Metallacyclopeptides: Artificial analogues of naturally occurring peptides

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Naturally occurring metalloproteins contain metal ions either to introduce a special reactivity or to stabilize a peptide structure. Since the early 1990s, chemists have been trying to use metal coordination for the fixation of short artificial peptides in well-defined cyclic structures. In this tutorial review a survey of the general approaches towards metallacyclopeptides as small cyclic peptide derivatives or as a part of a bigger α-helix (or β-sheet) structure is given. In three case studies it is shown how naturally occurring compounds can be mimicked by metal coordination to non-natural peptide derivatives.

1. Introduction

"Peptides" represent an important class of natural products which are crucial for the action of biological systems. Although their architecture can be considered as a biopolymer of amino acid monomers, their function largely depends on their ability to adopt well defined conformations ("secondary structures"). These lead to specific relative orientations of reactive side-chain functionalities to each other. The conformations are stabilized by the interplay of an ensemble of weak attractive (and sometimes repulsive) inter- and intramolecular forces (*e.g.* hydrogen bonding, electrostatics, disulfide bridges, or metal coordination).[1]

Typical structural motifs are the α-helix or the parallel and anti-parallel β-sheet as well as α-, β-, or γ-turns (see Fig. 1). In each of these structures, the amino acids have to adopt special conformations which can be described by the Ramachandran parameters Φ and Ψ. These dihedral angles (Φ = (CO)–CHR–NH–(CO), Ψ = NH–(CO)–(CHR)–NH) show typical values for the different structural motifs (*e.g.* α-helix: $\Phi = -57°$, $\Psi = -47°$).[1]

Transformation of one form of peptide into another can have dramatic consequences on the properties. For example an α-helix/β-sheet transition is discussed as the initial step in the precipitation of plagues from the β-*amyloid peptide*, which is responsible for *Alzheimers* disease.[2]

Besides the large peptidic units which are found *e.g.* in enzymes, many small peptides exist which often possess

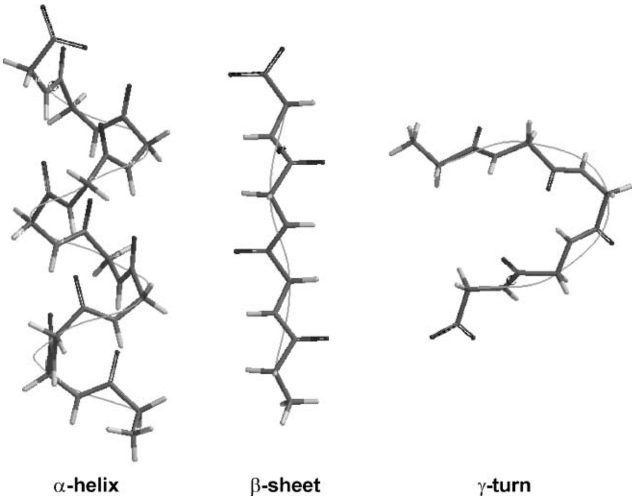

Fig. 1 Representative secondary peptide structures: α-helix, β-sheet, γ-turn.

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remarkable biological activity. Cyclic fixation induces conformational strain, which leads to compounds with well defined structures. They are preorganized for a specific biological activity.

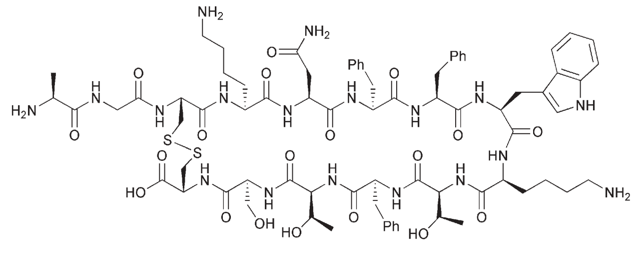
For preparative chemists it is challenging to prepare building blocks which can be introduced into peptides and which support the formation of a specific secondary structure. In many cases this is done by the use of covalent connections between different positions in the peptide leading to constraints within the peptide sequence and thus fixes the conformations of the strand.

As an example, *Somatostatin* is a heterodetic cyclic 14-mer-peptide which is produced by the hypothalamus and is a potent inhibitor for the release of several hormones (*e.g. Somatotropin*). Additionally it also regulates many other biological processes. Thorough investigations showed that only the Phe-Trp-Lys-Thr sequence is responsible for the activity.

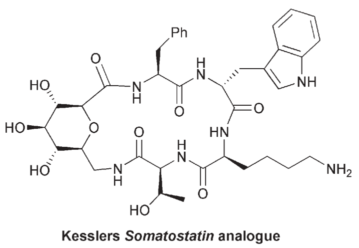
The structure of *Somatostatin* is shown in Fig. 2 as well as that of a cyclic analogue, which inhibits the release of growth hormones in mice. The analogue represents a macrocycle with an incorporated carbohydrate unit. Notice, that the L-tryptophane of the natural derivative is substituted by the D-configured amino acid. This results in a higher activity.[3]

Non-covalent interactions also can be used to control the secondary structures of peptides. Thus, an α-helix can be stabilized by attractive interactions between appropriate side-chain functionalities of amino acids in the *i* and *i + 4* position. Both units are located on the same face of the helix and therefore the interaction is maximized if the helical conformation is adopted by the peptide. Even a simple cation–π interaction between a phenyl group attached to the *i* and an ammonium moiety at the *i + 4* position can enhance the stability of the α-helix (Fig. 3).[4]

In this review, it will be shown that metal coordination is a powerful tool in stabilising specific conformations in artificial peptidic systems. In addition three case studies will be discussed, in which metallacyclopeptides are obtained, that mimic naturally occurring peptide structures. At the present time we are coming close to obtaining metallapeptides which are able to act specifically with biological systems. Many problems had to be solved on the way. However, this article summarizes the preliminary investigations on how artificial peptide microstructures can be fixed by metal coordination to mimic natural examples.



Somatostatin



Kesslers *Somatostatin* analogue

Fig. 2 *Somatostatin* and an artificial analogue.

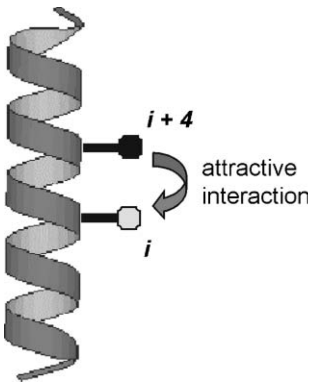


Fig. 3 Attractive interactions which can stabilize an α-helix.

Herein, we do not discuss biological activities or functions of the artificial metallapeptides. The purpose of this article is to gain a deeper understanding of the influence of metal coordination on the secondary structures of peptides. This should help in the understanding of the structural chemistry of the much more complicated naturally occurring systems.

2. General approaches to metallacyclopetides

In the simplest case a peptide secondary structure can be stabilized by direct binding of a metal ion to the peptide strand. Gas phase investigations with *e.g.* an Ala_{20} peptide show, that the helicity of the peptide strand is enhanced in the presence of metal ions like the sodium cation. Here metal coordination takes place at the carbonyl oxygen atoms of the amide units of the peptide.[5] A more directed binding of metals can be obtained, if the peptide bears coordinating units for metal cations either at the amino acid side chains or at the termini of the strand.

