β-Peptidic Secondary Structures Fortified and Enforced by Zn^{2+} Complexation – On the Way to β-Peptidic Zinc Fingers?

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Dedicated to Professor *Dieter Enders* on the occasion of his 60th birthday

The correlation between β^2-, β^3-, and $\beta^{2,3}$-amino acid-residue configuration and stability of helix and hairpin-turn secondary structures of peptides consisting of homologated proteinogenic amino acids is analyzed (*Figs. 1–3*). To test the power of Zn^{2+} ions in fortifying and/or enforcing secondary structures of β-peptides, a β-decapeptide, **1**, four β-octapeptides, **2**–**5**, and a β-hexadecapeptide, **10**, have been devised and synthesized. The design was such that the peptides would *a*) fold to a *14*-helix (**1** and **3**) or a hairpin turn (**2** and **4**), or form neither of these two secondary structures (*i.e.*, **5**), and *b*) carry the side chains of cysteine and histidine in positions, which will allow Zn^{2+} ions to use their extraordinary affinity for RS^- and the imidazole N-atoms for stabilizing or destabilizing the intrinsic secondary structures of the peptides. The β-hexadecapeptide **10** was designed to *a*) fold to a turn, to which a *14*-helical structure is attached through a β-dipeptide spacer, and *b*) contain two cysteine and two histidine side chains for Zn complexation, in order to possibly mimic a Zn-finger motif. While CD spectra (*Figs. 6–8* and *17*) and ESI mass spectra (*Figs. 9* and *18*) are compatible with the expected effects of Zn^{2+} ions in all cases, it was shown by detailed NMR analyses of three of the peptides, *i.e.*, **2**, **3**, **5**, in the absence and presence of $ZnCl_2$, that *i*) β-peptide **2** forms a hairpin turn in H_2O, even without Zn complexation to the terminal β^3hHis and β^3hCys side chains (*Fig. 11*), *ii*) β-peptide **3**, which is present as a *14*-helix in MeOH, is forced to a hairpin-turn structure by Zn complexation in H_2O (*Fig. 12*), and *iii*) β-peptide **5** is poorly ordered in CD_3OH (*Fig. 13*) and in H_2O (*Fig. 14*), with far-remote β^3hCys and β^3hHis residues, and has a distorted turn structure in the presence of Zn^{2+} ions in H_2O, with proximate terminal Cys and His side chains (*Fig. 15*).

1. Introduction. – The functional properties of proteins depend upon their three-dimensional structures, which arise because particular sequences of amino acids in polypeptides fold to generate, from linear chains, compact three-dimensional

[1]) Part of the Ph.D. thesis of *G. L.*, ETH-Zürich, Dissertation No. 15429 (2004).
[2]) Part of the projected Ph.D. theses of *R. M.* and of *A. W.*, ETH-Zürich.
[3]) Postdoctoral Fellowship at ETH 2004/2005, financed by the *Deutsche Forschungsgemeinschaft* and *Swiss National Science Foundation* (Project No. 200020-109065).
[4]) On leave from *Pfizer*, R & D, Nerviano (MI), Italy, 2002/2003.
[5]) Postdoctoral Fellowship, ETH-Zürich, 2002–2004, financed by *Swiss National Science Foundation* (Project No. 200020-100182).

domains[6]). The folded domains can serve as modules for building up large assemblies (quaternary structures) such as virus particles or muscle fibres, or they can provide specific catalytic or binding sites, as found in enzymes or proteins that carry oxygen (*e.g.*, haemoglobin) or that regulate the function of DNA (*e.g.*, transcription factors). It is noteworthy that proteins, as a class of macromolecules, are unique in being able to recognize and interact with most diverse molecules.

At present, only proteins and nucleic acids are known to fold to specific, compact tertiary structures, inspiring many scientists to study the fundamental principles that control the construction and function of peptides. *De novo* protein design offers an excellent way to study the fundamental forces that cause protein folding and protein–protein interactions [1–3]. In addition, the increasing rate of protein–structure determination[7]) is providing insight into features that stabilize the three-dimensional structures of proteins. As a consequence of this better understanding, there is additional interest in the design of unnatural, bio-inspired polymers that fold to predictable tertiary structures with the goal of mimicking 'α-proteins', to some extent [4–9]. In the quest to understand, control, and mimic the actions of proteins, many chemists have first turned to the more basic idea of mimicking secondary structures. Unnatural oligomers that can fold into compact, well-ordered structures might allow chemists to not only duplicate nature by developing functional macromolecules, but also to transcend nature by making molecules with 'superior' properties. These compounds can offer novel structures, improved bio-availability, and greater thermodynamic stability[8]). In this respect, β-peptides are among the most thoroughly characterized type of unnatural oligomers, which have been developed within the last decade. β-Peptides have particular appeal for extending our understanding of protein structure and stabilization into the realm of folded, abiological polymers, since β-amino acids represent the smallest step away from α-amino acids in the backbone unit. As a result of the enhanced structural diversity of β-amino acids, the resulting peptides have been found to fold into a wider variety of secondary structures that range from helical to pleated sheet or turn conformations, adopting more stable motifs in comparison with their α-counterparts [5]. While potentially pharmaceutically useful β-peptides are likely to be short and of low molecular weight [11], the natural expansion of our fundamental research on the folding and function of β-peptides is to assemble larger sequences capable of forming tertiary structures, *i.e.*, 'β-proteins'.

Albeit natural proteins typically require >100 residues to display stable tertiary structures, the careful choice of α-amino acids has permitted the construction of tertiary-structured oligomers with as few as 23 amino acid residues [12]. Recent advances in solid-phase peptide synthesis (SPPS) have allowed the preparation of long-chain β-oligopeptides of up to 17 residues by automated SPPS [13] and 24 residues by the manual method [14], and have enabled the successful assembly of β-peptides containing all β-

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[6]) In other words, protein tertiary structures derive from the assembly of elements of secondary structure (helices, sheets, and turns) through control of the noncovalent forces that govern folding and self-assembly processes (*i.e.*, electrostatic, hydrogen, and *Van der Waals* bonds).

[7]) Over the past 30 years, the structures of thousands of proteins have been solved, and the sequences of more than 500,000 have been determined!

[8]) For reviews on synthetic, unnatural mimics of natural protein secondary structures, see [5–10].

amino acids with 'proteinogenic' side chains [15–17]. Moreover, the recent success in achieving the 'native chemical ligation' of two unprotected β-peptide strands [18] has paved the way towards this goal. The first step toward creation of a specific tertiary structure with β-amino acid oligomers has been achieved by *Gellman* and co-workers [19], in synthesizing an amphiphilic 3_{14} helix that undergoes self-association in aqueous solution to form tetrameric and hexameric aggregates[9]). Similarly, *Seebach* and co-workers [18a] have described the aggregation behavior of a β-heneicosapeptide that could, in principle, also fold into an amphiphilic 3_{14}-helical structure[10]). By a parallel approach, *Cheng* and *DeGrado* [22] synthesised a two-helix bundle derived from β-amino acids, in which the helices are held together through a disulfide bond by long-range tertiary contacts (*i.e.*, hydrophobic interactions and salt bridges). As an extension to this concept, *Diederichsen* and co-workers synthesized β-peptides containing nucleobase recognition units in the side chains [23–26], designed to fold into a 3_{14}-helical structure. The nucleobase pairing can organize these β-peptide helices, gaining more control over the geometry, stoichiometry, and specificity of self-association [27][28][11]).

We have decided to approach the goal of generating tertiary structures from a different point of view. Many natural proteins have recruited metal ions as intrinsic parts of their structures. Metal ions serve a variety of functions in proteins, the most important of which is to enhance the structural stability of the protein in the conformation required for biological function and/or to take part in the catalytic processes of enzymes. Among the possible metal ions, we envisaged the use of Zn^{2+} ions for the complexation of histidine- and cysteine-containing β-oligopeptides, with the ultimate goal of mimicking the structure of the so-called $\beta\beta\alpha$-Zn-finger proteins, a class of transcription factors that function by recognition of specific DNA sequences.

Among the numerous possible approaches to induce and reinforce intramolecular interactions, the use of a metal ion capable of binding to certain functional groups in a peptide is interesting for at least two reasons: *a*) ion complexation is commonly used in nature to stabilize protein structures, and *b*) it takes the correct positioning of only a few amino acids in order to reinforce secondary structures such as a helix or a turn by metal-ion binding. In the world of α-peptides, two histidines in positions i and $i+4$, at the C-terminus as well as in the middle of a short peptide, can induce nucleation and stabilization of a helical conformation upon addition of metal ions such as Zn^{2+}, Cd^{2+}, Cu^{2+}, Pd^{2+}, and Ni^{2+} [30–37]. A similar approach can be used to stabilize turn secondary structures. *Searle* and co-workers [38] synthesized short α-peptides with a proline residue to induce a reverse turn, and two or three histidines for metal binding

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[9]) Pioneering work by *DeGrado* and co-workers [1][2][20], and *Mutter* and co-workers [21] has shown that identifying conventional peptides (α-amino acid residues) that form amphiphilic helices and self-associate in small clusters constitutes the initial phase in a 'hierarchic' design strategy for helix-bundle tertiary structure.

[10]) To date, β-peptides containing up to 20 amino acid residues have been structurally characterized [16].

[11]) It should be noted that *Zuckermann* and co-workers [29] identified amphiphilic peptoid sequences that can bind a small dye molecule, forming a noncovalent self-assembling macromolecular structure and thus providing evidence that it is not only β-peptides that could make promising candidates for mimicking nature.

on both strands near the N- and C-termini. They found a substantial stabilizing effect of the hairpin structure by complexation with Zn^{2+} ions[12]).

To the best of our knowledge, no example of a β-peptidic secondary structure, stabilized by metal-ion complexation is so far known. In contrast, it has been established that the 3_{14}-helical conformation of β-peptides can be stabilized by a disulfide bridge [42][43], by salt bridges between ornithine, arginine or lysine, and glutamic acid side chains [22][44–46], by the insertion of pre-organized β-amino acids [6][47], and by macrodipole–charge interactions [48]. Less well-explored is the world of the hairpin structures of β-peptides. *Balaram* and co-workers [49–51] prepared mixed α/β-peptides in which a D-proline and a glycine unit induced a so-called reverse turn of two peptide strands. *Gellman* and co-workers used a D-Pro-Xxx linker, where Xxx was 1,2-diamino-1,1-dimethylethyl [52] or α-hydroxy acetate [53], for the construction of mixed α/β-peptides with parallel and antiparallel hairpin structures, respectively. Additionally, they applied a template formed by two nipecotic acid (*i.e.*, β^2-homoproline) units to enforce the formation of a hairpin secondary structure in a mixed α/β- [54] and in a β-tetrapeptide [55–57][13]). Finally, it was found by the *Seebach* group [59][60] that the insertion of a β^2/β^3-amino acid dimer segment into the middle of a β-hexapeptide with two sheet-inducing *u*-$\beta^{2,3}$-amino acid residues on each *terminus* caused the folding to a hairpin in methanol (NMR analysis) and probably also in aqueous solution (CD analysis; *Fig. 1*).

To gather information about the metal-ion complexation of β-peptides we decided to study the influence of a Zn^{2+} ion in stabilizing or destabilizing secondary structures of β-peptides in H_2O, a solvent in which unfolding may occur most easily [44][45][61–64].

2. Design and Synthesis of the β-Peptides for Zn^{2+} Complexation. – To verify the Zn^{2+}-complexing ability in a β-peptidic *14*-helix, we have synthesized [65] the β^3-decapeptide **1** with two β^3hHis residues in the 6- and 9-position, which are in juxtaposition (distance *ca.* 4.8 Å) on the surface of a 3_{14}-helix, and with salt-bridge-forming side chains of Glu and Asp, also in *i* and $i+3$ positions, a feature that was shown to stabilize this helix in aqueos solution [44][45][66] (*Fig. 2*). Indeed, CD measurements clearly indicate enhanced helicity in the presence of Zn^{2+} ions in aqueos buffer solution [65], a helix fortification by complexation.

For Zn^{2+} complexes with turn structure, we have designed four β-octapeptides which would be suitable to test the power of Zn^{2+} complexation and Zn^{2+} bonding to the imidazole unit of the His side chain and S^- of a Cys side chain. The structural features of the β-peptidic *14*-helix, hairpin turn, and pleated sheet are outlined in *Fig. 3*.

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[12]) *Imperiali* and co-workers [39] have inserted unnatural multidentate amino acids, which bear bipyridyl and phenanthryl groups as side chains, in order to tune the affinity towards various metal ions. However, it looks as if metal coordination alone is not sufficient for the folding of the peptide; there must be additional effects, for instance, steric and conformational ones, to stabilize the folded structure [40]. For a review on the metal-assisted stabilization of peptide microstructures by peptide/metal-ligand hybrids, see [41].

[13]) A reverse-turn mimic composed of two β-amino acid residues, (*R*)-nipecotic acid and (*S*)-nipecotic acid, was successfully incorporated by *Raines* and co-workers [58] into the enzyme ribonuclease A (RNase A). The modification increased the conformational stability of the protein, whilst maintaining its full enzymatic activity.

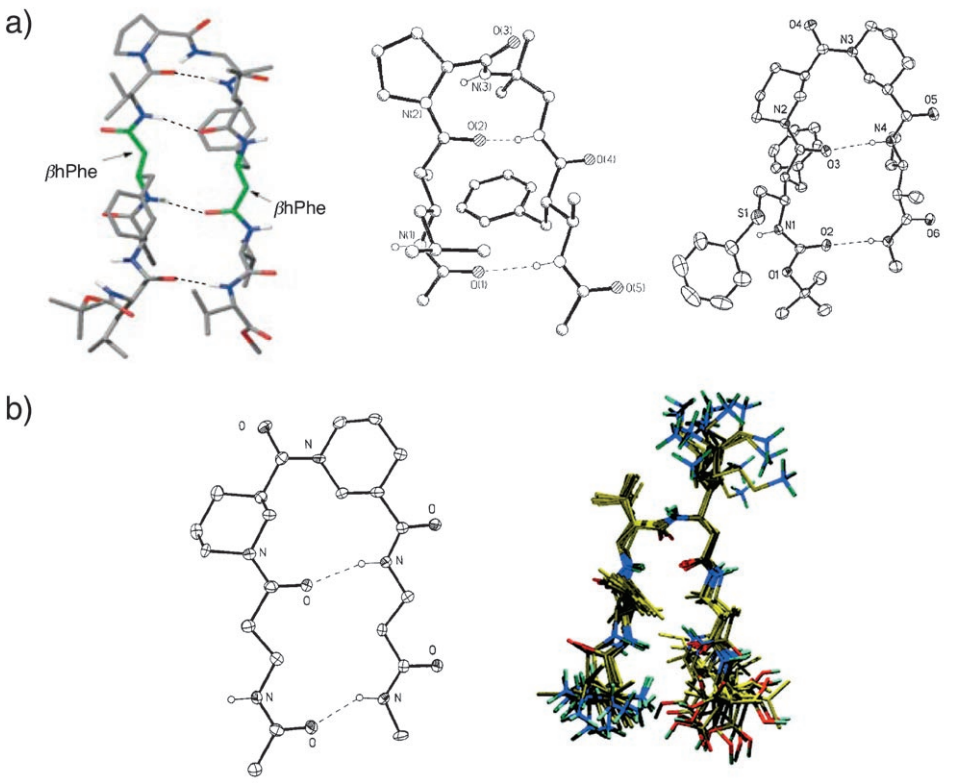


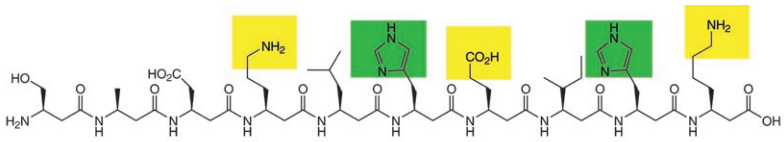
Fig. 1. *X-Ray crystal and NMR solution structures of* a) *mixed α/β-hairpins* [50][52][53] *and* b) *of β-peptide hairpins* [55][56][59]

The spiraling of the β-peptide backbone to the 3_{14}-helix – the alternative *12/10*-helix [67–70] is disregarded here – and/or the folding to a hairpin turn can be prevented by certain substitution patterns of the β-amino-acid residues (*Fig. 3*)[14]).

The octapeptides all contain a central dimer segment of (*S*)-β^2hVal-β^3hLys, which is known to induce formation of a ten-membered H-bonded ring, similar to that of an α-peptidic β_{II}-turn, but which also fits into a *14*-helix. At the N-terminus of the β-octapeptides, we placed a β^3hHis, at the C-terminus a β^3hCys, so that Zn^{2+} ions would be able to force these termini into close proximity for complexing and binding (*Fig. 4*).

The remaining pairs of amino acids βhXaa^2/βhXaa^3 and βhXaa^6/βXaa^7 were chosen such that there would be *i*) a 'natural' [59][60] hairpin turn in the absence of Zn^{2+} ion (β-octapeptide **2**), or *ii*) a preferred *14*-helical structure of a sequence which would also be able to form a turn (β-octapeptide **3**) [67], or *iii*) a novel type of β^2/β^3-amino acid sequence with two (*R*)-β^2-homo-amino acid residues, containing no hairpin 'violation'

[14]) For extensive discussions of the secondary structures of β- and γ-peptides in relation to the constitution, and absolute and relative configuration of their building blocks, see the reviews [5][71] and refs. cit. therein.



1 [β^3-decapeptide carrying β^3hOrn^4, β^3hGlu^7, and β^3hLys^{10} for salt-bridge formation and β^3hHis^6 and β^3hHis^9 for Zn^{2+} complexation]

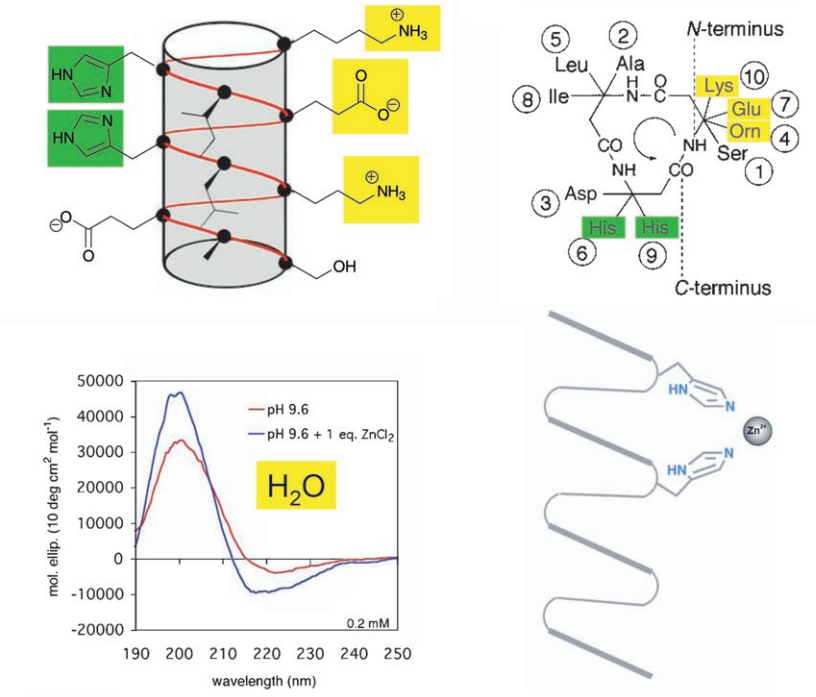


Fig. 2. *A β^3-decapeptide designed to have a salt-brigde-stabilized and Zn^{2+}-complexing helical structure to promote folding in aqueous solution as demonstrated by CD spectroscopy* [65]

and being unable to form a *14*-helix (β-octapeptide **4**), or *iv*) a diastereoisomer of **3**, which is neither capable of folding to a *14*-helix nor to a hairpin turn containing two violating β^3-homo-amino acids for both secondary structures (β-octapeptide **5**).

The β^3-, β^2-, and $\beta^{2,3}$-homo-amino acid derivatives required for the sytheses of the decapeptide **1** and the four octapeptides **2**–**5** are collected in *Fig. 5*, with references to literature where their preparation is described. In some cases, not previously reported protecting-group combinations are employed (see the *Exper. Part*).

Peptide coupling was achieved by manual solid-phase synthesis on *Wang* resin[15]) to which the first amino acid Fmoc-β^3hCys(Tr)-OH (for **2**–**5**) or Fmoc-β^3hLys(Boc)-OH

[15]) The synthesis and CD spectra in the presence and absence of Zn^{2+} ion of the octapeptide **5** have been described by us in a preliminary communication [65].

