Modulation of Receptor and Receptor Subtype Affinities Using Diastereomeric and Enantiomeric Monosaccharide Scaffolds as a Means to Structural and Biological Diversity. A New Route to Ether Synthesis

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We show that carbohydrates constitute an attractive source of readily available, stereochemically defined scaffolds for the facile attachment of side chains contained in genetically encoded and other amino acids. β-D- and β-L-glucose, L-mannose, and the 6-deoxy-6-N-analogue of β-D-glucose have been employed to synthesize peptidomimetics that bind the SRIF receptors on AtT-20 mouse pituitary cells, five cloned human receptor subtypes (hSSTRs), and the NK-1 receptor. The affinity profile of various sugar-based ligands at the hSSTRs is compared with that of SRIF. Compound **19** bound hSSTR4 with a K_i of 100 nM. Subtle structural changes affect affinities. Evidence is presented that suggests that one compound (**8**) binds both the AtT-20 cell receptors and the five hSSTRs via a unique mode. The SARs of the glycosides at SRIF receptors differ markedly from those at the NK-1 receptor. For example a 4-benzyl substituent is important for SRIF receptor binding, but the 4-desbenzyl analogue **27** was highly potent (IC_{50} of 27 nM) at the NK-1 receptor. A new, nonbasic method for the synthesis of base-sensitive ethers from primary and secondary alcohols is also described.

Introduction

The discovery of peptidomimetics[1] has been the goal of research in many laboratories for a number of years. Naturally occurring mimetics include the agonist morphine known since antiquity. The latter was recognized as a peptidomimetic only after the discovery of the enkephalins in 1975.[2] Statine, a component of such natural products as pepstatin and the didemnins, was also slow to be recognized as a peptidomimetic. That this simple secondary alcohol actually mimics a dipeptide was reported independently by Marciniszyn,[3] Marshall,[4] and Rich.[5] Rich and his collaborators and others made statine the focal point in aspartate protease inhibitor design. The screening of diverse sources of natural products and of sample collections also provided mimetics of peptide hormones and neurotransmitters. These screening leads, typified by asperlicin,[6] reveal no obvious structural resemblance to the peptidal ligands.

The design of peptidomimetics has included pseudopeptides, retro-inverso peptides, and mimics of β-turns.[7] Our search for mimetics of biologically active peptides generated structures that lack the amide backbone but retain peptidal side chains required for receptor binding.[12] The tactic of replacing the amide backbone by a nonpeptidal scaffold devoid of H-bonding capability with the receptor can succeed only if the hydrogen bonds between the amide backbone of the peptide ligand and that of its receptor are not required for biological activity. That such interaction may not be needed was first suggested by Urry and Walter during their investigations of oxytocin analogues in 1971.[8] Four years later, the discovery[2] that morphine binds the enkephalin receptors supported the Urry/Walter speculation.

The use of a novel scaffold for peptidomimetic design was first proposed by Farmer[9] in 1980. He suggested, but did not explore, the use of cyclohexane. In 1986 Bélanger and Dufresne,[10] in a publication that was widely overlooked, successfully implemented the concept by attaching appropriate side chains to the bicyclo[2.2.2]-octane scaffold. Four years later, also unaware of this work, Olson et al.[11] and our laboratory[12] described the successful application of the same underlying concept, using cyclohexane and β-D-glucose, respectively, as the amide surrogates. Unlike earlier scaffolds designed to mimic β-turns,[1,7] our strategy, like that of Bélanger[10] and Olson,[11] employed biological assays rather than physical measurements to validate the design. This paradigm increases the synthetic challenge because the side chains needed to bind the receptor must be incorporated into the ligand. Recently, Patchett, Smith, and their collaborators have reported a potent orally active growth hormone secretagogue, L-163,191,[13] a peptidomimetic of the GH-releasing peptide (GHRP-6),[14] employing the"privileged structure" concept, a phrase coined by Evans et al.[6b]

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Our choice of a sugar scaffold was based on molecular modeling[12a] which suggested that appropriately substituted positions 2, 1, and 6 of β-D-glucose may mimic the *i*, *i* + 1, and *i* + 2 positions, respectively, of the cyclic hexapeptide *c*-(Pro-Phe-D-Trp-Lys-Thr-Phe) (L-363,301, **1**, Figure 1),[15] a potent somatostatin (SRIF) agonist. In addition, the potency-enhancing Phe between Thr and Pro of this peptide overlaps the benzyl substituent in the 4-position of the sugars. Importantly, **3** and **4** bound the mixture of SRIF receptors on AtT-20 cells with affinities of about 10 μM. Compound **4** was also tested in a functional assay and found to act as a partial agonist.[12b]

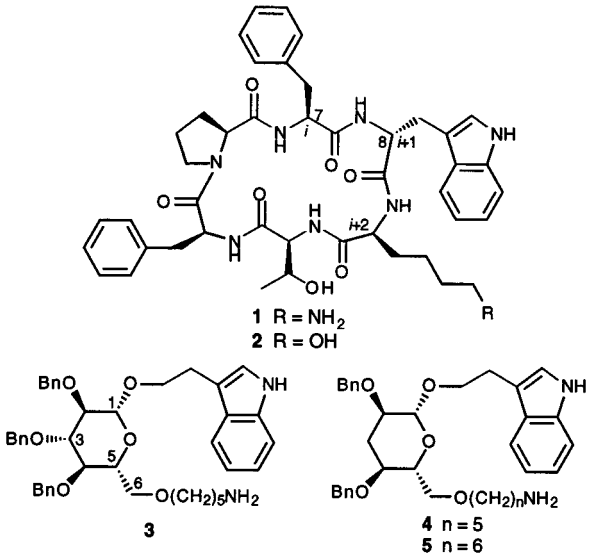


Figure 1. Structures of *c*-hexapeptides **1** and **2** and sugar-based mimetics **3**, **4**, and **5**.

