26] Doughty, M. B.; Chaurasia, C. S.; Li, K., Benextramine-neuropeptide Y receptor interactions: contribution of the benzylic moieties to [3H]neuropeptide Y displacement activity. *J. Med. Chem.* **1993,** 36, 272-9.

[27] Chaurasia, C.; Misse, G.; Tessel, R.; Doughty, M. B., Nonpeptide Peptidomimetic Antagonists of the Neuropeptide Y Receptor: Benextramine Analogs with Selectivity for the Peripheral Y_2 Receptor. *J. Med. Chem.* **1994,** 37, 2242-8.

[28] Buschauer, A., Synthesis and in vitro pharmacology of arpromidine and related phenyl(pyridylalkyl)guanidines, a potential new class of positive inotropic drugs. *J. Med. Chem.* **1989,** 32, 1963-70.

[29] Michel, M. C.; Moersdorf, J. P.; Engler, H.; Schickaneder, H.; Ahrens, K. H. Use of guanidine derivatives for the manufacture of a medicament with neuropeptide Y-antagonistic activity. 448765, **1991**.

[30] Michel, M. C.; Motulsky, H. J., He 90481: A compeptitive nonpeptidergic antagonist at neuropeptide Y receptors. *Ann. N. Y. Acad. Sci.* **1990,** 611, 392-4.

[31] Michel, M. C.; Dove, S.; Buschauer, A., Arpromidine and related histamine H_2 agonists as chemical leads for the development of non-peptide neuropeptide Y antagonists. *Arch. Pharm. (Weinheim, Ger.)* **1993,** 326, 664.

[32] Dove, S.; Michel, M. C.; Knieps, S.; Buschauer, A., Pharmacology and quantitative structure-activity relationships of imidazolylpropylguanidines with mepyramine-like substructures as non-peptide neuropeptide Y Y_1 receptor antagonists. *Can. J. Physiol. Pharmacol.* **2000,** 78, 108-15.

[33] Knieps, S.; Dove, S.; Michel, M. C.; Rottmeier, K.; Werner, W.; Bernhardt, G.; Buschauer, A., ω-Phenyl-ω-(2-pyridyl)alkyl-substituted bisguanidines are moderate neuropeptide Y antagonists. *Pharm. Pharmacol. Lett.* **1996,** 6, 27-30.

[34] Beck-Sickinger, A. G., The importance of various parts of the NPY molecule for receptor recognition. In *Neuropeptide Y and drug development*, Grundemar, L.; Bloom, S. R., Eds. Academic Press: London, San Diego, 1997; pp 107-26.

[35] Meister, A. Pharmacological characterization of new histamine and neuropeptide Y receptor ligands: set-up and validation of functional test models on isolated organs. doctoral thesis, University of Regensburg, Regensburg, Germany, 1999.

[36] Mueller, M.; Knieps, S.; Gessele, K.; Dove, S.; Bernhardt, G.; Buschauer, A., Synthesis and neuropeptide Y Y_1 receptor antagonistic activity of N,N-disubstituted ω-guanidino- and ω-aminoalkanoic acid amides. *Arch. Pharm. (Weinheim, Ger.)* **1997,** 330, 333-42.

[37] Doods, H. N.; Wienen, W.; Entzeroth, M.; Rudolf, K.; Eberlein, W.; Engel, W.; Wieland, H. A., Pharmacological characterization of the selective nonpeptide neuropeptide Y_1 receptor antagonist BIBP 3226. *J. Pharmacol. Exp. Ther.* **1995,** 275, 136-42.

[38] Wieland, H. A.; Willim, K. D.; Entzeroth, M.; Wienen, W.; Rudolf, K.; Eberlein, W.; Engel, W.; Doods, H. N., Subtype selectivity and antagonistic profile of the nonpeptide Y_1 receptor antagonist BIBP 3226. *J. Pharmacol. Exp. Ther.* **1995,** 275, 143-9.

[39] Doods, H. N.; Wieland, H. A.; Engel, W.; Eberlein, W.; Willim, K. D.; Entzeroth, M.; Wienen, W.; Rudolf, K., BIBP 3226, the first selective neuropeptide Y_1 receptor antagonist: a review of its pharmacological properties. *Regul. Pept.* **1996,** 65, 71-7.

[40] Aiglstorfer, I.; Uffrecht, A.; Gessele, K.; Moser, C.; Schuster, A.; Merz, S.; Malawska, B.; Bernhardt, G.; Dove, S.; Buschauer, A., NPY Y_1 antagonists: structure-activity relationships of arginine derivatives and hybrid compounds with arpromidine-like partial structures. *Regul. Pept.* **1998,** 75-76, 9-21.

[41] Abounader, R.; Villemure, J. G.; Hamel, E., Characterization of neuropeptide Y (NPY) receptors in human cerebral arteries with selective agonists and the new Y_1 antagonist BIBP 3226. *Br. J. Pharmacol.* **1995,** 116, 2245-50.

[42] Jacques, D.; Cadieux, A.; Dumont, Y.; Quirion, R., Apparent affinity and potency of BIBP3226, a non-peptide neuropeptide Y receptor antagonist, on purported neuropeptide Y Y_1, Y_2 and Y_3 receptors. *Eur. J. Pharmacol.* **1995,** 278, R3-5.

[43] Mollereau, C.; Gouarderes, C.; Dumont, Y.; Kotani, M.; Detheux, M.; Doods, H.; Parmentier, M.; Quirion, R.; Zajac, J. M., Agonist and antagonist activities on human $NPFF_2$ receptors of the NPY ligands GR231118 and BIBP3226. *Br. J. Pharmacol.* **2001,** 133, 1-4.

[44] Mollereau, C.; Mazarguil, H.; Marcus, D.; Quelven, I.; Kotani, M.; Lannoy, V.; Dumont, Y.; Quirion, R.; Detheux, M.; Parmentier, M.; Zajac, J. M., Pharmacological characterization of human $NPFF_1$ and $NPFF_2$ receptors expressed in CHO cells by using NPY Y_1 receptor antagonists. *Eur. J. Pharmacol.* **2002,** 451, 245-56.

[45] Malmström, R. E.; Balmer, K. C.; Lundberg, J. M., The neuropeptide Y (NPY) Y_1 receptor antagonist BIBP 3226: equal effects on vascular responses to exogenous and endogenous NPY in the pig in vivo. *Br. J. Pharmacol.* **1997,** 121, 595-603.

[46] Kask, A.; Rago, L.; Harro, J., Evidence for involvement of neuropeptide Y receptors in the regulation of food intake: studies with Y1-selective antagonist BIBP3226. *Br. J. Pharmacol.* **1998,** 124, 1507-15.

[47] Morgan, D. G. A.; Small, C. J.; Abusnana, S.; Turton, M.; Gunn, I.; Heath, M.; Rossi, M.; Goldstone, A. P.; O'Shea, D.; Meeran, K.; Ghatei, M.; Smith, D. M.; Bloom, S., The NPY Y_1 receptor antagonist BIBP 3226 blocks NPY induced feeding via a non-specific mechanism. *Regul. Pept.* **1998,** 75-76, 377-82.

[48] Iyengar, S.; Li, D. L.; Simmons, R. M., Characterization of neuropeptide Y-induced feeding in mice: do Y_1-Y_6 receptor subtypes mediate feeding? *J. Pharmacol. Exp. Ther.* **1999,** 289, 1031-40.

[49] Wieland, H. A.; Engel, W.; Eberlein, W.; Rudolf, K.; Doods, H. N., Subtype selectivity of the novel nonpeptide neuropeptide Y Y_1 receptor antagonist BIBO 3304 and its effect on feeding in rodents. *Br. J. Pharmacol.* **1998,** 125, 549-55.

[50] Dumont, Y.; Cadieux, A.; Doods, H.; Fournier, A.; Quirion, R., Potent and selective tools to investigate neuropeptide Y receptors in the central and peripheral nervous systems: BIBO3304 (Y_1) and CGP71683A (Y_5). *Can. J. Physiol. Pharmacol.* **2000,** 78, 116-25.

[51] Kask, A.; Harro, J., Inhibition of amphetamine- and apomorphine-induced behavioural effects by neuropeptide Y Y_1 receptor antagonist BIBO 3304. *Neuropharmacology* **2000,** 39, 1292-302.

[52] Polidori, C.; Ciccocioppo, R.; Regoli, D.; Massi, M., Neuropeptide Y receptor(s) mediating feeding in the rat: characterization with antagonists. *Peptides* **2000,** 21, 29-35.

[53] Rudolf, K.; Eberlein, W.; Engel, W.; Beck-Sickinger, A. G.; Wittneben, H.; Wieland, H. A.; H., D., BIBP3226, a potent and selective neuropeptide Y Y_1-receptor antagonist. Structure-activity studies and localization of the human Y_1 receptor binding site. In *Neuropeptide Y and drug development.*, Grundemar, L.; Bloom, S., Eds. Academic Press: London, San Diego, 1997; pp 175-90.

[54] Doods, H.; Gaida, W.; Wieland, H. A.; Dollinger, H.; Schnorrenberg, G.; Esser, F.; Engel, W.; Eberlein, W.; Rudolf, K., BIIE0246: A selective and high affinity neuropeptide Y Y_2 receptor antagonist. *Eur. J. Pharmacol.* **1999,** 384, R3-R5.

[55] Rudolf, K.; Eberlein, W.; Engel, W.; Mihm, G.; Doods, H.; Wieland, H.-A.; Willim, K.-D.; Krause, J.; Dollinger, H.; et al. Preparation of amino acid derivatives as neuropeptide Y antagonists. 9417035, **1994**.

[56] Antonsson, T.; Bergman, N.-A.; Linschoten, M.; Westerlund, C. Preparation and effect of diphenylacetylaminoalkylamides as neuropeptide Y antagonists. **2001**.

[57] Aiglstorfer, I.; Hendrich, I.; Moser, C.; Bernhardt, G.; Dove, S.; Buschauer, A., Structure-activity relationships of neuropeptide Y Y_1 receptor antagonists related to BIBP 3226. *Bioorg. Med. Chem. Lett.* **2000,** 10, 1597-600.

[58] Malmström, R. E., Neuropeptide Y Y_1 receptor mediated mesenteric vasoconstriction in the pig in vivo. *Regul. Pept.* **2000,** 95, 59-63.

[59] Malmström, R. E.; Alexandersson, A.; Balmer, K. C.; Weilitz, J., In vivo characterization of the novel neuropeptide Y Y_1 receptor antagonist H 409/22. *J. Cardiovasc. Pharmacol.* **2000,** 36, 516-25.

[60] Malmström, R. E., Pharmacology of neuropeptide Y receptor antagonists. Focus on cardiovascular functions. *Eur. J. Pharmacol.* **2002,** 447, 11-30.

[61] Daniels, A. J.; Chance, W. T.; Grizzle, M. K.; Heyer, D.; Matthews, J. E., Food intake inhibition and reduction in body weight gain in rats treated with GI264879A, a non-selective NPY-Y_1 receptor antagonist. *Peptides* **2001,** 22, 483-91.

[62] Kanno, T.; Kanatani, A.; Keen, S. L. C.; Arai-Otsuki, S.; Haga, Y.; Iwama, T.; Ishihara, A.; Sakuraba, A.; Iwaasa, H.; Hirose, M.; Morishima, H.; Fukami, T.; Ihara, M., Different binding sites for the neuropeptide Y Y_1 antagonists 1229U91 and J-104870 on human Y_1 receptors. *Peptides* **2001,** 22, 405-13.

[63] Palczewski, K.; Kumasaka, T.; Hori, T.; Behnke, C. A.; Motoshima, H.; Fox, B. A.; Le Trong, I.; Teller, D. C.; Okada, T.; Stenkamp, R. E.; Yamamoto, M.; Miyano, M., Crystal structure of rhodopsin: A G protein-coupled receptor. *Science* **2000,** 289, 739-45.

[64] Hutzler, C. Synthesis and pharmacological activity of new neuropeptide Y receptor ligands: from N,N-disubstituted alkanamides to highly potent argininamide-type Y_1 antagonists. Doctoral thesis, University of Regensburg, Regensburg, Germany, 2001.

[65] Hutzler, C.; Kracht, J.; Mayer, M.; Graichen, F.; Bauer, B.; Schreiber, E.; Bollwein, S.; Bernhardt, G.; Dove, S.; Fricker, G.; Buschauer, A., N^G-Acylated argininamides: highly potent selective NPY Y_1 receptor antagonists with special properties, Arch. Pharm. Pharm. Med. Chem. 334, Suppl. 2, 17 (2001). *Arch. Pharm. Pharm. Med. Chem.* **2001,** 334 Suppl. 2, 17.

[66] Moser, C.; Bernhardt, G.; Michel, J.; Schwarz, H.; Buschauer, A., Cloning and functional expression of the hNPY Y_5 receptor in human endometrial cancer (HEC-1B) cells. *Can. J. Physiol. Pharmacol.* **2000,** 78, 134-42.

[67] Serradeil-Le Gal, C., SR 120819A or the first generation of orally active Y_1 receptor antagonists. In *Neuropeptide Y and drug development*, Grundemar, L.; Bloom, S. R., Eds. Academic Press: London, San Diego, 1997; pp 157-74.

[68] Serradeil-Le Gal, C.; Valette, G.; Rouby, P.-E.; Pellet, A.; Oury-Donat, F.; Brossard, G.; Lespy, L.; Marty, E.; Neliat, G.; et al., SR120819A, an orally-active and selective neuropeptide Y Y_1 receptor antagonist. *FEBS Lett.* **1995,** 362, 192-6.

[69] Hipskind, P. A.; Lobb, K. L.; Nixon, J. A.; Britton, T. C.; Bruns, R. F.; Catlow, J.; Dieckman-McGinty, D. K.; Gackenheimer, S. L.; Gitter, B. D.; Iyengar, S.; Schober, D. A.; Simmons, R. M. A.; Swanson, S.; Zarrinmayeh, H.; Zimmerman, D. M.; Gehlert, D. R., Potent and Selective 1,2,3-Trisubstituted Indole NPY Y_1 Antagonists. *J. Med. Chem.* **1997,** 40, 3712-4.

[70] Zarrinmayeh, H.; Nunes, A. M.; Ornstein, P. L.; Zimmerman, D. M.; Arnold, M. B.; Schober, D. A.; Gackenheimer, S. L.; Bruns, R. F.; Hipskind, P. A.; Britton, T. C.; Cantrell, B. E.; Gehlert, D. R., Synthesis and Evaluation of a Series of Novel 2-[(4-Chlorophenoxy)methyl]benzimidazoles as Selective Neuropeptide Y Y_1 Receptor Antagonists. *J. Med. Chem.* **1998,** 41, 2709-19.

[71] Zarrinmayeh, H.; Zimmerman, D. M.; Cantrell, B. E.; Schober, D. A.; Bruns, R. F.; Gackenheimer, S. L.; Ornstein, P. L.; Hipskind, P. A.; Britton, T. C.; Gehlert, D. R., Structure-activity relationship of a series of diaminoalkyl substituted benzimidazole as neuropeptide Y Y_1 receptor antagonists. *Bioorg. Med. Chem. Lett.* **1999,** 9, 647-52.

[72] Zarrinmayeh, H.; Zimmerman, D. M.; Cantrell, B. E.; Schober, D. A.; Bruns, R. F.; Gackenheimer, S. L.; Ornstein, P. L.; Hipskind, P. A.; Britton, T. C.; Gehlert, D. R., Structure-activity relationship of a series of diaminoalkyl substituted benzimidazole as neuropeptide Y Y1 receptor antagonists. *Bioorg. Med. Chem. Lett.* **1999,** 9, 647-52.

[73] Britton, T. C.; Spinazze, P. G.; Hipskind, P. A.; Zimmerman, D. M.; Zarrinmayeh, H.; Schober, D. A.; Gehlert, D. R.; Bruns, R. F., Structure-activity relationships of a series of benzothiophene-derived NPY Y_1 antagonists: optimization of the C-2 side chain. *Bioorg. Med. Chem. Lett.* **1999,** 9, 475-80.

[74] Wright, J.; Bolton, G.; Creswell, M.; Downing, D.; Georgic, L.; Heffner, T.; Hodges, J.; MacKenzie, R.; Wise, L., 8-Amino-6-(arylsulfonyl)-5-nitroquinolines: novel nonpeptide neuropeptide Y_1 receptor antagonists. *Bioorg. Med. Chem. Lett.* **1996,** 6, 1809-14.

[75] Blum, C. A.; Desimone, R.; Hutchison, A.; Peterson, J. M. Certain amido- and amino- substituted benzylamine derivatives; a new class of neuropeptide Y_1 specific ligands. **2000**.

[76] Peterson, J. M.; Blum, C. A.; Cai, G.; Hutchison, A. Certain substituted benzylamine derivatives: a new class of neuropeptide Y_1 specific ligands. **1996**.

[77] Rabinovich, A.; Mazurov, A.; Krokhina, G.; Ling, A. L.; Livnah, N.; Hong, Y.; Valenzuela, E.; Gregor, V.; Feinstein, R.; Polinsky, A.; May, J. M.; Hagaman, C.; Brown, M., Discovery of Neuropeptide Y Receptor Antagonists. In *Neuropeptide Y and drug development*, Grundemar, L.; Bloom, S., Eds. Academic Press: London, San Diego, 1997; pp 191-201.

[78] Shigeri, Y.; Ishikawa, M.; Ishihara, Y.; Fujimoto, M., A potent nonpeptide neuropeptide Y Y_1 receptor antagonist, a benzodiazepine derivative. *Life Sci.* **1998,** 63, PL 151-60.

[79] Murakami, Y.; Hagishita, S.; Okada, T.; Kii, M.; Hashizume, H.; Yagami, T.; Fujimoto, M., 1,3-disubstituted benzazepines as neuropeptide Y Y_1 receptor antagonists. *Bioorg. Med. Chem.* **1999,** 7, 1703-14.

[80] Murakami, Y.; Hara, H.; Okada, T.; Hashizume, H.; Kii, M.; Ishihara, Y.; Ishikawa, M.; Shimamura, M.; Mihara, S.-i.; Kato, G.; Hanasaki, K.; Hagishita, S.; Fujimoto, M., 1,3-Disubstituted benzazepines as novel, potent, selective neuropeptide Y Y_1 receptor antagonists. *J. Med. Chem.* **1999,** 42, 2621-32.

[81] Kanatani, A.; Hata, M.; Mashiko, S.; Ishihara, A.; Okamoto, O.; Haga, Y.; Ohe, T.; Kanno, T.; Murai, N.; Ishii, Y.; Fukuroda, T.; Fukami, T.; Ihara, M., A typical Y_1 receptor regulates feeding behaviors: effects of a potent and selective Y_1 antagonist, J-115814. *Mol. Pharmacol.* **2001,** 59, 501-5.

[82] Kanatani, A.; Kanno, T.; Ishihara, A.; Hata, M.; Sakuraba, A.; Tanaka, T.; Tsuchiya, Y.; Mase, T.; Fukuroda, T.; Fukami, T.; Ihara, M., The Novel Neuropeptide Y Y_1 Receptor Antagonist J-104870: A Potent Feeding Suppressant with Oral Bioavailability. *Biochem. Biophys. Res. Commun.* **1999,** 266, 88-91.

[83] Poindexter, G. S.; Bruce, M.; Johnson, G.; Kozlowski, M.; Leboulluec, K.; Monkovic, I.; Seethala, R.; Sloan, C. P. Dihydropyridine NPY antagonists: piperazine derivatives. EP 747356, **1996**.

[84] Poindexter, G. S.; Bruce, M. A.; LeBoulluec, K. L.; Monkovic, I.; Martin, S. W.; Parker, E. M.; Iben, L. G.; McGovern, R. T.; Ortiz, A. A.; Stanley, J. A.; Mattson, G. K.; Kozlowski, M.; Arcuri, M.; Antal-Zimanyi, I., Dihydropyridine neuropeptide Y Y_1 receptor antagonists. *Bioorg. Med. Chem. Lett.* **2002,** 12, 379-82.

[85] Sit, S. Y.; Huang, Y.; Antal-Zimanyi, I.; Ward, S.; Poindexter, G. S., Novel dihydropyrazine analogues as NPY antagonists. *Bioorg. Med. Chem. Lett.* **2002,** 12, 337-40.

[86] Poindexter, G. S.; Bruce, M. A.; Breitenbucher, J. G.; Higgins, M. A.; Sit, S. Y.; Romine, J. L.; Martin, S. W.; Ward, S. A.; McGovern, R. T.; Clarke, W.; Russell, J.; Antal-Zimanyi, I., Dihydropyridine neuropeptide Y Y_1 receptor antagonists 2. bioisosteric urea replacements. *Bioorg. Med. Chem.* **2004,** 12, 507-21.

[87] Malmström, R. E.; Balmer, K. C.; Weilitz, J.; Nordlander, M.; Sjolander, M., Pharmacology of H 394/84, a dihydropyridine neuropeptide Y Y_1 receptor antagonist, in vivo. *Eur. J. Pharmacol.* **2001,** 418, 95-104.

[88] Grouzmann, E.; Buclin, T.; Martire, M.; Cannizzaro, C.; Dorner, B.; Razaname, A.; Mutter, M., Characterization of a selective antagonist of neuropeptide Y at the Y_2 receptor. Synthesis and pharmacological evaluation of a Y_2 antagonist. *J. Biol. Chem.* **1997,** 272, 7699-706.

[89] Dumont, Y.; Cadieux, A.; Doods, H.; Pheng, L. H.; Abounader, R.; Hamel, E.; Jacques, D.; Regoli, D.; Quirion, R., BIIE0246, a potent and highly selective non-peptide neuropeptide Y Y_2 receptor antagonist. *Br. J. Pharmacol.* **2000,** 129, 1075-88.

[90] Malmström, R. E., Vascular pharmacology of BIIE0246, the first selective non-peptide neuropeptide Y Y_2 receptor antagonist, in vivo. *Br. J. Pharmacol.* **2001,** 133, 1073-80.

[91] Malmström, R. E., Existence of both neuropeptide Y, Y_1 and Y_2 receptors in pig spleen: evidence using subtype-selective antagonists in vivo. *Life Sci.* **2001,** 69, 1999-2005.

[92] Malmström, R. E.; Lundberg, J. N.; Weitzberg, E., Effects of the neuropeptide Y Y_2 receptor antagonist BIIE0246 on sympathetic transmitter release in the pig in vivo. *Naunyn. Schmiedebergs Arch. Pharmacol.* **2002,** 365, 106-11.

[93] Malmström, R. E.; Lundberg, J. O.; Weitzberg, E., Autoinhibitory function of the sympathetic prejunctional neuropeptide Y Y(2) receptor evidenced by BIIE0246. *Eur. J. Pharmacol.* **2002,** 439, 113-9.

[94] Smith-White, M. A.; Hardy, T. A.; Brock, J. A.; Potter, E. K., Effects of a selective neuropeptide Y Y_2 receptor antagonist, BIIE0246, on Y_2 receptors at peripheral neuroeffector junctions. *Br. J. Pharmacol.* **2001,** 132, 861-8.

[95] Dollinger, H.; Esser, F.; Mihm, G.; Rudolf, K.; Schnorrenberg, G.; Gaida, W.; Doods, H. N. Preparation of novel peptides for use as NPY antagonists. DE 19816929, **1999**.

[96] Esser, F.; Schnorrenberg, G.; Dollinger, H.; Gaida, W. Preparation of novel peptides for use as NPY antagonists. DE 19816932, **1999**.

[97] Rudolf, K.; Eberlein, W.; Engel, W.; Mihm, G.; Doods, H.; Willim, K.-D.; Krause, J.; Wieland, H.-A.; Schnorrenberg, G.; Esser, F.; Dollinger, H. Preparation of piperazine-containing peptidomimetics for use as NPY antagonists. DE 19816889, **1999**.

[98] Salaneck, E.; Holmberg, S. K.; Berglund, M. M.; Boswell, T.; Larhammar, D., Chicken neuropeptide Y receptor Y_2: structural and pharmacological differences to mammalian Y_2(1). *FEBS Lett.* **2000,** 484, 229-34.

[99] Berglund, M. M.; Fredriksson, R.; Salaneck, E.; Larhammar, D., Reciprocal mutations of neuropeptide Y receptor Y_2 in human and chicken identify amino acids important for antagonist binding. *FEBS Lett.* **2002,** 518, 5-9.

[100] Jablonowski, J. A.; Chai, W.; Li, X.; Rudolph, D. A.; Murray, W. V.; Youngman, M. A.; Dax, S. L.; Nepomuceno, D.; Bonaventure, P.; Lovenberg, T. W.; Carruthers, N. I., Novel non-peptidic neuropeptide Y Y_2 receptor antagonists. *Bioorg. Med. Chem. Lett.* **2004,** 14, 1239-42.

[101] Bonaventure, P.; Nepomuceno, D.; Mazur, C.; Lord, B.; Rudolph, D. A.; Jablonowski, J. A.; Carruthers, N. I.; Lovenberg, T. W., Characterization of N-(1-Acetyl-2,3-dihydro-1*H*-indol-6-yl)-3-(3-cyano-phenyl)-N-[1-(2-cyclopentyl-ethyl)-piperidin-4yl]acrylamide (JNJ-5207787), a small molecule antagonist of the neuropeptide Y Y_2 receptor. *J. Pharmacol. Exp. Ther.* **2004,** 308, 1130-7.

[102] Gerald, C.; Walker, M. W.; Criscione, L.; Gustafson, E. L.; Batzl-Hartmann, C.; Smith, K. E.; Vaysse, P.; Durkin, M. M.; Laz, T. M.; Linemeyer, D. L.; Schaffhauser, A. O.; Whitebread, S.; Hofbauer, K. G.; Taber, R. I.; Branchek, T. A.; Weinshank, R. L., A receptor subtype involved in neuropeptide-Y-induced food intake. *Nature* **1996,** 382, 168-71.

[103] Duhault, J.; Boulanger, M.; Chamorro, S.; Boutin, J. A.; Della Zuana, O.; Douillet, E.; Fauchere, J. L.; Feletou, M.; Germain, M.; Husson, B.; Vega, A. M.; Renard, P.;

Tisserand, F., Food intake regulation in rodents: Y_5 or Y_1 NPY receptors or both? *Can. J. Physiol. Pharmacol.* **2000,** 78, 173-85.

[104] Turnbull, A. V.; Ellershaw, L.; Masters, D. J.; Birtles, S.; Boyer, S.; Carroll, D.; Clarkson, P.; Loxham, S. J.; McAulay, P.; Teague, J. L.; Foote, K. M.; Pease, J. E.; Block, M. H., Selective antagonism of the NPY Y_5 receptor does not have a major effect on feeding in rats. *Diabetes* **2002,** 51, 2441-9.

[105] Kanatani, A.; Mashiko, S.; Murai, N.; Sugimoto, N.; Ito, J.; Fukuroda, T.; Fukami, T.; Morin, N.; MacNeil, D. J.; Van der Ploeg, L. H.; Saga, Y.; Nishimura, S.; Ihara, M., Role of the Y_1 receptor in the regulation of neuropeptide Y-mediated feeding: comparison of wild-type, Y_1 receptor-deficient, and Y_5 receptor-deficient mice. *Endocrinology* **2000,** 141, 1011-6.

[106] Ling, A. L., Neuropeptide Y receptor antagonists. *Expert Opin. Ther. Pat.* **1999,** 9, 375-84.

[107] Dax, S. L., Small-molecule neuropeptide Y Y_5 antagonists. *Drugs of the Future* **2002,** 27, 273-87.

[108] Fritz, J. E.; Kaldor, S. W.; Scott, W. L.; Lobb, K. L.; Hipskind, P. A.; Nixon, J. A. 3-Arylpropylamino Neuropeptide Y Receptor Antagonists. WO 9852890, **1998**.

[109] Gehlert, D. R.; Hipskind, P. A., Neuropeptide Y receptor antagonists in obesity. *Expert Opin. Invest. Drugs* **1997,** 6, 1827-38.

[110] Du, P.; Finn, J. M.; Jeon, Y. T.; Dhanoa, D. S.; Islam, I.; Gluchowski, C. Aryl sulfonamide and sulfamide derivatives and uses thereof. WO 9719682, **1997**.

[111] Rueeger, H.; Rigollier, P.; Yamaguchi, Y.; Schmidlin, T.; Schilling, W.; Criscione, L.; Whitebread, S.; Chiesi, M.; Walker, M. W.; Dhanoa, D.; Islam, I.; Zhang, J.; Gluchowski, C., Design, synthesis and SAR of a series of 2-substituted 4-amino-quinazoline neuropeptide Y Y5 receptor antagonists. *Bioorg. Med. Chem. Lett.* **2000,** 10, 1175-9.

[112] Rüeger, H.; Schmidlin, T.; Rigollier, P.; Yamaguchi, Y.; Tintelnot-Blomley, M.; Schilling, W.; Criscione, L. Quinazoline-2,4-diazirines as NPY receptor antagonists. WO 9720822, **1997**.

[113] Rüeger, H.; Rigollier, P.; Yamaguchi, Y.; Schmidlin, T.; Schilling, W.; Criscione, L.; Whitebread, S.; Chiesi, M.; Walker, M. W.; Dhanoa, D.; Islam, I.; Zhang, J.; Gluchowski, C., Design, synthesis and SAR of a series of 2-substituted 4-amino-quinazoline neuropeptide Y Y_5 receptor antagonists. *Bioorg. Med. Chem. Lett.* **2000,** 10, 1175-9.

[114] Criscione, L.; Yamaguchi, Y.; Mah, R.; Schmidlin, T.; Rüeger, H.; Schilling, W.; Tintelnot-Blomley, M.; Rigollier, P. Receptor Antagonists. WO 9720823, **1997**.

[115] Rüeger, H.; Schmidlin, T.; Rigollier, P.; Yamaguchi, Y.; Tintelnot-Blomley, M.; Schilling, W.; Criscione, L. Quinazoline derivatives useful as antagonists of NPY receptor subtype Y_5. WO 9720820, **1997**.

[116] Rüeger, H.; Schmidlin, T.; Rigollier, P.; Yamaguchi, Y.; Tintelnot-Blomley, M.; Schilling, W.; Criscione, L.; Stutz, S. Heteroaryl Derivatives. WO 9720821, **1997**.

[117] Criscione, L.; Rigollier, P.; Batzl-Hartmann, C.; Rüeger, H.; Stricker-Krongrad, A.; Wyss, P.; Brunner, L.; Whitebread, S.; Yamaguchi, Y.; Gerald, C.; Heurich, R. O.; Walker, M. W.; Chiesi, M.; Schilling, W.; Hofbauer, K. G.; Levens, N., Food intake in free-feeding and energy-deprived lean rats is mediated by the neuropeptide Y_5 receptor. *J. Clin. Invest.* **1998,** 102, 2136-45.

[118] Feletou, M.; Nicolas, J. P.; Rodriguez, M.; Beauverger, P.; Galizzi, J. P.; Boutin, J. A.; Duhault, J., NPY receptor subtype in the rabbit isolated ileum. *Br. J. Pharmacol.* **1999,** 127, 795-801.

[119] Feletou, M.; Rodriguez, M.; Beauverger, P.; Germain, M.; Imbert, J.; Dromaint, S.; Macia, C.; Bourrienne, A.; Henlin, J. M.; Nicolas, J. P.; Boutin, J. A.; Galizzi, J. P.; Fauchere, J. L.; Canet, E.; Duhault, J., NPY receptor subtypes involved in the contraction of the proximal colon of the rat. *Regul. Pept.* **1998,** 75-76, 221-9.

[120] Kask, A.; Vasar, E.; Heidmets, L. T.; Allikmets, L.; Wikberg, J. E., Neuropeptide Y Y_5 receptor antagonist CGP71683A: the effects on food intake and anxiety-related behavior in the rat. *Eur. J. Pharmacol.* **2001,** 414, 215-24.

[121] Lecklin, A.; Lundell, I.; Paananen, L.; Wikberg, J. E.; Mannisto, P. T.; Larhammar, D., Receptor subtypes Y_1 and Y_5 mediate neuropeptide Y induced feeding in the guinea-pig. *Br. J. Pharmacol.* **2002,** 135, 2029-37.

[122] Yokosuka, M.; Dube, M. G.; Kalra, P. S.; Kalra, S. P., The mPVN mediates blockade of NPY-induced feeding by a Y_5 receptor antagonist: a c-FOS analysis. *Peptides* **2001,** 22, 507-14.

[123] Della Zuana, O.; Sadlo, M.; Germain, M.; Feletou, M.; Chamorro, S.; Tisserand, F.; de Montrion, C.; Boivin, J. F.; Duhault, J.; Boutin, J. A.; Levens, N., Reduced food intake in response to CGP 71683A may be due to mechanisms other than NPY Y_5 receptor blockade. *Int. J. Obes. Relat. Metab. Disord.* **2001,** 25, 84-94.

[124] Pheng, L. H.; Quirion, R.; Iyengar, S.; Fournier, A.; Regoli, D., The rabbit ileum: a sensitive and selective preparation for the neuropeptide Y Y_5 receptor. *Eur. J. Pharmacol.* **1997,** 333, R3-5.

[125] Itani, H.; Ito, H.; Sakata, Y.; Hatakeyama, Y.; Oohashi, H.; Satoh, Y., Novel potent antagonists of human neuropeptide Y Y_5 receptors. Part 2: substituted benzo[a]cycloheptene derivatives. *Bioorg. Med. Chem. Lett.* **2002,** 12, 757-61.

[126] Itani, H.; Ito, H.; Sakata, Y.; Hatakeyama, Y.; Oohashi, H.; Satoh, Y., Novel potent antagonists of human neuropeptide Y Y_5 receptors. Part 3: 7-methoxy-1-hydroxy-1-substituted tetraline derivatives. *Bioorg. Med. Chem. Lett.* **2002,** 12, 799-802.

[127] Dax, S. L.; Lovenberg, T. W.; Baxter, E. W.; Carson, J. R.; Ludovici, D. W.; Mark, Y. N-Aralkylaminotetralins as ligands for the neuropeptide Y Y_5 receptor. WO 0020376, **2000**.

[128] Dax, S. L.; McNally, J. J. 3a,4,5,9b-tetrahydro-1H-benz[e]indol-2-yl amine-derived neuropeptide Y receptor ligands useful in the treatment of obesity and other disorders. WO 0068197, **2000**.

[129] Dax, S. L.; McNally, J. J.; Youngman, M. A. Amine and amide derivatives as ligands for the neuropeptide Y Y_5 receptor useful in the treatment of obesity and other disorders. WO 0109120, **2001**.

[130] McNally, J. J.; Youngman, M. A.; Lovenberg, T. W.; Nepomuceno, D.; Wilson, S.; Dax, S. L., N-acylated α-(3-pyridylmethyl)-β-aminotetralin antagonists of the human neuropeptide Y Y_5 receptor. *Bioorg. Med. Chem. Lett.* **2000,** 10, 1641-3.

[131] McNally, J. J.; Youngman, M. A.; Lovenberg, T. W.; Nepomuceno, D. H.; Wilson, S. J.; Dax, S. L., N-(sulfonamido)alkyl[tetrahydro-1*H*-benzo[e]indol-2-yl]amines: potent antagonists of human neuropeptide Y Y_5 receptor. *Bioorg. Med. Chem. Lett.* **2000,** 10, 213-6.

[132] Youngman, M. A.; McNally, J. J.; Lovenberg, T. W.; Reitz, A. B.; Willard, N. M.; Nepomuceno, D. H.; Wilson, S. J.; Crooke, J. J.; Rosenthal, D.; Vaidya, A. H.; Dax, S. L., α-Substituted N-(sulfonamido)alkyl-β-aminotetralins: potent and selective neuropeptide Y Y_5 receptor antagonists. *J. Med. Chem.* **2000,** 43, 346-50.

[133] Kawanishi, Y.; Takenaka, H.; Hanasaki, K.; Okada, T. Preparation of sulfonamides and sulfinamides as NPY Y_5 antagonists. WO 0137826, **2001**.

[134] Marzabadi, M. R.; Wong, W. C.; Noble, S. A. Preparation of benzo[2,3]thiepino[4,5-d]thiazol-2-ylaminoalkylsulfonamides and related compounds as selective neuropeptide Y (Y_5) antagonists. US 6124331, **2000**.

[135] Marzabadi, M. R.; Wong, W. C.; Noble, S. A.; Buhlmayer, P.; Rueger, H.; Yamaguchi, Y.; Schilling, W. Preparation of selective NPY (Y_5) antagonists and pharmaceutical compositions thereof for treating an abnormality modulated by human Y_5 receptor activity. WO 0102379, **2001**.

[136] Marzabadi, M. R.; Wong, W. C.; Noble, S. A.; Desai, M. N. Preparation of triazineamines, thiazolamines, and benzo[2,3]thiepino[4,5-d][1,3]thiazol-2-ylamines as selective neuropeptide Y (Y_5) antagonists. WO 0064880, **2000**.

[137] Schmidlin, T.; Rüeger, H.; Gerspacher, M. Preparation of condensed thiazolamines as neuropeptide Y_5 antagonists. WO 0164675, **2001**.

[138] Rüeger, H.; Gerspacher, M.; Buehlmayer, P.; Rigollier, P.; Yamaguchi, Y.; Schmidlin, T.; Whitebread, S.; Nuesslein-Hildesheim, B.; Nick, H.; Cricione, L., Discovery and SAR of potent, orally available and brain-penetrable 5,6-dihydro-4H-3-thia-1-aza-benzo[e]azulen- and 4,5-dihydro-6-oxa-3-thia-1-aza-benzo[e]azulen derivatives as neuropeptide Y Y_5 receptor antagonists. *Bioorg. Med. Chem. Lett.* **2004,** 14, 2451-7.

[139] Galiano, S.; Erviti, O.; Perez, S.; Moreno, A.; Juanenea, L.; Aldana, I.; Monge, A., Synthesis of new thiophene and benzo[*b*]thiophene hydrazide derivatives as human NPY Y_5 antagonists. *Bioorg. Med. Chem. Lett.* **2004,** 14, 597-9.

[140] Fauchere, J.-L.; Ortuno, J.-C.; Duhault, J.; Boutin, J. A.; Levens, N. Aminotriazole compounds useful as neuropeptide Y receptor ligands, process for their preparation, and pharmaceutical compositions containing them. EP 1044970, **2000**.

[141] Fukami, T.; Fukuroda, T.; Kanatani, A.; Ihara, M. Preparation of urea moiety-containing pyrazole derivatives as neuropeptide Y antagonists. WO 9824768, **1998**.

[142] Fukami, T.; Fukuroda, T.; Kanatani, A.; Ihara, M. Preparation of pyrazole derivatives for treatment of bulimia, obesity, and diabetes. WO 9825907, **1998**.

[143] Fukami, T.; Fukuroda, T.; Kanatani, A.; Ihara, M. Preparation of aminopyrazole derivatives for the treatment of bulimia, obesity, and diabetes. WO 9827063, **1998**.

[144] Fukami, T.; Fukuroda, T.; Kanatani, A.; Ihara, M. Preparation of novel aminopyrazole derivatives as neuropeptide Y antagonists. WO 9825908, **1998**.

[145] Fukami, T.; Kanatani, A.; Ishihara, A.; Ishii, Y.; MacNeil, D. J.; Van der Ploeg, L. H. T.; Ihara, M., Synthesis and pharmacology of potent and selective aminopyridine NPY Y_1 receptor antagonists. *Abstracts of Papers, 222nd ACS National Meeting, Chicago, IL, United States, August 26-30, 2001* **2001**, MEDI-282.

[146] Sato, N.; Takahashi, T.; Shibata, T.; Haga, Y.; Sakuraba, A.; Hirose, M.; Sato, M.; Nonoshita, K.; Koike, Y.; Kitazawa, H.; Fujino, N.; Ishii, Y.; Ishihara, A.; Kanatani, A.; Fukami, T., Design and Synthesis of the Potent, Orally Available, Brain-Penetrable Arylpyrazole Class of Neuropeptide Y_5 Receptor Antagonists. *J. Med. Chem.* **2003,** 46, 666-9.

[147] Kordik, C. P.; Lovenberg, T. W.; Reitz, A. B. Preparation of pyrazole carboxamides for the treatment of obesity and other disorders. WO 0069849, **2000**.

[148] Kordik, C. P.; Luo, C.; Zanoni, B. C.; Dax, S. L.; McNally, J. J.; Lovenberg, T. W.; Wilson, S. J.; Reitz, A. B., Aminopyrazoles with high affinity for the human neuropeptide Y_5 receptor. *Bioorg. Med. Chem. Lett.* **2001,** 11, 2283-6.

[149] Thurkauf, A.; Yuan, J.; Hutchison, A.; Blum, C.; Elliott, R. L.; Hammond, M. Preparation of diarylimidazoles as neuropeptide Y receptor ligands. WO 9901128, **1999**.

[150] Elliott, R. L.; Hammond, M.; Hank, R. F. 2-Pyridyl-4-heteroarylimidazoles for the treatment of obesity. WO 0066578, **2000**.

[151] Elliott, R. L.; Oliver, R. M.; Hammond, M.; Patterson, T. A.; She, L.; Hargrove, D. M.; Martin, K. A.; Maurer, T. S.; Kalvass, J. C.; Morgan, B. P.; DaSilva-Jardine, P. A.; Stevenson, R. W.; Mack, C. M.; Cassella, J. V., In vitro and in vivo characterization of 3-[2-[6-(2-tert-butoxyethoxy)pyridin-3-yl]-1H-imidazol-4-yl]benzonitrile hydrochloride salt, a potent and selective NPY Y_5 receptor antagonist. *J. Med. Chem.* **2003,** 46, 670-3.

[152] Blum, C. A.; Zheng, X.; De Lombaert, S., Design, synthesis, and biological evaluation of substituted 2-cyclohexyl-4-phenyl-1*H*-imidazoles: potent and selective neuropeptide Y Y_5-receptor antagonists. *J. Med. Chem.* **2004,** 47, 2318-25.

[153] Poindexter, G. S.; Antal, I.; Guipponi, L. M.; Stoffel, R. H.; Gillman, K.; Higgins, M. Imidazolone anorectic agents: II. phenyl derivatives. WO 9948888, **1999**.

[154] Poindexter, G. S.; Gillman, K. Imidazolone anorectic agents: III. heteroaryl derivatives. WO 9948873, **1999**.

[155] Poindexter, G. S.; Gillman, K. Imidazolone anorectic agents: I. acyclic derivatives. WO 9948888, **1999**.

[156] Gao, Y.-d.; McNeil, D.; Yang, L.; Morin, N. R.; Fukami, T.; Kanatani, A.; Fukuroda, T.; Ishii, Y.; Morin, M. Preparation of spiroindolines as Y_5 receptor antagonists. WO 0027845, **2000**.

[157] Fukami, T.; Kanatani, A.; Ishihara, A.; Ishii, Y.; Takahashi, T.; Haga, Y.; Sakamoto, T.; Itoh, T. Preparation of spiroisoindolinepiperidines, spiroisoquinolinepiperidines, spiroisobenzofuranpiperidines and related compounds. WO 0114376, **2001**.

[158] Poindexter, G. S.; Antal, I.; Giupponi, L. M.; Stoffel, R. H.; Bruce, M. NPY antagonists: spiroisoquinolinone derivatives. WO 0113917, **2001**.

[159] Dow, R. L.; Hammond, M. 4-aminopyrrolo[3,2-d]pyrimidines as neuropeptide Y receptor antagonists. US 6187778, **2001**.

[160] Norman, M. H.; Chen, N.; Han, N.; Liu, L.; Hurt, C.; Fotsch, C.; Jenkins, T.; Moreno, O. Preparation of bicyclic pyridine and pyrimidine derivatives as neuropeptide Y receptor antagonists. WO
9940091, **1999**.

[161] Norman, M. H.; Chen, N.; Chen, Z.; Fotsch, C.; Hale, C.; Han, N.; Hurt, R.; Jenkins, T.; Kincaid, J.; Liu, L.; Lu, Y.; Moreno, O.; Santora, V. J.; Sonnenberg, J. D.; Karbon, W., Structure-Activity Relationships of a Series of Pyrrolo[3,2-d]pyrimidine Derivatives and Related Compounds as Neuropeptide Y_5 Receptor Antagonists. *J. Med. Chem.* **2000,** 43, 4288-312.

[162] Carpino, P. A.; Hammond, M.; Hank, R. F. Amide derivatives useful as Neuropeptide Y (NPY) antagonists. EP 1033366, **2000**.

[163] Akwabi-Ameyaw, A.; Heyer, D.; Aquino, C.; Fang, J.; Ramanjulu, J.; Travis, B.; Burnette, T.; Tong, W.-Q.; Lyerly, D.; Daniels, A., Synthesis and SAR of substituted 5-acylamino benzimidazoles as potent neuropeptide Y Y_5 antagonists. *Abstracts of Papers, 222nd ACS National Meeting, Chicago, IL, United States, August 26-30, 2001* **2001**, MEDI-033.

[164] Fang, J.; Aquino, C.; Heyer, D.; Travis, B.; Burnette, T.; Tong, W.-Q.; Lyerly, D.; Daniels, A., 2-Aminobenzimidazoles as neuropeptide Y Y_5 antagonists: Solution phase synthesis and structure-activity relationships. *Abstracts of Papers, 222nd ACS National Meeting, Chicago, IL, United States, August 26-30, 2001* **2001**, MEDI-026.

[165] Heyer, D.; Akwabi-Ameyaw, A.; Aquino, C., Aminobenzimidazoles: A class of orally bioavailable and brain permeable neuropeptide Y Y_5 antagonists. *Abstracts of Papers, 222nd ACS National Meeting, Chicago, IL, United States, August 26-30, 2001* **2001**, MEDI-284.

[166] Heyer, D.; Marron, B.; Miyasaki, Y.; Daniels, A., Discovery of a novel series of benzimidazole-based neuropeptide Y Y_5 antagonists from a 7-TM targeted chemical library. *Abstracts of Papers, 222nd ACS National Meeting, Chicago, IL, United States, August 26-30, 2001* **2001**, MEDI-030.

[167] Linn, J.; Aquino, C.; Dezube, M., Benzimidazole neuropeptide Y Y_5 antagonists: rapid SAR development using a solid phasse approach. *Abstracts of Papers, 222nd ACS National Meeting, Chicago, IL, United States, August 26-30, 2001* **2001**, MEDI-027.

[168] Daniels, A. J.; Grizzle, M. K.; Wiard, R. P.; Matthews, J. E.; Heyer, D., Food intake inhibition and reduction in body weight gain in lean and obese rodents treated with GW438014A, a potent and selective NPY-Y_5 receptor antagonist. *Regul. Pept.* **2002,** 106, 47-54.

[169] Ramanjulu, J.; Aquino, C.; Palazzo, F.; Heyer, D.; Daniels, A.; Burnette, T., Synthesis and structure activity studies of bisheteroaryl guanidines as NPY-Y_5 antagonists. *Abstr. Pap. - Am. Chem. Soc.* **2001,** 221st, MEDI-210.

[170] Fukami, T.; Okamoto, O.; Fukuroda, T.; Kanatani, A.; Ihara, M. Preparation and formulation of aminopyridine derivatives as neuropeptide Y receptor antagonists. WO 9840356, **1998**.

[171] Tabuchi, S.; Itani, H.; Sakata, Y.; Oohashi, H.; Satoh, Y., Novel potent antagonists of human neuropeptide Y Y_5 receptor. Part 1: 2-oxobenzothiazolin-3-acetic acid derivatives. *Bioorg. Med. Chem. Lett.* **2002,** 12, 1171-5.

[172] Satoh, Y.; Hatori, C.; Ito, H., Novel potent antagonists of human neuropeptide Y-Y_5 receptor. Part 4: tetrahydrodiazabenzazulene derivatives. *Bioorg. Med. Chem. Lett.* **2002,** 12, 1009-11.

[173] Block, M. H.; Donald, C. S.; Foote, K. M.; Schofield, P.; Marsham, P. R. Preparation of carbazoles as neuropeptide Y_5 receptor ligand. WO 0107409, **2001**.

[174] Donald, C. S.; Foote, K. M.; Schofield, P.; Block, M. H. Carbazole derivatives and their use as neuropeptide Y_5 receptor ligands. WO 02051806, **2002**.

[175] Block, M. H.; Boyer, S.; Brailsford, W.; Brittain, D. R.; Carroll, D.; Chapman, S.; Clarke, D. S.; Donald, C. S.; Foote, K. M.; Godfrey, L.; Ladner, A.; Marsham, P. R.; Masters, D. J.; Mee, C. D.; O'Donovan, M. R.; Pease, J. E.; Pickup, A. G.; Rayner, J. W.; Roberts, A.; Schofield, P.; Suleman, A.; Turnbull, A. V., Discovery and optimization of a series of carbazole ureas as NPY Y_5 antagonists for the treatment of obesity. *J. Med. Chem.* **2002,** 45, 3509-23.

[176] Nishikawa, N.; Aoki, K.; Suzuki, M.; Tabata, Y.; Takei, N.; Tanaka, J.; Asai, K.; Ikekawa, A.; Kakui, N.; Ohsawa, F.; Sugai, M.; Takahashi, K. Tricyclic compounds. WO 0063171, **2000**.

[177] Connell, R. D.; Lease, T. G.; Ladouceur, G. H.; Osterhout, M. H. Amide derivatives and methods for using the same as selective neuropeptide Y receptor antagonists. US 6048900, **2000**.

[178] Fotsch, C.; Sonnenberg, J. D.; Chen, N.; Hale, C.; Karbon, W.; Norman, M. H., Synthesis and structure-activity relationships of trisubstituted phenyl urea derivatives as neuropeptide Y Y_5 receptor antagonists. *J. Med. Chem.* **2001,** 44, 2344-56.

[179] Aoki, K.; Nishikawa, N.; Ikekawa, A.; Iwao, E.; Osawa, F.; Takei, N.; Kakui, S.; Tanaka, J.; Tabata, Y.; Asai, K. Preparation of benzothiazolone derivatives having affinity to neuropeptide Y receptor. JP 2001122865, **2001**.

[180] Stamford, A. W.; Dugar, S.; Wu, Y.; Neustadt, B. Neuropeptide Y Y_5 receptor antagonists. WO 9964394, **1999**.

[181] Stamford, A. W.; Dugar, S.; Wu, Y.; Neustadt, B. Neuropeptide Y Y_5 receptor antagonists. US 6329395, **2001**.

[182] Stamford, A. W.; Dong, Y.; McCombie, S. Heteroaryl urea neuropeptide Y Y_5 receptor antagonists. WO 0249648, **2002**.

[183] Stamford, A. W.; Huang, Y.; Kelly, J. M.; Wu, Y.; Greenlee, W. J.; McCombie, S. Substituted urea neuropeptide Y Y_5 receptor antagonists. WO 0222592, **2002**.

[184] McCombie, S.; Stamford, A. W.; Huang, Y.; Kelly, J. M.; Wu, Y.; Grennlee, W. J. Substituted urea neuropeptide Y Y_5 receptor antagonists. US 2002165223, **2002**.

[185] Fukuroda, T.; Kanatani, A.; Fukami, T.; Ihara, M. Novel Neuropeptide Y Receptor Antagonists. WO 9915516, **2001**.

[186] Kanatani, A.; Ishihara, A.; Iwaasa, H.; Nakamura, K.; Okamoto, O.; Hidaka, M.; Ito, J.; Fukuroda, T.; MacNeil, D. J.; Van der Ploeg, L. H. T.; Ishii, Y.; Okabe, T.; Fukami, T.; Ihara, M., L-152,804: orally active and selective neuropeptide Y Y_5 receptor antagonist. *Biochem. Biophys. Res. Commun.* **2000,** 272, 169-73.

[187] Kirkpatrick, P., Dead end for NPY Y_5-receptor antagonists? *Nature Reviews Drug Discovery* **2002,** 1, 932-3.

[188] Parker, E.; Van Heek, M.; Stamford, A., Neuropeptide Y receptors as targets for anti-obesity drug development: perspective and current status. *Eur. J. Pharmacol.* **2002,** 440, 173-87.

3 Scope of the Thesis

Neuropeptide Y (NPY) is one of the most abundant neuropeptides in the central and peripheral nervous system. It is implicated in a variety of neurobiological functions, including cardiovascular regulation, control of food intake, anxiety, and memory retention. Several NPY receptor subtypes (Y_1, Y_2, Y_4, Y_5, and y_6), mediating the NPY induced effects, have been identified. However, the contributions of individual receptor subtypes to a certain physiological effect are not completely understood.

The goal of our project was the design and preparation of potent and selective NPY receptor ligands as useful pharmacological tools for the characterization of NPY Y_1 and Y_2 receptors. This includes the exploration of structure-activity relationships as well as investigations on the localization and distribution of NPY receptors in different tissues and organs, helping to elucidate the (patho-)physiological roles of the NPY receptor subtypes.

Based on the well established argininamide-type NPY Y_1 receptor antagonist BIBP 3226, we intended to develop fluorescence- or radiolabeled tracers — with special focus on centrally available ^{18}F-labeled PET ligands. Introduction of acyl sunbstituents in N^G-position of BIBP 3226 leads to equally potent, but less basic analogs. Due to the reduced basicity, these analogs occur at physiological pH to a substantial extent in the unprotonated, uncharged form, which facilitates the absorption from the gastrointestinal tract and the penetration across the blood-brain barrier. This observation led to the idea, to synthesize BIBP 3226 analogs, bearing an ω-aminoalkanoyl linker in N^G-position, which allows for the conjugation with labeling groups at the ω-amino function. A very common reagent for the preparation of ^{18}F-labeled compounds is succinimidyl 4-[^{18}F]fluorobenzoate. Therefore,

the preparation of N^G-[ω-(4-fluorobenzamido)alkanoyl]-substituted analogs was projected in order to identify model compounds for potential PET-ligands with high receptor affinity and favorable pharmacokinetic properties.

The synthesis of ω-aminoalkanoyl-substituted argininamides as building blocks for the preparation of labelled NPY receptor ligands turned out to be complicated due to the instability of the acylguanidine substructure under basic conditions. As the preparation of such intermediates is the key to synthesis of the tracer compounds, the kinetics of the decomposition reaction had to be studied in order to gain information on the stability of different linker groups depending on the structure and the experimental conditions.

BIIE 0246, a NPY Y_2 receptor selective antagonist, also comprises the argininamide substructure. Inspired by the results from the N^G-acylated Y_1 receptor selective argininamide-type antagonists, we intended to prepare a series of BIIE 0246 analogs with reduced basicity by introduction of electron withdrawing substituents in N^G-position, expecting to retain the antagonistic potency.

4 Overview Over the Synthetic Methods for the Preparation of NPY Y_1 and Y_2 Receptor Antagonistic Argininamides

Abstract **– Arginine is a proteogenic amino acid, bearing the highly basic guanidino group in the side chain. Due to the important role of arginine residues in many biological processes synthetic arginine derivatives and -mimetics are of special interest for medicinal and bioorganic chemistry. In this chapter we give attention to the chemical methods used for the preparation of neuropeptide Y antagonistic argininamides, including coupling techniques, protective group chemistry, and guanidinylation methods.**

1. Introduction

L-Arginine ((*R*)-2-amino-5-guanidinopentanoic acid, abbr. Arg, or R) is the most basic (pK_a = 12) amongst the 20 genetically encoded, proteogenic amino acids. At neutral pH the guanidino group in the arginine side chain is positively charged. Thus, arginine residues are prevalently located at the polar, hydrophilic surface of peptides and proteins[1] or in certain recognition sites, where they can form intermolecular high-energy interactions with carboxylates, phosph(on)ates or sulf(on)ates[2]. Thus, arginine residues often influence the tertiary structure of peptides and proteins, or are directly involved in molecular recognition processes. Arginine, together with tryptophan and tyrosine, are significantly enriched in "hot spots" of protein–protein heterodimers[3]. Numerous examples exist, which illustrate the importance of arginine residues for recognition processes in biological systems: For instance, the Arg-Gly-Asp (RGD) motif was identified as minimum pharmacophore for the binding to fibrinogen receptor binding sites. Thrombin, factor Xa, and trypsin are serine proteases, which bind arginine residues in their P_1

binding pocket. Certain arginine residues in neurotensin (NT)[4] and neuropeptide Y (NPY)[5] are crucial for receptor binding. Many interesting natural products with antifungal, antibiotic, and cytotoxic activities, isolated from diverse biological sources, comprise arginine substructures[6]. These examples show that the requirement to address an arginine binding site is a common task in the drug development process. Therefore, arginine derivatives or arginine mimetics are frequently lead structures in medicinal chemistry.

Different strategies for the preparation of arginine derivatives and mimetics have been developed. The major problems which arise from the presence of the extremely polar and basic guanidino functionality are the poor solubility in organic solvents and the residual nucleophilicity. To overcome these problems the use of N^G-protected starting materials has been advised.

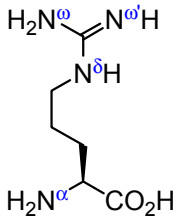


Fig. 1: Potential *N*-nucleophilic sites of arginine

However, an ideal protective group for the trifunctional guanidino group has not been found yet.[7] Alternative strategies are based upon the final introduction of the guanidino group (guanidinylation) into precursor compounds. The optimal strategy to meet a given synthetic goal has to be chosen carefully and the results are not completely predictable.

In quest of non-peptide NPY receptor ligands several series of arginine derivatives, which were intended to mimic the pharmacophoric C-terminus of NPY, were prepared in the laboratories at Boehringer Ingelheim. From these projects resulted the argininamide derivatives BIBP 3226 (**1**) and BIIE 0246 (**2**) which were described as potent NPY Y_1 and Y_2 selective antagonists, respectively (cf. Fig. 2). Based on the structures of BIBP 3226 and BIIE 0246 we prepared NPY receptor ligands with optimized properties for the development of pharmacological tools for *in vivo* and *in vitro* characterization of Y_1 and Y_2 receptors.

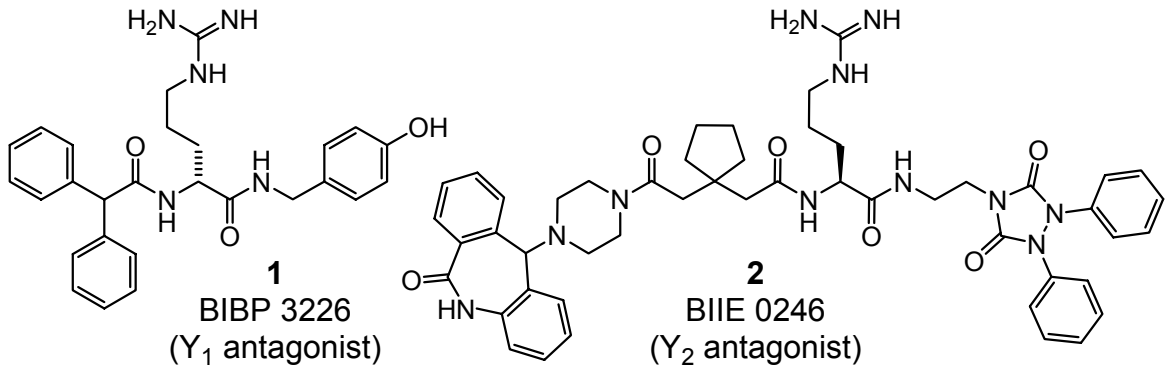


Fig. 2: Structures of Neuropeptide Y antagonists BIBP 3226 (**1**) and BIIE 0246 (**2**).

One of the tasks was to replace the basic guanidino side chain function with a less polar bioisostere, in order to enable the compounds to cross the blood brain barrier. For this reason we synthesized analogs with electron withdrawing substituents in N^G-position.

In the following we describe the common preparative methods in arginine chemistry and their application in the synthesis of arginine derivatives with neuropeptide Y antagonistic activity.

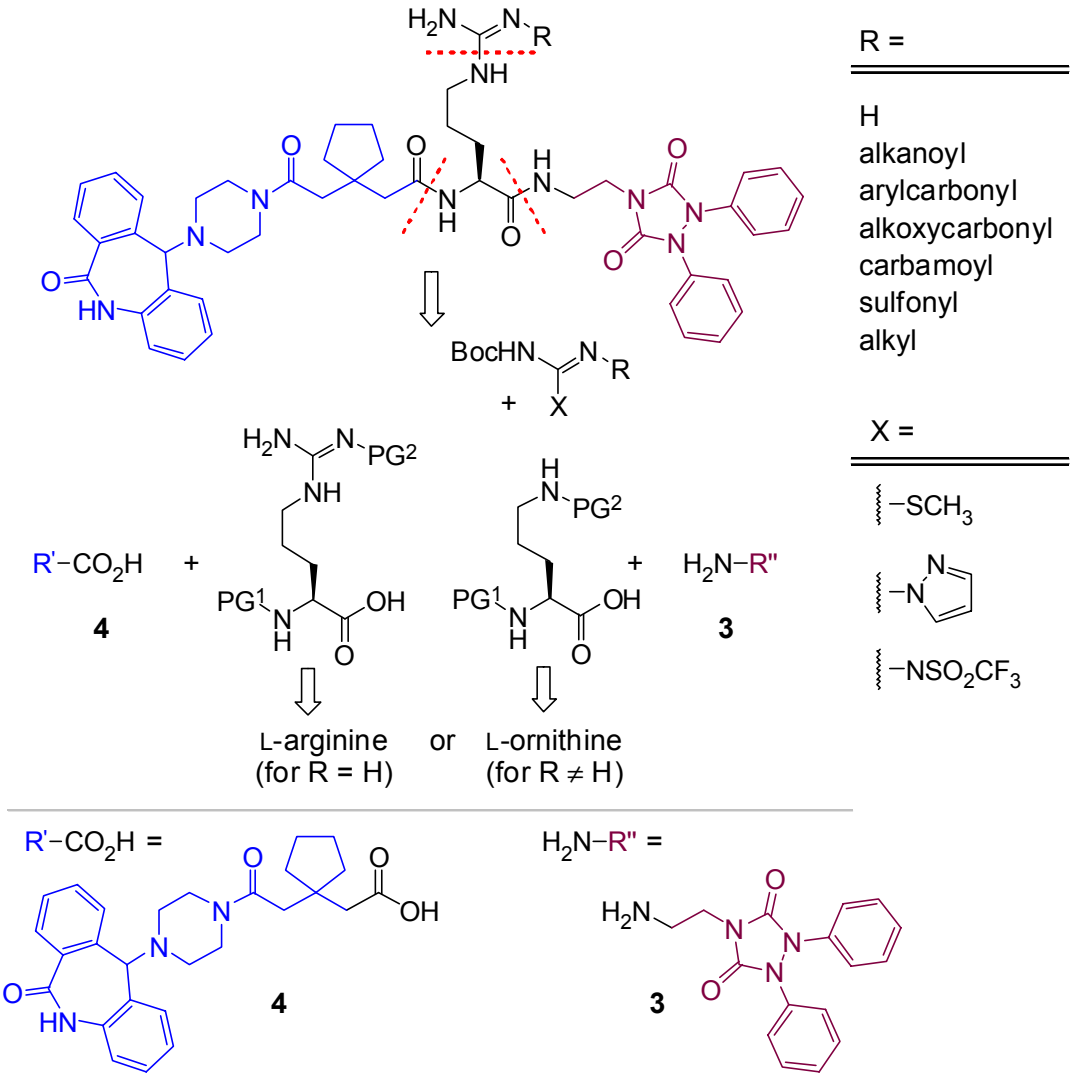
2. Retrosynthesis

Although BIBP 3226 (**1**) and BIIE 0246 (**2**) differ in the nature of the substituents and in the configuration at C^α, both NPY antagonistic argininamides are accessible via the same synthetic route. A suitably protected arginine building block is activated and coupled with a primary amine, deprotected at N^α, and subsequently acylated in the second peptide coupling step.

Since we were interested in arginine derivatives with diverse substituents in N^G-position, we first prepared the respective ornithinamides, which were then converted into the targeted N^G-substituted argininamides in a late guanidinylation step. Therefore, orthogonally protected ornithines, instead of arginines, were used as starting material.

2.1. BIIE 0246 and N^G-Substituted Analogs

BIIE 0246 (**2**) and its analogs consist of a central arginine residue, which is C-terminally amidated with the primary amine **3** and N^{α}-acylated with carboxyl component **4** (cf. Scheme 1).



Scheme 1: Retrosynthesis of BIIE 0246 and N^G-substituted analogs. PG^n = protective group(s).

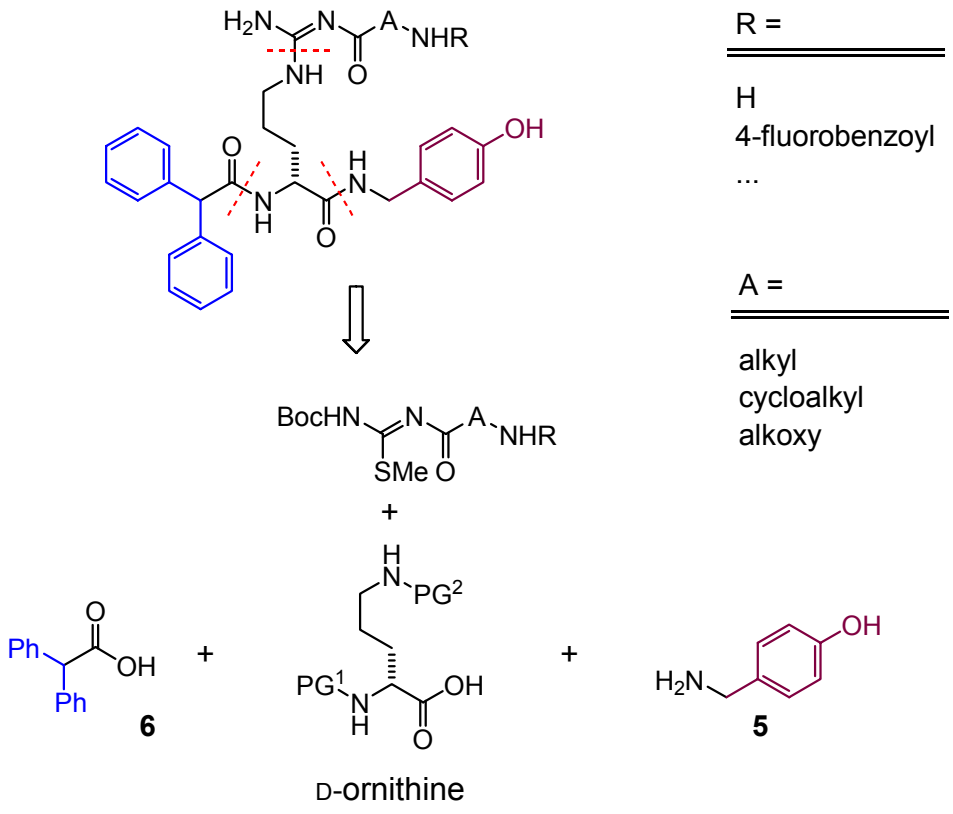
For the synthesis of unsubstituted BIIE 0246 (**2**), a suitably protected L-arginine derivative was used as starting material*. In contrast, N^G-substituted analogs of BIIE

* The most common arginine side chain protective groups are described in paragraph 4.

0246 were prepared from orthogonally protected L-ornithine. In both cases the respective amino acid building block was first coupled with amine **3** and, after deprotection, with carboxylic acid **4**. After the two coupling steps the side chain function was deblocked and finally transformed into the arginine derivatives by guanidinylation (cf. section 5).

2.2. N^G-Substituted BIBP 3226 Analogs

In the field of Y_1 antagonists our objective was to prepare N^G-(ω-aminoalkanoyl)-substituted analogs of BIBP 3226 with high affinity at the Y_1 receptor.



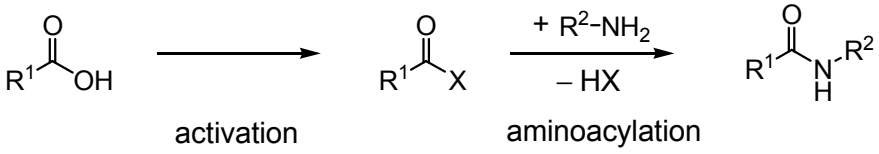
Scheme 2: Retrosynthetic analysis of ω-aminoacyl substituted BIBP 3226 analogs.

The terminal amino group was intended to act as a reactive group for the linkage of radio- or fluorescence labeled markers. The introduction of the ω-aminoalkanoyl

substituent was favorably done by guanidinylation chemistry (cf. Scheme 2). Starting point of our synthesis was orthogonally protected D-ornithine, which was first coupled with (O-protected) 4-hydroxybenzylamine (**5**), N^{α}-deprotected and subsequently acylated with 2,2-diphenylacetic acid (**6**). After removal of the N^{δ}-protective group, the resulting ornithinamide was converted into the desired arginine derivatives by guanidinylation using *N,N'*-disubstituted *S*-methylisothioureas.

3. Peptide Bond Formation

Due to its fundamental relevance in peptide chemistry the formation of the amide bond (-C(O)-NH-) is one of the best optimized reactions in synthetic chemistry and a broad repertory of methods is available today.



Scheme 3: Amide bond formation via activated carboxylic acid derivatives.

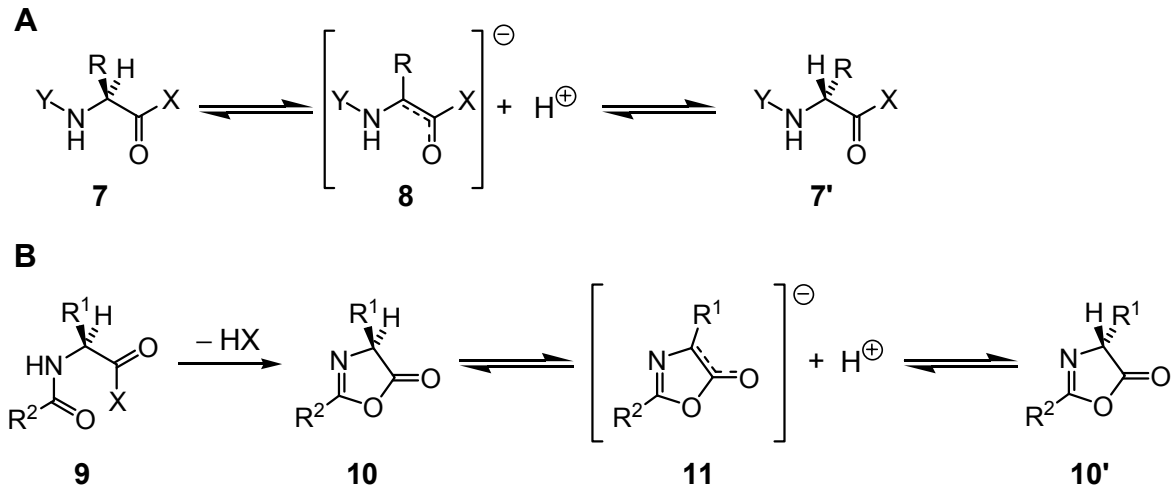
Coupling techniques which are suitable for peptide chemistry have to comply with the following particular requirements: excellent yields (especially important in solid phase peptide synthesis [SPPS]), good chemoselectivity (i.e. no side reactions in the presence of polyfunctional peptide fragments), and prevention of epimerization[†].

3.1. Epimerization

For amide coupling the carboxy function has to be converted into a more reactive derivative, which is capable of acylating amines (cf. Scheme 3). However, activation of the carboxy group leads to enhanced α-acidity compared to the free acid (X = OH) or peptide (X = NHR). In the presence of strong bases the epimeric amino acid

[†] In peptide chemistry the term racemization is often used for (partial) epimerization of asymmetric α-carbon atoms during peptide synthesis.

derivatives **7** and **7′** reversibly form the prochiral enolate **8**, which can be re-protonated from both stereoheterotopic sides (cf. route **A** in Scheme 4). In this manner, stereohomogeneous starting material can lose its optical purity.



Scheme 4: Epimerization mechanisms for carboxy activated amino acid derivatives and peptide fragments.

In practice, epimerization via enolate formation is negligible, when moderate bases are used and the electronegativity of X is not too high.

More relevant under peptide coupling conditions is epimerization via oxazol-5(4*H*)-ones (**10** and **10′**, cf. route **B** Scheme 4), which are easily deprotonated to give the quasi-aromatic anion **11**. Protonation of **11** from either *Re*- or *Si*-face leads to the epimeric forms **10** and **10′**. Since oxazol-5(4*H*)-ones (**10**, **10′**) are able to react with the amino component, to give the desired coupling product, peptides with correct constitution but non-uniform stereochemistry are obtained.

Fortunately, the rate of epimerization is greatly reduced, when R^2 is an alkoxy group (and not alkyl or aryl). For this reason peptide sequences in general are synthesized by stepwise elongation of C-terminal peptide fragments with activated, N^{α}-alkoxycarbonyl protected amino acid building blocks (C→N strategy). The standard protocols for the coupling of N^{α}-alkoxycarbonyl protected amino acids with amines are optimized to eliminate the risk of epimerization.

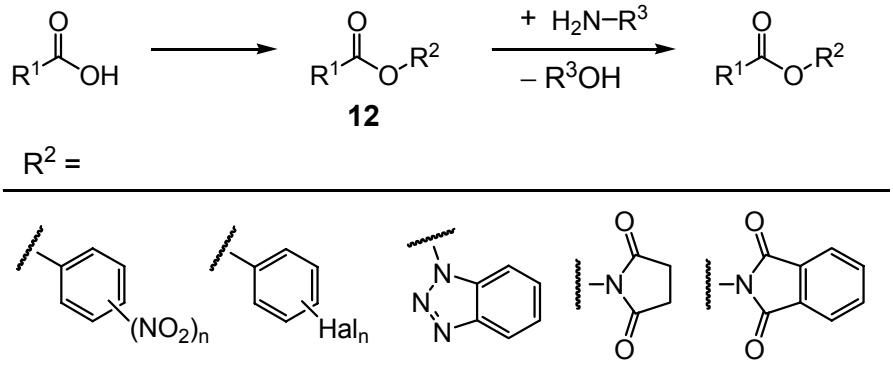
Regarding our syntheses, a risk of partial racemization is only conceivable for the coupling step of N^{α}-alkoxycarbonyl protected ornithine (or arginine) with the respective amine. Since we only used coupling techniques, which are not prone to cause epimerization, we can assume that our products maintained their optical purity. Exemplary stereochemical analysis of related coupling products in our lab gave no indication for the formation of the respective epimeric ornithinamides (or argininamides)[8, 9].

3.2. Coupling Reagents

Activation of a carboxy fragment and subsequent acylation of a nitrogen nucleophile was a recurring task in the synthesis of our target structures depicted in Scheme 1 and Scheme 2. In the following, the applied activation and coupling methods are briefly described.

3.2.1. *SUCCINIMIDYL ESTERS*[10]

In contrast to simple alkyl esters, esters with an electron-deficient substituent at the oxygen atom show enhanced reactivity towards amine nucleophiles. Acyl transferring reagents of this kind are called 'active esters' (**12**).



Scheme 5: Active esters commonly used for aminoacylation.

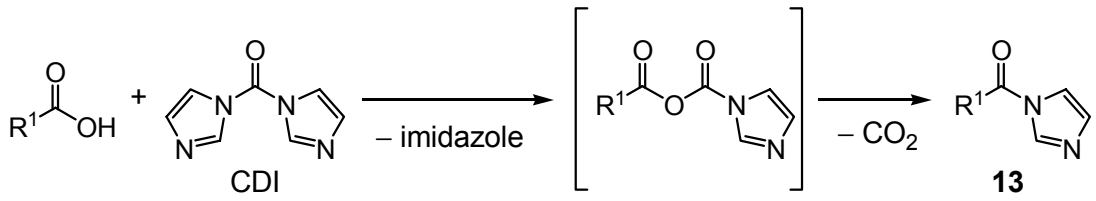
Beside the esters of polyhalogenated phenols, nitrophenols, 1,2,3-benzotriazol-1-ol, and *N*-hydroxyphthalimide, succinimidyl esters belong to the most popular compounds among the active esters (cf. Scheme 5). They can be prepared from the

corresponding carboxylic acid, an activating reagent (e.g. DCC), and *N*-hydroxysuccinimide in 1,4-dioxane, DME, or acetonitrile.

Since succinimidyl esters are stable[‡], isolable, and selective acylating reagents, they have found widespread application in organic-, peptide-, or bioconjugate chemistry. Due to their resistance to hydrolysis succinimidyl esters can also be used in aqueous solvent systems. *N*-Hydroxysuccinimide is the sole, water-soluble, and non toxic by-product formed during acylation of amines.

3.2.2. *1,1'-CARBONYLDIIMIDAZOLE*

1,1'-Carbonyldiimidazole[11, 12] (CDI) converts carboxylic acids into acylimidazoles (**13**), which are powerful acylating reagents (cf. Scheme 6). As CDI is moisture sensitive, anhydrous glassware and solvents have to be used. In general the acylimidazoles are not isolated; in fact the carboxylic acid is quantitatively converted to **13** with an equimolar amount of CDI, followed by addition of the amine. The completeness of the pre-activation is indicated by the end of carbon dioxide evolution (0.5–1 h). After acylation the by-products imidazole and CO_2 are quantitatively separated by simple aqueous workup. Pre-activation has to be performed in the absence of the amine, because CDI readily reacts with various nucleophiles. For the same reason, an excess of CDI has to be avoided.



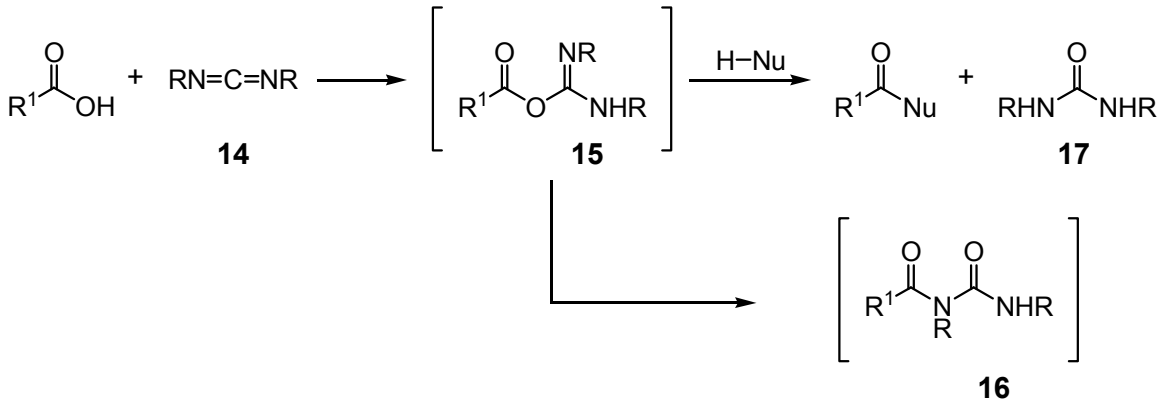
Scheme 6: In situ activation of carboxylic acids by conversion to acylimidazoles with 1,1'-carbonyldiimidazole (CDI).

Anderson et al. demonstrated the low level of epimerization when CDI was used in peptide coupling[13].

[‡] They can be recrystallized from hot alcohols and stored over longer periods of time.

3.2.3. CARBODIIMIDES

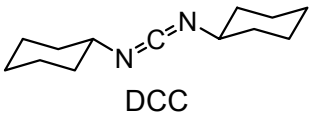
N,N'-Dialkylcarbodiimides (**14**, cf. Scheme 1) convert carboxylic acids into O-acylisoureas (**15**), which readily transfer the acyl group onto nucleophiles under concomitant release of *N,N'*-dialkylureas (**17**).



Scheme 7: Activation of carboxylic acid with carbodiimides.

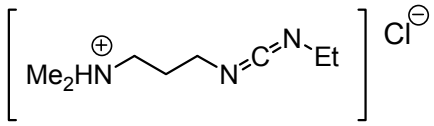
As intermediate **15** is highly reactive, there is a latent risk of side reactions. One of these complications is O→N acyl migration, which leads to the formation of non-reactive *N*-acylurea (**16**). A second drawback of the carbodiimide method is the epimerization tendency, when acylamino acids are activated with carbodiimides. Fortunately, both side reactions can be suppressed efficiently by performing the reaction at lower temperatures (~0 °C) and by addition of 1*H*-benzotriazol-1-ol (HOBt), *N*-hydroxysuccinimide (NHS) or related nucleophilic additives[14]. Presumably, in the presence of these auxiliary nucleophiles active esters are formed as selective, amino group acylating intermediates. Also copper salts have been described as additives efficiently preventing epimerization[15].

The choice of the carbodiimide is governed by the properties of the corresponding urea (**17**), which has to be separated from the coupling products.



DCC

18



EDC · HCl

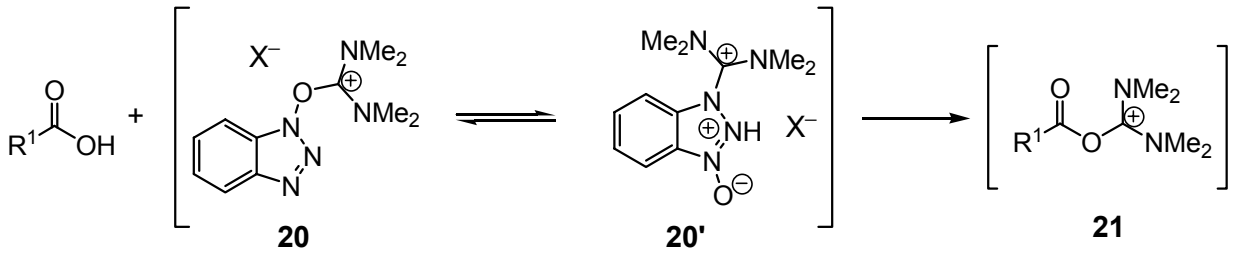
19

Fig. 3: Structures of dicyclohexylcarbodiimide (**18**, DCC) and *N*-[3-(dimethylamino)propyl]-*N'*-ethylcarbodiimide (**19**, EDC).

Probably, the most frequently used carbodiimide in peptide chemistry is dicyclohexylcarbodiimide (**18**, DCC, cf. Fig. 3). *N,N'*-Dicyclohexylurea is scarcely soluble in most organic solvents and can be separated by filtration. On the other hand, diisopropylcarbodiimide (DIC) is frequently used in solid phase peptide synthesis (SPPS), since *N,N'*-diisopropylurea is soluble in organic solvents. *N*-[3-(Dimethylamino)propyl]-*N'*-ethylcarbodiimide (**19**, EDC or WSC) is gaining increasing popularity because it leads to a water soluble urea product, which is removed by simple washing procedures.

