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DESIGN, SYNTHESIS, AND BINDING AFFINITY OF A NONPEPTIDE MIMIC OF SOMATOSTATIN

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Abstract: The tetrasubstituted xylofuranose **4** was synthesised as a potential nonpeptide mimic of somatostatin, based on conformational analysis of the endogenous ligand and molecular modelling studies. It displaced the radioligand [^{125}I-Tyr^3]-octreotide with an IC_{50} of 23 μM.

Somatostatin (SRIF) **1** is both a hypothalamic hormone and a peptidergic neurotransmitter. It has a wide diversity of biological effects, almost all of them inhibitory in nature[1]. Somatostatin itself, a tetradecapeptide most likely having a β-sheet conformation stabilised by six transannular hydrogen bonds, is of little therapeutic value because of its short plasma half-life[2]. Extensive chemical derivation of SRIF, coupled with structure-activity studies, led to highly potent, shorter, and conformationally restricted cyclic analogues with increased duration of action. Typical examples of such metabolically stable somatostatin analogues are the hexapeptide **2**[3] and the marketed drug Sandostatin® (octreotide, SMS 201-995) **3**[4] (Fig. 1).

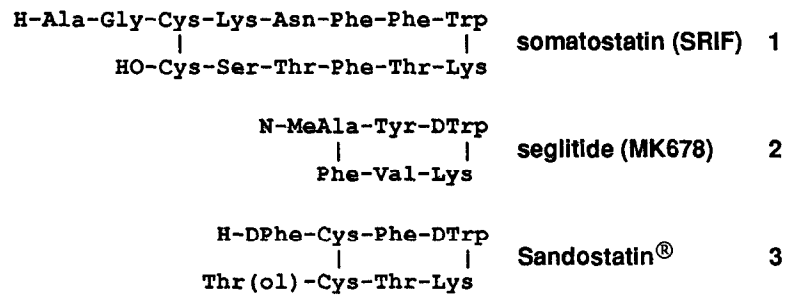


Figure 1

Although such analogues have proven their worth in therapy after parenteral application, they still leave much to be desired in terms of oral activity. Stimulated by recent reports of non-peptide ligands for various peptide receptors[5], we therefore initiated a program aimed at the discovery of somatostatin peptidomimetics. Although most of the successes in this field have been achieved through screening of natural products and/or collections of synthetic compounds, we decided on rational *de novo* design as our approach. Two main factors had to be taken into account: *a)* ^{1}H-NMR studies of SRIF and analogues suggest that in all biologically active analogues, the sequence Phe^7-Trp^8-Lys^9-Thr^{10} adopts a β-turn conformation[6] in which the sidechains of Trp and Lys are stacked and protrude as extensions of the arms of the β-sheet, and *b)* extensive structure-activity relationships suggest that the pharmacophore of SRIF comprises at least the contiguous Phe-Trp-Lys of the β-turn *plus* an additional lipophilic element such as Phe^6 and Phe^{11} of SRIF, the Phe in **2**, or the DPhe and cysteine bridge of **3**, respectively[3,4,7].

Hirschmann and coworkers recently described a SRIF-mimic based on a glucose skeleton which was reported[8] to bind to somatostatin receptors on AtT-20 cells with an IC_{50} of 1.3 μM. Upon modelling the conformation of this compound[9] (Fig 2b) and superposing it on the 5-12 fragment of a previously modelled conformation of SRIF[10] (Fig 2d), it seemed to us that the two benzyloxy groups most likely fit into the pocket occupied by Phe^6 and Phe^{11}. To our minds, a molecule capable of simultaneously interacting with the binding pocket of Phe^7 *and* of Phe^6/Phe^{11} of SRIF might have increased affinity. We accordingly designed the tetrasubstituted xylose derivative **4** (Fig 2c), in which the spatial relationship of the C-1 and C-5 substituents resembles that of the Lys and Trp sidechains of SRIF and the two benzyloxy moieties those of Phe^7 and Phe^{11} (Fig 2e).

