Peptide Folding Induces High and Selective Affinity of a Linear and Small β-Peptide to the Human Somatostatin Receptor 4

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β-Peptides with side chains in the 2- and 3-positions on neighboring residues (of (*S*) configuration) are known to fold and form a turn (similar to an α-peptidic β-turn). Thus, we have synthesized an appropriately substituted β-tetrapeptide derivative to mimic the hormone somatostatin in its binding to the human receptors $hsst_{1-5}$, which is known to rest upon a turn containing the amino acid residues Thr, Lys, Trp, and Phe. The *N*-acetyl-peptide amide Ac-β^3-HThr-β^2-HLys-β^3-HTrp-β^3-HPhe-NH_2 (**1**) indeed shows all characteristics of the targeted turn-mimic: Lys CH_2 groups are in the shielding cone of the Trp indole ring (by NMR analysis, Figure 2) and there is high and specific nanomolar affinity for $hsst_4$ receptor (Table 1). In contrast, the isomer **2** bearing the Lys side chain in 3-, rather than in the 2-position, has a 1000-fold smaller affinity to $hsst_4$. The syntheses of the required Fmoc-protected β-amino acids (**8**−**11**, **17**) are described (Schemes 1−3). Coupling of the β-amino acids was achieved by the manual solid-phase technique, on Rink resin.

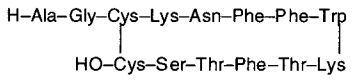
Introduction

Oligomers of β-amino acids (so-called β-peptides) have emerged recently as a new class of peptidomimetics. Small β-peptides of as few as six amino acids fold into helices and turns or form pleated sheet-type structures.[1] They have been shown to be resistant to the degradation by mammalian peptidase and protease enzymes.[2] Their building blocks, the β-amino acids, are nonmutagenic according to Ames' tests. Moreover, β-amino acids are commercially available, and the β-peptides can be synthesized by common solid-phase methods. These chemical, structural, and biological properties render them interesting candidates for use as peptidomimetics.

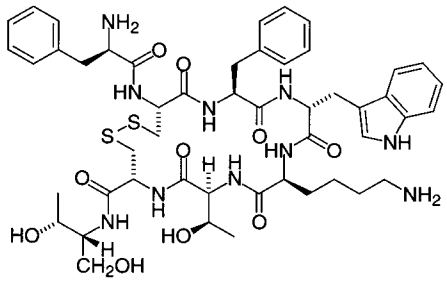
We were first to show that a β-peptide can display biological activity by binding to human receptors thus mimicking an α-peptide.[3] In addition, larger amphiphilic β-peptides have been demonstrated to inhibit cholesterol uptake.[4] DeGrado and Gellman and their respective co-workers have demonstrated antibiotic (and haemolytic) properties, again using amphiphilic β-peptides.[5] However, these large β−peptides (up to 18 amino acids) displayed unfavorable properties such as high polarity and high molecular masses.

We have thus embarked on a research project in order to demonstrate that a *small* β-peptide can achieve high binding to a given receptor with high binding constant. Moreover and in contrast to many peptidomimetics, which are heavily constrained in order to conformationally fit to a given receptor, we wanted to employ a linear and thus unconstrained β-peptide. The biological active conformation should therefore only be due to the *peptide folding* and not due to constraints by chemical bonding.

We chose, as a natural α-peptide hormone to be mimicked, somatostatin ($SRIF_{14}$), isolated in 1973 by Guillemin et al. from 500 000 hypothalami.[6] This peptide possesses various important biological functions, for example, the regulation of the release of growth hormone and insulin. Octreotide (SANDOSTATIN), a cyclic



Somatostatin, $SRIF_{14}$



Octreotide

octapeptide derivative of $SRIF_{14}$, is clinically in use for treatment of acromegaly, gastroentero pancreatic cancers, and other GIT indications. However, the elimination half-life is still rather short; it is therefore of interest to find nonpeptidic derivatives with higher bioavailability. In the past few years, a number of potent peptidomimetics have been discovered by chemists.[7]

The biologically active conformation of octreotide, a β-turn, was revealed by structure−activity relationships, the amino acids in the turn (Phe-Trp-Lys-Thr) are required for activity.[8] To enforce this turn, either

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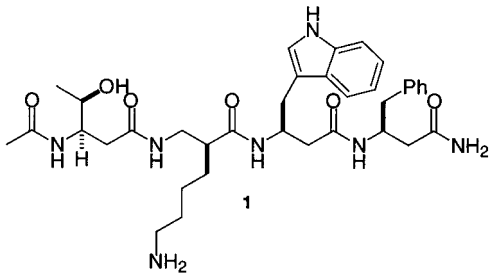
conformationally restricted building blocks have been incorporated into peptides or the peptides themselves have been cyclized. We sought to pursue a different approach, i.e., to design *nonconstrained, linear* β-peptides as analogues. The given strong folding preferences of β-amino acids should enforce a conformational preference of the backbone and thus increase binding to the receptor.

The incorporation of an α-branched (β^2)/β-branched (β^3) β-dipeptide motif or sequence (see Figure 1, left),[9] has been shown by NMR to induce a 10-membered turn in a β-peptide (see Figure 1, right). This turn is comparable to a so-called β-turn of α-peptides, both in size and orientation of the side chains (Figure 1, bottom). Note a difference lies in the orientation of the central amide bond that is reversed in the β-peptide (*retro*-β-peptide).

We have thus designed β-tetrapeptide **1** as a prospective somatostatin mimic by simply attaching the side chains of Lys and Trp to this β^2/β^3 motif. The other two amino acids necessary for binding were chosen as β^3-HPhe and β^3-HThr since modeling suggested favorable overlap of the hypothetical structure of β-peptide **1** with the solution structure of octreotide.



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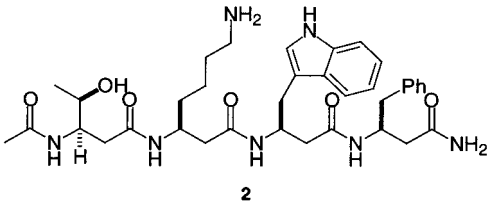

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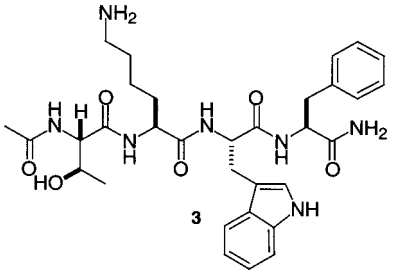


3

To investigate the folding of β-peptide **1** into the expected turn structure, we also prepared β-peptide **2** for comparison. This β-peptide with its all-β^3-amino acids lacking the β^2/β^3 motif is expected to fold, if at all, into a helical structure rather than a turn structure. Thus, its binding should be weaker. The α-peptide **3** was chosen as another compound to be compared with **1** for the demonstration of the folding of the β-tetrapeptide.

Synthesis

The most straightforward route to β^3-amino acids is the Arndt−Eistert reaction. Following a protocol developed earlier by our group,[10] the suitably protected (Fmoc/*t*-Bu-strategy) α-amino acid derivatives of Thr, Trp, Lys, and Phe were activated via their mixed anhydrides (*i*-BuOCOCl, NMM) and subsequently treated with excess of an ethereal diazomethane solution (Scheme 1). The resulting diazoketones **4**, **6**, and **7** (which can be stored for several months at −20 °C) were isolated in good yields (80−95%); however, the Trp-derived diazoketone **5** (with Boc protection of the indolyl group) was isolated in only 50% yield. (The corresponding yield of the conversion of Trp without Boc protection of the indole is still much poorer.) These compounds were then decomposed, using catalytic amounts of silver salts in the presence of water as a nucleophile, to give the β-amino acids **8**−**11** in moderate to good yields after flash chromatography.

