Solid-Phase Synthesis of Peptide – Metal-Complex Conjugates

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Sandra

&

meiner Familie

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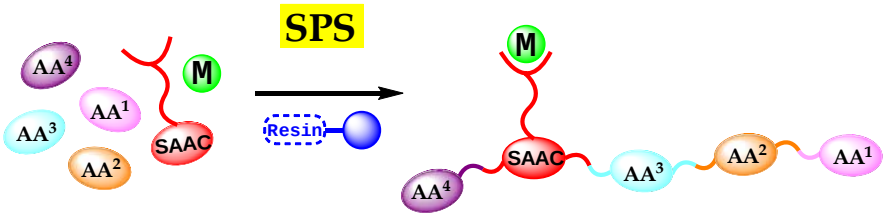
A. Introduction*

Graphical Abstract:



Solid-phase synthesis is a convenient and established method for the preparation of peptide based compounds. However, solid-phase synthesis of inorganic complexes is a rather new discipline and was established by Heinze, Metzler-Nolte, Reedijk and others.[1] Earlier attempts to use solid-phase synthesis, e.g. to obtain (2,2´bispyridine)-dichloro complexes of platinum(II) by Gallop, failed at the cleavage step,[2] due to the more labile metal-ligand bonds of organometallic building blocks compared to typical covalent bonds of organic molecules. Coordination and organometallic chemistry on solid-phase were typically studied in the context of catalyst performance.[3] Recently, solid-phase synthesis using insoluble resins as solid support was used to synthesize metal complexes based on peptide backbone ligands. These coordination compounds find applications in biochemistry as well as in medicinal chemistry. Resin-bound chelates were prepared in such a manner that upon the addition of suitable metal salts the target metal complexes were selectively released from the resin and used e.g. in fluorescence or radio imaging or oligonucleotide DNA/RNA binding studies. Other approaches incorporated beforehand prepared metal-complex building blocks in solid-phase peptide synthesis which leads e.g. to peptide-platinum complex conjugates with anticancer activity. This versatile approach to incorporate pendant protected amino acid functionalities, offers several advantages over solution phase or post solid-phase peptide synthesis conjugation.[4] It provides the flexibility to incorporate a metal ion chelator with exclusive site specificity in any amino acid sequence, not just terminally or at one or more lysine or cysteine side chains.[5] Additionally, peptides are often prepared most effective via automated solid-phase synthesis.

* This introduction is published as a micoreview: G. Dirscherl, B. König *Eur. J. Org. Chem.* **2007**, in press.

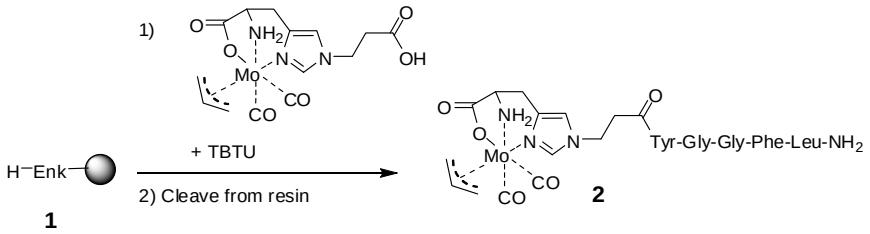
Scope and Limitations

This introduction will summarize recent reports on the preparation of metal complex – peptide conjugates by solid-phase synthesis methods. The focus of the introduction lies on the synthetic methodology to prepare the building blocks and peptides rather than applications of the metal complex – peptide conjugates. The survey is structured by the metal ions used for complex formation, and the discussion distinguish between examples of solid phase ligand synthesis with subsequent metallation[6] and the use of metal containing amino acids for synthesis.

1. Chromium, Molybdenum and Tungsten (Group 6) Metal Complex – Peptide Conjugates

1.1 N_δ,*N*,*O*-L-Histidinate (His) molybdenum conjugate

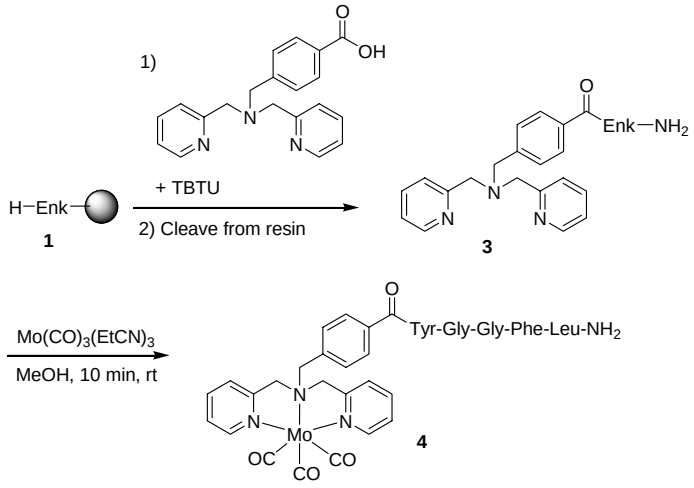
Metzler-Nolte and co-workers reported oligopeptide bioconjugates with organometallic Mo carbonyl complexes.[7] The conjugates were prepared in excellent yield and purity by two different solid-phase synthesis strategies. In one approach the neuropeptide enkephalin (enk) Tyr-Gly-Gly-Phe-Leu, which is a natural ligand to the opiate receptor, was synthesized by standard Fmoc solid-phase methods on NovaSyn TGA resin with an HMBA linker. The metal complex Mo(N_ε-$C_2H_4CO_2H$-His)(allyl)$(CO)_2$ was coupled to the resin-bound, fully deprotected enkephalin **9** and afterwards cleaved from the resin by treatment with saturated NH_3 solution in MeOH (Scheme 1).



Scheme 1. Solid-phase synthesis of Mo(His)Enk conjugate **2** using a metal complex acid.

1.2 Bis(2-picolyl)amine (bpa) molybdenum conjugate

In case when the attachment of a metal complex to the peptide on the solid support is not desirable, e.g. with radioactive metal isotopes, an innocent anchoring group can be attached to the peptide during solid-phase synthesis. The ligand–peptide conjugate is then cleaved from the resin, purified and the metal label is only added in solution immediately prior to use of the bioconjugate. Metzler-Nolte et al. provided an example for this procedure using the $Mo(CO)_3$ fragment and bpa as a ligand (Scheme 2).[7]

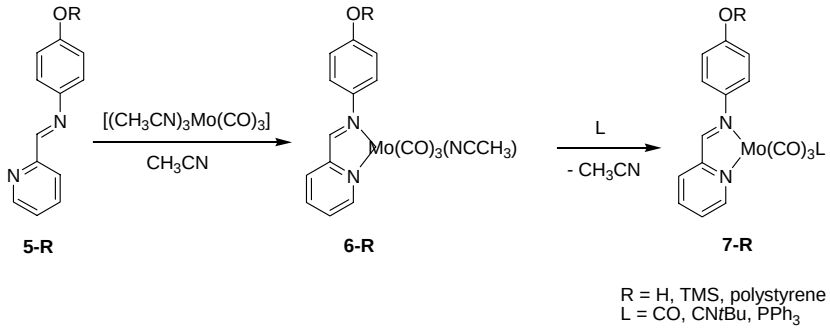


Scheme 2. Synthesis of Mo(bpa)-Enk conjugate **4** by subsequent metallation.

1.3 Bidentate schiff base metal conjugates

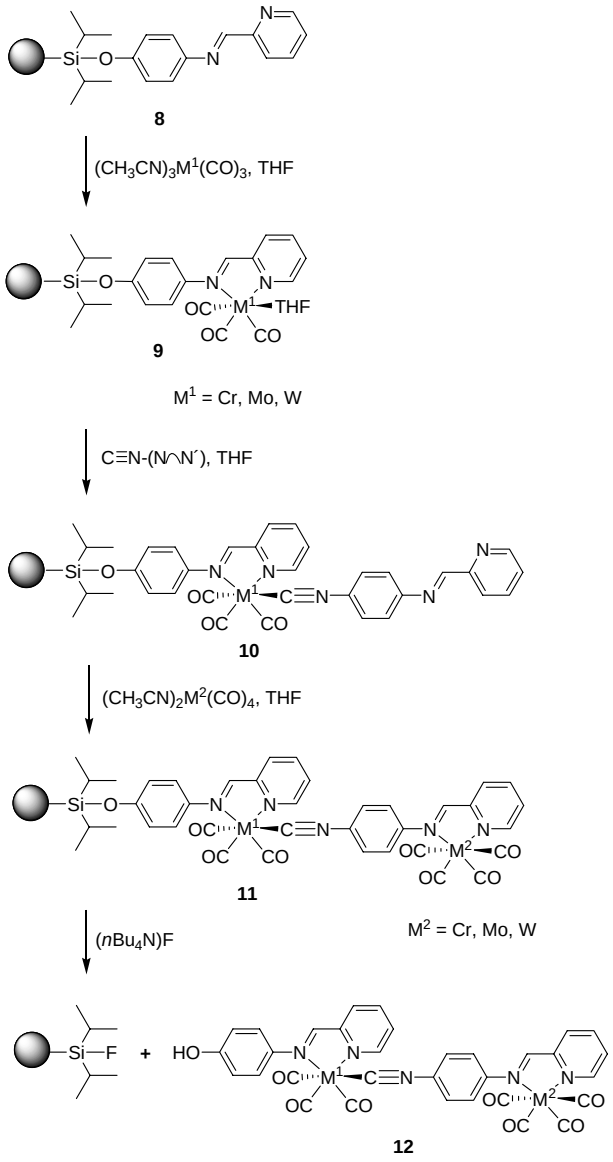
A solid-phase synthesis approach for molybdenum carbonyl complexes was developed by Heinze (Scheme 3).[8] We include this example, although neither peptide coupling nor metallated amino acids are used, because it illustrates that complex organometallic transformations are possible on solid support. A specific resin and linker system allows coordination and organometallic chemistry under solid-phase reaction conditions and the cleavage of the metal complex from the solid support. Bidentate Schiff base **5-R** was used as the ligand. The phenolic hydroxyl group allows the attachment to the solid support. A silyl ether[9] based linker was chosen due to its stability under basic and acidic conditions and the possibility to cleave with fluoride ions, which are expected to be unreactive towards most metal complexes. In solution high temperature and rather harsh oxidative reaction conditions are necessary to synthesize the desired tricarbonyl compounds. Such harsh conditions have to be avoided in solid-phase chemistry with polystyrene resins as the molybdenum precursors can react with the aromatic

residues of the support. Heinze and co-workers used $[(CH_3CN)_3Mo(CO)_3]$ as a $Mo(CO)_3$ source and under mild reaction conditions the intensely blue coloured complexes **6-R – 7-R** formed rapidly and cleanly in excellent yields. However, acetonitrile, a rather poor solvent for resin-swelling, had to be used in a mixture with toluene. Otherwise the complexation led to formation of the immobilised tetracarbonyl complex instead of the desired tricarbonyl complex. The cleavage was performed with tetra-*n*-butylammonium fluoride in dichloromethane and resulted in deeply coloured solutions of the deprotonated complexes.



Scheme 3. Synthesis of molybdenum tricarbonyl complexes on solid support.

Heinze et al. used their molybdenum carbonyl complexes, as the molybdenum-carbonyl and molybdenum-isonitrile bonds are substitutionally inert metal-ligand bonds, to synthesize di- and trimetallic homonuclear complexes (Scheme 4).[10] Finally mixed-metal dinuclear complexes prepared from chromium, molybdenum and tungsten and a directional bridging ligand were assembled stepwise on solid-phase and cleaved from the support.[11]



Scheme 4. Synthesis of mixed-metal dinuclear complexes on solid support.

Solution synthesis, although straightforward, requires purification of the products and intermediates, which is rather difficult, and makes this approach less suitable for longer-chain complexes. The solid-phase synthesis needs more reaction steps (ligand immobilisation and product release) and differently optimised reaction conditions. However, it is much easier to accomplish, and solubility problems and purification of intermediates can be disregarded.

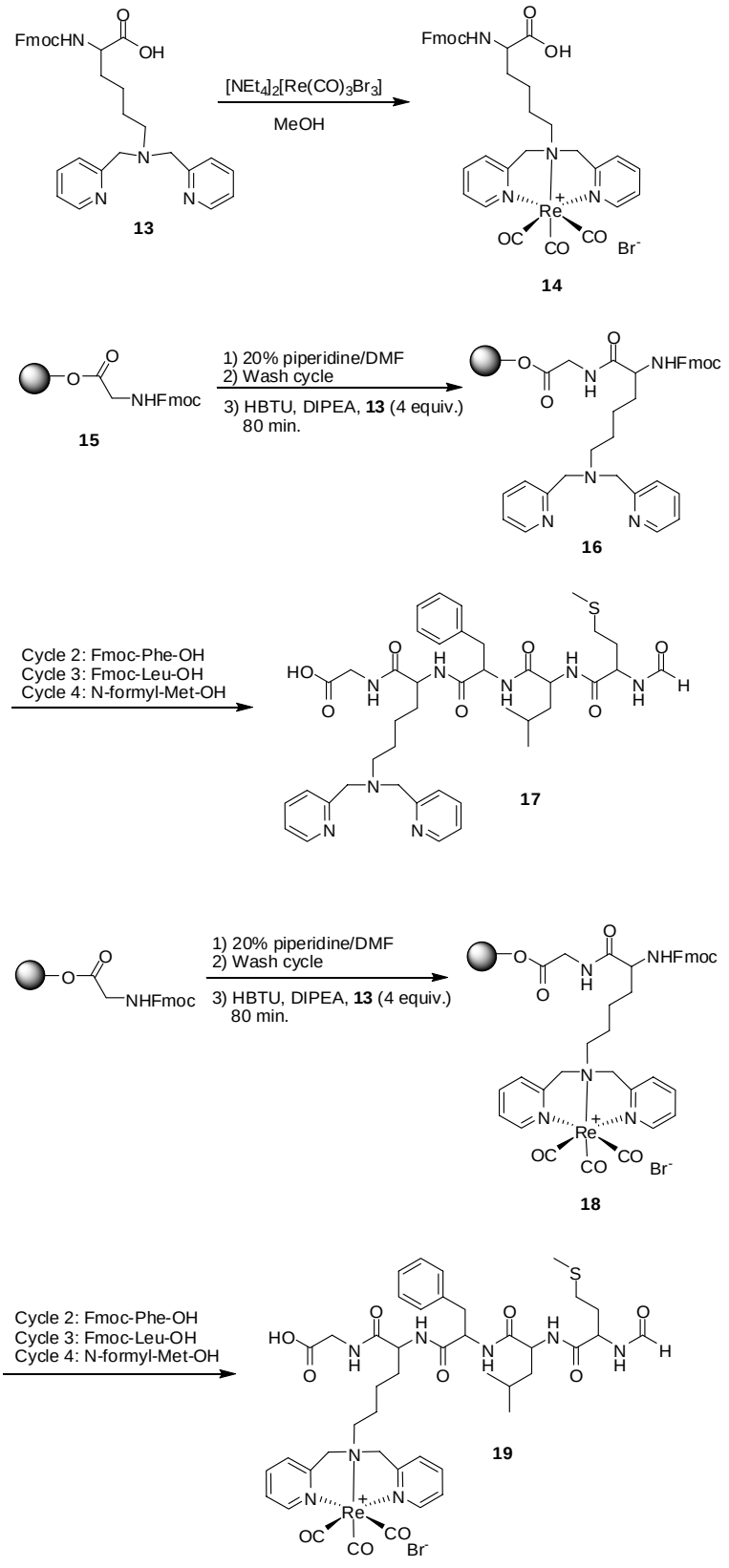
2. Manganese, Technetium and Rhenium (Group 7) Metal Complex – Peptide Conjugates

The manganese family comprises the most used metals for peptide complexation. Its applications range from rhenium and technetium labeled radiopharmaceuticals[12] to organometallic PNA oligomers with rhenium and their interaction with complementary DNA and to peptide-manganese complexes with catalytic activity.

The transition metals technetium and rhenium are among the most commonly used radioisotopes in medicine due to the favorable emission energies and decay properties of radioactive isotopes Tc-99m, Re-186 and Re-188.[13] As a result, methods of attaching these radionucleides to peptide sequences have been developed. Solid-phase synthesis strategy was employed to optimize the receptor binding affinity and biodistribution of technetium labeled peptides[14] as it allows the preparation of analogues of a particular peptide-ligand bioconjugate in parallel.[15]

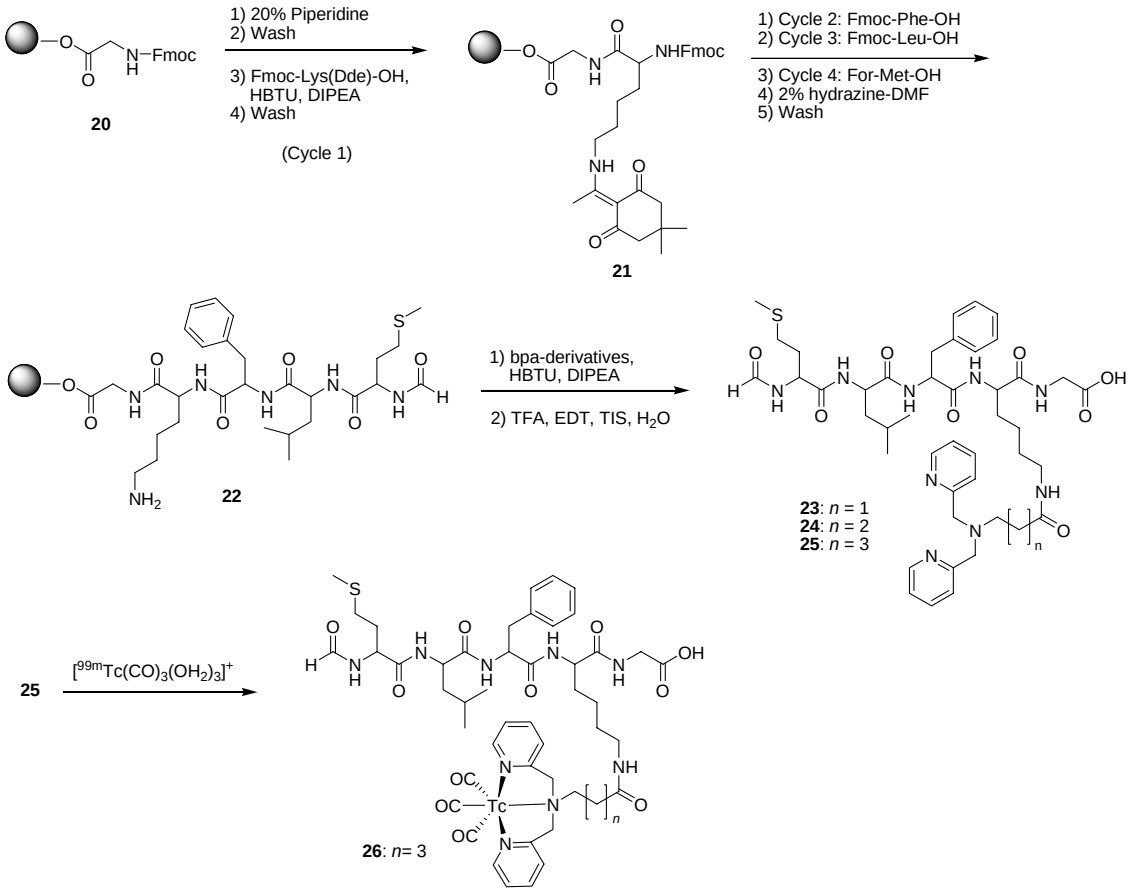
2.1 Bpa metal conjugate

Valliant and co-workers prepared the single amino acid chelate (SAAC) **17** and Re-SAAC-peptide derivatives **19** using solid-phase synthesis.[16] Fmoc protected dipyridyl chelate **13** and its Re complex **14** were incorporated into the growing peptide linked to a SASRIN resin using HBTU as the coupling agent (Scheme 5).

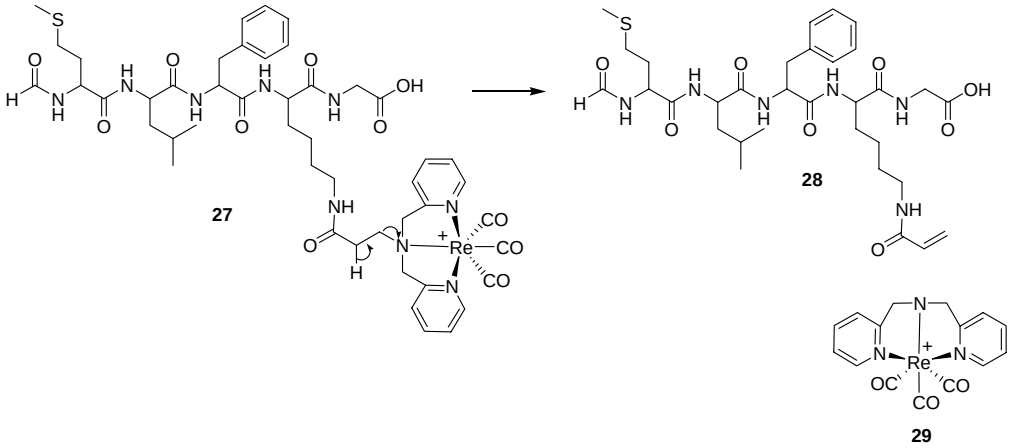


Scheme 5. Solid-phase synthesis of ligand **17** and rhenium complex **19** using a metallated amino acid.

In 2005 Valliant et al.[17] published a solid-phase methodology which aimed to incorporate lysine into the backbone of a peptide in such a manner that the ε-nitrogen could be selectively liberated and a metal-bpa-chelate added, while the peptide was still linked to the resin. Dde was used as lysine side chain protecting group, because it is stable to the conditions used in typical Fmoc solid-phase synthesis, and it can be selectively liberated without affecting Boc protecting groups.[18] This approach is applicable to bifunctional chelating systems containing a pendent acid group. After the removal of the Dde protecting group, a series of dipyridyl amine ligands **23**-**25** with linker arms varying in length were coupled to the resin-bound peptides using HBTU and DIPEA (Scheme 6). However, stable Tc(I) and Re(I) complexes were not obtained for all of the ligands. In case of peptide conjugate **27** degradation is likely caused by elimination to give an α,β-unsaturated amide **28**, which concomitantly results in liberation of a neutral metal complex **29** (Scheme 7).



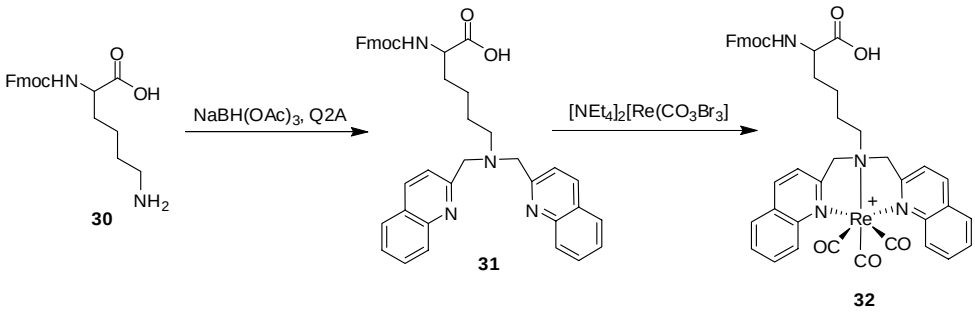
Scheme 6. Synthesis of peptide-technetium conjugate **26** by metallation after solid-phase ligand preparation.



Scheme 7. Proposed mechanism for the degradation of the Re(I)-peptide conjugate **27**.

2.2 Quinoline-2-aldehyde (Q2A) metal conjugate

To obtain a fluorescent SAAC-type Re-complex with retaining its ability to bind ^{99m}Tc, Valliant et al. reacted Fmoc-L-lysine with Q2A in the presence of $Na(OAc)_3BH$ to yield the bifunctional ligand **31** (Scheme 8).[19] The objective was to develop a method for preparing bioconjugates that can deliver the ligand to specific receptors. The SAACQ ligand and the SAACQ-Re complex represent such amino acid analogues which can be incorporated in peptide sequences by solid-phase peptide synthesis. The rhenium complex **32**, prepared by complexation with $Re(CO_3)Br_3$, was integrated in the peptide fMLF (*N*-formyl-L-methionine-L-leucine-L-phenylalanine), a targeting sequence which has been used to guide radiopharmaceuticals to the formyl peptide receptor (Figure 1). The work is an example of the use of metal containing amino acids in solid phase peptide synthesis.



Scheme 8. Synthesis of fluorescent SAAC-type Re-complex **32**.

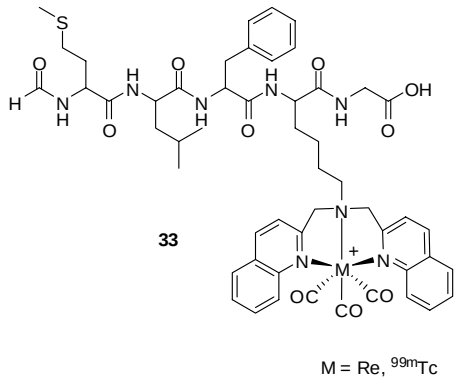
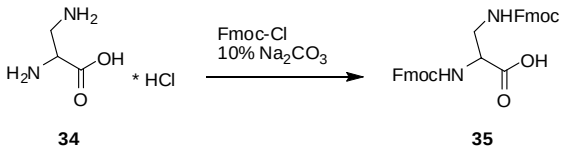


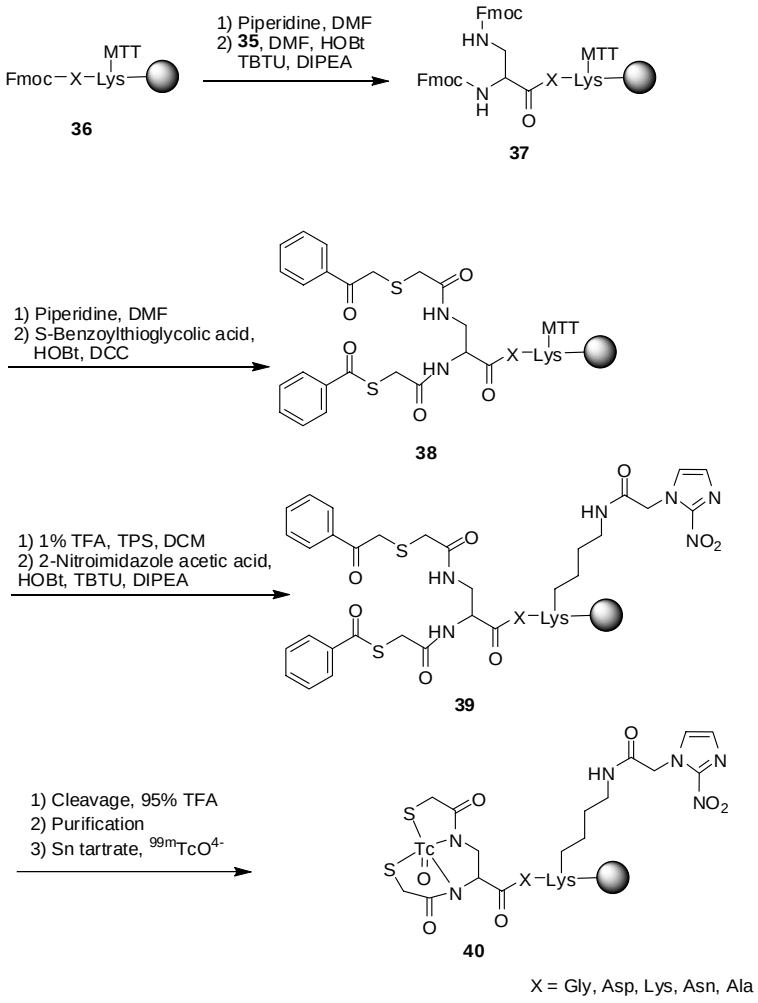
Figure 1. Bioconjugate complex fMLF[(SAACQ-M(CO)$_3$)$^+$]G **33**.

2.3 N_xS_y Metal conjugates

Many studies have shown that ligand systems containing nitrogen and thiol sulphur atoms are effective for the coordination of Tc and Re.[20] In 1997 Quinn and co-workers appended a rhenium-bound peptide to the N-terminus of receptor binding α-melanocyte stimulating hormone fragments as the last step of a conventional solid-phase peptide synthesis.[21] This diaminedithiol (N_2S_2) chelator was also assembled at the N-terminus of short peptides in a two-step procedure by Gariépy et al.[22] The deprotected terminal amino group was first reacted with di-Fmoc-diaminopropionic acid **35** (Scheme 9) and the two protected amino groups were then simultaneously deprotected and subsequently reacted with S-benzoylthiolglycolic acid to generate a protected N_2S_2 chelator **38**. The resulting constructs were cleaved from the resin support and labelled with ^{99m}Tc-pertechnetate (Scheme 10).

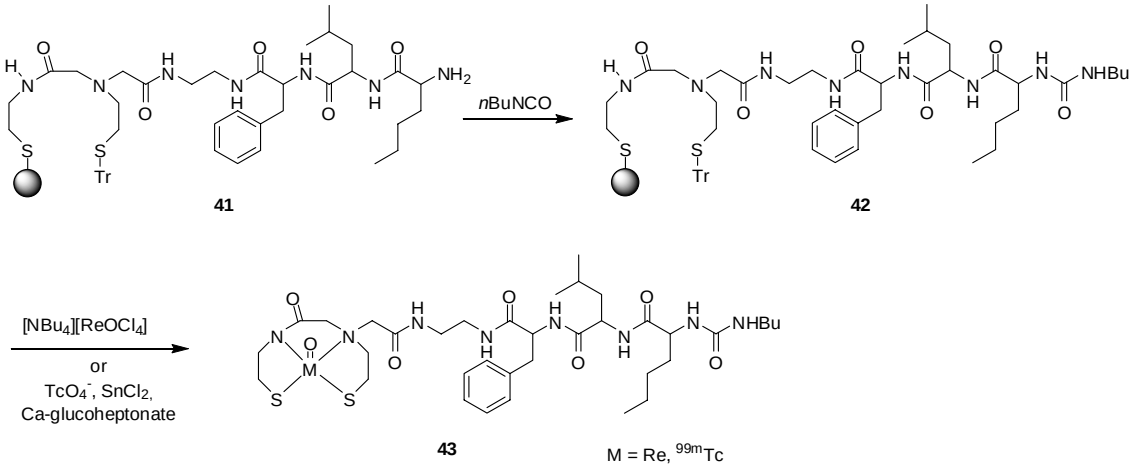


Scheme 9. Preparation of di-Fmoc protected amino acid derivative **35**.



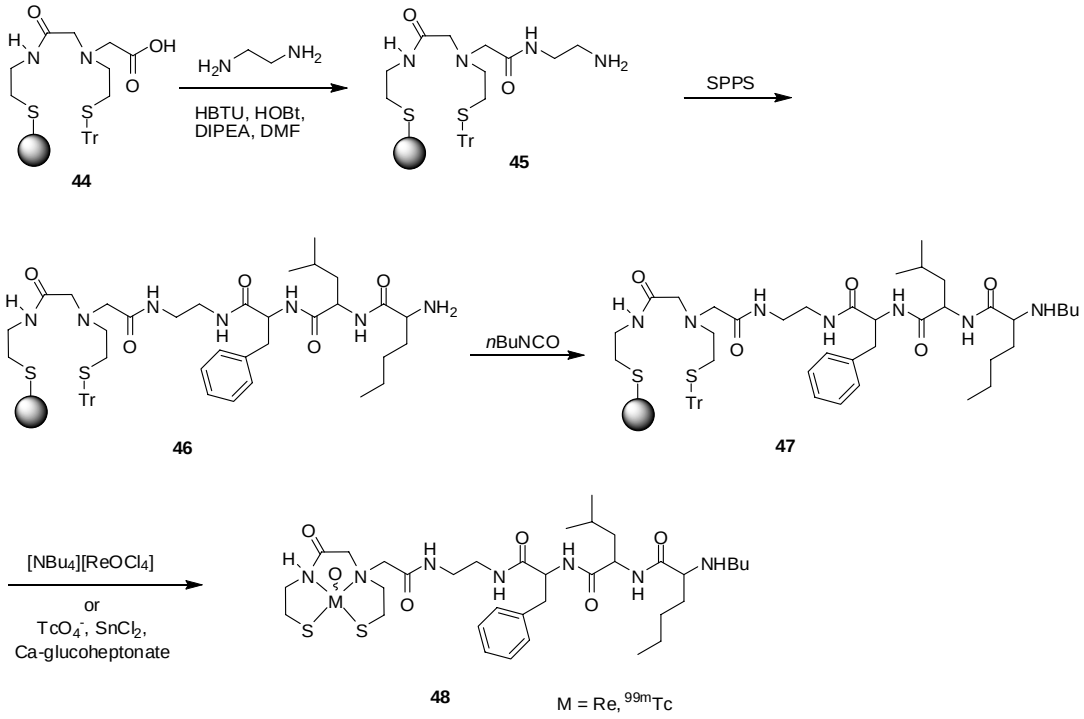
Scheme 10. Synthetic scheme for the preparation of $N_2S(benzoyl)_2$-containing peptides by ligand synthesis on solid support and subsequent metallation.

Okarvi used a pre-labeling method[23] in which the radionucleide binds to the chelate in a separate step prior to the attachment of a peptide.[24] Monoamide monoamine (MAMA)[25] forms neutral, stable and well defined complexes with both Tc(V) and Re(V), and it can be easily derivatized, regioselectively and with a wide range of different functional groups.[26] Resin bound peptide-MAMA conjugates were prepared in such a manner that upon the addition of suitable Re(V) and Tc(V) precursor **42** the target metal complexes **43** were selectively released from the resin.[27]



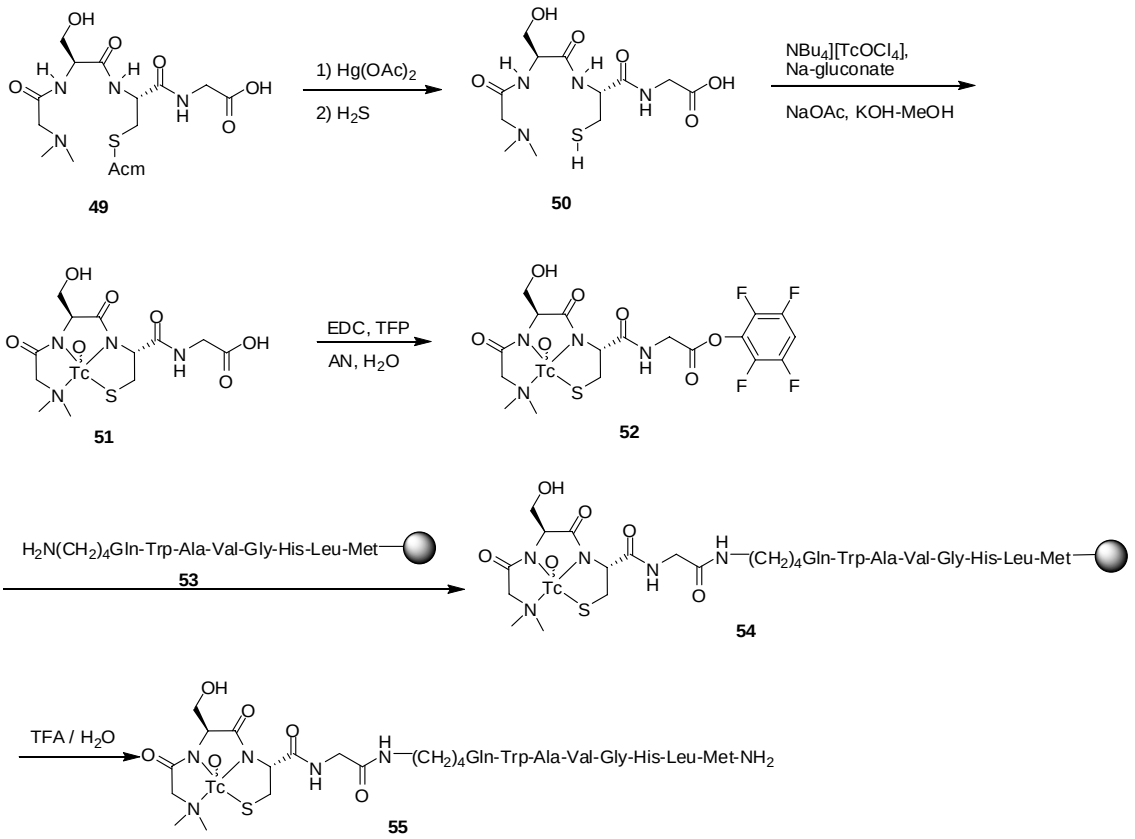
Scheme 11. Synthesis of Re(V) and Tc(V) peptide conjugates on solid support; release from resin occurs on metallation.

Although it is conceivable to build peptides using the carboxylic acid funtionalized MAMA derivative, automated peptide synthesis is typically performed starting from a primary amine using Fmoc-protected amino acids. Valliant et al. therefore prepared a primary amine functionalized MAMA chelate by coupling a diamine to the carboxylic acid of **44**[25] (Scheme 12). Compound **45** was used to synthesize a model peptide with standard Fmoc/HBTU protection and coupling methods. The peptide-functionalized resin **47** was subsequently treated with [TBA][$ReOCl_4$] and heating released the complex **48** from the resin. The target peptide is the *n*-butyl urea derivative of Phe-Leu-Nle, which is an antagonist for the formyl peptide receptor (FPR).[28]This ligand is of interest because radiolabeled compounds that are capable of binding selectively to the FPR on white blood cells can be used to image sites of infection and inflammation.[29]



Scheme 12. Synthesis of Re (V) and Tc(V)-Mama peptide conjugates **48**; metallation releases the complex from the solid support.

Valliant and co-workers also prepared a bombesin derived peptide-^{99m}Tc chelate conjugate **55** using solid-phase synthesis methodology. [30] Bombesin is a 14-amino acid peptide hormone.



Scheme 13. Solid phase synthesis of a N_2SN' technetium chelate peptide conjugate **55** derived from bombesin using metal containing amino acid **52** for peptide coupling.

The reported approach involved linking a prefabricated bifunctional N_2SN' technetium chelate complex **51** to a resin bound peptide sequence **53** derived from bombesin, which has been shown to bind to the gastrin-releasing peptide (GRP) receptor. Bombesin (BBN) is an analogue of human GRP that binds to GRP receptors (GRPr) with high affinity and specificity.[31] The GRPr is overexpressed on a variety of human cancer cells, including prostate, breast, lung, and pancreatic cancers. The synthesis of a series of bombesin derivatives was reported by Hofman et al.[32] They describe the design of BBN agonist analogues in which the radiometal chelate is linked either directly to the N-terminal amine group of BBN[7-14]NH_2 **56** (Figure 2) or via hydrocarbon spacer groups **57** – **60** (Figure 3). In a "posttransmetalation" manner, ^{99m}Tc was introduced to the triamido-thiol (N_3S) bifunctional chelating agent and the effects of varying the length of hydrocarbon spacer groups were determined (Scheme 14).

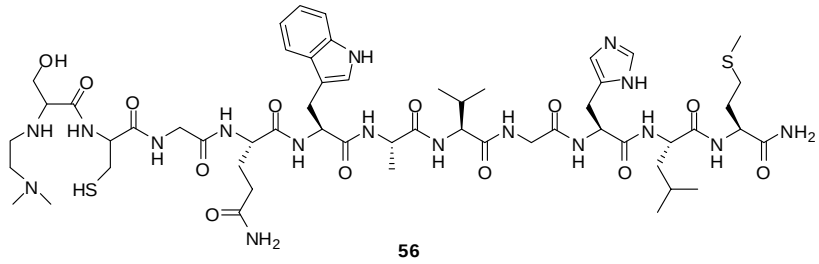


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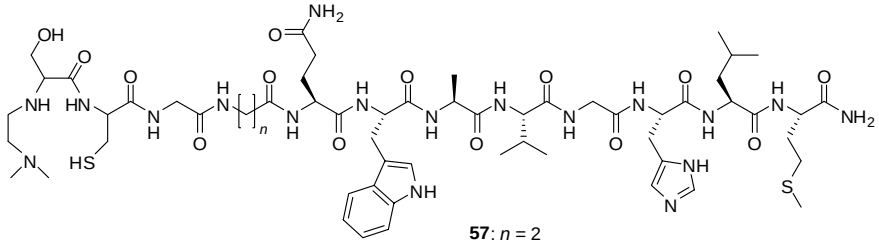
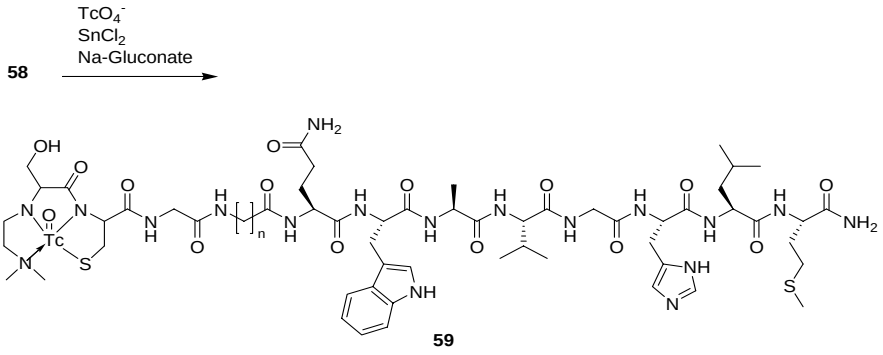
Figure 2. Radiometal chelate linked directly to the N-terminal amine group of BBN[7-14]NH_2.



57: *n* = 2
58: *n* = 4
59: *n* = 7
60: *n* = 10

Figure 3. Radiometal chelate linked to the N-terminal amine group of BBN[7-14]NH_2 via hydrocarbon spacer groups.

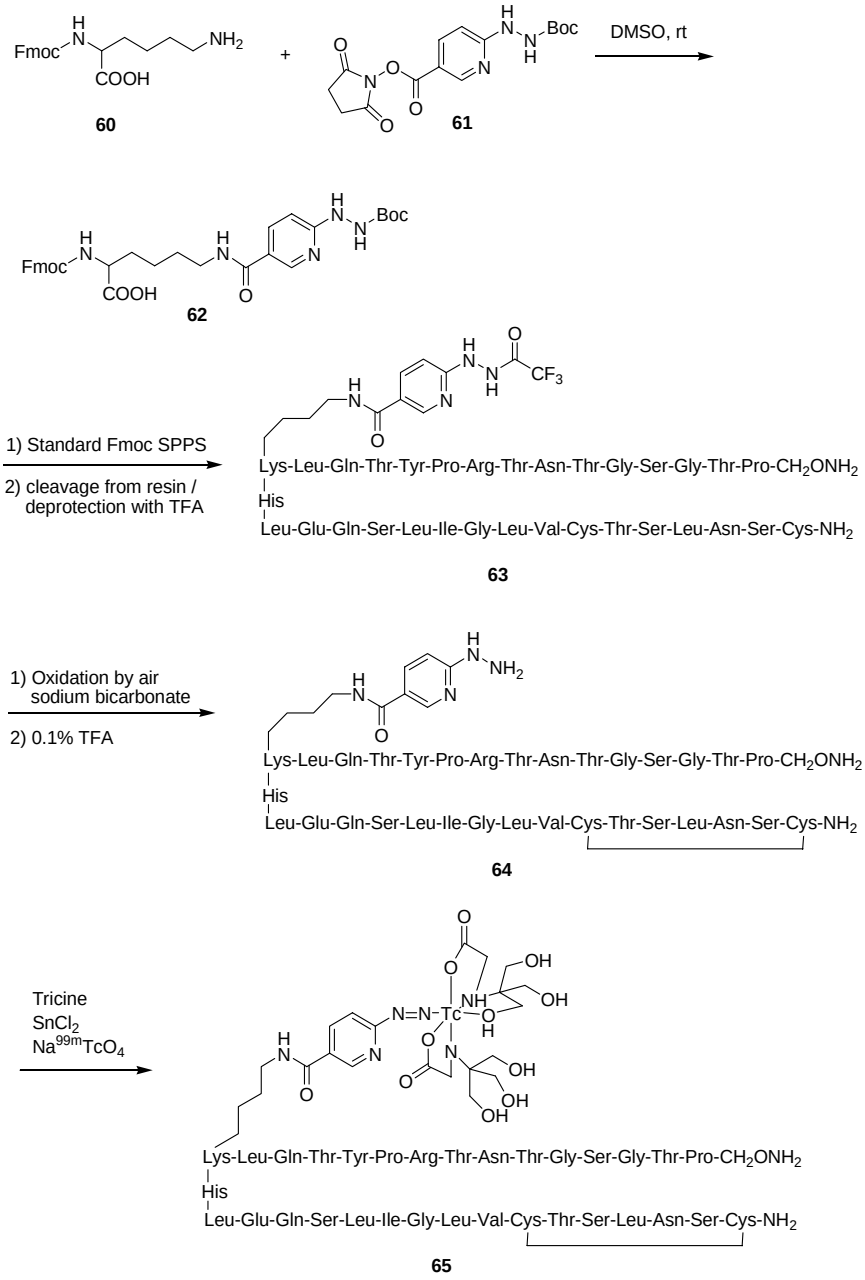


Scheme 14. Posttransmetalation of triamido-thiol bifunctional chelate with ^{99m}Tc.

2.4 Hydrazinonicotinyl acid (HYNIC) technetium conjugate

Blower and co-workers recently described a novel solid-phase synthesis approach in which a HYNIC derivative **61** of Fmoc-lysine was used as a metal-binding amino acid analogue.[33] The N-protected HYNIC derivative was successfully incorporated in a

bioactive peptide using standard Fmoc solid-phase peptide chemistry. Fmoc-*N*-ε-(Hynic-Boc)-Lys is a highly versatile technetium-binding amino acid and it was used to synthesize a technetium-99m-labeled salmon calcitonin with the HYNIC-linked amino acid in place of lysine-18. α-Fmoc-protected lysine **60** was treated with the NHS (*N*-hydroxysuccinimide) ester of Boc-protected HYNIC **61** to give the α-Fmoc-protected amino acid **62.** A trifluoroacetate group protected the HYNIC during alkaline oxidation to the cyclic disulfide and was readily removed by mild acid treatment. After deprotection and cleavage of the 32-amino acid sequence from the resin the peptide **63** was oxidized with air in 0.1 M $NaHCO_3$ under high dilution to form the respective disulfide-cyclized peptide **64**. After removal of the TFA-protecting group the peptide conjugate was labeled with Tc-99m.

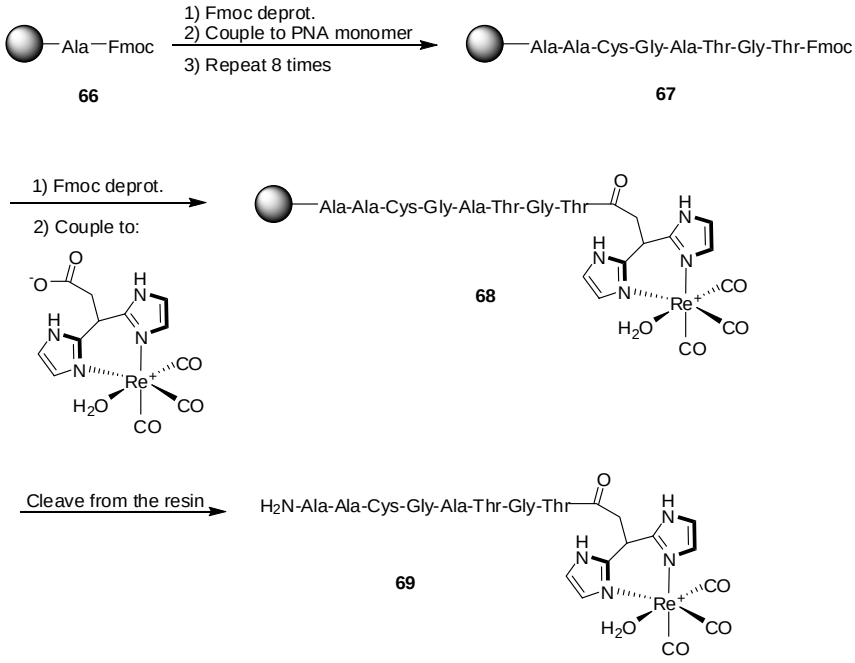


Scheme 15. Synthesis of Fmoc lysine-HYNIC derivative **62** and its use in peptide synthesis and subsequent Tc-99m labeling.

2.5 3,3-Bis(2-imidazolyl) propionic acid (bip-OH) rhenium conjugate

Metzler-Nolte et al. have reported the preparation of an organometallic metal-PNA conjugate.[34] Solid-phase synthesis was used to couple $Re(bip)(CO)_3$ fragments to PNA decamers on Tentagel resin with PAL linker and their interaction with complementary

DNA was studied. Such metal-PNA conjugates are of interest for the detection of complementary DNA or RNA due to the excellent hybridization properties of PNA.

