Linear, Peptidase-Resistant β^2/β^3-Di- and α/β^3-Tetrapeptide Derivatives with Nanomolar Affinities to a Human Somatostatin Receptor

Preliminary Communication

by **Dieter Seebach***, **Magnus Rueping**[1]), **Per I. Arvidsson**[2]), **Thierry Kimmerlin**[1]), **Peter Micuch**, and **Christian Noti**[3])

Laboratorium für Organische Chemie der Eidgenössischen Technischen Hochschule, ETH-Hönggerberg, Wolfgang-Pauli-Strasse 10, CH-8093 Zürich

and

Daniel Langenegger and **Daniel Hoyer**

Nervous System Research, S-386-745, *Novartis Pharma AG*, CH-4002 Basel

N-Acyl-β^2/β^3-dipeptide-amide somatostatin analogs, **5**–**8**, with β^2-HTrp-β^3-HLys ('natural' sequence) and β^2-HLys-β^3-HTrp (*retro*-sequence) have been synthesized (in solution). Depending on their relative configurations and on the nature of the terminal *N*-acyl and terminal *C*-amino group, the linear β-dipeptide derivatives have affinities for the human receptor hsst 4, ranging from 250 to >10000 nanomolar (*Fig. 3*). Also, *N*-Ac-tetrapeptide amides **9** and **10**, which contain one α- and three β-amino acid residues (*N*-β-α-β-β-*C*), have been prepared (solid-phase synthesis), with the natural (Phe, Trp, Lys, Thr) and the *retro*-sequence (Thr, Lys, Trp, Phe) of side chains and with two different configurations, each, of the two central amino acid residues. The novel 'mixed', linear α/β-peptides have affinities for the hsst 4 receptor ranging from 23 to >10000 nanomolar (*Fig. 4*), and, like 'pure' β-peptides, they are completely stable to a series of proteolytic enzymes. Thus, the peptidic turn of the cyclic tetradecapeptide somatostatin (*Fig. 1*) can be mimicked by simple linear di- and tetrapeptides. The tendency of β-dipeptides for forming hydrogen-bonded rings is confirmed by calculations at the B3LYP/6-31G(d,p) level (*Fig. 2*). The reported results open new avenues for the design of low-molecular-weight peptidic drugs.

β-Peptides have recently emerged as a promising class of peptidomimetics for medicinal chemistry. The ability of β-peptides (for reviews, see [1]) to adopt secondary structures such as helices [1], sheets [2a][3], and, especially, turns [3] suggests that these compounds might be structural and functional mimics of natural peptides. The potential of such mimics is evident from the facts that *a*) these secondary structures can be readily designed [1–5], and that *b*) the β-peptides are completely stable against proteolytic degradation *in vitro* and *in vivo* [2a][6].

So far, β-peptides, designed to form amphiphilic helices, have been shown to inhibit an intestinal membrane-bound cholesterol- and lipid-transporting protein [5]. Also, similar peptides have been shown to possess antimicrobial, and sometimes, hemolytic activities [7].

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[2]) Postdoctoral fellow at ETH-Zürich (2000/2001), financed by the *Swedish Foundation for International Cooperation in Research and Higher Education* (*STINT*).

[3]) Work partially carried out in advanced organic laboratory course at ETH.

The hypothalamic hormone somatostatin, **1** (*Fig. 1*), is a cyclic α-tetradecapeptide that possesses a number of important biological functions [8], such as the inhibition of growth-hormone (GH) release from the anterior pituitary [8b], and the pancreatic secretion of glucagon and insulin [8c]. It is also known to be involved in the regulation of gastrin secretion from the gut [9]. Furthermore, $SRIF_{14}$ (SRIF = Somatotropin Release Inhibiting Factor) acts as a neurotransmitter in the central nervous system [10], where it modulates numerous processes, including the release of other neurotransmitters [11]. Five different human somatostatin receptors (hsst 1–5) [12] have been cloned and characterized as G-protein-coupled transmembrane receptors [13]. So far, the physiological function of only two, hsst 2 (GH regulation) and hsst 5 (insulin regulation), are known. Receptors hsst 2 and hsst 5 also mediate the antiproliferative effect of $SRIF_{14}$ on tumor cell growth [14].