Fig. 4 shows a schematic representation of a peptide, which bears two metal coordinating moieties. In the absence of metals this peptide should adopt a random coil structure.

If appropriate metal ions are added, different conformations of the strand can be frozen and if the binding sites are located at the *i* and *i + 4* (or *i + 5*) position of the strand, an α-helix should be stabilized. It is also possible, that a β-sheet structure is obtained, if the binding sites are more separated. Binding sites at the termini of the peptide could lead to a macrocycle which bears a peptidic turn/loop structure. In all of the three cases metal directed cyclization leads to conformational restrictions in the strand and thus to well defined structures.[6]

In the simplest case, the metal binding site can be a side chain functionality of a naturally occurring amino acid (*e.g.* histidine, cysteine, aspartic or glutaric acid, lysine, serine, tyrosine).[7]

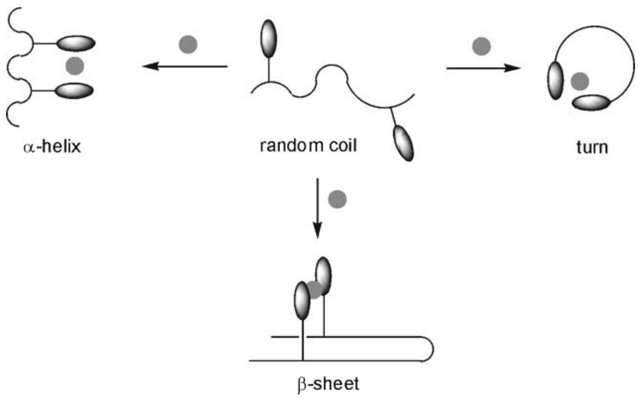


Fig. 4 Different possibilities of metal induced folding of peptides.

Imidazole as metal binding site

Histidine bears an imidazole unit in its side chain, which *via* its nitrogen lone pair is able to coordinate to metal ions. In many metalloenzymes the histidine residue acts as an anchor to bind metal ions. Ghadiri *et al.* prepared the peptide acetyl-AEAAAKHAAAHEAAAKA-CONH$_2$ **1**, which exhibits histidine residues in the 7- and 11-positions of the sequence (*i*, *i* + *4*). CD spectroscopy shows that this peptide adopts a random coil structure. However, upon coordination of the $[Ru(NH_3)_4]^{2+}$ unit to the histidine residues, the peptide adopts an α-helical structure which by CD spectroscopy was shown to be present in 45%.[8]

Searle *et al.* designed the peptide acetyl-HKHYTVSING-KKITVHI-OMe **2** with histidine residues in the 3- and 16-positions. Upon zinc(II) complexation the β-sheet-type hairpin structure which is shown in Fig. 5 is formed.[9]

The short pentapeptide acetyl-HAAAH-NH$_2$ was introduced by Fairlie *et al.* to form a macrocyclic metal complex with the "Pd(en)$^{2+}$"-moiety fixing the peptide in a conformation which corresponds to one turn of an α-helix **3a**.[10,11] NMR spectroscopic investigations showed that this structural motif indeed is adopted. However, more thorough investigations revealed that the α-helix **3a** is present in solution in about 45% while the γ-turn **3b** is observed in 55%.[12]

The presented examples show, that histidine is a valuable building block, in the design of different kinds of peptides which adopt different structures upon metal binding. This depends on the amino acid sequence, on the position of the histidine residues, and on the kind of metal ion or metal complex fragment.

Pyridine, 2,2′-bipyridine and 2,2′:6′,2″-terpyridine as metal binding sites

Pyridines are heterocyclic compounds which like imidazoles are good ligands for metal ions. Therefore pyridine moieties as well as chelating bipyridine or terpyridine can be introduced into peptides as "unnatural" metal binding sites.

For example, the bis(pyridinyl) terminated Leu-Ala-Leu derivative **4** is easily prepared by solid phase synthesis. Reaction of **4** with Pd(en)(NO$_3$)$_2$ (en = ethylene diamine) results in the formation of a mixture of the 1 : 1 and 2 : 2 macrocycles [Pd(en)(**4**)]$_n$ (*n* = 1, 2, Fig. 6). Due to the

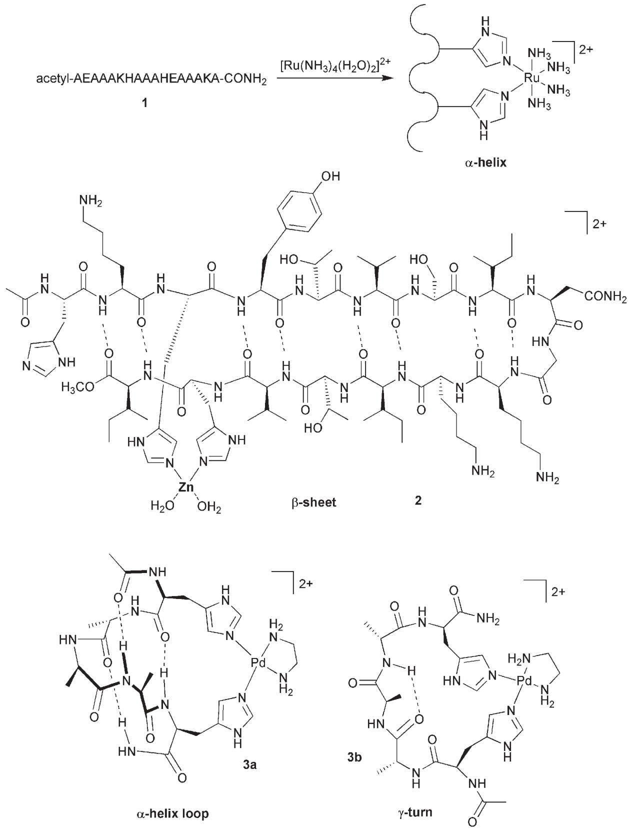


Fig. 5 Metallacyclopeptides with imidazole ligands.

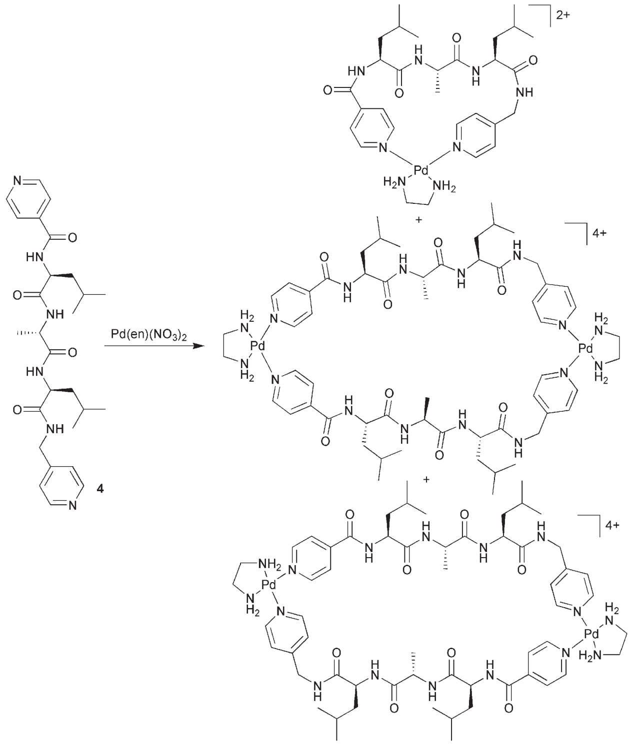


Fig. 6 Metallacyclopeptides with pyridine ligands.

