The Cyclo-β-Tetrapeptide (β-HPhe-β-HThr-β-HLys-β-HTrp): Synthesis, NMR Structure in Methanol Solution, and Affinity for Human Somatostatin Receptors[1])

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The known solid-state structure (*Fig. 1*, top) of cyclo(β-HAla)$_4$ was used to model the structure of the title compound **1** as a prospective somatostatin mimic (*Fig. 1*, bottom). The synthesis started with the *N*-protected natural amino acids Boc-Phe-OH, Boc-Trp-OH, Boc-Lys(2-Cl-Z)-OH, and Boc-Thr(OBn)-OH, which were homologated to the corresponding β-amino-acid derivatives (*Scheme 1*) and coupled to the β-tetrapeptide Boc-β-HTrp-β-HPhe-β-HThr(OBn)-β-HLys(2-Cl-Z)-OMe (**16**); the (N-Me)-β-HThr-(N-Me)-β-HPhe analog **17** was also prepared. C- and N-terminal deprotection and cyclization through the pentafluorophenyl ester gave the insoluble β-tetrapeptide with protected Thr and Lys side chains (**18**). Solubilization and debenzylation could only be effected in LiCl-containing THF (*ca.* 10% yield; with *ca.* 55% recovery). HPLC Purification provided a sample of the title compound **1**, the structure of which, as determined by NMR-spectroscopy (*Fig. 2*, left) was drastically different from the 'theoretical' model (*Fig. 1*). There is a transannular H-bond dividing the macrocyclic 16-membered ring, thus forming a ten- and a twelve-membered H-bonded ring, the former mimicking, or actually being superimposable on, an α-peptidic so-called β-turn. Still, the four side chains occupy equatorial positions on the ring, as planned, albeit with somewhat different geometry as compared to the 'original'. The *cyclo*-β-tetrapeptide has micromolar affinities to the human somatostatin receptors (hsst 1–5). Thus, we have demonstrated for the first time that it is possible to mimic a natural peptide hormone with a small β-peptide. Furthermore, we have discovered a simple way to construct the ubiquitous β-turn motif with β-peptides (which are known to be stable to mammalian peptidases).

Introduction. – Oligomers of β-amino acids (β-peptides) have received much attention in the realm of artificial compounds that fold into compact and well-defined secondary structures. This is due to their ability to display a large variety of different secondary structures with just one type of building block (see reviews: [2–4]). After the initial discovery of the 3_{14} helix without [5] and with conformational restraints [6], two other types of helices have been disclosed [7–9]. Two different turns [7][8], parallel [5] and antiparallel [10–12] sheets, as well as meander-type secondary structures [13][14] have been found in solution and in the solid state.

Cyclic β-peptides, however, have received less attention. Several cyclic β-peptides have been prepared [5][15–18], but structure determination was hampered by the poor solubility of these compounds. It was powder X-ray diffraction that led to the

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[1]) Partially published in a preliminary communication [1].

[2]) Part of the projected dissertation of *K. G.*, ETH-Zürich.

[3]) Part of the Diplomarbeit (master thesis) of *M. E.*, ETH-Zürich 1998.

assignment of the solid-state conformation of three cyclo-β-tetrapeptides [19][4]) (see the structure of the (all-*S*)-cyclo(β-HAla)$_4$ (**A**) in *Fig. 1*, top). They are characterized by a tubular stacking with an infinite network of pleated-sheet-type H-bonds (so called *peptide nanotubes*). This nanotube formation has been proposed to promote single-channel ion transport through phospholipid bilayers [18]. Only very recently, the first NMR structure in water of the cyclo-β-tripeptide ((β-HGlu)$_3$) with carboxylate side chains has been reported, being characterized by an all-up arrangement of the C=O groups [17].

β-Peptides are resistant to degradation by mammalian proteolytic enzymes [20][21], and their building blocks, the β-amino acids, have been shown to be non-mutagenic in

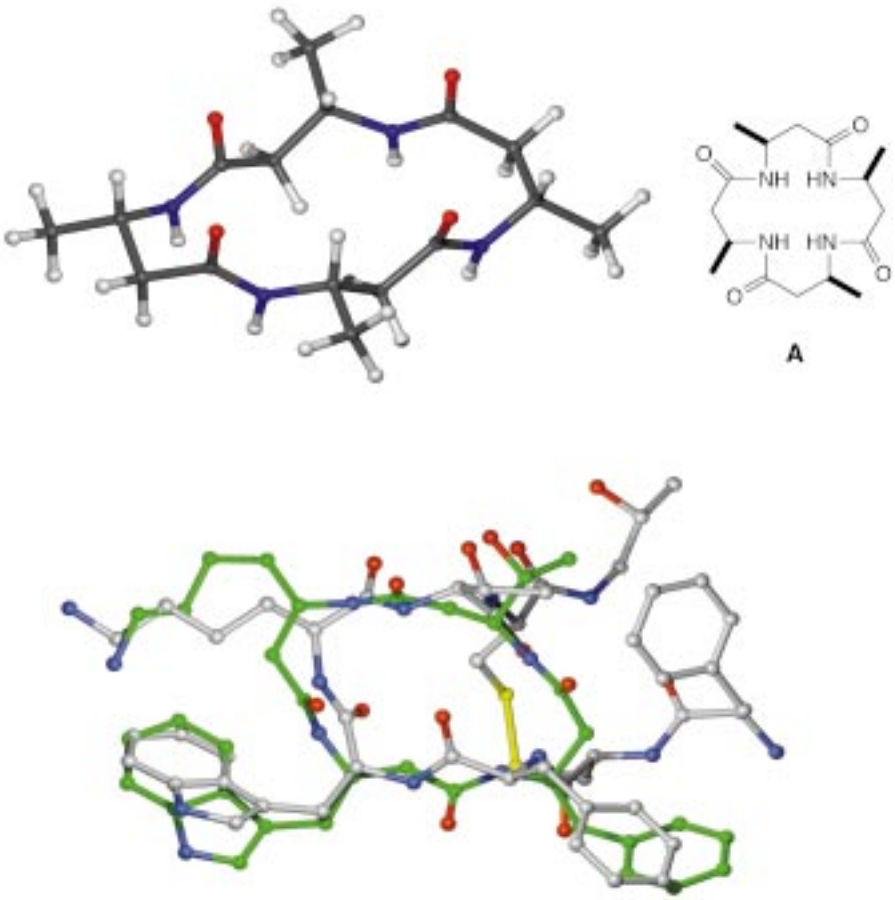

Fig. 1. Top: *Powder X-ray structure of (all-*S*)-cyclo(β-HAla)$_4$.* Bottom: *Top view of the superposition of the solution structure of octreotide* [41] (C-atoms in gray) *and a model of* **1** *derived from the powder X-ray structure of cyclo-β-tetrapeptide* **A** (C-atoms in green). The overlap of the side chains indicates good mimicry of the somatostatin pharmacophore by **1**.

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[4]) The rings of the three diastereoisomers of (*S*,*S*,*S*,*S*), and (*R*,*S*,*R*,*S*) and (*R*,*R*,*S*,*S*) configurations have different shapes and different relative geometries of the equatorial side chains, with all four C=O groups being involved in intermolecular H-bonding.

the *Ames* test [20]. β^3-Amino acids (two synthetic steps from the corresponding amino acids [22]), as well as the β-peptides (obtainable by solution- or solid-phase coupling methods [23]) are readily available.

All these features make β-peptides promising candidates for the use as peptidomimetics in medicinal chemistry[5]). However, before our preliminary communication [1], nothing had been known in the literature about the potential of β-peptides as biologically and pharmacologically active compounds. In this paper, we describe in full detail the design, synthesis, and biological evaluation of a β-peptide mimicking a natural α-peptide. Furthermore, we disclose the first solution structure of a cyclo-β-tetrapeptide, which turns out to be different from the known solid- state structures.

Results and Discussion. – *Target and Design.* There are few α-peptide hormones that have been studied as intensively as the tetradecapeptide *somatostatin* (SRIF: Somatotropin Release Inhibiting Factor). After its isolation in 1973 from 500,000 hypothalami [24], it has been characterized as a potent inhibitor of growth hormone (GH) from the anterior pituitary [24]. In addition, it also inhibits the pancreatic secretion of glucagon and insulin [25], as well as the secretion of gastrin from the gut [26]. Furthermore, SRIF acts as a neurotransmitter in the central nervous system and peripheral tissue, where it modulates several processes, such as smooth muscle motor activity [27] and the release of other neurotransmitters [28][29]. Five different somatostatin receptors (sst 1–5) have been identified and characterized as G-protein-coupled transmembrane receptors [30]. So far, the physiological function of only two, sst2 (mediation of the release of GH) and sst5 (inhibition of the release of insulin), are known.

All these physiological functions render SRIF very important for the treatment of several diseases. Many analogues have been synthesized and some are in clinical use, *e.g.*, octreotide (*Sandostatin*®) by *Novartis* [31][32]. It is applied in the treatment of acromegaly and of certain gastro-entero-pancreatic tumors. Its elimination half-life from the blood serum (90 min) is still rather short. It is, therefore, of great interest to find non-peptide analogues that mimick SRIF or octreotide[6]). For these reasons, we chose somatostatin as the target to demonstrate the potential of β-peptides as mimetics of natural α-peptide hormones.

The structure of octreotide has been studied both in the solid state [37] and in solution [38–41] (*Fig. 1*). All structures are characterized by a type II′ β-turn spanning Trp4 and Lys5. Furthermore, it is known that Phe3 and Thr6 are important for biological activity[7]). Computer-aided modelling revealed similarities between the powder X-ray structure of cyclo-β-tetrapeptides (*Fig. 1*, top) and type II′ β-turns of α-peptides. Thus, we have attached the side chains necessary for biological activity to the β-peptide backbone of the solid-state structure that led to the cyclo-β-tetrapeptide **1**, the side chains of which in the 'theoretical' model overlapped well with the solution structure of octreotide (*Fig. 1*, bottom). In addition, we intended to synthesize an *N*-

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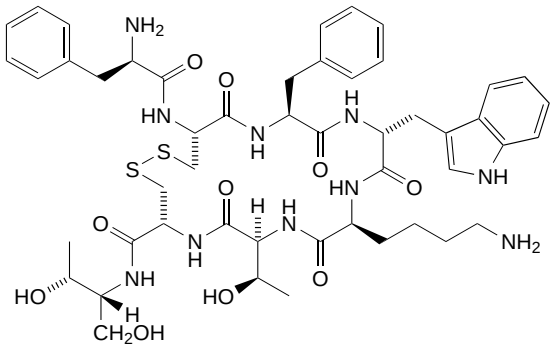
5) There are many β-amino acids that are part of natural products, especially of marine origin. Furthermore, they have been introduced in pharmaceutically active compounds for a long time to increase stability against proteolytic degradation or to induce certain structural elements. For references, see [2].

6) Recently, several groups reported non-peptidic compounds that mimick SRIF [33–36].