An important advantage of the use of a monosaccharide scaffold is the availability of a large repertory of readily available isomeric sugars with well-defined stereochemistry, facilitating diversity in three-dimensional space. Further, appending side chains onto sugar scaffolds can utilize well-precedented etherification reactions, whereas hydrocarbon scaffolds, such as cyclohexane or benzene, require the use of more difficult carbon−carbon bond forming methods for introduction of the requisite side chains. We now report inter alia that diastereomeric, enantiomeric, and other sugar congeners can be employed to modulate receptor and receptor subtype affinities and that unexpectedly diverse sugar scaffolds can be used in a library mode to gain information about the bioactive conformation of peptides.

Binding Affinities at AtT-20 Cell Receptors

Use of Nonpeptide Peptidomimetics To Elucidate the Bioactive Conformation of L-363,301.[12d] Somatostatin, a cyclic tetradecapeptide isolated, characterized and synthesized at the Salk Institute by Guillemin, Rivier, Vale, and their collaborators,[16] has attracted attention for its therapeutic potential.[17,18] Veber and his collaborators[19] demonstrated that Phe[7] of somatostatin has an axial disposition. Similarly, several bicyclic, conformationally constrained peptidal SRIF analogues[19a] containing an axial Phe[7] were found to be potent ligands. In contrast, when we began to explore β-D-glucose as a scaffold, it had not been possible to establish the conformation of Phe[7] of L-363,301 (**1**).[20] Having shown previously[12c] that the 2-benzyl group of **3** mimics Phe[7] of the cyclic hexapeptide **1**, we employed the mannose scaffold, with its axial C(2) hydroxyl, to gain information about the conformation of Phe[7] of the peptide **1**. The design of an appropriate mannose derivative required use of an L sugar (previously we had utilized only D sugars) to maintain the side chain in the 2-position in the same orientation as that of the Phe[7] of somatostatin. As a first step we had to demonstrate that the L-glucose-based enantiomer of **3** (**6**) can bind the SRIF receptors on AtT-20 cells. This proved to be the case since **6** bound the receptors on AtT-20 cells, although with somewhat lower affinity than **3** (Figures 4 and 10, below). Since both D- and L-glucosides have equatorial side chains, we had predicted this retention of activity. We then prepared the L-mannose derivative **7**. It was pleasing that mannoside **7** bound the SRIF receptor on AtT-20 cells with an affinity about twice that of **3** (Figure 4). Taken together, these results suggest that an axial substituent in the 2-position of the sugar enhances potency at SRIF receptors and that the Phe[7] in L-363,301 (**1**), the point of departure for our glycoside mimetics, is also axial (Figure 2).[21,22] *These results imply that the flow of information between peptide and peptidomimetic can be bidirectional.*[23] Finally, the work shows that the stereochemical diversity of readily available monosaccharides represents an important advantage of carbohydrate-based scaffolds over hydrocarbon scaffolds.[24,25]

Alternate Binding Mode of Monosaccharide 8 at the SRIF Receptor. We have previously shown[12c] that SARs in the SRIF-derived cyclic hexapeptides parallel those of our β-D-glycosides at the AtT-20 receptors. We now report[12d] one striking exception to this generalization. It was shown both at the Salk Institute and at Merck that an L- or D-Trp residue in position 8 of SRIF and of the *c*-hexapeptides (SRIF numbering) is required for activity. Therefore we had synthesized **8** as a negative control. Unexpectedly, this compound, in which a methoxy group replaced the Trp-mimicking side chain in **3**, proved to be one of our more potent ligands at AtT-20 cell membranes (IC_{50} = 5.9 μM for **8** vs 15.0 μM for **3**). We now suggest that **8** binds the SRIF receptors on AtT-20 cells in a manner different from our other active glycosides. The alternate binding mode, generated via rotation about the axis A_r (Figure 3) in which the 4-benzyloxy group replaces the indole ring, appeared attractive. Several experimental results support this hypothesis. First, the 4-deoxy desindole analogue **9** was found to be inactive.[12c] More significant experiments in support of the proposed alternate binding mode are described below.

Replacement of Phe[7] by His in cyclic hexapeptides and replacement of the 2-benzyl of **5** by the imidazole moiety enhanced potency by factors of 1.6 and 3.5, respectively.[12c] If **8** binds the SRIF receptors like the other sugars, then introduction of the imidazole residue in the 2-position should enhance activity. In fact analogue **10** (Figure 4) proved to be inactive. If, on the other hand, the alternate binding mode hypothesis is correct, one would expect the isomeric 3-imidazole analogue at least to

