Peptide/Metal-Ligand Hybrids for the Metal-Assisted Stabilization of Peptide-Microstructures

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Abstract: The incorporation of metal binding sites into peptides is an elegant method for the stabilization of peptide microstructures. In order to do this, first amino acid derivatives have to be synthesized which bear metal ligand moieties like bipyridines, phosphines, or catechols. By standard peptide coupling reactions those building blocks can be incorporated into peptide strands. Examples from the literature show that, depending on the system, different peptide structures are stabilized by addition of metal ions to appropriate artificial peptides. Thus, conformationally fixed α-helix-, β-sheet- or various turn/loop-motifs can be obtained.

1 Introduction

Approximately 20 amino acids are the basis for Mother Nature to develop the fascinating world of living organisms. This means, that the combination of the 20 different moieties not only has to enable different sequences of amino acid residues in more or less linear peptides and proteins, but there has also to be a high structural diversity to obtain all those impressive ‘micro-machines’ (enzymes, proteins …) which can arrange themselves and interact with each other to lead to cells and finally to large living creatures like plants, fish, birds, or even mammals. Many scientists are inspired by this fact to study the fundamental principles, which control the construction and the function of peptides. To focus on the conformation at every single amino acid is as important as the investigation of primary, secondary, or tertiary structures of proteins and the folding mechanisms related to this. Hydrophobicity and hydrophilicity influence protein structures as does π-stacking, electrostatic, or hydrogen bonding interactions. Metal-coordination is also an important motif to fix well defined spatial orientations of peptide strands to each other or control the specific folding of one strand.

The zinc finger (Figure 1) is an impressive example of the influence of metal coordination on protein folding, which occurs in a variety of nucleic acid binding and gene regulatory proteins.

1 Introduction

Figure 1 Representation of the zinc finger domain of the transcription factor IIIA. Amino acids with an R substituent represent relatively variable residues.

Binding of zinc(II) ions by two cysteine and two histidine residues in appropriate positions of a peptide leads to a specific folding. The Cys-AA-AA-Cys sequence (AA represents a relatively variable amino acid residue) induces a short β-sheet structure, which is followed by an α-helix that contains two coordinating histidine residues separated by three amino acids. This example not only shows that metal ions can induce peptide-microstructures...
(which may possess some special properties such as nucleic-acid-binding). It also demonstrates, that nature already has some amino acids available (like histidine or cysteine), that are able to coordinate metals by their side chain functionalities. Asparaginic and glutamic acid, lysine, serine, or tyrosine are further amino acids with coordination ability (Figure 2).

In order to prepare artificial metalloproteins with a metal-stabilized microstructure, we can make use of the pool of 20 amino acids and prepare unnatural peptide sequences with side chain donor functionalities in appropriate positions for metal binding. On the other hand, we can introduce unnatural metal binding sites and attach them to peptide strands. Addition of metal ions now will control or at least strongly influence the folding of the peptide.

Numerous peptide/metal ligand hybrids have been described in the literature and were used for different purposes (e.g. Lewis acid catalysis). However, this review will focus on peptides with two non-natural binding sites for metal ions, which upon metal complexation adopt a well-defined secondary peptide structure.

2 Peptide/Metal Ligand Hybrids

Peptide/metal ligand hybrids may possess a great variety of different binding sites for metals. Actually, they are all based on donor atoms with the most prominent being nitrogen, phosphorus, and oxygen. The following discussion is based on the kind of donor atom present.

Biographical Sketches

Markus Albrecht was born in 1964 and studied Chemistry in Würzburg and Münster (Dr. rer. Nat. with Prof. Gerhard Erker). After one year as a postdoctoral fellow in the laboratories of Prof. Kenneth N. Raymond in Berkeley he moved to the University of Karlsruhe and completed his habilitation in 1997. His research was honoured with the ‘ADUC-Jahrespreis für Habilitanden’ and he was a Heisenberg-fellow of the DFG. Recently he obtained the ‘Landeslehrpreis 2002 des Landes Baden-Württemberg’. Since April 2002 he is Professor of Organic Chemistry at the RWTH-Aachen.