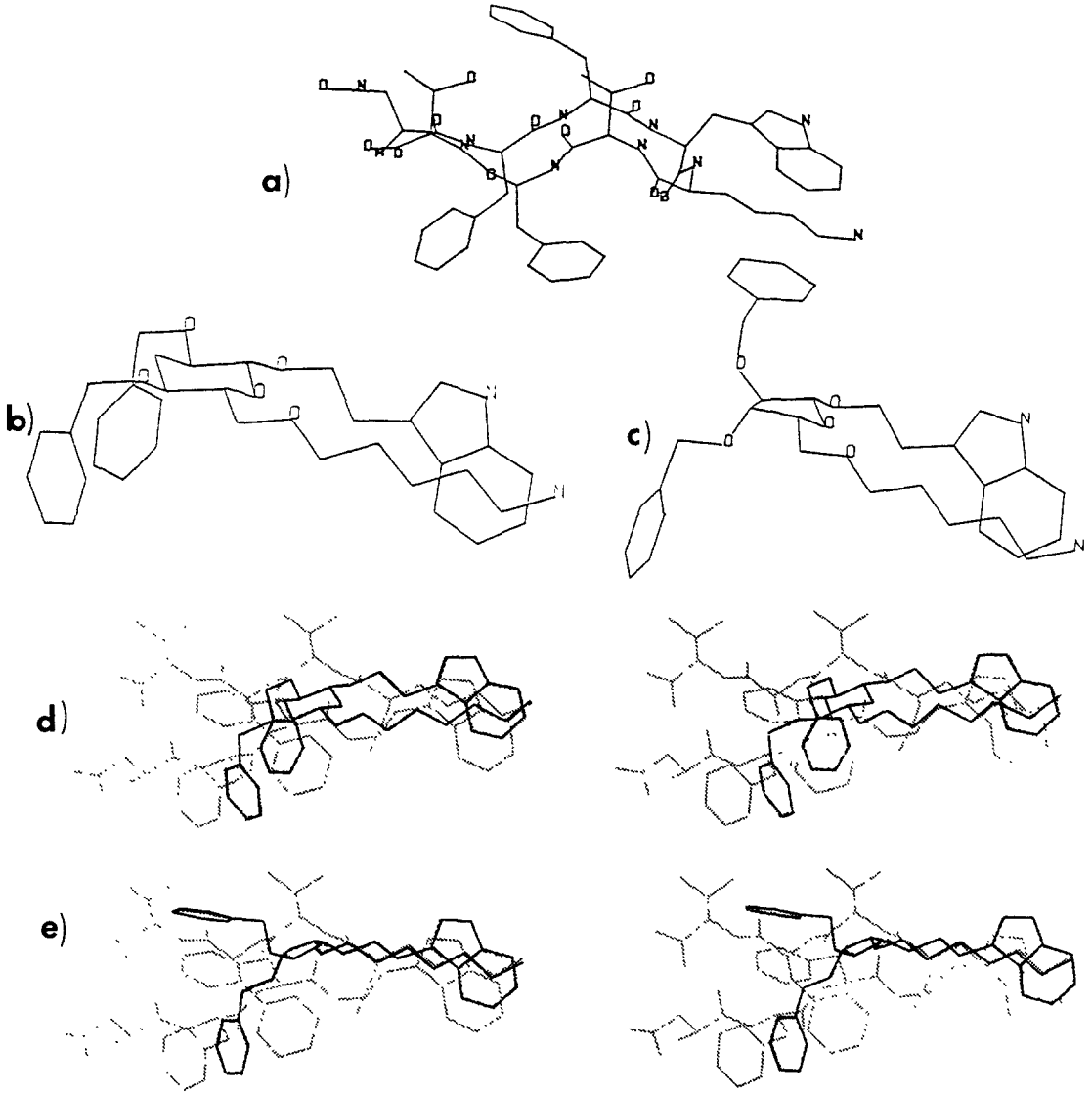
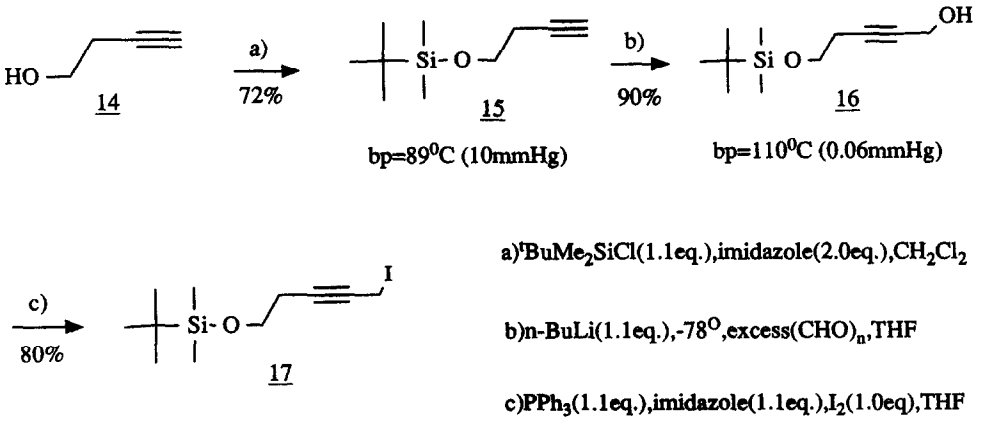