3.2.4. *URONIUM (GUANIDINIUM) SALTS*

Closely related to reactive O-acylisourea intermediate **15**, formed by carbodiimide activation, is intermediate **21**, which results from the reaction of a carboxylic acid with 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyl uronium salt **20** (cf. Scheme 8). As **21** is rapidly reacting with nucleophiles, excess HOBt is added as auxiliary coupling reagent, preventing side reactions and epimerisation.



20 **20'** **21**

HBTU ($X^- = PF_6^-$), TBTU ($X^- = BF_4^-$)

Scheme 8: HBTU and TBTU as carboxyl activating reagents.

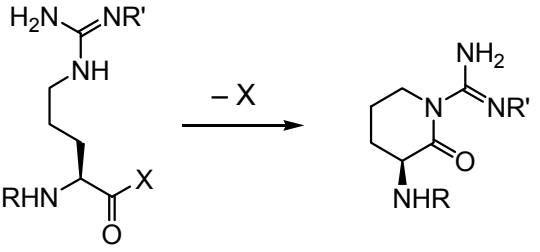
However, TBTU and HBTU are able to convert amines into tetramethylguanidines, pre-activation is not necessary in the presence of HOBt.

Inconsistent with the traditional uronium nomenclature the guanidinium structure **20'** was figured out by X-ray crystallography and NMR techniques[16] to be the authentic constitution of supposed **20**.

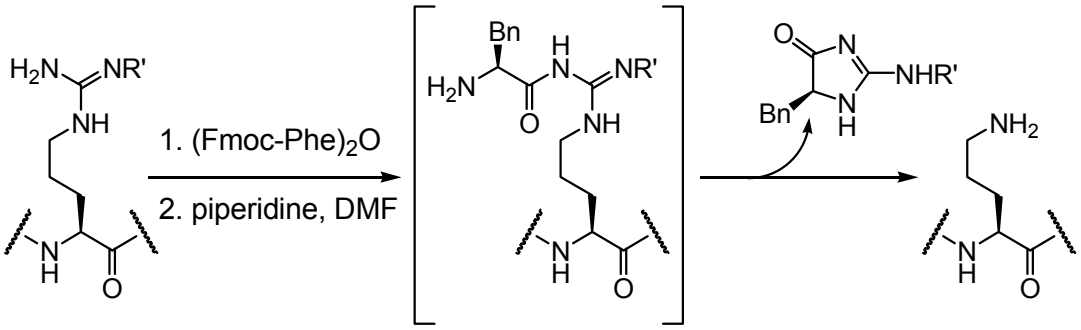
4. Protective Group Chemistry

4.1. Arginine Side Chain Protection

In general the nucleophilic character of the arginine side chain has to be masked in order to avoid unwanted side reactions during activation and coupling steps. One of the possible complications is the intramolecular δ-lactam formation resulting from nucleophilic attack of the N^{δ}-nitrogen at the activated carboxylic group (cf. Scheme 9). This side reaction is impending whenever the N^{δ}-nitrogen is unsubstituted.



Scheme 9: δ-Lactam formation of carboxy-activated arginine derivatives.



Scheme 10: N^{ω}-Acylation of arginine derivatives and subsequent degradation to ornithine derivatives.

Another risk is the intermolecular N^{ω}-acylation of insufficiently protected arginine residues by activated amino acid derivatives. Subsequently, N^{ω}-(α-aminoalkanoyl)-

substituted arginines degrade under formation of the parent ornithines and 2-amino-4*H*-imidazol-4(5*H*)-ones (cf. Scheme 10).

4.1.1. *PROTONATION*

Under common coupling conditions acylation of the arginine side chain is largely prevented by protonation of the guanidino function. Chain elongation steps of peptide fragments with *N*-terminal, protonated arginine residues have been performed successfully (cf. synthesis of secretin[17]). However, the poor solubility of arginine salts in organic solvents limits the practicability of this approach.

For the original synthesis of BIIE 0246, described in the patent specification[18], Cbz-protected arginine hydrochloride was used as arginine building block. The reported yield for the coupling of Z-Arg(HCl)-OH with HCl salt of 4-(2-aminoethyl)-1,2-diphenyl-1,2,4-triazolidine-3,5-dione was 45 % using CDI in DMF. Our own attempts to couple N^{α}-protected arginine hydrochlorides gave only poor results, due to the limited solubility, incomplete conversion and problems with the isolation and purification of the product.

4.1.2. N^{G}-*NITRO-PROTECTED ARGININES*

Boc-Arg(NO_2)-OH and Z-Arg(NO_2)-OH are commercially available, inexpensive arginine building blocks. The N^{G}-nitro group is stable against TFA, HBr/CH_3CO_2H or alkaline conditions. Liquid HF, Zn/CH_3CO_2H, or — most favorable — catalytic hydrogenolysis are suitable cleavage conditions. Still, $N^{\omega'}$-nitro protection does not completely exclude δ-lactam formation in the coupling step[19]. Sometimes deprotection under reductive methods (Zn/CH_3CO_2H or H_2/Pd) is incomplete and leads to only partially reduced by-products (e.g. N^{G}-aminoarginines)[7].

4.1.3. N^{ω}-*ALKOXYCARBONYL-PROTECTED ARGININES*

$N^{\omega,\omega'}$- or $N^{\delta,\omega}$-bis(alkoxycarbonyl)-substituted arginine derivatives are sufficiently protected against acylation. Among the urethane-type protective groups, which were applied in peptide chemistry to block the arginine side chain, are 1-Adoc, Alloc, Boc, and Cbz (= Z).

While Z-Arg(δ,ω-$Adoc_2$)-OH, Boc-Arg(δ,ω-$Alloc_2$)-OH, and Z-Arg(δ,ω-Z_2)-OH can be prepared from X-Arg-OH (X = Boc, Cbz) and the alkyl haloformates Adoc-F, Alloc-Cl, and Cbz-Cl, respectively[7], the reaction of X-Arg-OH with excess Boc_2O leads to a mixture of the ω,ω'- and the δ,ω-bis(Boc) regio-isomers, the latter being unstable[20]. X-Arg(ω,ω'-Boc_2)-OH (X = Boc, Cbz) is best prepared by guanidinylation of the corresponding ornithine precursors (cf. section 4.2).

Z-Arg(Z_2)-OH or its active esters are excellent building blocks for the incorporation of arginine residues. After subsequent, simultaneous cleavage of the Cbz-groups by catalytic hydrogenation the guanidine residue is shielded by protonation during the acylation of N^{α}.

We adapted this approach for the synthesis of N^{G}-unsubstituted BIIE 0246 and analogs. The coupling of Z-Arg(Z_2)-OSu with 4-(2-aminoethyl)-1,2-diphenyl-1,2,4-triazolidine-3,5-dione proceeded smoothly and yielded a pure, crystalline product. After removal of the Cbz groups by catalytic transfer hydrogenation using formic acid in the presence of Pd on carbon, the subsequent N^{α}-acylation yielded BIIE 0246.

Unfortunately, HPLC analysis of the final product revealed the presence of a closely related by-product with a molecular mass corresponding to that of BIIE 0246 plus 2 units. Analysis of the ESI mass spectra showed that the intensity of the MH+2 peak exceeds the expected value for a normal isotope distribution. This phenomenon was observed for all isolated intermediate products after the hydrogenation step. Obviously, the 1,2-diphenylurazole moiety is not inert towards catalytic hydrogenation. Therefore, protective groups which require hydrogenolytic cleavage can not be applied for the preparation of BIIE 0246 analogs with the 1,2-diphenyl urazole substructure.

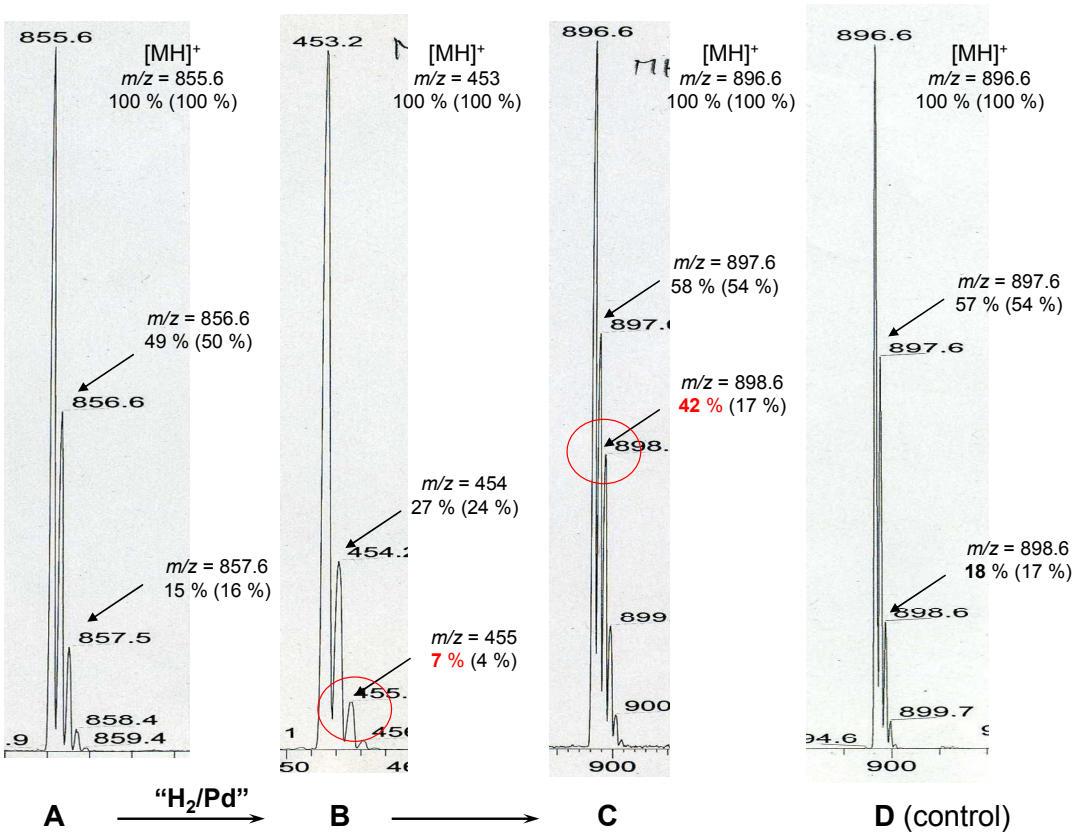
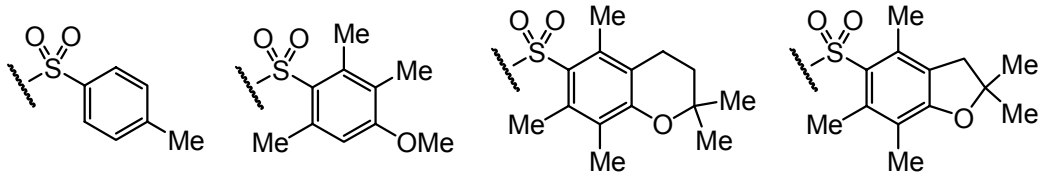


Fig. 4: Relative intensities of ESI-MS peaks (in brackets: calculated values for isotope peaks of pure compound). After hydrogenolytic deprotection of **A** the MH + 2 peaks of the following products **B** and **C** are too intensive, which indicates the presence of a hydrogenated by-product. **A**: N^{α},N^{δ},N^{ω}-tris-(benzyloxycarbonyl)-*N*-(2-(1,2-diphenyl-1,2,4-triazolidine-3,5-dione-4-yl)ethyl)argininamide; **B**: *N*-(2-(1,2-diphenyl-1,2,4-triazolidine-3,5-dione-4-yl)ethyl)argininamide; **C**: contaminated BIIE 0246 (**2**); **D**: pure BIIE 0246 (**2**) (control).

4.1.4. N^{ω}-ARENESULFONYL-PROTECTED ARGININES

The 4-toluenesulfonyl group (Tos) was largely used for the protection of the arginine side chain in peptide synthesis in solution or on solid support. Though, the N^{G}-p-toluenesulfonyl substitution almost completely masks the basic and nucleophilic character of the guanidino function, the harsh conditions required for its removal (HF/anisole or Na/NH_3) distract chemist from the application of the Tos protective group. In recent years many electron-rich arylsulfonyl protective groups have been developed for the protection of the arginine side chain. Mtr, Pmc, and Pbf (cf.

Scheme 11) can be cleaved off using TFA and have become standard arginine side chain protective groups in Fmoc/^{t}Bu chemistry.



Scheme 11: Arenesulfonyl-based arginine side chain protective groups: 4-toluenesulfonyl (Tos), 4-methoxy-2,3,6-trimethylbenzenesulfonyl (Mtr), 2,2,5,7,8-pentamethylchroman-6-sulfonyl (Pmc), and 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pbf).

Fmoc-Arg(Pbf)-OH was successfully used in our lab to prepare BIIE 0246 and analogs (cf. chapter 7).

4.2. Orthogonal N^{α}-/ Side Chain Protection (Tactics)

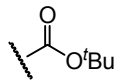
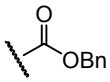
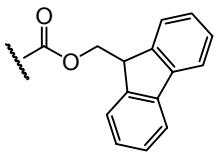
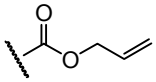
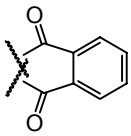
Our synthetic scheme required the temporary protection of the α-amino group. N^{α}-Protection had to be orthogonal to the groups used for the protection of the side chain function. Furthermore, cleavage conditions had to be compatible with functional groups in other portions of the molecule.

The protective groups used for the preparation of N^{G}-unsubstituted BIIE 0246 analogs are described in section 4.1. For the synthesis of substituted argininamides we had to start with orthogonally protected ornithine.

In Table 1 some properties of commonly used amino protective groups are shown. The protective groups applicable for our purpose not only had to be orthogonal to each other, but also they had to be compatible with other functional groups present in the molecule. The Cbz group could not be used for the synthesis of BIIE 0246 (**2**) analogs, because the 1,2-diphenyl-1,2,4-triazolidine-3,5-dione moiety is sensitive to hydrogenolysis (*vide infra*). Moreover, the diarylmethylpiperazino substructure is assumed to be sensitive towards strong acids and catalytic hydrogenolysis. However, 50 % TFA in CH_2Cl_2 was tolerated. Therefore, the Boc group was chosen as

temporary N^{α}-protection; as acid resistant N^{δ}-protective group we successfully utilized the phthaloyl (Pht) group.

Table 1: Properties of some conventional amino protective groups.

	Boc	Cbz	Fmoc	Alloc	Pht
					
A	Boc_2O [273]	Cbz–Cl [39] Cbz-OSu [162]	Fmoc–Cl [357] Fmoc-OSu [870]	Alloc-Cl [56] $Alloc_2O$ [2718]	EtOC(O)NPht (Nefken's reagent) [140]
B	TFA/DCM 1:1 or HCl/org. solv. [1-3 h]	Pd (black) or Pd (C) + H_2 (or HCO_2H or HCO_2^-) [2-12 h]	piperidine/DMF or Et_2NH/DMF etc. [1-10 min]	$(PPh_3)_4Pd^0$ + Bu_3SnH (or R_3SiH, Nu[1]) [1 h]	1. N_2H_5OH, 2. H^+ or (PhN_2H_3,MeN_2H_3, NH_2OH) [1-24 h]
C	CO_2, isobutene	CO_2, toluene	CO_2, dibenzofulvene and amine adducts	CO_2, $Nu–C_3H_5$	2,3-dihydro-phthalazine-1,4-dione
D	Alkylation of nucleophilic sites with Me_3C^+.	Not completely inert to prolonged exposure to TFA.	Ideal PG in SPPS; in solution phase synthesis separation of dibenzofulvene adducts can be difficult.	Use of metal based catalyst and reagent(s). Expensive Pd catalyst.	Base sensitivity. Separation of by-product can be laborious.

A: standard reagent(s) for introduction [€/mole];
B: standard cleavage conditions [typical reaction times],
C: by-products formed during cleavage reaction;
D: comments.

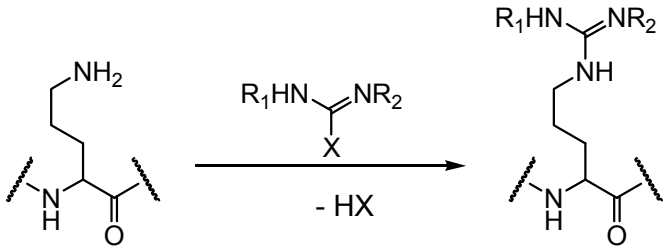
[1] other nucleophiles e.g. dimedone, DABCO etc.

In case of the BIBP 3226 (**1**) analogs we decided to use *tert*-butyl as permanent protective group for the reactive phenolic hydroxyl function. Hence, we used Cbz instead of Boc as N^{α}-protection and Pht as N^{δ}-protection. The introduction of the phthaloyl group is facile and inexpensive. The Pht group is characterized by its excellent acid resistance and inertness against catalytic hydrogenolysis. On the other hand the phthaloyl protective group is labile under alkaline conditions and in the

presence of nucleophiles. The standard cleavage procedure uses hydrazine[21] as deblocking reagent. However, in some cases the separation of the resulting phthaloylhydrazine can cause difficulties. During our efforts to synthesize (*R*)-*N*-(4-*tert*-butoxybenzyl)-N^{α}-diphenylacetylornithinamide (**23**) we encountered problems to isolate the free amine in high yields. Attempts to apply alternative procedures using methylhydrazine[22, 23], phenylhydrazine[24-26] or hydroxylamine[27] failed. As alternative starting materials Fmoc-D-Orn(Z)-OH and Fmoc-Orn(Boc)-OH can be used for the synthesis of substituted analogs of **1** and **2**, respectively, avoiding the use of the phthaloyl protective group.

5. Arginines from Ornithines (Guanidinylation Chemistry)

N^{G}-Substituted arginine derivatives are advantageously prepared by guanidinylation of the corresponding ornithine precursors (cf. Scheme 12).



Scheme 12: Conversion of ornithine derivatives into arginine derivatives by amidine group transferring reagents (guanidinylation).

For the amine – guanidine transformation numerous methods have been developed. The most common reagents for this purpose are of one the following types: thioureas and *S*-methylisothioureas, 1*H*-pyrazole-1-carboxamidines, and triflylguanidines.

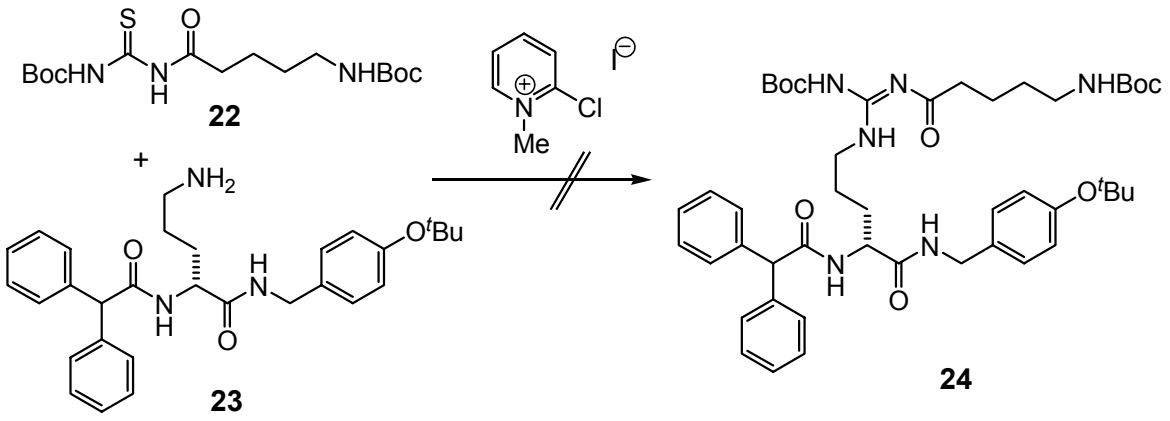
5.1. Thioureas

One of the advantages of the thiourea method is the accessibility of *N,N'*-disubstituted thioureas by the reaction of amines with isothiocyanates. Thus, a wide range of different, substituted guanidines is accessible. The reaction of thioureas

with amines is promoted by electrophilic agents, which convert the sulfur atom into a good leaving group. Desulfurization leads to an intermediate carbodiimide which readily reacts with primary or secondary amines to yield the desired guanidines[28]. The most common desulfurizing agents for this purpose are metal ions (Hg^{2+}, Cu^{2+}), 1-methyl-2-chloropyridinium iodide (Mukaiyama's reagent)[29] or EDC[30].

The reactivity of thiourea is enhanced by electron withdrawing subtituents at the nitrogen atom(s). Therefore, thioureas substituted with urethane-type protective groups (e.g. Boc, Cbz) are often used as efficient guanidinylating reagents. An additional advantage is that isolation and purification steps are facilitated due to the less polar character of the resulting protected guanidines. The free guanidines subsequently can be obtained using standard deprotection methods[§].

Serious side reactions which can occur are: i) reaction of the amine (or other nucleophilic sites in the substrate) with Mukaiyama's reagent or EDC (especially when resin bound thioureas are employed) and b) decomposition of the intermediately formed carbodiimide, when unreactive or sterically hindered amines are used.



Scheme 13: Guanidinylation of ornithinamide **23** with thiourea **22** and Mukaiyama's reagent did not yield the arginine derivative **24**.

[§] Hydrogen chloride (in organic solvent) does not reliably remove Boc groups from the guanidino nitrogen. However, in TFA/CH_2Cl_2 1:1 (v/v) cleavage is complete after 2–3 hours.

We probed the applicability of the thiourea/Mukaiyama's reagent method for the preparation of ω-aminoacyl substituted BIBP 3226 (**1**) analogs. Thiourea fragment **22** was prepared in three steps and used in combination with 1-methyl-2-chloropyridinium iodide as guanidinylating reagent. Although the reagents were consumed, it was not possible to isolate the desired product **24**; in fact, degradation and other side reactions led to a complex mixture of products.

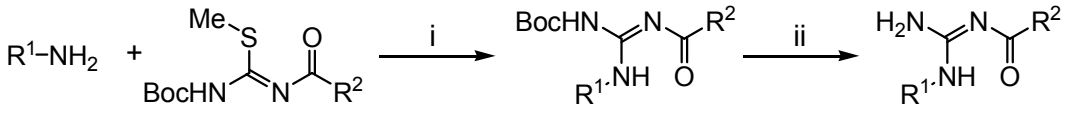
5.2. *S*-Methylisothioureas

2-Alkylisothioureas have been applied as amidine source for the guanidinylation of amines since the beginning of the 20th century. Methylation of thiourea with dimethylsulfate or methyliodide yields *S*-methylisothiourea, a basic compound, which is obtained in crystalline form as isothiouronium sulfate or iodide, respectively. *N*- and *N,N'*-protected derivatives of *S*-methylisothiourea have found widespread application as soluble, easily accessible, and efficient reagents for the preparation of protected guanidines from amines. *N,N'*-Bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea and *N,N'*-bis(benzyloxycarbonyl-*S*-methylisothioureas are commercially available guanidinylation reagents in common use. The reaction can optionally be supported by DMAP[31] catalysis or addition of stoichiometric amounts of Hg^{2+} salts[32]. Analogous reactions on solid support are described in literature[33].

According to this methodology we developed a novel protocol for the preparation of N^{ω}-acyl-substituted arginine derivatives, based on the use of *N*-acyl-*N'*-*tert*-butoxycarbonyl-*S*-methylisothioureas. Therefore, *S*-methylisothiouronium iodide** was mono-Boc-protected with hypostoichiometric amounts of Boc_2O in CH_2Cl_2 in the presence of triethylamine. Subsequently, the *N*-*tert*-butoxycarbonyl-*S*-methylisothiourea can be acylated under a broad variety of conditions. Even ω-*tert*-butoxycarbonylamino acids as acyl fragments were unproblematic. The thus obtained *N*-acyl-*N'*-*tert*-butoxycarbonyl-*S*-methylisothioureas were allowed to react

** The use of *S*-methylisothiouronium sulfate — instead of the iodide — leads to poor results due to insufficient solubility of the sulfate.

with the appropriate ornithinamides in the presence of one equivalent of $HgCl_2$ and two equivalents of triethylamine in DMF at ambient temperature (cf. Scheme 14). Guanidinylation proceeds smoothly and the products were obtained in good yield and excellent purity. This reaction was the most versatile and satisfactory method for the preparation of N^G-acyl-substituted analogs of BIBP 3226 (**1**) and BIIE 0246 (**2**) among all tested procedures.



Scheme 14: Preparation of Acylguanidines from *N*-acyl-*N'*-*tert*-butoxycarbonyl-*S*-methylisothioureas. Conditions: i: $HgCl_2$, NEt_3, DMF, r.t. ii: TFA/CH_2Cl_2 1:1 (v/v).

5.3. 1*H*-Pyrazole-1-carboxamidines

In 1953 Scott et al.[34] found that the reaction of amines with 3,5-dimethyl-1*H*-pyrazole-1-carboxamidine (**25a**, cf. Fig. 5) nitrate resulted in the formation of guanidinium nitrates. Four decades later, Bernatowicz et al.[35] suggested 1*H*-pyrazole-1-carboxamidine hydrochloride as convenient reagent for the conversion of amines into guanidines.

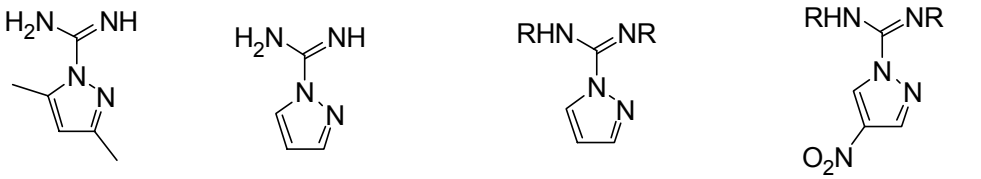
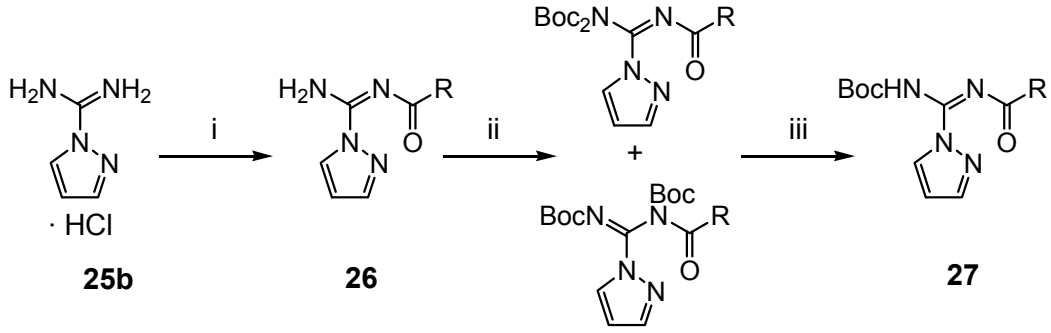


Fig. 5: 1*H*-Pyrazole-1-carboxamidine derivatives as guanidinylation reagents.

As in case of the thioureas and isothioureas, diacylation considerably enhances reactivity[36]. *N,N'*-Bis(*tert*-butoxycarbonyl)- and *N,N'*-bis(benzyloxycarbonyl)-1*H*-pyrazole-1-carboxamidine (**25c,d**) are commercially available, popular guanidinyl-

ation reagents, frequently used in peptide synthesis. Reactivity can be further improved by introduction of an electron-withdrawing nitro substituent in 4-position of the 1*H*-pyrazole heterocycle (**25e**[37]).

By contrast, *N*-monoacylated derivatives are unreactive[36]. Therefore, we had to use *N*-acyl-*N'*-*tert*-butoxycarbonyl-substituted 1*H*-pyrazole-1-carboxamidine derivatives (**27**, cf. Scheme 15) for the synthesis of N^G-acylated argininamides. Unfortunately, acylation of the monoacylated intermediate (**26**) happens only under forcing conditions. NaH[36], LiH[38], or LiHMDS[39] were used as strong bases to generate the corresponding anion, which was acylated by anhydrides or acyl chlorides. Alternatively, per-*tert*-butoxycarbonylation of *N*-monoacylated 1*H*-pyrazole-1-carboxamidines can be performed using Boc_2O/DMAP (cat.).



Scheme 15: Preparation of *N*-*tert*-butoxycarbonyl-*N'*-acyl-1*H*-pyrazole-1-carboxamidines. Conditions: i: DIPEA, R-C(O)X (acyl chloride, anhydride, or activated carboxylate); ii: Boc_2O/DMAP (10 mol-%); iii: $Mg(ClO_4)_2$, THF, 50 °C.

Treatment with $Mg(ClO_4)_2$ in THF at 50 °C selectively removes one Boc group from the disubstituted nitrogen atom yielding the *N*-acyl-*N'*-*tert*-butoxycarbonyl-1*H*-pyrazole-1-carboxamidines (**27**)[40]. By analogy with this procedure we prepared some of the N^G-acylated analogs of BIIE 0246 and BIBP 3226 (see Chapter 6 and 7).

Though the guanidinylation efficacy was satisfactory, the cumbersome preparation makes reagents of chemotype **27** less attractive. Especially, the presence of additional functional groups in the acyl fragment, as in our ω-(*tert*-butoxycarbonylamino)alkanoyl spacers, turned out to be problematic.

5.4. 1-Triflylguanidines

Recently, Feichtinger et al. introduced a novel type of guanidinylation reagents, the dialkoxycarbonyl protected trifluormethansulfonyl guanidines[41, 42]. In a comparative study **28a** proved to be superior to most other guanidinylation reagents[42].

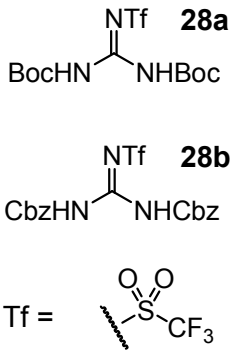
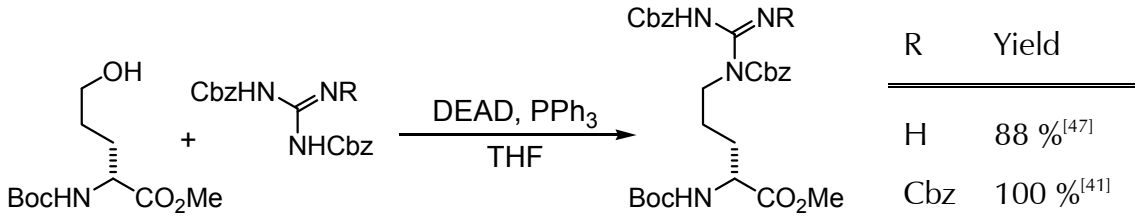


Fig. 6: Dialkoxycarbonyl protected triflylguanidines as guanidinylation reagents.

Schmuck et al. successfully prepared guanidinocarbonyl pyrroles as artificial receptors for carboxylate ions by activation of *N*-(1*H*-pyrrole-2-carbonyl)-*N'*-*tert*-butoxycarbonyl guanidine with trifluormethanesulfonic anhydride and subsequent guanidinylation of the amine component[43]. We adapted this approach for the synthesis of N^G-substituted **1** without isolation of the *N,N'*-diacyl-*N*-triflylguanidine. In our hands the method was successful for the synthesis of a 4-fluorobenzoyl substituted analog of BIBP 3226, but failed for the introduction of a 5-*tert*-butoxycarbonylaminopentanoyl spacer.

6. Arginines from Isoglutaminols

Acidic N-H nucleophiles (e.g. sulfonamides, iminodicarbonates, or cyclic imides) can be successfully alkylated with aliphatic alcohols under Mitsunobu conditions[44, 45]. Dodd and Kozikowski describe the preparation of protected alkylguanidines from alcohols using the Mitsunobu protocol[46]. Also arginine derivatives have been synthesized by this methodology[41, 47] (cf. Scheme 16). This prompted us to develop a new synthetic route to N^G-acyl substituted arginine derivatives via isoglutaminol **29** (cf. Scheme 17), which was synthesized starting from the N^α-benzyloxycarbonyl protected γ-monoester of D-glutamic acid (Z-D-Glu(OMe)-OH).

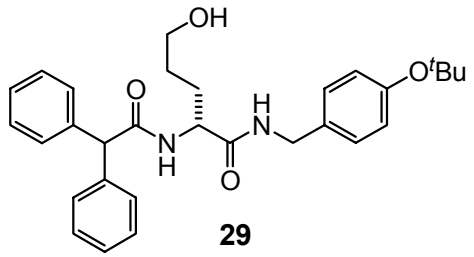


R	Yield
H	88 %[47]
Cbz	100 %[41]

Scheme 16: Synthesis of arginine derivatives under Mitsunobu conditions using protected guanidines. (Yields from cited literature).

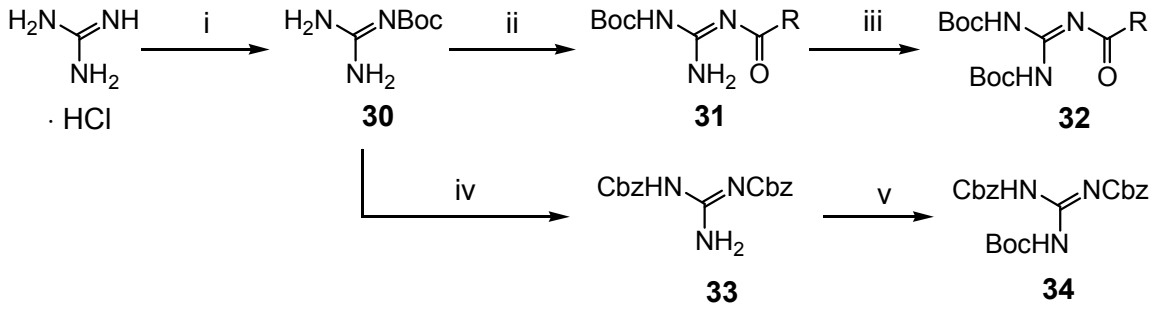
Coupling of Z-D-Glu(OMe)-OH with 4-*tert*-butoxybenzylamine, hydrogenolytic deprotection, and acylation with activated diphenylacetic acid led to the isoglutamine ester, which was reduced to alcohol **29** with $NaBH_4$/LiCl.

The protected guanidines were prepared by stepwise acylation of guanidine hydrochloride (cf. Scheme 18). 1-*tert*-Butoxycarbonyl guanidine is readily acylated under various conditions. In contrast, introduction of a third electron withdrawing substituent requires more forcing conditions (e.g. deprotonation with sodium hydride or DMAP catalysis).



29

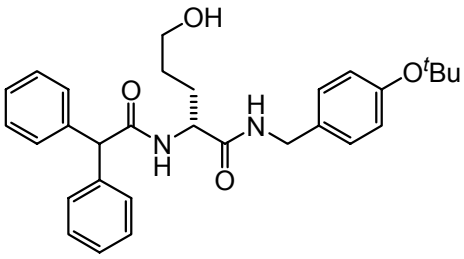
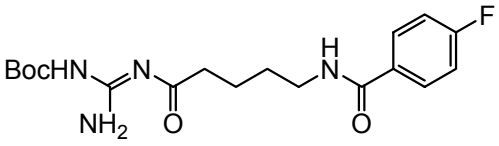
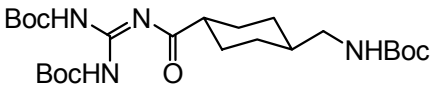
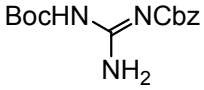
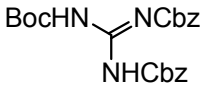
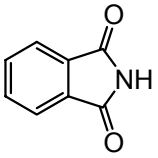
Scheme 17: Building block for the synthesis of N^G-substituted BIBP 3226 analogs by Mitsunobu protocols.



Scheme 18: Preparation of protected guanidines. Reagents and conditions: i: Boc_2O, 4N NaOH; ii: RCO_2H/CDI; iii: Boc_2O/DMAP; iv: CbzOSu; v: NaH, CbzCl, – 45 °C.

Unfortunately, reaction of **29** with protected guanidines **31** or **32** did not yield the desired substituted argininamides (cf. Table 2).

Table 2: Reaction of isoglutaminol **29** with various N-nucleophiles under Mitsunobu conditions.

alcohol + reagents	nucleophile	yield[1]
 + DIAD, $^{n}Bu_3P$		0 %
		0 %
		0 %
		60 %
		80 %

[1] isolated yield of Mitsunobu coupling product.

The only isolated product had a molecular mass reduced by 18 Da compared to that of **29**, obviously resulting from intramolecular condensation. Apparently the diacylated guanidines **31** are not active enough to compete with the nucleophilic sites in the alcohol **29** (e.g. the amide N–H's). On the other hand reaction with phthalimide proceeded smoothly, affording the phthaloyl protected ornithinamide, which could be deprotected and submitted to guanidinylation as described in section 5. The reactivities of N–H nucleophiles in Mitsunobu reactions are correlated to their pK_a[48], the more acidic compounds giving better yields. Presumably, the acidities of compounds **31** are insufficient.

7. Reference List

[1] Tsai, C. J.; Lin, S. L.; Wolfson, H. J.; Nussinov, R., Studies of protein-protein interfaces: a statistical analysis of the hydrophobic effect. *Protein Sci.* **1997,** 6, 53-64.

[2] Schug, K. A.; Lindner, W., Noncovalent Binding between Guanidinium and Anionic Groups: Focus on Biological- and Synthetic-Based Arginine/Guanidinium Interactions with Phosph[on]ate and Sulf[on]ate Residues. *Chem. Rev.* **2005,** 105, 67-114.

[3] Bogan, A. A.; Thorn, K. S., Anatomy of hot spots in protein interfaces. *J. Mol. Biol.* **1998,** 280, 1-9.

[4] Vincent, J.-P.; Mazella, J.; Kitabgi, P., Neurotensin and neurotensin receptors. *Trends Pharmacol. Sci.* **1999,** 20, 302-9.

[5] Beck-Sickinger, A. G.; Wieland, H. A.; Wittneben, H.; Willim, K. D.; Rudolf, K.; Jung, G., Complete L-alanine scan of neuropeptide Y reveals ligands binding to Y_1 and Y_2 receptors with distinguished conformations. *Eur. J. Biochem.* **1994,** 225, 947-58.

[6] Berlinck, R. G. S.; Kossuga, M. H., Natural guanidine derivatives. *Nat. Prod. Rep.* **2005,** 22, 516-50.

[7] Thamm, P.; Kolbeck, W.; Musiol, H.-J.; Moroder, L., Synthesis of Peptides In *Houben-Weyl Science of Synthesis*, Goodman, M.; Felix, A.; Moroder, L.; Toniolo, C., Eds. Thieme: 2002; Vol. E22a.

[8] Aiglstorfer, I. Argininamide und Argininamid-Analoga: Synthese und Struktur-Wirkungsbeziehungen neuer Neuropeptid Y Y_1-Rezeptorantagonisten. Ph.D. Thesis, Universität Regensburg, **1999**.

[9] Graichen, F. Neuropeptid Y Y_1-Rezeptorantagonisten der Argininamid-Reihe: Entwicklung von Synthesemethoden an polymeren Trägern und Strategien zur Herstellung von Radioliganden. Ph.D. Thesis, University of Regensburg, Regensburg, **2002**.

[10] Anderson, G. W.; Zimmermann, J. E.; Callahan, F. M., The Use of Esters of N-Hydroxysuccinimide in Peptide Synthesis. *J. Am. Chem. Soc.* **1964,** 86, 1839-42.

[11] Staab, H. A., Synthesen mit heterocyclischen Amiden. *Angew. Chem.* **1962,** 74, 407-23.

[12] Staab, H. A., Reaktionsfähige heterocyclische Diamide der Kohlensäure. *Liebigs Ann. Chem.* **1957,** 609, 75-83.

[13] Anderson, G. W.; Paul, R., *N,N'*-Carbonyldiimidazole - A new Reagent for Peptide Synthesis. *J. Am. Chem. Soc.* **1958,** 80, 4423.

[14] Wünsch, E.; Dress, F., Zur Synthese des Glucagons. X. Darstellung der Sequenz 22-29. *Chem. Ber.* **1966,** 99, 110-20.

[15] Miyazawa, T.; Otomatsu, T.; Fukui, Y.; Yamada, T.; Kuwata, S., Effect of copper(II) chloride on suppression of racemization in peptide synthesis by the carbodiimide method. *Int. J. Pept. Protein Res.* **1992,** 39, 237-44.

[16] Abdelmoty, I.; Albericio, F.; Carpino, L. A.; Foxman, B. M.; Kates, S. A., Structural studies of reagents for peptide bond formation: Crystal and molecular structures of HBTU and HATU. *Lett. Pept. Sci.* **1994,** 1, 57-67.

[17] Jaeger, G.; Koenig, W.; Wissmann, H.; Geiger, R., Synthesis of secretin. *Chem. Ber.* **1974,** 107, 215-31.

[18] Dollinger, H.; Esser, F.; Mihm, G.; Rudolf, K.; Schnorrenberg, G.; Gaida, W.; Doods, H. N. Preparation of novel peptides for use as NPY antagonists. DE 19816929, **1999**.

[19] Jakubke, H.-D., *Peptide: Chemie und Biologie*. 1 ed.; Spektrum Akademischer Verlag Heidelberg, Berlin, Oxford, **1996**.

[20] Grønvald, F. C.; Johansen, N. L.; Lundt, B. F., Synthesis of Z-Arg(Boc)$_2$-OH. In *Peptides 1980, Proceedings of the 16th European Peptide Symposium*, Brunfeldt, K., Ed. pp 111-5.

[21] Ing, H. R.; Manske, R. H. F., A Modification of the Gabriel Synthesis of Amines. *J. Org. Chem.* **1926**, 2348-52.

[22] Smith, A. L.; Hwang, C.-K.; Pitsinos, G. R.; Scarlato, G. R.; Nicolaou, K. C., Enantioselective Total Synthesis of (-)-Calicheamicinone. *J. Am. Chem. Soc.* **1992,** 114, 3134-6.

[23] Hubschwerlen, C.; Specklin, J.-L.; Doyon, J. G.; Paquette, L. A., (3*S*,4*S*)-3-Amino-1-(3,4-dimethoxybenzyl)-4-[(*R*)-2,2-Dimethyl-1,3-Dioxolan-4-yl]-2-Azetidinone. *Org. Synth.* 72, 14.

[24] Schumann, I.; Boissonnas, R. A., Synthèse de la L-valyl-L-(δ-carbobenzoxy)-ornithyl-L-leucyl-D-phénylalanyl-L-proline. *Helv. Chim. Acta* **1952,** 35, 2237-41.

[25] Schumann, I.; Boissonnas, R. A., Splitting of N-phthalyl Groups of Amino-acids with Phenylhydrazine. *Nature* **1952,** 169, 154-5.

[26] Schumann, I.; Boissonnas, R. A., Scission, par la phénylhydrazine, du groupe «phtalyle» d'acides aminés et de peptides N-phtalylés. *Helv. Chim. Acta* **195,** 35, 2235-7.

[27] Neunhoeffer, O.; Lehmann, G.; Haberer, D.; Steinle, G., Die Hydroxylaminolyse *N*-substituierter Phthalimide, ein Verfahren zur Darstellung von Peptiden und N-Hydroxy-peptiden. *Justus Liebigs Ann. Chem.* **1968,** 712, 208-13.

[28] Kim, K. S.; Qian, L., Improved method for the preparation of guanidines. *Tetrahedron Lett.* **1993,** 34, 7677-80.

[29] Yong, Y. F.; Kowalski, J. A.; Lipton, M. A., Facile and Efficient Guanylation of Amines Using Thioureas and Mukaiyama's Reagent. *J. Org. Chem.* **1997,** 62, 1540-2.

[30] Poss, M. A.; Iwanowicz, E.; Reid, J. A.; Lin, J.; Gu, Z., A mild and efficient method for the preparation of guanidines. *Tetrahedron Lett.* **1992,** 33, 5933-6.

[31] Gers, T.; Kunce, D.; Markowski, P.; Izdebski, J., Reagents for Efficient Conversion of Amines to Protected Guanidines. *Synthesis* **2004**, 37-42.

[32] DeMong, D. E.; Williams, R. M., The asymmetric synthesis of (2*S*,3*R*)-capreomycidine. *Tetrahedron Lett.* **2001,** 42, 3529-32.

[33] Dodd, D. S.; Wallace, O. B., Solid-phase synthesis of *N,N'*-substituted guanidines. *Tetrahedron Lett.* **1998,** 39, 5701-4.

[34] Scott, F. L.; O'Donovan, D. G.; Reilly, J., Studies in the Pyrazole Series. III. Substituted Guanidines. *J. Am. Chem. Soc.* **1953,** 75, 4053-4.

[35] Bernatowicz, M. S.; Wu, Y.; Matsueda, G. R., 1*H*-Pyrazole-1-carboxamidine hydrochloride an attractive reagent for guanylation of amines and its application to peptide synthesis *J. Org. Chem.* **1992,** 57, 2497-502.

[36] Bernatowicz, M. S.; Wu, Y.; Matsueda, G. R., Urethane protected derivatives of 1-guanylpyrazole for the mild and efficient preparation of guanidines. *Tetrahedron Lett.* **1993,** 34, 3389-92.

[37] Yong, Y. F.; Kowalski, J. A.; Thoen, J. C.; Lipton, M. A., A new reagent for solid and solution phase synthesis of protected guanidines from amines. *Tetrahedron Lett.* **1999,** 40, 53-6.

[38] Drake, B.; Patek, M.; Lebl, M., A Convenient Preparation of Monosubstituted *N,N'*-di(Boc)-Protected Guanidines. *Synthesis* **1994**, 579-82.

[39] Ghosh, A. K.; Hol, W. G. J.; Fan, E., Solid-Phase Synthesis of *N*-Acyl-*N*′-Alkyl/Aryl Disubstituted Guanidines. *J. Org. Chem.* **2001,** 66, 2161-4.

[40] Dowle, M. D.; Howes, P. D.; Robinson, J. E.; Trivedi, N. Process for the Preparation of Butoxycarbonylimino Compounds and Intermediates Therefor. WO0078723, **2000**.

[41] Feichtinger, K.; Sings, H. L.; Baker, T. J.; Matthews, K.; Goodman, M., Triurethane-Protected Guanidines and Triflyldiurethane-Protected Guanidines: New Reagents for Guanidinylation Reactions. *J. Org. Chem.* **1998,** 63, 8432-9.

[42] Feichtinger, K.; Zapf, C.; Sings, H. L.; Goodman, M., Diprotected Triflylguanidines: A New Class of Guanidinylation Reagents. *J. Org. Chem.* **1998,** 63, 3804-5.

[43] Schmuck, C.; Bickert, V., *N*′-alkylated guanidiniocarbonyl pyrroles: new receptors for amino acid recognition in water. *Org. Lett.* **2003,** 5, 4579-81.

[44] Wisniewski, K.; Koldziejczyk, A. S.; Falkiewicz, B., Applications of the Mitsunobu reaction in peptide chemistry. *J Pept Sci* **1998,** 4, 1-14.

[45] Hughes, D. L., Progress in the Mitsunobu Reaction. A Review. *Org. Prep. Proced. Int.* **1996,** 28, 129-64.

[46] Dodd, D. S.; Kozikowski, A. P., Conversion of Alcohols to Protected Guanidines Using the Mitsunobu Protocol. *Tetrahedron Lett.* **1994,** 35, 977-80.

[47] Ohshima, T.; Gnanadesikan, V.; Shibuguchi, T.; Fukuta, Y.; Nemoto, T.; Shibasaki, M., Enantioselective Syntheses of Aeruginosin 298-A and Its Analogues Using a Catalytic Asymmetric Phase-Transfer Reaction and Epoxidation. *J. Am. Chem. Soc.* **2003,** 125, 11206-7.

[48] Koppel, I.; Koppel, J.; Degerbeck, F.; Grehn, L.; Ragnarsson, U., Acidity of imidodicarbonates and tosylcarbamates in dimethyl sulfoxide. Correlation with yields in the Mitsunobu reaction. *J. Org. Chem.* **1991,** 56, 7172-4.

5 On the stability of 1-(ω-aminoalkanoyl)-guanidines under alkaline conditions

The decomposition of some N^G-(ω-aminoalkanoyl)argininamides, which are key intermediates for the preparation of radiolabeled and fluorescent NPY receptor ligands, prompted us to synthesize a small series of simple 1-(ω-aminoalkanoyl)guanidines, and to investigate these model compounds for stability in alkaline aqueous buffers. The degradation of acylguanidines was monitored by time resolved UV-spectroscopy. From the decay of the absorbance of the acylguanidine group at approx. 230 nm a (pseudo-)first order rate constants was derived. The most labile compound, 1-(5-aminopentanoyl)guanidine, decomposed with a half life of 19 s to yield piperidin-2-one (pH 10.4 at 25 °C). In contrast the half life of 1-(6-aminohexanoyl)guanidine is 7.7 h, which is comparable to the hydrolysis of acetylguanidine ($t_{1/2}$ = 9.6 h) in alkaline solution.

1. Introduction

Guanidines and guanidine derivatives are among the strongest non-anionic organic bases. The corresponding guanidinium ions are featuring the ability to form tight complexes with carboxylates, phosphonates, and sulfonates[1]; hence, the guanidine substructure plays an important role for molecular recognition processes in numerous biological and artificial systems. Numerous natural products and synthetic drugs comprising the guanidine motif are known[2]. The genetically coded amino acid arginine with its guanidino side chain is present in many proteins and peptides. Arginine residues are frequently found in the interaction sites of proteins[3].

Due to its strong basicity ($pK_a \approx 12$ of the conjugate acids) guanidines are positively charged at physiological pH. This is a major disadvantage with respect to the pharmacokinetic profiles of guanidine derived drug candidates. Therefore, many efforts have been undertaken to find less polar bioisosteric replacements for the guanidine motif (e.g. lit.[4-6]).

By introduction of an electron withdrawing acyl substituent at the guanidino nitrogen the pK_a value can by reduced by 4–5 orders of magnitude[7, 8]. Interestingly, N^G-acyl substituents do not necessarily impair the carboxylate binding properties of guanidines: Schmuck et al. successfully developed artificial carboxylate receptors on the basis of the acylguanidine motif[9-16]. Adang et al. report on thrombin inhibitors which bind with an acylguanidine moiety to the S_1 pocket[17].

In our lab we discovered that the neuropeptide Y Y_1 receptor antagonistic potency of the argininamide derivative BIBP 3226[18] can be enhanced by introduction of acyl substituents at the guanidine nitrogen[8, 19]. Therefore, acylguanidines can be considered as promising candidates for guanidine bioisosteres.

Based on this concept, we tried to synthesize N^G-(ω-aminoalkanoyl) substituted argininamides as precursors for radio- or fluorescence labeled neuropeptide Y receptor ligands. However, it turned out that the ω-amino group can dramatically accelerate the decomposition of these compounds, depending on length and nature of the spacer. In this work we report on the degradation rate and half lives of different ω-aminoalkylguanidines in alkaline buffer.

2. Results and discussion

In the course of our efforts to label N^G-(5-aminopentanoyl)-substituted argininamide derivatives in the presence of a base, we had to notice that exclusively the N^G-unsubstituted compounds could be isolated. This supported the idea that the acyl-N^G bond was cleaved by intramolecular attack of the terminal amino function at the carbonyl C-atom. In order to test this hypothesis we synthesized 1-(5-

aminopentanoyl)-guanidine (**1d**) as model compound for investigations on stability. 1-(5-aminopentanoyl)guanidine (**1d**) was accessible by condensation of CDI-activated 5-*tert*-butoxycarbonylaminopentanoic acid with 1-*tert*-butoxycarbonyl-guanidine and subsequent deprotection with 50 % TFA in CH_2Cl_2 (cf. Scheme 2). The obtained ditrifluoroacetate is sufficiently stable in acidic solutions.

Opportunely, acylguanidines in general exhibit strong UV-absorbance around 230 nm[20]. This property allowed us to monitor the kinetics of the degradation of **1d** in alkaline buffers by time-resolved UV-spectroscopy. Fig. 1 shows the UV spectra of diluted solutions of **1d** in borate buffer (pH = 10.4) after defined time intervals (Δt = 12 s).

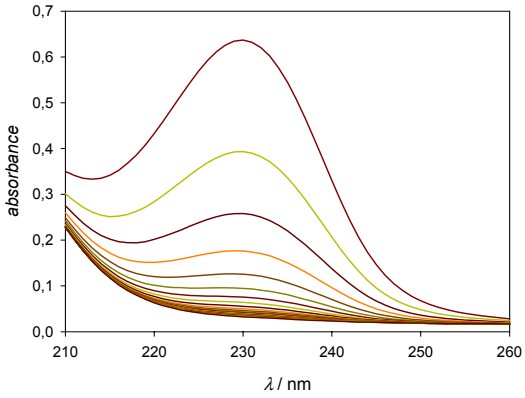
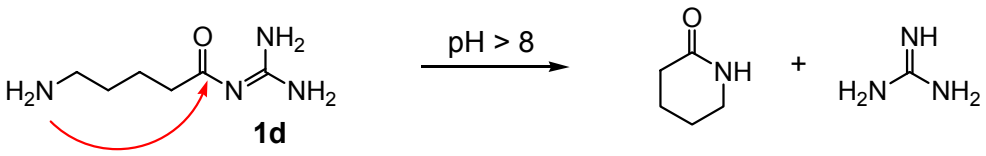


Fig. 1: Time dependent decay of UV-absorbance of 1-(5-aminopentanoyl)guanidine (1d) in borate buffer (pH 10.4) at 25 °C (Δt = 12 s).

Remarkably, the absorption of the acylguanidine moiety was extinguished after only a few minutes. As acyl-guanidines in general are stable and isolable compounds, we concluded that the presence of the terminal amino group dramatically enhances the rate of decomposition by intramolecular nucleophilic attack (6-*exo-trig* ring closure). And in fact piperidin-2-one (δ-lactam) was identified as the degradation product of 1-(5-aminopentanoyl)guanidine (**1d**) in alkaline solution by means of TLC and RP-HPLC (cf. Scheme 1).



Scheme 1: Degradation of 1-(5-aminopentanoyl)guanidine (**1d**) yielding δ-lactam and unsubstituted guanidine.

According to the Lambert-Beer Law, absorbance A is directly proportional to the concentrations c_i of the absorbing molecules. Therefore, the time-dependent variation of A is a quantitative measure for the progression of the degradation reaction. For first-order reactions the correlation of absorbance A and the observed rate constant k_{obs} of the reaction is given by eq. (1)*.

$$\frac{A - A_\infty}{A_o - A_\infty} = e^{-k_{obs} \cdot t} \tag{1}$$

$$-\ln \frac{A - A_\infty}{A_o - A_\infty} = k_{obs} \cdot t \tag{2}$$

A_0 and A_∞ represent the absorbance values at the beginning and at the end (*i.e.* after complete conversion) of the reaction. The time course of the degradation of 1-(5-aminopentanoyl)guanidine at various pH is depicted in Fig. 2.

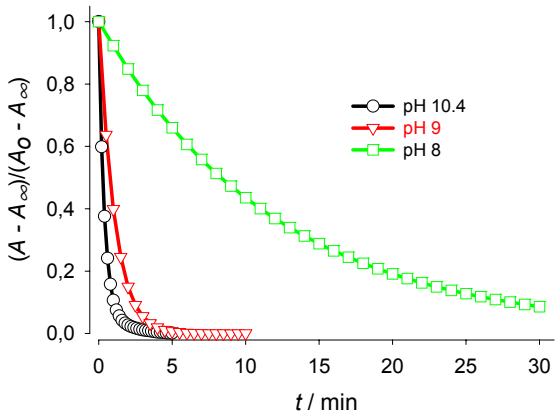


Fig. 2: Degradation of 1-(5-aminopentanoyl)guanidine (**1d**) in different buffers monitored by UV-spectroscopy at 230 nm.

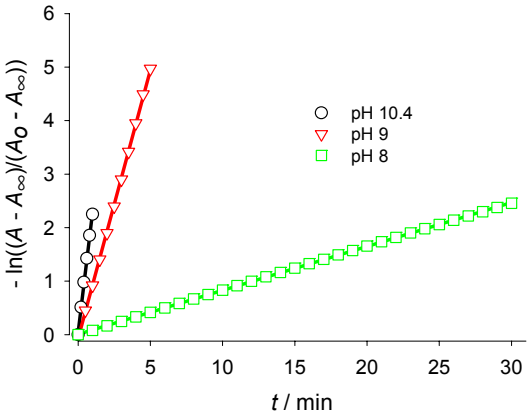


Fig. 3: Determination of first-order rate constants as slope of the linearized plots according to eq. 2.

The rapid decay of the acylguanidine absorbance at 230 nm can be quantified by evaluation of the rate constant and the half life. The respective linear plots are shown in Fig. 3. In the first half-life periods excellent linearity is observed

* For more details see appendix.

(correlation coefficients > 0.99), indicative of first-order kinetics of the decomposition of **1d**. The observed initial rate constants and half-lives for the decay of the UV-absorbance of 1-(5-aminopentanoyl)guanidine (**1d**) at pH 8, 9, and 10.4 are given in Table 1. The half-lives increase from 19 s at pH 10.4 to 42 s at pH 9 and 18.4 min at pH 8. The observation that degradation is decelerated at lower pH can be attributed to the lower concentration of the deprotonated form of **1d**, which can be considered the reactive species. Alternatively, certain modes of base-catalysis can be taken into account. Furthermore, it has to be regarded that 1-(ω-aminoalkanoyl)guanidines comprise two basic centers, the terminal primary amine ($pK_a \approx 10$) and the acylguanidine moiety ($pK_a \approx 8$). Whereas protonation of the guanidino residue enhances the reactivity of the electrophilic center and the quality of the leaving group, only the non-protonated, free amine is nucleophilic.

Table 1: Initial (pseudo-)first-order rate constants and half lives of 1-(5-aminopentanoyl)guanidine at different pH and 25 °C.

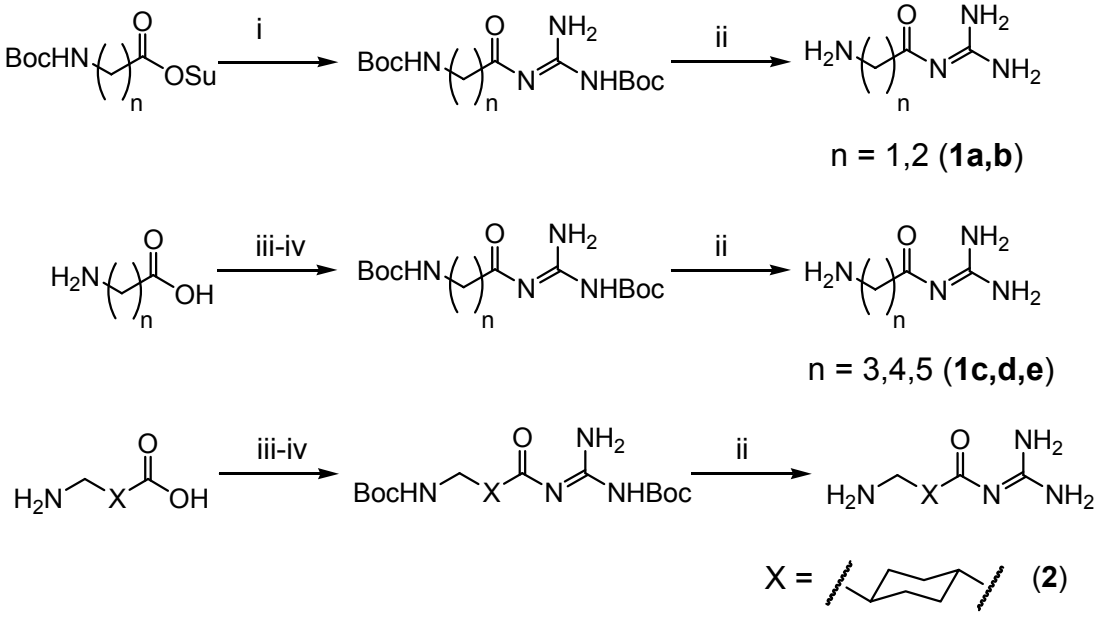
	time range* / min	k_{obs}/ min^{-1}	$t_{1/2}$
pH 8	0…30	0.082	18.4 min
pH 9	0…5	1.00	42 s
pH 10.4	0…1	2.25	19 s

* Data points within this time range were considered for linear regression and calculation of initial rate constants.

Protonation of the N^G-nitrogen causes a hypsochromic shift of the absorbance maxima to about 205 nm. As the degradation is almost completely prevented by protonation, compound **1b** (as well as related structures) can be stored very well as TFA-salts.

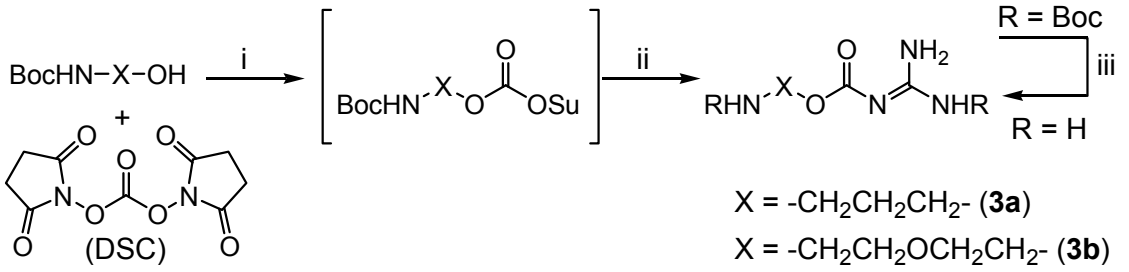
In quest of alternative, more stable spacers we tested various ω-aminoalkanoyl-guanidines of various chain lengths or with a cyclic scaffold for their stability in alkaline buffers. The compounds were prepared by acylation of 1-*tert*-butoxycarbo-

nylguanidine, which is accessible by treating guanidine hydrochloride with a hypostoichiometric amount of Boc_2O in 4 M NaOH[21]. The *N*-*tert*-butoxycarbonyl protected amino acids were either applied as succinimidyl esters, or activated with CDI, and coupled with 1-*tert*-butoxycarbonylguanidine. The resulting blocked aminoalkanoyl guanidines were deprotected with 50 % TFA in CH_2Cl_2 (cf. Scheme 2).



Scheme 2: Synthesis of 1-(ω-aminoalkanoyl)guanidines. i: 1-Boc-guanidine, THF. ii: TFA/DCM 1:1 (v/v).iii: $NaHCO_3$, Boc_2O, dioxane/water; iv: 1. CDI, DMF; 2. 1-Boc-guanidine.

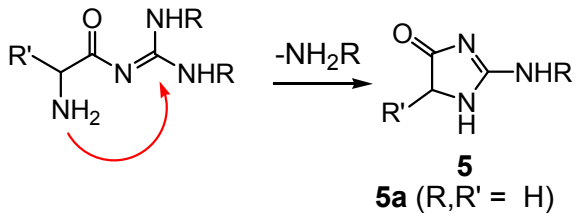
As further spacer variant we probed ω-aminoalkoxycarbonylguanidines, which were prepared from *N*-*tert*-butoxycarbonyl protected amino alcohols. The alcohols were treated with disuccinimidyl carbonate (DSC) yielding the corresponding activated mixed carbonates which were allowed to react with 1-*tert*-butoxycarbonylguanidine (cf. Scheme 3).



Scheme 3: Preparation of 1-(ω-aminoalkoxycarbonyl)guanidines. i: TEA, MeCN; ii: 1-Boc-guanidine, DMF; iii: TFA/DCM 1:1 (v/v).

The degradation rate in alkaline borate buffer (pH 10.4) for all ω-amino-acyl-guanidines (**1–3**) was determined using time-resolved UV-spectroscopy. Results are shown in Table 2. The slow reaction of acetylguanidine (entry **4**) and 4-(amino-methyl)cyclohexanecarbonylguanidine (**2**), which are unable to form lactams, reveals that there must be an alternative degradation pathway for acylguanidines — most probably hydrolytic cleavage[20, 22].

In case of the glycylguanidine (**1a**) the formation of an α-lactam is very implausible. Nevertheless, **1a** is reacting several fold faster than acetylguanidine, which indicates that the α-amino substituent must be participating in the degradation reaction. Rink et al.[23] found that N^{ω}-(α-Fmoc-aminoacyl)arginines form 2-amino-1*H*-imidazol-4(5*H*)-ones (**5**) when the Fmoc group is cleaved off in the presence of an excess of piperidine (cf. Scheme 4).



Scheme 4: Intramolecular reaction of 1-(α-amino-acyl)guanidines[23].

The UV spectrum of the cyclic acyl-guanidine **5a** shows an absorption maxima at 225 nm (in aqueous phosphate buffer at pH = 12) — but with a slightly lower molar extinction compared to acyclic acylguani-dines[22]. These data are in good agreement with the slight hypsochromic shift (227 vs. 224 nm) and the minor hypochromism we observed for the reaction of **1a** in

alkaline buffer. Thus, the formation of **5a** is the most probable mechanism for the degradation of **1a**.

Within our series of linear 1-(ω-aminoalkanoyl)guanines the β-alanyl (**1b**) and the 6-aminohexanoyl (**1e**) substituted guanidines exhibit the lowest degradation rates (cf. Table 2). The half lives for **1b** and **1e** are approximately 4 and 8 hours, respectively. Obviously, the formation of corresponding β- or ε-lactams is significantly less favored than the formation of the δ-lactam, which proceeds within minutes.

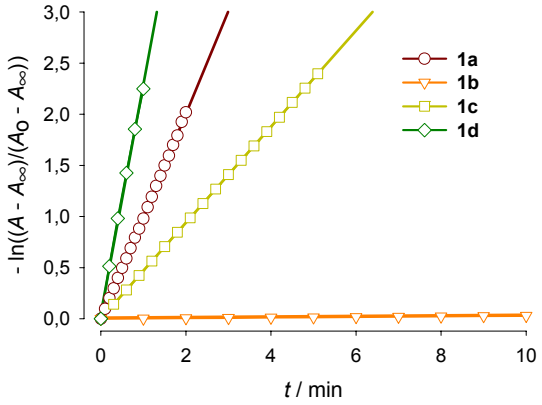


Fig. 4: Determination of (pseudo-)first-order rate constants for the degradation of short linear ω-aminoalkanoyl substituted guanidines by linear regression.

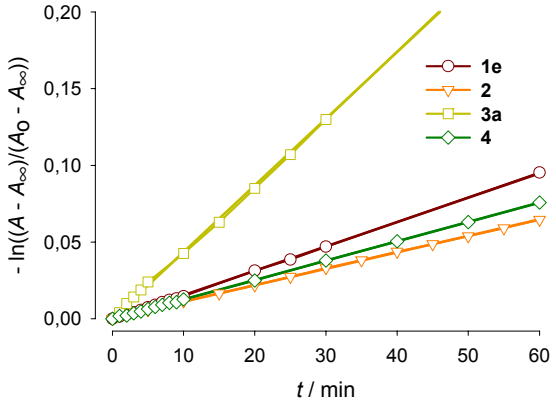
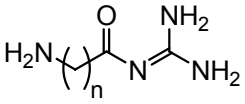
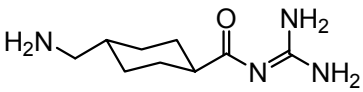
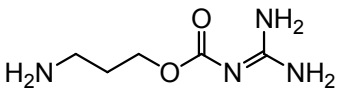
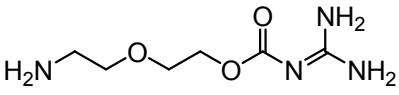
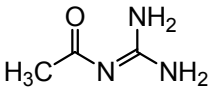


Fig. 5:Determination of (pseudo-)first-order rate constants for the slow degradation of various acyl guanidines by linear regression.

For 1-(β-aminopropanoyl)guanidine (**1b**) the formation of a cyclic acylguanidine is less likely than in case of **1a**. The expected absorption maximum at 233 nm, corresponding to, 2-amino-5,6-dihydropyrimidin-4(1*H*)-one, could not observed; moreover, the hypothetical product is known to be unstable in alkaline solution ($t_{1/2}$ = 4 h at pH 12)[22]. 1-(4-Aminobutanoyl)guanidine (**1c**), which can form a five-membered γ-lactam, decomposes at a comparably rapid rate as **1a** and **1d**. The most stable linear ω-aminoalkanoyl substituted guanidine in our series is **1e**, the decomposition of which proceeds only slightly faster than the alkaline hydrolysis of acetylguanidine (**4**).

Very surprising is the huge difference in the reaction rates of **1d**, the most labile compound, and **1e**, the most resistant compound within our series, since they differ only by one methylene group. Obviously, there is a substantial difference in the tendency to form 6-membered and 7-membered lactams from 1-(ω-aminoalkanoyl)guanidines. In contrast to N^G-(5-aminopentanoyl)-substituted argininamides, the N^G-(6-aminohexanoyl)-substituted analogs could successfully be acylated with active esters at the terminal primary amino function, due to their enhanced durability (cf. chapter 6).

Table 2: Degradation of various acylguanidines in alkaline buffer (pH 10.4) at 25 °C; (pseudo-) first-order rate constants and half-lives.

No.		λ_{max} / nm	k_{obs} / min^{-1}	$t_{1/2}$
1a	n = 1	227	0.97	43 s
1b	n = 2	230	0.028	4.1 h
1c	n = 3	230	0.44	96 s
1d	n = 4	230	2.25	19 s
1e	n = 5	230	0.0015	7.7 h
2		231	0.0009	13 h
3a		215	0.0043	2.7 h
3b		215	0	∞
4		228	0.0012	9.6 h

ω-Aminoalkoxycarbonyl substitued guanidines, which were taken into account as alternative spacer groups, are considerably more stable than the corresponding ω-aminoalkanoylguanidines. *tert*-Butoxycarbonylguanidine and 1-[2-(2-aminoethoxy)-ethyloxycarbonyl]guanidine (**3b**) were completely stable under the reaction conditions. The absorption maxima of the alkoxycarbonylguanidines are at shorter wavelengths (typically 215–225 nm) than those of the acylguandines.

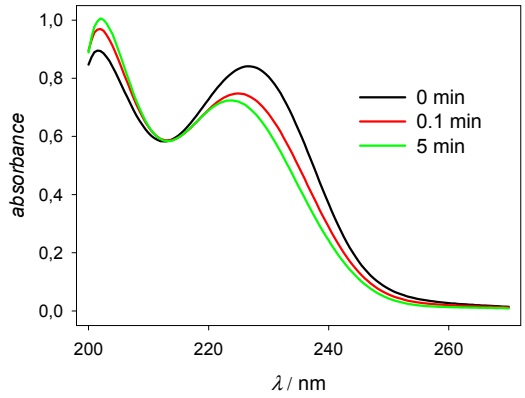


Fig. 6: Time-dependent UV-absorption of 1-glycylguanidine (1a) and the emerging product(s) in borate buffer (pH 10.4) at 25 °C.

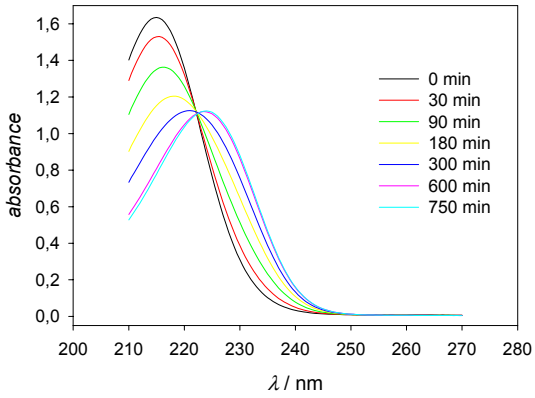


Fig. 7: Time-dependent UV-absorption of 1-(3-aminopropyloxycarbonyl)guanidine (3a) and the emerging product(s) in borate buffer (pH 10.4) at 25 °C.

In contrast alkoxycarbonylguanidine **3a**, which corresponds to the highly reactive acylguanidine **1d** (same number of heavy atoms in chain), reacts with a half-life of 2.7 hours. In principle the decomposition of both **3a** **1d** can be facilitated in a similar way by intramolecular nucleophilic attack ('6-exo-trig') of the terminal amino group. However, the time-dependent absorption spectrum of **3a** in alkaline buffer shows the dissapearence of the initial maximum at 215 nm and simultaneously the appearance of a new maximum at 224 nm. Since carbamates exhibit no absorbance in this region, the formation of a cyclic six-membered carbamate is implausible, whereas the preservation of the chromophoric acylguanidine substructure is more obvious. This can (only) be explained by a rearrangement of 1-(3-aminopropyloxy-carbonyl)guanidine **3a** to 1-(3-hydroxypropylaminocarbonyl)guanidine by initial

nucleophilic attack of the terminal amino group at the acyl-carbon atom and subsequent cleavage of the ester bond. Amidinourea shows a strong absorption maximum at approx. 220–225 nm[24], which is in good agreement with the spectrum of the decomposition product of **3a**. However, mechanistic details were beyond the scope of this study.

3. Conclusion

Acylguanidines are promising, less polar bioisosteres of the strongly basic guanidino group. However, acylguanidines tend to decompose when subjected to alkaline conditions. The alkaline hydrolysis of acetylguanidine proceeds with a half-life of 9.6 h. Decomposition can be extremely accelerated if an intramolecular nucleophilic attack is possible, as demonstrated by the cleavage of 1-(5-aminopentanoyl)-guanidine, which is completely converted to δ-lactam within minutes in alkaline solution. However, there are pronounced differences in the reaction rates of linear 1-(ω-aminoalkanoyl)guanidines depending on the length of the chain; half-lives vary from 19 s for 1-(5-aminopentanoyl)guanidine to 7.7 h for 1-(6-aminohexanoyl)-guanidine.

Compared to aminoalkanoylguanidines, the analogous aminoalkoxycarbonyl-substituted guanidines are considerably more stable towards alkaline hydrolysis. In general the oxa analogues are inert in aqueous buffer at pH 10.4 and 25 °C. However, the reaction can be enabled by intramolecular nucleophiles in appropriate distance to the electrophilic center; e.g. 1-(3-aminopropyloxy-carbonyl)guanidine reacted with a half life of 2.7 h.

On one hand, the degradation pathways described in this chapter should be taken into account in the design and synthesis of aminoalkanoylguanidines such N^{ω}-substituted argininamides, which are useful building blocks for the preparation of fluorescent and radiolabeled neuropeptide Y receptor antagonists. On the other hand, the 5-aminopentanoyl spacer could potentially serve as an easily cleavable

linker for the immobilization of guanidines on solid support or as tunable protecting group for the guanidino function based on the principle of 'assisted cleavage'.

4. Experimental section

4.1. General conditions

Chemicals were purchased from the following suppliers: Acros Organics (Geel, Belgium), IRIS Biotech GmbH (Marktredwitz, Germany), Mallinckrodt Baker (Deventer, NL), 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).

Buffer solutions were prepared according to the procedures given in the European Pharmacopoeia, 4th edition, 2002.

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 $\delta = 0$ ppm) to all samples.

Multiplicities are specified with the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (for broad signal), as well as combinations thereof. Mass spectrometry analysis (MS) was performed on a Finnigan SSQ 710A (CI-MS (NH_3)) and on a Finnigan ThermoQuest TSQ 7000 (ESI-MS) spectrometer. UV-spectroscopy was performed using a Varian Cary 100 Conc UV/Vis spectrophotometer. 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 ninhydrin spray, or ammonium molybdate/cerium(IV) sulphate solution.

Analytical reversed phase HPLC (RP-HPLC) was performed on a system from Thermo Separation Products (Egelsbach, Germany) equipped with a SN 400 controller, a P4000 pump, an AS3000 auto sampler and a Spectra Focus UV-Vis detector. Stationary phase was an Eurosphere-100 C-18 (250 × 4.0, 5 μm) column (Knauer, Berlin, Germany) thermostated at 45 °C. As mobile phase gradients of acetonitrile/0.1 % aq. TFA were used (flow rate: 0.7 ml·min^{-1}). Absorbance was detected at 210 nm.

Relative molecular weights (in Da) are given in parentheses behind the formula.

4.2. Preparation of 1-Boc-3-acyl substituted guanidines

1-*tert*-Butoxycarbonylguanidine[25] – Boc_2O (27.4 g, 0.125 mol) in 1,4-dioxane (160 mL) was added in a dropwise fashion to a stirred solution of guanidine hydrochloride (15.0 g, 0.157 mol) in 4 M NaOH (80 mL), cooled to 0 °C. The mixture was allowed to warm to ambient temperature, and stirring was continued for 16 h. The solvents were removed under reduced pressure and the residue was suspended in water and sonicated for 10 min. The white solid was collected on a filter, suspended in diethyl ether, sonicated for 10 min, filtered and subsequently dried in a desiccator over P_4O_{10}. The product was isolated as a white solid in 73 % yield (14.5 g). Mp > 184 °C (dec.); ^{1}H-NMR (300 MHz, DMSO-d_6): δ_H = 1.34 (s, 9H, Boc), 6.81 (brs, 4H, -N<u>H</u>'s) ppm; 28.1 (Boc), 75.4 (Boc), 162.6 (<u>C</u>=X), 163.2 (<u>C</u>=X) ppm. $C_6H_{13}N_3O_2$ (159.19).

5-(*tert*-Butoxycarbonylamino)pentanoic acid – To a vigorously stirred solution of 5-aminopentanoic acid (2.93 g, 25.0 mmol) and $NaHCO_3$ (5.26 g, 62.5 mmol) in water (50 mL) was added Boc_2O (6.55 g, 30.0 mmol) in 1,4-dioxane (50 mL), and

stirring was continued overnight. The resulting mixture was extracted with diethyl ether (50 mL, discarded) and carefully acidified with 2 M hydrochloric acid to pH 2. The aqueous layer was extracted with ethyl acetate (3 × 30 mL) and the combined extracts were washed with brine and dried over anhydrous Na_2SO_4. The volatiles were removed under reduced pressure and the residue was crystallized from diethyl ether/n-pentane. Yield: 4.48 g (82 %, as white powder); mp 50–52 °C (lit.[26] mp 47–50 °C). $C_{10}H_{19}NO_4$ (217.26).

In a similar manner was prepared **6-(*tert*-butoxycarbonylamino)hexanoic acid** in 84 % yield as white powder, mp 35–37 °C (lit.[27] mp 31–32 °C). $C_{11}H_{21}NO_4$ (231.29). **4-(*tert*-butoxycarbonylamino)butanoic acid** (Boc-4-Abu-OH) in 83 % yield as white solid, mp 56–57 °C (lit.[28] mp 50–52 °C). $C_9H_{17}NO_4$ (203.24), and ***trans*-4-[(*tert*-butoxycarbonylamino)methyl]cyclohexanecarboxylic acid** in 95 % yield as white powder, mp 132–134 °C (lit.[29] mp 135 °C). $C_{13}H_{23}NO_4$ (257.33).

[2-(2-Hydroxyethoxy)ethyl]carbamic acid *tert*-butyl ester – A solution of 2-(2-aminoethoxy)ethanol (1.05 g, 10.0 mmol) and K_2CO_3 in 10 mL water was treated with Boc_2O (2.18 g, 10.0 mmol) in acetone (10 mL). The mixture was stirred vigorously for 16 h at ambient temperature. The product was extracted with ethyl acetate and the combined extracts were washed with brine and dried over anhydrous Na_2SO_4. The solvents were evaporated and the residual colorless oil was dried in vacuum. Yield: 1.95 g (95 %); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.32 (s, 9H, Boc), 3.20 (m, 2H, -NHC$\underline{H}_2$-), 3.38–3.48 (m, 4H, -C$\underline{H}_2$OC$\underline{H}_2$-), 3.61 (m, 2H, -C$\underline{H}_2$OH) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 28.3 (Boc), 40.5 (-NH$\underline{C}H_2$-), 61.4 (-$\underline{C}H_2$OH), 70.2 (-NHCH$_2\underline{C}H_2$O-), 72.2 (-NHCH$_2$CH$_2$O$\underline{C}H_2$-), 79.3 (Boc), 156.2 (Boc) ppm; CI-MS (NH_3) *m/z* (%): 223 (21) $[M+NH_4]^+$, 206 (33) $[MH]^+$, 167 (100) $[M + NH_4 – C_4H_8]^+$, 150 (25) $[MH – C_4H_8]^+$. $C_9H_{19}NO_4$ (205.25).

3-Hydroxypropylcarbamic acid *tert*-butyl ester – To a solution of 3-aminopropan-1-ol (0.75 g, 10.0 mmol) and DIPEA (1.29 g, 10.0 mmol) in anhydrous CH_2Cl_2 (10 mL) was added Boc_2O (2.40 g, 11.0 mmol) in CH_2Cl_2 (10 mL). After stirring overnight the solution was washed with 1 M aq. NH_4Cl, 5 % aq. $KHSO_4$, and brine.

The organic layer was separated, dried over anhydrous Na_2SO_4 and, evaporated to dryness. Yield: 1.30 g (74 %) as colorless oil; ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.44 (s, 9H, Boc), 1.68 (m, 2H, $HOCH_2C\underline{H}_2CH_2NH$-), 3.27 (m, 2H, $HOCH_2CH_2C\underline{H}_2NH$-), 3.66 (m, 2H, $HOC\underline{H}_2CH_2CH_2NH$-) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 28.4 (Boc), 32.7 ($HOCH_2\underline{C}H_2CH_2NH$-), 37.2 ($HO\underline{C}H_2$-), 59.3 (-$\underline{C}H_2NH$Boc-), 79.7 (Boc), 157.1 (Boc) ppm. $C_8H_{17}NO_3$ (175.23).

1-*tert*-Butoxycarbonyl-2-[2-(*tert*-butoxycarbonylamino)acetyl]guanidine – Boc-Gly-OSu (0.34, 1.26 mmol) and 1-*tert*-butoxycarbonylguanidine (0.20 g, 1.26 mmol) were dissolved in THF (5 mL) and stirred for 20 h at ambient temperature. The mixture was filtered and the filtrate was concentrated under reduced pressure. The residual product was redissolved in CH_2Cl_2 and washed with aq. Na_2CO_3 and brine. The organic layers was separated, dried over anhydrous Na_2SO_4, and evaporated to dryness. Yield: 0.35 g (88 %); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.45 (s, 9H, Boc), 1.49 (s, 9H, Boc), 3.84 (m, 2H, -C$\underline{H}_2$NHBoc) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 28.0 (Boc), 28.3 (Boc), 46.5 (-$\underline{C}H_2$NHBoc), 79.7 (Boc), 82.7 (Boc), 155.8 (N$\underline{C}$(X)X), 156.8 (N$\underline{C}$(X)X), 158.8 (N$\underline{C}$(X)X), 176.9 ($\underline{C}$=O) ppm; CI-MS (NH_3) *m/z* (%): 317 (100) $[MH]^+$, 261 (11) $[MH - C_4H_8]^+$, 217 (16) $[MH - Boc]^+$. $C_{13}H_{24}N_4O_5$ (316.35).