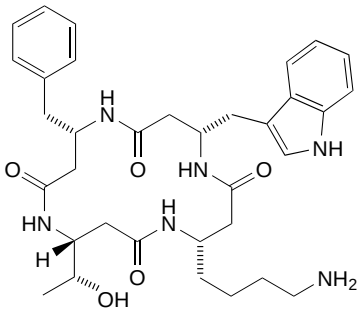
7) Most α-peptide SRIF analogues with biological activity contain the amino-acid sequence Phe-Trp-Lys-Thr [32].



Octreotide, Sandostatin®

H–Ala–Gly–Cys–Lys–Asn–Phe–Phe–Trp
HO–Cys–Ser–Thr–Phe–Thr–Lys

Somatostatin, SRIF



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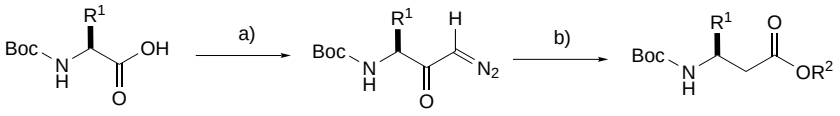
methylated derivative in order to circumvent possible solubility problems, often encountered in peptide chemistry (especially for cyclic β-peptides [15][16]).

Synthesis. We chose the Boc/benzyl (Bn) protecting-group strategy for the synthesis of **1** in solution. The Boc-protected β^3-amino acids **4**–**9** were prepared by the *Arndt-Eistert* method [22][42], *via* the corresponding diazo ketones **3a**–**d** (*Scheme 1*). To our surprise, and in contrast to earlier observations [22], Trp derivatives **3c** and **6** (containing an unprotected indole heterocycle) were easily isolated in good yields. In addition, we prepared the *N*-methylated building blocks **10** and **11**, following a protocol developed for α-amino acids [43][44] that has been recently adapted for β-amino acids [15][45].

Boc Deprotection of **4** and subsequent conventional peptide coupling to **6** with *N*-[3-(dimethylamino)propyl]-*N*′-ethylcarbodiimide hydrochloride (EDC)/1-hydroxy-1*H*-benzotriazole (HOBt) furnished the β-dipeptide **12** (85%). Similarly, **8** was deprotected and coupled with **9** and **11** to give the β-dipeptide **13** and the *N*-methylated derivative **14**. Removal of the *N*-terminal protecting group of **14** and coupling with **10** proceeded smoothly in 79% yield to give the β-tripeptide **15**[8]). Fragment coupling of *C*-terminally deprotected **12** with the *N*-terminally deprotected **13** gave β-tetrapeptide

[8]) The *N*-methylation appeared to be no obstacle to peptide-bond formation.

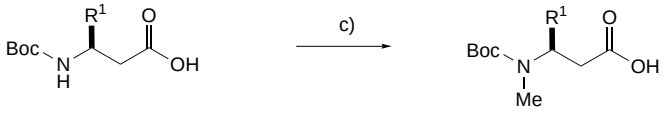
Scheme 1. *Preparation of β-Amino-Acid Building Blocks* **4**–**11**.



2 **3**

2, 3	R^1
a	Bn
b	$(CH_2)_4NH$(2-Cl-Z)
c	(1*H*-Indol-3-yl)methyl
d	(*R*)-1-(Benzyloxy)ethyl

4 R^1 = Bn, R^2 = Me
5 R^1 = Bn, R^2 = H
6 R^1 = (1*H*-indol-3-yl)methyl, R^2 = H
7 R^1 = $(CH_2)_4NH$(2-Cl-Z), R^2 = H
8 R^1 = $(CH_2)_4NH$(2-Cl-Z), R^2 = Me
9 R^1 = (*R*)-1-(Benzyloxy)ethyl, R^2 = H



5 R^1 = Bn
9 R^1 = (*R*)-1-(Benzyloxy)ethyl

10 R^1 = Bn
11 R^1 = (*R*)-1-(Benzyloxy)ethyl

a) $ClCO_2Et$, Et_3N, THF, then CH_2N_2, Et_2O; **3a**: 86%, **3b**: 70%, **3c**: 63%, **3d**: 55%. b) CF_3CO_2Ag (cat.), Et_3N, THF, R^2OH; **4**: 98%, **5**: 44%, **6**: 84%, **7**: 91%, **8**: 81%, **9**: 88%. c) NaH, MeI, THF; **10**: 92%, **11**: 83%.

16 in 93% yield. However, the analogous coupling of **15** to the doubly N-methylated β-tetrapeptide **17** proceeded with a yield of only 66%. The *N*-Me groups increase the solubility in organic solvents tremendously, making the isolation and purification of **17** much more facile than that of **16**.

β-Tetrapeptide **16** was deprotected at the C-terminus and allowed to react with pentafluorophenol and EDC, the resulting active ester was, after Boc deprotection, cyclized (*Hünig*'s base in MeCN at 70°) in dilute solution to furnish the fully protected cyclo-β-tetrapeptide **18** (38%; see *Scheme 2*). This compound is essentially insoluble in all solvents commonly used in peptide chemistry. Being aware of our previous work on the solubilization by Li-salts of peptides in organic solvents [17][46], we dissolved **18** in THF with 6 equiv. of LiCl and carried out the hydrogenolysis (Pd on charcoal/H_2). After purification by RP-HPLC, the deprotected cyclo-β-tetrapeptide **1** was obtained in only poor yield (9%), however, more than 50% of the starting material were recovered.

In contrast to the non-*N*-methylated β-peptide, the much more soluble *N*-methylated β-peptide **17** could not be cyclized *via* deprotection and pentafluorophenyl-ester formation. Also, other cyclization methods common in peptide chemistry, such as the use of diphenylphosphoryl azide (DPPA) in DMF [9]) or the application of HATU/HOAt[10]), were not successful. This observation is in agreement with the problems

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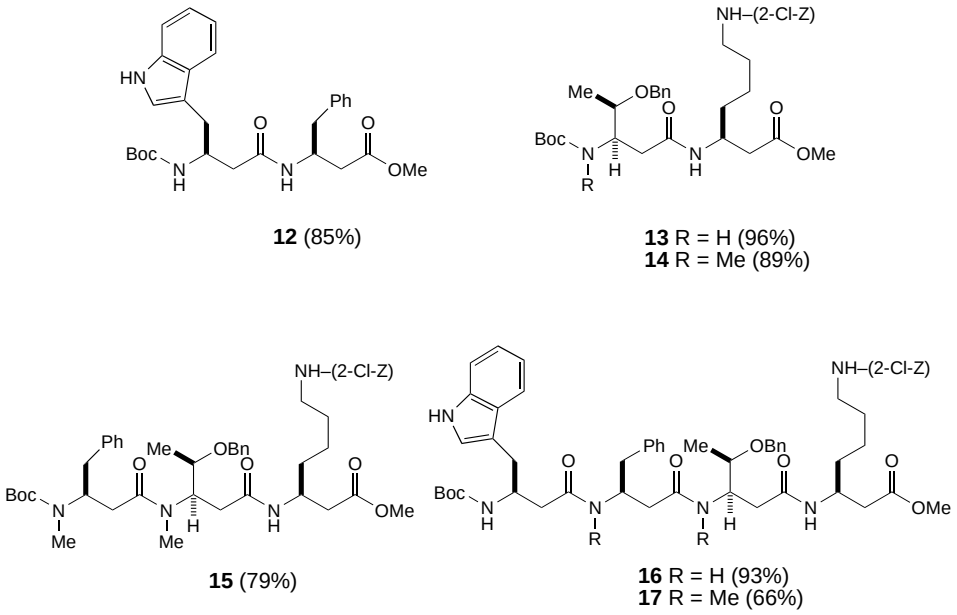
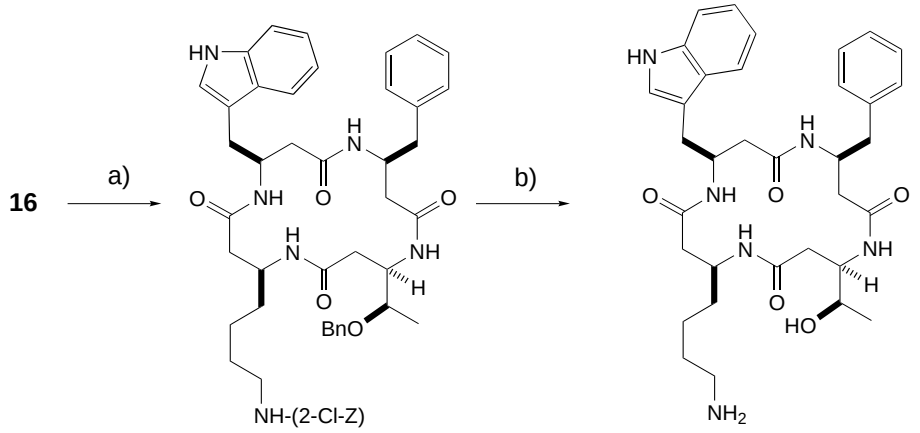
[9]) See, for example, [47].

[10]) HATU: *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, HOAt: 1-hydroxy-7-azabenzotriazole; see, for example, [18].



Scheme 2. *Synthesis of Cyclo-β-tetrapeptide* **1**.



a) i) 50 equiv. NaOH, H_2O, CF_3CH_2OH, ii) EDC, C_6F_5OH, DMF, iii) CF_3CO_2H, $HSCH_2CH_2SH$, CH_2Cl_2, iv) $EtN(i\text{-}Pr)_2$, MeCN, 70°, syringe pump (38% from **16**). b) *Ca*. 6 equiv. LiCl, THF, Pd/C, H_2; 9% (53% starting material recovered).

encountered during attempted synthesis of cyclic *N*-methylated β^3-HAla peptides [15]. Steric effects do not play a role, since the nucleophilic amino group of β^3-HTrp is not methylated. A possible reason might be the (helical) *preorganization* of non-*N*-methylated β-peptides that facilitates cyclization. Of course, substituents on the amide

N-atom have an impact on the conformation of any peptide[11]), and, thus, influence its reactivity.

Biological Investigations. The affinity of the cyclo-β-tetrapeptide **1** for the five different human somatostatin receptors (hsst 1–5, expressed in CHO or CCL-39 cell lines)[12]) was measured by radioligand-binding assays (*Table 1*). Displacement experiments for the specific binding of [^{125}I]LTT-SRIF$_{28}$ to these receptors were carried out as previously described, and the affinities are reported as p$K_d \pm$ SEM values. The data in *Table 1* clearly show that **1** has affinity, although the concentrations are in the micromolar and not in the nanomolar range as for octreotide[13]).