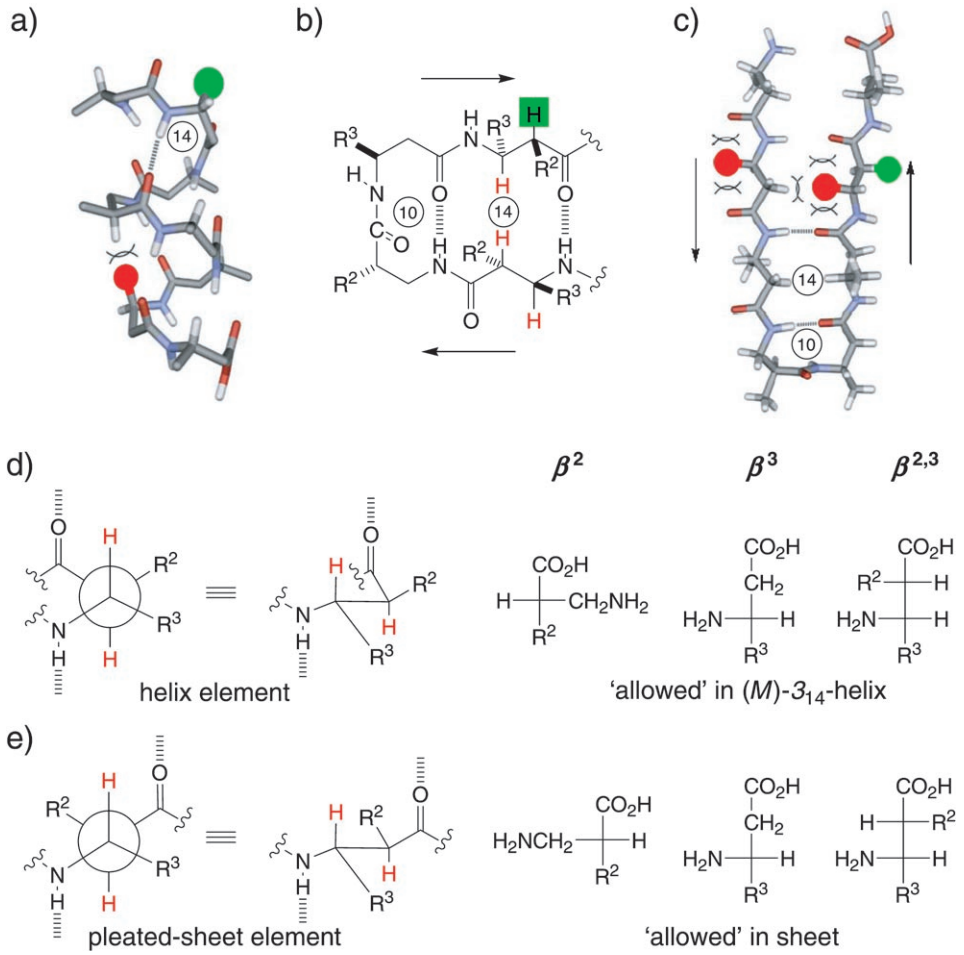
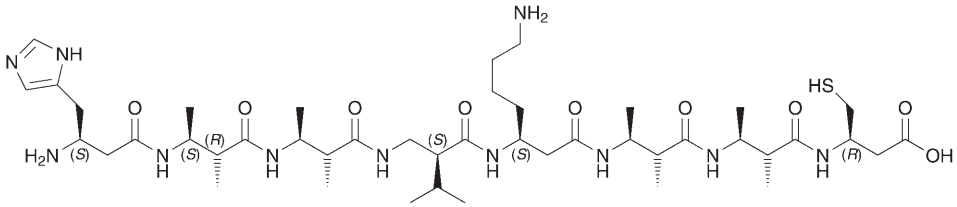


Fig. 3. *(*M*)-14-Helix* (*a*) *and hairpin turn* (*b* and *c*) *of β-peptides with allowed and forbidden β-homo-amino-acid constitutions and configurations* (*d* and *e*). All positions labelled with red H are 'forbidden' for non-H-atoms (exceptions may be F and OH [72] in the *β*-peptidic *14*-helix and pleated sheet). For the simple antiparallel sheet of the *β*-peptidic hairpin turn, the same rules apply as for the pleated sheet, except that in its C-terminal part an (*S*)-*β*^{2}Xaa would be allowed (green labelling in *b* and *c*); no $A^{1,3}$ repulsion on the 'outside'). A *Fischer* projection is used for the formulae of the *β*-amino acids because, according to the *CIP* convention, amino acids of the same chirality would be assigned opposite stereodescriptors (*cf.* (*S*)-*β*^{2}hAla and (*R*)-*β*^{2}hSer, or (*S*)-*β*^{3}hLeu and (*R*)-*β*^{3}hVal). The *α*-substituted *β*-amino acids fitting into the *14*-helix are 'safely' assigned *erythro* (*Fischer*) or *anti* (*Masamune*), those fitting into the pleated sheet *threo* or *syn*; the *like*/*unlike* assignment (*Prelog–Seebach*) for the *α*-branched *β*-amino acids may, again, be confusing when it comes to functionalized sidechains (of Ser, Thr, Cys).

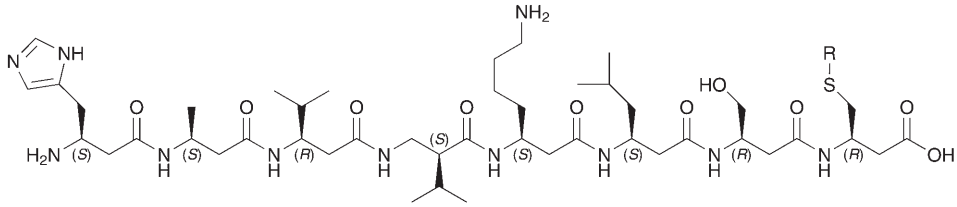
(for **1**) was attached with a loading efficiency between 55 and 85%. Sequences of Fmoc removal and coupling led to the resin-bound *β*-peptide derivatives **1** and **3**–**5**, whereby the N-terminal residues with Ser (for **1**) and His side chains (for **2**–**5**) were introduced

H-β^3hHis-(βhXaa)$_2$-(*S*)-β^2hVal-β^3hLys-(βhXaa)$_2$-β^3hCys-OH

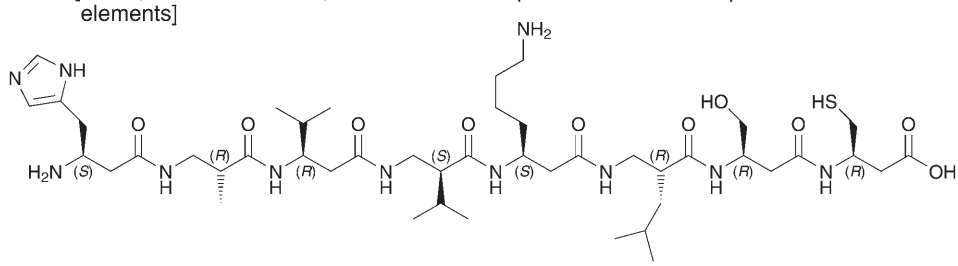
Zn^{2+}-complexing | turn-inducing dipeptide segment also fitting in an (*M*)-*14*-helix | Zn^{2+}-binding



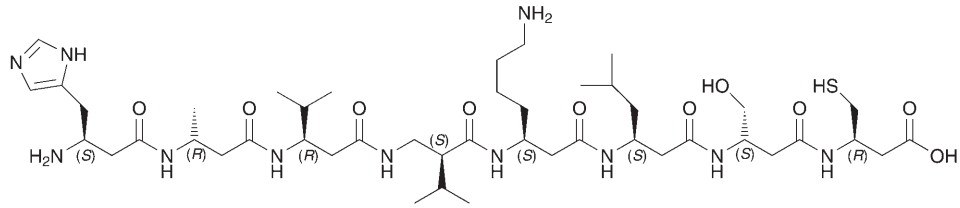
2 [hairpin structure, turn with antiparallel sheet; can not fold to a *14*-helix; four helix-breaking (2*R*,3*S*)-$\beta^{2,3}$Xaa]



3 [R = H, *14*-helix structure, no violation of hairpin structure with antiparallel sheet elements]

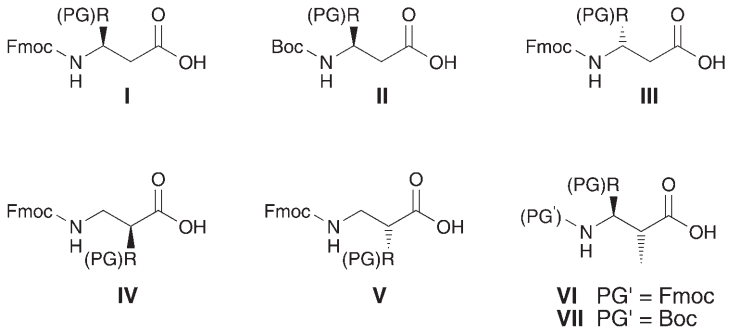


4 [no violation of hairpin structure; can not fold to an (*M*)-*14*-helix; two helix-breaking β^2hXaa (β^2hAla^2, β^2hLeu^6); can probably also not fold to *12/10*-helix because of 'wrong' configuration of the amino acid residues 2 and 6 [16][67 – 69]]



5 [diastereoisomer of **3**; can neither fold to a *14*-helix nor to a hairpin turn; two violating βhXaa in both cases ((*R*)-β^3hAla^2, (*S*)-β^3hSer^7)]

Fig. 4. *β-Octapeptides with terminal Zn^{2+}-binding amino acids β^3hHis and β^3hCys.* Compounds **2**, **3**, and **4** can possibly form a hairpin structure (turn with antiparallel sheet). Peptide **3** can fold to a 3_{14}-helix. β-Octapeptide **5** is violating both, the rules for *14*-helix- and for hairpin-structure formation (*cf. Fig. 3*).



	I												
β^3hXaa	Ala*	Arg*	Asn*	Asp*	Cys	His	Glu*	Ile*	Leu*	Lys*	Orn	Ser*	Val*
PG	---	Pmc	Tr	^{t}Bu	Tr	Tr	^{t}Bu	---	---	Boc	Boc	^{t}Bu	---
Ref.	[73]	[74]		[75]	[76]	[76]	[73]	[75][77]	[78]	[73]	[73]	[61]	[73]	[73]

	II			III		IV
βXaa	β^3hHis	β^3hLys	β^3hSer	(*R*)-β^3hAla	(*S*)-β^3hSer	(*S*)-β^2hVal
PG	Tr	2-Cl-Z	^{t}Bu	---	^{t}Bu	---
Ref.	[76]	[61]		[78]		[73]

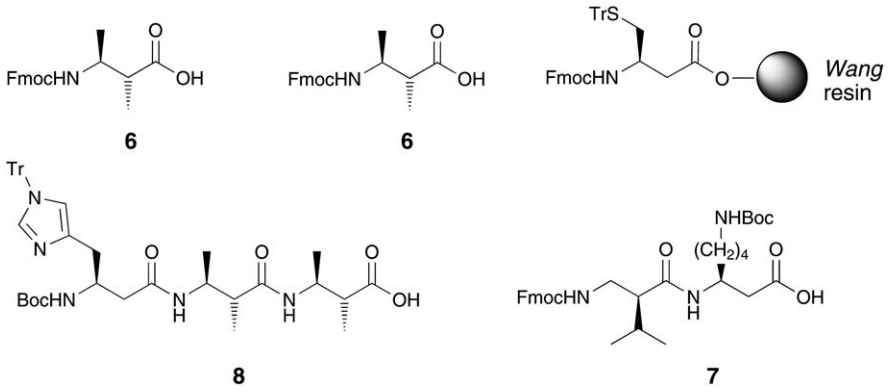
	V		VI	VII
βXaa	β^2hAla*	β^2hLeu	(2*R*,3*S*)-$\beta^{2,3}$hAla(αMe)	(2*R*,3*S*)-$\beta^{2,3}$hAla(αMe)
PG	---	---	---	---
Ref.	[79]	[80]	[81]	[82]

Fig. 5. *The β^2-, β^3- and $\beta^{2,3}$-homo-amino acid derivatives for the syntheses of β-peptides* **1**–**5**, *and* **10** (see *Sect. 6*) *with references to procedures for their preparation.* The asterisked (*) derivatives are commercially available (we thank *Fluka AG*, CH-Buchs, for discount prizes). Abbreviations are explained in the *Exper. Part*.

with *N*-Boc-protection, in order to avoid a final, N-terminal Fmoc deprotection. The β-peptide **2** with its built-in turn- and sheet-forming structural features could not be synthesized by single-amino-acid coupling: it was impossible to attach the eighth β-amino acid moiety (β^3hHis)[16])[17]). Thus, we resorted to manual solid-phase *fragment* coupling, a method which we have successfully applied previously [69][84]. After two coupling steps of the *Wang*-resin-anchored β^3hCys(Tr) with Fmoc-[(2*R*,3*S*)-$\beta^{2,3}$hAla(αMe)]-

[16]) We have noticed over and over again that coupling and Fmoc deprotection become slow when the chain lengths of β-peptides reach a range of six to eight residues in solid-phase syntheses. As suggested before, this is probably due to folding of the chain [5].

[17]) In a paper describing the synthesis of β-hexapeptides with hairpin forming structural features, *Langenhan* and *Gellman* mention that they were not able to 'prepare β-octapeptide hairpins' [83].



OH (**6**), the turn-inducing dipeptide segment **7** was attached, followed by the N-terminal tripeptide fragment **8**.

The dipeptide **7** was obtained from Boc-β^3hLys(2Cl-Z)-OBn and Fmoc-(*S*)-β^2hVal-OH, with subsequent hydrogenolytic debenzylations and Boc protection of the Lys side-chain functionality. The tripeptide **8**, on the other hand, was prepared from the known Boc-[(2*R*,3*S*)-$\beta^{2,3}$hAla(αMe)]-OH and its benzyl ester ($\rightarrow$ Boc-[(2*R*,3*S*)-$\beta^{2,3}$hAla(αMe)]$_2$-OBn), with subsequent Boc deprotection, coupling with Boc-β^3hHis(Tr)-OH and C-terminal debenzylation. By this route to the β-octapeptide **2**, we avoided highly insoluble fragments and intermediates. All couplings were carried out with HATU/*Hünig* base in DMF. Attachment of the central dipeptide **7** and of the N-terminal tripeptide **8** required stringent conditions: heating to 50° in the presence of LiCl [85–88] was required after a partially unsuccessful coupling at room temperature. Compound **2** was also difficult to isolate in pure form, and we were happy to eventually obtain a few milligrams of it! For more details, see the *Exper. Part* and for references to procedures for the preparation of the various known building blocks, see *Fig. 5*.

After deprotective removal from the resin, the five β-peptides **1**–**5** were purified by preparative HPLC, identified by high-resolution mass spectrometry and by NMR spectroscopy, and characterized by analytical HPLC and by CD measurements. The β-octapeptides **2**–**5** with C-terminal β^3hCys residues are more or less sensitive to oxidative dimerization, a process that can be used deliberately for purification purposes, and that is readily reversed by reductive disulfide cleavage. For an NMR investigation of the β-peptide **3** in the absence of Zn^{2+} ions, we have converted the SH group of β^3hCys[8] to a MeSS group to prevent disulfide formation during sample preparation and in the course of the measurements (*vide infra*)[18]).

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[18]) A first hint to Zn^{2+} complexation was provided by the following experiment with β-octapeptide **5**: an aqueous solution (*Tris* buffer at pH 7.3) of the peptide was kept for up to 15 h in the absence and presence of an equimolar amount of $ZnCl_2$. RP-HPLC Analysis showed that there was no decrease at all of the peptide concentration in the Zn^{2+}-containing solution, while the content of **5** decreased slowly in the Zn-free solution, indicating a stabilization by Zn^{2+} complexation.

3. CD Spectra of the β-Peptides 1–5 in MeOH and H_2O with and without Added Zn Salts. – Since all five peptides were isolated as their trifluoroacetate salts, *i.e.*, **1** with 5 TFAs, **2**–**5** with 3 TFAs each, it is not surprising that the CD spectra measured in MeOH without and with added $ZnCl_2$ differ only slightly: the histidine(s) side chains are protonated, and the imidazolinium cations can, of course, not act as ligands of Zn^{2+} ions (for the β-peptide **5**, see Fig. 3 in our preliminary communication [65]). The same is true for buffered aqueous solutions at low pH, as was shown for β-octapeptide **2** (*Fig. 6*, *a*).

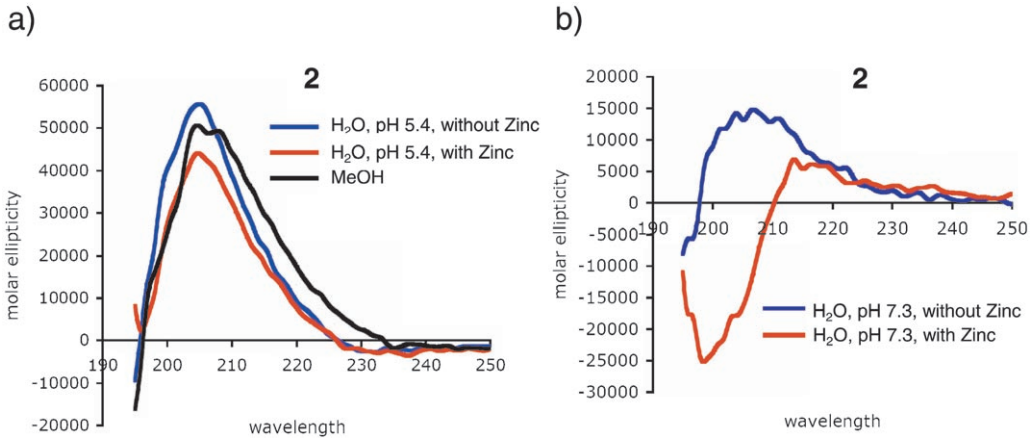

Fig. 6. *Non-normalized CD spectra of 0.2-mM solutions in MeOH and H_2O of the β-octapeptide* **2**·*3 TFA with and without added $ZnCl_2$. a*) CD Curves in MeOH (*black*), in H_2O (pH 5.4) without $ZnCl_2$ (*blue*) and with $ZnCl_2$ (*red*) (*Tris* buffer): protonation of the imidazole groups prevents Zn^{2+}-complexation. *b*) CD Curves in H_2O at pH 7.3 without $ZnCl_2$ (*blue*) and with $ZnCl_2$ (*red*): drastic change with red-shift and intensity decrease of positive band and new strong short-wavelength negative *Cotton* effect.

In contrast, dramatic changes of the CD pattern take place when $ZnCl_2$ is added to aqueous solutions of the β-peptides **2**–**5**, buffered at higher pH values. When going from *Tris*-buffered solutions of pH 5.4 to pH 7.3 with β-peptide **2**, the intensity of the positive CD band near 205 nm decreases to a fourth, and when $ZnCl_2$ is added at this higher pH value, there is a further collapse of intensity, accompanied by a shift of the positive band to longer wavelength (*ca.* 215 nm) and simultaneously an intensive negative (!) band appears near 200 nm (see *Fig. 6*, *b*).

β-Peptide **3** exhibits the expected [4][5] CD spectrum of a β-peptidic *14*-helix in MeOH solution (intensive extrema near 215 and 195 nm, with zero-crossing at 205 nm, see black curve in *Fig. 7*, *a*). In H_2O, the trough at 215 nm, which may be considered characteristic of the *14*-helix, disappears almost completely. A weaker, positive band between 200 and 205 nm is observed in H_2O, and in *Tris* buffer at pH 7.3, and when $ZnCl_2$ is added, there is a further intensity decrease. The curves seen in the presence of 0.5, 1.0, and 2.0 equiv. of $ZnCl_2$ (*Fig. 7*, *a*) are compatible with a 1 : 1 complex between peptide **3** and Zn^{2+} ion, which had also been shown for β-peptide **5** [65].

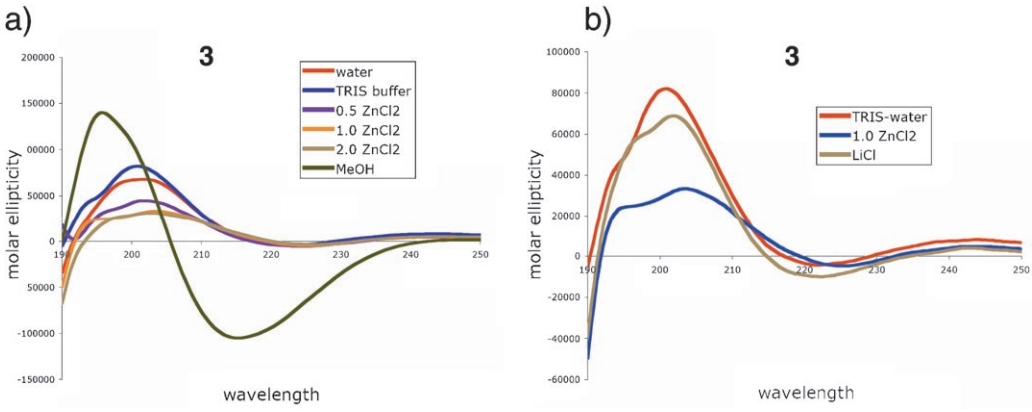

Fig. 7. *Non-normalized CD spectra of β-octapeptide* **3**·*3 TFA* (2.0 mM). *a*) In MeOH (*green*), in H_2O (*red*), and in *Tris*-buffered aqueous solution (pH 7.3; *blue*); addition of 0.5 (*violet*), 1.0 (*orange*), and 2.0 equiv. $ZnCl_2$ (*brown*) leads to decreased ellipticity. *b*) Comparison of the CD curves in *Tris*-buffered H_2O (*red*), in H_2O/1 equiv. $ZnCl_2$ (*blue*), and in H_2O/LiCl (*brown*; ionic strength 6.0 mM) shows a Zn^{2+}-specific effect on the intensity (*blue*) of the band at 200–205 nm.

To make sure that the observed large change of the CD spectrum upon addition of $ZnCl_2$ to the buffered aqueous solution of peptide **3** is not just a consequence of increased ionic strength of the solution, we have also measured the spectrum in the presence of 1 equiv. of LiCl; this caused only a slight change of the spectrum, as compared to the effect of $ZnCl_2$ (*Fig. 7*, *b*).