Fmoc-β^2-HLys(Boc)-OH (**18**) was prepared by the diastereoselective amidomethylation first described by

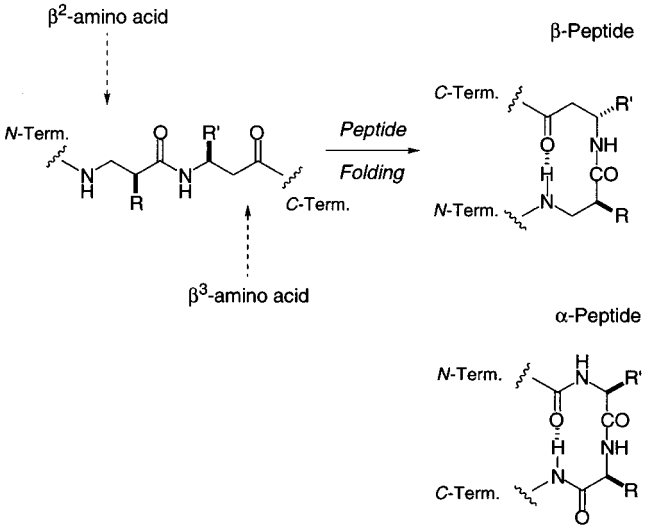
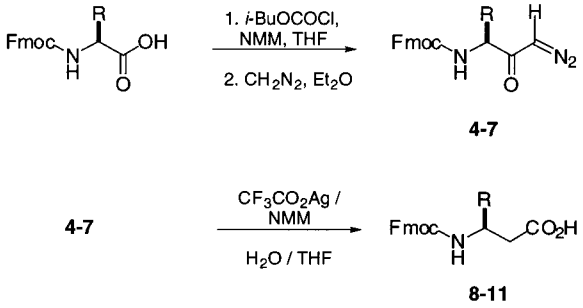


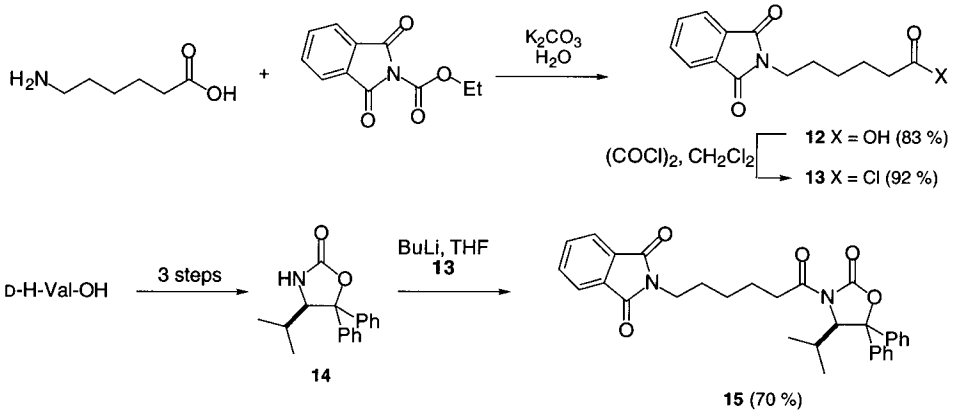
Figure 1. The use of the β^2/β^3 motif in a β-peptide (left) leads to a 10-membered H-bonding ring (right), as has been demonstrated previously by us. This turn is comparable to a β-turn of an α-peptide both in size and orientation of the side chains, the central amide bond being reversed however.

Scheme 1

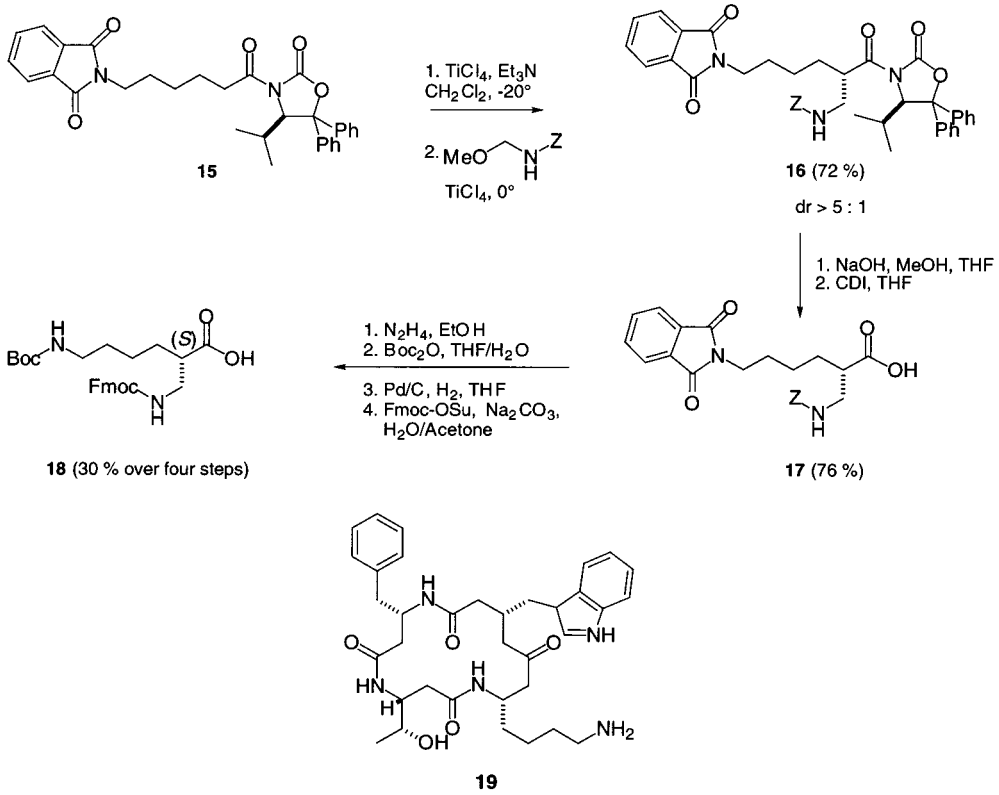


R	Diazoketone	Yield (%)	Fmoc-β3-Hxaa-OH	Yield (%)
(t-BuO)MeCH	**4**	80	**8**	75
(Boc-indol-3yl)CH_2	**5**	50	**9**	71
Boc$NH(CH_2)_4$	**6**	95	**10**	60
$PhCH_2$	**7**	86	**11**	65

Scheme 2



Scheme 3



Evans and co-workers and later by our group.[11] We used a modified Evans auxiliary (**14**) which has been shown to possess several advantages, such as higher crystallinity and better selectivity.[11,12] It is commercially available under the name DIOZ and is readily prepared from Boc-(D)-valine methylester and phenyl Grignard reagent in two step.

First, we had to choose the right protecting group for the stereoselective preparation of Fmoc-β^2-HLys-OH. As has been shown by preliminary experiments, *N*-carbamate protecting groups are in general not stable under the reaction conditions used for amidomethylation via Ti-enolate. Therefore, *N*-carbamate protection was rejected and the phthaloyl group was chosen since it is chemically inert in the amidomethylation via Ti-enolates and orthogonal to protecting groups such as Fmoc and Boc, commonly used in peptide chemistry. Hence, the commercially available 6-aminocaproic acid was N-terminally protected in good yield (83%), using the Nefkens' reagent[13] to give compound **12** which was subsequently transformed to the acid chloride **13** with oxalyl chloride in nearly quantitative yield (Scheme 2). The chiral auxiliary **14** was deprotonated at 0 °C with BuLi (the *gem*-diphenyl moiety protects the carbonyl group in the oxazolidinone from the attack) and acylated by acid chloride **13**. The reaction can be readily carried out on a 50 mmol scale, and **15** was isolated after FC in 70% yield.