Table 1. *Comparison of the Affinitiy Values of* **1** *and Octreotide by Radioligand Binding Assays* (displacement experiments of the specific binding of [^{125}I]-LTT-SRIF$_{28}$) *with the Five Different Human Somatostatin Receptors* (hsst 1–5) *expressed in CCL-39* (Chinese Hamster Lung Fibroblast) *or CHO* (Chinese Hamster Ovary) *Cell Lines.* The values are reported as p$K_d \pm$ SEM of 4 independent determinations, the values for octreotide are taken from [69]. The affinities of **1** are in the micromolar range, at least an order of magnitude lower than those of octreotide.

Receptor	β-Peptide **1**	Octreotide
hsst1 (CCL-39)	4.85 ± 0.04	6.65
hsst2 (CCL-39)	4.44 ± 0.09	9.19
hsst3 (CHO)	5.48 ± 0.01	7.88
hsst4 (CCL-39)	5.00 ± 0.05	6.40
hsst5 (CCL-39)	3.73 ± 0.15	7.17

Structure Determination by NMR Spectroscopy. Having the first soluble cyclo-β-tetrapeptide **1** in hand, we thought that an NMR structure determination might provide insights into its hitherto unknown conformation and, thus, explain the reduced affinity as compared to octreotide. All resonances in the ^{1}H-NMR spectrum obtained at 500 MHz in CD_3OH at 298 K were assigned by DQF-COSY and TOCSY experiments (*Table 2*), and it was possible to extract the *J* values of all backbone H-atoms directly from the 1D-NMR spectrum.

From the spectrum, two important facts became immediately evident: *a*) Investigations on octreotide demonstrated that the aromaticity of the indole ring leads to a shift of the resonances of the lysine side-chain protons to lower frequencies (higher fields), since both side-chains are in close proximity [38–41]. This phenomenon was not observed in the ^{1}H-NMR spectrum of **1**, indicating a lack of closeness of the Trp and Lys side-chains. *b*) The J(NH, H–C(β)) values show that the solution conformation differs drastically from that of the 'theoretical' model (see *Fig. 1*). According to this model, four equal J(NH, H–C(β)) values would have been expected, however, two large (β^3-HPhe, β^3-HThr), one medium (β^3-HTrp), and one small (β^3-HLys) were measured. In addition, the backbone conformation around the central C(α)–C(β) bond for the lysine residue must be different from those of the other three β-amino acids, as can be judged from the J(H–C(α),H–C(β)) values.

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11) For 'normal' peptides, a reversed situation is often encountered: while regular α-peptides cyclize in poor yields, their *N*-methylated counterparts give higher cyclization yields (see, for example, [48]).

12) For abbreviations: see the legend of *Table 1* and *Exper. Part.*

13) The molecular mass of **1** is only 60% of octreotide: **1** (618.77), octreotide (1019.24), and somatostatin (1637.88)

Table 2. *Assignment of ^{1}H-NMR Resonances* (500 MHz, CD_3OH, 298 K) *by a Combination of COSY and TOCSY Experiments.* The *J* values were extracted from the 1D spectrum.

	Thr	Lys	Phe	Trp
NH	7.22	8.22	7.66	8.35
H–C(β)	4.01	3.42	4.36	4.36
H–C(α)	2.42	2.49	2.35	2.39
H′–C(α)	2.18	2.14	2.24	2.31
H–C(γ)	3.73	1.65	2.71	2.87
H′–C(γ)	–	1.53	2.65	3.07
Me	1.10	–	–	–
H–C(δ)	–	1.51	–	–
H′–C(δ)	–	1.51	–	–
H–C(ε)	–	1.62	–	–
H′–C(ε)	–	1.62	–	–
H–C(ϕ)	–	2.95	–	–
H′–C(ϕ)	–	2.89	–	–
J(NH, H–C(β))	9.5	5.2	8.6	7.0
J(H–C(β), H–C(α))	12.9	5.7	11.2	11.7
J(H–C(β), H–C(α))	3.3	3.3	2.3	2.3

Additional information was obtained from a ROESY spectrum. The NOEs were extracted and classified into three intensity categories based on their relative crosspeak volumes (*Table 3*). These NOEs and all ^3J values for the backbone protons have been used as restraints in molecular-dynamics calculations by the QUANTA package. The resulting conformation, in agreement with all experimental values, is shown in *Fig. 2*.

The conformation of the cyclo-β-tetrapeptide **1** is characterized by an intramolecular H-bond that divides the 16-atom backbone into a ten-membered and a twelve-membered H-bonded ring. The ten-membered ring mimicks a natural α-peptide β-turn and is very similar to the ring observed in the 12/10/12-helix [7][8], in a hairpin [12], and in a $\beta^{2,2}$-peptide [13]. However, there is no reason obvious to us why the H-bond should form from NH of β^3-HTrp to the C=O of β^3-HPhe. In addition, the other

Table 3. *NOEs Extracted from the 150-ms ROESY Spectrum* (500 MHz, CD_3OH, 298 K). Intraresidual NOEs from H–C(β) to NH were not classified due to COSY artefacts.

Atom	Residue	Atom 2	Residue	Intensity
NH	β^3-HTrp	H–C(β)	β^3-HLys	s
NH	β^3-HTrp	H–C(α)	β^3-HLys	s
NH	β^3-HTrp	H′–C(α)	β^3-HLys	w
NH	β^3-HTrp	H–C(α)	β^3-HTrp	s
NH	β^3-HTrp	H′–C(α)	β^3-HTrp	m
NH	β^3-HLys	H–C(α)	β^3-HLys	m
NH	β^3-HLys	H–C(β)	β^3-HThr	s
NH	β^3-HLys	H–C(α)	β^3-HThr	s
NH	β^3-HLys	H′–C(α)	β^3-HThr	m
NH	β^3-HPhe	H′–C(α)	β^3-HPhe	s
NH	β^3-HPhe	H–C(α)	β^3-HTrp	s
NH	β^3-HPhe	H′–C(α)	β^3-HTrp	s
NH	β^3-HThr	H′–C(α)	β^3-HThr	m
NH	β^3-HThr	H–C(α)	β^3-HPhe	s
NH	β^3-HThr	H′–C(α)	β^3-HPhe	s

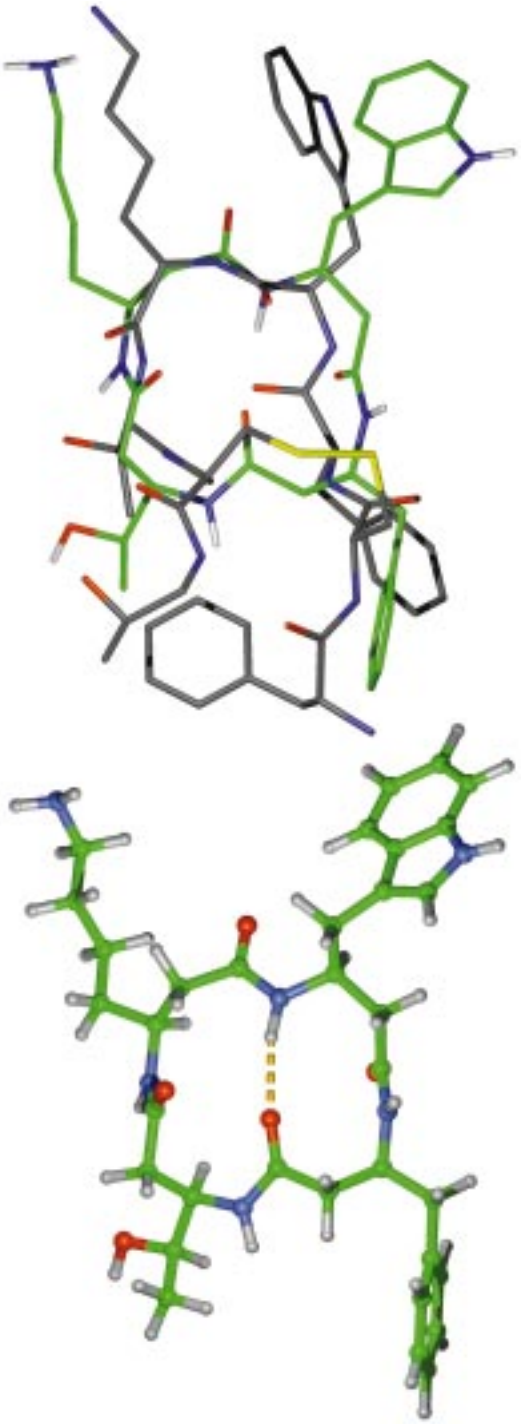

Fig. 2. *Model of the solution structure of the cyclo-β-tetrapeptide* **1** (left; C-atoms in green) *and its superposition with the solution structure of octreotide* (right; C-atoms in gray) [41]. An intramolecular H-bond from the NH of β^3-HTrp to the C=O of β^3-HPhe is formed, dividing the 16-atom backbone into a twelve- and a ten-membered H-bonded ring. The other two amide groups are pointing up and down, reducing the resulting dipole moment of the molecule. All side chains are lateral, which results in a flat molecular shape. The superposition with the solution structure of octreotide displays no close proximity of the Lys and Trp side-chains, which might explain the affinity pattern towards the somatostatin receptors.

two amide groups are pointing up and down, thus further reducing the net dipole moment of the molecule. All side chains occupy lateral positions on the macrocyclic ring, which results in a flat ring shape.

The superposition with octreotide (*Fig. 2*, right) shows that the overlap of the side chains is reduced as compared to the model (*Fig. 1*). This might explain the weaker binding of compound **1**, compared to octreotide, although the receptor-bound conformation of both compounds might be different [49]. The 'fit' of the side chains might have been increased if the intramolecular H-bond had been formed between NH of β^3-HLys and C=O of β^3-HTrp, mimicking a β-turn with the side chains of Lys and Trp.

This is the first example of a solution structure of a cyclo-β-tetrapeptide. Of course, we should not conclude that, in general, all cyclo-β-tetrapeptides exhibit such H-bonding patterns. However, if one compares this solution structure to the solid state conformation of cyclo(β^3-HAla)$_4$ (**A**; *Fig. 1*, top), there is only the fundamental difference of the H-bonding pattern, while the overall shape of the molecules is flat, and side-chain orientation is comparable. Cyclic α-peptides (starting with pentapeptides, which have a comparable ring size) often exhibit intramolecular H-bonds[14]).

CD Spectroscopy. CD-Spectroscopy is a valuable tool for analysis of secondary structure in a straightforward manner, once a correlation between the observed pattern and the structure in solution is established[15]). Although CD spectroscopy never proves structure, it is complementary to NMR spectroscopy. The CD spectrum of **1** in MeOH (*Fig. 3*) displays a single maximum at *ca.* 200 nm, and a zero crossing at 193 nm. This pattern, which is very similar to that observed for the *12*/*10*/*12* helix [7][8] and also for the β-peptide hairpin [12][53], has been assigned to ten- and/or twelve-membered H-bonded rings. This spectrum thus further corroborates the structure derived from NMR spectroscopy.

However, this observed CD pattern differs from that observed for **A** (in trifluoroethanol), which displays a minimum at 215 nm, a zero crossing at 207 nm and a maximum at 196 nm [15]. This suggests that different cyclo-β-tetrapeptides fold into different conformations in solution.