Patrick Stortz was born in 1974 in Oberkirch (Black Forest). He studied Chemistry at the University of Karlsruhe and received his diploma in 2001. In 2002 he moved to the RWTH-Aachen and currently is working on metal-stabilized cyclopeptide-mimetics.

Rainer Nolting was born in 1975 in Bühl (Black Forest) and studied Chemistry in Karlsruhe where he obtained his diploma in 2002. In 2002 he moved to Aachen to work on amino acid bridged ligands for metalloenzyme models.
2.1 Pyridine-Based (and Related) Ligands

sp²-Hybridized nitrogen atoms are extensively used and studied donors for the coordination of metal ions. The zinc finger, which was discussed in the introduction of this article, is a nice example of a protein in which two histidine imidazoles coordinate to zinc. In artificial systems this can be used to stabilize non-natural secondary peptide structures (Figure 3).

Searle et al. described derivative 1, in which the coordination of two histidine units (being residues number 3 and 16 of a heptadecapeptide) bind to Zn(II) and stabilize the β-sheet moiety of a β-hairpin motif. Just recently an approach to 2,2':6',6''-terpyridine and 2,2'-bipyridine substituted tyrosine derivatives was described by Ziessel as shown in Scheme 1.

As a starting material the N- and C-terminal protected iodo-tyrosine 4 was subjected to a Sonogashira–Hagihara Pd/Cu-catalyzed coupling reaction with the terpyridine 5 and bipyridine-substituted alkynes 6 to obtain the terpyridine derivative 7 in 42% yield, while the bipyridine 8 was formed in 57% yield.

Figure 3  A zinc(II) stabilized β-hairpin 1, a Pd(II) stabilized α-helical turn 2 and a bipyridine based β-sheet motif 3 which were used to investigate electron transfer reactions.

Fairlie et al. describe compound 2 in which the two histidine residues of Ac-His-(Ala)₂·His-NH₂ coordinate to the Pd(II)en fragment and a single helical turn of an α-helix is stabilized by this coordination. The structure was elucidated thorough 2D-NMR spectroscopy.

In compound 3 a short β-sheet domain is formed by binding two Val-Val as side chains to one heterocycle of 2,2'-bipyridine (bipy) and this non-natural donor moiety is coordinated to the ruthenium(II) bis(bipyridine) fragment. Coordination of the carboxylate terminus of one of the peptide strands to CoIII(NH₃)₅ allows the investigation of electron transfer along β-sheet peptides.

To allow a systematic study of bipyridine containing peptides and their folding behavior in the presence of metal ions, synthetic strategies for the preparation of unnatural bipyridine based amino acids have to be developed.

2.1.1 Preparation of Bipyridine Amino Acids

Phenylalanine derivatives in which the phenyl group is substituted by pyridine are well known and commercially available. However, bipyridine based amino acids are less common and have to be prepared in order to incorporate them into metal-binding peptides.
The bipyridine derivative 12 bearing an α-methylene amino acid in the 6-position was prepared by asymmetric alkylation of the benzophenone imine of the glycine t-butyester 15 with the alkyl bromide 16 under phase-transfer conditions. (8S,9R)-(−)-N-Benzylchinchinodinium chloride 17, which was introduced by O’Donnell 17 was used as a chiral phase-transfer catalyst. The alkylation proceeds in 83% yield with an ee of 53%. However, after crystallization the protected amino acid derivative 18 is obtained in 99% ee. The amino acid 12 is obtained quantitatively as the HCl adduct by removal of the protecting groups with 6 N HCl (Scheme 2).14