The *β*-peptides **4** (turn 'allowed', *14*-helix 'forbidden') and **5**, the diastereoisomer of **3**, (neither 'fits' in a turn nor in the helix) showed a similar change when going from MeOH to H_2O solution (*Fig. 8*): decreased intensity of the positive *Cotton* effect between 200 and 205 nm. Addition of $ZnCl_2$ caused a red-shift to 215 nm, with further decreased intensity, in the case of *β*-peptide **5** (*Fig. 8*, *b*), while $ZnCl_2$ shut down essentially all chiroptical activity of *β*-peptide **4** in the range of measurement (250–195 nm; *Fig. 8*, *a*)[19]).

In summary, the CD spectra demonstrate that there are interactions between the *β*-peptides **1**–**5** and Zn^{2+} ions in aqueous buffer solution of pH values above 7 (where histidine side chains are available for complexation). Since almost all *β*-peptides show a more or less strong *Cotton* effect between 210 and 195 nm, in MeOH and in H_2O, irrespective of the NMR-solution structures in these solvents (*14*- and *10*/*12*-helix, hairpin turn, strand, and those of unknown structures[20])), the spectra reported here do not provide any structural information, nor do they lend themselves for further qualitative assignments. On the other hand, the changes of some CD spectra observed with increasing Zn^{2+} concentrations are compatible with the formation of 1:1 complexes[21]).

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[19]) In all of our work on chiral *β*- and *γ*-peptides, we have, so far, observed total lack of CD activity only with the γ^4-peptides [89].

[20]) See the discussion in Sect. 7.6 of [5].

[21]) For the application of the so-called molar-ratio method [90] to *β*-peptide **5**, see [65]; *cf.* also *Fig. 7*, *a*.

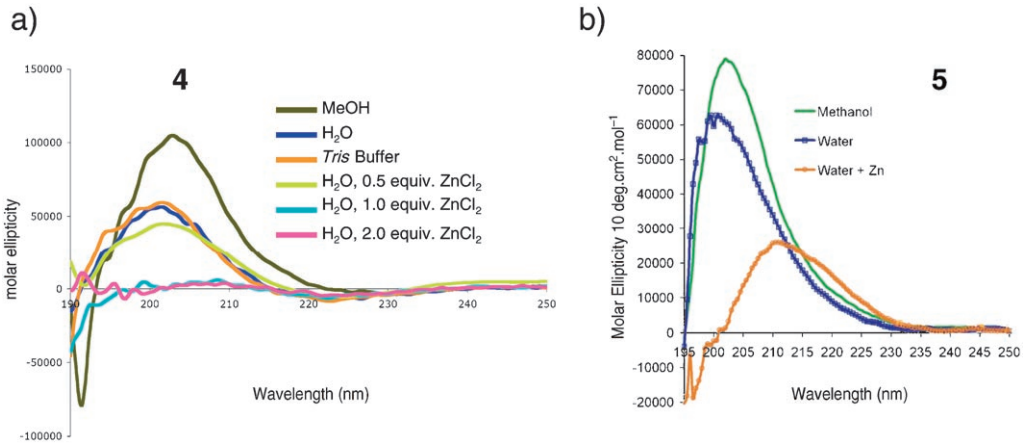

Fig. 8. *CD Spectra of the β-peptides* **4** (*a*) *and* **5** (*b*) *in MeOH, H_2O, and $H_2O/ZnCl_2$* (buffered at pH 7.3). Annihilation of the one and only CD band between 250 and 195 nm of **4** by addition of $ZnCl_2$ (*a*). Reduction of intensity and red-shift by addition of $ZnCl_2$ to the aqueous solution of β-octapeptide **5** (*b*).

4. Electrospray Mass Spectra (ESI-MS) of the β-Peptides 1 and 5. – Another method that can provide information about the complexation stoichiometry of peptide–Zn^{2+} complexes is electrospray ionization mass spectrometry (ESI-MS). Indeed, mass spectrometry represents a relatively new technique for the study of non-covalent interactions and is becoming more and more accepted by the scientific community due to the numerous 'successful' reports that involve the use of ESI-MS for such studies[22]). The gentleness of the electrospray process allows intact protein complexes to be directly detected by mass spectrometry, reflecting, to some extent, the nature of the interaction found in the condensed phase. Additionally, in comparison to other established methods that have been applied for the study of macromolecular interactions, such as spectroscopic approaches (*e.g.*, CD, light scattering, fluorescence, differential scanning calorimetry, and isothermal titration calorimetry [100]), mass spectrometry offers advantages in speed and sensitivity[23]). A number of peptide–metal and protein–metal complexes have been studied by ESI-MS both qualitatively and quantitatively [101–103].

To confirm the formation of β-peptide–Zn^{2+} 1:1 complexes, as suggested by the CD measurements, we have investigated[24]) the lyophilized peptides **1** and **5** also by ESI-MS (aqueous $AcONH_4$ buffer solution of pH 7.6 in the absence and presence of $Zn(OAc)_2$[25]) (*Fig. 9*).

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[22]) For reviews on the direct observation of non-covalent protein complexes by ESI-MS, see [91–99].

[23]) For a comparison of the merits and disadvantages of mass spectrometry with respect to other biophysical methods, such as NMR, X-ray crystallography, and other spectroscopic techniques, see [92].

[24]) The determination of binding constants (**1**·Zn^{2+}, **5**·Zn^{2+}) has been published seperately [104].

[25]) For the MS measurements, $Zn(OAc)_2$, rather than $ZnCl_2$, was used because of aggregation problems with the latter salt during the ESI process.

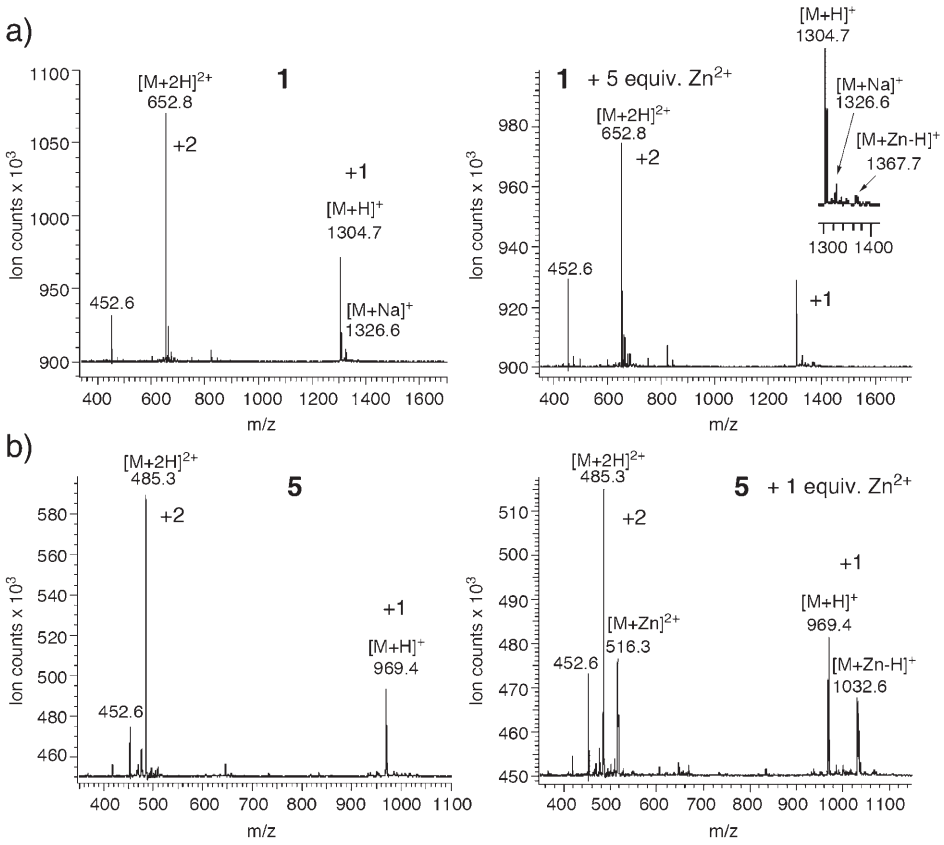

Fig. 9. *ESI Mass spectra of β-peptides* **1** *and* **5** (20 mM) *in AcONH₄ buffer solution* (20 mM) *in the absence and presence of Zn(OAc)₂. a*) ESI Mass spectrum of **1** (*left*) with molecular-ion peaks $[M+H]^+$, $[M+2H]^{2+}$, and $[M+Na]^+$; spectrum of **1** obtained in the presence of 5 equiv. of $Zn(OAc)_2$ (*right*) with an additional Zn^{2+}-peptide peak of low intensity. *b*) ESI Mass spectrum of **5** with molecular ion peaks $[M+H]^+$ and $[M+2H]^{2+}$ (*left*); addition of 1 equiv. of $Zn(OAc)_2$ (*right*) leads to strong signals of the 1:1 Zn complex. Internal standard: peptide GGYR of *m/z* 452.

The ESI mass spectra of the β-peptides **1** and **5** are characterized by peaks of the singly and doubly charged molecular cations, and no fragmentations were detectable under the experimental conditions (*Fig. 9*). In the case of **1**, a Na adduct was also detected. Upon addition of $Zn(OAc)_2$, new peaks were observed, which corresponded to 1:1 peptide–Zn^{2+} complexes; no other species could be detected even after addition of up to 10 equiv. of Zn^{2+}. This provides evidence for **1** and confirms for **5** the 1:1 stoichiometry of the peptide–Zn^{2+} complexes[26]). It should be mentioned that addition of Zn^{2+} ions to the peptide solutions caused a decrease in the ion response, leading to spectra with signals of diminished intensities. Indeed, it is well-known that the presence

[26]) Specificity of the complexes was demonstrated by control experiments with bradykinin (RPPGFSPFR).

of buffer additives or high salt concentrations used to fix the ionic strength may decrease the signal sensitivity and even lead to a complete extinction of ESI signals [105].

Titration experiments monitored by ESI-MS [106–113] with the β-peptides **1** and **5**, and $Zn(OAc)_2$ provided values for the dissociation constants in the order of *ca.* 20 μM[24]), which is in the same range as those reported for α-peptides[27]).

The fact that both CD titration[21]) and ESI-MS measurements are only compatible with a 1:1 Zn^{2+} complex of **5** may be rationalized with the presence of structures such as those shown in *Fig. 10*, *a* and *b*.

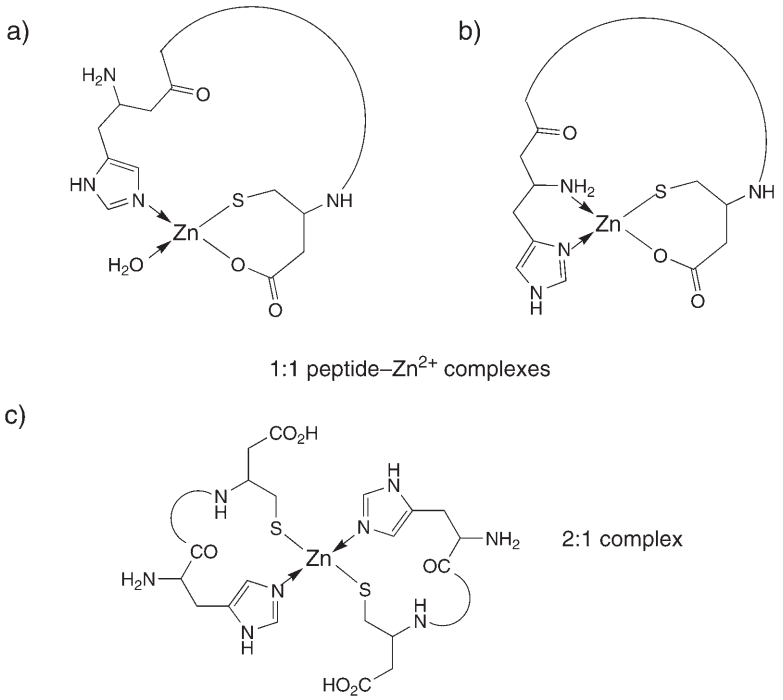

Fig. 10. *Schematic models for a 1:1* (*a* and *b*) *and a 2:1 complex* (*c*) *of the β-peptide* **5** *with a* Zn^{2+} *ion in* H_2O. The imidazole N-atom will be available for complexation at physiological pH values, and the terminal amino N-atom under slightly basic conditions.

5. NMR Analysis of the Solution Structures of β-Peptides 2, 3, and 5. – While CD and MS spectra can give valuable hints to secondary structures and metal complexes of peptides, we have come to rely on detailed structure analyses by the high-resolution techniques 2D-NMR spectroscopy and X-ray diffraction for the determination of β-peptidic secondary structures[20]). Thus, full NMR analyses (DQF-COSY, TOCSY, HMBC, ROESY measurements) and interpretation of the data according to the

[27]) For K_d values from 0.6 to 300 μM of metal complexes of helical and hairpin-turn α-peptides containing cysteine and histidine residues, see [114–116].

XPLOR protocol were performed for the β-peptides **2** (in H_2O, without and with $ZnCl_2$), and **3** and **5** (in MeOH and in H_2O, without and with $ZnCl_2$).

5.1. *The Turn β-Octapeptide* **2**. This peptide was designed to fold to a hairpin turn, just like the previously reported β-hexapeptide **9** with sheet-enforcing $\beta^{2,3}$-residues of *ul*-configuration [59][60] (*Fig. 11*, *a*). So far, no β-peptidic turn has been identified in aqueous solution [59][60][83]. The four $\beta^{2,3}$-residues in compound **2** were expected to stabilize such a turn sufficiently to be observed in H_2O. Thus, we determined the NMR-solution structure of this β-octapeptide in H_2O in buffered solution of pH 5.4. As can be seen from *Fig. 11*, *b*, **2** is present as a hairpin turn under these conditions, with the terminal residues somewhat less well-ordered than the central part containing the ten-membered H-bonded ring and the first *trans*-catenary H-bond, which is part of a 14-membered ring of an antiparallel sheet-structural element. As expected, addition of $ZnCl_2$ at this pH does not cause significant changes of the structure (*Fig. 11*, *c*): the imidazole group is protonated, even if this would lead to a Zn^{2+}-binding to the termini of the octapeptide **2**, we would not expect a dramatic structural change, since the turn is intrinsically preformed and does not require stabilization by complexation[28]).

5.2. *The Helix β-Octapeptide* **3**. The situation is fundamentally different with the β-octapeptide **3**: in MeOH it should fold to a *14*-helix, into which its one and only β^2-amino acid residue of (*S*)-configuration fits (*cf. Fig. 3*) ; in H_2O, we expect loss of helical structure (*cf. Fig. 7*), but if Zn^{2+} complexation with the terminal side chains of β^3hCys and β^3hHis provides enough binding enthalpy, a hairpin turn would be enforced; there is no turn-violating residue (*Fig. 3*) in this β-peptide. To prevent complications due to disulfide formation of **3**, R=H, during NMR sample preparation and during NMR measurements, we converted the cystein SH group to a mixed disulfide group (**3**, R = MeS)[29]). The helical structure of **3** in MeOH, as suggested by the typical CD spectrum (*Fig. 7*, *a*; green curve), was confirmed by the NMR analysis (*Fig. 12*, *a* and *b*). A well-ordered (*M*)-helix is seen in MeOH, with the N-terminal imidazolyl and MeSS groups far remote (*ca.* 20 Å).

When dissolved in H_2O, peptide **3** (R=H) with free SH group loses (most of) its helical structure, according to the CD spectra (*Fig. 7*, *a* and *b*; H_2O and *Tris* buffer pH 7.3), and we do not know its structure under these conditions. But we do know by NMR analysis that this β-peptide exists in a hairpin-turn secondary structure in H_2O in the presence of 1 equiv. of $ZnCl_2$ (*Fig. 12*, *c*). The imidazolyl and the SH groups are close to each other, and NOEs across the two strand parts confirm the turn structure.

5.3. *The 'Impossible' β-Octapeptide* **5**. Finally, it was especially important to look at the NMR structure of the 'impossible' β-octapeptide **5**, in which two centers of **3** are inverted, and which is, therefore, predicted not to be capable of forming either a helix or a turn (*Fig. 4*). Three detailed NMR analyses of **5** were performed: in CD_3OH, in H_2O (KH_2PO_4 buffer + 10% D_2O, pH 7.6), and in H_2O in the presence of Zn^{2+} ions ((D_{11})*Tris* buffer, 1 equiv. of $ZnCl_2$, 10% D_2O, pH 7.2).

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[28]) It was not possible to record an NMR spectrum at higher pH because the peptide starts precipitating from the aqueous solution above pH 5.4.

[29]) We thank Dr. *Thierry Kimmerlin* for his assistance in the preparation of this mixed disulfide.

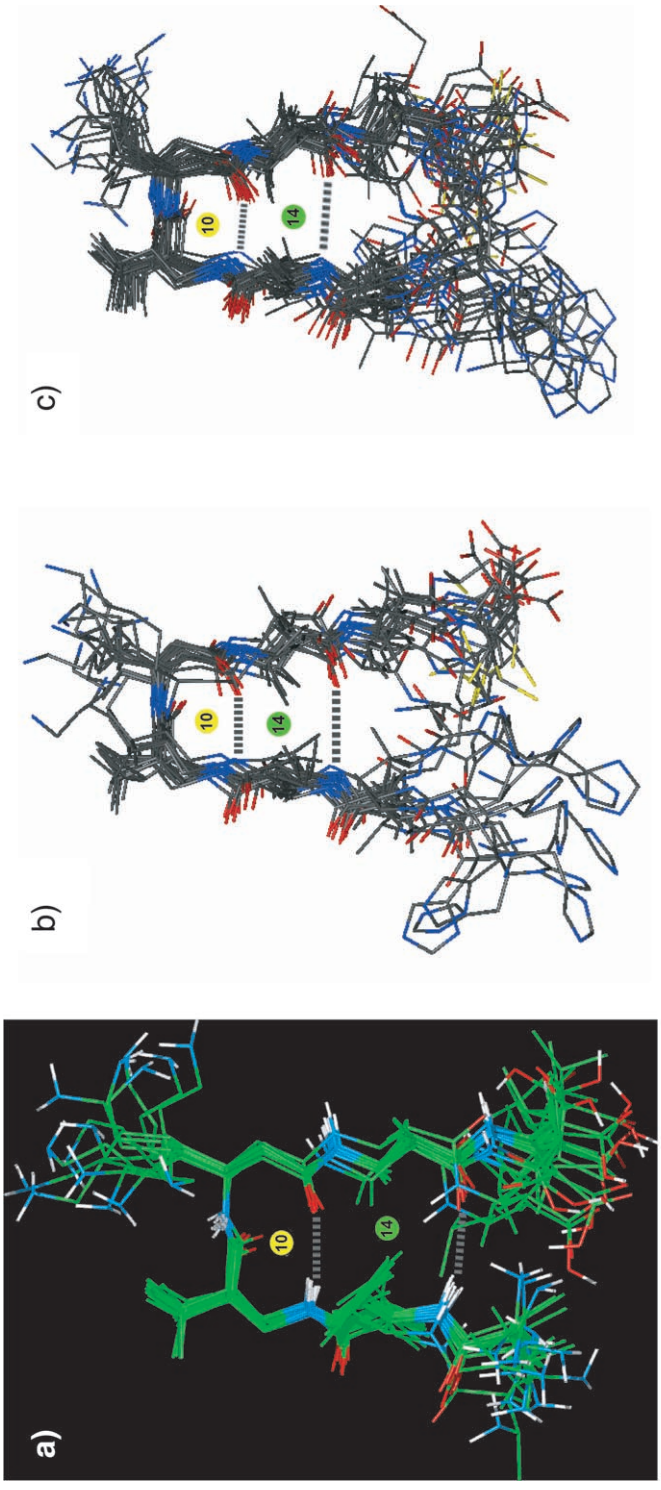

Fig. 11. *β-Peptidic Hairpin Turns. a)* The previously reported β-hexapeptide **9** with four strand-enforcing $\beta^{2,3}$-amino acids (in CD_3OH). *b)* The β-octapeptide **2** with four strand-inducing $\beta^{2,3}$-amino acid residues (in H_2O, pH 5.4 buffer). *c)* Peptide **2** ((D_{11})*Tris*-buffered in H_2O at pH 5.4) in the presence of 1 equiv. of $ZnCl_2$. See *Fig. 15,c*, for a structural proposal for the isomer **5**.