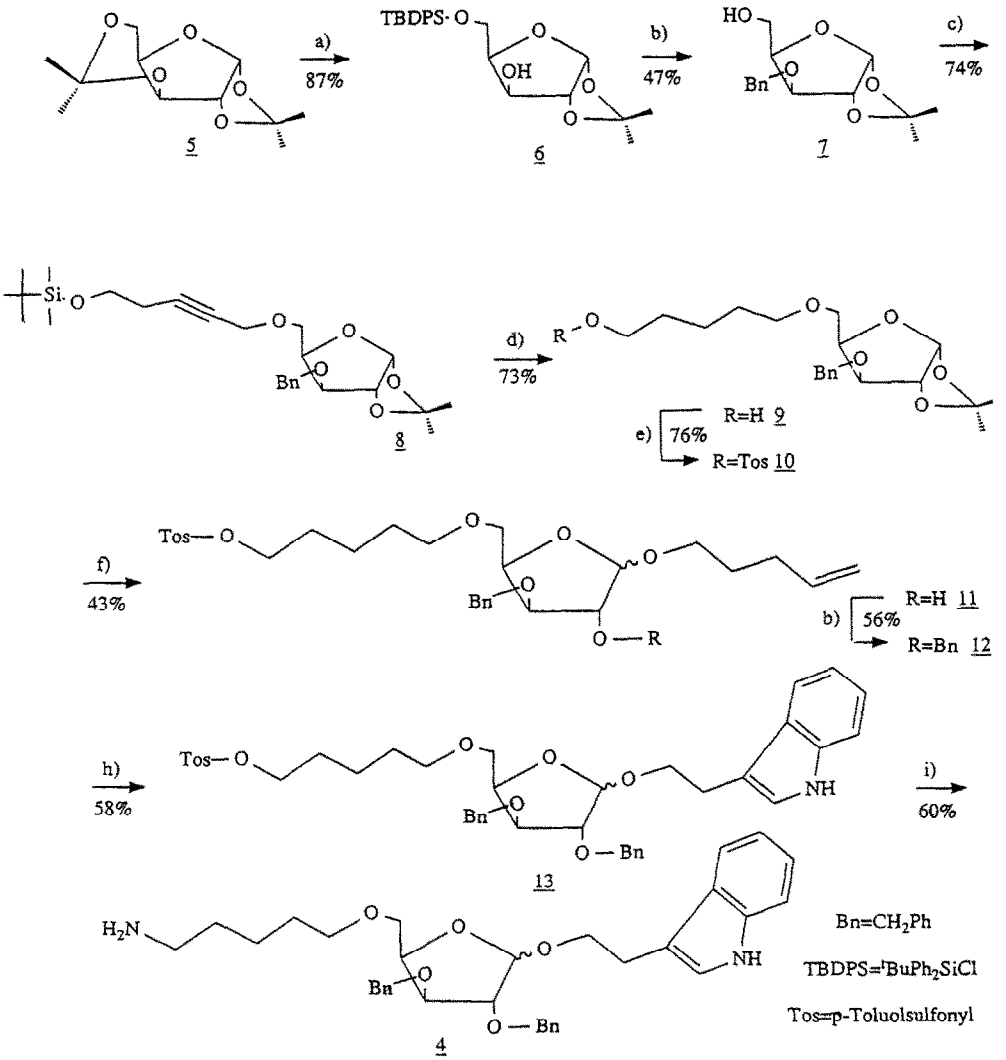