The bipyridine 13 is synthesized following a similar strategy as for the preparation of 12. Alkylation of imine 15 with bipyridine derivative 19 in the presence of 17 as phase-transfer catalyst provided 20 in 85% yield with an ee of 40–66%. However, the acidic hydrolysis of 20 with HCl was followed by addition of methanol and esterification to obtain enantiomerically enriched 21. Chemoenzymatic reaction with alkaline protease resulted in the formation of 13 and its enantiomer as the methyl ester 22 (Scheme 3). 15

Finally, derivative 14 was prepared following Erlenmeyer’s method starting from aldehyde 23 and glycine derivative 24 to obtain 25 (75% yield) followed by reductive cleavage of the aza-lactone with red phosphorus and HI with methanolic work up (77% yield). The obtained racemic ester 26 again is cleaved by alkaline protease to yield amino acid 14 and the methyl ester of its enantiomer 27 (Scheme 4).15

Phenanthroline derivatives were also obtained by kinetic resolution of appropriate amino acid esters with alkaline protease.18 A related derivative is the α,α’-substituted protected amino acid 28. Compound 28 is prepared starting from phenanthroline 29 by oxidation with KMnO4 to obtain a diazafluorenone, which is condensed with benzyl amine. The imine 30 is the starting material for the preparation of 28.19

As shown in Scheme 5, the imine 30 is deprotonated by reaction with NaHMDS and the resulting anion 31, described by the two different mesomorphic forms, is trapped by reaction of CIC(=O)OCH2Ph. Thus, benzyl ester 28 is
formed in good yields by C–C coupling on the 5-membered ring. Protecting group transformations can be performed on 28 and it is possible to introduce the amino acid into peptides. However, due to the constraints introduced by the 5-membered ring, the donor ability of the ‘bipyridine moiety’ should not be as good as in unstrained derivatives.\(^a\)

Besides the \(\alpha\)-amino acid bipyridine derivatives, other similar compounds \(32a, b, 33\) can be made, which bear the amino and the carboxylate units largely separated.

For the preparation of the bipyridino-amino acid \(32a\), first the pyridine building blocks \(35\) (from \(34\)) and \(37\) (from \(36\)) have to be synthesized (Scheme 6). The C–C coupling step is realized by a Stille reaction between triflate \(35\) and tin reagent \(37\) to afford bipyridine \(38\) in 73\% yield. Final reduction of the nitro moiety leads to the methyl ester of the amino acid \(32a\).\(^b\) Alternatively the ethyl ester of the acid \(32b\) can be prepared by desymmetrization of diester \(39\) by successive reaction with hydrazinehydrate, aqueous \(\text{NaNO}_2\) and \(\text{HCl}\), with ethanol in refluxing xylene to obtain \(40\) in 66\% yield. The urethane moiety of \(40\) is cleaved by reaction with \(\text{NaOH}\) and acidification with \(\text{HCl}\) affords \(32b\) in 89\%\(^c\).

4,4′-Dimethyl-2,2′-bipyridine (\(41\)) is the starting material for the preparation of amino acid \(33\). First the two methyl groups have to be successively oxidized to obtain \(42\) bearing one carboxylic acid and one aldehyde functionality. The aldehyde is transformed into an amino methyl unit by condensation with hydroxylamine followed by reduction (\(\text{Pd/C, H}_2\)) to afford \(33\) (Scheme 7).\(^d\)

As can be seen from the examples presented, a series of unnatural amino acids with bipyridine metal-binding sites can be prepared. Incorporation of those into peptide strands should allow the binding of the strands to metal ions.

### 2.1.2 Preparation of Peptide Derivatives and their Metal Complexes

The bipyridine/amino acid derivatives obtained now can be assembled onto peptide strands and the coordination chemistry of the ligand-bearing peptides can be investigated with respect to the stabilization of well-defined peptide structures.