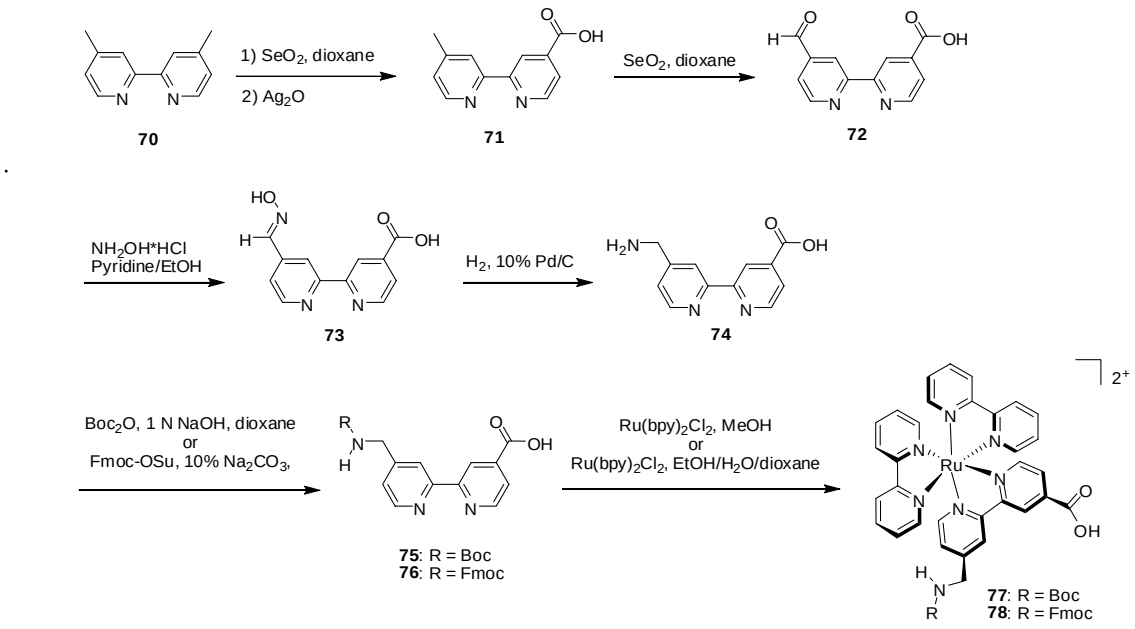


Scheme 16. Synthesis of rhenium-PNA conjugate applying a rhenium carboxylic acid to solid-phase peptide synthesis.

3. Iron, Ruthenium and Osmium (Group 8) Metal Complex – Peptide Conjugates

3.1 4´-Aminomethyl-2,2´-bipyridyl-4-carboxylic acid (Abc) ruthenium conjugate

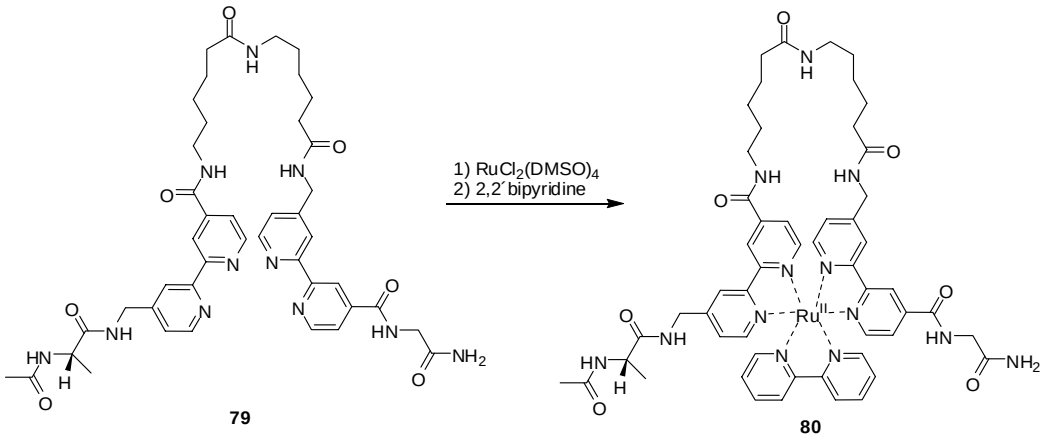
Tris-diimine metal complexes of 4´-aminomethyl-2,2´-bipyridyl-4-carboxylic acid (Abc) are of interest, since they possess a number of favourable properties including high stability, inertness to ligand exchange reactions, tuneable electronic structures, long lifetimes in fluid solution, and high quantum yields. Site-specific labeled ruthenium oligonucleotides were prepared by DNA solid-phase synthesis using a ruthenium-nucleoside phosphoramidite,[35] but this example lies not in the scope of this review. Another approach used bipyridyl amino acids and in particular Boc and Fmoc-protected Abc, which were incorporated into a hexapeptide.[36]



Scheme 17. Synthesis and metal complexation of Abc **74** and Boc/Fmoc protected derivatives.

Solid-phase synthesis of these metallopeptides was performed on MBHA resin using BOP and ByBOP as coupling reagents to provide high-affinity binding sites for ruthenium(II). Metal complexation occurred in solution followed by cleavage of the peptide from the solid support. The Abc residue bears the bipyridyl group not in a side chain but in the main peptide chain and is used as a tetradentate ligand to octahedrally coordinate and asymmetrically encapsulate a ruthenium(II) ion, creating a novel

peptide-caged redox-active metal complex. To prepare the Abc **74**, a dual oxidation strategy was employed (Scheme 17). First, 4,4′-dimethyl-2,2′-bipyridine **70** was selectively oxidized to the 4′-monocarboxylic acid derivative **71**. Second, the 4′-methyl group of **71** was oxidized with excess selenium dioxide to the aldehyde acid 4′-formyl-2′2-bipyridine-4-carboxylic acid **72**. Oxime formation with hydroxyl-amine in ethanol/pyridine smoothly converted **72** into compound **73**. Lastly, oxime acid **73** was transformed into the desired amino acid Abc **74** by catalytic hydrogenation. Amino acid **74** was converted into both Boc and Fmoc-derivatives for use in solid-phase peptide synthesis. Treatment of the Abc•HCl salt with di-(tert-butyl)dicarbonate provided Boc-Abc-OH **75** and similarly the reaction of Abc•HCl with Fmoc-succinimide furnished Fmoc-Abc-OH **76**. The metal complexation properties of bipyridyl solid-phase peptide synthesis of building blocks **75** and **76** were confirmed by the synthesis of their respective ruthenium(II) octahedral mixed-ligand complexes. Reaction of **75** and **76** with dichlorobis(2,2′-bipyridine)ruthenium(II) (Rub_2Cl_2) gave the bis-heteroleptic complexes **77** and **78**. To demonstrate the utility of Abc **74** in solid-phase peptide synthesis, a heptapeptide containing two Abc residues was synthesized to serve as a tetradentate caging peptide ligand for ruthenium(II) ions (Scheme 18). Two aminohexanoic acid residues (Ahx) were arranged as a bridging tether just long enough to form *cis*-bridged meridonal metal complexes. The C-terminal Gly residue was included to facilitate attachment of the bipyridine **77** or **78** to the sterically hindered MBHA resin, since direct coupling of Abc-OH to MBHA resin proved sluggish.

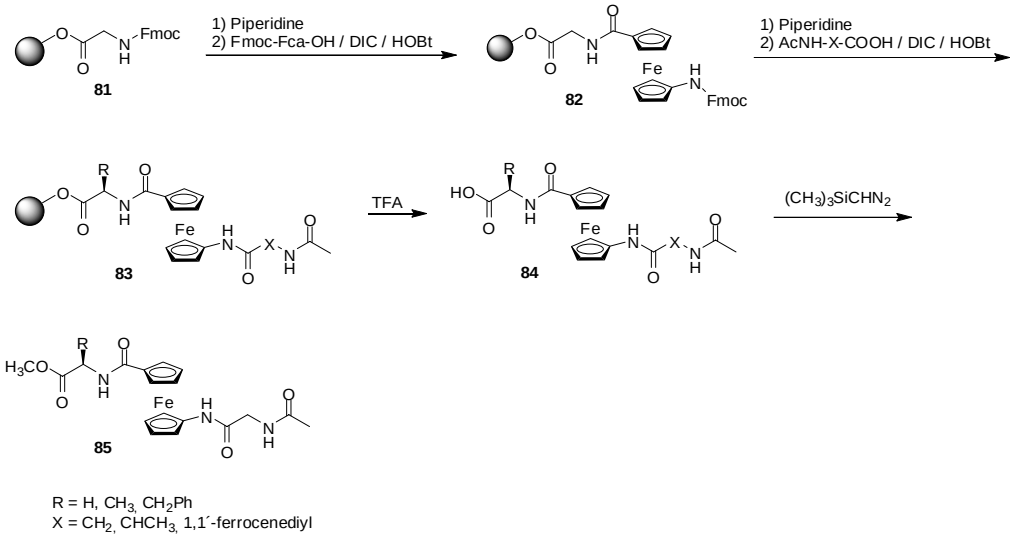


Scheme 18. Preparation of heteroleptic tris(bipyridyl) complex Ru^{II}(Aha)(bpy).

The acetylated hexapeptide amide Aha **79** was prepared by Boc/TFA strategy from Boc-Abc-OH **75** and other Boc amino acids using conventional reagents and procedures for manual solid-phase peptide synthesis. Coupling times and yields of **77** to the Gly-MBHA resin were remarkably improved by addition of stoichiometric amounts of the acylation catalyst DMAP. Following the assembly, apopetide **79** was cleaved from the resin with anhydrous HF and subsequent conversion of $Ru^{II}(Aha)Cl_2$ to the heteroleptic tris(bipyridyl) complex $Ru^{II}(Aha)(bpy)$ **80** was performed in solution.

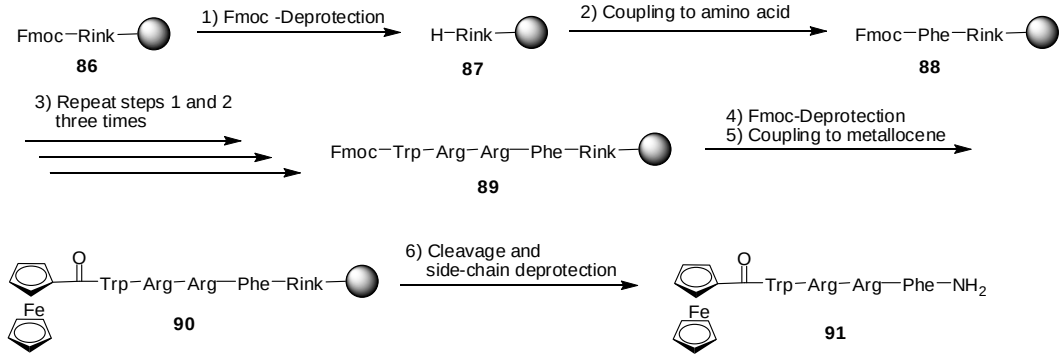
3.2 Metallocene (ferrocene)[37] conjugate

Ferrocene-containing tripeptides with one or two ferrocene building blocks were prepared by solid-phase peptide synthesis.[38] Heinze et al. incorporated the solid-phase peptide synthesis-compatible ferrocene building block Fmoc-protected 1´-aminoferrocene-1-carboxylic acid (Fca)[39] into the backbone of tripeptides. The coupling was performed using DIC/HOBt for activation and TentaGel-Wang, which turned out to be superior to polystyrene/divinyl resin, as solid support. Cleavage of the resulting tripeptides from the support with trifluoroacetic acid gave the mono- (Scheme 19) or diferrocene peptides. Reversible on-bead oxidation allows switching between the neutral ferrocene (low-affinity state) and charged ferrocenium ion (high affinity state), which results in superior anion-binding affinities.



Scheme 19. Synthesis of ferrocene-containing tripeptides with ferrocene building block via solid-phase peptide synthesis.

Metallocene-modified tri- to penta-peptides were identified to have antibacterial activities,[40] although the highest activity is still one order of magnitude lower than the minimum inhibitory concentration (MIC) values found for most naturally occurring antimicrobial peptides (AMPs). First Metzler-Nolte and co-workers synthesized metallocene-peptide bioconjugates where the amino acid sequence ranged from three to five residues by solid-phase peptide synthesis. The ferrocene and the cobaltocenium groups were introduced at the *N*-terminus by reacting ferrocene carboxylic acid hexafluorophosphate with the free amino group of the peptide **87**, while the peptide was attached to the solid support. Attention has to be taken during the cleavage from the Rink amide resin. Decomposition, that is loss of a ferrocenoyl moiety, occurs when TFA/H_2O/TIS cleavage mixture is used. However, this problem can be circumvented by the use of phenol rather than water.



Scheme 20. Solid-phase peptide synthesis of metallocene-peptide bioconjugates.

Later, Metzler-Nolte and co-workers hoped to arrive at small, readily available artificial AMPs with activity comparable to the best naturally occurring AMPs by adding metallocenes to more active peptide sequences.[41] Arg- and Trp-containig hexapeptide sequences which were shown to have good antibacterial properties[42] were selected and modified by replacing the N-terminal amino acid with a ferrocenyl (and a cobaltocenium) group. The metallocene peptide conjugates were prepared on Rink amide resin whereas the ferrocene carboxylic acid was attached by forming an amide bond with the free N-terminal amino group of the solid support. The ferrocene moiety is stable towards deprotection reagents and to resin cleavage, however, the ferrocenoyl peptides are only stable when phenol rather than water is used in the cleavage mixture.

Indeed, the activity of the resulting metallocene-pentapeptide conjugate [Fe(Cp)(C_5H_4)-C(O)-WRWRW-NH_2] **93** increased and was even better than 20 amino acid naturally occurring pilosulin, which was used as a positive control.

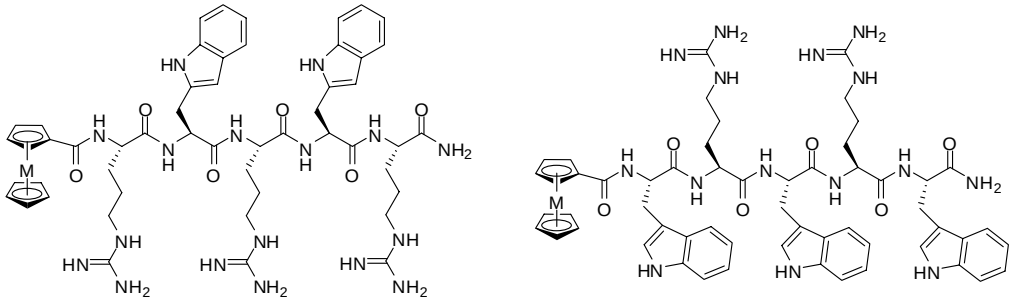


Figure 4. Metallocene-pentapeptide conjugate **92** and **93**.

4. Cobalt and Rhodium (Group 9) Metal Complex – Peptide Conjugates

4.1 Metallocene (cobaltocenium) conjugate

Although much work has been devoted to ferrocene bioconjugates,[35,43] the closely related cobaltocenium group has received considerably less attention although the cobaltcenium cation has a much higher redox potential and better chemical stability than ferrocene. Its unique electrochemical properties have, however, been exploited in enzyme biosensors and DNA detection,[44] and in a more recent study on the cellular uptake and directed nuclear delivery of a cobaltocenium-NLS peptide bioconjugate.[45] The lipophilic nature of the ferrocenyl moiety acts as a mimic for the bulky Trp residue, whereas the positively charged cobaltocenium moiety is isostructural to the neutral ferrocene thus allowing an assessment of additional positive charge, and thus acts as a bulky Arg-mimetic.[46] Capping the *N*-terminus of Arg- and Trp-containing hexapeptide sequences results in a net loss of one unit of positive charge in the case of the ferrocenoyl bioconjugates, but the cobaltocenium analogues retain the overall charge of the peptide, which is favorable for their antibacterial activity.

Metzler-Nolte and co-workers reported the first nonradioactive organometallic–peptide conjugate[47] which specifically delivers the organometallic species into the nucleus of a cell. Solid-phase peptide synthesis was used to prepare the cobaltocenium conjugate of a nuclear localization signal peptide. The cobaltocenium–NLS conjugate significantly accumulates in the nucleus of HepG2 cells. The heptapeptide H-Pro-Lys-Lys-Lys-Arg-Lys-Val-OH[48] was chosen as the antigen NLS which serves as an "address label" for proteins, and indicates their destination as being the cell nucleus. In addition, this heptapeptide enables the active transport of a variety of substrates through the nuclear pore complex.[49] An additional protected lysine residue was introduced at the *N*-terminus of the NLS peptide using Wang resin as solid support. Fluorescein isothiocyanate (FITC) was used as a label to visualize the metal conjugate inside the cells. For this purpose, the *N*-terminal lysine residue was modified with a Mtt protecting group. After coupling of the cobaltocenium moiety to the peptide, the *N*-terminal Fmoc-protecting group was cleaved and cobaltocenium carboxylic acid was coupled by use of TBTU. Cleavage from the resin and deprotection of all amino acid side chains were

accomplished by concentrated trifluoroacetic acid yielding the cobaltocenium-NLS peptide bioconjugate **94** (Figure 5).

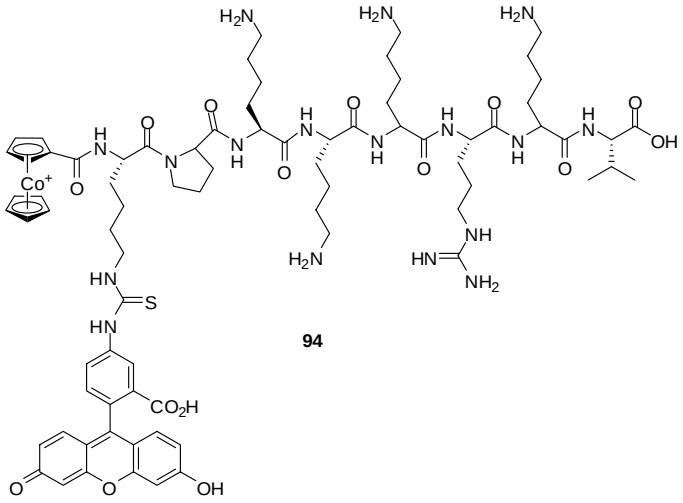


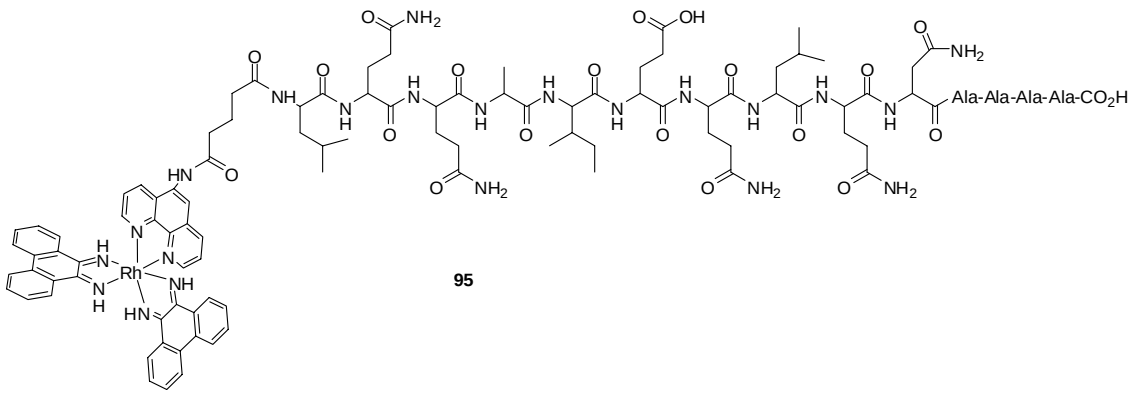
Figure 5. Cobaltocenium-NLS peptide bioconjugate **94**.

4.2 Phenanthrenequinone diimine (phi) rhodium conjugate

Barton and co-workers have focused on the development of peptide conjugates of rhodium(III) complexes as models for sequence-selective DNA binding proteins.[50] For this issue, a family of rhodium-peptide complexes (Figure 6) was synthesized by coupling short oligopeptides to the intercalating $([Rh(phi)_2(phen')]^{3+}$ (phi = phenanthrenequinone diimine; phen = phenanthroline) to explore whether the side-chain functionalities of small peptides may be used to augment metal complex recognition.[51] To summarize this work, DNA site-specificity depends on the peptide side-chain functional groups. Moreover, the phi complexes of rhodium cleave DNA upon photoactivation.

Barton and co-workers used two complementary solid-phase peptide synthesis strategies for the covalent attachment of phi complexes of rhodium(III) complexes to a specific site on synthetic peptides.[52] All natural amino acids except methionine were used in the synthesis, and peptides ranging from 5 to 30 amino acids were successfully coupled to the rhodium complex by standard solid-phase synthesis. The metal-peptide conjugates were either synthesized using the coordination method or by direct coupling.

In the coordination strategy the chelating ligand is first coupled onto the amino terminus of the peptide on the resin. Then, the resin-bound peptide containing the chelating ligand is reacted with $[Rh(phi)_2(DMF)_2](OTf)_3$, in a manner similar to the synthesis of the parent rhodium complex. In the direct coupling strategy**,** the coordinatively saturated metal complex is assembled first. Then the functionalized metal complex and the terminal amine of the peptide bound to the resin are condensed in a way that is analogous to the addition of another residue to the growing peptide chain. Several conditions for the synthesis were examined where peptides are constructed using both Fmoc and *t*-Boc methodologies and manual as well as automated solid-phase techniques. Furthermore, a range of coupling agents was examined using both strategies. To summarize, in the case of the coordination method, several different coupling reagents were used with similar success. These reagents include DCC/HOBt, DSC, TBTU, and TSTU. With the direct coupling method, Barton et al. observed that the presence of the metal centre makes the coupling reaction less efficient. The metal-peptide complexes are more difficult to be cleaved off the resin than the peptide alone. Several linkages to the resins such as MBHA, PAM, and PEG-PAM were also examined, but variation in the linker does not affect the yield of the cleaved product. The presence of the metal complex, does, however, significantly decrease the overall yield; furthermore it tends to inhibit the coupling reaction since coordination on the resin is of lower efficiency than the coordination of the metal complex alone in solution. In conclusion, both strategies offer distinct advantages over solution phase methods, in that functionalization of side chains is precluded. Thus, selective attachment of the metal centre to a specific residue or to the N-terminus can be reliably accomplished.

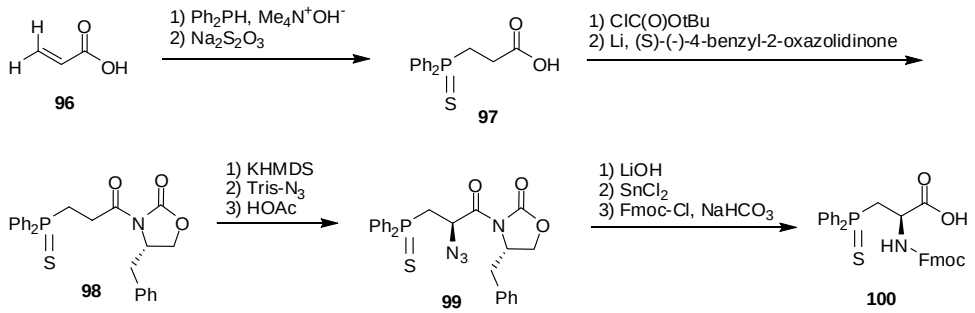


95

Figure 6. Phenanthrenequinone diimine rhodium peptide conjugate.

4.3 Diphenylphosphineoserine (Pps) rhodium conjugate

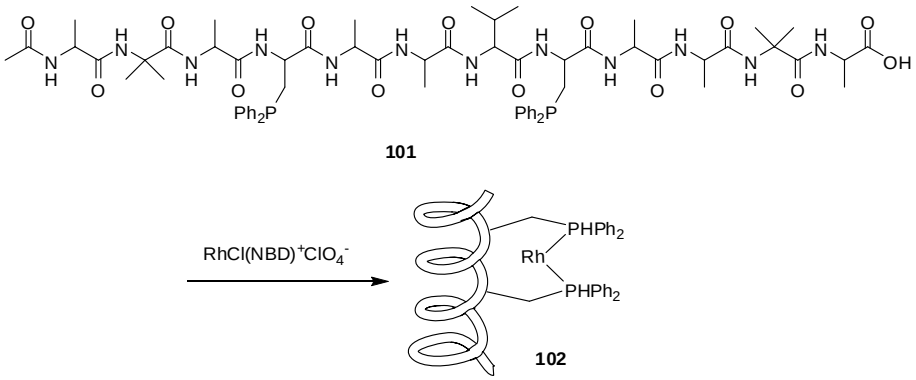
Gilbertson et al. reported over the last decade important examples of resin bound peptide based phosphine transition metal complexes.[53] Rhodium was used to prepare the first peptide-phosphine-metal complexes. For the incorporation of a phosphine-containing amino acid building block it was necessary to prevent the undesirable formation of phosphine oxide. To overcome this problem, a temporary conversion of the phosphine to the phosphine sulphide[54] gave rise to an amino acid that could be used in standard coupling procedures. The best route to the required amino acid involved the use of Evans´chiral oxazolidinone chemistry (Scheme 21). Addition of diphenylphosphine to acrylic acid proceeded smoothly using tetramethylammonium hydroxide as a base. Treatment with sodium thiosulfate converted the phosphine to the phosphine sulphide **97**. Acid **97** is then converted to the amino acid by formation of the oxazolidinone **98**. Cleavage of the chiral auxiliary and reduction of the azide **98** with tin(II) chloride gives amino acid **99** which is finally converted to the Fmoc protected amino acid **100** ready for peptide synthesis.



Scheme 21. Synthesis of a phosphine-containing Fmoc protected amino acid building block for use in solid-phase peptide synthesis.

Once the desired peptide was assembled, the phosphine was regenerated by desulfurization with Raney nickel.[55] The phosphine-containing amino acids were incorporated in *i*, *i* + 4 position to stabilize helix formation and thus be able to chelate one metal ion between them (Scheme 22). The peptide conjugate was synthesized by standard Fmoc solid-phase peptide synthesis on Wang resin and the diphenylphosphineoserine (Pps) was incorporated as a dipeptide with alanine (Fmoc-

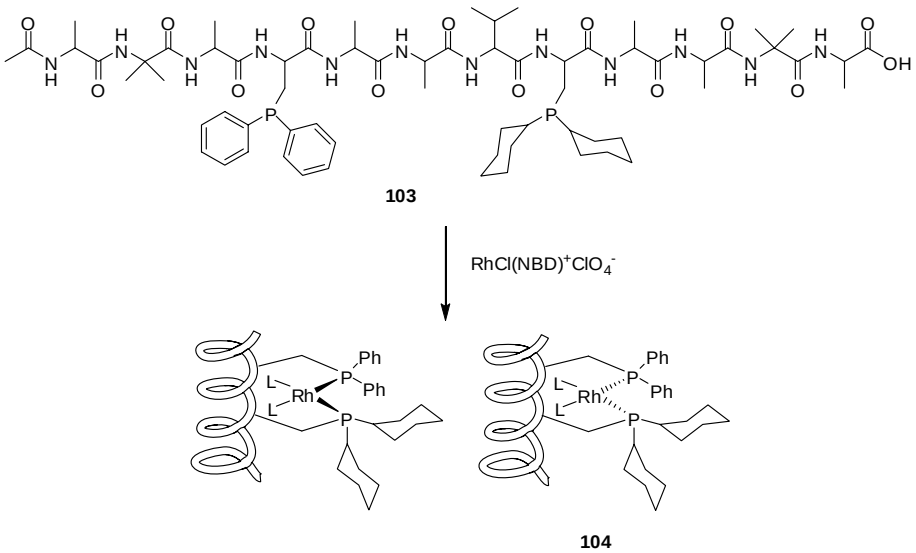
Pps(sulphide)-Ala-OPfp).[56] The resulting bis-phosphine ligand **101** was complexed with rhodium by stirring with $RhCl(NBD)^{+}ClO_4^{-}$ (NBD = norbonadiene).



Scheme 22. Complexation of the bis-phosphine ligand **101** with a rhodium-salt.

4.4 Mixed bidentate Pps,Cps-based rhodium conjugate

The diphenylphosphineoserine (Pps) group was later incorporated into a 12-residue peptide **103** along with a dicyclohexylphosphineoserine (Cps) (Scheme 23).[57] The synthesis of the peptide conjugate, as well as the rhodium complexation, was done as described before, but on polystyrene resin.

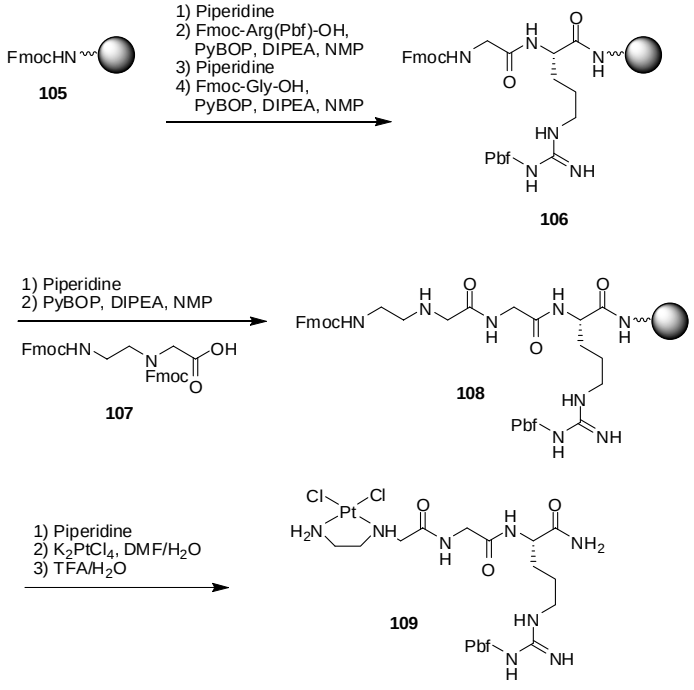


Scheme 23. Mixed bidentate Pps,Cps-based rhodium conjugates.

5. Nickel, Palladium, Platinum (Group 10) Metal Complex – Peptide Conjugates

5.1 Ethylenediamine platinum conjugate

In 2000 Reedijk et al. reported the first synthesis of a trimeric arginine-containing peptide-dichloroplatinum(II) complex with potential antitumor activity by solid-phase synthesis.[58] An ethylene diamine moiety, which serves as a platinum-chelating ligand was tethered to a resin-bound arginine-glycine dipeptide. The solid-phase peptide synthesis was performed on Rink amide resin with commercially available protected amino acids Fmoc-Arg(Pbf)-OH and Fmoc-Gly-OH following a standard Fmoc protocol.[59] Fmoc-protected *N*-2-aminoethyl-glycine derivative **107** was then condensed to the dipeptide **106** followed by platination of the ethylenediamine moiety, subsequent deprotection and release from the solid support (Scheme 24). Preliminary resin-cleavage experiments with TFA/H_2O/(TIS) led to metallic platinum and free ligand probably due to the reduction of the coordinated $PtCl_2$ moiety by the scavenger TIS. However, nearly quantitative complexation was achieved by treatment with excess K_2PtCl_4 in DMF/H_2O followed by a resin cleavage with TFA/H_2O.

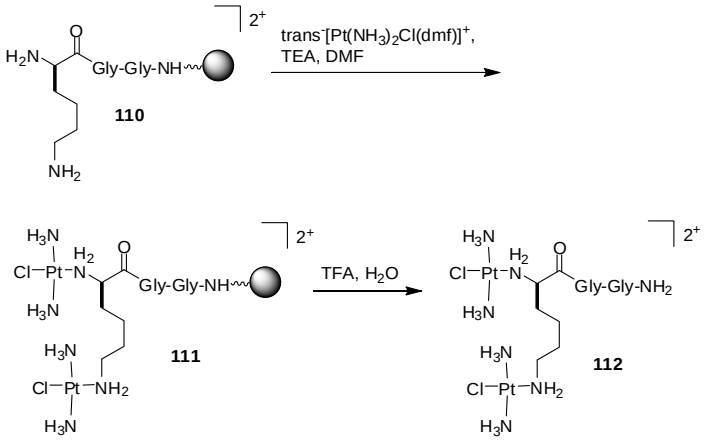


Scheme 24. Solid-phase synthesis of platinum complex **109**.

Later, Reedijk and co-workers examined the scope and generality of the solid-phase platination approach preparing a six by six array of individual dichloroplatinum peptide analogues.[60] The parallel solid-phase peptide synthesis of dichloroplatinum-peptide array was performed on Rink amide resin with six natural amino acids on an automated synthesizer. Unfortunately, these platinum peptide complexes showed no use as cytotoxic agents, but only demonstrated the utility of solid-phase peptide synthesis for the preparation of platinum drugs. However, in a subsequent publication,[61] Reedijik et al. reported on cytotoxic platinum tripeptide complexes, although the highest activity, which was measured for the tripeptide conjugate containing the Gly-Gly dipeptide, was still lower than cisplatin.

5.2 Dinuclear $N_{\alpha,\varepsilon}$-L-lysine platinum conjugate

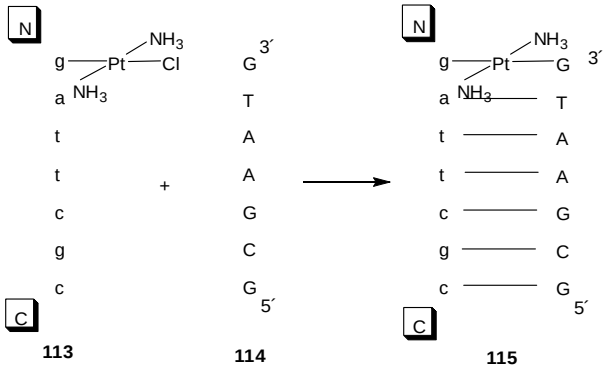
As an extension of these studies, Reedijk et al. described the first solid-phase peptide synthesis of dinuclear lysine bridged platinum(II) complexes.[62] Platination of the lysine was achieved with 5 fold excess of activated *trans*-platin to give the immobilized compound **111** (Scheme 25). To avoid strong acidic cleavage conditions considering the moderate stability of the immobilized platinum complex **111**, Rink amide MBHA was used in combination with the 2-chlorotrityl linker, which allow mild cleavage conditions.



Scheme 25. Solid-phase synthesis of platinum complex **112** by metallation on solid support.

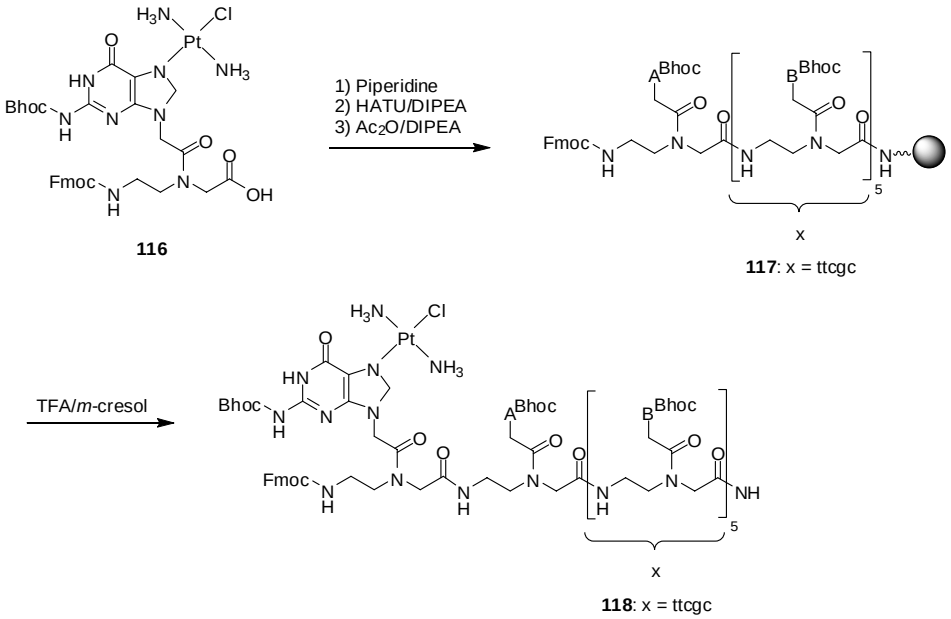
Both linkers were suitable for the solid-phase peptide synthesis of dinuclear trans-platinum complexes. Biological testing of the platinum complexes showed their potential as anticancer agents. However, compared to cisplatin, compound **112** revealed a 60 fold decrease in activity.

Metal complexes of suitable geometry and coordination properties are promising cross-linking agents.[63] One application of metal complex cross-linking is to increase the affinity of an antisense oligonucleotide to its target.[64] Lippert et al. used this strategy in a model cross-linking reaction of the monofunctional *trans*-Pt-modified PNA oligomer *trans*-$[(NH_3)_2Pt(g\text{-}N7\text{-}attcgc)Cl]^+$ **113** with its complementary deoxyoligonucleotide 5´d(GCGAATG) **114** (Scheme 26).[65] The *trans*-Pt(II)-modified building block **116** was synthesized by the reaction of *trans*-$[Pt(NH_3)_2Cl(DMF)]BF_4$ with Fmoc/Bhoc-PNA G.



Scheme 26. Cross-linking reaction of PNA **113** with DNA **114**.

Building block **116** was then coupled to the Rink amide-bound fully protected PNA oligomers using the coupling reagent HATU. Removal of the Bhoc protecting groups along with the release from the solid support was effected with TFA/*m*-cresol (Scheme 27). In summary, this methodology allows the preparation of monofunctional *trans*-Pt(II)-modified mixed pu/pym PNA oligomers, which have shown to cross-link sequence-specifically with a target oligonucleotide.

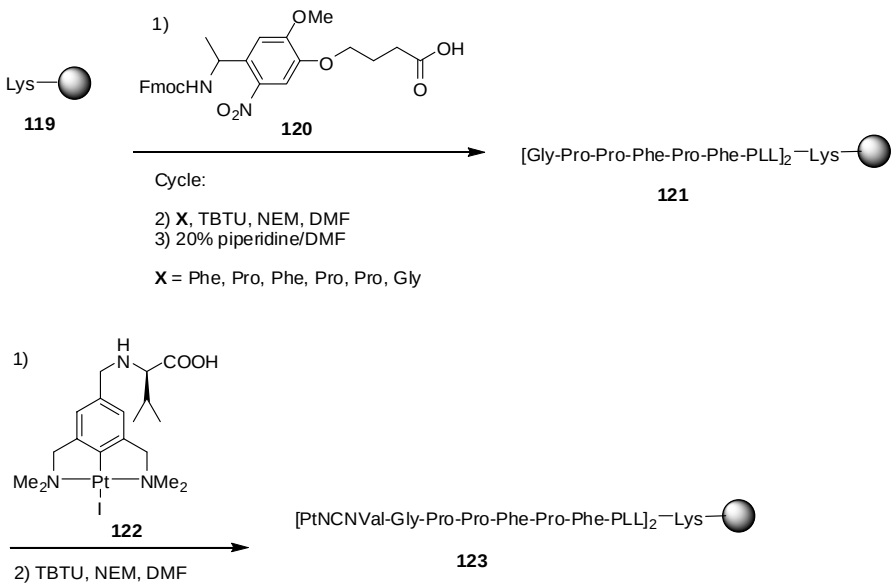


Scheme 27. Solid-phase synthesis of a monofunctional *trans*-Pt^{II}-modified PNA oligomere.

5.3 Tetradentate monoanionic "pincer" NCN [$C_6H_2(CH_2NMe_2)_2$-2,6-R-4)$^-$] platinum conjugate

Van Koten et al reported a robust organoplatinum(II) biomarker which can be incorporated in peptides using standard solid-phase coupling techniques.[66] The biomarker-containing peptides can be identified by the addition of an aqueous KI_3 solution causing visually detectable coloured resin beads. For the almost instantaneous change of colour from colourless to deep purple, capping of only 6 % of the available amine termini of the resin bound peptide is sufficient. Furthermore, this colouration process is reversible by washing with DMF/Et_3N or DMF/morpholine solutions. $PEGA_{1900}$ resin (a copolymer of bis(2-aminopropyl)poly-(ethylene glycol)/acrylamide) was chosen as the solid support since it combines good characteristics for organic synthesis and screening in aqueous buffer solution, which is required for a biomarker-function in solid-phase screening assays. First a lysine residue was coupled to the resin to double its loading capacity. The peptide sequence Gly-Pro-Pro-Phe-Pro-Phe was synthesized on a photolabile linker[67], using syringe technology[68] and Fmoc/OPfp-derivatized amino acids, which were activated with Dhbt-OH. Finally, the *N*-protected

platinum(II) biomarker **122** was attached to the *N*-terminus of the resin-bound peptide **121** using TBTU and NEM activation.



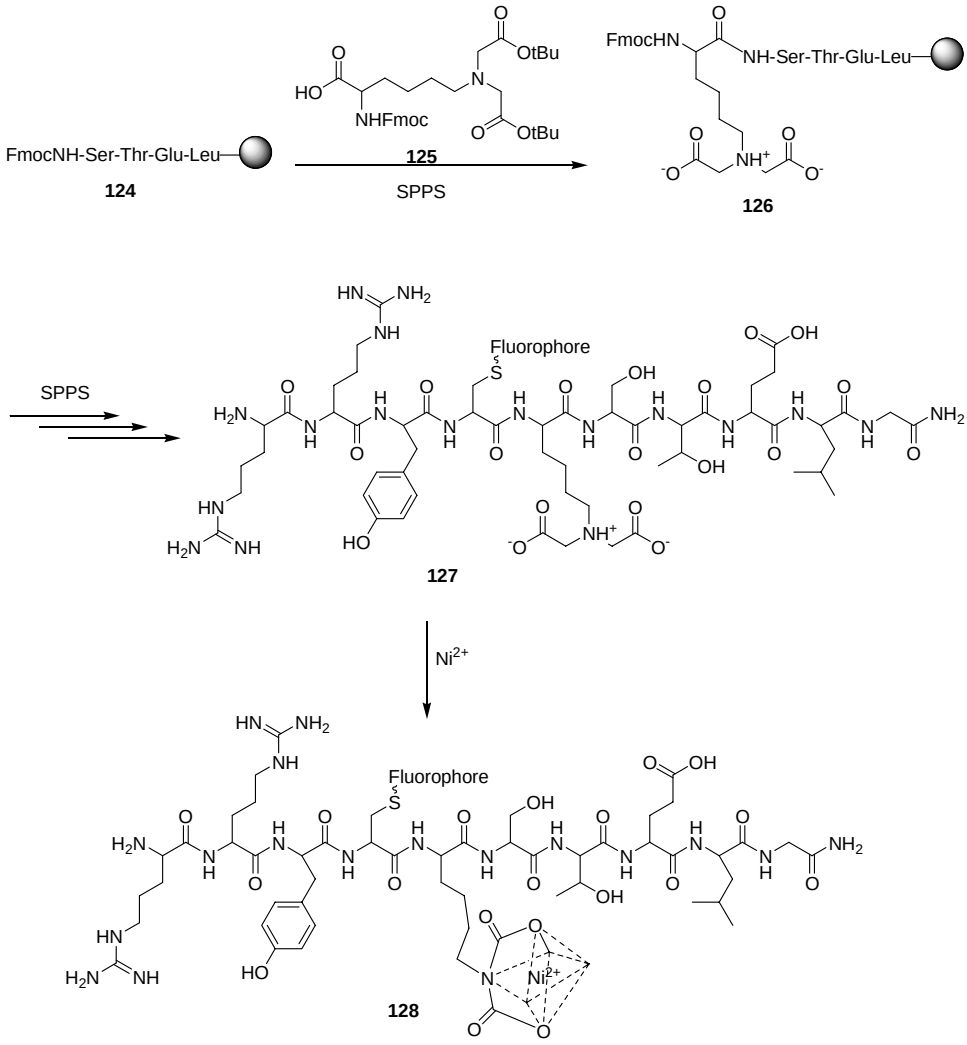
Scheme 28. Solid-phase synthesis of a platinum-biomarker-containing peptide using metal containing amino acid **122**.

5.4 Iminodiacetic acid (IDA) nickel conjugate

Metallopeptides of the general form Ni(II)AA_1-AA_2-His[69] are used in biochemical analysis of protein-nucleic acid and protein-protein interactions.[70] Long and co-workers prepared two libraries derived from AA_1-AA_2-His sequence in which the first and the second positions of the peptide ligand were varied.[71] Standard *t*-Boc protocols on methylbenzydryl amine (MBHA) resin were used including all possible combinations of 18 natural α-amino acids excluding Cys and Trp to prevent disulfide formation and partial DNA intercalation[72] of these residues. The optimized metallopeptide Ni(II)-Pro-Lys-His was found to cleave DNA one order of magnitude better than Ni(II)-Gly-Gly-His.

Tampé and co-workers synthesized a metal-chelating amino acid building block for synthetic receptors.[73] Such synthetic receptors bearing an IDA-chelate were employed as metal ion sensors and as receptors for histidine-tagged proteins. Standard solid-phase peptide synthesis was used to incorporate the SAAC **125** into a polypeptide (Scheme 29). The peptide conjugate was further labeled with fluorescein at a cysteine residue[74] to

signal metal-ion binding. After release of the IDA-peptide **127** from the resin, it was treated with Ni^{2+} and several experiments were performed which demonstrated a strong binding to imidazole.

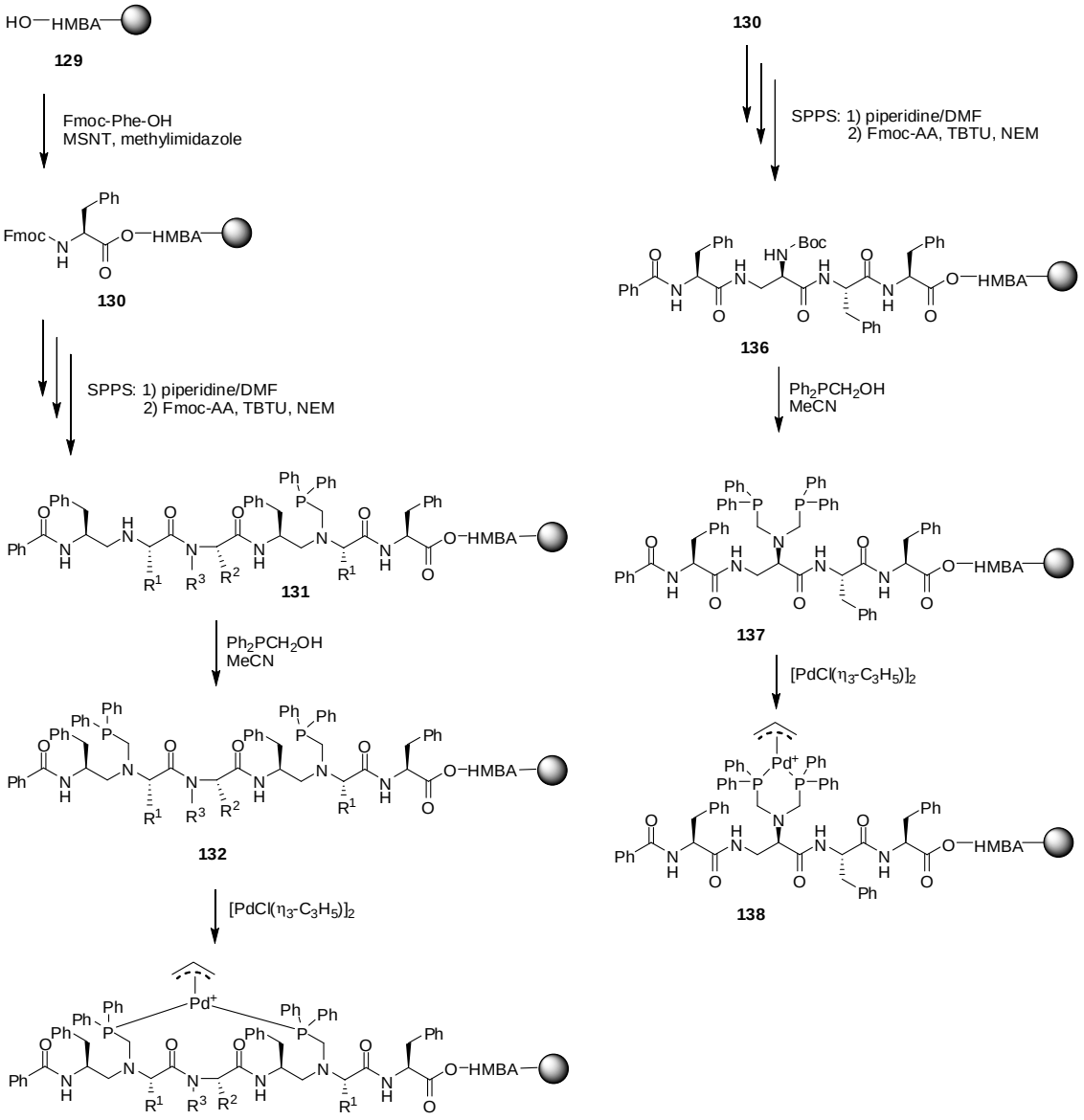


Scheme 29. Solid-phase peptide synthesis of fluorescein-labeled IDA peptide 127 and subsequent metallation in solution to nickel complex **128**.

5.5 Bidentate phosphine palladium conjugates

Palladium(II) allyl complexes were prepared by Meldal and co-workers from resin bound ligands to demonstrate their catalytic properties.[75] The palladium complexes **133-135** and **138** were synthesized on solid support using Fmoc protected amino acids and Fmoc protected amino aldehydes. Phosphine moieties were introduced by

phosphinomethylation of the free amines as the final solid-phase synthetic step, prior to complexation with palladium. $PEGA_{1900}$ resin[76] was selected due to its excellent swelling properties in organic solvents, as well as in water. After the $PEGA_{1900}$ resin was functionalized with Fmoc-glycine by TBTU activation and subsequent Fmoc-deprotection with piperidine, the HMBA linker was introduced by TBTU. The HMBA linker can be efficiently cleaved under mild conditions and is also suitable for on-bead NMR analysis, since it possesses no stereocenter making the analysis more difficult.

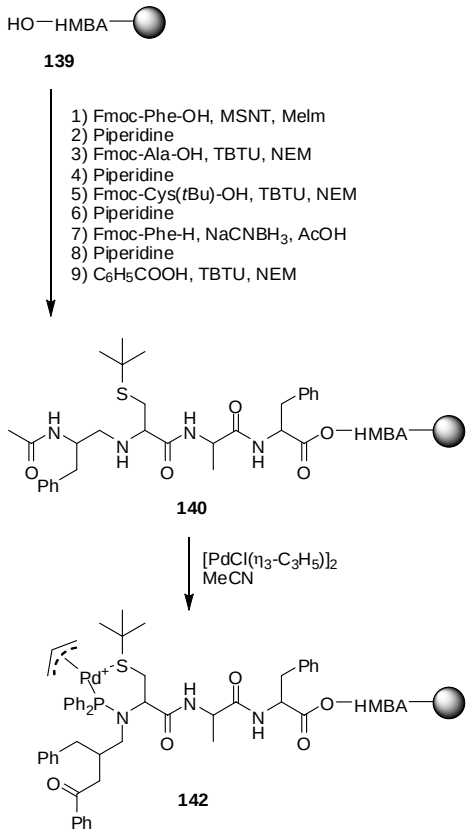


Scheme 30. Formation of palladium(II) allyl complexes on solid support.