Conclusion. – We have shown for the first time that it is possible to mimic a natural α-peptide hormone with a small β-peptide. This was not at all obvious, since in β-peptides not only the amide bonds but also the side chains are shifted compared to their natural analogs. While some proteins (*e.g.*, proteases) do not recognize β-peptides as substrates, the somatostatin receptors *do*! It is now in the hands of medicinal chemists to exploit these specific properties of β-peptides, and to explore their scope and limitations as peptidomimetics. This search is facilitated by the ease of the synthesis of the building blocks as well as of β-peptides. In addition, there are various possibilities for optimization of receptor binding by simple variation of the substitution pattern of the side chains on the β-amino-acid backbone [54]. All these features make β-peptides promising candidates for peptidomimetics.

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14) For an early review by *Kessler*, see [49].

15) For work on β-peptides, see [2][8][50–53].

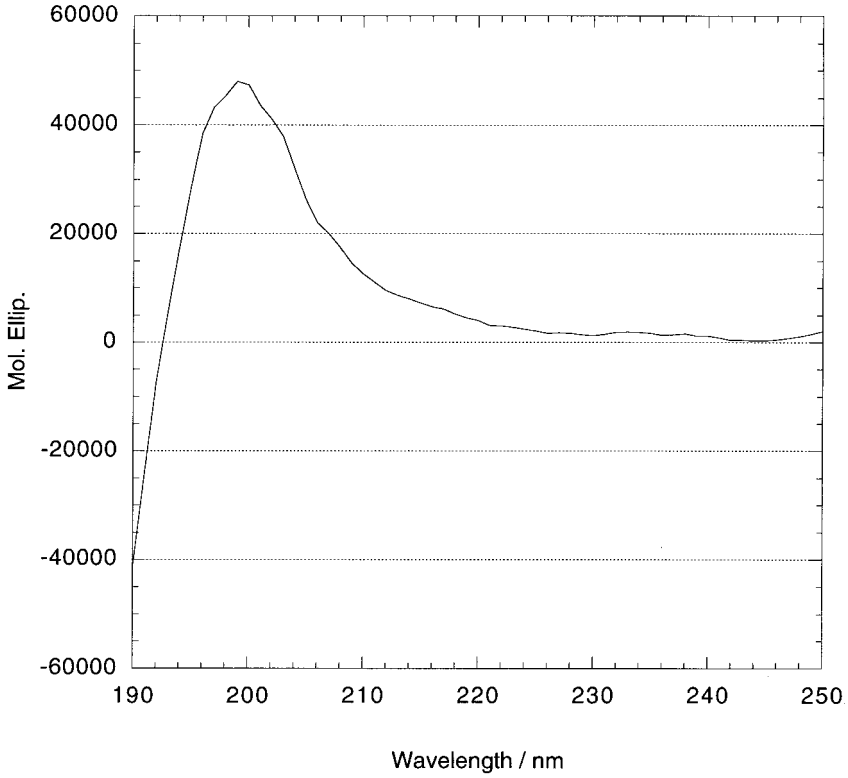

Fig. 3. *CD Spectrum of β-peptide* **1** *in MeOH* ($c = 0.323$ mM, molar ellipticity in 10 deg cm^2 mol^{-1}).

chemistry. Generous supply of TFA by *Solvay* and of Phe and Trp by *Degussa* are greatly appreciated, as is the ongoing financial support from *Novartis Pharma* and *Agro*, Basel. *M. E.* thanks the DAAD for a grant and the *Studienstiftung des Deutschen Volkes* for generous support.

Experimental Part

1. *General*. Abbreviations: Boc: (*tert*-butoxy)carbonyl, EDC: *N*-[3-(Dimethylamino)propyl]-*N*′-ethylcarbodiimide hydrochloride, FC: flash chromatography, GP: General Procedure, HOBt: 1-hydroxy-1*H*-benzotriazole, h.v.: high vacuum, 0.01 – 0.1 Torr, *β*-HXxx: *β*-homoamino acid, NMM: *N*-methylmorpholine, SEM: standard error, TFA: trifluoroacetic acid, Z: benzyloxycarbonyl, 2-Cl-Z: (2-chlorobenzyloxy)carbonyl. THF was freshly distilled over K under Ar before use. Et_3N was distilled over CaH_2. Solvents for chromatography and workup were distilled over *Sikkon (Fluka)*. Boc-Amino acids were purchased from *Senn* or were prepared according to the literature [55]. All other reagents were used as received from *Fluka*. All reactions were carried out under Ar. All given temp. refer to the bath temp. TLC: *Merck* silica gel *60 F$_{254}$* plates; detection with UV or dipping into a soln. of anisaldehyde (9.2 ml), AcOH (3.75 ml), conc. H_2SO_4 (12.5 ml), and EtOH (338 ml), followed by heating. FC: *Fluka* silica gel 60 (40 – 63 mm); at *ca.* 0.3 bar. Anal. HPLC: *Knauer* HPLC system (pump type 64, *EuroChrom 2000* integration package, degaser, UV detector (variable-wavelength monitor)), *Macherey-Nagel C$_{18}$* column (*Nucleosil 100-7 C$_{18}$* (250 × 4 mm)), solvent *A:* 1% TFA in H_2O, solvent *B:* MeCN. Prep. HPLC: *Knauer* HPLC system (pump type *64*, programmer *50*, UV detector (variable-wavelength monitor)), *Macherey-Nagel C$_{18}$* column (*Nucleosil 100-7 C$_{18}$* (250 × 21 mm)). M.p.: *Büchi-510* apparatus; uncorrected. Optical rotations: *Perkin-Elmer 241* polarimeter (10 cm, 1 ml cell) at r.t. CD: *Jobin-Yvon-Mark III*, 1-mm cell length. IR Spectra: *Perkin-Elmer-782* spectrophotometer. NMR Spectra: *Bruker AMX 500* (^{1}H: 500 MHz, ^{13}C: 125 MHz), *AMX 400* (^{1}H: 400 MHz, ^{13}C: 100 MHz), *ARX 300* (^{1}H: 300 MHz),

Varian Gemini 300 (^{1}H: 300 MHz, ^{13}C: 75 MHz), or *Gemini 200* (^{1}H: 200 MHz, ^{13}C: 50 MHz); chemical shifts (δ) in ppm downfield from $SiMe_4$ (= 0 ppm); *J* values in Hz. *Hitachi Perkin-Elmer RHU-6M* (FAB, in a 3-nitrobenzyl-alcohol matrix) spectrometer; in *m/z* (% of basis peak). Elemental analyses were performed by the Microanalytical Laboratory of the Laboratorium für Organische Chemie, ETH-Zürich.

2. *Preparation of Diazo Ketones. General Procedure 1* (*GP 1*). *Caution:* The generation and handling of CH_2N_2 requires special precautions [56][57]. The *N*-Boc-protected α-amino acid was dissolved in THF (0.2M) under Ar. The soln. was cooled to $-15°$, and Et_3N (1 equiv.) and ClC(O)OEt (1 equiv.) were added. After 15 min, the suspension was allowed to warm to 0°, and a soln. of CH_2N_2 in Et_2O was added until a strong yellow color persisted. The suspension was allowed to warm to r.t. and stirred for 3 h. The excess of CH_2N_2 was destroyed by the addition of few drops of AcOH. The mixture was diluted with H_2O and the bulk of THF evaporated. After addition of Et_2O, the mixture was washed with sat. $NaHCO_3$, NH_4Cl, and NaCl solns. The org. layer was dried ($MgSO_4$) and evaporated. FC or recrystallization gave the pure diazo ketones.

3. *Homologation of the Diazo Ketones to the Corresponding β-Amino Acids. General Procedure 2* (*GP 2*). The diazo ketone was dissolved in THF/H_2O 9:1 (*v*/*v*) (0.25M). Under the exclusion of light, the soln. was cooled to *ca.* $-25°$, and a soln. of CF_3CO_2Ag (0.11 equiv.) in Et_3N (2.3 equiv.) was added. The mixture was allowed to warm up to r.t. and stirred for 3 h in the dark. THF was evaporated, and the residue was dissolved in Et_2O. The β-amino acid was extracted with sat. $NaHCO_3$ soln., and the H_2O-layer was carefully acidified at 0° to pH 2–3 and extracted with Et_2O. The combined org. layers were dried ($MgSO_4$) and evaporated. The resulting β-amino acids were used without further purification.

4. *Homologation of the Diazo Ketones to the Corresponding β-Amino Acid Methyl Esters. General Procedure 3* (*GP 3*). The diazo ketone was dissolved in MeOH (0.25M). Under the exclusion of light, the soln. was cooled to *ca.* $-25°$, and a soln. of CF_3CO_2Ag (0.11 equiv.) in Et_3N (2.3 equiv.) was added. The mixture was allowed to warm up to r.t. and stirred for 3 h in the dark. The solvent was evaporated, and the residue was dissolved in Et_2O. The org. layer was washed with sat. $NaHCO_3$, NH_4Cl, and NaCl solns., dried ($MgSO_4$), and evaporated.

5. N-*Methylation of Boc-β-Amino Acids. General Procedure 4* (*GP 4*). The β-amino acid was dissolved in THF (0.1M), MeI (8 equiv.) was added, the soln. was cooled to 0°, and NaH (3 equiv.) was added in portions. The mixture was allowed to warm to r.t. and stirred for 22 h, then cooled to $-10°$ and excess NaH was hydrolyzed with ice. The solvent was evaporated, and the residue was dissolved in H_2O. The aq. phase was washed with Et_2O (the pH was adjusted to *ca.* 2 with sat. aq. $KHSO_4$ soln.) and extracted with Et_2O. The org. phase was washed with 0.5M HCl soln. and dried ($MgSO_4$). The solvent was removed under reduced pressure to yield the Boc-protected *N*-methyl-β-amino acid which was used without further purification.

6. *Boc Deprotection with TFA. General Procedure 5* (*GP 5*). The Boc-protected compound was dissolved in CH_2Cl_2 (0.25M) and cooled to 0°. The same amount (*v*/*v*) of TFA was added, and the mixture was stirred for 1 h at 0° and for 1 h at r.t. The solvent was evaporated, the residue was twice taken up in $CHCl_3$ and evaporated. The resulting trifluoroacetate was dried under h.v. and used without further purification.

7. *Peptide Coupling with EDC and HOBt. General Procedure 6* (*GP 6*). The trifluoroacetate of the amino fragment was dissolved in $CHCl_3$ (0.5M) and cooled to 0°. Et_3N (4 equiv.), HOBt (1.2 equiv.), a soln. of the Boc-protected fragment (1 equiv.) in $CHCl_3$ (0.25M), and EDC (1.2 equiv.) were added, and the resulting mixture was stirred for 30 min at 0°. The mixture was then allowed to warm up and stirred for additional 12 h (TLC control). The mixture was diluted with $CHCl_3$ and was washed with 1M HCl, sat. $NaHCO_3$, and sat. NaCl solns. The org. layer was dried ($MgSO_4$) and evaporated, and the resulting peptides were either purified or used without further purification.