Similarly was prepared **1-*tert*-butoxycarbonyl-2-[3-(*tert*-butoxycarbonylamino)-propanoyl]guanidine** from Boc-β-Ala-OSu in 79 % yield; ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.43 (s, 9H, Boc), 1.47 (s, 9H, Boc), 2.61 (m, 2H, -C$\underline{H}_2CH_2$NHBoc), 3.42 (m, 2H, -C$\underline{H}_2$NHBoc) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 28.1 (Boc), 28.4 (Boc), 35.9 (-$\underline{C}H_2CH_2$NHBoc), 38.1 (-$\underline{C}H_2$NHBoc), 79.4 (Boc), 80.7 (Boc), 155.8 (N$\underline{C}$(X)X), 159.0 (N$\underline{C}$(X)X), 160.2 (N$\underline{C}$(X)X), 178.1 ($\underline{C}$=O) ppm; CI-MS (NH_3) *m/z* (%): 331 (100) $[MH]^+$, 275 (15) $[MH - C_4H_8]^+$, 231 (8) $[MH - Boc]^+$. $C_{14}H_{26}N_4O_5$ (330.38).

1-*tert*-Butoxycarbonyl-2-[4-(*tert*-butoxycarbonylamino)butanoyl]guanidine – To a stirred solution of 4-(*tert*-butoxycarbonylamino)butanoic acid (1.02 g, 5.0 mmol) in dry DMF (5 mL) was added CDI (0.81 g, 5.0 mmol) under exclusion of moisture.

After 45 min 1-*tert*-butoxycarbonylguanidine (0.80 g, 5.0 mmol) was added and stirring was continued for 16 h. The resulting mixture was diluted with ethyl acetate (60 mL) and washed with water 5 % aq. $KHSO_4$, 5 % aq. $KHCO_3$, and brine. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. The product was purified by flash chromatography eluting with $CHCl_3$/ethyl acetate. Yield: 0.30 g (44 %); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.43 (s, 9H, Boc), 1.49 (s, 9H, Boc), 1.86 (m, 2H, -CH_2C$\underline{H}_2$$CH_2$NHBoc), 2.49 (t, ^3J = 7.2 Hz, 2H, -C$\underline{H}_2$$CH_2$$CH_2$NHBoc), 3.18 (m, 2H, -$CH_2$$CH_2C\underline{H}_2$NHBoc) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 25.1 (-CH_2C$\underline{H}_2$$CH_2$NHBoc), 28.1 (Boc), 28.4 (Boc), 35.0 (-C$\underline{H}_2$$(CH_2)_2$NHBoc), 39.6 (-$(CH_2)_2C\underline{H}_2$NHBoc), 79.3 (Boc), 81.9 (Boc), 156.0 (NC(X)X), 157.8 (NC(X)X), 163.9 (NC(X)X), 177.4 (C=O) ppm; ESI-MS (+p) *m/z* (%): 345 (100) $[MH]^+$, 289 (37) $[MH - C_4H_8]^+$, 245 (15) $[MH - Boc]^+$. $C_{15}H_{28}N_4O_5$ (344.41).

In similar manner were prepared:

1-*tert*-Butoxycarbonyl-2-[5-(*tert*-butoxycarbonylamino)pentanoyl]guanidine in 36 % yield; ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.44 (s, 9H, Boc), 1.48 (s, 9H, Boc), 1.50–1.60 (m, 2H, -$(CH_2)_2$C$\underline{H}_2$$CH_2$NHBoc), 1.62–1.72 (m, 2H, -CH_2C$\underline{H}_2$$(CH_2)_2$-NHBoc), 2.46 (t, ^3J = 7.3 Hz, 2H, -C$\underline{H}_2$$(CH_2)_3$NHBoc), 3.13 (m, 2H, -$(CH_2)_3C\underline{H}_2$-NHBoc) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 21.9 (-CH_2C$\underline{H}_2$$(CH_2)_2$NHBoc), 28.2 (Boc), 28.4 (Boc), 29.4 (-$(CH_2)_2$C$\underline{H}_2$$CH_2$NHBoc), 37.3 (-C$\underline{H}_2$$(CH_2)_3$NHBoc), 40.1 (-$(CH_2)_3C\underline{H}_2$NHBoc), 79.2 (Boc), 80.0 (Boc), 156.0 (NC(X)X), 158.8 (NC(X)X), 161.7 (NC(X)X), 177.3 (C=O) ppm; ESI-MS (+p) *m/z* (%): 718 (12) $[2M + H]^+$, 359 (100) $[MH]^+$. $C_{16}H_{30}N_4O_5$ (358.43)

1-*tert*-Butoxycarbonyl-2-[6-(*tert*-butoxycarbonylamino)hexanoyl]guanidine in 77 % yield; ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.35 (m, 2H, -C$\underline{H}_2$$(CH_2)_2$NHBoc), 1.44 (s, 9H, Boc), 1.47 (s, 9H, Boc), 1.50 (m, 2H, -C$\underline{H}_2$$CH_2$NHBoc) 1.66 (m, 2H, -C$\underline{H}_2$$CH_2$C(O)-), 2.38 (t, ^3J = 7.3 Hz, 2H, -C$\underline{H}_2$C(O)-), 3.10 (-C$\underline{H}_2$NHBoc) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 24.4 (-C$\underline{H}_2$-), 26.2 (-C$\underline{H}_2$-), 28.2 (Boc), 28.4 (Boc), 29.8 (-CH_2-), 37.4 (-C$\underline{H}_2$C(O)-), 40.3 (-C$\underline{H}_2$NHBoc), 79.1 (Boc), 79.7 (Boc), 156.0

(N<u>C</u>(X)X), 159.1 (N<u>C</u>(X)X), 162.1 (N<u>C</u>(X)X), 177.3 (<u>C</u>=O) ppm; ESI-MS (+p) *m/z* (%): 373 (100) $[MH]^+$, 317 (10) $[MH - C_4H_8]^+$, 273 (15) $[MH - Boc]^+$. $C_{17}H_{32}N_4O_5$ (372.46).

1-*tert*-Butoxycarbonyl-2-[*trans*-4-(*tert*-butoxycarbonylaminomethyl)cyclohexanecarbonyl]guanidine in 84 % yield; ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 0.99 (m, 2H, 3/5-H ax. -C_6H_{10}-), 1.40 (m, 2H, 3/5-H eq. -C_6H_{10}-), 1.44 (s, 9H, Boc), 1.48 (s, 9H, Boc), 1.50 (m, 1H, 4-H -C_6H_{10}-), 1.86 (m, 2H, 2/6-H ax. -C_6H_{10}-), 1.97 (m, 2H, 2/6-H eq. -C_6H_{10}-), 2.23 (m, 1H, 1-H -C_6H_{10}-), 2.98 (m, 2H, -CH_2NHBoc) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 28.2 (Boc), 28.4 (Boc), 29.5 (C-2/6 -C_6H_{10}-), 29.8 (C-3/5 -C_6H_{10}-), 37.6 (C-4 -C_6H_{10}-), 46.3 (C-1 -C_6H_{10}-), 46.5 (-<u>C</u>H_2NHBoc), 79.2 (Boc), 79.9 (Boc), 156.1 (N<u>C</u>(X)X), 158.8 (N<u>C</u>(X)X), 162.0 (N<u>C</u>(X)X), 182.4 (<u>C</u>=O) ppm; ESI-MS (+p) *m/z* (%): 797 (13) $[2M + H]^+$, 399 (100) $[MH]^+$, 343 (5) $[MH - C_4H_8]^+$, 299 (5) $[MH - Boc]^+$. $C_{19}H_{34}N_4O_5$ (398.50)

1-*tert*-Butoxycarbonyl-2-[2-[2-(*tert*-butoxycarbonylamino)ethoxy]ethoxycarbonyl]guanidine – A solution of 2-[-2-(*tert*-butoxycarbonylamino)ethoxy]ethanol (1.03 g, 5.0 mmol) in anhydrous acetonitrile was treated with disuccinimidyl carbonate (1.92 g, 7.5 mmol) and triethylamine (2.1 mL, 15.0 mmol) under exclusion of moisture. After stirring overnight the solvents were removed under reduced pressure and the residue was re-dissolved in ethyl acetate. The solution was washed with 5 % aq. $KHCO_3$ and brine and dried over anhydrous Na_2SO_4. The volatiles were evaporated and the residual material was dried in vacuum. The crude product was dissolved in anhydrous DMF (10 mL) and 1-*tert*-butoxycarbonylguanidine was introduced. The mixture was stirred for 20 h at ambient temperature. Subsequently, ethyl acetate (50 mL) was added and the resulting solution was washed with water, 5 % aq. $KHCO_3$, 5 % aq. $KHSO_4$, and brine and dried over anhydrous Na_2SO_4. The volatiles were removed under reduced pressure and the crude product was submitted to flash chromatography (eluent: $CHCl_3$/ethyl acetate 1:1 v/v). Yield: 1.07 g (55 %); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H =1.44 (s, 9H, Boc), 1.51 (s, 9H, Boc), 3.32 (m, 2H, -C<u>H</u>$_2$NHBoc), 3.54 (m, 2H, -OC<u>H</u>$_2$CH_2OC(O)-), 3.69 (m, 2H,

-OCH$_2$CH$_2$NHBoc), 4.29 (m, 2H, -OCH$_2$CH$_2$OC(O)-) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 28.4 (Boc), 29.0 (Boc), 40.3 (-CH$_2$NHBoc), 60.4 (-CH$_2$OC(O)-), 68.7 (-OCH$_2$CH$_2$OC(O)-), 70.3 (-OCH$_2$CH$_2$NHBoc), 79.2 (Boc), 84.0 (Boc), 153.5 (NC(X)X), 156.0 (NC(X)X), 158.1 (NC(X)X), 160.4 (NC(X)X) ppm; ESI-MS (+p) *m/z* (%): 391 (100) $[MH]^+$, 291 (25) $[MH - Boc]^+$. $C_{16}H_{30}N_4O_7$ (390.43).

Similarly was prepared **1-*tert*-butoxycarbonyl-2-{[3-(*tert*-butoxycarbonylamino)-propyl]oxycarbonyl}guanidine** from 3-(*tert*-butoxycarbonylamino)propan-1-ol in 63 % yield: ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.44 (s, 9H, Boc), 1.51 (s, 9H, Boc), 1.88 (m, 2H, -CH$_2$CH$_2$NHBoc), 3.20 (m, 2H, -CH$_2$NHBoc), 4.19 (m, 2H, -OCH$_2$(CH$_2$)$_2$NHBoc) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 28.0 (Boc), 28.4 (Boc), 29.1 (-CH$_2$CH$_2$NHBoc), 37.2 (-CH$_2$NHBoc), 63.7 (-OCH$_2$(CH$_2$)$_2$NHBoc), 79.3 (Boc), 84.3 (Boc), 153.7 (NC(X)X), 156.0 (NC(X)X), 157.9 (NC(X)X) ppm; ESI-MS (+p) *m/z* (%): 743 (5) $[2M + Na]^+$, 721 (10) $[2M + H]^+$, 383 (15) $[MNa]^+$, 361 (100) $[MH]^+$, 261 (15) $[MH - Boc]^+$. $C_{15}H_{28}N_4O_6$ (360.41).

4.3. Preparation of stock solutions of 1-acyl substituted guanidines

10 μmol of the respective Boc-protected precursor were dissolved in 1 mL freshly distilled CH_2Cl_2 and treated with TFA (1 mL). After 2 h the volatiles were removed under reduced pressure and the residue was dried in vacuum. The product was redissolved in 1.0 mL acetonitrile (HPLC grade) and stored as $1.0 \cdot 10^{-2}$ M stock solution of the respective TFA salt at – 20 °C.

4.4. Kinetic measurements:

Two quartz cuvettes were filled with 2.5 mL buffer solution and a volume of 25 μL was taken from each cuvette; the withdrawn volume was replaced by acetonitrile (reference cell) or $1.0 \cdot 10^{-2}$ M stock solution of the respective acylguanidine TFA-salt. The cuvettes were capped and shaken rapidly in order to avoid fluctuations in concentration. Bubbles were eliminated and the cuvettes were placed into the optical path of the instrument. Absorption spectra in the range from 200 to 260 nm

were recorded in predefined time intervals. Cuvettes were thermostated at 25 °C throughout the whole measurement.

5. References

[1] Schug, K. A.; Lindner, W., Noncovalent binding between guanidinium and anionic groups: focus on biological- and synthetic-based arginine/guanidinium interactions with phosph[on]ate and sulf[on]ate residues. *Chem. Rev.* **2005**, 105, 67-114.

[2] Berlinck, R. G. S.; Kossuga, M. H., Natural guanidine derivatives. *Nat. Prod. Rep.* **2005**, 22, 516-50.

[3] Bogan, A. A.; Thorn, K. S., Anatomy of hot spots in protein interfaces. *J. Mol. Biol.* **1998**, 280, 1-9.

[4] Hartman, G. D.; Egbertson, M. S.; Halczenko, W.; Laswell, W. L.; Duggan, M. E.; Smith, R. L.; Naylor, A. M.; Manno, P. D.; Lynch, R. J.; Zhang, G.; et al., Non-peptide fibrinogen receptor antagonists. 1. Discovery and design of exosite inhibitors. *J. Med. Chem.* **1992**, 35, 4640-2.

[5] Lawson, C. E.; Kinney, W. A.; Costanzo, M. J.; Hoekstra, W. J.; Kauffman, J. A.; Luci, D. K.; Santulli, R.; Tounge, B. A.; Yabut, S. C.; Andrade-Gordon, P.; Maryanoff, B. E., Structure-Function Study of Quinazolinone-Based Vitronectin Receptor ($\alpha_v\beta_3$) Antagonists: Computer-Assisted Analysis of Ligand-Receptor Interactions. *Letters in Drug Design and Discovery* **2004**, 1, 14-8.

[6] Polla, M. O.; Tottie, L.; Norden, C.; Linschoten, M.; Musil, D.; Trumpp-Kallmeyer, S.; Aukrust, I. R.; Ringom, R.; Holm, K. H.; Neset, S. M.; Sandberg, M.; Thurmond, J.; Yu, P.; Hategan, G.; Anderson, H., Design and synthesis of potent, orally active, inhibitors of carboxypeptidase U (TAFIa). *Bioorg. Med. Chem.* **2004**, 12, 1151-75.

[7] Bream, J. B.; Lauener, H.; Picard, C. W.; Scholtisyk, G.; White, T. G., Substituted Phenylacetylguanidines: A new Class of Atihypertensive Agents. *Arzneim.-Forschung* **1975**, 25, 1477-82.

[8] Hutzler, C. Synthese und pharmakologische Aktivität neuer Neuropeptide Y Rezeptorliganden: Von *N,N*-disubstituierten Alkanamiden zu hochpotenten Y_1-Antagonisten der Argininamid-Reihe. Ph.D. Thesis, Universität Regensburg, **2001**.

[9] Schmuck, C., Carboxylate binding by 2-(guanidiniocarbonyl)pyrrole receptors in aqueous solvents: improving the binding properties of guanidinium cations through additional hydrogen bonds. *Chemistry* **2000**, 6, 709-18.

[10] Schmuck, C.; Bickert, V., *N'*-alkylated guanidiniocarbonyl pyrroles: new receptors for amino acid recognition in water. *Org. Lett.* **2003**, 5, 4579-81.

[11] Schmuck, C.; Geiger, L., Dimerization of a guanidiniocarbonyl pyrrole cation in DMSO that can be controlled by the counteranion. *Chem. Commun.* **2004**, 1698-9.

[12] Schmuck, C.; Geiger, L., Dipeptide binding in water by a de novo designed guanidiniocarbonylpyrrole receptor. *J. Am. Chem. Soc.* **2004**, 126, 8898-9.

[13] Schmuck, C.; Machon, U., Amino Acid Binding by 2-(Guanidiniocarbonyl)pyridines in Aqueous Solvents: A Comparative Binding Study Correlating Complex Stability with Stereoelectronic Factors. *Chemistry - A European Journal* **2005**, 11, 1109-18.

[14] Schmuck, C.; Rehm, T.; Grohn, F.; Klein, K.; Reinhold, F., Ion pair driven self-assembly of a flexible bis-zwitterion in polar solution: formation of discrete nanometer-sized cyclic dimers. *J. Am. Chem. Soc.* **2006**, 128, 1430-1.

[15] Schmuck, C.; Schwegmann, M., A naked-eye sensing ensemble for the selective detection of citrate—but not tartrate or malate—in water based on a tris-cationic receptor. *Org. Biomol. Chem.* **2006**, 4, 836-8.

[16] Schmuck, C.; Wienand, W., Highly stable self-assembly in water: ion pair driven dimerization of a guanidiniocarbonyl pyrrole carboxylate zwitterion. *J. Am. Chem. Soc.* **2003**, 125, 452-9.

[17] Adang, A. E. P.; Lucas, H.; de Man, A. P. A.; Engh, R. A.; Grootenhuis, P. D. J., Novel acylguanidine containing thrombin inhibitors with reduced basicity at the P_1 moiety. *Bioorg. Med. Chem. Lett.* **1998**, 8, 3603-8.

[18] Rudolf, K.; Eberlein, W.; Wieland, H. A.; Engel, W.; Willim, K. D.; Entzeroth, M.; Wienen, W.; Beck-Sickinger, A. G.; Doods, H., The first highly potent and selective non-peptide NPY Y_1 receptor antagonist: BIBP 3226. *Eur. J. Pharm.* **1994**, 271, R11-3.

[19] Brennauer, A.; Dove, S.; Buschauer, A., Structure-Activity Relationships of Nonpeptide Neuropeptide Y Receptor Antagonists. In *Neuropeptide Y and Related Peptides*, Michel, M. C., Ed. Springer: Berlin, Heidelberg, New York, 2004; Vol. 162, pp 505-46.

[20] Greenhill, J. V.; Ismail, J. M.; Edwards, P. N.; Taylor, P. J., Conformational and Tautomeric Studies of Acylguanidines. Part 1. Synthesis, Ultraviolet Spectroscopy, Tautomeric Preference, and Site of Protonation in Some Model compounds. *J. Chem. Soc. Perkin Trans. II* **1985**, 1255-74.

[21] Zapf, C. W.; Creighton, C. J.; Tomioka, M.; Goodman, M., A novel traceless resin-bound guanidinylating reagent for secondary amines to prepare *N,N*-disubstituted guanidines. *Org. Lett.* **2001**, 3, 1133-6.

[22] Matsumoto, K.; Rapoport, H., The Preparation and Properties of some Acylguanidines. *J. Org. Chem.* **1968**, 33, 552-8.

[23] Rink, H.; Sieber, P.; Raschdorf, F., Conversion of N^G-urethane protected arginine to ornithine in peptide solid phase synthesis. *Tetrahedron Lett.* **1984**, 25, 621-4.

[24] Takimoto, M., Ultraviolet absorption spectra and structure of cyanamide derivatives. *Nippon Kagaku Zasshi* **1964**, 85, 159-68.

[25] Feichtinger, K.; Zapf, C.; Sings, H. L.; Goodman, M., Diprotected Triflylguanidines: A New Class of Guanidinylation Reagents. *J. Org. Chem.* **1998**, 63, 3804-5.

[26] Norma G., D.; Eric M., G.; Miguel A., O. Mercaptoalkanamide Enkephalinase Inhibitors. EP0136883, **1985**.

[27] Donati, D.; Morelli, C.; Porcheddu, A.; Taddei, M., A New Polymer-Supported Reagent for the Synthesis of β-Lactams in Solution. *J. Org. Chem.* **2004**, 69, 9316-8.

[28] Fumo, G.; Neckers, L. Methods of reducing cellular activity or concn. of mutant kit protein involved in cancer using inhibitors of HSP90. WO 2004037978, **2004**.

[29] Svahn, C. M.; Merenyi, F.; Karlson, L.; Widlund, L.; Gralls, M., Tranexamic acid derivatives with enhanced absorption. *J. Med. Chem.* **1986**, 29, 448-53.

6 Towards the Development of NPY Y_1-Receptor Selective Tracers

The first highly potent and selective non-peptidic neuropeptide Y Y_1 receptor antagonist, the D-argininamide derivative BIBP 3226 is inactive after oral administration and does not penetrate the blood brain barrier. Recently we reduced the basicity of the arginine side chain in BIBP 3226 by introduction of electron withdrawing substituents at the guanidino nitrogen atom. Surprisingly, some N^G-acylated argininamides proved to be even more potent Y_1R antagonists than the unsubstituted parent compound. Based on this concept we prepared N^G-(ω-aminoalkanoyl) substituted analogs as precursors for the preparation of fluorescence or radiolabeled (^{3}H, ^{125}I, ^{18}F) ligands for the investigation of Y_1 receptors *in vivo* and *in vitro*, respectively.

Key step for the preparation of the N^G-acylated argininamides was the guanidinylation of the respective ornithine precursor with *N*-acyl-*N'*-*tert*-butoxycarbonyl-*S*-methylisothioureas in the presence of one equivalent of $HgCl_2$.

Y_1R affinity, chemical resistance and physicochemical properties were influenced by the chemical nature of the linker. In this context, the 6-aminohexanoyl spacer turned out to be the optimal choice. Preliminary results with ^{18}F- or fluorescence labeled N^G-(ω-aminoalkanoyl) substituted analogs of BIBP 3226 confirm that this class of compounds serves as valuable precursors for the development of Y_1-selective tracers.

1. Introduction

Fluorescence- or radiolabeled receptor ligands are valuable pharmacological tools for the *in vitro* and/or *in vivo* characterization of their biological targets. Their application includes binding assays, investigations on the localization and

distribution of receptors in tissues (e.g. imaging techniques such as PET, autoradiography, or optical imaging using a CCD camera), cell biology of receptor proteins (e.g. fluorescence microscopy, investigations on internalization or oligomerization), and pharmacokinetic studies (ADMET). Beside their use as pharmacological tools, radiolabeled receptor selective compounds can also be used for diagnostic and therapeutic purposes.

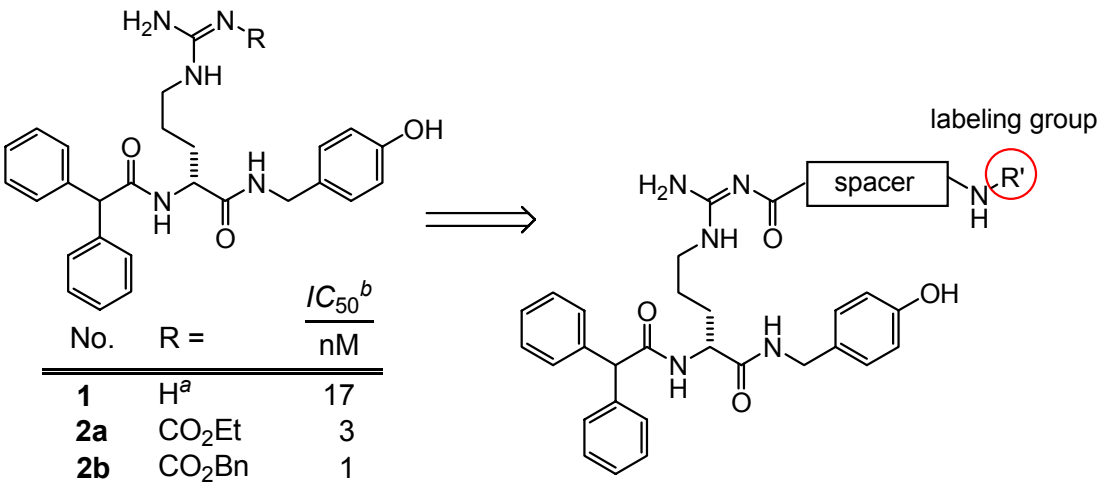
So far, mainly agonistic peptides have been used for the preparation of radio[1]- or fluorescence[2, 3]-labeled NPY receptor ligands. However, peptidic ligands bear the risk of being modified by the action of peptide recognizing enzymes in biological systems. For instance, full length NPY is cleaved by the action of dipeptidyl peptidase IV; the resulting fragment NPY(3-36) is still a full agonist at the Y_2 receptor, but substantially less active at the Y_1 receptor[4, 5]. Moreover, peptides often have a very low bioavailability and are generally unable to cross the blood-brain barrier.

In case of agonistic ligands the possibility of desensitization and receptor internalization has to be taken into account. Labeled compounds which are internalized together with the receptor can no longer be replaced by other competitive ligands, unless they are able to diffuse freely through cell membranes. The fraction of labeled ligands trapped in the cell cannot be displaced. In ligand binding assays this worsens the signal to noise ratio. On the other hand, for imaging studies internalization of receptor-tracer complexes is desirable, since the target-selective enrichment of labeled compounds in intracellular compartments leads to a more stable and durable signal.

The Y_1R selective ligand [^{3}H]BIBP 3226 was the only labeled non-peptide NPY antagonist described in literature so far[6]. However, BIBP 3226 (**1**) has unfavorable physicochemical properties and is not centrally available due to the strongly basic guanidino group, which is protonated at physiological pH.

As described earlier we found that N^G-acylated analogs of BIBP 3226 are remarkably less basic but show comparable or even enhanced receptor affinity[7] (cf. chapter 2). These findings prompted us to synthesize analogs bearing a N^G-(ω-

aminoalkanoyl) linker allowing for the introduction of fluorescence- or radiolabeled (^{3}H, ^{125}I, ^{18}F) electrophiles at the terminal amino group (cf. Scheme 1).



No.	R =	IC_{50}[b] nM
1	H[a]	17
2a	CO_2Et	3
2b	CO_2Bn	1
2c	COEt	1

[a] BIBP 3226
[b] for inhibition of NPY induced $[Ca^{2+}]_i$ releasein HEL cells

Scheme 1: Design of model compounds for potential Y_1 antagonistic tracers derived from N^G-acylated analogs of BIBP 3226 (**1a**).

As the "cold" form of potential [^{18}F]-labeled PET-ligands we prepared 4-fluorobenzoylated analogs comprising different linker groups. In addition to a high receptor affinity *in vivo* applicable PET-ligands must have suitable physicochemical properties — especially when imaging of the CNS is intended. These properties all depend on the choice of the spacer group.

Carefully optimized Y_1-selective PET-ligands will valuably contribute to the elucidation of NPY receptor distribution in the brain and other tissues.

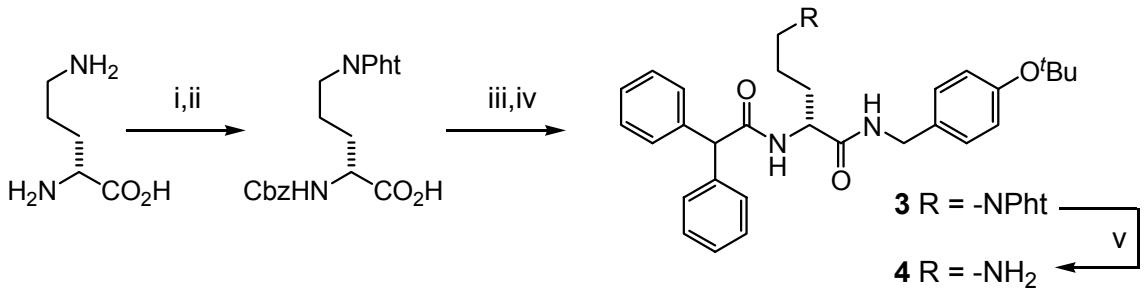
Recently, overexpression of Y_1 receptors in breast carcinomas and other tumors was described[8-10]. Therefore, Y_1 receptor selective tracers are also of interest as potential diagnostic tools.

2. Results and Discussion

2.1. Synthetic Chemistry

2.1.1. SYNTHESIS OF CENTRAL ORNITHINE BUILDING BLOCK

The N^G-substituted D-argininamides were prepared by guanidinylation of the parent D-ornithinamide **4** (cf. Scheme 2). For the preparation of **4**, D-ornithine, was orthogonally protected as Z-D-Orn(Pht)-OH (via Z-D-Orn(Boc)-OH) and coupled with 4-*tert*-butoxybenzylamine using DCC/HOBt.

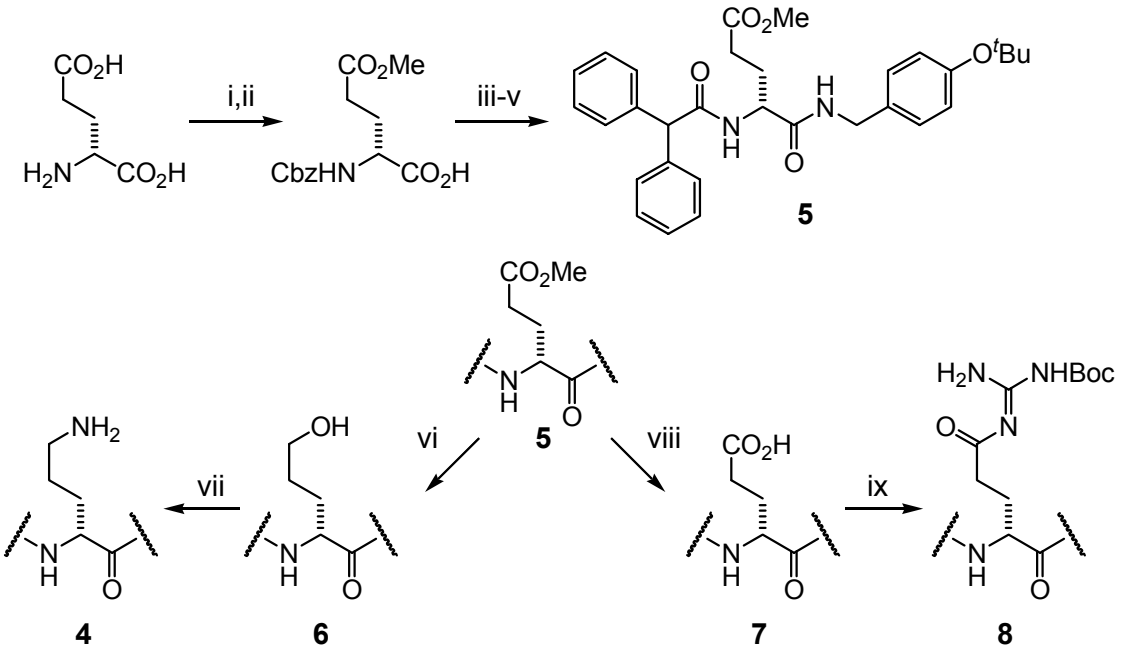


Scheme 2: Preparation of ornithinamide **4**. Reagents and conditions: i: 1. $Cu(OAc)_2$, NaOH, Boc_2O, acetone/water, 2. 8-quinolinol, Na_2CO_3, CbzOSu; ii: 1. HCl/ethyl acetate, 2. $NaHCO_3$, EtOC(O)NPht, 1,4-dioxane/water; iii: DCC/HOBt, 4-*tert*-butoxybenzylamine, CH_2Cl_2; iv: 1. Pd/C, HCO_2K, MeOH, 2. Ph_2CHCO_2Su; v: 1. N_2H_5OH, MeOH/THF, 2. H^+.

Removal of the Cbz group by catalytic hydrogenolysis and acylation with succinimidyl diphenylacetate afforded the N^δ-phthaloyl protected ornithine derivative **3**. Dephthaloylation was achieved by hydrazinolysis using hydrazine hydrate and subsequent treatment with aq. $KHSO_4$.

4-*tert*-Butoxybenzylamine was obtained from 4-Hydroxybenzonitril *via* etherification with isobutene or TBTA[11] and reduction with $LiAlH_4$.

As novel variant of the N^G-acylarginine motif we synthesized the 2-amino-5-guanidino-5-oxopentanamide analog **13**, starting from γ-methyl D-glutamate (cf. Scheme 3). Coupling of Z-D-Glu(OMe)-OH with 4-*tert*-butoxybenzylamine, hydrogenolytic deprotection, and acylation with activated diphenylacetic acid led to the isoglutamine ester **5**, which was saponified giving the free acid **7**.



Scheme 3: Isoglutamine derivative **3** as building block for the preparation of BIBP 3226 analogs. Reagents and conditions: i: TMS-Cl, MeOH; ii: Cbz-OSu, K_2CO_3, acetone/H_2O; iii: 1.CDI, DMF; 2. 4-*tert*-butoxybenzylamine; iv: HCO_2NH_4, Pd/C, MeOH; v: succinimidyl diphenylacetate, THF; vi: $NaBH_4$, LiCl, THF/EtOH; vii: 1. Phthalimide, DIAD, PBu_3, 2. N_2H_5OH, MeOH/THF; viii: KOH, EtOH/H_2O; ix: EDC/HOBt, 1-Boc-guanidine, DMF.

The carboxylate group of **7** was activated with EDC/HOBt and coupled with 1-*tert*-butoxycarbonylguanidine. Final TFA-mediated cleavage of the *tert*-butyl and the Boc protective group in **8** yielded (*R*)-2-(2,2-diphenylacetamido-5-guanidino-*N*-(4-hydroxybenzyl)-5-oxopentanamide.

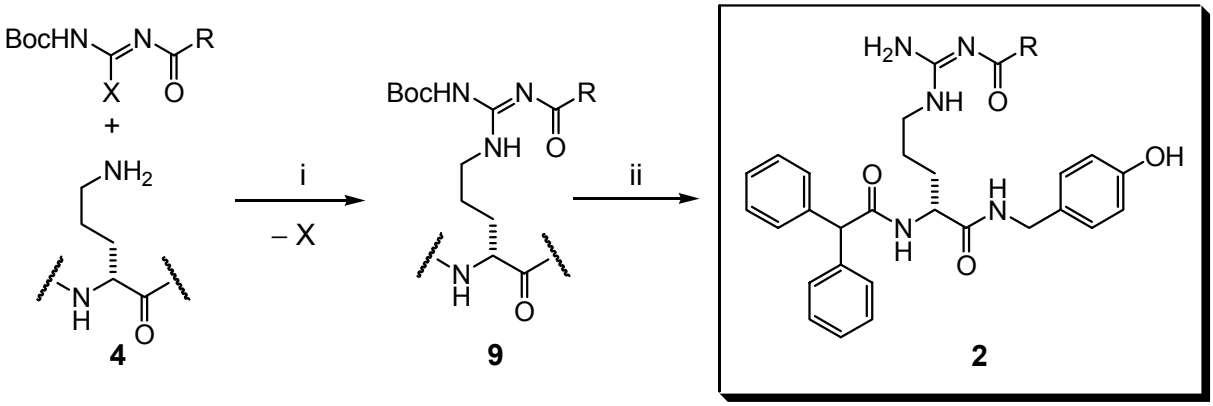
Moreover, ester **5** can be "redirected" to the ornithinamide route by reduction to the respective alcohol **6** using $NaBH_4$/LiCl followed by reaction with phthalimide under Mitsunobu conditions to give the phthaloyl protected ornithine building block **3**.

2.1.2. GUANIDINYLATION

For the preparation of N^G-substituted argininamides the ornithinamide **4** was treated with the pertinent substituted guanidinylation reagents (Scheme 4).

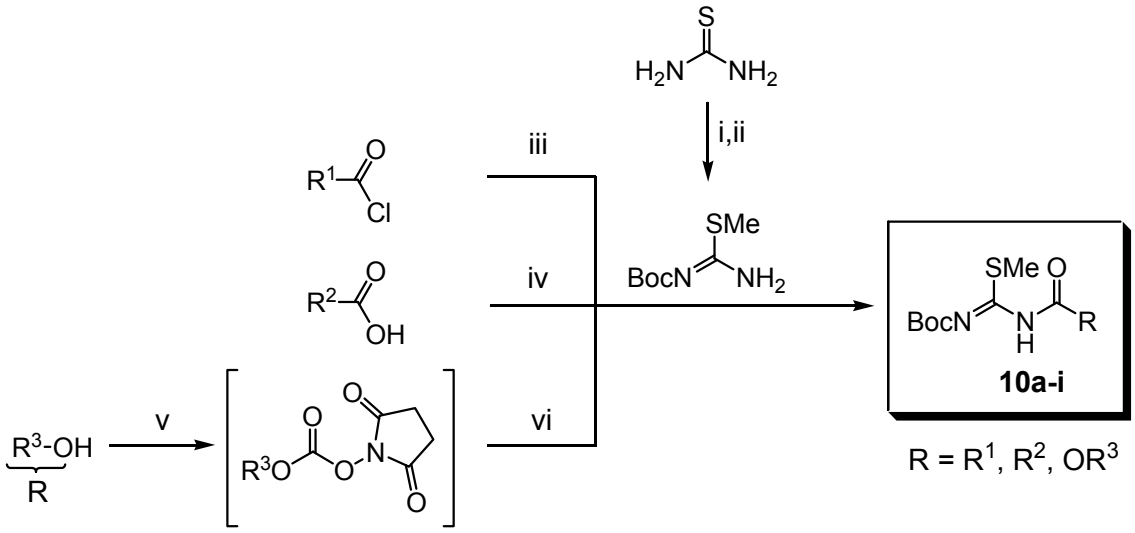
Subsequent deprotection with TFA afforded the targeted N^G-substituted BIBP 3226 analogs (**2**).

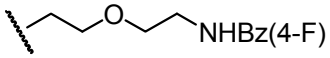
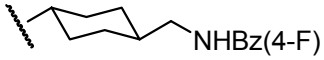
Guanidinylation with *N'*-substituted *N*-*tert*-butoxycarbonyl-*S*-methylisothioureas (method **A**) in the presence of $HgCl_2$[12] turned out to be the most convenient method for the synthesis of alkanoyl and alkoxycarbonyl substituted argininamides. Alternatively, we also guanidinylated **4** with substituted 1*H*-pyrazole-1-carboxamidines (method **B**)[13] and triflylguanidines (method **C**)[14, 15].



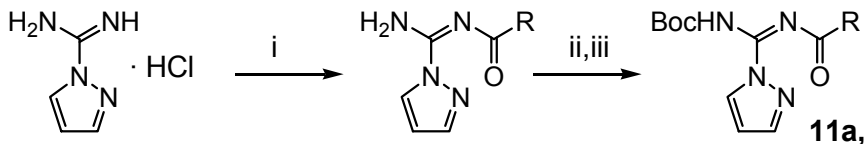
Scheme 4: Gyanidinylation of D-ornithinamide building block **4** and release of N^G-acylated BIBP 3226 analogs by deprotection. i: Method **A**: X = -SMe, $HgCl_2$, NEt_3, DMF, method **B**: X = 1*H*-pyrazole-1-yl, MeCN, method **C**: -NHTf, NEt_3, CH_2Cl_2; ii: TFA/CH_2Cl_2 1:1 (v/v).

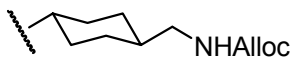
For the preparation of the guanidinylation reagents, the respective ω-amino acids or ω-amino alcohols were Boc-protected or 4-fluorobenzoylated and coupled to *N*-*tert*-butoxycarbonyl-*S*-methylisothiourea (cf. Scheme 5) or 1*H*-pyrazole-1-carboxamidine hydrochloride.



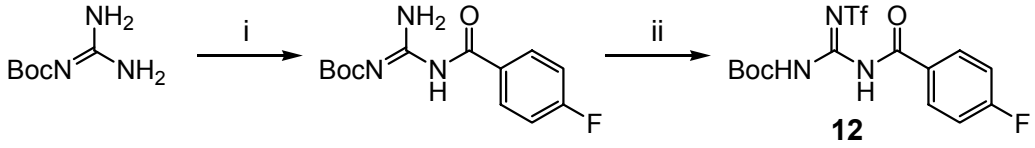
No.	R^1 =	No.	R^2 =	No.	R^3 =
10a	$-(CH_2)_2Ph$	**10b**	$-(CH_2)_4NHBoc$	**10g**	$-(CH_2)_3NHBoc$
		10c	$-(CH_2)_5NHBoc$	**10h**	$-(CH_2)_3NHBz(4\text{-}F)$
		10d	$-(CH_2)_4NHBz(4\text{-}F)$	**10i**	
		10e	$-(CH_2)_5NHBz(4\text{-}F)$		
		10f			

Scheme 5: Preparation of *N*-acyl-*N'*-*tert*-butoxycarbonyl-*S*-methylisothioureas **10a-i**. Reagents and conditions: i: CH_3I, EtOH, 1h reflux; ii: Boc_2O (0.8 eq.), NEt_3, CH_2Cl_2, 0 °C → r.t.; iii: DIPEA, CH_2Cl_2; iv: EDC/HOBt, TBTU/HOBt, or CDI; v: Disuccinimidyl carbonate[16], NEt_3, MeCN; vi: NEt_3, CH_2Cl_2.



No.	R =
11a	$-(CH_2)_4NPht$
11b	

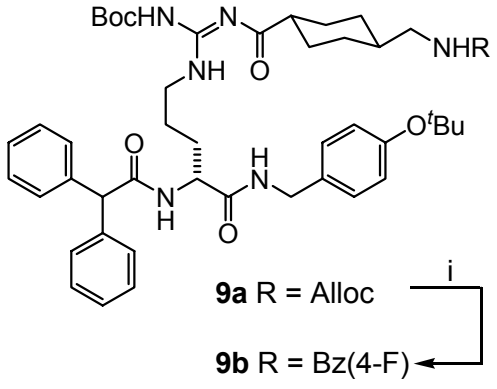
Scheme 6: Synthesis of the *N*-acyl-*N'*-*tert*-butoxycarbonyl-1*H*-pyrazole-1-carboxamidines **11a** and **b**. Reagents and conditions: i: RCO_2H, CDI, DIPEA, CH_2Cl_2; ii: Boc_2O (2 eq.), DMAP (10 mol-%), CH_2Cl_2; iii: $Mg(ClO_4)_2$, THF, 50 °C;



Scheme 7: Activation of *N*-*tert*-butoxycarbonyl-*N*′-(4-fluorobenzoyl)guanidine as triflyl guanidine **12**. Reagents and conditions: i: 4-fluorobenzoic acid, CDI, DMF; iii: Tf_2O, NEt_3, CH_2Cl_2, – 78 °C.

The *N*-monoacylated 1*H*-pyrazole-1-carboxamidines were activated by introduction of an additional Boc group in *N*′-position (cf. Scheme 6).

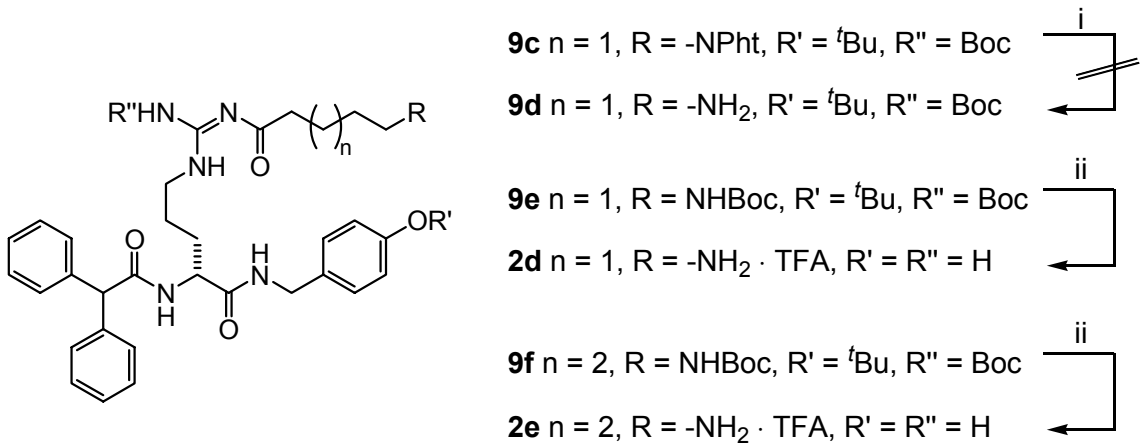
N-*tert*-Butoxycarbonyl-*N*′-(4-fluorobenzoyl)guanidine was converted into the triflyl guanidine and used for the guanidinylation of **4** without isolation (cf. Scheme 7).



Scheme 8: In situ[17] acylation of Alloc protected N^G-(ω-amino)acylated argininamide (**7a**). Reagents and conditions: i: $Pd(PPh_3)_4$ (cat.), DABCO, succinimidyl 4-fluorobenzoate, CH_2Cl_2.

Beside the N^G-[ω-(4-fluorobenzamido)-alkanoyl] substituted argininamides, which are model compounds for potential PET-ligands, we wanted to prepare ω-aminoalkanoyl substituted analogs, having a free terminal amino group, where any arbitrary (electrophilic) labeling group can be attached. For instance, **9a** was Alloc-deprotected in the presence of succinimidyl 4-fluorobenzoate, yielding the 4-fluorobenzoylated compound **9b** (cf. Scheme 8).

In contrast, dephthaloylation of compound **9c** by hydrazinolysis did not yield the desired compound **9d** but the respective N^G-unsubstituted argininamide (cf. Scheme 9).



Scheme 9: Preparation of N^G-(ω-aminoalkanoyl) substituted argininamides. Reagents and conditions: i: 1. N_2H_5OH, 2. H^+, H_2O; ii: TFA/CH_2Cl_2 1:1 (v/v).

It turned out that 5-aminopentanoyl substituted guanidines are rapidly cleaved under basic conditions by intramolecular δ-lactam formation (see chapter 5). The TFA salt of 5-aminopentanoyl compound **2d** could be obtained by treatment of **9e** with 50 % TFA in CH_2Cl_2. However, derivatization of the amino group in **2d** was impractical, since the conversion of the ammonium group to the free amine by addition of base, instantaneously induced the degradation reaction. Surprisingly, the higher homolog **2e** exhibited a markedly reduced tendency to decompose and was successfully acylated. N^G-(ω-Aminoalkoxycarbonyl) substituted argininamides were also sufficiently stable but they were not further developed due to their slightly lower Y_1R antagonistic potency.

2.2. NPY Y_1 Receptor Antagonistic Activity

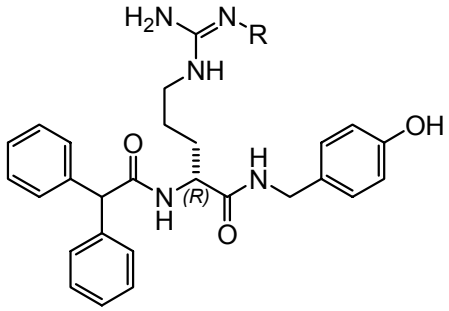
High receptor affinity of labeled ligands is a prerequisite for their applicability as tracers. HEL cells, expressing the Y_1 receptor, are a well established pharmacological model for the investigation of Y_1R ligands. Addition of NPY leads to a Y_1R mediated mobilization of calcium ions from intracellular stores[18]. The agonist-induced increase in intracellular Ca^{2+} concentration can be monitored by fluorimetric

techniques using Ca^{2+} sensitive fluorescent dyes. Thus, HEL cells suit for ligand binding as well as for functional assays.

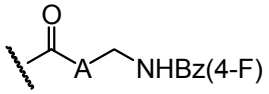
In order to assess the suitability of our model compounds as Y_1R-binding PET-ligands, we determined their Y_1 antagonistic activity in a spectrofluorimetric Ca^{2+} assay (using fura-2) as a (indirect) measure for their receptor affinity. Maximum Ca^{2+} response was provoked by addition of 10 nM pNPY. For the calculation of IC_{50} values 2-3 antagonist concentrations (B), which reduce the Ca^{2+} signal (P) to 20-80 % of the maximum response, were used in each assay. Linear plot of logit(P) versus log(B) gives log(IC_{50}) as the concentration (in log units) where the regression line intersects the abscissa (logit(P) = 0). At least three independent experiments with an SEM (standard error of the mean) < 10 % were carried out for each antagonist.

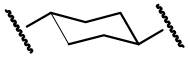
To our surprise the observed antagonistic activities of the N^G-[ω-(4-fluorobenzamido)-alkyl] substituted argininamides show a pronounced dependence on the structure of the incorporated spacer (cf. Table 1). Based on the crystal structure of bovine rhodopsin and receptor mutagenesis results, computer models for the binding mode of BIBP 3226 and related compounds have been developed (cf. chapter 2). The guanidino moiety of the ligand is suggested to interact with a highly conserved Asp residue in the upper

Table 1: Y_1-Antagonistic Activity of some N^G-substituted (*R*)-argininamides.



No.	R =	IC_{50} [a] / nM
1	H	17
2f	-C(O)CH_2CH_2Ph	0.4
2g	Bz(4-F)	90



	A =	
2h	-$(CH_2)_3$-	23
2i	-$(CH_2)_4$	30
2j		200
2k	-O$(CH_2)_2$-	100
2l	-O$(CH_2)_2$O$(CH_2)_2$-	75

[a] for inhibition of NPY induced $[Ca^{2+}]_i$ release in HEL cells.

part of TM6, close to the extracellular domains. Therefore, we hypothesized that spacer and labeling group are arranged outside the binding pocket and that their implication in receptor binding is only secondary. By virtual ligand docking using our Y_1R model a 5-aminopentanoyl linker was proposed[19]. Indeed, the respective 4-fluorobenzoyl substituted derivative **2h** proved to be almost as potent as BIBP 3226. Moreover, as demonstrated in our lab, **2h** is able to penetrate across the blood brain barrier[20].

However, 5-aminopentanoyl substituted guanidines turned out to be very labile, due to rapid intramolecular aminolysis resulting in the formation of δ-lactam (cf. chapter 5). Therefore, the 5-aminopentanol spacer was inappropriate for the preparation of labeled ligands.

In order to prevent intramolecular lactamization we synthesized compound **2j**, bearing a cycloalkyl spacer. But, presumably due to the bulkiness of the cyclohexyl moiety, the potency of **2j** was reduced by a factor of 10 compared to analog **2h**. Furthermore, too lipophilic tracers bear the risk of high plasma protein binding levels and hamper the recovery of the analyte from biological samples. Hence, we incorporated additional heteroatoms in the spacer, in order to lower the lipophilicity. Unfortunately, the respective compound **2l** was not sufficiently potent.

As the alkoxycarbonylguanidines turned out to be considerably more resistant than the corresponding alkanoylguanidines, we prepared analog **2k** which comprises the same number of heavy atoms within the spacer chain as the potent analog **2h**. But again the antagonistic activity of **2k** was not sufficient.

Investigations on the degradation rates of acylguanidines showed that the 6-(aminohexanoyl)guanidine is markedly more stable than the lower homolog (cf. chapter 5). Thus, compound **2i** combines chemical stability with acceptable antagonistic activity (about equipotent to BIBP 3226). In fact, preliminary PET-studies with ^{18}F labeled N^G-(6-aminohexanoyl) substituted BIBP 3226 showed promise[21]. In addition, first fluorescence labeled Y_1 antagonists have been prepared from N^G-(ω-aminoalkanoyl) substituted analogs[3].

In general, the alkanoyl substituted (*R*)-argininamides are more potent than the respective alkoxycarbonyl substituted analogs with the same number of heavy atoms. For instance the 3-phenylpropionyl substituted analog **2f** is twice as potent as the benzyloxycarbonyl substituted analog **2b** and forty-times more potent than the parent compound BIBP 3226 (**1**) in our assay. The high antagonistic activity of **2f** gives rise to the consideration to synthesize the [^{125}I]-labeled 3-(4-hydroxy-3-iodophenyl)propionyl (Bolton-Hunter) modified analog as a potential high affinity Y_1-selective radioligand.

On the basis of the high Y_1R affinity of N^G-acylated BIBP 3226 analogs, novel strategies for the preparation of Y_1R selective radioligands by direct N^G-acylation without spacer were developed in our group.

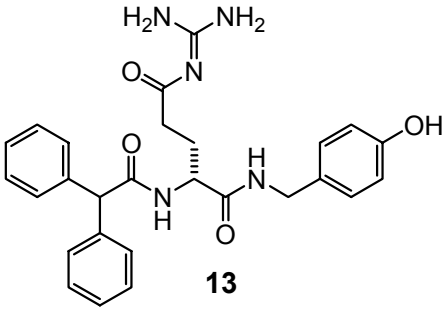


Fig. 1: Structure of guanidino-carbonyl derivative **13**.

In such manner, the highly potent [^{3}H]-labeled Y_1-antagonist N^G-[^{3}H]-propionyl BIBP 3226 was prepared (cf. **2c**)[21]. In contrast, the directly N^G-(4-fluorobenzoyl) substituted analog **2g** was not sufficiently active.

A tremendous loss in activity was observed for (*R*)-2-(2,2-diphenylacetamido-5-guanidino-*N*-(4-hydroxybenzyl)-5-oxopentanamide (**13**, cf. Fig. 1, IC_{50} = 2200 nM), which comprises a "wrong" orientation of the acylguanidine motif.

3. Conclusion

N^G-(ω-Aminoalkanoyl) substituted BIBP 3226 analogs are promising precursors for the preparation of radio- and fluorescence labeled Y_1 receptor selective antagonists. However, the choice of the linker group is critical with respect to chemical stability, physicochemical properties, receptor affinity, and pharmacokinetics of the tracer. Within the series of synthesized compounds, the 6-aminohexanoyl substituted

analog exhibited the most promising properties. This building block allowed for the preparation the first non-peptidic Y_1 receptor selective tracers.

4. Experimental section

4.1. Chemistry

4.1.1. GENERAL CONDITIONS

Chemicals were purchased from the following suppliers: Acros Organics (Geel, Belgium), Advanced ChemTech, Inc. (Hatley St George, UK), Alfa Aesar GmbH & Co. KG (Karlsruhe, Germany), Bachem AG (Bubendorf, Switzerland), IRIS Biotech GmbH (Marktredwitz, Germany), Mallinckrodt Baker (Deventer, NL), Merck KGaA and Merck Biosciences GmbH* (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); solvents were dried by refluxing over sodium hydride† (diethyl ether, DME, 1,4-dioxane, MTBE, THF, and toluene) or phosphorous(V) oxide (acetonitrile, chloroform, dichloromethane, 1,2-dichloroethane). Dried solvents were stored over micro sieves (4 Å) under protective gas. DMF (H_2O < 0.01 %) was purchased from Sigma-Aldrich Chemie GmbH. Triethyl amine and DIPEA were distilled over calcium hydride.

Nuclear Magnetic Resonance spectra were recorded on an Avance-300, Avance-400, or Avance-600 NMR spectrometer from Bruker BioSpin GmbH (Rheinstetten, Germany). Tetramethylsilane was added as internal standard (chemical shift $\delta = 0$ ppm) to all samples.

---

* formerly Novabiochem

† until positive sodium/benzophenone reaction.

Multiplicities are specified with the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (for broad signal), as well as combinations thereof. In certain cases 2D-NMR techniques (HSQC, HMQC, HMBC, COSY) were used to assign ^{1}H and ^{13}C chemical shifts.

Mass spectrometry analysis (MS) was performed on a Finnigan MAT 95 (PI-EIMS 70 eV, HR-MS), a Finnigan SSQ 710A (PI-EIMS 70 eV, CI-MS (NH_3)) and on a Finnigan ThermoQuest TSQ 7000 (ESI-MS, APCI-MS) spectrometer. Infrared spectra (IR) were recorded on a BRUKER TENSOR 27 spectrometer equipped with an ATR (attenuated total reflexion) unit from Harrick Scientific Products Inc. (Ossining/NY, US). UV-spectroscopy was performed using a Varian Cary 100 Conc UV/Vis spectrophotometer. Melting points (mp) were measured on a BÜCHI 530 or on an electrically heated copper block apparatus from Pefra (Germany) using open capillaries and are uncorrected.

Merck Silica Gel 60 (particle size 0.040–0.063 mm) was used for flash column chromatography; vacuum flash chromatography was carried out using Merck silica gel 60 H (particle size 90 % < 0.045 mm). Elemental analyses were carried out in the department of microanalysis, Regensburg. Compounds were dried in vacuo (0.1–1Torr) at room temperature or with heating up to 50 °C for at least 24 h prior to submission for elemental analysis. 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, ninhydrin spray, or ammonium molybdate/cerium(IV) sulfate solution.

Analytical reversed phase HPLC (RP-HPLC) was performed on a system from Thermo Separation Products (Egelsbach, Germany) equipped with a SN 400 controller, a P4000 pump, an AS3000 auto sampler and a Spectra Focus UV-Vis detector. As stationary phase a Nucleodur 100-5 C-18 (250 × 4.0 mm, 5 μm, flow rate: 0.8 ml · min^{-1}, Macherey-Nagel, Düren, Germany), a Luna C-18 (150 × 4.6 mm, 3 μm, flow rate: 0.7 ml · min^{-1}, Phenomenex, Aschaffenburg, Germany) or an Eurosphere-100 C-18 (250 × 4.0 mm, 5 μm, flow rate: 0.7 ml · min^{-1}, Knauer,

Berlin, Germany) column was used. As mobile phase mixtures of acetonitrile and 0.05 % aqueous trifluoroacetic acid (TFA) at a flow rate of 0.7–0.8 ml · min^{-1} were used. Absorbance was detected at 210 and 254 nm.

Relative molecular weights (in Da) are given in parentheses behind the formula.

4.1.2. *PREPARATION OF 4-(TERT-BUTOXY)BENZYLAMINE*

4-*tert*-Butoxybenzonitrile From 4-hydroxybenzonitrile and TBTA[11]. 1. *tert*-butyl 2,2,2-trichloroacetimidate (TBTA) – To a stirred solution of trichloroacetonitrile (72.20 g, 0.5 mmol) in anhydrous diethyl ether (100 mL), chilled in an ice-bath, was slowly added a solution of potassium *tert*-butoxide (6.10 g, 50.0 mmol) in *tert*-butanol (100 mL) and anhydrous diethyl ether (100 mL) under an inert atmosphere (N_2). The cooling bath was removed and stirring was continued for 1 h. Subsequently, the mixture was heated to reflux for an additional h. After cooling to ambient temperature, volatiles were evaporated and the residual oil was diluted with n-pentane (40 mL). Solid by-products, which had precipitated, were removed by filtration, and the filtrate was concentrated. The pure TBTA (74.2 g, 68 %) was obtained by distillation under reduced pressure (bp 72–75 °C at 18–20 mm Hg) as colorless liquid, which solidifies in a refrigerator and has a characteristic odor. ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.55 (s, 9H, -C(C$\underline{H}_3$)$_3$), 8.22 (s, 1H, -N$\underline{H}$) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 27.3 (-C(C$\underline{H}_3$)$_3$), 84.0 (-$\underline{C}$(CH$_3$)$_3$), 93.0 (-$\underline{C}$Cl$_3$), 160.6 (-$\underline{C}$(NH)O^{t}Bu) ppm.

4-Hydroxybenzonitrile (5.96 g, 50.0 mmol) was dissolved in anhydrous diethyl ether (50 mL) and cyclohexane (100 mL) under an inert atmosphere (N_2). TBTA (43.70 g, 200.0 mmol) and boron trifluoride etherate ($BF_3 \cdot Et_2O$, 1.0 mL) were added to the stirred solution and stirring was continued for 22 h. Some solid $NaHCO_3$ was added and the mixture was filtered through a short plug of silica. The solids were washed with a small amount of cyclohexane and the combined filtrates concentrated under reduced pressure. The residual liquid was diluted with CH_2Cl_2 and washed with 1 M

NaOH. The organic layer was separated, dried over anhydrous Na_2SO_4, and evaporated, yielding 4-*tert*-butoxybenzonitril as a colorless liquid (3.73 g, 43 %).

From 4-hydroxybenzonitrile and isobutene – Isobutene was generated by heating a stirred mixture of *tert*-butanol (14.92 g, 0.2 mol), acetic anhydride (40.84 g, 0.4 mol)[‡] and montmorillonit KSF[22] (4.0 g) in a 1 liter flask equipped with a reflux condenser[§]. The gases, leaving the condenser were introduced into a cold trap, cooled with a dry ice/acetone mixture. After about 30 min, gas evolution had ceased, and an amount of 9.0 g (80 %) of liquid isobutene had accumulated in the cold trap.

4-Hydroxybenzonitrile (5.96 g, 50 mmol) was placed in an autoclavable glass bottle with screw top and dissolved in 60 mL CH_2Cl_2/THF 5:1 (v/v) and the resulting solution was cooled to – 18 °C. Liquefied isobutene (22.4 g, 0.4 mol) and 3 drops of conc. H_2SO_4 were added. The bottle was tightly sealed, wrapped in a towel and kept for 5 d at ambient temperature. Before opening, the bottle was again cooled to – 18 °C. Solid K_2CO_3 was introduced and the excess isobutene was distilled in a cold trap. Then the solvents were evaporated and the residue was dissolved in MTBE, washed with 2 M aq. NaOH and brine and dried over K_2CO_3. After removal of the solvents the product was purified by vacuum flash chromatography. Yield: 2.08 g (24 %); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.42 (s, 9H, ^{t}Bu), 7.04 (m, 2H, 2/6-H ArO^{t}Bu), 7.56 (m, 2H, 3/5-H ArO^{t}Bu) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 28.8 (^{t}Bu), 80.2 (^{t}Bu), 105.7 (C-4 ArO^{t}Bu), 119.1 (-CN), 123.0 (C-2/6 ArO^{t}Bu), 133.4 (C-3/5 ArO^{t}Bu), 159.9 (C-1 ArO^{t}Bu) ppm; EI-MS (70 eV) m/z (%): 175 (10) $M^{\cdot+}$, 160 (25) $[M - CH_3]^+$, 120 (88) $[M - C_4H_7]^+$, 119 (100) $[M - C_4H_8]^+$, 102 (7) $[M - OC_4H_9]^+$. $C_{11}H_{13}NO$ (175.23).

4-*tert*-Butoxybenzylamine[23] – To a mechanically stirred suspension of $LiAlH_4$ (1.52 g, 40.0 mmol) in anhydrous diethyl ether (20 mL) was slowly added a solution of 4-

[‡] or an equivalent amount of *tert*-butyl acetate
[§] *cave*: gas evolution can become vigorous

tert-butoxybenzonitrile (3.50 g, 20.0 mmol) in abs. diethyl ether (40 mL). The reaction mixture was then refluxed for 3 h. After cooling to ambient temperature the reaction was quenched by careful addition of water (1.5 mL), 15 % aq. NaOH (1.5 mL), and again water (4.2 mL). Inorganic salts were removed by vacuum filtration and washed with diethyl ether. The combined filtrate and washings were concentrated and to the residue was added water and 5 % aq. $KHSO_4$, until a pH of 2–3 was adjusted. The aqueous solution was washed with MTBE (discarded) and then alkalified with 2 M NaOH. The product was extracted with CH_2Cl_2 (4 × 40 mL). The extracts were pooled and dried over anhydrous K_2CO_3. After evaporation to dryness the product was obtained as yellowish oil in 92 % yield (3.29 g). The amine was dissolved in diethyl ether (1.5 mL per mmol substrate) and treated with 1 eq. acetic acid in diethyl ether (0.66 m). The *tert*-butoxybenzylammonium acetate, which precipitated as fine, white powder, was collected on a sintered filter and dried in vacuo. The free amine was obtained by partitioning the acetate between 2 M aq. NaOH and CH_2Cl_2 as colorless oil. ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.33 (s, 9H, ^{t}Bu), 1.59 (brs, 2H, -N$\underline{H}_2$), 3.81 (s, 2H, -C$\underline{H}_2$NH$_2$), 6.95 (m, 2H, 2/6-H ArO^{t}Bu), 7.19 (m, 2H, 3/5-H ArO^{t}Bu) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 28.8 (^{t}Bu), 45.9 (-$\underline{C}$H$_2$NH$_2$), 78.3 (^{t}Bu), 124,3 (C-2/6 ArO^{t}Bu), 127,5 (C-3/5 ArO^{t}Bu), 138,2 (C-3/5 ArO^{t}Bu), 154.1 (C-1 ArO^{t}Bu) ppm; CI-MS (NH_3) m/z (%): 180 (11) $[MH]^+$, 163 (100) $[MH - NH_3]^+$. $C_{11}H_{17}NO$ (179.26).

4.1.3. *PREPARATION OF ACTIVE ESTERS*

***N*-Succinimidyl 2,2-diphenylacetate** – Diphenylacetic acid (10.61 g, 50.0 mmol) and *N*-hydroxysuccinimide (5.86 g, 51.0 mmol) were dissolved in 100 mL THF/CH_2Cl_2 4:1 (v/v). The resulting solution was chilled in an ice-water bath and treated with DCC (10.31 g, 50 mmol). After the DCC had dissolved, a white precipitate began to form and the mixture was placed in a refrigerator for 16 h. The solids were filtered off, and washed with CH_2Cl_2. The filtrate was concentrated and the residue was recrystallized from hot 2-propanol yielding 13.31 g (86 %) fine

colorless needles. Mp 120–121 °C (lit.[24] mp 120–122 °C); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 2.77 (s, 4H, -C(O)C$\underline{H}_2$C$\underline{H}_2$C(O)-), 5.35 (s, 1H, -C$\underline{H}$Ph$_2$), 7.27 – 7.39 (m, 10H, Ph$_2$) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 25.7 (-C(O)$\underline{C}$H$_2$$\underline{C}H_2$C(O)-), 54.0 (-$\underline{C}HPh_2$), 127.9 (Ph), 128.7 (Ph), 128.8 (Ph), 136.7 (Ph), 168.1 (-$\underline{C}$(O)CHPh$_2$), 168.9 ($\underline{C}$=O imide) ppm. $C_{18}H_{15}NO_4$ (309.32).

***N*-Succinimidyl 4-fluorobenzoate** – To a stirred solution of *N*-hydroxysuccinimide (5.75 g, 50.0 mmol) and DIPEA (17.07 mL, 100.0 mmol) in $CHCl_3$ (100 mL), cooled in an ice-water bath, was slowly added 4-fluorobenzoylchloride (5.91 mL, 50.0 mmol). After stirring for 16 h at ambient temperature the solution was washed with water, 5 % aq. $KHCO_3$, and brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Recrystallization from hot 2-propanol yielded 8.78 g (74 %) *N*-succinimidyl 4-fluorobenzoate as colorless needles. Mp 108–109 °C (lit.[25] mp 113–114 °C); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 2.92 (s, 4H, -C(O)C$\underline{H}_2$C$\underline{H}_2$C(O)-), 7.22 (m, 2H, ArF), 8.18 (m, 2H, ArF) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 25.7 (-C(O)$\underline{C}$H$_2$$\underline{C}H_2$C(O)-), 116.3 ($^2J_{C,F}$ = 22.2 Hz), 121.4 ($^4J_{C,F}$ = 2.9 Hz), 133.4 ($^3J_{C,F}$ = 9.7 Hz), 160.9 (-$\underline{C}$(O)C$_6$H$_4$F), 166.9 ($^1J_{C,F}$ = 257.9 Hz), 169.2 ($\underline{C}$=O imide) ppm. EI-MS (70 eV) m/z (%): 237 (1) $M^{\cdot+}$, 123 (100) [F-C$_6$H$_4$CO]$^+$, 95 (18) [F-C$_6$H$_4$]$^+$. $C_{11}H_8FNO_4$ (237.18).

*4.1.4. PREPARATION OF THE ORNITHINE PRECURSOR **4**:*

(*R*)-N^{α}-Benzyloxycarbonyl-N^{δ}-*tert*-butoxycarbonylornithine (Z-D-Orn(Boc)-OH) was prepared from D-ornithine hydrochloride according to a known procedure[26] in excellent yield and purity. – To a stirred solution of D-ornithine hydrochloride (8.43 g, 50 mmol) in 2 M NaOH (50 mL) was added a solution of $Cu(CH_3CO_2)_2 \cdot H_2O$ in water (25 mL) followed by a solution of Boc_2O (14.37 g, 65.0 mmol) in acetone (100 mL). After 24 h an additional portion of acetone (50 mL) was added and stirring was continued for 20 h. The resulting blue precipitate was filtered by suction and washed with acetone/water 2:1 (v/v, 100 mL) and water (2 × 250 mL). The air-dried material was washed with diethyl ether/petroleum ether 1:1 (v/v) and dried in

vacuum to yield 11.01 g (84 %) of bis(N^{δ}-*tert*-butoxycarbonylornithine) copper (II) complex ([H-D-Orn(Boc)O]$_2$Cu). Finely powdered [H-D-Orn(Boc)O]$_2$Cu (7.80 g, 14.9 mmol) was suspended in acetone (30 mL) and stirred vigorously for 15 min. Water (30 mL) was added and stirring was continued for 10 min. Then, 10 % aq. Na_2CO_3 solution (60 mL) and 8-quinolinol (4.48 g, 30.9 mmol) were added. After 1 h a solution of *N*-(benzyloxycarbonyloxy)-succinimide (7.47 g, 30.0 mmol) in 54 mL acetone/water 5:4 (v/v) was poured into the reaction mixture. 1h later, stirring was terminated and the precipitate of copper quinolinate was filtered off and washed with water. The filtrates and washings were combined and acetone was removed in vacuum. The residual solution was washed with dichloromethane (3 × 40 mL, discarded) and carefully acidified to pH 2 with 1 M HCl. The product was extracted with ethyl acetate (3 × 50 mL); the extracts were pooled, washed with 0.25 M HCl and brine, dried over anhydrous Na_2SO_4, and evaporated to dryness. Recrystallization from ethyl acetate/hexane 3:5 (v/v) afforded 9.19 g (84 %) Z-D-Orn(Boc)OH. mp 101–102 °C (lit[27] mp 101 °C). $C_{18}H_{26}N_2O_6$ (366.41).

(*R*)-N^{α}-Benzyloxycarbonyl-N^{δ}-phthaloylornithine (Z-D-Orn(Pht)-OH) – Z-D-Orn-(Boc)OH (17.3 g, 47 mmol) was dissolved in 100 mL of a saturated solution of hydrogen chloride in ethyl acetate and stirred vigorously. After 1 h, the precipitated solid was collected on a sintered filter and washed with petroleum ether. After drying in vacuum, the obtained Z-D-Orn-OH · HCl (10.59 g, 35.0 mmol) was suspended in 100 mL water in a 0.5 liter flask and stirred intensely, while $NaHCO_3$ (6.0 g, 71.4 mmol) was added in small portions. To the resulting clear solution was added *N*-ethoxycarbonyl phthalimide (7.67 g, 35.0 mmol) in 1,4-dioxane (70 mL) and stirring was continued for 1 h. The mixture was acidified with 1 M HCl and extracted with ethyl acetate (3 × 75 mL). The combined extracts were washed with brine and dried over anhydrous Na_2SO_4. After removal of the solvent the residue was dissolved in acetone (250 mL) and treated with 7.0 g (35 mmol) DCHA. The resulting precipitate was collected on a sintered filter and washed with acetone and diethyl ether. The DCHA salt was partitioned between diluted sulfuric acid and

CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4, diluted with hexane, and concentrated until a white solid precipitated. The product was filtered off, washed with petroleum ether, and dried in vacuum yielding 10.52 g (76 %) Z-D-Orn(Pht)-OH. Mp 126–127 °C (hexane/CH_2Cl_2, lit[28] mp 127–129 °C (ethyl acetate)). ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.63 – 2.03 (m, 4H, -$C^\beta\underline{H}_2C^\gamma\underline{H}_2$-), 3.70 (m, 2H, -$C^\delta\underline{H}_2$-), 4.44 (m, 1H, -$C^\alpha\underline{H}$-), 5.08 (s, 2H, -$C\underline{H}_2$OPh), 5.54 (d, ^3J = 8.2 Hz, -N$\underline{H}$-), 7.20–7.40 (m, 5H, -Ph), 7.69 (dd, ^4J = 3.0 Hz, ^3J = 5.3 Hz, 2H, -NPht), 7.82 (dd, ^4J = 3.0 Hz, ^3J = 5.3 Hz, 2H, -NPht) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 24.7 ($\underline{C}^\gamma$), 29.7 ($\underline{C}^\beta$), 37.4 ($\underline{C}^\delta$), 53.4 ($\underline{C}^\alpha$), 67.2 (-O$\underline{C}H_2$Ph), 123.3 ($\underline{C}$-4, -NPht), 128.1 (Cbz), 128.2 (Cbz), 128.5 (Cbz), 132.0 ($\underline{C}$-3, -NPht), 134.0 ($\underline{C}$-5, -NPht), 136.0 (Cbz), 156.2 (-NH$\underline{C}$(O)O-), 168.5 ($\underline{C}$=O -NPht), 175.9 (-$\underline{C}O_2$H) ppm; ESI-MS (+p) m/z (%): 435 (30) $[MK]^+$, 414 (80) $[MNH_4]^+$, 397 (100) $[MH]^+$, 353 (50) $[MH - CO_2]^+$. $C_{21}H_{20}N_2O_6$ (396.39).

(*R*)-N^α-Benzyloxycarbonyl-N^δ-phthaloyl-*N*-(4-*tert*-butoxybenzyl)ornithinamide – A suspension of Z-D-Orn(Pht)OH (7.93 g, 20.0 mmol) and HOBt · H_2O (3.22 g, 21.0 mmol) in CH_2Cl_2 (100 mL) was cooled in an ice-bath and treated with finely grounded DCC (4.13 g, 20.0 mmol) and a solution of 4-*tert*-butoxybenzylamine (3.58 g, 20.0 mmol) in CH_2Cl_2 (80 mL). The resulting solution was stirred for 0.5 h at 0 °C and for further 16 h at ambient temperature. The precipitate formed was removed by filtration and washed with CH_2Cl_2; the clear filtrate was washed with water, solutions of 5 % aq. $KHSO_4$, 5 % aq. $KHCO_3$, and brine. The combined organic layers were dried over anhydrous Na_2SO_4 and diluted with n-hexane. Upon removal of CH_2Cl_2 a white solid formed, which was collected on a sintered filter, washed with n-hexane and dried in vacuum. The product obtained weighed 9.39 g (84 % yield). mp 135–136 °C (petroleum ether/ethyl acetate); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.32 (s, 9H, -C($C\underline{H}_3$)$_3$), 1.55–1.82 (m, 4H, -$C^\beta\underline{H}_2C^\gamma\underline{H}_2$-), 3.60–3.90 (m, 2H, -$C^\delta\underline{H}_2$-), 4.27–4.52 (m, 3H, -$C\underline{H}_2$ArO^{t}Bu and -$C^\alpha\underline{H}$-), 5.07 (s, 2H, -$C\underline{H}_2$OPh), 5.65 (d, ^3J = 8.3 Hz, -N$\underline{H}$-), 6.69 (m, 1H, -N$\underline{H}$-), 6.89 (m, 2H, 2/6-H ArO^{t}Bu), 7.30 (m, 3/5-H ArO^{t}Bu), 7.28–7.38 (m, 5H, -Ph), 7.69 (dd, ^4J = 3.0 Hz, ^3J = 5.7 Hz, 2H,

-NPht), 7.76 (dd, ^4J = 3.0 Hz, ^3J = 5.7 Hz, 2H, -NPht) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 24.7 (C$^{\gamma}$), 28.8 (^{t}Bu), 30.7 (C$^{\beta}$), 36.6 (C$^{\delta}$), 43.1 (-CH_2ArO^{t}Bu), 53.4 (C$^{\alpha}$), 67.0 (-OCH_2Ph), 78.5 (^{t}Bu), 123.3 (C-4 Pht), 124.3 (C-2/6 ArO^{t}Bu), 128.0 (C-3/5 ArO^{t}Bu), 128.1 (Ph), 128.2 (Ph), 128.5 (Ph), 131.9 (C-3 Pht), 132.6 (C-4 ArO^{t}Bu), 134.1 (C-5, -NPht), 136.2 (C-1 Ph), 154.7 (C-1 ArO^{t}Bu), 156.4 (-OC(O)NH-), 168.7 (C=O Pht), 171.5 (C=O amide) ppm; ESI-MS (+p) m/z (%): 580 (25) $[MNa]^+$, 558 (100) $[MH]^+$; analysis calcd. for $C_{32}H_{35}N_3O_6 \cdot 0.25\ H_2O$: C 68.37, H 6.38, N 7.46 %; found: C 68.33, H 5.84, N 7.23 %. $C_{32}H_{35}N_3O_6$ (557.64).

(*R*)-*N*$^{\alpha}$-(2,2-Diphenylacetyl)-*N*$^{\delta}$-phthaloyl-*N*-(4-*tert*-butoxybenzyl)ornithinamide (**3**) – To palladium on carbon (2 g), carefully moistened with formic acid (2 mL) under an atmosphere of nitrogen, was added (5.58 g, 10.0 mmol) in 1,4-dioxane (20 mL) and a solution of potassium formate (80 mL, 0.5 mol·L^{-1}) in methanol. The mixture was vigorously stirred at ambient temperature until the starting material was completely consumed (monitored by TLC). A solution of succinimidyl 2,2-diphenylacetate (3.09 g, 10.0 mmol) in 1,4-dioxane (50 mL) was added and stirring was continued for additional 16 h. Palladium on carbon was removed by filtration through a pad of Celite® and washed with 1,4-dioxane. The filtrate was concentrated in vacuum, diluted with ethyl acetate, and washed with water, solutions of 5 % aq. $KHSO_4$, 5 % aq. $KHCO_3$, and brine. The combined organic layers were dried over anhydrous Na_2SO_4 and evaporated. The product was purified using vacuum flash chromatography, eluting with $CHCl_3$ (stabilized with 1 % ethanol). The product was obtained as white powder after crystallization from $CHCl_3$/hexane. (yield: 5.29 g, 86 %). mp 110–112 °C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.32 (s, 9H, ^{t}Bu), 1.50–1.80 (m, 4H, -C$^{\beta}H_2$C$^{\gamma}H_2$-), 3.60–3.90 (m, 2H, -C$^{\delta}H_2$-), 4.20–4.50 (m, 2H, -CH_2Ar), 4.75–4.85 (m, 1H, -C$^{\alpha}$H-), 4.94 (s, 1H, -CHPh$_2$), 6.60 (m, 1H, -NH-), 6.68 (m, 1H, -NH), 6.87 (m, 2/6-H ArO^{t}Bu), 7.08 (m, 3/5-H ArO^{t}Bu), 7.20–7.40 (m, 10H, Ph), 7.65–7.80 (m, 4H, Pht) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 24.8 (C$^{\gamma}$), 28.8 (^{t}Bu), 30.3 (C$^{\beta}$), 36.6 (C$^{\delta}$), 43.0 (-CH_2ArO^{t}Bu), 51.7 (C$^{\alpha}$), 58.8 (-CHPh$_2$), 78.5 (^{t}Bu), 123.3 (C-4 Pht), 124.3 (C-2/6 ArO^{t}Bu), 127.3

(C-3/5 ArO^{t}Bu), 128.2 (Ph), 128.7 (Ph), 128.8 (Ph), 131.9 (C-3 Pht), 132.6 (C-4 ArO^{t}Bu), 134.1 (C-5 Pht), 139.0 (C-1 Ph), 154.7 (C-1 ArO^{t}Bu), 168.7 (C=O Pht), 171.2 (C=O), 172.4 (C=O) ppm; ESI-MS (+p) m/z (%) : 618 (100) $[MH]^+$; analysis calcd. for $C_{38}H_{39}N_3O_5$: C 73.88, H 6.36, N 6.80 %; found: C 73.63, H 6.32, N 6.80 %. $C_{38}H_{39}N_3O_5$ (617.73).

(*R*)-*N*$^\alpha$-(2,2-Diphenylacetyl)-*N*-(4-*tert*-butoxybenzyl)ornithinamide (**4**) – A solution of **3** (1.23 g, 2.0 mmol) in 9 mL THF/methanol 2:1 (v/v) was treated with hydrazine hydrate (0.2 mL, 4.0 mmol) and stirred overnight at ambient temperature. After addition of 5 % aq. $KHSO_4$ solution (13 mL) stirring was continued for further 2 h. Thereafter, organic solvents were evaporated in vacuum and the residual suspension alkalified with 2 M NaOH solution. The solution was extracted with $CHCl_3$ (3 × 30 mL) and the combined extracts were dried over anhydrous K_2CO_3. After evaporation a dry foam was obtained, which was further purified by vacuum flash chromatography (eluent: $CHCl_3$/methanol/NH_4OH 100:10:1 v/v), yielding 0.76 g (78 %) of the title compound as a white solid. ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.33 (s, 9H, ^{t}Bu), 1.36–1.46 (m, 2H, -C$^\gamma$H$_2$-), 1.66–1.86 (m, 4H, -C$^\beta$H$_2$- , -NH$_2$), 2.54–2.69 (m, 2H, -C$^\delta$H$_2$-), 4.19–4.38 (m, 2H, -CH$_2$Ar), 4.53 (m, 1H, -C$^\alpha$H-), 4.92 (s, 1H, -CHPh$_2$), 6.84–7.12 (m, 4H, ArO^{t}Bu), 7.16–7.43 (m, 10H, Ph$_2$) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 28.3 (C$^\gamma$), 28.8 (^{t}Bu), 30.2 (C$^\beta$), 41.3 (C$^\delta$), 42.9 (-CH$_2$ArO^{t}Bu), 53.0 (C$^\alpha$), 58.8 (-CHPh$_2$), 78.5 (^{t}Bu), 124.3 (C-2/6 ArO^{t}Bu), 127.3 (C-3/5 ArO^{t}Bu), 128.2 (Ph), 128.7 (Ph), 128.8 (Ph), 132.9 (C-4 ArO^{t}Bu), 139.1 (C-1 Ph), 154.6 (C-1 ArO^{t}Bu), 171.3 (C=O), 172.4 (C=O) ppm; ESI-MS (+p) m/z (%): 488 (100) $[MH]^+$, 975 (13) $[2MH]^+$; $C_{30}H_{37}N_3O_3$ (487.63).

