New Open-Chain and Cyclic Tetrapeptides, Consisting of α-, β^2-, and β^3-Amino-Acid Residues, as Somatostatin Mimics – A Survey

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Dedicated to Prof. *Elias J. Corey* on the occasion of his 80th birthday

Cyclo-β-tetrapeptides are known to adopt a conformation with an intramolecular transannular hydrogen bond in solution. Analysis of this structure reveals that incorporation of a β^2-amino-acid residue should lead to mimics of 'α-peptidic β-turns' (*cf.* **A**, **B**, **C**). It is also known that short-chain mixed β/α-peptides with appropriate side chains can be used to mimic interactions between α-peptidic hairpin turns and G protein-coupled receptors. Based on these facts, we have now prepared a number of cyclic and open-chain tetrapeptides, **7**–**20**, consisting of α-, β^2-, and β^3-amino-acid residues, which bear the side chains of Trp and Lys, and possess backbone configurations such that they should be capable of mimicking somatostatin in its affinity for the human SRIF receptors ($hsst_{1-5}$). All peptides were prepared by solid-phase coupling by the Fmoc strategy. For the cyclic peptides, the three-dimensional orthogonal methodology (*Scheme 3*) was employed with best success. The new compounds were characterized by high-resolution mass spectrometry, NMR and CD spectroscopy, and, in five cases, by a full NMR-solution-structure determination (in MeOH or H_2O; *Fig. 4*). The affinities of the new compounds for the

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receptors $hsst_{1-5}$ were determined by competition with [^{125}I]LTT-$SRIF_{28}$ or [^{125}I][Tyr^{10}]-CST_{14}. In *Table 1*, the data are listed, together with corresponding values of all *β*- and *γ*-peptidic somatostatin/*Sandostatin*® mimics measured previously by our groups. Submicromolar affinities have been achieved for most of the human SRIF receptors $hsst_{1-5}$. Especially high, specific binding affinities for receptor $hsst_4$ (which is highly expressed in lung and brain tissue, although still of unknown function!) was observed with some of the *β*-peptidic mimics. In view of the fact that numerous peptide-activated G protein-coupled receptors (GPCRs) recognize ligands with turn structure (*Table 2*), the results reported herein are relevant far beyond the realm of somatostatin: many other peptide GPCRs should be 'reached' with *β*- and *γ*-peptidic mimics as well, and these compounds are proteolytically and metabolically stable, and do not need to be cell-penetrating for this purpose (*Fig. 5*).

1. Introduction. – In a series of publications, we have shown that the affinity of the peptide hormone somatostatin for the human receptors $hsst_{1-5}$ can be imitated by a number of *β*- and *γ*-peptide derivatives consisting of four or two amino-acid residues, respectively [1–6]. The most potent mimic turned out to be a specific 15-nM agonist of the somatostatin $hsst_4$ receptor [5][7]. Highest concentrations of this receptor are found in brain and lung tissues, but no physiological correlate has been clearly established so far [8]. The peptide was shown to be bioavailable *p. o.*, to be excreted with a half-life of *ca.* 8 h, and to be enzymatically and metabolically stable in rats [7][9]. These properties render small *β*-peptidic compounds promising candidates for drug-discovery programs.

Somatostatin (a cyclic disulfide of a tetradecapeptide) [8] and the drug *Sandostatin*® (octreotide, an octapeptide analog; *Fig. 1*) [10] are thought to interact with the $hsst_{1-5}$ receptors mainly by inserting a so-called '*β*'-turn substructure, carrying a lysine and a tryptophan side chain (*i.e.*, **A**), into a pocket of the G protein-coupled somatostatin receptor [8].

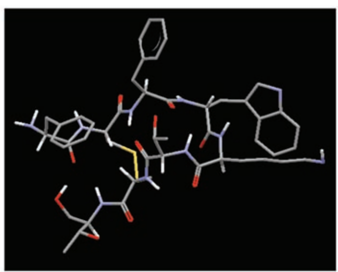
Our work with *β*-peptidic somatostatin mimics was primarily aimed at imitating the turn structure (see **B** and **C**, and compare with **A** [11]). On the other hand, we have made observations that shed some doubt upon the generally accepted view that a '*β*'-turn with the two characteristic side chains of Trp and Lys is a prerequisite for a somatostatin peptidomimetic to bind to SRIF receptors: *i*) the most potent *β*-peptidic mimic which we have disclosed [5][7], so far, and which is mentioned above, actually contains the *α*-peptidic D-Trp building block in the 2-position (*i.e.*, **1**) and cannot possibly fold to a turn structure resembling **A** or **B**. *ii*) Our first somatostatin-mimicking compound (3–200 μM) was a cyclo-$β^3$-tetrapeptide, **2**, the NMR-solution structure, **D**, of which in (D_3)MeOH carries the side chains of Trp and Lys too far apart and not in the correct relative arrangement in space [1][2]. *iii*) A *γ*-dipeptide, **3**, was found to bind to various human SRIF receptors (*ca.* 1 μM), even when its lysine NH_2 or tryptophan NH group carried bulky, hydrophobic protecting groups R^{1-3} ($PhCH_2$, $(PhCH_2)_2$ or mesitylenesulfonyl (2,4,6-$Me_3C_6H_2-SO_2$), resp.), which would have been expected not to fit into pockets 'made for' $(CH_2)_4NH_2$ or 1*H*-indol-3-yl [6].

The goal of the present investigations was to test some new types of cyclic and turn-forming open-chain *β*- and *β*/*α*-mixed peptides as somatostatin mimetics[8]). Inspection

[8]) For a series of papers, in which open-chain, and cyclic and bicyclic analogs of somatostatin are described, and tested for their affinities for the $hsst_{1-5}$ receptors, see [12]. In these extensive investigations, the authors have also incorporated non-proteinogenic amino acids, and they have determined numerous NMR-solution structures.

SOMATOSTATIN (SRIF14)

Octreotide
SANDOSTATIN®



X-ray structure of octreotide

Fig. 1. *Molecular formulae of somatostatin* (SRIF-14) *and* Sandostatin®, *and X-ray crystal structure of* Sandostatin® [11]

of the NMR-solution structure **D** of the cyclo-β^3-tetrapeptide **2**, which could be considered as a kind of bicyclic arrangement with a trans-annular H-bond forming a ten- and a twelve-membered ring, would suggest that incorporation of a neighboring pair of a β^2- and a β^3-amino-acid residue in the ten-membered ring should result in an ideal β-turn-mimicking structure (see arrows in **D**). Of course, the cyclo-β-tetrapeptide can form this combination of ten- and twelve-membered H-bonded rings in two ways,

depending upon which pair of C=O and N−H bonds on opposite sides of the 16-membered macrocyclic ring will turn inwards to form a H-bond. To learn about the rules, according to which this 'bicyclic' secondary structure is formed, we decided to prepare several different cyclo-β-tetrapeptides of type **E**, which have the β^3-β^2-section carrying a Lys and a Trp side chain in common. Encouraged by the fact that in open-chain compounds, such as **1**, replacement of a β- by an α-amino acid gave more potent agonists, we have also envisaged cyclotetrapeptides of type **F**, 15-membered macrocycles consisting of three β- and one α-amino-acid residue.

E

F

G
a hexapeptide

H
a heptapeptide

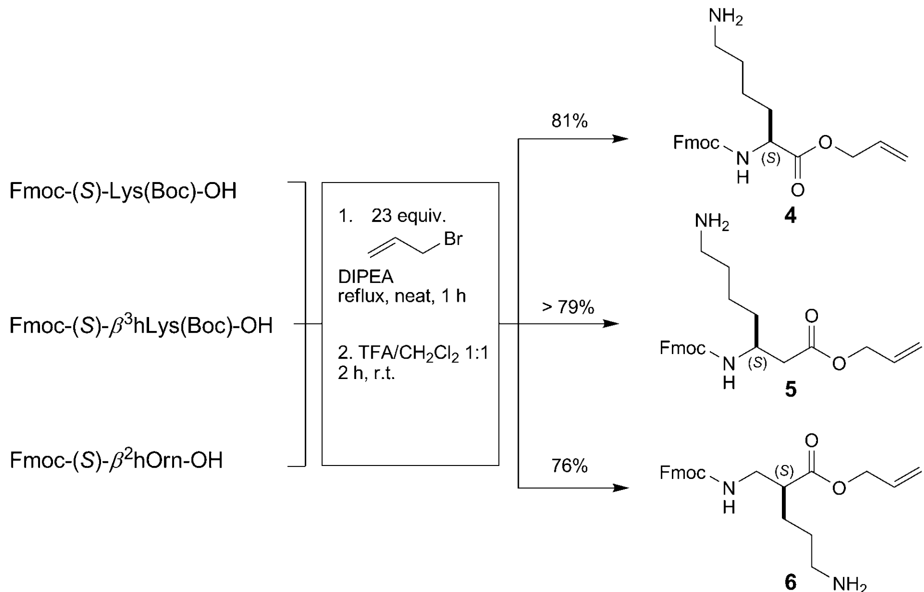
Along a different line of work, we had studied open-chain 'mixed' peptides consisting of a total of six or seven α- and β-amino-acid residues. This has led to the discovery of turn structures **G** and **H**, which are stabilized more by backbone conformational preferences than by formation of the nine- or ten-membered H-bonded ring in the actual turn section [13]. We wondered whether such turns would also be formed by suitably composed tetrapeptides, and decided to test such turn formation by placing Lys and Trp side chains in the central section of 'mixed' β/α-peptides for somatostatin mimicking. If this were successful, there would also be the benefit of providing less expensive somatostatin mimics, because the cost of α-peptidic building blocks for peptide synthesis is by far lower than that for the homologous β-peptidic building blocks. The enzymatic stability, on the other hand, may not be as high as that of pure β-peptides, except if there is an N-terminal β-amino-acid residue [14].

2. Synthesis of the Cyclic and Open-Chain β- and β/α-Mixed Tetrapeptides. – All assemblies of the tetrapeptides were performed with *N*-Fmoc-protected amino acids, with acid-labile protection of the side-chain functional groups, employing various resins for the manual solid-phase peptide synthesis (SPPS). Most of the required building blocks are commercially available and were used as purchased[9]). For applying the so-

[9]) We gratefully acknowledge discount prices for the β^3-amino-acid derivatives from *Sigma-Aldrich* (*Fluka*, CH-Buchs). We thank Dr. *T. Kimmerlin* (*Novartis*, Vienna) for samples of β^2-amino-acid derivatives.

called three-dimensional orthogonal solid-phase (Fmoc/*t*-Bu or Boc/allyl) strategy [15][16] (*vide infra*), we have prepared the Fmoc-(*S*)-Lys, the Fmoc-(*S*)-β^3hLys, and the Fmoc-(*S*)-β^2hOrn[10]) allyl esters **4**–**6** as TFA salts (*Scheme 1*).

Scheme 1. *Preparation of the Allyl Esters of Fmoc-(*S*)-Lys, Fmoc-(*S*)-β^3hLys, and Fmoc-(*S*)-β^2hOrn.* DIPEA = EtN(^{i}Pr)$_2$ (*Hünig*'s base), TFA = CF_3COOH.



2.1. *The Cyclotetrapeptides* **7**–**14**. – Numerous cyclo-β-peptides consisting of up to eleven β-amino-acid residues (up to 44-membered rings) have been reported in the literature[11]). As early as 1979, *Rothe* and *Mühlhausen* [18] described the formation and isolation of -(βhGly)$_n$-, $n = 2-11$, by cyclo-oligocondensation of the amino acid H-βhGly-OH[12])[13]). Cyclic peptides containing single β-amino acids have also been described [12][33]. There are numerous methods of cyclizing peptides [17], the classical procedure being the activation of the C-terminal amino-acid residue, which leads to cyclization, usually under high-dilution conditions[14])[15]).

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[10]) Fmoc-(*S*)-β^2hOrn was also prepared as part of an other project (*cf. Footnote 19*).

[11]) For some reviews on the synthesis of cyclic peptides, see [17].

[12]) This β-amino acid is commonly called β-alanine.

[13]) Specific examples of cyclo-β-peptides -(βhXaa)$_n$-: $n = 2$ [18–20], $n = 3$ [21–27], $n = 4$ [2][20][23][28–31], $n = 5$ [31], $n = 6$ [22][23][32].

[14]) The azide activation [34] is still used, employing a 'modern' reagent (diphenylphosphonyl azide (DPPA)) [35]. Activation by the *Schmidt* method (pentafluorophenol esters [17c][36]) was applied in our own previous work on cyclo-β-peptides [1][2][23][24]. Typical activation for peptide coupling, such as HATU, can also be used to induce cyclization [37].

[15]) In a recent work, there is the statement that *'it is known that the cyclization rate of a linear peptide sequence is somewhat unpredictable and may well be sequence-dependent'* [35a], with reference to *Freidinger*, one of the masters of peptide chemistry [38].

7 **8**

9 **10**

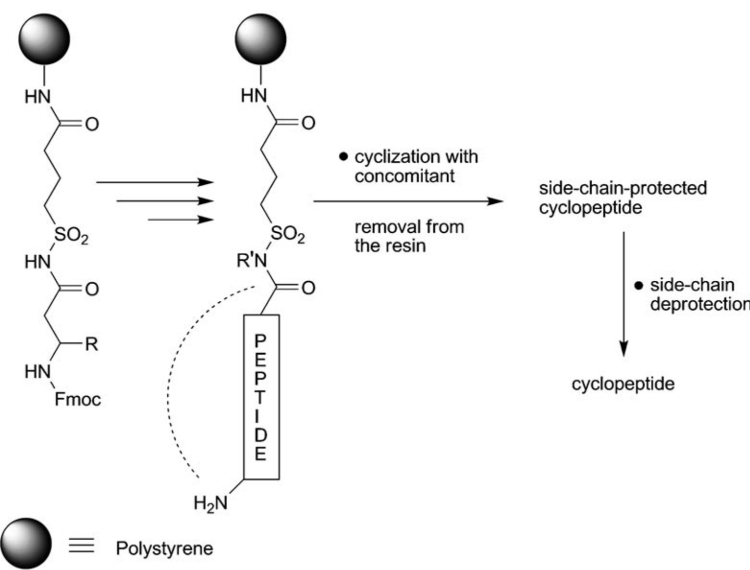
11 **12**

13 **14**

Since the early 1990s, the on-resin cyclization has been in use[16]). It was first applied with the *DeGrado–Kaiser* [40] oxime resin [41][42], later by the *Kenner–Ellman* sulfonamide '*safety-catch*' method [43][44][17]), and more recently by side-chain anchoring (of a CO_2H or an NH_2 group)[18]) (see outlines in *Schemes 2* and *3*).

We first applied the sulfonamide methodology (*Scheme 2*) to the synthesis of a number of cyclo-β-tetrapeptides[19]), with disappointing results; thus, cyclo-β-tetrapeptide **7** was isolated as a mixture of two epimers in 6.5% yield. The two compounds were epimeric at the stereogenic center of the β^2hTrp residue. After purification, the isomer **7** with (*S*)-configuration at this center was isolated in *ca.* 2.7% yield (see *Exper. Part*). The side-chain anchoring method turned out to be far superior in our hands in this case. Thus, the other cyclopeptides **8**, **9**, **11**–**14** were all prepared through anchoring the α- or βh-lysine ω-NH_2 group by reaction with chloro-trityl resin, assembling the tetrapeptide chain with *N*-Fmoc-protected amino acids (Trp and Lys side chains *t*-Bu- and Boc-protected, resp.), cyclizing on-resin, and removing the cyclopeptides with simultaneous

Scheme 2. *Cyclopeptide Head-to-Tail Synthesis by the* Kenner–Ellman *Safety Catch Methodology* [43]. The 4-sulfamoyl-butyryl AM resin is commercially available. For 'activation' of the acyl-sulfonamide group, the sulfamoyl N-atom is alkylated (R' = Me, CH_2CN). In the cyclization step, the peptide is removed from the resin, with the side-chain functional-group protection still in place [44]. This method was applied to the synthesis of the cyclo-β-tetrapeptide **7**.



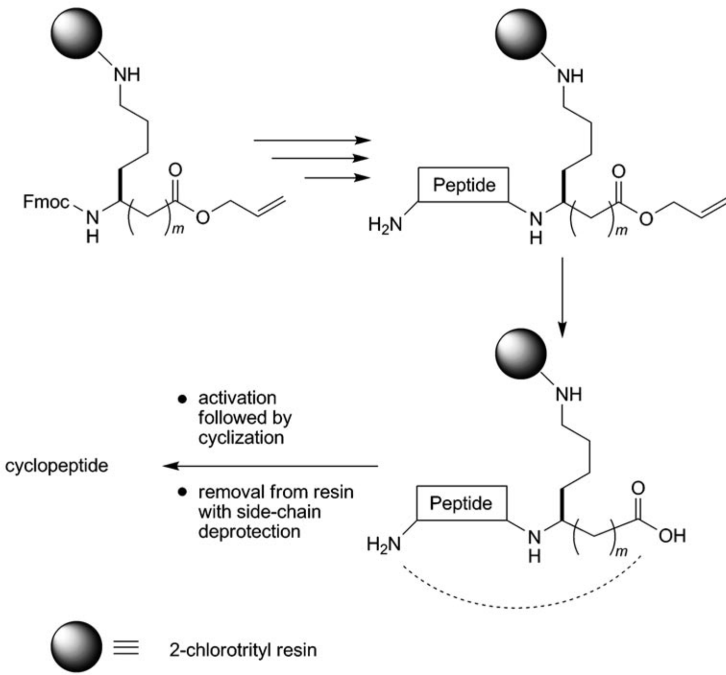
[16]) One of the first to recognize the *quasi*-high-dilution effect with polymer-bound reagents and intermediates was *Patchornik* [39].