8. *Synthesis of Cyclo-β-tetrapeptide* **1**. tert-*Butyl* N-*[(1S)-1-benzyl-3-diazo-2-oxopropyl]carbamate* (**3a**) [22]: 15.9 g (60 mmol) of Boc-Phe-OH (**2a**), 5.75 ml (1 equiv.) of ClC(O)OEt, and 8.4 ml (1 equiv.) of Et_3N were transformed according to *GP 1*. Recrystallization (AcOEt/pentane) gave **3a** (15.0 g, 86%). Yellow solid. R_f (Et_2O/pentane 1:1) 0.4. M.p. 95–96° ([22]: M.p. 69–70°). $[\alpha]_D = -11.8$ ($c = 1.00$, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$): 1.41 (*s*, *t*-Bu); 3.02 (*d*, $J = 6.8$, CH_2); 4.42 (br., CHN); 5.11 (br., NHBoc); 5.22 (br., CHN_2); 7.18–7.33 (*m*, 5 arom. H).

2-Chlorobenzyl N-*((5S)-5-[[(*tert-*Butoxy)carbonyl]amino}-7-diazo-6-oxoheptyl)carbamate* (**3b**): 25 g (60.3 mmol) of Boc-Lys(N^ε-2-Cl-Z)-OH (**2b**), 7.9 ml (1 equiv.) of ClC(O)O^{i}Bu, and 8.0 ml (1 equiv.) of Et_3N were transformed according to *GP 1*. FC (Et_2O/pentane 3:1) gave **3b** (18.5 g, 70%). Yellow solid. R_f (AcOEt/pentane 4:9) 0.34. M.p. 72–74°. $[\alpha]_D = -18.0$ ($c = 1.03$, $CHCl_3$). IR ($CHCl_3$): 3446*w*, 3008*w*, 2960*w*, 2940*w*, 2110*s*, 1800*w*, 1711*s*, 1642*m*, 1500*m*, 1446*w*, 1368*m*, 1248*w*, 1163*w*, 1042*w*, 859*w*, 630*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.36–1.83 (*m*, 3 CH_2, *t*-Bu); 3.17–3.23 (*m*, NCH_2CH_2); 4.14 (br., CHN); 4.91 (br., NHBoc); 5.21 (*m*, NH, $PhCH_2$); 5.45 (*s*, CHN_2); 7.23–7.29 (*m*, 2 arom. H); 7.35–7.43 (*m*, 2 arom. H). ^{13}C-NMR (75 MHz, $CDCl_3$):

22.33; 28.36; 29.55; 32.10; 40.56; 53.97; 57.34; 64.05; 80.20; 127.11; 129.51; 129.61; 129.77; 130.01; 133.83; 134.57; 155.86; 193.48. FAB-MS: 877.2 (1.7, $[2M+H]^+$), 439.1 (23.1, $[M+H]^+$), 339.0 (24.7, $[M-Boc+2H]^+$), 311.1 (27.8 $[M-N_2-Boc+2H]^+$). Anal. calc. for $C_{20}H_{27}N_4O_5Cl$ (438.91): C 54.73, H 6.20, N 12.76; found: C 54.65, H 6.17, N 12.50.

tert-*Butyl* N-*{(1S)-3-Diazo-1-[(1*H*-3-indolyl)methyl]-2-oxopropyl}carbamate* (**3c**): 20 g (65.7 mmol) of Boc-Trp-OH (**2c**), 8.6 ml (1 equiv.) of ClC(O)O^{i}Bu, and 9.0 ml (1 equiv.) of Et_3N were transformed according to *GP 1*. FC (Et_2O/pentane 2:1) gave **3c** (13.6 g, 63%). Yellow solid. R_f (Et_2O/pentane 1:1) 0.43. M.p. 53–54°. $[\alpha]_D = +6.0$ ($c = 1.00$, $CHCl_3$). IR ($CDCl_3$): 3689*w*, 3477*m*, 3428*w*, 3008*w*, 2980*w*, 2110*s*, 1706*s*, 1636*m*, 1495*m*, 1456*w*, 1368*s*, 1165*m*, 1092*w*, 1044*w*, 1011*w*, 857*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.43 (*s*, *t*-Bu); 3.19–3.21 (*m*, CH_2–C(1)); 4.52 (br., CHN); 5.19 (br., NHBoc, CHN_2); 7.02–7.37 (*m*, 4 arom. H); 7.62 (*d*, $J = 7.5$, 1 arom. H); 8.28 (br, NH of indole). ^{13}C-NMR (75 MHz, $CDCl_3$): 28.33; 54.32; 57.92; 80.07; 110.35; 111.26; 118.83; 119.77; 122.29; 123.10; 127.52; 136.20; 155.32; 194.29. FAB-MS: 329.5 (60.7, $[M+H]^+$), 328.5 (37.7, M^+), 154.2 (100.0). Anal. calc. for $C_{17}H_{20}N_4O_3$ (328.37): C 62.18, H 6.14, N 17.06; found: C 61.97, H 6.37, N 16.93.

tert-*Butyl* N-*{(1S)-3-Diazo-1-[(1*R*)-1-(benzyloxy)ethyl]-2-oxopropyl}carbamate* (**3d**): 30.4 g (98 mmol) of Boc-Thr(OBn)-OH (**2d**), 10.3 ml (98 mmol) of ClC(O)OEt, and 10.5 ml (93.5 mmol) of Et_3N were transformed according to *GP 1*. Recrystallization (AcOEt/pentane) gave **3d** (17.9 g, 55%). R_f (AcOEt/pentane 1:2) 0.82. M.p. 104–105°. $[\alpha]_D = -39.0$ ($c = 0.80$, $CHCl_3$). IR ($CHCl_3$): 3435*w*, 3008*w*, 2981*w*, 2111*s*, 1710*m*, 1635*m*, 1494*m*, 1454*w*, 1368*m*, 1162*m*, 1072*w*, 867*w*, 624*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.21 (*d*, $J = 6.2$, Me); 1.46 (*s*, *t*-Bu); 4.15–4.23 (*m*, CHN, CHOBn); $\nu_A = 4.47$, $\nu_B = 4.57$ (*AB*, $J_{AB} = 11.5$, OCH_2Ph); 5.44 (*d*, $J = 7.8$, NHBoc); 5.58 (*s*, CHN_2); 7.28–7.38 (*m*, 5 arom. H). ^{13}C-NMR (75 MHz, $CDCl_3$): 16.25; 28.33; 54.22; 62.03; 71.50; 74.55; 80.15; 127.82; 128.41; 137.98; 155.81; 193.26. FAB-MS: 334.2 (55.3, $[M+H]^+$), 90.8 (100.0, $[C_7H_7]^+$). Anal. calc. for $C_{17}H_{23}N_3O_4$ (333.39): C 61.25, H 6.95, N 12.60; found: C 61.27, H 7.02, N 12.55.

*Methyl (3*S*)-3-{[(*tert-*Butoxy)carbonyl]amino}-4-phenylbutanoate* (Boc-β^3-HPhe-OMe, **4**) [22]: 3.0 g (10.4 mmol) of **3a**, 0.25 g (1.14 mmol) of CF_3CO_2Ag, and 3.4 ml (24 mmol) of Et_3N were reacted according to *GP 3*. FC (Et_2O/pentane 3:5) gave **4** (3.0 g, 98%). Colorless wax. R_f (AcOEt/pentane 1:1) 0.77. $[\alpha]_D = -9.7$ ($c = 1.00$, $CHCl_3$) ([22]: $[\alpha]_D = -10.0$ ($c = 0.99$, $CHCl_3$)).

*(3*S*)-3-{[(*tert-*Butoxy)carbonyl]amino}-4-phenylbutanoic Acid* (Boc-β^3-HPhe-OH, **5**) [58]: 20.9 g (72 mmol) of **3a** were reacted according to *GP 2*. Recrystallization (AcOEt/pentane) gave **5** (8.9 g, 44%). Colorless solid. M.p. 108–109° ([58]: 100–105°).

*(3*S*)-3-{[(*tert-*Butoxy)carbonyl]amino}-4-(1*H*-indol-3-yl)butanoic Acid* (Boc-β^3-HTrp-OH, **6**): 9.0 g (27 mmol) of **3c**, 0.65 g (2.9 mmol) of CF_3CO_2Ag, and 8.8 ml (63 mmol) of Et_3N were reacted according to *GP 2*. Drying under h.v. gave **6** (7.2 g, 84%). Colorless foam. M.p. 144–145°. $[\alpha]_D = -12.1$ ($c = 1.00$, $CHCl_3$). IR ($CHCl_3$): 3674*w*, 3476*m*, 3007*w*, 2981*w*, 1706*s*, 1504*m*, 1456*m*, 1418*w*, 1393*w*, 1368*m*, 1166*m*, 1090*w*, 1047*w*, 869*w*, 622*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.42 (*s*, *t*-Bu); 2.48–2.64 (*m*, 2 H–C(2)); 3.00–3.13 (*m*, 2 H–C(4)); 4.31 (br., CHN); 5.01 (br., NHBoc); 7.04–7.22 (*m*, 3 arom. H); 7.36 (*d*, $J = 7.8$, 1 arom. H); 7.65 (*d*, $J = 7.5$, 1 arom. H); 8.14 (br., NH of indole). ^{13}C-NMR (75 MHz, $CDCl_3$): 28.35; 30.01; 37.99; 47.99; 79.62; 111.16; 111.61; 118.94; 119.61; 122.15; 123.02; 127.65; 136.27; 155.58; 176.06. FAB-MS: 319.2 (48.3, $[M+H]^+$), 318.2 (55.1, M^+), 307.1 (100.0). Anal. calc. for $C_{17}H_{22}N_2O_4$ (318.37): C 64.13, H 6.96, N 8.80; found: C 64.05, H 6.92, N 8.71.

*(3*S*)-3-{[(*tert-*Butoxy)carbonyl]amino}-7-({[(2-chlorobenzyl)oxy]carbonyl}amino)heptanoic Acid* (Boc-β^3-HLys(N$^\varepsilon$-2-Cl-Z)-OH, **7**), [59]. 2.0 g (4.3 mmol) **3b**, 0.11 g (0.5 mmol) CF_3CO_2Ag and 1.5 ml (11 mmol) Et_3N were transformed according to *GP 2*. Drying under h.v. and precipitation with pentane gave **7** (1.8 g, 91%). Colorless powder. $[\alpha]_D = -12.9$ ($c = 0.98$, $CHCl_3$). ([59]: $= -11.4$ ($c = 1.19$, $CHCl_3$)). ^{1}H-NMR (200 MHz, $CDCl_3$): 1.32–1.62 (*s*, 3 CH_2, *t*-Bu); 2.57 (*d*, $J = 5.4$, $CH_2C(O)$); 3.23 (*q*, $J = 6.4$, NCH_2CH_2); 3.91 (br., CHN); 4.96 (br., 2 NH); 5.23 (*s*, CH_2(2-Cl-Ph)); 7.26–7.36 (*m*, 2 arom. H); 7.38-7.47 (*m*, 2 arom. H).