*4.1.5. PREPARATION OF (R)-ISOGLUTAMINOL DERIVATIVE **6**:*

(*R*)-γ-Methyl *N*-(benzyloxycarbonyl)glutamate[29] – To a stirred suspension of D-glutamic acid (10.00 g, 68.0 mmol) in methanol (200 mL) was added chlorotrimethylsilane (18.9 mL, 150.0 mmol). After 0.1 h, the mixture was concentrated under reduced pressure to one tenth of its volume, and the residue

was poured into ethyl acetate (500 mL). The precipitate was collected on a sintered filter, washed with ethyl acetate, diethyl ether, and petroleum ether and dried in vacuum. Yield: 10.88 g (81 %) (*R*)-glutamic acid γ-methyl ester hydrochloride. The free amino acid was obtained as a white solid, which precipitated from a methanolic solution of the hydrochloride after addition of pyridine. ^{1}H-NMR (300 MHz, D_2O, neutral betaine): δ_H = 2.15 (m, 2H, -C$^{\beta}$H$_2$-), 2.56 (m, 2H, -C$^{\gamma}$H$_2$-), 3.70 (s, 3H, -CH$_3$), 3.76 (m, 1H, -C$^{\alpha}$H-) ppm; ^{13}C-NMR (300 MHz, D_2O): δ_C = 25.4 (C$^{\gamma}$), 29.6 (C$^{\beta}$), 52.3 (-CH$_3$), 53.9 (C$^{\alpha}$), 173.9 (-CO$_2$Me), 175.27 (-CO$_2$H) ppm; ESI-MS (+p) m/z (%): 162 (100) $[MH]^+$, 323 (18) $[2M + H]^+$. $C_6H_{11}NO_4$ (161.16).

(*R*)-γ-Methyl glutamate hydrochloride (10.88 g, 55.1 mmol) was dissolved in water (200 mL), and potassium carbonate (19.0 g, 137.5 mmol) was introduced carefully. To the resulting mixture a solution of *N*-(benzyloxycarbonyloxy)succinimide (15.0 g, 60.2 mmol) in acetone (200 mL) was added. The solution was stirred at room temperature for 2 h, then washed with ether (400 mL), and acidified with conc. HCl to pH 1. The resulting solution was extracted with ethyl acetate (3 × 50 mL), and the combined extracts were washed with brine, dried over anhydrous Na_2SO_4, and evaporated to give (*R*)-γ-methyl *N*-(benzyloxycarbonyl)glutamate as colorless oil. In order to remove traces of α,γ-dimethyl diester, the product was dissolved in diethyl ether (50 mL) and treated with an ethereal solution of DCHA (1.01 eq., 7.40 mL DCHA in 20 mL). The precipitated dicyclohexylammonium salt was filtered with suction, washed with ether, and air-dried. To recover the purified free acid, the finely powdered DCHA salt was partitioned between diluted sulfuric acid and ethyl acetate (3 × 50 mL); the ethyl acetate extracts were combined, washed with water, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure affording a colorless oil, which slowly solidified (10.78 g, 66 %). ^{1}H-NMR (300 MHz, $CHCl_3$): δ_H = 2.04 and 2.24 (m, 2H, -C$^{\beta}$H$_2$-, diast.), 2.46 (m, 2H, -C$^{\gamma}$H$_2$-), 3.66 (s, 3H, -CH$_3$), 4.43 (m, 1H, -C$^{\alpha}$H-), 5.11 (s, 2H, -OCH$_2$Ph), 5.58 (d, ^{3}J = 8.0 Hz, 1H, -NH-), 7.34 (m, 5H, -Ph) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 27.3 (C$^{\beta}$), 30.1 (C$^{\gamma}$), 52.0 (-

$\underline{C}H_3$), 53.2 ($\underline{C}^{\alpha}$), 67.3 (-O$\underline{C}H_2$Ph), 128.2 (Ph), 128.3 (Ph), 128.6 (Ph), 136.0 (C-1 Ph), 156.2 (-NH$\underline{C}$(O)OPh), 173.6 (-$\underline{C}O_2$Me), 176.0 (-$\underline{C}O_2$H) ppm. ESI-MS (+p) m/z (%): 296 (100) $[MH]^+$, 313 (94) $[M + NH_4]^+$, 251 (37) $[MH - CO_2]^+$. $C_{14}H_{17}NO_6$ (295.29).

(*R*)-Methyl 4-(benzyloxycarbonylamino)-5-(4-*tert*-butoxybenzylamino)-5-oxo-pentanoate – (*R*)-γ-Methyl *N*-(benzyloxycarbonyl) glutamate (10.94 g, 37.0 mmol) was placed in a hot-air dried flask with stirring bar and dissolved in dry DMF (40 mL) under an inert atmosphere (N_2). CDI (6.01 g, 37.1 mmol) was added in one portion and the mixture was stirred magnetically at ambient temperature. After 45 min, when CO_2 evolution had ceased, 4-(*tert*-butoxy)benzylamine (6.65 g, 37.1 mmol) was added and stirring was continued for 18 h. The reaction mixture was poured into ice-water (300 mL), giving a white precipitate, which was collected on a sintered filter, washed with water, and air-dried. The solid product was dissolved in ethyl acetate (200 mL) and the resulting solution washed with 5 % aq. $KHSO_4$, water, and brine, and dried over anhydrous Na_2SO_4. The solvent was removed in vacuum and the raw product purified by vacuum flash chromatography (eluent petroleum ether/ethyl acetate 4:3). Yield: 12.60 g (75 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.32 (s, 9H, ^{t}Bu), 1.96 and 2.14 (m, 2H, -C$^{\beta}\underline{H}_2$-, diast.), 2.42 (m, 2H, -C$^{\gamma}\underline{H}_2$-), 3.63 (s, 3H, -C$\underline{H}_3$), 4.27 (m, 1H, -C$^{\alpha}\underline{H}$-), 4.36 (d, ^3J = 5.5 Hz, 2H, -NHC$\underline{H}_2$ArO^{t}Bu), 5.06 (s, 2H, -OC$\underline{H}_2$Ph), 5.77 (d, ^3J = 8.0 Hz, 1H, -N$\underline{H}$-), 6.92 (m, 2H, 2/6-H ArO^{t}Bu), 7.13 (m, 2H, 3/5-H ArO^{t}Bu), 7.32 (m, 5H, Ph) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 28.2 ($\underline{C}^{\gamma}$), 28.8 (^{t}Bu), 30.1 ($\underline{C}^{\beta}$), 43.1 (-$\underline{C}H_2$ArO^{t}Bu), 51.9 (-$\underline{C}H_3$), 54.2 ($\underline{C}^{\alpha}$); 67.1 (-O$\underline{C}H_2$Ph), 78.6 (^{t}Bu), 124.4 (C-2/6 ArO^{t}Bu), 128.1 (C-3/5 ArO^{t}Bu), 128.2 (Ph), 128.3 (Ph), 128.6 (Ph), 132.6 (C-4 ArO^{t}Bu), 136.1 (C-1 Ph), 154.8 (C-1 ArO^{t}Bu), 156.3 (-NH$\underline{C}$(O)OPh), 171.0 (-$\underline{C}O_2$Me), 173.9 (-$\underline{C}O_2$H) ppm; ESI-MS (+p) m/z (%): 457 (100) $[MH]^+$ 474 (28) $[M + NH_4]^+$. $C_{25}H_{32}N_2O_6$ (456.53).

(*R*)-Methyl 5-(4-*tert*-butoxybenzylamino)-4-(2,2-diphenylacetamido)-5-oxopentanoate (**5**) – 10 % Pd-C (1.0 g) was placed in a flask filled with argon and carefully moistened with formic acid. A solution of (*R*)-methyl 4-(benzyloxycarbonylamino)-

5-(4-*tert*-butoxybenzylamino)-5-oxopentanoate (3.29 g, 7.2 mmol) in methanolic ammonium formate (50 mL, c = 0.5 mol·L^{-1}) was introduced and the mixture was stirred vigorously until the starting material was consumed as indicated by TLC. Pd-C was removed by filtration through a pad of Celite®; the filtrate was concentrated under reduced pressure, and the residue taken up in $CHCl_3$. Portions of 5 % aq. $KHSO_4$ solution were added and the mixture was shaken in a separatory funnel, until the aqueous layer had a pH value of 8–9. The phases were separated and the chloroform layer was dried over anhydrous Na_2SO_4. After removal of the solvent, 2.11 g (6.5 mmol) of (*R*)-methyl 4-amino-5-(4-*tert*-butoxybenzylamino)-5-oxopentanoate were obtained. This material was dissolved in THF (30 mL) and treated with succinimidyl 2,2-diphenylacetate (2.01 g, 6.5 mmol). The reaction mixture was stirred magnetically and kept at about 45 °C in a temperature bath. After 16 h the mixture was concentrated at reduced pressure and diluted with ethyl acetate (50 mL). The organic phase was washed with 1 M aq. NH_4Cl, 5 % aq. $KHSO_4$, and brine, separated, dried over anhydrous Na_2SO_4, and evaporated. The residue was purified by vacuum flash chromatography (eluent: $CHCl_3$) and recrystallization from hot ethyl acetate/hexane or chloroform/hexane. Yield: 2.78 g (75 % over two steps). mp 129–130 °C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.32 (s, 9H, ^{t}Bu), 1.75–2.50 (m, 4H, -C$^\beta$H$_2$C$^\gamma$H$_2$-), 3.59 (s, 3H, -CH$_3$), 4.25 (m, 2H, -NHCH$_2$ArO^{t}Bu), 4.58 (m, 1H, -C$^\alpha$H-), 4.89 (s, 1H, -CHPh$_2$), 6.81 (d, ^3J = 7.7 Hz, 1H, -N$^\alpha$H-), 7.00 (t, ^3J = 5.8 Hz, 1H, -NHCH$_2$ArO^{t}Bu), 6.89 (m, 2H, 2/6-H ArO^{t}Bu), 7.05 (m, 2H, C-3/5 ArO^{t}Bu), 7.15–7.33 (m, 10H, Ph) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 27.6 (C$^\gamma$), 28.9 (^{t}Bu), 30.2 (C$^\beta$), 42.9 (-CH$_2$ArO^{t}Bu), 51.9 (-CH$_3$), 52.7 (C$^\alpha$), 58.7 (Ph$_2$CH-), 78.5 (^{t}Bu), 124.3 (C-2/6 ArO^{t}Bu), 127.4 (C-3/5 ArO^{t}Bu), 128.1 (Ph), 128.8 (Ph), 128.9 (Ph), 132.7 (C-4 ArO^{t}Bu), 139.0 (C-1 Ph), 154.7 (C-1 ArO^{t}Bu), 170.8 (-CO$_2$Me), 172.5 (C=O amide), 173.8 (C=O amide) ppm; ESI-MS (+p) m/z (%): 517 (100) $[MH]^+$, 534 (12) $[M + NH_4]^+$, 1033 (8) $[2M + H]^+$; analysis calcd. for $C_{31}H_{36}N_2O_5$: C 72.09, H 7.02, N 5.42 %; found: C 71.63, H 7.18, N 5.29 %. $C_{31}H_{36}N_2O_5$ (516.63).

(*R*)-*N*-(4-*tert*-Butoxybenzyl)-2-(2,2-diphenylacetamido)-5-hydroxypentanamide (**6**) – **5** (2.29 g, 4.4 mmol) was dissolved in dry THF (10 mL) under an inert atmosphere (Ar). To the stirred solution was added lithium chloride (0.37 g, 8.8 mmol), sodium borohydride (0.33 g, 8.8 mmol), and ethanol (10 mL). After 16 h the reaction mixture was diluted with ethyl acetate and washed with 1 M aq. NH_4Cl solution and brine. The ethyl acetate layer was separated, dried over anhydrous Na_2SO_4, and rotary evaporated. The compound was dried in vacuum, yielding a white, chromatographically pure (TLC, chloroform/methanol 20:1 v/v), amorphous solid (2.0 g, 93 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.31 (s, 9H, ^{t}Bu), 1.43 (m, 2H, -C$^\gamma$H$_2$-), 1.63–1.88 (m, 2H, -C$^\beta$H$_2$-), 3.50 (m, 2H, -C$^\delta$H$_2$-), 4.21 (m, 2H, -OCH$_2$ArO^{t}Bu), 4.63 (m, 1H, -C$^\alpha$H-), 4.90 (s, 1H, Ph$_2$CH-), 6.87 (m, 2H, 2/6-H ArO^{t}Bu), 6.96 (d, 3*J* = 8.0 Hz, 1H, -N$^\alpha$H-), 7.02 (m, 2H, 3/5-H ArO^{t}Bu), 7.15–7.30 (m, 10H, Ph), 7.33 (t, 3*J* = 5.8 Hz, 1H, -NHCH$_2$ArO^{t}Bu); ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 28.2 (C$^\gamma$), 29.1 (^{t}Bu), 30.0 (C$^\beta$), 43.1 (-CH$_2$ArO^{t}Bu), 53.2 (C$^\alpha$), 58.9 (Ph$_2$CH-), 62.4 (C$^\delta$), 78.8 (^{t}Bu), 124.5 (C-2/6 ArO^{t}Bu), 127.6 (C-3/5 ArO^{t}Bu), 128.4 (Ph), 129.0 (Ph), 129.1 (Ph), 133.1 (C-4 ArO^{t}Bu), 139.3 (C-1 Ph), 154.9 (C-1 ArO^{t}Bu), 171.7 (C=O amide), 172.7 (C=O amide) ppm; ESI-MS (+p) m/z (%): 489 (100) $[MH]^+$, 978 (8) $[2M + H]^+$. $C_{30}H_{36}N_2O_4$ (488.62).

(*R*)-N^α-(2,2-Diphenylacetyl)-N^δ-phthaloyl-*N*-(4-*tert*-butoxybenzyl)ornithinamide (**3**) from isoglutaminol **6**: – Isoglutaminol derivative **6** (0.44 g, 0.9 mmol) and phthalimide (0.20 g, 1.4 mmol) were dissolved in 5 mL dry THF under an inert atmosphere (N_2). Tri-n-butylphosphine (0.7 mL of a 2.0 mol·L^{-1} solution in toluene, 1.4 mmol) was introduced and the mixture cooled in an ice-water bath. Under vigorous stirring a solution of DIAD (0.7 mL of a 2.0 mol·L^{-1} solution in toluene, 1.4 mmol) was added drop by drop. Stirring was continued and the mixture was allowed to warm to ambient temperature. After 16 h the reaction was quenched by addition of some drops of methanol and the mixture was concentrated under reduced pressure; the residue was poured into petroleum ether/diethyl ether 1:1 (v/v) (40 mL). The precipitate was filtered off, washed with petroleum ether/diethyl

ether 1:1 (v/v) (10 mL) and dried in vacuum. Yield: 0.34 g (61 %). Analytical data vide infra.

(*R*)-5-(4-*tert*-Butoxybenzylamino)-4-(2,2-diphenylacetamido)-5-oxopentanoic acid (**7**) – **5** (0.52 g, 1.0 mmol) was dissolved in ethanol (5 mL) and water (2 mL) and treated with 0.5 M KOH (2.0 mL). After 16 h stirring at ambient temperature, the mixture was diluted with water (50 mL), acidified with 5 % aq. $KHSO_4$ and extracted in ethyl acetate (3 × 20 mL). The organic extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness. Yield: 0.48 g (96 %). ^{1}H-NMR ($CDCl_3$, 300 MHz): δ_H = 1.30 (s, 9H, -C(C$\underline{H}_3$)$_3$), 1.45–2.45 (m, 4H, -C$^\beta\underline{H}_2$C$^\gamma\underline{H}_2$-), 4.25 (m, 2H, -C$\underline{H}_2$ArO^{t}Bu), 4.49 (brm, 1H, -C$^\alpha\underline{H}$-), 4.96 (s, 1H, Ph$_2$C$\underline{H}$-), 6.89 (m, 2H, 2/6-H ArO^{t}Bu), 7.07 (m, 2H, 3/5-H ArO^{t}Bu), 7.14–7.36 (m, 10H, Ph) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 27.7 (C$^\beta$), 28.8 (^{t}Bu), 34.5 (C$^\gamma$), 43.0 (-C$\underline{H}_2$ArO^{t}Bu), 52.5 (C$^\alpha$), 58.4 (Ph$_2$C$\underline{H}$-), 78.8 (^{t}Bu), 124.3 (C-2/6 ArO^{t}Bu), 127.3 (C-3/5 ArO^{t}Bu), 128.1 (Ph), 128.8 (Ph), 128.9 (Ph), 132.5 (C-4 ArO^{t}Bu), 138.8 (C-1 Ph), 154.6 (C-1 ArO^{t}Bu), 171.3 (C=O amide), 173.3 (C=O amide), 175.9 (-C$\underline{O}_2$H) ppm; ESI-MS (+p) m/z (%): 503 (100) $[MH]^+$, 520 (35) $[MNH_4]^+$, 531 (15) $[M_1H]^+$ (M_1: byproduct ethyl ester). $C_{30}H_{34}N_2O_5$ (502.60).

(*R*)-*N*-(4-*tert*-butoxybenzyl)-2-(2,2-Diphenylacetamido)-5-(*N*^{2}-*tert*-butoxycarbonylguanidino)-5-oxopentanamide (**8**) – To a magnetically stirred solution of **7** (0.50 g, 1.0 mmol), 1-*tert*-butoxycarbonylguanidine (0.16 g, 1.0 mmol) and HOBt · H_2O (0.16 g, 1.0 mmol) in DMF (5 mL), chilled in an ice-bath, was added EDC · HCl (0.19 g, 1.0 mmol). After 1 h, the cooling bath was removed and stirring was continued for 16 h. The mixture was diluted with ethyl acetate (40 mL) and washed with water, 5 % aq. K_2CO_3 solution, 5 % aq. $KHSO_4$ solution, and brine; the organic layers were dried over anhydrous Na_2SO_4 and the volatiles were removed in vacuum. The product was purified by flash chromatography (eluent: chloroform/MeOH 5:1 (v/v)) affording 0.29 g (45 %) of a white solid. ^{1}H-NMR (300 MHz, DMSO-d_6): δ_H = 1.27 (s, 9H, ^{t}Bu), 1.40 and 1.41 (isomers, 2 s, 9H, ^{t}Bu), 1.74–2.04 (m, 2H, -C$^\beta\underline{H}_2$-), 2.17 and 2.33 (2 t, ^3J = 7.7 Hz, 2H, -C$^\gamma\underline{H}_2$-, diast.), 4.21

(m, 2H, -NHC<u>H</u>$_2$ArO^{t}Bu), 4.30 (m, 1H, -C$^\alpha$<u>H</u>-), 5.08 and 5.10 (isomers, 2 s, 1H, -C<u>H</u>Ph$_2$), 6.88 (m, 2H, 2/6-H ArO^{t}Bu), 7.11 (m, 2H, 3/5-H ArO^{t}Bu), 7.17–7.35 (m, 10H, Ph), 8.29 (t, ^3J = 5.8 Hz, 1H, -N<u>H</u>-), 8.42 (t, ^3J = 5.8 Hz, 1H, -N<u>H</u>), 8.49 (d, ^3J = 7.9 Hz, 1H, -N$^\alpha$<u>H</u>-), 8.74 (m, 2H, -N<u>H</u>$_2$), 10.89 (brs, 1H, -N<u>H</u>) ppm; ^{13}C-NMR (300 MHz, DMSO-d$_6$): δ_C = 26.9 (-<u>C</u>$^\beta$H$_2$-), 27.8 (Boc), 28.4 (^{t}Bu), 31.4 (-<u>C</u>$^\gamma$H$_2$-), 41.4 (-C<u>H</u>$_2$ArO^{t}Bu), 52.1 (-<u>C</u>$^\alpha$H-), 55.9 (-<u>C</u>HPh$_2$), 77.6 (Boc, ^{t}Bu), 123.5 (C-2/6 ArO^{t}Bu), 126.5 (C-4 Ph), 127.6 (C-3/5 ArO^{t}Bu), 128.1 and 128.4 (C-2/3/5/6 Ph), 133.6 (C-4, ArO^{t}Bu), 140.2 (C-1 Ph), 153.6 (C-1 ArO^{t}Bu), 154.5 (-NH<u>C</u>(NH)N-), 158.4 (Boc), 170.9 (<u>C</u>=O amide), 171.2 (<u>C</u>=O amide), 172.8 (<u>C</u>=O) ppm; ESI-MS (+p) m/z (%): 644 (100) [MH]$^+$; $C_{36}H_{45}N_5O_6$ (643.77).

(*R*)-2-(2,2-Diphenylacetamido-5-guanidino-*N*-(4-hydroxybenzyl)-5-oxopentan-amide (**13**) – **8** (200 mg, 0.31 mmol) was dissolved in 5 mL TFA/CH_2Cl_2 1:1 (v/v) and stirred at ambient temperature for 3 h. The volatiles were removed under reduced pressure and the crude product was submitted to flash chromatography (eluent: CH_2Cl_2/MeOH/TFA (5 % in CH_2Cl_2) 100:10:1). Yield: 0.15 g (98 %). ^{1}H-NMR (300 MHz, DMSO-d$_6$): δ_H = 1.81–2.06 (m, 2H, -C$^\beta$<u>H</u>$_2$-), 2.22 and 2.43 (2 t, ^3J = 7.6 Hz, 2H, -C$^\gamma$<u>H</u>$_2$-, diast.), 4.13 (m, 2H, -N<u>H</u>CH$_2$ArOH), 4.29 (m, 1H, -C$^\alpha$<u>H</u>-), 5.08 and 5.11 (isomers, 2 s, 1H, -C<u>H</u>Ph$_2$), 6.68 (m, 2H, 2/6-H ArOH), 7.01 (m, 2H, 3/5-H ArOH), 7.14–7.36 (m, 10H, Ph), 8.25 (t, ^3J = 5.8 Hz, 1H, -N<u>H</u>), 8.36 (t, ^3J = 5.8 Hz, 1H, -N<u>H</u>), 8.52 (d, ^3J = 7.9 Hz, 1H, -N$^\alpha$<u>H</u>-), 8.94 (d, ^3J = 5.9 Hz, 2H, -N<u>H</u>$_2$), 11.75 and 11.80 (2 s, 1H, -N<u>H</u>, isomers) ppm; ^{13}C-NMR (300 MHz, DMSO-d$_6$): δ_C = 26.2 (-<u>C</u>$^\beta$H$_2$-), 32.6 (-<u>C</u>$^\gamma$H$_2$-), 41.5 (-C<u>H</u>$_2$ArOH), 51.8 (-<u>C</u>$^\alpha$H-), 55.9 (-<u>C</u>HPh$_2$), 114.9 (C-2/6 ArOH), 126.5 (C-4 Ph), 128.1 and 128.4 (C-2/3/5/6 Ph), 128.3 (C-3/5 ArOH), 139.9 (C-1 Ph), 154.5 (-NH<u>C</u>(NH)N-), 156.2 (C-1 ArOH), 158.9 (C-4 ArOH), 170.6 (<u>C</u>=O amide), 171.0 (<u>C</u>=O amide), 174.2 (NC(N)N<u>C</u>(O)-) ppm; ESI-MS (+p) m/z (%): 488 (100) [MH]$^+$. $C_{27}H_{29}N_5O_4$ (487.55).

4.1.6. *PREPARATION OF N-PROTECTED ω-AMINO ACIDS AND ω-AMINO ALCOHOLS*

5-Phthalimidopentanoic acid – *N*-Ethoxycarbonylphthalimid (11.40 g, 51.0 mmol) was introduced into a stirred solution of 5-aminopentanoic acid (5.86 g, 50.0 mmol) and Na_2CO_3 (5.41 g, 51.0 mmol) in water (200 mL). After 45 min the reaction mixture was filtered and the filtrate was acidified with 6 M hydrochloric acid; the resulting precipitate was collected on a filter, washed with water and dried. The product was recrystallized from toluene/n-hexane and EtOH/H_2O and dried in a desiccator over phosphorous pentoxide. Yield: 9.33 g (75 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.72 (m, 4H, -$CH_2C\underline{H}_2C\underline{H}_2CH_2$-), 2.41 (t, ^3J = 7.0 Hz, 2H, -$(CH_2)_3C\underline{H}_2CO_2H$), 3.71 (t, ^3J = 6.9 Hz, 2H, -$(CH_2)_3C\underline{H}_2$NPht), 7.72 (dd, ^3J = 3.0 Hz, ^3J = 5.5 Hz, 2H, Pht), 7.85 (dd, ^3J = 3.0 Hz, ^3J = 5.5 Hz, 2H, Pht) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 21.8 (-$\underline{C}H_2(CH_2)_2$NPht), 27.9 (-$\underline{C}H_2CH_2$NPht), 33.4 (-$\underline{C}H_2CO_2H$), 37.4 (-$\underline{C}H_2$NPht), 123.3 (Pht), 132.0 (Pht), 134.0 (Pht), 168.4 (Pht), 179.0 (-$\underline{C}O_2H$) ppm. $C_{13}H_{13}NO_4$ (247.25).

5-(*tert*-Butoxycarbonylamino)pentanoic acid – To a vigorously stirred solution of 5-aminopentanoic acid (2.93 g, 25.0 mmol) and $NaHCO_3$ (5.26 g, 62.5 mmol) in water (50 mL) was added Boc_2O (6.55 g, 30.0 mmol) in 1,4-dioxane (50 mL) and stirring was continued overnight. The resulting mixture was extracted with diethyl ether (50 mL, discarded) and carefully acidified with 2 M hydrochloric acid to pH 2. The aqueous layer was extracted with ethyl acetate (3 × 30 mL) and the combined extracts were washed with brine and dried over anhydrous Na_2SO_4. The volatiles were removed under reduced pressure and the residue was crystallized from diethyl ether/n-pentane. Yield: 4.48 g (82 %) as white powder. Mp 50–52 °C (lit.[30] mp 47–50 °C). $C_{10}H_{19}NO_4$ (217.26); in a similar manner was prepared **6-(*tert*-butoxycarbonylamino)hexanoic acid**. Yield: 4.84 g (84 %) as white powder. Mp 35–37 °C (lit.[31] mp 31–32 °C). $C_{11}H_{21}NO_4$ (231.29).

***trans*-4-(Allyloxycarbonylaminomethyl)cyclohexanecarboxylic acid** – To a solution of *trans*-4-aminomethylcyclohexanecarboxylic acid (3.14 g, 20.0 mmol) in 4 M aq. NaOH (5 mL), chilled to 0 °C, were alternately added allylchloroformate (2.12 mL,

20.0 mmol) and 4 M aq. NaOH (5 mL) in small portions under vigorous stirring over a period of 30 min. After stirring for additional 30 min the solution was extracted with diethyl ether (20 mL, discarded). The aqueous layer was carefully acidified with conc. hydrochloric acid and extracted with CH_2Cl_2 (3 × 25 mL). The combined extracts were dried over anhydrous Na_2SO_4, diluted with n-hexane and concentrated at atmospheric pressure. The resulting white solid was filtered, washed with petroleum ether and dried in vacuum. Yield: 4.48 g (93 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 0.97 (m, 2H, ax. cyclohexyl), 1.33–1.57 (m, 3H, cyclohexyl), 1.84 (m, 2H, cyclohexyl), 2.05 (m, 2H, cyclohexyl), 2.25 (m, 1H, cyclohexyl), 3.05 (m, 2H, -C$\underline{H}_2$NHAlloc), 4.56 (m, 2H, -OC$\underline{H}_2$CH=CH_2), 5.21 (m, 1H, -OCH_2CH=CH(E)-$\underline{H}$), 5.30 (dd, ^2J = 1.6 Hz, ^3J = 17.2 Hz, 1H, -OCH_2CH=CH(Z)-$\underline{H}$), 5.92 (m, 1H, -OCH_2C$\underline{H}$=CH_2) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 28.2 (C-2/6 -$C_6H_{10}CO_2H$), 29.5 (C-3/5 -$C_6H_{10}CO_2H$), 37.6 (C-4 -$C_6H_{10}CO_2H$), 43.0 (C-1 -$C_6H_{10}CO_2H$), 46.9 (-$\underline{C}H_2$NHAlloc), 65.6 (-O$\underline{C}H_2$CH=CH_2), 117.7 (-OCH_2CH=$\underline{C}H_2$), 132.9 (-$OCH_2\underline{C}$H=CH_2), 156.5 (-NH$\underline{C}OOC_3H_5$), 181.4 (-$\underline{C}O_2H$) ppm; ESI-MS (+p) m/z (%): 259 (50) $[MNH_4]^+$, 242 (55) $[MH]^+$, 182 (100) $[MH - C_2H_4O_2]^+$. $C_{12}H_{19}NO_4$ (241.28).

***tert*-Butyl 3-hydroxypropylcarbamate** – To a stirred solution of 3-aminopropan-1-ol (0.75 g, 10.0 mmol) and DIPEA (1.29 g, 10.0 mmol), in CH_2Cl_2 (10 mL) was slowly added Boc_2O (2.40 g, 11.0 mmol) in CH_2Cl_2 (10 mL). After stirring overnight the resulting solution was washed with 1 M aq. NH_4Cl, 5 % aq. $KHSO_4$, 5 % aq. $KHCO_3$, and brine. The organic layers were dried over anhydrous Na_2SO_4 and evaporated yielding *tert*-butyl 3-hydroxypropylcarbamate as colorless oil (1.30 g, 74 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.45 (s, 9H, Boc), 1.68 (m, 2H, -$OCH_2C\underline{H}_2CH_2$NHBoc), 3.27 (t, ^3J =5.3 Hz, 2H, -C$\underline{H}_2$NHBoc), 3.66 (t, ^3J = 5.1 Hz, 2H, HOC$\underline{H}_2$-) ppm; CI-MS (NH_3) m/z (%): 193 (41) $[MNH_4]^+$, 176 (86) $[MH]^+$, 137 (100) $[MNH_4 - C_4H_8]^+$, 120 (34) $[MH - C_4H_8]^+$. $C_8H_{17}NO_3$ (175.23).

***tert*-Butyl 2-(2-hydroxyethoxy)ethylcarbamate** – Boc_2O (2.18 g, 10.0 mmol) in acetone (10 mL) was added to a vigorously stirred solution of 2-(2-

aminoethoxy)ethanol (1.05 g, 10.0 mmol) and (1.38 g, 10.0 mmol) K_2CO_3 in water (10 mL). After 16 h the solution was extracted with ethyl acetate (3 × 20 mL). The combined extracts were washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Yield: 1.95 g (95 %) as colorless oil. ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.32 (s, 9H, Boc), 3.20 (m, 2H, BocNHC$\underline{H}_2$-), 3.38–3.48 (m, 4H, -C$\underline{H}_2$OC$\underline{H}_2$-), 3.61 (m, 2H, -C$\underline{H}_2$OH) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 28.3 (Boc), 40.5 (BocNH$\underline{C}H_2$-), 61.4 (-$\underline{C}H_2$OH), 70.2 (BocNHCH$_2\underline{C}H_2$O-), 72.2 (-O$\underline{C}H_2CH_2$OH), 79.3 (Boc), 165.2 (Boc) ppm; CI-MS (NH_3) m/z (%): 223 (21) $[MNH_4]^+$, 206 (33) $[MH]^+$, 167 (100) $[MNH_4 - C_4H_8]^+$, 150 (25) $[MH - C_4H_8]^+$. $C_9H_{19}NO_4$ (205.25).

4.1.7. PREPARATION OF N-4-FLUOROBENZOYL ω-AMINO ACIDS AND ω-AMINO ALCOHOLS

5-(4-Fluorobenzamido)pentanoic acid – 5-aminopentanoic acid (0.35 g, 3.0 mmol) in THF (10 mL) was treated with DIPEA (0.51 mL, 3.0 mmol) and succinimidyl 4-fluorobenzoate (0.71 g, 3.0 mmol) in DMF (3 mL) and stirred overnight. The mixture was diluted with ethyl acetate (50 mL), washed with 5 % aq. $KHSO_4$ and brine and dried over anhydrous Na_2SO_4. Evaporation of the solvents under reduced pressure afforded a white solid (0.52 g, 72 %). ^{1}H-NMR (300 MHz, DMSO-d_6): δ_H = 1.45–1.61 (m, 4H, -CH$_2$C$\underline{H}_2$C$\underline{H}_2$CH$_2$CO$_2$H), 2.51 (m, 2H, (4-F)BzNHC$\underline{H}_2$-), 3.26 (m, 2H, -C$\underline{H}_2$CO$_2$H), 7.29 (2/6-H ArF), 7.91 (3/5-H ArF), 8.49 (t, ^3J = 5.8 Hz, 1H, -N$\underline{H}$) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 21.9 (-$\underline{C}$H$_2$CH$_2$CO$_2$H), 28.5 (-CH$_2\underline{C}$H$_2$CH$_2$CH$_2$CO$_2$H), 33.2 (-$\underline{C}$H$_2$CO$_2$H), 38.8 (-$\underline{C}$H$_2$NH-), 115.0 (d, $^2J_{C,F}$ = 21.9 Hz), 129.6 (d, $^3J_{C,F}$ = 8.9 Hz), 131.0 (d, $^4J_{C,F}$ = 2.9 Hz), 163.6 (d, $^1J_{C,F}$ = 248.1 Hz), 164.9 (-$\underline{C}$(O)C$_6$H$_4$F), 174.3 (-$\underline{C}$O$_2$H) ppm; ESI-MS (+p) m/z (%): 240 (100) $[MH]^+$, 479 (15) $[2M + H]^+$. $C_{12}H_{14}FNO_3$ (239.24).

6-(4-Fluorobenzamido)hexanoic acid – 6-aminohexanoic acid (0.33 g, 2.5 mmol) was dissolved in a solution of $NaHCO_3$ (0.42 g, 5.0 mmol) in water (5 mL) and combined with a solution of succinimidyl 4-fluorobenzoate (0.59 g, 2.5 mmol) in acetonitrile (5 mL). After stirring overnight the mixture was acidified with 2 M

hydrochloric acid and extracted with ethyl acetate (2 × 25 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Yield: 0.52 g (82 %). ^{1}H-NMR (300 MHz, DMSO-d_6): δ_H = 1.32 (m, 2H, -$(CH_2)_2$C$\underline{H}_2$$(CH_2)_2$-), 1.44–1.60 (m, 4H, -$CH_2C\underline{H}_2$$CH_2C\underline{H}_2$$CH_2$-), 2.21 (t, ^3J = 7.3 Hz, 2H, -CH_2C$\underline{H}_2$$CO_2$H), 3.24 (m, 2H, -$CH_2C\underline{H}_2$NH-), 7.29 (m, 2H, 2/6-H ArF), 7.91 (m, 2H, 3/5-H ArF), 8.48 (t, ^3J = 5.5 Hz, 1H, -NH-), 12.03 (brs, 1H, -$CO_2$$\underline{H}$) ppm; ^{13}C-NMR (300 MHz, DMSO-d_6): δ_C = 24.1 (-$\underline{C}H_2CH_2CO_2H$), 25.9 (-$(CH_2)_2$$\underline{C}H_2$$(CH_2)_2$-), 28.7 (-$\underline{C}H_2CH_2NH$-), 33.5 (-$\underline{C}H_2CO_2H$), 39.0 (-$\underline{C}H_2NH$-), 115.0 (d, $^2J_{C,F}$ = 21.6 Hz), 129.6 (d, $^3J_{C,F}$ = 8.9 Hz), 131.0 (d, $^4J_{C,F}$ = 2.9 Hz), 163.6 (d, $^1J_{C,F}$ = 248.2 Hz), 164.9 (-$\underline{C}$(O)C_6H_4F), 174.4 (-$\underline{C}O_2H$) ppm; EI-MS (70 eV) m/z (%): 253 (10) $M^{\cdot+}$, 194 (10) [M – CH_2CO_2H]$^+$, 152 (12) [F-C_6H_4C(O)NH=CH_2]$^+$, 140 (4) [F-C_6H_4C(O)NH_3]$^+$, 123 (100) [F-C_6H_4CO]$^+$. $C_{13}H_{16}FNO_3$ (253.27).

***trans*-4-(4-Fluorobenzamidomethyl)cyclohexanecarboxylic acid** – *trans*-4-(Aminomethyl)cyclohexanecarboxylic acid (0.47 g, 3.0 mmol) in THF (10 mL) was treated with DIPEA (0.51 mL, 3.0 mmol) and succinimidyl 4-fluorobenzoate (0.71 g, 3.0 mmol) in DMF (3 mL) and stirred overnight. The mixture was diluted with ethyl acetate (50 mL), washed with 5 % aq. $KHSO_4$ and brine and dried over anhydrous Na_2SO_4. Evaporation of the solvents under reduced pressure afforded a white solid (0.75 g, 89 %). ^{1}H-NMR (300 MHz, DMSO-d_6): δ_H = 0.96 (m, 2H, 3/5-H ax. cyclohexyl), 1.26 (m, 2H, 3/5-H eq. cyclohexyl) 1.50 (m, 1H, 4-H, cyclohexyl), 1.77 (m, 2H, 2/6-H ax. cyclohexyl), 1.96 (m, 2H, 2/6-H eq. cyclohexyl), 2.14 (m, 1H, 1-H cyclohexyl), 3.11 (m, 2H, -C$\underline{H}_2$NH-), 7.28 (m, 2H, 2/6-H ArF), 7.91 (m, 2H, 3/5-H ArF), 8.47 (t, ^3J = 5.7 Hz, 1H, -N$\underline{H}$-) ppm; ^{13}C-NMR (300 MHz, DMSO-d_6): δ_C = 28.2 (C-2/6 cyclohexyl), 29.5 (C-3/5 cyclohexyl), 36.9 (C-4 cyclohexyl), 42.4 (C-1 cyclohexyl), 45.2 (-NHC$\underline{H}_2$-), 115.0 (d, $^2J_{C,F}$ = 21.6 Hz), 129.7 (d, $^3J_{C,F}$ = 9.0 Hz), 131.0 (d, $^4J_{C,F}$ = 2.9 Hz), 163.6 (d, $^1J_{C,F}$ = 248.0 Hz), 165.1 (-$\underline{C}$(O)C_6H_4F), 176.7 (-$\underline{C}O_2H$) ppm; EI-MS (70 eV) m/z (%): 279 (8) $M^{\cdot+}$, 152 (24) [F-C_6H_4C(O)NH=CH_2]$^+$, 140 (49) [F-C_6H_4C(O)NH_3]$^+$, 123 (100) [F-C_6H_4CO]$^+$. $C_{15}H_{18}FNO_3$ (279.31).

4-Fluoro-*N*-(3-hydroxypropyl)benzamide – To a stirred solution of 3-aminopropan-1-ol (0.75 g, 10.0 mmol) and DIPEA (1.29 g, 10.0 mmol), in CH_2Cl_2 (10 mL) was slowly added succinimidyl 4-fluorobenzoate (2.37 g, 10.0 mmol) in CH_2Cl_2 (10 mL). After stirring overnight the resulting solution was washed with water, 5 % aq. $KHSO_4$, 5 % aq. $KHCO_3$ and brine. The organic layers were dried over anhydrous Na_2SO_4 and evaporated yielding 0.20 g (10 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.80 (m, 2H, -$CH_2C\underline{H}_2CH_2OH$), 3.61 (m, 2H, -$CH_2CH_2C\underline{H}_2NH$-), 3.73 (t, ^3J = 5.5 Hz, 2H, -$CH_2CH_2C\underline{H}_2OH$), 7.09 (m, 2H, 2/6-H ArF), 7.80 (m, 2H, 3/5-H ArF) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 31.9 (-$CH_2\underline{C}H_2CH_2OH$), 37.6 (-$CH_2CH_2\underline{C}H_2NH$-), 60.1 (-$CH_2CH_2\underline{C}H_2OH$), 115.0 (d, $^2J_{C,F}$ = 22.1 Hz), 129.3 (d, $^3J_{C,F}$ = 8.8 Hz), 130.3 (d, $^4J_{C,F}$ = 2.9 Hz), 164.8 (d, $^1J_{C,F}$ = 252.1 Hz), 167.5 (-$\underline{C}(O)C_6H_4F$) ppm; EI-MS (70 eV) m/z (%): 197 (5) $M^{\bullet+}$, 179 (10) $[M - H_2O]^+$, 153 (16) $[M - C_2H_4O]^+$, 123 (100) $[F\text{-}C_6H_4CO]^+$. $C_{10}H_{12}FNO_2$ (197.21).

***N*-[2-(2-Hydroxyethoxy)ethyl]-4-fluorobenzamide** – 2-(2-Aminoethoxy)ethanol-amin (2.10 g, 20.0 mmol) and succinimidyl 4-fluorobenzoate (4.74 g, 20.0 mmol) were combined in ethyl acetate (75 mL) and stirred for 16 h at ambient temperature. The resulting solution was washed with water, 5 % aq. K_2CO_3, 5 % aq. $KHSO_4$ and brine, dried over anhydrous Na_2SO_4 and evaporated. The crude product was purified by vacuum flash chromatography, eluting with petroleum ether/ethyl acetate. Yield: 1.9 g (42 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 3.60 (m, 2H, -$OCH_2C\underline{H}_2NH$-), 3.65 (m, 4H, -$C\underline{H}_2OC\underline{H}_2$-), 3.74 (m, 2H, -$OCH_2C\underline{H}_2OH$), 7.07 (m, 2H, 2/6-H ArF), 7.81 (m, 2H, 3/5-H ArF) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 40.0 (-$OCH_2\underline{C}H_2NH$-), 61.7 (-$OCH_2\underline{C}H_2OH$), 69.8 (-$O\underline{C}H_2CH_2NH$-), 72.2 (-$O\underline{C}H_2CH_2OH$), 115.0 (d, $^2J_{C,F}$ = 22.0 Hz), 129.5 (d, $^3J_{C,F}$ = 8.9 Hz), 130.3 (d, $^4J_{C,F}$ = 3.1 Hz), 164.7 (d, $^1J_{C,F}$ = 252.0 Hz), 167.0 (-$\underline{C}(O)C_6H_4F$) ppm; EI-MS (70 eV) m/z (%): 228 (3) $M^{\bullet+}$, 165 (25) $[F\text{-}C_6H_4C(O)NHC_2H_3]^+$, 123 (100) $[F\text{-}C_6H_4CO]^+$. $C_{11}H_{14}FNO_3$ (227.23).

4.1.8. PREPARATION OF GUANIDINYLATION REAGENTS

4.1.8.1. N,N'-disubstituted 1H-pyrazole-1-carboxamidines:

1*H*-Pyrazole-1-carboxamidine hydrochloride – To a vigorously stirred solution of aminoguanidine carbonate (27.22 g, 0.20 mol) in water (50 mL) and conc. hydrochloric acid (34 mL), gently warmed to 40 °C, was slowly added 1,1,3,3-tetramethoxypropane (34.48 g, 0.21 mol) over a period of 3 h. The mixture was diluted with acetone (250 mL) and concentrated under reduced pressure until a crop of crystals had formed. After cooling to ambient temperature the crystals were collected on a sintered filter, washed with acetone and dried. Yield: 22.0 g (75 %). mp 155–157 °C (lit[32] mp 165–166 °C) ^{1}H-NMR (300 MHz, DMSO-d_6) δ_H = 6.80 (dd, ^3J = 1.6, ^3J = 2.9 Hz, 1H, 4-H), 8.10 (d, ^3J = 1.5 Hz, 1H, pyrazole), 8.93 (d, ^3J = 2.9 Hz, 1H, pyrazole), 9.49 (brs, 2H, -N$\underline{H}_2$), 9.76 (brs, 2H, -N$\underline{H}_2$) ppm. $C_4H_7ClN_4$ (146.58).

***N*-(5-Phthalimidopentanoyl)-1*H*-pyrazole-1-carboxamidine** – 5-Phthalimidopentanoic acid (3.17 g, 15.0 mmol) was dissolved in anhydrous CH_2Cl_2 (50 mL) and treated with CDI (2.43 g, 15.0 mmol) under exclusion of moisture. After stirring for 45 min 1*H*-pyrazole-1-carboxamidine hydrochloride (2.20 g, 20.0 mmol) and DIPEA (5.14 mL, 30.0 mmol) were added and stirring was continued for 16 h. The resulting solution was washed with 5 % aq. $KHSO_4$ and brine, dried over anhydrous Na_2SO_4 and evaporated. Recrystallization from hot MTBE – ethyl acetate afforded 4.0 g (79 %) product. mp 110–111 °C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.76 (m, 4H, -$CH_2C\underline{H}_2C\underline{H}_2CH_2$NPht), 2.54 (m, 2H, -$C\underline{H}_2CH_2CH_2CH_2$NPht), 3.74 (-$CH_2CH_2CH_2C\underline{H}_2$NPht), 6.42 (dd, ^3J = 1.6 Hz, ^3J = 2.7 Hz, 1H, pyrazole), 7.70–7.74 (m, 2H, Pht, and 1H, pyrazole), 7.84 (dd, ^3J = 1.6 Hz, ^3J = 2.7 Hz, 2H, Pht), 8.43 (d, ^3J = 2.7 Hz, 1H, pyrazole), 9.82 (brs, 2H, -N$\underline{H}_2$) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 22.7 (-$CH_2\underline{C}H_2CH_2CH_2$NPht), 28.2 (-$CH_2CH_2\underline{C}H_2CH_2$NPht), 37.8 (-$\underline{C}H_2CH_2CH_2CH_2$NPht), 40.6 (-$CH_2CH_2CH_2\underline{C}H_2$NPht), 109.1 (C-4 pyrazole), 123.2 (Pht) 128.8 (C-5 pyrazole), 132.1 (Pht), 133.9 (Pht), 143.7 (C-3 pyrazole), 153.6

(NC(N)N), 168.4 (Pht), 188.9 (C=O) ppm; ESI-MS (+p) m/z (%): 340 (100) $[MH]^+$. $C_{17}H_{17}N_5O_3$ (339.35).

***N,N'*-Bis(*tert*-butoxycarbonyl)-*N*-(5-phthalimidopentanoyl)-1*H*-pyrazole-1-carboxamidine** – *N*-(5-Phthalimidopentanoyl)-1*H*-pyrazole-1-carboxamidine (3.39 g, 10.0 mmol) and Boc_2O (5.46 g, 25.0 mmol) were dissolved in dry CH_2Cl_2 (50 mL) and treated with DMAP (0.30 g). After stirring overnight at ambient temperature the reaction mixture was washed with water (twice), 5 % aq. $KHSO_4$, 5 % aq. $KHCO_3$ and brine. The CH_2Cl_2 phase was dried over anhydrous Na_2SO_4, decolorized with activated charcoal and filtered through a pad of silica. The volatiles were removed and the residual product was purified by vacuum flash chromatography (eluent: petroleum ether/ethyl acetate 10:1 to 5:1 v/v). Yield 2.39 g (44 %). ^{1}H-NMR (300 MHz, $CDCl_3$) for the major isomer: δ_H = 1.37 (s, 9H, Boc), 1.51 (s, 9H, Boc), 1.66–186 (m, 4H, -CH_2CH_2C$H_2$$CH_2$NPht), 3.02 (m, 2H, -C$H_2$$CH_2$$CH_2$$CH_2$NPht), 3.72 (t, ^3J = 6.5 Hz, 2H, -$CH_2$$CH_2$$CH_2CH_2$NPht), 6.43 (dd, ^3J = 1.1 Hz, ^3J = 2.7 Hz, 1H, pyrazole), 7.67 (d, ^3J = 1.1 Hz, 1H, pyrazole), 7.68–7.88 (m, 4H, Pht), 8.25 (d, ^3J = 2.7 Hz, 1H, pyrazole) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 21.5 (C^{β}), 27.0 (C^{γ}), 27.6 (Boc), 27.9 (Boc), 36.2 (C^{α}), 37.7 (C^{δ}), 83.0 (Boc), 85.0 (Boc), 110.1 (C-3 pyrazole), 123.2 (Pht), 129.1 (C-5 pyrazole), 132.2 (Pht), 133.9 (Pht), 142.3 (C-3 pyrazole), 144.0 (XC(X)N), 148.7 (XC(X)N), 156.6 (XC(X)N), 168.4 (Pht), 173.1 (C=O) ppm; ESI-MS (+p) m/z (%): 540.2 (100) $[MH]^+$, 557.3 (97) $[M + NH_4]^+$, 440.1 (23) $[MH - Boc]^+$. $C_{27}H_{33}N_5O_7$ (539.58).

***N*-*tert*-Butoxycarbonyl-*N'*-(5-phthalimidopentanoyl)-1*H*-pyrazole-1-carboxamidine** (**11a**) – To a solution of *N,N'*-bis(*tert*-butoxycarbonyl)-*N*-(5-phthalimidopentanoyl)-1*H*-pyrazole-1-carboxamidine (0.89 g, 1.65 mmol) in THF (10 mL) was added $Mg(ClO_4)_2$ (18 mg), and the mixture was placed in a warm water bath (50–60 °C) and stirred for 2 h. The THF was removed under reduced pressure and the residue was taken up in CH_2Cl_2 (40 mL). The solution was washed with water, dried over anhydrous Na_2SO_4 and concentrated in vacuum. Purification by vacuum flash chromatograhy (eluent: petroleum ether/ethyl acetate) afforded 0.65 g (89 %) **11a** as

a white powder. ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.49 (s, 9H, Boc), 1.65–1.90 (m, 4H, -$CH_2C\underline{H}_2C\underline{H}_2CH_2$NPht), 2.60 (m, 2H, -$C\underline{H}_2(CH_2)_3$NPht), 3.73 (t, ^3J = 6.5 Hz, 2H, -$CH_2CH_2CH_2C\underline{H}_2$NPht), 6.45 (dd, ^3J = 1.1 Hz, ^3J = 2.7 Hz, 1H, pyrazole), 7.63 (d, ^3J = 1.1 Hz, 1H, pyrazole), 7.68–7.88 (m, 4H, Pht), 8.31 (d, ^3J = 2.7 Hz, 1H, pyrazole) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 21.5 ($\underline{C}^{\beta}$), 27.8 ($\underline{C}^{\gamma}$), 28.1 (Boc), 36.6 ($\underline{C}^{\alpha}$), 37.2 ($\underline{C}^{\delta}$), 81.6 (plus additional signal for minor tautomer at 83.5, Boc), 110.0 (C-3 pyrazole), 123.3 (Pht), 128.9 (C-5 pyrazole), 132.0 (Pht), 134.0 (Pht), 142.7 (C-3 pyrazole), 149.4 (C_q), 168.4 (Pht), 170.0 (C_q), 174.0 ($\underline{C}$=O) ppm; ESI-MS (+p) m/z (%): 440 (100) $[MH]^+$, 407 (77) $[X + NH_4]^+$; $C_{22}H_{25}N_5O_5$ (439.46).

Similarly was prepared ***N-tert*-butoxycarbonyl-*N'*-[*trans*-4-(allyloxycarbonyl-aminomethyl)cyclohexanecarbonyl]-1*H*-pyrazole-1-carboxamidine** (**11b**). Yield: 0.40 g (71 %). $C_{21}H_{31}N_5O_5$ (433.50).

4.1.8.2. N,N'-Disubstituted guanidines:

***N-tert*-Butoxycarbonylguanidine**[33] – Boc_2O (27.4 g, 0.125 mol) in 1,4-dioxane (160 mL) was dropped to a stirred solution of guanidine hydrochloride (15.0 g, 0.157 mol) in 4 M NaOH (80 mL), cooled to 0 °C. The mixture was allowed to warm to ambient temperature and stirring was continued for 16 h. The solvents were removed under reduced pressure and the residue was suspended in water and sonicated for 10 min. The white solid was collected on a filter, suspended in diethyl ether, sonicated for 10 min, filtered and subsequently dried in a desiccator over P_4O_{10}. The product was isolated as a white solid in 73 % yield (14.5 g). Mp > 184 °C (decomp.); ^{1}H-NMR (300 MHz, DMSO-d_6): δ_H = 1.34 (s, 9H, Boc), 6.81 (brs, 4H, -N$\underline{H}$'s) ppm; 28.1 (Boc), 75.4 (Boc), 162.6 ($\underline{C}$=X), 163.2 ($\underline{C}$=X) ppm. $C_6H_{13}N_3O_2$ (159.19).

***N-tert*-Butoxycarbonyl-*N'*-(4-fluorobenzoyl)guanidine** – 4-Fluorobenzoic acid (0.70 g, 5.0 mmol) was dissolved in dry DMF (10 mL) and activated with CDI (0.81 g, 5.0 mmol). After 45 min *N-tert*-butoxycarbonylguanidine (0.79 g, 5.0 mmol) was introduced and the reaction mixture was stirred overnight. DMF was removed

under reduced pressure and the residue was dissolved in CH_2Cl_2 (25 mL) washed with 5 % aq. $KHSO_4$, and brine, and dried over anhydrous Na_2SO_4. After evaporation the product remained as colorless oil which slowly solidified in the refrigerator. Recrystallization from methanol afforded 0.84 g (60 %) crystalline product. mp 141–142 °C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.40 (s, 9H, Boc), 7.12 (m, 2H, 2/6-H ArF), 8.21 (m, 2H, 3/5-H ArF), 9.40 (brs, 3H, -NH's) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 27.8 (Boc), 84.0 (Boc), 115.3 (d, $^2J_{C,F}$ = 22.1 Hz), 131.6 (d, $^3J_{C,F}$ = 9.1 Hz), 132.1 (C_q), 153.4 (N<u>C</u>(O)O), 158.9 (N<u>C</u>(N)N), 165,6 (d, $^1J_{C,F}$ = 253.6 Hz), 175.7 (Ar<u>C</u>O) ppm; Cl-MS (NH_3) m/z (%): 282 (100) $[MH]^+$, 226 (10) $[MH - {}^t\!Bu]^+$, 182 (20) $[MH - Boc]^+$; $C_{13}H_{16}FN_3O_3$ (281.28).

4.1.8.3. <u>*N,N'-Disubstituted S-methylisothioureas:*</u>

***S*-Methylisothiouronium iodide** – A suspension of thiourea (7.61 g, 0.1 mol) in ethanol (100 mL) was placed in a 500 mL flask equipped with reflux condenser and stirring bar. The mixture was stirred and cooled in an ice-water bath; iodomethane (14.2 g, 0.1 mol) was carefully added through the condenser and the mixture was heated to reflux. After 1 h the heater was turned off and stirring was continued overnight. The mixture was concentrated under reduced pressure to a volume of about 15 mL and diluted with diethyl ether (150 mL). The resulting crystalline product was collected on a sintered filter, washed with diethyl ether (2 × 50 mL) and dried in vacuum. Yield: 21.3 g (98 %). mp 114–115 °C (lit.[34] mp 117 °C). $C_2H_7IN_2S$ (218.06).

***N*-*tert*-butoxycarbonyl-*S*-methylisothiourea** – To a vigorously stirred suspension of S-methylisothiouronium iodide (18.5 g, 85.0 mmol) in CH_2Cl_2 (75 mL) surrounded by an ice-water bath was added triethylamine (11.9 mL, 85.0 mmol). To the clear solution Boc_2O (16.4 g, 75.0 mmol), dissolved in CH_2Cl_2 (75 mL), was added slowly over a period of 1 h. The cooling bath was removed and stirring was continued overnight. The reaction mixture was diluted with CH_2Cl_2 and washed with water, 5 % aq. $KHSO_4$, 5 % aq. $KHCO_3$ and brine and dried over anhydrous Na_2SO_4. n-

Hexane (100 mL) was added and the solution was concentrated in a rotary evaporator until the product precipitated. The mixture was cooled and the crystalline product was collected on a filter and dried in vacuum. Yield: 10.9 g (76 %). mp 76–78 °C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ_H = 1.40 (s, 9H, Boc), 2.31 (s, 3H, -SC<u>H</u>$_3$), 8.56 (brs, 2H, -N<u>H</u>$_2$) ppm; ^{13}C-NMR (300 MHz, DMSO-d_6): δ_C = 12.7 (S<u>C</u>H$_3$), 27.8 (Boc), 77.7 (Boc), 160.6 (Boc), 171.4 (N<u>C</u>(N)SMe) ppm; EI-MS (70 eV) m/z (%): 190 (17) $M^{\cdot+}$, 175 (50) $[M - CH_3]^+$, 134 (44) $[M - C_4H_8]^+$, 57 (100) $[C_4H_9]^+$. $C_7H_{14}N_2O_2S$ (190.26).

***N*-*tert*-Butoxycarbonyl-*N'*-(3-phenylpropanoyl)-*S*-methylisothiourea** (**10a**): To a solution of *N*-*tert*-butoxycarbonyl-*S*-methylisothiourea (0.76 g, 4.0 mmol) in dry CH_2Cl_2 (10 mL) was added DIPEA (4.0 mmol) in CH_2Cl_2 (10 mL). A solution of 3-phenylpropanoyl chloride (4.0 mmol) in CH_2Cl_2 (5 mL) was introduced and the reaction mixture was mechanically shaken overnight. The solution was washed with water, 5 % aq. $KHSO_4$, 5 % aq. $KHCO_3$, and brine, and dried over anhydrous Na_2SO_4. The solution is diluted with n-hexane and CH_2Cl_2 is evaporated. The solid product which precipitated from the cold solution was collected on a filter and dried in vacuum. ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.51 (s, 9H, Boc), 2.41 (s, 3H, -SC<u>H</u>$_3$), 2.80 (t, ^3J = 7.6 Hz, 2H, -COC<u>H</u>$_2$CH$_2$Ph), 3.01 (t, ^3J = 7.6 Hz, 2H, -COCH$_2$C<u>H</u>$_2$Ph), 7.15–7.34 (m, 5H, Ph) ppm; CI-MS (NH_3) m/z (%): 323 (100) $[MH]^+$, 223 (23) $[MH\text{-Boc}]^+$. $C_{16}H_{22}N_2O_3S$ (322.42).

***N*-*tert*-Butoxycarbonyl-*N'*-(5-*tert*-butoxycarbonylaminopentanoyl)-*S*-methylisothiourea** (**10b**) – 5-*tert*-Butoxycarbonylaminopentanoic acid (0.43 g, 2.0 mmol) and *N*-*tert*-butoxycarbonyl-*S*-methylisothiourea (0.38 g, 2.0 mmol) in THF (5 mL) were treated with HOBt · H_2O (0.31 g, 2.0 mmol) and EDC · HCl (0.38 g, 2.0 mmol). After stirring for 16 h ethyl acetate (40 mL) was added and the solution was washed with water, 5 % aq. $KHSO_4$, 5 % aq. $KHCO_3$, and brine and dried over anhydrous Na_2SO_4. Evaporation of the volatiles afforded 0.75 g (96 %) of a colorless oil. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.44 (s, 9H, Boc), 1.52 (s, 9H, Boc), 1.54 (m, 2H, -CH$_2$CH$_2$C<u>H</u>$_2$CH$_2$NHBoc), 1.70 (m, 2H, -CH$_2$C<u>H</u>$_2$CH$_2$CH$_2$NHBoc), 2.42 (s, 3H,

-SC$\underline{H}_3$), 2.50 (t, ^3J = 7.2 Hz, 2H, -C$\underline{H}_2$CH$_2$CH$_2$CH$_2$NHBoc), 3.14 (m, 2H, -C$\underline{H}_2$NHBoc), 4.62 (brs, 1H, -N$\underline{H}$) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 14.7 (-S$\underline{C}$H$_3$), 21.8 (-CH$_2\underline{C}$H$_2$CH$_2$CH$_2$NHBoc), 28.0 (Boc), 28.4 (Boc), 29.4 (-CH$_2$CH$_2\underline{C}$H$_2$CH$_2$NHBoc), 30.8 (-$\underline{C}$H$_2$(CH$_2$)$_3$NHBoc), 40.1 (-$\underline{C}$H$_2$NHBoc), 77.3 (Boc), 79.2 (Boc), 156.0 (Boc), 169.4 (N$\underline{C}$(SMe)N), 171.3 ($\underline{C}$=O) ppm; CI-MS (NH_3) m/z (%): 390 (100) $[MH]^+$, 290 (27) [MH – Boc]$^+$. $C_{17}H_{31}N_3O_5S$ (389.51).

***N*-*tert*-Butoxycarbonyl-*N'*-(6-*tert*-butoxycarbonylaminohexanoyl)-*S*-methylisothiourea** (**10c**) – To a stirred solution of 6-(*tert*-butoxycarbonylamino)hexanoic acid (1.16 g, 5.0 mmol), HOBt · H_2O (0.76 g, 5.0 mmol) and DIPEA (1.71 mL, 5.0 mmol) in DMF (10 mL) was added TBTU (1.61 g, 5.0 mmol). *N*-*tert*-Butoxycarbonyl-*S*-methylisothiourea (0.95 g, 5.0 mmol) was added and stirring was continued for 3 h. The solution was diluted with ethyl acetate (80 mL) and washed with 5 % aq. $KHSO_4$, 5 % aq. $KHCO_3$, and brine, dried over anhydrous Na_2SO_4, and evaporated. Crystallization from n-pentane/CH_2Cl_2 afforded 1.72 g (85 %) product as a white solid. ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.40–1.60 (m, 4H, -CH$_2$CH$_2$C$\underline{H}_2\underline{C}H_2CH_2$NHBoc), 1.44 (s, 9H, Boc), 1.52 (s, 9H, Boc), 1.70 (m, 2H, -CH$_2\underline{C}$H$_2$(CH$_2$)$_3$NHBoc), 2.40 (s, 3H, -SC$\underline{H}_3$), 2.45 (t, ^3J = 7.3 Hz, 2H, -C$\underline{H}_2$(CH$_2$)$_4$NHBoc), 3.12 (m, 2H, -(CH$_2$)$_4\underline{C}$H$_2$NHBoc), 4.56 (brs, 1H, -N$\underline{H}$) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 14.5 (-S$\underline{C}$H$_3$), 24.4 (-CH$_2\underline{C}$H$_2$(CH$_2$)$_3$NHBoc), 26.2 (-CH$_2$CH$_2\underline{C}$H$_2$CH$_2$CH$_2$NHBoc), 28.0 (Boc), 28.4 (Boc), 29.8 (-$\underline{C}$H$_2$CH$_2$NHBoc), 37.1 (-$\underline{C}$H$_2$(CH$_2$)$_4$NHBoc), 40.4 (-(CH$_2$)$_4\underline{C}$H$_2$NHBoc), 77.2 (Boc), 79.1 (Boc), 156.0 (Boc), 169.3 (N$\underline{C}$(SMe)N), 171.1 ($\underline{C}$=O) ppm; ESI-MS (+p) m/z (%): 404 (100) $[MH]^+$, 304 (45) [MH – Boc]$^+$. $C_{18}H_{33}N_3O_5S$ (403.54).

***N*-*tert*-Butoxycarbonyl-*N'*-[3-(*tert*-butoxycarbonylamino)propyloxycarbonyl]-*S*-methylisothiourea** (**10g**) – 3-(*tert*-butoxycarbonylamino)propan-1-ol (0.94 g, 5.36 mmol) were dissolved in anhydrous acetonitrile and treated with DSC (2.06 g, 8.0 mmol) and triethylamine (2.23 mL, 16.0 mmol). The reaction mixture was stirred overnight at ambient temperature. Acetonitrile was removed under reduced pressure and the residue was taken up in ethyl acetate (50 mL). The ethyl acetate

layer was washed with aq. $NaHCO_3$ solution, and brine, dried over anhydrous Na_2SO_4, and evaporated to dryness. The raw 3-(*tert*-butoxycarbonylamino)propyl succinimidyl carbonate was redissolved in CH_2Cl_2 (40 mL); triethylamine (1.11 mL, 8.0 mmol) and *N*-*tert*-butoxycarbonyl-*S*-methylisothiourea (1.02 g, 5.36 mmol) were introduced and the mixture was left overnight. The resulting solution was concentrated and diluted with ethyl acetate. The organic phase was washed with water, 5 % aq. $KHCO_3$, 0.6 % aq. acetic acid, and brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The product was purified by flash chromatography, eluting with petroleum ether/ethyl acetate 5:1 (v/v). Yield: 1.36 g (65 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.44 (s, 9H, Boc), 1.51 (s, 9H, Boc), 1.90 (m, 2H, -$OCH_2C\underline{H}_2CH_2NHBoc$), 2.41 (s, 3H, -$SC\underline{H}_3$), 3.24 (m, 2H, -$OCH_2CH_2C\underline{H}_2NHBoc$), 4.23 (t, ^3J = 6.2 Hz, 2H, -$OC\underline{H}_2CH_2CH_2NHBoc$), 4.81 (brs, 1H, -N<u>H</u>) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 14.6 (-$S\underline{C}H_3$), 28.0 (Boc), 28.4 (Boc), 29.1 (-$\underline{C}H_2CH_2NHBoc$), 37.6 (-$\underline{C}H_2NHBoc$), 64.0 (-$O\underline{C}H_2(CH_2)_2NHBoc$), 77.3 (Boc), 79.4 (Boc), 148.7 (N<u>C</u>(O)O), 156.0 (Boc), 156.1 (Boc), 172.7 (N<u>C</u>(SMe)N) ppm; ESI-MS (+p) m/z (%): 392 (100) $[MH]^+$. $C_{16}H_{29}N_3O_6S$ (391.48).

***N*-*tert*-Butoxycarbonyl-*N'*-[5-(4-fluorobenzamido)pentanoyl]-*S*-methylisothiourea** (**10d**) – 5-(4-Fluorobenzamido)pentanoic acid (0.24 g, 1.0 mmol) in dry CH_2Cl_2 (5 mL) was activated with CDI (0.16 g, 1.0 mmol). After stirring for 45 min *N*-*tert*-butoxycarbonyl-*S*-methylisothiourea (0.19 g, 1.0 mmol) was added and stirring was continued for 16 h. The solvent was removed and the residue redissolved in ethyl acetate (30 mL), washed with water, 5 % aq. $KHSO_4$, 5 % aq. $KHCO_3$ and brine, dried over anhydrous Na_2SO_4, and concentrated. Flash chromatography eluting with $CHCl_3$ afforded 50 mg (51 %) **10d** as a colorless oil. 1H-NMR (300 MHz, DMSO-d_6): δ_H = 1.41 (s, 9H, Boc), 1.45–1.62 (m, 4H, -$CH_2C\underline{H}_2C\underline{H}_2CH_2NHBz(4\text{-}F)$), 2.28 (s, 3H, -$SC\underline{H}_3$), 2.39 (m, 2H, -$C\underline{H}_2(CH_2)_3NHBz(4\text{-}F)$), 3.25 (m, 2H, -$(CH_2)_3C\underline{H}_2NHBz(4\text{-}F)$), 7.29 (m, 2H, 2/6-H ArF), 7.90 (m, 2H, 3/5-H ArF), 8.48 (t ^3J = 5.5 Hz, 1H, -N<u>H</u>), 11.20 (s, 1H, -N<u>H</u>) ppm; 13C-NMR (300 MHz, $CDCl_3$): δ_C = 13.8 (-$S\underline{C}H_3$), 21.6 (-$CH_2\underline{C}H_2(CH_2)_2NHBz(4\text{-}F)$), 27.6 (Boc), 28.3

(-$(CH_2)_2$C$H_2$$CH_2$NHBz(4-F)), 35.4 (-C$H_2$$(CH_2)_3$NHBz(4-F)), 38.7 (-$(CH_2)_3CH_2$NHBz(4-F)), 79.9 (Boc), 115.0 (d, $^2J_{C,F}$ = 21.8 Hz), 129.6 (d, $^3J_{C,F}$ = 8.9 Hz), 131.0 (d, $^4J_{C,F}$ = 2.8 Hz), 157.9 (Boc), 161.8 (NC(SMe)N), 163.6 (d, $^1J_{C,F}$ = 248.1 Hz), 164.9 (-C(O)C_6H_4F), 171.0 (C=O) ppm; CI-MS (NH_3) *m/z* (%): 412 (100) $[MH]^+$, 312 (5) $[MH - Boc]^+$. $C_{19}H_{26}FN_3O_4S$ (411.49).

***N*-*tert*-Butoxycarbonyl-*N'*-[6-(4-fluorobenzamido)hexanoyl]-*S*-methylisothiourea** (**10e**) – To a stirred solution of 6-(4-fluorobenzamido)hexanoic acid (0.51 g, 2.0 mmol), HOBt · H_2O (0.30 g, 2.0 mmol), DIPEA (0.34 mL, 4.0 mmol) and TBTU (0.63 g, 2.0 mmol) in DMF (5 mL) was added *N*-*tert*-butoxycarbonyl-*S*-methylisothiourea (0.38 g, 2.0 mmol) and stirring was continued for 2 h. The reaction mixture was poured into diluted HCl (pH > 2) and the product was extracted into ethyl acetate. The organic layers were combined, washed with 5 % aq. $KHCO_3$, and brine, and dried over anhydrous Na_2SO_4. The volatiles were removed under reduced pressure and the crude residue was purified by flash chromatography (eluent: petroleum ether/ethyl acetate 3:2 v/v). Yield: 0.53 g (62 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.45 (m, 2H, -$(CH_2)_2$C$H_2$$(CH_2)_2$NHBz(4-F)), 1.52 (s, 9H, Boc), 1.57 – 1.80 (m, 4H, -CH_2C$H_2$$CH_2CH_2$$CH_2$NHBz(4-F)), 2.40 (s, 3H, -SCH_3), 2.48 (t, ^3J = 7.0 Hz, 2H, -C$H_2$$(CH_2)_4$NHBz(4-F)), 3.03 (m, 2H, -$(CH_2)_4$CH_2NHBz(4-F)), 6.35 (brs, 1H, -NH), 7.10 (m, 2H, 2/6-H ArF), 7.80 (m, 2H, 3/5-H ArF), 12.48 (brs, 1H, -NH) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 14.6 (-SCH_3), 23.9 (-CH_2C$H_2$$(CH_2)_3$NHBz(4-F)), 26.2 (-$(CH_2)_2CH_2$$(CH_2)_2$NHBz(4-F)), 28.0 (Boc), 29.1 (-$(CH_2)_3$C$H_2$$CH_2$NHBz(4-F)), 37.2 (-C$H_2$$(CH_2)_4$NHBz(4-F)), 39.6 (-$(CH_2)_4CH_2$NHBz(4-F)), 77.3 (Boc), 115.5 (d, $^2J_{C,F}$ = 22.0 Hz), 129.2 (d, $^3J_{C,F}$ = 8.9 Hz), 130.8 (d, $^4J_{C,F}$ = 3.3 Hz), 164.6 (d, $^1J_{C,F}$ = 251.4 Hz), 166.5 (-C(O)C_6H_4F), 171.3 (C=O) ppm, signals for C=O (Boc) and NC(SMe)N not visible; ESI-MS (+p) m/z (%): 426 (100) $[MH]^+$, 326 (80) $[MH - Boc]^+$. $C_{20}H_{28}FN_3O_4S$ (425.52).

***N*-*tert*-Butoxycarbonyl-*N'*-[*trans*-4-(4-fluorobenzamidomethyl)cyclohexane-carbonyl]-*S*-methylisothiourea** (**10f**) – EDC · H_2O (0.19 g, 1.0 mmol) was added to a stirred solution of *trans*-4-(4-fluorobenzamido-methyl)cyclohexanecarboxylic acid

(0.28 g, 1.0 mmol), HOBt · H_2O (0.16 g, 1.0 mmol), and *N-tert*-butoxycarbonyl-*S*-methylisothiourea (0.19 g, 1.0 mmol) in DMF (5 mL), surrounded by an ice-water bath. Stirring was continued overnight while the cooling bath was allowed to warm to ambient temperature. The solution was diluted with ethyl acetate (40 mL) and washed with water, 5 % aq. $KHSO_4$, 5 % aq. $KHCO_3$, and brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was subjected to flash chromatography, eluting with $CHCl_3$. Yield: 0.37 g (89 %). ^{1}H-NMR (300 MHz, DMSO-d_6): δ_H = 0.98 (m, 2H, 3/5-H ax. cyclohexyl), 1.30 (m, 2H, 3/5-H eq. cyclohexyl), 1.42 (s, 9H, Boc), 1.50 (m, 1H, 4-H cyclohexyl), 1.82–2.00 (m, 4H, 2/6-H cyclohexyl), 2.28 (s, 3H, -SC<u>H</u>$_3$), 2.33 (m, 1H, 1-H cyclohexyl), 3.12 (m, 2H, -C<u>H</u>$_2$NH-), 7.29 (m, 2H, 2/6-H ArF), 7.92 (m, 2H, 3/5-H ArF), 8.46 (t, ^3J = 5.6 Hz, 1H, -N<u>H</u>Bz(4-F)), 11.28 (s, 1H, -N<u>H</u>Boc) ppm; ^{13}C-NMR (300 NHz, DMSO-d_6): δ_C = 13.8 (-S<u>C</u>H$_3$), 27.6 (Boc), 28.0 (C-2/6 cyclohexyl), 29.3 (C-3/5 cyclohexyl), 36.8 (C-4 cyclohexyl), 44.0 (C-1 cyclohexyl), 45.1 (-C<u>H</u>$_2$NH-), 79.9 (Boc), 115.0 ($^2J_{C,F}$ = 21.6 Hz), 129.7 ($^3J_{C,F}$ = 8.9 Hz), 131.0 ($^4J_{C,F}$ = 2.9 Hz), 158.1 (Boc), 162.6 (N<u>C</u>(SMe)N), 163.6 ($^1J_{C,F}$ = 248.2 Hz), 165.1 (-<u>C</u>(O)C_6H_4F), 173.7 (<u>C</u>=O) ppm; CI-MS (NH3) *m/z* (%): 452 (100) $[MH]^+$, 395 (16) $[MH - C_4H_8]^+$, 352 (28) $[MH - Boc]^+$, 296 (33).

***N-tert*-Butoxycarbonyl-*N'*-[3-(4-fluorobenzamido)propyloxycarbonyl]-*S*-methyl-isothiourea** (**10h**) was prepared from 3-(4-fluorobenzamido)propan-1-ol similarly to **10g**. Yield: 0.19 g (54 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.52 (s, 9H, Boc), 2.05 (m, 2H, -OCH$_2$C<u>H</u>$_2$CH$_2$NHBz(4-F)), 2.42 (s, 3H, -SC<u>H</u>$_3$), 3.59 (m, 2H, -OCH$_2$CH$_2$-C<u>H</u>$_2$NHBz(4-F)), 4.32 (t, ^3J = 5.9 Hz, -OC<u>H</u>$_2$CH$_2$CH$_2$NHBz(4-F)), 7.09 (m, 2H, ArF), 7.83 (m, 2H, ArF) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_H = 14.2 (-S<u>C</u>H$_3$), 28.0 (Boc), 28.7 (-OCH$_2$<u>C</u>H$_2$CH$_2$NHBz(4-F)), 37.5 (-O(CH$_2$)$_2$<u>C</u>H$_2$NHBz(4-F)), 64.7 (-O<u>C</u>H$_2$CH$_2$CH$_2$NH), 77.3 and 83.6 (Boc), 115.5 (d, $^2J_{C,F}$ = 21.9 Hz), 129.3 (d, $^3J_{C,F}$ = 8.9 Hz), 130.7 (d, $^4J_{C,F}$ = 3.1 Hz), 148.5 (-O<u>C</u>(O)N), 151.7 (-O<u>C</u>(O)N), 164.7 (d, $^1J_{C,F}$ = 251.7 Hz), 166.4 (-<u>C</u>(O)C_6H_4F) ppm; ESI-MS (+p) *m/z* (%): 414 (100) $[MH]^+$. $C_{18}H_{24}FN_3O_5S$ (413.46).

***N*-*tert*-Butoxycarbonyl-*N'*-(2-[2-(4-fluorobenzamido)ethoxy]ethoxycarbonyl)-*S*-methylisothiourea** (**10i**) was prepared from 2-[2-(4-fluorobenzamido)ethoxy]-ethanol similarly to **10g**. Yield: 0.19 g (54 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.51 (s, 9H, Boc), 2.37 (s, 3H, -SC$\underline{H}_3$), 3.68 (m, 4H, -OCH$_2$C$\underline{H}_2$OC$\underline{H}_2$CH$_2$N-), 3.77 (m, 2H, -OCH$_2$CH$_2$OCH$_2$C$\underline{H}_2$N-), 4.34 (m, 2H, -OC$\underline{H}_2$CH$_2$OCH$_2$CH$_2$N-), 7.10 (m, 2H, 2/6-H ArF), 7.82 (m, 2H, 3/5-H ArF) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 14.6 (-S$\underline{C}$H$_3$), 28.0 (Boc), 39.7 (-OCH$_2\underline{C}$H$_2$N-), 65.1 (-O$\underline{C}$H$_2$CH$_2$N-), 68.8 (-OCH$_2\underline{C}$H$_2$O-), 69.8 (-O$\underline{C}$H$_2$CH$_2$O-), 77.3 and 83.4 (Boc), 115.5 (d, $^{2}J_{C,F}$ = 21.7 Hz), 129.4 (d, $^{3}J_{C,F}$ = 8.9 Hz), 130.6 (d, $^{4}J_{C,F}$ = 3.1 Hz), 148.5 (-O$\underline{C}$(O)N-), 151.7 (-O$\underline{C}$(O)N-), 164.7 (d, $^{1}J_{C,F}$ = 251.5 Hz), 166.4 (-$\underline{C}$(O)C$_6$H$_4$F), 172.9 (N$\underline{C}$(SMe)N) ppm; ESI-MS (+p) m/z (%): 444 (85) $[MH]^+$, 344 (100) $[MH - Boc]^+$. $C_{19}H_{26}FN_3O_6S$ (443.49).

4.1.9. *GUANIDINYLATION OF ORNITHINE PRECURSOR **4** AND DEPROTECTION*

Guanidinylation – General Method **A**: A magnetically stirred solution of amine **4** and *N'*-substituted *N*-*tert*-butoxycarbonyl-*S*-methylisothiourea (1.0 eq.) in DMF (3 mL/0.20 mmol substrate) was treated with triethylamine (2.0 eq.) and $HgCl_2$ (1.0 eq.) and stirring was continued overnight. The mixture was diluted with ethyl acetate and filtered through a small pad of Celite® in order to remove insoluble mercury salts. The filtrate was diluted with a further portion of ethyl acetate, washed with solutions of 5 % aq. $KHSO_4$, 5 % aq. $KHCO_3$, and brine, dried over anhydrous Na_2SO_4, and evaporated. The products were purified by flash chromatography (eluent: CH_2Cl_2/methanol).

Deprotection – General Method **D**: The protected argininamide was dissolved in a 1:1 (v/v) mixture of trifluoroacetic acid and CH_2Cl_2 (approx. 2 mL/0.1 mmol substrate) and stirred for 2 h. The volatiles were rotary evaporated and the residue was submitted to flash chromatography eluting with mixtures of CH_2Cl_2 (or $CHCl_3$) and methanol, containing a small amount of 5 % trifluoroacetic acid in CH_2Cl_2.

4.1.10. AROYL- AND ARYLALKANOYL-SUBSTITUTED BIBP 3226 ANALOGS

(*R*)-*N*-(4-*tert*-Butoxybenzyl)-N^{ω}-*tert*-butoxycarbonyl-N^{α}-(2,2-diphenylacetyl)-$N^{\omega'}$-(3-phenylpropanoyl)argininamide – Yield (General Method **A**): 0.15 g (99 %). ESI-MS (+p) m/z (%): 762 (100) $[MH]^+$, 662 (8) $[MH - Boc]^+$. $C_{45}H_{55}N_5O_6$ (761.95).

(*R*)-N^{α}-(2,2-diphenylacetyl)-*N*-(4-hydroxybenzyl)-N^{ω}-(3-phenylpropionyl)-argininamide (**2f**) – Yield (General Method **D**): 0.10 g (96 %). mp > 103–104 °C decomp. ^{1}H-NMR (300 MHz, DMSO-d_6): δ_H = 1.32–1.79 (m, 4H, -C$^{\beta}$H$_2$C$^{\gamma}$H$_2$-), 2.77 (m, 2H, -CH$_2$CH$_2$Ph), 2.88 (m, 2H, -CH$_2$CH$_2$Ph), 3.23 (m, 2H, -C$^{\delta}$H$_2$), 4.15 (m, 2H, -CH$_2$ArOH), 4.34 (m, 1H, -C$^{\alpha}$H-), 5.13 (s, 1H, Ph$_2$CH-), 6.68 (m, 2H, 2/6-H ArOH), 7.01 (m, 2H, 3/5-H ArOH), 7.13–7.38 (m, 15H, Ph), 8.38 (t, ^3J = 5.8 Hz, 1H, -NHCH$_2$ArOH), 8.51 (d, ^3J = 8.1 Hz, 1H, -N$^{\alpha}$H-), 8.77 (m, 2H, -NH$_2$), 9.26 (m, 1H, -N$^{\delta}$H-), 11.97 (s, 1H, -NH-) ppm; ^{13}C-NMR (300 MHz, DMSO-d_6): δ_C = 24.3 (C$^{\gamma}$), 29.4 (C$^{\beta}$), 37.2 (-CH$_2$CH$_2$Ph), 37.6 (-CH$_2$CH$_2$Ph), 41.5 (-CH$_2$ArOH), 52.2 (C$^{\alpha}$), 55.8 (Ph$_2$CH-), 114.9 (C-2/6 ArOH), 126.3 (Ph), 128.1 (Ph), 128.1 (Ph), 128.2 (Ph), 128.3 (Ph), 128.4 (Ph), 129.0 (C-3/5 ArOH), 140.1 (C-1 Ph), 140.3 (C-1 Ph), 152 .7 (C_q), 156.2 (C_q), 159.6 (C_q), 170.9 (C=O amide), 170.9 (C=O amide), 174.4 (-NHC(NH)C(O)-) ppm; ESI-MS (+p) m/z (%): 606 (100) $[MH]^+$; RP-HPLC (Nucleodur100-5, gradient MeCN/0.05 % aq. TFA: 0 min 25:75, 25 min 65:35): k = 5.26 (t_R = 14.4 min); analysis calcd. for $C_{36}H_{39}N_5O_4 \cdot TFA \cdot H_2O$: C 61.86, H 5.74, N 9.49 %; found: C 61.85, H 5.55, N 9.55 %. $C_{36}H_{39}N_5O_4$ (605.73)

(*R*)-*N*-(4-*tert*-Butoxybenzyl)-N^{ω}-*tert*-butoxycarbonyl-N^{α}-(2,2-diphenylacetyl)-$N^{\omega'}$-(4-fluorobenzoyl)argininamide – Method **C**: A magnetically stirred solution of 1-*tert*-butoxycarbonyl-3-(4-fluorobenzoyl)guanidine (0.31 g, 1.10 mmol) and triethylamine (0.46 mL, 3.30 mmol) in dry CH_2Cl_2 (5 mL) was cooled to – 78 °C; trifluoromethanesulfonic anhydride (0.22 mL, 1.32 mmol) was added slowly under careful exclusion of moisture. After 0.5 h the cooling bath was removed and stirring was continued for additional 2 h. The reaction was quenched with 5 drops of water and a solution of **4** (0.49 g, 1.00 mmol) in CH_2Cl_2 (2 ml) was added. After stirring overnight the mixture was diluted with ethyl acetate (50 mL) and washed with a 5 %

aq. K_2CO_3 and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The product was purified by flash chromatography eluting with $CHCl_3$/ethyl acetate 5:1; yield 0.40 g (53 %) of a colorless solid. ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.32 (s, 9H, ^{t}Bu), 1.52 (s, 9H, Boc), 1.57–2.00 (m, 4H, -C$^\beta$H$_2$C$^\gamma$H$_2$-), 3.40–3.80 (m, 2H, -C$^\delta$H$_2$-), 4.17–4.45 (m, 2H, -NHCH$_2$ArO^{t}Bu), 4.52–4.69 (m, 1H, -C$^\alpha$H-), 5.07 (s, 1H, Ph$_2$CH-), 6.82–7.40 (m, 10H, Ph$_2$ and 2H, 2/6-H ArF and 4H, ArO^{t}Bu), 8.19 (m, 2H, 3/5-H ArF) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 25.5 (C$^\gamma$), 28.0 (^{t}Bu), 28.8 (Boc), 29.5 (C$^\beta$), 40.7 (C$^\delta$) 43.0 (-NHCH$_2$ArO^{t}Bu), 52.6 (C$^\alpha$), 58.7 (Ph$_2$CH-), 78.5 (^{t}Bu), 83.8 (Boc), 115.1 (d, $^2J_{C,F}$ = 21.5 Hz), 124.3 (C-2/6 ArO^{t}Bu), 127.4 (C-3/5 ArO^{t}Bu), 128.2 (Ph), 128.7 (Ph), 128.8 (Ph), 131.8 (d, $^3J_{C,F}$ = 9.0 Hz), 132.4 (d, $^4J_{C,F}$ = 1.8 Hz), 133.0 (C-4 ArO^{t}Bu), 138.8 (C-1 Ph), 153.3 (-NC(N)N-), 154.7 (C-1 ArO^{t}Bu), 156.6 (Boc), 162.5 (d, $^1J_{C,F}$ = 247.1 Hz), 167.0 (C=O Bz(4-F)), 170.9 (C=O), 172.5 (C=O) ppm; ESI-MS (+p) m/z (%): 752 (100) $[MH]^+$, 692 (45), 652 (75) $[MH - Boc]^+$. $C_{43}H_{50}FN_5O_6$ (751.89).