The key step, the diastereoselective Mannich-type reaction, is shown in Scheme 3. The Ti-enolate of **15** is allowed to react with the N,O-acetal $MeOCH_2NHZ$ to give the β^2-amino amide **16** in remarkable 72% yield. The diastereoselectivity was greater than 5:1 in favor of the diastereoisomer shown in Scheme 3, as deduced from the ^1H NMR spectrum of the crude product. (Due to overlap of the NMR signals, the diastereoselectivity was estimated on a very conservative basis.) The product **15** is not crystalline (colorless foam), and hence,

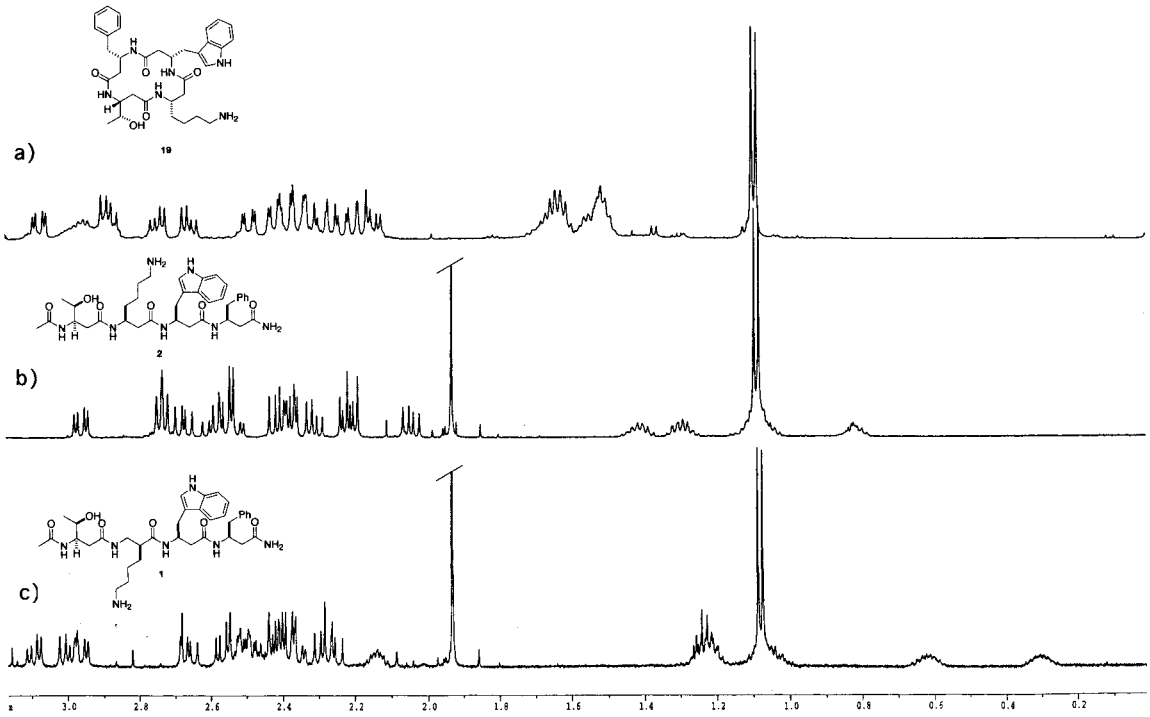


Figure 2. ^{1}H NMR spectra of β-peptide somatostatin analogues: (a) cyclo-β-tetrapeptide **19** (500 MHz, CD_3OH); (b) linear β^3-peptide **2** (500 MHz, D_2O); and (c) linear *retro*-β^2/β^3-peptide **1** (500 MHz, D_2O).

the separation of the minor epimer required flash chromatography. The absolute configuration of the newly formed stereogenic center was assigned to be (*S*) by analogy with results of previous amidomethylations.[10,11,14]

The cleavage of the chiral auxiliary proceeded smoothly in nearly quantitative yield using aqueous base. However, the phthaloyl group was not inert under the reaction conditions; the amide formed was readily cyclized to the imide **17** using 1,1′-carbonyldiimidazole (CDI) in THF (total yield over two steps, 76%). Hydrazinolysis of the phthaloyl group proceeded smoothly, and the resulting hydrochloride was Boc-protected with Boc_2O. The Z group was then hydrogenolytically cleaved, and the resulting amino group Fmoc-protected (Fmoc-OSu, basic water/acetone). The target building block **18** was purified by flash chromatography and isolated in 30% yield over four steps. The overall yield starting from 6-aminocaproic acid is only 9.2% (10 linear and 15 total steps). It was not possible to determine the enantiopurity of **18**; however, it is safe to state that this compound is highly enantioenriched, since no diastereoisomers of the β-peptides prepared from it have been detected by HPLC and NMR.

We chose solid-phase peptide synthesis as a straightforward method to obtain multimilligramm samples of the peptides **1**−**3**. The Rink amide resin[15] was selected in order to obtain the C-terminal amide of the target compounds. The first amino acid was attached to the Rink resin (after deprotection with piperidine/DMF), using the standard BOP/HOBt/EtN(*i*-Pr)$_2$ protocol. The amino acid on the solid support was deprotected (20% piperidine/DMF), and the chain elongation was achieved with BOP/HOBt/EtN(*i*-Pr)$_2$. After the last step, the Fmoc group was removed and the amino group acetylated (Ac_2O/DMF). The peptides were cleaved from the resin (CH_2Cl_2/TFA/TIS 90:9:1) and purified by RP-HPLC. The two β-peptides **1** and **2** and the α-peptide **3** have thus been isolated, their homogeneity was greater than 95% (as determined by RP-HPLC and NMR analysis), and their identity was confirmed by FAB-MS and NMR spectroscopy.

Structural Characterization

One-dimensional NMR spectroscopy can often provide first insights into the solution structure of peptides; this is particularly true for somatostatin and its analogues.[16] The aromaticity of the indole ring leads to a distinct shift of the C(γ)-H protons of the neighbor Lys side chain to lower frequencies, when these groups are close in space, e.g., in a β-turn.[17] Correlations have been made of this shift and the biological activity.[17] Although the receptor-bound conformation may be different, this correlation has been found to serve as a first hint for high affinity if low frequencies of the C(γ)-H are observed.

In Figure 2, the ^{1}H NMR spectra of β-peptides **19**, **1**, and **2** are shown. (The cyclic somatostatin mimicking β-peptide **19** was prepared earlier by us,[3] its NMR spectrum is shown for comparison purposes.) Both β-peptides composed entirely of β^3-amino acids display chemical shifts of the C(γ)-H protons at ca. 1.5 ppm (**19**) and at 1.3 and 1.4 ppm (**2**). However, it is surprising to see that the C(γ)-H protons in **1** are shifted down to 0.3 ppm and to 0.6 ppm. The respective values for the corresponding protons of somatostatin and of octreotide are 1.04 and 0.35 ppm/0.53 ppm, respectively.[17] This indicates the β-peptide **1** displays a conformational preference and, moreover, that its C(γ)-H protons are close in space to the indole ring.

Circular dichroism (CD) spectroscopy is only a low resolution technique to obtain information about the secondary structure of peptides, but it is complementary

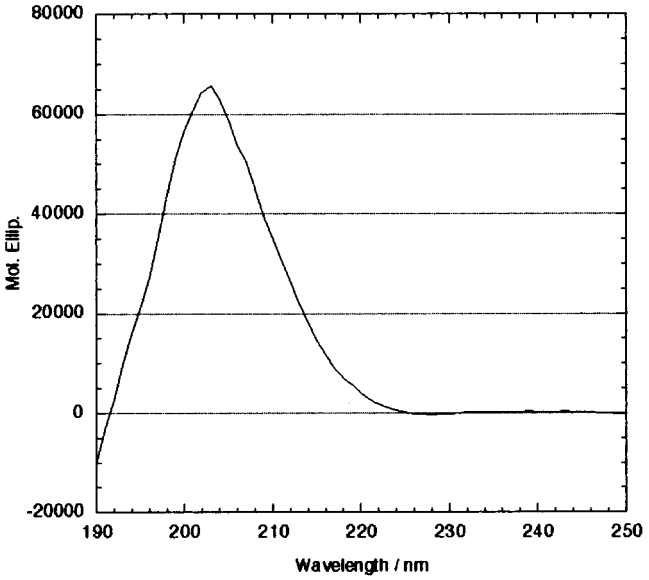


Figure 3. CD spectrum of β-peptide **1** in MeOH (0.2 mM concentration). This pattern has been observed before in β-peptides shown to form 10-membered H-bonding rings by NMR spectroscopy. This pattern supports the presence of such a turn in β-peptide **1**.