The first amino acid Fmoc-phenylalanine was attached to the HMBA linker by MSNT activation in dichloromethane (Scheme 30). For all the other couplings TBTU was sufficient. The resulting peptide-based bidentate phosphine palladium conjugates **132** and **137** were shown to be suitable for palladium catalyses of asymmetric allylic substitution reactions.

5.6 Bidentate P,S-based palladium conjugates

Recently, bidentate mixed heteroatom ligands have proven to be very successful for asymmetric organic synthesis.[77] One class of such ligands are P,S-ligands,[78] which have been successfully applied in palladium-catalyzed allylic substitution reaction.[79] Meldal et al.[80] expanded their above mentioned methodology for the solid-phase synthesis of peptide-based bidentate phosphine ligands to the solid-phase peptide synthesis of P,S-bidentate chelating palladium(II) complexes exploiting the readily available chiral pool of cysteine derivatives.

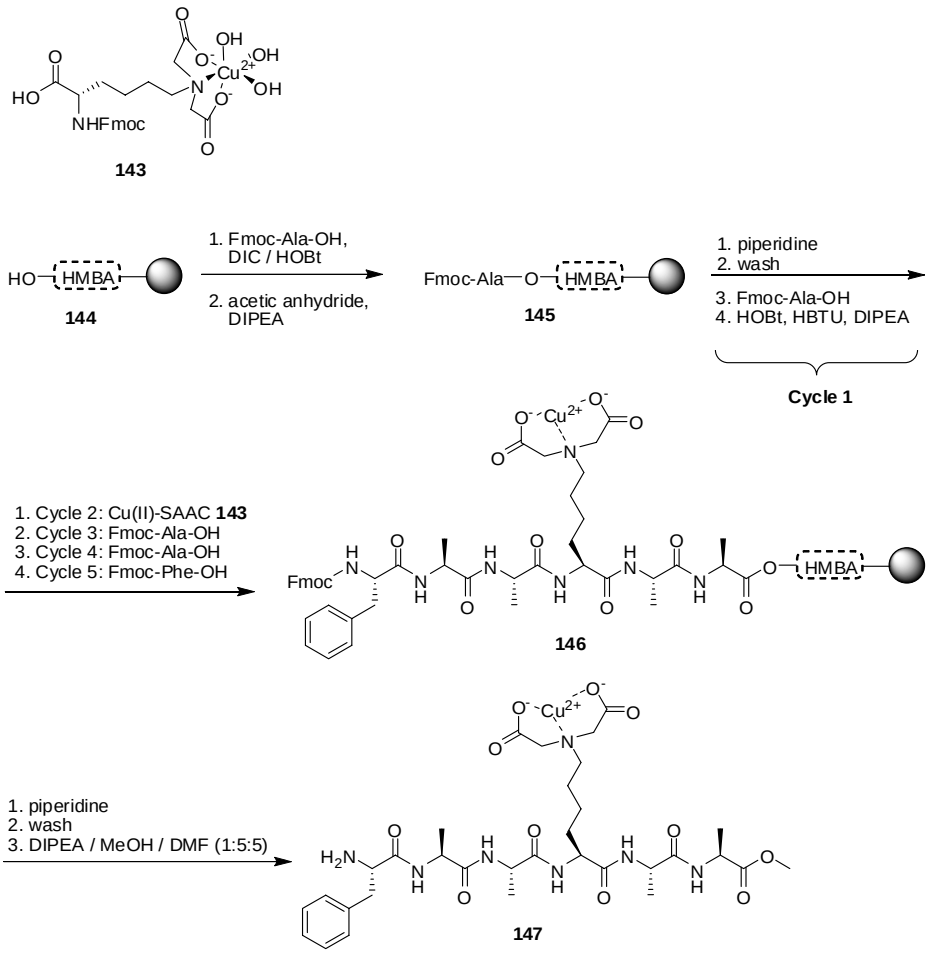


Scheme 31. Palladium catalyst derived from a solid-phase synthesized peptide scaffold and metallation on solid support.

6. Copper (Group 11) Metal Complex – Peptide Conjugates

6.1 IDA copper conjugate

Peptides with metal complexes in their side chains and peptide-metal complex conjugates have been used to enhance or control binding affinities and peptide conformations.[81] To extend the scope of solid-phase synthesis of peptide – metal complex conjugates König et al.[82] reported their preparation from modified amino acids bearing metal complex ligands or metal complexes in their side chains.



Scheme 32. Incorporation of Cu^{II}-IDA SAAC into a peptide sequence.

The IDA motif, known for its ability to bind imidazole residues and *N*-terminal His was chosen and converted into its copper complex as a SAAC. This modified amino acid **143** was incorporated into a peptide sequence using standard solid-phase peptide synthesis. The use of HMBA-AM resin allowed nucleophilic cleavage of the copper peptide conjugate from the resin without decomplexation.

6.2 Bis(2-picolyl)amine (bpa) metal conjugate

As discussed above, radioactive metals were successfully and extensively applied to radioimaging e.g. with ^{99m}Tc complexes. Thus control of cellular uptake and metal ion localization is a challenge for medicinal inorganic chemistry. However this concept is not widely applied to non-radioactive metals. Copper on the other hand plays an important role in cell regulating processes, but in certain cells there is not one single free copper ion.[83] Any Cu^{2+} ion is sequestered by so-called Cu chaperones, proteins which also serve to deliver the metal to specific Cu enzymes.[84] For such systems, Metzler-Nolte et al. proposed to use bioconjugates of metal-chelating ligands, linked to physiologically active peptides.[85] The preparation of metal-bpa complexes linked to amino acids and a cellular localization signal peptide, namely, a nuclear localization signal[86] (nls) was reported. The nls peptide Metzler-Nolte et al. used in their work is a heptapeptide with primary sequence H-Pro-Lys-Lys-Lys-Arg-Lys-Phe-OH and serves as a tag to proteins, indicating their destination in the nucleus of cells.[87] The nls-bpa bioconjugate **148** (Figure 7) was prepared by Fmoc solid-phase peptide synthesis on Rink amide resin with an acid labile linker and acid labile side chain protecting groups for amino acids Lys (Boc) and Arg (Pbf) were used. The peptide synthesis cycle was composed of Fmoc deprotection by piperidine and TBTU coupling. Metal complexation was carried out in aqueous solution with $Cu(NO_3)_2$. The formation of the complex **148-Cu** was immediately apparent by the deep blue colour of the solution due to a blue shift of the Cu d-d transition in the Cu(bpa) complex. The metal-peptide conjugates were

suggested as artificial metallochaperones, because they have the potential to deliver metal ions to specific compartments in the cell as determined by the peptide moieties.

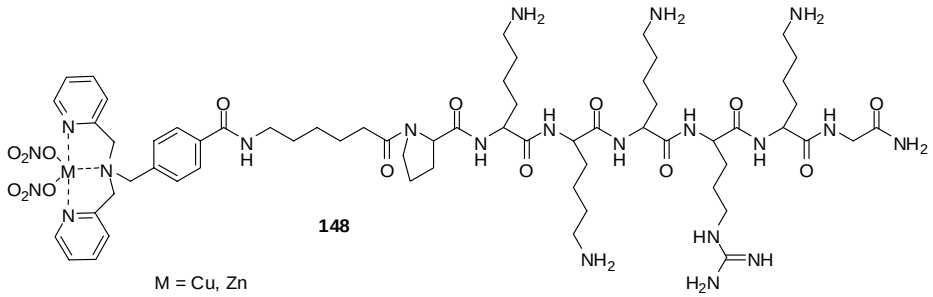


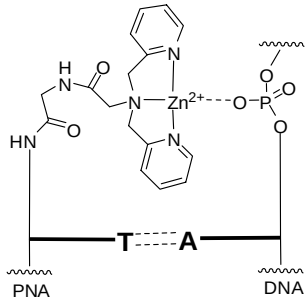
Figure 7. Structure of metal bioconjugates **148-M**. M = Cu(II) or Zn(II).

7. Zinc (Group 12) Metal Complex – Peptide Conjugate

7.1 Bpa zinc conjugate

Metzler et al. showed that also Zn^{2+} binds to their nls peptide conjugate **148**. The Cu(II) and Zn(II) complexes were characterized including X-ray structure analyses and the results indicated similar structural features of the transition metal complexes.

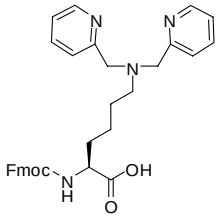
Kraemer and co-workers prepared conjugates of peptide acids (PNA) and metal binding ligands using solid-phase synthesis.[88] The ligands were attached to PNA via linkers of different length for optimization of metal complex–DNA interaction. Synthesis of conjugates was accomplished using sequential coupling/deprotection steps of the required number of Fmoc-Gly-OH building blocks to the terminal amino-group of Rink-resin bound PNA. Amination with bis-(2-picolyl)-amine, PNA deprotection and cleavage gave conjugates of 2-picolylamine. Equimolar concentrations of bioavailable metal ions, Ni^{2+}, Zn^{2+} and Cu^{2+} were used in the complexation step and the affinity of the metal-bpa conjugates to DNA (Figure 8) was shown to be strongly dependent upon the sort of the metal, in the order Ni^{2+}, $Zn^{2+} > Cu^{2+}$.



149

Figure 8. A proposed approach for metal-dependent binding of PNA probes to oligonucleotide targets.

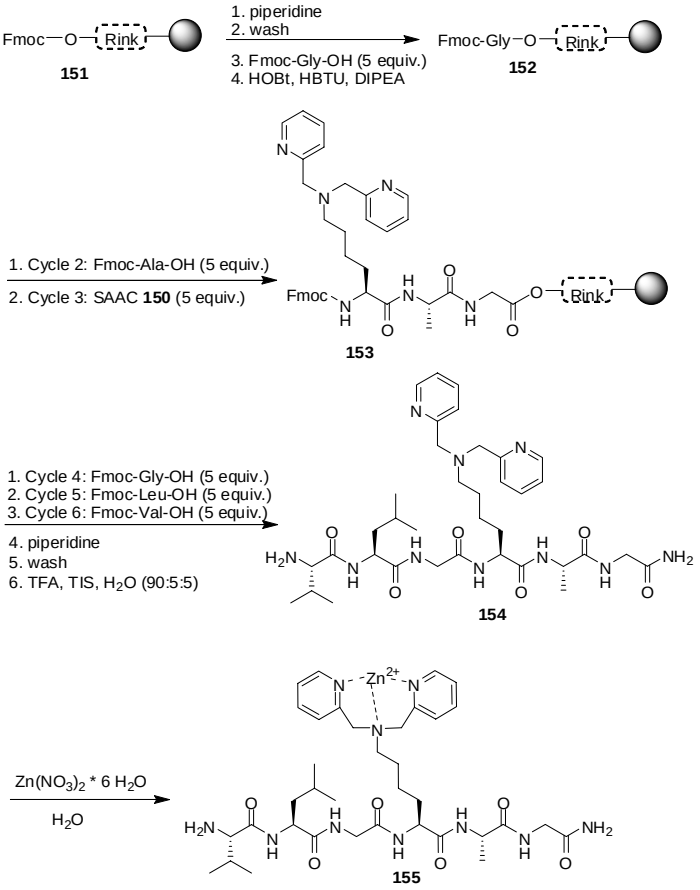
König et al. reported solid-phase peptide synthesis protocols where position and number of SAAC and metal complexes thereof may vary.[89] Peptide–metal complex conjugates were either obtained by incorporation of the metal coordinated SAAC followed by mild nucleophilic resin-cleavage or by complexation in metal salt solution after cleavage from the resin.



150

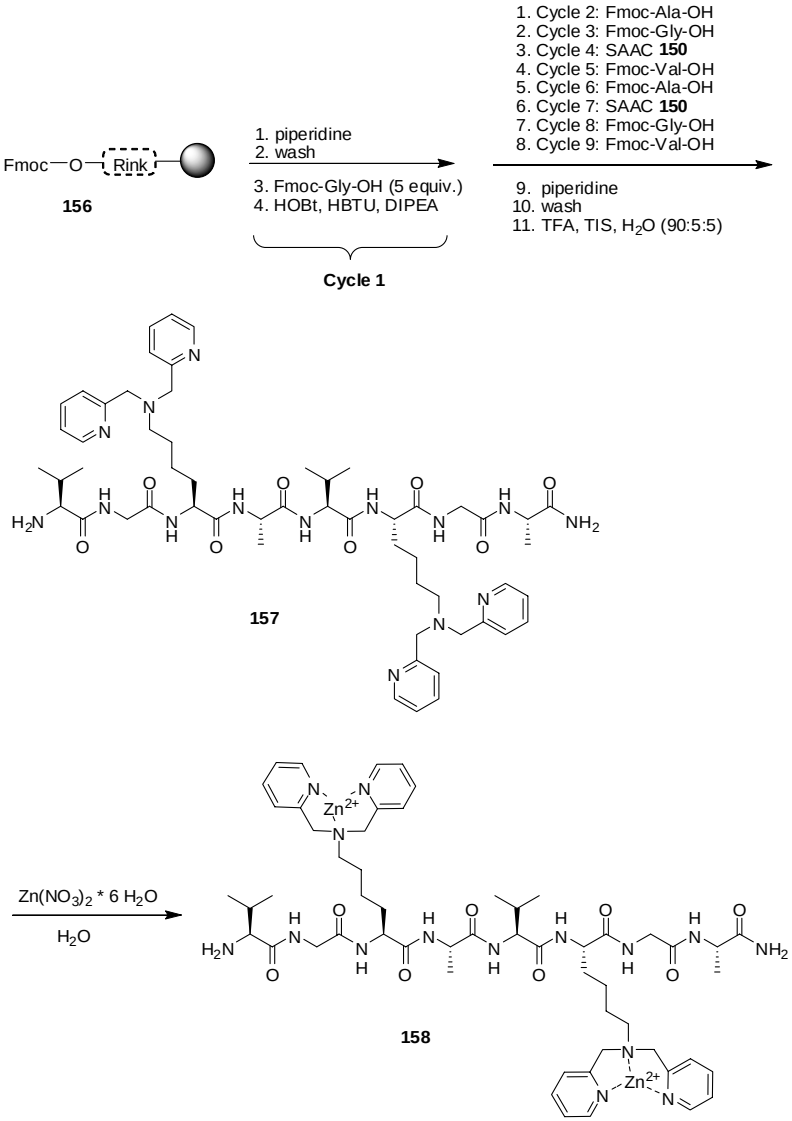
Figure 9. Bpa SAAC **150**.

A bpa containing peptide (Scheme 33) and a dinuclear peptide receptor (Scheme 34) based on the bpa-chelate were synthesized on Rink amide resin using solid-phase peptide synthesis. Fmoc protected aliphatic amino acids and SAAC **150** (Figure 9) were coupled with HBTU, HOBt and DIPEA in NMP/DMF using conventional frit-equipped syringe technique. After cleavage from the resin, the peptide conjugates were treated with $Zn(NO_3)_2$ in an aqueous solution.



151 **152** **153** **154** **155**

Scheme 33. Solid-phase peptide synthesis of peptide conjugate **154** and subsequent metallation to peptide metal complex **155** in solution.



Scheme 34. Solid-phase peptide synthesis of bis-bps ligand **157** and subsequent metallation to dinuclear peptide metal complex **158** in solution.

To expand the versatile solid-phase approach, an already metal coordinated bpa-derived Fmoc-amino acid **159** (Figure 10) was incorporated into the peptide chain.

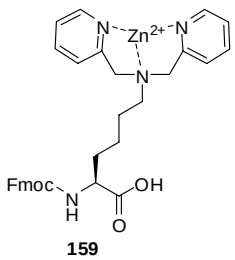
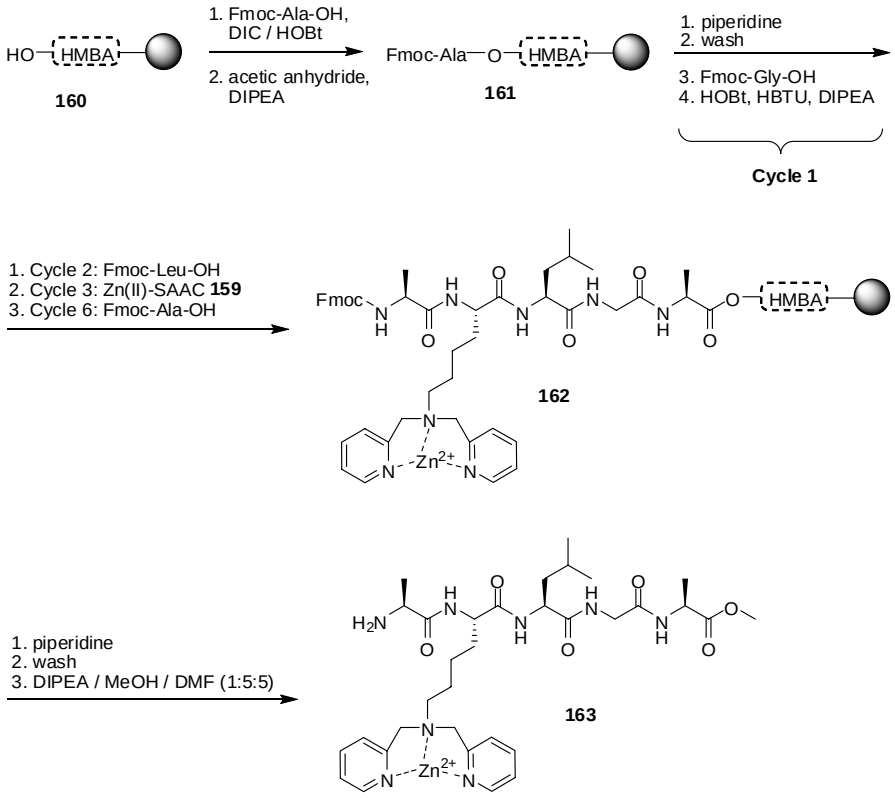


Figure 10. Zn^{II}-bpa SAAC **159**.

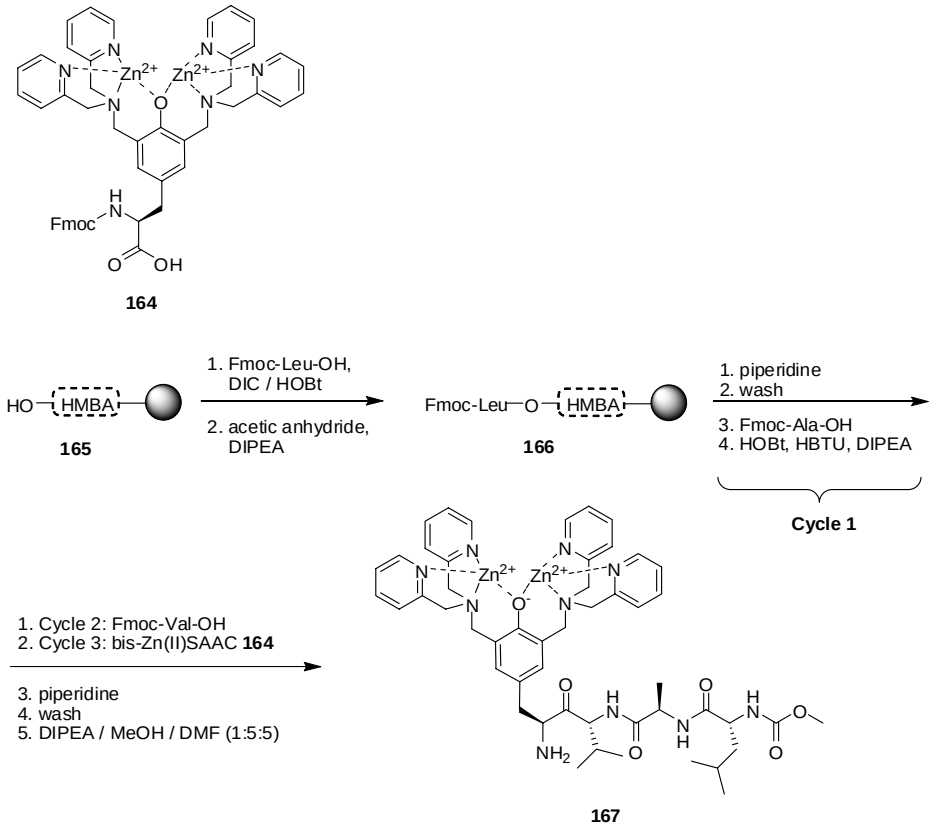
To avoid the loss of metal ions under acidic conditions, which are necessary to cleave from Rink amide resin, HMBA-AM was used as resin, as it allows nucleophilic cleavage of the peptide (Scheme 35).



Scheme 35. Direct solid-phase peptide synthesis of metal-peptide conjugate **163** on HMBA-AM resin using metal containing amino acid **159**.

7.2 Bis-bpa zinc conjugate

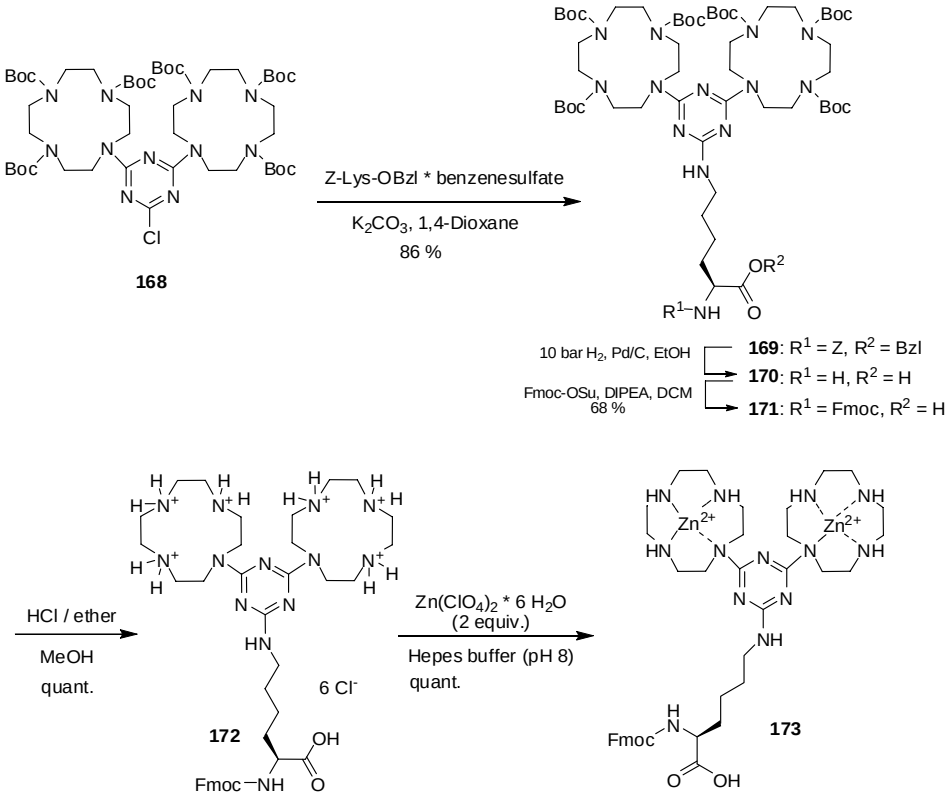
Bis(Zn^{II}-chloride)-SAAC **164** was also successfully used in solid-phase peptide synthesis. Cleavage with a solution of DIPEA/MeOH/DMF gave the metallated peptide bis-bpa-zinc complex **167**.



Scheme 36. Incorporation of bis(Zn^{II}-chloride)-SAAC **164** in a peptide sequence.

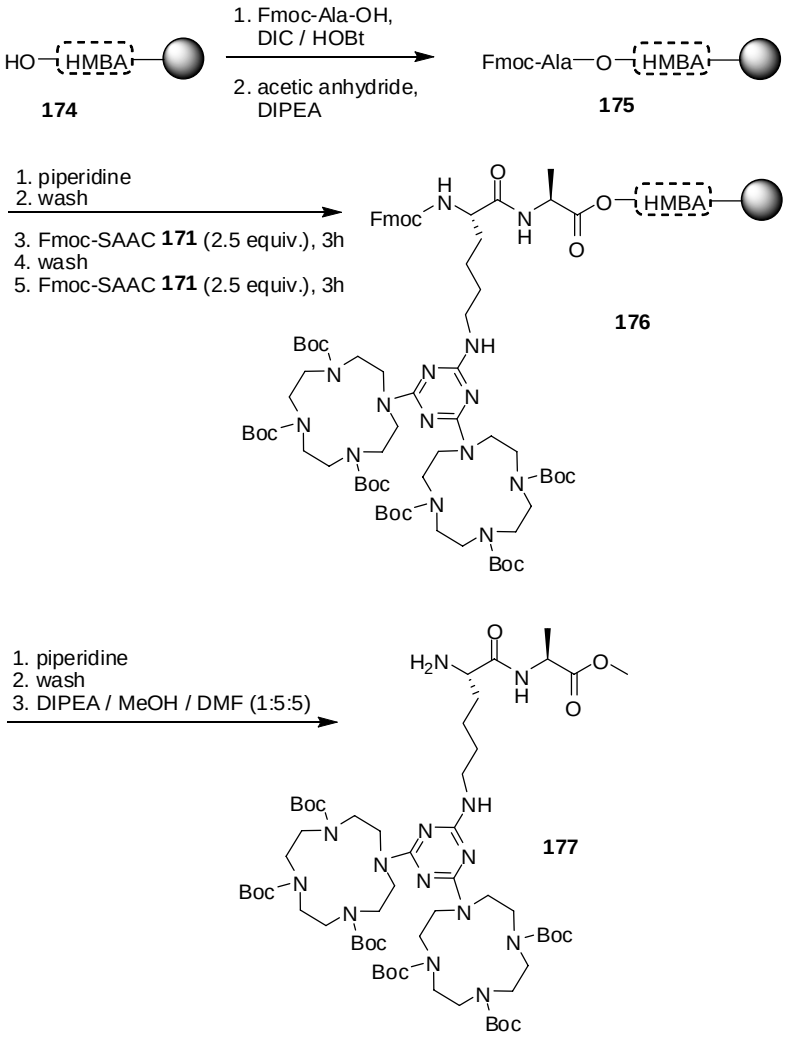
7.3 Bis-(1,4,7,10-tetraazacyclododecane) (bis-cyclene) zinc conjugate

The solid-phase synthesis of metal-complex containing peptides bearing a cyclene moiety has been performed.[90] An amino acid complex **173** was prepared from the previously reported triazene-bis-cyclen[91] **168** by reaction with α-amino *Z*-protected *L*-Lys-OBn (Scheme 37). Nucleophilic aromatic substitution gave compound **169** and the benzyl protecting groups were simultaneously removed by hydrogenation using 10% palladium on charcoal as catalyst. The complexation of the Fmoc protected cyclen ligand with Zn(II) required careful control of the reaction conditions.



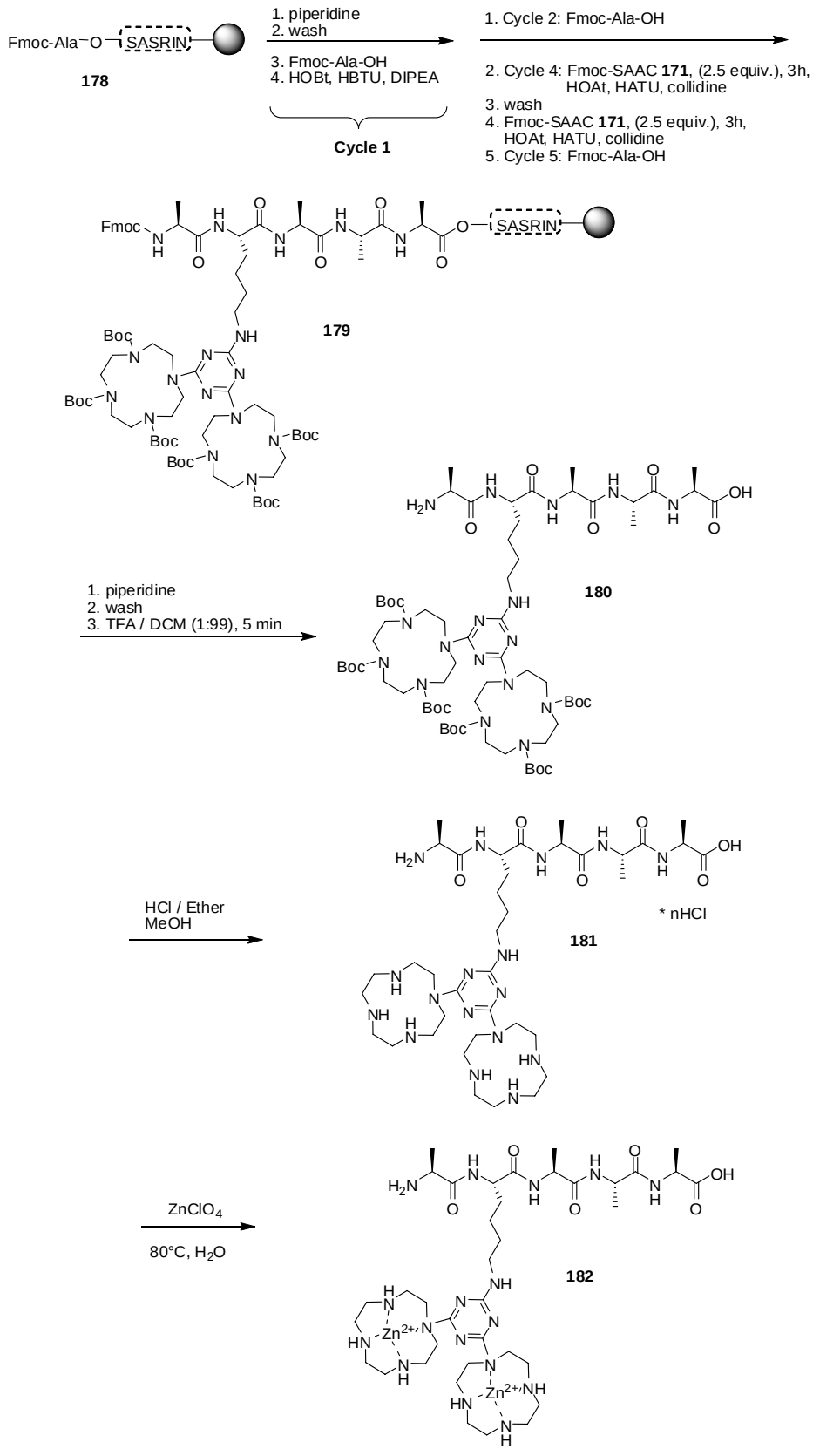
Scheme 37. Preparation of Fmoc-protected amino acid **171** and the bis(Zn^{II}-cyclen) SAAC **173**.

After Boc-deprotection with HCl saturated ether the complexation of the hydrochloride salt was achieved with Zn-salt in a buffered solution (Hepes buffer, pH 8). Preliminary attempts to couple amino acid **171** to aliphatic amino acids using HBTU, TBTU and DIPEA as coupling reagents failed. The more efficient reagent HOAt[92] was used instead of HOBt together with the onium salt HATU. DIPEA was exchanged by collidine,[93] a more suitable base for the HOAt reagent. By using HOAt/HATU/collidine, the coupling of **171** using two coupling cycles gave dipeptide **177** (Scheme 38).



Scheme 38. Solid-phase synthesis of dipeptide conjugate **177**.

A more extended peptide **180** was obtained on an Fmoc–Ala loaded SASRIN resin **178** using the same coupling conditions (Scheme 39). In the following steps, the Boc groups were cleaved with HCl saturated ether and the neutralized compound **181** was subsequently treated with $Zn(ClO_4)_2$ salt to obtain the peptide complex **182**.

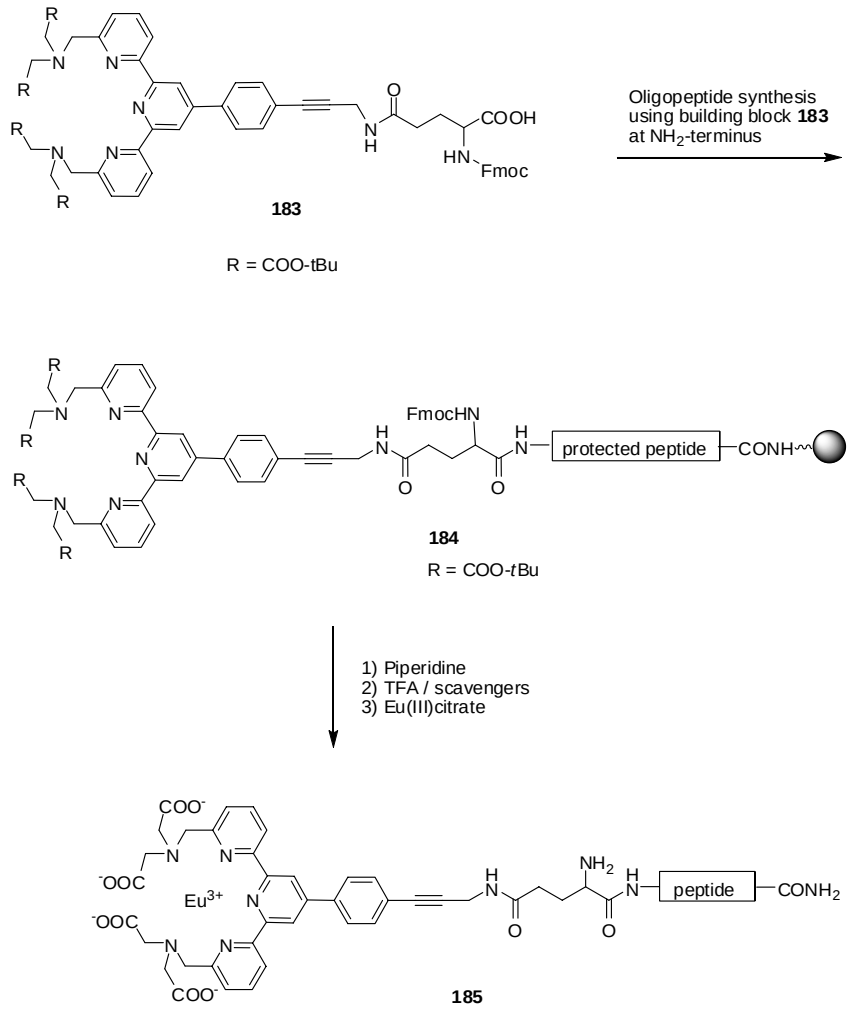


Scheme 39. Solid-phase synthesis of peptide **180** and peptide complex **182**.

8. Samarium, Europium, Terbium and Gadolinium (Lanthanides) Metal Complex – Peptide Conjugates

8.1 *N*-Isothiocyanatobenzyl)diethylenetriamine-*N,N´,N´´,N´´´*-tetrakis acetic acid metal conjugate

The chelates of certain lanthanides, such as Eu^{3+}, Tb^{3+}, Sm^{3+} and Dy^{3+}, have unique fluorescence properties, e.g. large Stoke´s shift, sharp emission peaks and exceptionally long decay times.[94] These properties are exploited in time-resolved fluorometry[95] (TRF). Oligopeptide conjugates were synthesized and used in a TRF-quenching assay (based on caspase-3, an enzyme that plays a key role in programmed cell death, or apoptosis.) and in a receptor binding assay (based on motilin, a polypeptide hormone secreted by Mo cells of the small intestine that increases the migrating myoelectric complex component of gastrointestinal motility and stimulates the production of pepsin).[96] Hovinen et al. described the synthesis of oligopeptide building blocks that allow for the introduction of lanthanide(III) chelates to synthetic oligopeptides using standard automated solid-phase peptide synthesis. The applicability of building block **183**[97] for oligopeptide derivatization was demonstrated with peptide sequences of motilin, substance-P, neurokinin-A and caspase-3 synthesized using Fmoc solid-phase peptide synthesis (Scheme 40). After the building block **183** was coupled to the amino terminus of the coding sequence using prolonged reaction time, but otherwise standard HBTU/HOBt conditions, the oligopeptide was released from the resin. Treatment of the deblocked oligomer with europium(III) citrate converted the conjugate to the corresponding europium peptide chelate **185**.



Scheme 40. Introduction of a luminescent europium(III) chelate by solid phase oligopeptide synthesis with N-terminal **183** and subsequent complex formation in solution.

Karvinen et al. have paved the way to a multiparametric caspase assay by characterizing fluorescence properties of a series of lanthanide (Ln^{3+}) chelates (Scheme 41) incorporated into peptides and testing their functionality in a caspase-3 assay.[98] Caspases are a group of cysteine proteases involved in apoptosis[99] and inflammatory reactions[100]. As the caspases and their substrates are a well-characterized[101] and an interesting group of enzymes as potential drug targets[102], Karvinen et al. have chosen them as a model system for the development of a multiparametric homogeneous time-resolved fluorescence quenching assay (TR-FQA). The principle of the enzymatic assay is shown in Figure 11.

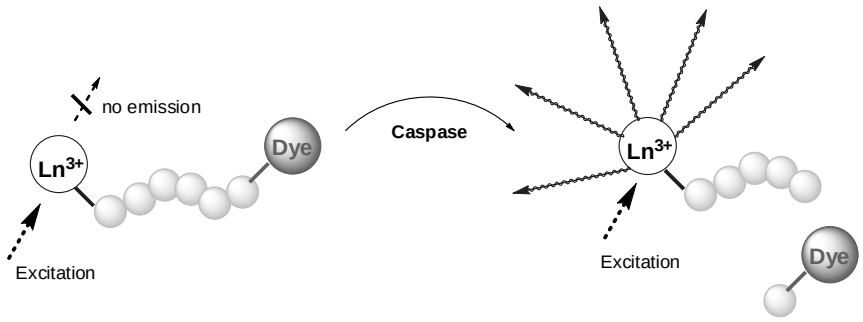
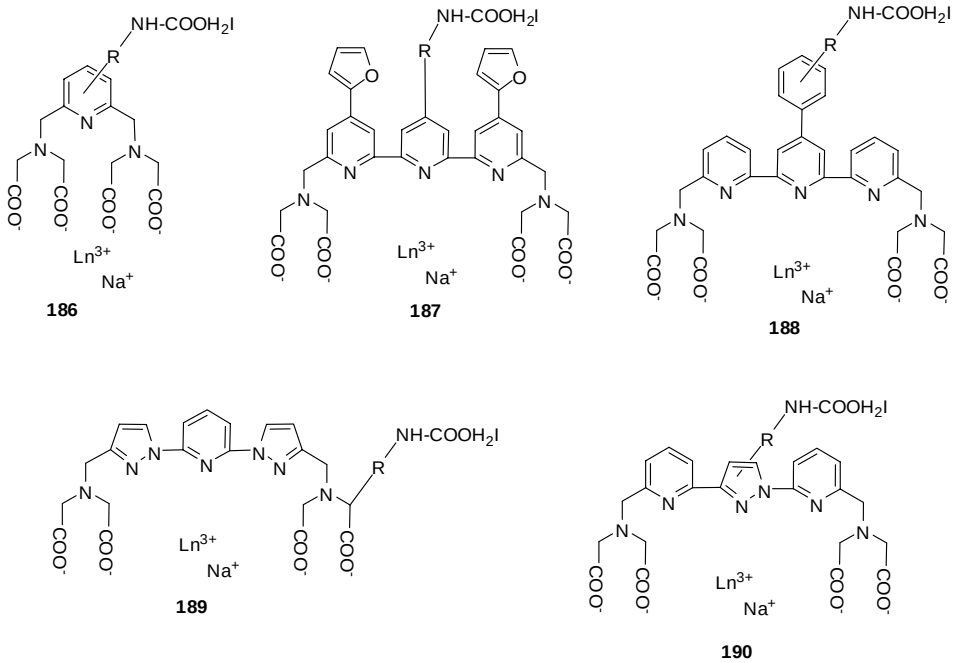


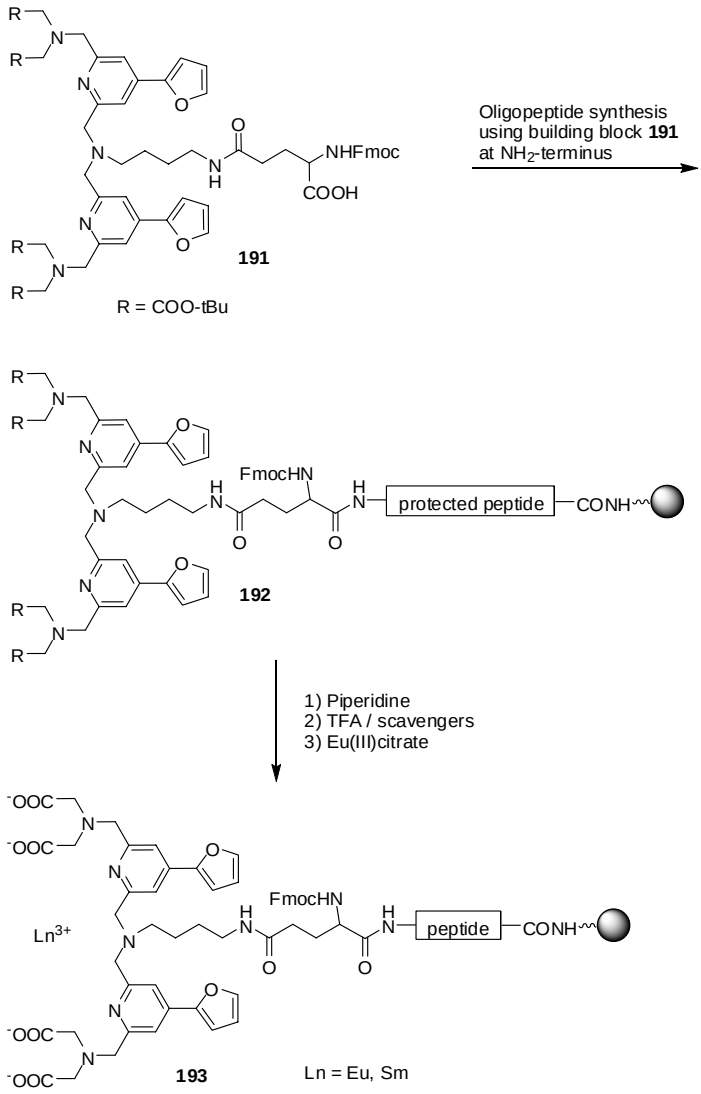
Figure 11. Principle of the TR-FQA caspase assay. The assay is based on recovery of the Ln^{3+} fluorescence after removal of the quencher by protease activity. QSY-7 ε-amino labeled lysine, inserted into a caspace recognition sequence, was used as quencher for all lanthanide chelates.

The homogeneous multiparametric assay was capable to measure the activity of three different caspases from one well using specific substrates labeled with europium, samarium, and terbium chelates. Although the quenching efficiencies were significantly lower than those observed earlier,[103] some of the chelates tested during this work proved to be extremely functional in TR-FQ assays and the technique might be adaptable to DNA assays.



Scheme 41. Simplified structures of the tested lanthanide chelates.

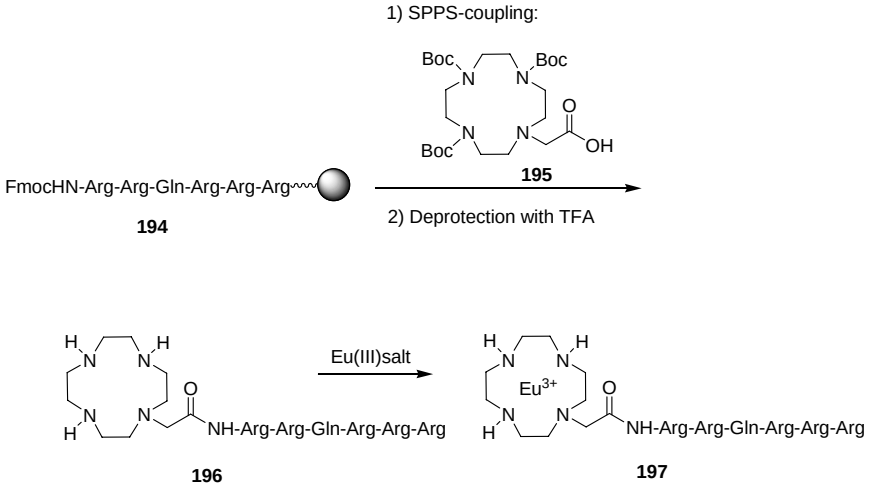
Hovinen et al. modified the synthesis of the building block **187**, that allows the introduction of photoluminescent europium(III) and samarium(III) chelates to synthetic oligopeptides on solid-phase using Fmoc chemistry. Oligopeptide synthesis and introduction of the Fmoc protected building block **191** to the growing peptide chain was performed as reported earlier.[104] Upon completion of the oligopeptide synthesis, the conjugates were converted to the corresponding lanthanide(III) chelates by treatment with the appropriate lanthanide(III) salt (Scheme 42).



Scheme 42. Introduction of luminescent lanthanide(III)-chelates to oligopeptides using building block **191**.

8.2 1,4,7,10-Tetraazacyclododecane (cyclene) europium conjugate

The metal coordinating ligand cyclene was attached to the arginine-rich region of the TAT-protein, (a transactivator of the HIV-1 infection, which is responsible for the replication and expression of HIV-1) and the lanthanide-complexes of the ligand-peptide conjugate were investigated in hydrolysis-cleavage experiments.[105] TAR-RNA of HIV-1 was chosen as the target for the hydrolysis studies, as it is recognized by the HIV-1 regulatory TAT.[106] The peptide-cyclene conjugate nonamer **196** with the attached cyclene moiety was synthesized by standard solid-phase peptide synthesis. In the last coupling step, the Boc-protected cyclene acetic acid **195**[107] was coupled to the *N*-terminus of the nonapeptide **194**. After subsequent cleavage from the resin by standard TFA conditions, the peptide **196** was incubated with Eu(III)-salt (Scheme 43). Surprisingly, the nonamer-cyclene conjugate without Eu(III) **196** gave selective and efficient cleavage at neutral pH and room temperature and the authors report those cleavage reactions are more efficient in the absence than in the presence of Eu(III).

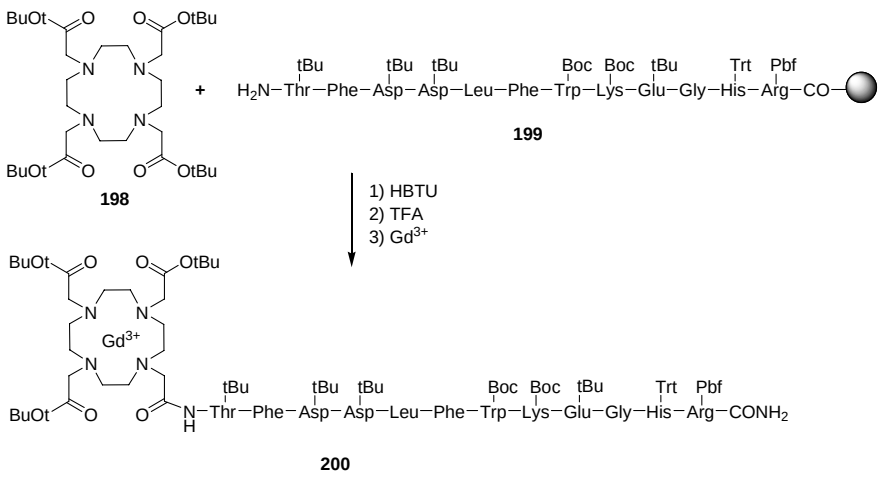


Scheme 43. Synthesis of peptide-cyclen conjugates by solid-phase synthesis and europium complex formation in solution.