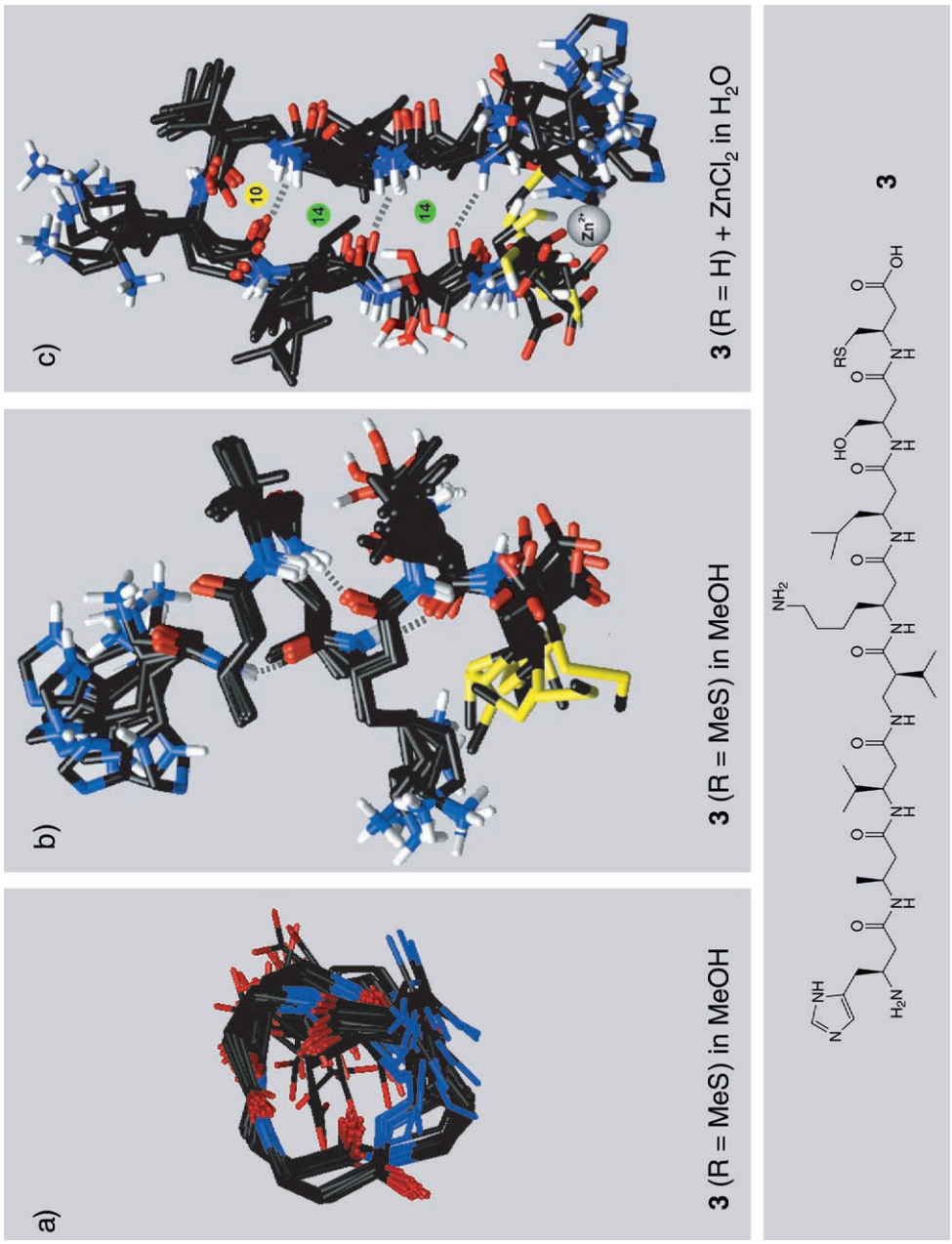

Fig. 12. *NMR-Solution structures of the β-octapeptide* **3**. The mixed disulfide **3**, R = MeS, is a helix in MeOH. *a*) View along the helix axis and *b*) side view of the *14*-helix. The *β*-peptide **3** with a non-protected thiol group, to which 1 equiv. of $ZnCl_2$ has been added (pH 7.3) is a hairpin in H_2O. *c*) The exact position of the Zn^{2+} ion is of course unknown, but it *must* be near the place, where we have drawn it: See *Fig. 15*, *c*, for a structural proposal for the isomer **5**.

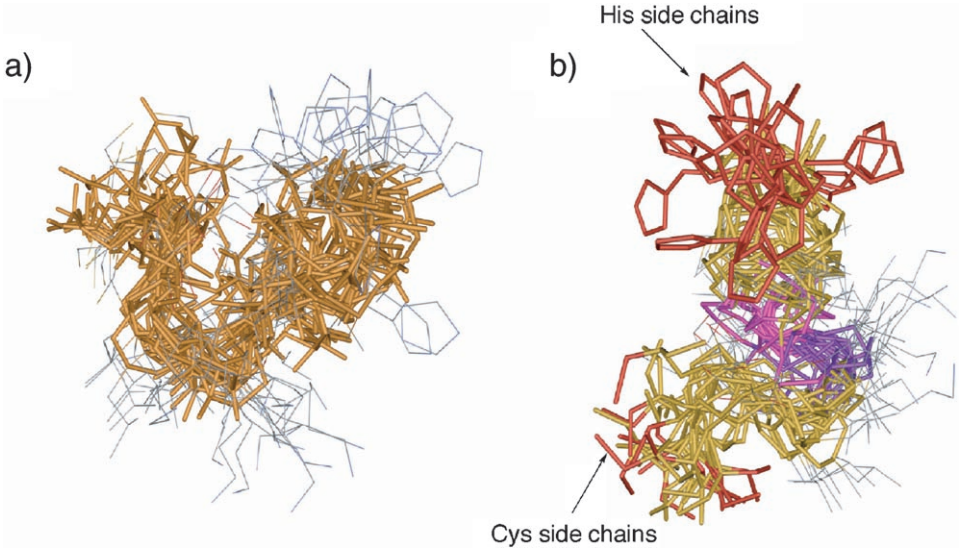

Fig. 13. *NMR-Solution structure of the β-octapeptide* **5** *in CD_3OH. a*) Bundle of 20 structures generated by simulated annealing; the peptide backbone (*orange*) is not well-defined. *b*) Presentation showing that the central β^2hVal-β^3hLys fragment (*purple* and *violet*) does not fold to a ten-membered H-bonded ring. Inversion of the configuration of β^3hAla^2 and β^3hSer^7 in peptide **3** (→ **5**) prevents the molecule from forming the *14*-helix (*cf. Fig. 12*, *b*).

For the peptide **5** measured in CD_3OH, a total of 107 NOEs were used as distance restraints in simulating annealing, according to the XPLOR protocol. This calculation yielded a set of 20 structures with low restraint violation and minimum energy (*Fig. 13*).

The structures give a rather poorly defined bundle that does not represent any of the known β-peptidic secondary structures. We cannot resist making the statement that the 'wrong' amino acid residues in β-peptide **5** ((*R*)-β^3hAla^2 and (*S*)-β^3hSer^7) convert the order of the *14*-helix of **3** to a real mess (compare *Fig. 12*, *b*, with *Fig. 13*). The four C-terminal residues fold into a vague helix, which is 'broken' by the β^2/β^3-fragment. It is worth noting that this fragment does not have a turn structure with a ten-membered H-bonded ring, but displays a conformation, in which the two side chains protrude in opposite directions. In addition, the histidine and cysteine side chains are far away from each other, precluding any Zn^{2+} complexation in this conformation.

For the peptide **5** measured in aqueous solution, a total of 101 NOEs were used for the simulation. In this calculation, two sets of conformations with low restraint violation were obtained, of which 15 structures correspond to conformation A (*Fig. 14*, *b*) and 5 to conformation B (*Fig. 14*, *c*). Again, the SH and the imidazole groups are far remote.

For the peptide **5**, measured in H_2O in the presence of $ZnCl_2$, a total of 71 NOEs were used for the simulation. The calculation yielded 17 structures with low restraint violation and minimum energy (*Fig. 15*). From the overlay of these structures, again, a poorly defined conformation results. The β-peptide backbone has changed its shape by the addition of Zn^{2+} ions, putting the terminal residues β^3hCys and β^3hHis

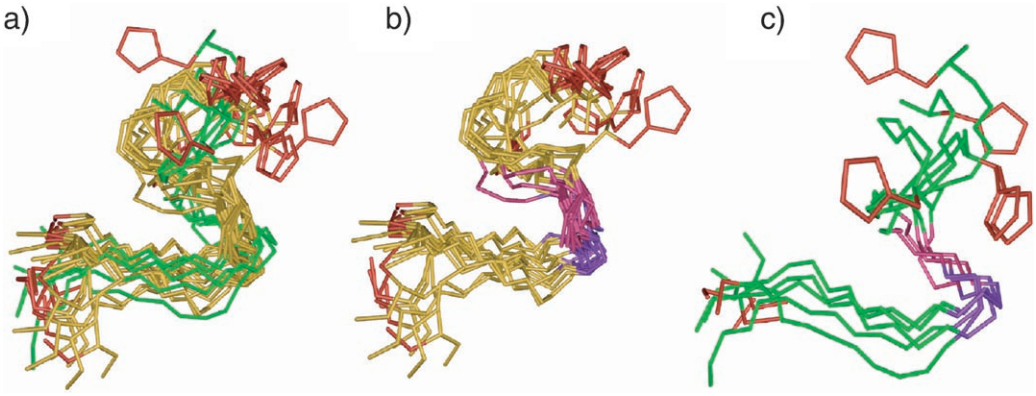

Fig. 14. *NMR-Solution structure of β-octapeptide* **5** *in H_2O solution.* Side chains other than those of histidine and cysteine are omitted for clarity. *a*) Bundle of 20 structures clustering into conformation A (*orange*) and B (*green*). *b*) Backbone of the 15 structures characterising conformation A with the β^2hVal-β^3hLys-fragment (*purple* and *violet*). *c*) Backbone of the five structures characterizing conformation B, which shows some similarities with the structure found in CD_3OH (*cf. Fig. 13*,*a*). Side chains of histidine and cysteine are indicated in *red*.

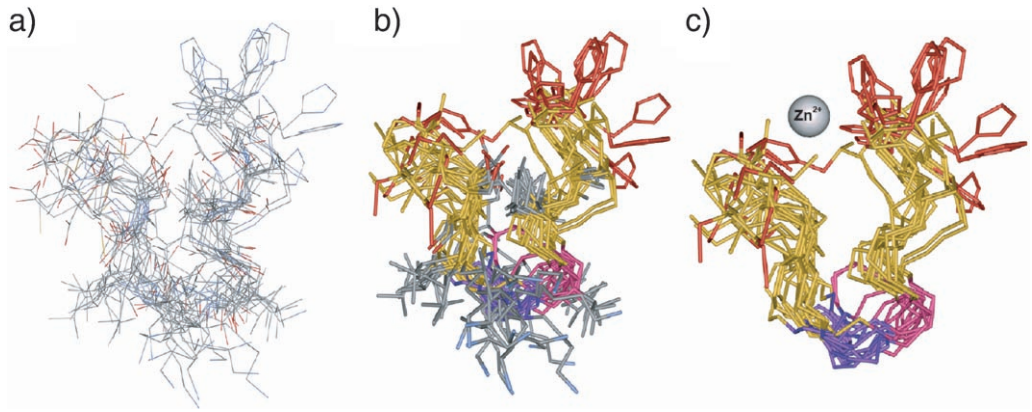

Fig. 15. *NMR-Solution structure of peptide* **5** *in H_2O in the presence of 1 equiv. of $ZnCl_2$* ((D_{11})*Tris* buffered at pH 7.2). *a*) Overlay of the 17 lowest-energy structures obtained by simulated annealing. The overlay of the peptide backbones (*orange*, *purple*, and *violet* in *b* and *c*) display the resulting hairpin-type confirmation, (with low definition!). The side chains of histidine and cysteine (*red* in *b* and *c*) are relatively near to each other and are propably coordinating the Zn^{2+} ion, which is suggestively drawn in *c*. The strand-'destroying' effect of the two *β*-amino acid residues 2 and 7 is nicely demonstrated by comparision with the Zn^{2+} complex of the diastereoisomer **3** in *Fig. 12*,*c*.

in relative proximity. It contains a hairpin structure, in which the β^2hVal-β^3hLys dimer fragment (*purple* and *violet* in *Fig. 15*,*b* and *c*) does not display the ten-membered H-bonded ring, as seen in the typical *β*-peptidic turns in *Figs. 11* and *12*.

Comparison of the bundles in *Fig. 12*,*c*, and *Fig. 15*,*c*, is a nice demonstration for the correctness of the 'rules for allowed and forbidden' positions of side chains on *β*-peptidic hairpin turns: in *β*-peptide **3**, there are no turn-violating residues, and Zn^{2+} cre-

ates a perfect hairpin turn with an antiparallel sheet attached. In the isomeric β-peptide **5**, the violating configuration of two amino-acid residues prevents proper folding to the β-peptidic turn. Still, the Zn^{2+} ion is powerful enough to pull the terminal residues together, in what one would call a highly distorted, twisted, and crooked backbone conformation.

6. Design, Synthesis, and CD and MS Characterization of a Possible, Zn-Finger-Mimicking β-Hexadecapeptide 10. – As an extension of the β-peptidic Zn^{2+}-binding turns described in the previous sections, we decided to combine two secondary structures through a linker in order to obtain a β-peptide that could fold into a tertiary structure. To achieve this goal, a helical and hairpin secondary structure containing two β-homohistidine and two β-homocysteine residues, respectively, were envisaged to be linked by a simple β-homoglycine amide bond, affording a peptide that could fold, with or without the help of a metal ion, into the so called $\beta\beta\alpha$ motif – an intrinsic structure of the Zn-finger proteins [117]. Zinc-finger domains are small metal-binding modules that are found in a wide range of gene regulatory proteins. Their functions are extraordinarily diverse and include DNA recognition, RNA packing, transcriptional activation, regulation of apoptosis, protein folding and assembly, and lipid binding [118]. The best known class is the Cys_2His_2 Zn finger that was first identified in transcription factor IIIA [117][119], and that has subsequently been found in hundreds of protein sequences [120]. The basic structure of the Cys_2His_2 Zn-finger protein is composed of one or multiple units of *ca.* 30 amino acids per finger in which a two-stranded antiparallel β-sheet and a so-called 'recognition helix' are held together by two pairs of highly conserved cysteine and histidine residues binding a Zn^{2+} ion in a tertiary structure. The Zn^{2+} ion does not play any catalytic role but 'only' a structural one (*cf.* allosteric ligands), maintaining the shape of the protein that binds to DNA to activate or deactivate genes. The three-dimensional structure characteristic of Cys_2His_2 domains is shown in *Fig. 16*, *a*. To achieve a possible mimic of such a tertiary structure, we have designed the β-hexadecapeptide **10**.

The following strategy was adopted for the design of **10**: at the N-terminus the first six residues were selected for the formation of a hairpin structure, with a turn-inducing β^2/β^3-fragment[30]). The next two amino acid residues (β^3hArg, βhGly) were to serve as a linker leading to the remaining eight residues for helix formation. One of the two β-homocysteines was placed on the turn-inducing fragment, the other one in position 2 for optimum distance to bind a Zn^{2+} ion. The two histidine residues were set at the C-terminus of the peptide chain, on the helical part, in i and $i+3$ positions (*i.e.*, in juxtaposition in the *14*-helical structure). A schematic presentation of the two secondary-structure parts is shown in *Fig. 16*, *b*.

The preparation of β-peptide **10** was achieved by first anchoring Fmoc-β^3hLys(Boc)-OH onto *Wang* resin and then attaching the other 15 amino acids in a manual synthesizer, according to the Fmoc strategy as for the β-peptides **1**–**5** (for details, see *Exper. Part*). The chromatograms of the crude product and of the pure β-

[30]) Apart from the N-terminal (*R*)-β^3hAla, there is no 'turn-violating' residue in this first part of the β-peptide (*cf. Fig. 3*).

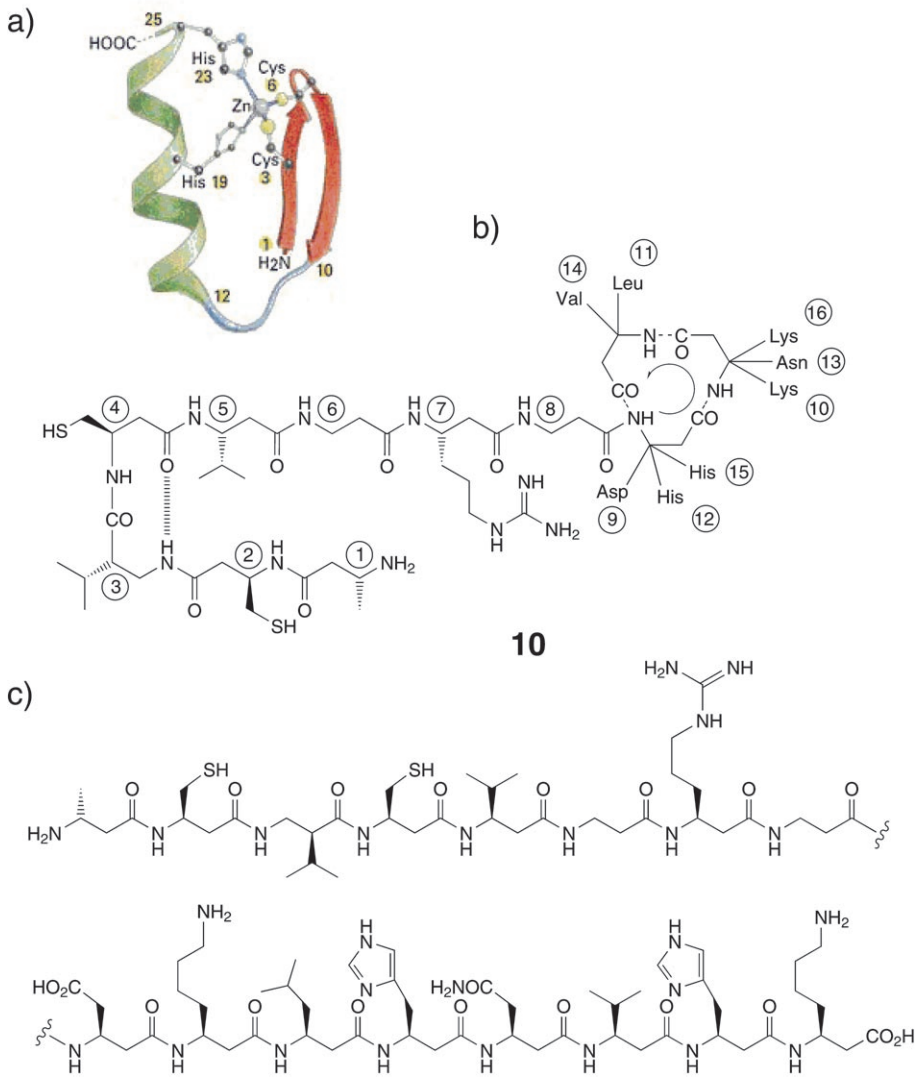

Fig. 16. *The α-peptidic Zn-finger motif and a possible β-peptidic mimic. a*) The schematic diagram of a Zn-finger domain highlighting the four metal-binding residues (taken from [121]). *b*) Presentation of the *β*-hexadecapeptide **10** in the expected secondary/tertiary structure(s), consisting of a hairpin turn and a *14*-helix, and the conventional formula of **10** (*c*).

peptide **10** after preparative HPLC isolation[31]) are shown in *Fig. 17*; the yield of a >98% pure sample was 41%.

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[31]) Interestingly, the major side product (starred HPLC peak in *Fig. 17*,*a*) was the 'capping' product formed due to incomplete coupling between *β*hGly[8] and *β*hArg[7], *i.e.*, the *β*-nonapeptide Ac-*β*hGly-*β*hAsp-*β*hLys-*β*hLeu-*β*hHis-*β*hAsn-*β*hVal-*β*hHis-*β*hLys-OH.

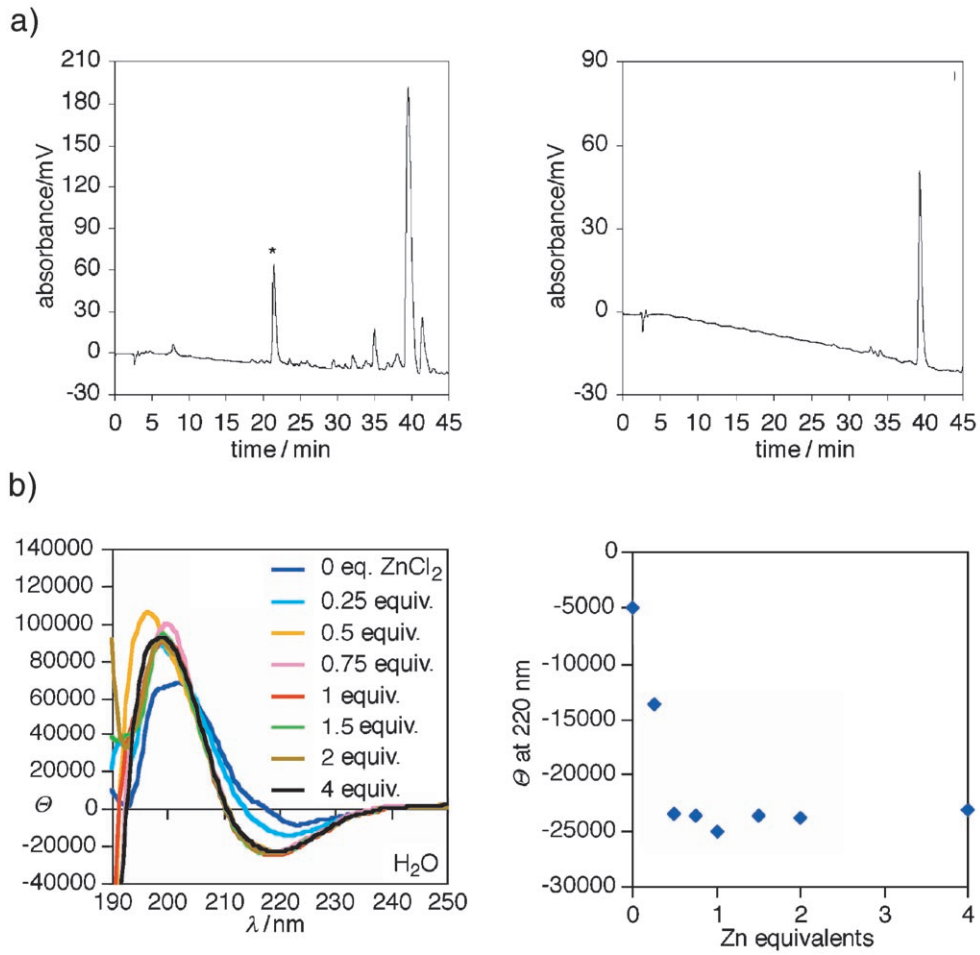

Fig. 17. *HPLC Traces and CD spectra of β-hexadecapeptide* **10**. *a*) Crude (*left*) and purified sample (*right*); for the starred-peak in the crude product, see *Footnote 31* in the text. *b*) Non-normalized CD spectra of **10** in H_2O (pH 7.5, 0.2 mM peptide, 20 mM *Tris* buffer) in the absence and presence of Zn^{2+} ions ($ZnCl_2$ added, *left*); shift of the negative *Cotton* effect to shorter wavelengths under the influence of increasing concentrations of Zn^{2+} ions (*right*).