*Methyl (3*S*)-3-{[(*tert-*Butoxy)carbonyl]amino}-7-({[(2-chlorobenzyl)oxy]carbonyl}amino)heptanoate* (Boc-β^3-HLys(N$^\varepsilon$-2-Cl-Z)-OMe, **8**) [60]: 2 g (4.6 mmol) of **3b** were reacted according to *GP 3*. FC (Et_2O/pentane) gave **8** (1.63 g, 81%). White solid. R_f (Et_2O) 0.67. M.p.: 77–79° ([60]: 77.5–79°). $[\alpha]_D = -16.7$ ($c = 1.03$, $CHCl_3$) ([60]: $[\alpha]_D = -15.5$ ($c = 1.00$, $CHCl_3$)). ^{1}H-NMR (300 MHz, $CDCl_3$): 1.34–1.63 (*m*, 3 CH_2, *t*-Bu); 2.51 (*d*, $J = 4.4$, 2 H–C(2)); 3.20 (*q*, $J = 6.5$, NCHCH_2); 3.67 (*s*, MeO); 3.90 (br., CHN); 4.85–5.0 (br., 2 NH); 5.21 (*s*, Cl$C_6H_4CH_2$); 7.24–7.28 (*m*, 2 arom. H); 7.35–7.43 (*m*, 2 arom. H).

*(3*R*,4*R*)-4-(Benzyloxy)-3-{[(*tert-*butoxy)carbonyl]amino}pentanoic Acid* (Boc-β^3-HThr(OBn)-OH, **9**): 15.4 g (42 mmol) of **3d**, 1.12 g (4.62 mmol) of CF_3CO_2Ag, and 15.0 ml (107 mmol) of Et_3N were reacted according to *GP 2*. Drying under h.v. gave **9** (13.1 g, 88%). Colorless glass. $[\alpha]_D = -1.0$ ($c = 1.30$, $CHCl_3$). IR ($CHCl_3$): 3439*w*, 2980*m*, 1710*s*, 1500*s*, 1455*w*, 1393*w*, 1368*m*, 1166*m*, 1098*w*, 908*w*, 863*w*, 617*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.22 (*d*, $J = 6.23$, Me); 1.45 (*s*, *t*-Bu); 2.52–2.69 (*m*, 2 H–C(2)); 3.65–3.73 (*m*, H–C(4)); 4.05–4.07 (*m*, CHN); $\nu_A = 4.39$, $\nu_B = 4.59$ (*AB*, $J_{AB} = 11.5$, PhCH_2); 5.11 (*d*, $J = 9.6$, NHBoc); 7.26–7.37 (*m*, 5

arom. H); 10.45 (br., OH). ^{13}C-NMR (75 MHz, $CDCl_3$): 17.03; 29.24; 38.00; 52.58; 71.95; 75.91; 80.46; 128.41; 128.60; 128.73; 129.26; 139.02; 156.79; 177.31. FAB-MS: 669.5 (4.1, $[2M+Na]^+$), 647.5 (5.1, $[2M+H]^+$), 346.2 (29.6, $[M+Na]^+$), 324.2 (53.9, $[M+H]^+$), 268.1 (73.8, $[M+H-{}^t\text{Bu}]^+$), 224.2 (100, $[M-\text{Boc}+2H]^+$). A small amount of **9** was treated with excess cyclohexylamine and recrystallized from MeOH. Anal. calc. for $C_{17}H_{25}NO_3\cdot C_6H_{13}N$ (422.56): C 65.38, H 9.06, N 6.63; found: C 65.21, H 9.17, N 6.61.

*(3*S*)-3-{[(*tert*-butoxy)carbonyl](methyl)amino}-4-phenylbutanoic Acid* (Boc-*N*-Me-β^3-HPhe-OH (**10**): 1.00 g (3.6 mmol) of **5** was reacted according to *GP 4* to yield **10** (968 mg, 92%). White powder. M.p. 78–81°. ^{1}H-NMR (200 MHz, $CDCl_3$, Rotamers!): 1.28–1.43 (br. *s*, *t*-Bu); 2.51–2.96 (*m*, 2 H–C(2), PhCH_2, MeN); 4.55 (br. *s*, NCH); 7.18–7.33 (*m*, 5 arom. H).

*(3*R*,4*R*)-4-(Benzyloxy)-3-{[(*tert*-butoxy)carbonyl](methyl)amino}pentanoic Acid* (Boc-*N*-Me-β^3-HThr-(OBn)-OH (**11**): 5.36 g (16.6 mmol) **9** was reacted according to *GP 4* to yield **11** (4.64 g, 83%). Yellow oil. ^{1}H-NMR (200 MHz, $CDCl_3$, Rotamers!): 1.19 (*d*, *Me*CH); 1.45 (*s*, *t*-Bu); 2.50–2.87 (*m*, 2 H–C(2), MeN); 3.64–3.83 (br., H–C(4)); 4.40–4.50 (br., CHN); $\nu_A = 4.34$, $\nu_B = 4.62$ (*AB*, $J_{AB} = 11.6$, PhCH_2); 7.26–7.38 (*m*, 5 arom. H).

Boc-β^3-HTrp-β^3-HPhe-OMe (**12**): 2.16 g (7.4 mmol) of **4** was reacted according to *GP 5*. The resulting trifluoroacetate was coupled, according to *GP 6*, with 2.34 g (7.4 mmol) of **6**. FC (AcOEt/pentane 2:1 to 4:1) gave **12** (3.1 g, 85%). Brownish solid. R_f (AcOEt/pentane 2:1) 0.46. M.p. 178–179°. $[\alpha]_D = -22.4$ ($c = 0.82$, $CHCl_3$). IR ($CHCl_3$): 3478*m*, 3428*m*, 3008*m*, 1700*s*, 1496*s*, 1456*m*, 1438*m*, 1367*m*, 1166*s*, 1090*w*, 1046*w*, 930*w*, 650*w*. ^{1}H-NMR (500 MHz, $CDCl_3$): 1.42 (*s*, *t*-Bu); $\nu_A = 2.26$, $\nu_B = 2.34$ (*ABM*, $J_{AB} = 14.9$, $J_{BM} = 5.8$, $J_{AM} = 5.3$, CH_2C(O)OMe); $\nu_A = 2.46$, $\nu_B = 2.50$ (*ABM*, $J_{AB} = 16.1$, $J_{BM} = 5.3$, $J_{AM} = 5.4$, CH_2C(O)N); 2.77–2.81 (*m*, 1 H, CH_2-Aryl); 2.88–2.94 (*m*, CH_2-Aryl); 3.03–3.10 (br., CH_2-Aryl); 3.67 (*s*, MeO); 4.14–4.21 (*m*, CHN); 4.43–4.55 (*m*, CHN); 5.43 (br., NHBoc), 6.15 (*d*, $J = 8.6$, NHCO); 6.97 (*d*, $J = 1.6$, 1 arom. H); 7.08–7.34 (*m*, 8 arom. H); 7.65 (*d*, $J = 7.8$, 1 H, indole), 8.19 (*s*, NH of indole). ^{13}C-NMR (125 MHz, $CDCl_3$): 28.43; 29.92; 37.10; 39.34; 39.92; 47.31; 48.78; 51.79; 79.21; 111.09; 112.14; 119.11; 122.05; 122.87; 126.77; 127.69; 128.63; 129.25; 136.25; 137.44; 155.58; 170.56; 172.11. FAB-MS: 1009.7 (8.62, $[2M+Na]^+$), 516.3, (58.4, $[M+Na]^+$), 494.3 (47.6, $[M+H]^+$), 416.2 (8.2, $[M-\text{Boc}+Na]^+$), 394.2 (66.2, $[M-\text{Boc}+2H]^+$), 376.1 (100.0). Anal. calc. for $C_{28}H_{35}N_3O_5$ (493.6): C 68.13, H 7.15, N 8.51; found: C 67.85, H 7.42, N 8.41.

Boc-β^3-HThr(OBn)-β^3-HLys(N$^\varepsilon$-2-Cl-Z)-OMe (**13**): 3.3 g (7.5 mmol) **8** was reacted according to *GP 5* and coupled, according to *GP 6*, with 2.44 g (7.5 mmol) of **9**. Drying under h.v. gave **13** (4.636 g, 96%), which was used without further purification. For anal. purposes, a small amount of **13** was recrystallized from MeOH. R_f (AcOEt) 0.63. M.p. 121–122°. $[\alpha]_D = -4.3$ ($c = 2.20$, $CHCl_3$). IR ($CHCl_3$): 3677*w*, 3436*m*, 3007*m*, 2979*m*, 2997*m*, 1711*s*, 1506*s*, 1439*m*, 1368*m*, 1248*m*, 1168*m*, 1061*m*, 864*w*, 653*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.18 (*d*, $J = 6.1$, Me); 1.26–1.14 (*m*, CH_2CH$_2$NH); 1.42 (*s*, *t*-Bu); 1.46–1.58 (*m*, 2 CH_2 (Lys)); 2.32–2.49 (*m*, 2 CH_2C(O)); 3.1–3.23 (*m*, NCH_2CH$_2$ (Lys)); 3.67 (*s*, MeO); 3.91–3.93 (*m*, CHN); 4.21–4.23 (*m*, CHN); $\nu_A = 4.39$, $\nu_B = 4.60$ (*AB*, $J_{AB} = 11.5$ PhCH_2); 5.12–5.19 (br., C*H*OBn, NHBoc); 5.19 (*s*, CH_2(2-Cl-C$_6$H$_4$)); 6.42 (*d*, $J = 7.6$, NH-(2-Cl-Z)); 7.22–7.44 (*m*, 9 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 16.13; 23.06; 28.37; 29.34; 33.57; 38.69; 39.91; 40.66; 45.77, 51.71; 52.47; 63.77; 71.09; 75.98; 79.52; 126.85; 127.76; 127.80; 128.23; 129.25; 129.46; 129.06; 133.43; 134.44; 138.28; 156.17; 156.28; 170.31; 172.03. FAB-MS: 1295.8 (18.2, $[2M]^+$), 670.38 (65.1, $[M+Na]^+$), 648.4 (86.5, $[M+H]^+$), 548.3 (100.0, $[M-\text{Boc}+2H]^+$). Anal. calc. for $C_{33}H_{46}N_3O_8Cl$ (648.19): C 61.15, H 7.15, N 6.48; found: C 61.10, H 7.24, N 6.46.