[17]) *Cf.* the use of a simple thioester linkage [45].

[18]) For some representative examples, see [31][46].

[19]) Besides the somatostatin-mimicking cyclopeptides described herein, we have also synthesized RGD-mimicking cyclopeptides, and, in all cases, the yields obtained with the '*safety catch*' method were disappointingly low.

Scheme 3. *Cyclopeptide Head-to-Tail Synthesis by the 'Three-Dimensional Orthogonal' Solid-Phase Methodology* (Fmoc/Boc, *t*-Bu/allyl) [31][46]. After the cyclization step, the cyclopeptide is removed from the resin with simultaneous deprotection of the side-chain functional groups. This method was applied to the synthesis of the cyclopeptides **8**–**14** in yields of 18, 27, 28, 5, 26, 25, and 27%, resp. $m = 0$: Lys, $m = 1$: β^3hLys. To some extent, the variation of the yields may also reflect the learning process of the experimentalist.



side-chain deprotection, as outlined in *Scheme 3*. The yields, after preparative HPLC purification, were mostly satisfying, in the range of 18 to 28%. Details are described in the *Exper. Part*. The same series of procedures was employed for the synthesis of peptide **10** containing an (*S*)-β^2hOrn residue.

2.2. *The Open-Chain Peptides* **15**–**20**. These peptides were prepared by solid-phase peptide synthesis (SPPS), using the Fmoc-protecting-group strategy and orthogonal acid-labile protection of the side-chain functional groups. The peptides were prepared on *Rink Amide* AM resin to afford the *C*-terminal amide necessary to provide the possibility of an additional H-bond with an *N*-terminal Ac group.

3. CD Spectra. – The CD spectra of the cyclic peptides **10**–**14** are shown in *Fig. 2*, and those of the open-chain peptides **15**–**20** in *Fig. 3*. These spectra should be considered as fingerprints of the corresponding compounds. As pointed out frequently [19][47], the CD spectra of β-peptides are not reliable, at this point, for structural correlations. Thus, the CD traces of cyclic peptides **10**–**14** have very different patterns (wavelength, sign, and intensity of the *Cotton* effects; see *Fig. 2*).

Of the open-chain peptides **15**–**20**, compound **15**, containing an N-terminal pyridine dicarboxylic acid moiety, shows a strong positive *Cotton* effect around 195 nm. The other tetrapeptides **16**–**20**, containing central lysine and tryptophane side chains, give rise to similar CD spectra (*Fig. 3*), irrespective of the particular nature of the

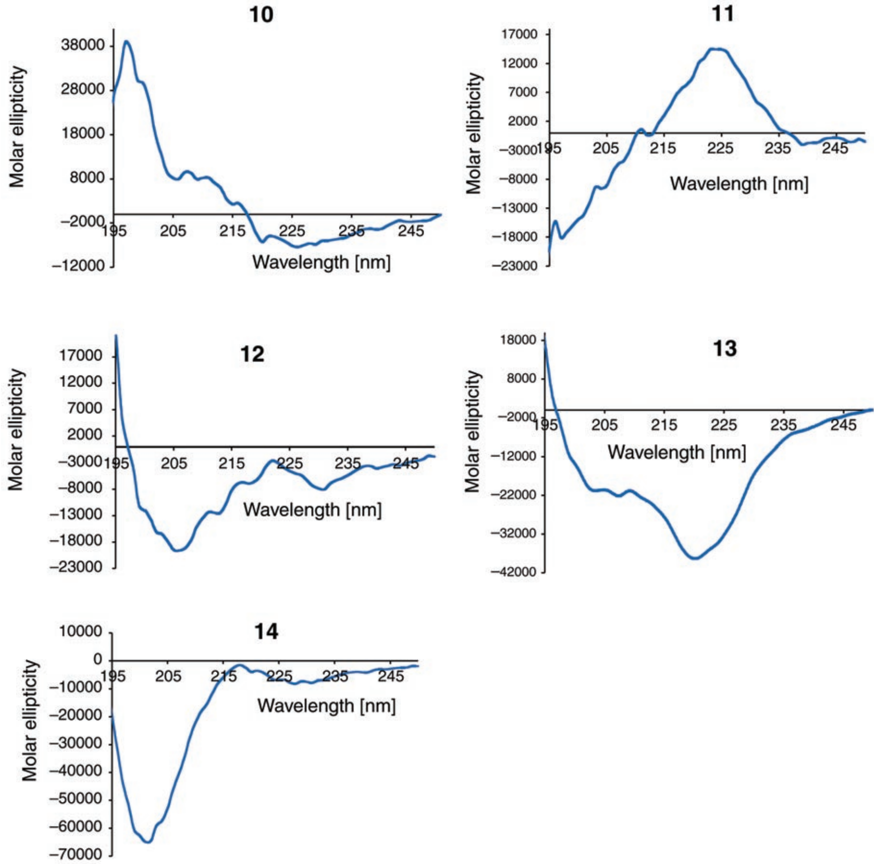


Fig. 2. *Normalized CD spectra of cyclic tetrapeptides* **10**–**14** *recorded in MeOH* (0.2 mM). The CD spectra of compounds **7**–**9** have not been recorded.

central unit, which is (*S*)-β^2hTrp-(*S*)-β^3hLys in **16**, (*S*)-β^2hLys-(*S*)-β^3hTrp in **17** and **18**, (*R*)-Lys-(*S*)-β^3hTrp in **19**, and (*R*)-Trp-(*S*)-β^3hLys in **20**; there is a strong positive *Cotton effect* near 205 nm and a weaker one near 230 nm[20]). Note that the Lys and Trp side chains are attached to two β-amino-acid residues in **16**, **17**, and **18**, and to an α- and a β-amino-acid residue in **19** and **20**. Still, the spectra have the same patterns.

4. NMR-Solution Structures of Somatostatin Analogs 7, 8, 9, 14, 16, and 17. – A detailed 2D-NMR-spectroscopic study was undertaken to deduce the conformational preferences of some of the tetrapeptides. The spectra of compounds **8** and **9** were recorded in H_2O/D_2O 95:5 at pH 6.8, those of the cyclic tetrapeptides **7** and **14**, as well as of the open-chain tetrapeptides **16** and **17** were recorded in CD_3OH. The complete assignment of all ^{1}H resonances was accomplished by a combination of DQF-COSY

[20]) In the spectrum of **18**, this second *Cotton* effect is at 220 nm!

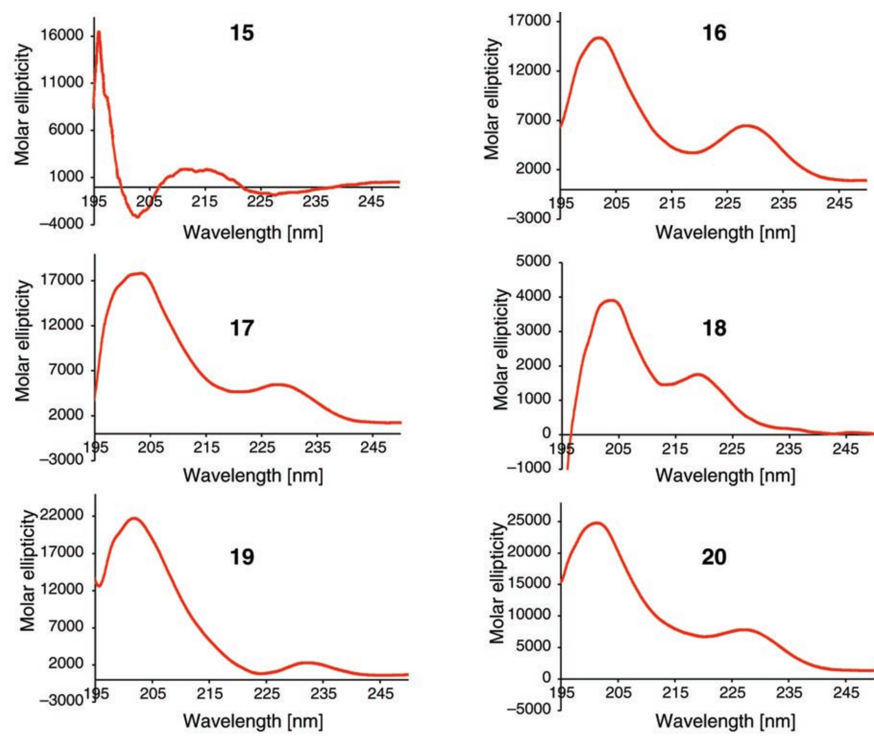


Fig. 3. *Normalized CD spectra of open-chain somatostatin analogs recorded in MeOH* (0.2 mM)

and TOCSY experiments. The sequence-specific assignment was further confirmed by a long-range heteronuclear correlation HMBC experiment. All the chemical shifts and $^3J(NH,H_\beta)$ values, as extracted from 1D spectra, are compiled in *Tables 3*, *5*, *6*, *8*, *10*, and *12* in the *Exper*. *Part.* Analysis of ^{1}H-NMR spectra provided an indication that the Trp and Lys side chains are in close proximity, a phenomenon which is commonly observed for somatostatin and its analogs: there is an upfield chemical shift for the δ-CH_2/ε-CH_2 resonances of the Lys side chains, caused by the aromatic anisotropy of the Trp indole ring. Such shifts have previously been correlated with the activity of SRIF analogues [6]. Although the conformation of a ligand bonded to a receptor could be somewhat different from that observed in solution, such shifts often provide the first clue for the affinity. Additional structural information about our compounds was obtained from ROESY experiments with $\tau_m = 300$ or 200 ms.

The NOE cross-peaks were integrated and subsequently converted into distance restraints with the two-spin approximation. The structures of the cyclic peptides were calculated by MD-simulated annealing employing the XPLOR protocol with NOE-derived distance restraints (see *Tables 4*, *7*, *9*, *11*, and *13* in the *Exper. Part*) and dihedral constraints derived from ^3J coupling constants. Each calculation started with an energy-minimized structure, and with a linear extended structure used in the case of the open-chain compounds. A total of 30 structures were calculated without violation > 0.2 Å of experimental constraints except one that was consistently violated by 0.3 Å (*Table 7*),

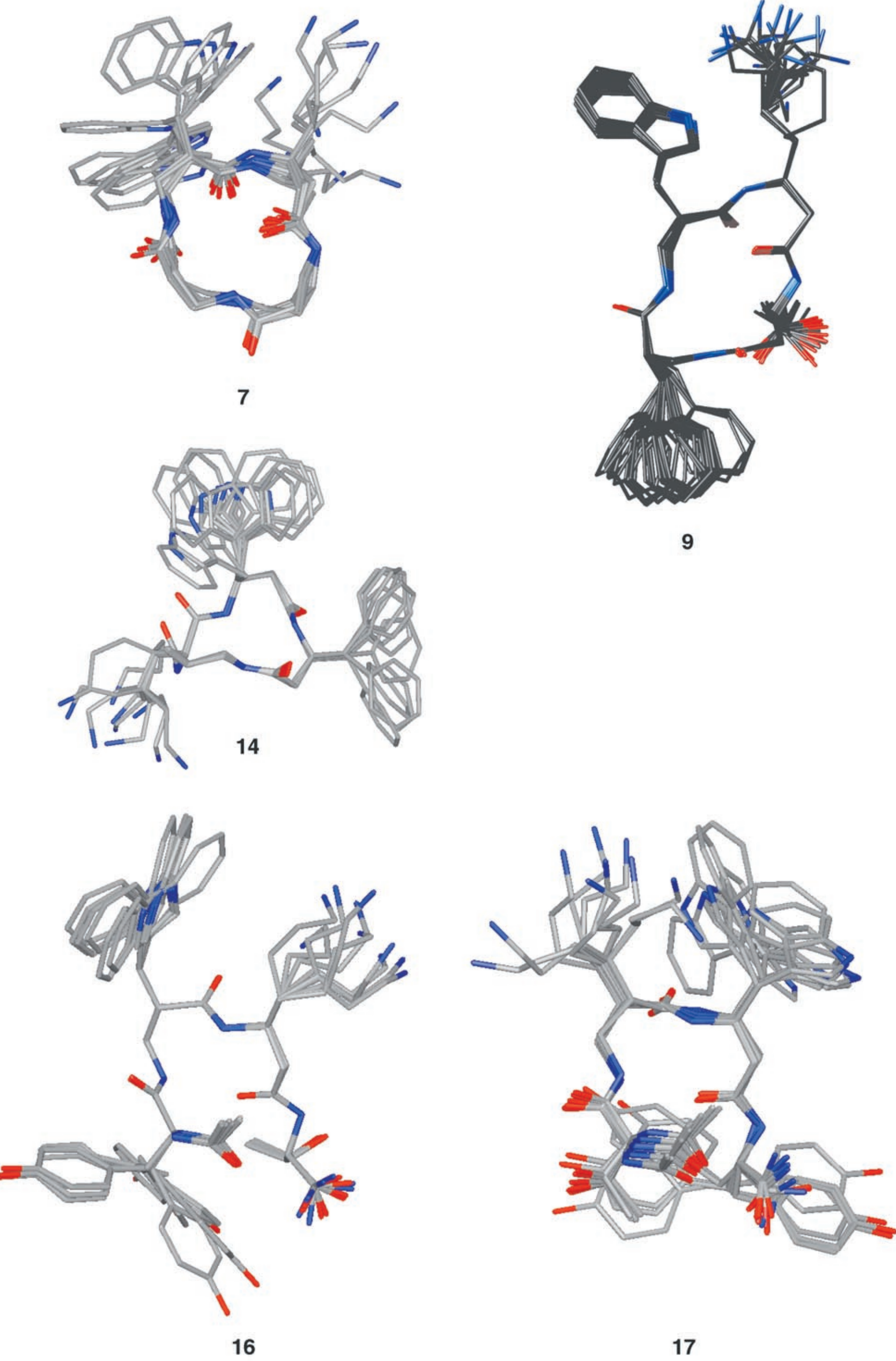


Fig. 4. *NMR Solution structures of the cyclic tetrapeptides* **7**, **9**, *and* **14**, *and of the open-chain tetrapeptides* **16** *and* **17**. Low-energy structures derived from SA annealing calculations are shown.

and the structures of lowest energy are shown in *Fig. 4*. The structure of compound **8** is not shown, because no full analysis was carried out in this case, due to the fact that all NOE measurements provided the same patterns as those observed for compound **9**[21])[22]).

The cyclo-β-tetrapeptides **7**–**9** have well-defined backbone conformations with ill-defined ten- and twelve-membered intramolecular H-bonded rings. All amide bonds adopt s-*trans*-arrangements. The two amide bonds not involved in H-bonding point approximately in opposite directions, perpendicular to the average plane of the ring. As expected [19][47][48], the β^2/β^3-unit forms a ten-membered H-bonded ring, which mimics the natural α-peptidic hormone β-turn, and which is similar to the ring observed in the *12/10*-helix [19]. The side chains of Lys and Trp occupy *equatorial*-type positions on the rings, separated by a distance of *ca*. 5.5 Å. The backbone conformation of peptide **7** is not rigid when compared to **8** and **9**. Its flexibility can be attributed to the presence of two βhGly moieties, which are known to be able to adopt more than one conformation [19][49][50].

The 'mixed' cyclotetrapeptide **14** containing an α-Lys residue forms a well-defined backbone conformation as well, but with no observable intramolecular H-bond. There is a distinct structure formed by the β^2/α-segment of this peptide. The overall shape is not flat but boat-like, with all amide bonds of s-*trans*-geometry. The C=O bonds of two adjacent amide groups in the structure of **14** point in one direction and the other two in nearly the opposite direction, similar to what was observed in the solid-state structure of (*R*,*R*,*S*,*S*)-cyclotetra-β-homoalanine, as determined from powder X-ray diffraction data [28a]. Also, the characteristic upfield NMR shift of the γ-CH_2 H-atoms in the Lys side chain is not observed for this mixed peptide **14**, implying that the side chains of Trp and Lys are not in close proximity (see *Fig. 4*).

The open-chain tetrapeptides **16** and **17** were designed to contain a well-established β-turn-inducing β^2/β^3-segment, with side chains of Trp and Lys, flanked by α-amino acids. Inspection of the ^{1}H-NMR spectra reveals the upfield shift for Lys δ-CH_2 H-atoms in **16**, whereas in **17** the upfield shift is less pronounced. The detailed analysis provided NMR-solution structures with well-ordered hairpins. In both structures, the βhTrp and βhLys side chains are proximal. A second possible H-bond between the terminal residues was not observed; these residues appear to be flexible [51]. Still, several long-range NOEs observed across the strands suggest the presence of the hairpin structure in the solution (see *Exper. Part*).

