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DESIGN, SYNTHESIS AND BINDING AFFINITIES OF NOVEL NON-PEPTIDE MIMICS OF SOMATOSTATIN/SANDOSTATIN®

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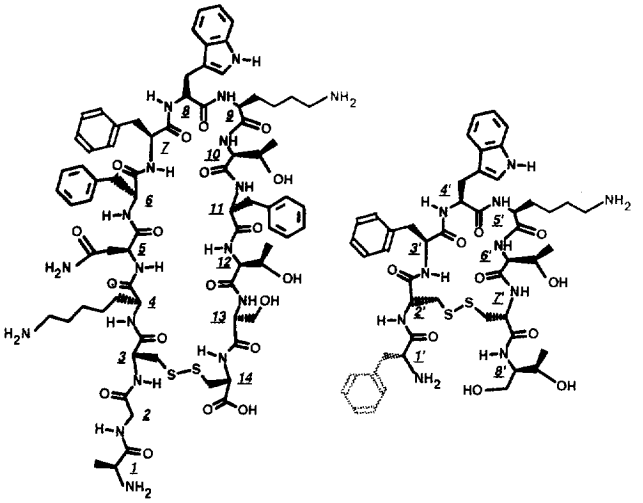
Abstract: Based on molecular modelling studies of Sandostatin®, sugar-based derivatives **I-VI** were prepared as potential non-peptide mimics of somatostatin/Sandostatin®. These compounds displaced 3-[^{125}I]-Tyr^{11}-SRIF-14 from the somatostatin receptor on membranes of rat cerebral cortex with IC_{50} values between 10 and 15 µM.

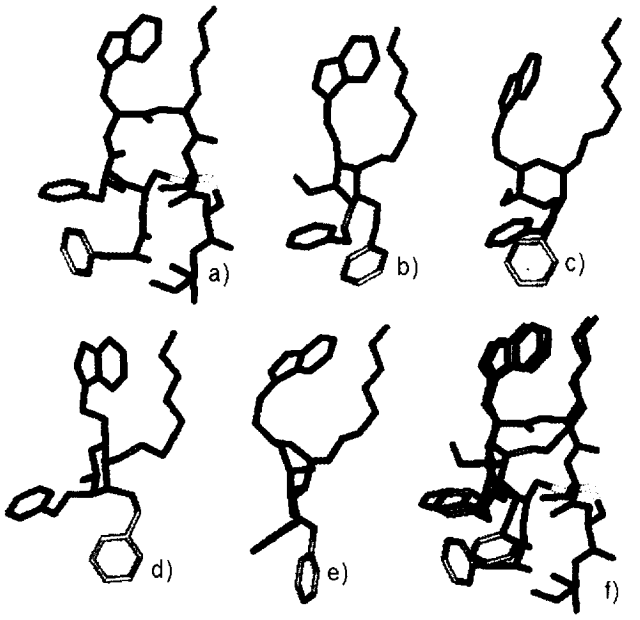
Introduction. Somatostatin-14 (SRIF-14, Scheme 1) is a cyclic tetradecapeptide which serves not only to regulate the release of growth hormone or other pituitary hormones, but also plays a role in neuronal transmission.[1] During the last few years, five SRIF receptor subtypes have been identified.[2] The possible therapeutic applications[3] of SRIF-14 are limited by its rapid proteolytic degradation resulting in poor oral bioavailibility and low selectivity. In order to circumvent these problems, several cyclic-peptides[4] and non-peptide mimics[5] of SRIF-14 have been prepared. One of them, Sandostatin® (Scheme 1) which has a longer half-life, a greater potency and a higher selectivity of action than native SRIF-14 is used routinely in the treatment of acromegaly, carcinomas and gastroenteropancreatic tumors[6]. Extensive investigations of the structure-activity relationships of the SRIF-14 peptide showed that the tetrapeptide sequence Phe^7-D-Trp^8-Lys^9-Thr^{10} is the most relevant for biological activity.[4a,7] From NMR studies, the peptide c(Pro-Phe-D-Trp-Lys-Thr-Phe) adopts a β II' turn about Trp^8-Lys^9 and a β VI turn about Phe^{11}-Pro^6.[8] This communication reports the design, synthesis and binding affinities of sugar-based derivatives **I-VI** (Schemes 3 and 4) which are novel non-peptide mimics of Sandostatin®.