(*R*)-*N*$^\alpha$-(2,2-Diphenylacetyl)-*N*$^{\omega'}$-(4-fluoro-benzoyl)-*N*-(4-hydroxybenzyl)arginin-amide (**2g**) – Yield (General Method **D**): 0.17 g (75 %). ^{1}H-NMR (600 MHz, DMSO-d$_6$, HSQC, COSY): δ_H = 1.50 (m, 2H, -C$^\gamma$H$_2$-), 1.60 and 1.73 (m, 2H, -C$^\beta$H$_2$-, diast.), 3.31 (m, 2H, -C$^\delta$H$_2$-), 4.16 (m, 2H, -NHCH$_2$ArOH), 4.36 (m, 1H, -C$^\alpha$H-), 5.13 (s, 1H, Ph$_2$CH-), 6.67 (m, 2H, 2/6-H ArOH), 7.01 (m, 2H, 3/5-H ArOH), 7.21 (m, 2H, 4-H Ph), 7.29 (m, 8H, 2/3/5/6-H Ph), 7.45 (m, 2H, 2/6-H ArF), 8.03 (m, 2H, 3/5-H ArF), 8.37 (t, ^3J = 5.7 Hz, 1H, -NHCH$_2$ArOH), 8.50 (d, ^3J = 8.1, 1H, -N$^\alpha$H-), 8.80 (brs, 2H, -NH$_2$), 9.12 (m, 1H, -N$^\delta$H-), 9.31 (brs, 1H, -NH), 11.45 (brs, 1H, -ArOH) ppm; ^{13}C-NMR (600 MHz, DMSO-d$_6$, HSQC): δ_C = 24.3 (C$^\gamma$), 29.4 (C$^\beta$), 40.7 (C$^\delta$), 41.6 (-NHCH$_2$ArOH), 52.3 (C$^\alpha$), 55.9 (Ph$_2$CH-), 115.0 (C-2/6 ArOH), 116.0 (d, $^2J_{C,F}$ = 22.0 Hz), 126.5 (C-4 Ph), 128.2 and 128.5 (C-2/3/5/6 Ph), 128.4 (C-3/5 ArOH), 129.1 (C-4 ArF), 131.2 (d, $^3J_{C,F}$ = 9.6 Hz), 140.3 (C-1 Ph), 153.5 (-NHC(N)N-), 156.2 (C-1 ArOH), 158.7 (C-4 ArOH), 165.2 (d, $^1J_{C,F}$ = 251.2 Hz),

166.7 (C=O Bz(4-F)), 170.9 ($Ph_2CHC(O)N^{\alpha}$-), 171.0 (-C(O)NCH_2ArOH) ppm; ESI-MS (+p) m/z (%): 596 (100) $[MH]^+$; RP-HPLC (Eurosphere-100, gradient MeCN/ 0.05 % aq. TFA: 0 min 25:75, 25 min 65:35): k = 5.52 (t_R = 15.7 min); HR-MS: calcd. for $C_{34}H_{34}FN_5O_4 \cdot H^+$: 596.2668, found: 596.2679.

4.1.11. ω-AMINOALKANOYL- AND ω-AMINOALKOXYCARBONYL- SUBSTITUTED BIBP 3226 ANALOGS

(*R*)-*N*-(4-*tert*-Butoxybenzyl)-N^{ω}-*tert*-butoxycarbonyl-N^{α}-(2,2-diphenylacetyl)-$N^{\omega'}$-(5-phthalimidopentanoyl)argininamide (**9c**) – Method **B**: To a stirred solution of *N*-*tert*-butoxycarbonyl-*N*′-(5-phthalimidopentanoyl)-1*H*-pyrazole-1-carboxamidine (0.76 g, 1.70 mmol) was added a solution of amine **4** (0.76 g, 1.56 mmol) in 5 mL acetonitrile and stirring was continued overnight. After removal of the solvent the residue was taken up in CH_2Cl_2 (50 mL) and washed with water, 5 % aq. $KHSO_4$ solution, and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. Vacuum flash chromatography (eluent: petroleum ether/ethyl acetate 4:3) afforded 0.90 g (67 %) of a white solid. ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.28 (s, 9H, ^{t}Bu), 1.38 (s plus additional signal for minor tautomer at 1.48, 9H, Boc), 1.35–1.98 (m, 4H, -$C^{\beta}H_2C^{\gamma}H_2$- and 4H, -$CH_2CH_2CH_2CH_2$NPht), 2.47 (t, ^3J = 6.9 Hz, 2H, -C(O)$CH_2(CH_2)_3$NPht), 3.22 (m, 2H, -$C^{\delta}H_2$-), 3.73 (t, ^3J = 6.5 Hz, 2H, -CH_2NPht), 4.36 (m, 2H, -CH_2ArO^tBu), 4.59 (m, 1H, -$C^{\alpha}H$-), 4.99 (s, 1H, Ph_2CH-), 6.86 (m, 1H, -NH), 6.90 (m, 2H, 2/6-H ArO^tBu), 7.09 (m, 2H, 3/5-H ArO^tBu), 7.16–7.38 (m, 10H, Ph), 7.62–7.93 (m, 4H, Pht), 8.98 (t, ^3J = 5.9 Hz, 1H, -NH), 12.41 (s plus additional signal for minor tautomer at 12.24, 1H, -NH) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): 21.7 (-$CH_2CH_2CH_2CH_2$NPht), 25.7 (C^{γ}), 27.8 (-CH_2CH_2NPht), 28.0 (C^{β}), 28.2 (Boc), 28.8 (^{t}Bu), 37.1 (-C(O)$CH_2(CH_2)_3$NPht), 37.3 (-CH_2NPht, 39.5 (C^{δ}), 42.9 (-CH_2ArO^tBu), 53.5 (C^{α}), 58.4 (Ph_2CH-), 78.5 (-CMe_3), 79.9 (-CMe_3), 123.3 (Pht), 124.3 (C-2/6 -ArO^tBu), 127.2 (C-4 Ph), 127.4 (C-3/5 ArO^tBu), 128.1 (Ph), 128.8 (Ph), 132.0 (Pht), 132.8 (C-4 ArO^tBu), 134.0 (Pht), 139.1 (C-1 Ph), 154.6 (C-1 ArO^tBu), 156.6 (C_q), 163.5 (C_q), 168.4 (C=O, Pht),

171.3 (C=O, amide), 172.4 (C=O, amide), 174.4 (N^G-$CO(CH_2)_4$-) ppm; ESI-MS (+p) m/z (%): 859 (100) $[MH]^+$, 759 (3) $[MH - Boc]^+$; analysis calcd. for $C_{49}H_{58}N_6O_8 \cdot 0.66\ H_2O$: C 67.57, H 6.87, N 9.65 %; found: C 67.53, H 6.37, N 9.53 %. $C_{49}H_{58}N_6O_8$ (859.02).

(*R*)-*N*-(4-*tert*-Butoxybenzyl)-N^{ω}-*tert*-butoxycarbonyl-N^{α}-(2,2-diphenylacetyl)-$N^{\omega'}$-[5-(*tert*-butoxycarbonylamino)pentanoyl]argininamide (**9e**) – Yield (General Method **A**): 90 mg (54 %). mp 70–72 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ_H = 1.26 (s, 9H, ^{t}Bu), 1.37 (s, 9H, Boc), 1.40 (s plus additional signal for minor tautomer at 1.47, 9H, Boc), 1.30–1.77 (m, 4H, -$C^{\beta}H_2C^{\gamma}H_2$-, 4H, -$CH_2CH_2CH_2CH_2NHBoc$), 2.44 (t, ^3J = 7.3 Hz, 2H, -$C(O)CH_2(CH_2)_3NHBoc$), 2.91 (m, 2H, -$(CH_2)_2CH_2NHBoc$), 3.24 (m, 2H, -$C^{\delta}H_2$-), 4.22 (m, 2H, -CH_2ArO^tBu), 4.35 (m, 1H, -$C^{\alpha}H$-), 5.12 (s, 1H, Ph_2CH-), 6.81 (t, ^3J = 5.7 Hz, 1H, -NHBoc), 6.86 (m, 2H, 2/6-H ArO^tBu), 7.10 (m, 2H, 3/5-H ArO^tBu), 7.15–7.39 (m, 10H, Ph), 8.33–8.56 (m, 2H, -NH's), 8.90 (t, ^3J = 5.5 Hz, 1H, -NH), 12.03 (s plus additional signal for minor tautomer at 12.41, 1H, -NH) ppm; ^{13}C-NMR (300 MHz, DMSO-d_6): δ_C = 21.2 (-$CH_2(CH_2)_2NHBoc$), 24.3 (C^{γ}), 27.5 (^{t}Bu), 28.2 (Boc), 28.4 (plus additional signal for minor tautomer at 27.9, Boc), 28.6 (-CH_2CH_2NHBoc), 29.6 (C^{β}), 36.3 (-$C(O)CH_2(CH_2)_3NHBoc$), 39.2 (-CH_2NHBoc), 39.6 (C^{δ}), 41.4 (-CH_2ArO^tBu), 52.3 (C^{α}), 55.8 (Ph_2CH-), 77.3 (Boc), 77.6 (^{t}Bu), 78.0 (plus additional signal for minor tautomer at 82.7, Boc), 123.5 (C-2/6 ArO^tBu), 126.4 (C-4 Ph), 127.6 (C-3/5 ArO^tBu), 128.1 and 128.4 (C-2/3/5/6 Ph), 133.6 (C-4 ArO^tBu), 140.2 (C-1 Ph), 153.7 (C-1 ArO^tBu), 155.1 (C_q), 155.5 (C_q), 162.8 (C_q), 170.8 (C=O amide), 171.2 (C=O amide), 174.3 (-NHC(NHBoc)NC(O)-) ppm; ESI-MS (+p) m/z (%): 829 (100) $[MH]^+$, 851 (8) $[MNa]^+$, 729 (3) $[MH - Boc]^+$; analysis calcd. for $C_{46}H_{64}N_6O_8 \cdot H_2O$: C 65.22, H 7.85, N 9.92 %; found: C 65.21, H 7.89, N 9.85 %. $C_{46}H_{64}N_6O_8$ (829.04).

(*R*)-*N*-(4-*tert*-Butoxybenzyl)-N^{ω}-*tert*-butoxycarbonyl-N^{α}-(2,2-diphenylacetyl)-$N^{\omega'}$-[6-(*tert*-butyloxycarbonylamino)hexanoyl]argininamide – Yield (General

Method **A**): 0.12 g (47 %). ESI-MS (+p) m/z (%): 843 (100) $[MH]^+$, 743 (10) [MH – Boc]$^+$. $C_{47}H_{66}N_6O_8$ (843.06).

(*R*)-*N*$^{\omega}$-(6-Aminohexanoyl)-*N*$^{\alpha}$-(2,2-diphenylacetyl)-*N*-(4-hydroxybenzyl)arginin-amide (**2e**) – Yield (General Method **D**): 50 mg (57 %). RP-HPLC (Nucleodur100-5; gradient MeCN/0.05 % aq. TFA: 0 min 25:75, 30 min 60:40): k = 3.78 (t_R = 11.0 min); ESI-MS (+p) m/z (%): 587 (55) $[MH]^+$, 314 (15) $[M + 2H + MeCN]^{2+}$, 294 (100) $[M + 2H]^{2+}$. $C_{33}H_{42}N_6O_4$ (586.72).

(*R*)-*N*-(4-*tert*-Butoxybenzyl)-*N*$^{\omega}$-*tert*-butoxycarbonyl-*N*$^{\alpha}$-(2,2-diphenylacetyl)-*N*$^{\omega'}$-{*trans*-4-[(allyloxycarbonylamino)methyl]-cyclohexane-carbonyl}argininamide (**9a**) – Method **B**: A solution of amine **4** (0.29 g, 0.60 mmol) and *N*-*tert*-butoxycarbonyl-*N*′-(*trans*-4-[(allyloxycarbonylamino)methyl]cyclo-hexane-carbonyl)-1*H*-pyrazole-1-carboxamidine (0.28 g, 0.65 mmol) in acetonitrile (5 mL) was stirred for 48 h at ambient temperature. After removal of the solvent in vacuum the residue was dissolved in CH_2Cl_2 (30 mL) and washed with water, 5 % aq. $KHSO_4$, 5 % aq. $KHCO_3$, and brine. The organic extracts were dried over anhydrous Na_2SO_4 and evaporated. The product was purified by vacuum flash chromatography eluting with $CHCl_3$/ethyl acetate. Yield: 0.32 g (63 %) as a white powder. ^{1}H-NMR (400 MHz, $CDCl_3$, HSQC, COSY): δ_H = 1.02 (m, 2H, -C_6H_{10}-), 1.31 (s, 9H, ^{t}Bu), 1.40 (s plus additional signal for minor tautomer at 1.51, 9H, Boc), 1.45 (m, 2H, -C$^{\gamma}$H$_2$-), 1.45 and 1.55 (m, 2H, -C$^{\beta}$H$_2$-), 1.50 (m, 1H, 4-H -C_6H_{10}-), 1.70 (m, 2H, -C_6H_{10}-), 1.86 (m, 2H, -C_6H_{10}-), 2.02 (m, 2H, -C_6H_{10}-), 2.23 (tt, $^3J_1$ = 3.3 Hz, $^3J_2$ = 12.0 Hz, 1-H -C_6H_{10}CO-), 3.05 (m, 2H, -CH_2NHAlloc), 3.24 (m, 2H, -C$^{\delta}$H$_2$-), 4.35 (m, 2H, -CH$_2$ArO^{t}Bu), 4.56 (m, 2H, -OCH$_2$CH=CH$_2$), 4.99 (s, 1H, Ph$_2$CH-), 5.21 (m, 1H, -OCH$_2$CH=C(E)-HH), 5.30 (m, 1H, -OCH$_2$CH=C(Z)-HH), 5.93 (m, 1H, -OCH$_2$CH=CH$_2$), 6.90 (m, 2H, 2/6-H ArO^{t}Bu), 7.09 (m, 2H, 3/5-H ArO^{t}Bu), 7.21 (m, 2H, 4-H Ph), 7.23 (m, 4H, Ph), 7.28 (m, 4H, Ph), 9.07 (t, ^3J = 5.7 Hz, 1H, -NH), 12.44 (s, plus additional signal for minor tautomer at 12.21, 1H, -NH) ppm; ^{13}C-NMR (400 MHz, $CDCl_3$, HSQC): δ_C = 26.0 (C$^{\beta}$), 28.1 (C$^{\gamma}$) 28.2 (plus additional signal for minor tautomer at 27.9, Boc), 28.4 (-CH$_2$-, -C_6H_{10}-), 28.9 (^{t}Bu),

29.4 (-$\underline{C}H_2$-, -C_6H_{10}-), 37.6 (C-4, -$C_6H_{10}CO$-), 39.7 ($\underline{C}^{\delta}$), 42.9 (-$\underline{C}H_2ArO^tBu$), 46.4 (C-1, -$C_6H_{10}CO$-), 47.0 (-$\underline{C}H_2NHAlloc$), 53.5 ($\underline{C}^{\alpha}$), 58.4 ($Ph_2\underline{C}H$-), 65.6 (-$O\underline{C}H_2CH{=}CH_2$), 77.3 (-$\underline{C}Me_3$), 78.5 (-$\underline{C}Me_3$), 117.7 (-CH=$\underline{C}H_2$), 124.3 (C-2/6 ArO^tBu), 127.3 (C-4 Ph), 128.1 (C-3/5 ArO^tBu), 128.8 (C-2/3/5/6 Ph), 132.9 (C-4 ArO^tBu), 139.2 (C-1 Ph), 154.7 (C-1 ArO^tBu), 156.4 ($\underline{C}$=O, Alloc), 156.7 (-N$\underline{C}$(N)N-), 171.3 ($Ph_2CH\underline{C}$(O)-), 172.4 (-$C^{\alpha}H\underline{C}$(O)-), 177.8 (-$C_6H_{10}\underline{C}$O-) ppm; ESI-MS (+p) m/z (%): 853 (100) $[MH]^+$, 753 $[MH - Boc]^+$. $C_{48}H_{64}N_6O_8$ (853.06).

(*R*)-*N*-(4-*tert*-Butoxybenzyl)-N^{ω}-*tert*-butoxycarbonyl-N^{α}-(2,2-diphenylacetyl)-$N^{\omega'}$-[3-(*tert*-butoxycarbonylamino)propyloxycarbonyl]argininamide – Yield (General Method **A**): 0.12 g (72 %). mp 88–90 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ_H = 1.26 (s, 9H, ^{t}Bu), 1.37 (s, 9H, Boc), 1.44 (m, 2H, -$C^{\gamma}\underline{H}_2$-), 1.48 (s plus additional signal for minor tautomer at 1.39, 9H, Boc), 1.52–1.82 (m, 2H, -$C^{\beta}\underline{H}_2$- and 2H, -$\underline{C}H_2CH_2NHBoc$), 2.97 (m, 2H, -$\underline{C}H_2NHBoc$), 3.26 (-$C^{\delta}\underline{H}_2$-), 3.94 (t, ^3J = 6.4 Hz, 2H, -$OC\underline{H}_2(CH_2)_2NHBoc$), 4.22 (m, 2H, -$\underline{C}H_2ArO^tBu$), 4.35 (m, 1H, -$C^{\alpha}\underline{H}$-), 5.12 (s, 1H, $Ph_2C\underline{H}$-), 6.86 (m, 2H, 2/6-H ArO^tBu), 6.91 (m, 1H, -N$\underline{H}$Boc), 7.10 (m, 2H, 3/5-H ArO^tBu), 7.16–7.38 (m, 10H, Ph), 8.36 (t, ^3J = 5.5 Hz, 1H, -$N^{\delta}\underline{H}$-), 8.49 (m, 2H, -N$\underline{H}$'s), 11.40 (s plus additional signal for minor tautomer at 11.70, 1H, -N$\underline{H}$) ppm; ^{13}C-NMR (300 MHz, DMSO-d_6): δ_C = 24.3 ($\underline{C}^{\gamma}$), 27.5 (^{t}Bu), 28.1 (Boc), 28.4 (plus additional signal for minor tautomer at 27.9, Boc), 28.9 (-$\underline{C}H_2CH_2NHBoc$), 29.6 ($\underline{C}^{\beta}$), 36.8 (-$\underline{C}H_2NHBoc$), 39.9 ($\underline{C}^{\delta}$), 41.4 (-$\underline{C}H_2ArO^tBu$), 52.3 ($\underline{C}^{\alpha}$), 55.8 ($Ph_2\underline{C}H$-), 62.4 (-$O\underline{C}H_2CH_2$-), 77.4 (Boc), 77.6 (^{t}Bu), 82.8 (plus additional signal for minor tautomer at 78.2, Boc), 123.5 (C-2/6 ArO^tBu), 126.4 (C-4 Ph), 127.6 (C-3/5 ArO^tBu), 128.1 and 128.4 (C-2/3/5/6 Ph), 133.7 (C-4 ArO^tBu), 140.3 (C-1 Ph), 151.9 (C_q), 153.6 (C-1 ArO^tBu), 155.2 (C_q), 155.5 (C_q), 163.2 (C_q), 170.8 ($\underline{C}$=O amide), 171.2 ($\underline{C}$=O amide) ppm; ESI-MS (+p) m/z (%): 831 (100) $[MH]^+$, 853 (5) $[MNa]^+$, 731 (2) $[MH - Boc]^+$; analysis calcd. for $C_{45}H_{62}N_6O_9 \cdot 0.5\ H_2O$: C 64.34, H 7.56, N 10.01 %; found: C 64.49, H 7.74, N 10.40 %. $C_{45}H_{62}N_6O_9$ (831.01).

4.1.12. ω-(4-FLUOROBENZAMIDO)ALKANOYL- AND –ALKOXYCARBONYL-SUBSTITUTED BIBP 3226 ANALOGS

(*R*)-*N*-(4-*tert*-Butoxybenzyl)-N^{ω}-*tert*-butoxycarbonyl-N^{α}-(2,2-diphenylacetyl)-N^{ω}-[6-(4-fluorobenzamido)hexanoyl]argininamide – Yield (General Method **A**): 0.19 g (73 %). ^{1}H-NMR (300 MHz, DMSO-d_6): δ_H = 1.26 (s, 9H, ^{t}Bu), 1.40 (s plus additional signal for minor tautomer at 1.47, 9H, Boc), 1.30–1.74 (m, 6H, -CH_2C$\underline{H}_2$C$\underline{H}_2$C$\underline{H}_2$$CH_2$- and 4H, -C$^{\beta}\underline{H}_2C^{\gamma}\underline{H}_2$-), 2.45 (t, ^3J = 7.3 Hz, 2H, -COC$\underline{H}_2$$(CH_2)_4$-), 3.16–3.33 (m, 2H, -$(CH_2)_4C\underline{H}_2$NH- and 2H, -C$^{\delta}\underline{H}_2$-), 4.22 (m, 2H, -C$\underline{H}_2$ArO^{t}Bu), 4.35 (m, 1H, -C$^{\alpha}\underline{H}$-), 5.13 (s, 1H, Ph_2C$\underline{H}$-), 6.86 (m, 2H, 2/6-H ArO^{t}Bu), 7.10 (m, 2H, 3/5-H ArO^{t}Bu), 7.15–7.37 (m, 10H, Ph_2 and 2H, 2/6-H ArF), 7.90 (m, 2H, 3/5-H ArF), 8.37–8.57 (m, 3H, -N$\underline{H}$'s), 8.91 (t, ^3J = 5.5 Hz, 1H, -N$\underline{H}$Bz(4-F)), 12.05 and 12.42 (2s, 1H, -N$\underline{H}$) ppm; ^{13}C-NMR (600 MHz, DMSO-d_6): δ_C = 23.6 (-$\underline{C}H_2$-), 25.0 ($\underline{C}^{\gamma}$), 25.7 (-$\underline{C}H_2$-), 27.9 (^{t}Bu), 28.4 (plus additional signal for minor tautomer at 27.5, Boc), 28.7 (-$\underline{C}H_2$-), 29.6 ($\underline{C}^{\beta}$), 36.6 (-$\underline{C}H_2$-), 38.9 (-$\underline{C}H_2$-), 39.6 ($\underline{C}^{\delta}$), 41.4 (-$\underline{C}H_2$ArO^{t}Bu), 52.3 ($\underline{C}^{\alpha}$), 55.8 ($Ph_2\underline{C}$H-), 77.6 (^{t}Bu), 78.0 (Boc), 115.0 (d, $^2J_{C,F}$ = 21.6 Hz), 123.5 (C-2/6 ArO^{t}Bu), 126.4 (C-4 Ph), 127.6 (C-3/5 ArO^{t}Bu), 128.1 and 128.4 (C-2/3/5/6 Ph), 129.6 (d, $^3J_{C,F}$ = 9.0 Hz), 131.0 (d, $^4J_{C,F}$ = 3.0 Hz), 133.6 (C-4 ArO^{t}Bu), 140.3 (C-1 Ph), 153.7 (C-1 ArO^{t}Bu), 155.1 (C_q), 163.6 (d, $^1J_{C,F}$ = 247.9 Hz), 162.8 (C_q), 164.9 ($\underline{C}$=O Bz(4-F)), 170.8 ($\underline{C}$=O amide), 171.2 ($\underline{C}$=O amide), 174.3 (-NHC(NH)N$\underline{C}$O-) ppm; ESI-MS (+p) m/z (%): 865 (100) $[MH]^+$, 765 (10) $[MH - Boc]^+$. $C_{49}H_{61}FN_6O_7$ (865.04).

(*R*)-*N*-(4-Hydroxybenzyl)-N^{α}-(2,2-diphenylacetyl)-N^{ω}-[6-(4-fluorobenzamido)-hexanoyl] argininamide (**2i**) – Yield (General Method **D**): 50 mg (88 %). ^{1}H-NMR (300 MHz, DMSO-d_6): δ_H = 1.13–1.80 (m, 6H, -CH_2C$\underline{H}_2$C$\underline{H}_2$C$\underline{H}_2$$CH_2$- and 4H, -C$^{\beta}\underline{H}_2C^{\gamma}\underline{H}_2$-), 2.44 (t, 2H, ^3J = 7.2 Hz, 2H, -COC$\underline{H}_2$$(CH_2)_4$-), 3.24 (m, 2H, -$(CH_2)_4C\underline{H}_2$NH- and 2H, -C$^{\delta}\underline{H}_2$-), 4.15 (m, 2H, -C$\underline{H}_2$ArO^{t}Bu), 4.34 (m, 1H, -C$^{\alpha}\underline{H}$-), 5.13 (s, 1H, Ph_2C$\underline{H}$-), 6.68 (m, 2H, 2/6-H ArOH), 7.01 (m, 2H, 3/5-H ArOH), 7.14–7.35 (m, 10H, Ph and 2H, 2/6-H ArF), 7.91 (m, 2H, 3/5-H ArF), 8.40 (t, ^3J = 5.7 Hz, 1H, -N$\underline{H}$$CH_2$ArOH), 8.46–8.56 (m, 2H, -N$\underline{H}$'s), 8.57–9.05 (brm, 2H, -N$\underline{H}$'s),

9.28 (m, 1H, -NH), 11.91 (s, 1H, -OH); ^{19}F-NMR (300 MHz, DMSO-d$_6$): δ_F = – 109.3 (m, -ArF), – 73.3 (s, TFA) ppm; ^{13}C-NMR (300 MHz, DMSO-d$_6$): δ_C = 23.5 (-CH$_2$-), 24.3 (C$^{\gamma}$), 25.7 (-CH$_2$-), 28.7 (-CH$_2$-), 29.3 (C$^{\beta}$), 36.0 (-CH$_2$-), 38.9 (-CH$_2$-), 40.3 (C$^{\delta}$), 41.5 (-CH$_2$ArOH), 52.2 (C$^{\alpha}$), 55.8 (Ph$_2$CH-), 114.9 (C-2/6 ArOH), 115.0 (d, $^2J_{C,F}$ = 20.9 Hz), 126.5 (C-4 Ph), 128.1 and 128.4 (C-2/3/5/6 Ph), 129.0 (C-3/5 ArOH), 129.6 (d, $^3J_{C,F}$ = 8.9 Hz), 131.0 (d, $^4J_{C,F}$ = 2.9 Hz), 140.3 (C-1 -Ph), 152.8 (-NC(N)N-) 156.2 (C-1 ArOH), 159.5 (C-4 ArOH), 163.6 (d, $^1J_{C,F}$ = 247.9 Hz), 164.9 (C=O Bz(4-F)), 170.9 (2× C=O amide), 175.2 (-NHC(NH)NCO-) ppm; ESI-MS (+p) m/z (%): 709 (100) [MH]$^+$; RP-HPLC (Nucleodur100-5, gradient MeCN/0.05 % aq. TFA: 0 min 25:75, 25 min 65:35): k = 5.9 (t_R = 15.8 min); analysis calcd. for $C_{40}H_{45}FN_6O_5 \cdot 0.5\ H_2O \cdot$ TFA: C 60.64, H 5.70, N 10.11 %; found: C 60.50, H 5.84, N 9.89 %. $C_{40}H_{45}FN_6O_5$ (708.82).

(*R*)-*N*-(4-*tert*-Butoxybenzyl)-*N*$^{\omega}$-*tert*-butoxycarbonyl-*N*$^{\alpha}$-(2,2-diphenylacetyl)-*N*$^{\omega'}$-{*trans*-4-[(4-fluorobenzamido)methyl]cyclohexanecarbonyl}argininamide (**9b**) from **9a** – To a magnetically stirred solution of **9a** (60 mg, 0.070 mmol) and succinimidyl 4-fluorobenzoate (47 mg, 0.20 mmol) in CH_2Cl_2 (2 mL) was added a 0.02 M solution of $Pd(PPh_3)_4$ (1.0 mL, 0.020 mmol) and DABCO (39 mg, 0.35 mmol) under an inert atmosphere. After stirring for 16 h the mixture was diluted with CH_2Cl_2 (25 mL) and washed with 5 % aq. $KHSO_4$, 5 % aq. $KHCO_3$, and brine. The CH_2Cl_2 extracts were dried over anhydrous Na_2SO_4 and concentrated. The residue was submitted to flash chromatography (eluent: $CHCl_3$/ethyl acetate) yielding 45 mg (72 %) of a colorless solid. From **4** (General Method **A**) – Yield: 0.12 g (67 %). ^{1}H-NMR (300 MHz, DMSO-d$_6$): δ_H = 1.03 (m, 2H, cyclohexyl), 1.25 (s, 9H, ^{t}Bu), 1.47 (s plus additional signal for minor tautomer at 1.40, 9H, Boc), 1.17–1.73 (m, 4H, -C$^{\beta}$H$_2$C$^{\gamma}$H$_2$- and 3H, cyclohexyl), 1.82 (m, 2H, cyclohexyl), 1.94 (m, 2H, cyclohexyl), 2.34 (m, 1H, cyclohexyl), 3.13 (m, 2H, -CH$_2$ NHBz(4-F)), 3.25 (m, 2H, -C$^{\delta}$H$_2$-), 4.22 (m, 2H, -CH$_2$ArO^{t}Bu), 4.34 (m, 1H, -C$^{\alpha}$H-), 5.12 (s, 1H, Ph$_2$CH-), 6.86 (m, 2H, 2/6-H ArO^{t}Bu), 7.09 (m, 2H, 3/5-H ArO^{t}Bu), 7.15–7.37 (m, 10H, Ph$_2$

and 2H, 2/6-H ArF), 7.92 (m, 2H, 3/5-H ArF), 8.37–8.57 (m, 3H, -NH's), 8.96 (t, ^3J = 5.5 Hz, 1H, -NHBz(4-F)), 12.17 and 12.48 (2s, 1H, -NH) ppm; ^{13}C-NMR (600 MHz, DMSO-d_6): δ_C = 24.9 (C^γ), 27.5 (plus additional signal for minor tautomer at 27.9, Boc), 28.0 and 29.2 (C-2/3/5/6 cyclohexyl), 28.4 (^{t}Bu), 29.6 (C^β), 36.7 (C-4 cyclohexyl), 39.7 (C^δ), 41.4 (-CH_2ArO^{t}Bu), 45.1 (C-1 cyclohexyl), 45.1 (-CH_2NHBz(4-F)), 52.3 (C^α), 55.8 (-CHPh$_2$), 77.6 (^{t}Bu), 82.6 (Boc, plus additional signal for minor tautomer at 78.1), 115.0 (d, $^2J_{C,F}$ = 21.7 Hz), 123.5 (C-2/6 ArO^{t}Bu), 126.4 (C-4 -Ph), 127.6 (C-3/5 ArO^{t}Bu), 128.0 (C-2/6 Ph), 128.4 (C-3/5 Ph), 129.7 (d, $^3J_{C,F}$ = 9.0 Hz), 131.0 (d, $^4J_{C,F}$ = 3 Hz), 133.6 (C-4, ArO^{t}Bu), 140.3 (C-1 Ph), 153.7 (C-1 ArO^{t}Bu), 155.3 (Boc), 163.0 (-NC(N)N-), 163.6 (d, $^4J_{C,F}$ = 248.4 Hz), 165.1 (C=O, Bz(4-F)), 170.8 (C=O, amide), 171.2 (C=O, amide), 176.9 (-NHC(NH)NC(O)-) ppm; ESI-MS (+p) m/z (%): 891 (100) $[MH]^+$, 913 (10) $[MNa]^+$, 791 (8) $[MH - Boc]^+$. $C_{51}H_{63}FN_6O_7$ (891.08).

(*R*)-N^α-(2,2-Diphenylacetyl)-N^ω-{*trans*-4-[(4-fluorobenzamido)methyl[cyclohexanecarbonyl}-*N*-(4-hydroxybenzyl)argininamide (**2j**) – Yield (General Method **D**): 66 mg (99 %). mp > 110 °C decomp. ^{1}H-NMR (300 MHz, DMSO-d_6): δ_H = 0.99 (m, 2H, cyclohexyl), 1.17–1.60 (m, 4H, -$C^\beta H_2C^\gamma H_2$- and 2H, cyclohexyl), 1.67 (m, 1H, cyclohexyl), 1.83 (m, 2H, cyclohexyl), 1.90 (m, 2H, cyclohexyl), 2.36 (m, 1H, cyclohexyl), 3.13 (m, 2H, -CH_2NHBz(4-F)), 3.25 (m, 2H, -$C^\delta H_2$-), 4.15 (m, 2H, -CH_2ArOH), 4.34 (m, 1H, -$C^\alpha H$-), 5.13 (s, 1H, Ph$_2$CH-), 6.68 (m, 2H, 2/6-H ArOH), 7.09 (m, 2H, 3/5-H ArOH), 7.15–7.35 (m, 10H, Ph$_2$ and 2H, 2/6-H ArF), 7.92 (m, 2H, 3/5-H ArF), 8.39 (t, ^3J = 5.7 Hz, 1H, -$N^\delta H$), 8.52 (m, 2H, -NH_2), 8.71 (brs, 2H, -OH), 9.16 (t, ^3J = 5.5 Hz, 1H, -NH), 11.67 (s, 1H, -NH) ppm; ^{13}C-NMR (300 MHz, DMSO-d_6): δ_C = 24.3 (C^γ), 27.8 and 29.2 (C-2/3/5/6 cyclohexyl), 29.3 (C^β), 36.7 (C-4 cyclohexyl), 40.4 (C^δ), 41.5 (-CH_2ArOH), 44.6 (C-1 cyclohexyl), 45.1 (-CH_2NHBz(4-F)), 52.2 (C^α), 55.8 (Ph$_2$CH-), 114.9 (C-2/6 ArOH), 115.0 ($^2J_{C,F}$ = 22.2 Hz), 126.5 (C-4 Ph), 128.1 and 128.3 (C-2/3/5/6 Ph), 128.4 (C-3/5 ArOH), 129.7 ($^3J_{C,F}$ = 9.0 Hz), 131.0 ($^4J_{C,F}$ = 2.9 Hz), 140.2 (C_q), 152.9 (C_q), 156.2 (C_q), 158.7 (C_q),

163.7 ($^1J_{C,F}$ = 248.3 Hz), 170.9 (C=O amide), 171.0 (C=O amide), 177.8 (-NC(N)NC(O)-) ppm; ESI-MS (+p) m/z (%): 735 (100) $[MH]^+$, 368 (4) $[M + 2H]^{2+}$; RP-HPLC (Luna, gradient MeCN/0.05 % aq. TFA: 0 min 25:75, 30 min 80:20): k = 4.87 (t_R = 19.9 min); analysis calcd. for $C_{42}H_{47}FN_6O_5 \cdot 1.5$ TFA $\cdot$ 0.5 H_2O: C 59.07, H 5.45, N 9.19 %; found: C 59.28, H 5.54, N 9.20 %. $C_{42}H_{47}FN_6O_5$ (734.86).

(*R*)-*N*-(4-*tert*-Butoxybenzyl)-N^{ω}-*tert*-butoxycarbonyl-N^{α}-(2,2-diphenylacetyl)-$N^{\omega'}$-{2-[2-(4-fluorobenzamido)ethoxy]ethoxycarbonyl}argininamide – Yield (General Method **A**): 0.49 g (87 %). ^{1}H-NMR (600 MHz, DMSO-d_6, HSQC, COSY): δ_H = 1.26 (s, 9H, t-Bu), 1.42 (m, 2H, -C$^{\gamma}$H$_2$-), 1.48 (s, plus additional signal for minor tautomer at 1.39, 9H, Boc), 1.53 and 1.65 (m, 2H, -C$^{\beta}$H$_2$-, diastereomeric), 3.24 (m, 2H, -C$^{\delta}$H$_2$-), 3.40 (m, 2H, -CH$_2$NHBz(4-F)), 3.53 (m, 2H, -OCH$_2$CH$_2$NHBz(4-F)), 3.61 (m, 2H, -C(O)OCH$_2$CH$_2$O-), 4.06 (m, 2H, -C(O)OCH$_2$CH$_2$O-), 4.22 (m, 2H, -CH$_2$ArO^{t}Bu), 4.35 (m, 1H, -C$^{\alpha}$H-), 5.13 (s, 1H, Ph$_2$CH-), 6.86 (m, 2H, 2/6-H ArO^{t}Bu), 7.10 (m, 2H, 3/5-H ArO^{t}Bu), 7.15–7.39 (m, 10H, Ph), 7.26 (m, 2H, 2/6-H ArF), 7.92 (m, 2H, 3/5-H ArF), 8.36 (t, ^3J = 5.7 Hz, 1H, -N$^{\delta}$H-), 8.43 (t, ^3J = 5.9 Hz, 1H, -NHCH$_2$ArO^{t}Bu), 8.48 (d, ^3J = 8.1 Hz, 1H, -N$^{\alpha}$H-), 8.54 (t, 1H, ^3J = 5.5 Hz, -NHBz(4-F)) ppm; ^{13}C-NMR (600 MHz, DMSO-d_6, HSQC, COSY): δ_C = 24.0 (-C$^{\gamma}$), (-C(O)OC(CH$_3$)$_3$), 26.8 (Boc, plus additional signal for minor tautomer at 26.4), 27.4 (^{t}Bu), 28.5 (C$^{\beta}$), 38.1 (-CH$_2$NHBz(4-F)), 38.9 (C$^{\delta}$), 40.3 (-CH$_2$ArO^{t}Bu), 51.3 (C$^{\alpha}$), 54.7 (-CHPh$_2$), 62.8 (-C(O)OCH$_2$CH$_2$O-), 67.2 (-C(O)OCH$_2$CH$_2$O-), 67.7 (-OCH$_2$CH$_2$NH-), 76.6 (^{t}Bu), 81.8 (Boc, plus additional signal for minor tautomer at 78.0), 114.0 (d, $^2J_{C,F}$ = 21.8 Hz), 122.4 (C-2/6 ArO^{t}Bu), 125.4 (C-4 Ph), 126.5 (C-3/5 ArO^{t}Bu), 127.3 (C-2/6 Ph), 127.4 (C-3/5 Ph), 128.6 (d, $^3J_{C,F}$ = 9.0 Hz), 129.7 (d, $^4J_{C,F}$ = 3 Hz), 132.6 (C-4 ArO^{t}Bu), 139.3 (C-1 Ph), 150.8 (C-1 ArO^{t}Bu), 152.6 (Boc), 154.3 (-NC(N)NC(O)O-), 162.0 (-NC(N)N-), 162.6 (d, $^1J_{C,F}$ = 247.9 Hz), 164.1 (C=O, Bz(4-F)), 169.8 (C=O amide), 170.1 (C=O amide) ppm; ESI-MS (+p) m/z (%): 883 (100) $[MH]^+$, 783 (35) [MH – Boc]$^+$. $C_{48}H_{59}FN_6O_9$ (883.02).

(*R*)-*N*$^{\alpha}$-(2,2-Diphenylacetyl)-*N*$^{\omega'}$-{2-[2-(4-fluorobenzamido)ethoxy]ethoxycarbonyl}-*N*-(4-hydroxybenzyl)argininamide (**2l**) – Yield (General Method **D**): 0.37 mg (98 %). mp 132–133 °C (decomp.) ^{1}H-NMR (400 MHz, DMSO-d_6, HSQC): δ_H = 1.45 (m, 2H, -C$^{\gamma}$H$_2$-), 1.56 and 1.69 (m, 2H, -C$^{\beta}$H$_2$-, diast.), 3.24 (m, 2H, -C$^{\delta}$H$_2$-), 3.43 (m, 2H, -CH$_2$NHBz(4-F)), 3.58 (m, 2H, -OCH$_2$CH$_2$NHBz(4-F)), 3.69 (m, 2H, -C(O)OCH$_2$CH$_2$O-), 4.15 (m, 2H, -CH$_2$ArO^{t}Bu), 4.31 (m, 2H, -C(O)OCH$_2$CH$_2$O-), 4.35 (m, 1H, -C$^{\alpha}$H-), 5.13 (s, 1H, -CHPh$_2$), 6.68 (m, 2H, 2/6-H ArOH), 7.00 (m, 2H, 3/5-H ArOH), 7.19–7.32 (m, 10H, Ph), 7.29 (m, 2H, 2/6-H (ArF)), 7.92 (m, 2H, 3/5-H ArF), 8.35 (t, ^3J = 5.7 Hz, 1H, -N$^{\delta}$H-), 8.48 (d, ^3J = 8.1 Hz, 1H, -N$^{\alpha}$H-), 8.56 (t, 1H, ^3J = 5.5 Hz, -NHBz(4-F)), 8.71 (t, ^3J = 5.9 Hz, 1H, -NHCH$_2$ArOH) ppm; ^{13}C-NMR (400 MHz, DMSO-d_6, HSQC): δ_C = 24.3 (C$^{\gamma}$), 29.2 (C$^{\beta}$), 39.0 (-CH$_2$NHBz(4-F)), 40.5 (C$^{\delta}$), 41.5 (-CH$_2$ArOH), 52.2 (C$^{\alpha}$), 55.8 (-CHPh$_2$), 65.5 (-C(O)OCH$_2$CH$_2$O-), 67.6 (-OCH$_2$CH$_2$NH-), 68.7 (-C(O)OCH$_2$CH$_2$O-), 114.9 (C-2/6 ArOH), 115.0 (d, $^2J_{C,F}$ = 22.8 Hz), 126.5 (C-4 Ph), 128.1 (C-3/5 Ph), 128.3 (C-2/6 Ph), 129.0 (C-3/5 ArOH), 129.7 (d, $^3J_{C,F}$ = 9.0 Hz), 130.7 (d, $^4J_{C,F}$ = 2.9 Hz), 140.3 (C-1 Ph), 152.7 (NC(N)N), 156.2 (C-1 ArOH), 158.3 (NC(O)O-), 158.8 (C-4 ArOH), 163.5 (d, $^1J_{C,F}$ = 251.3 Hz), 165.2 (-C(O)ArF), 170.9 (C=O amide, 2 signals) ppm; ESI-MS (+p) m/z (%): 727 (100) $[MH]^+$; RP-HPLC (Nucleodur 100-5, gradient MeCN/0.05 % aq. TFA: 0 min 25:75, 25 min 65:35): k = 5.05 (t_R = 13.9 min); analysis calcd. for $C_{39}H_{43}FN_6O_7 \cdot 2$ TFA $\cdot 2$ H_2O: C 52.12, H 4.98, N 8.48 %; found: C 52.02, H 4.60, N 8.37 %. $C_{39}H_{43}FN_6O_7$ (726.79).

(*R*)-*N*-(4-*tert*-Butoxybenzyl)-*N*$^{\omega}$-*tert*-butoxycarbonyl-*N*$^{\alpha}$-(2,2-diphenylacetyl)-*N*$^{\omega'}$-[3-(4-fluorobenzamido)propyloxycarbonyl]argininamide – Yield (General Method **A**): 0.18 g (62 %). ^{1}H-NMR (400 MHz, DMSO-d_6, HSQC, COSY, HMBC): δ_H = 1.26 (s, 9H, ^{t}Bu), 1.44 (m, 2H, -C$^{\gamma}$H$_2$-), 1.48 (s, plus additional signal for minor tautomer at 1.40, 9H, Boc), 1.55 and 1.67 (m, 2H, -C$^{\beta}$H$_2$-), 1.83 (m, 2H, -OCH$_2$CH$_2$CH$_2$NH-), 3.26 (m, 2H, -C$^{\delta}$H$_2$-), 3.31 (m, 2H, -OCH$_2$CH$_2$CH$_2$NH-), 4.02 (m, 2H, -OCH$_2$CH$_2$CH$_2$NH-), 4.22 (m, 2H, -NHCH$_2$ArO^{t}Bu), 4.36 (m, 1H, -C$^{\alpha}$H-),

5.13 (s, 1H, $Ph_2C\underline{H}$-), 6.85 (m, 2H, 2/6-H ArO^tBu), 7.09 (m, 2H, 3/5-H ArO^tBu), 7.21 (m, 2H, 4-H Ph), 7.27 (m, 2H, 2/6-H ArF), 7.28 (m, 8H, 2/3/5/6-H Ph), 7.90 (m, 2H, 3/5-H ArF), 8.35 (t, ^3J = 5.7 Hz, 1H, -$N^{\delta}\underline{H}$-), 8.43 (t, ^3J = 5.9 Hz, 1H, -$N\underline{H}CH_2ArO^tBu$), 8.47 (d, ^3J = 8.1 Hz, 1H, -$N^{\alpha}\underline{H}$-), 8.52 (t, ^3J = 5.5 Hz, 1H, -$N\underline{H}$Bz(4-F)) ppm; ^{13}C-NMR (400 MHz, DMSO-d_6, HSQC, COSY, HMBC): δ_C = 25.0 ($\underline{C}^{\gamma}$), 27.4 (^{t}Bu), 28.4 (Boc, plus additional signal for minor tautomer at 27.9), 28.4 (-$OCH_2\underline{C}H_2CH_2NH$-), 29.5 ($\underline{C}^{\beta}$), 36.2 (-$OCH_2CH_2\underline{C}H_2NH$-), 39.9 ($\underline{C}^{\delta}$), 41.1 (-$NH\underline{C}H_2ArO^tBu$), 52.3 ($\underline{C}^{\alpha}$), 55.9 ($Ph_2\underline{C}H$-), 62.3 (-$O\underline{C}H_2CH_2CH_2NH$-), 77.6 (^{t}Bu), 82.9 (Boc, plus additional signal for minor tautomer at 78.2), 115.0 (d, $^2J_{C,F}$ = 21.7 Hz), 123.4 (C-2/6 ArO^tBu), 126.4 (C-4 Ph), 127.5 (C-3/5 ArO^tBu), 128.0 and 128.4 (C-2/3/5/6 Ph), 129.6 (d, $^3J_{C,F}$ = 9.0 Hz), 130.9 (d, $^4J_{C,F}$ = 2.0 Hz), 133.6 (C-4, ArO^tBu), 140.2 (C-1 Ph), 151.9 (-$N\underline{C}(O)OCH_2$-), 153.7 (C-1 ArO^tBu), 155.3 (Boc), 163.2 (-$NH\underline{C}(N)N$-), 163.7 (d, $^1J_{C,F}$ = 247.9 Hz), 165.1 (-$HN\underline{C}(O)$ArF), 170.8 ($Ph_2CH\underline{C}(O)$-), 171.2 (-$C^{\alpha}H\underline{C}(O)$-) ppm; ESI-MS (+p) m/z (%): 853 (100) $[MH]^+$. $C_{47}H_{57}FN_6O_8$ (852.99).

(*R*)-*N*$^{\alpha}$-(2,2-Diphenylacetyl)-*N*$^{\omega}$-[3-(4-fluorobenzamido)propyloxycarbonyl]-*N*-(4-hydroxybenzyl)argininamide (**2k**) – Yield (General Method **D**): 0.12 g (87 %). mp > 105 °C (decomp.); ^{1}H-NMR (400 MHz, DMSO-d_6, HMQC): δ_H = 1.45 (m, 2H, -$C^{\gamma}\underline{H}_2$-), 1.55 and 1.69 (m, 2H, -$C^{\beta}\underline{H}_2$-, diast.), 1.90 (m, 2H, -$OCH_2C\underline{H}_2CH_2NH$-), 3.24 (m, 2H, -$C^{\delta}\underline{H}_2$-), 3.37 (m, 2H, -$OCH_2CH_2C\underline{H}_2NH$-), 4.15 (m, 2H, -$C\underline{H}_2ArOH$), 4.24 (t, ^3J = 6.4 Hz, 2H, -$OC\underline{H}_2CH_2CH_2NH$-), 4.33 (m, 1H, -$C^{\alpha}\underline{H}$-), 5.13 (s, 1H, $Ph_2C\underline{H}$-), 6.68 (m, 2H, 2/6-H ArOH), 7.00 (m, 2H, 3/5-H ArOH), 7.22 (m, 2H, 4-H Ph), 7.28 (m, 8H, 2/3/5/6-H Ph), 7.29 (m, 2H, 2/6-H ArF), 7.90 (m, 2H, 3/5-H ArF), 8.35 (t, ^3J = 5.8 Hz, 1H, -N$\underline{H}$-), 8.48 (d, ^3J = 8.1 Hz, 1H, -$N^{\alpha}\underline{H}$-), 8.57 (t, ^3J = 5.5 Hz, 1H, -N$\underline{H}$Bz(4-F)), 8.67 (t, ^3J = 5.7 Hz, 1H, -N$\underline{H}$-), 11.40 (s, 1H, -O$\underline{H}$) ppm; ^{13}C-NMR (400 MHz, DMSO-d_6, HSQC, COSY, HMBC): δ_C = 24.3 ($\underline{C}^{\gamma}$), 28.1 (-$CH_2\underline{C}H_2CH_2NH$-), 29.2 ($\underline{C}^{\beta}$), 35.7 (-$CH_2CH_2\underline{C}H_2NH$-), 40.6 ($\underline{C}^{\delta}$), 41.5 (-$\underline{C}H_2ArOH$), 52.2 ($\underline{C}^{\alpha}$), 55.8 ($Ph_2\underline{C}H$-), 64.4 (-$O\underline{C}H_2CH_2CH_2NH$-), 114.9 (C-

2/6 ArOH), 115.1 (d, $^2J_{C,F}$ = 21.9 Hz), 126.5 (C-4 Ph), 128.1 (Ph), 128.3 (Ph), 128.4 (C-3/5 ArOH), 129.7 (d, $^3J_{C,F}$ = 8.9 Hz), 130.8 (d, $^4J_{C,F}$ = 2.6 Hz), 140.3 (C-1 Ph), 152.8 (-NHC(NH)N-), 156.2 (C-1 ArOH), 158.4 (C=O), 158.7 (C-4 ArOH), 163.7 (d, $^1J_{C,F}$ = 248.3 Hz), 165.2 (F-ArCO), 170.9 (amide C=O, 2 signals) ppm; ESI-MS (+p) m/z (%): 697 (100) $[MH]^+$; RP-HPLC (Nucleodur 100-5, gradient MeCN/0.05 % aq. TFA: 0 min 25:75, 25 min 65:35): k = 5.33 (t_R = 14.6 min); analysis calcd. for $C_{38}H_{41}FN_6O_6 \cdot 1.5$ TFA $\cdot 1.5$ MeOH: C 55.73, H 5.34, N 9.18 %, found: C 55.53, H 5.40, N 9.11 %. $C_{38}H_{41}FN_6O_6$ (696.77).

4.2. NPY Y_1 Receptor Antagonistic Activity

Y_1R antagonistic activities (IC_{50} values) were determined by spectrofluorimetric measurement of the inhibition of pNPY-induced (10 nM) $[Ca^{2+}]_i$ mobilization in HEL cells[35, 36] (Fura assay). For details see ibid. and appendix.

5. Reference List

[1] Dumont, Y.; Chabot, J. G.; Quirion, R., Receptor autoradiography as mean to explore the possible functional relevance of neuropeptides: focus on new agonists and antagonists to study natriuretic peptides, neuropeptide Y and calcitonin gene-related peptides. *Peptides* **2004**, 25, 365-91.

[2] Mayer, M. Entwicklung fluorimetrischer Methoden zur Bestimmung der Affinität und Aktivität von Liganden G-Protein gekoppelter Rezeptoren. Ph.D. thesis, University of, Regensburg, **2002**.

[3] Schneider, E. Development of Fluorescence-Based Methods for the Determination of Ligand Affinity, Selectivity and Activity at G-Protein Coupled Receptors. Ph.D. thesis, University of Regensburg, Regensburg, **2005**.

[4] Mentlein, R., Dipeptidyl-peptidase IV (CD26)-role in the inactivation of regulatory peptides. *Regul. Pept.* **1999**, 85, 9-24.

[5] Mentlein, R.; Dahms, P.; Grandt, D.; Kruger, R., Proteolytic processing of neuropeptide Y and peptide YY by dipeptidyl peptidase IV. *Regul. Pept.* **1993**, 49, 133-44.

[6] Dumont, Y.; St-Pierre, J. A.; Quirion, R., Comparative autoradiographic distribution of neuropeptide Y Y_1 receptors visualized with the Y_1 receptor agonist [^{125}I][Leu^{31},Pro^{34}]PYY and the non-peptide antagonist [^{3}H]BIBP3226. *Neuroreport* **1996**, 7, 901-4.

[7] Hutzler, C. Synthese und pharmakologische Aktivität neuer Neuropeptide Y Rezeptorliganden: Von *N,N*-disubstituierten Alkanamiden zu hochpotenten Y_1-Antagonisten der Argininamid-Reihe. Ph.D. Thesis, University of Regensburg, Regensburg, **2001**.

[8] Amlal, H.; Faroqui, S.; Balasubramaniam, A.; Sheriff, S., Estrogen up-regulates neuropeptide Y Y_1 receptor expression in a human breast cancer cell line. *Cancer Res.* **2006**, 66, 3706-14.

[9] Reubi, J. C., Peptide receptors as molecular targets for cancer diagnosis and therapy. *Endocr. Rev.* **2003**, 24, 389-427.

[10] Russica, M.; Dozio, E.; Motta, M.; Magni, P., NPY family of peptides in endocrine, breast and prostate tumors. In *NPY Family of Peptides in Immune Disorders, Inflammation, Angiogenesis and Cancer*, Zukowska, Z.; Feuerstein, G. Z., Eds. Birhäuser Verlag: Basel (CH), 2005; pp 237-48.

[11] Armstrong, A.; Brackenridge, I.; Jackson, R. F. W.; Kirk, J. M., A new method for the preparation of tertiary butyl ethers and esters. *Tetrahedron Lett.* **1988**, 29, 2483-6.

[12] DeMong, D. E.; Williams, R. M., The asymmetric synthesis of (2*S*,3*R*)-capreomycidine. *Tetrahedron Lett.* **2001**, 42, 3529-32.

[13] Bernatowicz, M. S.; Wu, Y.; Matsueda, G. R., Urethane protected derivatives of 1-guanylpyrazole for the mild and efficient preparation of guanidines. *Tetrahedron Lett.* **1993**, 34, 3389-92.

[14] Feichtinger, K.; Sings, H. L.; Baker, T. J.; Matthews, K.; Goodman, M., Triurethane-Protected Guanidines and Triflyldiurethane-Protected Guanidines: New Reagents for Guanidinylation Reactions. *J. Org. Chem.* **1998**, 63, 8432-9.

[15] Feichtinger, K.; Zapf, C.; Sings, H. L.; Goodman, M., Diprotected Triflylguanidines: A New Class of Guanidinylation Reagents. *J. Org. Chem.* **1998**, 63, 3804-5.

[16] Ghosh, A. K.; Doung, T. T.; McKee, S. P.; Thompson, W. J., *N,N'*-dissuccinimidyl carbonate: a useful reagent for alkoxycarbonylation of amines. *Tetrahedron Lett.* **1992**, 33, 2781-4.

[17] Zorn, C.; Gnad, F.; Salmen, S.; Herpin, T.; Reiser, O., Deprotection of *N*-Alloc amines by Pd(0)/DABCO—an efficient method for in situ peptide coupling of labile amino acids. *Tetrahedron Lett.* **2001**, 42, 7049-53.

[18] Motulsky, H. J.; Michel, M. C., Neuropeptide Y mobilizes Ca^{2+} and inhibits adenylate cyclase in human erythroleukemia cells. *Am. J. Physiol.* **1988**, 255, E880-5.

[19] Graichen, F. Neuropeptid Y Y_1-Rezeptorantagonisten der Argininamid-Reihe: Entwicklung von Synthesemethoden an polymeren Trägern und Strategien zur Herstellung von Radioliganden. Ph.D. Thesis, University of Regensburg, Regensburg, **2002**.

[20] Brennauer, A.; Keller, M.; Freund, M.; Bernhardt, G.; Dove, S.; Buschauer, A., Towards the Development of Neuropeptide Y Y_1 Receptor Selective Tracers. In *"Frontiers in Medicinal Chemistry" Annual Meeting of the GDCh/DPhG - Fachgruppe Medizinische Chemie*, poster contribution: Leipzig, 2005.

[21] Keller, M., personal communication. In 2006.

[22] Li, T.-S.; Li, A.-X., Montmorillonite clay catalysis. Part 10: K-10 and KSF-catalysed acylation of alcohols, phenols, thiols and amines: scope and limitation. *J. Chem. Soc., Perkin Transactions 1* **1998**, 1913-8.

[23] Kruijtzer, J. A. W.; Hofmeyer, L. J. F.; Heerma, W.; Versluis, C.; Liskamp, R. M. J., Solid-Phase Syntheses of Peptoids using Fmoc-Protected N-Substituted Glycines: The Synthesis of (Retro)Peptoids of Leu-Enkephalin and Substance P. *Chemistry a European Journal* **1998**, 15, 1570-80.

[24] Rokita, S. E.; Zhou, Q. Recognition-driven alkylation of biopolymers. US2004072212, **2004**.

[25] Johnstrom, P.; Harris, N. G.; Fryer, T. D.; Barret, O.; Clark, J. C.; Pickard, J. D.; Davenport, A. P., ^{18}F-Endothelin-1, a positron emission tomography (PET) radioligand for the endothelin receptor system: radiosynthesis and in vivo imaging using microPET. *Clin Sci (Lond)* **2002**, 103 Suppl 48, 4S-8S.

[26] Wiejak, S.; Masiukiewicz, E.; Rzeszotarsk, B., Improved scalable syntheses of mono- and bis-urethane derivatives of ornithine. *Chem. Pharm. Bull. (Tokyo).* **2001**, 49, 1189-91.

[27] Tesser, G. I.; Schwyzer, R., Synthesis of 17,18-diornithine-beta-corticotropin-(1-24)-tetracosapeptide, a biologically active analogue of adrenocorticotropic hormone. *Helv. Chim. Acta* **1966**, 49, 1013-22.

[28] Maduskuie, T. P. j. Processes and Intermediate Compounds useful for the Preparation of Platelet Glycoprotein IIb/IIa Inhibitors. WO9426779, **1994**.

[29] Brook, M. A.; Chan, T. H., A simple procedure for the esterification of carboxylic acids. *Synthesis* **1983**, 201-3.

[30] Delaney, N. G.; Gordon, E. M.; Ondetti, M. A. Mercaptoalkanamide Enkephalinase Inhibitors. EP0136883, **1985**.

[31] Donati, D.; Morelli, C.; Porcheddu, A.; Taddei, M., A New Polymer-Supported Reagent for the Synthesis of β-Lactams in Solution. *J. Org. Chem.* **2004**, 69, 9316-8.

[32] Bredereck, H.; Effenberger, F.; Hajek, M., Darstellung von 1-Guanyl-pyrazol und Pyrazol-(1)-*s*-triazin. - Synthesen substituierter *s*-Triazine. *Chem. Ber.* **1965**, 98, 3178-86.

[33] Zapf, C. W.; Creighton, C. J.; Tomioka, M.; Goodman, M., A novel traceless resin-bound guanidinylating reagent for secondary amines to prepare *N,N*-disubstituted guanidines. *Org. Lett.* **2001**, 3, 1133-6.

[34] Bernthsen, A.; Klinger, H., Über Sulfinverbindunen des Thioharnstoffs. *Chem. Ber.* **1878**, 65, 1192.

[35] Geßele, K. Zelluläre Testsysteme zur pharmakologischen Charaterisierung neuer Neuropeptid Y Rezeptorantagonisten. Ph.D. thesis, University of Regensburg, Regensburg, **1998**.

[36] Mueller, M.; Knieps, S.; Gessele, K.; Dove, S.; Bernhardt, G.; Buschauer, A., Synthesis and neuropeptide Y Y_1 receptor antagonistic activity of *N,N*-disubstituted ω-guanidino- and ω-aminoalkanoic acid amides. *Arch. Pharm. (Weinheim, Ger.)* **1997**, 330, 333-42.

7 Synthesis and Y_2R Antagonistic Activity of N^{ω}-Substituted Argininamides

Abstract **– As a result of ligand based design the peptidomimetic argininamides BIBP 3226 and BIIE 0246 were identified as potent neuropeptide Y Y_1 and Y_2 receptor antagonists, respectively. In both classes of compounds the arginine side-chain is crucial for receptor binding. Introduction of electron-withdrawing substituents in N^{ω}-position of the Y_1-receptor selective (*R*)-argininamide BIBP 3226 resulted in novel compounds with reduced basicity and retained or even increased Y_1R antagonistic activity. In this study we demonstrated that the concept of using acylguanidines as bioisosteric replacement for the basic guanidine function can be succesfully applied to the synthesis of less polar Y_2 receptor antagonistic (*S*)-argininamides related to BIIE 0246.**

1. Introduction

As indicated by complete L-alanine scans the C-terminal residues Arg^{33}, Arg^{35}, and Tyr-$amide^{36}$ of neuropeptide Y are most crucial for binding to Y_1 and Y_2 receptors[1]. Based on this fact, different argininamide type mimics of the C-terminus of NPY were identified as high affinity NPY receptor ligands, e.g. BIBP 3226[2], an (*R*)-argininamide with nanomolar Y_1 receptor antagonistic activity, and BIIE 0246[3], an (*S*)-argininamide representing the only highly potent class of NPY Y_2 receptor antagonists known so far (cf. Fig. 1). Both compounds are used as standard Y_1 and Y_2 receptor antagonists, respectively. However, their *in vivo* application is limited due to the strongly basic guanidino group (pK_a value about 13) which is incompatible with oral bioavailability. As previously demonstrated in our group, the introduction of various electron withdrawing substituents at the N^G-position of **1** resulted in analogs with comparable or even increased Y_1 receptor antagonistic activity (cf.

chapters 2 and 6). The basicity of these derivatives is by 4-5 orders of magnitude lower than that of the parent argininmide, i.e. a considerable portion of the acylguanidines is uncharged at physiological pH. Moreover, such compounds are able to penetrate into the CNS and open a route to centrally available pharmacological tools.

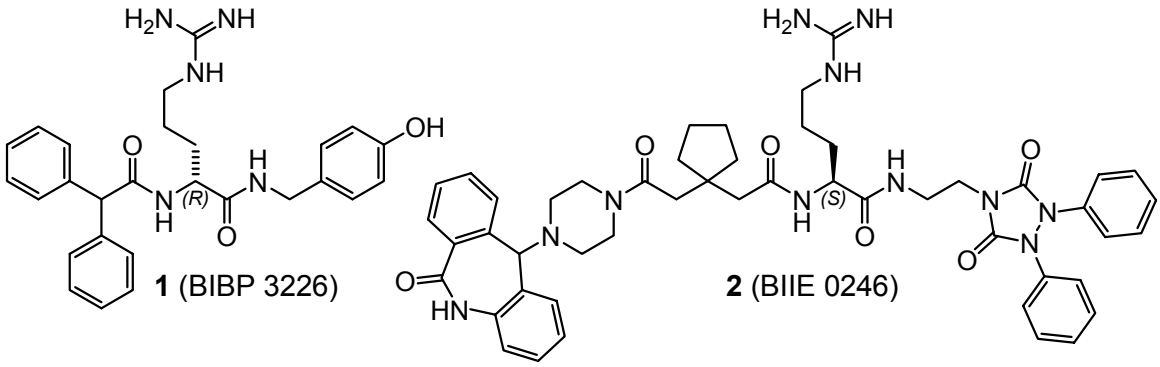


Fig. 1: Structures of Y_1 antagonistic (*R*)-argininamide BIBP 3226 (**1**) and Y_2 antagonistic (*S*)-argininamide BIIE 0246 (**2**).

Encouraged by these findings, we synthesized substituted (*S*)-argininamides related to BIIE 0246 with reduced basicity, aiming at developing potent Y_2 receptor antagonists with improved pharmacokinetic properties.

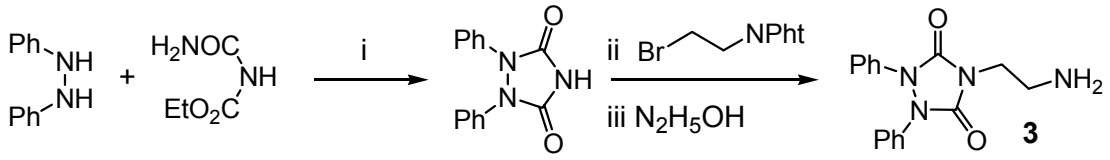
2. Results and Discussion

2.1. Chemistry

2.1.1 SYNTHESIS OF 4-(2-AMINOETHYL)-1,2-DIPHENYL-1,2,4-TRIAZOLIDINE-3,5-DIONE

The C-terminal amide nitrogen of all Y_2R antagonistic L-argininamides of the BIIE 0246 type is substituted with a 2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl

group[4]. The corresponding amine building block (**3**) was prepared by *N*-alkylation of deprotonated 1,2-diphenylurazole (1,2-diphenyl-1,2,4-triazolidine-3,5-dione) with *N*-(2-bromoethyl)phthalimide (cf. Scheme 1). Removal of the phthaloyl protective group by hydrazinolysis yielded the free amine **3**. 1,2-Diphenylurazole was obtained by condensation of 1,2-diphenylhydrazine and ethyl allophanate, which was prepared in large scale from urea and ethyl chloroformate[5].



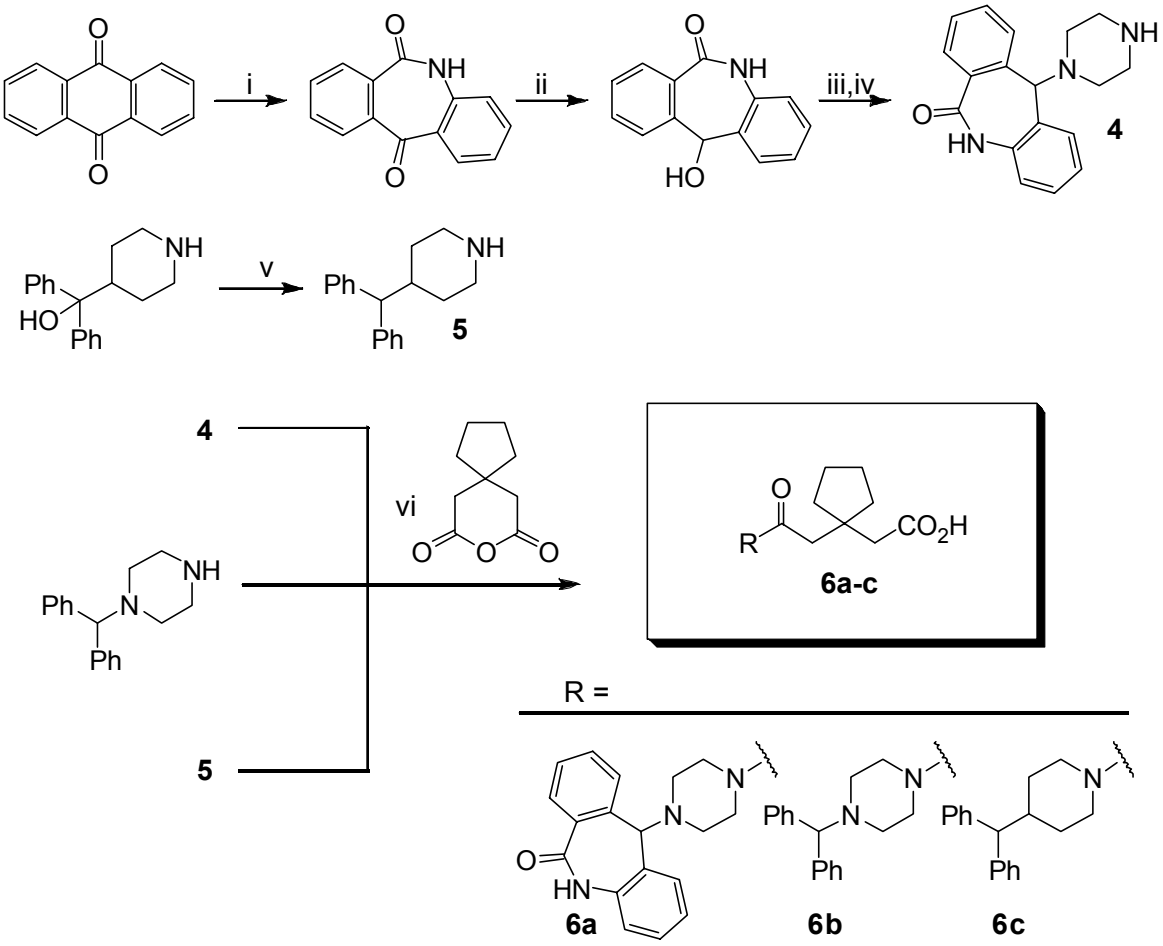
Scheme 1: Preparation of 4-(2-aminoethyl)-1,2-diphenyl-1,2,4-triazolidine-3,5-dione (**3**). Reagents and conditions: i: xylene, reflux, 24 h; ii: KO^{t}Bu, DMF, *N*-(2-bromoethyl)phthalimide; iii: 1. N_2H_5OH, THF/MeOH, 2. aq. H^+.

2.1.1. *SYNTHESIS OF 3,3-TETRAMETHYLENE GLUTARIC MONOAMIDES*

For the synthesis of BIIE 0246 and N^G-substituted analogs the carboxyl building block **6a** was required. Thus, anthraquinone was converted to 5*H*-dibenzo[*b,e*]-azepine-6,11-dione using NaN_3/H_2SO_4 (Schmidt reaction, cf. Scheme 2). The resulting ketone was reduced to the alcohol by $NaBH_4$ in refluxing ethanol. 11-Hydroxy-5,11-dihydro-dibenzo[*b,e*]azepin-6-one was converted to the 11-chloro compound using thionyl chloride. Subsequently, the alkyl chloride was allowed to react with an excess of piperazine yielding 11-piperazin-1-yl-5,11-dihydro-dibenzo[*b,e*]azepin-6-one (**4**). Lastly, reaction of piperazine **4** with 3,3-tetramethylene glutaric anhydride afforded **6a**.

In an analogous manner the alternative carboxyl components **6b** and **c** were prepared from 1-benzhydrylpiperazine and 4-benzhydrylpiperidine (**5**), respectively.

4-Benzhydrylpiperidine was obtained by careful reduction of diphenyl(piperidin-4-yl)methanol.

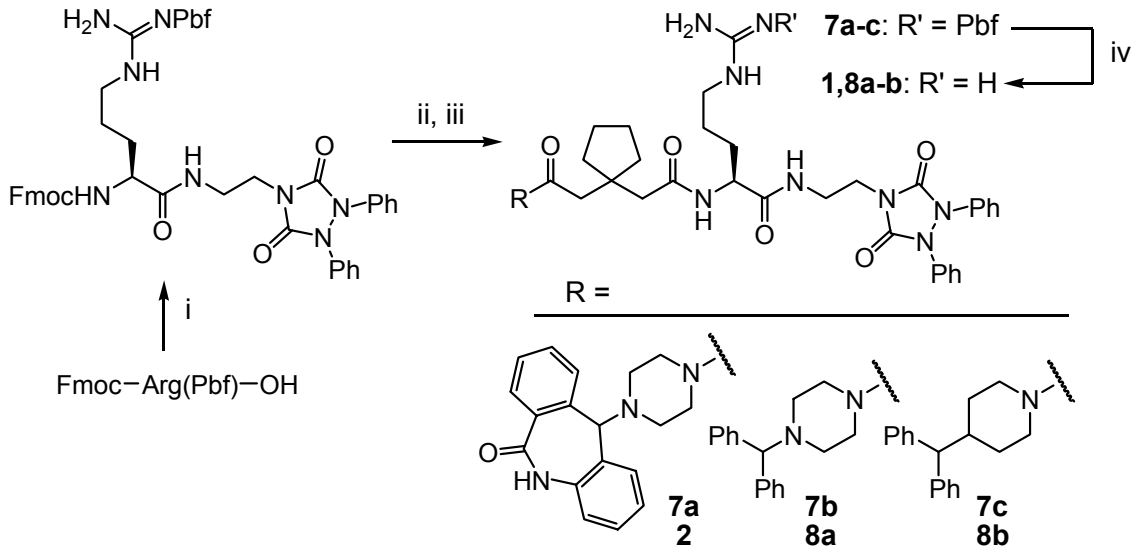


Scheme 2: Preparation of carboxyl components **6a-c**. Reagents and conditions: i: NaN_3, H_2SO_4, $CHCl_3$; ii: $NaBH_4$, EtOH; iii: $SOCl_2$, $CHCl_3$, iv: piperazine (5 eq.), 1,4-dioxane; v: $NaBH_4$, TFA, CH_2Cl_2; vi: 3,3-tetramethylene glutaric anhydride, CH_2Cl_2, r.t. overnight.

2.1.2. *SYNTHESIS OF N^G-UNSUBSTITUTED ARGININAMIDES*

The synthesis of the N^G-unsubstituted BIIE 0246 analogs is outlined in Scheme 3. As central L-aginine building block Fmoc-Arg(Pbf)-OH was used. Coupling with amine **3**, removal of the Fmoc protective group, and coupling with carboxyl fragments **6a-c**

yielded the protected argininamides, which were converted to the title compounds by final deprotection with 50 % TFA in CH_2Cl_2.



Scheme 3: Preparation of N^G-unsubstituted BIIE 0246 analogs. Reagents and conditions: i: **3**, EDC/HOBt, DMF; ii: Et_2NH, DMF; iii: **6a**,**b** or **c**, EDC/HOBt, DMF; iv: TFA/CH_2Cl_2 1:1 (v/v).

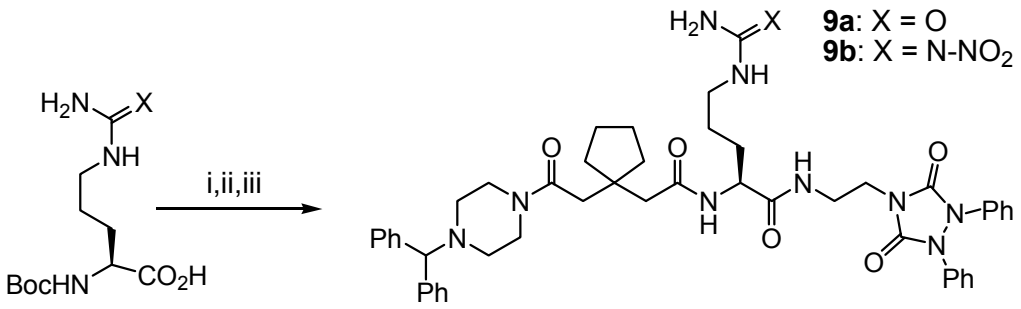
2.1.3. *SYNTHESIS OF AN N^G-TOSYLARGININAMIDE DERIVATIVE OF BIIE 0246*

The $N^{\omega'}$-tosyl* substituted analog **14l** was synthesized analogously to **7a**, but starting with Boc-protected $N^{\omega'}$-tosylarginine. Removal of the Boc group was performed in a saturated solution of HCl in ethyl acetate.

2.1.4. *SYNTHESIS OF CITRULLINAMIDES AND NITROARGININAMIDES DERIVED FROM THE ARGININAMIDE BIIE 0246*

The citrullinamide and $N^{\omega'}$-nitroarginine analogs **9a** and **b** were prepared from the corresponding N^{α}-Boc-protected amino acids as outlined in Scheme 4. For peptide coupling the building blocks were activated with CDI. After N^{α}-deprotection acylation with the succinimidyl ester of **6b** yielded the target compounds.

* For denomination of the nitrogen positions in the side chain of arginine with N^{δ}, N^{ω} and $N^{\omega'}$ cf. chapter 4, Fig. 1. "N^G- " is more general and refers to any of the guanidine-nitrogens.



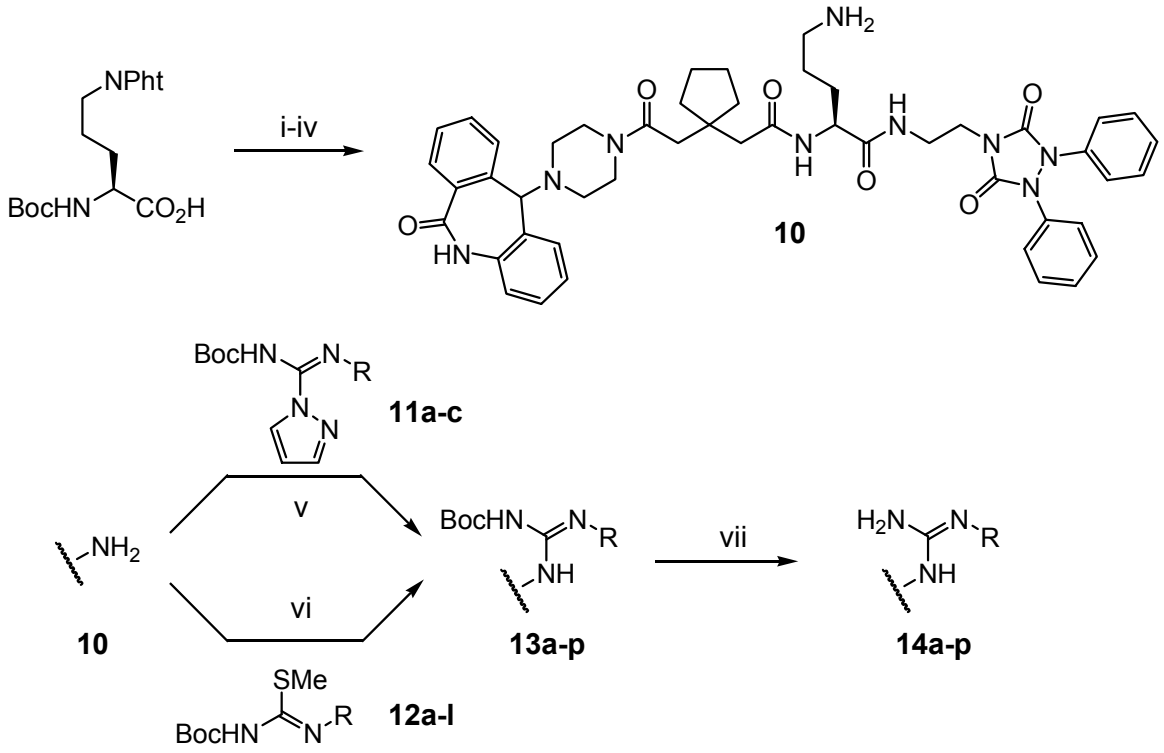
Scheme 4: Preparation of derivatives **9a,b**. Reagents and conditions: i: 1. CDI, CH_2Cl_2, 2. **3**; ii: TFA/CH_2Cl_2 1:1 (v/v); iii: succinimidyl 2-{1-[2-(4-benzhydrylpiperazin-1-yl)-2-oxoethyl]cyclopentyl} acetate, DIPEA, MeOH/THF.

2.1.5. *SYNTHESIS OF N^G-SUBSTITUTED BIIE 0246 ANALOGS*

The N^G-substituted argininamides were prepared by guanidinylation of the corresponding ornithinamide precursor **10** as outlined in Scheme 5. As orthogonal protective group for N^α and N^δ of the central ornithine residue we used the *tert*-butoxycarbonyl and the phthaloyl group, respectively.

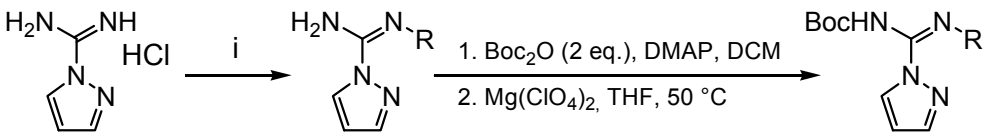
In the course of our efforts to find suitable methods for the preparation of N^G-acylated arginine derivatives we tested various guanidinylation variants based on literature methods. In modification of the frequently used bis(alkoxycarbonyl) protected isothioureas we developed *N*-acyl-*N'*-*tert*-butoxycarbonyl-*S*-methylisothioureas as novel reagents for the efficient and versatile synthesis of *N*-acyl-*N'*-alkyl substituted guanidines. Owing to their simple preparation by acylation of *N*-*tert*-butoxycarbonyl-*S*-methylisothiourea, we synthesized a small collection of various *N*-acyl-*N'*-*tert*-butoxycarbonyl-*S*-methylisothioureas (**12a-j**) using parallel synthesis techniques (cf. Table 1).

Alkylation of *N,N'*-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea under Mitsunobu conditions allows for the preparation of *N,N'*-dialkylguanidines by aminolysis of the obtained *N*-alkyl-*N,N'*-bis(*tert*-butoxycarbonyl)-*S*-methylisothioureas[6] (cf. compound **12k,l**). The building block **10** was treated with the isothioureas **12a-l** to obtain the corresponding argininamides (**14**) in good yield and high purity.