5. Receptor Studies. – As described in previous papers [1–6][52][53], the affinities of the new compounds **7**–**20** for the five recombinant human receptors ($hsst_{1-5}$) were determined by competition with [^{125}I]LTT-SRIF 28 from receptor proteins expressed in CCL-39 (Chinese Hamster Lung Fibroblast) cell lines. The results are collected in *Table 1*, together with the previously published affinities of somatostatin (*Entry 1*), *Sandostatin*® (*Entry 2*), and all the β- and γ-peptidic somatostatin mimics.

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[21]) After all, the only difference between the two compounds is replacement of a Me group (β^3hVal) in **8** by an OH group (β^3hThr) in **9**.

[22]) See the chemical shift data in the *Exper. Part*.

Inspection of the table reveals the following facts: *i*) there is no new affinity record in the low nM range, as observed with the β/α-mixed peptide **1** (*Entry 3*); *ii*) all new compounds **7**–**20** exhibit a single-digit μM affinity with at least one of the five receptors (*Entries 5–20*); *iii*) the expectation (see **D** in the *Introduction*) that a β^3-β^2-segment in the cyclic β-tetrapeptides could provide derivatives **7**–**10** mimicking the Trp-Lys turn structure of somatostatin better than the all-β^3-cyclotetrapeptide **2** has only partially come true: they[23]) have a better affinity only to $hsst_4$ (1.5–6.0 *vs.* 10 μM); *iv*) the mixed β/α-cyclotetrapeptides **11**–**14** (*Entries 10–14*) generally bind to the receptors $hsst_{1-4}$ almost equally well[24]) (2–10 μM) and somewhat more weakly (17–20 μM) to $hsst_5$; they are more promiscuous than the selective β^3/β^2-analogs **7**–**10**; *v*) the new mixed open-chain tetrapeptides **15**–**20** (*Entries 15–20*) have two (*i.e.*, **15**, **16**, **18**–**20**) or three (*i.e.*, **17**) single-digit μM affinities to receptors 1–4, but again bind more weakly to receptor 5, just like most other previously prepared open-chain tetrapeptides (*Entries 21–33*); *vi*) among these 'old' open-chain β-tetrapeptides, which contain no or only one α-amino-acid residue (Lys or Trp), there is a general preference for the SRIF receptor 4, *which is present in highest concentration in brain and lung tissue, and which is of hitherto unknown function* [8]; tetrapeptide **1**, a member of this group of compounds, *is orally bioavailable and completely cleared from rats in 3 days* [7] (*cf. Entries 3*, *21*, *31*); *vii*) remarkably, even β-dipeptide derivatives (*Entries 34–39*) may have sub-μM affinities for receptor 4 (*Entries 34* and *35*); *viii*) finally, the γ-dipeptide derivatives shown in *Entry 40* of *Table 1*, can also mimic somatostatin receptors with sub-μM affinities.

Conclusions and Outlook. – By the results reported herein, it is demonstrated that the incorporation of β^2-amino-acid residues into cyclo-β-tetrapeptides provides good 'β-turn' mimics, as evident from NMR structures and from the affinities for somatostatin receptors. Replacement of one β- by an α-amino-acid moiety in these cyclic tetrapeptides leads to *Sandostatin*® analogs of higher promiscuity among the five human receptors $hsst_{1-5}$. The open-chain mixed β/α-peptides of the type of amino-acid sequences, which had previously been shown to lead to new hairpin turn structures, have now been shown to have somatostatin-mimicking properties, if the side chains of Lys and Trp are placed in the proper positions, and with the 'right' backbone configurations. The new open-chain peptides do, however, not rival the previously prepared analogs (*Entries 3*, *21*, and *31* of *Table 1*) exhibiting nM affinities for the human somatostatin receptor $hsst_4$.

The demonstration of somatostatin mimicking by metabolically stable rationally designed β- and γ-peptidic compounds folding to hairpin secondary structures, and the discovery that one of these compounds is orally bioavailable in rats (25% in 15 min) has significance beyond the field of somatostatin: the interaction between peptide ligands with turn structures and their appropriate G protein-coupled receptors (GPCRs) is a

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[23]) An exception is *epi*-**7**, with the 'wrong' configuration of β^2hTrp.

[24]) *Cf.* the affinity of *ent*-octreotate towards all five receptors [53].

Table 1. *The Affinities of Cyclic Tetrapeptides, Open-Chain Tetrapeptides, β-Dipeptides, and γ-Peptides Compared with Those of Somatostatin* ($SRIF_{14}$), *Octreotide* (*Sandostatin*®), *and Peptide* **1** *by Radioligand Binding Assays* (competition experiments of the specific binding of [^{125}I] LTT-$SRIF_{28}$ or [^{125}I][Tyr^{10}]-CST_{14} with the five known human somatostatin receptors ($hsst_{1-5}$) expressed in CCL-39 (chinese hamster lung fibroblast) cell lines). The values are reported as $pK_d \pm$ SEM of at least three independent determinations. For *Entries 2–20*, *23–25*, *29*, *36*, and *40*, [^{125}I] LTT-$SRIF_{28}$ ligands were used, and for *Entries 1*, *21*, *22*, *26–28*, and *30–33*, [^{125}I][Tyr^{10}]-CST_{14} ligands were used as radioligands.

Entry	Peptide	$hsst_1$ pK_d	$hsst_2$ pK_d	$hsst_3$ pK_d	$hsst_4$ pK_d	$hsst_5$ pK_d	Ref.
1	$SRIF_{14}$ (Somatostatin)	9.08 (0.83 nM)	10.06 (0.087 nM)	9.67 (0.21 nM)	8.39 (4.07 nM)	9.01 (0.97 nM)	[52]
2	Octreotide (*Sandostatin*®)	6.65 (224 nM)	9.19 (0.645 nM)	7.88 (13.2 nM)	6.40 (398 nM)	7.17 (67.6 nM)	[52]

Table 1 (cont.)

Entry	Peptide	hsst$_1$ pK_d	hsst$_2$ pK_d	hsst$_3$ pK_d	hsst$_4$ pK_d	hsst$_5$ pK_d	Ref.
3[a])	Peptide **1**	4.73 (18.6 µM)	4.48 (33.11 µM)	4.58 (26.30 µM)	7.83 (14.8 nM)	4.73 (18.62 µM)	[4][5][7]
	Cyclic Tetrapeptides						
4	**2**	4.85 (14.1 µM)	4.44 (36.3 µM)	5.48 (3.3 µM)	5.00 (10.0 µM)	3.73 (186.2 µM)	[1]

Table 1 (cont.)

Entry	Peptide		hsst$_1$ pK_d	hsst$_2$ pK_d	hsst$_3$ pK_d	hsst$_4$ pK_d	hsst$_5$ pK_d	Ref.
5		**7**	< 5.00	5.05 (8.90 μM)	< 5.00	5.66 (2.20 μM)	< 5.00	this paper
6		*epi*-**7**	< 5.00	< 5.00	< 5.00	4.94 (11.5 μM)	< 5.00	this paper

Table 1 (cont.)

Entry	Peptide	hsst1 pK_d	hsst2 pK_d	hsst3 pK_d	hsst4 pK_d	hsst5 pK_d	Ref.
7	**8**	< 5.00	< 5.00	< 5.00	5.81 (1.54 μM)	< 5.00	this paper
8	**9**	< 5.00	< 5.00	< 5.00	5.34 (4.6 μM)	< 5.00	this paper

Table 1 (cont.)

Entry	Peptide	hsst$_1$ pK_d	hsst$_2$ pK_d	hsst$_3$ pK_d	hsst$_4$ pK_d	hsst$_5$ pK_d	Ref.
9	**10**	5.04 (9.12 μM)	4.99 (10.23 μM)	5.66 (2.18 μM)	5.22 (6.02 μM)	4.63 (23.44 μM)	this paper
10	**11**	5.05 (8.91 μM)	5.02 (9.54 μM)	5.85 (1.41 μM)	5.46 (3.46 μM)	4.76 (17.37 μM)	this paper

Table 1 (cont.)

Entry	Peptide		hsst$_1$ pK_d	hsst$_2$ pK_d	hsst$_3$ pK_d	hsst$_4$ pK_d	hsst$_5$ pK_d	Ref.
11		**12**	5.04 (9.12 μM)	5.01 (9.77 μM)	5.65 (2.23 μM)	5.20 (6.30 μM)	4.64 (22.90 μM)	this paper
12		**13**	5.08 (8.31 μM)	4.99 (10.23 μM)	5.69 (2.04 μM)	5.55 (2.81 μM)	4.59 (25.70 μM)	this paper

Table 1 (cont.)

Entry	Peptide		hsst$_1$ pK_d	hsst$_2$ pK_d	hsst$_3$ pK_d	hsst$_4$ pK_d	hsst$_5$ pK_d	Ref.
13		**14**	5.34 (4.57 µM)	5.04 (9.12 µM)	5.72 (1.90 µM)	5.31 (4.89 µM)	4.77 (16.98 µM)	this paper
14		*epi*-**14**	5.50 (3.16 µM)	5.04 (9.12 µM)	5.67 (2.13 µM)	5.28 (5.24 µM)	4.72 (19.05 µM)	this paper

Table 1 (cont.)

Entry	Peptide	hsst$_1$ pK_d	hsst$_2$ pK_d	hsst$_3$ pK_d	hsst$_4$ pK_d	hsst$_5$ pK_d	Ref.
	Open-Chain Tetrapeptides						
15[a])	**15**	< 5.07	< 5.00	5.80 (1.58 μM)	5.45 (3.54 μM)	< 5.00	this paper
16	**16**	< 5.24	4.65 (22.38 μM)	5.82 (1.50 μM)	5.53 (2.95 μM)	< 5.00	this paper

Table 1 (cont.)

Entry	Peptide		hsst$_1$ pK_d	hsst$_2$ pK_d	hsst$_3$ pK_d	hsst$_4$ pK_d	hsst$_5$ pK_d	Ref.
17		**17**	5.06 (8.70 µм)	< 5.0	5.82 (1.58 µм)	6.52 (0.30 µм)	< 5.00	this paper
18		**18**	< 5.14	< 5.04	5.82 (1.51 µм)	5.81 (1.54 µм)	< 5.00	this paper

Table 1 (cont.)

Entry	Peptide		hsst$_1$ pK_d	hsst$_2$ pK_d	hsst$_3$ pK_d	hsst$_4$ pK_d	hsst$_5$ pK_d	Ref.
19		**19**	< 5.56	4.75 (17.70 µM)	5.74 (1.81 µM)	5.45 (3.54 µM)	< 5.00	this paper
20		**20**	< 5.22	4.79 (16.20 µM)	5.75 (1.77 µM)	5.76 (1.73 µM)	< 5.00	this paper

Table 1 (cont.)

Entry	Peptide	hsst$_1$ pK_d	hsst$_2$ pK_d	hsst$_3$ pK_d	hsst$_4$ pK_d	hsst$_5$ pK_d	Ref.
21		4.88 (13.2 µM)	< 5.00	< 5.00	7.08 (83.1 nM)	< 5.00	[3]
22		< 4.00	< 4.00	< 5.00	< 4.00	< 4.00	[3]
23		4.25 (56.23 µM)	< 5	< 5	5.63 (2.34 µM)	< 5	[4][54]

Table 1 (cont.)

Entry	Peptide	$hsst_1$ pK_d	$hsst_2$ pK_d	$hsst_3$ pK_d	$hsst_4$ pK_d	$hsst_5$ pK_d	Ref.
24		< 4	< 5	< 5	6.45 (0.35 μM)	< 5	[54]
25		< 4	< 5	< 5	6.88 (0.13 μM)	< 5	[54]

Table 1 (cont.)

Entry	Peptide	$hsst_1$ pK_d	$hsst_2$ pK_d	$hsst_3$ pK_d	$hsst_4$ pK_d	$hsst_5$ pK_d	Ref.
26		<5	<5	<5	<5	<5	[49]
27[b])		<5	<5	<5	5.58 (2.63 μM)	<5	[4]

Table 1 (cont.)

Entry	Peptide	hsst$_1$ pK_d	hsst$_2$ pK_d	hsst$_3$ pK_d	hsst$_4$ pK_d	hsst$_5$ pK_d	Ref.
28[b])		< 5	< 5	< 5	< 5	< 5	[4]
29[a])		4.59 (25 μM)	3.82 (151.35 μM)	4.23 (58.88 μM)	6.47 (0.34 μM)	3.98 (104.71 μM)	[4][5][7]

Table 1 (cont.)

Entry	Peptide	hsst$_1$ pK_d	hsst$_2$ pK_d	hsst$_3$ pK_d	hsst$_4$ pK_d	hsst$_5$ pK_d	Ref.
30[a])		6.15 (0.7 µM)	5.20 (6.3 µM)	5.64 (2.3 µM)	5.56 (2.75 µM)	5.43 (3.71 µM)	[49]
31[a])		5.21 (6.16 µM)	5.58 (2.63 µM)	< 5	7.65 (22.38 nM)	< 5	[4]

Table 1 (cont.)

Entry	Peptide	$hsst_1$ pK_d	$hsst_2$ pK_d	$hsst_3$ pK_d	$hsst_4$ pK_d	$hsst_5$ pK_d	Ref.
32[a])		< 5	< 5	< 5	6.15 (0.70 μM)	< 5	[4]
33[a])		< 5	5.22 (6.02 μM)	< 5	6.16 (0.69 μM)	< 5	[49]

Table 1 (cont.)

Entry	Peptide	$hsst_1$ pK_d	$hsst_2$ pK_d	$hsst_3$ pK_d	$hsst_4$ pK_d	$hsst_5$ pK_d	Ref.
	β-Dipeptides						
34		4.93 (11.7 µM)	4.51 (30.90 µM)	4.68 (20.89 µM)	7.13 (74.13 nM)	4.57 (26.91 µM)	[4][5][7]
35		4.76 (17.37 µM)	5.51 (3.09 µM)	5.12 (7.58 µM)	6.29 (0.51 µM)	5.22 (6.02 µM)	[4][5][7]

Table 1 (cont.)

Entry	Peptide	$hsst_1$ pK_d	$hsst_2$ pK_d	$hsst_3$ pK_d	$hsst_4$ pK_d	$hsst_5$ pK_d	Ref.
36		4.75 (17.78 μM)	< 5	5.08 (8.31 μM)	5.58 (2.63 μM)	< 5	[54]
37					5.57 (2.70 μM)		[4]

Table 1 (cont.)

Entry	Peptide	$hsst_1$ pK_d	$hsst_2$ pK_d	$hsst_3$ pK_d	$hsst_4$ pK_d	$hsst_5$ pK_d	Ref.
38					5.0 (10.00 μM)		[4]
39					5.37 (4.26 μM)		[4]

Table 1 (cont.)

Entry	Peptide	hsst$_1$ pK_d	hsst$_2$ pK_d	hsst$_3$ pK_d	hsst$_4$ pK_d	hsst$_5$ pK_d	Ref.
	γ-Peptides						
40	**3**						
	R^1 = Mes, R^2 = R^3 = Bn, R^4 = Me	5.47 (3.38 μM)	< 5	5.53 (2.95 μM)	4.67 (21.37 μM)	4.49 (32.35 μM)	[6]
	R^1 = H, R^2 = R^3 = Bn, R^4 = Me	6.06 (0.87 μM)	< 5	5.89 (1.28 μM)	5.74 (1.81 μM)	5.01 (9.77 μM)	[6]
	R^1 = MeS, R^2 = Bn, R^3 = H, R^4 = Me	6.26 (0.55 μM)	5.17 (6.76 μM)	6.00 (1.0 μM)	5.92 (1.20 μM)	5.87 (1.34 μM)	[6]
	R^1 = H, R^2 = Bn, R^3 = H, R^4 = Me	5.61 (2.45 μM)	< 5	5.73 (1.86 μM)	5.66 (2.18 μM)	5.14 (7.24 μM)	[6]
	R^1 = MeS, R^2 = R^3 = R^4 = H	5.98 (1.04 μM)	5.01 (9.77 μM)	5.67 (2.13 μM)	5.79 (1.62 μM)	6.29 (0.51 μM)	[6]
	R^1 = R^2 = R^3 = R^4 = H	4.73 (18.62 μM)	2.81 (1.55 μM)	5.42 (3.80 μM)	5.44 (3.63 μM)	6.06 (0.87 μM)	[6]

[a]) The C-terminal β^3hThr-NH_2 moiety **I** tends to cyclize to a lactone **II**, which, in turn, may hydrolyze to the hydroxy acid **III**. The molecular weights of the amide and the acid differ only by one mass unit. This has led to wrong structural assignments in our previous papers [4][5][7], and some of the amide formulae shown here may actually also be wrong. The lactone or the hydroxy-acid form may prevail depending upon isolation conditions, even under HPLC conditions (see *Footnote 26* in the *Exper. Part*). [b]) The formulae of βhThr in [4] have been incorrectly drawn with (2*R*,3*S*) instead of (2*R*,3*R*).

I **II** **III**

rather general phenomenon. The list of these GPCRs is impressive (*Table 2*). They involve numerous important physiological activities[25]).

Besides being ubiquitous and important, these interactions are attractive targets for biomedical research for another reason: the turn-type peptides interacting with the GPCRs need not be cell-penetrating, since the site of interaction is in the transmembrane part of the receptor protein, which is facing extracellularly; the agonist–receptor interaction triggers intracellular signals and transduction pathways (see the outline for somatostatin in *Fig. 5*).