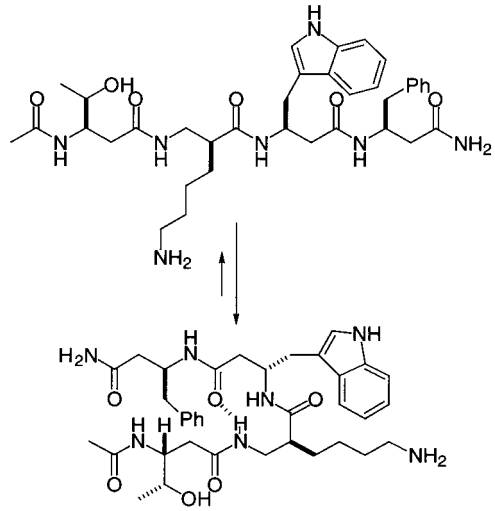


Figure 4. NMR and CD spectroscopy provide evidence for the folding propensities of β-peptide **1**, such that the linear β-peptides are folding to a turn structure (bottom).

to NMR spectroscopy. Many correlations between the patterns of CD spectra and the solution structure obtained by 2D-NMR have been established. The measured CD spectra corroborate the above experimental findings. While the α-peptide **3** and the linear β^3-peptide **2** do *not* show any Cotton effect in the CD spectrum (data not shown), the linear β^2/β^3-peptide **1** displays a very distinct CD spectrum (Figure 3). A single, positive maximum at 203 nm is observed in methanol solution. This spectrum is very similar to the spectrum observed for another β-peptide turn whose structure was solved by NMR-spectroscopy. Although a CD spectrum never really is proof for a structure, the Cotton effect exhibited by β-peptide **1** is an indication that it folds preferentially into a hairpin-type turn structure with a 10-membered H-bonded ring in solution (see the schematic drawing in Figure 4, bottom).

Table 1. pK_D ± SEM Values for β-Peptides **1**−**3** at the Five Human Somatostatin Receptors (hsst) Expressed in CCL-39 Cells Measured by Radioligand Binding Assays Using LTT-CST-14-(Tyr10) as Radioligand[a]

hsst	**1**	**2**	**3**	octreotide	$SRIF_{14}$
1	4.88 ± 01.4	<4	<4	6.41	9.08
2	<5	<4	<4	9.11	10.06
3	<5	<5	<5	8.60	9.67
4	7.08 ± 0.10	<4	<5	5.76	8.39
5	<5	<4	<4	7.31	9.01

[a] Determined from at least three different experiments. Data for somatostatin and octreotide are taken from the literature.[18]

Such a hairpin-type structure should result in increased binding of β-peptide **1** to the somatostatin receptors as compared to the other linear analogues **2** and **3**.

Biological Evaluation

In Table 1 we compare the affinity profiles of the peptides **1**−**3** with those of octreotide and SRIF-14 at the five human recombinant SRIF receptors using [^{125}I]-[Tyr^{10}]CST_{14} as the radioligand.[18,19] Several conclusions can be drawn from these data: (i) the α peptide **3** and the linear β^3 peptide **2** show very low affinity for all five human SRIF receptors, i.e., the K_D values are higher than 10 or even 100 μM. (ii) By contrast, the β^2/β^3 peptide **1** shows significant and selective affinity for one of the human SRIF receptors, namely sst_4. The measured affinity 83 nM is surprisingly high. (iii) The selectivity of β^2/β^3 peptide **1** for the human sst_4 receptor with respect to the other SRIF receptors is at least 100-fold. (iv) Apparently, a simple shift of the Lys side chain of peptide **2** from C(β) to C(α) in peptide **1** results in a nearly 1000-fold increase in affinity for the human sst_4 receptor.

Moreover, a comparison of the affinity of β-peptide **1**, somatostatin, and octreotide for $hsst_4$ reveals that the affinity of **1** is ca. 20 times higher than octreotide but ca. 20 times lower than somatostatin. The molecular function of $hsst_4$ is yet unkown. It is speculated in the literature that this receptor may be important in the treatment of glaucoma related diseases.[20]

The comparison to other nonpeptide ligands[20−22] is difficult since different assays have been used. These compounds generally display higher binding constants. We think, however, that it should be possible to increase the binding constants of our first-generation ligand **1**. Such design efforts are currently underway in our laboratory.

Conclusion

We have succeeded in designing and synthesizing the low molecular weight, linear β-peptide **1** mimicking the natural hormone somatostatin. The use of the β^2/β^3 motif is thought to lead to a folding preference comparable to a β-turn, which is supported by CD and NMR spectroscopy. β-Peptide **1** was shown by radioligand binding assays to display a K_D of 83 nM for the binding to $hsst_4$. It is selective for this receptor over the other four hsst by at least 2 orders of magnitude. Moreover, it is spectacular to see that the shifting of one single side chain from the β-carbon (as in **2**) to the α-carbon (as in **1**) leads to a 1000-fold more potent derivative! This result thus demonstrates that small β-peptides

with their strong folding preferences may be used as pharmaceutically active compounds.

Experimental Section

General. Abbreviations: Boc (*tert*-butoxycarbonyl), BOP (benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium-hexafluorophosphate, CD (circular dichroism), EDC ((*N*-(3-dimethylaminopropyl)-*N*'-ethyl-carbodiimide)hydrochloride), FC (flash chromatography), Fmoc (9-fluorenylmethoxycarbonyl), 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), R_t (retention time), TFA (trifluoroacetic acid), TNBS (2,4,6-trinitrobenzensulfonic acid), Z (benzyloxycarbonyl). 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). Fmoc-amino acids were purchased from Senn. All other reagents were used as received from Fluka. All reactions were carried out under Ar. All given temperatures refer to the bath temperature. TLC: Merck silica gel 60 F254 plates; detection with UV or dipping into a solution of anisaldehyde (9.2 mL), AcOH (3.75 mL), concentrated H_2SO_4 (12.5 mL), and EtOH (338 mL), followed by heating. FC: Fluka silica gel 60 (40−63 μm); 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: CH_3CN. 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)). Mp: Büchi-510 apparatus; uncorrected. Optical rotations: Perkin-Elmer 241 polarimeter (10 cm, 1 mL cell) at room temperature. IR spectra: Perkin-Elmer-782 spectrophotometer, intensities classified w (weak), m (medium), and s (strong). 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). Mass spectra: Hitachi Perkin-Elmer RHU-6M (FAB, in a 3-nitrobenzyl alcohol matrix) spectrometer; in *m*/*z* (% of basis peak). CD: Jobin-Yvon-Mark-III, 1 mm cell length. Elemental analyses were performed by the Microanalytical Laboratory of the Laboratorium für Organische Chemie, ETH-Zürich.

Methods for Biological Evaluation: (A) Human Recombinant Receptors Expressed in CCL39 Cells. CCL39 cells (established line of Chinese hamster lung fibroblasts; American Type Culture Collection) were cultured as previously described[18] and used for stable expression of the $hsst_{1-5}$ receptor genes. Transfection method and G418-selection have been described in detail in previous publications.[18] Receptor expression of single cell-derived colonies was tested by radioligand binding as described.[18] 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 °C for 5 min at 2500*g*. The cell pellet was either stored at −80 °C or directly used. The cells were resuspended in binding assay buffer (10 mM HEPES, pH 7.5, 0.5% (w/v) bovine serum albumin = BSA) by homogenization with the Polytron at 50 Hz for 20 s. In competition experiments, cell homogenates ($hsst_1$ and $hsst_2$: ca. 1.5 × 105; $hsst_3$ and $hsst_5$: 0.75 × 105; $hsst_4$: 4.5 × 105 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 mg/ mL bacitracin). A total of 150 mL of cell homogenate were incubated with 50 mL of 25−50 pM [^{125}I][Tyr^{10}]CST_{14} and 50 mL of assay buffer in the absence (total binding) or presence competing ligands or 1 μM $SRIF_{14}$ (nonspecific binding). Reactions were terminated by vacuum filtration, and bound radioactivity was measured in a Packard TopCount.