A well-established procedure for the preparation of peptides is solid-phase synthesis which was introduced by Merrifield in 1963.\(^2\)\(^d\) This is a superior synthetic method for the preparation of long peptides, which bear ligand units. Actually, for short peptides the solution phase synthesis also might be appropriate.\(^\)\(^e\)

The bipyridine \(33\) is incorporated into an unnatural linear peptide-type derivative by solid-phase synthesis using the methylbenzhydrylamine resin (MBHA) and successive coupling and deprotection with Boc protected building blocks. Thus, \(33\) is attached to the strand by the use of DMAP, benzotriazol-1-yloxy-tris(pyrrolidino)phosphonium salt (pyBop), \(N\)-methylmorpholine (NMM) and 1-hydroxybenzotriazole (HOBT) as coupling reagents. The resin bound linear strand \(43\) is cleaved with \(\text{HF}\) to obtain the random coil peptide \(44\) in 97\% yield. Cleavage of \(44\) by the \([\text{Ru(bipy)}]\)\(^2\) complex fragment leads to structural fixation and coordination compound \(45\) is obtained (Scheme 8). The only fragment of stereochemical information is introduced by the alanine residue, therefore no significant chiral induction takes place at the metal complex unit and four major and two minor isomers (\(\Lambda\) versus \(\Delta\) and meridional versus facial) are observed.\(^f\)

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\(^{a}\) Merrifield in 1963.\(^\)\(^d\) This is a superior synthetic method for the preparation of long peptides, which bear ligand units. Actually, for short peptides the solution phase synthesis also might be appropriate.\(^\)\(^e\)

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**Scheme 5**

**Scheme 6**

**Scheme 7**
Kelly uses the bipyridine building-blocks 46a, b for incorporation of the metal-binding site into a peptide strand as shown in Scheme 9. Compounds 46 are easily prepared from 6,6'-diamino-2,2'-bipyridine (47) by reaction with one equivalent of 2,2'-dimethyl-malonic monoethyl ester to yield unsymmetric 48 in 40% yield. Coupling of the remaining free amine 48 with N-Boc protected amino acids followed by cleavage of the ethyl ester afforded 46a and 46b in 42% and 80% yield, respectively.24

Peptide synthesis is performed using the MBHA resin to obtain peptide 49, which in solution adopts a random coil structure. Upon addition of Cu(II) salts, square planar copper complexes 50 are formed in which the copper coordination enforces a cis-orientation of the peptide strands attached to the bipyridine and a β-sheet secondary structure is induced. The Cu(II) coordination chemistry was thoroughly investigated by using simple bipyridine model compounds as ligands.24

Metal coordination can be used to support some structural preference, which already is embedded in the peptide strand but due to low stabilizing energies is not adopted. Imperiali investigated short linear peptides, which possess an internal sequence that preferentially forms a turn structure. Additionally, the turn structure is stabilized by coordination of metals to the bipyridines at the termini of the peptide.16,18,25

The hexapeptides 51a and 51b were prepared using an automated solid-phase peptide synthesizer with Fmoc-PAL-PEG-PS resin and either benzotriazol-1-yloxy-tris(dimethylamino)phosphonium salt (Bop), NMM, HOBT or diisopropycarbodiimide (DIC), HOBT, or Hüning base as coupling reagents. The proline-D-amino acid dipeptide 51a, b is able to adopt a turn structure and this structure is stabilized by binding of metal ions to the bipyridine units (Figure 5). However, the complexation constants KD of Zn(II) as well as Co(II) complexes show that in 51a the turn structure is more stabilized than in 51b.19,25

Figure 5 Stabilization of a turn structure.
The same principle of ‘turn-stabilization’ was used in the case of compound 52 shown in Figure 6, which was prepared by solid-phase synthesis, incorporating the bipyridine 32 into the peptide. Here, fluorescence of the anthracene moiety is quenched upon complex formation and stabilization of the turn structure. In the corresponding uncomplexed ‘random coil’ peptide fluorescence occurs.16