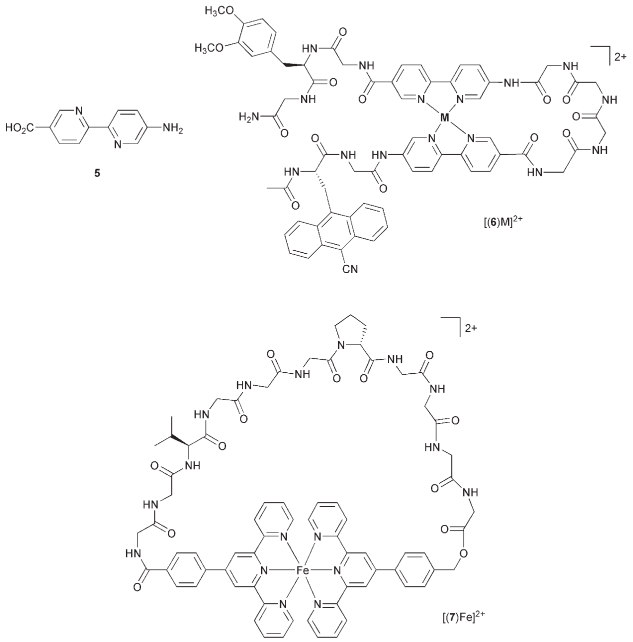


Fig. 7 Bipyridine and terpyridine metallacyclopeptides.

directionality of the peptide strands two different "regioisomers" of the 2 : 2 complex can be obtained. However, all isomers are metallacyclopeptides, although the formation of the mononuclear isomer would be preferred.[13]

Imperiali *et al.* prepared the 2,2′-bipyridine amino acid **5** (Fig. 7) and incorporated it into the short peptide **6**. Upon coordination of different metal ions (Zn^{2+}, Co^{2+}) fluorescence quenching between the terminal veratrole and anthracene groups occurs due to the formation of the metallacyclopeptide [(**6**)M]$^{2+}$. Upon metal binding a triglycine peptidic unit is forced into a loop type conformation.[14]

Constable *et al.* used terpyridines as terminating groups for the peptide **7** and the corresponding metallamacrocycle [(**7**)Fe]$^{2+}$ was prepared by reaction with iron(II) ions. Hereby proline is introduced as a central amino acid to support the turn formation upon metal complexation. The complex [(**7**)Fe]$^{2+}$ is formed spontaneously in solution but decomposes upon prolonged standing.[15]

Phosphane units as metal binding sites

Phosphorus is the higher homologue of nitrogen and as well is a good donor atom for the formation of coordination compounds. Gilbertson *et al.* introduced diphenylphosphano serine as an artificial amino acid building block for the synthesis of functionalized peptides.[16]

As depicted in Fig. 8 a short peptide which bears two diphenylophosphano serine units in *i* and *i + 4* position is able to bind rhodium(I). It is assumed, that rhodium coordination and macrocycle formation induces an α-helical structure at the peptide. This unit also can be generated bound to a resin and then can be used in heterogeneous hydrogenation reactions.[17]

In a similar way, the functionalized resin **8** (Fig. 9) is able to bind palladium with formation of a metallamacrocycle, which then can be used for asymmetric allylic substitution reactions.[18]

Catecholates as metal binding sites

Siderophores are a class of naturally occurring compounds which are produced from microorganisms to solubilize metal ions. One frequently found binding site in siderophores is the catechol moiety.[19]

In compound **9**, two catechol units are attached to the termini of a Val-Val-Val tripeptide. Reaction of this tripeptide with titanium(IV) ions leads to complexes in which three

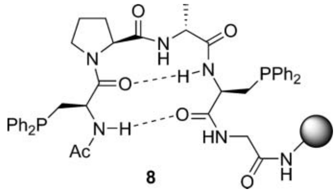


Fig. 9 A diphenylphosphanoserine containing turn structure.

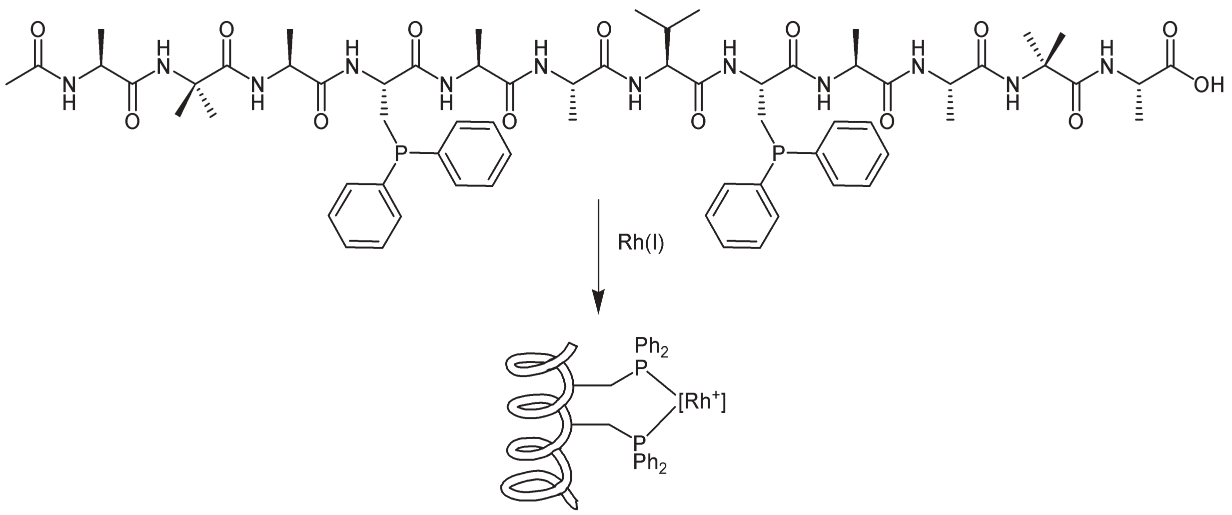
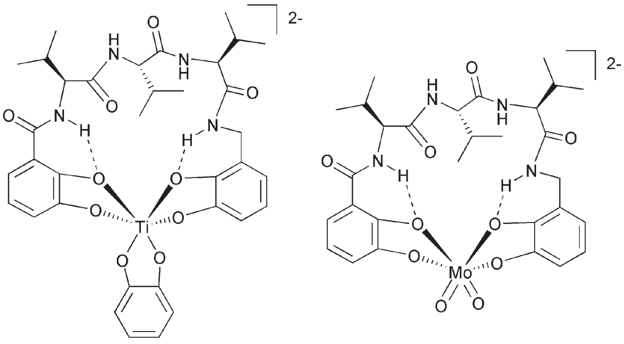


Fig. 8 Induction of an α-helix by binding of rhodium(I) to phosphanoserine residues.