(B) Binding. Competition curves from experiments performed in triplicate determination were analyzed as described previously, by nonlinear regression curve fitting with the computer program SCTFIT.[19] 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.

(C) Ligands. $SRIF_{14}$ (Ala-Gly-c[Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys]-OH) was purchased from Bachem AG (Bubendorf, Switzerland); octreotide (SMS 201−995 = Sandostatin; D-Phe-c[Cys-Phe-D-Trp-Lys-Thr-Cys]-Thr-OH) was from Novartis Pharma (Basel, Switzerland); the radioligand [^{125}I][Tyr^{10}]CST_{14} = (Pro-c[Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-(^{125}I-Tyr)-Ser-Ser-Cys]-Lys) was custom labeled by ANAWA AG (Wangen, Switzerland).

General Procedure for the Preparation of *N*-Fmoc-Protected Diazoketones: Procedure A.[10] *Caution*: The generation and handling of diazomethane requires special precautions.[23] The *N*-Fmoc-protected α-amino acid was dissolved in THF (0.2 M) and cooled to −25 °C. After addition of Et_3N (1 equiv) and $ClCO_2i$-Bu (1 equiv), the mixture was stirred for 15 min. The resulting suspension was allowed to warm to 0 °C. A solution of CH_2N_2 in Et_2O was added until the rich yellow color persisted. Stirring was continued overnight as the mixture was allowed to warm to room temperature. Excess of CH_2N_2 was destroyed by addition of a few drops of AcOH. The suspension was diluted with AcOEt to the double volume and then washed with saturated $NaHCO_3$, saturated NH_4Cl, and saturated NaCl solutions. The organic phase was dried ($MgSO_4$) and evaporated under reduced pressure. Recrystallization or FC afforded the pure diazoketone.

General Procedure for the Silver Salt-Catalyzed Wolff Rearrangement of Diazoketones to *N*-Fmoc-Protected β^3-Amino Acids: Procedure B.[10] The diazoketone was dissolved in THF (0.25 M) containing 10% H_2O and then cooled to 0 °C. A solution of CF_3COOAg (0.11 equiv) in NMM (2.5 equiv) was added, and the resulting mixture allowed to warm to room temperature in 8 h in the dark. After removal of the bulk of THF, the mixture was diluted with 0.5 M NaOH solution and washed with Et_2O. The aqueous phase was carefully adjusted to pH 2 at 0 °C with 1 M HCl and extracted with AcOEt. The organic phase was dried ($MgSO_4$) and evaporated under reduced pressure. FC afforded the pure β^3-amino acids.

General Procedure for the Fmoc Solid-Phase Synthesis: Procedure C. The first β-amino acid (Fmoc-protected) was attached to the Rink amide resin according to the method developed in our group.[24] Initial loading of the resin was measured by UV determination of the 9-fluorenylmethylpiperidine after cleavage of the Fmoc group with piperidine.[25] Peptides were synthesized using DMF as solvent in a three-way stopcock peptide vessel under Ar. Protected β-amino acids were coupled in 3-fold excess in the presence of 3-fold excess of BOP and HOBt as activating reagents and 9-fold excess of $(i\text{-Pr})_2EtN$. The Fmoc protecting group was removed by treatment with 20% piperidine in DMF (30 mL/mmol, 2 × 15 min). The completion of the coupling was monitored by the TNBS test.[26] The Fmoc-deprotected peptide resin was washed (30 mL/mmol) with DMF/CH_2Cl_2 1:1 (5 × 3 min) and treated successively with $(i\text{-Pr})_2EtN$ (20 equiv) and Ac_2O (10 equiv) in DMF/CH_2Cl_2 1:1 (2 mL) under Ar bubbling for 10−15 min. Monitoring of the acetylation was performed using the TNBS test.[26] The resin was then washed (30 mL/mmol) with DMF (6 × 3 min), CH_2Cl_2 (3 × 3 min), and Et_2O (5 × 1 min) and dried under h.v. for 12 h. The dry Fmoc-deprotected Rink amide peptide resin was then first treated with a mixture of $CH_2Cl_2/CF_3CO_2H/(i\text{-Pr})_3SiH$ 90:9:1 (20 mL/mmol, 3 × 2 min), and then with a mixture of $CH_2Cl_2/CF_3CO_2H/(i\text{-Pr})_3SiH$ 95:4:1 (20 mL/mmol, 3 × 2 min), allowing the solvent to pass through the resin slowly. Excess CF_3CO_2H/ CH_2Cl_2 was evaporated, and deprotection was completed by stirring the oily residue in 95% CF_3CO_2H/CH_2Cl_2 for 1 h. The solvent was evaporated, co-evaporated whith CH_2Cl_2, and dried under h.v. The precipitate which formed upon addition of cold Et_2O to the oily residue was collected by filtration or centrifugation. This precipitate was then purified by RP-HPLC.

(*S*)-1-Diazo-3-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}-4-(*tert*-butoxy)pent-2-one (4). Fmoc-Thr(*t*-Bu)-OH (4.47 g, 11.26 mmol) was transformed according to the general procedure A. The crude product was then purified by FC on silica gel (Et_2O/pentane 1:2) to give **4** (3.76 g, 80%); yellow foam. R_f (Et_2O/pentane 1:2): 0.13. $[\alpha]_D = +1.5$ ($c = 1$, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 1.08 (d, $J = 6.5$ Hz, 3H, Me), 1.22 (s, 9H, *t*-Bu), 4.13 (br, 2H, CH_2CO), 4.23 (t, $J = 6.7$ Hz, 1H, C*H*CH_2O), 4.40−4.54 (m, 2 H), 5.6 (s, 1H, CHN_2), 5.82 (d, $J = 6.5$ Hz, 1H, NH), 7.30−7.43 (m, 4 arom. H), 7.61 (d, $J = 7.2$ Hz, 2 arom. H), 7.77 (d, $J = 7.4$ Hz, 2 arom. H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 18.8, 28.3, 47.3, 54.8, 63.6, 66.7, 67.1, 74.7, 120.0, 125.0, 125.1, 127.0, 127.1,127.7, 127.8, 141.4, 143.8, 156.1. IR ($CHCl_3$): 3419*w*, 3007*w*, 2977*m*, 2110*s*, 1716*s*, 1633*m*, 1497*s*, 1450*w*, 1365*s*, 1327*w*, 1248*w*, 1150*w*, 1075*w*, 1029*w*. FAB-MS: 421 $[MH]^+$. Anal. ($C_{24}H_{27}N_3O_4$) C, H, N.

(*S*)-1-Diazo-3-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}-4-{*N*-[(*tert*-butoxy)carbonyl]indol-3-yl}butan-2-one (5). Fmoc-Trp(Boc)-OH (4.98 g, 9.49 mmol) was transformed according to the general procedure A. The crude product was then purified by FC on silica gel (Et_2O/pentane 1:2 to 1:1) to give **5** (2.69 g, 50%); yellow foam. R_f (Et_2O/pentane 1:2): 0.16. $[\alpha]_D = -5.4$ ($c = 1$, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 1.66 (s, H, *t*-Bu), 3.17 (d, 2H, $J = 6.5$ Hz, CH_2-indol-3-yl), 4.20 (t, 1H, $J = 6.7$ Hz, CH_2CH), 4.44 (br, 1H, C*H*CH_2O), 4.60 (br, 1H, NHC*H*CO), 5.12 (s, 1H, CHN_2), 5.49 (br, 1H, NH), 7.23−7.61 (m, 9 arom. H), 7.76 (d, $J = 7.5$ Hz, 3 arom. H), 8.13 (d, $J = 8.2$ Hz, 1 arom. H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.3, 28.2, 47.2, 54.8, 57.6, 66.9, 83.9, 115.0, 115.4, 119.0, 120.0, 122.8, 124.3, 124.7, 125.0, 125.1, 127.1, 127.7, 130.2, 135.5, 141.4, 143.7, 149.5, 155.8, 192.8. IR ($CHCl_3$): 3423*w*, 3007*w*, 2112*s*, 1724*s*, 1638*m*, 1502*m*, 1452*s*, 1369*s*, 1258*m*, 1156*m*, 1085*m*, 1020*w*. Anal. ($C_{32}H_{30}N_4O_5$) C, H, N.