8.3 1,4,7,10-Tetraazacyclododecane-*N*,*N´*,*N´´*,*N´´´*-tetraacetic acid (DOTA) gadolinium conjugate

Several types of ligands including DOTA, DTPA, NOTA and TETA,[108] have been attached to peptides. DOTA is of particular interest, since this macrocyclic ligand forms

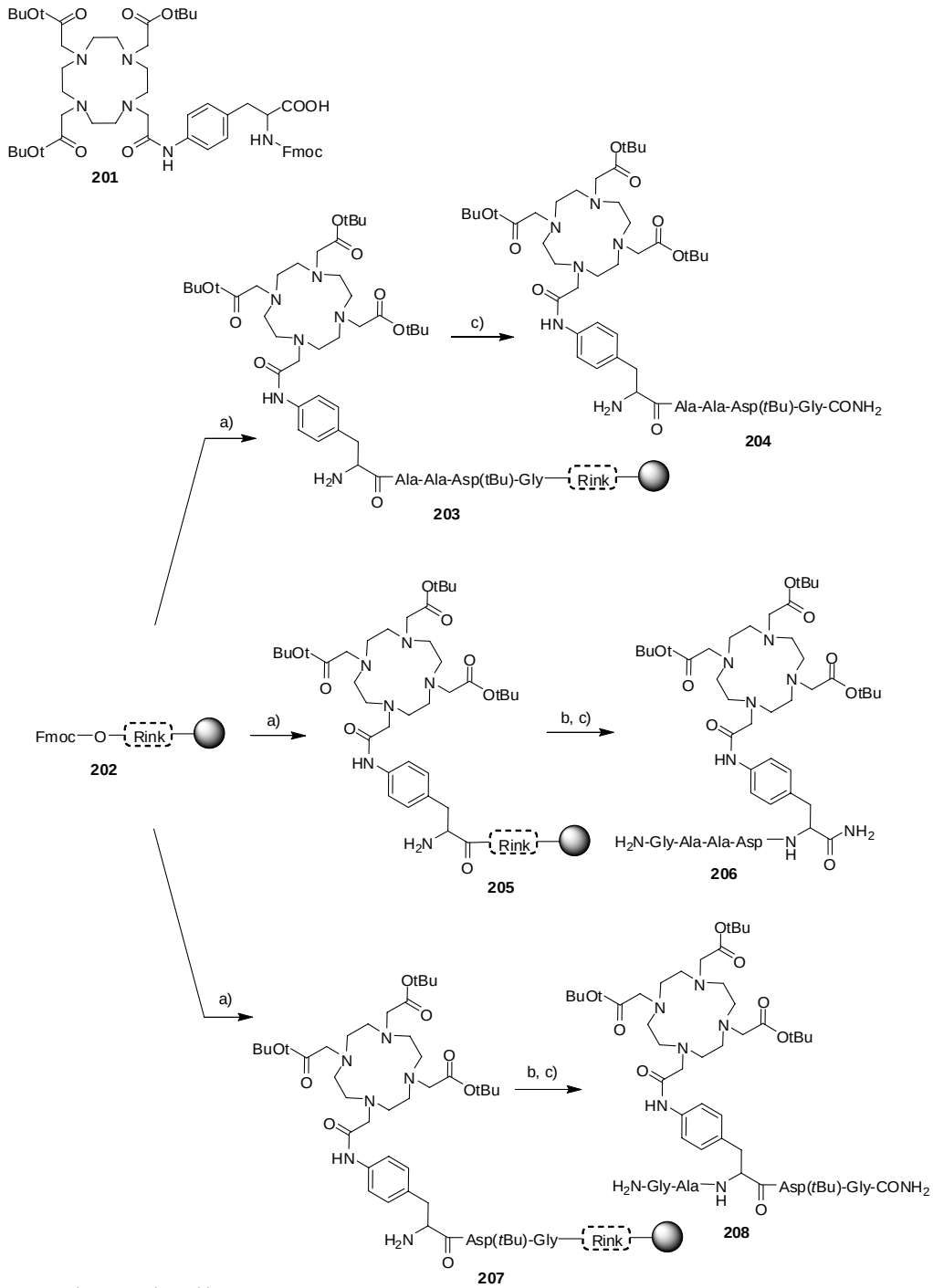
complexes with a variety of metal ions with exceptionally high binding affinities and kinetic stabilities.[109] Sherry et al. prepared Gd^{3+}-G80BP (*B*inding *P*eptide) using solid-phase peptide synthesis (Scheme 44) and demonstrated that magnetic resonance imaging (MRI) can detect the binding event of a Gd^{3+}-DOTA-labeled peptide (Gd^{3+}-G80BP) to its target protein Gal-80[110] (Gal-80 is a protein involved in regulation of galactose metabolism).[111] In a later work, Sherry et al. modified the Gal-80 binding peptide TFDDLFWKEGHR by introducing a DOTA-chelating group at three different residues (Scheme 45).[112] Conjugation of DOTA to the N-terminus of the resin bound peptide was accomplished using DOTA-tris(^{t}Bu) ester and standard Fmoc solid-phase peptide synthesis. Attempts to add subsequent amino acids beyond DOTA using even either HATU or TFFH as coupling agents were not successful. These coupling agents are reported to have superior coupling capabilities over HBTU. A report by Lewis et al. showed that Fmoc-DOTA-lysine can be introduced into a peptide sequence using a XAL-PEG-PS resin.[113] XAL-PEG-PS solid supports are prepared by grafting soluble polar PEG chains onto microporous polystyrene-co-divinylbenzene and have been shown to be superior to conventional resins for the synthesis of hydrophobic peptides.[114]



Scheme 44. Solid-phase peptide synthesis of Gd^{3+}-G80BP **200**.

However, Sherry et al. used in their work the more reactive activated amino acid Fmoc-pentafluorophenyl ester to couple successfully the remaining amino acids to the *endo*-DOTA peptides. After addition of Gd^{3+} to each peptide-DOTA conjugate, competitive binding experiments showed that the *exo*-peptide labeled with Gd^{3+}-DOTA at the

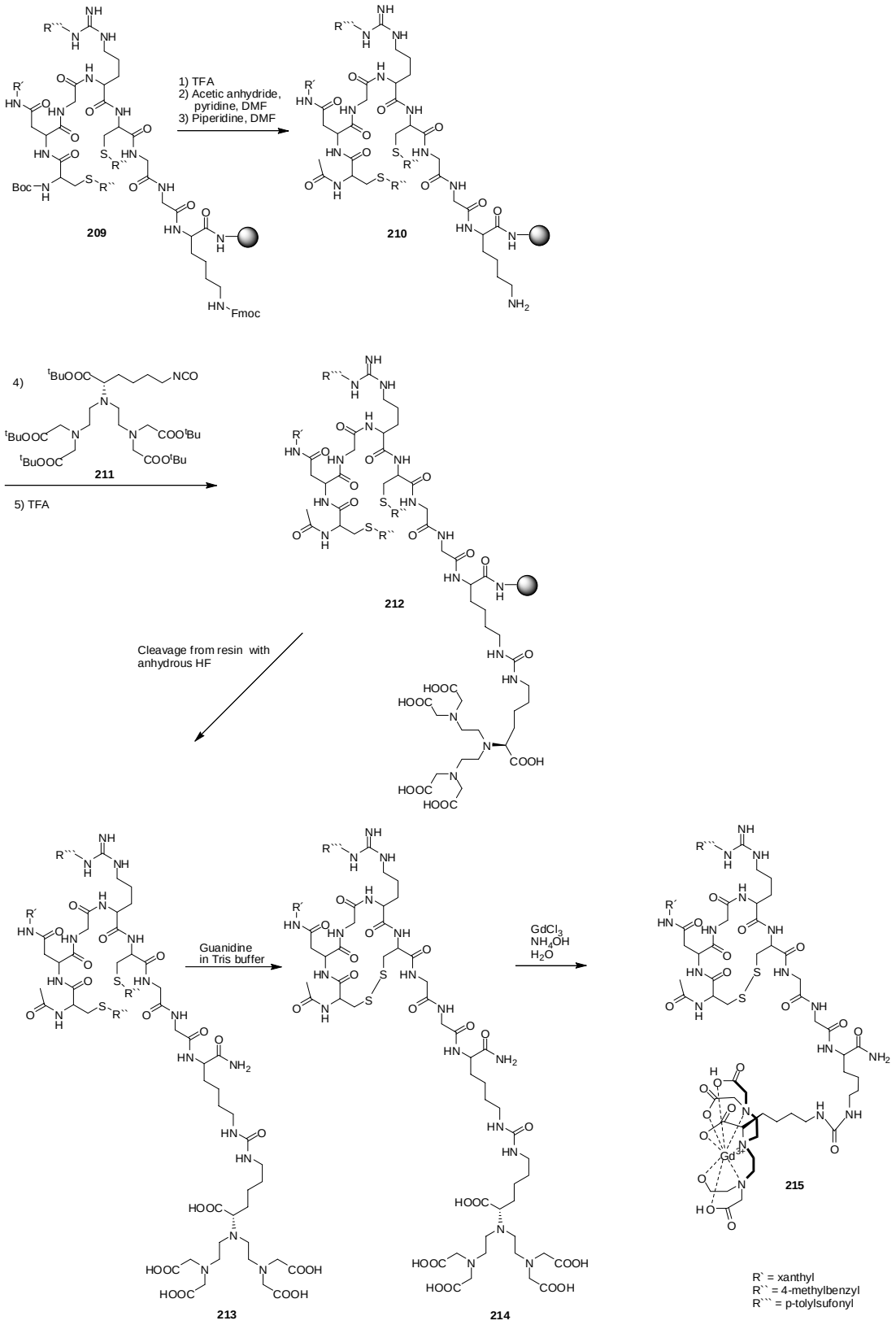
N-terminal had a reasonable affinity for Gal-80, while those peptides labelled with Gd^{3+}-DOTA at *endo*-positions within the peptide sequence had no detectable binding affinity for Gal-80.



Scheme 45. Solid-phase peptide synthesis of three pentapeptides with variable DOTA position.

8.4 Diethylenetriamine pentaacetic acid (DTPA) gadolinium conjugate

Gadolinium complexes of DTPA are widely employed as contrast agents in medicinal imaging.[115] The effectiveness of Gd^{III}DTPA-based contrast agents can be improved by incorporating target-specific oligopeptides to induce accumulation of MRI probes in the tissue of interest.[116] A cyclic peptide containing the Cys–Asn–Gly–Arg–Cys (CNGRC) sequence (cNGR) was identified as a targeting unit for the aminopeptidase CD13 that is overexpressed on endothelial cells during angiogenesis.[117] Hackeng and Meijer et al.[118] designed a cNGR-Gd^{III}DTPA complex **215** composed of the cNGR targeting domain and a Gd^{III}DTPA complex for imaging of angiogenesis (Scheme 46). The gadolinium chelate was introduced at the ε-amine of the lysine side chain of the peptide **210**. For this purpose, an isocyanate-functionalized lysine-based DTPA pentaester **211** was coupled to the resin bound peptide **210**. Solid-phase peptide synthesis and HBTU activation procedure for Boc chemistry on a MBHA resin[119] was applied to synthesize side chain protected BocCNGRCGGK(Fmoc)-MBHA **209** containing the target-specific NGR sequence. The conversion of the amine functionalized DTPA[120] into the corresponding isocyanate **211** was achieved with di-*tert*-butyl tricarbonate, which is a versatile reagent for the quantitative conversion of primary amines into isocyanates under mild reaction conditions.[121] The DTPA-functionalized oligopeptide **212** was obtained by reaction of the lysine side-chain ε-amine group with an excess of isocyanate-functionalized DTPA analogue **211**. After quantitative formation of the disulfide bridge by oxidation, the gadolinium complex **215** was prepared by adding gadolinium chloride to a solution of peptide conjugate **214** in water.



Scheme 46. Synthesis of cNGR-Gd^{III}DTPA complex **215** by solid phase synthesis of the ligand and subsequent gadolinium comple formation in solution.

9. Conclusions

The discussed examples of metal complex – peptide conjugates synthesized on solid phase show that a wide variety of different structures is already accessible by the developed methods. Procedures are in many cases different compared to standard SPPS protocols to address the special requirements of ligand and complex stability. Both general strategies, the synthesis of peptide – ligand conjugates and complexation with excess metal ions on solid support or the incorporation of an amino acid complex in the growing immobilized peptide chain have their specific advantages and limitations. While complexation of peptide – ligand conjugates is synthetically more facile in many cases, it does not allow the specific preparation of bi- or oligonuclear complexes with different metal ions. This is in principle possible with artificial metal complex amino acids, if they are kinetically and thermodynamically sufficiently stable and introduced in the right order. However, all reaction conditions of the subsequent peptide synthesis including deprotection and cleavage steps must be compatible with the stability of the complexes. With further advancements of the methodology the preparation of peptide metal complex conjugates by automated solid phase synthesis will surely become more common. However, the special conditions which are necessary for the formation of various metal complex types and their individual stability profile will always call for specific protocols.

10. Abbreviations

Bhoc	N-Benzhydryloxycarbonyl
Boc	*tert*-Butoxy carbonyl
BOP	Benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate
ByPOP	Benzotriazole-1-yl-oxy-tris-pyrralidino-phosponiumhexafluorophosphate
DCC	N,N‘-Dicyclohhexylcarbodiimide
Dde	1-(4,4-Dimethyl-2,6 dioxocyclohexyldiene)ethyl
Dhbt-OH	3,4-Dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine
DIC	N,N‘-Diisopropylcarbodiimide
DIPEA	N,N-Diisopropylethylamine
DMAP	4-(N,N-Dimethylamino)-pyridine
DMF	Dimethylformamide
DNA	Deoxyribonucleic acid
DSC	N,N-Disuccinimidyl carbonate
EDTA	Ethylenediamine tetraacetic acid
FITC	Fluorescein isothiocyanate
HATU	2-(1H-7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate methanaminium
HBTU	2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronoium hexafluorphosphate
HMBA	Hydroymethylbenzoic acid
HMBA-AM	4-Hydroxymethylbenzoic acid AM
HOAt	1-Hydroxy-7-azabenzotriazole
HOBt	1-Hydroxybenzotriazole
MBHA	4-Methylbenzhydrylamine
MeOH	Methanol
MSNT	1-(Mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole

Mtt	4-Methyl trityl
NBD	4-Halo-7-nitrobenzo-2-oxa-1,3-diazole
NEM	N-Ethylmorpholine
NHS	N-Hydroxysuccinimide
NMP	N-Methyl pyrrolidone
OPfp	(Acetic acid) pentafluorophenyl ester
PAL linker	5-(Aminomethyl-3,5-dimethoxyphenoxy)-pentanoic acid
PAM resin	para-Hydroxymethylphenylacetamidomethyl polystyrene
Pbf	2,2,4,6,7-Pentamethyldihydrobenzofuran-5-sulfonyl
PEG	Polyethylene glycol
PNA	Peptide nucleic acid
Pu	Purine
Pym	Pyrimidine
SAAC	Single Amino Acid Chelate
SASRIN	Super Acid Sensitive Resin
SPPS	Solid-phase peptide synthesis
TBTU	2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate
TFA	Trifluoroacetic acid
TFFH	N,N,N‘,N‘-Tetramethylfluoroformamidinium hexafluorophosphate
TIS	Triisopropylsilane
TMS	Trimethylsilane
TSTU	N,N,N’,N’-Tetramethyl-O-(succinimidyl)uronium tetrafluoroborate
XAL	Xanthenyloxyalkylamide

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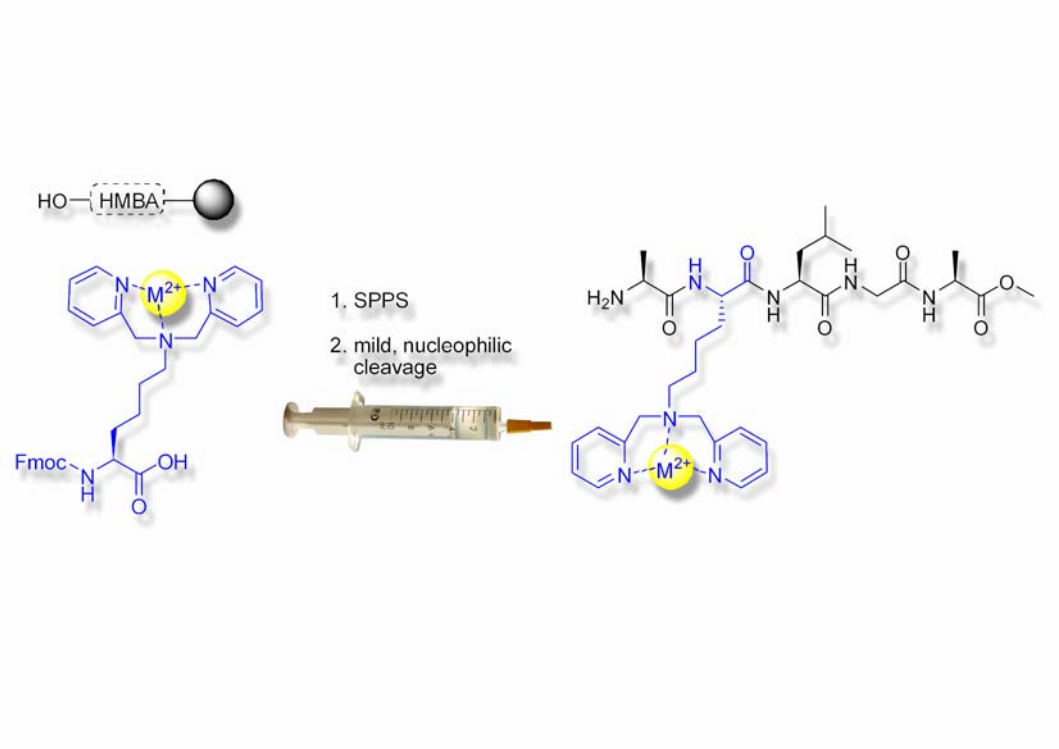
B. Main Part

1. Solid Phase Synthesis of Metal-Complex containing Peptides*

Graphical Abstract



In this chapter is reported the synthesis of Fmoc protected single amino acid chelates (SAAC) and their metal complexes. The modified amino acids are suitable for solid phase peptide synthesis. The use of 4-hydroxymethylbenzoic acid AM (HMBA-AM) resin allows the nucleophilic cleavage of the peptide – metal complexes from the resin without decomplexation.

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1.1 Introduction

Peptides with metal complexes in their side chains and peptide-metal complex conjugates have been used to enhance or control binding affinities and peptide conformations. Banjerjee and Stephenson et al. have reported the solid-phase synthesis of peptides bearing 2,2′-dipicolylamine single amino acid chelate (dpa-SAAC) for peptide based technetium and rhenium radiopharmaceuticals.[1] Metzler-Nolte and co-workers prepared N-terminally modified bioconjugates by a series of dpa based copper or zinc complexes for potential applications as artificial metallochaperones.[2] Recent reports on dpa-SAAC describe a more flexible preparation of peptide–Re and peptide–Tc ligand conjugates with non-terminal SAAC.[3] However, the reported syntheses of peptide – metal complex conjugates on solid phase are either limited to conjugation of the metal complex to the N-terminus of the resin-bound peptide or focus on the coordination of $Tc(CO)_3$ and $Re(CO)_3$.[4] To extend the scope of solid phase synthesis of peptide – metal complex conjugates, we report here their preparation from modified amino acids bearing metal complex ligands or metal complexes in their side chains. The modified amino acids can be incorporated at any position into the peptide during synthesis and the use of 4-hydroxymethylbenzoic acid AM (HMBA-AM) resin allows nucleophilic cleavage of the peptide from the resin avoiding decomplexation.

1.2 Results and discussion

1.2.1 Metal chelates

We have selected the iminodiacetic acid (IDA) motif as metal complex binding sites, known for its ability to bind imidazole residues and N-terminal His, and triazen-bis-zinc-cyclen, dipyridylmethyl amine (dpa) and bis-diypridylmethyl amine (bis-dpa) zinc complexes, which show affinity to phosphate groups. First we discuss the synthesis of the modified amino acids bearing the respective ligand or complex, and describe then their use in solid phase peptide synthesis.

1.2.2 Synthesis of Fmoc-amino acids with ligand or metal complex side chain

The synthesis of Fmoc protected IDA amino acid **1** (Figure 1) has been described by reductive amination of *tert*-butyl 2-oxoacetate,[5] or the nucleophilic substitution of the lysine side chain as reported by Tampé.[6] After optimization of the reaction conditions of hydrogenolytic cleavage of the *Z*-protecting group and Fmoc-protection using the more reactive Fmoc-OSu instead of Fmoc-Cl, **1** was obtained in good yield from the latter method. The ligand was converted into its copper complex using either two equivalents of $Cu_2(OH)_2CO_3$ or a stoichiometric amount of $CuCl_2$ with one equivalent of base. In both cases, mass spectrometric analysis (ESMS) confirmed the complete formation of complex **2**.

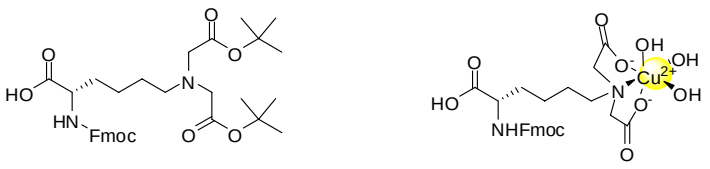
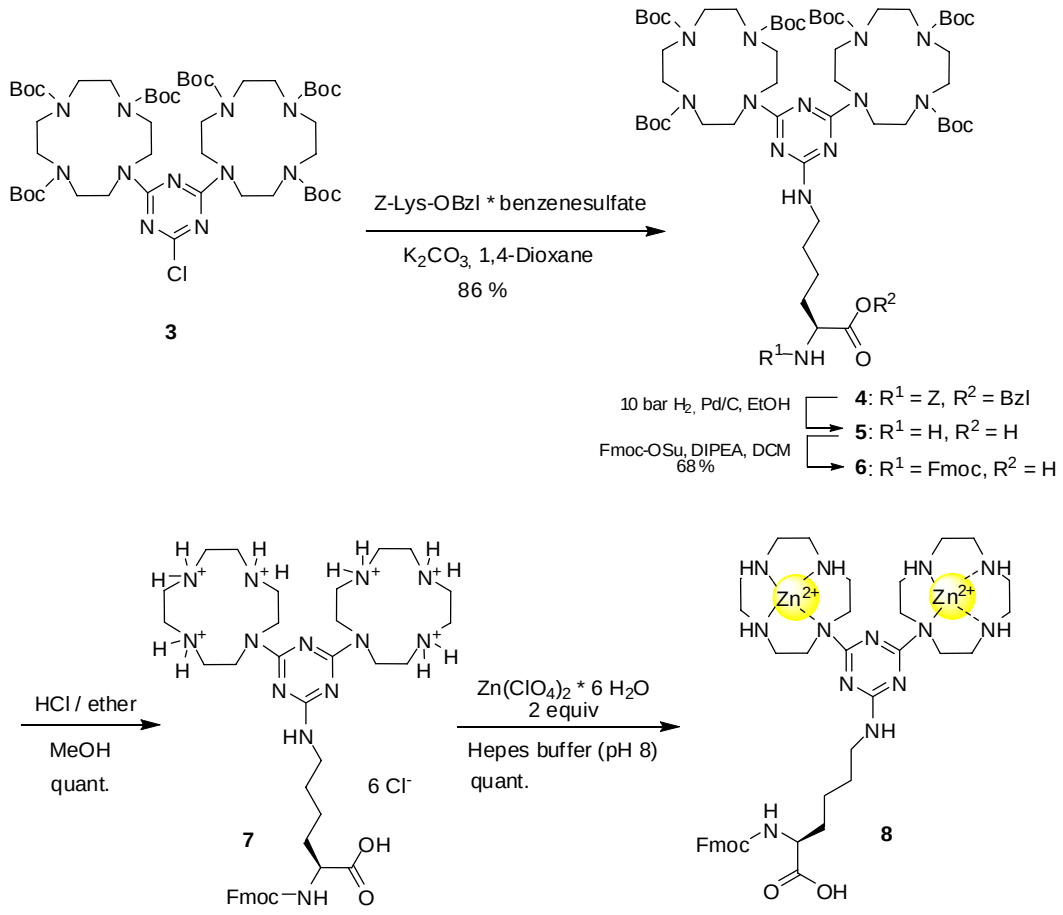


Figure 1. Fmoc-protected IDA-amino acid **1** and its copper(II) complex **2**.

Amino acid complex **8** was prepared from the previously reported triazene-bis-cyclen **3**[7] by reaction with α-amino *Z*-protected *L*-Lys-OBn (Scheme 1). The nucleophilic aromatic substitution gave compound **4** in 86 % yield and Z and benzyl protecting

groups were simultaneously removed by hydrogenation using 10% palladium on charcoal as catalyst. Fmoc protection gave compound **6**. The complexation of the cyclen ligands with Zn(II) requires careful control of the reaction conditions. After Boc-deprotection with HCl saturated ether the hydrochloride salt **7** must be neutralized by base for metal ion complexation and the complexation step typically requires elevated temperatures,[8] conditions which may cleave the Fmoc group. Ion exchange chromatography for deprotonation of the hydrochloride salt was therefore not applicable and a buffered solution was used for complexation. A clean twofold Zn(II)-complexation was achieved in Hepes buffer (pH 8) with $Zn(ClO_4)_2 \cdot 6\ H_2O$ yielding the protected amino acid **8**.



Scheme 1. Preparation of Fmoc-protected amino acid **6** and the bis(Zn^{II}-cyclen) compound **8**.

Amino acid **9** with a tridentate donor side chain was prepared according to a literature procedure[9] from Fmoc-lysine hydrochloride pyridine-2-carboxyaldehyde by reductive

amination with $NaBH(OAc)_3$.[10] The dpa complexes **10** are obtained quantitatively using stoichiometric amounts of the appropriate metal salts in a water/methanol solution.

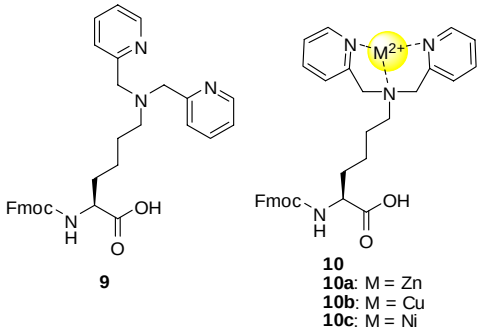
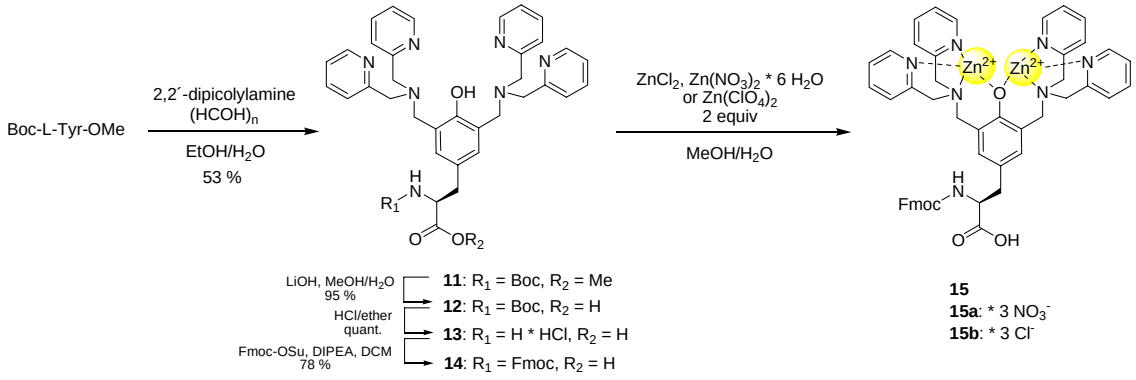


Figure 2. Dpa amino acid **9** and dpa metal complexes **10** (M = Zn, Cu, Ni).

The synthesis of amino acids **15** with binuclear Zn(II) dpa complex starts from Boc-L-tyrosine methyl ester (Scheme 2). The Mannich reaction with dpa and paraformaldehyde afforded ligand **11**.[11] Saponification and subsequent reprotection resulted in the desired amino acid **14**. The complexation of **14** with 2 equivalents of either $Zn(NO)_3 \bullet 6\ H_2O$ or $ZnCl_2$ gave dpa metal complexes **15**†.



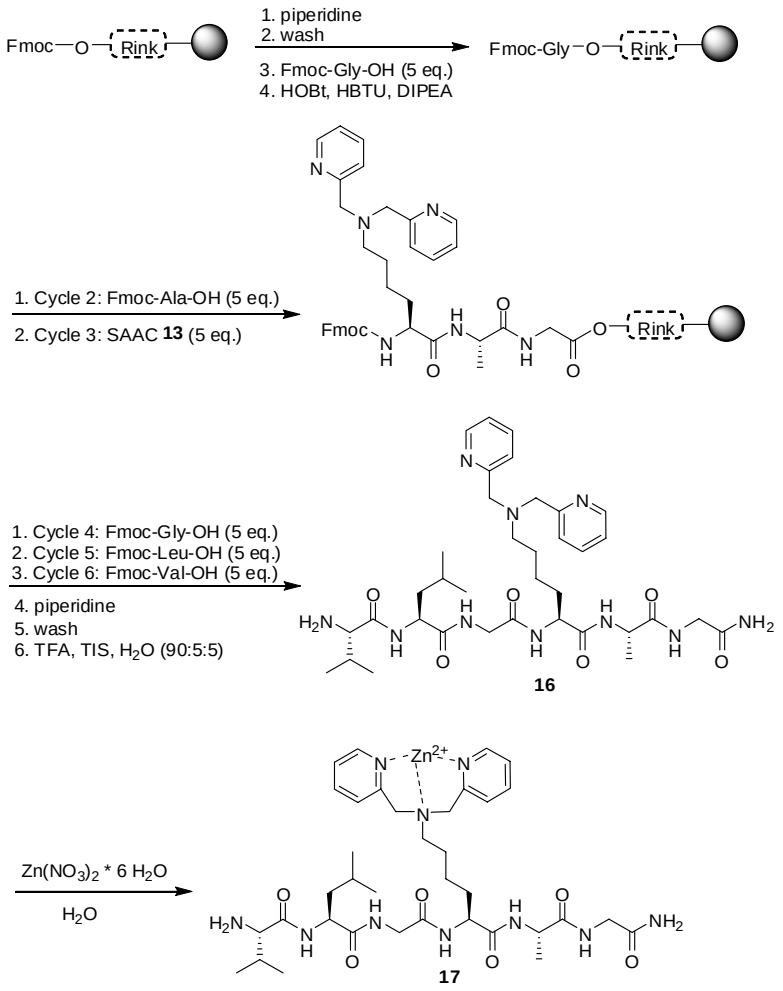
Scheme 2. Synthesis of bis-dpa amino acid **14** and bis-dpa metal complexes **15**.

† Bis-dpa metal complex **15b** was synthesized by Knape Robert, University of Regensburg.

1.2.3 Solid Phase Peptide Synthesis

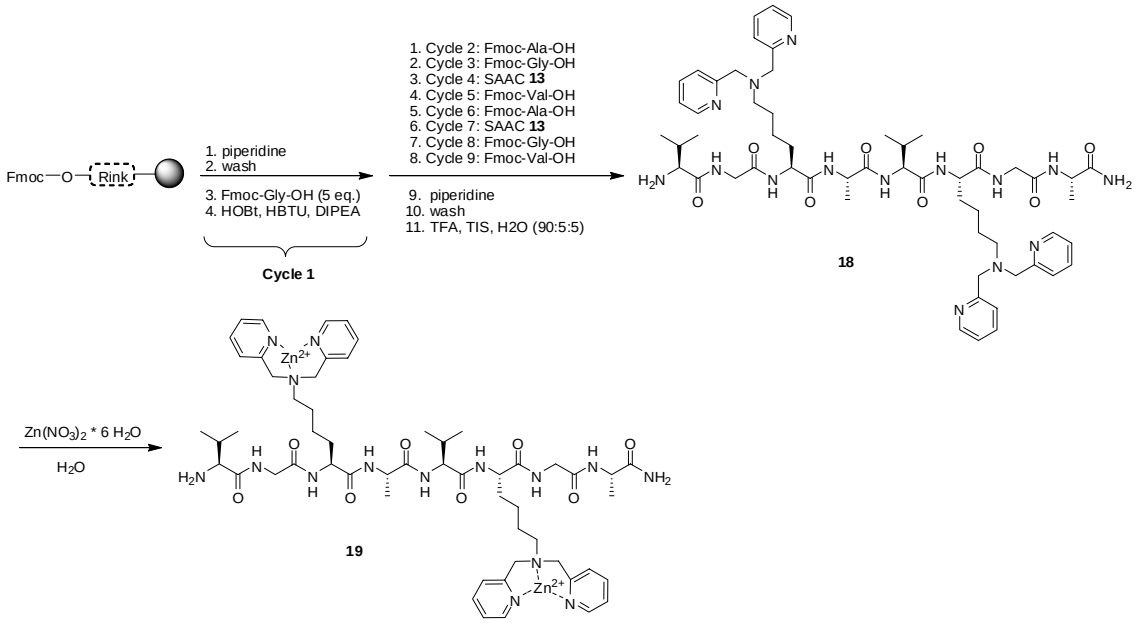
1.2.3.1 Synthesis of dpa-containing peptides

As a first peptide compound **16** was prepared (Scheme 3). After complete characterization by 2-dimensional NMR, peptide **16** was complexed in solution. This approach allowed the use of the versatile Rink amide MBHA resin, a resin based on MBHA with a modified Rink amide linker as an ideal tool for Fmoc SPPS, providing peptide amides in high yields and purities.[12] After Fmoc deprotection, the Fmoc protected aliphatic amino acids glycine and alanine were coupled by HBTU, HOBt and DIPEA in NMP/DMF using the conventional frit-equipped syringe technique. All coupling steps where carried out only once. Standard deprotection and washing cycles as outlined in Scheme 3 were performed after each coupling step. The SAAC **9** was then coupled using the same coupling reagents and equivalents of reagents. The reaction was completed after 3 hours indicated by a negative Kaiser test[13] and the deprotected N-terminus was subsequently coupled to Fmoc protected amino acids glycine, leucine and valine. Final Fmoc deprotection completes the solid-phase synthesis and gave peptide **16** after cleavage from the resin with 90% TFA, 5% TIS, 5% H_2O. After subsequent precipitation using cold diethyl ether and centrifugation (see experimental part for details), the peptide conjugate was dissolved in water, lyophilized and characterized by ESI-MS and 2-dimensional NMR. 26 mg of the target peptide sequence **16** were obtained analytically pure. Thus, peptide **16** was treated with $Zn(NO_3)_2 \cdot 6\ H_2O$ (1 equiv) to obtain exclusively metal bound peptide conjugate **17** exclusively.



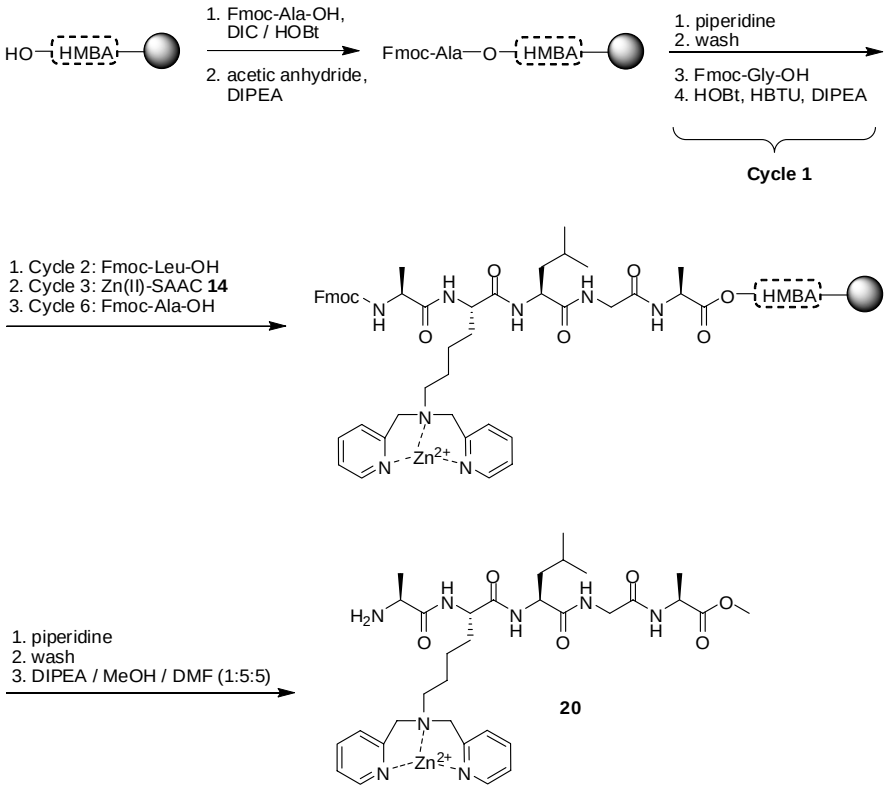
Scheme 3. Solid-Phase Synthesis of peptide conjugate **16** and peptide metal complex **17**.

The same approach was used to prepare the dinuclear peptide receptor **19** (Scheme 4). Peptide **18** was first synthesised on Rink amide MBHA resin. After cleavage from the resin, 16 mg of the target peptide sequence **18** were obtained in > 95% purity as verified by NMR. 2-Dimensional NMR allowed the complete assignment of all resonances. Thus, peptide **18** was treated with $Zn(NO_3)_2 \bullet 6\ H_2O$ (1 equiv) to obtain metal bound dinuclear peptide receptor **19.**



Scheme 4. SPRS of dinuclear peptide metal complex **19**.

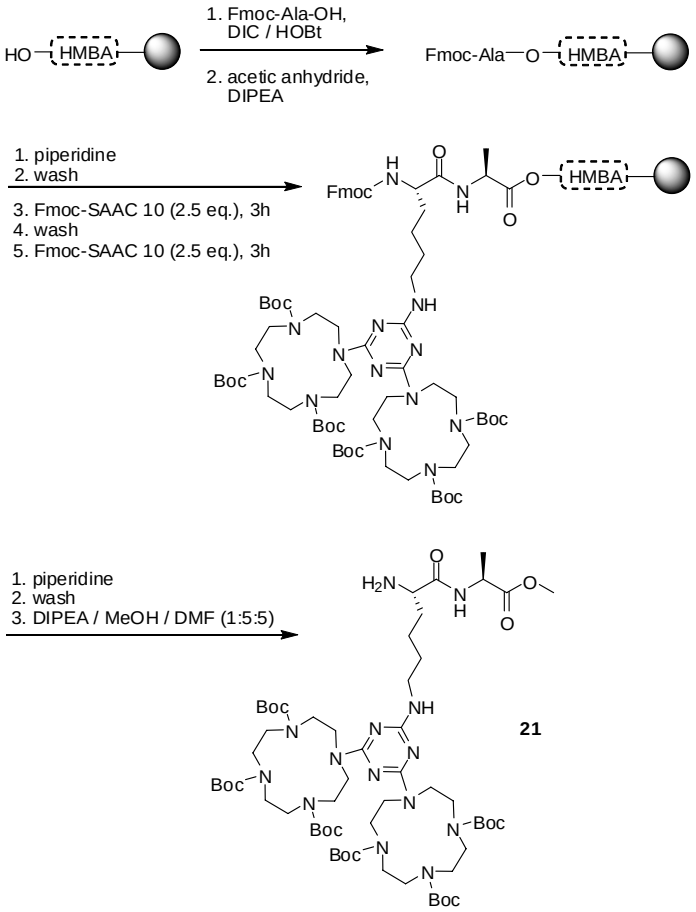
In the synthesis of peptide **20**, the already metal coordinated Fmoc-Dpa complex **10a** was incorporated into the peptide chain (Scheme 5). To avoid the loss of metal ions under acidic conditions, which are necessary to cleave from Rink amide resin, HMBA-AM was used as the resin as it allows nucleophilic cleavage of the peptide from the resin. Otherwise the same procedure as above was used yielding peptide **20** in > 95% purity as verified via NMR.



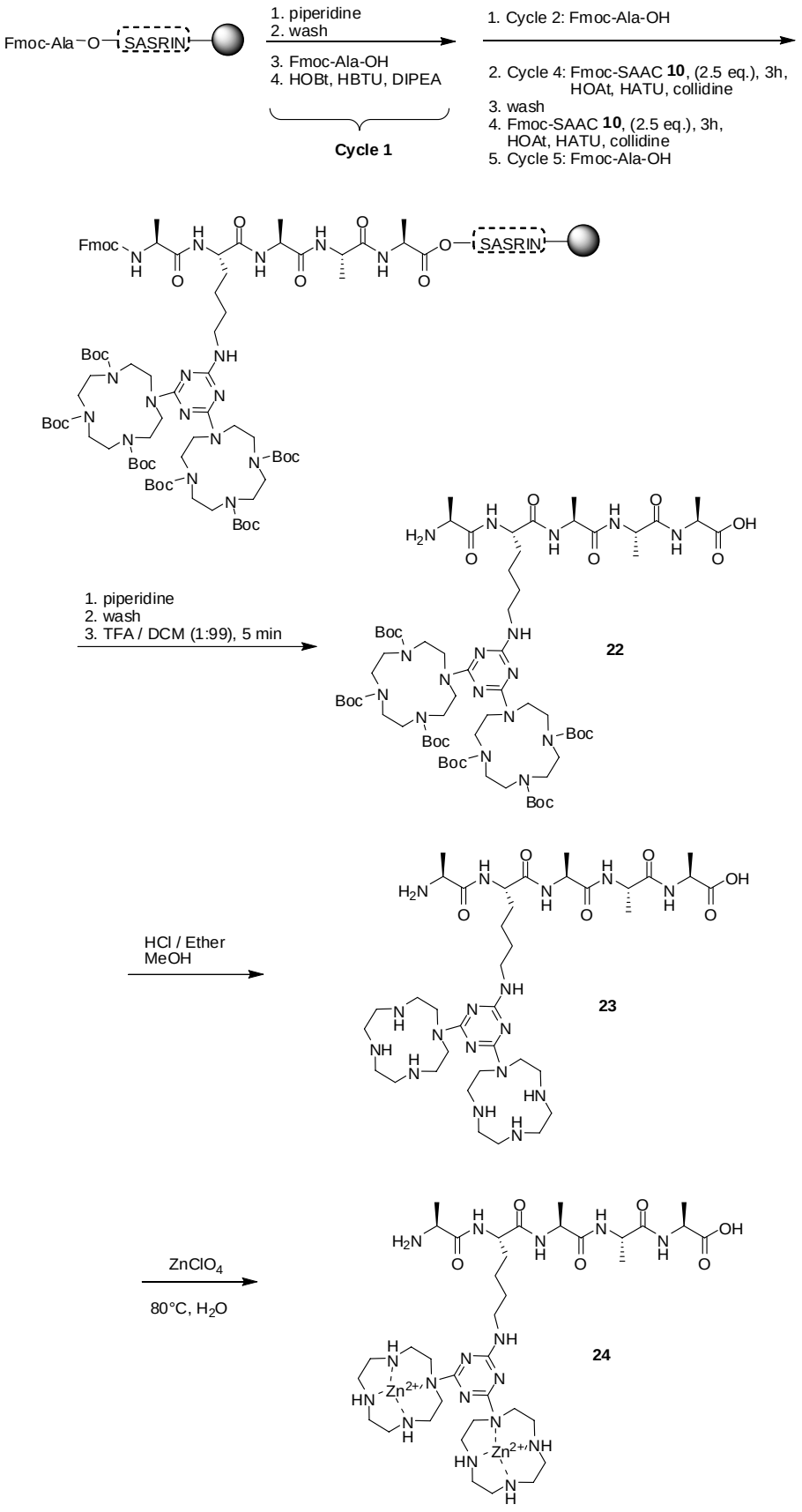
Scheme 5. Direct SPRS of **20** on HMBA-AM resin

1.2.3.2 Synthesis of peptides containing bis-zinc-cyclen amino acid **8**

First attempts to couple amino acids **8** or **6** in solid phase protocols to aliphatic amino acids using HBTU,[14] TBTU and DIPEA as coupling reagents failed. Therefore the more efficient reagent HOAt was used instead of HOBt together with the onium salt HATU. DIPEA was exchanged by collidine,[15] a more suitable base for the HOAt reagent. Using HOAt (2.5 eq.), HATU (2.5 eq.) and collidine (5 eq.) the coupling of **6** (2 x 2.5 eq.) using two coupling cycles gave dipeptide **21** (Scheme 6). A more extended peptide **22** was obtained on an Fmoc-Ala loaded SASRIN resin using the same coupling conditions (Scheme 7). In both cases the solely observed molecular ions in electro-spray mass spectrometry were only consistent with the mass of the desired compounds. In the following step, the Boc groups were cleaved with HCl saturated ether and the neutralized compound **23** was subsequently treated with $Zn(ClO_4)_2$ salt to obtain the peptide complex **24**.



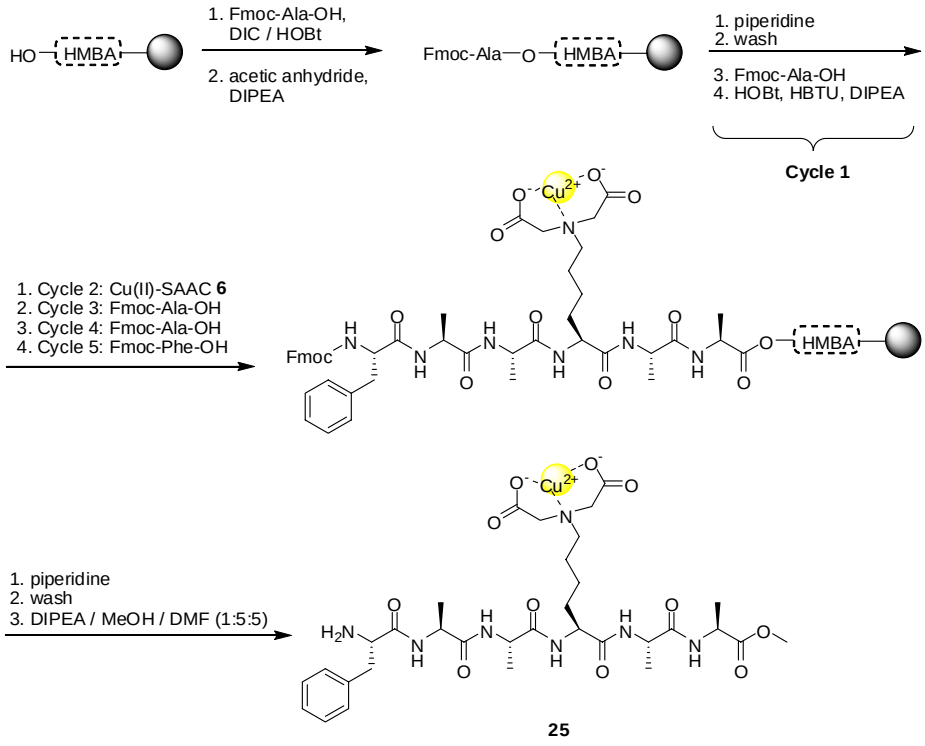
Scheme 6. Solid-phase synthesis of dipeptide conjugate **21**.



Scheme 7. Solid-phase synthesis of peptide **22** and peptide complex **24**.

1.2.3.3 Synthesis of peptides containing IDA amino acid **2**

To illustrate the incorporation of the IDA amino acid metal complex **2** into a short peptide sequence by a solid phase protocol peptide **25** was prepared on HMBA-AM resin using the standard coupling conditions as outlined in examples of the dpa-chelate (Scheme 8). Electro-spray mass analysis confirmed the formation of the desired compound.

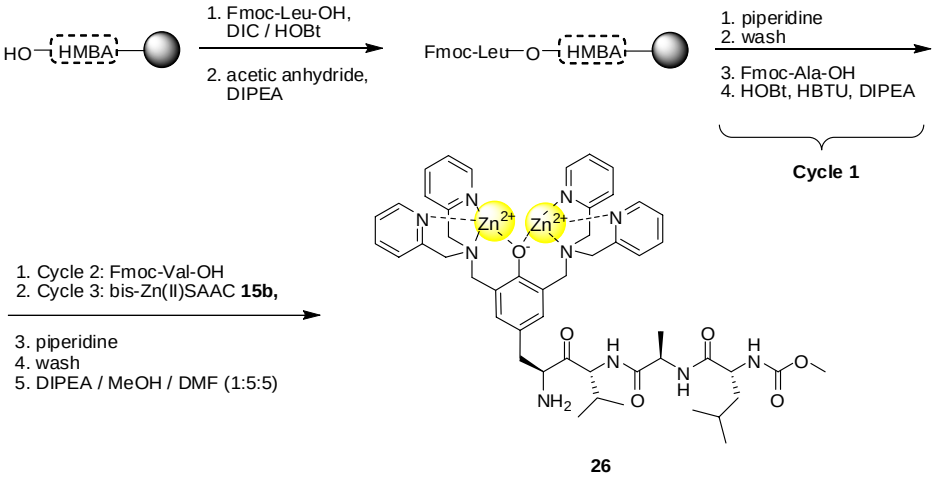


Scheme 8. SPRS of peptide metal complex **25**.

1.2.3.4 Synthesis of peptides containing bis-dpa-zinc amino acid **15**

The first attempt at incorporating **15** into a peptide backbone was as the bis(Zn^{II}-nitrate)-SAAR **15a** derived from complexation of **14** with $Zn(NO)_3 \bullet 6\ H_2O$. Due to the poor solubility of the metal complexes in NMP or DMF, the coupling was not efficient enough to give pure products. Fortunately, bis-Zn^{II}-SAAR **15b**, having chloride counter

ions, was more soluble in NMP and the synthesis of **26**‡ on HMBA-AM resin was successful using standard HOBt/TBTU conditions. Electro-spray mass analysis confirmed the clean formation of the desired compound.



Scheme 9. SPRS of peptide metal complex **26**.

‡ Peptide metal complex **26** was synthesized by Knape Robert.

1.3 Conclusion

In conclusion, we have reported the synthesis of Fmoc protected SAAC and their use in solid-phase synthesis. Peptide – metal complex conjugates were either obtained by incorporation of the metal coordinated SAAC followed by mild nucleophilic resin-cleavage or by complexation in metal salt solution after cleavage from the resin. Our reported solid phase peptide synthesis protocols are suitable for automation and the position and number of the modified amino acid within the peptide chain may vary. This allows the synthesis of libraries of modified peptides with metal complexes as binding sites in synthetic receptors or as paramagnetic labels.

1.4 Experimental

1.4.1 General procedure of solid phase peptide synthesis

Loading of the HMBA-AM resin:

HMBA-AM resin was added to a syringe with filter suited for SPPS, suspended in DMF and shaken for 30 min. After the DMF was drained off by vacuum, a solution of the appropriate Fmoc-AA (4 eq) in a 0.5 M HOBt-solution and 4 eq of N, N´-diisopropylcarbodiimide (DIPCI) were added. After 1 hour DIPEA (5 eq) was added and the so equipped syringe was shaken over night. The resin was washed 3 times each with DMF, DCM and Et_2O and dried under vacuum.

Photometric determination of the loading of the HBBA-AM resin with the first AA:

To a weighed sample of the resin was added a 20 % solution of piperidine in DMF (v/v) and the mixture was shaken for 30 min. A diluted sample of the filtrate was used to record the absorbance of the corresponding 9-methylene-9-fluorene at 266, 289 and 300 nm. With the appropriate extinction coefficients $\varepsilon(266) = 17500\ M^{-1}cm^{-1}$, $\varepsilon(289) = 5800\ M^{-1}cm^{-1}$ and $\varepsilon(300) = 7800\ M^{-1}cm^{-1}$ the loading was determined using the Lambert-Beer equation.

Capping:

Prior to acetylation the resin was swollen in DMF for 30 min. The solvent was filtered of and acetic anhydride (10 eq based on the difference in substitution-loading) and DIPEA (10 eq) were added. After 40 min the solution was drained off and the resin was washed 3 times each with DMF, DCM and Et_2O and dried under vacuum.