Boc-N-*Me-β^3-HThr(OBn)-β^3-HLys(*N$^\varepsilon$*-2-Cl-Z)-OMe* (**14**): 4.19 g (9.5 mmol) of **8** was reacted according to *GP 5* and coupled, according to *GP 6*, with 3.19 g (9.5 mmol) of **11**. FC (CH_2Cl_2/MeOH 20:1) gave **14** (5.6 g, 89%). Colorless Oil. R_f (CH_2Cl_2/MeOH 20:1) 0.30. IR ($CHCl_3$): 3449*w*, 3008*m*, 2977*m*, 1724*s*, 1675*s*, 1514*s*, 1440*m*, 1367*m*. ^{1}H-NMR (200 MHz, $CDCl_3$, Rotamers!): 1.17 (*d*, $J = 5.8$, MeCH); 1.21–1.48 (*m*, 3 CH_2, *t*-Bu); 2.37–2.87 (*m*, 2 CH_2C(O), MeN); 3.12–3.33 (br. *m*, NHCH_2); 3.69 (br. *s*, MeO, C*H*OBn); 4.18–4.30 (br. *m*, 2 CHN); $\nu_A = 4.33$, $\nu_B = 4.62$ (*AB*, $J_{AB} = 11.6$, PhCH_2); 5.22 (br., NH); 5.22 (*s*, CH_2-Aryl); 6.22, 6.42 (br. *d*, rotamers, NH); 7.24–7.46 (*m*, 9 arom. H). FAB-MS: 684.32 (4.1, $[M+Na]^+$), 662.33 (28.0, $[M+H]^+$), 562.27 (100.0, $[M-\text{Boc}+2H]^+$).

Boc-N-*Me-β^3-HPhe*-N-*Me-β^3-HThr(OBn)-β^3-HLys(*N$^\varepsilon$*-2-Cl-Z)-OMe* (**15**): 2.04 g (3.1 mmol) of **14** was reacted according to *GP 5* and coupled, according to *GP 6*, with 861 mg (3.1 mmol) of **10**. FC (80 g, AcOEt/CH_2Cl_2 1:1) gave **15** (2.01 g, 79%). Colorless foam. R_f (AcOEt/CH_2Cl_2 1:1) 0.29. ^{1}H-NMR (300 MHz, $CDCl_3$, Rotamers!): 1.12 (*d*, *Me*CH); 1.13–1.58 (br. *m*, 3 CH_2, *t*-Bu); 2.30–3.00 (br. *m*, 3 CH_2C(O), 2 MeN, PhCH_2); 3.05–3.22 (br. *m*, NHCH_2); 3.64 (br. *s*, MeO, CHOBn); 4.05–4.40 (br., 2 CHN); 4.50–4.60 (br. *m*, CHN); $\nu_A = 4.29$, $\nu_B = 4.57$ (*AB*, $J_{AB} = 11.5$, PhCH_2); 5.17–5.19 (*m*, NH(2-Cl-Z)); 5.19 (*s*, CH_2-Aryl); 5.57, 6.30, 6.58, 6.79 (br. *m*, rotamers, NH); 7.10–7.41 (*m*, 14 arom. H). FAB-MS: 859.1 (6.0, $[M+Na]^+$), 837.2 (19.5, $[M+H]^+$), 737.2 (100.0, $[M-\text{Boc}+2H]^+$).

*Boc-β³-HTrp-β³-HPhe-β³-HThr(OBn)-β³-HLys(*N$^{\varepsilon}$*-2-Cl-Z)-OMe* (**16**): 1.6 g (3.25 mmol) of **12** and 0.65 g (16 mmol) of NaOH were dissolved in 25 ml of MeOH/3 ml of H_2O, and the reaction mixture was stirred for 24 h at r.t. Additional 0.6 g (16 mmol) of NaOH were added, and the mixture was stirred overnight at 40° (TLC control). The peptide was precipitated by the addition of 1M HCl and collected by filtration. Drying under h.v. over P_2O_5 gave the peptide acid Boc-β³-HTrp-β³-HPhe-OH (1.54 g, 98%), which was used without further purification. Peptide ester **13** (2.094 g, 3.2 mmol) was deprotected according to *GP 6* and coupled with 1.54 g (3.28 mmol) of Boc-β³-HTrp-β³-HPhe-OH according *GP 7*. Precipitation gave **16** (3.01 g, 93%). Colorless solid. R_f (AcOEt) 0.30. Anal. RP-HPLC (50–100% *B* in 10 min, then 100% *B*): t_R 12.4 min. M.p. 195–196° (dec.). $[\alpha]_D = -7.3$ ($c = 0.55$, AcOH). IR (KBr): 3650*w*, 3303*s*, 3059*m*, 3028*m*, 2931*m*, 2857*m*, 1683*s*, 1646*s*, 1534*s*, 1440*m*, 1366*m*, 1342*m*, 1248*s*, 1163*s*, 1099*m*, 1020*m*, 854*w*, 806*w*, 741*s*, 699*m*, 668*m*, 591*m*, 508*w*, 431*w*. ^{1}H-NMR (500 MHz, $CD_3OD/CDCl_3$ 1:4): 1.12 (*d*, $J = 6.2$, Me); 1.25–1.52 (*m*, 3 CH_2 (Lys), *t*-Bu); 2.21–2.46 (*m*, 4 $CH_2C(O)$); $\nu_A = 2.77$, $\nu_B = 2.85$ (*ABM*, $J_{AB} = 13.9$, $J_{AM} = 6.9$, $J_{BM} = 6.7$, CH_2-Aryl); 2.89–2.90 (*m*, 1 H, CH_2-Aryl); 2.97 (br., 1 H, CH_2-Aryl); 3.12 (*t*, $J = 6.7$, 2 H); 3.61 (*s*, MeO); 3.67–3.86 (*m*, C*H*OBn); 4.11–4.13 (br., 2 CHN); 4.23 (br., CHN); 4.36–4.38 (*m*, CHN); $\nu_A = 4.40$, $\nu_B = 4.57$ (*AB*, $J_{AB} = 11.2$ PhCH_2); 5.17 (*s*, CH_2(2-Cl-Ph)); 5.21 (br., NH-(2-Cl-Z)); 7.01–7.41 (*m*, 18 arom. H); 7.60–7.62 (*m*, 1 arom. H (indole)). ^{13}C-NMR (125 MHz, $CD_3OD/CDCl_3$ 1:4): 16.06; 18.01; 23.24; 28.47; 29.45; 30.28; 33.91; 38.74; 39.16; 39.70; 40.27; 40.74, 46.40; 47.77; 48.60; 51.40; 51.85; 63.93; 71.48; 75.56; 79.53; 111.27; 111.41; 118.95; 119.14; 121.72; 123.59; 126.77; 126.88; 127.07; 127.90; 128.10; 128.25; 128.65; 128.72; 129.45; 129.50; 129.59; 133.43; 134.61; 136.58; 137.93; 138.22 ;156.19; 157.09; 171.24; 171.61 (2 C); 172.41. FAB-MS: 1031.9 (51.1, $[M + Na]^+$), 1009.9 (100.0, $[M + H]^+$), 909.9 (59.5, $[M - Boc + 2\,H]^+$), 307.2 (100.0).

Boc-β³-HTrp-N-*Me-β³-HPhe*-N-*Me-β³-HThr(OBn)-β³-HLys(*N$^{\varepsilon}$*-2-Cl-Z)-OMe* (**17**): 3.47 g (4.1 mmol) of **15** was reacted according to *GP 5* and coupled, according to *GP 6*, with 1.32 g (4.1 mmol) of **6**. FC (AcOEt) gave **17** (2.85 g, 66%). Colorless foam. R_f (AcOEt) 0.3. $[\alpha]_D = -5.55$ ($c = 1.035$, MeOH). ^{1}H-NMR (300 MHz, $CDCl_3$, Rotamers!): 1.13 (*d*, *Me*CH); 1.13–1.48 (br. *m*, 3 CH_2, *t*-Bu); 2.30–3.00 (br. *m*, 4 $C(O)CH_2$, 2 MeN, PhCH_2, CH_2-indole); 3.02–3.17 (br. *m*, NHCH_2); 3.64 (br. *s*, MeO, CHOBn); 4.04–4.38 (br. *m*, 4 CHN); $\nu_A = 4.27$, $\nu_B = 4.59$ (*AB*, $J_{AB} = 11.5$, $PhCH_2$); 5.19 (*m*, NH(2-Cl-Z)); 5.19 (*s*, CH_2-Aryl); 5.55, 6.39, 6.59, 6.93–7.00 (br. *m*, rotamers, NH); 7.04–7.40 (*m*, 18 arom. H); 7.58–7.64 (*m*, 1 arom. H). FAB-MS: 1060.0 (11.5, $[M + Na]^+$), 1037.8 (58.2, $[M + H]^+$), 937.6 (100.0, $[M - Boc + 2\,H]^+$). Anal. calc. for $C_{57}H_{73}N_6O_{10}Cl$ (1037.02): C 65.98, H 7.09, N 8.10; found: C 65.81, H 7.26, N 7.90.