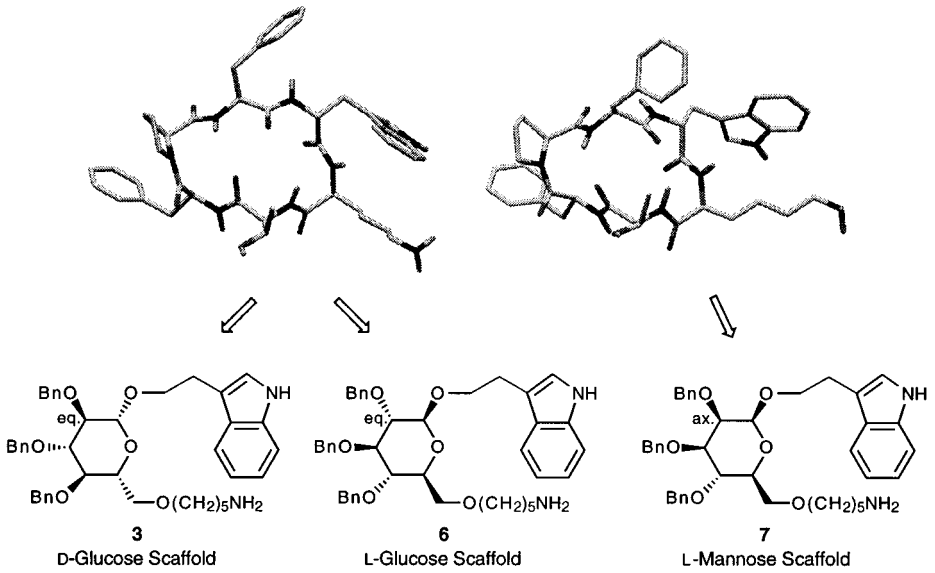


Figure 2. Correlation of the Phe[7] orientation in L-363,301 (**1**) and the C(2) benzyl of the glycosides.

retain comparable activity. This proved to be the case; **11** had an IC_{50} of 5.6 μM, identical with that of **8** (5.9 μM).

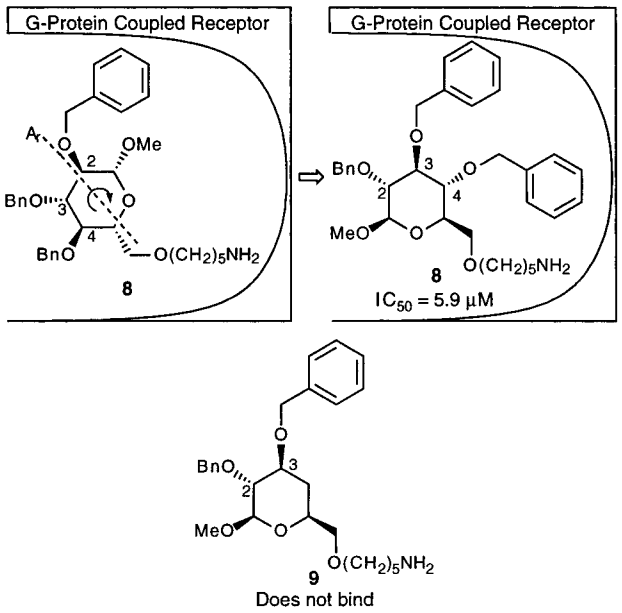


Figure 3. Alternate binding mode of **8** at the SRIF receptor.

The concept that structurally closely related compounds possessing similar biological properties can bind proteins differently is not new. Ringe and collaborators,[26] for example, showed convincingly via X-ray crystallography that chemically closely related inhibitors of the enzyme elastase nevertheless bind the protease differently. As Ringe has pointed out, the phenomenon is likely to be more common than is generally realized.[27] This is not surprising because the conformation of a complex of a ligand with an enzyme or receptor "will minimize the energy of the complex, but not necessarily that of its components".[7b]

G-protein-coupled receptors in the lipid bilayer such as the SRIF receptors are neither crystalline nor readily available. Therefore we have no X-ray data to support the alternate binding mode. We believe, however, that taken together the observed binding affinities of **9**, **10**, and **11** at AtT-20 cells are consistent with the alternate binding mode hypothesis. The orientation of the C(4) benzyl group relative to the Lys-mimicking side chain in the X-ray structure of our only crystalline intermediate (**12**) also supports the hypothesis (Figure 5), as does the difference in affinity pattern at hSSTRs of **8** compared with that of **3**, **4**, **6**, and **7** (see below). We do not propose that **3** and **8** bind exactly to the same sites. Nevertheless, that both compounds displace the same probe, [^{125}I-Tyr^{11}]SRIF, with micromolar affinities suggests that both are likely to target the same binding pocket which also binds SRIF.

Compound	AtT-20 cells IC_{50} (μM)
3	15.0
4	8.4
5	7.0
6	47.0
7	8.0
8	5.9
9	DNB[a]
10	DNB[a]
11	5.6

[a]DNB = Did Not Bind

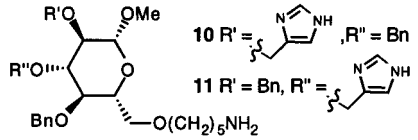


Figure 4. Binding affinities of sugar analogues at AtT-20 receptors.

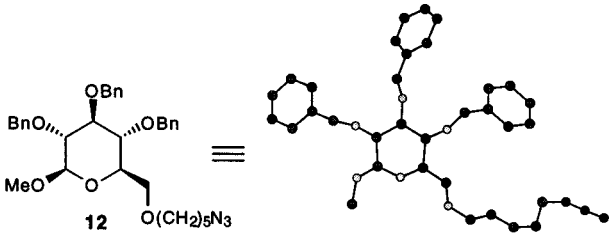
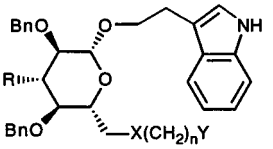


Figure 5. X-ray structure of **12**, the azido precursor of **8**.

