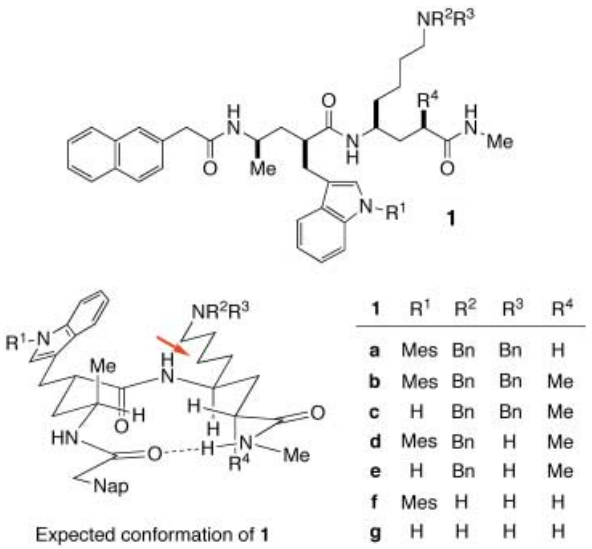

1	R¹	R²	R³	R⁴
a	Mes	Bn	Bn	H
b	Mes	Bn	Bn	Me
c	H	Bn	Bn	Me
d	Mes	Bn	H	Me
e	H	Bn	H	Me
f	Mes	H	H	H
g	H	H	H	H

Scheme 1. Structural formulae of the γ-peptides **1**. In the expected conformation of **1** the red arrow points to a CH_2 group of the $H_2N(CH_2)_4$ unit, which is placed inside the shielding cone of the aromatic indole ring. Mes = mesitylenesulfonyl, Bn = benzyl, Nap = naphthyl.

Somatostatin Mimics

Design and Synthesis of γ-Dipeptide Derivatives with Submicromolar Affinities for Human Somatostatin Receptors

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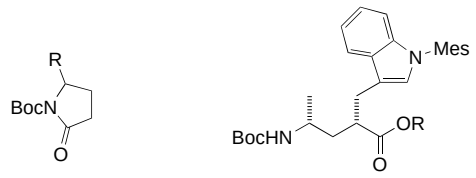
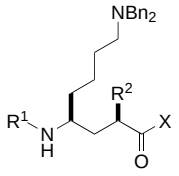
In a previous paper we have shown that simple *N*-acyl-γ-dipeptide amides that resemble a βII′ turn of an α-peptide can be designed to form a turn structure in solution (NMR) and in the solid state (X-ray).[1,2] To see whether such a turn could also be used to mimic a peptide, the biological activity of which rests upon a turn structure carrying functionalized side chains, we have now synthesized compounds **1a–g** (Scheme 1), with the side chain of tryptophan in the γ^2 position of the first and of lysine in the γ^4 position of the second γ-amino acid, and have tested their affinities for the human somatostatin receptors $hsst_{1-5}$.[3–6]

The synthesis of γ-dipeptide derivatives **1** commenced with the *N*-Boc-γ-lactams **2** and **3** (Boc = *tert*-butoxycarbonyl), readily available from the corresponding commercial (*R*)-Ala and (*S*)-Lys acids by known procedures.[1,7] Ring opening (with the Lys derivative after change of side-chain protection, →**4**), and esterification with $Me_3Si(CH_2)_2OH$ provided the (*R*)-Boc-γ^4-hhAla and Boc-γ^4-hhLys(Bn_2) esters, which were



(*R*)-**2**, R = Me
(*S*)-**3**, R = $(CH_2)_4$NH(2-Cl-Z)
(*S*)-**4**, R = $(CH_2)_4NBn_2$

5, R = $(CH_2)_2SiMe_3$
6, R = H



7, R^1 = Boc, R^2 = Me, X = $O(CH_2)_2SiMe_3$
8, R^1 = R^2 = H, X = NHMe
9, R^1 = H, R^2 = Me, X = NHMe

doubly deprotonated and alkylated with 1-mesitylenesulfonyl-3-bromomethylindole and MeI to give the *unlike* $\gamma^{2,4}$-amino acid derivatives **5** and **7**, respectively. The ester group in compound **5** with Trp side chain was cleaved (Bu_4NF, →**6**), and the lysine-derived esters were converted to the methylamides **8** and **9** without and with 2-methyl substitution, respectively (1. Bu_4NF, 2. $MeNH_2$, 3. F_3CCO_2H). Coupling of the two γ-amino acid derivatives (**6** + **8** and **6** + **9**), removal of the Boc groups, and acylation with 2-naphthylacetic acid[8] (4-methylmorpholine, 1-hydroxy-1*H*-benzotriazole, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) produced the side-chain-protected *N*-acyl-dipeptide amides **1a** and **1b**. Deprotection procedures ($MeSO_3H$, F_3CCO_2H, and Pd/C, H_2) led to the various partially or fully deprotected γ-dipeptide derivatives **1c–1g**. All compounds were purified and fully characterized by elemental analyses, specific optical rotations, circular dichroism (CD), IR, and NMR spectroscopy, and mass spectrometry.