In a series of papers, bipyridines were attached to the termini of specific linear peptides, which adopt a random coil structure. Upon coordination to metals, formation of a triple-helix bundle is induced by attractive interaction of hydrophobic faces of the helices and template formation by the metal.26–32 In Figure 7 an example from Ghadiri et al. 53 is depicted. The ‘helical wheel’ shows the presence of a hydrophillic (Gln, Glu and Lys residues) and a hydrophobic (Ala and Leu residues) face to the helix.30

When a trisbipyridine complex of ligands like 53 is formed with Fe(II), Co(III), Ru(II), or Ni(II) first a random coil structure is obtained as an intermediate which by weak hydrophobic/hydrophobic interactions rearranges to form the triple-helix bundle (Figure 7).30

Shepartz et al. prepared terpyridine substituted peptides that dimerize upon addition of Fe(II) ions and act as metallo-zipper models of bZIP proteins and bind to DNA.33,34

2.2 Phosphane-Based Ligands

Serine 54 was introduced for the incorporation of phosphane ligands into peptides and amino acids.35 However, to prevent oxidation of the phosphane unit, the corresponding sulfide 55 can be used for synthesis and at the end of the sequence the sulfur is removed by reduction with Raney-Ni.36

Compound 55 was prepared from acrolein 56, which was reacted with diphenylphosphane in the presence of base and the appropriate phosphane. After oxidation by Na2S2O3 57 is obtained in 96% yield. An oxazolidinone moiety is attached as chiral auxiliary to furnish 58 in 84% yield and an azide is introduced by use of trisylazide (TrisN3). After separation of the diasteromers, 59 is obtained as the major isomer in 85%. Finally the chiral auxiliary is removed again and the amine is protected with Fmoc to obtain the protected phosphino serine 55 in 83% yield. The building block 55 is inserted into the peptide 60 by solid-phase synthesis on Wang resin using Fmoc-coupling strategies. In the final step the sulfur is removed by reaction with Raney-Ni to obtain peptide 61. Upon addition of a precursor for Rh(I) ions, chelation takes place and the peptide is fixed in an α-helical structure 62 (Scheme 10).35

If the reduction of the sulfide and the metal complexation is performed with the peptide still attached to the resin, a solid-phase bound hydrogenation catalyst is obtained, however, only poor yield low ee’s resulted from catalysis.37

Expanding this concept, the resin bound peptide 63 (Figure 8) was found as a component of a combinatorial library and with Pd(II) resulted in ee’s > 60% in the asymmetric addition of dimethylmalonate to cyclopentenyl acetate.36

Figure 6 A metal stabilized peptide turn structure, which can be used for metal sensing by fluorescence quenching.

Figure 7 Triple-helix bundles induced by metal coordination in combination with hydrophobic interactions.

Figure 8
2.3 Catechol-Based Ligands

Besides nitrogen- or phosphorous-based ligands it is also possible to use oxygen donors for the metal-directed stabilization of peptide-microstructures. In nature, the siderophores are a prominent class of metal binding compounds, which are based on amino acids and on oxygen donors. The siderophores are responsible for the take up and transport of metal ions through membranes.\(^\text{39}\) Mainly catecholates, hydroxamates, and hydroxyacids are found as the ligand units.\(^\text{40}\) In Enterobactin (Figure 9) Fe(III) can be effectively encapsulated by three well preorganized catecholate units which are attached to a macrocyclic trilactone L-serine backbone.\(^\text{41}\)

The siderophores will not be the focus of this article and in the next two chapters (2.3.1 / 2.3.2) we will discuss ‘unnatural’ amino acid or peptide/catechol hybrids, which were investigated by us.