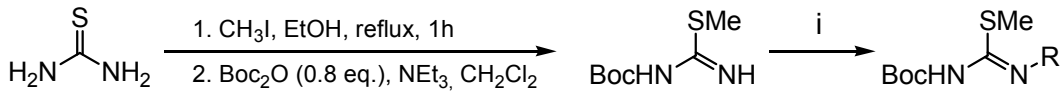
Scheme 5: Preparation of N^G-substituted argininamides. Reagents and conditions: i: **3**, DCC, HOBt, THF, 0 °C → r.t.; ii: HCl in ethyl acetate; iii: **6a**, EDC · HCl, HOBt, DIPEA, MeCN, 0 °C → r.t.; iv: 1. N_2H_5OH, MeOH, 2. aq. H^+; v: THF, r.t., 2d; vi: $HgCl_2$, DMF, r.t.; vii: TFA/CH_2Cl_2 1:1 (v/v).

Table 1: Preparation of the required guanidinylation reagents **11a-c** and **12a-l**.

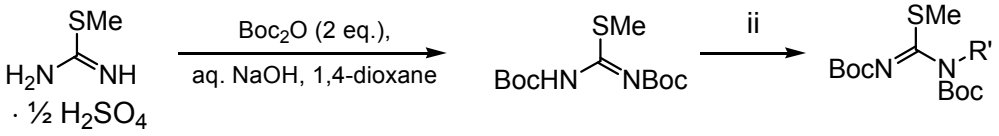


No.	R	Acylation conditions i:	Yield
11a	-CO_2Me	R-Cl, DIPEA, CH_2Cl_2.	88 %
11b	-CO_2Bn		87 %
11c	-COPh		80 %

(Table 1 continued)



No.	R	Acylation conditions i:	Yield
12a	-CO_2Et	R-Cl, DIPEA, CH_2Cl_2	90 %
12b	-$CO_2(CH_2)_3NHBoc$	[$HO(CH_2)_3NHBoc$ + DSC], NEt_3, CH_2Cl_2	65 %
12c	-$COCH_2CH_2$	R_2O, DIPEA, CH_2Cl_2	95 %
12d	-$COCH_2Ph$	R-Cl, DIPEA, CH_2Cl_2	70 %
12e	-$COCH_2OPh$		71 %
12f	-$COCH_2CH_2Ph$		81 %
12g	-$CO(CH_2)_5NHBoc$	R-OH, HOBt, TBTU, DIPEA, DMF	85 %
12h	-$CO(CH_2)_5NHBz$(4-F)		62 %
12i	-SO_2Me	Cl-SO_2Me, DIPEA, CH_2Cl_2	99 %
12j	-CONHEt	OCNEt, CH_2Cl_2	99 %



No.	R'	Alkylation conditions ii:	Yield
12k	-CH_2CH_3	R'-OH, DIAD, PBu_3, THF	31 %
12l	-CH_2Ph		55 %

Satisfactory results were also obtained using the alternative guanidinylation reagents, the *N*-acyl-*N'*-*tert*-butoxycarbonyl-1*H*-pyrazole-1-carboxamidines **11a-c**. However, the preparation of these reagents was less convenient and the use of Boc_2O/DMAP, required for the *tert*-butoxycarbonylation of the mono-acyl intermediate, is incompatible with various functional groups. The syntheses of the required guanidinylation reagents are summarized in Table 1.

Finally, the Boc-protected argininamides obtained by guanidinylation were deprotected with 50 % TFA in dichloromethane to yield the N^G-monoacylated argininamides. All compounds were characterized by NMR, ESI-MS, RP-HPLC and elemental analysis.

2.2. NPY Y_2 Receptor Antagonistic Activities

All compounds were tested for their efficiency to suppress NPY-induced mobilization of calcium ions from intracellular stores in CHO cells stably expressing hY_2 receptors. Cell clones giving a robust $[Ca^{2+}]_i$ response upon agonistic activation were obtained by co-transfection of the CHO-hY2 cells with the gene for the chimeric G-protein G_{qi5}[7]. The antagonistic activity of our compounds was determined in a spectrofluorimetric assay using the ratiometric calcium indicator dye fura-2. Maximum Ca^{2+} response was provoked by addition of 70 nM pNPY. (For further details see appendix.)

In addition, some of our compounds were intensively studied by Ziemek[7] in various *in vitro* assays: Binding constants were determined in a flow cytometric binding assay using transfected CHO cells, stably expressing the hY_2R, and fluorescence labeled NPY (cy5-pNPY, 5 nM). Inhibition of the NPY induced Ca^{2+} response was also measured in a flow cytometric experiment using fluo-4 as Ca^{2+} sensitive fluorescent dye and in a luminescence based assay using CHO-hY_2-K9-qi5-K9-mtAEQ-A7cells, which are expressing apoaequorin, a protein with Ca^{2+} dependent luciferase activity[7].

2.2.1. *DIPHENYLMETHYLPIPERAZINE AND –PIPERIDINE ANALOGS OF BIIE 0246*

Two basic arginine residues, Arg^{33} and Arg^{35}, in the C-terminus of NPY have been identified as crucial for high Y_2 receptor affinity[1]. The NPY mimetic argininamides of the BIIE 0246-type also comprise two basic centers, namely the guanidino group in the arginine side chain and the tertiary amino nitrogen in the piperazine ring. The 4-diphenylmethylpiperidine analog **8b** was synthesized in order to elucidate, if the

basic piperazine nitrogen of BIIE 0246 significantly contributes to receptor recognition. The impact of the replacement of the tricyclic dibenzo[*b,e*]azepine-6-one moiety by the diphenylmethyl motif on receptor affinity was probed with analog **8a**. Both modifications led to only moderate changes in Y_2R antagonistic activity; depending on the assay[†] a slight increase or decrease in potency was observed for **8a** compared to **2**. Analog **8b**, which is lacking the basic piperazine nitrogen, showed the same IC_{50} values as **8a** and a slightly decreased K_i value (cf. Table 2). Thus, it can be concluded that the presence of the second basic center is not essential for receptor recognition.

Table 2: Binding constants and antagonistic potencies of various BIIE 0246 analogs with reduced basicity in different assays[7].

	K_i /nM	IC_{50}/ nM		
No.	flow cytometric binding assay[a]	fluorimetric Ca^{2+} assay (fura-2)[b,d]	aequorin assay[b,d]	flow cytometric Ca^{2+} assay (fluo-4)[b,c]
2	2.6	38.4	50.9	20.4
8a	6.8	13.9	73.4	5.3
8b	41.6	10.4	69.1	3.9
9a	976.2	128.3	359.4	101.1
9b	2065	162.1	781.5	168.7

[a] displacement of 5 nM cy5-labeled NPY from CHO-hY_2-K9 cells; [b] inhibition of $[Ca^{2+}]_i$ mobilization induced with 70 nM pNPY; [c] determined with CHO-hY_2-K9-qi5-K9; [d] determined with CHO-hY_2-K9-qi5-K9-mtAEQ-A7cells.

[†] BIIE 0246 has the tendency to adsorb to the surfaces of plastic materials (microplates, cups etc.) used in the bio-assays. This — together with differing incubation times — may explain the apparent inconsistencies in the pharmacological constants of the BIIE 0246 type antagonists comparing results from different assays.

In contrast the importance of the guanidino group was demonstrated by the citrulline and $N^{\omega'}$-nitroarginine derivatives **9a** and **b**, which have substantially lower Y_2R affinities than argininamide **8a**.

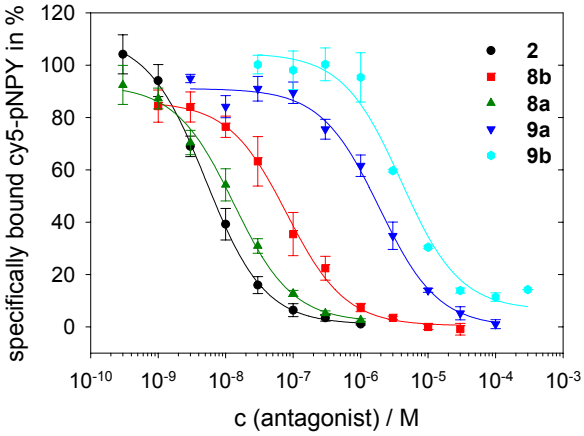


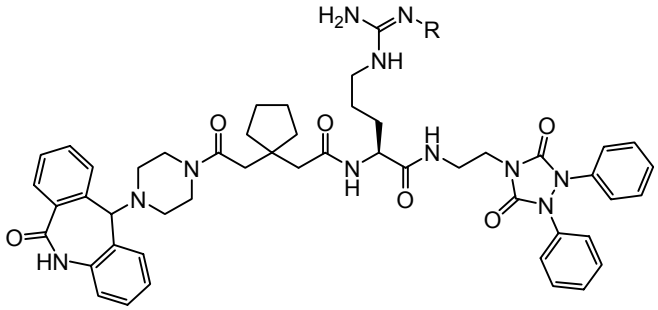
Fig. 2: Competition assay with 5 nM cy5-pNPY in presence of various concentrations of antagonists; unspecific binding was determined in the presence of 1 μM pNPY (mean values ± SEM, n=3) (reproduced from lit.[7]).

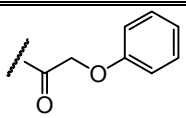
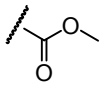
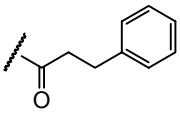
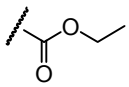
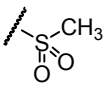
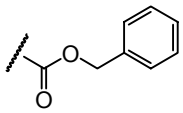
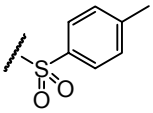
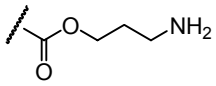
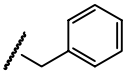
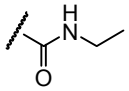
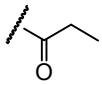
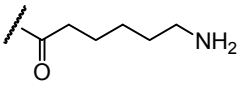
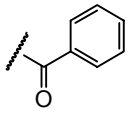
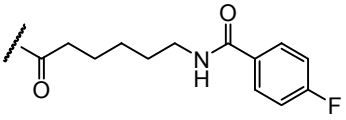
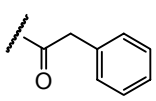
2.2.2. N^G-SUBSTITUTED BIIE 0246 ANALOGS

N^G-Substituted analogs **14a-p** suppressed NPY-induced calcium signals in CHO cells stably expressing hY_2 receptors, with IC_{50} values given in Table 3. The results indicate that the NPY Y_2 receptor tolerates electron withdrawing substituents in N^G-position — except for sulfonyl groups (cf. **12k**,**l**). The poor antagonistic activities of the N^G-sulfonyl substituted analogs reveal a) that the functionality in the arginine side-chain is crucial for affinity and b) that obviously a certain level of "residual guanidine basicity" is required, which is extinguished by too electronegative substituents.

Alkoxycarbonyl, alkylaminocarbonyl, and alkanoyl substituents have comparable influence on the potency of the Y_2R antagonistic argininamides; the respective compounds display activities ranging from about 70–200 % of the IC_{50} value of the unsubstituted antagonist **2**. An exception within our series of compounds is **14o**, which was 4-times more potent in the spectrofluorimetric Ca^{2+} assay than BIIE 0246 (**2**), the most potent Y_2 antagonist published in literature. Possibly, additional polar groups in an appropriate distance can confer additional receptor affinity. Interestingly, N^G-alkyl substituents (cf. **14m**,**n**) also do not alter the receptor binding capability of BIIE 0246 considerably.

Table 3: Y_2R antagonistic activities of N^G-substituted BIIE 0246 analogs in the spectrofluorimetric Ca^{2+} assay.



No.	R =	IC_{50} [b] / nM	No.	R=	IC_{50} [b] / nM
2[a]		38	**14i**		78
14a		73	**14j**		35
14b		73	**14k**		3 000
14c		45	**14l**		> 10 000
14d		56	**14m**		25
14e		92	**14n**		70
14f		38	**14o**		5
14g		69	**14p**		43
14h		27			

[a] BIIE 0246; [b] for inhibition of NPY-induced $[Ca^{2+}]_i$ mobilization in CHO-hY_2-K9-qi5-K9-mtAEQ-A7cells

2.2.3. *ACYLGUANIDINES AS LESS POLAR BIOISOSTERIC REPLACEMENT FOR GUANIDINES*

As known from supramolecular chemistry, acylguanidines (like guanidines) have the ability to form a tight complexes with carboxylate groups[8-15].

Receptor mutagenesis results and molecular modeling studies reveal, that the guanidino group of the Y_1R antagonistic (*R*)-argininamide BIBP 3226 (**1**)[2] and its N^G-acylated analogs interacts with a highly conserved Asp residue in TM6 (cf. chapter 3) of the Y_1 receptor. In the Y_2 receptor an Asp residue is found in the corresponding position, suggesting that the guanidine moiety binds to similar pockets in both receptors. Obviously, both receptors tolerate acylguanidines as less basic replacement for the guanidine motif.

Recently, Xie et al.[16] reported that acylguanidine analogs of impromidine (IMP) and arpromidine (ARP), two guanidine type histamine receptor ligands, are similarly potent agonists compared to IMP and ARP at the gpH_2R. All these results support the hypothesis that acylguanidines are potential guanidine bioisosteres, avoiding the adverse physicochemical and pharmacokinetic properties of the strongly basic guanidine function.

3. Summary and Conclusion

We established a straight-forward synthetic route to N^G-acylargininamide-type Y_2 receptor antagonists. One of the key steps was the guanidinylation of the respective ornithinamide with *N'*-substituted *N-tert*-butoxycarbonyl-*S*-methylisothioureas. The resulting N^G-substituted (*S*)-argininamides related to BIIE 0246 showed Y_2 antagonistic activities comparable to that of the parent compound — with exception of N^G-sulfonyl substituted analogs, which are substantially less potent. Therefore, the acylguanidine substructure can be regarded as a useful guanidine substitute for the synthesis of arginine derived NPY receptor ligands.

4. Experimental section

4.1. Synthetic Chemistry

4.1.1. GENERAL CONDITIONS

Chemicals were purchased from the following suppliers: Acros Organics (Geel, Belgium), Advanced ChemTech, Inc. (Hatley St George, UK), Alfa Aesar GmbH & Co. KG (Karlsruhe, Germany), Bachem AG (Bubendorf, Switzerland), IRIS Biotech GmbH (Marktredwitz, Germany), Mallinckrodt Baker (Deventer, NL), Merck KGaA and Merck Biosciences GmbH[‡] (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); solvents were dried by refluxing over sodium hydride[§] (diethyl ether, DME, 1,4-dioxane, MTBE, THF, and toluene) or phosphorous(V)-oxide (acetonitrile, chloroform, dichloromethane, 1,2-dichloro-ethane). Dried solvents were stored over micro sieves (4 Å) under protective gas. DMF ($H_2O < 0.01$ %) was purchased from Sigma-Aldrich Chemie GmbH. Triethyl amine and DIPEA were distilled from calcium hydride.

Parallel syntheses were performed in a Synthesis 1 Liquid 12 reactor from Heidolph, (Schwabach, Germany).

Nuclear Magnetic Resonance (^{1}H-NMR and ^{13}C-NMR) spectra were recorded on an Avance-300, Avance-400, or Avance-600 NMR spectrometer from Bruker BioSpin GmbH (Rheinstetten, Germany). Tetramethylsilane was added as internal standard (chemical shift $\delta = 0$ ppm) to all samples.

Multiplicities are specified with the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (for broad signal), as well as combinations

[‡] formerly Novabiochem
[§] until sodium/benzophenone reaction was positive

thereof. If atoms are specified with numbers, e.g. 4-H or C-2, the numbering is relative to substituent/heteroatom with highest priority *within* denoted subsystem. In certain cases 2D-NMR techniques (HSQC, HMQC, HMBC, and COSY) were used to assign ^{1}H and ^{13}C chemical shifts.

Mass spectrometry analysis (MS) was performed on a Finnigan MAT 95 (PI-EIMS 70 eV, HR-MS), a Finnigan SSQ 710A (PI-EIMS 70 eV, CI-MS (NH_3) and on a Finnigan ThermoQuest TSQ 7000 (ESI-MS, APCI-MS) spectrometer. Infrared spectra (IR) were recorded on a BRUKER TENSOR 27 spectrometer equipped with an ATR (attenuated total reflexion) unit from Harrick Scientific Products Inc. (Ossining/NY, US). UV-spectroscopy was performed using a Varian Cary 100 Conc UV/Vis spectrophotometer. Melting points (mp) were measured on a BÜCHI 530 or on an electrically heated copper block apparatus from Pefra (Germany) using open capillaries and are uncorrected.

Merck Silica Gel 60 (particle size 0.040–0.063 mm) was used for flash column chromatography; vacuum flash chromatography was carried out using Merck silica gel 60 H (particle size 90 % < 0.045 mm). The department of microanalysis Regensburg carried out elemental analysis. Compounds were dried in vaccum (0.1–1Torr) at room temperature or with heating up to 50 °C for at least 24 h prior to submission for elemental analysis. 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 ninhydrin spray, or ammonium molybdate/cerium(IV) sulfate solution.

Analytical reversed phase HPLC (RP-HPLC) was performed on a system from Thermo Separation Products (Egelsbach, Germany) equipped with a SN 400 controller, a P4000 pump, an AS3000 auto sampler and a Spectra Focus UV-Vis detector. Stationary phase was a Nucleodur 100-5 C-18 (250 × 4.0 mm, 5 μm) column (Macherey-Nagel, Düren, Germany) thermostated at 30 °C. As mobile phase the following gradient of acetonitrile and 0.05 % aqueous trifluoroacetic acid was used: 0 min 15:85, 35 min 95:5, 40 min 95:5, 42 min 15:85, 50 min 15:85

(flow rate: 0.8 ml·min^{-1}). Absorbance was detected at 210 nm. An ethanolic solution of the analyte (c = 10 mM) was diluted with MeCN/0.05 % TFA to a concentration of approx. 50 μM; injection volume was 100 μL.

Relative molecular weights (in Da) are given in parentheses behind the formula.

4.1.2. PREPARATION OF AMINE COMPONENT 4-(2-AMINOETHYL)-1,2-DIPHENYL-1,2,4-TRIAZOLIDINE-3,5-DIONE (3)

Ethyl allophanate – A mixture of ethyl chloroformate (95.3 mL, 1 mol) and urea (126.0 g, 2.1 mol) was placed in a round bottom flask fitted with a reflux condenser and heated on a water-bath for 3 h. Water (200 mL) was added to the warm mixture and the resulting suspension was stirred for 15 min. The mass was filtered with suction, washed with water, and recrystallized from hot ethanol affording the pure product as a colorless crystalline powder (yield: 62 g, 47 %). Mp 189 °C (lit.[5] 189–192 °C); ^{1}H NMR (300 MHz, DMSO-d_6): δ_H = 1.20 (t, 3H, ^3J = 7.1 Hz, -C$\underline{H}_3$), 4.10 (q, 2H, ^3J = 7.1 Hz, -C$\underline{H}_2$CH$_3$), 7.13 (brs, 1H, -N$\underline{H}_2$), 7.23 (brs, 1H, -N$\underline{H}_2$), 9.83 (s, 1H, -N$\underline{H}$-) ppm; ^{13}C NMR (300 MHz, DMSO-d_6): δ = 14.1 (-$\underline{C}$H$_3$), 61.0 (O$\underline{C}$H$_2$-), 153.6 ($\underline{C}$=O), 154.4 ($\underline{C}$=O) ppm. $C_4H_8N_2O_3$ (132.12).

1,2-Diphenyl-1,2,4-triazolidine-3,5-dione – 1,2-Diphenylhydrazine (18.4 g, 0.1 mol) and ethyl allophanate (13.2 g, 0.1 mol) were heated in xylene (300mL) under reflux for 20 h. The mixture was allowed to cool to ambient temperature and then poured into 300 mL petroleum ether. The product was collected on a filter and re-dissolved in acetone (400 mL). Insoluble by-products were filtered off and the filtrate concentrated to about 200 mL. After addition of water (200 mL) crystals begin to form. After complete crystallization the product was separated by filtration, washed with water, and dried in vacuum affording 14.0 g (55 %) pale yellow crystals. Mp 216–217 °C (lit.[4] mp 216–218 °C); IR (KBr) ν = 3400 (m, -NH), 3167 (m), 3062 (m, C_{ar}-H), 1778 (s), 1729 (s), 1595 (s), 1390 (s) cm^{-1}; ^{1}H NMR (250 MHz, DMSO-d_6): δ = 7.15–7.40 (m, 10H, $\underline{H}_{ar}$), 12.01 (brs, 1H, -N$\underline{H}$) ppm; EI-MS (70 eV) *m/z* (%): 253 (100) M$^{\cdot+}$, 105 (91), 77 (89), 91 (84); analysis calcd. for $C_{14}H_{11}N_3O_2$: C

66.40, H 4.38, N 16.59 %; found: C 66.47, H 4.44, N 17.02 %. $C_{14}H_{11}N_3O_2$ (253.26).

4-(2-Phthalimidoethyl)-1,2-diphenyl-1,2,4-triazolidine-3,5-dione – 1,2-Diphenyl-1,2,4-triazolidine-3,5-dione (2.53 g, 10.0 mmol) were dissolved in 30 mL DMF and the solution was cooled in an ice-water bath. KO^{t}Bu (1.23 g, 11.0 mmol) was added under stirring. After the KO^{t}Bu was completely dissolved, 2-bromoethylphthalimid (2.79 g, 11.0 mmol) was introduced and the mixture heated under reflux for 5 h. The resulting mixture was concentrated under reduced pressure and the residue was suspended in an aq. solution of K_2CO_3. The product was collected on a sintered filter, washed with water, and dried in vacuum at 50 °C. Recrystallization from 2-propanol afforded 3.88 g (91 %) of the title compound as a colorless, crystalline solid. Mp 169–170 °C; IR (KBr) ν = 3657 (w), 2953 (w), 1769, 1717 (s), 1442 (s); ^{1}H-NMR (250 MHz, DMSO-d_6): δ_H = 3.93–4.10 (m, 4H, -C$\underline{H}_2$C$\underline{H}_2$-), 7.10–7.40 (m, 10H, Ph), 7.50–7.80 (m, 4H, Pht) ppm; EI-MS (70 eV) *m/z* (%): 426 (100) $M^{\cdot+}$, 253 (92) 1,2-diphenylurazol$^{\cdot+}$, 183 (75) $[Ph_2N_2H]^+$. $C_{24}H_{18}N_4O_4$ (426.42).

4-(2-Aminoethyl)-1,2-diphenyl-1,2,4-triazolidine-3,5-dione (**3**) – 4-(2-Phthalimidoethyl)-1,2-diphenyl-1,2,4-triazolidine-3,5-dione (7.78 g, 18.2 mmol) was dissolved in 150 mL THF/methanol 2:1 (v/v) and treated with (2.50 g, 50 mmol) hydrazine hydrate. The mixture was stirred at ambient temperature for 16 h. 2 M hydrochloric acid was added and stirring was continued for 2h. The organic solvents were removed under reduced pressure and the residual suspension was diluted with water (90 mL) and alkalified with 2 M aq. NaOH. The aqueous phase was extracted with CH_2Cl_2 (3 × 80 mL); the extracts were pooled, dried over K_2CO_3 and evaporated to dryness. The crude product was suspended in ethanol and treated with 1 M HCl in diethyl ether (20 mL). The precipitate was collected on a filter, washed with petroleum ether, and dried. Yield: 5.10 g (85 %) of the HCl salt. In order to obtain the free amine base, the hydrochloride was partitioned between 2 M aq. NaOH and CH_2Cl_2. The CH_2Cl_2 extracts were dried over anhydrous K_2CO_3 and concentrated. The residue was dried in vacuum and digested with hot MTBE

whereupon the product separated as white powder. The product was filtered off and recrystallized from hot cyclohexane. Mp 95–95.5 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ_H = 1.61 (brs, 2H, -N<u>H</u>$_2$), 2.79 (t, ^3J = 6.5 Hz, 2H, -CH$_2$C<u>H</u>$_2$NH$_2$), 3.54 (t, ^3J = 6.5 Hz, 2H, -C<u>H</u>$_2$CH$_2$NH$_2$), 7.18–7.28 (m, 2H, Ph), 7.33–7.43 (m, 8H, Ph) ppm; ESI-MS (+p) *m/z* (%): 297 (100) $[MH]^+$, 593 (3) $[2M+H]^+$ analysis calcd. for $C_{16}H_{16}N_4O_2$: C 64.85, H 5.44, N 18.91 %; found: C 65.08, H 5.32, N 18.73 %. $C_{16}H_{16}N_4O_2$ (296.32).

4.1.3. PREPARATION OF CARBOXYLIC ACID COMPONENTS **6A-C**

5*H*-Dibenzo[*b,e*]azepine-6,11-dione – A mechanically stirred mixture of 9,10-anthraquinone (20.8 g, 0.1 mol), sodium azide (7.8 g, 0.12 mol) and $CHCl_3$ was cooled in an ice-bath, while concentrated sulfuric acid (60 mL) was added dropwise. After completion of the addition the mixture was heated under reflux for 10 h. The cold mixture was poured into a chilled sodium carbonate solution (10 % w/w, 750 mL) in small portions. The mixture was alkalized with concentrated ammonia solution and allowed to stand until the precipitate had settled on the ground. The supernatant clear solution was decanted and replaced by methanol (300 mL). The raw product was collected on a filter, washed with methanol and ether and dissolved in boiling acetic acid. Insoluble by-products were removed by filtering the hot suspension. After cooling, the product was separated by filtration and dried in vacuum at 60 °C affording fine, pale yellow crystals (13.2 g, 59 %). Mp 244–245 °C (acetic acid, lit.[17] mp 250–251 °C); ^{1}H-NMR (250 MHz, DMSO-d_6): δ_H = 7.27 (m, 1H, <u>H</u>$_{ar}$), 7.38 (m, 1H, <u>H</u>$_{ar}$), 7.62 (m, 1H, <u>H</u>$_{ar}$), 7.75 (m, 1H, <u>H</u>$_{ar}$), 7.80–7.92 (m, 3H, <u>H</u>$_{ar}$), 8.20 (m, 1H, <u>H</u>$_{ar}$), 11.13 (s, 1H, -N<u>H</u>) ppm. $C_{14}H_9NO_2$ (223.23).

11-Hydroxy-5,11-dihydrodibenzo[*b,e*]azepin-6-one – Sodium borohydride (3.0 g, 80 mmol) was slowly added to a suspension of 5*H*-dibenzo[*b,e*]azepine-6,11-dione (9.0 g, 40 mmol) in ethanol (500 mL). After complete addition the mixture was heated under reflux for 1 h. The mixture was concentrated in vacuum to about 100 mL, treated with ammonium chloride solution and neutralized with hydrochloric

acid. The colorless precipitate was filtered, washed with water, methanol, and petroleum ether, and dried in vacuum (yield: 8.5 g, 94 % as a white powder). Mp 247–248 °C (lit.[18] mp 262–264 °C); ^{1}H-NMR (250 MHz, DMSO-d_6): δ_H = 5.65 (s, 1H, 11-H), 6.40 (s, 1H, -O<u>H</u>), 7.0–8.0 (m, 8H, <u>H</u>$_{ar}$), 10.5 (s, 1H, -N<u>H</u>-) ppm. $C_{14}H_{11}NO_2$ (225.24).

11-Chloro-5,11-dihydrodibenzo[*b,e*]azepin-6-one – To a suspension of 8.5 g (37.5 mmol) 11-hydroxy-5,11-dihydrodibenzo[*b,e*]azepin-6-one in $CHCl_3$ (200 mL) was added thionyl chloride (10 mL) and the reaction mixture was heated under reflux for 30 min. The mixture was concentrated under reduced pressure and treated with n-hexane (100 mL). The product was collected on a filter, washed with n-pentane and dried in vacuum (yield: 8.8 g, 96 %). Mp 218–220 °C (benzene, lit.[19] mp 226 °C); ^{1}H-NMR (300 MHz, DMSO-d_6): δ_H = 5.68 (s, 1H, 11-H), 7.0–8.0 (m, 8H, <u>H</u>$_{ar}$), 10.84 (s, 1H, -N<u>H</u>) ppm. $C_{14}H_{10}ClNO$ (243.69).

11-Piperazin-1-yl-5,11-dihydrodibenzo[*b,e*]azepin-6-one (**4**) – A solution of 11-chloro-5,11-dihydrodibenzo[*b,e*]azepin-6-one (8.81 g, 36.2 mmol) in 150 mL 1,4-dioxane was added slowly to a stirred solution of piperazine (15.6 g, 5 eq.) in 300 mL 1,4-dioxane. To complete the reaction, the mixture was heated under reflux for 30 min. After cooling, the solvent was removed under reduced pressure and the residue partitioned between water (150 mL) and $CHCl_3$ (100 mL). The aqueous phase was extracted with two further portions of $CHCl_3$ and the combined extracts were washed with aq. K_2CO_3 solution and dried over anhydrous K_2CO_3. After evaporation of the volatiles the raw product was purified by vacuum flash chromatography (impurities were eluted with acetonitrile, the desired amine with an acetonitrile/methanol 2:1 mixture containing 0.1 % NEt_3) yield: 7.00 g (66 %) as a white powder. Mp > 260 °C (decomp.); ^{1}H NMR (300 MHz, DMSO-d_6): δ_H = 2.31 (m, 4H, -C<u>H</u>$_2$CH$_2$NH-), 2.85 (m, 4H, -CH$_2$C<u>H</u>$_2$NH-), 4.40 (s, 1H, Ar-C<u>H</u>-Ar), 7.0–7.8 (m, 8H, <u>H</u>$_{ar}$), 10.38 (s, 1H, -N<u>H</u>-); ^{13}C NMR (300 MHz, DMSO-d_6): δ_C = 42.7 (-NH<u>C</u>H$_2$CH$_2$NR-), 47.4 (-NHCH$_2$<u>C</u>H$_2$NR-), 72.8 (C-11), 121.3 (<u>C</u>$_{ar}$H), 123.7 (<u>C</u>$_{ar}$H), 127.6 (<u>C</u>$_{ar}$H), 128.0 (<u>C</u>$_{ar}$H), 128.4 (<u>C</u>$_{ar}$H), 129.8 (<u>C</u>$_{ar}$H), 130.3 (<u>C</u>$_{ar}$H), 130.6 (<u>C</u>$_{ar}$),

131.3 ($\underline{C}_{ar}H$), 131.5 ($\underline{C}_{ar}$), 136.0 ($\underline{C}_{ar}$), 141.5 ($\underline{C}_{ar}$), 168.0 ($\underline{C}$=O) ppm; MS (CI) *m/z* (%): 294 (100) $[MH]^+$; analysis calcd. for $C_{18}H_{19}N_3O \cdot 0.3\ H_2O$: C 72.36, H 6.61, N 14.07 %, found: C 72.39, H 6.33, N 13.74 %. $C_{18}H_{19}N_3O$ (293.36).

2-(1-{2-Oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetic acid (**6a**) 11-Piperazin-1-yl-5,11-dihydrodibenzo[*b,e*]-azepin-6-one (4.19 g, 14.3 mmol) in 50 mL anhydrous CH_2Cl_2 was slowly added to a stirred and chilled (0 °C) solution of 3,3-tetramethyleneglutaric anhydride (2.41 g, 14.3 mmol) in 100 mL anhydrous CH_2Cl_2. After stirring overnight at ambient temperature the reaction mixture was filtered and the filtrates were concentrated to dryness. The residue was subjected to vacuum flash chromatography eluting with mixtures of $CHCl_3$/acetonitrile. Yield: 6.52 g (99 %). Mp 153–157 °C (ethyl acetate, lit.[4] mp 154–157 °C); ^{1}H-NMR (300 MHz, DMSO-d_6): δ_H = 1.53 (m, 8H, cyclopentyl), 1.90–2.10 (m, 4H, 3/5-H piperazine), 2.42 (s, 2H, >C(O)C$\underline{H}_2$-), 2.45 (s, 2H, -C$\underline{H}_2CO_2H$), 3.29 (m, 4H, 2/6-H piperazine), 4.24 (s, 1H, dibenzazepin-11-yl), 7.00–7.74 (m, 8H, H_{ar}), 10.35 (s, 1H, -N$\underline{H}$), 11.97 (brs, 1H, -$CO_2\underline{H}$) ppm; ^{13}C-NMR (300 MHz, DMSO-d_6): δ_C = 23.3 (C-3/4 cyclopentyl), 37.3 (C-2/5 cyclopentyl), 38.5 (>NC(O)$\underline{C}H_2$-), 40.6 and 45.1 (C-2/6 piperazine), 41.5 (-$\underline{C}H_2CO_2H$), 42.8 (C-1 cyclopentyl), 50.6 and 51.1 (C-3/5 piperazine), 73.6 (C-11 dibenzazepin-11-yl), 121.2 ($\underline{C}_{ar}H$), 123.5 ($\underline{C}_{ar}H$), 127.5 ($\underline{C}_{ar}H$), 127.8 ($\underline{C}_{ar}H$), 128.2 ($\underline{C}_{ar}H$), 129.7 ($\underline{C}_{ar}H$), 130.3 ($\underline{C}_{ar}H$), 131.0 ($\underline{C}_{ar}$), 131.2 ($\underline{C}_{ar}H$), 131.5 ($\underline{C}_{ar}$), 136.0 ($\underline{C}_{ar}$), 141.9 ($\underline{C}_{ar}$), 167.9 ($\underline{C}$=O lactam), 169.3 ($\underline{C}$(O)N), 173.3 (-$\underline{C}O_2H$) ppm; ESI-MS (-p) *m/z* (%): 460 (100) $[M - H]^-$; analysis calcd. for $C_{27}H_{31}N_3O_4 \cdot EtOH$ C 68.61, H 7.35, N 8.28 %, found: C 68.70, H 7.42, N 8.28 %. $C_{27}H_{31}N_3O_4$ (461.55).

2-{1-[2-(4-Benzhydrylpiperazin-1-yl)-2-oxoethyl]cyclopentyl}acetic acid (**6b**) – 3,3-Tetramethyleneglutaric anhydride (8.41 g, 50.0 mmol) was dissolved in 300 mL anhydrous CH_2Cl_2, and cooled in an ice-water bath. A solution of *N*-benzhydrylpiperazine (12.62 g, 50.0 mmol) in anhydrous CH_2Cl_2 (500 mL) was added dropwise while the mixture was stirred intensely. After stirring overnight at ambient temperature the solvent was removed; the residual colorless oil was

dissolved in warm MTBE-cyclohexane. The product separated as a white solid, when the mixture was allowed to cool to ambient temperature under vigorous stirring. After complete precipitation the product was collected on a filter, dried, and recrystallized from THF/cyclohexane. Yield: 19.0 g (90 %). Mp 132–133 °C (lit.[4] mp 67–72 °C); ^{1}H-NMR (300 MHz, DMSO-d_6): δ_H = 1.54 (m, 8H, cyclopentyl), 2.24 (m, 4H, 3/5-H piperazine), 2.43(-C<u>H</u>$_2$CO$_2$H), 2.48 (>NC(O)C<u>H</u>$_2$-), 3.46 (brs, 4H, 2/6-H piperazine), 4.29 (Ph$_2$C<u>H</u>-), 7.19 (m, 2H, 4-H Ph), 7.29 (m, 4H, 2/6-H Ph), 7.43 (m, 4H, 3/5-H Ph), 12.00 (brs, 1H, -CO$_2$<u>H</u>) ppm; ^{13}C-NMR (300 MHz, DMSO-d_6): δ_C = 23.4 (C-3/4 cyclopentyl), 37.3 (C-2/5 cyclopentyl), 38.5 (>NC(O)<u>C</u>H$_2$-), 40.8 and 45.3 (C-2/6 piperazine), 41.6 (-<u>C</u>H$_2$CO$_2$H), 42.9 (C-1 cyclopentyl), 51.5 (C-3/5 piperazine), 74.7 (Ph$_2$<u>C</u>H-), 126.8 (C-4 Ph), 127.5 (C-2/6 Ph), 128.5 (C-3/5 Ph), 169.5 (>N<u>C</u>(O)CH$_2$-), 173.4 (-CH$_2$<u>C</u>O$_2$H) ppm; CI-MS (NH_3) *m/z* (%): 421 (94) $[MH]^+$, 253 (100) $[Ph_2CH - C_4H_8N_2H_2]^+$, 186 (69) $[C_9H_{12}O_3H]^+$; analysis calcd. for $C_{27}H_{31}N_3O_4$: C 74.26, H 7.67, N 6.66. %, found: C 73.94, H 7.67, N 6.80 %. $C_{27}H_{31}N_3O_4$ (420.54).

4-Benzhydrylpiperidine – Sodium borohydride, $NaBH_4$ (1.13 g, 30.0 mmol) was slowly introduced into 25 mL chilled (0 °C) TFA. After stirring for 30 min a solution of diphenyl(piperidin-4-yl)methanol (1.33 g, 5.0 mmol) in anhydrous CH_2Cl_2 (10 mL) was added in a dropwise manner over a period of 30 min. The cooling bath was removed and stirring was continued overnight. By careful addition of 2 M aq. NaOH the pH was adjusted to alkaline and the mixture was extracted with CH_2Cl_2. The extracts were pooled, dried over anhydrous K_2CO_3, and evaporated. The raw product was purified by vacuum flash chromatography, eluting with $CHCl_3$/methanol, containing 1 % methanolic NH_3 (ca. 7 M). Yield: 0.79 g (63 %). The product was crystallized from hot n-hexane. Mp 100–101 °C (lit.[20] mp 99 °C); ^{1}H-NMR (300 MHz, DMSO-d_6): δ_H = 0.93 (m, 2H, 3/5-H ax.), 1.34 (m, 2H, 3/5-H eq.), 2.24 (m, 1H, 4-H), 2.40 (m, 2H, 2/6-H ax.), 2.84 (m, 2H, 2/6-H eq.), 3.52 (m, 1H, Ph$_2$C<u>H</u>-), 7.12 (m, 2H, 4-H Ph), 7.25 (m, 4H, 2/6-H Ph), 7.35 (m, 4H, 3/5-H Ph) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 32.1 (C-3/5), 39.1 (C-4), 46.0 (C-2/6), 58.5

($Ph_2\underline{C}H$-), 125.8 (C-4 Ph), 127.8 (C-2/6 Ph), 128.3 (C-3/5 Ph) ppm; CI-MS (NH_3) *m/z* (%): 252.3 (100) [MH^+]; analysis calcd. for $C_{18}H_{21}N \cdot 0.1\ H_2O$: C 85.39, H 8.44, N 5.53 %, found: C 85.40, H 8.08, N 5.50 %. $C_{18}H_{21}N$ (251.37).

2-{1-[2-(4-Benzhydrylpiperidin-1-yl)-2-oxoethyl]cyclopentyl}acetic acid (**6c**) – A solution of 4-benzhydrylpiperidine (251 mg, 1.0 mmol) and DIPEA (172 µL, 1.0 mmol) in CH_2Cl_2 (5 mL) was cooled in an ice-water bath and stirred magnetically. 3,3-Tetramethyleneglutaric anhydride (168 mg, 1.0 mmol) in anhydrous CH_2Cl_2 (5 mL) was added dropwise and stirring was continued overnight. The mixture was diluted with CH_2Cl_2 (50 mL), washed with 5 % aq. $KHSO_4$ and brine, dried over anhydrous Na_2SO_4, and evaporated. After drying in vacuum the product was obtained as colorless solid (390 mg, 93 %). Mp 171–173 °C; ^{1}H-NMR (600 MHz, DMSO-d_6, HSQC): δ_H = 0.89 and 0.99 (m, 2H, 3/5-H ax. piperidine), 1.42 (m, 2H, 3/5-H eq. piperidine), 1.52 (m, 4H, 2/5-H cyclopentyl), 1.55 (m, 4H, 3/4-H cyclopentyl), 2.43 (m, 2H, -$C\underline{H}_2CO_2H$), 2.46 (m, 2H, >NC(O)$C\underline{H}_2$-), 2.46 (m, 1H, 4-H piperidine), 2.48 and 4.35 (m, 2H, 2-H piperidine), 2.93 and 3.87 (m, 2H, 6-H piperidine), 3.56 (d, ^3J = 11.2 Hz, 1H, $Ph_2C\underline{H}$-), 7.14 (m, 2H, 4-H Ph), 7.27 (m, 4H, 2/6-H Ph), 7.37 (m, 4H, 3/5-H Ph), 12.10 (brs, 1H, -$CO_2\underline{H}$) ppm; ^{13}C-NMR (600 MHz, DMSO-d_6, HSQC): δ_C = 23.4 (C-3/4 cyclopentyl), 30.6 and 31.4 (C-3/5 piperidine), 37.3 (C-2/5 cyclopentyl), 38.4 (C-4 piperidine), 38.7 (>NCO$\underline{C}H_2$-), 40.8 and 45.2 (C-2/6 piperidine), 41.7 (-$\underline{C}H_2CO_2H$), 43.0 (C-1 cyclopentyl), 57.4 ($Ph_2\underline{C}H$-), 125.9 (C-4 Ph), 127.8 (C-2/6), 128.4 (C-3/5), 143.8 (C-1 Ph), 169.2 (>N$\underline{C}$(O)CH_2-), 173.5 (-$\underline{C}O_2H$) ppm; ESI-MS (+p) *m/z* (%): 420 (100) $[MH]^+$, 442 (3) $[MNa]^+$; analysis calcd. for $C_{27}H_{33}NO_3 \cdot 0.2\ H_2O$: C 76.63, H 7.96, N 3.31 %, found: C 76.77, H 8.07, N 3.18 %. $C_{27}H_{33}NO_3$ (419.56).

*4.1.4. PREPARATION OF ORNITHINAMIDE **10***

***(S)*-N^{α}-*tert*-Butoxycarbonyl-N^{δ}-phthaloylornithine (Boc-Orn(Pht)-OH)** – To a clear solution of ornithine hydrochloride (16.85 g, 0.10 mol) and NaOH (8.0 g, 0.20 mol) in 175 mL water was added $CuSO_4 \cdot 5\ H_2O$ (12.5 g, 0.05 mol) in 175 mL

water. After stirring for 15 min $NaHCO_3$ (10.0 g, 0.12 mol) and *N*-ethoxycarbonyl phthalimide were introduced and the mixture was intensely stirred for an additional h. The resulting blue precipitate of $[H\text{-}Orn(Pht)O]_2Cu$ was collected on a sintered filter, washed with water, ethanol, $CHCl_3$ and diethyl ether, and dried in vacuum. To the finely grounded blue powder was added 6 M hydrochloric acid (150 mL) and the suspension was stirred vigorously for 1 h. The solid material was separated by filtration, washed with 6 M hydrochloric acid and air-dried. The crude H-Orn(Pht)OH · HCl was dissolved in warm methanol (420 mL); the solution was filtered and the filtrate was diluted with ethyl acetate (420 mL). The crystalline precipitate was collected on a filter, washed with ethyl acetate, and dried. A second crop of crystals was attained after reducing the volume of the mother liquor. Yield in total: 20.3 g (68 %).

H-Orn(Pht)OH · HCl (20.3 g, 68.0 mmol) was dissolved in 250 mL water; $NaHCO_3$ (12.60 g, 0.15 mol) was added in small portions. Subsequently, a solution of Boc_2O (15.27 g, 70.0 mmol) in 1,4-dioxane (700 mL) was introduced and the reaction mixture was stirred for 3 h. The solution was washed with diethyl ether (100 mL, discarded) and carefully adjusted to pH 2 with 2 M hydrochloric acid. The product was extracted with ethyl acetate (3 × 200 mL); the combined extracts were dried over anhydrous Na_2SO_4 and the solvents were removed under reduced pressure. The product was crystallized from toluene/petroleum ether. Recrystallization from ethyl acetate/hexane did not alter the melting point. Overall yield: 17.64 g (49 %). Mp 126–127 °C (lit.[21] mp 142–145 °C); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.43 (s, 9H, Boc), 1.60–2.02 (m, 4H, -C$^\beta$H$_2$C$^\gamma$H$_2$-), 3.73 (t, ^3J = 6.6 Hz, 2H, -C$^\delta$H$_2$-), 4.08–4.60 (m, 1H, -C$^\alpha$H-), 5.13 (d, ^3J = 7.8 Hz, 1H, -NH-), 7.71 (dd, ^4J = 3.0 Hz, ^3J = 5.4 Hz, 2H, Pht), 7.84 (dd, ^4J = 3.0 Hz, ^3J = 5.4 Hz, 2H, Pht) ppm. $C_{18}H_{22}N_2O_6$ (362.38).

(*S*)-*N*$^\alpha$-*tert*-Butoxycarbonyl-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)-ethyl]-*N*$^\delta$-phthaloylornithinamide – Boc-Orn(Pht)-OH (4.33 g, 11.9 mmol) and HOBt · H_2O (1.84 g, 12.0 mmol) were dissolved in 50 mL THF and cooled to 0 °C.

DCC (2.46 g, 11.9 mmol) was added in one batch, followed by 4-(2-aminoethyl)-1,2-diphenyl-1,2,4-triazolidine-3,5-dione (3.54 g, 11.9 mmol). The reaction mixture was stirred and kept at 0 °C for 2 h; the cooling bath was removed and stirring was continued for 20 h. The dicyclohexylurea (DCU) was removed by filtration and the filtrate concentrated in vacuum. The residue was re-dissolved in ethyl acetate and washed with water, 5 % aq. $KHCO_3$, 5 % aq. $KHSO_4$, and brine. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. The crude product was purified by vacuum flash chromatography eluting with $CHCl_3$/acetonitrile. Yield: 6.3 g (83 %). Mp 130 °C (decomp., sintering > 90 °C). ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.41 (s, 9H, Boc), 1.18–1.71 (m, 4H, -C$^\beta$H$_2$C$^\gamma$H$_2$-), 3.50–3.75 (m, 2H, -C$^\delta$H$_2$- and 2H, -NHCH$_2$CH$_2$N<), 3.82 (t, ^3J = 5.3 Hz, 2H, -NHCH$_2$CH$_2$N<), 4.24 (m, 1H, -C$^\alpha$H-), 5.08 (d, ^3J = 8.4 Hz, 1H, -NH-), 6.80 (t, ^3J = 5.7 Hz, 1H, -NH-), 7.12–7.23 (m, 2H, Ph), 7.25–7.37 (m, 8H, Ph), 7.71 (dd, ^4J = 3.1 Hz, ^3J = 5.5 Hz, 2H, Pht), 7.84 (dd, ^4J = 3.1 Hz, ^3J = 5.5 Hz, 2H, Pht) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 24.7 (C$^\gamma$), 28.3 (Boc), 30.0 (C$^\beta$), 36.8 (-NHCH$_2$CH$_2$N<), 38.0 (C$^\delta$), 40.1 (-NHCH$_2$CH$_2$N<), 53.0 (C$^\alpha$), 79.9 (Boc), 121.9 (C-2/6 Ph), 123.2 (Pht), 126.7 (C-4 Ph), 129.1 (C-3/5 Ph), 132.2 (Pht), 134.0 (Pht), 136.4 (C-1 Ph), 152.9 (C=O urazol), 155.8 (Boc), 168.7 (Pht), 172.6 (C=O amide) ppm; ESI-MS (+p) *m/z* (%): 663 (30) $[MNa]^+$, 641 (95) $[MH]^+$, 585 (20) $[MH - C_4H_8]^+$, 541 (100) $[MH - Boc]^+$; analysis calcd. for $C_{34}H_{36}N_6O_7 \cdot H_2O$: C 61.99, H 5.82, N 12.76 %, found: C 62.35, H 5.68, N 12.85 %. $C_{34}H_{36}N_6O_7$ (640.69).

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^\alpha$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]-*N*$^\delta$-phthaloylornithinamide – (*S*)-*N*$^\alpha$-*tert*-Butoxycarbonyl-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^\delta$-phthaloylornithinamide (7.00 g, 10.9 mmol) was dissolved in a saturated solution of HCl in ethyl acetate (25 mL). After stirring for 2 h the reaction mixture was poured into 300 mL of petroleum ether. The solid product was separated by filtration, washed with diethyl ether and

n-pentane, and dried in vacuum. Yield: 6.18 g (98 %) N^{α}-deprotected ornithinamide.

2-(1-{2-Oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)-piperazin-1-yl]ethyl}cyclopentyl)acetic acid (**6a**, 4.85 g, 10.5 mmol), HOBt · H_2O (1.61 g, 10.5 mmol) and DIPEA (1.80 mL, 10.5 mmol) were dissolved in 50 mL acetonitrile and cooled to 0 °C. EDC · HCl (2.00 g, 10.5 mmol) and subsequently a solution of (*S*)-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{δ}-phthaloyl ornithinamide · HCl (6.18 g, 10.7 mmol) and DIPEA (1.80 mL, 10.5 mmol) in 25 mL acetonitrile were added. The reaction mixture was stirred for 16 h, during which time the cooling bath was allowed to come up to ambient temperature. Acetonitrile was rotary evaporated and the residue was re-dissolved in CH_2Cl_2. The solution was washed with water, 5 % aq. $KHSO_4$, and brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified using vacuum flash chromatography (eluent: $CHCl_3$/MeOH 100:1 v/v). Yield: 9.66 g, (93 %). Mp 160 °C with sintering and gas evolution > 120 °C; ^{1}H-NMR (400 MHz, DMSO-D_6, HMQC, COSY): δ_H = 1.34–1.52 (m, 4H, 2/5-H, cyclopentyl), 1.51 (m, 4H, 3/4-H, cyclopentyl), 1.41 and 1.60 (m, 2H, -C$^{\beta}$H$_2$-), 1.53 (m, 2H, -C$^{\gamma}$H$_2$-), 2.01 (m, 4H, 3/5-H piperazine), 2.20 (m, 2H, -CH$_2$C(O)N$^{\alpha}$H-), 2.42 (m, 2H, >NC(O)CH$_2$-), 3.25 and 3.28 (m, 4H, 2/6-H piperazine), 3.25 and 3.40 (m, 2H, -NHCH$_2$CH$_2$N<), 3.47 (t, ^3J = 6.5 Hz, 2H, -C$^{\delta}$H$_2$NPht), 3.58 (t, ^3J = 6.0 Hz, 2H, -NHCH$_2$CH$_2$N<), 4.14 (m, 1H, -C$^{\alpha}$H-), 4.22 (s, 1H, 11-H dibenzazepin-11-yl), 7.05 (m, 1H, H$_{ar}$), 7.09 (m, 1H, H$_{ar}$), 7.22 (m, 2H, -Ph), 7.24 (m, 1H, H$_{ar}$), 7.32 (m, 1H, H$_{ar}$), 7.36 (m, 8H, -Ph), 7.39 (m, 2H, H$_{ar}$), 7.48 (m, 1H, H$_{ar}$), 7.72 (m, 1H, H$_{ar}$), 7.83 (m, 2H, Pht), 7.84 (m, 2H, Pht), 7.94 (d, ^3J = 8.1 Hz, 1H, -N$^{\alpha}$H-), 8.17 (t, ^3J = 6.0 Hz, 1H, -C(O)NHCH$_2$CH$_2$-), 10.33 (s, 1H, -C(O)NH- lactam) ppm; ^{13}C-NMR (400 MHz, DMSO-D_6, HMQC, COSY): δ_C = 23.1 (C-3/4 cyclopentyl), 24.7 (C$^{\gamma}$), 29.1 (C$^{\beta}$), 36.0 (-NHCH$_2$CH$_2$N<), 37.0 (C$^{\delta}$), 37.0 (C-2/5 cyclopentyl), 38.3 (>NCOCH$_2$-cyclo-C_5H_8-), 39.6 (-NHCH$_2$CH$_2$N<), 42.8 (-CH$_2$C(O)N$^{\alpha}$H-), 43.9 (C-1

cyclopentyl), 45.4 (C-2/6 piperazine), 50.7 (C-3/5 piperazine), 51.9 ($\underline{C}^{\alpha}$), 73.6 (C-11 dibenzazepin-11-yl), 121.2 ($\underline{C}_{ar}H$), 122.5 (Ph), 122.9 (Pht), 123.6 ($\underline{C}_{ar}H$), 126.5 (Ph), 127.6 ($\underline{C}_{ar}H$), 127.8 ($\underline{C}_{ar}H$), 128.3 ($\underline{C}_{ar}H$), 128.9 (Ph), 129.8 ($\underline{C}_{ar}H$), 130.4 ($\underline{C}_{ar}H$), 131.1 ($\underline{C}_{ar}$), 131.2 ($\underline{C}_{ar}H$), 131.5 (Pht), 131.6 ($\underline{C}_{ar}$), 134.3 (Pht), 136.1 ($\underline{C}_{ar}$), 136.5 (Ph), 142.0 ($\underline{C}_{ar}$), 152.5 (-N($\underline{C}$(O)NPh)$_2$), 167.7 ($\underline{C}$=O Pht), 168.0 ($\underline{C}$=O lactam), 169.8 (-$\underline{C}$ON<), 171.1 (-$\underline{C}$ON$^{\alpha}$H-), 171.8 (-$\underline{C}$ONHC$_2$H$_4$N<) ppm; ESI-MS (+p) *m/z* (%): 985 (100) $[MH]^+$; analysis calcd. for $C_{56}H_{57}N_9O_8 \cdot CHCl_3$: C 62.04, H 5.30 N 11.46 %, found: C 61.66, H 5.49, N 11.54 %. $C_{56}H_{57}N_9O_8$ (984.11).

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]ornithinamide (**10**) – (3.94 g, 4.0 mmol) were dissolved in methanol 40 mL and treated with hydrazine hydrate (0.48 mL, 10.0 mmol). After stirring for 16 h a solution of $KHSO_4$ (2.72 g, 20.0 mmol) in water (20 mL) was added and stirring was continued for additional 3 h. The precipitated solid was filtered off and washed with methanol. The filtrates and washings were pooled and the methanol was removed under reduced pressure. The residue was made alkaline with 5 % aq. K_2CO_3 and extracted with CH_2Cl_2 (3 × 75 mL). The combined extracts were dried over anhydrous K_2CO_3 and evaporated to dryness. The crude product was purified by vacuum flash chromatography eluting with $CHCl_3$/methanol mixtures containing 1 % formic acid. The consistent fractions were combined and reduced to a volume of 200 mL; the solution was washed with an aq. solution of K_2CO_3, water and brine. The organic layer was dried over anhydrous K_2CO_3 and evaporated. Yield: 4.40 g (87 %). ^{1}H-NMR (400 MHz, DMSO-d_6, HMQC, COSY): δ_H = 1.26 (m, 2H, -C$^{\gamma}\underline{H}_2$-), 1.41–1.53 (m, 4H, 2/5-H cyclopentyl), 1.54 (m, 4H, 3/4-H cyclopentyl), 1.37 and 1.60 (m, 2H, -C$^{\beta}\underline{H}_2$-), 2.03 (m, 4H, 3/5-H piperazine), 2.23 (m, 2H, -C$\underline{H}_2$C(O)N$^{\alpha}$H-), 2.41 (m, 2H, -C$^{\delta}\underline{H}_2$NH$_2$-), 2.47 (m, 2H, >NC(O)C$\underline{H}_2$-), 3.29 and 3.31 (m, 4H, 2/6-H piperazine), 3.25 and 3.44 (m, 2H, -NHC$\underline{H}_2$CH$_2$N<), 3.60 (t, ^3J = 6.0 Hz, 2H, -NHCH$_2$C$\underline{H}_2$N<), 4.09 (m, 1H, -C$^{\alpha}\underline{H}$-), 4.23 (s, 1H, 11-H dibenzazepin-11-yl), 7.05 (m, 1H, H_{ar}), 7.09 (m, 1H, H_{ar}), 7.22

(m, 2H, Ph), 7.24 (m, 1H, H_{ar}), 7.32 (m, 1H, H_{ar}), 7.38 (m, 8H, Ph), 7.39 (m, 2H, H_{ar}), 7.48 (m, 1H, H_{ar}), 7.72 (m, 1H, H_{ar}), 7.94 (d, ^3J = 8.1 Hz, 1H, -N$^{\alpha}$H-), 8.17 (t, ^3J = 6.0 Hz, 1H, -C(O)NHCH$_2$CH$_2$-), 10.33 (brs, 1H, -C(O)NH- lactam) ppm; ^{13}C-NMR (400 MHz, DMSO-d$_6$, HMQC): δ_C = 23.1 (C-3/4 cyclopentyl), 29.1 (C$^{\beta}$), 29.2 (C$^{\gamma}$), 35.8 (-NHCH$_2$CH$_2$N<), 37.1 (C-2/5 cyclopentyl), 38.4 (>NC(O)CH$_2$-), 39.6 (-NHCH$_2$CH$_2$N<), 40.8 and 45.4 (C-2/6 piperazine), 40.9 (C$^{\delta}$), 42.7 (-CH$_2$C(O)N$^{\alpha}$H-), 44.0 (C-1, cycolpentyl), 50.7 (C-3/5 piperazine), 52.0 (C$^{\alpha}$), 73.7 (C-11 dibenzazepin-11-yl), 121.2 (C_{ar}H), 122.5 (Ph), 123.6 (C_{ar}H), 126.5 (Ph), 127.6 (C_{ar}H), 127.8 (C_{ar}H), 128.3 (C_{ar}H), 128.9 (Ph), 129.8 (C_{ar}H), 130.4 (C_{ar}H), 131.1 (C_{ar}), 131.2 (C_{ar}H), 131.6 (C_{ar}), 136.1 (C_{ar}), 136.5 (Ph), 142.0 (C_{Ar}), 152.5 (-N(C(O)NPh)$_2$), 168.0 (C=O lactam), 169.8 (-CON<), 171.1 (-CON$^{\alpha}$H-), 172.3 (-CONHC$_2$H$_4$N<) ppm; ESI-MS (+p) *m/z* (%): 854 (100) [MH]$^+$, 876 (6) [MNa]$^+$. $C_{48}H_{55}N_9O_6$ (854.01).

4.1.5. PREPARATION OF N^G-UNSUBSTITUTED ARGININAMIDES

(*S*)-*N*$^{\omega}$-2,3-Dihydro-2,2,4,6,7-pentamethylbenzofuran-5-sulfonyl-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-[(9*H*-fluoren-9-yl)methyloxycarbonyl]argininamide – Fmoc-Arg(Pbf)-OH (3.24 g, 5.0 mmol) was dissolved in 10 mL DMF and cooled to 0 °C. To the stirred solution was added i) 5 mL 1 M HOBt · H_2O in DMF, ii) 1.48 g (5.0 mmol) 4-(2-aminoethyl)-1,2-diphenyl-1,2,4-triazolidine-3,5-dione, and iii) 10 mL 0.5 M EDC in DMF. The reaction mixture was stirred for 16 h in which the cooling bath was allowed to come to ambient temperature. Ethyl acetate (150 mL) was added and the organic solution was washed with 5 % aq. $KHSO_4$, 5 % aq. $KHCO_3$ and brine. After evaporation the residual product was purified by vacuum flash chromatography eluting with $CHCl_3$/methanol. Yield: 3.96 g (85 %). Mp 109–110 °C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.25–1.80 (m, 4H, -C$^{\beta}$H$_2$C$^{\gamma}$H$_2$-), 1.41 (s, 6H, -OC(CH$_3$)$_2$-), 2.06 (s, 3H, -CH$_3$), 2.51 (s, 3H, -CH$_3$), 2.59 (s, 3H, -CH$_3$), 2.90 (s, 2H, H_2-3 benzofurane), 3.44 and 3.75 (m, 4H, -NHCH$_2$CH$_2$N<), 4.11 (t, ^3J = 7.1 Hz, 1H, Fmoc), 4.26 (m, 1H,

-C^{α}H-) 4.29 (d, ^3J = 7.1 Hz, 2H, Fmoc), 5.85 (d, ^3J = 7.9 Hz, 1H, -NH), 6.28 (s, 2H, -NH_2), 7.12 (m, 2H, Fmoc), 7.17–7.41 (m, 10 H, Ph and 2H, Fmoc), 7.54 (m, 2H, Fmoc), 7.61 (brs, 1H, -NH), 7.72 (m, 2H, Fmoc) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 12.5 (Pbf), 18.0 (Pbf), 19.4 (Pbf), 25.3 (C^{γ}), 28.6 (Pbf), 30.2 (C^{β}), 37.7 (-NHCH_2CH_2N<), 39.9 (C^{δ}), 40.6 (-NHCH_2CH_2N<), 43.2 (Pbf), 47.1 (Fmoc), 53.7 (C^{α}), 70.0 (Fmoc), 86.4 (Pbf), 117.6 (Pbf), 119.9 (Fmoc), 122.5 (Ph), 124.7 (Pbf), 125.2 (Fmoc), 126.9 (Ph), 127.0 (Fmoc), 127.7 (Fmoc), 129.1 (Ph), 132.4 (Pbf), 136.2 (Ph), 138.5 (Pbf), 141.2 (Fmoc), 143.7 (Pbf), 143.9 (Fmoc), 153.0 (-NCONPh-), 156.3 (-NC(N)N), 156.4 (Fmoc), 158.9 (Pbf), 172.8 (C=O amide) ppm; ESI-MS (+p) *m/z* (%): 927 (100) $[MH]^+$, 949 (12) $[MNa]^+$; analysis calcd. for $C_{50}H_{54}N_8O_8S$: C 64.77, H 5.87, N 12.09 %, found: C 64.61, H 5.88, N 12.17 %. $C_{50}H_{54}N_8O_8S$ (927.08).

Preparation of N^{ω}-Pbf protected argininamides 7a-c – General procedure: (*S*)-N^{ω}-2,3-Dihydro-2,2,4,6,7-pentamethylbenzofuran-5-sulfonyl-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{α}-[(9*H*-fluoren-9-yl)methyloxycarbonyl]-argininamide was dissolved in 10 % Et_2NH in DMF (5 mL/mmol substrate). After stirring for 10 min at ambient temperature the volatiles were removed under reduced pressure and the residue was purified by flash chromatography eluting with CH_2Cl_2/methanol containing 1 % of 7 M NH_3 in methanol.

The N^{α}-deprotected argininamide (211 mg, 0.30 mmol) was combined with the carboxylic acid component (0.30 mmol) **6a**, **b** or **c**, respectively, and dissolved in 3 mL DMF. To the stirred solution was added 1 M HOBt · H_2O in DMF (0.30 mL, 0.30 mmol) and the mixture was surrounded by an ice-water bath. A solution containing 0.5 mol·L^{-1} of EDC · HCl and DIPEA in DMF was added (0.60 mL, 0.30 mmol), and stirring was continued overnight, whereupon the cooling bath was allowed to warm to ambient temperature. The reaction mixture was diluted with ethyl acetate (40 mL) and washed with 5 % aq. $KHSO_4$, 5 % aq. $KHCO_3$ and brine. After drying over anhydrous Na_2SO_4 the organic layer was concentrated under reduced pressure, and

the crude products were submitted to flash chromatography (eluent: CH_2Cl_2/methanol 10:1 v/v).

(2*S*)-*N*$^{\omega}$-(2,3-Dihydro-2,2,4,6,7-pentamethylbenzofuran-5-sulfonyl)-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)-acetyl]argininamide (**7a**). Yield: 140 mg (41 %); ESI-MS (+p) *m/z* (%): 1148.8 (100) $[MH]^+$, 941.6 (33) $[MH - C_{14}H_9NO]^+$. $C_{62}H_{73}N_{11}O_9S$ (1148.38).

(*S*)-*N*$^{\omega}$-(2,3-Dihydro-2,2,4,6,7-pentamethylbenzofuran-5-sulfonyl)-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-(2-{1-[2-(4-benzhydryl-piperazin-1-yl)-2-oxoethyl]cyclopentyl}acetyl)argininamide (**7b**). Yield: 220 mg (66 %); ESI-MS (+p) *m/z* (%): 1107.7 (100) $[MH]^+$, 941.6 (12) $[MH - Ph_2C]^+$. $C_{61}H_{74}N_{10}O_8S$ (1107.37).

(*S*)-*N*$^{\omega}$-(2,3-Dihydro-2,2,4,6,7-pentamethylbenzofuran-5-sulfonyl)-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-(2-{1-[2-(4-benzhydryl-piperidin-1-yl)-2-oxoethyl]cyclopentyl}acetyl)argininamide (**7c**). Yield: 180 mg (54 %); ESI-MS (+p) *m/z* (%): 1106.7 (100) $[MH]^+$. $C_{62}H_{75}N_9O_8S$ (1106.38).

Preparation of *N*$^{\omega}$-Pbf deprotected argininamides 2, 8a and 8b – General procedure: *N*$^{\omega}$-Pbf protected argininamides **7a-c** were dissolved in 3 mL TFA/CH_2Cl_2 1:1 (v/v) and stirred for 1.5 h. The reaction mixture was diluted with 10 mL CCl_4 and the volatiles were rotary evaporated. The crude products were purified by flash chromatography eluting with $CHCl_3$/methanol/5 % TFA in CH_2Cl_2 7.5:1:0.075 (v/v/v).

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide (**2**). Yield: 130 mg (95 %). ^{1}H-NMR (600 MHz, DMSO-d_6, HSQC): δ_H = 1.44 (m, 2H, -C$^{\gamma}$$\underline{H}_2$-), 1.40 and 1.65 (m, 2H, -C$^{\beta}$$\underline{H}_2$--), 1.40 – 1.55 (m, 4H, 2/5-H cyclopentyl), 1.55 (m, 4H, 3/4-H cyclopentyl), 2.11

(m, 4H, 3/5-H piperazine), 2.24 and 2.30 (m, 2H, -C$\underline{H}_2$CON$^{\alpha}$H-), 2.46 and 2.52 (m, 2H, >NCOC$\underline{H}_2$-cyclo-C_5H_8-), 3.00 (m, 2H, -C$^{\delta}\underline{H}_2$-), 3.32 (m, 4H, 2/6-H piperazine), 3.29 and 3.38 (m, 2H, -NHC$\underline{H}_2$CH$_2$N<), 3.59 (m, 2H, --NHCH$_2$C$\underline{H}_2$N<), 4.14 (m, 1H, -C$^{\alpha}\underline{H}$-), 4.30 (m, 1H, 11-H dibenzazepin-11-yl), 7.03 (m, 1H, H_{ar}), 7.11 (m, 1H, H_{ar}), 7.21 (m, 2H, Ph), 7.22 (m, 1H, H_{ar}), 7.32 (m, 1H, H_{ar}), 7.37 (m, 8H, Ph), 7.38 (m, 2H, H_{ar}), 7.45 (m, 1H, H_{ar}), 7.73 (m, 1H, H_{ar}), 8.02 (d, ^3J = 7.5 Hz, 1H, -N$^{\alpha}\underline{H}$-), 8.32 (t, ^3J = 5.6 Hz, 1H, -CON$\underline{H}$C$_2$H$_4$N-), 10.40 (s, 1H, -C(O)N$\underline{H}$- lactam) ppm; ^{13}C-NMR (600 MHz, DMSO-d$_6$, HSQC): δ_C = 23.2 (C-3/4 cyclopentyl), 24.9 (C$^{\gamma}$), 28.6 (C$^{\beta}$), 36.0 (-NH$\underline{C}$H$_2$CH$_2$N<), 37.1 (C-2/5 cyclopentyl), 38.5 (>NCO$\underline{C}$H$_2$-cyclo-C_5H_8-), 39.6 (-NHCH$_2\underline{C}$H$_2$N<), 40.1 (C$^{\delta}$), 41.9 and 45.5 (C-2/6 piperazine), 42.7 (-$\underline{C}$H$_2$CON$^{\alpha}$H-), 43.9 (C-1 cyclopentyl), 50.7 (C-3/5 piperazine), 51.9 (C$^{\alpha}$), 73.7 (C-11 dibenzazepin-11-yl), 121.3 (C$_{ar}$H), 122.7 (Ph), 123.8 (C$_{ar}$H), 126.6 (Ph), 127.8 (2 C$_{ar}$H), 128.3 (C$_{ar}$H), 128.9 (Ph), 129.7 (C$_{ar}$H), 130.4 (C$_{ar}$H), 131.1 (C$_{ar}$H), 131.4 (C$_{ar}$), 136.3 (C$_{ar}$), 136.4 (C$_{ar}$), 143.4 (C$_{ar}$), 152.5 (-N$\underline{C}$ONPh-), 156.9 (-NC(N)N-), 168.2 (C=O lactam), 169.9 (-$\underline{C}$ON<), 171.2 (-$\underline{C}$ON$^{\alpha}$H-), 171.9 (-$\underline{C}$ONHC$_2$H$_4$N<) ppm; ESI-MS (+p) *m/z* (%): 896.6 (100) [MH]$^+$, 689 (5) [MH – 207]$^+$; RP-HPLC: k' = 6.3 (t_R = 16.9 min); analysis calcd. for $C_{49}H_{57}N_{11}O_6 \cdot 4\ H_2O \cdot 5$ HCl: C 51.16, H 6.13, N 13.40 %, found: C 51.12, H 6.25, N 13.08 %. $C_{49}H_{57}N_{11}O_6$ (896.05)

(*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-(2-{1-[2-(4-benzhydrylpiperazin-1-yl)-2-oxoethyl]cyclopentyl}acetyl)argininamide (**8a**). Yield: 200 mg (93 %). ^{1}H-NMR (400 MHz, DMSO-d$_6$, HSQC): δ_H = 1.43 (m, 2H, -C$^{\gamma}\underline{H}_2$-), 1.40 and 1.65 (m, 2H, -C$^{\beta}\underline{H}_2$-), 1.41–1.58 (m, 4H, 2/5-H cyclopentyl), 1.55 (m, 4H, 3/4-H cyclopentyl), 2.30 (m, 2H, -C$\underline{H}_2$CON$^{\alpha}$H-), 2.47 and 2.56 (m, 2H, >NCOC$\underline{H}_2$-cyclo-C_5H_8-), 3.02 (m, 2H, -C$^{\delta}\underline{H}_2$-), 3.08 (m, 4H, 3/5-H piperazine), 3.40 and 4.42 and 3.85 and 4.12 (m, 4H, 2/6-H piperazine), 3.29 and 3.39 (m, 2H, -NHC$\underline{H}_2$CH$_2$N<), 3.59 (m, 2H, -NHCH$_2$C$\underline{H}_2$N<), 4.15 (m, 1H, -C$^{\alpha}\underline{H}$-), 5.69 (m, 1H, Ph$_2$C$\underline{H}$-), 7.22 (m, 2H, Ph), 7.34 (m, 2H, Ph), 7.38 (m, 8H, Ph), 7.42 (m, 4H,

Ph), 7.94 (m, 4H, Ph), 8.02 (d, ^3J = 8.4 Hz, 1H, -N$^{\alpha}$H-), 8.20 (m, 1H, -CONHC$_2$H$_4$N-) ppm; ^{13}C-NMR (400 MHz, DMSO-d$_6$, HSQC): δ_C = 23.2 (C-3/4 cyclopentyl), 24.9 (C$^{\gamma}$), 28.6 (C$^{\beta}$), 36.1 (-NHCH$_2$CH$_2$N<), 37.3 (C-2/5 cyclopentyl), 38.3 (>NCOCH$_2$-cyclo-C$_5$H$_8$-), 39.4 (-NHCH$_2$CH$_2$N<), 39.8 (C$^{\delta}$), 36.9 and 41.5 (C-2/6 piperazine), 42.3 (-CH$_2$CON$^{\alpha}$H-), 43.8 (C-1 cyclopentyl), 50.9 (C-3/5 piperazine), 51.8 (C$^{\alpha}$), 74.0 (Ph$_2$CH-), 122.7 (Ph), 126.6 (Ph), 128.3 (Ph), 128.8 (2C Ph), 129.2 (Ph), 136.4 (Ph), 138.2 (Ph), 152.5 (-NCONPh-), 156.9 (-NC(N)N-), 170.1 (-CON<), 171.2 (-CON$^{\alpha}$H-), 172.0 (-CONHC$_2$H$_4$N<) ppm; ESI-MS (+p) *m/z* (%): 855.5 (100) [MH]$^+$, 689 (20) [MH – Ph$_2$C]$^+$, 428 (20) [MH$_2$]$^{2+}$; RP-HPLC: k' = 5.52 (t_R = 15.0 min); analysis calcd. for C$_{48}$H$_{58}$N$_{10}$O$_5$ · 5 H$_2$O · 2 HCl: C 56.63, H 6.93, N 13.76 %, found: C 56.34, H 6.77, N 13.42 %. C$_{48}$H$_{58}$N$_{10}$O$_5$ (855.04).

(*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-(2-{1-[2-(4-benzhydrylpiperidin-1-yl)-2-oxoethyl]cyclopentyl}acetyl)argininamide (**8b**)

Yield: 150 mg (95 %). ^{1}H-NMR (400 MHz, DMSO-d$_6$, HSQC): δ_H = 0.96 and 1.40 (m, 4H, 3/5-H piperidine), 1.41 (m, 2H, -C$^{\gamma}$H$_2$-), 1.41 and 1.63 (m, 2H, -C$^{\beta}$H$_2$-), 1.41–1.54 (m, 4H, 2/5-H cyclopentyl), 1.54 (m, 4H, 3/4-H cyclopentyl), 2.26 (m, 2H, -CH$_2$CON$^{\alpha}$H-), 2.46 (m, 1H, 4-H piperidine), 2.48 (m, 2H, >NCOCH$_2$-cyclo-C$_5$H$_8$-), 2.92 and 3.90 and 3.82 and 4.37 (m, 4H, 2/6-H piperidine), 3.00 (m, 2H, -C$^{\delta}$H$_2$-), 3.28 and 3.37 (m, 2H, -NHCH$_2$CH$_2$N<), 3.56 (m, 1H, Ph$_2$CH-), 3.59 (m, 2H, -NHCH$_2$CH$_2$N<), 4.16 (m, 1H, -C$^{\alpha}$H-), 7.13 (m, 2H, Ph), 7.22 (m, 2H, Ph), 7.26 (m, 4H, Ph), 7.36 (m, 4H, Ph), 7.38 (m, 8H, Ph), 7.86 (d, ^3J = 7.7 Hz, 1H, -N$^{\alpha}$H-), 8.17 (t, ^3J = 5.8 Hz, 1H, -CONHC$_2$H$_4$N-) ppm; ^{13}C-NMR (400 MHz, DMSO-d$_6$, HSQC): δ_C = 23.2 (C-3/4 cyclopentyl), 24.7 (C$^{\gamma}$), 28.6 (C$^{\beta}$), 31.3 (C-3/5 piperidine), 36.0 (-NHCH$_2$CH$_2$N<), 37.1 (C-2/5 cyclopentyl), 38.5 (>NCOCH$_2$-cyclo-C$_5$H$_8$-), 39.4 (-NHCH$_2$CH$_2$N<), 39.8 (C-4 piperidine), 40.1 (C$^{\delta}$), 40.8 and 45.4 (C-2/6 piperidine), 42.8 (-CH$_2$CON$^{\alpha}$H-), 44.0 (C-1 cyclopentyl), 51.8 (C$^{\alpha}$), 57.2 (Ph$_2$CH-), 122.6 (Ph), 125.8 (Ph), 126.5 (Ph), 127.7 (Ph), 128.3 (Ph), 128.9 (Ph), 136.4 (Ph), 143.8 (Ph), 152.5 (-NCONPh-), 156.9 (-NC(N)N-), 169.6

(-CON<), 171.2 (-CON$^{\alpha}$H-), 171.9 (-CONHC$_2$H$_4$N<) ppm; ESI-MS (+p) *m/z* (%): 854.5 (100) $[MH]^+$; RP-HPLC: k' = 8.9 (t_R = 22.9 min); analysis calcd. for $C_{49}H_{59}N_9O_5 \cdot 3\ H_2O \cdot 3$ HCl: C 57.84, H 6.74, N 12.39 %, found: C 57.74, H 6.94, N 12.23 %. $C_{49}H_{59}N_9O_5$ (854.05).

4.1.6. PREPARATION OF N^{ω}-TOSYL ARGININAMIDE

(*S*)-*N*$^{\alpha}$-*tert*-Butoxycarbonyl-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)-ethyl]-*N*$^{\omega}$-(*p*-toluenesulfonyl)argininamide – Boc-Arg(Tos)-OH (477 mg, 1.0 mmol) and 4-(2-aminoethyl)-1,2-diphenyl-1,2,4-triazolidine-3,5-dione (296 mg, 1.0 mmol) were dissolved in 3 mL DMF and cooled at 0 °C. To the stirred mixture was added i) a 1 M solution of HOBt · H_2O in DMF (1.0 mL, 1.0 mmol), ii) a 0.5 M solution of EDC · HCl and DIPEA (2.0 mL, 1.0 mmol) in DMF. Stirring was continued overnight while the reaction mixture was allowed to come up to ambient temperature. The resulting solution was diluted with ethyl acetate (40 mL) and washed with 5 % aq. $KHSO_4$, 5 % aq. $KHCO_3$ and brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. After flash chromatography (eluent: $CHCl_3$/methanol 20:1 v/v) the product was obtained as a white solid in 58 % yield (410 mg). Mp 169–171 °C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.39 (s, 9H, Boc), 1.40–1.75 (m, 4H, -C$^{\beta}$H$_2$C$^{\gamma}$H$_2$-), 2.38 (s, 3H, -CH$_3$), 3.04 (m, 2H, -C$^{\delta}$H$_2$), 3.43–3.77 (m, 4H, -NHCH$_2$CH$_2$N<), 4.27 (m, 1H, -C$^{\alpha}$H-), 5.46 (d, ^3J = 6.9 Hz, 1H, -NH), 6.43 (brs, 2H, -NH$_2$), 7.10–7.42 (m, 10H, Ph and 2H, Tos), 7.52 (brs, 1H, -NH), 7.74 (m, 2H, Tos) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 21.5 (-CH$_3$), 25.1 (C$^{\gamma}$), 28.4 (Boc), 28.8 (C$^{\beta}$), 37.6 (-NHCH$_2$CH$_2$N<), 40.6 (-NHCH$_2$CH$_2$N<), 79.8 (Boc), 122.6 (C$_{ar}$-H), 126.1 (C$_{ar}$-H), 126.9 (C$_{ar}$-H), 129.1 (C$_{ar}$-H), 129.3 (C$_{ar}$-H), 136.2 (C$_{ar}$), 140.5 (C$_{ar}$), 142.2 (C$_{ar}$), 153.0 (C=O, imide), 155.8 (C=X), 156.7 (C=X) (C=O amide signal not visible) ppm; ESI-MS (+p) *m/z* (%): 729 (10) $[MNa]^+$, 707 (100) $[MH]^+$, 651 (10) $[MH - C_4H_8]^+$. $C_{34}H_{42}N_8O_7S$ (706.81).

(2*S*)-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-

yl]ethyl}cyclopentyl)acetyl]-N^{ω}-(*p*-toluenesulfonyl)argininamide (**14l**) – (*S*)-N^{α}-*tert*-Butoxycarbonyl-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{ω}-(*p*-toluenesulfonyl)argininamide (283 mg, 0.40 mmol) was dissolved in a saturated solution of HCl in ethyl acetate (3 mL). After stirring for 1 h the precipitated solids were separated by decanting, washed with petroleum ether, and recrystallized from CH_2Cl_2/hexane, and dried in vacuum. The vacuum-dried deprotected argininamide was dissolved in 5 mL DMF and treated with **6a** (185 mg, 0.40 mmol), DIPEA (68 µL, 0.40 mmol), and 1 M HOBt · H_2O (0.4 mL, 0.40 mmol) in DMF. The solution was stirred magnetically and cooled at 0 °C. A 0.5 M solution of EDC · HCl and DIPEA in DMF (0.8 mL, 0.40 mmol) was added, and stirring was continued for 16 h, during which time the mixture was allowed to warm to ambient temperature. Ethyl acetate (70 mL) was added and the resulting solution was washed with water, 5 % aq. $KHSO_4$, 5 % aq. $KHCO_3$ and brine. The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash chromatography eluting with CH_2Cl_2/methanol 20:1 (v/v). ^{1}H-NMR (600 MHz, DMSO-d_6, HSQC): δ_H = 1.35 (m, 2H, -C$^{\gamma}$H$_2$-), 1.37 and 1.56 (m, 2H, -C$^{\beta}$H$_2$--), 1.40–1.56 (m, 4H, 2/5-H cyclopentyl), 1.54 (m, 4H, 3/4-H cyclopentyl), 2.03–2.06 (m, 4H, 3/5-H piperazine), 2.22 and 2.30 (m, 2H, -CH$_2$CON$^{\alpha}$H-), 2.30 (s, 3H, -$SO_2C_6H_4CH_3$), 2.41 and 2.50 (m, 2H, >NCOCH$_2$-cyclo-C_5H_8-), 2.95 (m, 2H, -C$^{\delta}$H$_2$-), 3.28 and 3.30 (m, 4H, 2/6-H piperazine), 3.29 and 3.41 (m, 2H, -NHCH$_2$CH$_2$N<), 3.60 (m, 2H, -NHCH$_2$CH$_2$N<), 4.12 (m, 1H, -C$^{\alpha}$H-), 4.21 (s, 1H, 11-H dibenzazepin-11-yl), 6.56 and 6.70 (brs, 2H, -N$^{\omega}$H$_2$), 7.05 (m, 1H, H_{ar}), 7.10 (m, 1H, H_{ar}), 7.20 (m, 2H, Ph), 7.24 (m, 1H, H_{ar}), 7.26 (m, 2H, Tos), 7.33 (m, 1H, H_{ar}), 7.37 (m, 4H, Ph), 7.38 (m, 4H, Ph), 7.39 (m, 2H, H_{ar}), 7.47 (m, 1H, H_{ar}), 7.64 (m, 2H, Tos), 7.72 (m, 1H, H_{ar}), 7.92 (d, ^3J = 8.0 Hz, 1H, -N$^{\alpha}$H-), 8.19 (t, ^3J = 6.0 Hz, 1H, -CONHC$_2$H$_4$N-), 7.3–7.4 (brm, 1H, -N$^{\delta}$H), 10.33 (s, 1H, -C(O)NH- lactam) ppm; ^{13}C-NMR (600 MHz, DMSO-d_6, HSQC): δ_C = 20.8 (-$SO_2C_6H_4CH_3$), 23.2 (C-3/4 cyclopentyl), 25.6 (C$^{\gamma}$), 28.9 (C$^{\beta}$), 36.0 (-NHCH$_2$CH$_2$N<), 37.2 (C-2/5

cyclopentyl), 38.5 (>NCO$\underline{C}H_2$-cyclo-C_5H_8-), 39.6 (-NHCH$_2\underline{C}H_2$N<), 40.3 ($\underline{C}^{\delta}$), 40.7 and 45.5 (C-2/6 piperazine), 42.8 (-$\underline{C}H_2CON^{\alpha}H$-), 44.1 (C-1 cyclopentyl), 50.8 and 51.2 (C-3/5 piperazine), 51.9 ($\underline{C}^{\alpha}$), 73.8 (C-11 dibenzazepin-11-yl), 121.2 ($\underline{C}_{ar}H$), 122.6 (Ph), 123.6 ($\underline{C}_{ar}H$), 125.5 (Tos), 126.6 (Ph), 127.8 (2 $\underline{C}_{ar}H$), 128.3 ($\underline{C}_{ar}H$), 128.9 (Tos), 128.9 (Ph), 129.8 ($\underline{C}_{ar}H$), 130.4 ($\underline{C}_{ar}H$), 131.2 ($\underline{C}_{ar}H$), 131.6 ($\underline{C}_{ar}$), 136.1 ($\underline{C}_{ar}$), 136.6 ($\underline{C}_{ar}$), 141.0 (Tos), 141.6 (Tos), 142.1 ($\underline{C}_{ar}$), 152.6 (-N$\underline{C}$ONPh-), 156.6 (N$\underline{C}$(N)N), 168.2 ($\underline{C}$=O lactam), 169.9 (-$\underline{C}$ON<), 171.2 (-$\underline{C}ON^{\alpha}H$-), 172.1 (-$\underline{C}$ONHC$_2H_4$N<) ppm; ESI-MS (+p) *m/z* (%): 1050.6 (100) $[MH]^+$, 1072.5 (30) $[MNH_4]^+$, 843.5 (35) $[MH - 207]^+$; RP-HPLC: $k' = 9.1$ ($t_R = 23.2$ min); analysis calcd. for $C_{56}H_{63}N_{11}O_8S \cdot H_2O \cdot HCl$: C 60.88, H 6.02, N 13.95 %, found: C 61.17, H 6.02, N 14.03 %. $C_{56}H_{63}N_{11}O_8S$ (1050.23).