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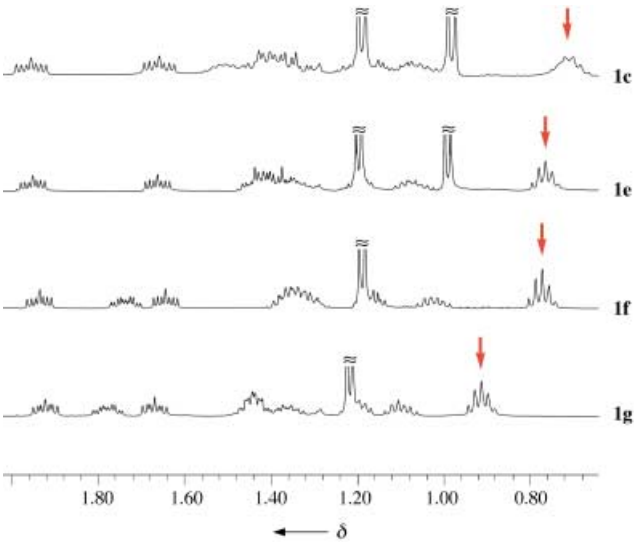

Figure 1. High-field part of the 500 MHz ^{1}H NMR spectra of the γ-dipeptides **1c, 1e, 1f**, and **1g** in CD_3OD. The red arrows point to high-field-shifted $N(CH_2)_4$ resonances.

A typical feature of the turn structure in somatostatin and its analogues is the juxtaposition of the tryptophan and lysine side chains, which places CH_2 groups of the $H_2N(CH_2)_4$ unit inside the shielding cone of the aromatic indole ring (NMR shifts between $\delta = 0.8$ and 0.3 ppm are observed).[9] High-field sections of the NMR spectra of four γ-dipeptide amides, shown in Figure 1, in which CH_2 signals appear between $\delta = 0.9$ and 0.6 ppm, confirm the proximity between the corresponding side chains, and are thus compatible with a turn conformation of these compounds. The CD spectra of the *N*-naphthylacetyl dipeptide amides **1** exhibit an intensive negative Cotton effect near 200 nm ([Θ] up to 70000 deg cm^2 dmol^{-1}), with a weaker and broader peak near 220 nm ([Θ] up to 30000 deg cm^2 dmol^{-1}) (Figure 2); this CD pattern may be taken as another piece of evidence for the presence of a secondary structure.

Probably the most stringent test of the γ-dipeptide structure is the affinity for somatostatin receptors. Binding affinities for the five cloned human receptors $hsst_{1-5}$, expressed in CCL-39 cell lines, were determined by displacement of [^{125}I]LTT-SRIF$_{28}$ from these receptor proteins.[10] While the fully protected γ-dipeptide **1d** binds to $hsst_1$ and $hsst_3$ with remarkable K_D values of 0.55 and 1.00 μM, respectively, the partially and the fully deprotected γ-dipeptide derivatives **1f** and **1g** bind to $hsst_5$ with K_D values of 0.51 and 0.87 μM, respectively (Table 1). Intriguingly, the highest affinities (**1d**/$hsst_1$, **1f**/$hsst_5$) are observed when the side chain functional groups (3-indolylmethyl and $(CH_2)_4NH_3^+$) are protected by bulky aromatic moieties (*N*-mesitylenesulfonyl and/or -benzyl)!

The results presented here are confirmative, surprising, and promising; they demonstrate that a 14-amino-acid cyclic disulfide hormone, somatostatin, can be mimicked by a simple, designed, low-molecular-weight, open-chain γ-dipeptide derivative (cf. **1g**) that contains only three amide bonds; they suggest that hitherto unknown hydrophobic pockets are present in the receptors ($hsst_1$, $hsst_3$, and $hsst_5$), which supposedly house the turn-bound Trp and Lys side chains (cf. **1c, 1d, 1f**); and they promise a potential of γ-peptides for the development of peptidase-resistant[11] peptidomimetic drugs.

Table 1: pK_D Values for γ-peptides **1b–1g** at the five hsst receptors expressed in CCL-39 cells and measured by radioligand binding assays with [^{125}I]LTT-SRIF$_{28}$ as radioligand.[a],[10]

Receptor	1b	1c	1d	1e	1f	1g	Octreotide[b]	SRIF$_{14}$[c]
$hsst_1$	5.47	6.06	6.26	5.61	5.98	4.73	6.45	9.08
$hsst_2$	< 5	< 5	5.17	< 5	5.01	2.81	9.11	10.06
$hsst_3$	5.53	5.89	6.00	5.73	5.67	5.42	8.60	9.67
$hsst_4$	4.67	5.74	5.92	5.66	5.79	5.44	5.76	8.39
$hsst_5$	4.49	5.01	5.87	5.14	6.29	6.06	7.31	9.01

[a] Submicromolar affinities are highlighted in red. [b] Sandostatin. [c] Somatostatin.

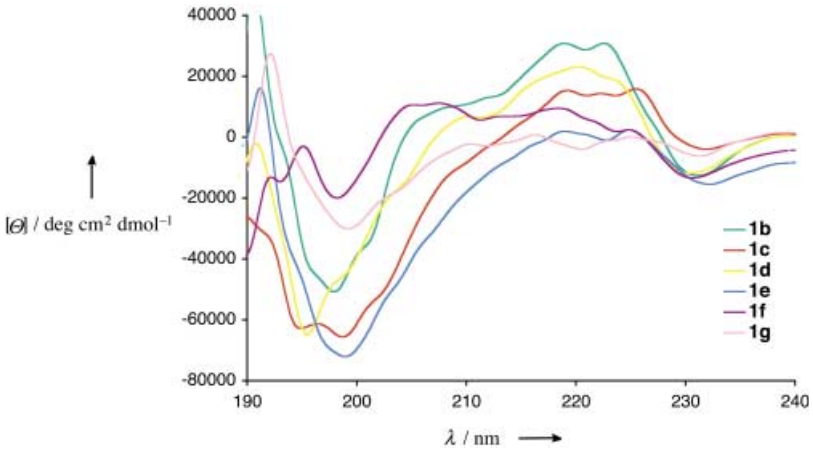

Figure 2. Nonnormalized CD spectra in MeOH (0.2 mM) of the γ-dipeptide derivatives **1b–1g**.

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