The CD spectrum in H_2O of *β*-peptide **10** shows an increased intensity of the negative *Cotton* effect and shift towards the wavelength typical of *14*-helical structures upon addition of Zn^{2+} ions at pH 7.5 (*cf.* the 'helical' *β*-peptide **1** [65]; *Fig. 17*,*b*, *left*); as pointed out above, the CD spectrum is not useful for assigning turn structures; the titration with $ZnCl_2$ is compatible with the formation of a 1:1 complex. This is confirmed by the ESI-MS measurements (*Fig. 18*).

At this stage, no information about the *structure* of the complex formed between *β*-peptide **10** and Zn^{2+} ion is available. We will have to wait for the result of an NMR investigation to see whether the designed mimicking of a natural Zn-finger structure

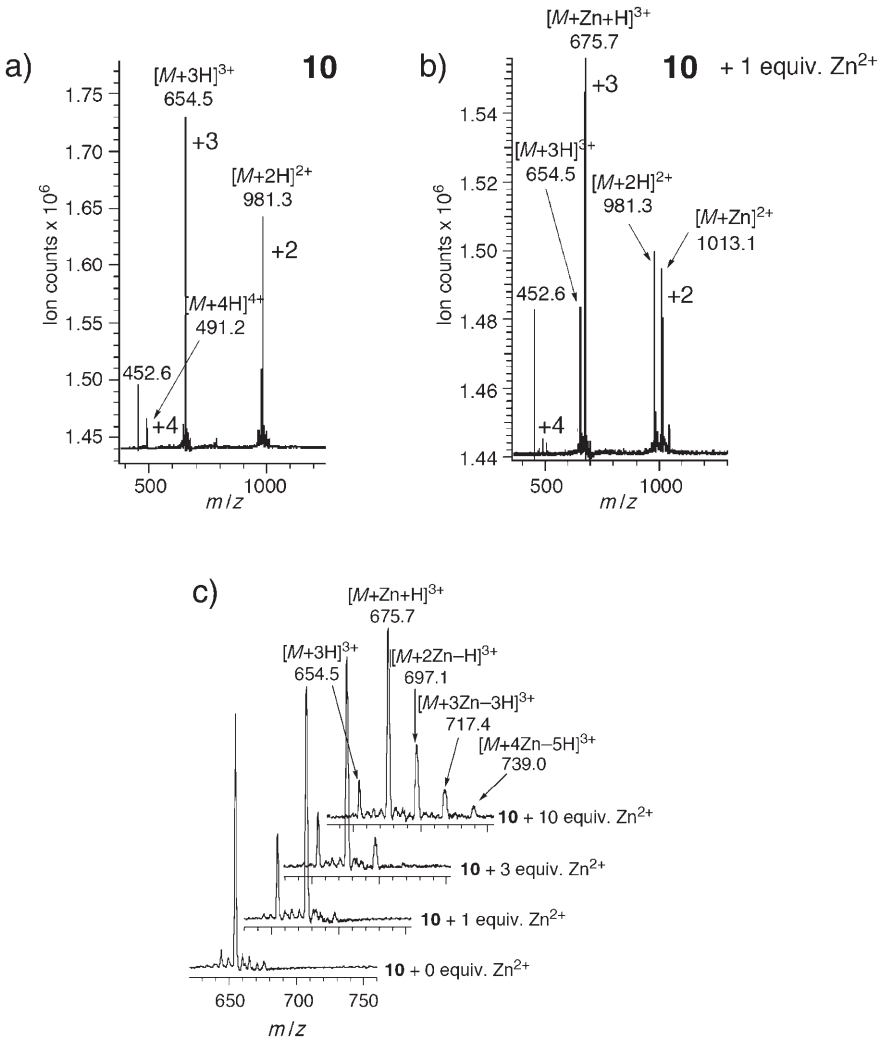

Fig. 18. *ESI Mass Spectra of the β-hexapeptide* **10** *in the absence* (*a*) *and in the presence of Zn(OAc)*$_2$ (*b* and *c*).

has been successful[32]). It must be pointed out that we have 'designed' the Zn-finger mimic **10** simply by using a ball-and-stick model. β-Peptide **10** must, therefore, be con-

[32]) Crystallization experiments with the β-peptides **5** and **10** were unsuccessful, so far. With a *Tris* buffer solution (pH 7, 1 equiv. $ZnCl_2$, *ca.* 25 mg of peptide/ml), in the presence of $AcONH_4$ (5–55%) and of various salts, buffer additives, and polymers, *ca.* 500 crystallization experiments were set up (hanging-drop technique). Over a long period of time, crystal formation was checked, in vain. The experiments were carried out in collaboration with Dr. *Klaus Piontek* and Dr. *Thomas Choinowski* of the Department of Biology, ETH-Zürich, and at the *Synchrotrone* in Grenoble (France).

sidered a first attempt, which will be followed by more sophisticated approaches. Hereby, the lessons we have learned from studying the simple β-peptides **1**–**5** will be of instrumental value.

7. Conclusions. – Zn^{2+} Ions are able to stabilize helical and hairpin-turn structures of β-peptides in H_2O, even at the low concentrations of CD and NMR measurements, if Cys and His side chains are positioned properly in the peptide sequence. The free energy of peptide–Zn^{2+} complex formation (including the affinity of Zn^{2+} for S^- of β^3hCys and for the imidazole N-atom of β^3hHis) is large enough to overcome enthalpic and entropic losses of the β-peptidic backbone and of solvation. This is not trivial in view of the fact that similar effects have been observed with α-peptides and proteins, because the stability of β-peptidic secondary structures is much larger than that of their α-peptidic counterparts [5]. The conformational stability of the sp^3-sp^3-C,C-bonds in the β-peptidic residues has been shown to be a major contribution to the stabilization of the corresponding secondary structures [16], so that the forces involved in their distortions by Zn^{2+} ions must be larger than with α-peptides.

With the Zn-stabilized structures in H_2O, described herein, new avenues of constructions in the field of β-peptidic chemistry have been opened (see the proposed Zn-finger mimic and analogues thereof, the NMR structure of which we are about to solve).

Experimental Part

1. *General. Abbreviations.* 2-Cl-Z: 2-Chlorobenzyloxycarbonyl, Bn: benzyl, Boc: (*tert*-(butoxy)carbonyl), DBU: 1,8-diazabicyclo[5.4.0]undec-7-ene, DMAP: 4-dimethylaminopyridine, EDT: ethane-1,2-dithiol, ESI: electron spray ionization, FC: flash chromatography, Fmoc: [(9*H*-fluoren-9-yl)methoxy]carbonyl, HATU: *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, HR: high resolution, h.v.: high vacuum (0.01–0.1 Torr), MALDI: matrix assisted laser desorption ionization, MeIm: 1-methyl-1*H*-imidazole, MSTN: 1-(mesitylene-2-sulfonyl)-3-nitro-1*H*-1,2,4-triazole, NMM: *N*-methylmorpholine, PMC: 2,2,5,7,8-pentamethylchroman-6-sulfonyl, SPS: solid phase synthesis, TFA: CF_3COOH, TNBS: 2,4,6-trinitrobenzene sulfonic acid, Tr: trityl (=triphenylmethyl).

Solvents for chromatography and workup procedures were distilled from *Sikkon* (anh. $CaSO_4$; *Fluka*) and from KOH (Et_2O). Amino acids were purchased from *Fluka* or *Senn*. LiCl was dried under h.v. at 150° for 4 h. Fmoc-D-β^3hSer(*t*-Bu)-OH was prepared from Fmoc-D-Ser(*t*-Bu)-OH using a method from *Sewald* and co-workers [75]. Boc-β^3hSer(*t*-Bu)-OH was synthesized from Boc-Ser(*t*-Bu)-OH according to one of our protocols [82]. All other reagents were used as received from *Fluka* or *Aldrich*. TLC: *Merck* silica gel *60 F_{254}* plates; detection with UV, anisaldehyde soln. (9.2 ml of anisaldehyde, 12.5 ml of conc. H_2SO_4, 3.75 ml of AcOH, 340 ml of EtOH), or 'Mostain' soln. (25 g of phosphormolybdic acid, 10 g of $Ce(SO_4)_2 \cdot H_2O$, 60 ml of conc. H_2SO_4, 940 ml of H_2O), FC: *Fluka* silica gel *60* (40–63 μm); at *ca.* 0.3 bar. IR Spectra: *Perkin-Elmer 1600 FT-IR* spectrophotometer. NMR Spectra: *Bruker AMX II 500* (^{1}H: 500 MHz, ^{13}C: 125 MHz), *AMX-400* (^{1}H: 400 MHz, ^{13}C: 100 MHz), *AMX-300* (^{1}H: 300 MHz, ^{13}C: 75 MHz), *Varian Mercury XL 300* (^{1}H: 300 MHz, ^{13}C: 75 MHz); chemical shifts δ in ppm downfield from internal Me_4Si (=0 ppm); *J* values in Hz. MS: *IonSpec Ultima 4.7 T FT* Ion Cyclotron Resonance (ICR, HR-MALDI, in 2,5-dihydroxybenzoic acid matrix) spectrometer; in *m*/*z* (% of basis peak). Elemental analyses were performed by the Microanalytical Laboratory of the Laboratorium für Organische Chemie, ETH-Zürich.

2. *Synthesis of the Building Blocks* **7** *and* **8**. 2.1. *Boc Deprotection. General Procedure 1* (*GP 1*). *GP 1a*. The Boc-protected amino acid was dissolved in TFA (5 ml/1 mmol) at 0°. Stirring for 0.5 h at 0° and for 1.5 h at r.t., concentration under reduced pressure, co-evaporation with toluene (2×) and drying under h.v. yielded the crude TFA salt which was used without further purification.

GP 1b. As *GP 1a* except that a mixture of TFA (5 ml/1 mmol) and CH_2Cl_2 (5 ml/1 mmol) was used, and the mixture was stirred for 0.5 h at 0° and of 2 h at r.t.

2.2. *Peptide Coupling with HATU: General Procedure 2* (*GP2*). The appropriate *N*-deprotected amino acid (1 equiv.) was dissolved in CH_2Cl_2 (20 ml/1 mmol) and cooled in an ice-bath. To the mixture was successively added NMM (3 equiv.) and the *N*-protected amino acid (1 equiv.–1.3 equiv.). To this soln., HATU (1.2 equiv.) was added, and the mixture was allowed to warm up to r.t. overnight. The mixture was then diluted with CH_2Cl_2 and washed with aq. KH_2PO_4 soln. (1M), aq. K_2HPO_4 soln. (1M), and brine. The org. phase was dried ($MgSO_4$), and the solvent was removed under reduced pressure. The crude peptide was purified by FC (silica gel).

2.3. *Preparation of* **7**. *Boc-β³hLys(2-Cl-Z)-OBn.* To a soln. of Boc-β³hLys(2-Cl-Z)-OH (747 mg, 6 mmol) in DMF (6 ml) under N_2, Cs_2CO_3 (737 mg, 2.26 mmol) and BnBr (0.31 ml, 2.61 mmol) were added by cooling with a water bath. After stirring overnight at r.t., H_2O (20 ml) was added. The aq. phase was extracted with AcOEt (3×40 ml), the combined org. phases were washed with brine (2× 20 ml), dried (Na_2SO_4), and the solvents were removed. After FC (hexane/AcOEt 2:1) and drying under h.v. at 50° overnight, Boc-β³hLys(2-Cl-Z)-OBn (1.81 g, 98%) was isolated. Colorless solid. M.p. 86–87°. R_f (hexane/AcOEt 2:1) 0.36. $[\alpha]_D^{r.t.} = -15.2$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3010*w*, 2940*w*, 1720*s*, 1505*s*, 1455*w*, 1370*m*, 1165*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.15 (*m*, Me_3C, 3 CH_2); 2.54 (br. *d*, $J = 5.5$, CH_2); 3.16 (br. *d*, $J = 5.7$, NCH_2); 3.91 (br. *s*, NCH); 4.98 (*m*, 2 NH); 5.09, 5.12 (*AB* system, $J = 12.3$, 1 H each, OCH_2Aryl); 5.20 (*s*, OCH_2Aryl); 7.21–7.43 (*m*, 9 arom. H). ^{13}C-NMR (75 MHz, $CDCl_3$): 22.9 (CH_2); 28.2 (Me); 29.3, 34.0, 39.3, 40.6 (CH_2); 47.2 (CH); 63.7, 66.3 (CH_2); 79.2 (C); 126.7, 128.2, 128.5, 129.1, 129.3, 129.6 (CH); 133.3, 134.2, 135.6, 155.3, 156.1, 171.3 (C). MALDI-MS: 557.2 (17, $[M+K]^+$), 541.2 (34, $[M+Na]^+$), 519.2 (7, $[M+H]^+$), 419.2 (100, $[M-C_5H_7O_2]^+$). MALDI-HR-MS: 541.2067 ($[M+Na]^+$, $C_{27}H_{35}N_2O_6Na^+$; calc. 541.2076). Anal. calc. for $C_{27}H_{35}ClN_2O_6$ (519.04): C 62.48, H 6.80, N 5.40; found: C 62.22, H 6.87, N 5.60.

Fmoc-β²hVal-β³hLys(2-Cl-Z)-OBn. Boc-β³hLys(2-Cl-Z)-OBn (519 mg, 1.00 mmol) was Boc-deprotected according to *GP 1b*. The resulting TFA salt was coupled with Fmoc-β³Val-OH (388 mg, 1.10 mmol) according to *GP 2* overnight. FC (CH_2Cl_2/MeOH 30:1) yielded Fmoc-β²hVal-β³hLys(2-Cl-Z)-OBn (633 mg, 84%, purity >90%). For anal. purposes, a sample was recrystallized (hexane/CH_2Cl_2). Colorless solid. M.p. 133°. R_f (CH_2Cl_2/MeOH 30:1) 0.23. $[\alpha]_D^{r.t.} = +7.4$ ($c = 0.5$, $CHCl_3$). IR ($CHCl_3$): 3010*w*, 1720*s*, 1665*m*, 1515*s*, 1450*m*. ^{1}H-NMR (400 MHz, (D_6)DMSO): 0.83 (*d*, $J = 6.5$, Me); 0.89 (*d*, $J = 6.8$, Me); 1.13–1.46 (*m*, 3 CH_2); 1.67–1.77 (*m*, Me_2CH); 2.10–2.18 (*m*, CH); 2.37 (*dd*, $J = 8.1$, 15.1, 1 H, CH_2); 2.60 (*dd*, $J = 6,1$, 15.1, 1 H, CH_2); 2.95 (br. *d*, $J = 3.5$, $(CH_2)_3CH_2N$); 3.03–3.12 (*m*, 1 H, CH_2N); 3,19–3.28 (*m*, 1 H, CH_2N); 4.04–4.15 (*m*, CHN); 4.15–4.24 (*m*, $CHCH_2O$); 5.08 (*s*, OCH_2Aryl); 5.05, 5.07 (*AB* system, $J = 12.3$, 1 H each, OCH_2Aryl); 7.08 (*t*, $J = 5.8$, NH); 7.25–7.49 (*m*, 13 arom. H, NH); 7.67 (*d*, $J = 7.6$, 2 arom. H); 7.76 (*d*, $J = 8.1$, NH); 7.87 (*d*, $J = 7.5$, 2 arom. H). ^{13}C-NMR (75 MHz, $CDCl_3$): 20.0, 20.9 (Me); 22.9 (CH_2); 28.3 (CH); 29.2, 33.6 39.2, 40.5, 41.1 (CH_2); 46.0, 47.2, 54.3 (CH); 63.8, 66.6, 66.6 (CH_2); 119.9, 125.1, 126.8, 127.0, 127.6, 128.4, 128.5, 129.3, 129.4, 129.6 (CH); 133.4, 134.3, 135.4, 141.2, 144.0, 156.2, 156.5, 171.5, 173.9 (C). ESI-MS (pos. mode): 792.0 (6, $[M+K]^+$), 776.0 (100, $[M+Na]^+$), 754.0 (56, $[M+H]^+$). MALDI-HR-MS: 776.3058 ($[M+Na]^+$, $C_{43}H_{48}N_3O_7ClNa^+$; calc. 776.3073). Anal. calc. for $C_{43}H_{48}ClN_3O_7$ (754.32): C 68.47, H 6.41, N 5.57; found: C 68.19, H 6.49, N 5.62.

Fmoc-β²hVal-β³hLys(Boc)-OH (**7**). Fmoc-β²hVal-β³hLys(2-Cl-Z)-OBn (377 mg, 0.50 mmol) was dissolved in a mixture from EtOH (12 ml) and CH_2Cl_2 (12 ml) under N_2, and Pd/C (10%, 75 mg, 0.07 mmol) and AcOH (0.08 ml) were added. The apparatus was evacuated and flushed with H_2 (3×), and the soln. was stirred under H_2 (balloon). After 26 h, the soln. was filtered through *Celite*, which was washed with MeOH, and the combined solns. were concentrated under reduced pressure. The crude compound was suspended in AcOEt (100 ml), and the org. phase was extracted three times with aq. AcOH (5%, 100 ml, 50 ml, 25 ml). The combined aq. phases were lyophilized. To the resulting white solid suspended in MeCN (10 ml), sat. aq. $NaHCO_3$ soln. (10 ml) and Boc_2O (142 mg, 0.65 mmol) were added.

After stirring overnight, an aq. citric acid soln. (10%, 40 ml) was added. The aq. phase was extracted with AcOEt (3×50 ml), the combined org. phases were washed with brine (40 ml), dried ($MgSO_4$), and the solvent was removed. Washing with AcOEt (30 ml) yielded **7** (168 mg, 56%). Colorless solid. M.p. 173–174°. R_f (hexane/AcOEt/AcOH 50:50:1) 0.14. $[\alpha]_D^{r.t.} = +29.2$ ($c = 0.2$, MeOH). IR ($CHCl_3$): 3010*w*, 1710*s*, 1510*m*, 1450*w*, 1365*w*. ^{1}H-NMR (300 MHz, (D_6)DMSO): 0.83 (*d*, $J = 6.9$, Me); 0.89 (*d*, $J = 6.6$, Me); 1.13–1.49 (*m*, 3 CH_2); 1.35 (*s*, Me_3C); 1.64–1.76 (*m*, Me_2CH); 2.06–2.16 (*m*, CH); 2.22 (*dd*, $J = 8.2$, 15.4, 1 H, CH_2); 2.40–2.53 (*m*, 1 H, CH_2); 2.84 (br. *d*, $J = 6.0$, $(CH_2)_3CH_2N$); 3.00–3.13 (*m*, 1 H, CH_2N); 3.17–3.28 (*m*, 1 H, CH_2N); 3.95–4.10 (*m*, CHN); 4.19 (br. *s*, $CHCH_2O$); 6.71 (*t*, $J = 5.4$, NH); 7.14 (*t*, $J = 5.5$, NH); 7.26–7.45 (*m*, 4 arom. H); 7.68 (*d*, $J = 7.4$, 2 arom. H); 7.64–7.75 (*m*, NH); 7.88 (*d*, $J = 7.4$). ^{13}C-NMR (75 MHz, CD_3OD): 21.0, 21.7 (Me); 24.7 (CH_2); 29.1 (Me_3C); 30.0 (CH); 30.8, 35.1 41.1, 41.5, 42.8 (CH_2); 47.9, 50.4, 55.7 (CH); 68.3 (CH_2); 80.2 (C); 121.2, 126.5, 126.6, 128.5, 129.1 (CH); 142.9, 145.7, 158.8, 159.0, 175.4, 176.4 (C). ESI-MS (pos. mode): 634.0 (18, $[M+K]^+$), 618.1 (100, $[M+Na]^+$), 596.2 (17, $[M+H]^+$), 496.1 (14, $[M-C_5H_7O_2]^+$). MALDI-HR-MS: 618.3143 ($[M+Na]^+$; $C_{33}H_{45}N_3O_7Na^+$; calc. 618.3150).