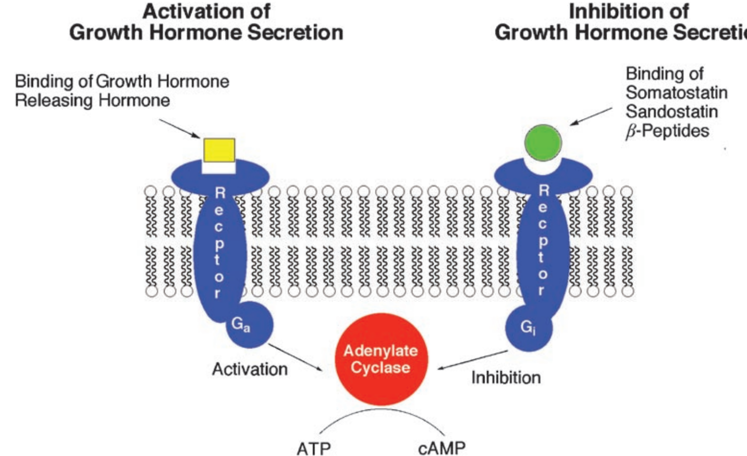

Fig. 5. *Schematic presentation of the interaction between a ligand* (peptide) *and G protein-coupled receptor, illustrated for somatostatin*

Following our successful mimicking of the interaction of the peptide hormone somatostatin with its receptors, by simple di- and tetrapeptides, containing β- or γ-amino-acid residues, the search for turn mimics of other GPCR peptide ligands is promising. Syntheses of such peptides are in progress in our laboratories.

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[25]) The title and some introductory statements of the 2005 review article by *Fairlie and* co-workers [55] emphasize the importance of peptide-turn-GPCR interactions: '*Over One Hundred Peptide-Activated G Protein-Coupled Receptors Recognize Ligands with Turn Structure. – Sequencing of the human genome has so far revealed between 700 and 1000 human genes that encode G protein-coupled receptors and, although many more may be added to this list, this is already the largest group of membrane-spanning surface receptors on human cells. Around 60% of known GPCRs are thought to be olfactory or sensory receptors associated with smell, taste, vision, etc., leaving at least 300–400 GPCRs that are nonsensory in function, including 175* orphan *receptors for which an endogenous ligand is still to be identified. Almost half of all registered pharmaceuticals today exert therapeutic effects by binding to GPCRs, yet they target only 30 GPCRs and only a few of those are peptide-activated GPCRs*'.

Table 2. *Peptide Hormones/Neurotransmitters* (ligands) *with Turn Structure, Which Are Recognized by G Protein-Coupled Receptors* (GPCRs). With minor modifications, the list is taken from [55] and presents the state of knowledge as of 2005. Many of the interactions involve β-turns of the type discussed herein (*cf. Footnote 25*).

Mammalian GPCR-Binding Peptide Hormones
Adrenomedullin
Agouti Protein and Agouti-Related Peptide
Amylin
Apelins
Angiotensins
Bombesin, Neuromedin B, and Gastrin Releasing Peptide
Bradykinin
Calcitonin
Calcitonin Gene-Related Peptide
β-Casomorphin
Chemokines
Cholecystokinin and Gastrin
Chorionic Gonadotropin
Complement Factor C5a
Complement Factor C3a
Corticotropin-Releasing Factor
Dynorphin A
Endomorphin
β-Endorphin
Endothelins
[Met]-Enkephalin and [Leu]-Enkephalin
Follicle-Stimulating Hormone
N-Formyl Peptides
Galanin and Galanin-Like Peptide
Ghrelin
Glucagon
Glucagon-Like Peptides **1** and **2**
Glucose-Dependent Insulinotropic Polypeptide
Glycoprotein Hormone: FSH, LH/HCG, TSH
Gonadotropin-Releasing Hormone
Growth Hormone-Releasing Hormone
Lutropin
Melanin-Concentrating Hormone
Melanocortins and Corticotropin
Motilin
Neuropeptide AF and Neuropeptide FF
Neuropeptide Y, Peptide YY, and Pancreatic Polypeptide
Neurotensin and Neuromedin N
Nociceptin
Orexin A and B
Oxytocin
Parathyroid Hormone
Pituitary Adenylate Cyclase Activating Peptide
Prolactin-Releasing Peptide
Protease-Activated Receptors: Thrombin, Trypsin
Relaxins

Table 2 (cont.)

Somatostatins/Cortistatins
Tachykinins: Substance P, Neurokinin A, and Neurokinin B
Thyrotropin
Thyrotropin-Releasing Hormone
Tuberoinfundibular Peptide
Urocortin, Urocortin II, and Urocortin III
Urotensin II
Vasoactive Intestinal Peptide
Vasopressin and Oxytocin
Nonmammalian Peptide Hormones
Alpha Factors
Neuropeptide F

Experimental Part

General. Et_3N and DIPEA were distilled from CaH_2 and stored over KOH. Protected Fmoc-amino acids were purchased from *Fluka*. *Rink Amide/Amide AM* resin was purchased from *Novabiochem*. Fmoc-(*S*)-β^2hTrp(Boc)-OH [56], Fmoc-(*R*)-β^2hTrp(Boc)-OH, Fmoc-(*S*)-β^2hLys(Boc)-OH [56][57], and Fmoc-(*S*)-β^2hPhe-OH [57] were synthesized according to literature procedures. *Abbreviations:* Boc: (*tert*-butoxy)carbonyl, CD: circular dichroism, DIPCDI: 1,3-diisopropylcarbodiimide, DIPEA: Et-N(^{i}Pr)$_2$, DMAP: 4-(dimethylamino)pyridine, DPA: pyridine-2,6-dicarboxylic acid, Fmoc: (9*H*-fluoren-3-yl)methoxycarbonyl, HATU: *O*-(7-azabenzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate, h.v.: high vacuum (0.01 – 0.1 Torr), MALDI: matrix-assisted laser-desorption ionization, MeIm: 1-methyl-1*H*-imidazole, NMM: *N*-methylmorpholine, SPPS: solid-phase peptide synthesis, TFA: CF_3COOH, TIS: (i-Pr)$_3$SiH, TNBS: 2,4,6-trinitrobenzenesulfonic acid, βhXaa: β-homoamino acid. NMR Spectra: chemical shifts δ are given in ppm relative to resonances of solvent (^{1}H: 3.31 ppm for CD_3OD; ^{13}C: 49.15 ppm for CD_3OD), coupling constants *J* are given in (Hz). The multiplicities of ^{13}C-NMR signals were determined by the DEPT technique; DEPT: + = primary or tertiary (positive DEPT signal), − = secondary (negative DEPT signal); C_q = quaternary C-atoms. MS: *VG Tribrid* (EI), *Bruker Reflex* (MALDI), or *IonSpec Ultima 4.7 TFT* Ion Cyclotron Resonance (ICR, MALDI-HR-MS, in a 2,5-dihydroxybenzoic acid matrix) mass spectrometer; in *m*/*z* (% of basis peak). Anal. HPLC: performed on a *Merck* HPLC system (*LaChrom*, pump type *L-7150*, UV detector *L-7400*, Interface *D-7000*, HPLC Manager *D-7000*). Prep. HPLC: *Merck/Hitachi* HPLC system (pump type *L-6250*, UV detector *L-4000*) or *Merck* HPLC system (*LaChrom*, pump type *L-7150*, UV detector *L-7400*, Interface *D-7000*, HPLC Manager *D-7000*). TFA for anal. and prep. RP-HPLC was UV-grade quality (> 99% GC). Lyophilization: *Hetosicc* cooling condenser with h.v. pump to obtain the peptides as their TFA salts.

Allyl Ester Preparation, General Procedure 1 (*GP 1*). The Fmoc-protected amino acid (1 equiv.) was added to a mixture of allyl bromide (23 equiv.) and DIPEA (2 equiv.), and the soln. was refluxed for 1 h. Upon cooling, the soln. was diluted with AcOEt, washed with 0.1% HCl and sat. aq. NaCl, and the org. layer was separated and concentrated to dryness. The resulting residue was treated with TFA (5 ml) in CH_2Cl_2 (5 ml) and stirred at r.t. for 2 h. Evaporation of the solvent, followed by flash chromatography (CH_2Cl_2/MeOH 95/5), afforded the pure allyl ester as TFA salt.

Loading of Kenner*'s Sulfonamide 'Safety-Catch' Linker: General Procedure 2* (*GP 2*). In a dried manual SPPS reactor, the soln. of the Fmoc-protected β-amino acid (4 equiv.), MeIm (4 equiv.), and DIPCDI (4 equiv.) in CH_2Cl_2/DMF (2 : 2) was added to the pre-swollen resin (CH_2Cl_2 for 1 h), and the suspension was mixed by Ar bubbling for 18 h at r.t. The resin was then filtered, washed with DMF (4 ml, 4 × 1 min) and CH_2Cl_2 (4 ml, 4 × 1 min), and dried under h.v. overnight. The loading was determined by measuring the absorbance of the benzofulvene piperidine adduct: two aliquots of the Fmoc-amino-acid resin were weighted exactly (m_1(resin) and m_2(resin) [mg]) and suspended in an exact amount of piperidine soln. (20% in DMF) in volumetric flasks ($V_1 = V_2 = 10$ ml). After 30 min, the mixtures were

transferred to a UV cell, and the absorbance (*A*) was measured relative to a blank piperidine soln. (20% in DMF) at 290 nm. The concentrations (c_1 and c_2 [mM]) of the benzofulvene – piperidine adduct in soln. were determined using a calibration curve [58]. 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 amino acid).

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

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

Fmoc Deprotection: General Procedure 3 (*GP 3*). The Fmoc deprotection was carried out using 20% piperidine in DMF (4 ml, 4 × 10 min) under N_2 bubbling. After filtration, the resin was washed with DMF (5 ml, 3 × 1 min) and CH_2Cl_2 (5 ml, 5 × 1 min).

Coupling of Amino Acids on Sulfamoyl/Wang/Rink *Amide*/Rink *Amide AM Resin: General Procedure 4* (*GP 4*). Fmoc Deprotection was carried out according to *GP 3*. SPPS was continued by sequential incorporation of Fmoc-protected amino acids. For each coupling step, the resin was treated with a soln. of Fmoc-protected amino acid (3 equiv.), HATU (2.9 equiv.), and DIPEA (6 equiv.) in DMF (5 ml) for 1 – 2 h. Monitoring of the coupling reaction was performed with the TNBS test [59]. In case of a positive TNBS test (indicating incomplete coupling), the suspension was allowed to react for a further 1 – 2 h, or retreated with the same Fmoc-protected β-amino acid (2 equiv.) and coupling reagents. After complete coupling, the resin was washed with DMF (5 ml, 5 × 1 min). The cycle was then repeated until all remaining amino acids were incorporated.

Activation of Sulfamoyl Resin, ICH_2CN Method (*Cyanomethylation*): *General Procedure 5* (*GP 5*). The peptide resin was Fmoc-deprotected according to *GP 3* and then washed with CH_2Cl_2 (4 ml, 4 × 1 min). The resin-bound peptide with free N-terminal amino group was then treated with trityl chloride (4 equiv.) and DIPEA (6 equiv.) in CH_2Cl_2 (5 ml) to furnish the N-terminal trityl derivative. After washing with CH_2Cl_2 (4 ml, 4 × 1 min), the resin was suspended in CH_2Cl_2 (4 ml), and DIPEA (10 equiv.) and ICH_2CN (25 equiv., prefiltered through an alumina plug) were added. The reaction flask was then shielded from light, and the resin was agitated for 24 h, filtered, and washed with CH_2Cl_2 (4 ml, 4 × 1 min) and DMF (4 ml, 4 × 1 min).

Cyclization Method and Cleavage from Sulfamoyl Resin: General Procedure 6 (*GP 6*). After cyanomethylation (*GP 5*), the resin was treated with 1% TFA and 5% TIS in CH_2Cl_2 to remove the trityl protecting group. Cyclization and cleavage was accomplished by treatment of the peptide – resin with DIPEA (4 equiv.) in DMF for 18 h at r.t. The side-chain-protected cyclic peptide was passed through a short flash column (eluting with CH_2Cl_2 and MeOH) to remove the nonpolar impurities. The solvent was removed under reduced pressure, and the peptide was dried under h.v. for 12 h.

Side-Chain Deprotection and Cleavage of Peptides from Wang/Rink *Amide*/Rink *Amide AM Resin: General Procedure 7a* (*GP 7a*). The dry peptide – resin was treated in a dry SPPS reactor with a soln. of TFA/TIS/H_2O (95 : 2.5 : 2.5, 10 ml) for 3 h. The resin was filtered, washed with TFA (2 × 5 ml), and the org. phase was concentrated under reduced pressure. The crude peptide, which formed upon addition of cold Et_2O to the oily residue, was collected, dried under h.v., and stored at $-20°$ before being purified by RP-HPLC.

General Procedure 7b (*GP 7b*). Identical to *GP 7a* but using TFA/TIS/H_2O/phenol 90 : 2.5 : 5 : 2.5 (10 ml) as the cleavage soln.

HPLC Analysis and Purification of Peptides: General Procedure 8 (*GP 8*). RP-HPLC Analysis was performed with a *Macherey-Nagel C_{18}* column (*Nucleosil 100-5 C_{18}* (250 × 4 mm)) or a *Macherey-Nagel* C_8 column (*Nucleosil 100-5 C_8* (250 × 4 mm)) using a linear gradient of *A* (MeCN) and *B* (0.1% TFA in H_2O) at a flow rate of 1 ml/min with UV detection at 220 nm. Retention time (t_R) in min. Aliquots of the crude products were purified by prep. RP-HPLC with a *Macherey-Nagel C_{18}* column (*Nucleosil 100-7 C_{18}* (250 × 21 mm)) or a *Macherey-Nagel C_8* column (*Nucleosil 100-7* C_8 (250 × 21 mm)), using a gradient of

A and *B* at a flow rate of 10 ml/min with UV detection at 220 nm. Lyophilization gave the pure peptide as a TFA salt (purity > 95%).

Loading of Fmoc-Amino-Acid Allyl Esters onto Chlorotrityl Resin: General Procedure 9 (*GP 9*). A soln. of Fmoc-amino-acid-allyl ester (*GP1*, 3 equiv.) and DIPEA (4 equiv.) in THF (4 ml) was added to previously swollen (4 ml dry THF, 1 h) chlorotrityl-resin (1.6 mmol/g) in a dried manual SPPS reactor and mixed by Ar bubbling for 2 h. The resin was washed with DMF (4 ml, 4 × 1 min) and CH_2Cl_2 (4 ml, 4 × 1 min). The unreacted chlorotrityl groups were 'capped' by treatment with CH_2Cl_2/MeOH/DIPEA 17:1:2 (4 ml, 2 × 2 min). After washing with CH_2Cl_2 (4 ml, 4 × 1 min), the resin was dried under h.v. overnight, and the resin loading was determined by the procedure outlined in *GP 2*.

Removal of Allyl Protecting Group: General Procedure 10 (*GP 10*). After the last amino-acid coupling (SPPS reactor), the resin was dried under h.v. for 4 h and re-swollen in CH_2Cl_2 (5 ml, 1 h) under Ar. A soln. of $PhSiH_3$ (24 equiv.) in CH_2Cl_2 (2 ml) was added, and the resin was shaken for 10 min under Ar. A soln. of $Pd(PPh_3)_4$ (0.1 equiv.) in dry CH_2Cl_2 (2 ml) was added, and, after shaking for 40 min, the resin was washed with CH_2Cl_2 (4 ml, 4 × 1 min). Treatment of the resin with $PhSiH_3/Pd(PPh_3)_4$ was repeated once again, and the resin was washed with CH_2Cl_2 (4 ml, 4 × 1 min) and DMF (4 ml, 4 × 1 min). Fmoc Deprotection was then carried out according to *GP 3*.

Peptide Cyclization: General Procedure 11 (*GP 11*). The peptide – resin was treated with a soln. of HATU (1.5 equiv.) and DIPEA (2 equiv.) in DMF (5 ml), and gently stirred using Ar bubbling at r.t. for 4 h. The resin was then washed with DMF (4 ml, 4 × 1 min) and CH_2Cl_2 (4 ml, 4 × 1 min), and dried under h.v. overnight (SPPS reactor).

Anchoring of N-*Fmoc-Protected Amino Acids on a* Rink *Amide Resin: General Procedure 12a* (*GP 12a*). The resin was placed into a dried manual SPPS reactor, swollen in DMF (20 ml/g resin) for 1 h, and washed with DMF (6 × 5 ml). The resin was deprotected according to *GP 3*, and the first amino acid was attached according to *GP 4*.

General Procedure 12b (*GP 12b*). Same as *GP 12a* except that, after attaching the first amino acid, the resin was 'capped' according to *GP 13*.

'Capping' of Free Amino Groups: General Procedure 13 (*GP 13*). The peptide – resin was covered with DMF (20 ml/g resin), and unreacted amino groups were 'capped' by treatment with 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 (SPPS reactor). 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).

Benzyl Deprotection: General Procedure 14 (*GP 14*). The Bn-protected peptide was dissolved in MeOH (5 ml) under N_2, and Pd/C (10% (*w/w*)) was added. The apparatus was evacuated, flushed with H_2 (3 ×), and the soln. was stirred under H_2 (balloon) for 12 h. The soln. was filtered through *Celite*, which was washed twice more with MeOH, and the combined solns. were concentrated under reduced pressure.