The various functions of somatostatin in biological systems make the receptors of this peptidic hormone important targets to search for agonists and antagonists in the treatment of several human diseases. However, the use of somatostatin itself is hampered by the disadvantages of having a short half-life in blood serum (< 3 min) and of being nonselective. To circumvent these problems, an immense search for analogs has commenced, and some of these are in clinical use, *e.g.*, octreotide (*Sandostatin*®) **2** is applied in the treatment of acromegaly and of certain gastroentero pancreatic tumors [15]. However, since the half-life of octreotide is also rather short (90 min), further developments of new $SRIF_{14}$ mimics remain a challenging research topic in industry and academia.

The pharmacophore of somatostatin is known to involve the sequence Phe[7]-Trp[8]-Lys[9]-Thr[10], which comprises a type-II′ β-turn spanning the Trp-Lys fragment. It was also shown that the Trp[8] and Lys[9] residues are essential for activity, whereas the Phe[7] and Thr[10] residues can undergo minor 'revisions', provided that residues with hydrophobic side chains are next to Trp, and those with hydrophilic side chains next to Lys [16]. With this in mind, we have previously constructed β-peptidic $SRIF_{14}$ analogs: in a first attempt, the β^3-homologs of Phe, Tyr, Lys, and Thr [17a,b] were incorporated in a cyclic β-tetrapeptide, but a much better affinity for one of the receptors (hsst 4) was obtained with an open-chain β-tetrapeptide, notably, containing a central β^2/β^3-peptidic segment (*cf.* **3** and **4**) [17c]. We knew that such a segment would promote turn formation (*cf.* the *12/10* helix formed by β-peptides built of β^2- and β^3-amino acids [2d,f]).

In the present study, we examined *a*) whether simple β-dipeptide derivatives, which contain a β^2- and a β^3-amino acid residue with the correct side chains, would result in formation of a turn, *i.e.*, in affinity to somatostatin receptor(s)[4]), and *b*) whether a β^2-amino acid in the tetrapeptidic somatostatin analogs can be replaced by an α-amino acid residue.

In a first attempt to scrutinize the intrinsic potential of the β^2/β^3-structural element for forming a turn, we used a computational approach [19a]. High-level *ab-initio* calculations (HF and DFT) showed that an *N*-acylated β^2-HAla-β^3-HAla dipeptide bearing a terminal *C*-methylamino group is flexible, but that the conformations having a ten- or a twelve-membered H-bonded ring are indeed preferred over alternative

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[4]) During the course of our work, it has been reported that certain α-dipeptide derivatives show a high affinity for the hsst 2 receptor [18].