2.4. *Preparation of* **8**. *Boc-(2*R,*3*S*)-β^{2,3}hAla(α*Me*)-OBn*. To a soln. of Boc-(2*R*,3*S*)-β^{2,3}hAla(α*Me)-OH (1.30 g, 6.00 mmol) in DMF (15 ml) under N_2, Cs_2CO_3 (2.54 g, 7.80 mmol) and BnBr (1.07 ml, 9.01 mmol) were added by cooling with a water bath. After stirring overnight at r.t., H_2O (50 ml) was added. The aq. phase was extracted with AcOEt (3×50 ml), the combined org. phases were washed with brine (2×50 ml), dried (Na_2SO_4), and the solvents were removed. After FC (hexane/AcOEt 4:1) (2*R*,3*S*)-β^{2,3}hAla(α*Me)-OBn (1.81 g, 98 %) was isolated. Colorless solid. M.p. 66–67°. R_f (hexane/AcOEt 4:1) 0.29. $[\alpha]_D^{r.t.} = -25.0$ ($c = 0.9$, $CHCl_3$). IR ($CHCl_3$): 3010*w*, 2980*m*, 1710*s*, 1500*s*, 1455*m*, 1390*w*, 1370*m*, 1165*s*, 1060*w*, 1015*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.09 (*d*, $J = 6.8$, Me); 1.18 (*d*, $J = 7.2$, Me); 1.43 (*s*, Me_3C); 2.60–2.75 (*m*, CH); 3.81–3.98 (*m*, CH); 4.83 (br. *d*, $J = 7.9$, NH); 5.08, 5.09 (*AB* system, $J = 12.2$, 1 H each, OCH_2Ph); 7.26–7.40 (*m*, 5 arom. H). ^{13}C-NMR (75 MHz, $CDCl_3$): 13.3, 17.2 (Me); 28.1 (Me_3C); 44.4, 48.3 (CH); 78.8 (C); 127.9, 128.0, 128.3 (CH); 135.7, 155.0, 173.9 (C). ESI-MS (pos. mode): 346.2 (8, $[M+K]^+$), 330.2 (100, $[M+Na]^+$), 308.2 (1, $[M+H]^+$), 274.1 (15), 230.1 (5). Anal. calc. for $C_{17}H_{25}NO_4$ (307.39): C 66.43, H 8.20, N 4.56; found: C 66.39, H 8.02, N 4.56.

*Boc-[(2*R,*3*S*)-β^{2,3}hAla(α*Me)-(2*R,*3*S*)-β^{2,3}hAla(α*Me)]-OBn*. Boc-(2*R*,3*S*)-β^{2,3}hAla(α*Me)-OBn (614 mg, 2.00 mmol) was Boc-deprotected according to *GP 1a*. The resulting TFA salt was coupled with (2*R*,3*S*)-β^{2,3}hAla(Me)-OBn (435 mg, 2.00 mmol) according to *GP 2*. FC (CH_2Cl_2/MeOH 30:1) yielded Boc-[(2*R*,3*S*)-β^{2,3}hAla(α*Me)-(2*R*,3*S*)-β^{2,3}hAla(α*Me)]-OBn (719 mg, 88%). Colorless solid. M.p. 172–173°. R_f (CH_2Cl_2/MeOH 30:1) 0.22. $[\alpha]_D^{r.t.} = -40.5$ ($c = 0.6$, $CHCl_3$). IR ($CHCl_3$): 2980*m*, 1705*s*, 1500*s*, 1455*m*, 1370*m*, 1165*s*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.07 (*d*, $J = 6.7$, Me); 1.08 (*d*, $J = 7.2$, Me); 1.10 (*d*, $J = 6.8$, Me); 1.14 (*d*, $J = 7.0$, Me); 1.43 (*s*, Me_3C); 2.17–2.30 (*m*, CH); 2.55–2.64 (*m*, CH); 3.65–3.75 (*m*, NCH); 4.11–4.20 (*m*, NCH); 5.09, 5.13 (*AB* system, $J = 12.2$, 1 H each, OCH_2Ph); 7.28–7.40 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, CD_3OD): 14.0, 15.5, 18.6, 19.4 (Me); 28.8 (Me_3C); 46.2, 48.2, 48.3, 50.0 (CH); 67.5 (CH_2); 79.9 (C); 129.3, 129.5, 129.6 (CH); 137.6, 158.0, 175.9, 176.9 (C). ESI-MS (pos. mode): 444.9 (5, $[M+K]^+$), 429.2 (100, $[M+Na]^+$), 407.5 (6, $[M+H]^+$). Anal. calc. for $C_{22}H_{34}N_2O_5$ (406.52): C 65.00, H 8.43, N 6.89; found: C 65.16, H 8.49, N 6.96.

*Boc-β^{3}hHis(Tr)-(2*R,*3*S*)-β^{2,3}hAla(α*Me)-(2*R,*3*S*)-β^{2,3}hAla(α*Me)-OBn*. Boc-[(2*R*,3*S*)-β^{2,3}hAla(α*Me)-(2*R*,3*S*)-β^{2,3}hAla(α*Me)]-OBn (407 mg, 1.00 mmol) was Boc-deprotected according to *GP 1a*. The resulting TFA salt was coupled with Boc-β^{3}hHis(Tr)-OH (665 mg, 1.30 mmol) according to *GP 2*. FC (CH_2Cl_2/MeOH 10:1) yielded Boc-β^{3}hHis(Tr)-(2*R*,3*S*)-β^{2,3}hAla(α*Me)-(2*R*,3*S*)-β^{2,3}hAla(α*Me)-OBn (611 mg, 76%, purity, >90%). For anal. purposes, a sample was washed twice with toluene. Colorless solid. Degradation >185°. R_f (CH_2Cl_2/MeOH 10:1) 0.45. $[\alpha]_D^{r.t.} = -17.0$ ($c = 0.5$, $CHCl_3$). IR ($CHCl_3$): 3005*m*, 1700*s*, 1655*s*, 1495*s*, 1450*m*, 1365*w*, 1310*w*, 1170*s*, 1130*m*. ^{1}H-NMR (400 MHz, (D_6)DMSO): 0.91 (*d*, $J = 7.1$, Me); 0.92 (*d*, $J = 7.2$, Me); 1.00 (*d*, $J = 6.7$, Me); 1.04 (*d*, $J = 7.0$, Me); 1.28 (*s*, Me_3C); 2.13–2.25 (*m*, CH, CH_2); 2.49–2.58 (*m*, CH, CH_2); 3.83–3.90 (*m*, NCH); 3.91–3.96 (*m*, NCH); 4.00–4.07 (*m*, NCH); 5.05, 5.08 (*AB* system, $J = 12.4$, 1 H each, OCH_2Ph); 6.55 (*d*, $J = 8.6$, NH); 6.62 (*s*, 1 arom. H); 7.06–7.10 (*m*, 6 arom. H); 7.23 (*s*, 1 arom. H); 7.29–7.42 (*m*, 14 arom. H); 7.59 (*d*, $J = 9.1$, NH); 7.78 (*d*, $J = 8.8$, NH). ^{13}C-NMR (100 MHz, (D_6)DMSO): 13.4, 15.4, 18.5, 19.1 (Me); 28.1 (Me_3C); 32.6, 40.6 (CH_2); 44.4, 45.8, 45.9, 46.4, 47.9 (CH); 65.5 (CH_2); 74.3, 77.2 (C); 118.6, 127.8, 127.9, 127.9, 128.0, 128.3, 129.2

(CH); 136.0 (C); 137.5, 137.8 (C, CH); 142.3, 154.5, 169.4, 173.5, 173.9 (C). ESI-MS (pos. mode): 821.9 (36, $[M+Na]^+$), 799.9 (100, $[M+H]^+$). MALDI-HR-MS: 822.4211 ($[M+Na]^+$, $C_{48}H_{57}N_5O_6Na^+$; calc. 822.4201.

*Boc-β³hHis(Tr)-(2*R*,3*S*)-β²,³hAla(αMe)-(2*R*,3*S*)-β²,³hAla(αMe)-OH* (**8**). Boc-β³hHis(Tr)-(2*R*,3*S*)-β²,³hAla(αMe)-(2*R*,3*S*)-β²,³hAla(αMe)-OBn (520 mg, 0.65 mmol) was dissolved in MeOH (20 ml) under N_2, and Pd/C (10%, 52 mg, 0.05 mmol) was added. The apparatus was evacuated and flushed with H_2 (3×), and the soln. was stirred under H_2 (balloon) for 2.5 h. Subsequent filtration through *Celite*, washing with MeOH, and concentration under reduced pressure yielded **8** (380 mg, 82%, purity >90%), which was used without further purification. For anal. purposes, a sample was washed twice with toluene and once with AcOEt. Colorless solid. Degradation >177°. R_f (CH_2Cl_2/MeOH 10:1) 0.16. $[\alpha]_D^{r.t.} = +6.9$ ($c = 0.3$, MeOH). IR ($CHCl_3$): 3015*m*, 3010*m*, 1700*s*, 1655*s*, 1495*s*, 1445*m*, 1370*w*, 1165*s*. ^{1}H-NMR (300 MHz, (D_6)DMSO): 0.92 (br. *d*, $J = 6.9$, 2 Me); 0.99 (*d*, $J = 6.6$, Me); 1.00 (*d*, $J = 6.3$, Me); 1.27 (*s*, Me_3C); 2.10–2.60 (*m*, 2 CH, 2 CH_2); 3.80–3.98 (*m*, 3 NCH); 6.56–6.64 (*m*, NH); 6.61 (*s*, 1 arom. H); 7.04–7.12 (*m*, 6 arom. H); 7.23 (*s*, 1 arom. H); 7.32–7.44 (*m*, 9 arom. H); 7.63 (*d*, $J = 8.8$, NH); 7.80 (*d*, $J = 8.8$, NH). ^{13}C-NMR (75 MHz, (D_6)DMSO): 14.6, 15.8, 19.0, 19.4 (Me); 28.4 (Me_3C); 32.9, 40.9 (CH_2); 45.1, 46.2, 46.3, 46.7, 48.2 (CH); 74.6, 77.5 (C); 118.8, 128.1, 128.3, 129.4 (CH); 137.7, 138.0 (C, CH); 142.5, 154.8, 169.7, 173.6, 176.3 (C). ESI-MS (pos. mode): 732.5 (5, $[M+Na]^+$), 710.4 (100, $[M+H]^+$). MALDI-HR-MS: 710.3899 ($[M+H]^+$, $C_{41}H_{52}N_5O_6^+$; calc. 710.3912).

3. *Peptide Synthesis.* 3.1. *Anchoring of* N-*Fmoc-Protected β-Amino Acids on* Wang *Resin; Determination of Loading. General Procedure 3* (*GP 3*). Esterification of the Fmoc-protected β^3-amino acid with *Wang* resin was performed according to [122], by the MSNT/MeIm method. The resin was placed into a dried manual SPS reactor, swelled in CH_2Cl_2 (20 ml/g resin) for 30 min and in DMF (20 ml/g resin) for other 30 min, or only in CH_2Cl_2 (20 ml/g resin) for 1 h, and washed with CH_2Cl_2. In a separate dry round-bottomed flask equipped with magnetic stirrer, the Fmoc-protected amino acid (5 equiv.) was dissolved in dry CH_2Cl_2 (3 ml/mmol), then MeIm (3.75 equiv.) and MSNT (5 equiv.) were added under Ar. Stirring was continued until the MSNT was dissolved. Thereafter, the soln. was transferred with a syringe to the reaction vessel containing the resin and mixed by Ar bubbling for 2–4 h. Subsequently, the resin was filtered, washed with CH_2Cl_2 (20 ml/g resin, 5×1 min) or DMF (6 ml, 5×1 min) and CH_2Cl_2 (6 ml, 5×1 min), and dried under h.v. for 24 h. The resin substitution was determined by measuring the absorbance of the dibenzofulvene-piperidine adduct: two aliquots of the Fmoc-amino acid resin were weighed exactly (m_1(resin) and m_2(resin)) and suspended in piperidine (20%) in DMF, in a volumetric flasks ($V_1 = V_2 = 10$ ml). After 30–40 min, the mixtures were transferred to a UV cell and piperidine (20%) to another UV cell (blank), and the absorbance (A) was measured at 290 nm. The concentrations (c_1 and c_2, [mM]) of the benzofulvene-piperidine adduct in soln. were determined using a calibration curve [122]. The loading (Subst.) was then calculated according to *Eqn. 1*:

$$\text{Subst}_n\ [\text{mmol/g resin}] = c_n \cdot V_n / \{m_n(\text{resin}) - [c_n \cdot V_n \cdot (\text{MW}-18)/1000]\} \quad (1)$$

(MW = molecular weight of the Fmoc-protected β-amino acid)

The yield for the attachment to the resin (loading yield) was determined by *Eqn. 2*:

$$\text{Loading yield} = [(\text{Subst}_1 + \text{Subst}_2)/2]/\text{Subst}_{\text{theor.}} \quad (2)$$

3.2. *Capping. General Procedure 4* (*GP 4*). *GP 4a.* The peptide-resin was covered with DMF (20 ml/g resin), and unreacted OH groups were capped by using Ac_2O (10 equiv.) and DMAP (0.1 equiv.) dissolved in DMF (0.1 ml/mmol Ac_2O) for 1–2 h under Ar bubbling. The resin was then washed with DMF (20 ml/g resin, 5×1 min) and with CH_2Cl_2 (20 ml/g resin, 5×1 min).

GP 4b. As in *GP 4a*, except that Ac_2O (1 ml, 9.1 mmol) in DMF (5 ml) and DMAP (60 mg, 0.5 mmol, added in 0.5 ml DMF) were used.

3.3. *Fmoc-Deprotection. General Procedure 5* (*GP 5*). *GP 5a*. The Fmoc deprotection was carried out using 20% piperidine in DMF (20 ml/g resin, 2×10 min), DBU/piperidine/DMF 1:1:48 (20 ml/g resin, 3×10 min), and 20% piperidine in DMF (20 ml/g resin, 1×10 min) under Ar bubbling. After filtration, the resin was washed with DMF (20 ml/g resin, 5×1 min) and CH_2Cl_2 (20 ml/g resin, 5×1 min).

GP 5b. The Fmoc group was removed by treating the resin with 20% piperidine in DMF (4 ml, 4× 10 min) under Ar bubbling. After filtration, the resin was washed with DMF (5 ml, 4×1 min).

GP 5c. As in *GP 5b*, except that a soln. of 2% DBU, 2% piperidine in DMF/sat. LiCl soln. (5 ml, 4×10 min) was used.

3.4. *Coupling of the β-Amino Acids on* Wang *Resin. General Procedure 6* (*GP 6*). *GP 6a.* The Fmoc deprotection was carried out according to *GP 5a*. Solid-phase synthesis was continued by sequential incorporation of Fmoc-protected amino acids. For each coupling step, the resin was treated with a soln. of the Fmoc-protected β^3-amino acid (3 equiv.), HATU (2.9 equiv.), and $EtN(i\text{-}Pr)_2$ (6 equiv.) in DMF/CH_2Cl_2 1:1 (20 ml/g resin) for 30–60 min. Monitoring of the coupling reaction was performed with the TNBS test [123]. In the case of a positive TNBS test (indicating incomplete coupling), the suspension was allowed to react further for 0.5–4 h or, after filtration, the peptide-resin was treated again with the same Fmoc-protected amino acid (1–3 equiv.), and with the coupling reagents. When Fmoc-protected β^2-amino acids were employed, 2 equiv. of amino acid were used and coupled 3× for 30 min each, in order to prevent epimerization. The resin was then filtered and washed with DMF (20 ml/g resin, 5×1 min) and CH_2Cl_2 (20 ml/g resin, 5×1 min) prior to the following deprotection step. For the last coupling either Boc- or Fmoc-protected β^3-amino acids were used. In the second case, the Fmoc group of the last amino acid was removed as previously described, and the resin was washed with DMF (20 ml/g resin, 5×1 min) and with CH_2Cl_2 (20 ml/g resin, 5×1 min), and dried under h.v. for 2 h.

GP 6b. Coupling was performed as in *GP 6a*, except that *i*) the amino acid was dissolved in the minimal amount of DMF, *ii*) in the case of a positive TNBS test, the suspension was filtered, and treated again with a freshly prepared soln. of the same *N*-Fmoc amino acid (1.5 equiv.) and coupling reagents, *iii*) the resin was only washed with DMF (5 ml, 4×1 min), and *iv*) the same conditions were used for β^2- and β^3-amino acids.

GP 6c. As in *GP 6b*, except that 4 equiv. of the *N*-Fmoc-protected amino acid or the peptide derivative, 3.7 equiv. of HATU and 8 equiv. of DIPEA were used.

3.5. Wang *Resin Cleavage and Final Deprotection. General Procedure 7 (GP 7). GP 7a.* The cleavage from the resin and the peptide deprotection were performed according to [124]. The dry peptide-resin was suspended in a soln. of $TFA/H_2O/(i\text{-}Pr)_3SiH$ 95:2.5:2.5 (10 ml) for 2 h. The resin was removed by filtration, washed with TFA (2×), and the org. phase concentrated under reduced pressure. The resulting oily residue was treated with cold Et_2O, and the formed precipitate separated. The crude β-peptide was dried under h.v. and stored at −20° before purification.

GP 7b. As in *GP 7a*, except that a soln. of $TFA/H_2O/EDT/(i\text{-}Pr)_3SiH$ 94:2.5:2.5:1 (10 ml) was used.

3.6. *HPLC Analysis and Purification of β-Peptides. General Procedure 8* (*GP 8*). RP-HPLC Analysis was performed on a *Macherey-Nagel* C_8 column (*Nucleosil 100-5* C_8 (250×4 mm)) by using a linear gradient of *A*: 0.1% TFA in H_2O and *B*: MeCN at a flow rate of 1 ml/min with UV detection at 220 nm. t_R in min. Crude products were purified by prep. RP-HPLC on a *Macherey-Nagel* C_8 column (*Nucleosil 100-7* C_8 (250×21 mm)) using gradient of *A* and *B* at a flow rate of 17–20 ml/min with UV detection at 220 nm and then lyophilized.

*H-(*R*)-β³hSer-(*S*)-β³hAla-(*R*)-β³hAsp-(*S*)-β³hOrn-(*S*)-β³hLeu-(*S*)-β³hHis-(*S*)-β³hGlu-(*R*)-β³hIle-(*S*)-β³hHis-(*S*)-β³hLys-OH* (**1**). Fmoc-(*S*)-β^3hLys(Boc)-OH (651 mg, 1.35 mmol) was loaded onto the *Wang* resin (300 mg, 0.9 mmol/g, 100–200 mesh) according to *GP 3* for 2 h. Loading was estimated as 0.75 mmol/g (83%), corresponding to 0.225 mmol of Fmoc-(*S*)-β^3hLys(Boc)-OH. After capping (*GP 4a*), the peptide synthesis was performed according to *GP 6a*. Treatment of the peptide-resin according to *GP 7a* afforded the crude peptide **1** (419 mg) as a TFA salt. Purification of a part of the crude peptide (20 mg) by RP-HPLC (5–15% *B* in 40 min, 18 ml/min) according to *GP 8* yielded the TFA salt of **1** (10 mg). Calculated overall yield 50%. White solid. RP-HPLC (10–20% *B* in 40 min) t_R 35.29, purity >98%. CD (0.14 mM in MeOH): $+2.2\cdot10^5$ (196.5 nm); $-9.1\cdot10^4$ (215 nm). IR (neat): 3277*w*, 3086*w*, 2962*w*, 2636*w*, 2324*w*, 1644*s*, 1538*m*, 1434*m*, 1386*w*, 1180*s*, 1127*s*, 974*w*, 837*m,* 798*m*, 721*s*. MALDI-TOF-MS: 1392.14 (20), 1369.31 (38), 1347.32 (54), 1325.37 (100), 1303.37 (74).

*H-(*S*)-β³hHis-(2*R*,3*S*)-β²,³hAla(αMe)-(2*R*,3*S*)-β²,³hAla(αMe)-(*S*)-β²hVal-(*S*)-β³hLys-(2*R*,3*S*)-β²,³h-Ala(αMe)-(2*R*,3*S*)-β²,³hAla(αMe)-(*R*)-β³hCys-OH* (**2**). The first β-amino acid Fmoc-(S)-β³hCys(Tr)-OH (600 mg, 1.00 mmol) was loaded onto the *Wang* resin (200 mg, 1 mmol/g, 0.2 mmol) according to *GP 3* for 2 h. Loading was estimated as 0.57 mmol/g (57%), corresponding to 0.114 mmol of anchored Fmoc-(*S*)-β³hCys(Tr)-OH. Then the resin was dried under h.v. overnight. Loaded *Wang* resin (160 mg, 0.057 mmol) was swelled in DMF for 1 h, capping was performed (*GP 4b*), and Fmoc-deprotected according to *GP 5b*. The second amino acid (2*R*,3*S*)-β²,³hAla(αMe)-OH (**6**) was coupled according to *GP 6c* and Fmoc-deprotected according to *GP 5b*. The third amino acid **6** was coupled according to *GP 6c* and Fmoc-deprotected according to *GP 5b*. The dipeptide **7** was coupled according to *GP 6c*, but, after 1.5 h, the TNBS test was still postive (indicating incomplete coupling). The coupling soln. was filtered off, and the resin was washed with 1 ml of DMF. The combined solns. were treated with HATU (80 mg, 0.21 mmol, 3.7 equiv.), EtN(i-Pr)$_2$ (0.04 ml, 0.234 mmol, 4 equiv.) and added to the resin. After 2 h, TNBS test was still positive. The resin was filtered off, washed with DMF (4 ml, 4×1 min), CH_2Cl_2 (4 ml, 4×1 min), and dried for 2 h at h.v. Then, the resin was transferred to a flask and preswelled in 5 ml of 0.8M LiCl soln. in DMF for 1 h. The soln. was removed from the resin, and dipeptide **7** (102 mg, 0.171 mmol, 3 equiv.) was dissolved in a minimum amount of 0.8M LiCl soln. in DMF, treated with 2.8 equiv. of HATU (61 mg, 0.16 mmol, 2.8 equiv.), 6 equiv. of EtN(i-Pr)$_2$ (0.06 ml, 0.35 mmol, 6 equiv.), and added to the resin. The soln. was heated to 50° under shaking for 4 h. The TNBS test was negative, the resin was transferred to the reactor and filtered. After washing with DMF (4×4 ml), the growing peptide was Fmoc-deprotected according to *GP 5b*. The tripeptide **8** was coupled according *GP 6c* using only 3 equiv. of **8** (121 mg, 0.17 mmol), HATU (61 mg, 0.16 mmol, 2.8 equiv.), and EtN-(i-Pr)$_2$ (0.06 ml, 0.35 mmol, 6 equiv.). After 2 h, the TNBS test was still positive. The resin was transferred to a flask and preswelled in 5 ml of 0.8M LiCl soln. in DMF for 1 h. The soln. was removed from the resin, and tripeptide **8** (121 mg, 0.17 mmol, 3 equiv.) was dissolved in 3 ml of 0.8M LiCl soln. in DMF, treated with HATU (61 mg, 0.16 mmol, 2.8 equiv.), EtN(i-Pr)$_2$ (0.06 ml, 0.35 mmol, 6 equiv.), and added to the resin. After addition of 1 ml of DMF, the soln. was heated to 50° under shaking for 4 h. The TNBS test was negative, and the resin was transferred to the reactor and filtered. After washing with DMF (4×4 ml) and CH_2Cl_2 (4×4 ml), the resin was dried under h.v. overnight. Treatment of the peptide-resin according to *GP 7b* afforded the crude peptide **2** (104 mg) as a TFA salt. Purification of a part of the crude peptide (73 mg) by RP-HPLC (5–95% *B* in 50 min: 5 min 5% *B*; 40 min 30% *B*; 50 min 95% *B*; 17 ml/min; t_R 18.7 min) according to *GP 8* yielded the TFA salt of **2** (14.6 mg). Calc. overall yield 29%. White solid. RP-HPLC (5–99% *B* in 30 min: 5 min 5% *B*; 20 min 30% B; 30 min 99% B; t_R 15.06 min), purity >95%. MALDI-HR-MS: 938.5839 ($[M+H]^+$, $C_{44}H_{80}N_{11}O_9S^+$: 938.5856).