NMR Measurements. The trifluoroacetate salt (H_3N^+/COOH form) of β-peptides **7** and **14**, and protected forms of peptides **16** and **17** were dissolved in CD_3OH (0.7 ml), and the trifluoroacetate salts (H_3N^+/COOH form) of **8** and **9** were dissolved in 5% D_2O in H_2O. All NMR spectra were acquired with presaturation of the solvent OH signal at 600 MHz (^1H)/150 MHz (^{13}C).

DQF-COSY Measurements were performed with coherence transfer selection by *z*-gradients. TOCSY: 80 ms DIPSI-2 spin lock (8.9 kHz). HSQC: with coherence transfer selection by *z*-gradients. HMBC with coherence transfer selection by *z*-gradients. ROESY: with 300 and 150 ms CW-spin lock (2.8 kHz), or 200 ms (10 kHz) for compounds **8** and **9**. Spectral width 6000 Hz, 2 k × 512 data points were acquired (64 scans/FID) with TPPI. Data processing: with $\cos^2$ window function to give 1 k × 1 k real data points. Polynomial baseline corrections were done in both dimensions.

Assignments and volume integration of ROESY cross-peaks were performed with the aid of SPARKY [60]. 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, and other groups of isochronous H-atoms, were corrected through division by the number of protons.

Simulated-Annealing (SA) *Structure Calculations.* Program XPLOR-NIH v2.9.7. [61]. The standard parameter and topology files of XPLOR-NIH (parallhdg.pro; topallhdg.pro) were modified to

accommodate β^3-amino-acid residues. Minimized extended *zig-zag* conformations were used as the starting structures. The SA calculation protocol (adopted from the torsional angle dynamics protocol of *Stein et al.* [62] included 4000 steps (0.015 ps each) of high-temp. torsional-angle dynamics at 20000 K, followed by 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 nonbonded interactions used were *Van der Waals* repel functions. For each compound, 30 structures were calculated. The list of distance constraints is given in *Tables 4*, *7*, *9*, *11*, and *13*.

Preparation of **4**–**20**. *Fmoc-Lys-OAll · TFA* (**4**). Fmoc-Lys(Boc)-OH (5 g, 10 mmol) was treated with allyl bromide and DIPEA according to *GP 1* to give **4** (4.2 g, 81%). Yellow oil. ^{1}H-NMR (300 MHz, CD_3OD): 1.39–1.88 (*m*, 3 CH_2); 2.87 (*t*, *J* = 7.5, C*H*$_2$NH$_2$); 4.16 (*m*, C*H*NH, CH of Fmoc); 4.31 (*m*, CH_2O of allyl); 4.58 (*d*, *J* = 5.4, CH_2O of Fmoc); 5.17 (*dd*, *J* = 10.5, 1.3, HC=CH*H*); 5.27 (*dd*, *J* = 1.4, 17.1, HC=C*H*H); 5.86 (*m*, *H*C=CH$_2$); 7.24–7.42 (*m*, 4 arom. H); 7.68 (*t*, *J* = 12.6, 6.5, 2 arom. H); 7.74 (*d*, *J* = 7.4, 2 arom. H). ^{13}C-NMR (75 MHz, CD_3OD): 23.6 (CH_2); 27.7 (CH_2); 31.7 (CH_2); 40.2 (CH_2); 47.91 (CH); 55.0 (CH); 66.5 (CH_2); 67.6 (CH_2); 118.3 (CH_2 of allyl); 120.5, 125.7, 127.7, 128.4 (4 CH of Fmoc); 132.8 (CH of allyl); 142.1, 144.7 (2 C of Fmoc); 158.2 (CONH); 173.0 (CO). MALDI-HR-MS: 409.2119 ($[M+H]^+$, $C_{24}H_{29}N_2O_4^+$; calc. 409.2127).

*Fmoc-(*S*)-β^{3}hLys-OAll · TFA* (**5**). Fmoc-(*S*)-β^3hLys(Boc)-OH (1 g, 2.07 mmol) was treated with allyl bromide and DIPEA according to *GP 1* to give **5** (872 mg, 79%). Yellow oil. ^{1}H-NMR (300 MHz, CD_3OD): 1.36–1.70 (*m*, 3 CH_2); 2.5 (*d*, *J* = 6.6, C*H*$_2$COO); 2.88 (*m*, C*H*$_2$NH$_2$); 3.96 (*m*, C*H*NH); 4.17 (*t*, *J* = 6.6, CH of Fmoc); 4.35 (*ddd*, *J* = 6.9, 10.5, 17.4, CH_2O of allyl); 4.54 (*d*, *J* = 5.4, CH_2O of Fmoc); 5.15 (*dd*, *J* = 10.5, 1.2, HC=CH*H*); 5.28 (*ddd*, *J* = 1.2, 3.0, 17.4, HC=C*H*H); 5.90 (*m*, *H*C=CH$_2$); 7.09 (*d*, *J* = 9.0, NH); 7.27–7.41 (*m*, 4 arom. H); 7.63 (*d*, *J* = 7.2, 2 arom. H); 7.79 (*d*, *J* = 7.2, 2 arom. H). ^{13}C-NMR (75 MHz, CD_3OD): 23.6 (CH_2); 27.9 (CH_2); 34.9 (CH_2); 40.4 (CH_2); 40.7 (CH_2); 48.0 (CH); 48.8 (CH); 66.1 (CH_2); 67.3 (CH_2); 118.2 (CH_2 of allyl); 120.7, 126.0, 127.9, 128.6 (4 CH of Fmoc); 133.4 (CH of allyl); 142.4, 145.0 (2 C of Fmoc); 158.2 (CONH); 173.4 (CO). MALDI-HR-MS: 423.2278 ($[M+H]^+$, $C_{25}H_{31}N_2O_4^+$; calc. 423.2283).

*Fmoc-(*S*)-β^{2}hOrn-OAll · TFA* (**6**). Fmoc-(*S*)-β^2hOrn(Boc)-OH[26]) (400 mg, 0.85 mmol) was treated with allyl bromide and DIPEA according to *GP 1* to give **6** (340 mg, 76%). Yellow oil. ^{1}H-NMR (300 MHz, CD_3OD): 1.54–1.75 (*m*, 4 CH_2); 2.66 (*m*, CHCO); 2.89 (*m*, C*H*$_2$NH$_2$); 3.30 (*m*, C*H*$_2$NH); 4.17 (*t*, *J* = 6.9, CH of Fmoc); 4.33 (*m*, CH_2O of allyl); 4.58 (*dd*, *J* = 1.2, 5.7, CH_2O of Fmoc); 5.18 (*dd*, *J* = 1.2, 10.9, HC=CH*H*); 5.29 (*dd*, *J* = 1.2, 17.4, HC=C*H*H); 5.92 (*m*, *H*C=CH$_2$); 7.26–7.40 (*m*, 4 arom. H); 7.61 (*d*, *J* = 7.5, 2 arom. H); 7.78 (*d*, *J* = 7.5, 2 arom. H). ^{13}C-NMR (75 MHz, $CDCl_3$): 24.6 (CH_2); 25.2 (CH_2); 27.5 (CH_2); 39.5 (CH_2); 41.1 (CH_2); 44.7 (CH); 46.8 (CH); 65.9 (CH); 67.3 (CH_2); 119.1 (CH_2 of allyl); 120.0, 124.8, 127.0, 127.8 (4 CH of Fmoc); 131.1 (CH of allyl); 142.2, 143.3 (2 C of Fmoc); 160.0 (CONH); 173.9 (CO). MALDI-HR-MS: 409.2122 ($[M+H]^+$, $C_{24}H_{29}N_2O_4^+$; calc. 409.2127).

*Cyclo((*S*)-β^{2}hTrp-(*S*)-β^{3}hLys-βAla-βAla)* (**7**). According to *GP 2*, sulfamoyl resin (1.1 mmol/g, 600 mg, 0.660 mmol) was coupled with Fmoc-βAla-OH (822 mg, 2.64 mmol). Loading was determined to be 66% corresponding to 0.22 mmol of Fmoc-βAla-OH bound to the resin. Fmoc-βAla-OH was then coupled according to *GP 4*, and a portion of the Fmoc-dipeptide–resin (442 mg, 0.11 mmol) was used for completion of the synthesis. Solid-phase synthesis was then continued until all amino-acid residues had been incorporated. The peptide–resin was then cyanomethylated according to *GP 5*, and the peptide was cyclized and cleaved from the resin according to *GP 6*. Deprotection of the side chains according to *GP 7a* gave the crude cyclic peptide. Purification by RP-HPLC (5–50% *A* in 30 min, C_8) according to *GP 8* yielded *epi*-**7** (4.95 mg, 3.8%) and **7** (3.56 mg, 2.7%), both as a TFA salt.

Compound **7**. Colorless solid. Anal. RP-HPLC (0–1% *A* in 10 min, 1–50% *A* in 40 min): t_R 29.13, purity > 95%. ^{1}H-NMR (600 MHz): see *Table 3*; for NOEs, see *Table 4*. ESI-HR-MS: 507.2690 ($[M+Na]^+$, $C_{25}H_{36}N_6NaO_4^+$; calc. 507.2696).

Compound epi-**7**. Colorless solid. Anal RP-HPLC (0–1% *A* in 10 min, 1–50% *A* in 40 min): t_R 23.27 min, purity > 95%.

*Cyclo((*S*)-β^{3}hVal-(*S*)-β^{2}hPhe-(*S*)-β^{2}hTrp-(*S*)-β^{3}hLys)* (**8**). Chlorotrityl resin (1.6 mmol/g, 150 mg, 0.24 mmol) was coupled with Fmoc-(*S*)-β^3hLysOAll (304.2 mg, 0.72 mmol) according to *GP 9*. Loading

[26]) Provided by *Novartis Pharma AG*, Basel.

Table 3. *^{1}H-NMR Chemical Shifts for β-Tetrapeptide* **7** *in CD_3OH*

Amino acid	NH	$CH_2(\alpha)$	$H-C(\beta)$	$H-C(\gamma)$, $Me-C(\gamma)$, $CH_2(\gamma)$	$H-C(\delta)$, $Me-C(\delta)$	$Me-C(\varepsilon)$
β^2hTrp[1]	8.1	2.80	3.28/3.59	2.84/3.00		
β^3hLys[2]	7.66	2.18/2.29	4.03	1.11/1.28	0.76	1.37
βhGly[3]	7.96	2.36	3.37			
βhGly[4]	8.09	2.41	3.42			

Table 4. *NOEs Observed in the 300-ms ROESY Spectrum of Peptide* **7** *in CD_3OH*

Residue	H-Atom	Residue	H-Atom	d_{NOE} [Å]	Residue	H-Atom	Residue	H-Atom	d_{NOE} [Å]
1	β1	1	β2	2.0	4	β[a])	4	HN	3.3
1	HN	1	β[a])	3.2	1	HN	2	HN	4.1
1	HN	1	γ[a])	3.9	1	β[a])	2	HN	3.8
1	HN	1	β[a])	3.2	1	ε	2	β	4.7
2	α[a])	2	β	3.1	1	ε	2	HN	4.0
2	α[a])	2	HN	3.5	1	γ[a])	2	HN	3.3
2	γ[a])	2	β	3.1	2	α[a])	3	HN	3.2
2	HN	2	β	3.0	2	β	3	β[a])	4.7
2	HN	2	δ[a])	3.6	2	β	3	HN	3.4
2	HN	2	γ[a])	3.4	2	HN	3	HN	4.8
3	α[a])	3	HN	3.6	2	β	4	HN	4.1
3	β[a])	3	HN	3.1					

[a]) Pseudoatom used for calculations.

was determined to be 42% corresponding to 0.1 mmol of Fmoc-(*S*)-β^3hLysOAll bound to the resin. The remaining amino acids were sequentially incorporated according to *GP 4*. Allyl deprotection was performed according to *GP 10*, and the peptide was cyclized according to *GP 11*. Cleavage of the peptide from the resin and side-chain deprotection was carried out according to *GP 7a*. Purification of the crude peptide by prep. RP-HPLC (15–45% *A* in 40 min, C_8) according to *GP8* yielded **8** (13 mg, 18%) as a TFA salt. Colorless solid. Anal. RP-HPLC (20–45% *A* in 40 min, C_8): t_R 11.95, purity > 95%. ^{1}H-NMR (600 MHz): see *Table 5*. MALDI-HR-MS: 617.3810 ($[M+H]^+$, $C_{35}H_{49}N_6O_4^+$; calc. 617.3815).

Table 5. *^{1}H-NMR Chemical Shifts for β-Tetrapeptide* **8** *in 95% H_2O/5% D_2O*

Amino acid	NH	$CH_2(\alpha)$	$H-C(\beta)$ (^3J(HN,βH))	$H-C(\gamma)$, $Me-C(\gamma)$, $CH_2(\gamma)$	$H-C(\delta)$, $Me-C(\delta)$	$H-C(\varepsilon)$	$H-C(\zeta)$
β^2hPhe[1]	7.95	2.74	3.15/3.30	2.64/2.75			
β^2hTrp[2]	7.53	2.49	2.97/3.28	2.49/2.73			
β^3hLys[3]	7.25	1.96/2.30	3.85 (10.0)	0.81/0.98	0.39/0.52	1.20	2.46, 2.55
β^3hVal[4]	7.59	2.10/2.41	3.57 (8.8)	1.71	0.76		

*Cyclo((*S*)-β^3hThr-(*S*)-β^2hPhe-(*S*)-β^2hTRp-(*S*)-β^3hLys)* (**9**). Chlorotrityl resin (1.6 mmol/g, 250 mg, 0.40 mmol) was coupled with Fmoc-(*S*)-β^3hLysOAll (507 mg, 1.2 mmol) according to *GP 9*. Loading was determined to be 46% corresponding to 0.186 mmol of Fmoc-(*S*)-β^3hLysOAll bound to the resin. The remaining amino acids were sequentially incorporated according to *GP 4*. Allyl deprotection was

performed according to *GP 10*, and the peptide was cyclized according to *GP 11*. Cleavage of the peptide from the resin and side-chain deprotection was carried out according to *GP 7a*. Purification of the crude peptide by prep. RP-HPLC (15–35% *A* in 55 min, C_8) according to *GP 8* yielded **9** as TFA salt (36.5 mg, 27%). Colorless solid. Anal RP-HPLC (0–1% *A* in 10 min, 1–50% *A* in 40 min, C_8): t_R 35.25, purity > 95%. ^{1}H-NMR (600 MHz): see *Table 6*; for NOEs, see *Table 7*. ^{13}C-NMR (75 MHz, CD_3OD): 19.5 (Me); 22.8 (CH_2); 27.5 (CH_2); 27.6 (CH_2); 34.9, 37.2, 39.9, 40.3, 41.9, 42.7, 42.9 54.7, 69.6 (C(α), C(β), C(γ)); 111.8, 112.4, 119.2, 119.3, 121.9, 123.8, 127.0, 128.3, 129.0, 129.6, 137.6, 140.0 (arom. C); 173.6, 174.5, 175.7, 175.9 (4 CO). MALDI-HR-MS: 619.3603 ($[M+H]^+$, $C_{34}H_{47}N_6O_5^+$; calc. 619.3608).

Table 6. *^{1}H-NMR Chemical Shifts for β-Tetrapeptide* **9** *in 95% H_2O/5% D_2O*

Amino acid	NH	$CH_2(\alpha)$	H–C(β) (^3J(HN,βH))	H–C(γ), Me–C(γ), $CH_2(\gamma)$	H–C(δ), Me–C(δ)	H–C(ε)	H–C(ζ)
β^2hPhe^1	7.99	2.72	3.16/3.32	2.63/2.74			
β^2hTrp^2	7.51	2.50	2.92/3.30	2.51/2.75			
β^3hLys^3	7.25	1.98/2.29	3.85 (9.90)	0.78/0.95	0.36/0.49	1.17	2.46, 2.54
β^3hThr^4	7.56	2.22/2.46	3.77 (8.70)	3.80	1.01		

Table 7. *NOEs Observed in the 200-ms ROESY Spectrum of Peptide* **9** *in 50 mM Na-Phosphate, pH 6.8, in 95% H_2O/5% D_2O*[a])[b])

Residue	H-Atom	Residue	H-Atom	d_{NOE} [Å]	Residue	H-Atom	Residue	H-Atom	d_{NOE} [Å]
1	NH	1	γ_{Re}	3.0	2	C(2)H	3	γ2	4.0
1	NH	1	γ_{Si}	3.5	2	C(2)H	3	δ1	4.0
1	NH	1	β1	3.0	2	C(2)H	3	δ2	4.0
1	NH	1	β2	3.0	2	C(4)H	2	γ_{Re}	3.0
1	NH	4	α_{Si}	2.5	2	C(4)H	2	γ_{Si}	3.0
1	NH	4	α_{Re}	3.0[c])	3	NH	2	α	2.5
1	NH	4	β or γ	2.5	3	NH	3	α *	3.0
1	C(2)H/C(6)H	1	β1	3.5	3	NH	3	γ1	3.5
1	C(2)H/C(6)H	1	β2	3.5	3	NH	3	γ2	3.5
2	NH	1	β1	3.5	3	NH	3	δ1	3.5
2	NH	1	β2	3.5	3	NH	3	δ2	3.5
2	NH	1	γ2	3.0	4	NH	3	α_{Re}	3.0
2	NH	2	α or γ *	3.0	4	NH	3	α_{Si}	2.5
2	C(2)H	2	β1	4.0	4	NH	3	β	3.5
2	C(2)H	2	β2	4.0	4	NH	4	α_{Si}	3.5
2	C(2)H	2	γ_{Re}	3.0	4	NH	4	α_{Re}	3.0
2	C(2)H	2	γ_{Si}	3.5	4	NH	4	β or γ	3.0
2	C(2)H	3	γ1	4.0	4	NH	4	δ-Me	3.0

[a]) Stereospecific assignments for each pair of possible assignments; structures were calculated and the assignment giving lower energies was selected. [b]) For **8**, same NOEs as for **9** were observed. [c]) This NOE is violated by 0.3 Å in the calculated structures.