[(**9**)Ti(cat)]$^{2-}$ [(**9**)MoO$_2$]$^{2-}$

Fig. 10 Metallacyclopeptides with catechol ligands.

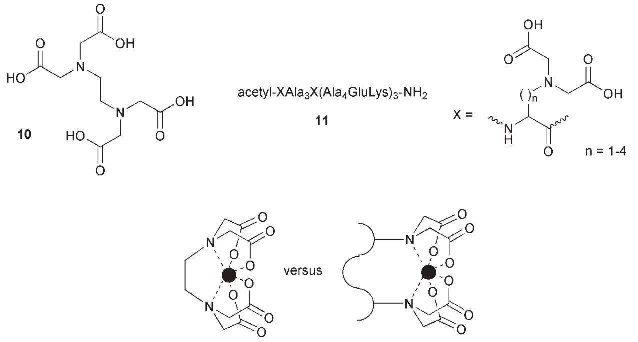


Fig. 11 Comparison of Hopkins' peptide derivative with EDTA.

ligands **9** bridge two metal ions. In addition, complexes are formed in which only one ligand **9** is bridging, while two others are each tetracoordinating to only one metal center and form loop-type macrocycles.[20]

If complex formation between one equivalent of titanium(IV) and one equivalent of **9** is performed in the presence of one equivalent of catechol, the complex [(**9**)Ti(cat)]$^{2-}$ is formed as the dominating product (Fig. 10). A similar macrocyclization proceeds quantitatively, if not titanium(IV), but Mo(VI)O$_2$ is used for the metal directed ring closure. Only one isomer of [(**9**)MoO$_2$]$^{2-}$ is observed which shows the induction of the stereochemistry at the molybdenum complex unit by the chiral peptide.[20]

8-Hydroxyquinolines as metal binding sites

8-Hydroxyquinoline moieties were introduced at the side chains of amino acids and were used for the sensing of divalent zinc Hereby a metallacyclopeptide with a β-turn sequence is stabilized in the sensor.[21]

Aminodiacetates as metal binding sites

EDTA **10** is one of the most powerful chelating agents for metal ions. Hopkins early on copied this unit to prepare peptides **11** which bear amino acids with *N*-diacetate side chains as residues in position *i* and *i + 4*. Combining the two side chains of those unnatural amino acids leads to a chelating unit which is very similar to EDTA. **11** was reacted with Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, or Cd^{2+}.

CD spectroscopic investigations showed that the metal ions are able to induce a helical conformation in the peptide **11** (see Fig. 11). In this respect specially cadmium(II) was highly effective. A strong negative Cotton effect at 222 nm was observed for **11** in the presence of cadmium(II) but not in its absence.[22,23]

Crown-ethers and related macrocycles as metal binding sites

In 1995 Voyer described crown ether functionalized amino acids **12** and their incorporation into the peptides BOC-Ala$_3$-**12**-Ala-**12**-Ala$_3$-NHC$_3$H$_7$ (**13**), BOC-Ala$_3$-**12**-Ala$_2$-**12**-Ala$_3$-NHC$_3$H$_7$ (**14**), and BOC-Ala$_3$-**12**-Ala$_3$-**12**-Ala$_3$-NHC$_3$H$_7$ (**15**). In the absence of metal ions the peptides adopt a β-sheet type structure (Fig. 12) which is not affected upon addition of sodium or potassium cations. However, caesium induces a transition to a turn type arrangement with peptide **14** but not with **13** or **15**.[24]

Caesium is able to form a sandwich complex with the crown ethers and thus leads to macrocyclization. In order to form the complex, peptide **14** has to adopt a β-turn. This observation was in contrast to the prediction, to stabilize an α-helix. Here the β-turn seems to be a favoured alternative.[24]

Just recently peptides with a helix–loop–helix motif were described which bear the triazanonane unit **16** (Fig. 13). Addition of zinc(II) ions stabilizes the α-helix and the resulting metal complex was used for transesterification reactions of phosphate esters.[25]

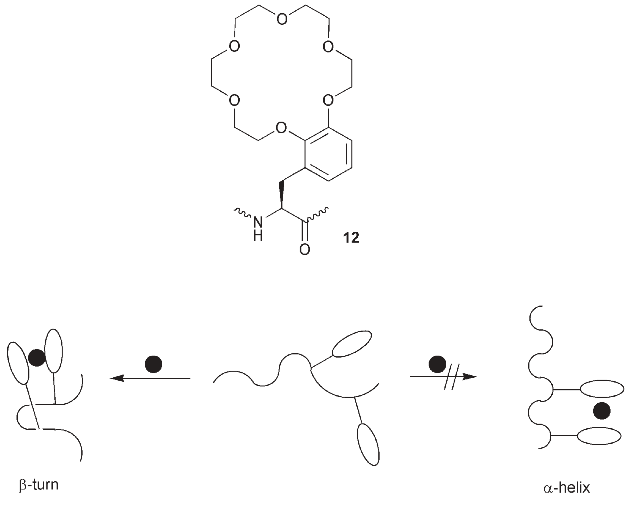


Fig. 12 Crown-ether functionalized peptides.

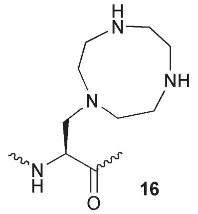


Fig. 13

As was described, different metal ligand units can be attached to peptides. In the presence of appropriate metal ions, often metallacyclopeptides are formed, in which a special conformation at a peptidic unit is fixed. A random coil peptide is rigidified upon complex formation. As shown, this can be used to obtain simple macrocycles, but α-helices or β-sheets also can be stabilized.

In the following, three case studies will be discussed, in which this principle is used to mimic naturally occurring peptides.

3. Case studies

3.1. Case study A: Mimicking cyclopeptides: *Segetalin A/B*, *Urotensin II*, and the RGD sequence

As was already mentioned above, peptide-bridged dicatechol ligands form metallacyclopeptides by reaction with the *cis*-molybdenum(VI)dioxo moiety in the presence of an appropriate base.[20] At this point it was of interest, if not only "innocent" peptides like Val-Val-Val can be introduced. The question was, if it would be possible to form metallacyclopeptides containing peptide sequences which are known from nature to be most active, if they are fixed in a loop type structure. A major goal of this study was to learn, if side chain functionalized amino acid residues like tryptophane, lysine or aspartic acid will interfere with metal coordination.

Segetalins (see Fig. 14) are a class of cyclopeptides which were isolated from the seeds of *Vaccaria segetalis*. These seeds are used in Chinese medicine for the treatment of breast infections, to promote milk secretion, to activate blood flow and to treat amenorrhea. In experiments with ovarectomized rats it was shown, that the estrogene like activity is due to *Segetalins A* and *B*, but not *C* and *D*.[26,27]

Comparing the structures of the *Segetalins A* and *B* it can be seen that both derivatives have the WAGV-tetrapeptide sequence in common. Therefore it is supposed that this sequence is responsible for the activity of the compounds. Furthermore, NMR spectroscopic structural studies show that the valine residue is differently orientated in the two compounds. Therefore, this residue should not be essential for the activity, the WAG sequence should be sufficient.