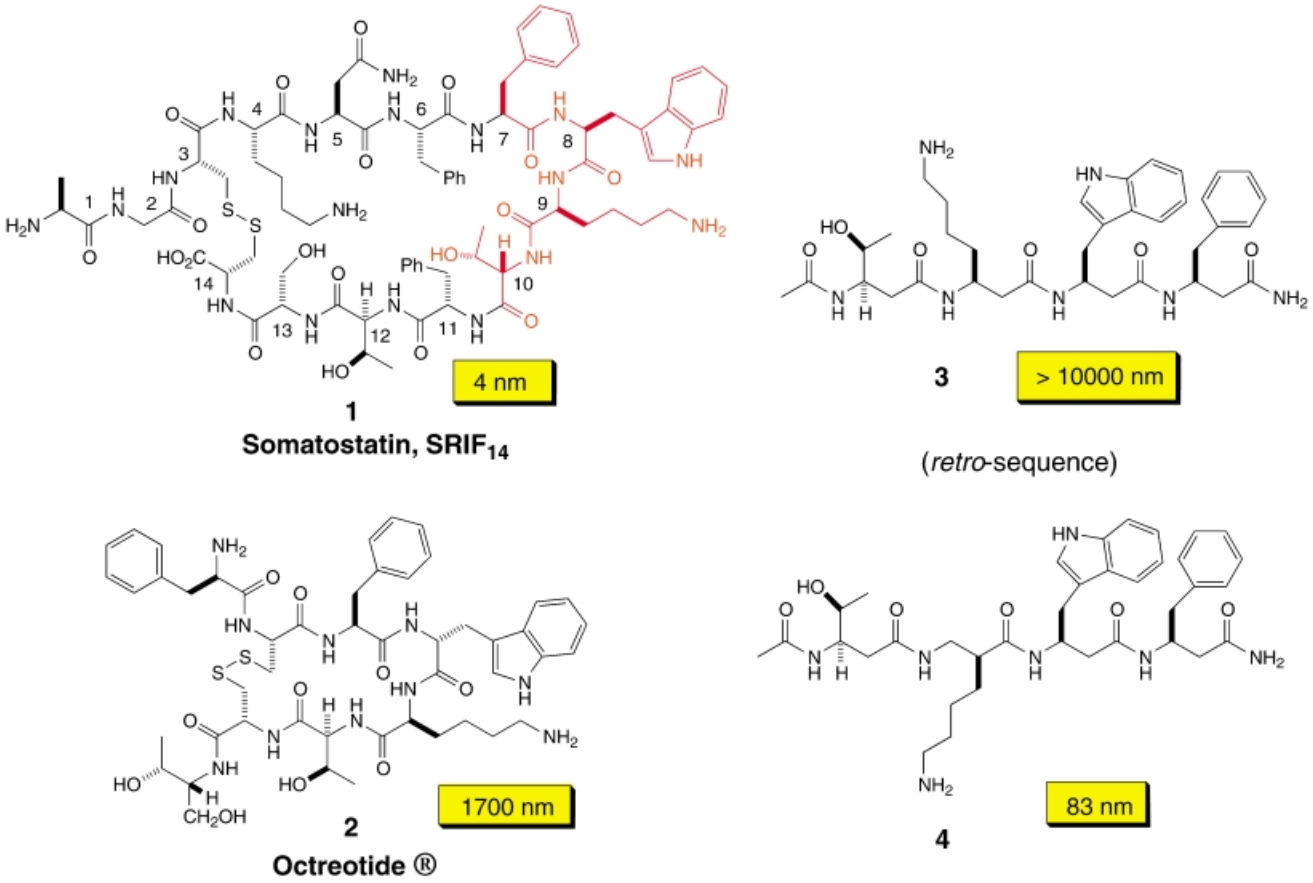

Fig. 1. *Somatostatin* (**1**), *octreotide* (**2**), *and two linear β-peptidic somatostatin analogs.* The molecular formulae of **1**, of **2**, and of the linear β-peptides **3** and **4** are shown together with the corresponding binding affinities to the cloned hsst 4 receptor. The sequence Phe-Trp-Lys-Thr labeled in red is decisive for the affinity of somatostatin to the hsst receptors, and the neighborhood of the Trp and Lys side-chains in a turn structure is evident from shielding in the NMR spectrum of the Lys CH_2 groups by the indole ring. In the β-peptides **3** and **4**, the side chains of Lys and Trp are arranged in reversed sequence, as compared to the natural somatostatin.