Figure 2. *a)* conformation of the fragment 5-12 of SRIF; *b)* conformation of the deoxyglucose mimetic; *c)* conformation of the xylose mimetic **4**; *d)* stereo view of the superposition of *b)* and SRIF; *e)* stereo view of the superposition of **4** and SRIF.

The synthesis of the desired 2-indolylethyl-5-O-(5-aminopentyl)-2,3-di-O-benzyl-D-xylofuranoside **4** is depicted in Scheme 2. Starting from the commercially available di-acetonide **5**, the alcohol **6** was obtained in two steps by a selective deprotection / selective monoprotection sequence[11]. Benzylation followed by fluoride cleavage of the silyl protecting group afforded the key intermediate **7**. In the next step the masked aminopentyl chain was introduced via the propargylic iodide **17** synthesised efficiently in three steps (57% overall yield) as shown in Scheme 1. The triple bond in **8** was selectively reduced in the presence of the benzyl ether with H_2 and $Pd/BaSO_4$ as catalyst and the resulting crude product was deprotected to give the alcohol **9**. The surprising hydrogenation of the acetylene **8** to the saturated compound with the above-mentioned poisoned catalyst can be explained by a change in the coordination of Pd due to the presence of the oxygen atoms of the sugar[12]. Protection / activation of **9** with tosyl-Cl afforded the corresponding tosylate **10** which was subsequently treated with pentenol in the presence of camphorsulphonic acid for 1h. The resulting hydroxypentenyl xyloside **11** was transformed intc the benzyl ether **12** which, thanks to the chemospecific activation of its anomeric centre by the pent-4-enyl group[13], underwent readily in situ glycoside exchange yielding **13** upon treatment first with Br_2 (1 eq.) and then with indol-3-yl ethanol. The above glycosidation procedure takes place under neutral, mild conditions compatible with the oxidisable and acid-sensitive alcohol used. The remainder of the synthesis was carried out via azide displacement of the tosylate followed by selective hydrogenation of the azide functionality with $Pd/CaCO_3$. The desired peptidomimetic **4** was thus obtained as a 40/60 α/β anomeric mixture[14].



Scheme 1



a)0.1N HCl,dioxane ; $^tBuPh_2SiCl$(1.1eq.),imidazole(2eq.),CH_2Cl_2 b)NaH(1.05eq.),PH_2CH_2Cl (1.1eq.),THF ; TBAF(1.1eq.),THF c)NaH(1.05eq.),17(1.2eq.),THF d)H_2,Pd/$BaSO_4$,MeOH, quinoline(1eq.) ; TBAF(1.1eq.),THF e)TosCl(1.5eq.),pyridine(2eq.),CH_2Cl_2 f)CSA(1eq.), 4-pentenol(5eq.),toluene(1h,80°C) h)Br_2(1eq.),CH_2Cl_2,0° after 10min Na_2CO_3(5eq.), indolyl-3-ethanol(5eq.),TBABr(1eq.),3h,rt i)NaN_3,MeOH/H_2O(1:1),Δ ; H_2,Pd/$CaCO_3$,MeOH

Scheme 2

Characterisation of the biological activity of **4** was performed by means of radioligand binding studies on rat cortex membranes using ^{125}I-Tyr^3-octreotide as ligand as previously described[15]. Incubations were performed in triplicate and experiments replicated at least once. Compound **4** displaced the radioligand from its receptor with an IC_{50} of 23 μM (pK_i = 4.73 ± 0.04, n = 4). Displacement was complete at 10^{-4} M and the form of the binding curve was consistent with competition for a single set of binding sites (Fig. 3). When measured under the same assay conditions, the deoxyglucose mimetic[8] had an IC_{50} of 16 μM (Fig. 3). The reason for the 10-fold greater potency reported for the latter compound on AtT-20 cells is presently unclear.