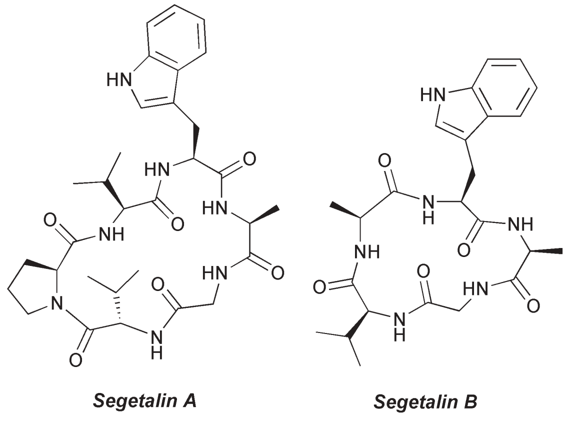


Fig. 14 *Segetalin A* and *B*.

Based on these results, it is interesting to prepare macrocyclic *Segetalin* analogous. This was done by Sonnet *et al.* in 2001 by the use of covalent macrocycle formation.[28]

Metallacyclopeptide analogues of the *Segetalins A/B* also can be prepared. The WAG tripeptide bridged ligand **17** (Fig. 15) was obtained by solution phase synthesis,[29] while the preparation of the corresponding tetrapeptide derivative **18** was achieved on a solid phase followed by deprotection in solution. The latter hereby has the advantage of a more simple reaction procedure, shorter reaction times, and higher yields.[30]

Both derivatives **17** and **18** smoothly form metallacyclopeptides $[(\mathbf{17})MoO_2]^{2-}$ and $[(\mathbf{18})MoO_2]^{2-}$ by reaction of the ligands with *cis*-dioxomolybdenum(VI) bis(acetylacetonate) in the presence of base. The mononuclear complexes $[(\mathbf{17})MoO_2]^{2-}$ and $[(\mathbf{18})MoO_2]^{2-}$ are characterized by NMR as well as FT-ICR ESI-MS.[29,30]

Urotensin II (Fig. 16) was originally isolated from the urophysis of the goby fish (*Gillichthys mirabilis*). Hereby it was obtained as a 12 mer, but its composition ranges from 11 amino acids in humans to 14 amino acids in mice. All the derivatives have the cysteine linked CFWKYC macrocycle in common. Goby *Urotensin II* probably is the most potent vasoconstrictor which is known. Therefore it recently was thoroughly investigated.[31]

Structure–function analysis showed that in particular the WKY front of the macrocyclic moiety is important for the biological activity and for the binding of *Urotensin II* to a G-protein-coupled receptor model. Therefore the WKY tripeptide is an interesting target to be incorporated into a metallamacrocycle.[32]

The dicatechol terminated WKY derivative **19** can be prepared easily by solid phase synthesis of the protected ligand and final deprotection with BBr_3. Formation of the

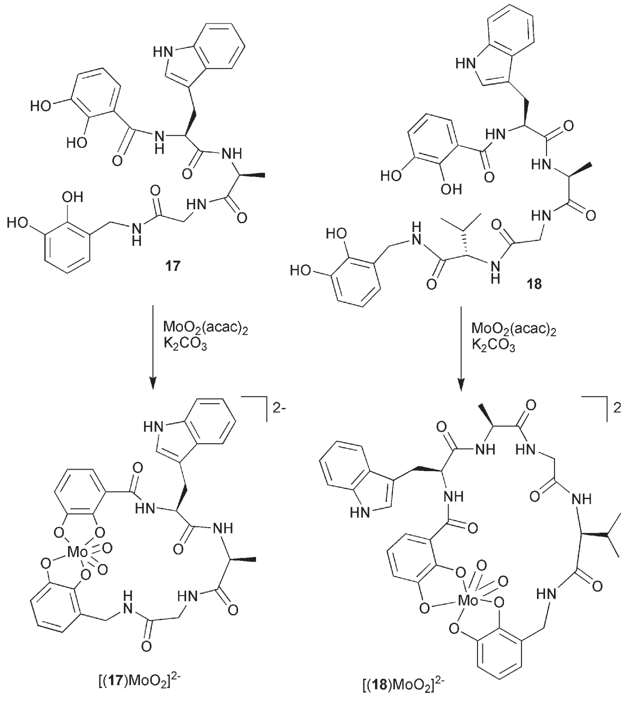


Fig. 15 *Segetalin*-type metallacyclopeptides.

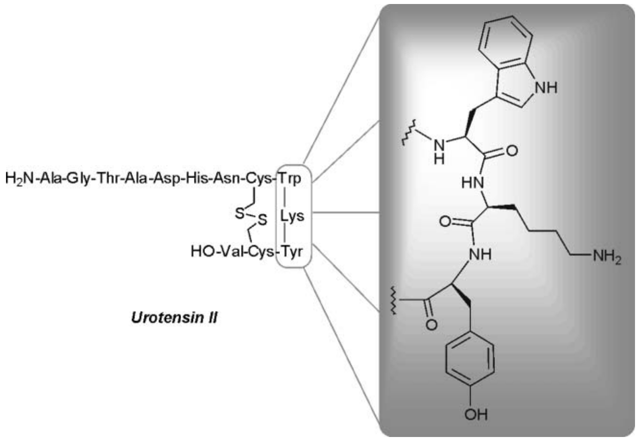


Fig. 16 *Urotensin II.*

metallacyclopeptide $[(\mathbf{19})MoO_2]^{2-}$ by reaction of **19** with $MoO_2(acac)_2$ (Fig. 17) in methanol in the presence of base can be followed by the colour change of the solution. After six days the macrocycle is obtained. The reaction time probably has to be long in order to obtain the thermodynamically favoured 1 : 1 macrocycle. NMR and FT-ICR ESI MS show the formation of the desired compound $[(\mathbf{19})MoO_2]^{2-}$.[33]

As a representative example, Fig. 18 shows a part of the ^1H NMR spectrum of **19** as well as of the complex $K_2[(\mathbf{19})MoO_2]$ in methanol-d_4. The signals of the "free" ligand are not well resolved due to conformational flexibility. However, upon complex formation, the conformation is fixed and well resolved signals are observed. This is demonstrated in Fig. 18 for the diastereotopic protons of the dihydroxybenzylamide unit of **19**, which split into two signals upon formation of the metallacyclopeptide $K_2[(\mathbf{19})MoO_2]$.