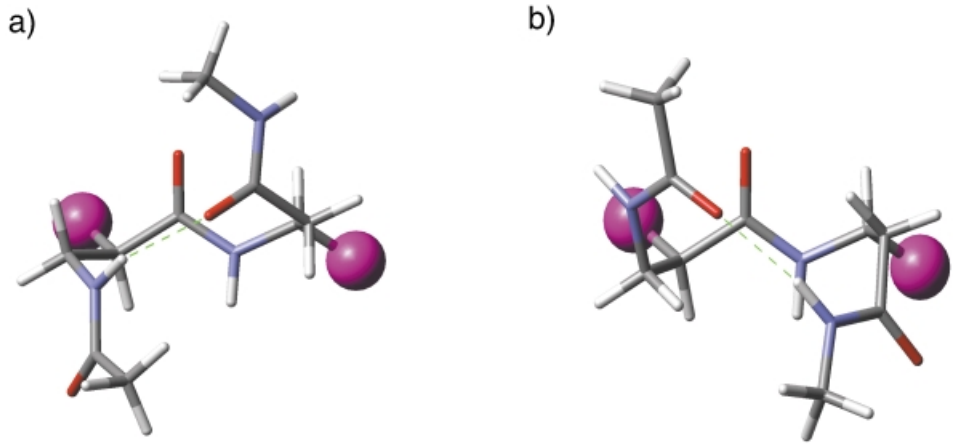

Fig. 2. *HF/6-31G(d,p)-Optimized geometries of the lowest-energy conformations of Ac-(*R*)-β²HAla-(*R*)-β³-HAla-NMe.* The ten-membered H-bonded ring conformer (*a*) is 0.5 kcal mol^{-1} more stable than the twelve-membered ring conformer (*b*) (B3LYP/6-31G(d,p)//HF/6-31G(d,p)). Other conformations, also having ten- and twelve-membered H-bonded rings, are found within 1 kcal mol^{-1} of these structures, while alternative conformations, such as eight-membered H-bonded rings and extended arrangements, are at least 3 kcal mol^{-1} higher in energy. The Me groups in the side chains are represented by red balls to highlight the positions of the side chains in these turns.

conformations by at least 3 kcal mol^{-1} (B3LYP/6-31G(d,p)), while the ten- and the twelve-membered rings are almost isoenergetic ($\pm$0.5 kcal mol^{-1}) (*Fig. 2*).

This preference is independent of the configurations at the two stereogenic centers; only the chirality of the β^3-amino acid determines the handedness of the resulting turn. Although the lowest-energy conformations (*Fig. 2*) have a more helical-turn than reverse-turn-type appearance, other conformers, also comprising ten- and twelve-membered H-bonded rings but with a more parallel arrangement of the backbone, were found with energies only 0.5–1 kcal mol^{-1} above the global minimum [19]. Thus, these calculations suggest that *the side chains of a β-dipeptide, built of a β²- and a β³-residue can have a turn-like conformation* (as part of a rapidly interconverting ensemble of different conformers).

Even though the conformational preference of an unbound molecule might be completely different from its biologically active conformation when bound to a receptor, these predictions encouraged us to synthesize – in solution – the potential β-peptidic somatostatin analogs composed of β^2-HLys [17c] and β^3-HTrp bearing a β-hydroxybutyryl at the N-terminus and a 2-phenylamino group at the C-terminus.

The peptides **5** and *epi*-**5** (*Fig. 3*) were tested for their binding to cloned human somatostatin receptors (hsst 1–5)[5]). Compound **5** shows a good binding to hsst 4 (K_D = 245 nM) comparable to β-tetrapeptide **4** (K_D = 83 nM), while the epimer *epi*-**5a** has a greatly reduced affinity ($K_D > 5$ μM).

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[5]) The binding affinities were determined by a radioligand binding assay, based on the displacement of [^{125}I][Tyr^{10}]CST$_{14}$ [20] from the corresponding receptors expressed in CCL-39 cells [17]. Since all peptides investigated in this study showed the highest affinity for the hsst 4 receptor, only the binding constants to hsst 4 will be presented in the present paper.