Role of the Lys[9]-Mimicking Side Chain in SRIF Receptor Binding. We previously reported that isomers of **3** and **5** in which the lysine-mimicking side chain is linked via nitrogen rather than oxygen (i.e., **13** and **14**; Figure 6) have affinities for the SRIF receptor that were about the same as those of **3** and **5**.[12c] We

Compound	AtT-20 cells IC_{50} (μM)
13	14.0
14	5.1
15	DNB[a]
16	13.0

[a]DNB = Did Not Bind



13 R = OBn, n = 5, X = NH, Y = OH
14 R = H, n = 6, X = NH, Y = OH
15 R = OBn, n = 5, X = N(Ac), Y = OH
16 R = OBn, n = 5, X = NH, Y = NH_2

Figure 6. Binding affinities of sugar analogues at AtT-20 receptors.

considered the possibility that the primary hydroxyls of **13** and **14** replace the binding interactions provided by the primary amines of **3** and **5**. This interpretation became unattractive when it was found that the cyclic hexapeptide **2**, in which the ϵ-amino group of lysine is replaced by a hydroxyl, and the N-acylated sugar **15** are both inactive. These results suggest that it is the secondary amines of **13** and **14** that make possible the binding of these two compounds to the SRIF receptors. For cyclic hexapeptides such as L-363,301 (**1**), the lysine binding region is postulated[28] to be on transmembrane helix no. 3 (TM3) typified by Asp^{122} for hSSTR2. This raises the question whether the amines of the N-linked sugars (**13** and **14**) interact with the same counterion (e.g., Asp^{122}) of the receptor, as the primary amines of **3** and **5,** or whether the secondary amines **13** and **14** interact with another dibasic amino acid. To address this question we synthesized diamine **16**. Since **13** and **16** have identical affinities for the AtT-20 cells, we suggest that Asp^{122} is positioned so it can form a salt bridge either with the primary amines of **3** and **4** or with the secondary amines of **13** and **14** as depicted in Figure 7.

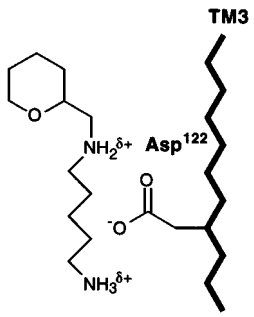
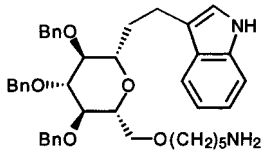


Figure 7. Formation of a hypothetical salt bridge between Asp^{122} on TM3 of hSSTR2 with either primary or secondary amines of the glycosides.

Unsolved Problem of the Indole-Mimicking Side Chain in SRIF Receptor Binding. Although we have achieved submicromolar affinities at certain human receptor subtypes with **16** and **19** (see below), we have not yet achieved the subnanomolar affinities displayed by SRIF and L-363,301. A combination of ^1H NMR and biological studies has established[19b] a direct relationship between potency of peptidal SRIF analogues and the observed shielding of the γ-methylene protons of Lys^9 by Trp^8. Thus D-Trp replacement enhances the potency of SRIF by an order of magnitude[29] and also generates a dramatic upfield shift in the γ-methylene protons.[19b] None of our sugars showed such a shielding effect, suggesting that the spatial relationship of the Trp- and Lys-mimicking side chains is not optimal. In the initial design of our sugars, typified by **3** and **4**, an ethylene group was inserted between the anomeric oxygen and the indole ring to prevent gramine-type fragmentation.[30] Hoping to enhance affinity, we subsequently undertook the synthesis of the deoxy analogue **17** (Figure 8), since this compound is not subject to such fragmentation. Unfortunately **17** (IC_{50} = 22 μM) did not improve the affinity at AtT-20 cells and also failed to display the desired chemical shift in the ^1H NMR. Therefore, optimization of the interactions of the side chains in positions 1 and 6 of the sugars remains an important objective for us.



(IC_{50} = 22 μM at AtT-20 receptors)

17

Figure 8. Structure of deoxy sugar analogue **17**.

Binding Affinities at Human SRIF Receptor Subtypes

In 1992 Bell[31] reported the cloning and expression of SRIF receptor subtypes; Reisine[32] used these subtypes to study the binding affinities of SRIF receptor ligands. Other laboratories have extended these investigations.[33] We now report the binding affinities of selected glycosides for the five known human receptor subtypes SSTR1 through SSTR5. We[12c] and others[33g] have previously cautioned that the affinity of SRIF for the mixture of SRIF receptors present on AtT-20 cells may depend in part on the subclone of the AtT-20 cell line and on the radioactive ligand employed.[34] Thus SRIF had an IC_{50} of 0.83 nM against [^{125}I-Tyr^{11}]SRIF and of 9.3 nM when [^{125}I]CGP-23996 (des-Ala^1, Gly^2-desamino-Cys^3-[Tyr^{11}]dicarba^{3,4}-somatostatin) was employed as the radio ligand.[12a] Since the AtT-20 cells are derived from *transformed mouse* cells they may contain receptor subtypes which have no counterparts among the five known *human* SSTRs. In our hands, SRIF-14 had the highest affinity (0.07 nM) for hSSTR2 and the lowest (2.7 nM) for hSSTR5 when [^{125}I-Tyr^{11}]SRIF-14 was the radioligand. This represents an attractive biological profile, because it has been suggested that SSTR2 controls the inhibition of the release of growth hormone[35] (GH) and glucagon,[36] whereas SRIF agonists are thought to inhibit insulin release via SSTR5.[36,37] The selective inhibition of the release of GH and of glucagon is thought to be desirable for the treatment of adult onset diabetes.[17] Bruns et al.[33h] have cautioned, however, that the assignment of a response to a single SSTR subtype remains tentative in the absence of subtype