2.3.1 Amino Acid-Bridged Dicatechol Ligands

Simple catechol units can be attached to the N- as well as to the C-terminus of amino acids or peptides by use of 2,3-dimethoxybenzoic acid (\(\text{64}\)) and 2,3-dimethoxybenzylamine (\(\text{65}\)) as terminal blocking agents (Scheme 11). After cleavage of the aryl-methyl ethers by standard procedures the catechol ligands are liberated.
Starting from 64, a typical peptide coupling procedure with DCC and N-hydroxysuccinimide (NHS) in dioxane leads to the glycine and alanine derivatives 66a and 66b in 81% or 87% yield, respectively. The coupling step is repeated and the amine 65 is attached to the C-terminus to yield 67a and 67b in 77% and 73% yield respectively.42

Due to solubility problems, the same coupling conditions cannot be applied to the more hydrophobic amino acids phenyl alanine, valine and leucine. Here HOBt and -(N-methyl)hydroxybenzotriazole-N,N,N',N'-tetramethyluronium-salt (HBTU) as coupling agent. This would call for DMF as solvent but acetonitrile proved to be a better alternative. This way, epimerization of the amino acids is avoided and solubility problems reduced to a minimum. Via this method it is possible to carry out coupling reactions at room temperature, with a simple work-up and high yields (> 90%).44

Metal complexes of the amino acid derivatives 68 can be obtained by reaction with Ti(IV) salt in the presence of base. Elemental analysis, ESI-MS and X-ray structural analysis show, that dinuclear coordination compounds 69 are formed, in which two metal centers are bridged by two ligands 68 and two coligands (methoxy or hydroxyl).45,46

Figure 11 shows as a representative example, the solid state structure of the dianionic alanyl bridged complex 69b, in which hydroxyl groups act as coligands.

In solution the chemistry of the complexes is quite complicated. Due to the directionality of the ligands 68 a parallel and an anti-parallel orientation of the two ligands can be adopted. Additionally, the ligands can wrap around the metals, forming a double-stranded helix with both metal centers similarly configured, or both metals possess opposite configuration and a non-helical structure is obtained. This, in combination with the chirality at the ligands, leads to seven different isomers, which indeed can be observed in the mixture of complexes formed under kinetic reaction conditions. However, leaving a solution of the isomeric complexes for some days at room temperature resulted in the formation of the thermodynamically favored major isomer. A combination of investigations using NMR spectroscopy, optical rotation, PM3 calculations, and conformational analysis following Ramachandran method47 shows that the favored isomer is the one in which both ligands 68 are orientated parallel with the N-terminus binding to a Λ-configured and the C-terminus to a Δ-configurated metal complex moiety. Only in this isomer, the configuration at the metal allows a ‘relaxed’ right-handed twist at the amino acids.45,46

Recent investigations show that it is possible to exchange the coligands of the complexes by other (maybe more reactive or functionalized) ones. Those ligands are fixed in a chiral pocket as is found in metallo-enzymes and it is hoped that a ‘functional’ metallo-enzyme-model can be developed based on ligands 68 and the complexes 69.43,44

Scheme 11

Due to solubility problems, the same coupling conditions cannot be applied to the more hydrophobic amino acids phenyl alanine, valine and leucine. Here HOBt and N-3-dimethylaminopropyl-N'-ethylcarbodiimide (EDC) in DMF have to be used as coupling reagents to obtain compounds 67c–e in 46% (c), 61% (d), or 59% (e) yield over two steps.42

The methyl ethers of 67a–e are cleaved in nearly quantitative yield by use of BBr3 to obtain derivatives 68 with free catechol units.42

Similarly the side-chain functionalized lysine derivative 68f and compound 68g (with an unnatural pyridyl amino acid), can be prepared by HOBt/EDC coupling in DMF followed by BBr3 deprotection.43

For the preparation of side-chain functionalized compounds 68h and 68i (Figure 10) different coupling conditions proved to be more appropriate, 1-

Figure 10

Figure 11

X-ray structure of the dianionic Ti(IV) complex 69b with two OH-coligands (hydrogen atoms are omitted for clarity).
2.3.2 Peptide Bridged Dicatechol Ligands