Molecular modelling studies. [9] The ^{1}H-NMR study[9a] of Sandostatin® was carried out in CD_3OH, at 273K, providing experimental data for the conformational analysis of this peptide. About 70 interproton distances and 10 angular constraints were obtained by measurement of n.O.e intensities and $^3J_{HH}$ couplings. The temperature dependence of the NH proton chemical shifts were also investigated, as an indication of existing CO...HN hydrogen bonds. These NMR constraints were then used in molecular dynamics simulations[9b]. The root mean square deviation (rmsd) obtained for the α carbon β turn skeleton was 0.5Å. Our studies indicated that residues Phe$^{3'}$-D-Trp$^{4'}$-Lys$^{5'}$-Thr$^{6'}$ (Scheme 1), were in a remarkably stable conformation, whereas the remaining

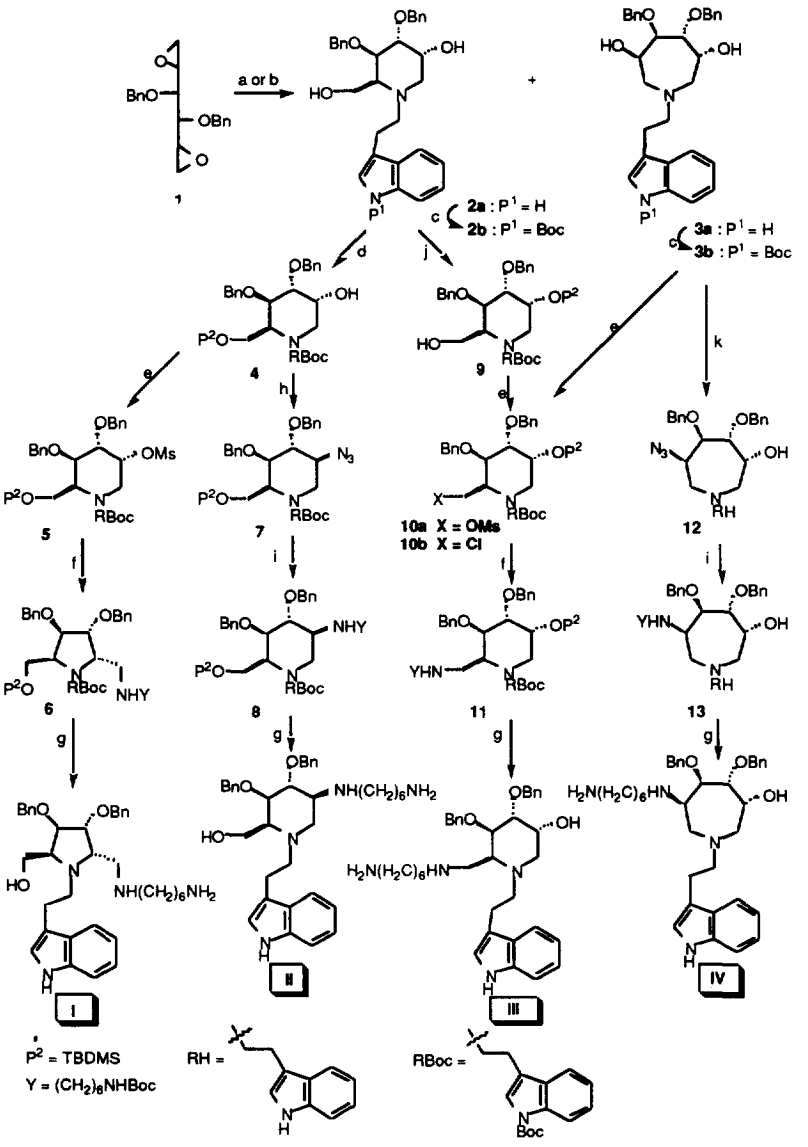


somatostatin (SRIF-14) **Sandostatin®**

Scheme 1: Chemical structures of SRIF-14 and Sandostatin®



Scheme 2: 3D structures of low-energy conformations of Sandostatin® (a), I (b), II (c), III (d), IV (e), and the superimposition of compound I and Sandostatin® (f)