specific *antagonists*. These authors also call attention to the discrepancies reported by different laboratories for the binding patterns at SSTRs for endogenous and synthetic ligands. As shown in Figure 9, in our hands, the affinity of SRIF-14 for the five hSSTRs decreases in the order SSTR2 ≫ SSTR1 > SSTR3 > SSTR4 and SSTR5. It is of interest to compare the profiles of **3**, **4**, **6**, and **7** with each other and with that of SRIF. As mentioned above, the dominant feature of the receptor subtype profile of SRIF-14 is its high affinity for SSTR2. As can be seen from the bar graphs (Figure 9), the profile of the four glycosides resemble each other more than they resemble SRIF. The most striking feature of all the glycosides reported herein is that they have a preference for SSTR4. To our knowledge no SRIF receptor ligand has heretofore revealed this property. This fact may make these glycosides a potential tool for gaining an understanding of the function of this receptor subtype, especially if one of these compounds proved to be an antagonist.

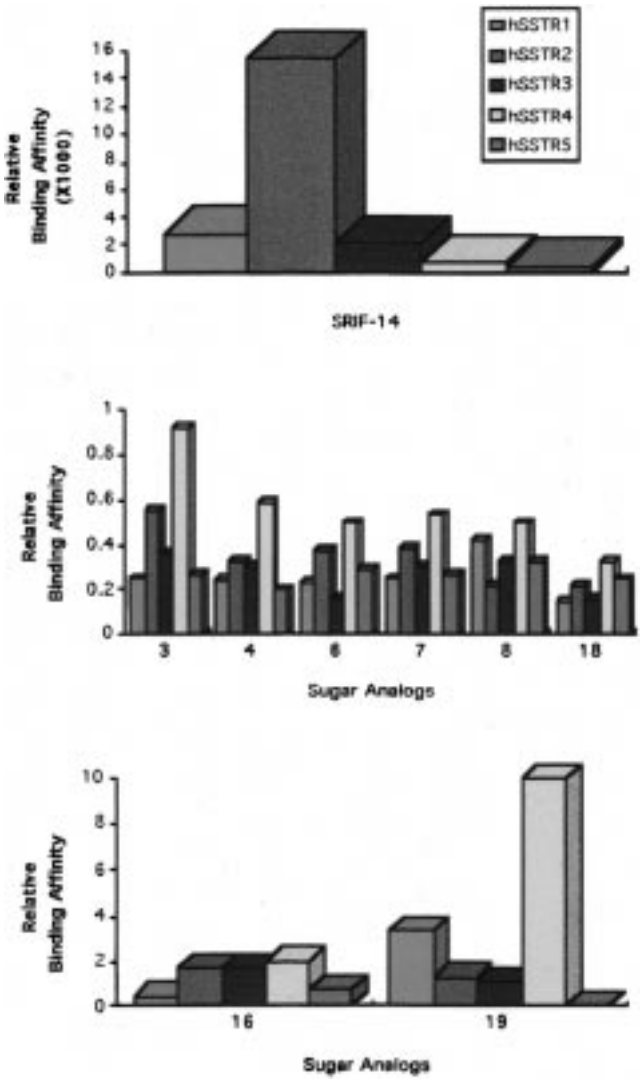


Figure 9. Comparison of binding affinity profiles of SRIF-14 and sugar analogues at somatostatin subtype receptors (note differences in scale).

That the affinity *pattern* of SRIF differs from that of the three sugars is not surprising if we consider that both Bruns *et al.*[33h] and Reisine and Bell[38] found major differences in their receptor subtype affinity patterns between SRIF-14 and MK-678 [*c*-(N-Me-Ala-Tyr-D-Trp-Lys-Val-Phe)],[39] a close analogue of L-363,301. It is pleasing that the affinity profiles of glycoside **3**, its 3-deoxy analogue **4,** and of the diastereomeric congener **7** at the five receptor subtypes resemble each other as shown by the bar graphs (Figure 9), because it is consistent with our interpretation of the SARs.[12c] Interestingly, **6**, the enantiomer of **3**, shares with **3**, **4**, and **7** high affinities for hSSTR2 and hSSTR4 and a low affinity for SSTR1, but **6** differs in that it has a higher relative affinity for SSTR5 and a lesser relative affinity for SSTR3 than the other three glycosides. This demonstrates that subtle changes in the scaffold affect the biological profile. Compound **18**, which possesses an imidazole substituent at C(2) mimicking the mutation Phe7His in SRIF, resembles **3**, **4**, and **7** in its binding affinity profile except for the fact that, like **6**, it has an atypically high affinity for hSSTR5.