The synthesis of simple peptide bridged dicatechol derivatives 70a–e proceeds very similarly to that described for the amino acid derivatives 68. However, if the desired peptide is not commercially available (or is too expensive), the compounds have to be prepared in a repetitive synthesis as shown in Scheme 12, 70f as a representative example.48

Scheme 12

The benzoic acid 64 is activated with EDC and HOBt and the dipeptide phenylalanyl-leucine is attached to obtain 71 which is not isolated but the procedure is repeated to obtain the tetrapeptide 72. Compound 72 again is used without purification and the benzylamine 65 is attached to the C-terminus and the methyl ethers are cleaved by reaction with BBr₃. The dicatechol 70f is obtained on a half-gram scale in 35% yield over four steps.48

In coordination studies the peptide-bridged derivatives 70 can lead to different types of metal complexes.

On the one hand the peptide moieties can adopt linear – ‘stretched’ – orientations in which a β-sheet-type structure is found. Here the ligands are well predisposed to act as bridging units between metal centers. On the other hand, both ligand units can bind to only one metal center and a loop- or turn-type structure is enforced at the peptide (Figure 11). If the sterically constrained Val-Val derivative 70b is reacted with Ti(IV) ions, a triple-stranded helicate-type complex is observed (first a mixture of isomers which transforms into only one thermodynamically favored species after two weeks in solution). The sterically highly demanding valine units prefer a stretched β-sheet structure and therefore the formation of a triple-stranded complex 73 (Figure 13) is favored.49

Figure 13

Peptides like alanyl-leucine or valyl-valyl-valine are less sterically demanding than valyl-valine. Therefore, those do not to adopt the linear (‘β-sheet’) arrangement but also can ‘bend around’ to bind with both ligand units to one metal center. This leads in coordination studies with ligands 70a–e and Ti(IV) ions to mixtures containing ‘β-sheet-type’ and ‘loop-type’ conformations of the peptides. However, by performing a coordination study of ligand 70d with Ti(IV) in the presence of catechol, the complex 74 can be observed as the major (but not only) product. By metal coordination of the ligand units the peptidic part of 74 is forced to adopt a turn- or loop-structure.49

Even more impressive is the complex formation of 70d (Figure 13) with cis-molybdenum(VI)dioxide. The metallacyclopeptide 75 is isolated as the only product, as a single stereoisomer. Cyclopeptides like 75 are obtained from ligands 70a–e with [MoVI O₂]²⁺.49

The formation of metallacyclopeptides by simple complexation of linear ligand substituted ‘random coil’ peptides to metal complexes opens up a way to stabilize
biologically active loop-type peptide structures in a simple but very effective way.

Segetalins were chosen as a model system to stabilize a naturally occurring biologically active cyclopeptide. The Segetalins A and B (shown in Figure 14) are cyclohexa- or cyclopentapeptides, respectively, which are found in the seeds of Vaccaria segetalis (Caryophyllacea) and were shown to possess an oestrogene-like activity. The seeds of Vaccaria segetalis are used in Chinese folk medicine to activate blood flow and promote milk secretion, and to treat amenorrhea and breast infections.50–52

![Figure 14](image)

**Figure 14** The Segetalins A and B.

Both, Segetalin A and B possess the Trp-Ala-Gly-Val sequence which is fixed in a loop-type structure. Therefore it is expected that this part of the macrocycle is responsible for the biological activity. However, NMR spectroscopic studies show that the vanyl isopropyl group of Segetalin A and B are orientated in different spatial directions.50–52 This implies that only the tripeptide Trp-Ala-Gly is biologically active.53 The dicatehol derivative 76, which bears the Trp-Ala-Gly sequence can be prepared easily by solution peptide coupling procedures following the Fmoc-strategy (Scheme 13).54