(*S*)-7-{[(*tert*-Butoxy-9-carbonyl]amino}-1-diazo-3-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}heptane-2-one (6). Fmoc-L-Lys(Boc)-OH (8.68 g, 18.5 mmol) was transformed according to general procedure A. The crude product was then purified by FC on silica gel (CH_2Cl_2/Et_2O 6:1 → 1:1) to give **6** (8.08 g, 89%). Light yellow solid. ^1H NMR in agreement with the literature.[10]

(*S*)-1-Diazo-3-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}-4-phenylbutan-2-one (7). Fmoc-Phe-OH (20.87 g, 53.83 mmol) was transformed according to general procedure A. The crude product was then purified by FC on silica gel (AcOEt/hexane 3:7) to give **7** (19.11 g, 86%); light yellow solid. ^1H NMR in agreement with the literature:[10] (m.p. 133−135 °C).

(*R*)-3-{[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}-4-(*S*)-(*tert*-butoxy)pentanoic Acid (8). Diazo ketone **4** (3.10 g, 7.37 mmol) was transformed according to general procedure B. The crude product was then purified by FC on silica gel (Et_2O/pentane 1:2 to 1:1) to give **8** (2.27 g, 75%); white foam. R_f (Et_2O/pentane 1:2): 0.23. $[\alpha]_D = +7.8$ ($c = 1$, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ 1.02 (d, $J = 5.9$ Hz, 3H, Me), 1.20 (s, 9H, *t*-Bu), 2.35 (m, 1H, CH_2CO), 2.61 (m, 1H, CH_2CO), 3.78 (br, 1H, C*H*NH), 4.00 (br, 1H, C*H*O*t*-Bu), 4.23 (br, 1H, C*H*CH_2O), 4.35 (d, $J = 6.2$ Hz, 2H, CH_2O), 7.28−7.40 (m, 4 arom. H), 7.65 (br, 2 arom. H), 7.79 (d, $J = 7.4$ Hz, 2 arom H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.8, 31.3, 37.7, 56.7, 70.1, 71.3, 77.7, 123.4, 128.8, 130.7, 131.3, 145.2, 147.7, 148.0, 160.9, 178.1. IR ($CHCl_3$): 3436*w*, 2977*m*, 1718*s*, 1507*s*, 1451*m*, 1374*w*, 1323*w*, 1256*w*, 1087*m*. FAB-MS: 434 $[MNa]^+$, 412 $[MH]^+$.

(*S*)-3-{[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}-4-{*N*-[(*tert*-butoxy)carbonyl]indol-3-yl}butanoic Acid (9). Diazo ketone **5** (1.0 g, 0.182 mmol) was transformed according to general procedure B. The crude product was then purified by recrystallization ($CHCl_3$/hexane) to give **9** (697 mg, 71%); white powder. $[\alpha]_D = -12.7$ ($c = 1$, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ 1.65 (s, 9H, *t*-Bu), 2.33−2.45 (m, 2H, CH_2CO), 2.61−2.85 (m, 1H, CHN), 3.07−3.19 (m, 2H, CH_2-indol-3-yl), 4.14−4.25 (m, 1H, C*H*CH_2O), 4.24−4.56 (m, 2H, CH_2O), 5.66 (d, $J = 8.4$ Hz, 1H, NH), 7.24−7.60 (m, 10 arom. H), 7.70−8.12 (m, 3 arom. H). IR ($CHCl_3$): 3432*w*, 2982*m*, 1722*s*, 1509*m*, 1452*m*, 1371*m*, 1309*w*, 1257*m*, 1157*m*, 1087*m*, 1045*w*. FAB-MS: 1081 $[2MH]^+$, 563 $[MNa]^+$, 540 $[MH]^+$. Anal. ($C_{32}H_{32}N_2O_6$) C, H, N.

(*S*)-7-{[(*tert*-Butoxy-9-carbonyl]amino}-3-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}heptanoic Acid (10). Diazo ketone **6** (7.40 g, 15.0 mmol) was transformed according to general procedure B. The crude product was then purified by FC on silica gel (AcOEt/pentane 1:1, 1% HOAc) and recrystalized ($CHCl_3$/hexane) to give **10** (4.33 g, 60%); white solid. ^1H NMR in agreement with the literature.[10]

(*S*)-3-{[(9′*H*-Fluoren-9′-ylmethoxy)carbonyl]amino}-4-phenylbutanoic Acid (11). Diazo ketone (**7**) (2.058 g, 5 mmol) was transformed according to general procedure B. Because of incomplete reaction (TLC-control), another 87 mg (0.39 mmol, 0.08 equiv) of CF_3COOAg in 1 mL (8.9 mmol, 1.8 equiv) of NMM were added, and stirring was continued for 12 h at room temperature. The crude product was then purified by FC on silica gel (CH_2Cl_2/MeOH 9:1) to give **7** (1.307 g, 65%); white powder. ^1H NMR in agreement with the literature.[10]

***N*,*N*-Phthalimidocaproic Acid (12).** To a solution of 6-aminocaproic acid (43.3 g, 330 mmol) in H_2O (500 mL) and Na_2CO_3 hydrate (94.42 g, 330 mmol) was added *N*-ethoxycarbonyl phthalimide (72.35 g, 330 mmol). The solution was stirred for 30 min and then acidified to pH 4 with 6 M HCl. The resulting precipitate was collected by filtration. Drying (h.v.) yielded **12** (71.79 g, 83%); white solid. Mp: 107−108 °C. ^1H NMR (200 MHz, $CDCl_3$): δ 1.21−1.41 (m, 2H, CH_2), 1.56−1.72 (m, 4H, CH_2), 2.24−2.31 (t, $J = 7.3$ Hz, 2H, C*H*$_2$COOH), 3.66 (t, $J = 7.1$ Hz, 2H, (Phth)NC*H*$_2$), 7.75−7.86 (m, 4 arom. H).

***N*,*N*-Phthalimidocaproyl Chloride (13).** Compound **12** (13.83 g, 52.9 mmol) was dissolved under Ar in CH_2Cl_2 (200 mL) and a few drops of dioxane. Oxalyl chloride (8.75 g, 68.9 mmol) was added, and the solution was stirred at room temperature for 4 h. The solvent was removed under reduced pressure. Drying (h.v.) yielded **13** (14.81 g, 92%) as colorless oil. ^1H NMR (200 MHz, $CDCl_3$): δ 1.27−1.41 (m, 2H, CH_2), 1.56−1.76 (m, 4H, CH_2), 2.84 (t, $J = 7.2$ Hz, 2H, CH_2CO), 3.61 (t, $J = 7.1$ Hz, 2H, (Phth)NC*H*$_2$), 7.63−7.78 (*m*, 4 arom. H).

(*R*)-4-(1-Methylethyl)-3-(1-oxo-5-phthalimidohexanoyl)-5,5-diphenyloxazolidin-2-one (15). To a suspension of (*R*)-4-(1-methylethyl)-5,5-diphenyloxazolidin-2-one (**14**)[11] (14.27 g, 50.8 mmol) in THF (200 mL) was slowly added BuLi (37 mL, 53.25 mmol) at 0 °C (ice bath). To the resulting clear solution was added compound **13** (17.03 g, 60.9 mmol) in one portion. The mixture was allowed to warm slowly to room temperature overnight, treated with saturated NH_4Cl solution, and diluted with Et_2O. The organic phase was washed with 1 M HCl (2 × 20 mL), 1 M NaOH (2 × 20 mL), and saturated NaCl solution, dried ($MgSO_4$), and evaporated. FC on silica gel (Et_2O/pentane 1:1) afforded **15** (18.55 g, 70%); white powder. Mp: 111−112 °C. R_f (Et_2O/pentane 1:1): 0.38. $[\alpha]_D = +155.45$ ($c = 1.12$, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 0.72 (d, $J = 6.5$ Hz, 3H, Me), 0.84 (d, $J = 6.8$ Hz, 3H, Me), 1.23−1.31 (m, 2H, CH_2), 1.50−1.65 (m, 4H, CH_2), 2.70−2.89 (m, 2H, CH_2CON), 3.60 (t, $J = 7.3$ Hz, 2H, (Phth)NC*H*$_2$), 5.34 (d, $J = 3.4$ Hz, 1H, C*H*-*i*-Pr), 7.21−7.47 (m, 10 arom. H), 7.63−7.67 (m, 2H, Phth), 7.67−7.78 (m, 2H, Phth). ^{13}C NMR (75 MHz, $CDCl_3$): δ 16.4, 21.8, 24.2, 26.2, 28.3, 29.8, 34.9, 37.7, 64.5, 89.3, 123.1, 125.6, 125.9, 127.9, 128.4, 128.6, 128.9, 132.1, 133.8, 138.1, 142.4, 152.9, 168.3, 172.8. IR ($CHCl_3$): 3011*w*, 2942*w*, 1778*s*, 1710*s*, 1493*w*, 1467*w*, 1397*m*, 1366*m*, 1175*m*, 1119*w*, 1048*w*. FAB-MS: 525 $[MH]^+$. Anal. ($C_{32}H_{32}N_2O_5$) C, H, N.