*Cyclo((*S*)-β²hOrn-(*S*)-β³hTrp-(*S*)-β³hPhe-(*S*)-β²hAla)* (**10**). Chlorotrityl resin (1.6 mmol/g, 150 mg, 0.24 mmol) was coupled with Fmoc-(*S*)-β^2hOrnOAll (336 mg, 0.82 mmol) according to *GP 9*. Loading was determined to be 37% corresponding to 0.09 mmol of Fmoc-β^2hOrnOAll bound to the resin. The remaining amino acids were sequentially incorporated according to *GP 4*. Allyl deprotection was performed according to *GP 10*, and the peptide was cyclized according to *GP 11*. Cleavage of the peptide

from the resin and side-chain deprotection was carried out according to *GP 7a*. Purification of the crude peptide by prep. RP-HPLC (20–50% *A* in 35 min, C_{18}) according to *GP 8* yielded **10** (17.6 mg, 28%) as a TFA salt. Colorless solid. Anal. RP-HPLC (5–50% *A* in 40 min, C_{18}): t_R 39.41, purity >95%. ^{1}H-NMR (300 MHz, CD_3OD): 0.28 (*d*, *J* = 6.4, Me); 1.55 (*m*, 2 CH_2); 2.30 (*m*, 3 H); 2.52 (*m*, 3 H); 2.78 (*dd*, *J* = 2.7, 6.9, CH_2); 2.89 (*t*, *J* = 6.4, CH_2); 2.96 (*t*, *J* = 7.2, CH_2); 3.13 (*dd*, *J* = 3.3, 13.8, CH_2); 3.35 (*q*, *J* = 13.2, C*H*$_2$NH); 4.35 (*m*, 2 C*H*NH); 6.98–7.34 (*m*, 9 arom. H); 7.59 (*d*, *J* = 7.8, 1 arom. H); 7.82 (*m*, NH). ^{13}C-NMR (75 MHz, CD_3OD): 16.2 (Me); 25.9 (CH_2); 27.3 (CH_2); 31.9 (CH_2); 40.2, 40.9, 41.1, 41.2, 41.5, 42.2, 43.2 (C(α), C(β)); 111.4, 112.0, 118.9, 119.4, 122.0, 124.1, 127.2, 128.4, 128.96, 128.97, 130.10, 130.11, 137.7, 138.8 (arom. C); 172.7, 174.9, 176.28, 176.29 (CO). MALDI-HR-MS: 575.3340 ([*M* + H]$^+$, $C_{32}H_{43}N_6O_4^+$; calc. 575.3345).

*Cyclo((*D*)-Trp-(*S*)-β^{3}hLys-βAla-βAla)* (**11**). Chlorotrityl resin (1.6 mmol/g, 250 mg, 0.40 mmol) was coupled with Fmoc-(*S*)-β^{3}hLysOAll (507 mg, 1.2 mmol) according to *GP 9*. Loading was determined to be 37% corresponding to 0.15 mmol of Fmoc-(*S*)-β^{3}hLysOAll bound to the resin. The remaining amino acids were sequentially incorporated according to *GP 4*. Allyl deprotection was performed according to *GP 10*, and the peptide was cyclized according to *GP 11*. Cleavage of the peptide from the resin and side-chain deprotection was carried out according to *GP7a*. Purification of the crude peptide by prep. RP-HPLC (5–50% *A* in 50 min, C_{18}) according to *GP 8* yielded **11** (4.6 mg, 5%) as a TFA salt. Colorless solid. Anal. RP-HPLC (5–50% *A* in 40 min): t_R 32.08, purity >95%. ^{1}H-NMR (300 MHz, CD_3OD): 1.25–1.65 (*m*, 3 CH_2); 2.13 (*m*, CH_2); 2.24–2.41 (*m*, 4 H); 2.85 (*t*, *J* = 7.2, CH_2); 3.10 (*m*, 3 H); 3.20–3.53 (*m*, 5 H); 4.09 (*m*, 1 H); 4.49 (*t*, *J* = 7.2, 1 H); 6.98–7.14 (*m*, 3 arom. H); 7.34 (*d*, *J* = 7.8, NH); 7.57 (*d*, *J* = 7.8, NH). MALDI-HR-MS: 471.2714 ([*M* + H]$^+$, $C_{24}H_{35}N_6O_4^+$; calc. 471.2719).

*Cyclo(Lys-(*S*)-β^{3}hTrp-βAla-βAla)* (**12**). Chlorotrityl resin (1.6 mmol/g, 250 mg, 0.40 mmol) was coupled with Fmoc-LysOAll (490.2 mg, 1.2 mmol) according to *GP 9*. Loading was determined to be 37.5% corresponding to 0.15 mmol of Fmoc-LysOAll bound to the resin. The remaining amino acids were sequentially incorporated according to *GP 4*. Allyl deprotection was performed according to *GP 10*, and the peptide was cyclized according to *GP 11*. Cleavage of the peptide from the resin and side-chain deprotection was carried out according to *GP 7a*. Purification of the crude peptide by prep. RP-HPLC (5–50% *A* in 45 min, C_{18}) according to *GP 8* yielded **12** (23.2 mg, 26%) as a TFA salt. ^{1}H-NMR (300 MHz, CD_3OD): 1.28–1.80 (*m*, 3 CH_2); 2.21 (*dd*, *J* = 3.2, 5.7, 1 H); 2.25 (*m*, 2 H); 2.35 (*dd*, *J* = 11.5, 15.0, 1 H); 2.45 (*dd*, *J* = 3.3, 15.0, 1 H); 2.49 (*dd*, *J* = 3.4, 15.0, 1 H); 2.85 (*t*, *J* = 7.5, CH_2); 2.89 (*dd*, *J* = 7.8, 14.6, C*H*H); 3.02 (*dd*, *J* = 5.0, 14.4, CH*H*); 3.20–3.58 (*m*, 2 CH_2); 4.18 (*m*, 1 H); 4.63 (*m*, 1 H); 6.97–7.33 (*m*, 3 arom. H); 7.59 (*dd*, *J* = 1.1, 7.5, 1 H); 7.77 (*d*, *J* = 6.0, 1 H); 7.93 (*d*, *J* = 7.5, NH); 8.12 (*t*, *J* = 5.9, NH). ^{13}C-NMR (75 MHz, CD_3OD): 23.3 (CH_2 of Lys); 27.5 (CH_2 of Lys); 31.1 (CH_2); 31.9 (CH_2); 36.2 (CH_2 of Gly); 36.4 (CH_2 of Gly); 36.5 (CH_2 of Gly); 37.2 (CH_2 of Gly); 40.0, 40.3 (C(α), C(β) of Trp); 54.2 (C(α) of Lys); 111.4, 111.8, 119.1, 119.3, 121.9, 124.0, 128.7, 137.7 (arom. C); 172.9, 173.4, 173.9, 174.3 (4 CO). MALDI-HR-MS: 471.2714 ([*M* + H]$^+$, $C_{24}H_{35}N_6O_4^+$; calc. 471.2718).

*Cyclo((*D*)-Trp-(*S*)-β^{3}hLys-(*S*)-β^{3}hThr-(*S*)-β^{2}hPhe)* (**13**). Chlorotrityl resin (1.6 mmol/g, 200 mg, 0.32 mmol) was coupled with Fmoc-(*S*)-β^{3}hLysOAll (422.5 mg, 0.96 mmol) according to *GP 9*. Loading was determined to be 41.6% corresponding to 0.133 mmol of Fmoc-(*S*)-β^{3}hLysOAll bound to the resin. The remaining amino acids were sequentially incorporated according to *GP 4*. Allyl deprotection was performed according to *GP 10*, and the peptide was cyclized according to *GP 11*. Cleavage of the peptide from the resin and side-chain deprotection was carried out according to *GP 7a*. Purification of the crude peptide by prep. RP-HPLC (20–40% *A* in 35 min, C_{18}) according to *GP 8* yielded **13** (24.4 mg, 25%) as a TFA salt. Colorless solid. Anal. RP-HPLC (5–50% *A* in 40 min): t_R 36.47, purity >95%. ^{1}H-NMR (300 MHz, DMSO): 0.92 (*d*, *J* = 6.3, Me); 1.00–1.44 (*m*, 2 CH_2); 2.14–3.16 (*m*, 12 H, CH, CH_2); 3.74 (*m*, CH_2); 4.49 (*q*, *J* = 6.9, CH); 5.32 (*s*, OH); 6.76 (*d*, *J* = 2.4, 1 H); 6.94–7.24 (*m*, 10 arom. H); 7.30 (*d*, *J* = 8.1, 1 arom. H); 7.44 (*d*, *J* = 7.8, 1 H); 7.66 (br., 3 NH); 7.82 (*m*, 2 NH). MALDI-HR-MS: 605.3446 ([*M* + H]$^+$, $C_{33}H_{45}N_6O_5^+$; calc. 605.3451).

*Cyclo(Lys-(*S*)-β^{3}hTrp-(*S*)-β^{3}hPhe-(*R*)-β^{2}hAla)* (**14**). Chlorotrityl resin (1.6 mmol/g, 150 mg, 0.24 mmol) was coupled with Fmoc-LysOAll (294 mg, 0.72 mmol) according to *GP 9*. Loading was determined to be 31% corresponding to 0.072 mmol of Fmoc-LysOAll bound to the resin. The remaining amino acids were sequentially incorporated according to *GP 4*. Allyl deprotection was performed according to *GP 10*, and the peptide was cyclized according to *GP 11*. Cleavage of the peptide from the

resin and side-chain deprotection was carried out according to *GP 7a*. Purification of the crude peptide by prep. RP-HPLC (10–50% *A* in 40 min, C_{18}) according to *GP 8* yielded besides the *epi*-**14** (5.1 mg, 10.3%) **14** (13.6 mg, 27%) both as a TFA salt.

Compound **14**. Colorless solid. Anal. RP-HPLC (0–1% *A* in 10 min, 1–50% *A* in 40 min): t_R 29.13, purity >95%. ^{1}H-NMR (600 MHz): see *Table 8*; for NOEs, see *Table 9*. ^{13}C-NMR (75 MHz, CD_3OH): 14.8 (Me); 23.3 (CH_2); 27.5 (CH_2); 31.0 (CH_2); 40.0, 40.3, 41.2, 41.4, 42.3, 42.5, 53.7 (C(α), C(β), C(γ)); 111.7, 111.9, 119.0, 119.4, 122.0, 123.9, 127.1, 128.5, 129.0, 130.0 (arom. CH); 137.7, 139.1 (C_q); 173.1, 173.7, 173.9, 176.7 (CO). MALDI-HR-MS: 575.3340 ([M+H]$^+$, $C_{32}H_{47}N_6O_4^+$; calc. 575.3345).

Compound epi-**14**. Colorless solid. Anal. RP-HPLC: t_R 38.76 min (5–50% *A* in 40 min), purity >95%.

Table 8. *^{1}H-NMR Chemical Shifts for β-Tetrapeptide* **14** *in CD_3OH*

Amino acid	NH	$CH_2(\alpha)$ (^3J(HN,αH))	H–C(β) (^3J(HN,βH))	H–C(γ), Me–C(γ), $CH_2(\gamma)$	H–C(δ), Me–C(δ)	Me–C(ε)
β^3hPhe^1	7.70	2.35/2.49	4.27 (8.58)	2.80/2.84		
β^3hAla^2	7.73	2.36	3.12/3.48	1.02		
Lys^3	8.03	4.23 (7.85)	1.57/1.85	1.37	1.60	2.87
β^4hTrp^4	7.80	2.50	4.48 (7.34)	2.94/3.05		

Table 9. *NOEs Observed in the 300-ms ROESY Spectrum of Peptide* **14** *in CD_3OH*

Residue	H-Atom	Residue	H-Atom	d_{NOE} [Å]	Residue	H-Atom	Residue	H-Atom	d_{NOE} [Å]
1	β	1	α_{Si}	2.6	3	$\gamma 2$	3	HN	3.5
1	β	1	α_{Re}	3.0	4	β	4	HN	2.8
1	β	1	γ[a])	3.2	4	$\gamma 1$	4	HN	3.1
1	β	1	HN	3.0	4	$\gamma 2$	4	HN	3.2
2	$\beta 1$	2	$\beta 2$	1.9	3	α	2	HN	2.7
2	α	2	γ[a])	2.9	2	HN	3	HN	2.9
3	α	3	$\gamma 1$	3.1	3	HN	4	HN	3.9
3	α	3	$\gamma 2$	3.2	1	β	4	HN	2.7
3	α	3	HN	2.9	2	γ[a])	4	β	5.3
3	$\beta 1$	3	$\beta 2$	2.0	2	γ[a])	4	HN	5.4
3	$\gamma 1$	3	HN	3.5	2	HN	4	β	4.0

[a]) Pseudoatom used for calculations.

*DPA-((*S*)-β^3hTyr-(*D*)-Trp-(*S*)-β^3hLys-(*S*)-β^3Thr-NH_2* (**15**). Fmoc-(*S*)-β^3hThr(*t*Bu)-OH (340 mg, 825 µmol) was loaded onto *Rink Amide* resin (500 mg, 550 µmol/g, 275 µmol, 100–200 mesh) according to *GP 12*. After capping (*GP 13*) and deprotection (*GP 3*), the resin was successively coupled with Fmoc-(*S*)-β^3hLys(Boc)-OH (398 mg, 825 µmol), Fmoc-(D)-Trp(Boc)-OH (434 mg, 825 µmol) and Fmoc-(*S*)-β^3hTyr(*t*Bu)-OH (390 mg, 825 µmol), and 6-[(benzyloxy)carbonyl]pyridine-2-carboxylic acid [63] (212 mg, 825 µmol) according to *GP 4*. The crude peptide was cleaved from the resin (*GP 7b*) and Bn-deprotected according to *GP 14*. Purification by prep. RP-HPLC (5–40% *A* in 50 min, C_{18}) according to *GP 8* yielded 40 mg (16%) of a compound corresponding to the lactone of **15**[27]) as colorless solid (RP-HPLC (5% *A* for 2 min then 5–40% *A* in 30 min, t_R 20.5), purity >98.5%) beside **15** (30 mg, 12%) as a TFA salt. Colorless solid. Anal. RP-HPLC (5% *A* for 2 min then 5–40% *A* in 30 min, C_{18}): t_R

[27]) We have observed that lactone formation can take place during the cleavage of β-peptides with C-terminal β^3hThr from the resin. Also, in urine and faeces of rats, by LC/MS techniques, various ratios of the lactone and the corresponding carboxylic acid were detected [4][5][7][54]. *cf. Footnote* in *Table 1*.

18.9 min, purity >97%. ^{1}H-NMR (500 MHz, CD_3OD): 0.94 (*m*, 2 H); 1.09 (*d*, *J* = 6.4, Me); 1.17 (*m*, 1 H); 1.31–1.49 (*m*, 3 H); 2.27 (*m*, 2 H); 2.38–2.51 (*m*, 2 H); 2.55 (*d*, *J* = 7.1, 2 H); 2.69 (*m*, 2 H); 2.83 (*m*, 2 H); 2.97 (*dd*, *J* = 7.3, 14.6, 1 H); 3.09 (*dd*, *J* = 7.3, 14.6, 1 H); 3.79 (*m*, CH); 3.97 (*m*, CH); 4.14 (*m*, CH); 4.48 (*t*, *J* = 7.3, CH); 4.64 (*m*, CH); 6.93 (*t*, *J* = 8.0, 1 arom. H); 7.04 (*m*, 4 arom. H); 7.23 (*d*, *J* = 8.1, 1 arom. H); 7.39 (*d*, *J* = 8.0, 1 arom. H); 7.58 (*d*, *J* = 9.1, NH); 7.68 (*d*, *J* = 8.6, NH); 8.06 (*t*, *J* = 7.8, 1 arom. H); 8.19 (*t*, *J* = 7.9, 2 arom. H). MALDI-MS: 825.3 (8, [*M* – H + K]$^+$), 810.3 (8, [*M* + Na]$^+$), 809.3 (18, [*M* – H + Na]$^+$), 789.4 (8), 788.4 (30, [*M* + H]$^+$), 787.4 (64, *M*$^+$), 772.3 (12), 771.3 (46), 770.3 (100, [*M* – 17]$^+$). HR-MS: 787.3551 ($C_{40}H_{49}N_7O_{10}^+$; calc. 787.3541).