4.1.7. PREPARATION OF CITRULLINAMIDE **9A** *AND NITRO-ARGININAMIDE* **9B**

N^{α}-*tert*-Butoxycarbonyl-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)-ethyl]citrullinamide – Under an inert atmosphere, Boc-Cit-OH (2.75 g, 10 mmol) was dissolved in 100 ml anhydrous CH_2Cl_2. CDI (1.78 g, 10.1 mmol) was added, and the clear solution was stirred at ambient temperature for 30 min. After addition of 4-(2-aminoethyl)-1,2-diphenyl-1,2,4-triazolidine-3,5-dione (2.96 g, 10 mmol) the mixture was stirred for further 12 h. Water (50 mL) was added, and the aqueous phase was carefully acidified with 5 % aq. $KHSO_4$. The layers were separated, and the aqueous phase was extracted with two further portions of CH_2Cl_2. The CH_2Cl_2 layers were pooled, dried over anhydrous Na_2SO_4, and evaporated to dryness. Yield: 4.34 g (78 %). Purification was carried out by vacuum flash chromatography over silica gel with CH_2Cl_2/methanol 20:1 to 10:1 (v/v). ^{1}H-NMR (300 MHz, DMSO-d_6): $\delta_H = 1.40$ (s, 9H, Boc), 1.43 (m, 2H, -C$^{\gamma}\underline{H}_2$-), 1.67 and 2.17 (m, 2H, -C$^{\beta}\underline{H}_2$-), 3.04 (m, 2H, -C$\underline{H}_2$N), 3.61 (m, 2H, -C$\underline{H}_2$N), 3.79 (t, $^3J = 5.1$ Hz, 2H, -C$\underline{H}_2$N), 4.13 (m, 1H, -C$^{\alpha}\underline{H}$-), 4.74 (brs, 2H, -NH$_2$), 5.37 (m, 2H, -NH$_2$) 7.15–7.40 (m, 10H, Ph) ppm; ^{13}C-NMR (300 MHz, DMSO-d_6): $\delta_C = 25.3$ ($\underline{C}^{\gamma}$), 28.4 (Boc), 30.5 ($\underline{C}^{\beta}$), 37.7 (-$\underline{C}H_2$N), 39.5 (-$\underline{C}H_2$N), 40.3 (-$\underline{C}H_2$N), 53.4 ($\underline{C}^{\alpha}$), 79.8 (Boc), 122. 3 (Ph), 126.9 (Ph),

129.2 (Ph), 136.2 (Ph), 153.0 (-NCONPh-), (a separate peak which could be assigned to the Boc C=O group was not detected), 159.5 (NC(O)N), 172 (-CONHC$_2$H$_4$N<) ppm; ESI-MS (+p) *m/z* (%): 592 (40) [MK]$^+$, 576 (100) [MNa]$^+$, 554 (12) [MH]$^+$, 454 (23) [MH – Boc]$^+$. $C_{27}H_{35}N_7O_6$ (553.61).

(2*S*)-*N*$^\alpha$-(2-{1-[2-(4-Benzhydrylpiperidin-1-yl)-2-oxoethyl]cyclopentyl}acetyl)-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]citrullinamide (**9a**) – **6b** (4.20 g, 10.0 mmol) and *N*-hydroxysuccinimide (1.26 g, 11.0 mmol) were dissolved in THF (100 mL) and cooled to 0 °C. DCC (2.06 g, 10.0 mmol) was added, and the reaction mixture was stirred for 16 h during which time the surrounding cooling bath came up to ambient temperature. The precipitated by-products were removed by filtration, and the filtrate was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 and washed with water and brine. The CH_2Cl_2 layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. The succinimidyl ester was used without further purification for the subsequent coupling reaction.

N$^\alpha$-*tert*-Butoxycarbonyl-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-citrullinamide was treated with TFA/CH_2Cl_2 1:1 (v/v, 5 mL/mmol substrate) and stirred over a period of 1 h. Afterwards the solution was diluted with the same volume of methanol, and the volatiles were removed under reduced pressure. The vacuum-dried deprotected citrullinamide (1.0 g, 1.4 mmol as di-TFA salt) was dissolved in methanol (10 mL) and treated with DIPEA (476 µL, 2.8 mmol). The active ester component (0.76 g, 1.4 mmol) in 10 mL THF was added, and the reaction mixture was stirred overnight. The solvents were removed under reduced pressure, and the residue was re-dissolved in CH_2Cl_2. The solution was transferred into a separatory funnel, and washed with 5 % aq. $KHSO_4$ and brine and dried over anhydrous Na_2SO_4. The solvent was rotary evaporated, and the crude product was purified by vacuum flash chromatography eluting with $CHCl_3$/MeOH. Yield: 660 mg (53 %). ^{1}H-NMR (400 MHz, DMSO-d$_6$, HSQC): δ_H = 1.34 (m, 2H, -C$^\gamma$H$_2$-), 1.41 and 1.59 (m, 2H, -C$^\beta$H$_2$-), 1.39–1.60 (m, 4H, 2/5-H cyclopentyl), 1.56 (m, 4H, 3/4-H cyclopentyl), 2.25 and 2.32 (m, 2H, -CH$_2$CON$^\alpha$H-), 2.49 and 2.59 (m, 2H,

>NCOC$\underline{H}_2$-cyclo-C_5H_8-), 2.93 (m, 2H, -$C^{\delta}\underline{H}_2$-), 2.94–3.19 (m, 4H, 3/5-H piperazine), 3.38 and 4.41 and 3.83 and 4.12 (m, 4H, 2/6-H piperazine), 3.28 and 3.40 (m, 2H, -NHC$\underline{H}_2CH_2N$<), 3.59 (m, 2H, -$NHCH_2C\underline{H}_2N$<), 4.14 (m, 1H, -$C^{\alpha}\underline{H}$-), 5.62 (m, 1H, $Ph_2C\underline{H}$-), 7.22 (m, 2H, Ph), 7.38 (m, 10H, Ph), 7.43 (m, 4H, Ph), 7.94 (m, 4H, Ph), 8.00 (m, 1H, -$N^{\alpha}\underline{H}$-), 8.24 (m, 1H, -CON$\underline{H}C_2H_4N$-) ppm; ^{13}C-NMR (400 MHz, DMSO-d_6, HSQC): δ_C = 23.2 (C-3/4 cyclopentyl), 25.8 ($\underline{C}^{\gamma}$), 28.8 ($\underline{C}^{\beta}$), 35.8 (-NH$\underline{C}H_2CH_2N$<), 37.1 (C-2/5 cyclopentyl), 38.5 (>NCO$\underline{C}H_2$-cyclo-C_5H_8-), 39.2 ($\underline{C}^{\delta}$), 39.5 (-$NHCH_2\underline{C}H_2N$<), 37.1 and 41.5 (C-2/6 piperazine), 42.5 (-$\underline{C}H_2CON^{\alpha}H$-), 43.8 (C-1 cyclopentyl), 51.0 (C-3/5 piperazine), 52.0 ($\underline{C}^{\alpha}$), 74.2 ($Ph_2\underline{C}H$-), 122.6 (Ph), 126.6 (Ph), 128.4 (Ph), 128.8 (Ph), 128.9 (Ph) 129.2 (Ph), 135.5 (Ph), 136.4 (Ph), 152.5 (-N$\underline{C}$ONPh-), 159.3 (-NC(N)N-), 170.2 (-$\underline{C}$ON<), 171.1 (-$\underline{C}ON^{\alpha}H$-), 172.1 (-$\underline{C}ONHC_2H_4N$<) ppm; ESI-MS (+p) *m/z* (%): 856.6 (100) $[MH]^+$; RP-HPLC: k' = 6.2 (t_R = 16.5 min); analysis calcd. for $C_{48}H_{57}N_9O_6 \cdot 2\ H_2O \cdot$ HCl: C 62.09, H 6.73, N 13.58 %, found: C 62.09, H 6.96, N 13.55 %. $C_{48}H_{57}N_9O_6$ (856.02).

Similarly was prepared **(*S*)-*N*$^{\alpha}$-(2-{1-[2-(4-benzhydrylpiperidin-1-yl)-2-oxoethyl]-cyclopentyl}acetyl)-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\omega}$-nitroargininamide** (**9b**) from Boc-Arg(NO_2)-OH. ^{1}H-NMR (400 MHz, DMSO-d_6, HSQC): δ_H = 1.43 (m, 2H, -$C^{\gamma}\underline{H}_2$-), 1.43 and 1.61 (m, 2H, -$C^{\beta}\underline{H}_2$-), 1.37–1.59 (m, 4H, 2/5-H cyclopentyl), 1.55 (m, 4H, 3/4-H cyclopentyl), 2.24 and 2.33 (m, 2H, -$C\underline{H}_2CON^{\alpha}H$-), 2.45 and 2.59 (m, 2H, >NCOC$\underline{H}_2$-cyclo-C_5H_8-), 3.05 (m, 2H, -$C^{\delta}\underline{H}_2$-), 2.92–3.15 (m, 4H, 3/5-H piperazine), 3.38 and 4.42 and 3.84 and 4.11 (m, 4H, 2/6-H piperazine), 3.29 and 3.39 (m, 2H, -NHC$\underline{H}_2CH_2N$<), 3.59 (m, 2H, -$NHCH_2C\underline{H}_2N$<), 4.14 (m, 1H, -$C^{\alpha}\underline{H}$-), 5.57 (m, 1H, $Ph_2C\underline{H}$-), 7.22 (m, 2H, Ph), 7.37 (m, 8H, Ph), 7.38 (m, 2H, Ph), 7.43 (m, 4H, Ph), 7.93 (m, 4H, Ph), 8.00 (brs, 1H, -$N^{\alpha}\underline{H}$-), 8.23 (m, 1H, -CON$\underline{H}C_2H_4N$-) ppm; ^{13}C-NMR (400 MHz, DMSO-d_6, HSQC): δ_C = 23.2 (C-3/4 cyclopentyl), 24.6 ($\underline{C}^{\gamma}$), 28.8 ($\underline{C}^{\beta}$), 36.2 (-NH$\underline{C}H_2CH_2N$<), 37.2 (C-2/5 cyclopentyl), 38.5 (>NCO$\underline{C}H_2$-cyclo-C_5H_8-), 39.5 (-$NHCH_2\underline{C}H_2N$<),

40.0 (C$^{\delta}$), 37.0 and 41.6 (C-2/6 piperazine), 42.5 (-CH$_2$CON$^{\alpha}$H-), 43.8 (C-1 cyclopentyl), 51.1 (C-3/5 piperazine), 52.0 (C$^{\alpha}$), 74.3 (Ph$_2$CH-), 122.6 (Ph), 126.6 (Ph), 128.4 (Ph), 128.8 (Ph), 128.9 (Ph) 129.2 (Ph), 135.4 (Ph), 136.4 (Ph), 152.5 (-NCONPh-), 159.2 (-NC(N)N-), 170.2 (-CON<), 171.2 (-CON$^{\alpha}$H-), 172.0 (-CONHC$_2$H$_4$N<) ppm; ESI-MS (+p) *m/z* (%): 900.6 (100) [MH]$^+$; RP-HPLC: $k' = 6.7$ (t_R = 17.6 min); analysis calcd. for $C_{48}H_{57}N_{11}O_7 \cdot 2\ H_2O \cdot HCl$: C 59.28, H 6.43, N 15.85 %, found: C 59.31, H 6.58, N 15.55 %. $C_{48}H_{57}N_{11}O_7$ (900,04).

4.1.8. GUANIDINYLATION METHODS – GENERAL PROCEDURES:

4.1.8.1. Using 1H-pyrazole-1-carboxamidines as guanidinylating reagents:

1*H*-Pyrazole-1-carboxamidine hydrochloride – To a vigorously stirred solution of aminoguanidine carbonate (27.22 g, 0.20 mol) in water (50 mL) and conc. hydrochloric acid (34 mL), gently warmed to 40 °C, was slowly added 1,1,3,3-tetramethoxypropane (34.48 g, 0.21 mol) over a period of 3 h. The mixture was diluted with acetone (250 mL) and concentrated under reduced pressure until a crop of crystals had formed. After cooling to ambient temperature, the crystals were collected on a sintered filter, washed with acetone, and dried. Yield: 22.0 g (75 %). mp 155–157 °C (lit.[22] mp 165–166 °C) ^{1}H-NMR (300 MHz, DMSO-d$_6$) δ_H = 6.80 (dd, $^3J_{4A}$ = 1.6, $^3J_{4B}$ = 2.9 Hz, 1H, 4-H), 8.10 (d, $^3J_{A4}$ = 1.5 Hz, 1H, pyrazole), 8.93 (d, $^3J_{B4}$ = 2.9 Hz, 1H, pyrazole), 9.49 (brs, 2H, -NH$_2$), 9.76 (brs, 2H, -NH$_2$) ppm. $C_4H_7ClN_4$ (146.58).

1*H*-Pyrazole-1-carboxamidine hydrochloride (733 mg, 5.0 mmol) was suspended in 10 mL abs. CH_2Cl_2. One equivalent of acylating reagent in 1 mL abs. CH_2Cl_2 and DIPEA (1.71 mL, 10.0 mmol) were added, and the resulting solution was shaken overnight. After washing with water and brine, and drying over anhydrous Na_2SO_4, CH_2Cl_2 was evaporated, and the residue was crystallized from n-hexane/CH_2Cl_2.

***N*-Benzyloxycarbonyl-1*H*-pyrazole-1-carboxamidine** – Yield: 1.06 g (87 %) as white, crystalline powder; ^{1}H-NMR (300 MHz, DMSO-d$_6$): δ_H = 5.13 (s, 2H, Cbz), 6.58 (dd, ^3J = 2.8 Hz, 1.6 Hz, 1H, pyrazole), 7.29–7.45 (m, 5H, Cbz), 7.92 (dd, ^3J

= 1.6 Hz, ^4J = 0.7 Hz, 1H, pyrazole), 8.45 (dd, ^3J = 2.8 Hz, ^4J = 0.7 Hz, 1H, pyrazole), 8.98 (brs, 2H, -N$\underline{H}_2$) ppm; CI-MS (NH_3) *m/z* (%): 245 (100) $[MH]^+$. $C_{12}H_{12}N_4O_2$ (244.25).

***N*-Methoxycarbonyl-(1*H*)-pyrazole-1-carboxamidine** – Yield: 0.74 g (88 %) as white, crystalline powder. ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 3.81 (s, 3H, -OC$\underline{H}_3$), 6.45 (dd, ^3J = 2.6 Hz, ^3J = 1.6 Hz, 1H, pyrazole), 7.71 (brs, 1H, pyrazole), 8.49 (brs, 1H, pyrazole) ppm; CI-MS (NH_3) *m/z* (%): 169 (100) $[MH]^+$. $C_6H_8N_4O_2$ (168.15).

***N*-Benzoyl-(1*H*)-pyrazole-1-carboxamidine** – Yield: 0.86 g (80 %) as white, crystalline powder. ^{1}H-NMR (300 MHz, $CDCl_3$, TMS): δ_H = 6.50 (dd, ^3J = 2.7 Hz, ^3J = 1.6 Hz, 1H, pyrazole), 7.40–7.62 (m, 3H, benzoyl), 7.76 (brs, 1H, pyrazole), 8.29 (m, 2H, benzoyl), 8.72 (brs, 1H, pyrazole) ppm; CI-MS (NH_3) *m/z* (%): 215 (100) $[MH]^+$. $C_{11}H_{10}N_4O$ (214.22).

Gyanidinylation – General procedure: The *N*-substituted 1*H*-pyrazole-1-carboxamidine derivatives (0.30 mmol) were dissolved in CH_2Cl_2 (1 mL) and treated with 1 M Boc_2O in CH_2Cl_2 (0.60 mL) and DMAP (0.03 mmol) in CH_2Cl_2 (1 mL). The reaction mixtures were shaken for 18 h at ambient temperature. The solutions were washed with 0.6 % aq. acetic acid and brine, dried over anhydrous Na_2SO_4, and evaporated. The residues were re-dissolved in THF (3 mL) and treated with $Mg(ClO_4)_2$ (7 mg, 0.03 mmol). After shaking for 2 h at 55 °C, the mixtures were allowed to cool to ambient temperature and **10** (213 mg, 0.25 mmol) in 2.5 mL THF was added. The reaction mixtures were shaken for 2 d. Ethyl acetate (25 mL) was added, and the solutions were washed with 5 % aq. $KHCO_3$ and brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The products were purified by flash chromatography (eluent: $CHCl_3$/methanol).

(2*S*)-$N^{\omega'}$-*tert*-Butoxycarbonyl-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{ω}-methoxycarbonyl-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide

(**13a**) – Yield: 210 mg (80 %); ESI-MS (+p) *m/z* (%): 1054.6 (100) $[MH]^+$, 747.5 (14) $[MH - C_{14}H_9NO]^+$, 528 (30) $[MH_2]^{2+}$. $C_{56}H_{67}N_{11}O_{10}$ (1054.20).

(2*S*)-*N*$^{\omega}$-Benzyloxycarbonyl-*N*$^{\omega'}$-*tert*-butoxycarbonyl-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]arginin-amide (**13c**) – Yield: 200 mg (73 %); ESI-MS (+p) *m/z* (%): 1130.8 (55) $[MH]^+$, 566 (30) $[MH_2]^{2+}$. $C_{62}H_{71}N_{11}O_{10}$ (1130.29).

(2*S*)-*N*$^{\omega}$-Benzoyl-*N*$^{\omega'}$-*tert*-butoxycarbonyl-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]-azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide (**13g**) – Yield: 260 mg (92 %); ESI-MS (+p) *m/z* (%): 1100.9 (100) $[MH]^+$, 551 (30) $[MH_2]^{2+}$. $C_{61}H_{69}N_{11}O_9$ (1100.27).

4.1.8.2. Using S-methylisothioureas as guanidinylating reagents:

***S*-Methylisothiouronium iodide** – A suspension of thiourea (7.61 g, 0.1 mol) in ethanol (100 mL) was placed in a 500 mL flask equipped with reflux condenser and stirring bar. The mixture was stirred and cooled in an ice-water bath; iodomethane (14.2 g, 0.1 mol) was carefully added through the condenser, and the mixture was heated to reflux. After 1 h the heater was turned off, and stirring was continued overnight. The mixture was concentrated under reduced pressure to a volume of about 15 mL and diluted with diethyl ether (150 mL). The resulting crystalline product was collected on a sintered filter, washed with diethyl ether (2 × 50 mL) and dried in vacuum. Yield: 21.3 g (98 %). mp 114–115 °C (lit.[23] mp 117 °C). $C_2H_7IN_2S$ (218.06).

***N*-*tert*-Butoxycarbonyl-*S*-methylisothiourea** – To a vigorously stirred suspension of *S*-methyl-isothiouronium iodide (18.5 g, 85.0 mmol) in CH_2Cl_2 (75 mL) surrounded by an ice-water bath was added triethylamine (11.9 mL, 85.0 mmol). To the clear solution Boc_2O (16.4 g, 75.0 mmol), dissolved in CH_2Cl_2 (75 mL), was added slowly over a period of 1 h. The cooling bath was removed, and stirring was continued

overnight. The reaction mixture was diluted with CH_2Cl_2 and washed with water, 5 % aq. $KHSO_4$, 5 % aq. $KHCO_3$, and brine and dried over anhydrous Na_2SO_4. n-Hexane (100 mL) was added, and the solution was concentrated in a rotary evaporator until the product precipitated. The mixture was cooled, and the crystalline product was collected on a filter and dried in vacuum. Yield: 10.9 g (76 %). Mp 76–78 °C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ_H = 1.40 (s, 9H, Boc), 2.31 (s, 3H, -SC$\underline{H}_3$), 8.56 (brs, 2H, -N$\underline{H}_2$) ppm; ^{13}C-NMR (300 MHz, DMSO-d_6): δ_C = 12.7 (S$\underline{C}H_3$), 27.8 (Boc), 77.7 (Boc), 160.6 (Boc), 171.4 (N-$\underline{C}$(=N)SMe) ppm; EI-MS (70 eV) *m/z* (%): 190 (17) $M^{\bullet+}$, 175 (50) $[M - CH_3]^+$, 134 (44) $[M - C_4H_8]^+$, 57 (100) $[C_4H_9]^+$. $C_7H_{14}N_2O_2S$ (190.26).

***N'*-Substituted *N*-*tert*-butoxycarbonyl-*S*-methylisothioureas** – To a solution of *N*-*tert*-butoxycarbonyl-*S*-methylisothiourea (0.76 g, 4.0 mmol) in 10 mL anhydrous CH_2Cl_2 was added DIPEA (4.0 mmol) in CH_2Cl_2 (10 mL). A solution of the respective acylating agent (4.0 mmol) in CH_2Cl_2 (5 mL) was introduced, and the reaction mixture was mechanically shaken overnight. The solution was washed with water, 5 % aq. $KHSO_4$, 5 % aq. $KHCO_3$, and brine and dried over anhydrous Na_2SO_4. The solution was diluted with n-hexane, and CH_2Cl_2 was evaporated. The solid product, which precipitated from the cold solution, was collected on a filter and dried in vacuum.

***N*-*tert*-Butoxycarbonyl-*N'*-ethoxycarbonyl-*S*-methylisothioura** (**12a**) – From ethyl chloroformate. Yield: 0.94 g (90 %, colorless oil); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.34 (t, ^3J = 7.1 Hz, 3H, -OCH$_2$C$\underline{H}_3$), 2.42 (s, 3H, -SC$\underline{H}_3$), 1.51 (s, 9H, Boc), 4.22 (q, ^3J = 7.1 Hz, 2H, -OC$\underline{H}_3$CH$_2$) ppm; CI-MS (NH_3) *m/z* (%): 263 (100) $[MH]^+$, 163 (23) $[MH - Boc]^+$. $C_{10}H_{18}N_2O_4S$ (262.33).

***N*-*tert*-Butoxycarbonyl-*N'*-benzyloxycarbonyl-*S*-methylisothioura** – From benzyl succinimidyl carbonate (Cbz-Osu). Yield: 1.11 g (86 %, colorless oil); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.50 (s, 9H, Boc), 2.41 (s, 3H, -SC$\underline{H}_3$), 5.20 (s, 2H, -C$\underline{H}_2$Ph), 7.28–7.46 (m, 5H, Ph) ppm; CI-MS (NH_3) *m/z* (%): 325 (100) $[MH]^+$, 225 (24) $[MH–Boc]^+$. $C_{15}H_{20}N_2O_4S$ (324.40).

***N*-*tert*-Butoxycarbonyl-*N'*-(2-phenylacetyl)-*S*-methylisothioura** (**12d**) – From 2-phenylacetyl chloride. Yield: 0.87 g (70 %, white solid); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.47 (s, 9H, Boc), 2.37 (s, 3H, -SC<u>H</u>$_3$), 3.77 (brs, 2H, -C<u>H</u>$_2$Ph), 7.18–7.42 (m, 5H, Ph) ppm; EI-MS (70 eV) *m/z* (%): 308 (4) $M^{\bullet+}$, 252 (15) $[M - C_4H_8]^+$, 217 (27) $[M - C_7H_7]^+$, 161 (83) $[M - C_7H_7 - C_4H_8]^+$, 57 (100) $[C_4H_9]^+$. $C_{15}H_{20}N_2O_3S$ (308.4).

***N*-*tert*-Butoxycarbonyl-*N'*-(2-phenoxyacetyl)-*S*-methylisothiourea** (**12e**) – From 2-phenoxyacetyl chloride. Yield: 0.92 g (71 %, white solid); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.52 (s, 9H, Boc), 2.40 (s, 3H, -SC<u>H</u>$_3$), 4.68 (brs, 2H, -C<u>H</u>$_2$OPh), 6.89–7.07 (m, 3H, Ph), 7.27–7.37 (m, 2H, Ph) ppm; EI-MS (70 eV) *m/z* (%): 324 (6) $M^{\bullet+}$, 267 (8), 251 (13), 224 (15), 175 (17), 161 (70), 57 (100). $C_{15}H_{20}N_2O_4S$ (324,40).

***N*-*tert*-Butoxycarbonyl-*N'*-(3-phenylpropionyl)-*S*-methylisothiourea** (**12f**) – From 3-phenylpropanoyl chloride. Yield: 1.04 g (81 %, as white solid); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.51 (s, 9H, Boc), 2.41 (s, 3H, -SC<u>H</u>$_3$), 2.80 (t, ^3J = 7.6 Hz, 2H, -C$^{\alpha}$<u>H</u>$_2$-), 3.01 (t, ^3J = 7.6 Hz, 2H, -C$^{\beta}$<u>H</u>$_2$-), 7.15–7.34 (m, 5H, Ph) ppm; CI-MS (NH_3) *m/z* (%): 323 (100) $[MH]^+$, 223 (24) $[MH - Boc]^+$. $C_{16}H_{22}N_2O_3S$ (322.42).

***N*-*tert*-Butoxycarbonyl-*N'*-propionyl-*S*-methylisothiourea** (**12c**) – To a solution of 0.38 g (2.0 mmol) *N*-*tert*-butoxycarbonyl-*S*-methylisothiourea in 5 mL CH_2Cl_2 was added DIPEA (0.34 mL, 2.0 mmol) and propionic anhydride (0.26 mL, 2.0 mmol). The mixture was stirred for 16 h at ambient temperature. The solution was diluted with ethyl acetate (40 mL), transferred into a separatory funnel, and washed with H_2O, 5 % aq. $KHCO_3$, 5 % aq. $KHSO_4$, and brine. The organic layer was separated, dried over anhydrous Na_2SO_4, and evaporated. Yield: 0.47 g (95 %, white solid); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.20 (t, ^3J = 7.5 Hz, 3H, -C(O)CH$_2$C<u>H</u>$_3$), 1.52 (s, 9H, Boc), 2.40 (s, 3H, -SC<u>H</u>$_3$), 2.48 (q, ^3J = 7.5 Hz, 2H, -C(O)C<u>H</u>$_2$CH$_3$), 12.4 (brs, 1H, -N<u>H</u>-) ppm; CI-MS (NH_3) *m/z* (%): 247 (100) $[MH]^+$, 191 (5) $[MH - C_4H_8]^+$, 147 (45) $[MH - Boc]^+$. $C_{10}H_{18}N_2O_3S$ (246.33).

***N*-*tert*-Butoxycarbonyl-*N'*-(ethylaminocarbonyl)-*S*-methylisothiourea** (**12j**) – To a solution of 0.38 g (2.0 mmol) *N*-*tert*-butoxycarbonyl-*S*-methylisothiourea in 5 mL CH_2Cl_2 was added ethylisocyanate (0.16 ml, 2.0 mmol). The mixture was stirred for 16 h at ambient temperature. The resulting solution was diluted with ethyl acetate (40 mL), transferred into a separatory funnel and washed with H_2O, 5 % aq. $KHCO_3$, 5 % aq. $KHSO_4$, and brine. The organic layer was separated, dried over anhydrous Na_2SO_4, and evaporated. Yield: 0.52 g (99 %, colorless oil); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.19 (t, ^3J = 7.3 Hz, 3H, -NHCH$_2$C<u>H</u>$_3$), 1.49 (s, 9H, Boc), 2.35 (s, 3H, -SC<u>H</u>$_3$), 3.28 (dq, ^3J= 7.3 Hz, ^3J= 1.3 Hz, 2H, -NHC<u>H</u>$_2$CH$_3$), 5.71 (brs, 1H, -N<u>H</u>Et), 12.41 (s, 1H, -N<u>H</u>-) ppm; CI-MS (NH_3) *m/z* (%): 262 (100) $[MH]^+$, 206 (2) $[MH - C_4H_8]^+$, 162 (28) $[MH - Boc]^+$. $C_{10}H_{19}N_3O_3S$ (261.34).

***N*-*tert*-Butoxycarbonyl-*N'*-methanesulfonyl-*S*-methylisothiourea** (**12i**) – To a solution of 0.38 g (2.0 mmol) *N*-*tert*-butoxycarbonyl-*S*-methylisothiourea in 5 mL CH_2Cl_2 was added DIPEA (0.34 mL, 2.0 mmol) and methanesulfonyl chloride (0.16 ml, 2.0 mmol). The mixture was stirred for 16 h at ambient temperature. The resulting solution was diluted with ethyl acetate (40 mL), transferred into a separatory funnel, and washed with H_2O, 5 % aq. $KHCO_3$, 5 % aq. $KHSO_4$, and brine. The organic layer was separated, dried over anhydrous Na_2SO_4, and evaporated. Yield: 0.55 g (99 %, slightly orange, amorphous solid); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.50 (s, 9H, Boc), 2.35 (s, 3H, -SC<u>H</u>$_3$) 3.10 (s, 3H, -SO$_2$C<u>H</u>$_3$), 10.1 (s, 1H, -N<u>H</u>-) ppm; CI-MS (NH_3) *m/z* (%): 286 (100) $[MNH_4]^+$, 269 (76) $[MH]^+$, 186 (23) $[MNH_4 - Boc]^+$, 169 (13) $[MH - Boc]^+$. $C_8H_{16}N_2O_4S_2$ (268.35).

***N,N'*-Bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea**[24] – *S*-Methylisothiouronium sulfate (2.78 g, 10.0 mmol) was dissolved in 60 mL water-dioxane 1:1 (v/v) and treated with 1 M aq. NaOH solution (20 mL) and di-*tert*-butyl dicarbonate (11.62 g, 50.0 mmol). After stirring for 16 h the precipitated product was filtered off and washed with a small amount of water; the filtrates were concentrated under reduced pressure to half the volume, and additional product was collected by filtration. The combined solid products were dried in a desiccator over P_4O_{10}. Yield:

4.39 g (76 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.52 (s, 9H, Boc), 1.53 (s, 9H, Boc), 2.41 (s, 3H, -SCH_3), 11.60 (brs, 1H, -N<u>H</u>-) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_c = 14.4 (-S<u>C</u>H_3), 27.4 (Boc), 28.0 (Boc), 82.0 (Boc), 85.2 (Boc), 146.8 (<u>C</u>=O, Boc), 171.5 (-<u>C</u>(SMe)-) ppm; CI-MS (NH_3) *m/z* (%): 291 (100) $[MH]^+$, 191 (25) [MH – Boc]$^+$. $C_{12}H_{22}N_2O_4S$ (290.38).

Alkylation of ***N,N'*-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea**[24] with alcohols under Mitsunobu conditions[6]: General procedure: – To a stirred solution of *N,N'*-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea (0.58 g, 2.0 mmol) and alcohol (2.0 mmol) in anhydrous THF (10 mL) was added a 2 M solution tri-n-butylphosphine in toluene (1.5 mL, 3.0 mmol) under inert conditions. A 2 M solution of DIAD in toluene (1.5 mL, 3.0 mmol) was introduced in a drop wise manner using a syringe. Stirring was continued for 16 h, and the resulting solution was diluted with ethyl acetate. The organic phase was washed with 5 % $KHSO_4$, 5 % $KHCO_3$, and brine, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The residual raw product was subjected to flash chromatography (eluent: petroleum ether/ethyl acetate 20:1, v/v).

***N,N'*-Bis(*tert*-butoxycarbonyl)-*N*-ethyl-*S*-methylisothiourea** (**12k**). Yield: 0.20 g (31 %, as colorless oil); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.24 (t, ^3J = 7.1 Hz, 3H, -CH_2C<u>H</u>$_3$), 1.48 (s, 9H, Boc), 1.51 (s, 9H, Boc), 2.39 (s, 3H, -SC<u>H</u>$_3$), 3.58 (q, ^3J = 7.1 Hz, 2H, -C<u>H</u>$_2$CH$_3$) ppm; CI-MS (NH_3) *m/z* (%): 319 (100) $[MH]^+$, 263 (1) [MH – C_4H_8]$^+$, 219 (43) [MH – Boc]$^+$, 163 (16) [MH – Boc – C_4H_8]$^+$, 119 (2) [MH – 2Boc]$^+$. $C_{14}H_{26}N_2O_4S$ (318.43).

***N,N'*-Bis(*tert*-butoxycarbonyl)-*N*-benzyl-*S*-methylisothiourea**[24] (**12l**). Yield: 0.42 g (55 %) as colorless oil. ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.40 (s, 9H, -C(C<u>H</u>$_3$)$_3$), 1.53 (s, 9H, -C(C<u>H</u>$_3$)$_3$), 2.29 (s, 3H, -SC<u>H</u>$_3$), 4.78 (s, 2H, -C<u>H</u>$_2$Ph), 7.21–7.41 (m, 5H, -Ph) ppm; CI-MS (NH_3) *m/z* (%): 381 (100) $[MH]^+$, 281 (43) [MH – Boc]$^+$, 225 (16) [MH – Boc – C_4H_8]$^+$, 181 (2) [MH – 2Boc]$^+$. $C_{19}H_{28}N_2O_4S$ (380.5).

***tert*-Butyl 3-hydroxypropylcarbamate** – To a stirred solution of 3-aminopropan-1-ol (0.75 g, 10.0 mmol) and DIPEA (1.29 g, 10.0 mmol), in CH_2Cl_2 (10 mL) was

slowly added Boc_2O (2.40 g, 11.0 mmol) in CH_2Cl_2 (10 mL). After stirring overnight the resulting solution was washed with 1M aq. NH_4Cl, 5 % aq. $KHSO_4$, 5 % aq. $KHCO_3$, and brine. The organic layers were dried over anhydrous Na_2SO_4 and evaporated yielding the product as colorless oil (1.30 g, 74 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.45 (s, 9H, Boc), 1.68 (m, 2H, -$OCH_2C\underline{H}_2CH_2NHBoc$), 3.27 (t, ^3J =5.3 Hz, 2H, $HOCH_2CH_2C\underline{H}_2NHBoc$), 3.66 (t, ^3J = 5.1 Hz, 2H, $HOC\underline{H}_2CH_2CH_2NHBoc$) ppm; CI-MS ($NH_3$) *m/z* (%): 193 (41) $[MNH_4]^+$, 176 (86) $[MH]^+$, 137 (100) $[MNH_4 - C_4H_8]^+$, 120 (34) $[MH - C_4H_8]^+$. $C_8H_{17}NO_3$ (175.23).

***N*-*tert*-Butoxycarbonyl-*N'*-[3-(*tert*-butoxycarbonylamino)propyloxycarbonyl]-*S*-methylisothiourea** (**12b**) – 3-(*tert*-Butoxycarbonylamino)propan-1-ol (0.94 g, 5.36 mmol) was dissolved in anhydrous acetonitrile and treated with DSC (2.06 g, 8.0 mmol) and triethylamine (2.23 mL, 16.0 mmol). The reaction mixture was stirred overnight at ambient temperature. Acetonitrile was removed under reduced pressure, and the residue was taken up in ethyl acetate (50 mL). The ethyl acetate layer was washed with aq. $NaHCO_3$ solution and brine, dried over anhydrous Na_2SO_4 and evaporated to dryness. The raw 3-(*tert*-butoxycarbonylamino)propyl succinimidyl carbonate was re-dissolved in CH_2Cl_2 (40 mL); triethylamine (1.11 mL, 8.0 mmol) and *N*-*tert*-butoxycarbonyl-*S*-methylisothiourea (1.02 g, 5.36 mmol) were introduced, and the mixture was left overnight. The resulting solution was concentrated and diluted with ethyl acetate. The organic phase was washed with water, 5 % aq. $KHCO_3$, 0.6 % aq. acetic acid and brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The product was purified by flash-chromatography, eluting with petroleum ether/ethyl acetate 5:1 (v/v). Yield: 1.36 g (65 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.44 (s, 9H, Boc), 1.51 (s, 9H, Boc), 1.90 (m, 2H, -$OCH_2C\underline{H}_2CH_2NHBoc$), 2.41 (s, 3H, -$SC\underline{H}_3$), 3.24 (m, 2H, -$OCH_2CH_2C\underline{H}_2NHBoc$), 4.23 (t, ^3J = 6.2 Hz, 2H, -$OC\underline{H}_2CH_2CH_2NHBoc$), 4.81 (brs, 1H, -N$\underline{H}$) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 14.6 (-$S\underline{C}H_3$), 28.0 (Boc), 28.4 (Boc), 29.1 (-$OCH_2\underline{C}H_2CH_2NHBoc$), 37.6 (-$OCH_2CH_2\underline{C}H_2NHBoc$), 64.0 (-$O\underline{C}H_2CH_2CH_2NHBoc$), 77.3 (Boc), 79.4 (Boc), 148.7 (N$\underline{C}$(O)O), 156.0 (Boc),

156.1 (Boc), 172.7 (N<u>C</u>(SMe)N) ppm; ESI-MS (+p) *m/z* (%): 392 (100) $[MH]^+$. $C_{16}H_{29}N_3O_6S$ (391.48).

6-(*tert*-Butoxycarbonylamino)hexanoic acid – To a vigorously stirred solution of 5-aminopentanoic acid (2.93 g, 25.0 mmol) and $NaHCO_3$ (5.26 g, 62.5 mmol) in water (50 mL) was added Boc_2O (6.55 g, 30.0 mmol) in 1,4-dioxane (50 mL), and stirring was continued overnight. The resulting mixture was extracted with diethyl ether (50 mL, discarded) and carefully acidified (pH 2) with 2 M hydrochloric acid. The aqueous layer was extracted with ethyl acetate (3 × 30 mL), and the combined extracts were washed with brine and dried over anhydrous Na_2SO_4. The volatiles were removed under reduced pressure, and the residue was crystallized from diethyl ether/n-pentane. Yield: 4.84 g (84 %, as white powder); mp 35–37 °C (lit.[25] mp 31–32 °C). $C_{11}H_{21}NO_4$ (231.29).

***N*-*tert*-Butoxycarbonyl-*N'*-[6-(*tert*-butoxycarbonylamino)hexanoyl]-*S*-methylisothiourea** (**12g**) – To a stirred solution of 6-(*tert*-butoxycarbonylamino)hexanoic acid (1.16 g, 5.0 mmol), HOBt · H_2O (0.76 g, 5.0 mmol) and DIPEA (1.71 mL, 5.0 mmol) in DMF (10 mL) was added TBTU (1.61 g, 5.0 mmol). *N*-*tert*-butoxycarbonyl-*S*-methylisothiourea (0.95 g, 5.0 mmol) was introduced, and stirring was continued for 3 h. The solution was diluted with ethyl acetate (80 mL) and washed with 5 % aq. $KHSO_4$, 5 % aq. $KHCO_3$ and brine, dried over anhydrous Na_2SO_4 and evaporated. Crystallization from n-pentane/CH_2Cl_2 afforded 1.72 g (85 %) product as a white solid. ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.40–1.60 (m, 4H, -$CH_2CH_2C\underline{H}_2C\underline{H}_2CH_2$NHBoc), 1.44 (s, 9H, Boc), 1.52 (s, 9H, Boc), 1.70 (m, 2H, -$CH_2C\underline{H}_2(CH_2)_3$NHBoc), 2.40 (s, 3H, -S$C\underline{H}_3$), 2.45 (t, ^3J = 7.3 Hz, 2H, -$C\underline{H}_2(CH_2)_4$NHBoc), 3.12 (m, 2H, -$(CH_2)_4C\underline{H}_2$NHBoc), 4.56 (brs, 1H, -N<u>H</u>) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 14.5 (-S<u>C</u>H_3), 24.4 (-$CH_2\underline{C}H_2(CH_2)_3$NHBoc), 26.2 (-$CH_2CH_2\underline{C}H_2CH_2CH_2$NHBoc), 28.0 (Boc), 28.4 (Boc), 29.8 (-$\underline{C}H_2CH_2$NHBoc), 37.1 (-$\underline{C}H_2(CH_2)_4$NHBoc), 40.4 (-$(CH_2)_4\underline{C}H_2$NHBoc), 77.2 (Boc), 79.1 (Boc), 156.0 (Boc), 169.3 (N<u>C</u>(SMe)N), 171.1 (<u>C</u>=O) ppm; ESI-MS (+p) *m/z* (%): 404 (100) $[MH]^+$, 304 (45) $[MH - Boc]^+$. $C_{18}H_{33}N_3O_5S$ (403.54).

6-(4-Fluorobenzamido)hexanoic acid – 6-Aminohexanoic acid (0.33 g, 2.5 mmol) was dissolved in a solution of $NaHCO_3$ (0.42 g, 5.0 mmol) in water (5 mL) and combined with a solution of succinimidyl 4-fluorobenzoate (0.59 g, 2.5 mmol) in acetonitrile (5 mL). After stirring overnight the mixture was acidified with 2 M hydrochloric acid and extracted with ethyl acetate (2 × 25 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Yield: 0.52 g (82 %). ^{1}H-NMR (300 MHz, DMSO-d_6): δ_H = 1.32 (m, 2H, -$(CH_2)_2C\underline{H}_2(CH_2)_2$-), 1.44–1.60 (m, 4H, -$CH_2C\underline{H}_2CH_2C\underline{H}_2CH_2$-), 2.21 (t, ^3J = 7.3 Hz, 2H, -$CH_2C\underline{H}_2CO_2H$), 3.24 (m, 2H, -$CH_2C\underline{H}_2NH$-), 7.29 (m, 2H, 2/6-H ArF), 7.91 (m, 2H, 3/5-H ArF), 8.48 (t, ^3J = 5.5 Hz, 1H, -NH-), 12.03 (brs, 1H, -$CO_2\underline{H}$) ppm; ^{13}C-NMR (300 MHz, DMSO-d_6): δ_C = 24.1 (-$\underline{C}H_2CH_2CO_2H$), 25.9 (-$(CH_2)_2\underline{C}H_2$-$(CH_2)_2$-), 28.7 (-$\underline{C}H_2CH_2NH$-), 33.5 (-$\underline{C}H_2CO_2H$), 39.0 (-$\underline{C}H_2NH$-), 115.0 (d, $^2J_{C,F}$ = 21.6 Hz), 129.6 (d, $^3J_{C,F}$ = 8.9 Hz), 131.0 (d, $^4J_{C,F}$ = 2.9 Hz), 163.6 (d, $^1J_{C,F}$ = 248.2 Hz), 164.9 (-$\underline{C}(O)C_6H_4F$), 174.4 (-$\underline{C}O_2H$) ppm; EI-MS (70 eV) *m/z* (%): 253 (10) $M^{\bullet+}$, 194 (10) $[M - CH_2CO_2H]^+$, 152 (12) $[F\text{-}C_6H_4C(O)NH{=}CH_2]^+$, 140 (4) $[F\text{-}C_6H_4C(O)NH_3]^+$, 123 (100) $[F\text{-}C_6H_4CO]^+$. $C_{13}H_{16}FNO_3$ (253.27).

***N*-*tert*-Butoxycarbonyl-*N'*-[6-(4-fluorobenzamido)hexanoyl]-*S*-methylisothiourea** (**12h**) – To a stirred solution of 6-(4-fluorobenzamido)hexanoic acid (0.51 g, 2.0 mmol), HOBt · H_2O (0.30 g, 2.0 mmol), DIPEA (0.34 mL, 4.0 mmol) and TBTU (0.63 g, 2.0 mmol) in DMF (5 mL) was added *N*-*tert*-butoxycarbonyl-*S*-methylisothiourea (0.38 g, 2.0 mmol) and stirring was continued for 2 h. The reaction mixture was poured into diluted HCl (pH > 2) and the product was extracted with ethyl acetate. The organic layers were combined, washed with 5 % aq. $KHCO_3$ and brine, and dried over anhydrous Na_2SO_4. The volatiles were removed under reduced pressure and the crude residue was purified by flash chromatography (eluent: petroleum ether/ethyl acetate 3:2 v/v). Yield: 0.53 g (62 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 1.45 (m, 2H, -$(CH_2)_2C\underline{H}_2(CH_2)_2$NHBz(4F)), 1.52 (s, 9H, Boc), 1.57–1.80 (m, 4H, -$CH_2C\underline{H}_2CH_2C\underline{H}_2CH_2$NHBz(4F)), 2.40 (s, 3H, -$SC\underline{H}_3$), 2.48 (t, ^3J = 7.0 Hz, 2H, -$C\underline{H}_2(CH_2)_4$NHBz(4F)), 3.03 (m, 2H, -$(CH_2)_4C\underline{H}_2$-

NHBz(4F)), 6.35 (brs, 1H, -NH), 7.10 (m, 2H, 2/6-H ArF), 7.80 (m, 2H, 3/5-H ArF), 12.48 (brs, 1H, -NH) ppm; ^{13}C-NMR (300 MHz, $CDCl_3$): δ_C = 14.6 (-S<u>C</u>H$_3$), 23.9 (-CH$_2$<u>C</u>H$_2$(CH$_2$)$_3$NHBz(4F)), 26.2 (-(CH$_2$)$_2$<u>C</u>H$_2$(CH$_2$)$_2$NHBz(4F)), 28.0 (Boc), 29.1 (-<u>C</u>H$_2$CH$_2$NHBz(4F)), 37.2 (-<u>C</u>H$_2$(CH$_2$)$_4$NHBz(4F)), 39.6 (-(CH$_2$)$_4$<u>C</u>H$_2$NHBz(4F)), 77.3 (Boc), 115.5 (d, $^2J_{C,F}$ = 22.0 Hz), 129.2 (d, $^3J_{C,F}$ = 8.9 Hz), 130.8 (d, $^4J_{C,F}$ = 3.3 Hz), 164.6 (d, $^1J_{C,F}$ = 251.4 Hz), 166.5 (-<u>C</u>(O)C$_6$H$_4$F), 171.3 (<u>C</u>=O) ppm (separate peaks corresponding to <u>C</u>=O (Boc) and N<u>C</u>(SMe)N could not be specified); ESI-MS (+p) *m/z* (%): 426 (100) [MH]$^+$, 326 (80) [MH-Boc]$^+$. $C_{20}H_{28}FN_3O_4S$ (425.52).

Guanidinylation of 10 with *S*-methylisothioureas

To a solution of ornithinamide **10** (213 mg, 0.25 mmol), substituted *S*-methylisothiourea (0.25 mmol), and triethylamine (69 μL, 0.50 mmol) in 3 mL DMF, was added $HgCl_2$ (68 mg, 0.25 mmol). After stirring overnight the resulting mixture was diluted with ethyl acetate (25 mL). Insoluble Hg-salts were separated by vacuum filtration through a short bed of Celite® in a Pasteur pipette plugged with some cotton wool and washed with ethyl acetate. The combined filtrates and washings were washed with 5 % aq. $KHSO_4$ and brine and dried over anhydrous Na_2SO_4. The products were purified by flash chromatography (eluent: $CHCl_3$/methanol or CH_2Cl_2/methanol).

(2*S*)-*N*$^{\omega'}$-*tert*-Butoxycarbonyl-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\omega}$-ethoxycarbonyl-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide (**13b**) – Yield: 260 mg (97 %); ESI-MS (+p) *m/z* (%): 1068.8 (100) [MH]$^+$, 535 (60) [MH$_2$]$^{2+}$. $C_{57}H_{69}N_{11}O_{10}$ (1068.23).

(2*S*)-*N*$^{\omega}$-[3-(*tert*-Butoxycarbonylamino)propyloxycarbonyl]-*N*$^{\omega'}$-*tert*-butoxycarbonyl-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]-ethyl}cyclopentyl)acetyl]argininamide (**13d**) – Yield: 260 mg (87 %); ESI-MS (+p) *m/z* (%):1198.0 (100) [MH]$^+$, 1097.8 (10) [MH – Boc]$^+$, 890.7 (27) [MH – Boc – $C_{14}H_9NO$]$^+$, 715.6 (15), 599.6 (60) [MH$_2$]$^{2+}$. $C_{63}H_{80}N_{12}O_{12}$ (1197.38).

(2*S*)-$N^{\omega'}$-*tert*-Butoxycarbonyl-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{ω}-methanesulfonyl-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide (**13k**) – Yield: 270 mg (99 %); ESI-MS (+p) *m/z* (%): 1074.7 (100) $[MH]^+$, 867.6 (35) $[MH - C_{14}H_9NO]^+$, 767.5 (20) $[MH - C_{14}H_9NO - Boc]^+$, 538.0 (38) $[MH_2]^{2+}$. $C_{55}H_{67}N_{11}O_{10}S$ (1074.25).

(2*S*)-$N^{\omega'}$-*tert*-Butoxycarbonyl-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{ω}-ethylaminocarbonyl-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide (**13e**) – Yield: 230 mg (86 %); ESI-MS (+p) *m/z* (%): 1067.7 (100) $[MH]^+$, 967.6 (5) $[MH - Boc]^+$, 860.6 (5) $[MH - C_{14}H_9NO]^+$, 760.5 $[MH - C_{14}H_9NO - Boc]^+$. $C_{57}H_{70}N_{12}O_9$ (1067.24).

(2*S*)-$N^{\omega'}$-*tert*-Butoxycarbonyl-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]-N^{ω}-(phenylacetyl)argininamide (**13h**) – Yield: 290 mg (99 %); ESI-MS (+p) *m/z* (%): 1114.8 (100) $[MH]^+$, 1014.7 (10) $[MH - Boc]^+$, 907.7 (8) $[MH - C_{14}H_9NO]^+$, 807.6 (65) $[MH - C_{14}H_9NO - Boc]^+$, 558 (30) $[MH_2]^{2+}$. $C_{62}H_{71}N_{11}O_9$ (1114.30).

(2*S*)-$N^{\omega'}$-*tert*-Butoxycarbonyl-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]- N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]-N^{ω}-(phenoxyacetyl)argininamide (**13i**) – Yield: 270 mg (96 %); ESI-MS (+p) *m/z* (%): 1130.8 (100) $[MH]^+$, 1030.6 (20) $[MH - Boc]^+$, 923.8 (3) $[MH - C_{14}H_9NO]^+$, 823.6 (65) $[MH - C_{14}H_9NO - Boc]^+$, 566.0 (30) $[MH_2]^{2+}$. $C_{62}H_{71}N_{11}O_{10}$ (1130.29).

(2*S*)-$N^{\omega'}$-*tert*-Butoxycarbonyl-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]-N^{ω}-(3-phenylpropanoyl)argininamide

(**13j**) – Yield: 260 mg (92 %); ESI-MS (+p) *m/z* (%): 1128.7 (50) $[MH]^+$, 565.0 (100) $[MH_2]^{2+}$. $C_{63}H_{73}N_{11}O_9$ (1128.32).

(2*S*)-*N*$^{\omega'}$-*tert*-Butoxycarbonyl-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]-*N*$^{\omega}$-propanoylargininamide (**13f**) – Yield: 240 mg (91 %); ESI-MS (+p) *m/z* (%): 1052.7 (60) $[MH]^+$, 527.0 (100) $[MH_2]^{2+}$. $C_{57}H_{69}N_{11}O_9$ (1052.23).

(2*S*)-*N*$^{\omega}$,*N*$^{\omega'}$-Bis(*tert*-butoxycarbonyl)-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\omega}$-ethyl-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide (**13n**) – Yield: 140 mg (50 %); ESI-MS (+p) *m/z* (%): 1124.8 (60) $[MH]^+$, 917.7 (8) $[MH - C_{14}H_9NO]^+$, 563.0 (100) $[MH_2]^{2+}$. $C_{61}H_{78}N_{11}O_{10}$ (1125.34).

(2*S*)-*N*$^{\omega}$-Benzyl-*N*$^{\omega}$,*N*$^{\omega'}$-bis(*tert*-butoxycarbonyl)-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)-piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide (**13m**) – Yield: 190 mg (64 %); ESI-MS (+p) *m/z* (%): 1186.9 (35) $[MH]^+$, 594.0 (100) $[MH_2]^{2+}$. $C_{66}H_{80}N_{11}O_{10}$ (1187.41).

(2*S*)-*N*$^{\omega}$-[6-(*tert*-Butoxycarbonylamino)hexanoyl)-*N*$^{\omega'}$-*tert*-butoxycarbonyl-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide (**13o**) – Yield: 260 mg (86 %); ESI-MS (+p) *m/z* (%): 1209.9 (50) $[MH]^+$, 605.5 (100) $[MH_2]^{2+}$. $C_{65}H_{84}N_{12}O_{11}$ (1209.44).

(2*S*)-*N*$^{\omega'}$-*tert*-Butoxycarbonyl-*N*$^{\omega}$-[6-(4-fluorobenzamido)hexanoyl]-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)-acetyl]argininamide (**13p**) – Yield: 260 mg (84 %); ESI-MS (+p) *m/z* (%): 1231.8 (55) $[MH]^+$, 616.5 (100) $[MH_2]^{2+}$. $C_{67}H_{79}FN_{12}O_{10}$ (1231.42).

4.1.9. $N^{\omega'}$-BOC DEPROTECTION – GENERAL PROCEDURE:

Deprotection was carried out in TFA-CH_2Cl_2 1:1 (v/v) (approx. 5 mL/0.2 mmol substrate). After stirring for 2 h CCl_4 (10 mL) was added, and the volatiles were removed under reduced pressure. The products were purified by flash chromatography eluting with $CHCl_3$/methanol containing 1 % (v/v) of 5 % TFA in CH_2Cl_2. The final products were obtained as white to slightly yellowish powders in yields ranging from 69 to 99 %. For combustion analysis samples of about 20 mg were re-dissolved in 0.5 mL methanol and treated with 0.25 mL 2 M HCl in diethyl ether. After 20 min diethyl ether (2.5 mL) was added, and the resulting suspension was centrifuged. The precipitated HCl salts were separated by decanting the supernatant and washed twice with 2.5 mL diethyl ether. Prior to analysis the samples were dried in vacuum at 50–60 °C for 24 h.

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{ω}-methoxycarbonyl-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide (**14a**): ^{1}H-NMR (600 MHz, DMSO-d_6, HSQC): δ_H = 1.44 (m, 2H, -C$^{\gamma}$H$_2$-), 1.40 and 1.65 (m, 2H, -C$^{\beta}$H$_2$--), 1.44–1.54 (m, 4H, 2/5-H cyclopentyl), 1.54 (m, 4H, 3/4-H cyclopentyl), 2.04 and 2.06 (m, 4H, 3/5-H piperazine), 2.21 and 2.31 (m, 2H, -CH$_2$CON$^{\alpha}$H-), 2.41 and 2.49 (m, 2H, >NCOCH$_2$-cyclo-C_5H_8-), 3.17 (m, 2H, -C$^{\delta}$H$_2$-), 3.24–3.28 (m, 4H, 2/6-H piperazine), 3.29 and 3.41 (m, 2H, -NHCH$_2$CH$_2$N<), 3.59 (t, ^3J = 5.9 Hz, 2H, -NHCH$_2$CH$_2$N<), 3.74 (s, 3H, -OCH$_3$), 4.15 (m, 1H, -C$^{\alpha}$H-), 4.29 (m, 1H, 11-H dibenzazepin-11-yl), 7.07 (m, 1H, H_{ar}), 7.11 (m, 1H, H_{ar}), 7.23 (m, 2H, Ph), 7.25 (m, 1H, H_{ar}), 7.34 (m, 1H, H_{ar}), 7.37 (m, 8H, Ph), 7.40 (m, 2H, H_{ar}), 7.50 (m, 1H, H_{ar}), 7.74 (m, 1H, H_{ar}), 8.04 (d, ^3J = 8.0 Hz, 1H, -N$^{\alpha}$H-), 8.28 (t, ^3J = 5.9 Hz, 1H, -CONHC$_2$H$_4$N-), 8.64 (m, 2H, -N$^{\omega}$H$_2$), 8.83 (t, ^3J = 5.6 Hz, 1H, -N$^{\delta}$H), 10.40 (s, 1H, -C(O)NH- lactam), 11.65 (s, 1H, -N$^{\omega'}$H) ppm; ^{13}C-NMR (600 MHz, DMSO-d_6, HSQC): δ_C = 23.2 (C-3/4 cyclopentyl), 24.2 (C$^{\gamma}$), 28.7 (C$^{\beta}$), 36.0 (-NHCH$_2$CH$_2$N<), 37.3 (C-2/5 cyclopentyl), 38.3 (>NCOCH$_2$-cyclo-C_5H_8-), 39.6 (-NHCH$_2$CH$_2$N<), 40.7 (C$^{\delta}$), 40.6 and 45.5 (C-2/6 piperazine), 42.8

(-$\underline{C}H_2CON^{\alpha}H$-), 44.0 (C-1 cyclopentyl), 50.8 and 51.3 (C-3/5 piperazine), 51.8 ($\underline{C}^{\alpha}$), 53.3 (-O$\underline{C}H_3$), 73.6 (C-11 dibenzazepin-11-yl), 121.4 ($\underline{C}_{ar}H$), 122.5 (Ph), 123.7 ($\underline{C}_{ar}H$), 126.6 (Ph), 127.9 ($\underline{C}_{ar}H$), 128.1 ($\underline{C}_{ar}H$), 128.5 ($\underline{C}_{ar}H$), 129.0 (Ph), 130.0 ($\underline{C}_{ar}H$), 130.5 ($\underline{C}_{ar}H$), 131.4 ($\underline{C}_{ar}H$), 131.6 ($\underline{C}_{ar}$), 136.1 ($\underline{C}_{ar}$), 136.5 ($\underline{C}_{ar}$), 141.8 ($\underline{C}_{ar}$), 152.6 (-N$\underline{C}$ONPh-), 152.9 (-XC(X)N-), 153.2 (-XC(X)N-), 168.0 ($\underline{C}$=O lactam), 169.9 (-$\underline{C}$ON<), 171.2 (-$\underline{C}ON^{\alpha}H$-), 171.9 (-$\underline{C}ONHC_2H_4N$<) ppm; ESI-MS (+p) *m/z* (%): 954.6 (100) $[MH]^+$, 477.9 (25) $[M+2H]^{2+}$; RP-HPLC: $k' = 6.7$ ($t_R = 17.8$ min); analysis calcd. for $C_{51}H_{59}N_{11}O_8 \cdot H_2O \cdot 2\ HCl$: C 58.61, H 6.08, N 14.75 %, found: C 58.69, H 6.37, N 14.76 %. $C_{51}H_{59}N_{11}O_8$ (954.08).

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{ω}-ethoxycarbonyl-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide (**14b**): ^{1}H-NMR (600 MHz, DMSO-d_6, HSQC): $\delta_H = 1.23$ (t, $^3J = 7.1$ Hz, 3H, -OCH$_2$C$\underline{H}_3$), 1.43 (m, 2H, -C$^{\gamma}\underline{H}_2$-), 1.43 and 1.62 (m, 2H, -C$^{\beta}\underline{H}_2$-), 1.40–1.55 (m, 4H, 2/5-H cyclopentyl), 1.54 (m, 4H, 3/4-H cyclopentyl), 2.04 and 2.06 (m, 4H, 3/5-H piperazine), 2.19 and 2.29 (m, 2H, -C$\underline{H}_2CON^{\alpha}H$-), 2.41 and 2.48 (m, 2H, >NCOC$\underline{H}_2$-cyclo-C_5H_8-), 3.16 (m, 2H, -C$^{\delta}\underline{H}_2$-), 3.28–3.31 (m, 4H, 2/6-H piperazine), 3.29 and 3.37 (m, 2H, -NHC$\underline{H}_2CH_2N$<), 3.59 (t, $^3J = 6.0$ Hz, 2H, -NHCH$_2$C$\underline{H}_2$N<), 4.14 (m, 1H, -C$^{\alpha}\underline{H}$-), 4.20 (q, $^3J = 7.1$ Hz, 2H, -OC$\underline{H}_2CH_3$), 4.24 (m, 1H, 11-H dibenzazepin-11-yl), 7.06 (m, 1H, H_{ar}), 7.09 (m, 1H, H_{ar}), 7.22 (m, 2H, Ph), 7.25 (m, 1H, H_{ar}), 7.33 (m, 1H, H_{ar}), 7.37 (m, 8H, Ph), 7.38 (m, 1H, H_{ar}), 7.40 (m, 1H, H_{ar}), 7.48 (m, 1H, H_{ar}), 7.72 (m, 1H, H_{ar}), 8.01 (d, $^3J = 8.0$ Hz, 1H, -N$^{\alpha}\underline{H}$-), 8.25 (t, $^3J = 5.8$ Hz, 1H, -CON$\underline{H}C_2H_4N$-), 8.30–8.80 (m, 2H, -N$^{\omega}\underline{H}_2$), 8.60 (t, $^3J = 5.6$ Hz, 1H, -N$^{\delta}\underline{H}$), 10.35 (s, 1H, -C(O)N$\underline{H}$- lactam), 11.31 (s, 1H, -N$^{\omega'}\underline{H}$) ppm; ^{13}C-NMR (600 MHz, DMSO-d_6, HSQC): $\delta_C = 13.8$ (-CH$_2$C$\underline{H}_3$), 23.3 (C-3/4 cyclopentyl), 24.2 ($\underline{C}^{\gamma}$), 28.6 ($\underline{C}^{\beta}$), 36.0 (-NH$\underline{C}H_2CH_2N$<), 37.3 (C-2/5 cyclopentyl), 38.3 (>NCO$\underline{C}H_2$-cyclo-C_5H_8-), 39.6 (-NHCH$_2\underline{C}H_2$N<), 40.6 ($\underline{C}^{\delta}$), 40.6 and 45.4 (C-2/6 piperazine), 42.8 (-$\underline{C}H_2CON^{\alpha}H$-), 44.0 (C-1 cyclopentyl), 50.8 and 51.3 (C-3/5 piperazine), 51.7 ($\underline{C}^{\alpha}$),

62.6 (-O<u>C</u>H_2CH_3), 73.6 (C-11 dibenzazepin-11-yl), 121.3 (<u>C</u>$_{ar}$H), 122.4 (Ph), 123.7 (<u>C</u>$_{ar}$H), 126.5 (Ph), 127.9 (<u>C</u>$_{ar}$H), 128.1 (<u>C</u>$_{ar}$H), 128.4 (<u>C</u>$_{ar}$H), 129.0 (Ph), 129.8 (<u>C</u>$_{ar}$H), 130.5 (<u>C</u>$_{ar}$H), 131.3 (<u>C</u>$_{ar}$H), 131.5 (<u>C</u>$_{ar}$), 136.0 (<u>C</u>$_{ar}$), 136.4 (<u>C</u>$_{ar}$), 141.9 (<u>C</u>$_{ar}$), 152.6 (-N<u>C</u>ONPh-), 152.5 (-NC(X)X-), 152.8 (-NC(X)X-), 168.0 (<u>C</u>=O lactam), 169.9 (-<u>C</u>ON<), 171.1 (-<u>C</u>ON$^{\alpha}$H-), 171.8 (-<u>C</u>ONHC$_2$H$_4$N<) ppm; ESI-MS (+p) *m/z* (%): 968.7 (100) $[MH]^+$; RP-HPLC: k' = 7.03 (t_R = 18.5 min); analysis calcd. for $C_{52}H_{61}N_{11}O_8 \cdot H_2O \cdot 2$ HCl: C 58.97, H 6.19, N 14.55 %, found: C 58.88, H 6.18, N 14.50 %. $C_{52}H_{61}N_{11}O_8$ (968.11).

(2*S*)-*N*$^{\omega}$-Benzyloxycarbonyl-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)-ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)-piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide (**14c**): ^{1}H-NMR (600 MHz, DMSO-d_6, HSQC): δ_H = 1.44 (m, 2H, -C$^{\gamma}$<u>H</u>$_2$-), 1.42 and 1.62 (m, 2H, -C$^{\beta}$<u>H</u>$_2$-), 1.38–1.55 (m, 4H, 2/5-H cyclopentyl), 1.54 (m, 4H, 3/4-H cyclopentyl), 2.06 and 2.08 (m, 4H, 3/5-H piperazine), 2.20 and 2.30 (m, 2H, -C<u>H</u>$_2$CON$^{\alpha}$H-), 2.41 and 2.48 (m, 2H, >NCOC<u>H</u>$_2$-cyclo-C$_5$H$_8$-), 3.16 (m, 2H, -C$^{\delta}$<u>H</u>$_2$-), 3.28–3.33 (m, 4H, 2/6-H piperazine), 3.29 and 3.37 (m, 2H, -NHC<u>H</u>$_2$CH$_2$N<), 3.59 (t, ^3J = 6.0 Hz, 2H, -NHCH$_2$C<u>H</u>$_2$N<), 4.15 (m, 1H, -C$^{\alpha}$<u>H</u>-), 4.28 (m, 1H, 11-H dibenzazepin-11-yl), 5.23 (s, -OC<u>H</u>$_2$Ph), 7.06 (m, 1H, H$_{ar}$), 7.10 (m, 1H, H$_{ar}$), 7.21 (m, 2H, Ph), 7.25 (m, 1H, H$_{ar}$), 7.33 (m, 1H, H$_{ar}$), 7.36 (m, 8H, Ph), 7.38 (m, 1H, H$_{ar}$), 7.40 (m, 1H, H$_{ar}$), 7.39–7.44 (m, 5H, Cbz), 7.50 (m, 1H, H$_{ar}$), 7.74 (m, 1H, H$_{ar}$), 8.00 (d, ^3J = 8.0 Hz, 1H, -N$^{\alpha}$<u>H</u>-), 8.25 (t, ^3J = 5.6 Hz, 1H, -CON<u>H</u>C$_2$H$_4$N-), 8.32–8.80 (m, 2H, -N$^{\omega}$<u>H</u>$_2$), 8.64 (t, ^3J = 5.4 Hz, 1H, -N$^{\delta}$<u>H</u>), 10.37 (s, 1H, -C(O)N<u>H</u>- lactam), 11.53 (s, 1H, -N$^{\omega'}$<u>H</u>) ppm; ^{13}C-NMR (600 MHz, DMSO-d_6, HSQC): δ_C = 23.1 (C-3/4 cyclopentyl), 24.2 (<u>C</u>$^{\gamma}$), 28.6 (<u>C</u>$^{\beta}$), 36.1 (-NH<u>C</u>H$_2$CH$_2$N<), 37.2 (C-2/5 cyclopentyl), 38.3 (>NCO<u>C</u>H$_2$-cyclo-C$_5$H$_8$-), 39.5 (-NHCH$_2$<u>C</u>H$_2$N<), 40.7 (<u>C</u>$^{\delta}$), 40.5 and 45.3 (C-2/6 piperazine), 42.8 (-<u>C</u>H$_2$CON$^{\alpha}$H-), 44.0 (C-1 cyclopentyl), 50.7 and 51.1 (C-3/5 piperazine), 51.8 (<u>C</u>$^{\alpha}$), 67.7 (-O<u>C</u>H$_2$Ph), 73.6 (C-11 dibenzazepin-11-yl), 121.3 (<u>C</u>$_{ar}$H), 122.5 (Ph), 123.7 (<u>C</u>$_{ar}$H), 126.5 (Ph), 127.7 (<u>C</u>$_{ar}$H), 127.9 (<u>C</u>$_{ar}$H), 128.4

($\underline{C}_{ar}H$), 128.4 (Cbz), 128.9 (Ph), 129.9 ($\underline{C}_{ar}H$), 130.5 ($\underline{C}_{ar}H$), 131.3 ($\underline{C}_{ar}H$), 131.5 ($\underline{C}_{ar}$), 134.9 (Cbz), 136.1 ($\underline{C}_{ar}$), 136.5 ($\underline{C}_{ar}$), 141.9 ($\underline{C}_{ar}$), 152.5 (-N$\underline{C}$ONPh-), 152.5 (-NC(X)X-), 152.8 (-NC(X)X-), 167.9 ($\underline{C}$=O lactam), 169.9 (-$\underline{C}$ON<), 171.1 (-$\underline{C}ON^{\alpha}H$-), 171.8 (-$\underline{C}ONHC_2H_4N$<) ppm; ESI-MS (+p) *m/z* (%): 1030.7 (60) $[MH]^+$, 516.0 (100) $[M + 2H]^{2+}$; RP-HPLC: $k' = 8.1$ (t_R = 20.8 min); analysis calcd. for $C_{57}H_{63}N_{11}O_8 \cdot H_2O \cdot 2$ HCl: C 61.06, H 6.02, N 13.70 %, found: C 61.03, H 6.32, N 13.70 %. $C_{57}H_{63}N_{11}O_8$ (1030.18).

(2*S*)-*N*$^{\omega}$-Benzoyl-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide (**14g**): ^{1}H-NMR (600 MHz, DMSO-d_6, HSQC): δ_H = 1.50 (m, 2H, -C$^{\gamma}\underline{H}_2$-), 1.44 and 1.67 (m, 2H, -C$^{\beta}\underline{H}_2$-), 1.36–1.56 (m, 4H, 2/5-H cyclopentyl), 1.54 (m, 4H, 3/4-H cyclopentyl), 2.06 and 2.08 (m, 4H, 3/5-H piperazine), 2.20 and 2.31 (m, 2H, -C$\underline{H}_2$CON$^{\alpha}$H-), 2.41 and 2.49 (m, 2H, >NCOC$\underline{H}_2$-cyclo-C_5H_8-), 3.25 (m, 2H, -C$^{\delta}\underline{H}_2$-), 3.27 and 3.32 (m, 4H, 2/6-H piperazine), 3.32 and 3.39 (m, 2H, -NHC$\underline{H}_2$CH$_2$N<), 3.60 (t, ^3J = 6.1 Hz, 2H, -NHCH$_2$C$\underline{H}_2$N<), 4.19 (m, 1H, -C$^{\alpha}\underline{H}$-), 4.28 (m, 1H, 11-H dibenzazepin-11-yl), 7.06 (m, 1H, H_{ar}), 7.09 (m, 1H, H_{ar}), 7.21 (m, 2H, Ph), 7.25 (m, 1H, H_{ar}), 7.33 (m, 1H, H_{ar}), 7.36 (m, 8H, Ph), 7.40 (m, 2H, H_{ar}), 7.48 (m, 1H, H_{ar}), 7.61 (m, 2H, Bz), 7.72 (m, 1H, Bz), 7.74 (m, 1H, H_{ar}), 7.95 (m, 2H, Bz), 8.02 (d, ^3J = 8.0 Hz, 1H, -N$^{\alpha}\underline{H}$-), 8.26 (t, ^3J = 5.7 Hz, 1H, -CON$\underline{H}C_2H_4$N-), 8.86 (brs, 2H, -N$^{\omega}\underline{H}_2$), 9.13 (t, ^3J = 5.4 Hz, 1H, -N$^{\delta}\underline{H}$), 10.35 (s, 1H, -C(O)N$\underline{H}$- lactam), 11.39 (s, 1H, -N$^{\omega'}\underline{H}$) ppm; ^{13}C-NMR (600 MHz, DMSO-d_6, HSQC): δ_C = 23.2 (C-3/4 cyclopentyl), 24.2 ($\underline{C}^{\gamma}$), 28.7 ($\underline{C}^{\beta}$), 36.1 (-NH$\underline{C}$H$_2$CH$_2$N<), 37.2 (C-2/5 cyclopentyl), 38.3 (>NCO$\underline{C}$H$_2$-cyclo-C_5H_8-), 39.5 (-NHCH$_2\underline{C}$H$_2$N<), 40.8 ($\underline{C}^{\delta}$), 40.5 and 45.3 (C-2/6 piperazine), 42.8 (-$\underline{C}$H$_2$CON$^{\alpha}$H-), 44.0 (C-1 cyclopentyl), 50.7 and 51.2 (C-3/5 piperazine), 51.7 ($\underline{C}^{\alpha}$), 73.6 (C-11 dibenzazepin-11-yl), 121.3 ($\underline{C}_{ar}H$), 122.5 (Ph), 123.7 ($\underline{C}_{ar}H$), 126.5 (Ph), 127.9 (2 $\underline{C}_{ar}H$), 128.0 (Bz), 128.4 ($\underline{C}_{ar}H$), 128.8 (Ph), 128.9 (Bz), 129.9 ($\underline{C}_{ar}H$), 130.5 ($\underline{C}_{ar}H$), 131.3 ($\underline{C}_{ar}H$), 131.5 ($\underline{C}_{ar}$), 133.9 (Bz), 136.1 ($\underline{C}_{ar}$), 136.5 ($\underline{C}_{ar}$), (further peaks

corresponding to C_{ar} remained unresolved), 152.5 (-N<u>C</u>ONPh-), 153.3 (-N<u>C</u>(N)N-), 167.9 (<u>C</u>=O lactam), 169.9 (-<u>C</u>ON<), 171.2 (-<u>C</u>ON$^{\alpha}$H-), 171.8 (-<u>C</u>ONHC$_2$H$_4$N<), 176.0 (Bz) ppm; ESI-MS (+p) *m/z* (%): 1000.7 (100) $[MH]^+$, 501.0 (30) $[MH_2]^{2+}$; RP-HPLC: $k' = 7.6$ (t_R = 19.7 min); analysis calcd. for $C_{56}H_{61}N_{11}O_7 \cdot H_2O \cdot 2$ HCl: C 61.64, H 6.01, N 14.12 %, found: C 61.51, H 6.27, N 14.07 %. $C_{56}H_{61}N_{11}O_7$ (1000.15).

(2*S*)-*N*$^{\omega}$-(3-Aminopropyloxycarbonyl)-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide (**14d**): ^{1}H-NMR (600 MHz, DMSO-d$_6$, HSQC): δ_H = 1.48 (m, 2H, -C$^{\gamma}$<u>H</u>$_2$-), 1.44 and 1.64 (m, 2H, -C$^{\beta}$<u>H</u>$_2$-), 1.39–1.56 (m, 4H, 2/5-H cyclopentyl), 1.54 (m, 4H, 3/4-H cyclopentyl), 1.94 (m, 2H, -OCH$_2$C<u>H</u>$_2$CH$_2$NH$_2$), 2.09 and 2.11 (m, 4H, 3/5-H piperazine), 2.21 and 2.31 (m, 2H, -C<u>H</u>$_2$CON$^{\alpha}$H-), 2.42 and 2.50 (m, 2H, >NCOC<u>H</u>$_2$-cyclo-C$_5$H$_8$-), 2.90 (m, 2H, -OCH$_2$CH$_2$C<u>H</u>$_2$NH$_2$), 3.18 (m, 2H, -C$^{\delta}$<u>H</u>$_2$-), 3.29 and 3.35 (m, 4H, 2/6-H piperazine), 3.33 and 3.39 (m, 2H, -NHC<u>H</u>$_2$CH$_2$N<), 3.60 (t, ^3J = 6.0 Hz, 2H, -NHCH$_2$C<u>H</u>$_2$N<), 4.16 (m, 1H, -C$^{\alpha}$<u>H</u>-), 4.22 (t, ^3J = 6.1 Hz, -OC<u>H</u>$_2$(CH$_2$)$_2$NH$_2$), 4.34 (m, 1H, 11-H dibenzazepin-11-yl), 7.07 (m, 1H, H$_{ar}$), 7.11 (m, 1H, H$_{ar}$), 7.22 (m, 2H, Ph), 7.26 (m, 1H, H$_{ar}$), 7.34 (m, 1H, H$_{ar}$), 7.37 (m, 8H, Ph), 7.40 (m, 2H, H$_{ar}$), 7.50 (m, 1H, H$_{ar}$), 7.74 (m, 1H, H$_{ar}$), 7.97 (brs, 3H, -(CH$_2$)$_3$NH$_3^+$), 7.99 (d, ^3J = 8.1 Hz, 1H, -N$^{\alpha}$<u>H</u>-), 8.26 (t, ^3J = 6.0 Hz, 1H, -CON<u>H</u>C$_2$H$_4$N-), 8.74 (brs, 2H, -N$^{\omega}$<u>H</u>$_2$), 9.04 (t, ^3J = 5.3 Hz, 1H, -N$^{\delta}$<u>H</u>), 10.36 (s, 1H, -C(O)N<u>H</u>- lactam), 11.99 (s, 1H, -N$^{\omega'}$<u>H</u>) ppm; ^{13}C-NMR (600 MHz, DMSO-d$_6$, HSQC): δ_C = 23.2 (C-3/4 cyclopentyl), 24.3 (<u>C</u>$^{\gamma}$), 26.0 (-OCH$_2$<u>C</u>H$_2$CH$_2$NH$_2$), 28.7 (<u>C</u>$^{\beta}$), 36.0 (-<u>C</u>H$_2$NH$_2$), 36.1 (-NH<u>C</u>H$_2$CH$_2$N<), 37.2 (C-2/5 cyclopentyl), 38.4 (>NCO<u>C</u>H$_2$-cyclo-C$_5$H$_8$-), 39.5 (-NHCH$_2$<u>C</u>H$_2$N<), 40.7 (<u>C</u>$^{\delta}$), 40.6 and 45.3 (C-2/6 piperazine), 42.8 (-<u>C</u>H$_2$CON$^{\alpha}$H-), 44.0 (C-1 cyclopentyl), 50.7 and 51.2 (C-3/5 piperazine), 51.8 (<u>C</u>$^{\alpha}$), 63.7 (-O<u>C</u>H$_2$(CH$_2$)$_2$NH$_2$), 73.6 (C-11 dibenzazepin-11-yl), 121.4 (<u>C</u>$_{ar}$H), 122.6 (Ph), 123.8 (<u>C</u>$_{ar}$H), 126.5 (Ph), 127.9 (<u>C</u>$_{ar}$H), 128.0 (<u>C</u>$_{ar}$H), 128.5

($\underline{C}_{ar}H$), 129.0 (Ph), 130.0 ($\underline{C}_{ar}H$), 130.5 ($\underline{C}_{ar}H$), 131.4 ($\underline{C}_{ar}H$), 131.6 ($\underline{C}_{ar}$), 136.1 ($\underline{C}_{ar}$), 136.5 ($\underline{C}_{ar}$), 141.5 ($\underline{C}_{ar}$), 152.6 (-N$\underline{C}$ONPh-), 152.8 (X$\underline{C}$(X)N), 153.1 (-X$\underline{C}$(X)N-), 167.9 ($\underline{C}$=O lactam), 170.0 (-$\underline{C}$ON<), 171.2 (-$\underline{C}$ON$^{\alpha}$H-), 171.9 (-$\underline{C}$ONHC$_2$H$_4$N<), ppm; ESI-MS (+p) *m/z* (%): 997.8 (60) $[MH]^+$, 790.6 (60) [MH – 207]$^+$, 499.5 (100) $[MH_2]^{2+}$; RP-HPLC: $k' = 5.44$ (t_R = 14.8 min); analysis calcd. for $C_{53}H_{64}N_{12}O_8 \cdot 5\ H_2O \cdot 3\ HCl \cdot CHCl_3$: C 49.28, H 5.90, N 12.78 %, found: C 49.17, H 6.90, N 12.65 %. $C_{53}H_{64}N_{12}O_8$ (997.15).