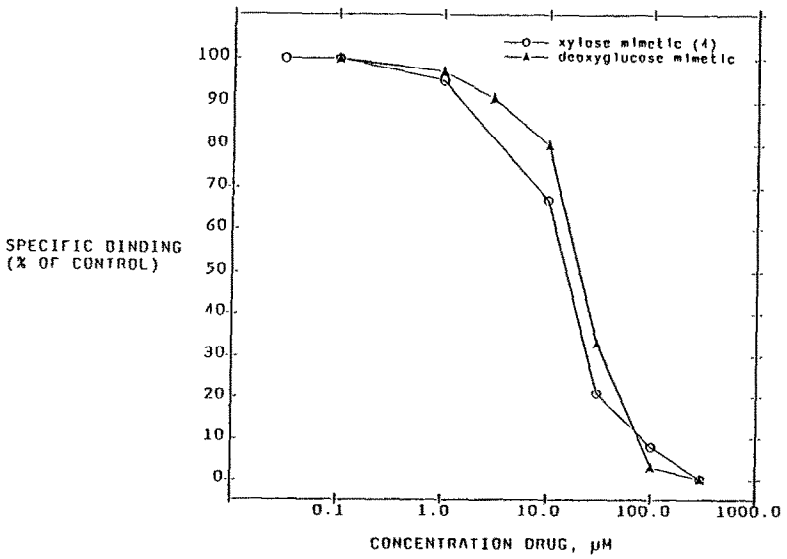


Figure 3

Discussion

The free energy change upon binding of a drug with its receptor contains both enthalpic and entropic terms ($\Delta G = \Delta H - T\Delta S$) and is related to the equilibrium binding constant by the equation $\Delta G = -1.4 \log K$; thus the free energy of binding for Sandostatin® **3** (IC_{50} = 0.46 nM) is about 13 kcal/mole. The experimentally observed affinity of **4** corresponds to a free energy of about 6.5 kcal/mole instead of the 12.5 kcal/mole which can be empirically calculated[16] for the sum of the contributions of the four substituents on the xylofuranose. This difference can be attributed to the change in entropy needed to attain the "stacking" association between indolyl and aminoalkyl substituents thought to be necessary for receptor binding. In arriving at our figure of 12.5 kcal/ mole for **4**, we used Andrews' recommended figure[16] of 0.7 kcal/mol loss in binding energy for each freely rotatable bond; others have suggested that this loss can be up to 1.5 kcal/mole per bond[17]. Since compound **4** has a total of 17 freely rotatable bonds, only a small increment in this entropic loss factor would rapidly reduce the predicted binding energy to that observed. Compounds having a central sugar moiety and long, flexible substituents are thus probably simply too floppy for high-affinity binding to somatostatin receptors.

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9. The molecular-modelling program packet SYBYL from Evans & Sutherland, Tripos Assoc. Inc., St.Louis, MO, was used. A geometry optimisation was performed with the force-field program MAXIMIN 2 (Tripos force field).
10. Modelled in the early 80's as an extended twisted β-sheet using a locally modified version of ECEPP (Momany, F. et al., *J. Phys. Chem.* **1975**, *79*, 2361; QCPE No. 286), incorporating H-bond and cyclisation constraints (Petcher, T.J., unpublished). The version we used incorporated an artificially reduced N-amide – N-amide 1-4 repulsive term (Momany, F. personal communication) to overcome problems in the modelling of β-turns seen in the then standard version of the program. Remodelling in SYBYL 5.4 using the Kollman all-atom force field yields an essentially similar conformation.
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