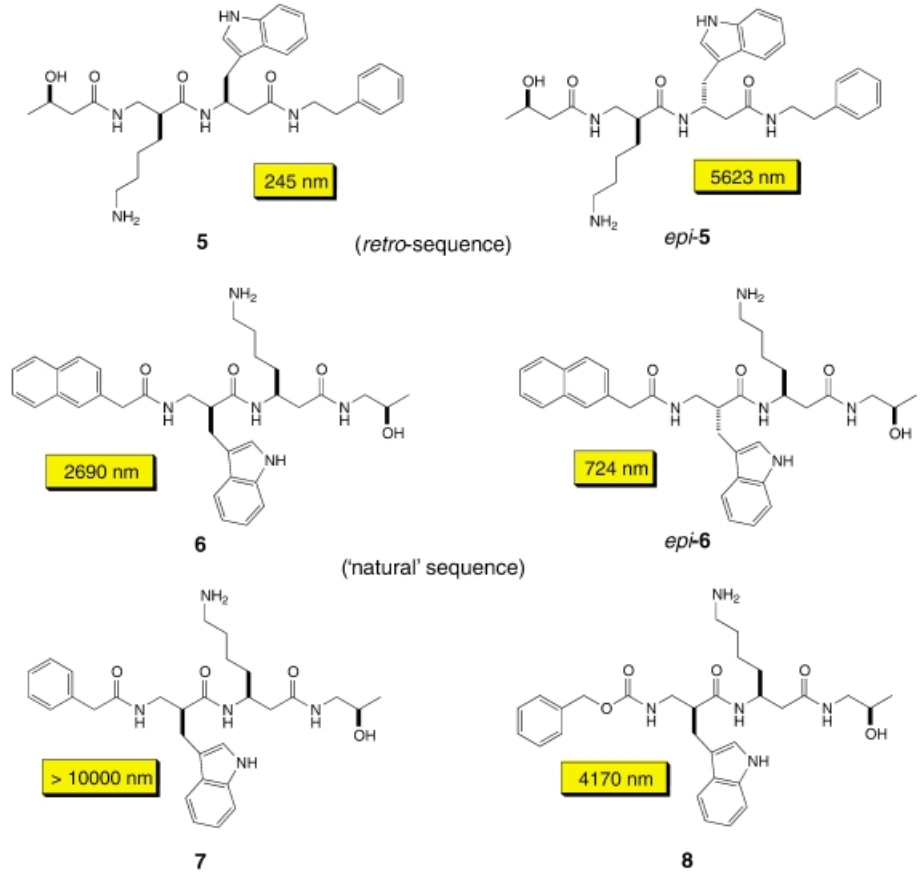

Fig. 3. *Somatostatin analogs* **5**–**8** *containing the* β^2*-HLys-*β^3*-HTrp and the* β^2*-HTrp-*β^3*-HLys sequences.* The molecular formulae of the dipeptide derivatives **5**–**8** are shown together with the corresponding binding affinities to the cloned hsst 4 receptor.

A second set of dipeptides **6**–**8** was then prepared, in which the side-chain positions of β-HLys and β-HTrp are interchanged (*Fig. 3*): they contain a β^2- rather than a β^3-HTrp and a β^3- rather than a β^2-HLys residue[6]). To keep the structures as similar as possible, aryl-substituted acetyl groups were attached to the N-terminus and 2-hydroxypropylamino groups to the C-terminus. The binding of these compounds to hsst 4 is clearly dependent on the nature of the hydrophobic group at the N-terminus: derivatives **6** and *epi*-**6** with a (naphthalen-2-yl)methyl group show a markedly stronger binding ($K_D = 2690$ nM and $K_D = 724$ nM) than the benzyl-substituted compound **7** ($K_D > 10$ μM) and the benzyloxy derivative **8** ($K_D = 4170$ nM). In contrast to the situation with the *retro*-β^2-HLys-β^3-HTrp series (**4**, **5**, and *epi*-**5**), inversion of the

[6]) β^2-HTrp has been obtained by alkylation of the *Evans* enolate of indole-3-propionic acid with bromoacetate and subsequent *Curtius* degradation.

configuration of the β^2-HTrp in the β^2-HTrp-β^3-HLys series (**6**, *epi*-**6**, **7**, and **8**) leads to the compound with the highest affinity.

We wondered whether it might be possible to replace a β^2- by an α-amino acid residue in the β-tetrapeptide of type **4** without losing hsst affinity[7]). Thus, mixed α/β^3-tetrapeptides **9**, *epi*-**9**, **10**, and *epi*-**10** were prepared – by solid-phase synthesis – and their affinities for the somatostatin receptors were investigated (*Fig. 4*)[8])[9])[10]).