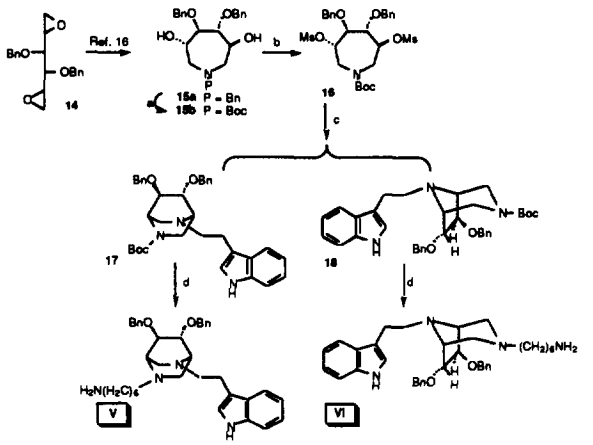
Scheme 3[18] : a) Tryptamine, $CHCl_3$, reflux, 50 and 45% for **2a** and **3a**, respectively b) Tryptamine, $HClO_4$, H_2O, 26 and 64% for **2a** and **3a**, respectively c) Boc_2O, NaOH, Bu_4NHSO_4, CH_2Cl_2, 100%. d) TBDMSCl, DMF, 71% from **2a**. e) MsCl, Et_3N, CH_2Cl_2 f) $H_2N(CH_2)_6NHBoc$, EtOH, 20°C, 93% for **4** --> **6**, 84% for **9** --> **11**; 60°C, 60% for **3b** --> **10b** g) HCl, AcOEt, 85, 55, 85, and 81 from **6**, **7**, **11** and **13**, respectively h) Ph_3P, DEAD, HN_3, THF, 0°C, 61%. i) H_2, Pd/C, EtOH, then $Br(CH_2)_6NHBoc$, $^iPr_2NEt$, EtOH reflux. j) *i*-TBDMSCl, DMF, 20°C, *ii*-AcOH, H_2O, THF, 60% from **2b**. k) Ph_3P, DEAD, HN_3, THF, mixture of **12** and of the corresponding azidomethyl-piperidine.

peptide sequence displayed considerable conformational flexibility. One of the most stable conformations is shown in Scheme 2. The calculated lowest-energy conformation is in good agreement with the recently described X-ray crystal structure. [10] Starting from this structural model and non-peptide mimics of somatostatin previously described[5], we designed compounds **I-IV** (Scheme 3) as potential non-peptide mimics of Sandostatin®. In compounds **I-IV** the N-alkylamino, N-alkyl-3-indolyl and the two alkylphenyl chains are expected to be in an adequate spatial disposition[11], and to retain some of the electronic features of Sandostatin®. These side chains could be superimposed onto the $Lys^{5'}$, D-$Trp^{4'}$, and $Phe^{3'}$moieties of Sandostatin® respectively (Scheme 2).

Synthesis of compounds I-IV. Compounds **I-IV** were prepared from the 1,2:5,6-di-anhydro-3,4-di-*O*-benzyl-D-mannitol **1** (Scheme 3). Aminoheterocyclization of **1** with tryptamine, according to the method previously reported[12] afforded the key intermediates **2a** and **3a**. *N*-Boc protection of the indole moiety of **2a** gave **2b**, as a common precursor of target molecules **I**, **II** and **III**. Selective silylation of the primary hydroxyl group of **2b**, and mesylation of the secondary hydroxyl afforded **5**, which was then subjected to nucleophilic displacement by *N*-Boc-1,6-hexyldiamine to give the pyrrolidine **6**, *via* an aziridinium salt. [13] After cleavage of both silylether and carbamates of **6**, the pyrrolidine-type scaffold **I** was isolated in 28% overall yield from **1**. Furthermore, the secondary hydroxyl group of **4** could be transformed into an azido group, with inversion of configuration, under Mitsunobu conditions in the presence of hydrazoic acid to give **7** (61% yield). [13] Catalytic reduction of the azido group into the amine followed by alkylation with 6-bromo-*N*-Boc-hexylamine, and removal of silylether and carbamates groups yielded the piperidine-type scaffold **II** (12% overall yield from **1**). The preparation of **III** from **2b** requires the transformation of the primary alcohol into a mesylate followed by nucleophilic displacement with *N*-Boc-1,6-hexyldiamine. For this transformation, better yields were obtained through silylation of the two hydroxyl groups of **2b**, followed by selective hydrolysis of the primary silylether and subsequent mesylation. In this manner, the piperidine-type scaffold **III** was isolated in 22% overall yield from **1**. [14] The azepane-type compound **IV** was obtained from the C_2-symmetric azepane **3a**. Treatment of **3a** under Mitsunobu conditions in the presence of hydrazoic acid afforded a mixture of the azidoazepane **12** and of the corresponding azidomethyl-piperidine, *via* an aziridinium intermediate. [15] Catalytic reduction of the azido group into the amine, and separation of the undesired aminomethyl-piperidine, followed by alkylation with 6-bromo-*N*-Boc-hexylamine and acidic cleavage of the Boc-protecting group, provided the azepane-type scaffold **IV** in 15% overall yield from **1**.