*H-(*S*)-β³hHis-(*S*)-β³hAla-(*R*)-β³hVal-(*S*)-β²hVal-(*S*)-β³hLys-(*S*)-β³hLeu-(*R*)-β³hSer-(*R*)-β³hCys-OH* (**3**). The first β-amino acid Fmoc-(*S*)-β³hCys(Tr)-OH (1.95 g, 3.25 mmol) was loaded onto the *Wang* resin (700 mg, 0.65 mmol) according to *GP 3* for 2.5 h. Loading was estimated as 0.63 mmol/g (68%), corresponding to 0.441 mmol of anchored Fmoc-(*S*)-β³hCys(Tr)-OH. After capping (*GP 4b*), the resin was dried under h.v. overnight. Loaded *Wang* resin (370 mg, 0.148 mmol) was swelled in DMF for 1 h and Fmoc-deprotected according to *GP 5b*. The second amino acid (Fmoc-(*R*)-β³hSer(tBu)-OH) was coupled according to *GP 6b* and Fmoc-deprotected according to *GP 5b*. The third amino acid (Fmoc-(*S*)-β³hLeu-OH) was coupled according to *GP 6b* and Fmoc-deprotected according to *GP 5b*. The fourth amino acid (Fmoc-(*S*)-β³hLys(Boc)-OH) was coupled according to *GP 6b* and Fmoc-deprotected according to *GP 5b*. The fifth amino acid (Fmoc-(*S*)-β²hVal-OH) was coupled according to *GP 6b* and Fmoc-deprotected according to *GP 5c*. The sixth amino acid (Fmoc-(*R*)-β³hVal-OH) was coupled according to *GP 6b* and Fmoc-deprotected according to *GP 5b*. The seventh amino acid (Fmoc-(*S*)-β³hAla-OH) was coupled according to *GP 6b* and Fmoc-deprotected according to *GP 5c*. The eighth amino acid (Fmoc-(*S*)-β³hHis(Tr)-OH) was coupled according to *GP 6b* for 2 h, but a positive TNBS test indicated incomplete coupling. Thus, another coupling was performed with 1.5 equiv. of this amino acid. Fmoc-Deprotection was carried out according to *GP 5c*. After washing with DMF (5 ml, 4×1 min), CH_2Cl_2 (5 ml, 4×1 min), and MeOH (4 ml, 4×1 min), the resin was dried under h.v. for 24 h. Treatment of the peptide-resin according to *GP 7b* afforded the crude peptide **3** (165 mg) as a TFA salt. Purification of a part of the crude peptide (40 mg) by RP-HPLC (5–95% *B* in 40 min: 5 min 5% *B*; 10 min 20% *B*; 30 min 27% *B*; 40 min 95% *B*; 18 ml/min; t_R 17.9 min) according to *GP 8* yielded

the TFA salt of **3** (18 mg) in >98% purity. Calc. overall yield 38%. White solid. ESI-HR-MS (pos. mode): 968.5961 ($[M+H]^+$, $C_{45}H_{82}N_{11}O_{10}S^+$; calc. 968.5967); 484.8017 ($[M+2\,H]^{2+}$, $C_{45}H_{83}N_{11}O_{10}S^{2+}$: calc. 484.8002).

*H-(*S*)-β³hHis-(*R*)-β²hAla-(*R*)-β³hVal-(*S*)-β²hVal-(*S*)-β³hLys-(*R*)-β²hLeu-(*R*)-β³hSer-(*R*)-β³hCys-OH* (**4**). The first β-amino acid Fmoc-(*S*)-β³hCys(Tr)-OH (1.95 g, 3.25 mmol) was loaded onto the *Wang* resin (700 mg, 0.65 mmol) according to *GP 3* for 2.5 h. Loading was estimated as 0.63 mmol/g (68%), corresponding to 0.441 mmol of anchored Fmoc-(*S*)-β³hCys(Tr)-OH. After capping (*GP 4b*), the resin was dried under h.v. overnight. Loaded *Wang* resin (370 mg, 0.148 mmol) was swelled in DMF for 1 h and Fmoc-deprotected according to *GP 5b*. The second amino acid (Fmoc-(*R*)-β³hSer(tBu)-OH) was coupled according to *GP 6b* and Fmoc-deprotected according to *GP 5b*. The third amino acid (Fmoc-(*R*)-β²hLeu-OH) was coupled according to *GP 6b* and Fmoc-deprotected according to *GP 5b*. The fourth amino acid (Fmoc-(*S*)-β³hLys(Boc)-OH) was coupled according to *GP 6b* and Fmoc-deprotected according to *GP 5b*. The fifth amino acid (Fmoc-(*S*)-β²hVal-OH) was coupled according to *GP 6b* and Fmoc-deprotected according to *GP 5c*. The sixth amino acid (Fmoc-(*R*)-β³hVal-OH) was coupled according to *GP 6b* and Fmoc-deprotected according to *GP 5b*. The seventh amino acid (Fmoc-(*R*)-β²hAla-OH) was coupled according to *GP 6b* and Fmoc-deprotected according to *GP 5c*. The eighth amino acid (Fmoc-(*S*)-β³hHis(Tr)-OH) was coupled according to *GP 6b* for 2 h and Fmoc-deprotected according to *GP 5b*. After washing with DMF (5 ml, 4×1 min), CH_2Cl_2 (5 ml, 4×1 min), and MeOH (4 ml, 4×1 min), the resin was dried under h.v. for 24 h. Treatment of the peptide-resin according to *GP 7b* afforded the crude peptide **4** (145 mg) as a TFA salt. RP-HPLC Purification of a part of the crude peptide (50 mg) according to *GP 8* (5–95% *B* in 40 min: 5 min 5% *B*; 10 min 17% *B*; 30 min 23% *B*; 40 min 95% *B*; 17 ml/min; t_R 15.2 min) yielded peptide **4** (20 mg) in 90% purity as its TFA salt. A further RP-HPLC purification of 10 mg of the above obtained peptide **4** according to *GP 8* but using a *Macherey-Nagel C₁₈* column (*Nucleosil 100-7 C₁₈* (250×21 mm)) and with only 0.5-mg injections (5–95% *B* in 40 min: 2 min 5% *B*; 8 min 15% *B*; 30 min 20% *B*; 40 min 95% *B*; 17 ml/min; t_R 16.7 min) afforded peptide **4** (3 mg) in >98% purity. Calc. overall yield 9%. White solid. ESI-HR-MS (pos. mode): 484.8017 ($[M+2\,H]^{2+}$, $C_{45}H_{83}N_{11}O_{10}S^{2+}$; calc. 484.8002).

H-(S)-β³hHis-(R)-β³hAla-(R)-β³hVal-(S)-β²hVal-(S)-β³hLys-(S)-β³hLeu-(S)-β³hSer-(R)-β³hCys-OH (**5**). Fmoc-(*R*)-β³hCys(Tr)-OH (809 mg, 1.35 mmol) was loaded onto the *Wang* resin (300 mg, 0.9 mmol/g, 100–200 mesh) according to *GP 3* for 2 h. Loading was estimated as 0.67 mmol/g (74%), corresponding to 0.20 mmol of Fmoc-(*R*)-β³hCys(Trt)-OH. After capping (*GP 4a*), peptide synthesis was performed according to *GP 6a*. Treatment of the peptide-resin according to *GP 7b* afforded the crude peptide **5** (267 mg) as a TFA salt. Purification of the crude peptide (30 mg) by RP-HPLC (5–20% *B* in 60 min, 20 ml/min) according to *GP 8* yielded the TFA salt of **5** (13 mg, 44%). White solid. RP-HPLC (5–20% *B* in 50 min): t_R 40.03, purity >98%. CD (0.165 mM in MeOH): $+9.5\cdot10^4$ (202 nm). IR (neat): 3281*w*, 3080*w*, 2962*w*, 2920*w*, 2851*w*, 2636*w*, 2315*w*, 1642*s*, 1544*m*, 1380*w*, 1303*w*, 1200*s*, 1132*s*, 969*w*, 836*w*, 798*w*, 721*m*. MALDI-MS: 1012 (15), 990 (44), 968 (100).

H-(R)-β³hAla-(R)-β³hCys-(S)-β²hVal-(R)-β³hCys-(R)-β³hVal-β³hGly-(S)-β³hArg-β³hGly-(R)-β³hAsp-(S)-β³hLys-(S)-β³hLeu-(S)-β³hHis-(S)-β³hAsn-(R)-β³hVal-(S)-β³hHis-(S)-β³hLys-OH (**10**). The first β-amino acid Fmoc-(*S*)-β³hLys(Boc)-OH (1.3 g, 2.7 mmol) was loaded onto the *Wang* resin (660 mg, 0.82 mmol/g, 100–200 mesh) according to *GP 3* for 2×2 h. Loading was estimated as 0.56 mmol/g (68%), corresponding to 0.37 mmol of anchored Fmoc-(*S*)-β³hLys(Boc)-OH. After capping (*GP 4a*), the resin was dried under h.v. for 12 h. Thereafter, the resin was weighed and divided into two equal parts. The peptide synthesis was performed according to *GP 6a* with 350 mg of resin (0.185 mmol). Starting from the eighth amino acid, double couplings followed by cappings with Ac_2O/DMAP was performed. Treatment of the peptide-resin according to *GP 7b* afforded the crude peptide **10** (520 mg) as a TFA salt. Purification of a part of the crude peptide (60 mg) by RP-HPLC (5–18% *B* in 50 min, 20 ml/min) according to *GP 8* yielded the TFA salt of **10** (23 mg). Calc. overall yield 41%. White solid. RP-HPLC (5–22% *B* in 46 min): t_R 39.28, purity >98%. CD (0.2 mM in MeOH): $+6.8\cdot10^4$ (202 nm); $-8.8\cdot10^3$ (223 nm). IR (neat): 3280*w*, 3080*w*, 2961*w*, 2920*w*, 2851*w*, 2636*w*, 2362*w*, 1644*s*, 1540*s*, 1427*m*, 1385*w*, 1266*w*, 1199*s*, 1179*s*, 1131*s*, 837*w*, 799*w*, 721*m*. MALDI-HR-MS: 1960.1367 ($[M+H]^+$, $C_{88}H_{155}N_{26}O_{20}S_2^+$; calc. 1960.1347).

4. *NMR Measurements.* 4.1. *General Remarks.* NMR Spectra of the free peptide **2** were collected in aq. soln. (H_2O, 10% D_2O, pH 5.4; see *Tables 1* and *2*) and in aq. soln. in the presence of $ZnCl_2$ (D_{11})*Tris* buffer + 1 equiv. $ZnCl_2$ + 10% D_2O, pH 5.4; see *Tables 3* and *4*). The free peptide **3** was measured in CD_3OH (see *Tables 5* and *6*) and in aq. soln. (D_{11})*Tris* buffer + 1 equiv. $ZnCl_2$ + 10% D_2O, pH 7.2; see *Tables 7* and *8*). Peptide **5** was measured in CD_3OH (see *Tables 9* and *10*) and H_2O (KH_2PO_4 buffer + 10% D_2O, pH 7.6; see *Tables 11* and *12*) solns., where as the complexation behavior was investigated in H_2O solution (D_{11})*Tris* buffer + 1 equiv. $ZnCl_2$ + 10% D_2O, pH 7.2; see *Tables 13* and *14*).

Table 1. *^{1}H- and ^{13}C-NMR Data and Assignments for β-Octapeptide* **2** *in H_2O* (n.a.: not available)

β-Amino acid	NH	$CH_2(\alpha)$	H–C(β) (^3J(HN,Hβ))	H–C(γ), Me–C(γ), $CH_2(\gamma)$	H–C(δ), Me–C(δ)	Me–C(ε)	C(α)	C(β)	C(γ)	C(δ)	C(ε)
β^3hHis[1]		2.67	3.94	3.15			39.32	50.45	30.15		
β^3-2MehAla[2]	8.04	*2.36*	3.9 (9.54)	1.02			48.5	50.30	16.5		
β^3-2MehAla[3]	8.06	2.3	3.95 (9.32)	1.01			49.03	50.35	20.9		
β^2hVal[4]	7.89	2.1	3.29/3.41	1.76	0.93		55.65	42.19	31.18	n.a.	
β^3hLys[5]	8.07	2.35	4.1 (8.8)	1.49	1.4	1.62	48.8	49.78	n.a.		29.14
β^3-2MehAla[6]	8.0	2.34	3.96 (9.17)	1.08			44.45	50.40	n.a.		
β^3-2MehAla[7]	8.09	2.33	3.98 (9.24)	1.1			44.26	50.42	20.9		
β^3hCys[8]	8.1	2.49	4.25 (8.95)	2.74			41.55	51.7	30.58		

Table 2. *NOE Distance Restraints for β-Octapeptide* **2** *in H_2O*

Residue	H-Atom[a])	Residue	H-Atom[a])	d_{NOE} [Å]	Residue	H-Atom[a])	Residue	H-Atom[a])	d_{NOE} [Å]
1	α*	2	β	2.6	8	β	8	NH	3.0
2	γ	2	HN	3.1	8	α	8	NH	3.2
4	α	4	β_{Si}	2.6	1	α*	2	NH	2.7
4	α	4	β_{Re}	2.9	1	α*	2	γ*	4.1
4	α	4	δ	3.1	2	α	3	NH	2.6
4	α	4	γ	3.0	3	α	4	NH	2.4
4	α	4	HN	3.1	4	γ	5	NH	3.4
4	γ	4	β_{Si}	2.9	5	α*	6	NH	2.6
4	NH	4	β_{Si}	3.0	5	β	6	NH	3.0
4	NH	4	β_{Re}	2.8	7	β	8	NH	3.2
4	NH	4	γ	3.6	7	α	8	NH N	2.4
5	α*	5	β	2.6	4	NH	6	β	3.4
5	β	5	ε*	3.2	3	α	6	β	2.5
5	β	5	HN	3.0	2	α	7	β	2.6
8	β	8	γ	3.0					

[a]) * = Pseudoatom used for calculations.

NMR Spectra of peptides **3** and **5** were acquired at 500 MHz (^{1}H)/125 MHz (^{13}C) and peptide **2** at 600 MHz (^{1}H)/150.9 MHz (^{13}C) with presaturation of the solvent signal; 90-K data points, 128 scans, 5.6-s acquisition time. {1H}-BB-decoupled ^{13}C-NMR: 80-K data points, 20-K scans, 1.3-s acquisition time, 1-s relaxation delay, 45° excitation pulse. Processed with 1.0-Hz exponential line broadening. The following spectra were used for the resonance assignments: [^{1}H,^{1}H]-DQF-COSY, [^{1}H,^{1}H]-TOCSY, [^{13}C,^{1}H]-HSQC, [^{13}C,^{1}H]-HMBC, [^{1}H,^{1}H]-ROESY. 2D-NMR: Solvent suppression with presat. DQF-COSY (500 MHz, CD_3OH) with pulsed-field gradients (PFG) for coherence pathway selection: acquisition: 2K(*t2*) × 512 (*t1*) data points. 10 scans per *t1* increament, 0.17-s acquisition time in *t2*; relax-

Table 3. *^{1}H- and ^{13}C-NMR Data and Assignments for β-Octapeptide* **2** *in $H_2O + ZnCl_2$*

β-Amino acid	NH	$CH_2(\alpha)$	H–C(β) (^3J(NH,Hβ))	H–C(γ), Me–C(γ), $CH_2(\gamma)$	H–C(δ), Me–C(δ)	Me–C(ε)	C(α)	C(β)	C(γ)	C(δ)	C(ε)
β^3hHis[1]		2.67	3.92	3.14			39.45	50.42	30.32		
β^3-2MehAla[2]	8.06	2.35	3.94 (9.17)	1.02			49.3	50.3	16.6		
β^3-2MehAla[3]	8.058	2.34	3.95 (9.32)	1.03			49.09	50.5			
β^2hVal[4]	7.9	2.17	3.28/3.43	1.76	0.87/0.93		55.81	42.12	31.12	22.23	
β^3hLys[5]	8.07	2.36	4.1 (8.66)	1.3/1.49	1.33/1.41	1.61	44.27	49.71	n.a		29.12
β^3-2MehAla[6]	8.04	2.35	3.96 (9.32)	1.05			49.1	50.25	20.92		
β^3-2MehAla[7]	8.0	2.37	3.98 (9.46)	1.11			49.2	50.2	20.94		

Table 4. *NOE Distance Restraints for β-Octapeptide* **2** *in H_2O with $ZnCl_2$*

Residue	H-Atom[a])	Residue	H-Atom[a])	d_{NOE} [Å]	Residue	H-Atom[a])	Residue	H-Atom[a])	d_{NOE} [Å]
1	α*	1	β	2.7	7	β	7	γ*	2.8
2	β	2	NH	3.0	7	γ*	7	NH	3.0
3	β	3	NH	3.0	8	α*	8	NH	2.8
4	α	4	β_{Si}	2.5	8	β	8	NH	3.0
4	α	4	β_{Re}	2.7	8	β	8	NH	3.2
4	α	4	δ*	2.9	1	α*	2	NH	2.7
4	α	4	γ	3.1	4	α	5	NH	2.2
4	α	4	NH	3.0	4	γ	5	NH	3.6
4	γ	4	β_{Si}	2.7	4	NH	3	α	2.2
4	NH	4	β_{Si}	2.8	5	β	6	γ	3.8
4	NH	4	β_{Si}	2.8	6	NH	5	β	3.0
4	NH	4	γ	3.9	7	γ*	8	β	4.0
4	α	5	β	2.7	8	NH	7	γ*	3.5
5	β	5	δ*	3.6	4	NH	6	β	3.6
5	ε*	5	NH	4.3	6	β	3	α	2.7
6	β	6	NH	3.0					

[a]) * = Pseudoatom used for calculations.

Table 5. *^{1}H- and ^{13}C-NMR Data and Assignments for β-Octapeptide* **3** *in MeOH* (n.a.: not available)

β-Amino acid	NH	$CH_2(\alpha)$	H–C(β) (^3J(NH,Hβ))	H–C(γ), Me–C(γ), $CH_2(\gamma)$	H–C(δ), Me–C(δ)	Me–C(ε)	C(α)	C(β)	C(γ)	C(δ)
β^3hHis[1]		2.58/2.87	3.95	3.03			39.41	51.26	31.5	
β^3hAla[2]	8.5	2.49/2.82	4.57 (9.1)	1.2			43.39	45.83	21.29	
β^3hVal[3]	8.2	2.43/2.81	4.04 (9.54)	1.74	0.92		38.82	53.57	n.a	23.28
β^2hVal[4]	8.29	2.76	2.9/3.9 (9.1)	1.75	0.95/1.0		53.02	40.61	31.1	21.32
β^3hLys[5]	8.36	2.44/2.56	4.42 (8.8)	1.64	1.49	1.66	40.7	47.78	28.95	24.13
β^3hLeu[6]	7.6	2.28	4.56 (9.46)	1.31/1.42	1.59	0.91	42.6	n.a.	n.a.	29.84
β^3hSer[7]	7.69	2.38/2.64	4.39 (8.6)	3.41/3.54			38.44	49.69	64.83	
β^3hCys[8]	7.92	2.46/2.69	4.62 (9.32)	2.85/2.96			n.a	48.08		

Table 6. *NOE Distance Restraints for β-Octapeptide* **3** *in MeOH*

Residue	H-Atom[a])	Residue	H-Atom[a])	d_{NOE} [Å]	Residue	H-Atom[a])	Residue	H-Atom[a])	d_{NOE} [Å]
1	β	1	α*	2.6	8	β	8	NH	3.0
2	β	2	γ*	2.9	8	γ*	8	NH	3.0
2	β	2	NH	2.9	1	α*	2	NH	2.5
2	γ*	2	NH	3.0	5	NH	4	α	2.2
3	β	3	γ	2.6	5	α*	6	NH	3.2
3	β	3	NH	2.9	6	α*	7	NH	2.5
3	α*	3	NH	2.3	7	α*	8	NH	2.8
3	γ	3	NH	3.1	2	γ*	5	β	2.9
4	α	4	γ	2.8	2	NH	5	β	3.0
4	β*	4	NH	2.7	5	NH	6	β	3.0
5	α*	5	NH	2.9	4	α	7	β	2.2
5	β	5	NH	2.9	4	β*	1	α*	2.9
5	β	5	γ*	2.4	5	β	7	β	3.1
6	β	6	γ*	2.6	4	NH	7	β	2.9
7	β	7	α*	2.6	5	β	2	α*	2.4
7	β	7	γ*	2.8	5	δ*	8	β	3.8
7	β	7	NH	3.0	5	NH	8	β	3.3
7	γ*	7	NH	3.4	6	β	3	α*	2.9
7	α*	7	NH	2.9	8	β	5	α*	3.2
8	β	8	γ	2.7					

[a]) * = Pseudoatom used for calculations.