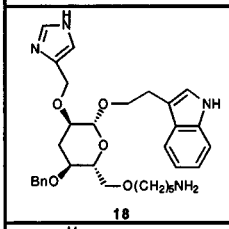
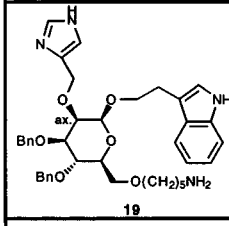
It was of particular interest to determine the profile of **8**. Although all compounds in Figure 10 have the highest affinity for hSSTR4, the *pattern* of affinities for **8** at the four remaining receptor subtypes differs markedly from that of compounds **3**, **4**, **6**, and **7**. *The differences are satisfying because they are consistent with the alternate binding mode hypothesis.*

We were also interested in the glycoside **16** in which a secondary amino nitrogen replaces the C(6) oxygen. On the basis of data reported at AtT-20 receptors (see above), it was not unexpected that its general profile resembles that of **3.** Moreover the average K_i's at the five hSSTRs for **3** and **16** are 2.7 and 1.0 μM, respectively, consistent with the view expressed above that the presence of two nitrogens in the C(6) side chains does not enhance affinity sufficiently to suggest the presence of a second salt bridge. It is of considerable interest that **16** displays submicromolar affinities at SSTRs 2, 3, and 4.

The most significant profile is that of **19** with K_i's of 100, 300, 800, and 900 nM at SSTRs 4, 1, 2, and 3, respectively. Interestingly, **19** failed to bind SSTR5, an appealing feature (see above). The IC_{50} of SRIF at AtT-20 receptors and the average K_i's at the five hSSTRs are about the same (0.83 and 1.05 nM) when [^{125}I-Tyr^{11}]-SRIF is used as the radioactive ligand, whereas the glycosidic peptidomimetics display higher affinities at selected human receptor subtypes than at the transformed mouse receptors on AtT-20 cells. That a K_i of 100 nM is obtainable at hSSTRs provides validation of our underlying design and casts some doubt on the suggestion[24] that free rotation of the side chains precludes submicromolar affinities.[40]

Divergent SARs at the SRIF and NK-1 Receptors

We previously reported the unexpected observation that **3** and **4** are potent antagonist ligands for the NK-1 receptor subtype of substance P. This had provided the first report that a Trp-mimicking side chain can enhance affinity of nonpeptidal ligands at the NK-1 receptor.[12b] Furthermore, these ligands also bind the β_2-adrenergic receptor as antagonists.[12b] In contrast, SRIF and L-363,301 do not bind either the NK-1 or the β_2-adrenergic receptors.[41] β-D-glycosides such as **3** or **4**

Compound	AtT-20 IC_{50} (μM)	hSSTR1 K_i (μM)[b]	hSSTR2 K_i (μM)[b]	hSSTR3 K_i (μM)[b]	hSSTR4 K_i (μM)[b]	hSSTR5 K_i (μM)[b]
3	15.0	4.0	1.8	2.8	1.1	3.7
4	8.4	4.1	3.1	3.3	1.7	5.1
6	47.0	4.4	2.7	6.4	2.0	3.4
7	8.0	4.0	2.6	3.3	1.9	3.9
8	5.9	2.4	4.6	3.0	2.0	3.0
16	13.0	2.1	0.6	0.6	0.5	1.3
 **18**	4.1	6.8	4.6	6.2	3.0	4.0
 **19**	-	0.3	0.8	0.9	0.1[c]	DNB[a]

[a] DNB = Did Not Bind; refers to compounds with K_i's > 10,000 nM at hSSTRs. [b] All compounds were examined over a range of concentrations from 0.01 nM to 10,000 nM using full log dilutions and seven point titrations to determine K_i values. All experiments were performed in triplicate with replicate values varying less than 10%. Compounds were tested in at least two completely separate experiments. Values reported here represent averages from pooled experiments. [c] For compound **19**, the results were least variable at the hSSTR4 (99 ± 7 nM).

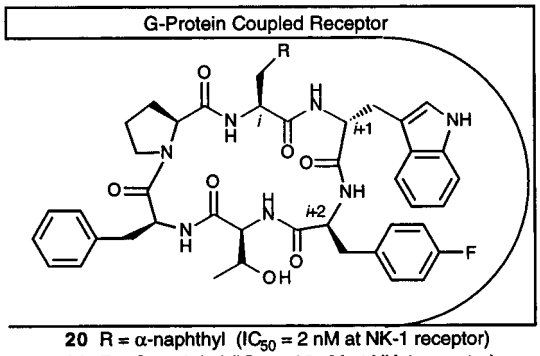
Figure 10. Comparison of binding affinities of selected sugar analogues at AtT-20 and human SRIF subtype receptors.

provided the *first direct evidence* that peptides such as SRIF and nonpeptidal ligands, typified by epinephrine, bind G-protein-coupled receptors in an analogous manner and that the binding sites of the SRIF, NK-1, and the β_2-adrenergic receptors have much in common. Subsequently we showed that this is indeed the case, since we converted the potent *c*-hexapeptide SRIF agonist L-363,301 (**1**) into the potent (2 nM) NK-1 receptor antagonist **20** with remarkable ease.[23] These experiments demonstrated that peptidomimetics can provide valuable information about structural similarities between different receptors *which cannot be obtained through studies with their endogenous ligands*.