(*R*)-3-((*S*)-2-{[(Benzyloxycarbonyl)amino]methyl}-1-oxo-3-phthalimidohexanoyl)-4-(1-methylethyl)-5,5-diphenyloxazolidin-2-one (16). To a solution of compound **15** (4.11 g, 7.84 mmol) in CH_2Cl_2 (40 mL) was added $TiCl_4$ (0.94 mL, 8.63 mmol) at −20 °C. Et_3N (1.20 mL, 8.63 mmol) was added to the yellow solution, and the resulting dark red solution was stirred at −20 °C for 30 min before addition of a solution of compound benzyl *N*-(methoxymethyl) carbamate (prepared according to ref 11) (1.68 g, 8.63 mmol) in CH_2Cl_2 (17 mL) and $TiCl_4$ (0.94 mL, 8.63 mmol). The mixture was stirred at 0 °C (ice bath) for 4 h and then treated with saturated NH_4Cl

solution (6 mL) diluted with Et_2O (20 mL). The organic phase was washed with 1 M HCl (2 × 10 mL), 1 M NaOH (2 × 10 mL), and saturated NaCl solution (10 mL), dried ($MgSO_4$), and evaporated. Purification by FC on silica gel (Et_2O/pentane 1:1) yielded **16** (3.93 g, 72%); white foam. R_f (Et_2O/pentane 1:1): 0.11. $[\alpha]_D = +47.50$ ($c = 1$, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.71 (d, $J = 6.8$ Hz, 3H, Me), 0.83 (d, $J = 7.2$ Hz, 3H, Me), 1.21−1.32 (m, 6H, CH_2), 1.95−2.00 (m, 1H, C*H*(CH_3)$_2$), 3.33−3.40 (m, 2H, NHC*H*$_2$), 3.41−3.54 (m, 2H, C*H*$_2$N(Phth), 3.85 (m, 1H, CHCO), 5.06 (s, 2H, PhC*H*$_2$), 5.22 (br, 1H, N*H*CH), 5.32 (d, $J = 3.4$ Hz, 1H, NC*H*i*Pr*), 7.26−7.48 (m, 15 arom. H), 7.71 (*m*, 2H, Phth), 7.82 (m, 2H, Phth). ^{13}C NMR (75 MHz, $CDCl_3$) δ 16.4, 21.6, 23.4, 28.2, 28.9, 29.6, 37.4, 42.8, 65.5, 66.7, 89.7, 123.2, 125.4, 125.7, 128.0, 128.2, 128.5, 128.9, 129.0, 132.2, 133.9, 142.3, 168.3, 174.6. IR ($CHCl_3$): 3453*w*, 3011*w*, 2945*w*, 1776*s*, 1711*s*, 1514*m*, 1450*w*, 1397*m*, 1366*m*, 1176*w*, 1051*w*. FAB-MS: 1375 [2*M*]$^+$, 688.4 [*M*H]$^+$. Anal. ($C_{41}H_{41}N_3O_7$) C, H, N.

(*S*)-2-{[(Benzyloxycarbonyl)amino]methyl}-6-{[(*N*,*N*-phthalyl)]amino} hexanoyl Acid (17). To a solution of compound **16** (1.14 g, 1.61 mmol) in MeOH/THF (3 mL/3 mL) was added 1 M aqueous NaOH solution (2 mL), and the mixture was stirred for 2.5 h at room temperature. THF was removed under reduced pressure, Et_2O (6 mL) was added, the suspension was stirred for 15 min, and filtered. The residue was washed with 1 M NaOH (5 mL), H_2O, Et_2O (5 mL), and pentane (5 mL) and dried (h.v.) to yield (*R*)-4-(1-methylethyl)-5,5-diphenyloxazolidin-2-one (**14**) (1.29 g, 80%) as white powder. The filtrate was diluted with Et_2O (5 mL), the aqueous phase was separated, the pH was adjusted to 1−2 with 6 M HCl, and the solution was extracted with Et_2O (2 × 20 mL). The combined organic layers were washed with saturated NaCl solution and dried ($MgSO_4$). The residue (0.591 g, 1.33 mmol) was dissolved in dry THF (10 mL), 1,1′-carbonyldiimidazole (0.647 g, 3.99 mmol) was added, and the solution was stirred at room temperature for 48 h. The solvent was removed under reduced pressure, and the residue was redissolved in 10% aqueous $NaHCO_3$ solution (10 mL). The solution was washed with Et_2O (2 × 10 mL) and then acidified to pH 1 by dropwise addition of 1 M HCl. The resulting white precipitate was extracted with AcOEt (3 × 10 mL), and the organic phases were washed with saturated NaCl solution and dried ($MgSO_4$) to yield (**17**) as a white foam (0.54 g, 95%). R_f (CH_2Cl_2/Et_2O/AcOH 4:1:0.2): 0.28. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.34−1.71 (m, 6H, CH_2), 2.61(br, 1H, C*H*COOH), 3.26−3.38 (m, 2H, C*H*$_2$-NH), 3.63 (t, $J = 7.15$ Hz, 2H, *CH*$_2$N(Phth)), 5.04 (s, 2H, CH_2-Ph), 7.65 (m, 2 arom. H), 7.85 (m, 2 arom. H).