*Ac-Tyr-(*S*)-β^2hTrp-(*S*)-β^3hLys-Thr-NH$_2$* (**16**). Fmoc-Thr(*t*Bu)-OH (138 mg, 426 µmol) was loaded onto the *Rink Amide* resin (200 mg, 710 µmol/g, 142 µmol, 100–200 mesh) according to *GP 12b*. After capping (*GP 13*) and deprotection (*GP 3*), the resin was successively coupled with Fmoc-(*S*)-β^3hLys(Boc)-OH (206 mg, 426 µmol), Fmoc-(*S*)-β^2hTrp(Boc)-OH (230 mg, 426 µmol), and Fmoc-Tyr(*t*Bu)-OH (165 mg, 426 µmol) according to *GP 4*. After final Fmoc deprotection (*GP 3*) and capping (*GP 13*), the crude peptide was cleaved from the resin (*GP 7b*) and purified by prep. RP-HPLC (5–50% *A* in 40 min, *C$_{18}$*, t_R 31.42 min) according to *GP 8* to yield **16** (67.2 mg, 61%) as a TFA salt. Colorless solid. Anal. RP-HPLC (5–50% *A* in 40 min, *C$_{18}$*): t_R 24.49 min, purity >98%. ^{1}H-NMR (500 MHz): see *Table 10*; for NOEs, see *Table 11*. ^{13}C-NMR (DEPT, 125 MHz, CD_3OH): 20.3 (+, Me); 22.6 (+, Me);

Table 10. *^{1}H-NMR Chemical Shifts for β-Tetrapeptide* **16** *in CD_3OH*

Amino acid	NH	H–C(α) (^3J(HN,αH))	H–C(β) (^3J(HN,βH))	H–C(γ), Me–C(γ), CH$_2$(γ)	H–C(δ), Me–C(δ)	H–C(ε), Me–C(ε)
Tyr[1]	8.12	4.56 (7.63)	2.78/2.97			
β^2hTrp[2]	8.23	2.85	3.14/3.55	2.80/2.93		
β^3hLys[3]	7.54	2.34	4.05 (8.95)	1.28/1.13	0.72	
Thr[4]	7.87	4.29 (8.29)	4.15	1.18		

Table 11. *NOEs Observed in the 300-ms ROESY Spectrum of Peptide* **16** *in CD_3OH*

Residue	H-Atom	Residue	H-Atom	d_{NOE} [Å]	Residue	H-Atom	Residue	H-Atom	d_{NOE} [Å]
1	α	1	β1	2.8	1	α	2	HN	2.3
1	α	1	β2	2.8	2	α	3	β	4.5
1	α	1	HN	2.9	2	α	3	HN	2.4
1	AcN	1	HN	3.0	2	β_{Re}	3	HN	3.1
2	β_{Si}	2	β_{Re}	1.9	2	HN	3	β	4.6
2	HN	2	β_{Si}	2.7	2	HN	3	HN	3.5
2	HN	2	β_{Re}	2.8	3	α[a])	4	HN	3.0
2	α	2	β_{Si}	2.5	3	β	4	HN	3.1
2	β_{Si}	2	γ1	2.5	3	HN	4	HN	3.9
2	β_{Si}	2	γ2	2.7	1	AcN	4	HN	5.3
3	β	3	α[a])	3.0	1	α	3	α[a])	4.1
3	β	3	HN	2.9	1	α	4	γ[a])	4.8
3	β	3	HNX[a])	4.5	1	α	4	HN	3.7
3	β	3	δ[a])	3.0	2	HN	4	α	4.4
4	α	4	γ[a])	3.1	2	HN	4	β	5.1
4	α	4	HN	2.8	2	HN	4	HN	4.2
4	β	4	HN	3.1	1	HN	4	α	5.2
4	γ[a])	4	HN	3.6					

[a]) Pseudoatom used for calculation.

23.1 (–, CH_2); 26.9 (–, CH_2); 27.9 (–, CH_2); 34.9 (–, CH_2); 38.4 (–, CH_2); 40.4 (–, CH_2); 42.8 (–, CH_2); 43.1 (–, CH_2); 47.9 (+, CH); 49.5 (+, CH); 56.8 (+, CH); 60.0 (+, CH); 68.4 (+, CH); 112.2 (+, arom. C); 113.3 (C_q, arom. C); 116.3 (+, 2 C, arom. C); 119.7 (+, arom. C); 119.8 (+, arom. C); 122.3 (+, arom. C); 124.2 (+, arom. C); 128.9 (C_q, arom. C); 129.1 (C_q, arom. C); 131.4 (+, 2 C, arom. C); 138.0 (C_q, arom. C); 157.3 (C_q, arom. C); 173.3 (C_q, C=O); 173.9 (C_q, C=O); 174.4 (C_q, C=O); 175.4 (C_q, C=O); 176.0 (C_q, C=O). MALDI: 704 (8), 689 (10), 688 (27), 668 (9), 667 (39), 666 (100), 648 (11). MALDI-HR-MS: 666.3615 (100, $[M+H]^+$, $C_{34}H_{48}N_7O_7^+$; calc. 666.3615), 688.3431 (27, $[M+Na]^+$, $C_{34}H_{47}N_7NaO_7^+$; calc. 688.3435).

*Ac-Thr-(*R*)-β²Lys-(*S*)-β³Trp-Tyr-NH₂* (**17**). Fmoc-Tyr(*t*Bu)-OH (148 mg, 321 μmol) was loaded onto the *Rink Amide* resin (150 mg, 0.71 mmol/g, 106.5 μmol) according to *GP 12a*. After Fmoc deprotection (*GP 3*), the resin was successively coupled with Fmoc-(*S*)-β^3hTrp(Boc)-OH (230 mg, 426 μmol), Fmoc-(*S*)-β^2hLys(Boc)-OH (200 mg, 426 μmol), and Fmoc-Thr(*t*Bu)-OH (138 mg, 426 μmol) according to *GP 4*. After final Fmoc deprotection (*GP 3*) and capping (*GP 13*), the crude peptide was cleaved from the resin (*GP 7a*) and purified by prep. RP-HPLC (5% *A* for 5 min, 5–50% *A* in 40 min, C_{18}) according to *GP 8* to yield **17** (24.6 mg, 30%) as a TFA salt. Colorless solid. Anal. RP-HPLC (5% *A* for 5 min, 5–50% *A* in 40 min, C_{18}): t_R 23.8, purity > 98%. ^{1}H-NMR (500 MHz): see *Table 12*; for NOEs, see *Table 13*. ^{13}C-NMR (150 MHz, DEPT): 20.5 (+, Me); 22.8 (+, Me); 26.0 (–, CH_2); 26.9 (–, CH_2); 31.9 (–, CH_2); 38.3 (–, CH_2); 40.6 (–, CH_2); 41.8 (–, CH_2); 42.9 (–, CH_2); 47.2 (+, CH); 50.0 (+, CH); 56.3 (+, CH); 61.3 (+, CH); 68.6 (+, CH); 112.4 (+, arom. C); 112.5 (C_q, arom. C); 116.4 (+, arom. C); 119.8 (C_q, arom. C); 119.9 (+, arom. C); 122.4 (+, arom. C); 124.7 (+, arom. C); 129.1 (C_q, arom. C); 129.2 (C_q, arom. C); 131.4 (+, arom. C); 138.3 (C_q, arom. C); 157.5 (C_q, arom. C); 173.5 (C_q, C=O); 173.7 (C_q, C=O); 174.0 (C_q, C=O); 175.2 (C_q, C=O); 176.6 (C_q, C=O). MALDI: 704.3 (28, $[M+K]^+$), 689.8 (37), 688.3 (100, $[M+Na]^+$), 667.4 (30), 666.4 (88, $[M+H]^+$), 648.4 (21). HR-MS: 688.3439 ($C_{34}H_{47}N_7NaO_7^+$; calc. 688.3435).

Table 12. *¹H-NMR Chemical Shifts for β-Tetrapeptide* **17** *in CD₃OH*

Amino acid	NH	H–C(α) (³J(HN,αH))	H–C(β) (³J(HN,βH))	H–C(γ), Me–C(γ), CH₂(γ)	H–C(δ), Me–C(δ)	H–C(ε), Me–C(ε)
Thr¹	7.96	4.23 (7.63)	4.15	1.19		
β²hLys²	8.24	2.25	3.02/3.38	1.28/1.39	n.a.	1.15/1.22
β³hTrp³	7.78	2.42/2.49	4.55 (8.73)	2.90		
Tyr⁴	8.00	4.52 (7.78)	2.83/2.98			

*Ac-(*R*)-β³hThr-(*S*)-β²hLys-(*S*)-β³hTrp-(*R*)-β³hPhe-NH₂* (**18**). Fmoc-(*R*)-β^3hPhe-OH (134 mg, 280 μmol) was loaded onto the *Rink Amide AM* resin (100 mg, 0.071 mmol, 0.71 mmol/g) according to *GP 12a*. The resin was then successively coupled with Fmoc-(*S*)-β^3hTrp(Boc)-OH (151 mg, 280 μmol), Fmoc-(*S*)-β^2hLys(Boc)-OH (103 mg, 210 μmol), and Fmoc-(*R*)-β^3hThr(*t*Bu)-OH (86 mg, 210 μmol) according to *GP 4*. After final Fmoc deprotection (*GP 3*) and capping (*GP 13*), the crude peptide was cleaved from the resin (*GP 7a*), and a portion (33 mg) was purified by prep. RP-HPLC (22–98% *A* in 50 min, C_{18}, 17 ml/min; t_R 19.67) according to *GP 8* to yield **18** (3.2 mg, 14%) as a TFA salt. Colorless solid. Anal. RP-HPLC (5–50% *A* in 45 min, C_{18}): t_R 26.29, purity > 98%. MALDI-HR-MS: 678.3984 ($[M+H]^+$, $C_{36}H_{52}N_7O_6^+$; calc. 678.3974).

*Ac-Thr-(*R*)-Lys-(*S*)-β³hTrp-Tyr-NH₂* (**19**). Fmoc-Tyr(*t*Bu)-OH (165 mg, 426 μmol) was loaded onto the *Rink Amide* resin (200 mg, 710 μmol/g, 142 μmol, 100–200 mesh) according to *GP 12b*. After capping (*GP 13*) and Fmoc deprotection (*GP 3*), the resin was successively coupled with Fmoc-(*S*)-β^3hTrp(Boc)-OH (230 mg, 426 μmol), Fmoc-(*R*)-Lys(Boc)-OH (200 mg, 426 μmol), and Fmoc-Thr(*t*Bu)-OH (138 mg, 426 μmol) according to *GP 4*. After final Fmoc deprotection (*GP 3*) and capping (*GP 13*), the crude peptide was cleaved from the resin (*GP 7b*) and purified by prep. RP-HPLC (5–50% *A* in 40 min, t_R 21.45 min) according to *GP 8* to yield **19** (58.3 mg, 53%) as a TFA salt. Colorless solid.

Table 13. *NOEs Observed in the 300-ms ROESY Spectrum of Peptide* **17** *in CD_3OH*

Residue	H-Atom	Residue	H-Atom	d_{NOE} [Å]	Residue	H-Atom	Residue	H-Atom	d_{NOE} [Å]
1	α	1	γ[a])	3.0	1	HN	2	HN	3.0
1	α	1	HN	3.0	1	α	2	α	5.0
1	β	1	γ[a])	3.0	2	α	3	β	4.9
1	AcN	1	α	4.9	2	α	3	HN	2.2
1	AcN	1	β	5.1	2	β_{Si}	3	HN	4.2
1	AcN	1	HN	3.0	2	β_{Re}	3	HN	3.7
2	α	2	β_{Si}	2.6	2	HN	3	β	4.8
2	α	2	β_{Re}	3.1	2	HN	3	HN	3.3
2	α	2	HN	3.2	3	α_{Si}	4	HN	2.8
2	β_{Si}	2	β_{Re}	1.9	3	α_{Re}	4	HN	2.7
2	$\gamma 1$	2	HN	3.9	3	β	4	HN	3.5
2	$\gamma 2$	2	HN	4.1	3	HN	4	HN	4.2
3	α_{Si}	3	HN	3.2	1	AcN	4	α	4.7
3	α_{Re}	3	HN	3.0	1	α	3	HN	4.5
3	β	3	γ[a])	3.1	1	α	4	$\beta 1$	4.6
3	β	3	HN	2.9	1	α	4	$\beta 2$	4.7
3	γ[a])	3	HN	3.0	1	α	4	HN	3.9
4	α	4	HN	2.8	2	HN	4	α	4.0
4	β[a])	4	$CONH_2$	3.8	1	α	4	$CONH_2$	4.8
1	α	2	HN	2.5	1	HN	4	α	4.0
1	β	2	HN	2.9	1	β	4	$\beta 1$	4.9
1	γ[a])	2	HN	4.2	1	β	4	$\beta 2$	4.9

[a]) Pseudoatom used for calculation.

Anal. RP-HPLC (5–50% *A* in 40 min): t_R 26.01 min, purity >98%. ^{1}H-NMR (500 MHz, CD_3OH): 1.24 (*d*, *J* = 6.1, Me); 1.29–1.36 (*m*, CH_2); 1.52–1.64 (*m*, CH, CH_2); 1.78–1.99 (*m*, CH); 1.91 (*s*, Me); 2.33 (*dd*, *J* = 4.3, 9.0, CH); 2.42 (*dd*, *J* = 6.9, 10.6, CH); 2.76–2.89 (*m*, 2 CH_2); 2.95 (*dd*, *J* = 3.8, 7.0, CH_2); 4.09–4.13 (*m*, CH_2); 4.40 (*dd*, *J* = 4.1, 6.9, CH_2); 4.54–4.59 (*m*, CH); 6.66 (*d*, *J* = 8.6, 2 arom. H); 6.99–7.02 (*m*, 4 arom. H); 7.07 (*t*, *J* = 7.0, 1 arom. H); 7.31 (*d*, *J* = 6.3, 1 arom. H); 7.59 (*d*, *J* = 7.8, 1 arom. H). ^{13}C-NMR (DEPT, 125 MHz, CD_3OH): 20.4 (+, Me); 22.6 (+, Me); 23.6 (−, CH_2); 27.8 (−, CH_2); 31.1 (−, CH_2); 31.3 (−, CH_2); 38.6 (−, CH_2); 40.5 (−, CH_2); 41.0 (−, CH_2); 50.3 (+, CH); 53.8 (+, CH); 55.5 (+, CH); 62.3 (+, CH); 68.1 (+, CH); 111.5 (+, arom. C); 112.2 (+, arom. C); 116.3 (+, 2 C, arom. C); 119.7 (+, arom. C); 119.9 (+, arom. C); 122.3 (+, arom. C); 124.7 (+, arom. C); 128.7 (C_q, arom. C); 129.2 (C_q, arom. C); 131.3 (C_q, 2 C, arom. C); 138.0 (C_q, arom. C); 157.4 (C_q, arom. C); 173.0 (C_q, C=O); 173.1 (C_q, C=O); 173.6 (C_q, C=O); 174.3 (C_q, C=O); 176.5 (C_q, C=O). MALDI: 675 (10), 674 (26), 654 (8), 653 (37), 652 (100), 650 (7), 649 (18), 608 (27), 607 (7), 456 (8), 260 (30). MALDI-HR-MS: 652.3445 (100, $[M+H]^+$, $C_{33}H_{46}N_7O_7^+$; 652.3459), 674.3300 (26, $[M+Na]^+$, $C_{33}H_{45}N_7NaO_7^+$; calc. 674.3278).