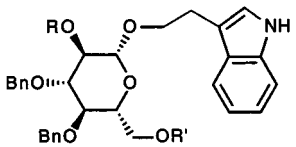
To explore further the relationship between peptidal and glycosidic ligands, we designed and synthesized sugars related to **20**[23] and **21** (IC_{50} 2 and 65 nM, respectively; Figure 11). One of them, glycoside **25**, was found to bind the NK-1 receptor with an IC_{50} of 209 nM, whereas analogues **22**, **23**, **24**, and **26**, containing a β-naphthyl substituent, did not bind the NK-1 receptors. The N-acylated derivative of **3**, compound **15**, had an IC_{50} of 60 nM at the NK-1 receptor, but did not bind some 65 other receptors, including the SRIF and β_2-adrenergic receptors.[12b]

Recently we also prepared the 4-unsubstituted analogue **27** (Figure 11) and found it to possess the highest affinity of any of our sugars for the NK-1 receptor (27 nM). In contrast, the 4-benzyl substituent is important for SRIF receptor binding. These results show that the monosaccharides can be both potent and specific.

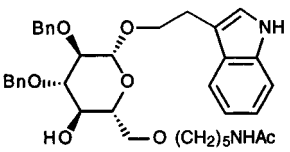
That seemingly small changes in the sugar scaffold can affect very differently the biological profile at the



20 R = α-naphthyl (IC_{50} = 2 nM at NK-1 receptor)
21 R = β-naphthyl (IC_{50} = 65 nM at NK-1 receptor)



22 R = CH_2-β-naphthyl, R' = *p*-F-benzyl (did not bind)
23 R = CH_2-β-naphthyl, R' = *p*-F-C_6H_4 (did not bind)
24 R = *p*-F-benzyl, R' = CH_2-β-naphthyl (did not bind)
25 R = *p*-F-benzyl, R' = $(CH_2)_5NHAc$ (IC_{50} = 209 nM)
26 R = CH_2-β-naphthyl, R' = $(CH_2)_5NHAc$ (did not bind)



27 (IC_{50} = 27 nM at NK-1 receptor)

Figure 11. *c*-Hexapeptides and sugar analogues that bind the NK-1 receptor.

SRIF and NK-1 receptors is also exemplified by the finding that going from the β-D-glucose scaffold (i.e., **3**) to the L-mannose scaffold enhanced affinity at the somatostatin receptors on AtT-20 cells by a factor of 2, but reduced the affinity at the NK-1 receptor from 150 nM to ≥1 μM. Similarly, changing to the C-glycoside **17** did not significantly alter affinity at the SRIF receptor but blocked binding at the NK-1 receptor.

Summary of Biological Results

The assay results show that modulations of the sugar scaffold, glycosidic linkers and of side chains can generate various levels of specificity and potency at different receptors and at receptor subtypes. *These results suggest that further exploration of the concept of attaching genetically encoded and uncoded amino acid side chains to privileged platforms holds the promise of preparing compounds with diverse biological profiles via library synthesis on solid support or in solution. We have initiated such programs.*

Preparative Experiments

A New Nonbasic Protocol for the Synthesis of Base-Sensitive Ethers. During the course of this work, we discovered that glycosides such as **28** (Figure 12) reacted with crude alkyl triflates to yield the desired ethers when a mixture of the two compounds is dried in vacuo prior to the addition of solvent and base. We believe that this reaction is not catalyzed by residual 2,6-di-*tert*-butyl-4-methylpyridine since purification by silica gel column chromatography to remove the 2,6-di-*tert*-butyl-4-methylpyridine was performed on representative triflates; use of triflates purified in this manner provided ethers in high yields! This method has been successfully applied to the synthesis of over 30 sugar-based ligands including those in which a primary and/or a secondary alcohol acted as the reacting nucleophile. Preliminary investigations have revealed that benzyl alcohol may also serve as a nucleophile. Attempts to use this method for disaccharide formation or phenolic etherification have failed. We are studying further the scope and mechanism of this method. This work will be published elsewhere.

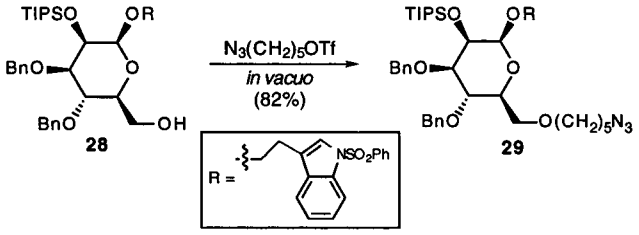


Figure 12. Representative example demonstrating the use of a novel etherification method.

Experimental Section[42]

Representative Procedure for Etherification of Glycosides: Preparation of 2-[*N*-(Phenylsulfonyl)indol-3-yl]-ethyl 2-*O*-(Triisopropylsilyl)-3,4-di-*O*-benzyl-6-*O*-(5′-azidopentyl)-β-L-mannopyranoside [(+)-29]. A solution of 5-azidopentanol (100 mg, 0.83 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (170 mg, 0.83 mmol) in dichloromethane (5 mL) at 0 °C was treated with trifluoromethanesulfonic anhydride (0.14 mL, 0.83 mmol). The reaction mixture was stirred at room temperature for 15 min, poured into water (50 mL), and extracted with dichloromethane (3 × 50 mL). The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo to a volume of ca. 100 mL. The concentration of this triflate solution was assumed to be 8 mM and was used directly in the following step. *Caution! Explosion hazard! Attempts to isolate neat azidoalkyl triflates have resulted in violent decomposition. These triflates should not be concentrated to dryness and should be used only in solution as described in this procedure.*

To alcohol (+)-**27** (0.133 g, 0.167 mmol) was added freshly prepared 5-azidopentyl trifluoromethanesulfonate (8 mM in dichloromethane, 25 mL, 0.2 mmol), and the reaction mixture was concentrated in vacuo and placed under high vacuum (ca. 1 mmHg) for 2−3 h. This process was repeated three additional times, and the reaction mixture was kept under high vacuum (ca. 1 mmHg) for 12 h. The residue was then dissolved in ethyl acetate, filtered through a plug of silica gel with ethyl acetate as eluant, and concentrated in vacuo. Flash chromatography (10:1 → 7:3 hexanes/ethyl acetate) afforded (+)-**29** (124 mg, 82% yield) and recovered (+)-**28** (21 mg).