The RGD sequence is also termed the "universal cell recognition sequence". It is able to bind to integrins which are located at the surface of cells and thus plays an important role in cell–cell or cell–matrix recognition or aggregation. Fig. 19 shows the ribbon type structure of *Echistatin* from *Echis carinatus*, which is an active ingredient in viper venom. This small protein contains a RGD sequence, which is located in a loop type region.[34]

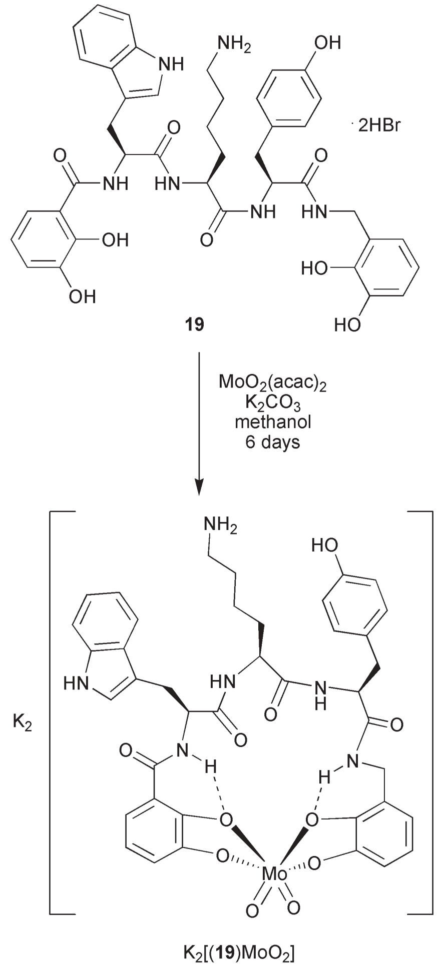


Fig. 17 A metallacyclic analogue of *Urotensin II.*

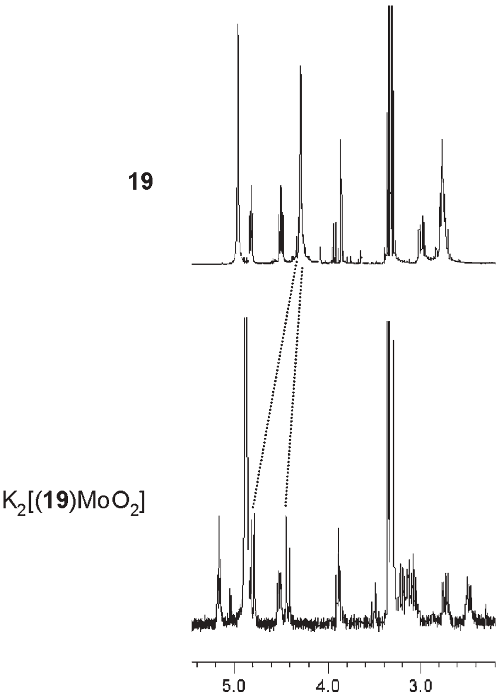


Fig. 18 Splitting of the signal of the diastereotopic benzylic protons of **19** upon coordination to MoO_2^{2+}.

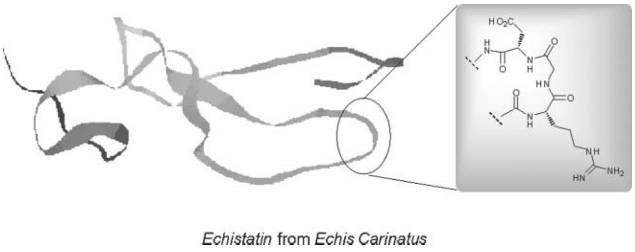
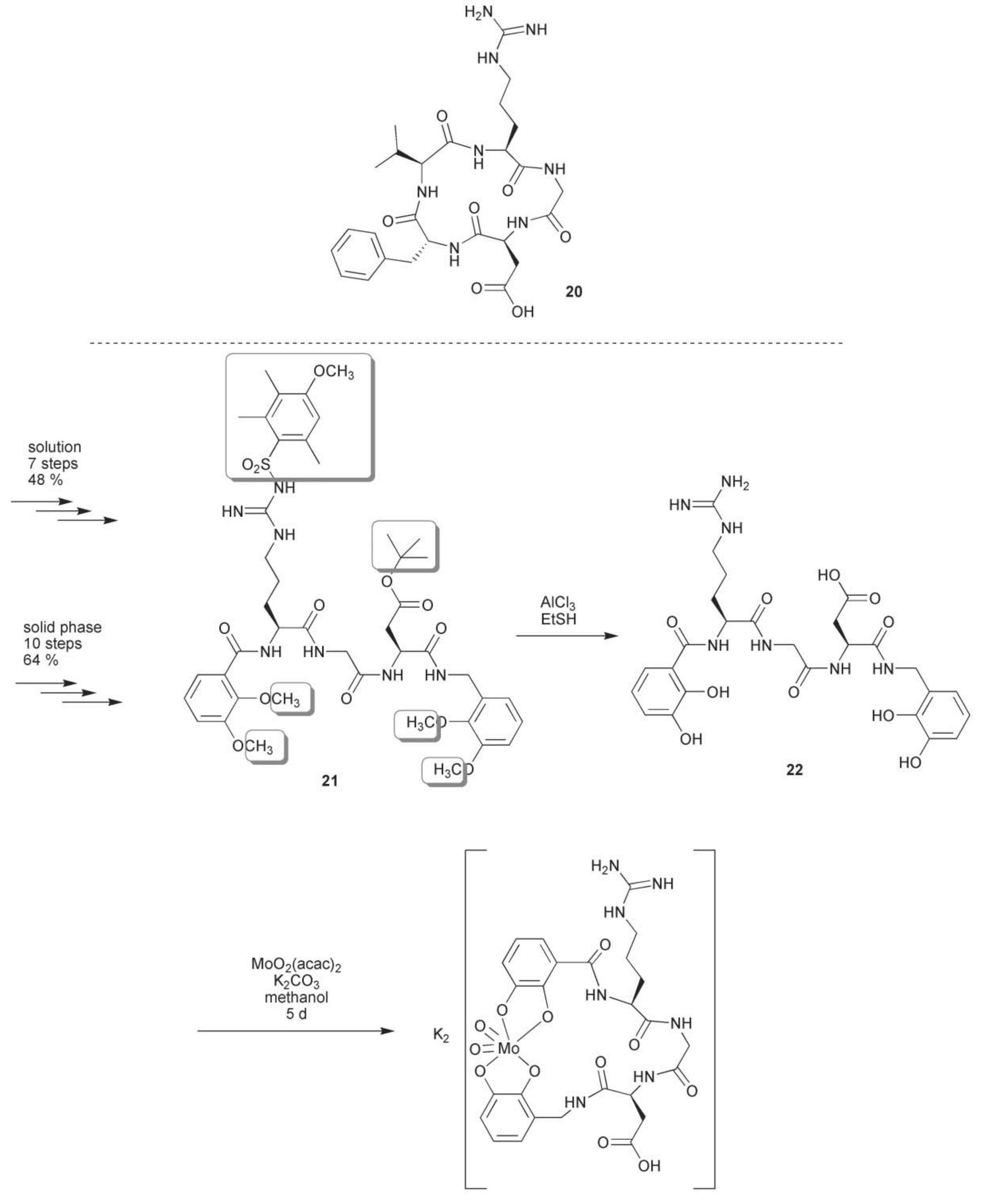


Fig. 19 *Echistatin*, exhibiting the RGD sequence in a loop-type arrangement.

Due to its special properties, the RGD sequence was thoroughly investigated and it could be shown that this tripeptide unit is most active for binding to *e.g.* $\alpha_v\beta_3$ *integrin*, if it is fixed in a loop type conformation.