Starting with amine 65, successively the Fmoc-protected amino acids glycine, alanine and tryptophane (with Boc protection at the side chain heterocycle) are attached using HBTU as coupling reagent. The Fmoc groups are removed by reaction with piperidine. Finally, benzoic acid 64 is attached to the N-terminus and the methyl ethers and the Boc group are cleaved with BBr_3 to obtain the peptide-bridged ligand 76 in 40% yield over eight steps. Complexation with cis-molybdenum(VI) dioxide smoothly leads to metallacyclopeptide 77, in which the biologically active front of Segetalins A/B is fixed in a loop type fashion. The conformation of this loop is close to the biologically active one.54

The preparation of the analogous tetrapeptide Trp-Ala-Gly-Val bridged 78 proved to be not as simple. Solubility problems during the synthesis forced a different strategy. Here the preparation of the ligand precursor 79 has to be performed by solid-phase synthesis.55

Use of the arylhydrazide linker 79 shown in Scheme 14 is an elegant way to build up the tetrapeptide at the solid-phase and to couple the N-terminal ligand unit upon cleavage from the solid support.56 Reaction of 80 with Cu(II) acetate and oxygen oxidizes the hydrazide unit to yield the intermediate 81 which immediately is attacked by the benzylamine 65.

After cleavage of the methyl ethers the ligand 78 is obtained. Preliminary coordination studies with 78 and cis-
molybdenum(VI)dioxide show the formation of a metal-lacyclopeptide which is isolated in 58% yield.  

3 Miscellaneous

There are many more examples in the literature in which metals influence the structure at peptides. However, herein we only wanted to give an overview on systems where binding sites from the ‘classical’ coordination chemistry are combined with amino acids and peptides.

Further classical ligand units for metal complexation would be crown ethers or EDTA. Some examples are depicted in Figure 15. In 1990 Hopkins described peptides which contain bis-acetylated amines on the side chains and which are able to bind to metals and thus stabilize an α-helical structure at the peptide. Hereby the relation between the distance of the amino acid residues and the length of the side chain is important for effective formation of the helix.

Coupling ferrocenyl bis(aminoc acid) with 7-azabenzotriazol-1-yl-oxy-tris(pyrrolidino)phosphonium salt (pyAop) leads to the formation of metal containing cyclopeptides and . The yield of the different oligomers is highly dependent on the conditions of the coupling reaction and can be up to 75% for and 68% for and (ratio of 3:1).

Shanzer described a series of tripodal helical iron-binders, which possess amino acid residues in the spacer. In the impressive example two different binding sites are incorporated which enable the ‘moving’ of a metal ion between those sites depending on its redox state.

4 Conclusion

In this article it is shown that metal coordination is a very effective tool to induce well-defined conformations at amino acids or at peptides. The stabilization of peptide microstructures is important because this might help more active and/or selective secondary structures at peptides compared to those at the ‘non-fixed’ ‘metal-free’ linear peptides to be obtained. The most important structural features of peptides are α-helices, β-sheets, and various turns and loops. As it is described in this article, starting with a random coil peptide strand we are able to induce all three of those important kinds of structures (Figure 16).

For the stabilization of α-helices, the amino acid residues, which bear ligand moieties have to be separated by three amino acid residues, so that one turn of the helix is stabilized by metal coordination and induces the helical structure of the chain.

Figure 15

Figure 16
β-Sheet structures are stabilized, if the ligating moieties are located in appropriate positions of a long linear peptide chain and an additional 'turn motif' helps to bring those moieties close to each other.

Turn- or loop-type structures are induced if chelating units are attached to short peptides, which are able to ‘bend around’ and to bind with both ligand moieties to only one metal ion.

The use of metal ions to build up big non-covalently linked architectures is already a well-studied field of supramolecular chemistry. However, the combination of metal coordination and secondary peptide structures opens up a way to develop new compounds with novel material properties or exciting biological activities.

The exploration of metal ligand/peptide hybrids and their coordination compounds just started but we expect it to have a bright future.

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