(+)-**29**: colorless oil; $[\alpha]^{20}_D$ +14.1° (*c* 1.41, $CHCl_3$); IR ($CHCl_3$) 3020 (m), 2950 (s), 2870 (s), 2100 (s), 1450 (s), 1370 (s), 1175 (s), 1120 (s), 690 (m), 590 (m) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.98 (d, *J* = 8.3 Hz, 1 H), 7.86 (d, *J* = 8.4 Hz, 2 H), 7.53−7.48 (m, 2 H), 7.42 (t, *J* = 7.8 Hz, 2 H), 7.38−7.22 (m, 13 H), 4.89 (d, *J* = 10.9 Hz, 1 H), 4.74 (d, *J* = 11.8 Hz, 1 H), 4.62 (d, *J* = 11.8 Hz, 1 H), 4.61 (d, *J* = 10.9 Hz, 1 H), 4.27 (m, 2 H), 4.14 (m, 1 H), 3.90 (t, *J* = 9.4 Hz, 1 H), 3.71 (dd, *J* = 16.6, 7.8 Hz, 1 H), 3.66 (d, *J* = 2.7 Hz, 2 H), 3.57 (dt, *J* = 9.3, 6.3 Hz, 1 H), 3.43 (m, 2 H), 3.34 (m, 1 H), 3.20 (t, *J* = 6.9 Hz, 2 H), 2.96 (m, 2 H), 1.57 (m, 4 H), 1.41 (m, 2 H), 1.15−1.04 (m, 21 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.5, 138.4, 135.2, 133.6, 131.0, 129.2, 128.3, 128.2, 128.1, 127.6, 127.4, 126.7, 124.7, 123.3, 123.1, 119.7, 119.4, 113.7, 100.8, 83.0, 75.8, 75.0, 74.6, 71.9, 71.3, 70.3, 70.2, 68.0, 51.4, 29.4, 28.8, 25.4, 23.4, 18.3 (2 C), 12.9; high-resolution mass spectrum (FAB, *m*-nitrobenzyl alcohol) *m/z* 933.4251 [(M + Na)$^+$; calcd for $C_{50}H_{66}N_4O_8SSi$: 933.4269].

Ligand Binding Assays at hSSTRs. All assays were performed in 96-well format using polypropylene plates. Membranes were isolated from CHO-K1 cells expressing the hSSTR1−5 and resuspended in an assay buffer as previously described by Raynor et al.[35] The assay buffer contained 50 mM Tris-HCl (pH 7.8), 1 mM EGTA, 5 mM $MgCl_2$, 10 μg/mL leupeptin, 10 μg/mL pepstatin, 200 μg/mL bacitracin, and 0.5 μg/mL aprotinin. Test compounds, membranes, and (3-[^{125}I]-iodotyrosyl^{11})somatostatin-14 were diluted in the assay buffer. The final assay volume was 200 μL, the final concentration of radiolabeled somatostatin was 0.1 nM, and the test compounds were examined over a range of concentrations from 0.01 to 10 000 nM. Assay components were incubated at room temperature for 45 min and then harvested onto Packard Unifilter GF/C plates pretreated with 0.1% polyethyleneimine. Plates were dried overnight at room temperature and then sealed and counted the next morning in a Packard Topcount Scintillation Counter using Microscint-20 scintillation fluid.

Binding assays at the AtT-20 SRIF receptors and the NK-1 receptors were carried out as previously described (see refs 12c and 43, respectively).

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ties, for assistance in obtaining NMR spectra, small molecule X-ray crystal structures, and high-resolution mass spectra, respectively.

Supporting Information Available: Detailed experimental procedures and complete spectral data for all intermediates and target compounds (91 pages). Ordering information is given on any current masthead page.

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(42) **Materials and Methods.** All solvents were either reagent grade or HPLC grade. Analytical thin-layer chromatography was performed on E. Merck 0.25 mm precoated silica gel 60 F_{254} plates. EM Science silica gel 60 (230−400 mesh ASTM) was used for flash chromatography. ^{1}H and ^{13}C NMR spectra were obtained with a Brucker AMX2-500 spectrometer. Chemical shifts are reported in δ values relative to internal chloroform (δ = 7.24 for proton spectra and δ = 77.0 for carbon spectra) or internal methanol (δ 3.30 for proton spectra and δ 49.0 for carbon spectra) Infrared spectra were determined using a Perkin-Elmer model 283B spectrophotometer using polystyrene as an external standard. High-resolution mass spectra were measured on a VG 70/70 Micromass or VG ZAB-E spectrometer. Optical rotations were measured on a Perkin-Elmer model 241 polarimeter. Microanalyses were performed at Robertson Microlit Laboratoires, Madison, NJ, or at the University of Pennsylvania. Somatostatin, leupeptin, aprotinin, and pepstatin were purchased from Sigma. Bacitracin was obtained from Bachem, and radiolabeled somatostatin-14 was purchased from Amersham.

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