Kessler *et al.* designed and synthesized a cyclic pentapeptide cyclo-(Arg-Gly-Asp-D-Phe-Val) **20** with high activity in $\alpha_v\beta_3$ binding.[35]

The ligand precursor **21** for the preparation of a metallamacrocycle which bears the RGD-sequence was prepared by solution as well as by solid phase synthesis (see Fig. 20). Hereby the preparation on a solid support again is superior. The procedures are much faster, work up is easier and the yields are better, although more reaction steps have to be performed. All protecting groups of **21** are removed



$K_2[(\mathbf{22})MoO_2]$

Fig. 20 Preparation of an RGD containing metallamacrocycle.

simultaneously by reaction with $AlCl_3$ and ethanethiol. Ligand **22** forms the desired macrocycle $K_2[(\mathbf{22})MoO_2]$ within 5 days in methanol.[33]

In summary, macrocyclic peptide metal complex conjugates which contain conformationally fixed peptide sequences can be obtained by the attachment of ligand units to the termini of the peptides followed by complex formation. In the discussed examples side chain functionalities of the peptides do not influence complex formation significantly. However, if donor groups are present (*e.g.* **19**, **22**) long reaction times are necessary in order to obtain the thermodynamically favored complexes. Probably initial binding of the side chains to the metal occurs by kinetic reaction control.[33]

Spectroscopic investigations reveal that the conformation of the peptides are fixed in the metallacyclopeptides, while in the "free" ligands they adopt a more random coil type structure.

The presented results show an interesting possibility for obtaining rigid peptide structures, which in the future might allow the synthesis of analogous derivatives which are well designed for specific properties.

3.2. Case study B: *Thermolysine*

Thermolysine is a thermostable endopeptidase with a molecular weight of 34600, which was first isolated from the thermophile *Bacillus thermoproteolyticus*. The structure has been determined and was refined to high resolution.[36]

The structure contains seven α-helical domains beside a region dominated by β-sheets (Fig. 21). One of the central helices bears two histidine residues in *i* and *i + 4* position, which bind zinc(II) ions. This metal ion is crucial for the peptidase activity of the enzyme. In addition the binding of zinc stabilizes the α-helical structure.

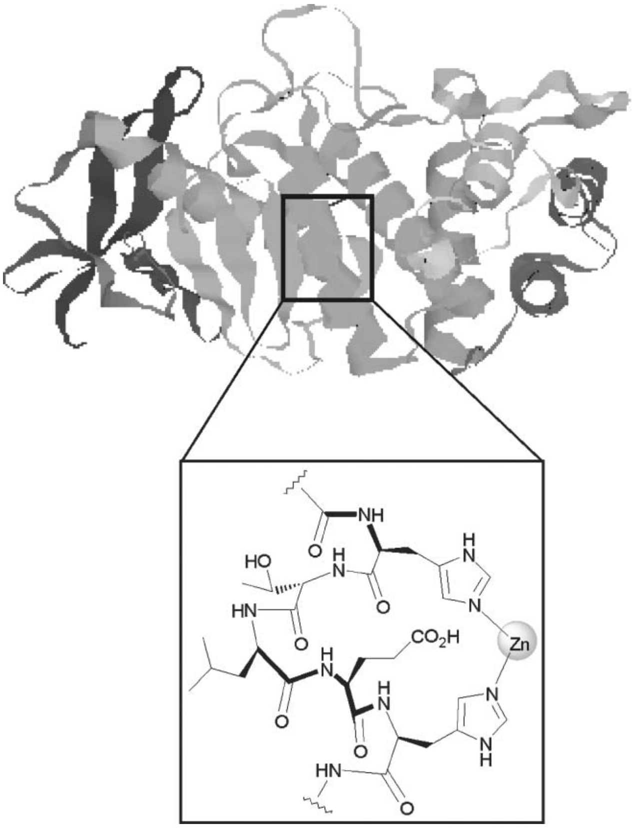


Fig. 21 *Thermolysine*.

Based on the earlier investigations to stabilize α-helical structures by coordination of the Pd(en) fragment to pentapeptides His-XXX-His, which resulted in complexes like **3a** and **3b**, Fairlie *et al.* described an approach, in which the "isolated" central helix of *Thermolysine* is not stabilized by zinc(II), but by "Pd(en)".

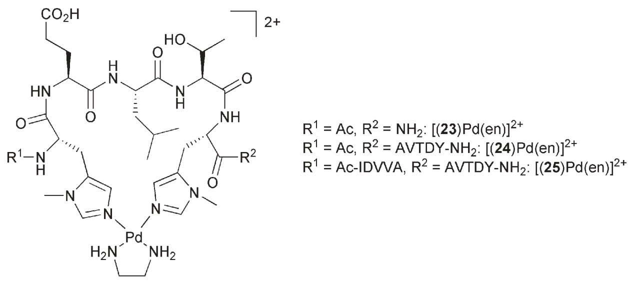
In order to obtain a palladium(II) stabilized α-helix, three peptides acetyl-His(Me)-Glu-Leu-Thr-His(Me)-NH_2 **23**, acetyl-His(Me)-Glu-Leu-Thr-His(Me)-Ala-Val-Thr-Asp-Tyr-NH_2 **24**, and acetyl-Ile-Asp-Val-Val-Ala-His(Me)-Glu-Leu-Thr-His(Me)-Ala-Val-Thr-Asp-Tyr-NH_2 **25** were prepared. All of them resemble parts of the central helix of *Thermolysine* with different lengths. As a variation, side chain methylated histidine was introduced instead of unsubstituted histidine in order to prevent cross linking upon metal coordination.[37]

The linear oligopeptides **23–25** (Fig. 22) adopt random coil structures in solution. Addition of $Pd(en)(NO_3)_2$ to the compounds **23–25** affords the metallacyclopeptides $[(\mathbf{23–25})Pd(en)]^{2+}$.

Thorough NMR investigations with the simple metallacyclopeptide $[(\mathbf{23})Pd(en)]^{2+}$ as well as with the bigger systems $[(\mathbf{24/25})Pd(en)]^{2+}$ indicate, that the smallest derivative adopts the structure of a single α-helical turn. In the bigger ones, the cyclo-(His(Me)-XXX-His(Me)-Pd(en)) unit is able to induce helicity throughout the strand. Helicity in the metallacyclopeptide enables it to act as a template and it is propagated at least through one additional helical turn at either side of the metallacycle.

In this case the simple metallacyclopeptide acts as a helical seed which is amplified throughout the peptide chain. Use of zinc instead of palladium, as it is found in the natural system, does not allow the observation of defined structures in solution. Zinc coordination probably is too labile and thus no defined structures are found.[37]

However, temporary binding of labile metal ions in a big biological system can add some stabilization energy to a given structure and thus might already be sufficient to stabilize this structure. As demonstrated by Fairlie for the α-helix of *Thermolysine* which bears the active unit, artificial systems can be designed that mimic the natural examples.



R^1 = Ac, R^2 = NH_2: $[(\mathbf{23})Pd(en)]^{2+}$
R^1 = Ac, R^2 = AVTDY-NH_2: $[(\mathbf{24})Pd(en)]^{2+}$
R^1 = Ac-IDVVA, R^2 = AVTDY-NH_2: $[(\mathbf{25})Pd(en)]^{2+}$

Fig. 22 Fairlie's *Thermolysine* analogue.