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\omega}$-methanesulfonyl-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide (**14k**): ^{1}H-NMR (600 MHz, DMSO-d$_6$, HSQC): δ_H = 1.36 (m, 2H, -C$^{\gamma}\underline{H}_2$-), 1.37 and 1.58 (m, 2H, -C$^{\beta}\underline{H}_2$--), 1.35–1.55 (m, 4H, 2/5-H cyclopentyl), 1.53 (m, 4H, 3/4-H cyclopentyl), 2.10 (m, 4H, 3/5-H piperazine), 2.21 and 2.29 (m, 2H, -C$\underline{H}_2$CON$^{\alpha}$H-), 2.43 and 2.52 (m, 2H, >NCOC$\underline{H}_2$-cyclo-C$_5$H$_8$-), 2.77 (s, 3H, -SO$_2$C$\underline{H}_3$), 2.95 (m, 2H, -C$^{\delta}\underline{H}_2$-), 3.28 and 3.38 (m, 4H, 2/6-H piperazine), 3.29 and 3.40 (m, 2H, -NHC$\underline{H}_2$CH$_2$N<), 3.59 (m, 2H, -NHCH$_2$C$\underline{H}_2$N<), 4.13 (m, 1H, -C$^{\alpha}\underline{H}$-), 4.40 (brm, 1H, 11-H dibenzazepin-11-yl), 7.08 (m, 1H, H_{ar}), 7.15 (m, 1H, H_{ar}), 7.22 (m, 2H, Ph), 7.22 (m, 1H, H_{ar}), 7.37 (m, 1H, H_{ar}), 7.37 (m, 8H, Ph), 7.40 (m, 2H, H_{ar}), 7.54 (m, 1H, H_{ar}), 7.79 (m, 1H, H_{ar}), 7.94 (d, ^3J = 8.0 Hz, 1H, -N$^{\alpha}\underline{H}$-), 8.19 (t, ^3J = 6.0 Hz, 1H, -CON$\underline{H}$C$_2$H$_4$N-), 8.32 (m, 1H, -N$^{\delta}\underline{H}$), 8.93 (brs, 2H, -N$^{\omega}\underline{H}_2$), 10.41 (s, 1H, -C(O)N$\underline{H}$- lactam), 11.13 (s, 1H, -N$^{\omega'}\underline{H}$) ppm; ^{13}C-NMR (600 MHz, DMSO-d$_6$, HSQC): δ_C = 23.3 (C-3/4 cyclopentyl), 25.4 ($\underline{C}^{\gamma}$), 29.9 ($\underline{C}^{\beta}$), 36.1 (-NH$\underline{C}$H$_2$CH$_2$N<), 37.2 (C-2/5 cyclopentyl), 38.5 (>NCO$\underline{C}$H$_2$-cyclo-C$_5$H$_8$-), 39.6 (-NHCH$_2\underline{C}$H$_2$N<), 39.7 ($\underline{C}^{\delta}$), 40.3 and 44.8 (C-2/6 piperazine), 41.4 (-SO$_2\underline{C}$H$_3$), 42.7 (-$\underline{C}$H$_2$CON$^{\alpha}$H-), 44.0 (C-1 cyclopentyl), 50.8 and 51.2 (C-3/5 piperazine), 52.0 ($\underline{C}^{\alpha}$), 73.4 (C-11 dibenzazepin-11-yl), 121.5 ($\underline{C}_{ar}$H), 122.6 (Ph), 123.9 ($\underline{C}_{ar}$H), 126.6 (Ph), 127.7 (2 $\underline{C}_{ar}$H), 128.4 ($\underline{C}_{ar}$H), 129.0 (Ph), 130.4 ($\underline{C}_{ar}$H), 130.8 ($\underline{C}_{ar}$H), 131.2 ($\underline{C}_{ar}$H), 131.5 ($\underline{C}_{ar}$), 136.2 ($\underline{C}_{ar}$), 136.5 ($\underline{C}_{ar}$), 152.6 (-N$\underline{C}$ONPh-), 156.6 (N$\underline{C}$(N)N), 167.5 ($\underline{C}$=O lactam),

170.0 (-CON<), 171.2 (-CON$^{\alpha}$H-), 172.1 (-CONHC$_2$H$_4$N<) ppm; ESI-MS (+p) *m/z* (%): 974.6 (100) $[MH]^+$, 991.6 (15) $[MNH_4]^+$, 996.6 (35) $[MNa]^+$; RP-HPLC: $k' = 7.3$ (t_R = 19.1 min); analysis calcd. for $C_{50}H_{59}N_{11}O_8S \cdot 0.5\ H_2O \cdot HCl$: C 58.90, H 6.03, N 15.11 %, found: C 58.77, H 6.02, N 14.96 %. $C_{50}H_{59}N_{11}O_8S$ (974.14).

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\omega}$-ethylamino-carbonyl-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)-piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide (**14e**): ^{1}H-NMR (600 MHz, DMSO-d$_6$, HSQC): δ_H = 1.04 (t, ^3J = 7.2 Hz, 3H, -NHCH$_2$CH$_3$), 1.38–1.54 (m, 4H, 2/5-H cyclopentyl), 1.44 (m, 2H, -C$^{\gamma}$H$_2$-), 1.44 and 1.63 (m, 2H, -C$^{\beta}$H$_2$-), 1.54 (m, 4H, 3/4-H cyclopentyl), 2.07 and 2.08 (m, 4H, 3/5-H piperazine), 2.20 and 2.30 (m, 2H, -CH$_2$CON$^{\alpha}$H-), 2.40 and 2.50 (m, 2H, >NCOCH$_2$-cyclo-C$_5$H$_8$-), 3.10 (m, 2H, -NHCH$_2$CH$_3$), 3.13 (m, 2H, -C$^{\delta}$H$_2$-), 3.29–3.32 (m, 4H, 2/6-H piperazine), 3.29 and 3.38 (m, 2H, -NHCH$_2$CH$_2$N<), 3.59 (t, ^3J = 6.1 Hz, 2H, -NHCH$_2$CH$_2$N<), 4.15 (m, 1H, -C$^{\alpha}$H-), 4.28 (m, 1H, 11-H dibenzazepin-11-yl), 7.06 (m, 1H, H$_{ar}$), 7.10 (m, 1H, H$_{ar}$), 7.22 (m, 2H, Ph), 7.25 (m, 1H, H$_{ar}$), 7.34 (m, 1H, H$_{ar}$), 7.36 (m, 4H, Ph), 7.37 (m, 4H, Ph), 7.40 (m, 2H, H$_{ar}$), 7.49 (m, 1H, H$_{ar}$), 7.73 (m, 1H, H$_{ar}$), 8.01 (d, ^3J = 7.3 Hz, 1H, -N$^{\alpha}$H-), 8.24 (t, ^3J = 5.7 Hz, 1H, -CONHC$_2$H$_4$N-), 8.42 (brs, 2H, -N$^{\omega}$H$_2$), 8.96 (m, 1H, -N$^{\delta}$H), 10.34 (s, 1H, -C(O)NH- lactam), 10.06 (s, 1H, -N$^{\omega'}$H) ppm; ^{13}C-NMR (600 MHz, DMSO-d$_6$, HSQC): δ_C = 14.5 (-CH$_2$CH$_3$), 23.2 (C-3/4 cyclopentyl), 24.4 (C$^{\gamma}$), 28.7 (C$^{\beta}$), 34.1 (-NHCH$_2$CH$_3$), 36.1 (-NHCH$_2$CH$_2$N<), 37.3 (C-2/5 cyclopentyl), 38.3 (>NCOCH$_2$-cyclo-C$_5$H$_8$-), 39.5 (-NHCH$_2$CH$_2$N<), 40.3 (C$^{\delta}$), 40.4 and 45.3 (C-2/6 piperazine), 42.8 (-CH$_2$CON$^{\alpha}$H-), 44.0 (C-1 cyclopentyl), 50.7 and 51.3 (C-3/5 piperazine), 51.7 (C$^{\alpha}$), 73.6 (C-11 dibenzazepin-11-yl), 121.3 (C$_{ar}$H), 122.5 (Ph), 123.7 (C$_{ar}$H), 126.5 (Ph), 128.0 (2 C$_{ar}$H), 128.5 (C$_{ar}$H), 128.9 (Ph), 129.9 (C$_{ar}$H), 130.5 (C$_{ar}$H), 131.3 (C$_{ar}$), 131.5 (C$_{ar}$H), 136.1 (C$_{ar}$), 136.4 (C$_{ar}$), 141.6 (C$_{ar}$), 152.5 (-NCONPh-), 153.5 (-NC(X)N-), 153.6 (-NC(X)N-), 167.9 (C=O lactam), 169.9 (-CON<), 171.2 (-CON$^{\alpha}$H-), 171.8 (-CONHC$_2$H$_4$N<) ppm; ESI-MS (+p) *m/z* (%): 967.7 (100)

$[MH]^+$; RP-HPLC: k' = 6.89 (t_R = 18.1 min); analysis calcd. for $C_{52}H_{62}N_{12}O_7 \cdot 1.5\ H_2O \cdot 2\ HCl$: C 58.53, H 6.33, N 15.76 %, found: C 58.54, H 6.57, N 15.77 %. $C_{52}H_{62}N_{12}O_7$ (967.12).

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b*,*e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]-N^{ω}-(phenylacetyl)argininamide (**14h**): ^{1}H-NMR (600 MHz, DMSO-d_6, HSQC): δ_H = 1.44 (m, 2H, -C$^{\gamma}$H$_2$-), 1.41 and 1.61 (m, 2H, -C$^{\beta}$H$_2$-), 1.37–1.51 (m, 4H, 2/5-H cyclopentyl), 1.51 (m, 4H, 3/4-H cyclopentyl), 2.06 and 2.08 (m, 4H, 3/5-H piperazine), 2.19 and 2.29 (m, 2H, -CH$_2$CON$^{\alpha}$H-), 2.40 and 2.48 (m, 2H, >NCOCH$_2$-cyclo-C$_5$H$_8$-), 3.14 (m, 2H, -C$^{\delta}$H$_2$-), 3.27 and 3.32 (m, 4H, 2/6-H piperazine), 3.28 and 3.37 (m, 2H, -NHCH$_2$CH$_2$N<), 3.59 (t, ^3J = 6.1 Hz, 2H, -NHCH$_2$CH$_2$N<), 3.76 (s, 2H, -CH$_2$Ph), 4.16 (m, 1H, -C$^{\alpha}$H-), 4.28 (m, 1H, 11-H dibenzazepin-11-yl), 7.06 (m, 1H, H$_{ar}$), 7.11 (m, 1H, H$_{ar}$), 7.21 (m, 2H, Ph), 7.25 (m, 1H, H$_{ar}$), 7.27 (m, 1H, -CH$_2$Ph), 7.28 (m, 2H, -CH$_2$Ph), 7.31 (m, 2H, -CH$_2$Ph), 7.33 (m, 1H, H$_{ar}$), 7.36 (m, 8H, Ph), 7.39 (m, 1H, H$_{ar}$), 7.41 (m, 1H, H$_{ar}$), 7.49 (m, 1H, H$_{ar}$), 7.74 (m, 1H, H$_{ar}$), 7.97 (d, ^3J = 8.0 Hz, 1H, -N$^{\alpha}$H-), 8.22 (t, ^3J = 5.5 Hz, 1H, -CONHC$_2$H$_4$N-), 8.77 (brs, 2H, -N$^{\omega}$H$_2$), 9.19 (t, ^3J = 5.2 Hz, 1H, -N$^{\delta}$H), 10.35 (s, 1H, -C(O)NH- lactam), 11.12 (s, 1H, -N$^{\omega'}$H) ppm; ^{13}C-NMR (600 MHz, DMSO-d_6, HSQC): δ_C = 23.2 (C-3/4 cyclopentyl), 24.1 (C$^{\gamma}$), 28.7 (C$^{\beta}$), 36.1 (-NHCH$_2$CH$_2$N<), 37.3 (C-2/5 cyclopentyl), 38.3 (>NCOCH$_2$-cyclo-C$_5$H$_8$-), 39.5 (-NHCH$_2$CH$_2$N<), 40.6 (C$^{\delta}$), 40.1 and 45.3 (C-2/6 piperazine), 42.7 (-CH$_2$Ph), 42.7 (-CH$_2$CON$^{\alpha}$H-), 44.0 (C-1 cyclopentyl), 50.7 and 51.2 (C-3/5 piperazine), 51.7 (C$^{\alpha}$), 73.6 (C-11 dibenzazepin-11-yl), 121.3 (C$_{ar}$H), 122.5 (Ph), 123.7 (C$_{ar}$H), 126.5 (Ph), 127.0 (-CH$_2$Ph), 127.7 (C$_{ar}$H), 128.0 (C$_{ar}$H), 128.3 (-CH$_2$Ph), 128.5 (C$_{ar}$H), 129.5 (-CH$_2$Ph), 129.9 (C$_{ar}$H), 130.0 (Ph), 130.5 (C$_{ar}$H), 131.4 (C$_{ar}$H), 131.4 (C$_{ar}$), 133.4 (C$_{ar}$), 136.1 (C$_{ar}$), 136.4 (C$_{ar}$), 141.6 (brs, C$_{ar}$), 152.5 (-NCONPh-), 152.9 (-NC(N)N-), 167.9 (C=O lactam), 169.9 (-CON<), 171.2 (-CON$^{\alpha}$H-), 171.8 (-CONHC$_2$H$_4$N<), 173.0 (-C(O)CH$_2$Ph) ppm; ESI-MS (+p) *m/z* (%): 1014.5 (100) $[MH]^+$; RP-HPLC: k'

= 7.8 (t_R = 20.3 min); analysis calcd. for $C_{57}H_{63}N_{11}O_7 \cdot H_2O \cdot 2$ HCl: C 61.95, H 6.11, N 13.94 %, found: C 62.13, H 6.37, N 13.96 %. $C_{57}H_{63}N_{11}O_7$ (1014.18).

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]-N^{ω}-(phenoxyacetyl)argininamide (**14i**): ^{1}H-NMR (600 MHz, DMSO-d_6, HSQC): δ_H = 1.45 (m, 2H, -C$^{\gamma}$H$_2$-), 1.43 and 1.63 (m, 2H, -C$^{\beta}$H$_2$-), 1.37–1.55 (m, 4H, 2/5-H cyclopentyl), 1.53 (m, 4H, 3/4-H cyclopentyl), 2.07 and 2.09 (m, 4H, 3/5-H piperazine), 2.20 and 2.30 (m, 2H, -CH$_2$CON$^{\alpha}$H-), 2.41 and 2.48 (m, 2H, >NCOCH$_2$-cyclo-C_5H_8-), 3.19 (m, 2H, -C$^{\delta}$H$_2$-), 3.29 and 3.33 (m, 4H, 2/6-H piperazine), 3.30 and 3.38 (m, 2H, -NHCH$_2$CH$_2$N<), 3.59 (t, ^3J = 6.0 Hz, 2H, -NHCH$_2$CH$_2$N<), 4.16 (m, 1H, -C$^{\alpha}$H-), 4.28 (m, 1H, 11-H dibenzazepin-11-yl), 4.82 (s, 2H, -CH$_2$OPh), 7.00 (m, 3H, 2/4/6-H -OPh), 7.06 (m, 1H, H_{ar}), 7.10 (m, 1H, H_{ar}), 7.22 (m, 2H, Ph), 7.26 (m, 1H, H_{ar}), 7.31 (m, 2H, 3/5-H -OPh), 7.34 (m, 1H, H_{ar}), 7.36 (m, 4H, Ph), 7.37 (m, 4H, Ph), 7.39 (m, 1H, H_{ar}), 7.40 (m, 1H, H_{ar}), 7.49 (m, 1H, H_{ar}), 7.74 (m, 1H, H_{ar}), 8.00 (d, ^3J = 8.0 Hz, 1H, -N$^{\alpha}$H-), 8.24 (t, ^3J = 5.4 Hz, 1H, -CONHC$_2$H$_4$N-), 8.86 (brs, 2H, -N$^{\omega}$H$_2$), 9.13 (t, ^3J = 5.4 Hz, 1H, -N$^{\delta}$H), 10.35 (s, 1H, -C(O)NH- lactam), 11.39 (s, 1H, -N$^{\omega'}$H) ppm; ^{13}C-NMR (600 MHz, DMSO-d_6, HSQC): δ_C = 23.1 (C-3/4 cyclopentyl), 24.1 (C$^{\gamma}$), 28.7 (C$^{\beta}$), 36.0 (-NHCH$_2$CH$_2$N<), 37.2 (C-2/5 cyclopentyl), 38.3 (>NCOCH$_2$-cyclo-C_5H_8-), 39.4 (-NHCH$_2$CH$_2$N<), 40.6 (C$^{\delta}$), 40.5 and 45.1 (C-2/6 piperazine), 42.8 (-CH$_2$CON$^{\alpha}$H-), 44.0 (C-1 cyclopentyl), 50.7 and 51.1 (C-3/5 piperazine), 51.7 (C$^{\alpha}$), 66.6 (-CH$_2$OPh), 73.6 (C-11 dibenzazepin-11-yl), 114.6 (C-2/6 -OPh), 121.3 (C$_{ar}$H), 121.7 (C-4 -OPh), 122.4 (Ph), 123.6 (C$_{ar}$H), 126.5 (Ph), 127.8 (C$_{ar}$H), 128.0 (C$_{ar}$H), 128.4 (C$_{ar}$H), 128.8 (Ph), 129.9 (C$_{ar}$H), 130.5 (C$_{ar}$H), 131.4 (C$_{ar}$H), 131.5 (C$_{ar}$), 136.1 (C$_{ar}$), 136.5 (C$_{ar}$), 142.0 (brs, C$_{ar}$), 152.5 (-NCONPh-), 152.4 (-NC(N)N-), 167.9 (C=O lactam), 169.9 (-CON<), 171.2 (-CON$^{\alpha}$H-), 171.8 (-CONHC$_2$H$_4$N<), 170.5 (-C(O)CH$_2$OPh) ppm; ESI-MS (+p) *m/z* (%): 1030.5 (100) $[MH]^+$; RP-HPLC: k' =

7.8 (t_R = 20.3 min); analysis calcd. for $C_{57}H_{63}N_{11}O_8 \cdot 1.5\ H_2O \cdot 2$ HCl: C 60.58, H 6.07, N 13.64 %, found: C 60.52, H 6.31, N 13.62 %. $C_{57}H_{63}N_{11}O_8$ (1030.18).

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]-N^{ω}-(3-phenylpropanoyl)argininamide (**14j**): ^{1}H-NMR (600 MHz, DMSO-d_6, HSQC): δ_H = 1.45 (m, 2H, -C$^{\gamma}$H$_2$-), 1.43 and 1.63 (m, 2H, -C$^{\beta}$H$_2$-), 1.37–1.55 (m, 4H, 2/5-H cyclopentyl), 1.53 (m, 4H, 3/4-H cyclopentyl), 2.09 and 2.11 (m, 4H, 3/5-H piperazine), 2.20 and 2.30 (m, 2H, -CH$_2$CON$^{\alpha}$H-), 2.41 and 2.50 (m, 2H, >NCOCH$_2$-cyclo-C_5H_8-), 2.73 (t, ^3J = 7.5 Hz, 2H, -CH$_2$CH$_2$Ph), 2.86 (t, ^3J = 7.5 Hz, 2H, -CH$_2$CH$_2$Ph), 3.15 (m, 2H, -C$^{\delta}$H$_2$-), 3.28 and 3.35 (m, 4H, 2/6-H piperazine), 3.30 and 3.39 (m, 2H, -NHCH$_2$CH$_2$N<), 3.59 (t, ^3J = 6.0 Hz, 2H, -NHCH$_2$CH$_2$N<), 4.16 (m, 1H, -C$^{\alpha}$H-), 4.31 (m, 1H, 11-H dibenzazepin-11-yl), 7.06 (m, 1H, H_{ar}), 7.12 (m, 1H, H_{ar}), 7.21 (m, 2H, Ph), 7.26 (m, 1H, H_{ar}), 7.34 (m, 1H, H_{ar}), 7.36 (m, 8H, Ph), 7.39 (m, 1H, H_{ar}), 7.40 (m, 1H, H_{ar}), 7.50 (m, 1H, H_{ar}), 7.76 (m, 1H, H_{ar}), 7.98 (d, ^3J = 8.0 Hz, 1H, -N$^{\alpha}$H-), 8.23 (t, ^3J = 6.0 Hz, 1H, -CONHC$_2$H$_4$N-), 8.73 (brs, 2H, -N$^{\omega}$H$_2$), 9.14 (t, ^3J = 5.6 Hz, 1H, -N$^{\delta}$H), 10.35 (s, 1H, -C(O)NH- lactam), 11.81 (s, 1H, -N$^{\omega'}$H) ppm; ^{13}C-NMR (600 MHz, DMSO-d_6, HSQC): δ_C = 23.1 (C-3/4 cyclopentyl), 24.1 (C$^{\gamma}$), 28.7 (C$^{\beta}$), 36.0 (-NHCH$_2$CH$_2$N<), 37.2 (C-2/5 cyclopentyl), 38.3 (>NCOCH$_2$-cyclo-C_5H_8-), 39.5 (-NHCH$_2$CH$_2$N<), 40.5 (C$^{\delta}$), 40.5 and 45.1 (C-2/6 piperazine), 42.8 (-CH$_2$CON$^{\alpha}$H-), 44.0 (C-1 cyclopentyl), 50.7 and 51.1 (C-3/5 piperazine), 51.7 (C$^{\alpha}$), 73.6 (C-11 dibenzazepin-11-yl), 121.3 (C$_{ar}$H), 122.5 (Ph), 123.8 (C$_{ar}$H), 126.5 (Ph), 128.0 (2 C$_{ar}$H), 128.4 (C$_{ar}$H), 128.8 (Ph), 129.9 (C$_{ar}$H), 130.6 (C$_{ar}$H), 131.4 (C$_{ar}$H), 131.5 (C$_{ar}$), 136.1 (C$_{ar}$), 136.5 (C$_{ar}$), 140.0 (C-1 -CH$_2$Ph), 142.0 (brs, C$_{ar}$), 152.5 (-NCONPh-), 152.7 (-NC(N)N-), 167.8 (C=O lactam), 169.9 (-CON<), 171.2 (-CON$^{\alpha}$H-), 171.8 (-CONHC$_2$H$_4$N<), 174.3 (-C(O)CH$_2$OPh) ppm; ESI-MS (+p) *m/z* (%): 1028.6 (100) $[MH]^+$; RP-HPLC: k' = 8.2 (t_R = 21.2 min); analysis calcd. for $C_{58}H_{65}N_{11}O_7 \cdot 1\ H_2O \cdot$

2 HCl: C 62.25, H 6.21, N 13.77 %, found: C 61.89, H 6.58, N 13.73 %. $C_{58}H_{65}N_{11}O_7$ (1028.21).

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]-N^{ω}-propanoylargininamide (**14f**): ^{1}H-NMR (600 MHz, DMSO-d_6, HSQC): δ_H = 1.01 (t, ^3J = 7.4 Hz, 3H, -C(O)$CH_2C\underline{H}_3$), 1.46 (m, 2H, -$C^{\gamma}\underline{H}_2$-), 1.44 and 1.64 (m, 2H, -$C^{\beta}\underline{H}_2$-), 1.41–1.56 (m, 4H, 2/5-H cyclopentyl), 1.54 (m, 4H, 3/4-H cyclopentyl), 2.13 and 2.14 (m, 4H, 3/5-H piperazine), 2.21 and 2.30 (m, 2H, -$C\underline{H}_2CON^{\alpha}H$-), 2.42 (q, ^3J = 7.4 Hz, 2H, -C(O)$C\underline{H}_2CH_3$), 2.42 and 2.50 (m, 2H, >NCO$C\underline{H}_2$-cyclo-C_5H_8-), 3.16 (m, 2H, -$C^{\delta}\underline{H}_2$-), 3.31–3.35 (m, 4H, 2/6-H piperazine), 3.31 and 3.39 (m, 2H, -NH$C\underline{H}_2CH_2N$<), 3.59 (t, ^3J = 6.0 Hz, 2H, -$NHCH_2C\underline{H}_2N$<), 4.15 (m, 1H, -$C^{\alpha}\underline{H}$-), 4.36 (brs, 1H, 11-H dibenzazepin-11-yl), 7.07 (m, 1H, H_{ar}), 7.12 (m, 1H, H_{ar}), 7.21 (m, 2H, Ph), 7.27 (m, 1H, H_{ar}), 7.35 (m, 1H, H_{ar}), 7.37 (m, 8H, Ph), 7.41 (m, 2H, H_{ar}), 7.51 (m, 1H, H_{ar}), 7.75 (m, 1H, H_{ar}), 8.01 (d, ^3J = 8.0 Hz, 1H, -$N^{\alpha}\underline{H}$-), 8.22 (t, ^3J = 5.8 Hz, 1H, -CON$\underline{H}C_2H_4N$-), 8.64 and 8.85 (brs, 2H, -$N^{\omega}\underline{H}_2$), 9.22 (t, ^3J = 5.5 Hz, 1H, -$N^{\delta}\underline{H}$), 10.36 (s, 1H, -C(O)N$\underline{H}$-lactam), 11.86 (s, 1H, -$N^{\omega'}\underline{H}$) ppm; ^{13}C-NMR (600 MHz, DMSO-d_6, HSQC): δ_C = 8.2 (-$CH_2\underline{C}H_3$), 23.2 (C-3/4 cyclopentyl), 24.3 ($\underline{C}^{\gamma}$), 28.8 ($\underline{C}^{\beta}$), 29.5 (-C(O)$\underline{C}H_2CH_3$), 36.1 (-NH$\underline{C}H_2CH_2N$<), 37.4 (C-2/5 cyclopentyl), 38.4 (>NCO$\underline{C}H_2$-cyclo-C_5H_8-), 39.6 (-$NHCH_2\underline{C}H_2N$<), 40.5 ($\underline{C}^{\delta}$), 40.5 and 45.2 (C-2/6 piperazine), 42.8 (-$\underline{C}H_2CON^{\alpha}H$-), 44.0 (C-1 cyclopentyl), 50.8 and 51.2 (C-3/5 piperazine), 51.8 ($\underline{C}^{\alpha}$), 73.6 (C-11 dibenzazepin-11-yl), 121.4 ($\underline{C}_{ar}H$), 122.6 (Ph), 123.8 ($\underline{C}_{ar}H$), 126.6 (Ph), 128.1 ($\underline{C}_{ar}H$), 128.3 ($\underline{C}_{ar}H$), 128.6 ($\underline{C}_{ar}H$), 129.0 (Ph), 130.1 ($\underline{C}_{ar}H$), 130.7 ($\underline{C}_{ar}H$), 131.6 ($\underline{C}_{ar}H$), 131.5 ($\underline{C}_{ar}$), 136.2 ($\underline{C}_{ar}$), 136.6 ($\underline{C}_{ar}$), 141.4 ($\underline{C}_{ar}$), 152.6 (-N$\underline{C}$ONPh-), 153.0 (-NC(N)N-), 167.8 ($\underline{C}$=O lactam), 170.0 (-$\underline{C}$ON<), 171.3 (-$\underline{C}ON^{\alpha}H$-), 171.9 (-$\underline{C}ONHC_2H_4N$<), 176.0 (-$\underline{C}$(O)Et) ppm; ESI-MS (+p) *m/z* (%): 952.6 (100) $[MH]^+$; RP-HPLC: k' = 7.1 (t_R = 18.1 min); analysis calcd. for $C_{52}H_{61}N_{11}O_7 \cdot 1.5\ H_2O \cdot 2$

HCl: C 59.36, H 6.32, N 14.65 %, found: C 59.48, H 6.48, N 14.65 %. $C_{52}H_{61}N_{11}O_7$ (952.11).

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\omega}$-ethyl-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide (**14n**): ^{1}H-NMR (600 MHz, DMSO-d_6, HSQC, COSY, HMBC): δ_H = 1.07 (t, ^3J = 7.2 Hz, 3H, -$CH_2C\underline{H}_3$), 1.40 (m, 2H, -$C^{\gamma}\underline{H}_2$-), 1.40 and 1.62 (m, 2H, -$C^{\beta}\underline{H}_2$-), 1.40–1.54 (m, 4H, 2/5-H cyclopentyl), 1.54 (m, 4H, 3/4-H cyclopentyl), 2.04 and 2.06 (m, 4H, 3/5-H piperazine), 2.19 and 2.31 (m, 2H, -$C\underline{H}_2CON^{\alpha}H$-), 2.41 and 2.49 (m, 2H, >$NCOC\underline{H}_2$-cyclo-C_5H_8-), 3.01 (m, 2H, -$C^{\delta}\underline{H}_2$-), 3.13 (m, 2H, -$CH_2CH_3$), 3.24–3.33 (m, 4H, 2/6-H piperazine), 3.30 and 3.37 (m, 2H, -$NHC\underline{H}_2CH_2N$<), 3.59 (t, ^3J = 6.0 Hz, 2H, --$NHCH_2C\underline{H}_2N$<), 4.14 (m, 1H, -$C^{\alpha}\underline{H}$-), 4.25 (m, 1H, 11-H dibenzazepin-11-yl), 7.06 (m, 1H, H_{ar}), 7.09 (m, 1H, H_{ar}), 7.22 (m, 2H, Ph), 7.25 (m, 1H, H_{ar}), 7.30–7.43 (m, 2H, -NH's), 7.33 (m, 1H, H_{ar}), 7.36 (m, 8H, Ph), 7.40 (m, 2H, H_{ar}), 7.45–7.54 (m, 2H, -NH's), 7.48 (m, 1H, H_{ar}), 7.72 (m, 1H, H_{ar}), 8.00 (d, ^3J = 8.1 Hz, 1H, -$N^{\alpha}\underline{H}$-), 8.24 (t, ^3J = 6.0 Hz, 1H, -$CONHC_2H_4N$-), 10.34 (s, 1H, -C(O)N$\underline{H}$- lactam) ppm; ^{13}C-NMR (600 MHz, DMSO-d_6, HSQC, COSY, HMBC): δ_C = 14.2 (-$CH_2\underline{C}H_3$), 23.2 (C-3/4 cyclopentyl), 25.0 ($\underline{C}^{\gamma}$), 28.7 ($\underline{C}^{\beta}$), 35.7 (-$\underline{C}H_2CH_3$), 36.1 (-$NH\underline{C}H_2CH_2N$<), 37.4 (C-2/5 cyclopentyl), 38.3 (>$NCO\underline{C}H_2$-cyclo-C_5H_8-), 39.5 (-$NHCH_2\underline{C}H_2N$<), 40.3 ($\underline{C}^{\delta}$), 40.6 and 45.3 (C-2/6 piperazine), 42.8 (-$\underline{C}H_2CON^{\alpha}H$-), 44.1 (C-1 cyclopentyl), 50.7 and 51.2 (C-3/5 piperazine), 51.9 ($\underline{C}^{\alpha}$), 73.7 (C-11 dibenzazepin-11-yl), 121.3 ($\underline{C}_{ar}H$), 122.6 (Ph), 123.7 ($\underline{C}_{ar}H$), 126.6 (Ph), 127.8 ($\underline{C}_{ar}H$), 128.0 ($\underline{C}_{ar}H$), 128.4 ($\underline{C}_{ar}H$), 129.1 (Ph), 130.0 ($\underline{C}_{ar}H$), 130.4 ($\underline{C}_{ar}H$), 131.4 ($\underline{C}_{ar}H$), 131.6 ($\underline{C}_{ar}$), 136.1 ($\underline{C}_{ar}$), 136.4 ($\underline{C}_{ar}$), 142.0 ($\underline{C}_{ar}$), 152.6 (-N$\underline{C}$ONPh-), 155.6 (-N$\underline{C}$(NEt)NH_2), 168.0 ($\underline{C}$=O lactam), 170.0 (-$\underline{C}$ON<), 171.3 (-$\underline{C}ON^{\alpha}H$-), 172.0 (-$\underline{C}ONHC_2H_4N$<) ppm; ESI-MS (+p) *m/z* (%): 924.6 (100) $[MH]^+$, 717.5 (20) $[MH - C_{14}H_{10}NO]^+$; RP-HPLC: k' = 6.7 (t_R = 17.7 min); $C_{51}H_{61}N_{11}O_6 \cdot 5\ H_2O \cdot 5$

HCl: C 51.19, H 6.40, N 12.88 %, found: C 51.46, H 6.40, N 12.59 %. $C_{51}H_{61}N_{11}O_6$ (952.11).

(2*S*)-*N*$^{\omega}$-Benzyl-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide (**14m**): ^{1}H-NMR (600 MHz, DMSO-d_6, HSQC, COSY, HMBC): δ_H = 1.40 (m, 2H, -C$^{\gamma}$H$_2$-), 1.40 and 1.62 (m, 2H, -C$^{\beta}$H$_2$-), 1.40–1.54 (m, 4H, 2/5-H cyclopentyl), 1.53 (m, 4H, 3/4-H cyclopentyl), 2.06 and 2.08 (m, 4H, 3/5-H piperazine), 2.19 and 2.31 (m, 2H, -CH$_2$CON$^{\alpha}$H-), 2.41 and 2.49 (m, 2H, >NCOCH$_2$-cyclo-C_5H_8-), 3.05 (m, 2H, -C$^{\delta}$H$_2$-), 3.24–3.33 (m, 4H, 2/6-H piperazine), 3.34 (m, 2H, -NHCH$_2$CH$_2$N<), 3.59 (t, ^3J = 6.0 Hz, 2H, -NHCH$_2$CH$_2$N<), 4.15 (m, 1H, -C$^{\alpha}$H-), 4.26 (m, 1H, 11-H dibenzazepin-11-yl), 4.39 (d, ^3J = 5.9 Hz, 2H, -CH$_2$Ph), 7.07 (m, 1H, H_{ar}), 7.10 (m, 1H, H_{ar}), 7.22 (m, 2H, Ph), 7.25 (m, 1H, H_{ar}), 7.26–7.31 (m, 4H, Bn), 7.30–7.43 (m, 2H, -NH's), 7.33 (m, 1H, H_{ar}), 7.36 (m, 8H, Ph), 7.40 (m, 2H, H_{ar}), 7.48–7.53 (m, 2H, -NH's), 7.50 (m, 1H, H_{ar}), 7.58 (m, 1H, Bn), 7.74 (m, 1H, H_{ar}), 7.98 (d, ^3J = 8.0 Hz, 1H, -N$^{\alpha}$H-), 8.23 (t, ^3J = 5.8 Hz, 1H, -CONHC$_2$H$_4$N-), 10.36 (s, 1H, -C(O)NH- lactam) ppm; ^{13}C-NMR (600 MHz, DMSO-d_6, HSQC, COSY, HMBC): δ_C = 23.2 (C-3/4 cyclopentyl), 25.0 (C$^{\gamma}$), 28.7 (C$^{\beta}$), 36.1 (-NHCH$_2$CH$_2$N<), 37.3 (C-2/5 cyclopentyl), 38.4 (>NCOCH$_2$-cyclo-C_5H_8-), 39.5 (-NHCH$_2$CH$_2$N<), 40.7 (C$^{\delta}$), 40.6 and 45.3 (C-2/6 piperazine), 42.8 (-CH$_2$CON$^{\alpha}$H-), 43.9 (-CH$_2$Ph), 44.0 (C-1 cyclopentyl), 50.7 and 51.7 (C-3/5 piperazine), 51.8 (C$^{\alpha}$), 73.5 (C-11 dibenzazepin-11-yl), 121.4 (C$_{ar}$H), 122.4 (Ph), 123.7 (C$_{ar}$H), 126.6 (Ph), 127.0 (Bn), 127.4 (Bn), 128.0 (2 C$_{ar}$H), 128.4 (C$_{ar}$H), 128.5 (Bn), 128.7 (C$_{ar}$H), 129.1 (Ph), 130.4 (C$_{ar}$H), 131.4 (C$_{ar}$H), 131.6 (C$_{ar}$), 136.1 (C$_{ar}$), 136.4 (C$_{ar}$), 137.2 (C$_{ar}$ Bn), 152.6 (-NCONPh-), 155.6 (-NC(NBn)NH$_2$), 167.9 (C=O lactam), 169.9 (-CON<), 171.1 (-CON$^{\alpha}$H-), 172.1 (-CONHC$_2$H$_4$N<) ppm; ESI-MS (+p) *m/z* (%): 986.5 (100) $[MH]^+$, 779.5 (13) $[MH - C_{14}H_{10}NO]^+$; RP-HPLC: k' = 7.5 (t_R = 19.6 min); analysis calcd. for $C_{56}H_{63}N_{11}O_6 \cdot 3$

$H_2O \cdot 2$ HCl: C 60.42, H 6.43, N 13.84 %, found: C 60.36, H 6.57, N 13.83 %. $C_{56}H_{63}N_{11}O_6$ (986.17).

(2*S*)-*N*$^{\omega}$-(6-Aminohexanoyl)-*N*-[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)-ethyl]-*N*$^{\alpha}$-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)-piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide (**14o**): ^{1}H-NMR (600 MHz, DMSO-d_6, HSQC): δ_H = 1.31 (m, 2H, -CO(CH$_2$)$_2$C$\underline{H}_2$(CH$_2$)$_2$NH$_2$), 1.49 (m, 2H, -C$^{\gamma}\underline{H}_2$-), 1.45 and 1.64 (m, 2H, -C$^{\beta}\underline{H}_2$-), 1.39–1.56 (m, 4H, 2/5-H cyclopentyl), 1.53 (m, 2H, -C$\underline{H}_2$CH$_2$NH$_2$), 1.54 (m, 2H, -COCH$_2$C$\underline{H}_2$-), 1.54 (m, 4H, 3/4-H cyclopentyl), 2.08 and 2.10 (m, 4H, 3/5-H piperazine), 2.21 and 2.30 (m, 2H, -C$\underline{H}_2$CON$^{\alpha}$H-), 2.41 (t, ^3J = 7.3 Hz, 2H, -C(O)C$\underline{H}_2$CH$_2$-), 2.41 and 2.49 (m, 2H, >NCOC$\underline{H}_2$-cyclo-C$_5$H$_8$-), 2.77 (m, 2H, -C$\underline{H}_2$NH$_2$), 3.18 (m, 2H, -C$^{\delta}\underline{H}_2$-), 3.31–3.33 (m, 4H, 2/6-H piperazine), 3.31 and 3.38 (m, 2H, -NHC$\underline{H}_2$CH$_2$N<), 3.60 (t, ^3J = 6.0 Hz, 2H, -NHCH$_2$C$\underline{H}_2$N<), 4.16 (m, 1H, -C$^{\alpha}\underline{H}$-), 4.32 (brs, 1H, 11-H dibenzazepin-11-yl), 7.06 (m, 1H, H$_{ar}$), 7.10 (m, 1H, H$_{ar}$), 7.22 (m, 2H, Ph), 7.25 (m, 1H, H$_{ar}$), 7.33 (m, 1H, H$_{ar}$), 7.37 (m, 8H, Ph), 7.39 (m, 1H, H$_{ar}$), 7.41 (m, 1H, H$_{ar}$), 7.49 (m, 1H, H$_{ar}$), 7.75 (m, 1H, H$_{ar}$), 7.84 (brs, 3H, -NH$_3^+$), 7.99 (d, ^3J = 8.0 Hz, 1H, -N$^{\alpha}\underline{H}$-), 8.25 (t, ^3J = 5.8 Hz, 1H, -CON$\underline{H}$C$_2$H$_4$N-), 8.61 and 9.15 (brs, 2H, -N$^{\omega}\underline{H}_2$), 9.47 (t, ^3J = 5.4 Hz, 1H, -N$^{\delta}\underline{H}$), 10.36 (s, 1H, -C(O)N$\underline{H}$- lactam), 12.30 (s, 1H, -N$^{\omega'}\underline{H}$) ppm; ^{13}C-NMR (600 MHz, DMSO-d_6, HSQC): δ_C = 23.2 (-COCH$_2$C$\underline{H}_3$), 23.2 (C-3/4 cyclopentyl), 24.2 (C$^{\gamma}$), 25.2 (-C$\underline{H}_2$CH$_2$CH$_2$NH$_2$), 26.6 (-C$\underline{H}_2$CH$_2$NH$_2$), 28.7 (C$^{\beta}$), 35.9 (-C(O)C$\underline{H}_2$-), 36.1 (-NHC$\underline{H}_2$CH$_2$N<), 37.2 (C-2/5 cyclopentyl), 38.5 (>NCOC$\underline{H}_2$-cyclo-C$_5$H$_8$-), 38.5 (-C$\underline{H}_2$NH$_2$), 39.6 (-NHCH$_2$C$\underline{H}_2$N<), 40.5 (C$^{\delta}$), 40.5 and 45.3 (C-2/6 piperazine), 42.8 (-C$\underline{H}_2$CON$^{\alpha}$H-), 44.0 (C-1 cyclopentyl), 50.6 and 51.2 (C-3/5 piperazine), 51.8 (C$^{\alpha}$), 73.6 (C-11 dibenzazepin-11-yl), 121.3 (C$_{ar}$H), 122.6 (Ph), 123.7 (C$_{ar}$H), 126.6 (Ph), 127.8 (C$_{ar}$H), 128.1 (C$_{ar}$H), 128.4 (C$_{ar}$H), 128.9 (Ph), 129.9 (C$_{ar}$H), 130.5 (C$_{ar}$H), 131.3 (C$_{ar}$H), 131.6 (C$_{ar}$), 136.1 (C$_{ar}$), 136.5 (C$_{ar}$), 141.5 (C$_{ar}$), 152.5 (-NCONPh-), 153.1 (-NC(N)N-), 167.9 (C=O lactam), 169.9 (-CON<), 171.2 (-CON$^{\alpha}$H-), 171.9 (-CONHC$_2$H$_4$N<), 175.3 (-CO(CH$_2$)$_5$-) ppm;

ESI-MS (+p) *m/z* (%): 1009.6 (32) $[MH]^+$, 802.5 (70) $[MH - 207]^+$, 505.4 $[MH_2]^+$; RP-HPLC (gradient MeCN/0.05 % aq. TFA: 0 min 15:85, 30 min 50:50): $k' = 9.6$ (t_R = 24.4 min); analysis calcd. for $C_{55}H_{68}N_{12}O_7 \cdot 5\ H_2O \cdot 5$ HCl: C 51.54, H 6.53, N 13.12 %, found: C 51.23, H 6.53, N 13.00 %. $C_{55}H_{68}N_{12}O_7$ (1009.20).

(2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{ω}-[6-(4-fluorobenzamido)hexanoyl]-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide (**14p**): ^{1}H-NMR (600 MHz, DMSO-d_6, HSQC): δ_H = 1.32 (m, 2H, -C<u>H</u>$_2$(CH$_2$)$_2$NH-), 1.48 (m, 2H, -C$^{\gamma}$<u>H</u>$_2$-), 1.44 and 1.63 (m, 2H, -C$^{\beta}$<u>H</u>$_2$-), 1.41–1.63 (m, 4H, 2/5-H cyclopentyl), 1.52 (m, 2H, -C<u>H</u>$_2$CH$_2$NHBz(4F)), 1.55 (m, 4H, 3/4-H cyclopentyl), 1.57 (m, 2H, -COCH$_2$C<u>H</u>$_2$-), 2.16 and 2.18 (m, 4H, 3/5-H piperazine), 2.21 and 2.32 (m, 2H, -C<u>H</u>$_2$CON$^{\alpha}$H-), 2.41 (t, ^3J = 7.3 Hz, 2H, -C(O)C<u>H</u>$_2$CH$_2$-), 2.42 and 2.50 (m, 2H, >NCOC<u>H</u>$_2$-cyclo-C$_5$H$_8$-), 3.17 (m, 2H, -C$^{\delta}$<u>H</u>$_2$-), 3.25 (m, 2H, -C<u>H</u>$_2$NHBz(4F)), 3.33–3.37 (m, 4H, 2/6-H piperazine), 3.31 and 3.40 (m, 2H, -NHC<u>H</u>$_2$CH$_2$N<), 3.60 (t, ^3J = 6.0 Hz, 2H, -NHCH$_2$C<u>H</u>$_2$N<), 4.17 (m, 1H, -C$^{\alpha}$<u>H</u>-), 4.43 (brs, 1H, 11-H dibenzazepin-11-yl), 7.08 (m, 1H, H$_{ar}$), 7.14 (m, 1H, H$_{ar}$), 7.22 (m, 2H, Ph), 7.28 (m, 1H, H$_{ar}$), 7.35 (m, 1H, H$_{ar}$), 7.37 (m, 8H, Ph), 7.41 (m, 1H, H$_{ar}$), 7.43 (m, 1H, H$_{ar}$), 7.52 (m, 1H, H$_{ar}$), 7.78 (m, 1H, H$_{ar}$), 7.98 (d, ^3J = 8.0 Hz, 1H, -N$^{\alpha}$<u>H</u>-), 8.24 (t, ^3J = 5.7 Hz, 1H, -CON<u>H</u>C$_2$H$_4$N-), 8.48 (t, ^3J = 5.5 Hz, 1H, -N<u>H</u>Bz(4F)), 8.68 and 8.81 (brs, 2H, -N$^{\omega}$<u>H</u>$_2$), 9.20 (t, ^3J = 5.6 Hz, 1H, -N$^{\delta}$<u>H</u>), 10.40 (s, 1H, -C(O)N<u>H</u>-lactam), 11.83 (s, 1H, -N$^{\omega'}$<u>H</u>) ppm; ^{13}C-NMR (600 MHz, DMSO-d_6, HSQC): δ_C = 23.3 (C-3/4 cyclopentyl), 23.6 (-COCH$_2$<u>C</u>H$_3$), 24.1 (<u>C</u>$^{\gamma}$), 25.7 (-<u>C</u>H$_2$CH$_2$CH$_2$NH-), 28.7 (<u>C</u>$^{\beta}$), 28.8 (-CH$_2$<u>C</u>H$_2$CH$_2$NH-), 36.0 (-C(O)<u>C</u>H$_2$-), 36.1 (-NH<u>C</u>H$_2$CH$_2$N<), 37.2 (C-2/5 cyclopentyl), 38.5 (>NCO<u>C</u>H$_2$-cyclo-C$_5$H$_8$-), 39.0 (-<u>C</u>H$_2$NHBz(4F)), 39.6 (-NHCH$_2$<u>C</u>H$_2$N<), 40.5 (<u>C</u>$^{\delta}$), 40.4 and 44.9 (C-2/6 piperazine), 42.8 (-<u>C</u>H$_2$CON$^{\alpha}$H-), 44.0 (C-1 cyclopentyl), 50.7 and 51.2 (C-3/5 piperazine), 51.7 (<u>C</u>$^{\alpha}$), 73.6 (C-11 dibenzazepin-11-yl), 115.0 (d, $^2J_{C,F}$ = 21.6 Hz), 121.3 (<u>C</u>$_{ar}$H), 122.6 (Ph), 123.8 (<u>C</u>$_{ar}$H), 126.5 (Ph), 128.1 (<u>C</u>$_{ar}$H), 128.4 (<u>C</u>$_{ar}$H), 128.7 (<u>C</u>$_{ar}$H), 128.9 (Ph), 129.7

(d, $^{3}J_{C,F}$ = 8.9 Hz), 130.2 (C$_{ar}$H), 130.7 (C$_{ar}$H), 131.1 (d, $^{4}J_{C,F}$ = 2.9 Hz), 131.5 (C$_{ar}$H), 131.1 (C$_{ar}$), 136.2 (C$_{ar}$), 136.5 (C$_{ar}$), 141.5 (C$_{ar}$), 152.5 (-NCONPh-), 152.9 (-NC(N)N-), 163.7 (d, $^{1}J_{C,F}$ = 248.0 Hz), 165.0 (-COC$_6$H$_4$F), 167.9 (C=O lactam), 170.0 (-CON<), 171.2 (-CON$^{\alpha}$H-), 171.9 (-CONHC$_2$H$_4$N<), 175.2 (-CO(CH$_2$)$_5$-) ppm; ESI-MS (+p) *m/z* (%): 1131.7 (100) $[MH]^+$, 924.6 (8) $[MH - 207]^+$; RP-HPLC: k' = 7.8 (t_R = 20.3 min); analysis calcd. for $C_{62}H_{71}FN_{12}O_8 \cdot 2\ H_2O \cdot 2$ HCl: C 60.04, H 6.26, N 13.56 %, found: C 60.27, H 6.55, N 13.51 %. $C_{62}H_{71}FN_{12}O_8$ (1131.30).

4.2. *In Vitro* Pharmacology

The methods for the *in vitro* pharmacological characterization of Y_2 receptor antagonist are described in detail by R. Ziemek[7]. The descriptions given in the following are adopted from ibid.

4.2.1. FLOW CYTOMETRIC BINDING ASSAY

In general, cells were grown to 80-90 % confluence, trypsinized and resuspended in Ham's F12 medium, containing 10 % FCS for the inactivation of trypsine. Cells were counted, centrifuged for 5 min at 1200 rpm (Minifuge RF, Heraeus, Hanau, Germany) and resuspended at a density of 10^6 cells/ml in binding buffer (Sheikh and Williams, 1990), containing 25 mM Hepes, 2.5 mM $CaCl_2$ and 1 mM $MgCl_2$ in millipore water, pH 7.4, supplemented with 1 % BSA and 0.1 mg/ml bacitracin (Sigma, Deisenhofen).

Cells were incubated at room temperature in 1.5 ml polypropylene tubes which were siliconized using Sigmacote™ (Sigma, Deisenhofen, Germany) to prevent protein adsorption. The incubation was accomplished with gentle shaking (120 rpm) in the dark to circumvent cell aggregation and photobleaching of the dye.

Samples were measured without further processing with a Becton Dickinson FACSCalibur™ flow cytometer; instrument settings were: FSC: E-1, SSC: 280 V FL4: 800 V, Flow: HI; measurement stopped when 20000 gated events were counted. The cells were gated and the geometric means of fluorescence were calculated

using the WinMDI software. For the screening of cell clones, 490 µl of cell suspension were incubated with 10 µl of cy5-pNPY (250 nM in 10 mM HCl plus 0.1 % BSA) for 60-90 minutes. Cell clones with high specific cell-bound fluorescence were expanded and analyzed in saturation experiments.

Association kinetics was determined by incubation of CHO-hY_2-K9 cells with 5 nM cy5-pNPY. Samples were taken at different time periods and measured.

For saturation experiments, 485 µl of cell suspension were added to 10 µl of cy5-pNPY and 5 µl of solvent resp. unlabelled pNPY (for the determination of total resp. unspecific binding). Cells were incubated for 120 min at room temperature.

Competition experiments were performed using 485 µl of cell suspension, 10 µl of cy5-pNPY (final concentration 5 nM) and 5 µl of test compound. Incubation time was 120 min at room temperature. The constant K_i for the inhibition of cy5-pNPY binding by unlabeled competitors was calculated from the concentration of unlabeled competitor, producing 50 % inhibition (IC_{50}) of the specific cy5-pNPY binding using the following relation $K_i = IC_{50} \cdot [K_d / (K_d + L)]$ where K_d is the dissociation constant and L the concentration of cy5-pNPY[26].

4.2.2. *SPECTROFLUORIMETRIC CA^{2+} ASSAY*

Y_2R antagonistic activities (IC_{50} values) were determined by spectrofluorimetric measurement of the inhibition of pNPY-induced (70 nM) mobilization of intracellular $[Ca^{2+}]_i$ in CHO-hY_2-K9-qi5-K9-mtAEQ-A7 cells[7]. For details see ibid. and appendix.

4.2.3. *AEQUORIN ASSAY*

A bioluminescent functional assay for the determination of Y_2 receptor antagonistic activity was developed by R. Ziemek[7] on the basis of CHO cells expressing the hY_2 receptor, a chimeric G-protein (G_{iq5}), and the bioluminescent protein aequorin, which has a Ca^{2+} dependent luciferase activity. For a detailed description see ibid.

4.2.4. *FLOW CYTOMETRIC Ca^{2+} ASSAY*

Cells (CHO-hY_2-K9-qi5) were grown for 2 days to 70-90 % confluence, trypsinized and detached with Ham's F12 supplemented with 10 % FCS to inactivate trypsine. Cells were counted in a hemocytometer, centrifuged for 5 min at 300 g at room temperature and resuspended at a density of 2.66 $\cdot 10^6$ cells / ml in loading buffer[27] containing 120 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$, 1.5 mM $CaCl_2$, 25 mM HEPES and 10 mM glucose at pH 7.4. For the preparation of the loading suspension, 3 μl of fluo-4-AM (Molecular Probes; 1 mM stock solution in anhydrous DMSO) were added to 5 μl of pluronic™ F-127 (Molecular Probes; 20 % stock solution in DMSO) and mixed carefully before addition of 1 ml of loading buffer containing 2 % BSA. 330 μl of loading suspension were added to 1 ml of cell suspension resulting in a cell number of $2 \cdot 10^6$ cells / ml and a dye concentration of 0.7 μM.

The cells were incubated in the dark for 30 min at room temperature and recentrifuged at 300 g for 5 min. After resuspension in loading buffer at a density of 0.5 - 1 $\cdot$ 10^6 cells/ml, the cells were incubated again for 30 min at room temperature in the dark; during this postincubation step, the fluo-4 AM-ester is intracellularly cleaved and thus the calcium indicator is trapped in the cell.

Measurements were performed in a purpose-built glass tube closed by a silicon septum as described [28]. This instrumentation allows injections into the samples during continuous flow cytometric measurements. A tube containing 1 ml of the cell suspension was connected with the flow cytometer under permanent stirring and the recording was started. Instrument settings were: FSC: E-1; SSC: 280; FL-1: 350; flow: high.

After 30 s of measurement of the basal fluorescence 10 μl of peptide agonist solution were injected with a hamilton syringe and data were recorded for another 90 s. The needle of the flow cytometer was washed with millipore water after each measurement. Raw data were first averaged with the WinMDI software and then exported to Sigma Plot™ 8.0.

Data were further smoothed (running average) with SigmaPlot™. The level of increase in fluorescence was calculated from the difference between the baseline (mean fluorescence of the first 25 s) and the highest value of the averaged curve. These amplitudes of the averaged signals were used to construct concentration-response curves. For the determination of EC_{50} values of agonists, every third measurement was a 100 % reference signal elicited with 1 μM pNPY.

Dilutions of Y_2 receptor antagonists were made in DMSO, and 10 μl of antagonist was preincubated with 990 μl of cell suspension for 1 min before the measurement. Calcium response was triggered with 10 μl of 70 μM pNPY in 10 mM HCl containing 0.1 % BSA. The 100 % reference signal was induced in every third measurement, too. In this case, cells were preincubated in the presence of the solvent without antagonist. EC_{50} and IC_{50} values were calculated with Sigma Plot™ (Version 8.0, SPSS Inc.) using the equation of the four parameter logistics function.

5. Reference List

[1] Cabrele, C.; Beck-Sickinger, A. G., Molecular characterization of the ligand-receptor interaction of the neuropeptide Y family. *J Pept Sci* **2000,** 6, 97-122.

[2] Rudolf, K.; Eberlein, W.; Wieland, H. A.; Engel, W.; Willim, K. D.; Entzeroth, M.; Wienen, W.; Beck-Sickinger, A. G.; Doods, H., The first highly potent and selective non-peptide NPY Y_1 receptor antagonist: BIBP 3226. *Eur. J. Pharm.* **1994,** 271, R11-3.

[3] Doods, H.; Gaida, W.; Wieland, H. A.; Dollinger, H.; Schnorrenberg, G.; Esser, F.; Engel, W.; Eberlein, W.; Rudolf, K., BIIE0246: A selective and high affinity neuropeptide Y Y_2 receptor antagonist. *Eur. J. Pharmacol.* **1999,** 384, R3-R5.

[4] Dollinger, H.; Esser, F.; Mihm, G.; Rudolf, K.; Schnorrenberg, G.; Gaida, W.; Doods, H. N. Preparation of novel peptides for use as NPY antagonists. DE 19816929, **1999**.

[5] Dains, F. B.; Wertheim, E., The action of ammonia and amines on the substituted ureas and urethanes. II. Allophanic ester. *J. Am. Chem. Soc.* **1920,** 42, 2303-9.

[6] Kim, H.-O.; Mathew, F.; Ogbu, C., A Convenient Synthesis of Disubstituted Guanidines via the Mitsunobu Protocol. *Synlett* **1999**, 193-4.

[7] Ziemek, R. Development of binding and functional assays for the neuropeptide Y Y_2 and Y_4 receptors. Ph.D. thesis, University of Regensburg, Regensburg, **2006**.

[8] Schmuck, C., Carboxylate binding by 2-(guanidiniocarbonyl)pyrrole receptors in aqueous solvents: improving the binding properties of guanidinium cations through additional hydrogen bonds. *Chemistry* **2000,** 6, 709-18.

[9] Schmuck, C.; Bickert, V., N'-alkylated guanidiniocarbonyl pyrroles: new receptors for amino acid recognition in water. *Org Lett* **2003,** 5, 4579-81.

[10] Schmuck, C.; Geiger, L., Dimerization of a guanidiniocarbonyl pyrrole cation in DMSO that can be controlled by the counteranion. *Chem Commun* **2004**, 1698-9.

[11] Schmuck, C.; Geiger, L., Dipeptide binding in water by a de novo designed guanidiniocarbonylpyrrole receptor. *J. Am. Chem. Soc.* **2004,** 126, 8898-9.

[12] Schmuck, C.; Machon, U., Amino Acid Binding by 2-(Guanidiniocarbonyl)pyridines in Aqueous Solvents: A Comparative Binding Study Correlating Complex Stability with Stereoelectronic Factors. *Chemistry - A European Journal* **2005,** 11, 1109-18.

[13] Schmuck, C.; Rehm, T.; Grohn, F.; Klein, K.; Reinhold, F., Ion pair driven self-assembly of a flexible bis-zwitterion in polar solution: formation of discrete nanometer-sized cyclic dimers. *J. Am. Chem. Soc.* **2006,** 128, 1430-1.

[14] Schmuck, C.; Schwegmann, M., A naked-eye sensing ensemble for the selective detection of citrate—but not tartrate or malate—in water based on a tris-cationic receptor. *Org Biomol Chem* **2006,** 4, 836-8.

[15] Schmuck, C.; Wienand, W., Highly stable self-assembly in water: ion pair driven dimerization of a guanidiniocarbonyl pyrrole carboxylate zwitterion. *J. Am. Chem. Soc.* **2003,** 125, 452-9.

[16] Xie, S. X.; Ghorai, P.; Ye, Q. Z.; Buschauer, A.; Seifert, R., Probing ligand-specific histamine H_1- and H_2-receptor conformations with N^G-acylated Imidazolylpropylguanidines. *J. Pharmacol. Exp. Ther.* **2006,** 317, 139-46.

[17] Jilek, J. O.; Pomykacek, J.; Svatek, E.; Seidlova, V.; Rajsner, M.; Pelz, K.; Hoch, B.; Protiva, M., Neurotropic and psychotropic substances. II. Morphanthridine and derivatives. A new synthesis of propazepine. *Collect. Czech. Chem. Commun.* **1965,** 30, 445-62.

[18] Dhainaut, A.; Regnier, G.; Atassi, G.; Pierre, A.; Leonce, S.; Kraus-Berthier, L.; Prost, J. F., New triazine derivatives as potent modulators of multidrug resistance. *J. Med. Chem.* **1992,** 35, 2481-96.

[19] Waring, W. S.; Whittle, B. A., Basic dihydromorphanthridinones with anticonvulsant activity. *J. Pharm. Pharmacol.* **1969,** 21, 520-30.

[20] Piantanida, M., Action of ammonia upon several 3-substituted 1,5-dibromopentanes. *J. Prakt. Chem.* **1939,** 153, 257-62.

[21] Kullmann, W., Design, synthesis, and binding characteristics of an opiate receptor mimetic peptide. *J. Med. Chem.* **1984,** 27, 106-15.

[22] Bredereck, H.; Effenberger, F.; Hajek, M., Darstellung von 1-Guanyl-pyrazol und Pyrazol-(1)-*s*-triazin. - Synthesen substituierter *s*-Triazine. *Chem. Ber.* **1965,** 98, 3178-86.

[23] Bernthsen, A.; Klinger, H., Über Sulfinverbindunen des Thioharnstoffs. *Chem. Ber.* **1878,** 65, 1192.

[24] Gers, T.; Kunce, D.; Markowski, P.; Izdebski, J., Reagents for efficient conversion of amines to protected guanidines. *Synthesis* **2004,** 1, 37-42.

[25] Donati, D.; Morelli, C.; Porcheddu, A.; Taddei, M., A New Polymer-Supported Reagent for the Synthesis of β-Lactams in Solution. *J. Org. Chem.* **2004,** 69, 9316-8.

[26] Cheng, Y.; Prusoff, W. H., Relationship between the inhibition constant (K1) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction. *Biochem. Pharmacol.* **1973,** 22, 3099-108.

[27] Geßele, K. Zelluläre Testsysteme zur pharmakologischen Charaterisierung neuer Neuropeptid Y Rezeptorantagonisten. Ph.D. thesis, University of Regensburg, Regensburg, **1998**.

[28] Schneider, E. Development of Fluorescence-Based Methods for the Determination of Ligand Affinity, Selectivity and Activity at G-Protein Coupled Receptors. Ph.D. thesis, University of Regensburg, Regensburg, **2005**.

8 Summary

Keywords: **Neuropeptide Y, NPY receptors, Y_1 receptor, Y_2 receptor, NPY receptor antagonists, argininamides, BIBP 3226, BIIE 0246, guanidinylation, acylguanidines.**

Neuropeptide Y (NPY), a 36 amino acid peptide, is widely distributed in the central and the peripheral nervous system, where it acts as a neurotransmitter and exhibits a large number of physiological activities, including the regulation of blood pressure, control of food intake, anxiety, and seizures. These effects are mediated by different NPY receptors (in mammals: Y_1, Y_2, Y_4, Y_5, and y_6), all members of the large superfamily of G-protein coupled receptors.

As shown by complete L-alanine scan, the C-terminal residues Arg^{35} and Tyr^{36} of NPY are of major importance for receptor recognition. Based on this pharmacophoric motif, two different non-peptide argininamide derivatives, BIBP 3226 and BIIE 0246, were identified as NPY Y_1 and Y_2 receptor selective antagonists, respectively. However, the strongly basic guanidino group of these argininamide-type antagonists, which is quantitatively protonated under physiological conditions, results in poor bioavailability and lack of access to the brain and limits the use of the compounds for *in vivo* studies. Interestingly, the introduction of acyl substituents at the guanidine nitrogen of BIBP 3226 ((*R*)-N^{α}-diphenylacetyl-*N*-(4-hydroxybenzyl)argininamide), leads to analogs with comparable or even increased NPY Y_1 receptor affinity. Thereby, the electron-withdrawing substituents in N^{G}-position reduce the basicity of the compounds and thus improve their pharmcokinetic properties. Molecular models of the binding mode of BIBP 3226 and related analogs suggest, that the guanidine moiety of the ligand is

interacting with a highly conserved aspartate residue in the transmembrane helix 6 (TM6) close to the extracellular space. In this model, flexible N^G-substituents are able to arrange in the extracellular loop region of the receptor, avoiding steric repulsions. These considerations encouraged us, to synthesize analogs of BIBP 3226 that are substituted with ω-aminoalkanoyl linkers in N^G-position, allowing for the introduction of fluorescence- or radio-labeled electrophiles at the terminal ω-amino group. The N^G-acyl substituted argininamides were prepared by guanidinylation of the respective ornithinamide precursor with *N*-acyl-*N'*-*tert*-butoxycarbonyl-*S*-methylisothioureas and subsequent deprotection. The ornithinamide building block was synthesized using standard peptide coupling and protective group manipulation protocols.

However, 1-(ω-aminoalkanoyl)guanidines turned out to be unstable in alkaline solution. For instance 1-(5-aminopentanoyl)guanidine degraded completely within a few minutes, resulting in the formation of guanidine and piperidin-2-one. As acylguanidines exhibit a strong UV-absorption at about 230 nm, we determined the (pseudo-) first order rate constants for the degradation of various ω-aminoalkanoyl- and ω-aminoalkoxycarbonyl-substituted guanidines by time-resolved UV-spectroscopy. Depending on the structure of the acylguanidine we observed half-lives between 19 s for 1-(5-aminopentanoyl)guanidine and 13 h for 1-[*trans*-4-(aminomethyl)cyclohexanecarbonyl]guanidine.

Beside the chemical resistance of the linker group, high receptor affinity and selectivity are crucial prerequisites for the usability of the labeled compounds. In addition, favorable pharmacokinetic properties are essential for *in vivo* studies. With intent to develop ^{18}F-labeled NPY Y_1 receptor selective PET (positron emission tomography) ligands, we synthesized several N^G-[ω-(4-fluorobenzamido)alkanoyl)]-substituted analogs of BIBP 3226 as model compounds. The antagonists were assayed for their potency to inhibit the NPY-induced increase in intracellular Ca^{2+} concentration in HEL cells. The 5-(4-fluorobenzamido)pentanoyl substituted analog

was about as potent as BIBP 3226 in our assay (IC_{50} = 23 nM vs. 17 nM), but the instability of the 5-aminopentanoyl linker made the labeling of the respective precursor impossible. The 6-(fluorobenzamido)hexanoyl substituted homolog was slightly less potent (30 nM), but the remarkably stable 6-aminohexanoyl linker makes this analog a promising candidate for the preparation of Y_1 receptor selective tracers.

As the argininamide substructure is also present in the Y_2 receptor antagonist BIIE 0246 ((2*S*)-*N*-[2-(3,5-Dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]-N^{α}-[2-(1-{2-oxo-2-[4-(6-oxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-11-yl)piperazin-1-yl]ethyl}cyclopentyl)acetyl]argininamide), we synthesized a series of N^{G}-substituted analogs, in order to find out, whether potent, but less polar Y_2 receptor selective ligands can be obtained by this strategy, too. The Y_2 receptor antagonistic activity was determined in a calcium assay using transfected CHO cells. The IC_{50} values for the N^{G}-acylated analogs were in the same range as for the unsubstituted compound BIIE 0246, indicating that the Y_2 receptor is also tolerating acyl-substituents in N^{G}-position. The N^{G}-(6-aminohexanoyl) substituted analog exhibited a lower IC_{50} value than BIIE 0246 — the most potent Y_2 receptor antagonist known in literature so far.

In summary, the acylguanidine moiety can be considered as a less polar, bioisosteric replacement for the strongly basic guanidine group in argininamide-type Y_1- and Y_2 receptor antagonists. N^{G}-Acylated argininamides are promising candidates for the development of non-peptide, centrally available neuropeptide Y receptor tracers. Thus, this class of compounds may be considered a source of versatile pharmacological tools for the characterization of NPY receptors *in vitro* and *in vivo*.

Appendix

1. List of Abbreviations and Acronyms

Ac	acetyl- (CH_3CO-)
Adoc	adamantyl-1-oxycarbonyl-
ADMET	absorption, distribution, metabolism, excretion, toxicology
Alloc	allyloxycarbonyl-
aq.	Aqueous
ax.	axial (orientation of substituents in alicyclics)
BBB	blood brain barrier
Bn	benzyl- ($PhCH_2$-)
Boc	*tert*-butoxycarbonyl-
Bu	butyl-
^{t}Bu	*tert*-butyl-
Bz	benzoyl- (PhCO-)
Bz(4-F)	4-fluorobenzoyl-
c	concentration
C_q	quaternary carbon atom (not covalently connected to hydrogen)
$[Ca^{2+}]_i$	intracellular calcium ion concentration
Cbz	benzyloxycarbonyl- (see also Z)
CDI	*N,N´*-carbonyldiimidazole
CI	chemical ionization (mass spectrometry)
CNS	central nervous system
COSY	correlated spectroscopy
d	doublet
δ	chemical shift

DABCO	1,4-diaza-bicyclo[2.2.2]octane
DCC	dicyclohexylcarbodiimide
DCHA	dicyclohexylamine
DCU	dicyclohexylurea
DEAD	diethyl azodicarboxylate
decomp.	decomposition
DIAD	diisopropyl azodicarboxylate
DIC	diisopropylcarbodiimide
diast.	diastereomeric, diastereomere(s)
DIPEA	*N*-ethyl-diisopropylamine
DME	ethylene glycol dimethyl ether (monoglyme)
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
DMSO-d_6	per-deuterated dimethyl sulfoxide
DSC	disuccinimidyl carbonate
EDC (· HCl)	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (hydrochloride)
EI	electron impact ionization (mass spectrometry)
eq.	equivalent(s) or equatorial (orientation of) substituents in alicyclics
ESI	electrospray ionization (mass spectrometry)
Et	ethyl-
Fmoc	fluorenylmethyloxycarbonyl-
h	hour(s)
HMBC	heteronuclear multiple bond correlation
HSQC	heteronuclear single quantum coherence
HOBt (· H_2O)	1-hydroxybenzotriazole (hydrate)
IC_{50}	i) antagonist concentration which suppresses 50 % of an agonist induced effect or ii) receptor ligand concentration that inhibits binding of labeled ligand by 50 %.
^nJ	coupling constant for geminal (n = 2), vicinal (n = 3) etc. coupling

k'	retention faktor (chromatography) or rate constant (kinetics)
k_{obs}	macroscopic rate coefficient
LiHMDS	lithium hexamethyldisilazide (lithium bis(trimethylsilyl)amide)
m	multiplet
M	molar ($mol \cdot dm^{-3}$)
Me	methyl-
mol	mole(s)
mp	melting point
MTBE	*tert*-butyl-methyl ether
N^G, N^G	guanidino-nitrogen (cp. N^ω, $N^{\omega'}$ nomenclature for arginines)
Pbf	2,3-dihydro-2,2,4,6,7-pentamethylbenzofuran-5-sulfonyl-
PET	positron emission tomography
Ph	phenyl-
PG	protective group
Pht	phthaloyl-
ppm	parts per million
q	quartet
s	singlet
SPPS	solid phase peptide synthesis
t	triplet
t	time
TBTA	*tert*-butyl-2,2,2-trichloroacetimidate
TBTU	2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate
TEA	triethylamine (NEt_3)
Tf	trifluoromethanesulfonyl-
TFA	trifluoroacetic acid
THF	tetrahydrofurane

TLC	thin-layer chromatography
Tos (or Ts)	4-toluenesulfonyl-
Y_n or Y_nR	NPY receptor subtypes, n = 1, 2, 4, 5, (6); sometimes with prefix h (human), m (mouse), r (rat), gp (guinea pig), p (porcine) etc.
Z	benzyloxycarbonyl- (see also Cbz)

2. Calculation of First Order Rate Constants

Eq. (1) describes the monomolecular conversion of reactant R into products P_i; k is the first order rate constant and ν_i are stoichiometric factors.

$$R \xrightarrow{k} \sum_i \nu_i P_i \tag{1}$$

According to Lambert-Beer's Law, the absorbance A of the reaction mixture is given by:

$$A = d \cdot \sum_i \varepsilon_i \cdot c_i \tag{2},$$

where d is the optical path length through the absorbing sample, ε_i are the molar extinction coefficients, and c_i are the concentrations of the spectroscopically active species. In our case A at time t can be expressed as:

$$A = \varepsilon_R \cdot c_R + \sum \nu_i \cdot \varepsilon_i \cdot c_i \tag{3}$$

We assume that at the beginning of the reaction only reactant R in the initial concentration c_o and no Products P_i are present; furthermore, we define the extent of reaction λ ($\lambda \cdot 100 =$ %-conversion); hence, eq. (3) can be written as:

$$A = (1-\lambda) \cdot \varepsilon_R \cdot c_o + \lambda \cdot c_o \cdot \sum \nu_i \cdot \varepsilon_i \tag{4a}$$

$$A = \varepsilon_R \cdot c_o + \lambda \cdot c_o (\sum \nu_i \cdot \varepsilon_i - \varepsilon_R) \tag{4b}$$

For $\lambda = 0$ (beginning of reaction) and $\lambda = 1$ (complete consumption of reactant), eq. (4b) simplifies to:

$$A(\lambda = 0) = A_o = \varepsilon_R \cdot c_o \tag{5}$$

$$A(\lambda = 1) = A_\infty = c_o \sum \nu_i \cdot \varepsilon_i \tag{6}$$

A, A_o and A_∞ result directly from the UV measurement. A_o is the initial and A the current absorbance; A_∞ is the absorbance after complete reaction—in practice A_∞ is taken after at least five to eight half times (96.9…99.6 % conversion).
We can form the differences $A - A_\infty$ and $A_o - A_\infty$ and obtain:

$$\begin{aligned} A - A_\infty &= \varepsilon_R \cdot c_o + \lambda \cdot c_o (\sum \nu_i \cdot \varepsilon_i - \varepsilon_R) - c_o \sum \nu_i \cdot \varepsilon_i \\ &= (1 - \lambda) \cdot (\varepsilon_R \cdot c_o - c_o \sum \nu_i \cdot \varepsilon_i) \end{aligned} \tag{7}$$

$$A_o - A_\infty = \varepsilon_R \cdot c_o - c_o \sum \nu_i \cdot \varepsilon_i \tag{8}$$

By division of (7) by (8) we achieve expression (9), which correlates the advancement of the reaction with experimentally accessible quantities.

$$\frac{A - A_\infty}{A_o - A_\infty} = 1 - \lambda \tag{9}$$

As $1 - \lambda$ equals the ratio of current and initial concentration, we can phrase:

$$c_E = (1 - \lambda) \cdot c_o \quad \text{hence:} \quad \frac{c_E}{c_o} = 1 - \lambda \quad \text{and} \quad \frac{c_E}{c_o} = \frac{A - A_\infty}{A_o - A_\infty} \tag{10}$$

The change of concentration in first-order reactions is described by the following integral rate law:

$$\frac{c}{c_o} = e^{-k \cdot t} \tag{11}$$

The combination of eqns. (10) and (11) affords:

$$\frac{A - A_\infty}{A_o - A_\infty} = e^{-k \cdot t} \tag{12}$$

$$\boxed{-\ln \frac{A - A_\infty}{A_o - A_\infty} = k \cdot t} \tag{13}$$

Linearization of eq. (12) provides the rate constant *k* as the slope of the line (eq. 13). In practice, *k* results from a linear regression run.

Note that molar extinction coefficients and initial concentrations are not required for the determination of (first-order) rate constants.

3. Spectrofluorimetric Ca^{2+} Assay for the Determination of NPY Antagonistic Activities

3.1. Experimental Procedure of the Spectrofluorimetric Ca^{2+} Assay

The spectrofluorimetric calcium assay with the ratiometric Ca^{2+} indicator fura-2 was performed as described for HEL cells by Gessele[1]. Cells were grown to 70-80 % confluence, trypsinized and resuspended in FCS containing medium for trypsine inactivation. Cells were counted, centrifuged at 300 g for 5 min and resuspended at $1.3 \cdot 10^6$ cells/ml in loading buffer. 0.75 ml cell suspension were added to 0.25 ml of loading suspension containing 20 mg of BSA, 5 μl of pluronic F-127 (20 % in DMSO) and 4 μl of fura-2/AM (Molecular Probes; 1 mM in anhydrous DMSO) in 1 ml of loading buffer. The addition of pluronic F-127 facilitates the solubilization of the lipophilic calcium indicator dye and the following dye loading as described for fluo-3 in [2] and [3]. Final concentrations were: $1 \cdot 10^6$ cell/ml, 1 μM fura-2/AM, 0.2 % DMSO and 0.025 % pluronic F-127. The cells were incubated for 30 min at room temperature in the dark, centrifuged and resuspended in the same volume of loading buffer. In order to achieve complete intracellular cleavage of the AM-ester, the cells were incubated for additional 30 min in the dark, washed twice with loading buffer and resuspended at a density of $1 \cdot 10^6$ cells/ml.

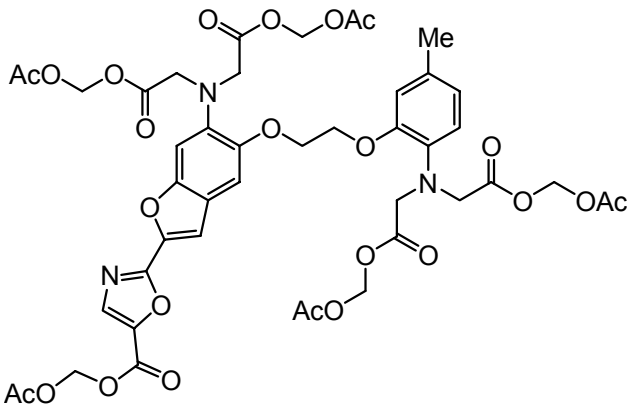


Fig. 1: Structure of the fura-2 AM ester. The lipophilic ester is able to diffuse through cell membranes. Intracellular esterases convert the acetoxymethyl esters to the active Ca^{2+} indicator dye with free carboxylate groups. In the polar, carboxylate form fura-2 is no longer able to leave the cell and thus accumulates in the cytosol.

For the measurement, 1 ml of the cell suspension was transferred into a cuvette containing 1 ml of loading buffer under stirring. The baseline was recorded for 30 s before the agonist was added. Antagonists were added to the cell suspension 1 min before the calcium signal was triggered by the addition of a fixed concentration of agonist. Every third measurement was a reference signal; for the determination of IC_{50} values of antagonists, the reference signal was elicited in the absence of antagonist. Instruments settings were λ_{ex} = 340 and 380 nm (alternating) with slit = 10 nm and λ_{em} = 510 nm with slit = 10 nm. Stirring was low and temperature was 25 °C.

The ratio R of fluorescence intensity at 510 nm after excitation at 340 and 380 nm was used for the calculation of the calcium concentration according to the Grynkiewicz equation [4]:

$$\left[Ca^{2+}\right] = K_D \cdot \frac{(R - R_{min})}{(R_{max} - R)} \cdot SFB$$

The K_D value is the dissociation constant of the fura-2-Ca^{2+} complex. R_{max} is the fluorescence ratio in presence of a saturating Ca^{2+} concentration, determined after the addition of 10 µl of digitonin solution (2% in water, Sigma), which caused lysis of the cells and saturation of the dye with the calcium ions of the loading buffer. R_{min} is the ratio in absence of free Ca^{2+}, determined after the addition of 50 µl of EGTA solution (600 mM in 1 M tris buffer, pH 8.7) to the lysed cells. The correction factor SFB is the ratio of fluorescence intensity at 510 nm after excitation at 380 nm of the Ca^{2+} free and Ca^{2+} saturated dye.

3.2. Calculation of IC_{50} values

IC_{50} values were calculated from at least two antagonist concentrations [B], inhibiting the NPY stimulated increase in intracellular $[Ca^{2+}]_i$ between 20 and 80 %. The mean percentual inhibition values P with SEM < 10 %, determined from 2-3

independent experiments, performed on different days, were logit transformed, according to the equation

$$\text{logit}(P) = \log \frac{P}{100 - P}$$

and IC_{50} values were from the plot logit (P) versus log [B] with the slope n according to

$$\log \frac{P}{100 - P} = n \cdot \log[\text{B}] - n \cdot \log IC_{50}$$

by linear regression[5].

3.3. Reference List

[1] Geßele, K. Zelluläre Testsysteme zur pharmakologischen Charaterisierung neuer Neuropeptid Y Rezeptorantagonisten. Ph.D. thesis, University of Regensburg, Regensburg, **1998**.

[2] M. E. Granados, E. S., A. Saavedra-Molina,, Use of pluronic acid F-127 with Fluo-3/AM probe to determine intracellular calcium changes elicited in bean protoplasts. *Phytochemical Analysis* **1997**, 8, 204-8.

[3] Kao, J. P.; Harootunian, A. T.; Tsien, R. Y., Photochemically generated cytosolic calcium pulses and their detection by fluo-3. *J. Biol. Chem.* **1989**, 264, 8179-84.

[4] Grynkiewicz, G.; Poenie, M.; Tsien, R. Y., A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J. Biol. Chem.* **1985**, 260, 3440-50.

[5] Mueller, M.; Knieps, S.; Gessele, K.; Dove, S.; Bernhardt, G.; Buschauer, A., Synthesis and neuropeptide Y Y_1 receptor antagonistic activity of *N,N*-disubstituted ω-guanidino- and ω-aminoalkanoic acid amides. *Arch. Pharm. (Weinheim, Ger.)* **1997**, 330, 333-42.

4. List of Publications and Abstracts

Brennauer, A., Dove, S., Buschauer, A.: *Structure–Activity Relationships of Non-peptide Neuropeptide Y Receptor Antagonists*, in **Hanbook of Experimental Pharmacology**, Vol. 162, chapter 18, Springer, Berlin Heidelberg, **2004**, pp. 505-546.

Ziemek, R., Brennauer, A., Schneider, E., Cabrele, C., Beck-Sickinger, A.G., Bernhardt, G., Armin Buschauer, A.: *Fluorescence- and luminescence-based methods for the determination of affinity and activity of neuropeptide Y Y_2 receptor ligands,* **Eur. J. Pharmacol.** (2006), doi:10.1016/j.ejphar.2006.08.075

Brennauer, A., Keller, M., Freund, M., Graichen F., Hutzler C., Ziemek, R., Bernhardt, G., Dove, S., Buschauer, A.: *Guanidine Replacement in NPY Receptor Ligands: Synthesis of N^G-Acylargininamides as Y_1 and Y_2 Receptor Antagonists*, **Conference of the German Chemical Society (GDCh), section „Medicinal Chemistry", Frankfurt/Main (Germany) 2006.**

Keller, M., Brennauer, A., Freund, M., Bernhardt, G., Dove, S., Beck-Sickinger, A.G., Buschauer, A.: *Synthesis and Characterization of Radiolabeled NPY Y_1 Receptor Antagonists,* **Conference of the German Chemical Society (GDCh), section „Medicinal Chemistry", Frankfurt/Main (Germany) 2006.**

Brennauer, A., Keller, M., Freund, M., Bernhardt, G., Dove, S., Buschauer, A.: *Towards the Development of Neuropeptide Y Y_1 Receptor Selective Tracers*, **Conference of the German Chemical Society (GDCh), section „Medicinal Chemistry", Leipzig (Germany) 2005.**

Brennauer, A., Freund, M., Graichen, F., Keller, M., Schneider, E.,Bernhardt, G., Dove, S., Buschauer, A.: *N^G-(ω-aminoalkanoyl)- arginine- amides: Building Blocks for the Synthesis of Fluorescence- or Radiolabeled Neuropeptide Y Receptor Ligands*, **2nd International Summer School „Medicinal Chemistry", Regensburg (Germany) 2004.**

Ziemek, R., Brennauer, A., Bernhardt, G., Cabrele, C., Beck-Sickinger, A.G., Buschauer, A.: *Fluorescence Based Determination of Affinity and Activity at the Human Neuropeptide Y Y_2 Receptor Using Flow Cytometry,* **2nd International Summer School „Medicinal Chemistry", Regensburg (Germany) 2004.**

Brennauer, A., Dove, S., Buschauer, A., *Synthesis of L-Argininamides related to BIIE 0246 as Potential NeuopeptideY Y_2 Receptor Antagonists*, **1st International Summer School „Medicinal Chemistry", Regensburg (Germany) 2002**.

5. Curriculum Vitae

Name:	Albert Brennauer
Date of birth:	January 15th 1973
Place of birth:	Schongau
Nationality:	german
Marital Status:	married, one son, born on July 5th 2000)

Education

1983–1992	Dominikus-Zimmermann Gymnasium, Landsberg/Lech
07/1992	School graduation (Abitur)

Professional Training

10/1992–12/1993	Community Service (Zivildienst) at mental-health charity "Herzogsägmühle" (Innere Mission München), Germany.
10/1994–01/2000	**M.Sc.** (Diplom) in Chemistry, University of Regensburg
04/1999–01/2000	**Master Thesis** (Diplomarbeit) in Medicinal Chemistry Department of Medicinal Chemistry, University of Regensburg. Advisor: Prof. Dr. Stefan Dove Title of thesis: *Molecular Modelling and Quantitative Structure-Activity Relationships of peptide and non-peptide Neuropeptide Y Y_2 Receptor Ligands*.
06/2000–09/2006	**PhD program** at the Department of Medicinal Chemistry, University of Regensburg. Advisor: Prof. Dr. Armin Buschauer Title of the project: *Design and Synthesis of N^G-substituted Argininamides as Neuropeptide Y Receptor Antagonists*
since 04/2002	Associate member of the Research training Group (Graduiertenkolleg GRK 760): *Medicinal Chemistry: Molecular Recognition—Ligand-Receptor Interactions*