General coupling procedure for aliphatic amino acids:

All peptides were synthesised using 2 / 5 mL frit syringes filled with 50 / 100 mg of resin. Fmoc-AA-loaded HMBA-AM resin, Rink Amide MBHA resin or Fmoc-AA-loaded SASRIN resin was added to a frit-syringe, suspended in DMF and allowed to swell for 30 min. Prior to the amino acid coupling the Fmoc protecting group of the growing peptide chain was cleaved using the procedure described beneath. Fmoc protected aliphatic amino acids (5 eq) in 0.45 M HOBt-solution, 5 equivalents of a 0.44

M HBTU[§] or TBTU solution in DMF and 10-fold excess of DIEA as a 1.2 M solution in DMF were added in succession. The syringe was subsequently shaken for 40 min. Following filtration, the resin was washed 4 times with DMF. Fmoc cleavage was brought about through the addition of 40 % (v/v) piperidine-DMF solution and shaking for 3 min followed by the addition of a 20 % (v/v) piperidine-DMF solution and shaking for 10 min. The solution was drained of, the deprotected resin bound peptide was washed 6 times with DMF and subsequently coupled to the next Fmoc protected amino acid or Fmoc protected SAAC.

General coupling procedure for SAAC and receptor units:

SAAC **9**, SAAR **10a**, SAAR **2** and SAAR **15b** were coupled using the same conditions as for the aliphatic amino acids.

The coupling procedure of BC-SAAC **6** was performed twice. Two times 2.5-fold excess of BC-SAAC **6** was dissolved in NMP and activated by sequential addition of 1 eq HOAT (0.5 M in NMP) and 1 eq HATU (0.5 M in NMP) and 2.5 eq collidine.

Cleavage from the solid support (Rink Amid):

Cleavage from the solid support was performed using a TFA cocktail containing TFA/TIS/H_2O (90:5:5 v/v/v) for 3 hours. In order to precipitate the peptide, cold Et_2O was added and the resulting heterogeneous solution centrifuged for 10 min at -4°C. The supernatant was decanted and the resulting pellet was washed with colt Et_2O. This procedure was repeated several times before the precipitate was dissolved in water and lyophilized.

Cleavage from the solid support (HMBA-AM):

Best results were achieved when adding DIEA/MeOH/DMF (1:5:5 v/v/v) to the preswollen resin and cleaving over night. If the peptide precipitates by adding cold Et_2O, resulting heterogeneous solution was centrifuged for 10 min at -4°C. The supernatant was decanted and the resulting pellet was washed with colt Et_2O. This procedure was repeated several times before the precipitate was dissolved in water and lyophilized.

[§] Abbreviations not defined in the text: HBTU = *N*-[(1*H*-benzoltriazol-1-yl)(dimethylamino)methylene]*N*-methylmethanaminium hexafluorophosphate *N*-oxide, HOBt = 1-Hydroxybenzotriazole; DIPEA = diisopropylethylamine; TBTU = Benzotriazol-1-yl-tetramethyluronium tetrafluoroborate, HOAt = 4-Hyddroxypyrazolo[3,4-d]pyrimidine, HATU = O-(7-Azabenzotriazole-1-yl)-N, N,N'N'-tetramethyluronium hexafluorophosphate, DIC = 1,3 – Diisopropylcarbodiimide.

If the precipitation fails, the filtrate is evaporated to dryness on a rotary evaporator, dissolved in water and lyophilized.

Cleavage from the solid support (SASRIN):

The preswollen peptide resin was treated with 1% TFA/DCM (15 mL/g) for 3 min. The cleavage solution was sucked into a vessel containing 2 eq of pyridine (per eq of TFA) and a little methanol (1 mL/10 mL solution). The treatment was repeated with further portions of 1% TFA/DCM. Volatile compounds of the collected filtrates were removed in vacuum and the residue was redissolved in Et_20 and removed under reduced pressure. This procedure was repeated several times before the precipitate was dissolved in little methanol and precipitated with cold Et_2O.

1.4.2 Synthesis and characterisation of compounds

Cu-IDA-complex 2

Compound **1** was first treated with HCl saturated ether to obtain the free iminodiacetic acid functionality: Compound **1** (1.62 g, 2.72 mmol) was dissolved in DCM and cooled to 0 °C using an ice bath. To this mixture 30 mL of HCl saturated ether was added. The mixture was allowed to warm to room temperature and stirred over night. The reaction progress was controlled by ^{1}H-NMR. The mixture was concentrated under reduced pressure and dried under high vacuum to obtain product **1a** as colorless solid in quantitative yield and was subsequently used for complexation. mp: 135 °C; $[a]^{20}_D$ = -13.9 ° (c = 0.13 in MeOH); IR (KBr disk): $\tilde{\nu}$ [cm^{-1}] = 3419, 2953, 2619, 1735, 1528, 1250, 740; MS (ESI, MeOH + 10 mmol/L NH_4OAc): e/z (%) = 485 (100) [MH^+], 619.4 (7) [MNa^+]. EA ($C_{25}H_{28}N_2O_8$ + 3 H_2O) calculated (%): C 55.74, H 6.37, N 5.20. Found: C 55.18, H 6.21, N 4.96.

Fmoc-IDA-OH **1a** (930 g, 1.79 mmol) and $Cu_2(OH)_2CO_3$ (395 mg, 1.79 mmol) were suspended in H_20/MeOH solution (30 mL, 1:1). The mixture was stirred for 3 h at 70 °C and the resulting blue solution was decanted from the residual $Cu_2(OH)_2CO_3$. MeOH was removed under vacuum and the remaining aqueous solution was lyophilized yielding **2** as a blue solid in quantitative yield. mp: decompostition at 135 °C; IR (KBr disk): $\tilde{\nu}$ [cm^{-1}] = 3414, 2945, 1707, 1622, 1583, 1400, 740; MS (ESI,

H_2O/MeCN/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 544 (100) $[M - H^+]^-$, 580 (35) $[M + Cl^-]^-$, 1091 (8) $[2M - H^+]^-$.

1,4,7-Tri-tert-butyl 10,10'-(6-(6-(benzyloxy)-5-(benzyloxycarbonylamino)-6 oxo-hexylamino)-1,3,5-triazine-2,4-diyl)-bis-(1,4,7,10-tetraazacyclododecane-1,4,7 tricarboxylate) 4

To a solution of Z-Lys-OBzl benzenesulfate (1.96 g, 371 mmol) and K_2CO_3 (1.03 g, 7.42 mmol) was added bis-Boc-cyclen triazin **3**[16] (3.73 g, 3.53 mmol) and the reaction mixture was refluxed for 3 d. The filtrate was concentrated under reduced pressure and the crude compound was purified by silica gel column (eluent: $CHCl_3$/MeOH = 95:5; R_f = 0.73) to give **4** as a white solid (4.19 g, 3.01 mmol, 85 %). mp: 140 °C; $[a]^{20}_D$ = -3.4 (c = 0.01 in $CHCl_3$); ^{1}H-NMR (600 MHz, d-DMSO): δ = 1.13-1.55 (m, 58 H, Boc-CH_3, Lys-$CHCH_2CH_2$, $CHCH_2CH_2CH_2$), 1.57-1.74 (m, 2 H, Lys-$CHCH_2$), 3.06-3.78 (m, 34 H, Cyclen-CH_2, $CHCH_2CH_2CH_2CH_2$), 4.00-4.08 (m, 1 H, CH), 4.98-5.07 (m, 2 H, H-Bzl), 5.10 (s, 2 H, H-Z) 6.64 (bs, 1 H, NH), 7.27-7.38 (m, 10 H, H-Aryl), 7.73 (d, 1 H, NH); ^{13}C-NMR (150 MHz, d-DMSO, HSQC, HMBC): δ = 22.9 (−, C-3), 27.9 (+, 12 C, Boc), 28.0 (+, 6 C, Boc), 28.9 (−, C-4), 30.5 (−, C-2), 40.0 (−, C-5), 49.4 (−, 16 C, Cyclen), 54.0 (+, CH), 65.5 (−, CH_2Bzl), 66.8 (−, CH_2Z), 78.9 (C_{quat}, Boc), 79.0 (C_{quat}, Boc), 79.1 (C_{quat}, 2 C, Boc), 79.3 (C_{quat}, Boc), 79.4 (C_{quat}, Boc), 127.7 (C_{quat}, 2 C, Aryl), 127.7 (C_{quat}, 2 C, Aryl), 127.8 (C_{quat}, Aryl), 127.9 (C_{quat}, Aryl), 128.3 (C_{quat}, 2 C, Aryl), 128.3 (C_{quat}, 2 C, Aryl), 155.5 (C_{quat}, 3 C, triazin), 156.1 (C_{quat}, ester), 172.2 (C_{quat}, carbamate); IR (KBr disk): $\tilde{\nu}$ [cm^{-1}] = 3441, 2974, 2932, 2360, 2342, 1560, 1542, 1410, 1366, 1250, 1166; MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc) : e/z (%) = 1391.2 (100) $[MH^+]$, 646.1 (26) $[M + 2H^+ - Boc]^{2+}$, 704.7 (7) $[MH^+ + NH_4^+]^{2+}$.

2-(((9H-Fluoren-9-yl)methoxy)carbonylamino)-6-(4,6-bis(4,7,10-tris(*tert*-butoxy-carbonyl)-1,4,7,10-tetraazacyclododecan-1-yl)-1,3,5-triazin-2-ylamino)hexanoic acid 6

Fmoc-OSuc (405 mg, 1.20 mmol) and DIPEA (155 µL, 1.20 mmol) were added in succession to a suspension of **5** (1.54 g, 1.10 mmol) in DCM (100 mL). The reaction mixture was stirred at room temperature for 12 h. It was extracted with an aqueous solution of NaH_2PO_4 (100 mM, 3 x 40 mL, pH 5.0) and the organic phase was washed

with water, then dried over anhydrous magnesium sulphate and the solvent was removed under vacuum. The crude product was purified by silica column chromatography (eluent: $CHCl_3$/MeOH = 95:5; R_f = 0.38) to give **6** (1052 mg, 0.76 mmol, 68 %) as a white solid solid. mp: 102 °C; $[a]^{20}_D$ = +25.0 (c 0.008 in $CDCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$, COSY, HSQC, HMBC): δ = 1.35-1.48 (m, 54 H, Boc), 1.49-165 (m, 4 H, H-3, H-4), 1.76-1.98 (m, 2 H, H-2), 2.99-3.95 (m, 34 H, H-cylen, H-5), 4.13-4.23 (m, 1 H, H-10), 4.26-4.44 (m, 3 H, H-1, H-9), 6.07 (bs, 1 H, NH), 7.21-7.28 (m, 2 H, H-B), 7.31-7.38 (m, 2 H, H-C), 7.54-7.63 (m, 2 H, H-A), 7.71 (d, 2 H, ^3J = 7.3 Hz, H-D); ^{13}C-NMR (100 MHz, $CDCl_3$, HSQC, HMBC): δ = 22.0 (−, C-3/4), 28.5 (+, 12 C, Boc), 28.5 (+, 6 C, Boc), 30.9 (−, C-3/4), 31.9 (−, C-2), 40.1 (−, C-5), 47.2 (+, C-10), 50.2 (−, 16 C, Cyclen), 54.3 (+, C-1), 66.9 (−, C-9), 80.0 (C_{quat}, 6 C, Boc), 119.9 (C_{quat}, 2 C, C-D), 125.3 (C_{quat}, 2 C, C-A), 127.0 (C_{quat}, 2 C, C-B), 127.6 (C_{quat}, 2 C, C-C), 141.2 (C_{quat}, 4 C, Fmoc), 143.9 (C_{quat}, 2 C, Fmoc), 144.1 (C_{quat}, 2 C, Fmoc), 156.1 (C_{quat}, triazin), 156.8 (C_{quat}, 2 C, triazin), 156.8 (C_{quat}, ester), 175.2 (C_{quat}, acid); IR (KBr disk): $\tilde{\nu}$ [cm^{-1}] = 3433, 2974, 2932, 1693, 1542, 1411, 1366, 1250, 1165; MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc) : e/z (%) = 703.7 (100) $[MH^+ + NH_4^+]^{2+}$, 1389.1 (33) $[MH^+]$, 645.2 (21) $[M + 2H^+ - Boc]^{2+}$, 617.1 (21) $[MH^+ + 2H^+ - Boc - \Delta C_4H_8]^{2+}$, 695 (10) $[(MH^+ + 2H^+)]^{2+}$. EA ($C_{70}H_{109}N_{13}O_{16}$ + 5 H_2O) calculated (%): C 56.84, H 8.12, N 12.32: Found: C 56.80, H 7.86, N 12.03.

Bis-Zn-cyclen-complex 8

Hepes buffer (10 mM, pH 8) was prepared and 10 mL where heated to 80 °C in a round-bottom flask. L-Tyr(tri-Boc-bis-Cyc)-OH **7** (500 mg, 0.54 mmo) and $Zn(ClO_4)_2$ (402 mg, 1.08 mmol) where each dissolved in 10 mL of Hepes-buffer and added dropwise and simultaneously under stirring. The reaction mixture was stirred for further 3 h at 80°C and at room temperature over night. Compound **8** was obtained as a white solid after lyophilization.

MS (ESI, H_2O/MeCN/MeOH + 10 mmol/L NH_4OAc): e/z (%) = 456.8 (100) $[K^{4+} - 2H^+]^{2+}$, 476.4 (24) $[K^{4+} + Cl^- - H^+]^{2+}$.

Fmoc-bpa-Zn$(NO_3)_2$ 10a

To the SAAC **9** (850 mg, 1.54 mmol) in 80 mL MeOH was added a solution of $Zn(NO_3)_2 \cdot 6\ H_2O$ (458 mg, 1.54 mmol) in 20 ml H_20 and the resulting solution was stirred for 1 h. The MeOH was removed under vacuum and the remaining aqueous solution was lyophilized yielding **10a** as a white solid in quantitative yield. mp**:** decomposition at 150 °C; $[a]^{20}_D$ = -27.2 ° (c = 0.007 in MeOH); IR (KBr disk): $\tilde{\nu}$ [cm^{-1}] = 3416, 3066, 2944, 1711, 1609, 1415, 1383, 1312, 1026, 763; MS (ESI, H_2O/MeCN): m/z (%) = 613 (100) [Fmoc-BPA-Zn^{2+} - H^+]$^+$, 1229 (10) [2(Fmoc-BPA-Zn^{2+}) - $3H^+$]$^+$;

2-{[(9H-Fluoren-9-yl)methoxy]carbonylamino}-3-{3,5-bis[(bis-pyridin-2-ylmethyl-amino)methyl]-4-hydroxyphenyl}propanoic acid 14

DIPEA (0.68 mL, 4.0 mmol) and Fmoc-OSuc (236 mg, 0.70 mmol) were added successively to a suspension of **13** (520 mg, 0.48 mmol) in DCM (50 mL). The reaction mixture was stirred at room temperature for 12 h. The mixture was extracted with an aqueous solution of NaH_2PO_4 (100 mM, 3 x 20 mL, pH 5.0) and the organic phase was washed with water, then dried over anhydrous magnesium sulphate and the solvent removed under vacuum. The crude product was purified by silica column chromatography (eluent: $CHCl_3$/MeOH = 95:5; R_f = 0.05) to give **14** (309 mg, 0.37 mmol, 78 %) as a pale yellow solid. mp**:** decomposition 88°C; $[a]^{20}_D$ = +41.5 ° (c = 0.02 in $CHCl_3$); IR (KBr disk): $\tilde{\nu}$ [cm^{-1}] = 3414, 3252, 3057, 3057, 2924, 2822, 2362, 1714, 1592. ^{1}H-NMR (600 MHz, $CDCl_3$, COSY, HSQC, HMBC): δ = 2.97 (dd, 1 H, ^2J = 13.2 Hz, ^3J = 5.0 Hz, TyrCHCH_2), 3.11 (dd, 1 H, ^2J = 12.7 Hz, ^3J = 2.5 Hz, TyrCHCH_2), 3.48- 3.50 (m, 2 H, TyrCH_2N), 3.76-3.86 (m, 10 H, Pyr-CH_2-N, TyrCH_2N), 4.11-4.16 (m, 1 H, Fmoc-CH), 4.17-4.22 (m, 1 H, Fmoc-CH_2), 4.26-4.31 (m, 1 H, TyrCH), 4.32-4.37 (m, 1 H, Fmoc-CH_2), 5.95 (d, 1 H, ^3J = 5.1 Hz, NH), 6.90 (bs, 2 H, CH-phenol), 7.10-7.14 (m, 4 H, pyr), 7.19-7.27 (m, 6 H, pyr, aryl), 7.34-7.39 (m, 2 H, aryl) 7.52-7.60 (m, 6 H, pyr, aryl), 7.74 (d, 2 H, ^3J = 7.5 Hz, aryl), 8.61-8.70 (m, 4 H, pyr), 10.96 (bs, 1 H, Tyr-OH); ^{13}C-NMR (151 MHz, $CDCl_3$, HSQC, HMBC): δ = 37.6 (−, CH_2), 47.4 (+, CH), 54.8 (−,2 C, CH_2), 57.4 (+, CH), 59.6 (−, 4 C, CH_2), 66.2 (−, CH_2), 119.8 (+, 2 C, CH), 122.3 (+, 4 C, CH), 122.9 (C_{quat}, 2 C), 123.5 (+, 4 C, CH), 125.3 (+, 2 C, CH), 127.0 (+, 2 C, CH), 127.5 (+, 2 C, CH), 129.2 (C_{quat}, 1 C), 131.3 (+, 2 C, CH), 136.7 (+,

4 C, CH), 141.2 (C_{quat}, 2 C), 144.3 (C_{quat}, 1 C), 149.8 (+, 4 C, CH), 154.2 (C_{quat}, 1 C), 155.3 (C_{quat}, carbamate), 158.3 (C_{quat}, 4 C), 176.6 (C_{quat}, acid); MS (ESI, DCM/MeCN/H_2O + 10 mmol/L TFA) : *m/z* (%) = 413 (100) [M + $2H^+$], 826.4 (30) [MH^+]. HRMS calculated for $C_{50}H_{48}N_7O_5$ [MH^+]: 826.3765; found: 826.3765 ± 5.8 ppm.

Bis-bpa-Zn(Cl)-complex 15b

The free ligand **14** (320 mg, 0.39 mmol) was dissolved in methanol (25 mL). To this solution, $ZnCl_2$ (52.8 mg, 0.39 mmol) in 5 mL H_2O was added and the mixture was stirred at room temperature for 3 h. Methanol was then removed under reduced pressure and the remaining aqueous solution was lyophilized to give the desired zinc coordinated complex as a white solid in quantitative yield. mp: decomposition > 170 °C; $[a]^{20}_D$ = +45.0 ° (c = 0.01 in $CHCl_3$); IR (KBr disk): $\tilde{\nu}$ [cm^{-1}] = 3422, 3055, 2921, 1713, 1606, 1526; MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): e/z (%) =1070.4 (100) [K^{3+} + $2CH_3COO^-]^+$, 1010.3 (45) [K^{3+} - H^+ + $CH_3COO^-]^+$, 505.4 (6) [K^{3+} - $H^+]^{2+}$.

Peptide 16

The synthesis was performed manually in a 5 mL syringe equipped with porous filter using Fmoc protected Rink amide resin (100 mg, subst.: 0.7 mmol/g). SPPS of Fmoc protected aliphatic amino acids and SAAC **9** according to the specific stated procedure provided peptide **16** as a white solid. ^{1}H-NMR (600 MHz, DMSO-d_6, COSY, HSQC, HMBC): δ = 0.85 (d, 3 H, ^3J = 6.7 Hz, H-29a), 0.83 (d, 3 H, ^3J = 6.9 Hz, H-24a), 0.81 (d, 3 H, ^3J = 6.7 Hz, H-29b), 0.80 (d, 3 H, ^3J = 6.9 Hz, H-24b), 1.22 (d, 3 H, ^3J = 7.06 Hz, H-11), 1.23-1.32 (m, 2 H, H-3), 1.42-1.46 (m, 2 H, H-22), 1.47-1.52 (m, 1 H, H-2a), 1.54-1.59 (m, 1 H, H-23), 1.60 – 1.67 (m, 1 H, H-2b), 1.69-1.76 (m, 2 H, H-4), 1.90-1-96 (m, 1 H, H-28), 3.06-3.13 (m, 2 H, H-5), 3.52-3.62 (m, 2 H, H-14), 3.68 (dd, 1 H, ^3J = 5.8 Hz, ^2J = 16.7 Hz, H-18a), 3.74 (dd, 1 H, ^3J = 5.6 Hz, ^2J = 16.7 Hz, H-18a), 4.06 (dd, 1 H, $^3J_1$ = 6.9 Hz, $^3J_2$ = 8.9 Hz, H-27), 4.18-4.22 (m, 1 H, H-1), 4.31-4.40 (m, 2 H, H-21, H-10), 4.50 (bs, 7 H, H-7), 7.00 (s, 1 H, H-31a), 7.33 (s, 1 H, H-31b), 7.41-7.45 (m, 2 H, H-B), 7.51 (d, 2 H, ^3J = 7.9 Hz, H-D), 7.67 (d, 1 H, NH-30), 7.87 (dd, 1 H, $^3J_1$ = 7.7 Hz, $^3J_2$ = 7.7 Hz, H-Ca), 7.87 (dd, 1 H, $^3J_1$ = 7.7 Hz, $^3J_2$ = 7.7 Hz, H-Cb), 7.96 (d, 1 H, NH-25), 8.00-8.08 (m, 2 H, NH-13, NH-19), 8.19 (d, 1 H, ^3J = 7.7 Hz, NH-16),

8.55 (d, 1 H, ^3J = 7.3 Hz), 8.61-8.63 (m, 2 H, H-A); ^{13}C-NMR (150 MHz, DMSO-d_6, HSQC, HMBC): δ = 18.0 (+, C-29a), 18.4 (+, C-11), 19.2 (+, C-29b), 21.5 (+, C-24a), 22.4 (−, C-3), 23.1 (+, C-24b), 23.1 (−, C-4), 24.1 (+, C-23), 30.4 (+, C-28), 31.2 (−, C-2), 40.0 (−, C-14), 40.1 (−, C-22), 41.8 (−, C-18), 48.3 (+, C-10), 51.1 (+, C-21), 52.5 (+, C-1), 53.8 (−, C-5), 56.9 (−, 4 C, C-7), 57.5 (+, C-27), 123.9 (+, 2 C, C-B), 124.7 (+, 2 C, C-D), 137.6 (+, 2 C, C-C), 149.1 (+, 2 C, C-A), 150.1 (C_{quat}, 2 C, C-E), 165.5 (C_{quat}, C-8), 168.4 (C_{quat}, C-20), 171.4 (C_{quat}, C-17), 171.7 (C_{quat}, C-26), 171.8 (C_{quat}, C-12), 172.8 (C_{quat}, C-15); MS (ESI, DCM/MeOH + NH_4OAc): e/z (%) = 363.2 (100) $[M + 2H^+]^{2+}$, 725.6 (71) $[MH^+]$.

Peptide complex 17

To a solution of peptide **16** (20 mg, 0.03 mmol) in 10 mL H_2O was added $Zn(NO_3)_2 \cdot 6H_2O$ (8.4 mg, 0.03 mmol). After the mixture was stirred over night a white solid was obtained after lyophilization. ^{1}H-NMR (600 MHz, DMSO-d_6, COSY, HSQC, HMBC): δ = 0.80-0.86 (m, 12 H, H-29, H-24), 1.08-1.19 (m, 2 H, H-4), 1.12 (d, ^3J = 7.0 Hz, 3 H, H-11), 1,42-1.48 (m, 5 H, H-22, H-2a, H-3), 1.53-1.61 (m, 2 H, H-2b, H-23), 1.91-1.97 (m, 1 H, H-28), 2.56-2.62 (m, 2 H, H-5), 3.53-3.75 (m, 4 H, H-14, H-18), 4.00 (d, 2 H, ^2J = 16.0 Hz, H-7a), 4.07 (dd, ^3J = 9.0, ^3J = 6.8, 1 H, H-27), 4.17 (m, 1 H, H-1), 4.24 (dd, ^2J = 16.0, ^4J = 2.3, 2 H, H-7b), 4.30-4.40 (m, 2 H, H-10, H-21), 7.02 (s, 1 H, N-H), 7.34 (s, 1 H, N-H), 7.59-7.66 (m, 2 H, H-B, H-D), 7.93-7.99 (m, 1H, N-H), 8.05 (t, ^3J = 5.5, 1 H, N-H), 8.08-8.12 (m, 2 H, H-C), 8.15 (d, 1 H, ^3J = 7.7 Hz, N-H), 8.50 (d, ^3J = 7.2 Hz, 1 H, NH), 8.71 (d, ^3J = 4.8 Hz, 2 H, H-A); ^{13}C-NMR (150 MHz, DMSO-d_6, HSQC, HMBC): δ = 18.0 (+, C-29a), 18.5 (+, C-11), 19.2 (+, C-29b), 21.5 (+, C-24a), 22.8 (−, C-3), 23.1 (+, C-24b), 23.1 (−, C-4), 24.1 (+, C-23), 30.4 (+, C-28), 31.5 (−, C-2), 40.1 (−, C-14), 40.8 (−, C-22), 41.8 (−, C-18), 48.3 (+, C-10), 51.1 (+, C-21), 52.6 (+, C-1), 54.9 (−, C-5), 56.7 (−, C-7), 57.4 (+, C-27), 124.2 (+, 2 C, C-B), 124.6 (+, 2 C, C-D), 140.7 (+, 2 C, C-C), 147.8 (+, 2 C, C-A), 155.1 (C_{quat}, 2 C, C-E), 165.4 (C_{quat}, C-8), 168.4 (C_{quat}, C-20), 171.4 (C_{quat}, C-17), 171.7 (C_{quat}, C-26), 171.7 (C_{quat}, C-12), 172.7 (C_{quat}, C-15); MS (ESI, H_2O/NH_4OAc): e/z (%) = 394.3 (100) $[M]^{2+}$, 901.4 (14) $[M^{2+} + TFA]^+$, 787.4 (8) $[M^{2+} - H^+]^+$, 850.4 (3)$[M^{2+} + NO_3^-]^+$.

Peptide 18

The synthesis was performed manually in a 5 mL syringe equipped with porous filter using Fmoc protected Rink amide resin (50 mg, subst.: 0.7 mmol/g). SPPS of Fmoc protected amino acid monomers and SAAC **9** according to the specific stated procedure provided compound **18** as a white solid. ^{1}H-NMR (600 MHz, DMSO-d_6, COSY, HSQC, HMBC): δ = 0.75-0.83 (dd, 6 H, $^{3}J_1$ = 6.7 Hz, $^{3}J_2$ = 6.9 Hz, H-21, H-21'), 0.94 (d, 6 H, ^{3}J = 6.7 Hz, H-35, H-35'), 1.17 (d, 3 H, ^{3}J = 7.2 Hz, H-11), 1.20 (d, 3 H, ^{3}J = 7.2 Hz, H-25), 1.20-1.32 (m, 4 H, H-3), 1.55-1.42 (m, 2 H, H-2´a, H-2'b), 1.55-1.67 (m, 2 H, H-2a, H-2'a), 1.69-1.78 (m, 4 H, H-4), 1.82 (s, 1 H, H-36), 1.85 (s, 1 H, H-36), 1.92-1.99 (m, 1 H, H-20), 2.01-2.07 (m, 1 H, H-34), 3.04-3.12 (m, 4 H, H-5), 3.62-3.66 (m, 1 H, H-30b), 3.67-3.76 (m, 2 H, H-14b, H-30a), 3.94 (dd, 1 H, ^{3}J = 16.6 Hz, ^{2}J = 6.1 Hz, H-14a), 4.11-4.23 (m, 3 H, H-33, H-10, H-19), 4.28-4.34 (m, 2 H, H-24, H-1), 4.49 (m, 8 H, H-7), 7.00 (s, 1 H, H-8), 7.29-7.33 (m, 1 H, H-8), 7.42-7.45 (m, 4 H, H-B), 7.53 (d, 4 H, ^{3}J = 7.9 Hz, H-D), 7.67 (dd, 1 H, ^{3}J = 27.7 Hz, ^{4}J = 8.7 Hz, H-22), 7.86-7.90 (m, 4 H, H-C), 7.91 (d, 1 H, ^{3}J = 7.4 Hz, H-12), 8.08-8.21 (m, 4 H, H-15, H-26, H-28, H-31), 8.61-8.66 (m, 4 H, H-A); ^{13}C-NMR (150 MHz, DMSO-d_6, HSQC, HMBC): δ = 17.7 (+, ^{i}Pr), 17.7 (+, C-11), 18.2 (+, ^{i}Pr), 18.2 (+, C-25), 19.1 (+, ^{i}Pr), 22.3 (−, 2 C, C-3), 23.1 (−, C-4), 29.7 (+, C-34), 30.5 (+, C-20), 31.3 (−, C-2), 31.7 (−, C-2'), 41.6 (−, C-14), 41.9 (−, C-30), 48.0 (+, C-10), 48.2 (+, C-24), 52.0 (+, C-1), 52.5 (+, C-33), 53.7 (−, C-5), 56.9 (−, C-7), 57.3 (+, C-19), 123.9 (+, C-B), 124.7 (+, C-D), 137.6 (+, C-C), 149.1 (+, C-A), 151.4 (C_{quat}, C-E), 174.1 (C_{quat}, C-9), 167.9 (C_{quat}, C-16), 168.1 (C_{quat}, C-13), 168.1 (C_{quat}, C-29), 170.8 (C_{quat}, C-18), 171.0 (C_{quat}, C-27), 171.5 (C_{quat}, C-32), 171.7 (C_{quat}, C-23), 174.1 (C_{quat}, C-9); MS (ESI, H_2O/NH_4OAc): e/z (%) = 365 (100) [M + 3 H^+]$^{3+}$, 547 (24) [M + 2 H^+]$^{2+}$, 1092.7 (0.3) [MH^+].

Peptide complex 19

To a solution of peptide **18** (10 mg, 0.01 mmol) in 10 mL H_2O was added $Zn(NO_3)_2 \cdot 6H_2O$ (5.6 mg, 0.02 mmol). After the mixture was stirred over night a white solid was obtained after lyophilization. MS (ESI, H_2O/MeOH + NH_4OAc): e/z (%) = 407.3 (100) [M^{4+} - H^+]$^{3+}$, 608.9 (74) [M^{4+} - 2H^+]$^{2+}$, 365.8 (40) [(M^{4+} - 2Zn^{2+} + 3H^+)]$^{3+}$.

Peptide complex 20

The synthesis was performed manually in a 5 mL syringe equipped with porous filter using HMBA-AM resin (100 mg, subst.: 0.83 mmol/g). SPPS of Fmoc protected amino acids and SAAR **10a** according to the specific stated procedure provided compound **20** as a white solid. ^{1}H-NMR (600 MHz, DMSO-d_6, COSY, HSQC, HMBC): δ = 0.80 (d, 3 H, ^{3}J = 6.7 Hz, H-18´), 0.85 (d, 3 H, ^{3}J = 6.5 Hz, H-18), 1.08 (d, 3 H, ^{3}J = 6.9 Hz, H-11), 1.15-1.28 (m, 2 H, H-3), 1.25 (d, 3 H, ^{3}J = 7.3 Hz, H-25), 1.38-1.50 (m, 5 H, H-2a, H-4, H-21), 1.52-1.62 (m, 2 H, H-2b, H-17), 2.39 (t, 2 H, ^{3}J = 7.2 Hz, H-5), 3.21-3.40 (m, 1 H, H-10), 3.60 (s, 3 H, OMe), 3.66-3.71 (m, 4 H, H-7, H-16), 4.18-4.29 (m, 3 H, H-24, H-15, H-1), 7.20-7.23 (m, 2 H, H-B), 7.50 (d, 2 H, ^{3}J = 7.7 Hz, H-D), 7.74 (dd, 1 H, $^{3}J_1$ = 6.7 Hz, $^{3}J_2$ = 7.7 Hz, H-Ca), 7.74 (dd, 1 H, $^{3}J_1$ = 7.7 Hz, $^{3}J_2$ = 7.7 Hz, H-Cb), 7.81-7.96 (m, 1 H, C-14), 8.05 (d, 1 H, ^{3}J = 7.1 Hz, NH-8), 8.11 (d, 1 H, $^{3}J_1$ = 6.9, H-23), 8.16 (t, 1 H, ^{3}J = 5.9, H-20), 8.43-8.47 (m, 2 H, H-A); ^{13}C-NMR (150 MHz, DMSO-d_6, HSQC, HMBC): δ 17.0 (+, C-25), 21.5= (+, H-11), 21.6 (+, C-18´), 22.9 (+, C-18), 24.1 (−, C-17), 26.3 (−, C-4), 32.3 (−, C-2), 40.4 (−, C-21), 41.6 (−, C-16), 47.5 (+, C-24), 50.1 (+, C-10), 51.3 (+, C-1), 51.8 (+, C-15), 51.8 (+, OMe), 53.5 (−, 4 C, C-7), 122.0 (+, 2 C, C-B), 122.4 (+, 2 C, C-D), 136.4 (+, 2 C, C-C), 148.7 (+, 2 C, C-A), 159.5 (C_{quat}, 2 C, C-E), 168.5 (C_{quat}, C-22), 171.8 (C_{quat}, C-13), 172.1 (C_{quat}, C-19), 172.9 (C_{quat}, C-26), 175.5 (C_{quat}, C-9); MS (ESI, H_2O/MeOH + NH_4OAc): e/z (%) = 359.1 (100) $[M]^{2+}$, 717.3 (14) $[M^{2+} - H^{+}]^{+}$, 352.1 (30) [M - OMe + OH]$^{2+}$, 703.3 (20) [(M - OMe + OH) - H^{+}]$^{+}$.

Dipeptide 21

The synthesis was performed manually in a 5 mL syringe equipped with porous filter using HMBA-AM resin (100 mg, subst.: 1.1 mmol/g). SPPS of Fmoc protected amino acids and SAAC **6** along with HOAt and HATU as coupling reagents according to the specific stated procedure provided compound **21** as a white solid. MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): e/z (%) = 626.6 (100) $[M + 2H^{+}]^{2+}$, 1252.1 (29) $[MH^{+}]$, 1220.1 (4) $[MH^{+} - CH_3OH]$.

Peptide 22

The synthesis was performed manually in a 5 mL syringe equipped with porous filter using SASRIN resin (100 mg). SPPS of Fmoc protected amino acids and SAAC **6** along with HOAt and HATU as coupling reagents according to the specific stated procedure provided compound **22** as a white solid. MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): e/z (%) = 726.1 (100) $[M + 2H^+]^{2+}$, 1451.4 (20) $[MH^+]$, 1463.2 (4) $[M + Na^+]$.

Peptide complex 25

The synthesis was performed manually in a 5 mL syringe equipped with porous filter using HMBA-AM resin (100 mg, subst.: 0.83 mmol/g). SPPS of Fmoc protected amino acids and SAAR **2** according to the specific stated procedure provided compound **25** as a white solid. MS (ESI, H_2O/MeOH + NH_4OAc): e/z (%) = 441 (8) $[(M + 2H^+)]^{2+}$, 882 (8) $[MH^+]$, 904 (6) $[M + Na^+]$.

Peptide complex 26

The synthesis was performed manually in a 5 mL syringe equipped with porous filter using HMBA-AM resin (100 mg, subst.: 1.1 mmol/g). SPPS of Fmoc protected amino acids and SAAR **15b** according to the specific stated procedure provided compound **26** as a white solid. MS (ESI, H_2O/MeOH + NH_4OAc): e/z (%) = 543.4 (100) $[K^{3+} + CH_3COO^-]^{2+}$, 1145.6 (50) $[K^{3+} + 2CH_3COO^-]^+$.

2-{[(9H-Fluoren-9-yl)methoxy]carbonylamino}-6-[bis(2-*tert*-butoxy-2 oxoethyl)amino] hexanoic acid 1

Improved synthesis according to *ChemBioChem* **2003**, *4*, 1340-1344: Fmoc-OSuc (1.96 g, 5.80 mmol) and DIPEA (1.00 mL, 5.80 mmol) were added to a solution of *N*,*N*-bis(*tert*-butyloxycarbonylmethyl)-L-lysine (2.13 g, 5.69 mmol) in dry CH_2Cl_2 (50 mL) and the reaction mixture was stirred over night at room temperature under a nitrogen atmosphere. The volatile compounds were removed under reduced pressure and the crude product was redissolved in CH_2Cl_2 (50 mL) and washed with an aqueous solution of NaH_2PO_4 (100 mM, 3 x 20 mL, pH 5.0). The organic phase was dried over anhydrous magnesium sulphate and the solvent was removed under vacuum. The crude

product was purified by silica column chromatography with $CHCl_3$/MeOH (3:1) as eluent. Yield: 2.44 g (72 %). mp: 50 °C; $[a]^{20}_D$ = 12.5° (c = 0.01 in $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.24-1.55 (m, 4 H, CHC*H*$_2$CH$_2$, CHCH$_2$C*H*$_2$), 1.44 (s, 18 H, Boc), 1.68-1.99 (m 2 H), 2.57-2.87 (m, 2 H), 3.51 (bs, 4 H, N-(CH$_2$)$_2$), 4.20 (t, 1 H, ^3J = 7.0 Hz, C*H*-Fmoc), 4.29-4.50 (m, 3 H, CH*2*-Fmoc, Lys-CH), 5.73 (bs, 1 H, NH), 7.27-7.33 (m, 4 H, Fmoc), 7.38 (t, 2 H, Fmoc), 7.52-7.63 (m, 2 H, Fmoc), 7.74 (d, 2 H, Fmoc); MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): e/z (%) = 597.4 (100) [MH^+], 619.4 (5) [MNa^+].

2-Amino-6-(4,6-bis(4,7,10-tris(tert-butoxycarbonyl)-1,4,7,10-tetraazacyclododecan-1-yl)-1,3,5-triazin-2-ylamino)hexanoic acid 5

Z-L-Tyr(tri-Boc-Cyc)-OBzl **4** (4.17 g, 3.0 mmol) was dissolved in EtOH (100 mL) and a spatula Tipp of 10% Pd/C was added. The reaction mixture was stirred in an autoclave under a hydrogen atmosphere at 10 bar pressure for 18 h. The catalyst was filtered off using celite and the filtrate was concentrated under vacuum. **5** (2.98 g, 2.56 mmol, 85 %) was obtained as a colourless solid and was subsequently used in the next step without further purification.
^{1}H-NMR (300 MHz): δ = 1.41 (s, 36 H, Boc), 1.45 (s, 18 H, Boc), 1.54-1.72 (m, 4 H, LysCHCH$_2$C*H*$_2$CH$_2$, LysCHCH$_2$CH$_2$C*H*$_2$), 1.94-2.10 (m, 2 H, CH$_2$-N), 3.17-3.91 (35 H, cylen, C*H*), 8.43 (bs, 2 H, NH_2) 8.82-9.75 (bs, 1 H, OH); MS (ESI, DCM/MeOH): *m/z* (%) = 1157.1 (21) [MH^+], 584.1 (100) [$M+2H^+]^{2+}$.

10,10'-(6-(5-(((9H-Fluoren-9-yl)methoxy)carbonylamino)-5-carboxypentylamino)-1,3,5-triazine-2,4-diyl)diaza-1,4,7-triazoniacyclododecane-1,4,7-triium 7

Fmoc-L-Tyr(tri-Boc-bis-Cyc)-OH **6** (620 mg, 0.45 mmol) was dissolved in MeOH, treated with trifluoroacetic acid (38 mmol) and stirred at room temperature for 24 h. The volatile compounds were removed under vacuum affording the fully deprotected compound as a white solid in sufficient purity for use in subsequent step. ^{1}H-NMR (300 MHz, MeOD): δ = 1.24-1.38 (m, 1 H), 1.44-1.82 (m, 3 H), 1.86-2.04 (m, 1 H), 2.95-3.43 (m, 35 H), 4.12-4.25 (m, 3 H), 4.29-4.40 (m, 1 H), 7.25-7.34 (m, 2 H), 7.35-7.44 (m, 2 H), 7.59-7.70 (m, 2 H), 7.80 (d, 2 H, ^3J = 7.7 Hz)

2-(((9*H*-Fluoren-9-yl)methoxy)carbonylamino)-6-(bis(pyridin-2-ylmethyl)amino)hexanoic acid 9[**]

DIPEA (131 mg, 1.02 mmol) was added to a suspension of Fmoc-L-Lys(HCl)-OH (405 mg, 1.00 mol) in 15 mL DCM. 2-Pyridinecarboxaldehyde (307 mg, 2.87 mmol) was added and the resulting mixture was stirred for 15 min. Sodium triacetoxyborohydride (890 mg, 4.20 mmol) was added and the resulting cloudy yellow solution was stirred for 90 h at ambient temperature under a nitrogen atmosphere. The organic phase was separated and the aqueous phase was extracted 3 times with chloroform/isopropanol 3:1. The organic phases were combined and washed with brine then dried with $MgSO_4$. The solvent was removed under reduced pressure to give brown oil which was purified by column chromatography on silica gel (7:3 chloroform/methanol). Concentration of the appropriate fractions gave the dipyridylmethyl derivative of Fmoc-L-lysine **9** as a colourless solid (465 mg, 85%). mp: 79 °C; ^{1}H NMR (300 MHz, d_6-DMSO): δ = 8.45 (d, ^3J = 4.1, 2 H, aryl), 7.90-7.83 (m, 2 H, aryl), 7.74-7.64 (m, 4 H, aryl), 7.51-7.46 (m, 2 H, aryl), 7.42-7.35 (m, 2 H, aryl), 7.30-7.17 (m, 4 H, aryl), 6.93 (bs, 1 H, OH), 6.40 (bs, 1 H, N-H), 4.33-4.16 (m, 3 H, CH, CH_2), 3.83-3.65 (m, 4 H, 2x CH_2), 2.43-2.36 (m, 2 H, CH_2), 1.70-1.16 (m, 6 H, 3x CH_2); MS (ESI, CH_2Cl_2/MeOH + NH_4Ac): e/z (%) = 551.5 (100) [MH^+]; HRMS ($C_{33}H_{34}N_4O_4$) calculated 551.2658 found 551.2653 ± 0.96 ppm.

Fmoc-bpa-$CuCl_2$ 10b

To the SAAC **9** (466 mg, 0.85 mmol) in 50 mL MeOH was added a solution of $NiC_4H_6O_4 \cdot 4\ H_2O$ (210 mg, 0.85 mmol) in 20 ml H_20 and the resulting solution was stirred over night. The MeOH was removed under vacuum and the remaining aqueous solution was lyophilized yielding **10b** as a green solid in quantitative yield. mp**:** decomposition > 200 °C; $[a]^{20}_D$ = -26.0° (c = 0.008 in MeOH); IR (KBr disk): $\tilde{\nu}$ [cm^{-1}] = 3423, 3080, 2939, 1713, 1603, 1442, 1410, 762; MS (ESI, H_2O/MeOH): m/z (%) = 612.1 (100) [Fmoc-BPA-Cu^{2+} - H^+]$^+$, 648.1 (30) [Fmoc-BPA-Cu^{2+} + Cl^-]$^+$, 1261.5 (10) [2Fmoc-BPA-Cu^{2+} + Cl^- - $2H^+$]$^+$, 1223.5 (6) [2Fmoc-BPA-Cu^{2+} - $3H^+$]$^+$.

[**] The compound was synthesized according to a similar, at that time unpublished procedure of Dr. Kate Jolliffe from the University of Sydney, Australia and was later published: Levada, M. K.; Banerjee, S. R.; Maresca, K. P.; Babich, J. W.; Zubieta, J. *Synthesis* **2004**, 11, 1759-1766.

Fmoc-bpa-Ni(ac)$_2$ 10c

To the SAAC **9** (466 mg, 0.85 mmol) in 50 mL MeOH was added a solution of $NiC_4H_6O_4 \cdot 4H_2O$ (210 mg, 0.85 mmol) in 20 ml H_20 and the resulting solution was stirred over night. The MeOH was removed under vacuum and the remaining aqueous solution was lyophilized yielding **10c** as a green solid in quantitative yield. mp: decomposition > 200 °C; $[a]^{20}_D$ = -26.0° (c = 0.008 in MeOH); IR (KBr disk): $\tilde{\nu}$ [cm^{-1}] = 3423, 3080, 2939, 1713, 1603, 1442, 1410, 762; MS (ESI, H_2O/MeOH): m/z (%) = 607 (100) [Fmoc-BPA-Ni^{2+} - H$^+$]$^+$.

3-{3,5-Bis[(bis-pyridin-2-ylmethyl-amino)methyl]-4-hydroxyphenyl}-2-(tert-butoxycarbonylamino)propionic acid methyl ester 11[††]

Boc-L-Tyr-OMe (300 mg, 1.02 mmol) and 1 M HCl (0.25 mL) were added to a suspension of paraformaldehyde (100 mg, 3.35 mmol) and 2, 2´-dipicolylamine (508 mg, 2.55 mmol) in ethanol (2.0 mL) and water (6.0 mL). The solution was refluxed for 36 hours, then cooled to room temperature and neutralized with Na_2CO_3. The neutral product was extracted into chloroform, dried with magnesium sulphate, and the solvent was evaporated to yield a yellow oil. The crude compound was purified by silica gel column (eluent: EtOH/EtOAc = 1:1, R_f = 0.41) to give **11** as a yellow solid (377 mg, 0.53 mmol, 52 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.32 (s, 9 H, H-Boc), 2.86-3.02 (m, 2 H, CH_2Tyr), 3.61 (s, 3 H, OCH_3), 3.79 (s, 4 H, Tyr-CH_2N), 3.88 (s, 8 H, Pyr-CH_2-N), 5.23 (m, 1 H, C*H*CH_2Tyr), 7.01 (s, 2 H, Tyr-aryl), 7.09-7.16 (m, 4 H, pyr), 7.48 (d, 4 H, *J* = 8.0), 7.56-7.64 (m, 4 H, Pyr), 8.46-8.64 (m, 4 H, Pyr); MS (ESI, DCM/MeCN/H_2O + 10 mmol/L TFA) : *m/z* (%) = 718.4 (100) [M+2H$^+$]$^{2+}$, 359.8 (31) [MH$^+$], 331.8 (23) [M+2H$^+$-C_4H_8]$^{2+}$.

3-{3,5-Bis[(bis-pyridin-2-ylmethyl-amino)methyl]-4-hydroxyphenyl}-2-(tert-butoxycarbonylamino)propanoic acid 12

Lithium hydroxide (25.2 mg, 1.05 mmol) was dissolved in water (5 mL) and added dropwise to a solution of **11** (0.50 g, 0.70 mmol) in water/methanol (25 %, 50 mL). The

[††] Sun, L.; Burkitt, M.; Tamm, M.; Raymond, M. K.; Abrahamsson, M.; LeGourrierec, D.; Frapart, Y.; Magnuson, A.; Kenez, P. H.; Brandt, P.; Tran, A.; Hammarstroem, L.; Styring, S.; Aakermark, B. *J. Am. Chem. Soc.* **1999,** *121*, 6834-6842. Jiang, H.; O'Neil, E. J.; DiVittorio, K. M.; Smith, B. D. *Org. Lett.* **2005,** *7*, 3013-3016.

mixture was stirred at room temperature for 12 h. The solvent was removed under reduced pressure and the residue was redissolved in water. The pH value was adjusted to 5 by $NaH_2PO_4 \cdot H_2O$ and the product was extracted in DCM. The organic layer was washed with brine, dried over anhydrous magnesium sulphate and concentrated under reduced pressure to obtain **12** as white solid (471 mg, 0.67 mmoL, 95 %). ^{1}H-NMR (600 MHz, d_6-DMSO, HSQC, HMBC): δ = 1.18 (bs, 9 H, Boc), 2.73 (dd, ^2J = 13.8 Hz, ^3J = 10.0 Hz, 1 H, TyrCHCH_2), 2.91 (dd, ^2J = 14.0 Hz^3J = 4.5 Hz, 1, H, TyrCHCH_2), 3.66 (s, 4 H, TyrCH_2N), 3.76 (s, 8 H ,Pyr-CH_2-N), 4.08-4.11 (m, 1 H, TyrCH), 6.96 (d, ^3J = 8.6 Hz, 1 H, NH), 7.07 (s, 2 H, CH-phenol), 7.21-7.24 (4 H, pyr), 7.44 (d, 4 H, ^3J = 7.8 Hz, pyr), 7.68-7.71 (m, 4 H, pyr), 8.47-8.48 (m, 4 H, pyr); ^{13}C-NMR (151 MHz, d_6-DMSO, HSQC, HMBC): δ = 28.0 (+, 3 C, Boc), 35.9−, CH_2), 54.0 (−, 2 C, CH_2), 55.2 (+, CH), 58.8 (−, 4 C, CH_2), 77.8 (C_{quat}, Boc), 122.2 (+, 4 C, CH), 122.8 (+, 4 C, CH), 123.2 (C_{quat}, 2 C), 127.2 (C_{quat}, 1 C), 129.8 (+, 2 C, CH), 136.6 (+, 4 C, CH), 148.6 (+, 4 C, CH), 154.0 (C_{quat}, 1 C), 155.3 (C_{quat}, carbamate), 158.5 (C_{quat}, 4 C), 173.7 (C_{quat}, acid); IR (KBr disk): $\tilde{\nu}$ [cm^{-1}] = 3256, 3057, 3008, 2974, 2927, 2821, 1706, 1672, 1592, 1478, 1434, 1365, 1290, 1250; MS (ESI, MeCN/TFA): *m/z* (%) = 704.4 (100) [MH$^+$], 352.8 (10) [M+2H$^+$]$^{2+}$.

2-Amino-3-3,5-bis{[bis(pyridin-2-ylmethyl)amino]methyl}-4-hydroxyphenyl-propanoic acid 13

To an ice cooled solution of **12** (340 mg, 0.48 mmol) in DCM (50 mL) was added dropwise a HCl saturated Et_2O-solution. The suspension was stirred for 30 min at 0°C and the organic phase was evaporated by stirring the solution over night at room temperature. The residue was taken up in water and lyophilized. Product **13** (520 mg) was obtained as a white solid in quantitative yield. ^{1}H-NMR (600 MHz, d_6-DMSO, HSQC, HMBC): δ = 3.08-2.98 (m, 2 H, TyrCHCH_2), 3.77 (bs, 4 H, TyrCH_2N), 4.14-4.17 (m, 1 H, TyrCH), 4.29 (bs, 8 H, Pyr-CH_2-N), 7.16 (s, 2 H, CH-phenol), 7.84 (t, ^3J = 6.6 Hz, 4 H, pyr), 8.10 (d, ^3J = 8.1 Hz, 4 H, pyr), (8.39-8.42 (m, 4 H, pyr), 8.77 (d, ^3J = 4.8 Hz, 4 H, pyr); ^{13}C-NMR (151 MHz, d_6-DMSO, HSQC, HMBC): δ = 34.5 (−, CH_2), 53.1 (+, CH), 53.3 (−, 2 C, CH_2), 55.2 (−, 4 C, CH_2), 123.2 (C_{quat}, 2 C), 125.6 (+, 4 C, CH), 126.2 (C_{quat}, 1 C), 126.8 (+, 4 C, CH), 132.7 (+, 2 C, CH), 142.3 (+, 4 C, CH), 144.9 (+, 4 C, CH), 152.8 (C_{quat}, 1 C), 153.5 (C_{quat}, 4 C), 170.3 (C_{quat}, acid); IR (KBr

disk): $\tilde{\nu}$ [cm^{-1}] = 3056, 2926, 1734, 1615, 1528, 1463, 1288, 1227, 767; MS (ESI, H_2O/MeCN): *m/z* (%) = 604.4 (100) [MH^+], 302.8 (17) [$M+2H^+]^{2+}$, 626.4 (5) [MNa^+].