(*S*)-7-{[(*tert*-Butoxy)carbonyl]amino}-3-{[(9*H*-fluoren-9-ylmethoxy)carbonyl] amino}heptanoic Acid (18). To a solution of compound **17** (540 mg, 1.27 mmol) in EtOH (6 mL) was added 1 M methanolic hydrazine solution (2 mL), and the mixture was heated under reflux for 30 min. The solvent was removed under reduced pressure, and the white residue was redissolved in 2 M HCl (5 mL) and heated at 50 °C for 15 min. The precipitate was collected by filtration to give (*S*)-2-{-[(benzyloxycarbonyl)amino]methyl}amino hexanoyl acid hydrochloride (378 mg, 90%) which was used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.48 (m, 6H, CH_2), 2.42 (t, $J = 7.0$ Hz, 1H, *CH*COOH), 2.87 (t, $J = 7.20$ Hz, 2H, *CH*$_2$NH), 3.21 (d, $J = 7.20$ Hz, 2H, *CH*$_2$NH); 5.11 (s, 2H, CH_2O), 7.42−7.45 (br, 4 arom. H). (*S*)-2-{[(Benzyloxycarbonyl)-amino]methyl}amino hexanoyl acid hydrochlride (380 mg, 1.146 mmol) was dissolved in 1 M NaOH (2.4 mL), a solution of Boc_2O (530 mg, 2.46 mmol) in dioxane (0.5 mL) was added, and the resulting mixture was stirred for 10 h at room temperature. The solution was then washed with pentane (3 × 5 mL), the pH was carefully adjusted to 1−2 with 1 M HCl, and the resulting white suspension was extracted with AcOEt (3 × 10 mL). The organic phases were dried ($MgSO_4$) and evaporated to yield (*S*)-6-{[(*tert*-butoxy)carbonyl]amino}-2-{-[(benzyloxycarbonyl)amino]methyl}-hexanoyl acid (384 mg, 85%). This compound (186.1 mg, 0.726 mmol) was dissolved in THF (5 mL), and Pd/C (10 wt %) was added. The apparatus was evacuated and flushed with H_2. The mixture was stirred at room temperature for 4 h under H_2 (1 bar). Filtration through Celite and concentration under reduced pressure yielded (*S*)-6-{[(*tert*-butoxy)carbonyl]amino}aminohexanoyl acid (160.3 mg, 86%). This compound (160.3 mg, 0.625 mmol) was dissolved in 0.15 aqueous Na_2CO_3 solution (5 mL) and was treated with a solution of Fmoc-OSu (253 mg, 0.750 mmol) in acetone (2 mL). The mixture was then concentrated under reduced pressure, diluted with H_2O (5 mL), and extracted with Et_2O (2 × 10 mL). The aqueous phase was carefully adjusted to pH 2−3 at 0 °C with 1 M HCl and extracted with AcOEt (3 × 10 mL). The organic phases were dried ($MgSO_4$) and evaporated. The crude product was purified by FC with CH_2-Cl_2/MeOH 9:1 to give **18** (230.5 mg, 30% based on **17**); white foam. R_f (CH_2Cl_2/MeOH 9:1): 0.31. $[\alpha]_D = +4.8$ ($c = 0.54$, $CHCl_3$). ^{1}H NMR (300 MHz, CD_3OD): δ 1.27−1.57 (m, 6H, CH_2), 1.42 (s, 9H, *t*-Bu), 2.57 (t, 1H, CHCO), 3.01 (t, $J = 6.7$ Hz, 2H, CH_2N), 3.24−3.31 (m, 2H, C*H*$_2$NH), 4.18 (t, $J = 7.2$ Hz, 1H, C*H*CH_2O), 4.31 (d, $J = 6.8$ Hz, 2H, CHC*H*$_2O$), 7.27−7.40 (m, 4 arom. H), 7.63 (d, $J = 7.2$ Hz, 2 arom. H), 7.78 (d, $J = 7.5$ Hz, 2 arom. H). ^{13}C NMR (75 MHz, CD_3OD): δ 14.5, 23.7, 25.4, 28.9, 30.4, 30.9, 32.8, 43.4, 47.0, 67.8, 79.9, 121.0, 126.3, 128.2, 128.8, 142.6, 145.3, 158.6, 158.9, 178.2. IR ($CHCl_3$): 3448*w*, 2943*w*, 1707*s*, 1513*m*, 1454*m*, 1367*w*, 1261*m*, 1166*w*, 1090*w*, 1008*w*.

Ac-(*S*)-Thr-(*S*)-Lys-(*S*)-Trp-(*S*)-Phe-NH$_2$ (3). The rink amide resin (208.4 mg, 141.7 μmol) was loaded with Fmoc-Phe-OH (164.7 mg, 425.1 μmol) according to general procedure C. Loading: 0.52 mmol/g (76%), corresponding to 108 μmol of anchored Fmoc-Phe-OH. Synthesis, N-acetylation, and cleavage according to general procedure C yielded the crude α-tetrapeptide **3** (77.9 mg, 86%). Purification by RP-HPLC (30−60% B in 20 min, C_8) yielded **3** (27.3 mg, 41%); white fluffy solid. Purity: >95% (HPLC). RP-HPLC (30−60% B in 20 min; C_8): t_R 9.18. FAB-MS: 1243 [2*M* + H]$^+$, 622 [*M*H]$^+$. ^{1}H NMR (500 MHz, CD_3OD): δ 1.14 (d, $J = 6.4$ Hz, 3H, Me), 1.45−1.56 (m, 6H, CH_2), 2.07 (s, 3H, Me), 2.74−2.77 (m, 2H, CH_2-Ph), 2.85−2.87 (m, 2H, CH_2ind), 3.01−3.20 (m, 3H, C*H*$_2$NH$_2$, C*H*OH), 4.13−4.15 (m, 1H, NHC*H*), 4.24−4.26 (m, 1H, NHC*H*), 4.50−4.54 (m, 2H, 2 × NHC*H*), 7.05−7.40 (m, 8 arom. H), 7.55 (d, $J = 7.8$ Hz, 2 arom. H).

Ac-(*R*)-β^3-HThr-(*S*)-β^3-HLys-(*S*)-β^3-HTrp-(*S*)-β^3-HPhe-NH$_2$ (2). The rink amide resin (360 mg, 244 μmol) was loaded with **10** (430 mg, 1.08 mmol) according to general procedure C. Loading: 0.63 mmol/g (92%), corresponding to 227 μmol of anchored Fmoc-β^3-HPhe-OH. Synthesis, N-acetylation, and cleavage according to general procedure C yielded the crude β^3-tetrapeptide **2** (141 mg, 91%). Purification by RP-HPLC (10−40% B in 25 min, C_8) yielded **2** (70 mg, 46%); white fluffy solid. Purity: >95%. RP-HPLC (10−30% B in 25 min; C_8): t_R 23.68. FAB-MS: 700 [MNa]$^+$, 678 [MH]$^+$. ^{1}H NMR (500 MHz, CD_3OD): δ 0.79−0.82 (m, 2H, CH_2), 1.07 (d, $J = 6.4$ Hz, 3H, Me), 1.29−1.32 (m, 2H, CH_2), 1.38−1.43 (m, 2H, CH_2), 2.01−2.69 (m, 12H, 4 x CH_2, CH_2Ph, CH_2ind), 2.73 (t, 2H, $J = 7.7$ Hz, *CH*$_2$NH$_2$), 2.94−2.98 (m, 1H, C*H*OH), 3.75−3.77 (m, 1H, NHC*H*), 4.01−4.04 (m, 1H, NHC*H*), 4.31−4.34 (m, 1H, NHC*H*), 4.49−4.52 (m, 1H, NHC*H*), 7.05−7.27 (m, 6H, arom.), 7.46 (d, $J = 8.2$ Hz, 2 arom. H), 7.53 (d, $J = 8.1$ Hz, 2 arom. H).

Ac-(*R*)-β^3-HThr-(*S*)-β^2-HLys-(*S*)-β^3-HTrp-(*S*)-β^3-HPhe-NH$_2$ (1). The Rink amide resin (42.1 mg, 28 μmol) was loaded with **10** (45,1 mg, 112 μmol) according to general procedure C. Loading: 0.53 mmol/g (79%), corresponding to 22 μmol of anchored Fmoc-β^3-HPhe-OH. Synthesis, N-acetylation, and cleavage according to general procedure C yielded the crude mixed-tetrapeptide **1** (15.5 mg, 85%). Purification by RP-HPLC (10−30% B in 30 min, C_8) yielded **1** (5.2 mg, 35%); white fluffy solid. Purity: >95%. RP-HPLC (10−30% B in 30 min; C8): t_R 23.14. FAB-MS: 700 [MNa]$^+$, 678 [MH]$^+$. ^{1}H NMR (500 MHz, CD_3OD): δ 0.26−0.34 (m, 2H, CH_2), 0.58−0.65 (m, 2H, CH_2), 1.04 (d, $J = 6.4$ Hz, 3H, Me), 1.16−1.22 (m, 2H, CH_2), 1.89 (s, 3H, Me), 2.10−2.64 (m, 8H, 3 × CH_2CO, C*H*CO, C*H*OH), 2.78−2.94 (m, 2H, CH_2ind), 3.03−3.07 (m, 4H, *CH*$_2$NH$_2$, CH_2Ph), 3.70−3.74 (m, 1H, NHC*H*), 3.98−4.01 (m, 2H, NHC*H*$_2$), 4.32−4.35 (m, 1H, NHC*H*), 4.47−4.51 (m, 1H, NHC*H*), 6.92−7.22

(m, 6 arom. H), 7.40 (d, J = 8.1 Hz, 2 arom. H), 7.48 (d, J = 7.8 Hz, 2 arom. H).

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