Synthesis of compounds V-VI. In a continuing effort to design structurally divergent classes of compounds acting on the somatostatin receptor, we also envisaged a new class of non-peptide mimics in which the scaffold unit is substituted by a rigid bicyclic system such as the 2,5-diazabicyclo[2.2.2]octane backbone (compounds **V** and **VI**, Scheme 4). The bicyclic derivatives **V** and **VI** were prepared starting from the C_2-symmetric azepane **15a**, which was easily obtained by *N*-heterocyclization of the 1,2:5,6-dianhydro-3,4-di-*O*-benzyl-L-iditol **14** with benzylamine

(Scheme 4). [16] Selective hydrogenolysis of the benzyl-nitrogen bond in presence of di-*tert*-butyl dicarbonate, followed by mesylation led to **16** (95% yield from **15a**). Treatment of **16** with an excess of tryptamine in ethanol gave a mixture of **17** and **18**, which were isolated in 60% and 15% yield respectively. [17] Finally, acidic cleavage of the Boc protecting group, followed by alkylation with 6-bromo-*N*-Boc-hexylamine and subsequent acidic cleavage of the carbamate afforded the bicyclic derivatives V or VI (33 and 8% overall yield respectively from **14**).



Scheme 4[18] : a) H_2, $Pd(OH)_2/C$, Boc_2O. b) MsCl, Et_3N, CH_2Cl_2, 95% from **15a**. c) Tryptamine, EtOH reflux, 60 and 15% for **17** and **18**, respectively d) *i)* HCl, AcOEt, 100%, *ii)* $Br(CH_2)_4NHBoc$, $^iPr_2NEt$, EtOH reflux, *iii)* HCl, AcOEt, 88%.

Binding. [19] Binding assays have shown a weak affinity of compounds I-VI for somatostatin receptor on membranes of rat cerebral cortex (3-[^{125}I]-Tyr^{11}-SRIF-14, IC_{50} = 14, 11, 12, 10, 15 and 12µM respectively *versus* 0.0002µM for the SRIF-14 itself).

Conclusion. The sugar-based derivatives I-VI represent novel scaffolds targeted towards the somatostatin receptor. They displayed moderate affinities to the SRIF-14 receptors (IC_{50} ~ 12 µM), regardless of the size (five to seven member rings) or the rigidity of the nitrogen heterocycle-based scaffolds used. These results show that the orientations of the various side chains are quite similar whatever the scaffold used. Taken together, our results, and those concerning other sugar-based derivatives[5a-d] and more recently a benzodiazepinone derivative[5e] exhibiting affinities for the SRIF-14 receptor strongly support the validity of the concept of the utilization of non-peptide scaffolds in the synthesis of non-peptide molecules as peptide analogues.

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14. The piperidine **III** can be obtained in better yield from the azepane **3b** by treatment with mesylchloride to give **10b** (60% yield, for analogous ring contraction, see ref. 15), and subsequent nucleophilic substitution with *N*-Boc-1,6-hexyldiamine (86%).
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17. The formation of these bridged compounds can be interpreted as an intramolecular displacement of the mesylate by the cyclic nitrogen atom to give an aziridinium intermediate, the nucleophilic opening of which gives rise to the formation of two isomers with a piperidine or an azepane skeleton, followed by an intramolecular displacement of the remaining mesylate by the secondary amine, see ref 15.
18. All new compounds gave spectral data (^{1}H-NMR, ^{13}C-NMR, MS) in accord with the assigned structure, and satisfactory combustion analysis or accurate mass measurement.
19. Binding assays with the sugar derivative **I-VI** and SRIF-14 were performed as reported by Srikant et *al.* (Srikant, C. B., Patel, Y.C.; *Proc. Natl. Acad. Sci. USA*, **1981**, 78, 3930) with slight modifications. The crude synaptic membranes were obtained from cerebral cortex of adult male Sprague-Dawley rats (180-200g) and suspended in 50mM HEPES/KOH buffer at pH 7.5. The 200µL incubation mixture included HEPES buffer containing 10mg/mL bovine serum albumin, 5mM $MgCl_2$, 500U/mL aprotinin, 0.02µg/mL PMSF and 0.02µg/ml bacitracin, 0.1nM 3-[^{125}I]-Tyr^{11} SRIF-14 (Amersham) and 25µg membranes. To measure nonspecific binding, 1µM unlabelled SRIF-14 was added to the above mixture. The incubation was maintained at 25°C for 60 minutes and stopped by vacuum filtration. Data obtained with different concentrations of sugar derivatives and SRIF-14 were used to generate inhibition curves. The calculated IC_{50} values which are the mean of at least three determinations each with six concentrations of the test compound in triplicate.

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