*Cyclo(β³-HTrp-β³-HPhe-β³-HThr(OBn)-β³-HLys(*N$^{\varepsilon}$*-2-Cl-Z))* (**18**): 2.5 g (2.49 mmol) of **16** and 6.0 g (150 mmol) of NaOH were suspended in 20 ml of H_2O and 25 ml of TFE, and the suspension was stirred for 2 d at r.t. and for another 24 h at 45°. The peptide acid was precipitated with 1M HCl soln., washed with H_2O and MeOH, and dried under h.v. The resulting β-peptide acid was dissolved in 100 ml of DMF and 50 ml of $CHCl_3$, and was treated at r.t. with 2.0 g (10 mmol) of EDC and 1.0 g (5.4 mmol) of C_6F_5OH. Molecular sieves (4 Å) were added to the mixture. After 24 h, additional EDC (1.0 g, 5 mmol) and C_6F_5OH (0.5 g, 2.7 mmol) were added. The solvent was evaporated after 48 h (TLC control), and the residue was triturated with MeOH, centrifuged and washed with H_2O. The resulting β-peptide acid ester (2.85 g) was treated with a mixture of TFA/ethane-1,2-dithiol/CH_2Cl_2 (5:4:2) under Ar at 0° (0.5M). The mixture was stirred for 10 min at 0°, and the solvent was removed under h.v. The oily residue was co-evaporated twice with $CHCl_3$ and dried for 3 h under h.v. The residue was subsequently dissolved in MeCN (0.025M) and was slowly added (syringe pump) to a soln. of 0.43 ml (2.4 equiv.) of EtN(i-Pr)$_2$ in MeCN (3.3 mM) at 70° during 16 h. A colorless precipitate was observed after 2 h. After completion of the addition, the mixture was stirred for additional 2 h and cooled to r.t. under stirring. The precipitate was collected by filtration and washed with MeCN and MeOH. Drying under h.v. gave **18** (0.84 g, 38%). Colorless powder. M.p. 255–260° (dec.). CD: ($c = 0.2$ mmol/ml, CF_3CH_2OH): no extrema observed. IR (KBr): 3752*w*, 3300*s*, 3060*m*, 2929*m*, 2858*m*, 1656*s*, 1547*s*, 1455*s*, 1440*s*, 1377*m*, 1368*m*, 1261*s*, 1141*m*, 1090*m*, 1060*m*, 1026*m*, 813*w*, 746*s*, 699*s*, 599*m*, 427*w*. ^{1}H-NMR (500 MHz, 24 mg **18** + 5 mg LiCl, 0.75 ml of (D_8)THF): 1.00 (*d*, $J = 6.4$, Me); 1.37–1.62 (*m*, 3 CH_2); $\nu_A = 2.08$, $\nu_B = 2.39$ (*AB*, $J_{AB} = 12.5$, CH_2); $\nu_A = 2.25$, $\nu_B = 2.30$ (*AB*, $J_{AB} = 10.8$, CH_2); 2.52 (*t*, $J = 12.6$, CH_2); 2.61–2.72 (*m*, CH_2); 2.86 (*dd*, $J = 9.2$, 13.8, 1 H, CH_2); 3.00–3.19 (*m*, 3 CH_2); 3.68–3.71 (*m*, CHO); $\nu_A = 4.54$, $\nu_B = 4.88$ (*AB*, $J_{AB} = 12.0$, CH_2OBn); 4.53 (*m*, CHN); 4.72 (*t*, $J = 8.3$, CHN); 4.96 (br., CHN); 5.09 (br., CHN); 5.17 (*s*, CH_2(2-Cl-C_6H_4)); 6.68 (*t*, $J = 5.7$, NH-(2-Cl-Z)); 6.92 (*t*, $J = 7.2$, 1 arom. H); 6.98 (*t*, $J = 7.2$, 1 arom. H); 7.06 (*t*, $J = 7.3$, 1 arom. H); 7.13–7.28 (*m*, 12 arom. H); 7.36 (*d*, $J = 7.6$, 1 arom. H); 7.42 (*d*, $J = 7.3$, 2 arom. H); 7.49 (*d*, $J = 7.2$, 1 arom. H); 7.65 (*d*, $J = 7.9$, 1 arom. H); 8.90 (*d*, $J = 6.9$, NH); 8.97 (*d*, $J = 8.1$, 2 NH); 9.17 (*d*, $J = 9.0$, NH); 10.18 (br., NH). ^{13}C-NMR (75 MHz, 24 mg **18** + 5 mg LiCl, 0.75 ml (D_8)THF): 11.65; 20.67; 26.88; 28.59; 31.71; 32.14; 37.10; 37.89; 38.62; 39.17; 42.89; 44.71; 46.53; 47.28; 60.24; 63.94; 67.76; 71.19; 108.32; 108.72; 115.81; 116.37; 118.28; 120.76; 123.56; 124.30; 124.43; 125.32 (2 C);

125.53; 125.61; 126.48; 126.57; 126.99; 130.45; 132.99; 134.36; 136.45; 137.24; 153.66; 170.04 (2 C); 170.41; 170.99. MALDI-TOF-MS: 899.8 ($[M+Na]^+$), 877.6 ($[M+H]^+$).

Cyclo(β^3-HTrp-β^3-HPhe-β^3-HThr-β^3-HLys) (**1**): 200 mg (0.23 mmol) of **18** were dissolved in 13 ml of THF by the addition of 60 mg (1.2 mmol) of LiCl; 250 mg of Pd/C (10%) were added under Ar. The apparatus was evacuated and flushed with H_2 three times. The mixture was stirred for 24 h, few drops of MeOH and *ca.* 100 mg LiCl (2.5 mmol) were added, and the mixture was stirred for another 24 h. The residue was filtered and washed with a soln. of LiCl in THF. The combined org. layers were evaporated and dissolved in 6 ml of MeOH. Purification by prep. RP-HPLC (C_{18}; 10 min 0% *B*; 20 min 0–35% *B*; at 30% *B* isocratic until product completely eluted; then 9.5 min 35–60% *B*; 5 min 60–98% *B*; 10 min 98% *B*): 106 mg (53%) of **18** and 12 mg (9%) of **1**. Anal. RP-HPLC (C_{18}; gradient 5 min 20–30% *B*; 10 min 30–45% *B*; 5 min 45–100% *B*; 5 min 100% *B*): t_R 12.8 min. $[\alpha]_D = +96$ ($c = 0.25$, MeOH). ^{1}H-NMR (500 MHz, CD_3OH): See *Table 2*. ^{13}C-NMR (125 MHz, CD_3OH): 20.38; 22.88; 27.07; 31.40; 34.77; 40.72; 40.89; 41.07; 42.38; 45.25; 51.29; 55.40; 69.74; 112.04; 112.33; 119.72 (2 C); 122.37; 124.62; 127.62; 129.34; 129.42 (2 C); 129.45; 130.58 (2 C); 130.62; 138.30; 139.27; 172.83; 173.30; 175.19; 175.20. FAB-MS: 641.5 (43.8, $[M+Na]^+$), 619.5 (100.0, $[M+H]^+$).

9. *NMR Analysis of Cyclo-β-tetrapeptide* **1**. *Sample:* 3 mg of **1** dissolved in 0.6 ml of CD_3OH. *1D-NMR (AMX 500):* ^{1}H-NMR (500 MHz): suppression of the CD_3OH signal by presat.; 90 K data points, 128 scans, 5.6-s acquisition time. *2D-NMR:* Solvent suppression by presat. DQF.COSY (500 MHz, CD_3OH) with pulsed field gradients (PFG) for coherence pathway selection [61]: acquisition: 4 K (t_2) × 512 (t_1) data points. 8 scans per t_1 increment, 0.33-s acquisition time in t_2; relaxation delay 2.0 s. TPPI Quadrature detection in ω_1. Processing: Zero filling and FT to 1 K × 1 K real/real data points after multiplication with $\sin^2$ filter shifted by $\pi/3$ in ω_2 and $\pi/2$ in ω_1. TOCSY (DIPSI-2 SL; 10 kHz) [62] (500 MHz, CD_3OH): acquisition: 2 K (t_2) × 1 K (t_1) data points. 32 scans per t_1 increment, mixing time 125 ms, TPPI quadrature detection. Processing: Zero filling and FT to 1 K × 1 K real/real data points after multiplication with $\sin^2$ filter shifted by $\pi/3$ in ω_2 and $\pi/2$ in ω_1. ROESY [63] (500 MHz, CD_3OH): acquisition: a ROESY spectrum with a mixing time of 150 ms was acquired. Solvent suppression by presat., CW-spin lock (3.8 kHz) between trim pulses, 2 K (t_2) × 480 (t_1) data points, 96 scans per t_1 increment. 0.16-s acquisition time in t_2, other parameters identical to DQF.COSY. Processing: Zero filling and FT to 2 K × 2 K real/real data points after multiplication by $\cos^2$ filter in ω_2 and in ω_1. Baseline correction with 3rd degree polynomial in both dimensions.

10. *Structure Determination with Restrained Molecular Dynamics (RMD)*. Based on the powder X-ray structure of cyclo(β^3-HAla)$_4$ [19], a model of **1** was generated using the QUANTA program package, with the Lys side chain NH_2 bearing no charge. Fifteen NOEs (*cf. Table 3*) were classified in three distance categories according to their relative cross-peak volume (determined by integration): s (strong), m (medium), and w (weak). Upper boundary distance values of 3, 3.5 and 4.5 Å, and lower boundary distance values of 1.9 Å were assigned to these three categories. The $H_2C(\alpha)$ of Thr, Trp, and Phe were stereospecifically assigned by their distinct coupling constants to H–C(β) after inspection of a model of the structure. The H′–C(α) (see *Table 2*) of Thr, Trp, and Phe correspond to the H_{Re} protons, which show large *J*-values to their H–C(β). These 15 distance restraints were used together with 8 dihedral restraints for all NH–C(β)H and C(β)H–C(α)H angles, obtained *via Karplus*-type equations [64][65], in RMD with the QUANTA program package and the CHARMm force field. The resulting model, which is in agreement with all experimentally determined values, is depicted in *Fig. 2*.

11. *Biological Evaluation of Cyclo-β-tetrapeptide* **1**. 11.1. *Human Recombinant Receptors Expressed in CCL39 or CHO Cells.* CCL39 Cells (established line of Chinese hamster lung fibroblasts; American Type Culture Collection) or CHO (hsst3 receptors in Chinese hamster oocytes, American Type Culture Collection) were cultured as described in [66][67], and used for stable expression of the human sst1–5 receptor genes. Transfection method and G418 selection have been described in detail in [66][67]. Receptor expression of single cell-derived colonies was tested by radioligand binding as described in [66][67]. For crude membrane preparations, cells were harvested by washing with 10 mM HEPES, pH 7.5, scraping off the culture plates with 4 ml of the same buffer, and centrifugation at 4° for 5 min at 2500 × *g*. The cell pellet was either stored at −80° or directly used. The cells were resuspended in binding assay buffer (10 mM HEPES, pH 7.5, 0.5% (*w*/*v*)) bovine serum albumin by homogenization with the *Polytron* at 50 Hz for 20 s. In competition experiments, cell homogenates (hsst1 and hsst2: *ca.* 1.5×10^5; hsst3 and hsst5: 0.75×10^5; hsst4: 4.5×10^5 cells, depending on the expression level of each receptor) were used in assay buffer (10 mM HEPES, pH 7.5, 0.5% (*w*/*v*) bovine serum albumin, 5 mM $MgCl_2$, 5 μg/ml bacitracin). Cell homogenate (150 μl) was incubated with 50 μl of 25–50 pM [^{125}I]LTT-SRIF$_{28}$, and 50 μl of assay buffer in the absence (total binding) or presence competing ligands or 1 μM SRIF$_{14}$ (non-specific binding). Reactions were terminated by vacuum filtration and bound radioactivity was measured in a *Packard TopCount*.

11.2. *Binding*. Competition and saturation curves from experiments performed in triplicate determination were analyzed as described in [66][67]. Data were analyzed by non-linear regression curve fitting with the computer program SCTFIT [68]. The data are reported as pK_d values ($-$ log mol/l). Protein concentrations were determined by the method of *Bradford* with bovine serum albumin as a standard.

11.2. *Ligands.* $SRIF_{14}$ (Ala-Gly-cyclo(Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys)-OH) was purchased from *Bachem AG* (Bubendorf, Switzerland); octreotide (SMS 201-995; D-Phe-cyclo(Cys-Phe-D-Trp-Lys-Thr-Cys)-Thr-OH) was from *Novartis Pharma*, Basel; ([^{125}I]LTT-$SRIF_{28}$; Ser-Ala-Asn-Ser-Asn-Pro-Ala-Leu-Ala-Pro-Arg-Glu-Arg-Lys-Ala-Gly-cyclo(Cys-Lys-Asn-Phe-Phe-D-Trp-Lys-Thr-(^{125}I-Tyr)-Thr-Ser-Cys)-OH), the radioligand was custom labelled by *ANAWA AG* (Wangen, Switzerland).

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