3.3. Case study C: Zinc finger analogues

A natural example of how metal binding to peptides and formation of a metallamacrocycle can affect the biological activity of the protein can be found in the zinc finger motif. The zinc finger proteins adopt a random coil structure, when no metal is present. However, in the presence of zinc(II), the metal coordinates to two cysteine and two histidine residues and induces a β-sheet as well as an α-helix domain. The latter is able to bind to DNA and, *e.g.*, plays a crucial role in transcription factors.[38]

Due to its biological importance the zinc finger motif was intensely studied. Fig. 23 shows a schematic representation of the zinc finger domain with a bound zinc(II) cation. Without this cation the peptide adopts a random coil structure, which does not show any activity. However, upon coordination of the metal distinct peptide secondary structures are induced. Coordination of the metal to two cystein residues induces a β-sheet, which is followed by a turn and an α-helix. The latter is stabilized by binding of two histidine residues to the same metal. In an elegant study Berg compared this motif with different metalloenzyme structures and found that the metal directed formation of the β-sheet can be compared with the one which is observed in *Aspartate Transcarbamoylase* or in *Rubredoxin*. The fixation of the α-helix relates to the one found in *Thermolysine* or *Hemerythrin*. Combination of these binding motifs and addition of a loop-type part, leads to a structure very similar to the zinc finger motif.[39]

From over 100 zinc finger sequences found in data bases, the one with the sequence Lys-Tyr-Ala-**Cys**-Ala-Ala-**Cys**-Ala-Ala-Ala-Phe-Ala-Ala-Lys-Ala-Ala-Leu-Ala-Ala-**His**-Ala-Ala-Ala-**His**-Ala-Lys can be considered as the most minimalistic.

In 1991 a 26 amino acid peptide was designed which possessed in each position the amino acid residue which in the known zinc finger structures is found in this position most. The sequence Pro-Tyr-Lys-Cys-Pro-Glu-Cys-Gly-Lys-Ser-Phe-Ser-Gln-Lys-Ser-Asp-Leu-Val-Lys-His-Gln-Arg-Thr-His-Thr-Gly resulted. This peptide binds cobalt(II) or zinc(II) with dissociation constants of 60 nM or 2 pM, respectively. NMR spectroscopic investigations show, that upon metal coordination the typical “zinc finger” structure is adopted.[39]

Based on the zinc finger motif, Imperiali designed a short peptide which can be used for the sensing of zinc(II). As sensor β-aminoalanine was incorporated into the peptide at position 10 and then was dansylated to exhibit a fluorescing moiety (Fig. 24).

Without zinc this peptide adopts a random coil structure exposing the dansyl group to the polar surrounding and the fluorescence is quenched. Upon zinc(II) coordination the β-sheet/α-helix conformation of the strand is fixed and now the dansyl substituent is buried in an apolar region of the peptide. Fluorescence occurs and can be detected spectroscopically. This sensor is able to detect zinc(II) in a 0.1–1 μM concentration even in the presence of 0.5 M Na^+, 50 mM Mg^{2+}, and 100 μM Co^{2+}.[40]

4. Conclusion

Metal ions are able to bind to peptides. Furthermore, selective binding sites for specific metal ions can be introduced into peptides. If two ligand units are present, metal coordination can lead to the formation of metallamacrocycles. The

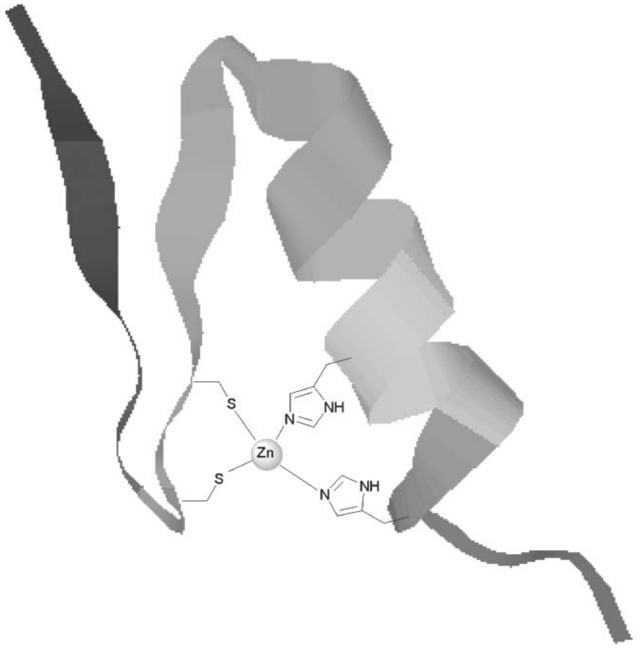


Fig. 23 The zinc finger motif.

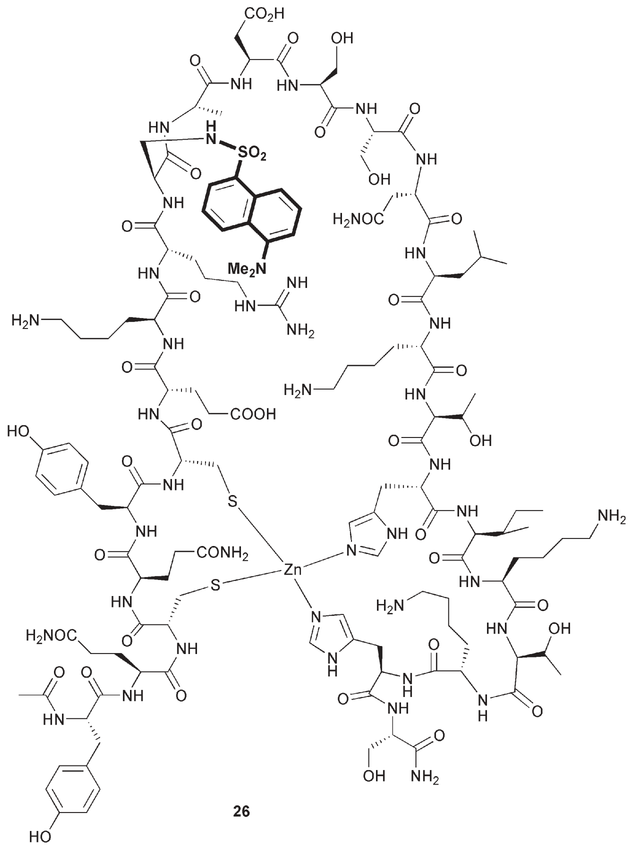


Fig. 24 A zinc finger analogue.

conformational fixation of the peptide then can induce a specific secondary peptide structure, a turn, a sheet, or a helix.

In this paper we presented examples for the formation of metallamacrocycles with well defined conformationally restricted structures. Different ligand units and different metal ions were presented and three case studies were discussed. Hereby the active sites of macrocyclic peptides (*Segetalins A/B*, *Urotensin II*, RGD), the central helix of *Thermolysine*, and the structure of the zinc finger motif were mimicked.

The presented method to use metal coordination as a tool for the conformational fixation of short cyclic peptide structures has the potential for application in bioorganic or medicinal chemistry in the future. However, before such derivatives can be used in binding studies with biological systems, some more knowledge on their properties and structures has to be gained and surely some adjustments of the ligands and/or the metal ions have to be done.

Finally, this work in the future might lead to the preparation of artificial proteins with specific functions by rational design.[41]

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