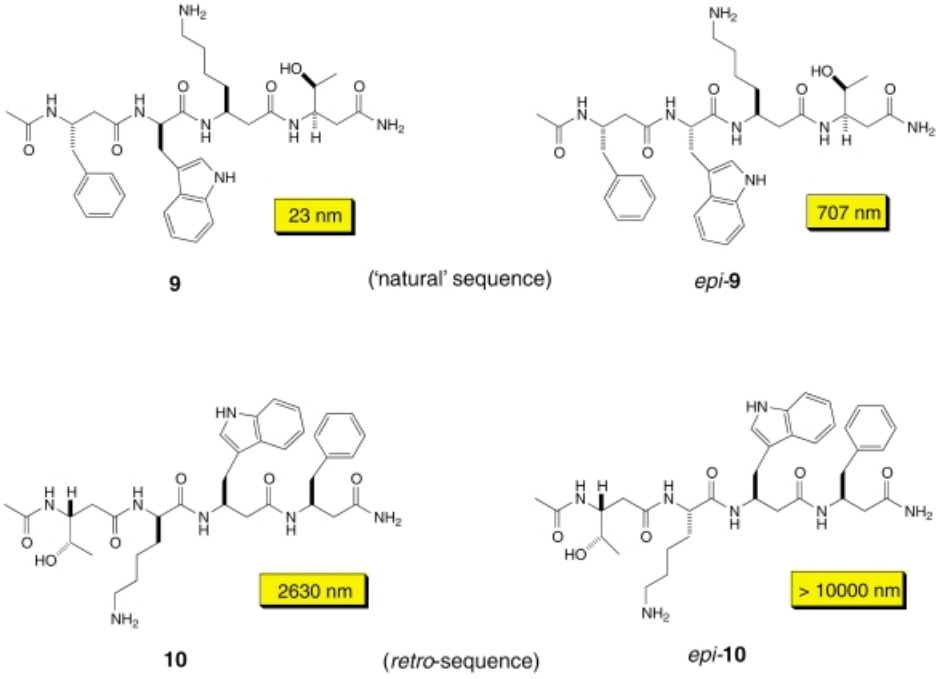

Fig. 4. *Somatostatin analogs* **9** *and* **10** *containing an α- and three β³-amino acids.* The molecular formulae of the tetrapeptides **9** and **10** are shown together with the corresponding binding affinities to the cloned hsst 4 receptor.

Surprisingly, two of these peptides had high affinity for the somatostatin hsst 4 receptor. Peptide **9** showed a 23 nM binding, *i.e.*, even higher than the all-β-tetrapeptide **4**, and is one of the most tightly binding so-called ligands for this receptor! Configurational inversion at the Trp residue (*cf. epi*-**9**) leads to a decrease of affinity ($K_D = 707$ nM), whereas reversing the sequence (*cf.* **10** ($K_D = 2630$ nM) and *epi*-**10** ($K_D > 10000$ nM)) causes a drastic drop of affinity. The large difference in affinity of

[7]) Indeed, a simple molecular-mechanics conformational search of the α/β^3-dipeptide Ac-(*R*)-Ala-(*S*)-β^3-HAla-NMe on the basis of the MMFF 94 force field predicts that nine- and eleven-membered H-bonded rings are among the lowest-energy conformers.

[8]) Since *Abderhalden* [21], the incorporation of β-amino acids into α-peptides was tested as a means of increasing proteolytic stability and of modifying structural and pharmacological properties [22].

[9]) Recently, *Gellman* and co-workers [23] have used cyclic β-amino acid residues to construct an otherwise α-peptidic haripin turn.

[10]) We have previously shown that the CD spectrum indicating the presence of a 3_{14}-helical structure of a β^3-hexapeptide changes drastically upon incorporation of a central Ala or Aib residue [2b].

these molecules indicates a complex relationship between the sequence and relative configuration of the side chains.

Since proteolytic stability is a prerequisite for possible pharmacological applications, we have tested the new peptides *in vitro* with several peptidases: pronase, proteinase K, carboxypeptidase A, chymotrypsin and trypsin[11]). All four compounds **9**, *epi*-**9**, **10**, and *epi*-**10** are stable against these peptide-cleaving enzymes for at least 48 h.

In conclusion, the study has shown that functional analogs of somatostatin, an α-tetradecapeptide, can be made from simple β^2-HLys-β^3-HTrp or β^2-HTrp-β^3-HLys dipeptides, properly functionalized at the N- and C-termini. Furthermore, it was demonstrated that the β^2-amino acid in the formerly reported β-tetrapeptide **4** can be successfully replaced by an α-amino acid. This modification does not seem to affect the proteolytic stability of the resulting compounds. Also, α-amino acids are, of course, much more readily available than β^2-amino acids, which, hitherto, are cumbersome to prepare. Thus, we expect that the findings reported here will be seminal for the development of peptidomimetics.

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