Bis-bpa-Zn(NO_3)-complex 15a

The free ligand **14** (150 mg, 0.18 mmol) was dissolved in methanol (20 mL). To this solution, $Zn(NO_3)_2$ (108 mg, 0.36 mmol) in 10 mL H_2O was added and the mixture was stirred at room temperature for 1 h. Methanol was then removed under reduced pressure and the remaining aqueous solution was lyophilized to give the desired zinc coordinated complex **15a** as a white solid in quantitative yield (209 mg). mp: decomposition > 200 °C; IR (KBr disk): $\tilde{\nu}$ [cm^{-1}] = 3428, 2924, 2855, 2426, 1707, 1609, 1477, 1441, 1384, 764; MS (ESI, H_2O/MeCN): *m/z* (%) = 507.3 (100) [$K^{3+} + CH_3COO^-]^{2+}$, 1010.3 (27) [$K^{3+} - H^+ + CH_3COO^-]^+$, 1070.3 (10) [$K^{3+} + 2CH_3COO^-]^+$.

Deprotected peptide 23

Peptide **22** was dissolved in MeOH and cooled to 0 °C in an ice bath. To the solution HCl saturated ether (0.7 mL/mmol Boc-group) was added. The mixture was allowed to warm to room temperature and was stirred over night. The solvent was evaporated. The volatile compounds were removed under vacuum affording the fully deprotected compound as a white solid in sufficient purity for use in subsequent step. MS (ESI, MeCN/TFA): e/z (%) = 425.8 (100) [$(M + 2H^+)]^{2+}$, 284.2 (36) [$(M + 3H^+)]^{3+}$, 850.7 (17) [MH^+].

Peptide complex 24

Peptide **23** was dissolved in H_2O and LiOH was added until the pH value was slightly basic (pH 8). To this solution was added 2 equivalents of $Zn(ClO4)_2$ and the mixture was stirred at 80°C over night. Liophilization gave peptide complex **24** as a white solid. MS (ESI, H_2O/MeOH + NH_4OAc): e/z (%) = 505.9 (100) [$M^{4+} - H^+ + Cl^-]^{2+}$, 517.9 (75) [$M^{4+} - H^+ + CH_3COO^-]^{2+}$, 537.9 (60) [$M^{4+} - H^+ + ClO_4^-]^{2+}$, 487.8 [$M^{4+} - 2H^+]^{2+}$.

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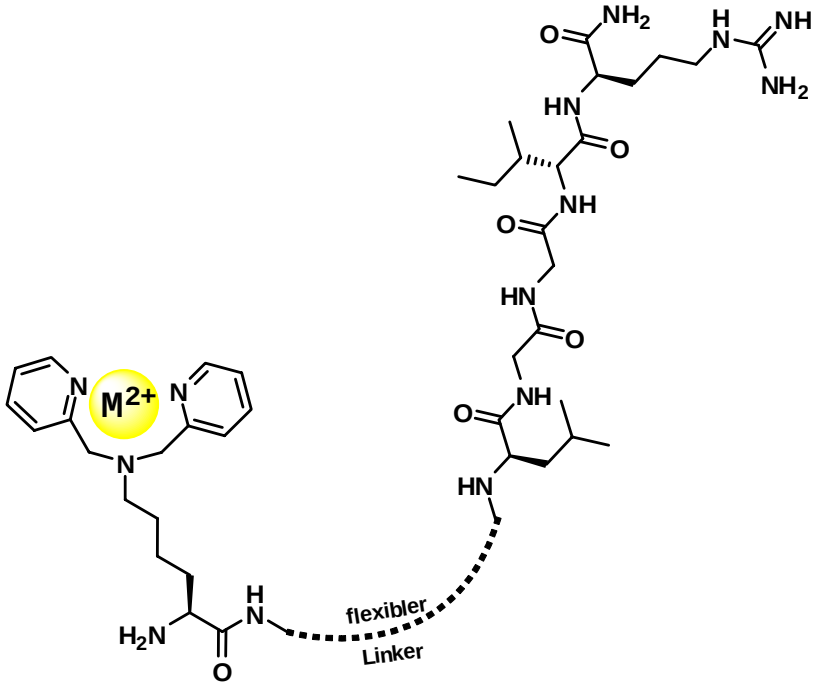
2. Modulation of the Ras-Effector Interaction by Structure Activity Relationship (SAR) Approach – Synthesis of a Hybridreceptor*

Graphical Abstract:



In this chapter, the methodology of − solid phase synthesis of metal-complex containing peptides − described in chapter 1 was successfully applied to the preparation of bidentate receptor ligand and its Zn^{II}- and Cu^{II}-complexes respectively. The SAR approach is used to guide the phosphate-binding bpa-derived metal-complex unit exclusively to the location of Ser39 close to γ-phosphate of RAS proteins due to the known and expected binding location of the pentapetide the Ras.

* All analytical measurements were performed by Ina Rosnizeck, Institute for Biophysics and Physical Biochemistry, University of Regensburg.

2.1 Introduction

The discovery of modulators of protein-protein interaction is an emerging field in drug design. One promising but still challenging approach in drug discovery is the direct protein-protein interaction by small molecules as it is demonstrated for the p53-MDM2 interaction.[1] Another mechanism in drug discovery is the stabilization of the inactive conformer of a protein by small molecules that is physically regulated by autoinhibition as for example for the neutral Wiskott-Aldrichsyndrome protein (N-WASP).[2] Kalbitzer et al. reported a novel principle where a stabilization of the very small population of excited states is influenced by the activity of small molecules.[3] When proteins require different conformations for their biological function, all these functional states have to coexist simultaneously in solution. However, the Gibbs free energy differences between the different states are rather high and thus the conformation with the lowest energy predominates in solution. However, the minor populated states with higher energy (excited states) can be stabilized as Kalbitzer stabilized the weak binding state of Ras-Mg^{2+}-GppNHp with Zn^{2+}-cyclen which should decrease the affinity of Ras to its main effector Raf. Unfortunately, the affinity of Zn^{2+}-cyclen is only in the milimolar concentration range. For an inhibitor of protein-protein interaction, the binding to an excited state of the protein should be stronger, at least in the micromolar to nonomolar concentration range. This encouraged us to search on the one hand for further metal complexes which will have a higher affinity to the weak effector binding state (state 1) in oncogenic variants of Ras. On the other hand, as a second binding site, peptide sequences were chosen from potentially peptide sequences of Ras binding proteins (SOS) and their binding (affinity and location) were examined. The overall strategy was to combine the two binding sites following the SAR strategy to enhance the binding to Ras-Mg^{2+}-GppNHp and hence interrupt Ras-Raf interaction.

2.2 Results and discussion

Binding of (substituted) bispicolylamine amine (bpa) metal-complexes to the different states of Ras in the complex with GppNHp

^{31}P NMR spectroscopy represents a method to identify compounds which preferentially bind to the "weak binding" conformation of activated Ras, the state 1, since the two conformational states of Ras can be directly observed by this method.[4] In collaboration with the group of H.R. Kalbitzer, it could be shown by ^{31}P NMR titration experiments that the metal-bpa (Figure 1) selectively binds to state 1 of Ras.

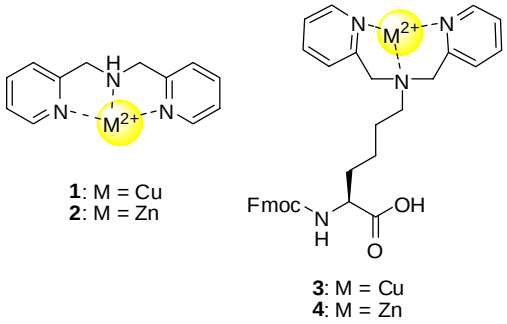


Figure 1. Structures of different metal-bpa complexes. Cu^{2+}-bpa **1**, Zn^{2+}-bpa **2**, Cu^{2+}-Fmoc-lys(bpa)-OH **3**, Zn^{2+}-Fmoc-lys(bpa)-OH **4**.

Binding of selected peptide sequences to the different states of Ras in the complex with GppNHp

A series of modified peptide sequences derived from the Ras interaction site of the Ras regulating protein (SOS)[5] was synthesized and the binding to Ras was evaluated by ^{1}H-^{15}N-HSQC titration experiments. Chemical shift mapping on the protein gave information about the localization of interaction between protein and ligand. Peptide **5** was found to bind in the expected region of the Ras.

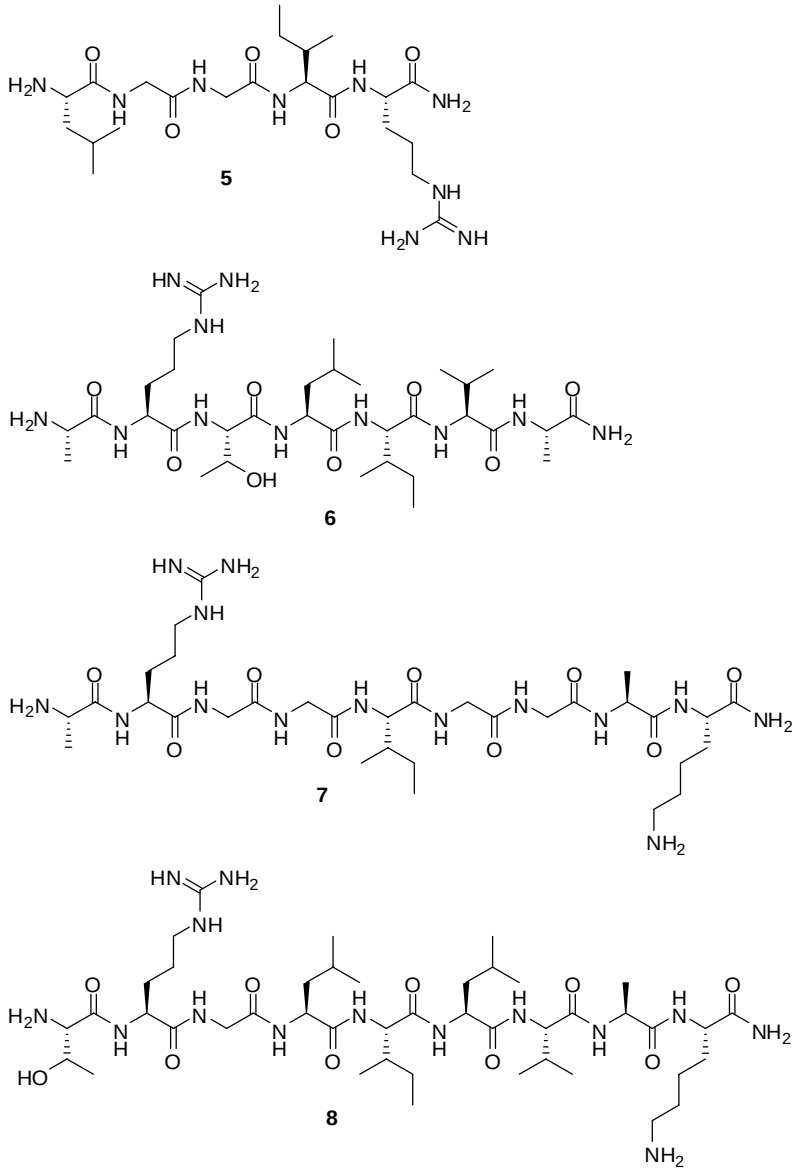


Figure 3. Structures of synthesized peptides with potential affinity to the Ras protein.

Design and synthesis of a hybridreceptor

The binding position of the peptide sequence **5** at Tyr64 and the position of Zn-bpa **2** at Ser39 close to γ-phosphate were examined using a crystal structure of the Ras. To combine these two binding sites, a distance between 23 and 25 Å has to be bridged. This linking part of the hybridreceptor was designed to be i) easy to synthesize, ii) flexible in its nature and iii) and offer a free carboxylic acid terminus as well as a Fmoc-protected amino terminus for assembling to the N-terminus of the pentapeptide **5** still bound to a resin using solid-phase peptide synthesis (SPPS). As a linker with an

appropriate length of 24 Å and distinct requirements compound **13** (Figure 4) was selected.

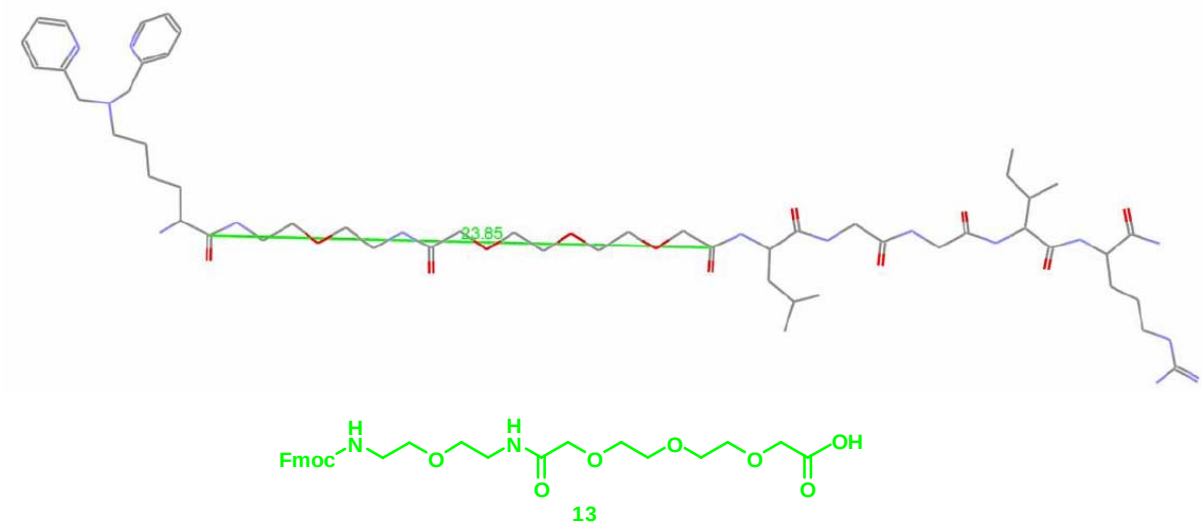
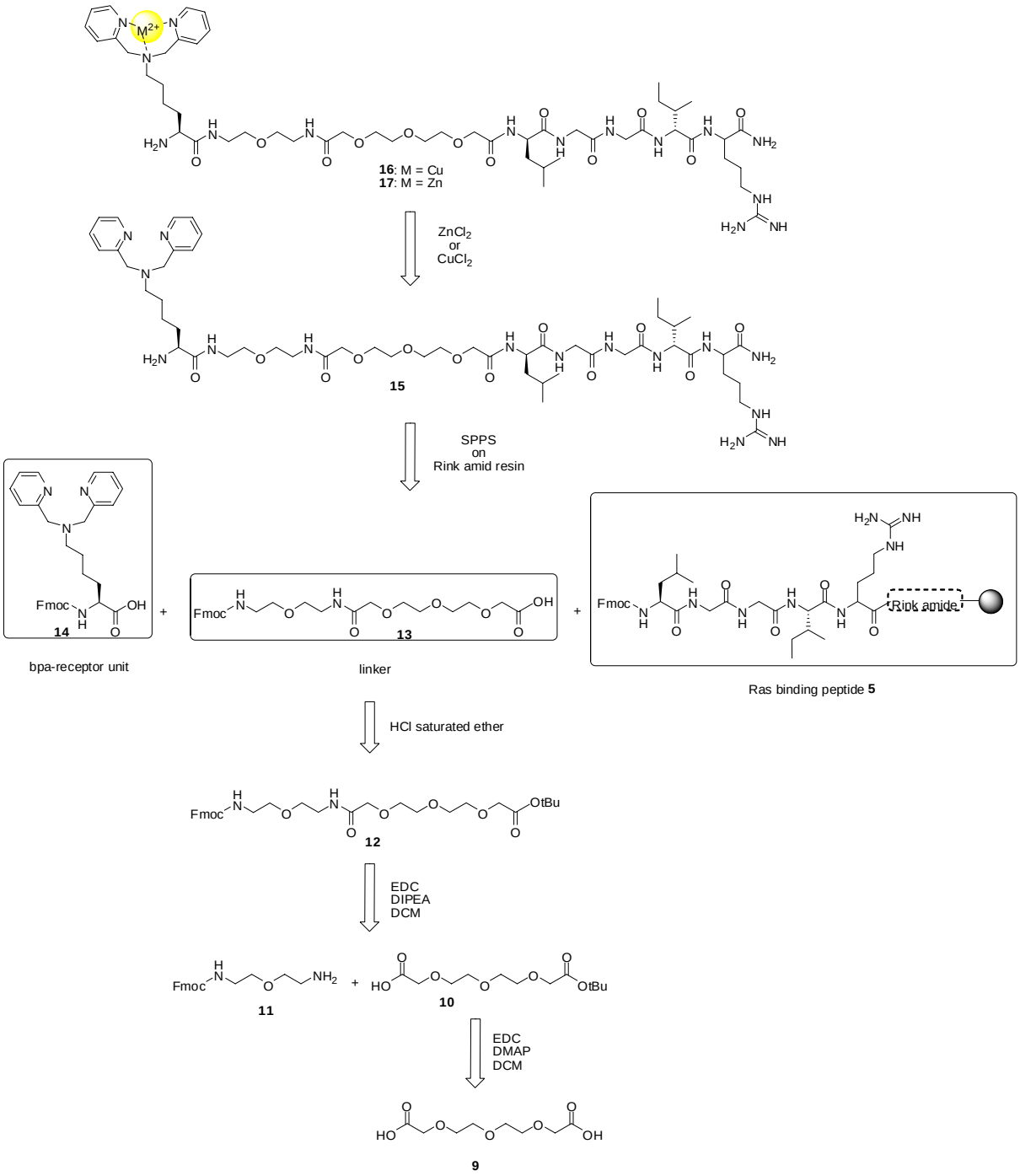


Figure 4. Linker design with an appropriate length of about 24 Å.

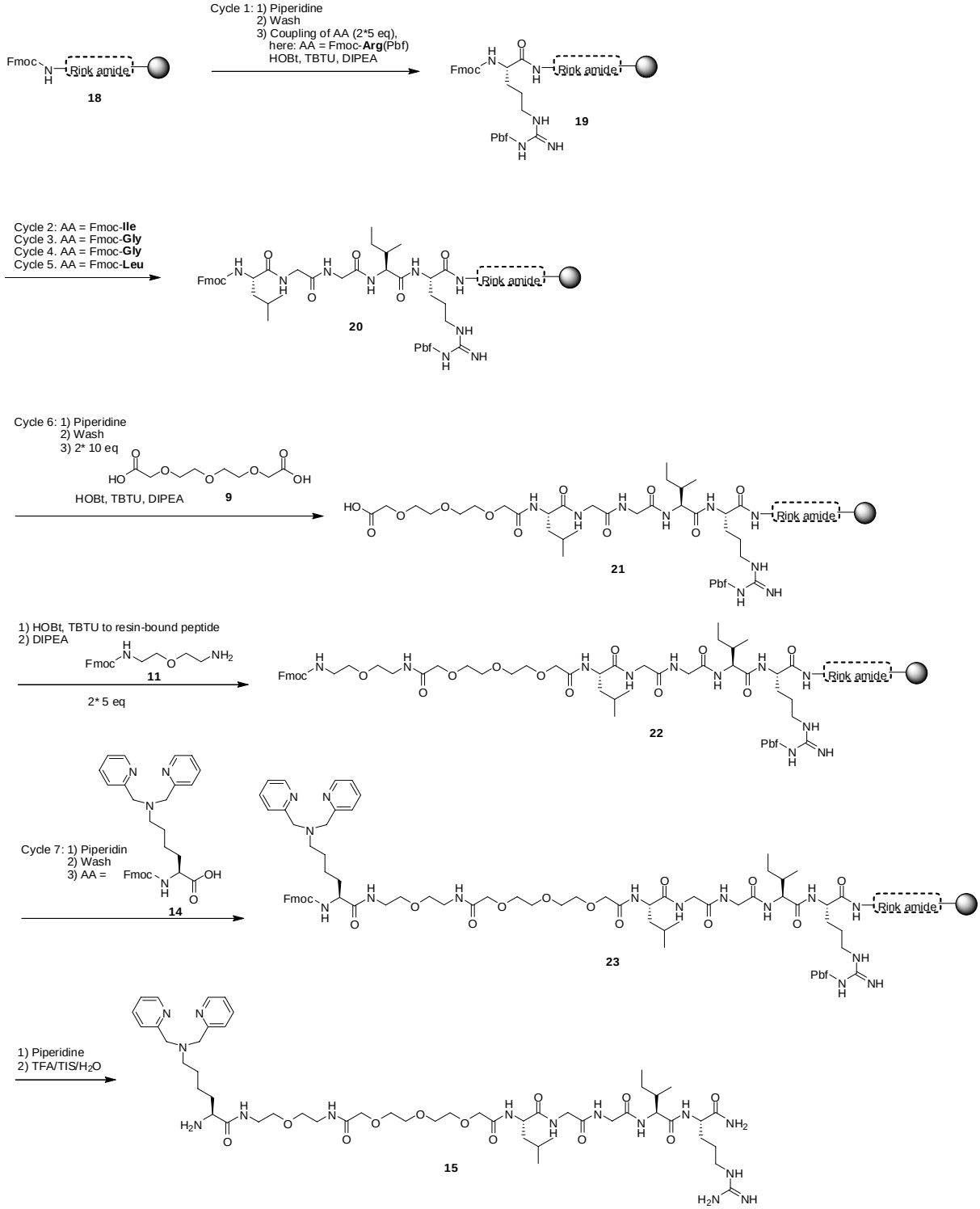
A retrosynthetic analysis of the hybridreceptor and in particular of the linker is given in Scheme 5.



Scheme 5. Retrosynthetic analysis of hybrid receptors **16** and **17**.

The hybrid receptor is composed of three units which where planed to be assembled on solid-phase. Compound **13** was prepared in solution in order to avoid the need of large excesses of compounds **10** and **11** if the linker would be prepared using SPPS. Unfortunately, the mono protection of the dicarboxylic acid **9** using 1 equivalent of *t*-

BuOH, 1.5 equivalents of EDC and 0.1 equivalent of DMAP in dichloromethane following a description of a patent was not successful.[6] Direct condensation of the diacid **9** with the Fmoc protected diamine **11** using standard solution peptide coupling conditions resulted in a complicated mixture of compounds and in a rather low yield of the desired linker precursor **12**. Therefore the linker **13** was also assembled in form of its two constituent parts **9** and **11** on solid-phase (Scheme 6). After purification of the hybrid receptor-ligand **15** by preparative HPLC, the bpa-function was complexed in solution with either $ZnCl_2$ or $CuCl_2$ to obtain the metal-complexed hybrid receptors **16** and **17**.



Scheme 6. Synthesis of the hybrid receptor-ligand **15** on solid-phase.

2.3 Conclusion and outlook

Substituted Zn^{II}- and Cu^{II}-bispicolylamine complexes were synthesized and indentified as a new class of coordination compounds that bind with high affinity to the Ras-bound nucleotide. In parallel, short peptide sequences derived from known complexes with Ras were synthesized and the affinity and binding sites on the Ras-surface was evaluated by co-workers of the Kalbitzer group. The methodology of − solid phase synthesis of metal-complex containing peptides − described in chapter 1 was successfully applied to the preparation of bidentate receptor ligand and their Zn^{II}- and Cu^{II}-complexes respectively.

Currently, the superior expected affinities due to the combination of these two different binding sites of these metal-complexed hybrid receptors are analysed in the Department of Biophysics and Physical Biochemistry of H.R. Kalbitzer and in the working group for Protein-Interaction of C. Herrmann. Detailed NMR-investigation, isothermal titration calorimetry as well as fluorescence based assays are used for binding studies.

2.4 Experimental

Bpa[7] and Fmoc-Lys(bpa)-OH[8] were prepared by literature known methods.

General notes:

With regard to NMR experiments of the metal-bpa complexes it was important to avoid even traces of uncomplexed metal-salts. Therefore, a slight excess of ligand was used in all complexation reactions and it was aspired to remove the excesses of free ligand after completion of the reactions by extensive washing procedures of insoluble metal complex in an organic solvent. Furthermore, metal salts and constituents of the linker were selected with respect to solubility in aqueous solution required for NMR experiments.

Bpa-Cu^{2+} 1

To a solution of bpa (50.0 mg, 0.25 mmol) in acetonitrile (20 mL) was added dropwise a solution of $CuCl_2 \cdot 2\ H_2O$ (37.5 mg, 0.22 mmol) in methanol (5 mL). After a few minutes, the copper complex **1** was precipitated and the reaction was stirred for further 30 min to complete the reaction. The product was filtered and washed 4 times with small amounts of cold acetonitrile. After drying under high vacuum, the pure product **1** was obtained as a blue solid in quantitative yield (73 mg, 0.22 mmol). MS (ESI, H_2O/MeOH + NH_4OAc): e/z (%) = 320.9 (100) $[M^{2+} + AcO^-]^+$, 260.8 (70) $[M^{2+} - H^+]^+$, 296.8 (13) $[M^{2+} + Cl^-]^+$, 199.9 (4) $[bpa + H^+]^+$. – Elemental analysis calcd. (%) for $C_{12}H_{13}Cl_2CuN_3$ (333.70): C 43.19, H 3.93, N 12.59; found C 43.19, H 3.97, N 12.70.

Bpa-Zn^{2+} 2

To a solution of bpa (50.0 mg, 0.25 mmol) in acetonitrile (20 mL) was added dropwise a solution of $ZnCl_2$ (30.0 mg, 0.22 mmol) in methanol (5 mL). After a few minutes, the copper complex **2** was precipitated and the reaction was stirred for further 30 min to complete the reaction. The product was filtered and washed 4 times with acetonitrile to get rid of the small excess of the dpa ligand. After drying under high vacuum, the pure product **2** was obtained as a white solid in quantitative yield (73 mg, 0.22 mmol). MS (ESI, H_2O/MeOH + NH_4OAc): e/z (%) = 321.9 (100) $[M^{2+} + AcO^-]^+$, 297.8 (13) $[M^{2+} +$

$Cl^-]^+$, 199.9 (11) $[bpa + H^+]^+$. – Elemental analysis calcd. (%) for $C_{12}H_{13}Cl_2N_3Zn$ (332.98): C 42.96, H 3.91, N 12.52; found C 42.30, H 3.98, N 12.34.

Fmoc-Lys-(bpa-Cu^{2+})-OH 3

To a solution of Fmoc-Lys(bpa)-OH (100 mg, 0.18 mmol) in H_2O (25 mL) was added dropwise a solution of $CuCl_2 \bullet 2\ H_2O$ (24.5 mg, 0.18 mmol) in methanol (15 mL). After stirring the reaction mixture for 30 min at room temperature, the methanol was removed under vacuum and the remaining aqueous solution was lyophilized yielding complex **3** as a blue solid in quantitative yield (117 mg, 0.18 mmol). MS (ESI, H_2O/MeOH): m/z (%) = 612.1 (100) $[M^{2+} - H^+]^+$, 648.1 (30) $[M^{2+} + Cl^-]^+$, 1261.5 (10) $[2M^{2+} + Cl^- - 2H^+]^+$, 1223.5 (6) $[2M\text{-}Cu^{2+} - 3H^+]^+$.

Fmoc-Lys-(bpa-Zn^{2+})-OH 4

To a solution of Fmoc-Lys(bpa)-OH (100 mg, 0.18 mmol) in H_2O (25 mL) was added dropwise a solution of $Zn(NO_3)_2 \bullet 6\ H_2O$ (24.5 mg, 0.18 mmol) in H_2O (10 mL). After stirring the reaction mixture for 30 min at room temperature, the aqueous solution was lyophilized yielding complex **4** as a white solid in quantitative yield (117 mg, 0.18 mmol).

General procedure for the synthesis of peptides 5 – 8

Peptides **5** – **8** were synthesized on an Advanced Chemtech 496 MOS synthesizer. Rink Amide MBHA resin (0.72 mmol/g) and Fmoc-SPPS strategy were used throughout all syntheses. Coupling was achieved by TBTU/HOBt activation and DIPEA was used as the base. HOBt was used as a 0.45 M solution, TBTU as a 0.44 M solution and DIPEA as a 1.2 M solution, all in DMF. The Fmoc protected amino acids were dissolved in NMP as 0.4 M solutions. All peptides were synthesized on 50 mg resin and two coupling cycles were performed. Prior to coupling, the resin was allowed to preswell in DMF for 30 min. Fmoc cleavage was brought about through the addition of 40 % (v/v) piperidine-DMF solution and shaking for 3 min followed by the addition of a 20 % (v/v) piperidine-DMF solution and shaking for 10 min. The solution was drained of, the deprotected resin bound peptide was washed 6 times with DMF and subsequently

coupled to the next Fmoc protected amino acid. Cleavage from the solid support was performed using a TFA cocktail containing TFA/TIS/H_2O (90:5:5 v/v/v) for 3 hours. In order to precipitate the peptide, cold Et_2O was added and the resulting heterogeneous solution centrifuged for 10 min at -4°C. The supernatant was decanted and the resulting pellet was washed with colt Et_2O. This procedure was repeated several times before the precipitate was dissolved in water and lyophilized. The peptides were analysed by ES-MS and HPLC-MS. Peptides **5** and **6** were purified by HPLC. As the yield of the peptides synthesized on the Advanced Chemtech 496 MOS synthesizer were not satisfied due to the occurrence of several fragments of the sequences, the amino acid sequence **20** for the hybrid receptor-ligand was synthesized manually using syringe technique and methodologies developed before.[9]

Leu-Gly-Gly-Ile-Arg-NH_2 5

Peptide **5** was synthesized following the general procedure. MS (ESI, AcN/TFA): e/z (%) = 257.6 (100) $[M^{2+} + 2H^+]^{2+}$, 514.4 (16) MH^+.

Ala-Arg-Thr-Leu-Ile-Val-Ala-NH_2 6

Peptide **6** was synthesized following the general procedure. MS (ESI, MeOH/AcN/TFA): e/z (%) = 371.8 (100) $[M^{2+} + 2H^+]^{2+}$, 742.6 (47) MH^+.

Hybridreceptor ligand 15

The synthesis was performed manually in a 10 mL syringe equipped with porous filter using Fmoc protected Rink amide MBHA resin **18** (200 mg, subst.: 0.72 mmol/g). Rink amide MBHA resin **18** was added to a frit-syringe, suspended in DMF and allowed to swell for 30 min. Prior to the first amino acid coupling the cleavage of the Fmoc-group was brought about through the addition of 40 % (v/v) piperidine-DMF solution and shaking for 3 min followed by the addition of a 20 % (v/v) piperidine-DMF solution and shaking for 10 min. The solution was drained off and the deprotected resin was washed 6 times with DMF. Fmoc protected amino acids (5 eq) arginine, *iso*-leucine, 2x glycine and leucine were coupled by HOBt/TBTU activation and DIPEA in NMP/DMF. More precisely, after the amino acids were dissolved in 5 equivalents of a 0.45 M HOBt-solution in DMF/NMP (4:1), 5 equivalents of a 0.44 M TBTU solution in DMF/NMP

(4:1) and 10-fold excess of DIPEA as a 1.2 M solution in NMP were added in succession. The syringe was subsequently shaken for each 120 min when coupling Fmoc-Arg as the first amino acid to the resin and when *iso*-leucine was coupled to the resin-bound bulky and polar arginine **19**. In case of Fmoc protected glycines and leucine, the syringe was shaken for 40 min. All coupling steps where carried out twice. Fmoc-deprotection and washing cycles as outlined above were performed after each coupling step. Two times 10 equivalents were used to couple 3,6,9-trioxaundecandisäure **9** to the resin bound amino acid sequence **20** in addition with the appropriate amount of coupling reagents. After shaking (2x 150 min), 5 equivalents of each HOBt-and TBTU-solutions were added to the resin-bound sequence **21** followed by the addition of 2[2-(Fmoc-amino)ethoxy]ethylamine hydrochloride **11** in a 1.2 M solution of DIPEA in NMP. The syringe was subsequently shaken (2x 120 min) and Fmoc-deprotection and washing cycles as outlined above were performed. Two times 5 equivalents were used to couple SAAC **14** to the resin-bound sequence **22** in addition with the appropriate amount of coupling reagents. After shaking (2x 150 min) the *N*-terminus was deprotected by piperidine-DMF solution and the final resin-bound hybridreceptor-ligand **23** was washed 4 times each with DMF, DCM and Et_2O. Cleavage from the solid support was performed using a TFA cocktail containing TFA/TIS/H_2O (90:5:5 v/v/v) for 3 hours. In order to precipitate the peptide, cold Et_2O was added and the resulting heterogeneous solution centrifuged for 10 min at -4°C. The supernatant was decanted and the resulting pellet was washed with colt Et_2O. This procedure was repeated several times before the precipitate was dissolved in water and lyophilized. The crude product was purified by preparative HPLC to obtain pure hybrid receptor ligand **15**. MS (ESI, AcN/TFA): e/z (%) = 372.4 (100) $[M + 3H^+]^{3+}$, 558.1 (38) $[M + 2H^+]^{2+}$, 1114.8 (2) MH^+. For copies of ^{1}H-NMR and ^{13}C-NMR spectra, see supporting information.

Copper(II)-hybrid receptor 16

To a solution of hybrid receptor-ligand **15** (15 mg, 13.5 μmol) in 10 mL H_2O was added 1 mL of a 12.1 mM solution of $CuCl_2 \cdot 2\ H_2O$ (2.1 mg, 12.1 μmol) in MeOH and the resulting blue solution was stirred at room temperature over night. MeOH was removed under vacuum and the remaining aqueous solution was lyophilized yielding 17 mg of **16**

as a blue solid in quantitative yield. MS (ESI, AcN/H_2O): e/z (%) = 392.7 (100) $[M^{2+} + H^+]^{3+}$, 588.7 (20) M^{2+}, 606.5 (10) $[M^{2+} + TFA]^{2+}$, 606.5 (8) $[M^{2+} + HCl]^{2+}$.

Zn^{2+}-hybridreceptor 17

To a solution of hybridreceptor-ligand **15** (7 mg, 6.3 μmol) in 5 mL H_2O was added 1 mL of a 5.7 mM solution of $ZnCl_2$ (0.77 mg, 5.7 μmol) in H_2O and the solution was stirred at room temperature over night. The aqueous solution was lyophilized yielding 8 mg of **17** as a white solid in quantitative yield. MS (ESI, AcN/H_2O + 10 mmol/L NH_4OAc): e/z (%) = 598.0 (100) M^{2+}, 558.0 (91) $[L + 2H^+]^{2+}$, 608.0 (37) $[M^{2+} + HCl]^{2+}$, 393.1 (31) $[M^{2+} + H^+]^{3+}$, 619.0 (30) $[M^{2+} + HOAc]^{2+}$, 646.0 (26) $[M^{2+} + TFA]^{2+}$, 372.4 (5) $[L + 3H^+]^{3+}$.

2.5 References

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University of Sydney, Australia. M. K. Levadala; S. R. Banerjee; K. P. Maresca; J. W. Babich; J. Zubieta *Synthesis* **2004**, 1759-1766.

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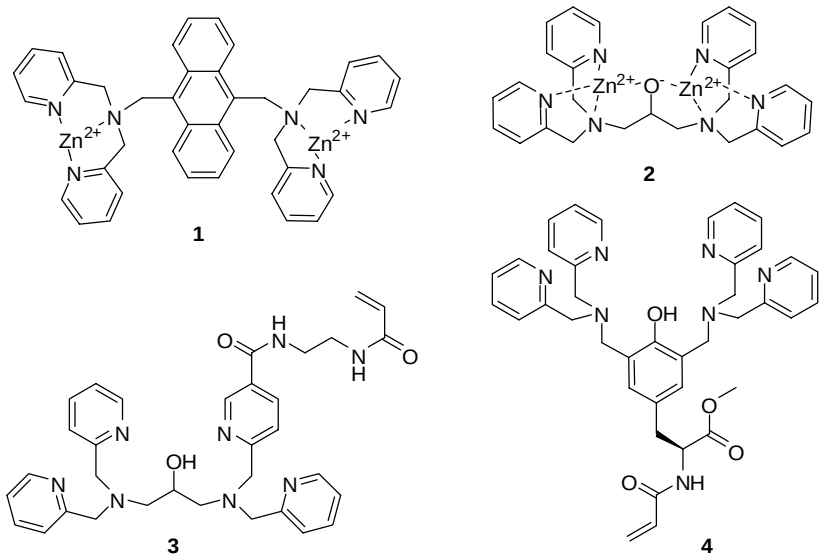
3. Enhancing the Separation of Phosphorylated Proteins in Gel Electrophoresis with Dinuclear Bispyridylmethylamine-Tyrosine-Acrylamide Complexes*

In this chapter is reported the facile preparation of phosphate bonding ligands from the amino acid tyrosine, formation of its zinc(II) and manganese(II) complexes and their use as mobility shift additive for the detection of phosphorylated proteins in SDA-PAGE gel electrophoresis.

* This chapter is aimed at publishing: G. Dirscherl, M. Schwab, W. Seufert, B. König, in preparation. SDS-PAGE gel electrophoreses were performed by Dr. M. Schwab, Institute of Biochemistry, Genetic and Microbiology.

3.1 Introduction

Post-translational modification of proteins by phosphorylation is fundamental for the regulation of biological processes, such as signal transduction, apoptosis, proliferation, differentiation and metabolism, in all living organisms.[1] Phosphorylation occurs on different amino acid residues. In eukaryotic cells, the hydroxyl groups of serine, threonine and tyrosine are phosphorylated.[2] Incorrect protein phosphorylation can cause severe disorders including cancer and neuropathogenesis. Methods for determining the phosphorylation status of proteins are thus very important for a better understanding of the molecular origin of diseases and biological and pathological processes. Historically, phosphorylation detection methods relay on either radioisotopes or phosphoamino acid-selective antibodies. More recently, specific dyes for phosphorylation detection in arrays,[3] biosensors for kinase activity[4] and artificial phosphoprotein sensors based on dinuclear Zn_2^{5+} or Zn_2^{4+} metal complexes (Scheme 1) have been described.



Scheme 1. Hamachi´s (top left) and Koike´s (top right) phosphate-binding dinuclear metal complexes **1** and **2**; polymerizable additives for the detection of phosphorylated proteins by mobility shift in gel electrophoresis: Phos-tag™ **3** (bottom left), a tyrosine-based ligand **4** (bottom right)

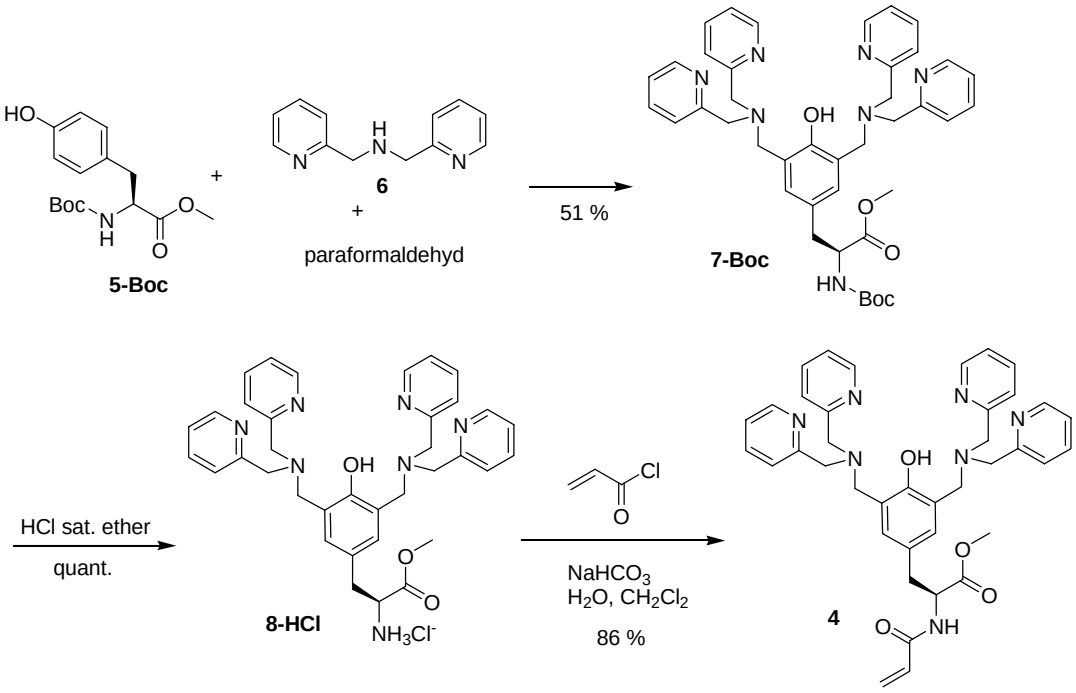
Koike et al. combined the specific reversible coordination of metal complexes to phosphorylated protein surfaces with SDS-PAGE gel electrophoresis, the standard biochemical technique for protein separation and analysis. A derivative **3** (called Phos-

tag^{TM} by the authors, Scheme 1) of their hydroxyl-bis-DPA (dipicolylamine) ligand bearing a polymerizable acryl amide moiety was immobilized within the polyacrylamide gel and subsequently complexed by zinc(II) or magnesium(II) ions.[5] In aqueous solution, the di-zinc(II) phos-tag complex strongly binds to phenyl phosphate (K_d = 2.5 • 10^{-8} mol/L) under physiological conditions whereas at pH > 9, the binding ability decreases. The dinuclear manganese(II) phos-tag complex captures *R*-OPO_3^{2-} anions preferentially, such as phosphoserine and phosphortyrosine at alkaline pH of ca. 9.[6] Such modified gels retain phosphorylated proteins stronger than corresponding non-phosphorylated proteins in SDS-PAGE gel electrophoresis which allows for their mobility shift detection. [7,8]

However, the preparation of compound **3** requires a multistep protocol.[9] In this chapter is reported the facile preparation of ligand **4** from the amino acid tyrosine, formation of its zinc(II) and manganese(II) complexes and their use as mobility shift additive for the detection of phosphorylated proteins in SDA-PAGE gel electrophoresis.

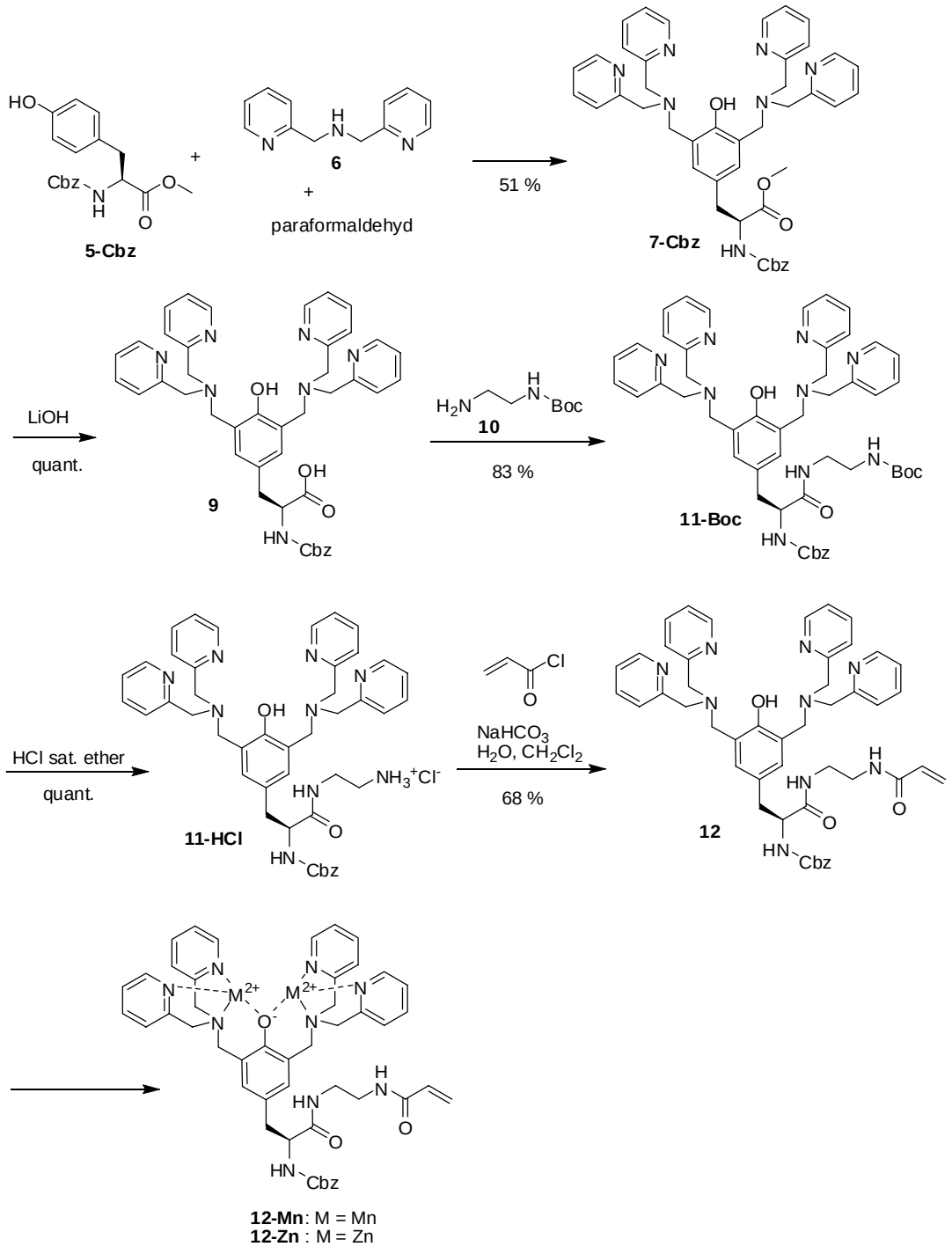
3.2 Results and discussion

The twofold Mannich reaction of Boc-Tyr-OMe **5-Boc**, dpa **6** and paraformaldehyde provides ligand **7-Boc** according to a literature known procedure in 51% yield.[10] The Boc protecting group is removed by treatment with HCl saturated ether and the crude product reacted with acrylchloride to give the target acrylamide-pendant ligand **4** in overall good yield.



Scheme 2. Synthesis of acrylamide-pendant ligand **4** in three steps from protected tyrosine

For some applications a modification of the acryl amide position may be desirable. Using **5-Cbz** as starting material, the analogous protected ligand **7-Cbz** is obtained from twofold Mannich reaction in the same yield. Saponification of the methyl ester with LiOH gave compound **9**, which was reacted with mono Boc-protected diethylenediamine under standard peptide coupling conditions to give amide **11-Boc**. Boc-deprotection and reaction with acrylic acid chloride gave ligand **12** in good overall yield. The ligand is converted into the di-manganese(II) or di-zinc(II) complex by treatment with the respective chloride salt in aqueous methanol solution.



Scheme 3. Synthesis of acrylamide-pendant ligand **12** and its di-manganese (**12-Mn**) and di-zinc (**12-Zn**) complexes

The ability of the compounds to specifically retain phosphorylated proteins was investigated in SDS-PAGE polyacrylamide gel electrophoresis, which was conducted according to Laemmli´s method.[11] Either acrylamide-pendant ligands **4** or **12** with two equivalents of $MnCl_2$ or the metal complexes **12-Mn** or **12-Zn** were added in different amounts to the acryl amide mixture before polymerisation of the separation gel. While there was no significant difference if the ligand was used in polymerization and its complex was formed subsequently or the metal complex was directly used in gel preparation, clearly only the manganese(II) complex led to retardation of phosphorylated proteins. For direct comparison, unphosphorylated trypsinogen was not affected whereas α-casein was dephosphorylated by treatment with λ-protein phosphatise and analysed by SDS-PAGE with and without the addition of 100μM of **12-Mn** to the acryl amide gel. Figure 1 clearly shows the stronger retardation of the phosphorylated α-casein in the presence of **12-Mn** in the gel. To investigate if the observed mobility change is caused by masking the phosphate charges or by altered interactions of the phosphorylated protein with the polymeric gel structure, complex **12-Mn** and or other coordination compounds (see supporting information for data) were added to the protein sample to be analyzed. After incubation the sample was subjected to standard SDS-PAGE gel electrophoresis. Even up to concentrations of 100 μM of added metal complex no mobility shift was detected in SDS-PAGE. This clearly shows that it is necessary to immobilize the metal complex phosphate binding during gel preparation.