*Ac-Tyr-(*R*)-Trp-(*S*)-β^{3}hLys-Thr-NH$_2$* (**20**). Fmoc-Thr(*t*Bu)-OH (138 mg, 426 μmol) was loaded onto the *Rink Amide* resin (200 mg, 710 μmol/g, 142 μmol, 100–200 mesh) according to *GP 12b*. After capping (*GP 13*) and Fmoc deprotection (*GP 3*), the resin was successively coupled with Fmoc-(*S*)-β^{3}hLys(Boc)-OH (206 mg, 426 μmol), Fmoc-(*R*)-Trp(Boc)-OH (224 mg, 426 μmol), and Fmoc-Tyr(*t*-Bu)-OH (165 mg, 426 μmol) according to *GP 4*. After final Fmoc deprotection (*GP 3*) and capping (*GP 13*), the crude peptide was cleaved from the resin (*GP 7b*) and purified by prep. RP-HPLC (5–50% *A* in 40 min, t_R 32.53 min) according to *GP 8* to yield **20** (61.1 mg, 57%) as a TFA salt. Colorless solid. Anal. RP-HPLC (5–50% *A* in 40 min): t_R 25.02 min, purity >98%. ^{1}H-NMR (500 MHz, CD_3OH): 1.17 (*d*, *J* = 6.4, Me); 1.20–1.32 (*m*, CH_2); 1.39–1.66 (*m*, 2 CH_2); 1.91 (*s*, Me); 2.35 (*dd*, *J* = 5.6, 9.6, CH); 2.56 (*dd*, *J* = 8.8, 11.2, CH); 2.65–2.74 (*m*, CH_2); 2.78–2.89 (*m*, CH_2); 2.97 (*dd*, *J* = 8.7, 11.8, CH); 3.22 (*dd*, *J* = 4.6, 7.4, CH); 4.09–4.13 (*m*, CH); 4.17–4.27 (*m*, CH); 4.29 (*d*, *J* = 4.0, CH); 4.35 (*t*, *J* = 7.5, CH);

4.50–4.52 (*m*, CH); 6.64 (*d*, $J = 8.6$, 2 arom. H); 6.91 (*d*, $J = 8.5$, 3 arom. H); 7.05 (*t*, $J = 7.0$, 1 arom. H); 7.07 (*t*, $J = 7.0$, 1 arom. H); 7.31 (*d*, $J = 8.8$, 1 arom. H); 7.53 (*d*, $J = 8.8$, 1 arom. H). ^{13}C-NMR (DEPT, 125 MHz, CD_3OH): 20.2 (+, Me); 22.5 (+, Me); 23.3 (−, CH_2); 27.9 (−, CH_2); 28.0 (−, CH_2); 35.0 (−, CH_2); 37.2 (−, CH_2); 40.6 (−, CH_2); 42.0 (−, CH_2); 48.3 (+, CH); 55.9 (+, CH); 57.8 (+, CH); 59.7 (+, CH); 68.5 (+, CH); 111.2 (C_q, arom. C); 112.4 (+, arom. C); 116.4 (+, 2 C, arom. C); 119.6 (+, arom. C); 119.9 (+, arom. C); 122.5 (+, arom. C); 124.6 (+, arom. C); 128.70 (C_q, arom. C); 128.74 (C_q, arom. C); 131.3 (+, 2 C, arom. C); 138.1 (C_q, arom. C); 157.4 (C_q, arom. C); 173.5 (C_q, C=O); 173.6 (C_q, C=O); 173.8 (C_q, C=O); 174.6 (C_q, C=O); 175.0 (C_q, C=O). MALDI: 675 (22), 674 (60), 654 (8), 653 (37), 652 (100), 634 (23), 456 (7), 260 (10). MALDI-HR-MS: 652.3445 (100, $[M+H]^+$, $C_{33}H_{46}N_7O_7^+$; 652.3459), 674.3294 (60, $[M+Na]^+$, $C_{33}H_{45}N_7NaO_7^+$; calc. 674.3278).

REFERENCES

[1] K. Gademann, M. Ernst, D. Hoyer, D. Seebach, *Angew. Chem., Int. Ed.* **1999**, *38*, 1223.
[2] K. Gademann, M. Ernst, D. Seebach, D. Hoyer, *Helv. Chim. Acta* **2000**, *83*, 16.
[3] K. Gademann, T. Kimmerlin, D. Hoyer, D. Seebach, *J. Med. Chem.* **2001**, *44*, 2460.
[4] D. Seebach, M. Rueping, P. I. Arvidsson, T. Kimmerlin, P. Micuch, C. Noti, D. Langenegger, D. Hoyer, *Helv. Chim. Acta* **2001**, *84*, 3503.
[5] C. Nunn, M. Rueping, D. Langenegger, E. Schuepbach, T. Kimmerlin, P. Micuch, K. Hurth, D. Seebach, D. Hoyer, *Naunyn-Schmiedebergs Arch. Pharmacol.* **2003**, *367*, 95.
[6] D. Seebach, L. Schaeffer, M. Brenner, D. Hoyer, *Angew. Chem., Int. Ed.* **2003**, *42*, 776.
[7] H. Wiegand, B. Wirz, A. Schweitzer, G. Gross, M. I. R. Perez, H. Andres, T. Kimmerlin, M. Rueping, D. Seebach, *Chem. Biodivers.* **2004**, *1*, 1812.
[8] D. Hoyer, G. I. Bell, M. Berelowitz, J. Epelbaum, W. Feniuk, P. P. A. Humphrey, A. M. Ocarroll, Y. C. Patel, A. Schonbrunn, J. E. Taylor, T. Reisine, *Trends Pharmacol. Sci.* **1995**, *16*, 86; E. E. Muller, V. Locatelli, D. Cocchi, *Physiol. Rev.* **1999**, *79*, 511; Z. Csaba, P. Dournaud, *Neuropeptides* **2001**, *35*, 1.
[9] H. M. Weiss, B. Wirz, A. Schweitzer, R. Amstutz, M. I. R. Perez, H. Andres, Y. Metz, J. Gardiner, D. Seebach, *Chem. Biodivers.* **2007**, *4*, 1413.
[10] W. Bauer, U. Briner, W. Doepfner, R. Haller, R. Huguenin, P. Marbach, T. J. Petcher, J. Pless, *Life Sci.* **1982**, *31*, 1133.
[11] E. Pohl, A. Heine, G. M. Sheldrick, Z. Dauter, K. S. Wilson, J. Kallen, W. Huber, P. J. Pfaffli, *Acta Crystallogr., Sect. D* **1995**, *51*, 48.
[12] C. R. R. Grace, J. Erchegyi, S. C. Koerber, J. C. Reubi, J. Rivier, R. Riek, *J. Med. Chem.* **2006**, *49*, 4487; C. R. R. Grace, L. Durrer, S. C. Koerber, J. Erchegyi, J. C. Reubi, J. E. Rivier, R. Riek, *J. Med. Chem.* **2005**, *48*, 523; C. R. R. Grace, S. C. Koerber, J. Erchegyi, J. C. Reubi, J. Rivier, R. Riek, *J. Med. Chem.* **2003**, *46*, 5606.
[13] D. Seebach, B. Jaun, R. Sebesta, R. I. Mathad, O. Flögel, M. Limbach, H. Sellner, S. Cottens, *Helv. Chim. Acta* **2006**, *89*, 1801.
[14] D. F. Hook, F. Gessier, C. Noti, P. Kast, D. Seebach, *ChemBioChem* **2004**, *5*, 691.
[15] S. A. Kates, N. A. Solé, C. R. Johnson, D. Hudson, G. Barany, F. Albericio, *Tetrahedron Lett.* **1993**, *34*, 1549.
[16] A. Trzeciak, W. Bannwarth, *Tetrahedron Lett.* **1992**, *33*, 4557.
[17] a) J. A. Davies, *Amino Acids, Peptides, Proteins* **2001**, *32*, 287; b) A. Janecka, M. Zubrzycka, T. Janecki, *J. Pept. Res.* **2001**, *58*, 91; c) U. Schmidt, J. Langner, *J. Pept. Res.* **1997**, *49*, 67.
[18] M. Rothe, D. Mühlhausen, *Angew. Chem., Int. Ed.* **1979**, *18*, 74.
[19] D. Seebach, A. K. Beck, D. J. Bierbaum, *Chem. Biodivers.* **2004**, *1*, 1111.
[20] P. W. Sutton, A. Bradley, J. Farràs, P. Romea, F. Urpí, J. Vilarrasa, *Tetrahedron* **2000**, *56*, 7947.
[21] D. N. J. White, C. Morrow, P. J. Cox, C. N. C. Drey, J. Lowbridge, *J. Chem. Soc., Perkin Trans. 2* **1982**, 239.
[22] J. Lowbridge, E. Mtetwa, R. J. Ridge, C. N. C. Drey, *J. Chem. Soc., Perkin Trans. 1* **1986**, 155.
[23] D. Seebach, M. Overhand, F. N. M. Kühnle, B. Martinoni, L. Oberer, U. Hommel, H. Widmer, *Helv. Chim. Acta* **1996**, *79*, 913; J. L. Matthews, M. Overhand, F. N. M. Kühnle, P. E. Ciceri, D. Seebach,

Liebigs Ann./Recl. **1997**, 1371; J. L. Matthews, K. Gademann, B. Jaun, D. Seebach, *J. Chem. Soc., Perkin Trans. 1* **1998**, 3331.
[24] K. Gademann, D. Seebach, *Helv. Chim. Acta* **2001**, *84*, 2924.
[25] F. Büttner, M. Erdélyi, P. I. Arvidsson, *Helv. Chim. Acta* **2004**, *87*, 2735.
[26] F. Fujimura, M. Fukuda, J. Sugiyama, T. Morita, S. Kimura, *Org. Biomol. Chem.* **2006**, *4*, 1896.
[27] S. Fournel, S. Wieckowski, W. Sun, N. Trouche, H. Dumortier, A. Bianco, O. Chaloin, M. Habib, J.-C. Peter, P. Schneider, B. Vray, R. E. Toes, R. Offringa, C. J. M. Melief, J. Hoebeke, G. Guichard, *Nat. Chem. Biol.* **2005**, *1*, 377; M. Habib, M. N. Rivas, M. Chamekh, S. Wieckowski, W. Sun, A. Bianco, N. Trouche, O. Chaloin, H. Dumortier, M. Goldman, G. Guichard, S. Fournel, B. Vray, *J. Immunol.* **2007**, *178*, 6700.
[28] a) D. Seebach, J. L. Matthews, A. Meden, T. Wessels, C. Baerlocher, L. B. McCusker, *Helv. Chim. Acta* **1997**, *80*, 173; b) H. C. Le, T. Hintermann, T. Wessels, Z. Gan, D. Seebach, R. R. Ernst, *Helv. Chim. Acta* **2001**, *84*, 208.
[29] T. D. Clark, L. K. Buehler, M. R. Ghadiri, *J. Am. Chem. Soc.* **1998**, *120*, 651.
[30] M. Royo, J. Farrera-Sinfreu, L. Solé, F. Albericio, *Tetrahedron Lett.* **2002**, *43*, 2029.
[31] F. Büttner, A. S. Norgren, S. Zhang, S. Prabpai, P. Kongsaeree, P. I. Arvidsson, *Chem. – Eur. J.* **2005**, *11*, 6145.
[32] S. A. W. Gruner, V. Truffault, G. Voll, E. Locardi, M. Stöckle, H. Kessler, *Chem. – Eur. J.* **2002**, *8*, 4366.
[33] F. Schumann, A. Müller, M. Koksch, G. Müller, N. Sewald, *J. Am. Chem. Soc.* **2000**, *122*, 12009; M. Sukopp, R. Schwab, L. Marinelli, E. Biron, M. Heller, E. Várkondi, A. Pap, E. Novellino, G. Kéri, H. Kessler, *J. Med. Chem.* **2005**, *48*, 2916; D. Zimmermann, E. W. Guthöhrlein, M. Malešević, K. Sewald, L. Wobbe, C. Heggemann, N. Sewald, *ChemBioChem* **2005**, *6*, 272; A. S. Norgren, F. Büttner, S. Prabpai, P. Kongsaeree, P. I. Arvidsson, *J. Org. Chem.* **2006**, *71*, 6814.
[34] a) M. Z. Magee, K. Hofmann, *J. Am. Chem. Soc.* **1949**, *71*, 1515; b) J. C. Sheehan, W. L. Richardson, *J. Am. Chem. Soc.* **1954**, *76*, 6329; c) R. Schwyzer, A. Tun-Kyi, *Helv. Chim. Acta* **1962**, *45*, 859; d) H. Gerlach, J. A. Owtschinnikow, V. Prelog, *Helv. Chim. Acta* **1964**, *47*, 2294.
[35] a) J. J. Wen, A. F. Spatola, *Tetrahedron Lett.* **2005**, *46*, 2499; b) S. Ueda, S. Oishi, Z.-X. Wang, T. Araki, H. Tamamura, J. Cluzeau, H. Ohno, S. Kusano, H. Nakashima, J. O. Trent, S. C. Peiper, N. Fujii, *J. Med. Chem.* **2007**, *50*, 192.
[36] U. Schmidt, H. Griesser, A. Lieberknecht, J. Talbiersky, *Angew. Chem., Int. Ed.* **1981**, *20*, 280; b) U. Schmidt, A. Lieberknecht, H. Griesser, J. Häusler, *Angew. Chem., Int. Ed.* **1981**, *20*, 281; c) U. Schmidt, A. Lieberknecht, J. Wild, *Synthesis* **1988**, 159.
[37] T. Jeremic, A. Linden, K. Moehle, H. Heimgartner, *Tetrahedron* **2005**, *61*, 1871.
[38] S. F. Brady, R. M. Freidinger, W. J. Paleveda, C. D. Colton, C. F. Homnick, W. L. Whitter, P. Curley, R. F. Nutt, D. F. Veber, *J. Org. Chem.* **1987**, *52*, 764; D. F. Veber, R. M. Freidinger, D. S. Perlow, W. J. Paleveda Jr., F. W. Holly, R. G. Strachan, R. F. Nutt, B. H. Arison, C. Homnick, W. C. Randall, M. S. Glitzer, R. Saperstein, R. Hirschmann, *Nature* **1981**, *292*, 55.
[39] A. Patchornik, M. A. Kraus, *Pure Appl. Chem.* **1975**, *43*, 503; A. Patchornik, 'Polymer Supported Reagents', in 'Modern Synthetic Methods 1976, Vol. 1', Ed. R. Scheffold, Sauerländer AG, Aarau, 1976, pp. 113–172.
[40] W. F. DeGrado, E. T. Kaiser, *J. Org. Chem.* **1980**, *45*, 1295.
[41] G. Ösapay, A. Profit, J. W. Taylor, *Tetrahedron Lett.* **1990**, *31*, 6121.
[42] H. Mihara, S. Yamabe, T. Niidome, H. Aoyagi, H. Kumagai, *Tetrahedron Lett.* **1995**, *36*, 4837, and other refs. by the *Mihara* group cited therein.
[43] G. W. Kenner, J. R. McDermot, R. C. Sheppard, *J. Chem. Soc., Chem. Commun.* **1971**, 636; B. J. Backes, J. A. Ellman, *J. Org. Chem.* **1999**, *64*, 2322.
[44] L. Yang, G. Morriello, *Tetrahedron Lett.* **1999**, *40*, 8197; T. D. Clark, M. Sastry, C. Brown, G. Wagner, *Tetrahedron* **2006**, *62*, 9533.
[45] L. S. Richter, J. Y. K. Tom, J. P. Burnier, *Tetrahedron Lett.* **1994**, *35*, 5547.
[46] D. A. Annis, O. Helluin, E. N. Jacobsen, *Angew. Chem., Int. Ed.* **1998**, *37*, 1907; M. Royo, J. Farrera-Sinfreu, L. Solé, F. Albericio, *Tetrahedron Lett.* **2002**, *43*, 2029; J. T. Lundquist, J. C. Pelletier, *Org. Lett.* **2002**, *4*, 3219; S. J. Reyes, K. Burgess, *Tetrahedron: Asymmetry* **2005**, *16*, 1061.

[47] R. I. Mathad, B. Jaun, O. Flögel, J. Gardiner, M. Löweneck, J. D. C. Codée, P. H. Seeberger, D. Seebach, M. K. Edmonds, F. H. M. Graichen, A. D. Abell, *Helv. Chim. Acta* **2007**, *90*, 2251.
[48] G. Lelais, D. Seebach, B. Jaun, R. I. Mathad, O. Flögel, F. Rossi, M. Campo, A. Wortmann, *Helv. Chim. Acta* **2006**, *89*, 361.
[49] M. Rueping, ETH Diss. No. 14677, ETH-Zürich, 2002.
[50] G. P. Dado, S. H. Gellman, *J. Am. Chem. Soc.* **1994**, *116*, 1054.
[51] G. Melacini, Q. Zhu, M. Goodman, *Biochemistry* **1997**, *36*, 1233; T. Hintermann, K. Gademann, B. Jaun, D. Seebach, *Helv. Chim. Acta* **1998**, *81*, 983.
[52] S. Siehler, K. Seuwen, D. Hoyer, *Naunyn-Schmiedebergs Arch. Pharmacol.* **1998**, *357*, 483; S. Siehler, K. Seuwen, D. Hoyer, *Naunyn-Schmiedebergs Arch. Pharmacol.* **1999**, *360*, 488.
[53] J. Gardiner, D. Langenegger, D. Hoyer, A. K. Beck, R. I. Mathad, D. Seebach, *Chem. Biodivers.* **2008**, *5*, 1213.
[54] T. Kimmerlin, ETH Diss. No. 14677, ETH Zürich, 2002.
[55] J. D. A. Tyndall, B. Pfeiffer, G. Abbenante, D. P. Fairlie, *Chem. Rev.* **2005**, *105*, 793.
[56] P. Micuch, D. Seebach, in 'Peptides: The Wave of the Future', Eds. M. Lebl, R. A. Houghten, American Peptide Society, San Diego, 2001, pp. 32–33; P. Micuch, D. Seebach, *Helv. Chim. Acta* **2002**, *85*, 1567.
[57] F. Gessier, L. Schaeffer, T. Kimmerlin, O. Flögel, D. Seebach, *Helv. Chim. Acta* **2005**, *88*, 2235.
[58] 'Fmoc Solid Phase Peptide Synthesis: A Practical Approach', Vol. 222, Eds. W. C. Chang and P. D. White, Oxford University Press, Oxford, 2000.
[59] W. S. Hancock, J. E. Battersby, *Anal. Biochem.* **1976**, *71*, 260.
[60] T. D. Goddard, D. G. Kneller, 'program SPARKY v3.110', University of California, San Francisco, 2004.
[61] C. D. Schwieters, J. J. Kuszewski, N. Tjandra, G. M. Clore, *J. Magn. Reson.* **2003**, *160*, 65.
[62] E. G. Stein, L. M. Rice, A. T. Brünger, *J. Magn. Reson.* **1997**, *124*, 154.
[63] J. Gardiner, A. V. Thomae, R. I. Mathad, D. Seebach, S. D. Krämer, *Chem. Biodivers.* **2006**, *3*, 1181.

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