Table 7. *^{1}H- and ^{13}C-NMR Data and Assignments for β-Octapeptide* **3** *in $H_2O + ZnCl_2$*

β-Amino acid	NH	$CH_2(\alpha)$	H–C(β) (^3J(HN,Hβ))	H–C(γ), Me–C(γ), $CH_2(\gamma)$	H–C(δ), Me–C(δ)	Me–C(ε)	C(α)	C(β)	C(γ)	C(δ)
β^3hHis[1]			3.56	2.74/2.89			44.12	51.72	32.3	
β^3hAla[2]	7.95	2.38	4.19	1.14			40.17	46.25	22.18	
β^3hVal[3]	7.89	2.26/2.44	3.99	1.69	0.82		41.12	55.01	34.16	23.68
β^2hVal[4]	7.92	2.11	3.12/3.49	1.74	0.85/0.92		55.98	44.40	30.96	22.46/25.09
β^3hLys[5]	8.03	2.36	4.14 (8.95)	1.48	1.25/1.33	1.62	41.14	49.54	35.42	24.85
β^3hLeu[6]	7.99	2.3	4.2 (9.1)	1.25/1.41	1.53	0.81/0.86	44.64	48.31	24.81	26.9
β^3hSer[7]	7.97	2.45	4.18	3.52/3.59			44.07	51.58	65.41	
β^3hCys[8]	7.85	2.52	4.05 (9.54)	2.61			43.74	53.80	31.74	

ation delay 2.0 s. TPPI quadrature detection in ω_1. Processing: zero filling and FT to 1K × 1K real/real data points after multiplication with a $\sin^2$ filter shifted by $\pi/2$ in ω_1. HSQC with PFG (500, 125 MHz, CD_3OH): acquisition: 2K(*t2*) × 512 (*t1*) data points, 48 scans per *t1*-increment. ^{13}C-GARP decoupling during *t2*. 0.17-s acq. time in *t2*. Processing: zero filling and FT to 1K × 1K real/real data points after multiplication with a $\sin^2$ filter shifted by $\pi/2$ in *t1* and sin filter shifted by $\pi/2$ in *t2*. HMBC with PFG (500, 125 MHz, CD_3OH): acquisition: delay for evolution of long-range antiphase magn.: 50 ms. No ^{13}C decoupling, otherwise identical to parameters for HSQC. Processing: zero filling and FT to 1K × 1K after multiplication with a $\cos^2$ filter in *t2* and a Gaussian filter in *t1*; power spectrum in both dimensions. ROESY [125] (500 MHz, CD_3OH): acquisition: a ROESY spectrum with a mixing time of 300 ms was acquired. CW-spin lock (2.7 kHz) between trim pulses, 2K(*t2*) × 512 (*t1*) data points, 64 scans per *t1* increment.

Table 8. *NOE Distance Restraints for β-Octapeptide* **3** *in* $H_2O + ZnCl_2$

Residue	H-Atom[a])	Residue	H-Atom[a])	d_{NOE} [Å]	Residue	H-Atom[a])	Residue	H-Atom[a])	d_{NOE}[Å]
1	α*	1	β	2.5	6	γ*	6	NH	3.2
1	β	1	γ*	2.7	7	β	7	NH	3.0
1	β	1	ε*	4.6	7	α*	7	NH	2.6
2	β	2	γ*	2.6	7	α*	7	β	3.1
2	β	2	α*	2.8	7	γ*	7	NH	2.9
2	β	2	NH	3.0	7	γ*	7	β	2.6
3	β	3	α*	2.8	8	β	8	α*	2.7
3	β	3	γ	2.6	8	α*	8	NH	2.6
3	α*	3	NH	2.7	8	β	8	NH	3.0
3	β	3	NH	3.0	8	β	8	α*	2.5
4	α	4	β*	2.6	8	γ*	8	NH	2.8
4	α	4	γ	2.8	1	α*	2	NH	2.6
4	α	4	NH	3.0	2	γ*	3	NH	4.6
4	β*	4	γ	2.7	2	β	3	NH	4.2
4	β*	4	NH	3.0	2	α*	3	NH	2.6
4	β*	4	δ*	3.5	3	β	4	NH	4.2
4	γ	4	NH	3.8	3	α*	4	NH	2.6
5	α*	5	NH	3.1	4	α	5	NH	2.5
5	β	5	NH	3.0	4	γ	5	NH	3.4
5	γ*	5	NH	2.6	5	NH	4	β*	4.4
5	β	5	α*	2.7	5	β	6	NH	3.8
5	β	5	NH	3.0	5	α*	6	NH	2.7
6	β	6	γ*	2.8	6	α*	7	NH	2.8
6	δ	6	NH	3.2	7	NH	6	β	4.2
6	β	6	α*	3.2	3	NH	7	β	3.2
6	α*	6	NH	2.6	2	NH	8	β	3.9
6	β	6	γ*	2.5	2	NH	8	γ*	3.8
6	β	6	NH	3.0	1	γ*	8	β	4.1

[a]) * = Pseudoatom used for calculations.

Table 9. *^{1}H- and ^{13}C-NMR Data and Assignments for β-Octapeptide* **5** *in MeOH*

β-Amino acid	NH	$CH_2(\alpha)$	H–C(β) (^3J(NH,Hβ))	H–C(γ), Me–C(γ), $CH_2(\gamma)$	H–C(δ), Me–C(δ)	Me–C(ε)	C(α)	C(β)	C(γ)	C(δ)	C(ε)
β^3hHis[1]		2.59	3.90	3.15			37.95	49.87	21.29		
β^3hAla[2]	8.18	2.40	4.27 (8.1)	1.0		1.20	43.40	44.75	20.83		
β^3hVal[3]	7.83	2.50/2.33	4.15 (9.7)	1.74		0.90	40.01	53.85	33.7	19.89	
β^2hVal[4]	8.09	2.04	3.61/3.15	1.82		0.99/0.93	55.32	41.08	29.65	20.76	
β^3hLys[5]	8.01	2.42/2.31	4.24 (8.9)	1.56	1.44	2.92	42.71	48.46	35.2	23.98	40.91
β^3hLeu[6]	7.91	2.36	4.32 (9.2)	1.49/1.28	1.678	0.92	38.98	46.68	44.92	28.36	
β^3hSer[7]	7.86	2.48	4.30 (8.8)	3.56			38.92	50.36	64.65		
β^3hCys[8]	8.10	2.55	4.27 (8.9)	2.70			38.12	50.38	29.08		

0.17-s acquisition time in *t2*, other parameters identical to DQF-COSY. Processing: zero filling and FT to 1K × 512K real/real data points after multiplication by a $\cos^2$ filter in *t2* and *t1*. Baseline correction with 3rd degree polynomial in both dimensions.

Table 10. *NOE Distance Restraints for β-Octapeptide* **5** *in d³-MeOH*

Residue	H-Atom[a])	Residue	H-Atom[a])	d_{NOE} [Å]	Residue	H-Atom[a])	Residue	H-Atom[a])	d_{NOE} [Å]
1	α*	1	γ*	3.2	7	NH	7	β	2.7
1	β	1	α*	2.3	7	β	7	γ*	2.3
1	β	1	γ*	2.3	8	β	8	γ*	2.3
2	γ*	2	γ*	2.4	8	NH	8	γ*	2.7
2	α	2	NH	2.6	8	NH	8	β	2.7
2	β	2	γ*	2.2	8	α*	8	γ*	2.5
2	β	2	NH	2.8	1	β	2	γ*	5.0
2	γ*	2	NH	2.7	1	β	2	NH	3.4
3	α*	3	β	2.3	2	γ*	3	β	4.7
3	α*	3	δ*	2.5	2	γ*	3	NH	3.8
3	α*	3	γ*	2.9	2	NH	3	δ*	3.9
3	β	3	δ*	2.2	2	NH	3	β	5.5
3	β	3	NH	2.9	3	β	4	β*	4.8
3	δ*	3	NH	2.6	3	β	4	NH	2.9
3	γ	3	NH	2.8	3	NH	4	NH	4.5
4	α	4	β	2.3	5	β	6	δ	2.9
4	α	4	NH	2.8	5	β	6	NH	3.0
4	γ	4	β	2.9	6	δ	7	NH	4.4
4	β*	4	NH	3.0	6	γ*	7	NH	4.6
4	β*	4	γ	2.6	5	γ*	6	NH	3.7
4	β*	4	NH	2.6	7	γ*	8	NH	3.9
4	γ	4	NH	3.6	1	γ*	3	β	4.3
5	γ*	5	α*	2.2	2	γ*	4	α	3.7
5	α*	5	NH	3.2	2	γ*	4	β*	4.5
5	α*	5	β	2.6	2	γ*	5	NH	5.0
5	α	5	NH	2.8	1	β	3	α*	4.7
5	γ*	5	β	2.4	5	NH	3	β	3.2
6	α*	6	β	2.2	1	β	3	δ*	3.7
6	α*	6	γ*	2.8	3	δ*	7	γ*	3.4
6	δ	6	β	2.8	3	γ	7	γ*	4.8
6	γ*	6	β	2.8	2	NH	4	α	5.0
6	ε*	6	β	2.3	4	α	7	β	3.0
7	γ*	7	β	2.2	4	α	7	γ*	5.5
7	NH	7	γ*	2.6					

[a]) * = Pseudoatom used for calculations.

Assignments and volume integration of ROESY cross-peaks were performed with the aid of SPARKY [125]. Distance constraints and error limits were generated from cross-peak volumes by calibration with known distances (two-spin approximation, ±20% error limits) through a python extension within SPARKY. The volumes of cross-peaks involving Me groups or other groups of isochronous H-atoms were corrected by division through the number of H-atoms.

4.2. *Generation of Distance Restraints.* ROESY Spectra in CD_3OH and H_2O with 300-ms mixing time were used. Possible contributions from spin diffusion were excluded from the generation of distance restraints. Also cross-peaks with signal-to-noise ratio ≤ 1 at mixing time of 300 ms and peaks overlapping more than 30% at their basis were exluded. A distance of 3 Å between backbone NH and HB, and of 1.9 Å between H_{ax}-αC and H_{eq}-αC were used as reference values to generate distance restraints with upper and lower bounds as ±20% of the calculated value. For distances involving Me groups, a multiplicity correction of 20% was applied.

Table 11. *^{1}H- and ^{13}C-NMR Data and Assignments for β-Octapeptide* **5** *in H_2O*

β-Amino acid	NH	$CH_2(\alpha)$	H–C(β) (^3J(NH,Hβ))	H–C(γ), Me–C(γ), $CH_2(\gamma)$	H–C(δ), Me–C(δ)	Me–C(ε)	C(α)	C(β)	C(γ)	C(δ)
β^3hHis[1]		2.55	3.78	2.93			38.47	50.35	40.98	
β^3hAla[2]	8.10	2.37	4.22 (9.1)			1.14	43.5	44.85	21.12	
β^3hVal[3]	7.86	2.46/2.24	4.00	1.65		0.78	40.12	54.11	33.32	
β^2hVal[4]	7.89	2.09	3.47/3.140	1.75		0.92/0.86	54.8	41.3	29.61	
β^3hLys[5]	8.02	2.36	4.13 (8.6)	1.51	1.63		43.4	48.53	34.35	23.75
β^3hLeu[6]	7.98	2.35	4.18 (8.3)	1.41/1.26	1.54	0.87/0.83	43.2	47.05	44.17	25.86
β^3hSer[7]	7.97	2.49/2.38	4.23 (10)	3.55			39.11	50.38	64.63	
β^3hCys[8]	8.00	2.45/2.40	4.19 (8.4)	2.72/2.60			42.41	51.31	29.59	

Table 12. *NOE Distance Restraints for β-Octapeptide* **5** *in H_2O Solution* (KH_2PO_4 buffer + 10% D_2O, pH 7.6) *without Zn^{2+}*

Residue	H-Atom[a])	Residue	H-Atom[a])	d_{NOE} [Å]	Residue	H-Atom[a])	Residue	H-Atom[a])	d_{NOE} [Å]
1	α*	1	β	2.4	6	γ*	6	β	2.8
1	α*	1	γ*	2.4	6	γ*	6	NH	2.8
1	β	1	γ*	2.4	6	γ*	6	δ	4.3
2	α*	2	NH	3.1	6	NH	6	β	2.8
2	β	2	γ*	2.3	7	α*	7	γ*	2.8
2	β	2	NH	3.7	7	β	7	α*	2.3
2	γ*	2	NH	3.4	7	β	7	γ*	2.3
3	α*	3	β	2.4	7	γ*	7	NH	3.0
3	α*	3	δ*	2.5	7	NH	7	β	3.0
3	α*	3	γ	2.9	8	β	8	γ*	2.7
3	α*	3	β	3.0	8	γ*	8	α*	3.4
3	α*	3	δ*	2.8	8	γ*	8	NH	4.3
3	α*	3	γ	3.2	8	γ*	8	NH	3.4
3	β	3	γ	2.4	1	α*	2	NH	3.7
3	β	3	NH	2.8	2	β	1	α*	3.5
3	δ*	3	γ	2.1	2	γ*	1	α*	4.0
3	δ*	3	NH	2.8	2	γ*	3	β	4.6
3	γ	3	NH	3.0	2	γ*	3	NH	4.1
4	α	4	β_{Si}	2.4	3	β	4	α	4.8
4	α	4	β_{Re}	3.0	3	NH	2	β	3.4
4	α	4	δ*	2.5	4	α	5	NH	2.1
4	α	4	γ	2.9	4	β_{Re}	5	NH	4.2
4	α	4	NH	3.1	4	δ*	5	NH	5.6
4	β_{Si}	4	β_{Re}	1.7	4	δ*	5	β	3.2
4	β_{Si}	4	δ*	3.4	4	γ	5	NH	3.7
4	β_{Si}	4	γ	3.1	1	α*	3	δ*	3.0
4	β_{Si}	4	NH	3.1	1	α*	3	γ	4.1
4	β_{Re}	4	δ*	3.3	1	γ*	3	δ*	3.8
4	β_{Re}	4	γ	2.7	1	γ*	4	δ*	4.7
4	β_{Re}	4	NH	2.7	1	γ*	5	γ*	3.3
4	δ*	4	γ	2.4	2	γ*	4	β_{Si}	4.4
4	δ*	4	NH	4.9	2	γ*	4	β_{Re}	5.3
4	δ*	4	β_{Re}	3.4	3	α*	1	α*	4.1

Table 12 (cont.)

Residue	H-Atom[a])	Residue	H-Atom[a])	d_{NOE} [Å]	Residue	H-Atom[a])	Residue	H-Atom[a])	d_{NOE} [Å]
4	δ*	4	γ	2.1	3	β	5	NH	4.0
4	γ	4	NH	3.5	3	β	6	α*	3.4
5	α*	5	β	2.3	3	δ*	1	β	3.4
5	α*	5	γ*	2.3	3	δ*	7	γ*	3.9
5	β	5	δ*	3.0	3	γ	7	γ*	4.7
5	β	5	γ*	2.3	4	β_{Re}	7	γ*	2.6
5	β	5	NH	2.6	4	δ*	1	γ*	3.4
5	NH	5	γ*	4.8	4	δ*	8	γ*	5.0
6	β	6	γ*	2.6	5	α*	3	β	3.3
6	δ*	6	β	2.9	7	β	4	α	5.0
6	δ*	6	γ*	3.2	7	γ*	3	α*	4.9
6	δ*	6	NH	3.0					
6	ε*	6	γ*	2.5					

[a]) * = Pseudoatom used for calculations.

Table 13. *^{1}H- and ^{13}C-NMR Data and Assignments for β-Octapeptide* **5** *in $H_2O + ZnCl_2$*

β-Amino acid	NH	$CH_2(\alpha)$	H–C(β) (^3J(NH,Hβ)	H–C(γ), Me–C(γ), $CH_2(\gamma)$	H–C(δ), Me–C(δ)	Me–C(ε)	C(α)	C(β)	C(γ)	C(δ)
β^3hHis[1]		2.567	3.80	3.03			42.18	50.01	41.02	
β^3hAla[2]	8.12	2.39	4.22 (8.9)	1.14			43.4	47.2	21.26	
β^3hVal[3]	7.84	2.47/2.27	4.00	1.66	0.786		40.18	54.05	33.32	21.06
β^2hVal[4]	7.93	2.092	3.53/3.09	1.75	0.93/0.86		54.97	41.31	29.62	20.0
β^3hLys[5]	8.031	2.37	4.14 (9.2)	1.50	1.42	1.64	39.16	48.4	34.4	23.81
β^3hLeu[6]	7.98	2.50	4.18 (8.6)	1.42/1.25	1.55	0.87/0.82	43.2	51.55	44.17	25.85
β^3hSer[7]	7.96	2.50	4.25 (8.68)	3.55			43.8	50.36	64.59	
β^3hCys[8]		2.45	4.17 (9.5)	2.68/2.64				51.55	30.02	

Table 14. *NOE Distance Restraints for β-Octapeptide* **5** *in H_2O Solution* ((D_{11})*Tris* buffer + 10% D_2O, pH 7.2) *in the Presence of 1 Equiv. of $ZnCl_2$*

Residue	H-Atom[a])	Residue	H-Atom[a])	d_{NOE} [Å]	Residue	H-Atom[a])	Residue	H-Atom[a])	d_{NOE} [Å]
1	α*	1	β	3.0	4	γ	4	NH	3.5
1	α*	1	ε*	3.8	6	β	6	γ*	2.4
1	α*	1	γ*	2.7	6	δ	6	NH	2.8
1	β	1	γ*	3.2	6	ε*	6	δ	2.0
1	ε*	1	γ*	3.2	6	ε*	6	γ*	2.3
2	α*	2	NH	2.8	6	γ*	6	NH	2.6
2	β	2	γ*	2.2	7	α*	7	β	2.4
2	β	2	NH	2.9	7	α*	7	γ*	2.7
2	γ*	2	NH	2.9	7	β	7	γ*	2.3
3	α*	3	β	2.3	7	β	7	NH	2.6
3	α*	3	δ	2.4	7	γ*	7	NH	2.8
3	α*	3	γ	2.7	1	α	2	NH	2.4

Table 14 (cont.)

Residue	H-Atom[a])	Residue	H-Atom[a])	d_{NOE} [Å]	Residue	H-Atom[a])	Residue	H-Atom[a])	d_{NOE} [Å]
3	α*	3	β	2.6	2	β	3	NH	3.3
3	α*	3	δ*	2.7	2	γ*	1	α*	4.0
3	α*	3	γ	3.2	2	γ*	1	ε*	4.4
3	α*	3	NH	2.4	2	γ*	3	β	4.3
3	β	3	δ*	2.3	2	γ*	3	NH	4.2
3	β	3	γ	2.3	2	NH	3	NH	5.0
3	β	3	NH	2.8	3	α*	4	NH	2.6
3	δ*	3	γ	2.0	3	β	4	NH	3.2
3	δ*	3	NH	2.8	3	δ*	2	NH	3.7
3	γ	3	NH	3.1	4	α	5	NH	2.1
4	α	4	β_{Si}	2.4	4	δ*	5	NH	3.3
4	α	4	β_{Re}	3.2	4	γ	5	NH	3.5
4	α	4	δ*	2.5	1	α*	3	δ*	3.0
4	α	4	γ	3.0	3	β	5	NH	3.9
4	α	4	NH	2.9	3	δ*	1	ε*	4.0
4	β_{Si}	4	β_{Re}	1.8	3	δ*	1	γ*	1.1
4	β_{Si}	4	δ*	2.4	3	δ*	7	γ*	3.2
4	β_{Si}	4	γ	3.0	3	γ	7	β	3.6
4	βsi	4	NH	2.9	3	γ	7	γ*	4.5
4	β_{Re}	4	γ	2.6	3	γ	7	γ*	4.5
4	β_{Re}	4	NH	2.8	3	γ	7	γ*	4.5
4	δ*	4	β_{Re}	3.3	3	γ	7	γ*	4.5
4	δ*	4	γ	2.2	3	γ	7	γ*	4.5

[a]) * = Pseudoatom used for calculations.

4.3. *Simulated Annealing (SA) Structure Calculations.* Program: XPLOR-NIH v2.9.7 [126]. The standard parameter and topology files of XPLOR-NIH (parallhdg.pro; topallhdg.pro) were modified to accommodate β^2- and β^3-amino acid residues. The SA calculation protocol (adopted from the torsional angle dynamics protocol of *Stein et al.* [127] included 4000 steps (0.015 ps each) of high temp. torsional angle dynamics at 20000 K, followed 4000 (0.015 ps) steps of slow cooling to 1000 K with torsion angle dynamics, 4000 steps (0.003 ps) of slow cooling with cartesian dynamics to 300 K and a final conjugate gradient minimization. The only non-bonded interactions used were *Van der Waals* repel functions. For each compound, 30 structures were calculated.

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Received October 19, 2005