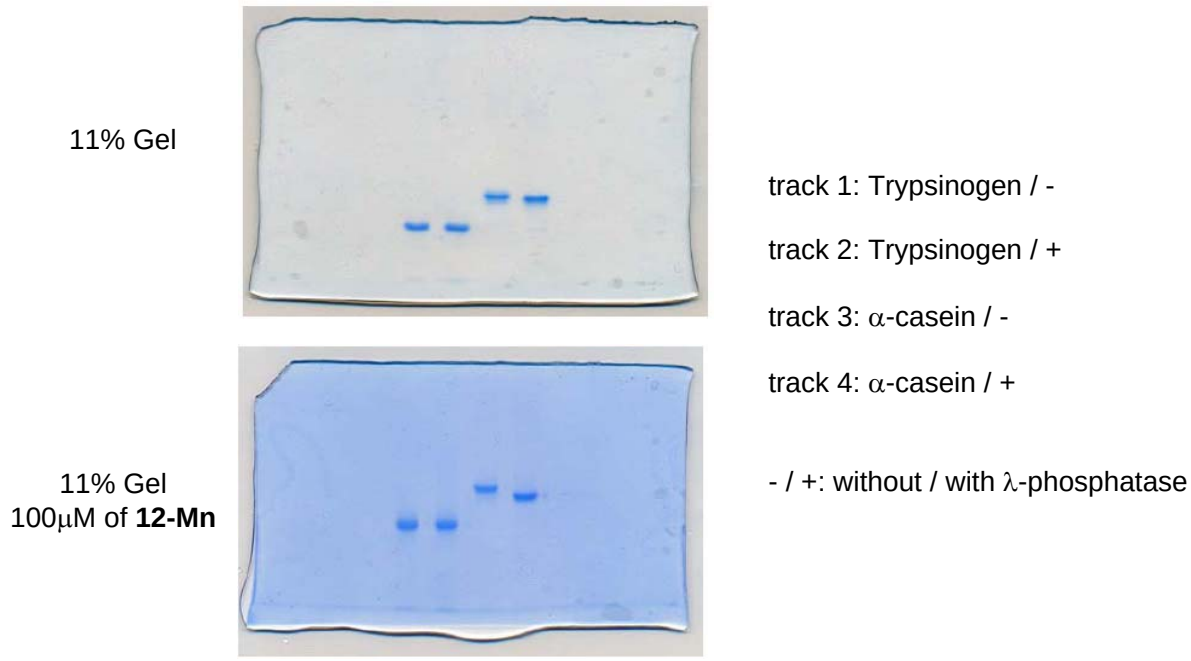


Figure 1. Specific mobility shift of phosphorylated α-casein compared to dephosphorylated α-casein in a gel containing **12-Mn**.

3.3 Conclusion

Complex **12-Mn** was successfully utilized as a gel additive to increase phosphate affinity in SDS-PAGE. The modified gel showed a distinct mobility shift of phosphorylated α-casein in comparison to the dephosphorylated protein. The stranger retention is caused by reversible interactions of the proteins phosphate groups with the metal complex binding sites immobilized in the gel. The investigated zinc(II) complexes were not able to induce specific mobility shifts. Overall, an easily accessible polymer additive as ligand or metal complex was synthesized, which allows the preparation of SDS-PAGE gels with enhanced resolution of phosphorylated versus non-phosphorylated proteins. If compared to the previously reported compounds, the facile synthetic modification of ligands **4** and **12** and the use as preformed di-manganese(II) complex **12-Mn** may be advantageous for specific tasks in bioseparation.

3.4 Experimental

Compounds **6,**[12] **7-Cbz**,[10] and **10,**[13] were prepared by literature known methods.

Cbz-*L*-Tyr 3-[3,5-bis (2,2´-dipicolylamine)methyl]-OMe (7-Cbz): 1 M HCl (5.0 mL) and Cbz-*L*-Tyr-OMe **5-Cbz** (6.59 g, 20.0 mmol) were added to a suspension of paraformaldehyde (1.97 g) and 2, 2´-dipicolylamine (9.85 g, 49.4 mmol) in ethanol (40 mL) and water (120 mL). The solution was refluxed for 36 h, then cooled to room temperature and neutralized with Na_2CO_3. The crude product was extracted with chloroform, the organic phase was dried over magnesium sulphate, and the solvent was evaporated to yield a yellow oil. The crude compound was purified by silica gel column (EtOH/EtOAc = 1:1, R_f = 0.51) to give **7-Cbz** as a yellow oil (7.9 g, 10.5 mmol, 53 %). $[a]^{20}_D$ = -3.7° (c = 0.007 in MeOH); ^{1}H NMR (600 MHz, $CDCl_3$) δ = 11.04 (s, 1 H, OH), 8.52 (d, $^3J_{H,H}$ = 4.12, 4 H, H-11), 7.60-7.55 (m, 4 H, H-13), 7.46-7.41 (m, 4 H, H-14), 7.30-7.25 (m, 3 H, H-Cbz), 7.23-7.20 (m, 2 H, H-Cbz), 7.13-7.08 (m, 4 H, H-12), 7.01 (s, 2 H, H-3, H-7), 5.64 (d, $^3J_{H,H}$ = 8.23, 1 H, N-H), 5.05-4.94 (m, 2 H, CH_2-Ph), 4.63-4.55 (m, 1 H, H-0), 3.86 (s, 8 H, H-9), 3.76 (s, 4 H, H-8), 3.64 (s, 3 H, OMe), 3.07-2.94 (m, 2 H, H-1); ^{13}C-NMR (600 MHz, $CDCl_3$): δ = 37.4 (−, C-1), 52.2 (+, Me), 54.7 (−, C-8), 55.1 (+, C-0), 59.6 (−, C-9), 66.8 (−, C-CH_2-Ph), 122.0 (+, C-12), 123.1 (+. C-14), 125.4 (+, C-4/6), 128.0 (+, C-Ph), 128.4 (+, C-Ph), 130.2 (−, C-7), 136.6 (+, C-13), 148.9 (+, C-11), 155.1 (C_{quat}, C-5), 155.8 (C_{quat}, Cbz-ester), 159.0 (C_{quat}, C-pyridine), 172.3 (C_{quat}, methyl-ester); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3057, 2951, 2824, 1716, 1690, 1591, 1475, 1434, 1200, 1124, 1150, 1001, 752; MS (ES-MS, CH_2Cl_2/MeOH + 10 mmol/L NH_4Ac): e/z (%) = 752.5 (100) [MH^+]; HR-MS calcd. for $C_{44}H_{46}N_7O_5$ [MH^+]: 752.3560, found: 752.3572 ± 1.54 ppm.

Acrylamido-*L*-Tyr 3-[3,5-bis (2,2´-dipicolylamine)methyl]-OMe (4): **7-Boc** (570 mg, 0.79 mmol) was dissolved in MeOH (20 mL) and cooled to 0 °C using an ice bath. To this mixture 5 mL of an HCl saturated Et_2O-solution was added and the mixture was allowed to warm to room temperature and stirred over night. The reaction progress was monitored by ^{1}H-NMR. The reaction mixture was concentrated under reduced pressure and the residue was taken up in water and lyophilized to give compound **8-HCl** (520

mg) as white solid in quantitative yield which was subsequently used in the next step without purification.

To a solution of **8-HCl** (300 mg, 0.42 mmol) in H_2O (20 mL) was added $NaHCO_3$ in small portions until the solution was slightly alkaline. The solution was cooled to 0°C with an ice-bath and a solution of acryl chloride (38 mg, 0.42 mmol) in dichloromethane (20 mL) was added dropwise. After stirring the reaction mixture for 15 min at 0°C, the reaction was stirred for an additional 1 h at room temperature. The organic layer was separated, washed with H_2O (3 x 10 mL) and dried over $MgSO_4$. The solvent was evaporated and the crude product purifiied using on flash-silica gel ($CHCl_3$:MeOH = 95:5, R_f = 0.05) yielding **4** (242 mg, 0.36 mmol, 86 %) as a white solid, m.p. = 63°C; $[a]^{20}_D$ = +21.0° (c = 0.006 in $CHCl_3$); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 2984, 2363, 1721, 1668, 1608, 1444, 1215, 745, 667, 632; MS (ES-MS, H_2O/MeOH + 10 mmol/L NH_4Ac): e/z (%) = 403.7 (100) $[M + 2H^+]^{2+}$, 726.5 (98) $[M + H]^+$, 749.5 (14) $[M + Na]^+$.

Benzyl 1-(2-acrylamidoethylamino)-3-(3,5-bis((bis(pyridin-2-ylmethyl)amino)methyl)-4-hydroxyphenyl)-1-oxopropan-2-ylcarbamate (12): Compound **11-Boc** (830 mg, 0.94 mmol) was dissolved in MeOH (20 mL) and cooled to 0 °C using an ice bath. To this mixture 6 mL of an HCl saturated Et_2O-solution was added and the mixture was allowed to warm to room temperature and stirred over night. The reaction progress was monitored by ^{1}H-NMR. The reaction mixture was concentrated under reduced pressure and the residue was taken up in water and lyophilized to obtain compound **11-HCl** as white solid in quantitative yield which was subsequently used in the next step without purification. MS (ES, H_2O/MeCN/TFA): m/z (%) = 390.9 (100) $[M + 2H]^{2+}$, 780.5 (12) $[M + H]^+$.

To a solution of **11-HCl** (973 mg, 0.94 mmol) in H_2O (30 mL) was added $NaHCO_3$ in small portions until the solution was slightly alkaline. The solution was cooled to 0°C with an ice-bath and a solution of acryl chloride (85 mg, 0.94 mmol) in dichloromethane (30 mL) was added dropwise. After stirring the reaction mixture for 15 min. at 0°C, the reaction was stirred for an additional 1 h at room temperature. The organic layer was separated, washed with H_2O (3 x 15 mL) and dried over $MgSO_4$. The solvent was evaporated and the crude product purification using column

chromatography on flash-silica gel ($CHCl_3$:MeOH = 95:5, R_f = 0.05) yielding **12** (536 mg, 0.64 mmol, 68 %) as a white solid, m.p. = 58°C; $[a]^{20}_D$ = +6.5° (c = 0.006 in MeOH); ^{1}H-NMR (600 MHz, DMSO, DQF-COSY, HSQC, HMBC): δ = 2.62-2.73 (m, 1 H, H-15A), 2.87-2.98 (m, 1 H, H-15B), 3.06-3.23 (m, 4 H, H-19, H-20), 3.65 (s, 4 H, H-7), 3.75 (s, 8 H, H-8), 4.11-4.26 (m, 1 H, H-16), 4.75-4.95 (m, 2 H, H-28), 5.56 (dd, $^3J_{H,H}$ = 9.64, $^2J_{H,H}$ = 2.68, 1 H, H-24cis), 6.06 (dd, $^3J_{H,H}$ = 17.08, $^2J_{H,H}$ = 2.68, 1 H, H-24trans), 6.17 (dd, $^3J_{H,H}$ = 17.08, $^3J_{H,H}$ = 9.64, 1 H, H-23), 7.03-7.13 (m, 3 H, H-31, H-32), 7.14-7.28 (m, 8 H, H-12, H-30, H-3, H-5), 7.39 (d, $^3J_{H,H}$ = 8.45, 1 H, H-25), 7.46 (d, $^3J_{H,H}$ = 7.68, 4 H, H-14), 7.60-7.77 (m, 4 H, H-13), 8.07 (t, $^3J_{H,H}$ = 4.48, 1 H, H-21), 8.11 (t, $^3J_{H,H}$ = 4.48, 1 H, H-18), 8.48 (d, $^3J_{H,H}$ = 10.92, 4 H, H-11), 10.91 (s, 1 H, OH); ^{13}C-NMR (600 MHz, DMSO): δ = 37.1 (−, C-15), 38.3 (−, C-19/20), 38.4 (−, C-19/20), 54.1 (−, C-7), 56.0 (+, C-16), 58.8 (−, C-8), 65.1 (−, C-28), 122.1 (+, C-12), 122.8 (+, C-14), 123.2 (C_{quat}, C-2/6), 125.1 (−, C-24), 127.3 (+, C-31), 127.5 (C_{quat}, C-4), 128.1 (+, C-30), 128.2 (+, C-32), 129.9 (+, C-3/5), 131.7 (+, C-23), 136.6 (+, C-13), 136.9 (C_{quat}, C-29), 148.7 (+, C-1), 154.0 (C_{quat}, C-1), 155.8 (C_{quat}, C-26), 155.8 (C_{quat}, C-9), 164.8 (C_{quat}, C-22), 171.7 (C_{quat}, C-17); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3060, 2928, 1715, 1659, 1591, 1535, 1476, 1433, 1234, 1048, 995, 753; MS (ES, MeCN/TFA): m/z (%) = 834.5 (10) $[M + H]^+$, 417.8 (100) $[M + 2H]^{2+}$; HR-MS calcd. for $C_{48}H_{52}N_9O_5$ $[MH^+]$: 834.4091, found: 834.4077 ± 1.73 ppm.

Compound 11-Boc: LiOH (35.9 mg, 1.50 mmol) was dissolved in H_2O (5 mL) and added dropwise to a solution of **7-Cbz** in water/methanol (25 %, 50 mL). The mixture was stirred at room temperature for 12 h. The completion of the reaction was monitored by ^{1}H-NMR. After the methanol was evaporated, the suspension was neutralized with a saturated NH_4Cl solution and extracted 3 times with dichloromethane. The combined organic layers were dried over anhydrous magnesium sulphate and concentrated under reduced pressure. Compound **9** was obtained as a yellow solid (494 mg, 0.67 mmol) in quantitative yield and was subsequently used in the next step without purification.

To a solution of **9** (146 mg, 0.20 mmol) and HOBt • H_2O (37 mg, 0.24 mmol) in DMF (20 mL) were added EDC (32.7 µL, 0.24 mmol) and *tert*-butyl 2-aminoethylcarbamate **10** (38.5 mg, 0.24 mmol) in DMF (5 mL) and the reaction was stirred for 1 h at room temperature and over night at 60°C. The solvent was removed in vacuum, the residue

was dissolved in dichloromethane (30 mL) and extracted with H_2O (3x 20 mL). Purification of the crude product using column chromatography on flash-silica gel ($CHCl_3$:MeOH = 95:5, R_f = 0.05) gave **11-Boc** (144 mg, 0.16 mmol, 83 %) as a colourless oil. $[a]^{20}_D$ = +10.7° (c = 0.007 in MeOH); ^{1}H-NMR (600 MHz, DMSO, DQF-COSY, HSQC, HMBC): δ = 1.24 (s, 9 H, Boc), 2.58-2.71 (m, 1 H, H-15A), 2.81-2.97 (m, 3 H, H-15B, H-20), 2.99-3.13 (m, 2 H, H-19), 3.71 (s, 4 H, H-7), 3.81 (s, 8 H, H-8), 4.08-4.24 (m, 1 H, H-16), 4.78-4.90 (m, 2 H, H-28), 6.63-6.78 (m, 1 H, H-21), 7.02-7.13 (m, 3 H, H-31, H-32), 7.14-7.30 (m, 8 H, H-12, H-30, H-3, H-5), 7.34 (d, $^3J_{H,H}$ = 8.86, 1 H, H-25), 7.43 (d, $^3J_{H,H}$ = 7.83, 4 H, H-14), 7.63-7.78 (m, 4 H, H-13), 7.95-8.09 (m, 1 H, H-18), 8.52-8.55 (m, 4 H, H-11); ^{13}C-NMR (600 MHz, DMSO): δ = 28.2 (+, Boc), 37.1 (−, C-15), 38.8 (−, C-19/20), 38.9 (−, C-19/20), 54.2 (−, C-7), 56.3 (+, C-16), 58.5 (−, C-8), 65.1 (−, C-28), 77.6 (C_{quat}, Boc), 122.3 (+, C-12), 122.9 (+, C-14), 127.3 (+, C-Ph), 127.4 (+, C-Ph), 127.5 (C_{quat}, C-2/6), 128.1 (−, C-2/6, C4), 130.3 (+, C-3/5), 136.7 (+, C-13), 136.9 (C_{quat}, C-29), 148.6 (+, C-1), 154.1 (C_{quat}, C-1), 155.5 (C_{quat}, C-26/27), 155.71 (C_{quat}, C-26/27), 157.8 (C_{quat}, C-9), 162.3 (C_{quat}, C-22), 171.5 (C_{quat}, C-17); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3273, 2932, 1703, 1671, 1591, 1531, 1476, 1365, 1250, 1169, 1049, 1026, 1005, 623; MS (ES, CH_2Cl_2/MeCN/TFA): m/z (%) = 440.8 (100) $[M + 2H^+]^{2+}$, 880.6 (38) $[MH^+]$; HR-MS calcd. for $C_{50}H_{59}N_9O_6$ $[MH^+]$: 880.4510, found: 880.4524 ± 1.58 ppm.

Complex 12-Mn

To a solution of compound **12** (50 mg, 0.06 mmol) in H_2O (15 mL) was added dropwise a solution of $MnCl_2$ • 4 H_2O (23.7 mg, 0.12 mmol) in methanol (5 mL). After stirring the reaction mixture for 30 min at room temperature, the methanol was removed under vacuum and the remaining aqueous solution was lyophilized yielding complex **12-Mn** as a white solid in quantitative yield, decomposition > 95 °C; $[a]^{20}_D$ = +6.7° (c = 0.003 in MeOH); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3279, 3057, 2928, 1657, 1602, 1535, 1472, 1433, 1244, 1014, 760; MS (ES-MS, MeOH + 10 mmol/L NH_4Ac): e/z (%) = 1060.3 (100) $[M^{3+} + 2CH_3COO^-]^+$, 500.8 (32) $[M^{3+} + CH_3COO^-]^{2+}$.

Complex 12-Zn

To a solution of compound **12** (50.0 mg, 0.06 mmol) in H_2O (15 mL) was added dropwise a solution of $ZnCl_2$ (16.4 mg, 0.12 mmol) in methanol (5 mL). After stirring the reaction mixture for 30 min at room temperature, the methanol was removed under vacuum and the remaining aqueous solution was lyophilized yielding complex **12-Zn** as a white solid in quantitative yield, decomposition > 135 °C; $[a]^{20}_D$ = -13.7° (c = 0.004 in MeOH); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3285, 3081, 2933, 1710, 1659, 1608, 1538, 1441, 1247, 1053, 1024, 763; MS (ES-MS, H_2O/MeOH + 10 mmol/L NH_4Ac): e/z (%) = 1078.3 (100) $[M^{3+} + 2CH_3COO^-]^+$, 509.8 (44) $[M^{3+} + CH_3COO^-]^{2+}$, 1000.3 (16) $[M^{3+} + 2OH^-]^+$.

Compound 14

To a solution of compound **7-Cbz** (100 mg, 0.13 mmol) in H_2O (25 mL) was added dropwise a solution of $MnCl_2 \cdot 4\ H_2O$ (52.6 mg, 0.27 mmol) in methanol (5 mL). After stirring the reaction mixture for 30 min at room temperature, the methanol was removed under vacuum and the remaining aqueous solution was lyophilized yielding complex **14** as a white solid in quantitative yield, decomposition > 110 °C; $[a]^{20}_D$ = +6.6° (c = 0.003 in MeOH); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3346, 2952, 1687, 1603, 1473, 1434, 1293, 1265, 1201, 1156, 1051, 758, 642; MS (ES-MS, H_2O/MeOH + 10 mmol/L NH_4Ac): e/z (%) = 752.5 (100) $[M^{3+} + 2CH_3COO^-]^+$.

Compound 15

To a solution of compound **7-Cbz** (100.0 mg, 0.13 mmol) in H_2O (25 mL) was added dropwise a solution of $ZnCl_2$ (36.3 mg, 0.27 mmol) in methanol (5 mL). After stirring the reaction mixture for 30 min at room temperature, the methanol was removed under vacuum and the remaining aqueous solution was lyophilized yielding complex **15** as a white solid in quantitative yield, decomposition > 103 °C; $[a]^{20}_D$ = -6.2° (c = 0.004 in MeOH); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3259, 3053, 2927, 1715, 1661, 1592, 1331, 1476, 1433, 1243, 1050, 759; MS (ES-MS, H_2O/MeOH + 10 mmol/L NH_4Ac): e/z (%) = 996.2 (100) $[M^{3+} + 2CH_3COO^-]^+$, 470.3 (3) $[M^{3+} + CH_3COO^-]^{2+}$.

SDS-PAGE gel electrophoresis conditions:

Untreated or phosphatase-treated proteins (2 µg each) were separated by SDS-PAGE and stained with RAPID Stain solution.

Phosphatase treatment:

100 µg protein + 500U λ-protein phosphatase (New England Biolabs) in 50 mM HEPES, pH 7.5, 5 mM DTT, 2 mM $MnCl_2$. Incubation for 1h at 30°C (without phosphatase, incubation only in buffer for 1h 30°C).

Reaction stopped by addition of 2x LSB (Laemmli sample buffer) and boiling for 10min.

Gels: 11% $MnCl_2$ or additionally 50 µM of Mn^{2+}-complex **14**.

3.5 References and notes

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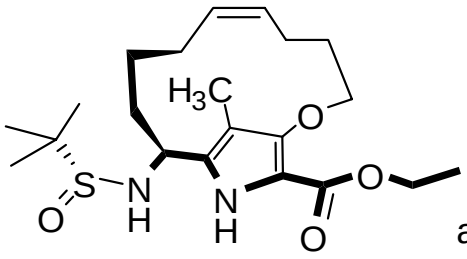
4. Synthesis of a Heterocyclic Ansa Pyrrol Amino Acid [*]

Graphical Abstract



In this chapter is reported the synthesis of a planar-chiral amino acid, in which the ansa-bridge is formed by an olefin ring closing metathesis.

[*] This chapter is aimed at publishing: G. Dirscherl, P. Rooshenas, P.R. Schreiner, F. Lamaty, B. König, in preparation.
Synthetic work was partially carried out during research periods at the University of Montpellier II, France. Energy barrier calculations were performed by P. Rooshenas, Institute of Organic Chemistry, University of Giessen, Germany.

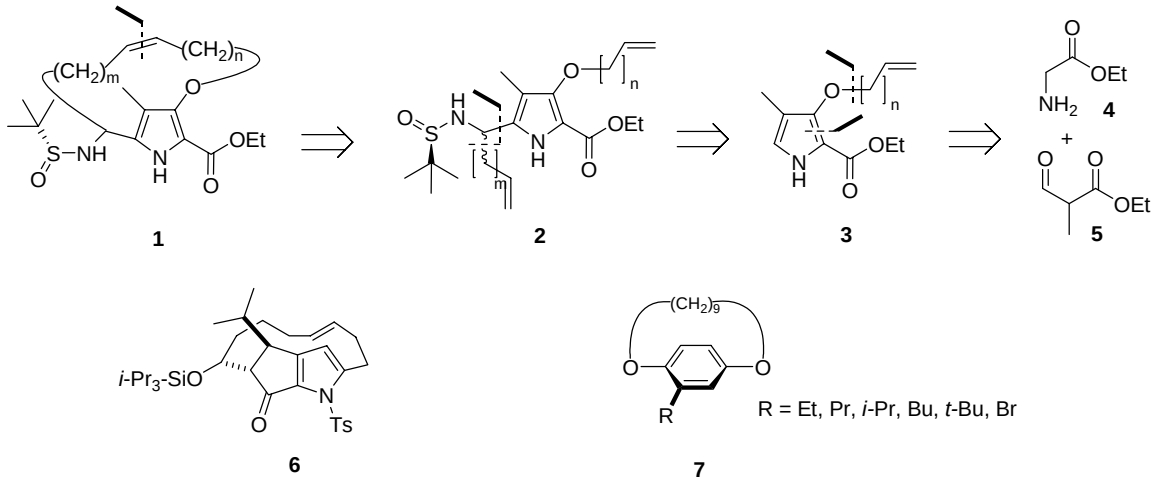
4.1 Introduction

Planar chirality can be described as a form of chirality originating from a helix. In addition, the terms “stereogenic center”, “axis” and “plane” are accepted. Thus, a chiral plane can be described as a planar arrangement of at least four atoms with a fifth or more centres placed outside of this plane.[1] A “chiral plane” arises therefore from the chirality resulting from the arrangement of out-of-plane groups with respect to a reference plane.[2] Structural studies of molecules possessing an element of planar chirality started in the 1940s with the synthesis and resolution of 1,12-dioxa[12]paracyclophane as the first chiral “ansa” compound.[3] Lüttringhaus was the first who realized and correctly predicted the possible existence of enantiomeric ansa compounds.[4]

Compounds with planar chirality are found among η^n-olefinmetal[5] and η^n-arenemetal complexes,[6] cyclophanes[7] and ansa compounds[8]. Although many of such planar chiral molecules have been synthesized, only one example of an amino acid with planar chirality exists, to the best of our knowledge. Pelter et al. prepared homochiral amino acids, where the chirality depends solely on the chirality of the [2.2]paracyclophane unit.[9] However, MOPAC calculation of simple peptides derived from this amino acid showed, that due to the close proximity of the amino and carboxyl groups, the chain was distorted, so that there was no hydrogen bonding between the second carbonyl and amide NH groups.[10]

We report here the synthesis of a planar-chiral amino acid, in which the ansa-bridge is formed by an olefin ring closing metathesis. Scheme 1 shows the general structure of the target heterocyclic amino acids **1** with planar chirality and the intended synthetic strategy consisting of ring closing metathesis (RCM) of suitable dienes **2** in the presence of Grubbs´ catalyst and the preparation of dienes from sulfinyl imines. RCM is an appropriate method for the synthesis of macrocycles[11] and is also employed as a key step for the synthesis of cyclophane derivatives.[12] The successful RCM to a 13-membered ring was reported in the synthesis of the macrotricyclic core of roseophilin **6**.[13] The formation of the rather strained ansa-chain in this target molecule was supported by conformational control in the precursor bringing the alkene moieties closer together and lowering the enthalpic barrier during ring-formation.

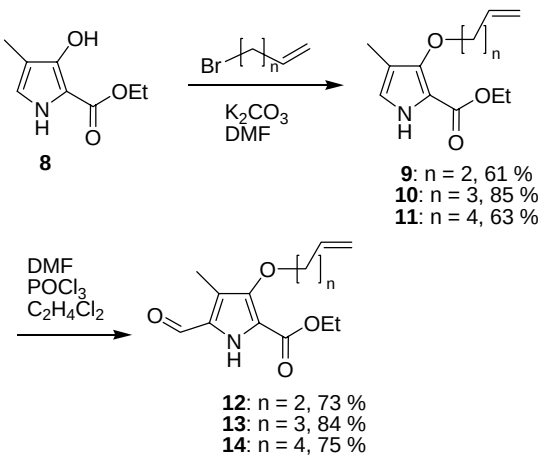
To allow resolution of enantiomers in planar chirality, the energy barrier of rotation must be high enough. Optimization on the target structures **1** using B31yp/6-31G and either CGST- or GIAO-method with B31yp/6-311G revealed, that the ansa-bridge of a compound with m = 3 and n = 3 should exhibit sufficient stability, due to the restricted aromatic ring flip.Therefore no racemisation within the range of thermal stability of the compound is expected.[14] Studies by König[15] on the influence of substitution on the rotational energy barrier of structurally related planar chiral cyclophanes **7** using dynamic enantioselective gas chromatography and computer simulation support our estimation for compound **1**: All substituents of compounds **7** were found to be too bulky to permit a rotation of the arene at the experimental conditions (133-145°C).



Scheme 1. Top: Retrosynthetic analysis of the target heterocylic amino acids **1** with planar chirality. Bottom: Roseophilin **6** and planar chiral cyclophanes **7**.

4.2 Results and discussion

In earlier studies, we have prepared hydroxypyrrole amino acids (HOPAS)[16] and incorporated them into small peptides with hairpin structures.[17] The heterocyclic amino acid is a dipeptide mimic and shows a peptide *ß*-sheet hydrogen bonding pattern. The chemistry of the HOPAS dipeptide mimic was extended by palladium catalysed allylation reaction, which allowed introducing a quaternary chiral center.[18] We now use the dipeptide mimic skeleton in the synthesis of a suitable precursor molecule for RCM. Ethyl 3-hydroxy-4-methyl-pyrrole-2-carboxylate **8** was prepared as starting material in three steps according to a literature procedure.[19] The first alkene functionality was introduced by alkylation of the hydroxyl group (Scheme 2). Williamson ether synthesis gave alkenyloxy pyrrole derivatives **9** – **11** in 61-85 % yield**,** using K_2CO_3 as base and DMF as solvent. Next, pyrrole-aldehydes **12** – **14** were prepared by Vilsmeier-Haack formylation of **9** – **11** and obtained crystalline in 73-84 % yield.

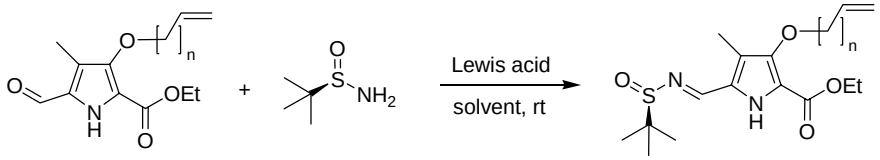


Scheme 2. Synthesis of pyrrole-aldehydes **12** – **14**

N-Sulfinyl imines are versatile intermediates in the asymmetric synthesis of chiral amines. Ellman and co-workers have employed Lewis acidic dehydrating agents $MgSO_4$, $CuSO_4$ and $Ti(OEt)_4$ (2 eq) for the condensations of (*R*)-*tert*-butanesulfinamide (**15**) with aldehydes.[20] Recently, the formation of sulfinimines by the catalytic action of $Yb(OTf)_3$ was achieved and reaction conditions extended to Ellman´s sulfinyl imines.[21] The different conditions were tested on pyrrole aldehyde **13**, giving best results with $Ti(OEt)_4$ to afford the (*R*)-*N*-*tert*-butanesulfinyl aldimines **16** – **18** in excellent yields

(Table 1). The structure of compound **17** was confirmed by an X-ray structure analysis (Figure 1).

Table 1. Synthesis of *N*-*tert*-butanesulfinyl aldimines **16** – **18** exploring Lewis acidic dehydrating agents $CuSO_4$, $Ti(OEt)_4$ and $Yb(OTf)_3$.



12: n = 2
13: n = 3
14: n = 4

15

16: n = 2
17: n = 3
18: n = 4

Entry	Aldehyde	Lewis Acid	Solvent	Product	Yield (%)
1	**13**	$CuSO_4$	CH_2Cl_2	**17**	36
2	**13**	$Yb(OTf)_3$	THF	**17**	0
3	**13**	$Ti(OEt)_4$	CH_2Cl_2	**17**	100
4	**12**	$Ti(OEt)_4$	CH_2Cl_2	**16**	92
5	**14**	$Ti(OEt)_4$	CH_2Cl_2	**18**	91

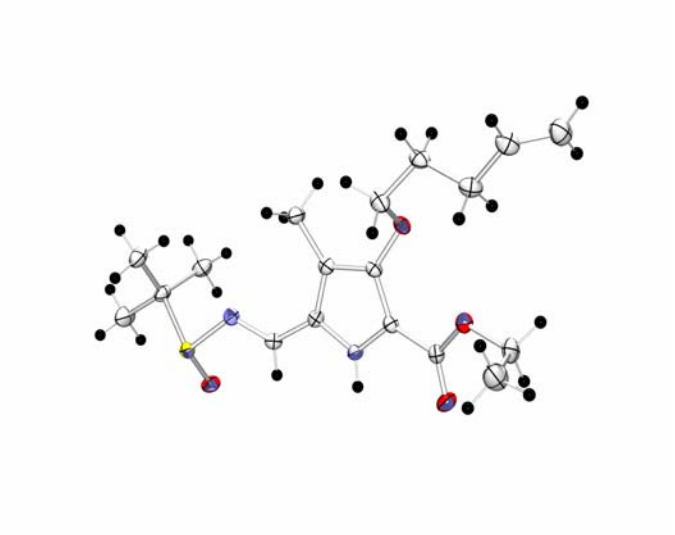
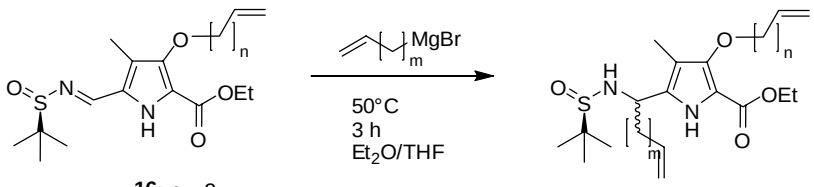


Figure 1. Structure of compound **17** in the solid state

A series of dienes with different chain length was prepared via nucleophilic addition of Grignard reagents to imines **16** – **18**. The nucleophilic additions of organomagnesium reagents to sulfinyl imines have been explored in detail by Ellman, Tang and others.[22]

General protocols are described for the addition of alkyl and aryl Grignard reagents to *N*-sulfinyl aldimines with high diastereoselectivity and good yields. Typically, about two equivalents of Grignard reagents are added at -78 or -48 °C to the *N*-sulfinyl imines to achieve addition, but in our case no nucleophilic addition of the organomagnesium reagents to the sulfinyl imine occurred in different solvents (THF toluene, diethyl ether, dichloromethane), even if the reaction temperature was allowed to reach room temperature. Starting material was recovered in all attempts. Only if the reaction mixture was heated to 50 °C nucleophilic addition occurred. The addition products were isolated in moderate to good chemical yields and *syn:anti* ratios of 9:1 (Table 2).

Table 2. Addition of unsaturated Grignard reagents to *N*-sulfinyl imines **16** – **18**



16: n = 2
17: n = 3
18: n = 4

19: n = 2, m = 2
20: n = 2, m = 3
21: n = 2, m = 4
22: n = 3, m = 2
23: n = 3, m = 3
24: n = 3, m = 4
25: n = 4, m = 2
26: n = 4, m = 3
27: n = 4, m = 4

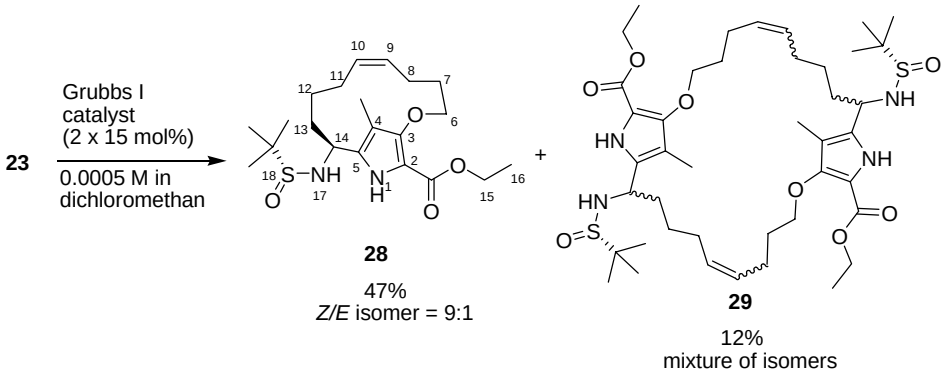
Entry	**Product**	**Imine**	**Yield**[a] **(%)**	**Diastereomeric ratio**[b] **(*syn:anti*)**
1	**19**	**16**	61	91:9
2	**20**	**16**	63	93:7
3	**21**	**16**	64	91:9
4	**22**	**17**	68	90:10
5	**23**	**17**	71	90:10
6	**24**	**17**	65	93:7
7	**25**	**18**	68	94:6
8	**26**	**18**	50	91:9
9	**27**	**18**	72	94:6

[a] Yields were determined by mass balance of purified material.
[b] Ratios of diastereomers were determined by ^{1}H NMR or HPLC analysis.

Ring closing diene metathesis has been applied in the synthesis of medium size rings (9 to 21-membered ring macrocycles), but yields and optimal conditions (choice of catalyst; solvent) vary and are still difficult to predict.[23] Therefore, the series of dienes

19 – **27**[24] with different side chain lengths (Table 2) were reacted with different catalysts. High dilution was used to avoid polymerization via acyclic diene metathesis. Commercially available Grubbs´ I, Grubbs´ II and 2nd generation Hoveyda-Grubbs´[25] catalysts were tested in dry and degassed dichloromethane and toluene.

The outcome of the RCM reactions was monitored by TLC, mass spectrometry and HPLC-MS analyses. Only a small fraction of all tested dienes gave macrocyclic products and the best conditions were found using the Grubbs I catalyst (2 x 15 mol%) in high dilution conditions (0.0005 M) in dichloromethane. The formation of the expected macrocyclic structure, although in minor amounts, was indicated by HPLC and mass spectrometry for dienes **23-Boc**, **24**, **25**, **26** and **27**, but only for **23** the HPLC-MS analysis confirmed sufficient product amounts for isolation. In the case of diene **23**, beside the 13-membered ansa-compound **28**, a 26-membered macrocycle **29** was obtained as a minor product.[26] Both products were isolated by preparative HPLC and investigated in detail by NMR spectroscopy. Isomeric products resulting from the minor *anti* diastereomer of **23** were detected analytically, but the amount was too small for isolation. The intramolecular cyclization of compound **23** to macrocycle **28** (yield: 47%) resulted in a 9:1 ratio of *Z/E* double bond isomers, inseparable by HPLC, favouring the *Z*-configuration.[27] The observed coupling constant (J = 10.8 Hz) of the olefinic protons in the ^{1}H-NMR spectrum confirms this assignment (see supporting information). The proton resonance in the open-chain precursor **23** has a chemical shift of δ = 5.86, while in the cyclic form this resonance is shifted downfield by $\Delta\delta$ = 0.8 - 0.6. The larger macrocyclic structure **29** (yield: 12%) was isolated as a mixture of stereoisomers (cis/trans double bonds; head to tail and head to head orientation). Under the reaction conditions and on standing the products loose their *tert*-butanesulfinamide group.



Scheme 3. Synthesis of 13-membered ansa-heterocyclic amino acid **28** by ring-closing olefin metathesis reaction of compound **23**.

The stereochemical analysis of the RCM reaction is hindered due to *syn/anti*-diastereomers of the starting material, diene **23,** and the formation of double bond *E/Z* isomers in the product. With the assumption that stable, non-interconverting atropisomers are formed, eight product stereoisomers are possible in total. However, the 9:1 ratio of the *syn/anti* stereoisomers and the *Z/E* double bond isomers lead to significantly different amounts of the product isomers, even in the absence of any stereoinduction in the RCM. Therefore the detection of all minor compounds is difficult or even impossible.

Detailed HPLC-MS analysis of compound **28** revealed four isomeric compounds: one major isomer, two minor isomers and one isomer in traces (see supporting information). The major isomer has 18-*R*, 14-*S* syn stereochemistry and Z-configuration of the 9,10-double bond as determined by NMR (see supporting information). Using a chiral HPLC column, the major isomer peak splits into two peaks in a ratio of about three to one. This may indicate a stereochemical induction of the sulfinylamine and C-14 stereocenters on the ring closing process.[28] However, the overall large number of possible isomers and small amount of product available did not allow elucidating and assigning the absolute configuration of the product isomers.

4.3 Conclusion

In conclusion, the synthetic route to ansa pyrrol amino acids is reported. The ansa-bridge is formed by an olefin ring closing metathesis reaction of diene precursors in the presence of Grubbs I catalyst. Diene precursors were in turn prepared by Grignard addition to pyrrol sulfinyl imines. Only some of the dienes give macrocyclic ring closing products, as identified by HPLC-MS analyses and yield and selectivity of the ring closing reaction is low. Only in the case of the 13-membered compound **28** sufficient material could be obtained by preparative HPLC separation to investigate its structure spectroscopically. Overall, the reported synthetic approach to ansa pyrrol amino acids is feasible, but suffers from poor efficiency of the ring closing metathesis reaction.

4.4 Experimental

Ethyl 3-(but-3-enyloxy-1*H*-pyrrole-2-carboxylate (9)

To a suspension of K_2CO_3 (364 mg, 2.63 mmol) in dry DMF (25 mL) was added ethyl 3-hydroxy-4-methyl-pyrrole-2-carboxylate **8** (420 mg, 2.48 mmol). After the reaction mixture was stirred for 10 min at room temperature, 4-bromo-1-butene (282 µL, 2.63 mmol) was added dropwise and the reaction was stirred for 40 h at 80°C. The reaction mixture was quenched with H_2O (100 mL) and extracted 7 times with each 15 mL of CH_2Cl_2. The collected organic layer was washed with each 25 mL of 0.5 m NaOH, H_2O und saturated $KHSO_4$ and dried over $MgSO_4$. The solvent was evaporated and the crude product purification using column chromatography on silica gel (PE:EtOAc = 8:2, R_f = 0.37) yielding **9** (339 mg, 1.52 mmol, 61 %) as a colourless oil. ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.35 (t, ^3J = 7.1, 3 H, H-12), 1.96 (d, ^4J = 0.8, 3 H, CH_3), 2.49 (ddt, ^3J = 13.6, ^3J = 6.8, ^2J = 1.4, 2 H, H-7), 4.04 (t, ^3J = 6.8, 2 H, H-6), 4.29 (q, ^3J = 7.1, 2 H, H-11), 5.00-5.19 (m, 2 H, H-9), 5.91 (ddt, ^3J = 17.2, ^3J = 10.3, ^3J = 6.8, 1 H, H-8), 6.54 (dd, ^3J= 3.4, ^4J = 0.7, 1 H, H-5), 9.03 (bs, 1H, NH); ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 8.4 (+, CH_3), 14.5 (+, C-12), 34.6 (−, C-7), 60.0 (−, C-11), 74.0 (−, C-6), 111.4 (C_{quat}, C-2), 113.2 (C_{quat}, C-4), 116.5 (−, C-9), 119.7 (+, C-5), 134.9 (+, C-8), 149.8 (C_{quat}, C-3), 160.7 (C_{quat}, C-10); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3318, 3078, 2981, 2933, 2873, 2744, 1668, 1285, 1028; MS (CI-MS, NH_3): m/z (%) = 224.2 MH^+ (100), 241.2 $[MNH_4]^+$ (37); HR-MS calcd. for $C_{12}H_{17}NO_3$ [$M^{+\bullet}$]: 223.1208; found: 223.1206 ± 0.6 ppm. – $C_{12}H_{17}NO_3$ (223.27).

Ethyl 4-methyl-3-(pent-4-enyloxy)-1*H*-pyrrole-2-carboxylate (10)

To a suspension of K_2CO_3 (1.62 g, 11.7 mmol) in dry DMF (75 mL) was added ethyl 3-hydroxy-4-methyl-pyrrole-2-carboxylate **8** (1.80 g, 10.6 mmol). After the reaction mixture was stirred for 10 min at room temperature, 5-bromo-1-pentene (1.39 mL, 11.7 mmol) was added dropwise and the reaction was stirred for 2 d at 80°C. The reaction mixture was quenched with H_2O (400 mL) and extracted 7 times with each 50 mL of CH_2Cl_2. The collected organic layer was washed with each 100 mL of 0.5 m NaOH, H_2O, saturated $KHSO_4$ and dried over $MgSO_4$. The solvent was evaporated and the crude product purification using column chromatography on silica gel (PE:EtOAc = 7:3,

R_f = 0.45) yielding **10** (2.14 g, 9.02 mmol, 85 %) as a colourless oil. ^{1}H-NMR (600 MHz, $CDCl_3$): δ = 1.35 (t, ^3J = 7.1, 3 H, H-13), 1.86 (m, 2 H, H-7), 1.99 (d, ^4J = 0.8, 3 H, CH_3), 2.28 (m, 2 H, H-8), 4.02 (t, ^3J = 6.5, 2 H, H-6), 4.31 (q, ^3J = 7.4, 2 H, H-12), 4.98 (ddt, ^3J = 10.2, ^3J = 2.0, ^4J = 1.3, 1 H, H-10cis), 5.05 (ddt, ^3J = 17.2, ^3J = 2.0, ^4J = 1.6, 1 H, H-10trans), 5.86 (ddt, ^3J = 17.2, ^3J = 10.2, ^3J = 6.6, 1 H, H-9), 6.57 (dq, ^3J = 3.4, ^4J = 0.8, 1 H, H-5), 8.58 (d, ^3J = 3.4, 1 H, NH); ^{13}C-NMR (150 MHz, $CDCl_3$): δ = 8.5 (+, CH_3), 14.5 (+, C-13), 29.4 (−, C-7), 30.2 (−, C-8), 60.0 (−, C-12), 74.2 (−, C-6), 111.5 (C_{quat}, C-2), 113.3 (C_{quat}, C-4), 114.8 (−, C-10), 119.6 (+, C-5), 138.2 (+, C-9), 149.9 (C_{quat}, C-3), 160.5 (C_{quat}, C-11); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3318, 3076, 2979, 2938, 2872, 2743, 1668 1285, 1029q1; MS (EI, 70 eV): m/z (%) = 237.1 (23) [$M^{+\bullet}$], 169.0 (26) [$M - C_4H_8]^{+\bullet}$ 123.0 (100) [$M - C_4H_8 - C_2H_6O]^{+\bullet}$; HR-MS calcd. for $C_{13}H_{19}NO_3$ [$M^{+\bullet}$]: 237.1369; found: 237.1363 ± 2.0 ppm. – $C_{13}H_{19}NO_3$ (237.30).

Ethyl 3-(hex-5-enyloxy)-4-methyl-1*H*-pyrrole-2-carboxylate (11)

To a suspension of K_2CO_3 (491 mg, 3.55 mmol) in dry DMF (15 mL) was added Ethyl 3-hydroxy-4-methyl-pyrrole-2-carboxylate **8** (600 mg, 3.55 mmol). After the reaction mixture was stirred for 10 min at room temperature, 6-bromo-1-hexene (460 µL, 3.55 mmol) was added drop wise and the reaction was stirred for 40 h at 80°C. The reaction mixture was quenched with H_2O (75 mL) and extracted 7 times with each 10 mL of CH_2Cl_2. The collected organic layer was washed with each 20 mL of 0.5 m NaOH, H_2O und saturated $KHSO_4$ and dried over $MgSO_4$. The solvent was evaporated and the crude product purification using column chromatography on silica gel (PE:EtOAc = 8:2, R_f = 0.40) yielding **6** (565 mg, 2.25 mmol, 63 %) as a colourless oil. ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.28 (t, ^3J = 7.1, 3 H, H-14), 1.46-1.58 (m, 2 H, H-8), 1.64-1.76 (m, 2 H, H-7) 1.92 (d, ^4J = 0.7, 3 H, CH_3), 2.00-2.11 (m, 2 H, H-9), 3.94 (t, ^3J = 6.5, 2 H, H-6), 4.25 (q, ^3J = 7.1, 2 H, H-13), 4.83-5.03 (m, 2 H, H-11), 5.76 (ddt, ^3J = 17.0, ^3J = 10.3, ^3J = 6.7,1 H, H-10), 6.50 (dd, ^3J = 3.4, ^4J = 0.7, 1 H, H-5), 8.82 (bs, 1H, NH); ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 7.5 (+, CH_3), 13.5 (+, C-14), 24.3 (−, C-8), 28.7 (−, C-7), 32.6 (−, C-9), 58.9 (−, C-13), 73.7 (−, C-6), 110.4 (C_{quat}, C-2), 112.2 (C_{quat}, C-4), 113.5 (−, C-11), 118.8 (+, C-5), 137.7 (+, C-10), 149.0 (C_{quat}, C-3), 150.7 (C_{quat}, C-12); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3315, 2978, 2936, 2868, 1666, 1285, 1028; MS (EI, 70 eV): m/z (%) = 251.3

(11) [$M^{+\bullet}$], 169.2 (36) [M – C_6H_{10}]$^{+\bullet}$, 123.0 (100) [M – C_6H_{10} – C_2H_6O]$^{+\bullet}$; HR-MS calcd. for $C_{14}H_{21}NO_3$ [$M^{+\bullet}$]: 251.1521; found: 251.15 ± 0.9 ppm. – $C_{14}H_{21}NO_3$ (251.32).

Ethyl 3-(but-enyloxy)-5-formyl-4-methyl-1*H*-pyrrole-2-carboxylate (12)

Compound **9** (220 mg, 0.99 mmol) in $C_2H_4Cl_2$ (10 mL) was added dropwise to an ice cooled solution of DMF (85.5 µL, 1.10 mmol) and $POCl_3$ (101 µL, 1.10 mmol) in $C_2H_4Cl_2$ (10 mL), the mixture was stirred for 30 min, another 30 min at room temperature and was refluxed for 24 h. H_2O (40 mL) and EtOAc (20 mL) were added to the cooled mixture and the aqueous layer was extracted 3 times with each 20 mL of EtOAc. The combined organic layers were washed 3 times with 10 % solution of Na_2CO_3 and dried over $MgSO_4$. The solvent was evaporated and the crude product was purified using column chromatography on flash-silica gel (PE:EtOAc = 8:2, R_f = 0.21) yielding **12** (182 mg, 0.72 mmol, 73 %), as white crystals, m.p. = 62 °C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.34 (t, ^3J = 7.1, 3 H, H-12), 2.23 (s, 3 H, CH_3), 2.37-2.56 (m, 2 H, H-7), 4.03 (t, ^3J = 6.7, 2 H, H-6), 4.32 (q, ^3J = 7.1, 2 H, H-11), 4.92-5.24 (m, 2 H, H-9), 5.72-6.06 (m, 1 H, H-8), 9.20 (bs, 1 H, NH), 9.69 (s, 1 H, CHO); ^{13}C-NMR (300 MHz, $CDCl_3$): δ = 6.9 (+, CH_3), 14.3 (+, C-12), 34.5 (−, C-7), 61.1 (−, C-11), 74.5 (−, C-6), 117.0 (−, C-11), 117.6 (C_{quat}, C-2), 122.6 (C_{quat}, C-4), 127.8 (C_{quat}, C-5), 134.5 (+, C-8), 149.1 (C_{quat}, C-3), 159.6 (C_{quat}, C-10), 179.1 (+, CHO); IR (KBr): $\tilde{\nu}$ [cm^{-1}] = 3442, 3261, 2982, 2928, 2861, 2263, 1676, 1280, 1023; MS (EI, 70 eV): m/z (%) = 251.2 (26) [$M^{+\bullet}$], 151.1 (100) [$M^{+\bullet}$ - $^{\bullet}$NH-(S=O)$(CH_3)_3$]; HR-MS calcd. for $C_{13}H_{17}NO_4$ [$M^{+\bullet}$]: 251.1158; found: 251.1162 ± 1.6 ppm.

Ethyl 5-formyl-4-methyl-3-(pent-4-enyloxy)-1*H*-pyrrole-2-carboxylate (13)

Compound **10** (1.94 g, 8.15 mmol) in $C_2H_4Cl_2$ (10 mL) was added dropwise to an ice cooled solution of DMF (698 µL, 8.97 mmol) and $POCl_3$ (821 µL, 8.97 mmol) in $C_2H_4Cl_2$ (20 mL), the mixture was stirred for 30 min, another 30 min at room temperature and was refluxed for 24 h. H_2O (80 mL) and EtOAc (40 mL) were added to the cooled mixture and the aqueous layer was extracted 3 times with each 70 mL EtOAc. The combined organic layers were washed 3 times with 10 % solution of Na_2CO_3 and dried over $MgSO_4$. The solvent was evaporated and the crude product was purified using column chromatography on flash-silica gel (PE:EtOAc = 9:1, R_f = 0.40)

yielding **13** (1.82 g, 6.87 mmol, 84 %) as white crystals, m.p. = 49 °C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.34 (t, ^{3}J = 7.2, 3 H, H-13), 1.83 (m, 2 H, H-7), 2.22 (m, 2 H, H-8), 2.24 (s, 3 H, CH_3), 3.98 (t, ^{3}J = 6.5, 2 H, H-6), 4.34 (q, ^{3}J = 7.2, 2 H, H-12), 4.96 (ddt, ^{3}J = 10.2, ^{3}J = 2.0, ^{4}J = 1.3, 1 H, H-10cis), 5.02 (ddt, ^{3}J = 17.1, ^{3}J = 2.0, ^{4}J = 1.6, 1 H, H-10trans), 5.82 (ddt, ^{3}J = 17.1, ^{3}J = 10.2, ^{3}J = 6.6, 1 H, H-9), 9.55 (bs, 1 H, NH), 9.71 (s, 1 H, CHO); ^{13}C-NMR (100 MHz, $CDCl_3$): δ= 7.0 (+, CH_3), 14.3 (+, C-13), 29.3 (−, C-7), 30.1 (−, C-8), 61.1 (−, C-12), 74.7 (−, C-6), 115.0 (+, C-10), 117.7 (C_{quat}, C-2), 122.4 (C_{quat}, C-4), 128.0 (C_{quat}, C-5), 137.9 (+, C-9), 149.2 (C_{quat}, C-3), 160.5 (C_{quat}, C-11), 179.4 (+, CHO); MS (CI, NH_3): m/z (%) = 283.3 (100) $[MNH_4]^+$, 266.2 MH^+ (57). – Elemental analysis calcd. (%) for $C_{14}H_{19}NO_4$ (265.31): C 63.36, H 7.22, N 5.28; found C 63.16, H 7.57, N 5.21.

Ethyl 5-formyl-3-(hex-5-enyloxy)-4-methyl-1*H*-pyrrole-2-carboxylate (14)

Compound **11** (315 mg, 1.25 mmol) in $C_2H_4Cl_2$ (10 mL) was added dropwise to an ice cooled solution of DMF (107 µL, 1.38 mmol) and $POCl_3$ (125 µL, 8.97 mmol) in $C_2H_4Cl_2$ (10 mL), the mixture was stirred for 30 min, another 30 min at room temperature and was refluxed for 24 h. H_2O (40 mL) and EtOAc (20 mL) were added to the cooled mixture and the aqueous layer was extracted 3 times with 20 mL of EtOAc. The combined organic layers were washed 3 times with 10 % solution of Na_2CO_3 and dried over $MgSO_4$. The solvent was evaporated and the crude product purification using column chromatography on flash-silica gel (PE:EtOAc = 9:1, R_f = 0.41) yielding **9** (265 mg, 0.95 mmol, 75 %) as white crystals, m.p. = 48 °C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.38 (t, ^{3}J = 7.1, 3 H, H-14), 1.48-1.87 (m, 4 H, H-7 and H-8), 2.05-2.20 (m, 2 H, H-9), 2.27 (s, 3 H, CH_3), 4.01 (t, ^{3}J = 6.6, 2 H, H-6), 4.36 (q, ^{3}J = 7.1, 2 H, H-13), 4.90-5.10 (m, 2 H, H-11), 5.72-5.93 (m, 1 H, H-10), 9.24 (bs, 1 H, NH), 9.73 (s, 1 H, CHO); ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 6.9 (+, CH_3), 14.4 (+, C-14), 25.3 (−, C-8), 29.5 (−, C-7), 33.5 (−, C-9), 61.1 (−, C-13), 75.3 (−, C-6), 114.7 (−, C-11), 117.6 (C_{quat}, C-2), 122.5 (C_{quat}, C-4), 127.8 (C_{quat}, C-5), 138.5 (+, C-10), 149.3 (C_{quat}, C-3), 159.7 (C_{quat}, C-12), 179.1 (+, CHO); IR (KBr): $\tilde{\nu}$ [cm^{-1}] = 3447, 3268, 2979, 2940, 2867, 2362, 1672, 1277, 1027; MS (EI, 70 eV): m/z (%) = 279.3 (5) [$M^{+\bullet}$], 197.1 (48) $[M - C_6H_{10}]^{+\bullet}$, 151.1 (100) $[M- C_6H_{10} - C_2H_6O]^{+\bullet}$; HR-MS calcd. for $C_{15}H_{21}NO_4$ [$M^{+\bullet}$]: 279.1471; found: 279.1475 ± 1.9 ppm. – $C_{15}H_{21}NO_4$ (279.34).

General procedure (GP1) for the synthesis of *tert*-butanesulfinyl imines

To a solution of the pyrrol aldehyde (1.0 eq) and $Ti(OEt)_4$ (2.0 eq) in dry dichloromethane (5 mL) was added (*R*)-*tert*-butanesulfinamide (1.2 eq) under dinitrogen, and the mixture was stirred at the given temperature and time. The reaction mixture was quenched with a mixture of saturated NH_4Cl solution (10 mL) and brine (10 mL) while vigorously stirred. The resulting suspension was filtered through a plug of Celite and the filter cake was washed well with EtOAc (30 mL). The filtrate was transferred to a separatory funnel, where the organic layer was washed 3 times with each 10 mL of brine. The brine layer was extracted once with a small volume of EtOAc and the combined organic portions were dried over $MgSO_4$, filtered and concentrated under vacuum. The sulfinyl imines were purified by silica gel chromatography if no other method is given.

(*R*)-Ethyl 3-(but-3-enyloxy)-4-methyl-5-((2-methylpropan-2-ylsulfinamido)methyl)-1*H*-pyrrole-2-carboxylate (16)

Compound **12** (251 mg, 0.64 mmol), (*R*)-*tert*-butanesulfinamide (**15**, 93 mg, 0.77 mmol) and $Ti(OEt)_4$ (292 mg, 1.28 mmol) in dry dichloromethane (5 mL) were allowed to react according to the GP1 at room temperature for 36 hours yielding 152 mg (0.43 mmol, 92 %, conversion corrected yield, 43 mg of starting material regained) of **16** (Et_2O: hexanes = 1:1; R_f = 0.22), as colourless crystals, m.p = 81 °C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.25 (s, 9 H, *tert*-Bu), 1.40 (t, ^3J = 7.1, 3 H, H-12), 2.21 (s, 3 H, CH_3), 2.52-2.55 (m, 2 H, H-7), 4.06 (t, ^3J = 6.8, 2 H, H-6), 4.39 (q, ^3J = 7.1, H-11), 5.09-5.21 (m, 2 H, H-9), 5.92-5.94 (m, 1 H, H-8), 8.47 (s, 1 H, CHN), 9.28 (bs, 1 H, NH); ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 7.5 (+, CH_3), 14.4 (+, C-12), 22.5 (+, *tert*-Bu), 34.5 (−, C-7), 57.8 (+, *tert*-Bu), 60.9 (−, C-11), 74.4 (−, C-6), 116.1 (C_{quat}, C-2), 116.9 (−, C-9), 120.2 (C_{quat}, C-4), 126.2 (C_{quat}, C-5), 134.5 (+, C-8), 149.5 (C_{quat}, C-3), 150.5 (+, CHN), 160.1 (C_{quat}, C-10); IR (KBr): $\tilde{\nu}$ [cm^{-1}] = 3447, 3256, 2980, 2959, 2926, 2868, 1701,1593, 1272, 1059, 744; MS (FAB+): m/z (%) = 355 (100) $[MH]^+$, 289 (43) $[M - C_4H_8]^+$; HR-MS calcd. for $C_{17}H_{27}N_2O_4S^+$: 355.1697; found: 355.1692 + 1.5 ppm. – $C_{17}H_{26}N_2O_4S$ (354.46).

(*R*)-Ethyl 4-methyl-5-[(2-methylpropan-2-ylsulfinamido)-methyl]-3-pent-4-enyloxy-1*H*-pyrrole-2-carboxylate (17)

Compound **13** (450 mg, 1.70 mmol), (*R*)-*tert*-butanesulfinamide (**15**, 247 mg, 2.04 mmol) and $Ti(OEt)_4$ (776 mg, 3.4 mmol) in dry dichloromethane were allowed to react according to the GP1 at 35°C for 48 hours yielding 625 mg (quantitative) of **17** (Et_2O: hexanes = 1:1; R_f = 0.32), as colourless crystals, m.p = 79 °C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.24 (s, 9 H, *tert*-Bu), 1.39 (t, ^3J= 7.1, 3 H, H-13), 1.80-1.93 (m, 2 H, H-7), 2.20 (s, 3 H, CH_3), 2.22-2.31 (m, 2 H, H-8), 4.01 (t, ^3J = 6.5, 2 H, H-6), 4.37 (dq, ^3J = 7.1, ^2J = 1,1, 2 H, H-12), 4.93-5.15 (m, 2 H, H-10), 5.86 (ddt, ^3J = 17.0, ^3J = 10.3, ^3J = 6.7, 1 H, H-9), 8.44 (s, 1 H, CHN), 9.11 (bs, 1 H, NH); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 7.4 (+, CH_3), 14.4 (+, C-13), 22.6 (+, *tert*-Bu), 29.3 (−, C-7), 30.1 (−, C-8), 57.9 (+, *tert*-Bu), 60.9 (−, C-12), 74.7 (−, C-6), 115.0 (−, C-10), 116.2 (C_{quat}, C-2), 120.3 (C_{quat}, C-4), 126.2 (C_{quat}, C-5), 138.0 (+, C-9), 149.6 (C_{quat}, C-3), 150.3 (+, CHN), 160.1 (C_{quat}, C-11); IR (KBr): $\tilde{\nu}$ [cm^{-1}] = 3437, 3227, 2980, 2935, 2869, 1693, 1265, 1057, 1025, 746; MS (EI, 70 eV): m/z (%) = 368.2 (6) [$M^{+\bullet}$], 312.0 (100) [$M - C_4H_8]^{+\bullet}$. – Elemental analysis calcd. (%) for $C_{18}H_{28}N_2O_4S$ (368.49): C 58.67, H 7.66, N 7.60; found C 58.61, H 7.68, N 7.31; Crystal data: $C_{18}H_{28}N_2O_4S$, M_r = 368.49, colourless flat prism, triclinic, space group P 1, a = 8.9068(9) Å, b = 10.5174(12) Å, c = 11.9303(12) Å, α = 71.989(12) °, β = 84.932(12) °, γ = 70.971(13) °, Z = 2, V = 1004.7(2) $Å^3$, D_x = 1.218 mg/m^3, μ = 0.184 mm^{-1}, F(000) = 396, crystal size 0.44 x 0.30 x 0.12 mm, θ-range for data collections 2.35 to 26.82 °, index ranges –11 <= h <= 11, -13 <= k <= 13, -15 <= l <= 15, reflections collected/unique 10549/7803 [R_{int} = 0.0218], data/restraints/parameters 7803/3/461, goodness-of-fit on F^2 0.993, final R indices [I>2σ(I)] R_1 = 0.0278, wR_2 = 0.0654 R indices (all data) R_1 = 0.0307, wR_2 = 0.0663, largest diff. peak and hole 0.328 and –0.136 e. $Å^{-3}$.

(*R*)-Ethyl 3-(hex-5-enyloxy)-4-methyl-5-((2-methylpropan-2-ylsulfinamido)methyl)-1*H*-pyrrole-2-carboxylate (18)

Compound **14** (240 mg, 0.86 mmol), (*R*)-*tert*-butanesulfinamide (**15**, 125 mg, 1.03 mmol) and $Ti(OEt)_4$ (392 mg, 1.72 mmol) in dry dichloromethane were allowed to react according to the GP1 at room temperature for 36 hours yielding 240 mg (92 %, conversion corrected yield, 48 mg of **14** regained) of **18** (Et_2O: hexanes = 1:1; R_f =

0.22), as white crystals, m.p = 75 °C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.26 (s, 9 H, *tert*-Bu), 1.40 (t, ^{3}J = 7.1, 3 H, H-14), 1.57-1.62 (m, 2 H, H-8), 1.77-1.83 (m, 2 H, H-7), 2.10-2.15 (m, 2 H, H-9), 2.21 (s, 3 H, CH_3), 4.01 (t, ^{3}J = 6.5, 2 H, H-6), 4.39 (q, ^{3}J = 7.1, 2 H, H-13), 4.96-5.07 (m, 2 H, H-11), 5.83-5.85 (m, 1 H, H-10), 8.46 (s, 1 H, CHN), 9.25 (bs, 1 H, NH); ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 6.7 (+, CH_3), 13.7 (+, C-14), 21.8 (+, *tert*-Bu), 24.6 (−, C-8), 28.9 (−, C-7), 32.8 (−, C-9), 57.1 (+, *tert*-Bu), 60.2 (−, C-13), 74.5 (−, C-6), 114.2 (−, C-11), 115.5 (C_{quat}, C-2), 119.6 (C_{quat}, C-4), 125.5 (C_{quat}, C-5), 137.8 (+, C-10), 149.0 (C_{quat}, C-3), 149.7 (+, CHN), 159.4 (C_{quat}, C-12); IR (KBr): $\tilde{\nu}$ [cm^{-1}] = 3445, 3120, 2989, 2939, 2866, 2701, 1693 1504, 1267, 1056,747; MS (FAB+): m/z (%) = 383 (100) $[MH]^+$, 326 (40) $[M^+ - C_4H_8]^+$; HR-MS calcd. for $C_{19}H_{31}N_2O_4S^+$: 382.2015; found: 383.2005 + 2.8 ppm.

General Procedure (GP2) for the Grignard addition to *N*-*tert*-butansulfinyl imines.
To a solution of the sulfinyl imine (1 eq) in THF, the appropriate Grignard reagent (2.5 - 3.3 equiv.) in dry Et_2O (5 ml) was added dropwise and the conversion was monitored by TLC. The reaction was stirred at 50 – 60°C for approximately 4 h. Upon reaction completion, the excess organometallic reagent was destroyed with sat. aqueous NH_4Cl (10 mL) and the resulting suspension was diluted with brine (10 mL). The suspension was filtered through a plug of Celite and the filter cake was washed with EtOAc (2 x 10 mL). The filtrate was transferred into a separatory funnel, the aqueous layer was washed with EtOAc (3 x 10 mL), organic layers were combined, dried over $MgSO_4$ and concentrated to afford the crude product. Diastereomeric ratios were determined by HPLC analyses or by NMR of the crude product. Purification was performed by flash-silica chromatography using Biotage SP4 chromatography system. Yields correspond to the mass balance of purified material.

(*R*)-Ethyl 3-(but-3-enyloxy)-5-(1-(1,1-dimethylethylsulfinamido)pent-4-enyl)-4-methyl-1*H*-pyrrole-2-carboxylate (19)
Sulfinyl imine **16** (44 mg, 0.12 mmol) and but-3-enylmagnesium bromide (0.24 ml, 1.3 M in Et_2O, 0.3 mmol) were reacted according to the GP2. Flash-silica chromatography (Biotage SP4 chromatography system, EtOAc : cyclohexane = 7:3) gave 30 mg (61%) of **19** in a diastereomeric *syn/anti* ratio of 91:9. The diastereomeric ratio was determined

by ^{1}H NMR. ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.18 (s, 9 H, $(CH_3)_3$), 1.31 (t, ^{3}J = 7.1, 3 H, H-12), 1.80-2.06 (m, 4 H, H-14, H-15), 1.90 (s, 3 H, CH_3), 2.40-2.52 (m, 2 H, H-7), 3.95-4.06 (m, 2 H, H-6), 4.20-4.32 (m, 2 H, H-11), 4.41-4.50 (m, 1 H, H-13), 4.90-5.16 (m, 4 H, H-9, H-17), 5.71 (dddd, ^{3}J = 16.9, ^{3}J = 10.3, ^{3}J = 6.5, ^{3}J = 6.1, 1 H, H-8), 5.80-5.96 (m, 1 H, H-16), 9.04 (bs, 1 H, N-H).

(*R*)-Ethyl 3-(but-3-enyloxy)-5-(1-(1,1-dimethylethylsulfinamido)hex-5-enyl)-4-methyl-1*H*-pyrrole-2-carboxylate (20)

Sulfinyl imine **16** (32 mg, 0.09 mmol) and pent-4-enylmagnesium bromide (0.25 ml, 0.94 M in Et_2O, 0.23 mmol) were reacted according to the GP2. Flash-silica chromatography (Biotage SP4 chromatography system, EtOAc : cyclohexane = 7:3) gave 24 mg (63%) of **20** in a diastereomeric *syn/anti* ratio of 93:7. The diastereomeric ratio was determined by ^{1}H NMR. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.14 (s, 9 H, $(CH_3)_3$), 1.29 (t, ^{3}J = 7.1, 3 H, H-12), 1.32-1.38 (m, 2 H, H-15), 1.88 (s, 3 H, CH_3), 1.92-2.01 (m, 2 H, H-16), 2.40-2.48 (m, 2 H, H-7), 3.99 (dt, ^{3}J = 6.7, ^{2}J = 2.3, 2 H, H-6), 4.16-4.29 (m, 2 H, H-11), 4.40 (ddd, ^{3}J = 7.3, ^{3}J = 7.3, J = 1.6, 1 H, H-13), 4.83-5.24 (m, 4 H, H-9, H-18), 5.65 (dddd, ^{3}J = 17.0, ^{3}J = 10.1, ^{3}J = 6.8, ^{3}J = 6.7, 1 H, H-8), 5.85 (dddd, ^{3}J = 17.1, ^{3}J = 10.3, ^{3}J = 6.8, ^{3}J = 6.7, 1 H, H-17), 9.01 (bs, 1 H, N-H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 7.5 (+, CH_3), 14.5 (+, C-12), 22.6 (+, $(CH_3)_3$, 25.2 (−, C-15), 33.2 (−, C-14), 34.6 (−, C-16), 35.5 (−, C-7), 52.1 (+, C-13), 55.7 (C_{quat}, C-$(CH_3)_3$), 60.0 (−, C-11), 74.1 (−, C-6), 110.5 (C_{quat}, C-2), 111.0 (C_{quat}, C-4), 115.2 (−, C-18), 116.6 (−, C-9), 131.5 (C_{quat}, C-5), 134.9 (+, C-8), 137.9 (+, C-17), 149.8 (C_{quart}, C-3), 160.4 (C_{quat}, C-11); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3448, 3255, 3077, 2979, 2929, 2866, 1665,1468, 1032, 911; MS (FAB+): m/z (%) = 425 (23) $[M+H]^+$, 304 (100) $[M^+ - NH\text{-}(S{=}O)(CH_3)_3]^+$; HR-MS calcd. for $C_{22}H_{37}O_4N_2S$: 425.2574; found: 425.2487 + 3.1 ppm.

(*R*)-Ethyl 3-(but-3-enyloxy)-5-(1-(1,1-dimethylethylsulfinamido)hept-6-enyl)-4-methyl-1*H*-pyrrole-2-carboxylate (21)

Sulfinyl imine **16** (40 mg, 0.11 mmol) and hex-5-enylmagnesium bromid (0.34 ml, 0.82 M in Et_2O, 0.28 mmol) were reacted according to the GP2. Flash-silica chromatography (Biotage SP4 chromatography system, EtOAc : cyclohexane = 7:3) gave 31 mg (64%) of **21** in a diastereomeric *syn/anti* ratio of 91:9. The diastereomeric ratio was determined

by ^{1}H NMR. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.11-1.36 (m, 4 H, H-15, H-16), 1.15 (s, 9 H, $(CH_3)_3$), 1.29 (t, ^{3}J = 7.1, 3 H, H-12), 1.67-1.83 (m, 2 H, H-14), 1.88 (s, 3 H, CH_3), 1.90-1.99 (m, 2 H, H-17), 2.44 (ddt, ^{3}J = 6.8, ^{3}J = 6.8, ^{2}J = 1.4, 2 H, H-7), 3.50 (d, ^{3}J = 2.2, 1 H, N-H), 3.94-4.05 (m, 2 H, H-69, 4.17-4.30 (m, 2 H, H-11), 4.35-4.44 (m, 1 H, H-13), 4.83-4.93 (m, 2 H, H-9), 4.98-5.12 (m, 2 H, H-19), 5.67 (dddd, ^{3}J = 17.0, ^{3}J = 10.2, ^{3}J = 6.7, ^{3}J = 6.7, 1 H, H-8), 5.86 (dddd, ^{3}J = 17.1, ^{3}J = 10.3, ^{3}J = 6.7, ^{3}J = 6.7, 1 H, H-18), 9.00 (bs, 1 H, H-pyrrole); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 7.5 (+, CH_3), 14.5 (+, C-12), 22.6 (+, $(CH_3)_3$, 25.4 (−, C-16), 28.4 (−, C-15), 33.4 (−, C-14), 34.6 (−, C-17), 35.9 (−, C-7), 52.1 (+, C-13), 55.7 (C_{quat}, C-$(CH_3)_3$), 59.9 (−, C-11), 74.1 (−, C-6), 110.5 (C_{quat}, C-2), 111.0 (C_{quat}, C-4), 114.7 (−, C-19), 116.6 (−, C-9), 131.6 (C_{quat}, C-5), 135.0 (+, C-8), 138.4 (+, C-18), 149.8 (C_{quart}, C-3), 160.4 (C_{quat}, C-11); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3422, 2979, 2929, 2861, 1647, 1465, 1280, 1032; MS (EI, 70 eV): m/z (%) = 438.2 (6) [$M^{+\bullet}$], 318.2 (100) [$M^{+\bullet}$ - $^{\bullet}$NBoc-(S=O)$(CH_3)_3$]; HR-MS calcd. for $C_{23}H_{38}N_2O_4S$ [$M^{+\bullet}$]: 438.2548; found: 438.2548 ± 1.9 ppm.

(*R*)-Ethyl 5-(1-(1,1-dimethylethylsulfinamido)pent-4-enyl)-4-methyl-3-(pent-4-enyloxy)-1*H*-pyrrole-2-carboxylate (22)

Sulfinyl imine **17** (50 mg, 0.14 mmol) and but-3-enylmagnesium bromide (0.28 ml, 1.3 M in Et_2O, 0.35 mmol) were reacted according to the GP2. Flash-silica chromatography (Biotage SP4 chromatography system, EtOAc : cyclohexane = 7:3) gave 40 mg (68%) of **22** in a diastereomeric *syn/anti* ratio of 90:7. The diastereomeric ratio was determined by ^{1}H NMR and HPLC. ^{1}H-NMR (600 MHz, $CDCl_3$): δ = 1.24 (s, 9 H, $(CH_3)_3$), 1.37 (t, ^{3}J = 7.1, 3 H, H-13), 1.84-1.89 (m, 2 H, H-7), 1.97 (s, 3 H, CH_3), 1.09-2.03 (m, 2 H, H-15), 2.04-2.12 (m, 2 H, H-16), 2.24-2.29 (m, 2 H, H-8), 3.55 (d, ^{3}J = 1.5, 1 H, N-H), 4.04 (dt, ^{2}J = 6.4, ^{3}J = 2.7, 2 H, H-6), 4.30-4.37 (m, 2 H, H-12), 4.53 (dt, ^{3}J = 7.1, ^{3}J = 1.9, 1 H, H-14), 5.00 (ddt, ^{3}J = 10.3, $^{2}J^{*}$ and $^{4}J^{*}$, 1 H, H-10cis), 5.03 (ddt, ^{3}J = 10.3, $^{2}J^{*}$ and $^{4}J^{*}$, 1 H, H-18cis), 5.05 (ddt, ^{3}J = 16.9, $^{2}J^{*}$ and $^{4}J^{*}$, 1 H, H-18trans), 5.07 (ddt, ^{3}J = 17.0, ^{2}J and $^{4}J^{*}$, 1 H, H-10trans), 5.78 (ddt, ^{3}J = 16.9, ^{3}J = 10.3, ^{3}J = 6.6, 1 H, H-17), 5.88 (ddt, ^{3}J = 17.0, ^{3}J = 10.3, ^{3}J = 6.7, 1 H, H-19), 8.98 (bs, 1 H, pyrrole-H); ^{13}C-NMR (150 MHz, $CDCl_3$): δ = 7.5 (+, CH_3), 14.5 (+, C-13), 22.6 (+, $(CH_3)_3$), 29.4 (−, C-7), 30.1 (−, C-16), 30.2 (−, C-8, 35.0 (−, C-15), 51.6 (+, C-14), 55.7 (C_{quat}, C-$(CH_3)_3$), 59.9 (−, C-12), 74.3 (−, C-6), 110.6 (C_{quat}, C-2), 111.1 (C_{quat}, C-4), 114.8 (−, C-10), 115.9 (,

C-18), 131.1 (C_{quat}, C-5), 137.0 (+, C-17), 138.2 (+, C-9), 150.0 (C_{quat}, C-3), 160.3 (C_{quat}, C-11); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3460, 3252, 3077, 2978, 2927, 2868, 1665 1468, 1032, 911; MS (EI, 70 eV): m/z (%) = 424.2 [$M^{+\bullet}$] (6), 304.2 [$M^{+\bullet}$ - $^{\bullet}NH$-(S=O)$(CH_3)_3$] (100), 367.8 [$M^{+\bullet}$ - $^{\bullet}C_4H_9$]; HR-MS calcd. for $C_{22}H_{37}N_2O_4S$ [MH^+]: 425.2474 found 425.2475 ± 0.3 ppm.

*coupling constants smaller than 2 Hz are not precise and are therefore not documented.

(*R*)-Ethyl 5-(1-(1,1-dimethylethylsulfinamido)hex-5-enyl)-4-methyl-3-(pent-4-enyloxy)-1*H*-pyrrole-2-carboxylate (23)

Sulfinyl imine **17** (100 mg, 0.27 mmol) and pent-4-enylmagnesium bromide (0.73 ml, 1.3 M in Et_2O, 0.90 mmol) were reacted according to the GP2. Flash-silica chromatography (Biotage SP4 chromatography system, EtOAc : cyclohexane = 7:3) gave 40 mg (71%) of **23** in a diastereomeric *syn/anti* ratio of 9:1. The diastereomeric ratio was determined by ^1H NMR and HPLC. ^1H-NMR (600 MHz, $CDCl_3$): δ = 1.20 (s, 9 H, $(CH_3)_3$), 1.35 (t, ^3J = 7.0, 3 H, H-13), 1.22-1.46 (m, 2 H, H-16), 1.77-1.93 (m, 4 H, H-7, H-15) 1.95 (s, 3 H, CH_3), 1.98-2.07 (m, 2 H, H-17), 2.22-2.29 (m, 2 H, H-8), 4.03 (m, 3 H, H-6, N-H), 4.37 (m, 2 H, H-12), 4.43-4.48 (m, 1 H, H-14), 4.90-5.10 (m, 4 H, H-10, H-19), 5.67-5.75 (m, 1 H, H-18), 5.80-5.90 (m, 1 H, H-9), 9.30 (s, 1 H, N-H); ^{13}C-NMR (150 MHz, $CDCl_3$): δ = 7.5 (+, CH_3), 14.5 (+, C-13), 22.6 (+, $(CH_3)_3$, 25.2 (−, C-16),29.4 (−, C-7), 30.2 (−, C-8), 33.2 (−, C-17), 35.7 (−, C-15), 52.4 (+, C-14), 55.8 (C_{quat}, C-$(CH_3)_3$), 60.0 (−, C-12), 74.3 (−, C-6), 110.4 (C_{quat}, C-2), 110.7 (C_{quat}, C-4), 114.8 (−, C-10), 115.1 (−, C-19), 132.0 (C_{quat}, C-5), 137.9 (+, C-18), 138.2 (+, C-9), 149.9 (C_{quart}, C-3), 160.6 (C_{quat}, C-11); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3458, 3252, 3075, 2976, 2928, 2866, 1665, 1468, 1273, 1032; MS (EI, 70 eV): m/z (%) = 438.2 (6) [$M^{+\bullet}$], 318.1 (100) [$M^{+\bullet}$-$^{\bullet}NH$-(S=O)$(CH_3)_3$]; HR-MS calcd. for $C_{23}H_{38}N_2O_4S$ [$M^{+\bullet}$]: 438.2555; found: 438.2550 ± 0.7 ppm.

(*R*)-Ethyl 5-(1-(1,1-dimethylethylsulfinamido)hept-6-enyl)-4-methyl-3-(pent-4-enyloxy)-1*H*-pyrrole-2-carboxylate (24)

Sulfinyl imine **17** (200 mg, 0.54 mmol) and hex-5-enylmagnesium bromide (1.27 mL, 0.94 M in Et_2O, 1.19 mmol) were reacted according to the GP2. Flash-silica chromatography (Biotage SP4 chromatography system, EtOAc : cyclohexane = 7:3)

gave 159 mg (65%) of **24** in a diastereomeric *syn/anti* ratio of 93:7. The diastereomeric ratio was determined by ^{1}H NMR. ^{1}H-NMR (600 MHz, $CDCl_3$): δ = 1.21 (s, 9 H, $(CH_3)_3$), 1.22-1.41 (m, 7 H, H-13, H-16, H-17), 1.77-1.91 (m, 4 H, H-7, H-15), 1.95 (s, 3 H, CH_3), 1.98-2.04 (m, 2 H, H-18), 2.21-2.32 (m, 2 H, H-8), 3,77-3.88 (m, 1 H, N-H), 3.94-4.05 (m, 2 H, H-6), 4.23-4.38 (m, 2 H, H-12), 4.41-4.51 (m, 1 H, H-14), 4.88-5.09 (m, 4 H, H-10, H-20), 5.69-5.78 (m, 1 H, H-19), 5.82-5.92 (m, 1 H, H-9), 9.23 (bs, 1 H, N-H); ^{13}C-NMR (150 MHz, $CDCl_3$): δ = 7.5 (+, CH_3), 14.5 (+, C-13), 22.6 (+, $(CH_3)_3$, 25.4 (−, C-17), 28.4 (−, C-16), 29.4 (−, C-7), 30.2 (−, C-8), 33.4 (−, C-18), 36.1 (−, C-15), 52.4 (+, C-14), 55.8 (C_{quat}, C-$(CH_3)_3$), 60.0 (−, C-12), 74.3 (−, C-6), 110.4 (C_{quat}, C-2), 110.8 (C_{quat}, C-4), 114.6 (−, C-10), 114.8 (, C-20), 132.0 (C_{quat}, C-5), 138.2 (+, C-19), 138.4 (+, C-9), 149.9 (C_{quart}, C-3), 160.6 (C_{quat}, C-11); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3458, 3252, 3075, 2976, 2928, 2866, 1665, 1469, 1280, 1033, 910, 733; MS (FAB+): m/z (%) = 453 (20) $[MH]^+$, 332 (100) $[M - NH\text{-}(S{=}O)(CH_3)_3]^+$; HR-MS calcd. for $C_{24}H_{41}N_2O_4S$ $[M^{+\bullet}]$: 453.2787; found: 453.2813 + 10 ppm.

(*R*)-Ethyl 5-(1-(1,1-dimethylethylsulfinamido)pent-4-enyl)-3-(hex-5-enyloxy)-4-methyl-1*H*-pyrrole-2-carboxylate (25)

Sulfinyl imine **18** (63 mg, 0.14 mmol) and but-3-enylmagnesium bromide (0.33 mL, 1.3 M in Et_2O, 0.41 mmol) were reacted according to the GP2. Flash-silica chromatography (Biotage SP4 chromatography system, EtOAc : cyclohexane = 7:3) gave 48 mg (68%) of **25** in a diastereomeric *syn/anti* ratio of 95:5. The diastereomeric ratio was determined by ^{1}H.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.23 (s, 9 H, $(CH_3)_3$), 1.28 (t, ^{3}J = 7.1, 3 H, H-14), 1.45-1.58 (m, 2 H, H-8), 1.62-1.75 (m, 2 H, H-7), 1.78-2.01 (m, 4 H, H-16 and H-17), 1.88 (s, 3 H, CH_3), 2.01-2.10 (m, 2 H, H-9), 3.85 (d, ^{3}J = 2.9, 1 H, N-H), 3.93 (t, ^{3}J = 6.8 2 H, H-6), 4.13-4.29 (m, 2 H, H-13), 4.36-4.48 (m, 2 H, H-15), 4.75-5.07 (m, 4 H, H-11 and H-19), 5.54-5.91 (m, 2 H, H-10 and H-18), 8.15 (bs, 1 H, pyrrole-H); ^{13}C-NMR (100 Hz $CDCl_3$): δ = 7.5 (+, CH_3), 14.5 (+, C-14), 22.6 (+, $(CH_3)_3$), 25.3 (−, C-8), 29.7 (−, C-7), 30.1 (−, C-17), 33.6 (−, C-9), 35.2 (−, C-16), 52.0 (+, C-15), 55.8 (C_{quat}, C-$(CH_3)_3$), 60.0 (−, C-13), 74.8 (−, C-6), 110.9 (C_{quat}, C-2), 111.1 (C_{quat}, C-4), 114.9 (−, C-11), 115.8 (−, C-19), 131.6 (C_{quat}, C-5), 137.1 (+, C-18), 138.7 (+, C-10), 150.0 (C_{quat}, C-3), 160.6 (C_{quat}, C-12); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3461, 3300, 2978, 2933, 2867, 1663,

1272, 1031, 909; MS (FAB+): m/z (%) = 439 (26) $[MH]^+$, 318 (100) [M - NH-(S=O)$(CH_3)_3$]$^+$; HR-MS calcd. for $C_{23}H_{39}O_4O_2S$: 439.2631; found: 439.2614 + 3.9 ppm.

(*R*)-Ethyl 5-(1-(1,1-dimethylethylsulfinamido)hex-5-enyl)-3-(hex-5-enyloxy)-4-methyl-1*H*-pyrrole-2-carboxylate (26)

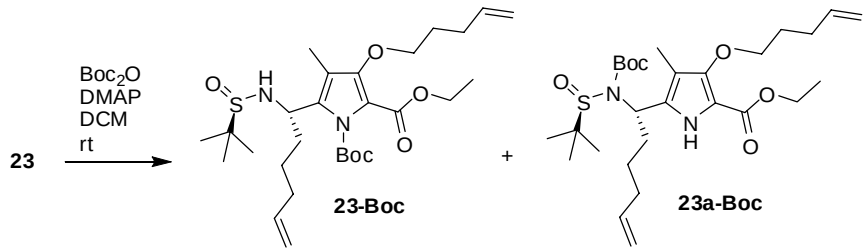
Sulfinyl imine **18** (63 mg, 0.14 mmol) and pent-4-enylmagnesium bromide (0.44 mL, 0.94 M in Et_2O, 0.41 mmol) were reacted according to the GP2. Flash-silica chromatography (Biotage SP4 chromatography system, EtOAc : cyclohexane = 7:3) gave 36 mg (50%) of **26** in a diastereomeric *syn/anti* ratio of 91:9. The diastereomeric ratio was determined by ^{1}H. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.14 (s, 9 H, $(CH_3)_3$), 1.18-1.41 (m, 2 H, H-17) 1.28 (t, ^3J = 7.1, 3 H, H-14), 1.46-1.57 (m, 2 H, H-8), 1.62-1.84 (m, 4 H, H-7 and H-16), 1.88 (s, 3 H, CH_3), 1.91-2.00 (m, 2 H, H-18), 2.01-2.10 (m, 2 H, H-9), 3.62 (d, ^3J = 2.5, 1 H, N-H), 3.88-3.98 (m, 2 H, H-6), 4.15-4.29 (m, 2 H, H-13), 4.36-4.45 (m, 1 H, H-15), 4.85-5.99 (m, 4 H, H-11 and H-20), 5.59-5.82 (m, 2 H, H-10 and H-19), 9.03 (bs, 1 H, pyrrole-H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 7.5 (+, CH_3), 14.5 (+, C-14), 22.6 (+, $(CH_3)_3$), 25.2 (−, C-17), 25.3 (−, C-8), 29.7 (−, C-7), 33.2 (−, C-18), 33.6 (−, C-9), 35.6 (−, C-16), 52.2 (+, C-15), 55.8 (C_{quat}, C-$(CH_3)_3$), 60.0 (−, C-13), 74.8 (−, C-6), 110.5 (C_{quat}, C-2), 110.9 (C_{quat}, C-4), 114.6 (−, C-11), 115.2 (−, C-20), 131.6 (C_{quat}, C-5), 137.9 (+, C-19), 138.7 (+, C-10), 150.0 (C_{quat}, C-3), 160.5 (C_{quat}, C-12); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3420, 3257, 2978, 2931, 2864, 1664, 1468, 1273, 1032; MS (FAB+): m/z (%) = 453 (20) $[MH]^+$, 332 (100) [M - NH-(S=O)$(CH_3)_3$]$^+$; HR-MS calcd. for $C_{24}H_{41}O_4O_2S$: 453.2787; found: 453.2807 + 4.5 ppm.

(*R*)-Ethyl 5-(1-(1,1-dimethylethylsulfinamido)hept-6-enyl)-3-(hex-5-enyloxy)-4-methyl-1*H*-pyrrole-2-carboxylate (27)

Sulfinyl imine **18** (63 mg, 0.16 mmol) and hex-5-enylmagnesium bromide (0.44 mL, 0.94 M in Et_2O, 0.41 mmol) were reacted according to the GP2. Flash-silica chromatography (Biotage SP4 chromatography system, EtOAc : cyclohexane = 7:3) gave 36 mg (72%) of **21** in a diastereomeric *syn/anti* ratio of 94:6. The diastereomeric ratio was determined by proton NMR. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.13 (s, 9 H, $(CH_3)_3$), 1.19-1.45 (m, 4 H, H-17 and 18) 1.28 (t, ^3J = 7.1, 3 H, H-14), 1.46-1.58 (m, 2 H, H-8), 1.60-1.84 (m, 4 H, H-7 and H-16), 1.88 (s, 3 H, CH_3), 1.90-2.11 (m, 4 H, H-9

and H-19), 3.72 (d, ^3J = 2.9, 1 H, N-H), 3.86-3.98 (m, 2 H, H-6), 4.16-4.31 (m, 2 H, H-13), 4.33-4.43 (m, 1 H, H-15), 4.80-5.01 (m, 4 H, H-11 and H-21), 5.59-5.83 (m, 2 H, H-10 and H-20), 9.17 (bs, 1 H, pyrrole-H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 7.5 (+, CH_3), 14.5 (+, C-14), 22.6 (+, $(CH_3)_3$), 25.3 (−, C-8), 25.4 (−, C-18), 28.4 (−, C-17), 29.7 (−, C-7), 33.4 (−, C-19), 33.7 (−, C-9), 36.1 (−, C-16), 52.3 (+, C-15), 55.8 (C_{quat}, C-$(CH_3)_3$), 59.9(−, C-13), 74.8 (−, C-6), 110.4 (C_{quat}, C-2), 110.8 (C_{quat}, C-4), 114.2 (−, C-11), 114.6 (−, C-21), 131.9 (C_{quat}, C-5), 138.4 (+, C-20), 138.7 (+, C-10), 150.0 (C_{quat}, C-3), 160.6 (C_{quat}, C-12); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3460, 3256, 3076, 2977, 2930, 2861, 1666, 1469, 1280, 1032, 994; MS (FAB+): m/z (%) = 467 (18) $[MH]^+$, 346 (100) [M - NH-(S=O)$(CH_3)_3]^+$; HR-MS calcd. for $C_{25}H_{42}O_4N_2S$: 466.2865; found: 466.2849 + 3.5 ppm.

(*R*)-1-*tert*-Butyl 2-ethyl 5-(1-(1,1-dimethylethylsulfinamido)hex-5-enyl)-4-methyl-3-(pent-4-enyloxy)-1*H*-pyrrole-1,2-dicarboxylate 23-Boc and (*R*)-Ethyl 5-(1-(*N*-(*tert*-butoxycarbonyl)-2-methylpropan-2-ylsulfinamido)hex-5-enyl)-4-methyl-3-(pent-4-enyloxy)-1*H*-pyrrole-2-carboxylate 23a-Boc



Scheme 4. Preparation of the Boc-protected diene **23**.

To a solution of **23** (130 mg, 0.30 mmol) in dry CH_2Cl_2 (20 mL) at room temperature DMAP (40.3 mg, 0.33 mmol) was added and the mixture was stirred for 10 min under dinitrogen atmosphere. $(Boc)_2O$ (131 mg, 0.60 mmol) was added and the mixture was stirred over night. The solution was washed with water (4x 10 mL) and dried over $MgSO_4$. The solvent was evaporated in vacuum and the two products were separated by column chromatography on silica gel (cyclohexane : EtOAc = 8:2, R_f = 0.13 and 0.47)

to give 44 mg of **23-Boc** (29 %) as a colourless oil and 99 mg of **23a-Boc** (61 %) as a colourless oil.

Compound 23-Boc: IR: $\tilde{\nu}$ [cm^{-1}] = 3450, 3302, 3077, 2979, 2934, 2870, 1713; MS (EI, 70 eV): m/z (%) = 538.1 (0.2) [$M^{+\bullet}$], 318.2 (100) [$M^{+\bullet}$ - $^{\bullet}$NBoc-(S=O)$(CH_3)_3$]; HR-MS calcd. for $C_{28}H_{46}N_2O_6S$ [$M^{+\bullet}$]: 538.3077; found: 538.3075 ± 1.2 ppm.

Compound 23a-Boc: ^{1}H-NMR (600 MHz, $CDCl_3$): δ = 1.15-1.23 (m, 2 H, H-16), 1.27 (s, 9 H, TBS), 1.34 (t, ^3J = 7.1, 3 H, H-13), 1.52 (s, 9 H, Boc), 1.69-1.79 (m, 1 H, H-15A), 1.80-1.87 (m, 2 H, H-7), 1.95-2.05 (m, 2 H, H-17), 2.02 (s, 3 H, CH_3), 2.19-2.29 (m, 2 H, H-8), 2.67-2.80 (m, 1 H, H-15B), 3.95-4.00 (m, 1 H, H-6A), 4.01-4.07 (m, 1 H, H-6B), 4.23-4.36 (m, 2 H, H-12), 4.57-4.65 (m, 1 H, H-14), 4.89-5.11 (m, 4 H, H-10, H-19), 5.71 (dddd, ^3J = 17.0, ^3J = 10.3, ^3J = 6.7, ^3J = 6.7, 1 H, H-18), 5.86 (dddd, ^3J = 17.0, ^3J = 10.3, ^3J = 6.7, ^3J = 6.7, 1 H, H-9), **9.70 (bs, 1 H, H-pyrrole)**; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 7.4 (+, CH_3), 14.5 (+, C-13), 22.8 (+, TBS, 26.3 (−, C-16), 28.3 (+, Boc), 29.5 (−, C-7), 30.2 (−, C-8), 33.2 (−, C-17), 33.5 (−, C-15), 45.5 (+, C-14), 59.6 (−, C-12), 60.4 (C_{quat}, TBS), 74.2 (−, C-6), 83.7 (C_{quat}, Boc), 110.3 (C_{quat}, C-2), 112.5 (C_{quat}, C-4), 114.7 (−, C-10), 114.9 (−, C-19), 130.8 (C_{quat}, C-5), 137.9 (+, C-18), 138.4 (+, C-9), 149.2 (C_{quart}, C-3), 154.5 (C_{quart}, C-Boc amide), 160.6 (C_{quat}, C-11); IR: $\tilde{\nu}$ [cm^{-1}] = 3450, 3397, 3077, 2978, 2932, 1694; MS (EI, 70 eV): m/z (%) = 538.1 (7) [$M^{+\bullet}$], 482.1 (13) [M – C_4H_8]$^{+\bullet}$, 318.2 (94) [$M^{+\bullet}$ - $^{\bullet}$NH-(S=O)$(CH_3)_3$], 276.1 (100) [$M^{+\bullet}$ - $^{\bullet}$NH-(S=O)$(CH_3)_3$ – C_2H_2O]; HR-MS calcd. for $C_{28}H_{46}N_2O_6S$ [$M^{+\bullet}$]: 538.3077; found: 538.3079 ± 0.4 ppm.

General procedure (GP3) for ring closing metathesis of sulfinyl imine dialkenes

Sulfinyl imine dienes were dissolved as a 0.0005 M solution in anhydrous and degassed CH_2Cl_2 under an argon atmosphere. Bis(tricyclohexylphosphine) benzylidine ruthenium(IV) dichloride (Grubb's I catalyst) (15 mol%), dissolved in anhydrous and degassed CH_2Cl_2 (50 mL), was added slowly over a period of 2 h by a syringe pump to the reaction mixture at 40 °C, and stirred for additional 24 h. Additional catalyst (15 mol%) was added and the mixture was refluxed for 1 d. After 50 equivalents of DMSO were added, the mixture was stirred at room temperature for 1d. Concentration under

reduced pressure and filtration through a pad of silica afforded the crude product, which was purified by HPLC.

Ethyl 11-((R)-1,1-dimethylethylsulfinamido)-15-methyl-2-oxa-13 azabicyclo-[10.2.1]pentadeca-1(14),6,12(15)-triene-14-carboxylate (28)

Compound **23** (20 mg, 0.05 mmol) was reacted following the GP3. Preparative HPLC afforded 7 mg of the ansa-bridged **28** (47%, conversion corrected yield, 5 mg of **23** regained) as an inseparable 9:1 *E:Z* mixture and 3.5 mg of the larger macrocycle **29** (12%), as a mixture of stereoisomers. **Compound 28**: ^{1}H-NMR (600 MHz, $CDCl_3$, DQF-COSY, HSQC, HMBC): $\delta = 0.84$-0.92 (m, 1 H, H-8A), 0.92-1.02 (m, 1 H, H-11A), 1.26 (s, 9 H, $(CH_3)_3$), 1.38 (t, 3 H, $^{3}J = 7.13$, H-16), 1.43-1.53 (m, 2 H, H-7A, H-8B), 1.55-1.63 (m, 1 H, H-13A), 1.65-1.75 (m, 2 H, H-11B, H-12A), 2.00-2.13 (m, 4 H, H-7B, CH_3), 2.15-2.21 (m, 2 H, H-12B, H-13B), 3.62 (bs, 1 H, N-H), 4.13-4.20 (m, 1 H, H-6A), 4.24-4.34 (m, 2 H, H-6B, H-15A), 4.43-4.49 (m, 1 H, H-15B), 4.54 (dd, 1 H, $^{2}J = 11.96$, $^{3}J = 5.26$, H-14), 5.13 (ddt, 1 H, $^{3}J = 10.8$, $^{3}J = 3.3$, $^{4}J = 2.0$, H-10), 5.21 (ddt, 1 H, $^{3}J = 10.8$, $^{3}J = 2.2$, $^{4}J = 2.2$, H-9), 9.01 (bs, 1 H, H-pyrrole); ^{13}C-NMR (assignment by HSQC, HMBC, 600 MHz, $CDCl_3$): $\delta = 9.1$ (+, CH_3), 14.5 (−, C-16), 22.6 (+, $(CH_3)_3$), 24.9 (−, C-11), 25.5 (−, C-12), 26.5 (−, C-8), 31.1 (−, C-7), 35.2 (−, C-13), 51.8 (+, C-14), 55.6 (C_{quat}, $C(CH_3)_3$), 59.9 (−, C-15), 70.9 (−, C-6), 111.8 (C_{quat}, C-2), 120.0 (C_{quat}, C-4), 128.5 (+, C-10), 130.2 (+. C-9), 131.0 (C_{quat}, C-5), 150.0 (C_{quat}, C-3), 160.6 (C_{quat}, C-ester); MS (ES, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 411.2 (100) $[M+H]^+$, 821.6 (13) $[2M + H]^+$; HR-MS calcd. for $C_{21}H_{34}N_2O_4S$ $[M^{+\bullet}]$: 410.2232; found: 410.2233 ± 1.5 ppm.

Compound 29: MS (ES, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 819.7 (100) $[M\text{-}H^+]^-$, 933.6 (10) $[M+TFA]^-$, 879.7 (9) $[M+CH_3COO^-]$, 855.6 (8) $[M+Cl^-]$.

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[24] The N-Boc protected derivative **23-Boc** was tested to explore the effect of the pyrrole amine function on the RCM reaction. However, reactions with and without N-Boc protection of the pyrrole nitrogen atom showed give equal results.

[25] This catalyst was tested to prevent the possible formation of a chelate intermediate between the *tert*-butyldimethylsilyl (TBS) protecting group and the ruthenium ion trapping the catalyst in an inactive form. This chelation should be avoided by the chelating effect of the isopropoxy-group of the 2nd generation Hoveyda-Grubb's catalyst: Zaja, M.; Connon, S. J.; Dunne, A. M.; Rivard, M.; Buschmann, N.; Jiricek, J.; Blechert, S. *Tetrahedron* **2003,** *59*, 6545-6558.

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[28] HPLC analysis of the RCM product from diene **23-Boc** on a chiral column showed four isomeric compounds (one major, three minor). This again indicates a stereochemical bias of the ring closing reaction.

C. Appendix

Publications

G. Dirscherl, R. Knape, P. Hanson, B. Koenig *Tetrahedron* **2007**, *63*, 4918-4928.

G. Dirscherl, B. König *Eur. J. Org. Chem.* **2007**, in press.

Conferences

COST-Meeting (Coopération européenne dans le domaine de la recherché scientifique et techinique) – Green Chemistry and Chemical Technology in Regensburg, Germany, June 2005.

Second World Congress on Synthetic Receptors, Salzburg, Austria, September 2005.

Second Summer School Medicinal Chemistry of the International Quality Network (IQN) in Regensburg, Germany October 2004.

Scholarships

EU-Scholarship for a *Short Term Scientific Mission* within COST in 2006.

EU-Scholarship for a *Short Term Scientific Mission* within COST in 2005.

EU-Scholarship within the *ERASMUS-Program* in 2002/03.

Oskar-Karl-Forster-Scholarship, University of Regensburg, 2002.

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04/2002		**Dechema e.V.** Kompaktkurs „Technische Chemie“	Frankfurt
09/2001	03/2002	**University of Bath** Studienaufenthalt	Bath / Großbritannien
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