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Synthesis and Binding Affinities of Novel SRIF-Mimicking β-D-Glucosides Satisfying the Requirement for a π-Cloud at C1

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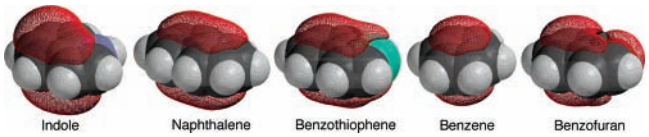
ABSTRACT



The synthesis of four bioactive analogues of the somatostatin (SRIF-14) mimetic, β-D-glucoside (+)-2, in which the C1 indole side chain is replaced with indole surrogates, has been achieved. These congeners, possessing the naphthyl, benzothiophene, benzyl, and benzofuran substituents, were predicted to satisfy the electrostatic requirements of the tryptophan binding pocket of SRIF. Unlike the previously described C4 picolyl and pyrazinyl congeners, these ligands bind the hSST4 receptor.

In 1987, we initiated a then conceptually novel approach for the discovery of nonpeptidal mimetics of cyclic peptides, as an alternative to random screening, that involved attaching relevant amino-acid-mimicking side chains to β-D-glucose via ether linkages.[1] Others have subsequently employed various monosaccharides as privileged scaffolds incorporating a β-turn.[2]

Early on, molecular modeling suggested that attachment of the indole and the lysine-mimicking side chains at the C1 and C6 positions of the sugar, respectively, would provide good overlap with those of Trp[8] and Lys[9] of somatostatin-14 (SRIF-14, **1**, Figure 1) in the critical $i + 1$ and $i + 2$ positions of the β-turn.[1a,3] Molecular modeling also demonstrated that the 2-benzyl substituent mimics the important Phe[7] of SRIF-14.

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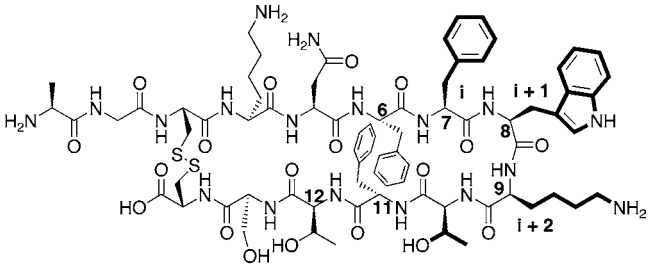
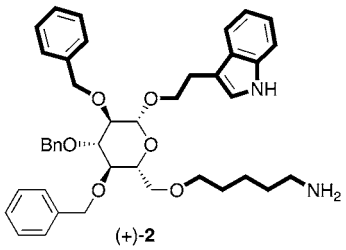
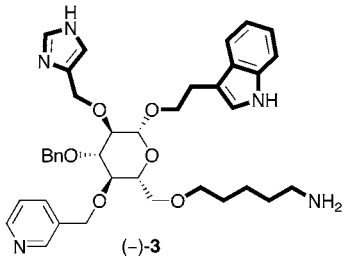


1 (SRIF-14)



(+)-**2**

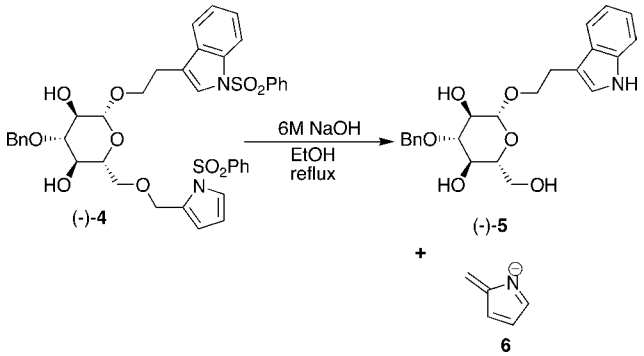


(−)-**3**

Figure 1. Structure of somatostatin (SRIF-14),[4] our initial designed monosaccharide-based mimetic (+)-**2**, and our best ligand at the hSST4 receptor (K_i = 53 nM) (−)-**3**.[3]

The initial design of (+)-**2** incorporated an ethylene rather than a methylene linker between the C1 indole and the anomeric oxygen to avoid a gramine fragmentation.[5] A reaction of this type was indeed observed on treatment of (−)-**4** with 6 M sodium hydroxide in ethanol at reflux (Scheme 1). All of our ligands described herein, including the best ligand (−)-**3** (Figure 1), incorporate an ethylene linker.[3]

Scheme 1. Gramine-Type Fragmentation of (−)-**4**



(-)-**4**

(-)-**5**

+

6

Early in the program, we serendipitously made the surprising observation that the indole ring of (+)-**2** can be replaced by a methoxy substituent, affording (+)-**7** without loss of affinity at the SRIF receptors of transformed AtT-20 cells[1a] (Figure 2b).

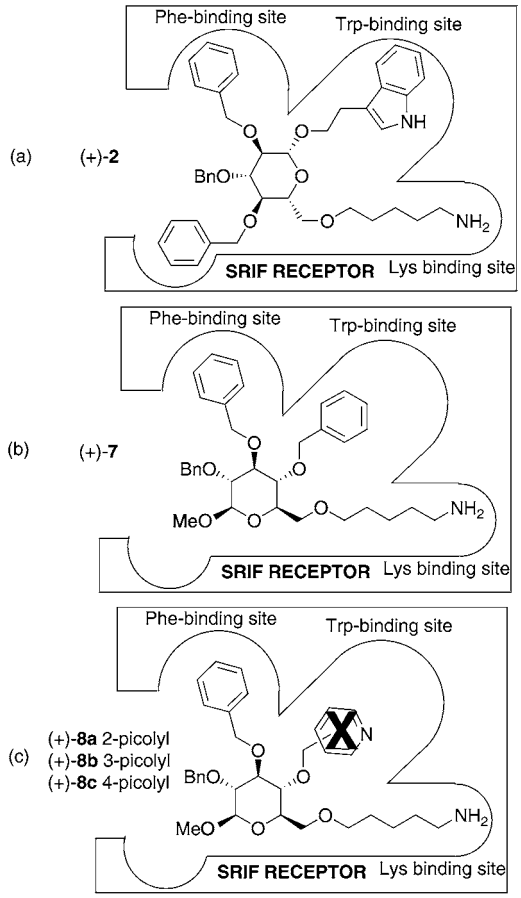


Figure 2. (a) Binding mode of (+)-**2** (K_i = 1490 nM). (b) The established binding mode for ligand (+)-**7**, lacking an indole side chain (K_i = 2857 nM). (c) 1-Methoxy congeners of (+)-**7**, with a C4 heterocyclic substituent, do not bind.[3]

Later, we observed only a modest loss in affinity for (+)-**7** vis-á-vis (+)-**2** at the hSST4 receptor subtype (the highest affinity human receptor subtype for our glycosides).[6] We explained the fact that a methoxy group can replace the Trp[8]-mimicking indole side chain by suggesting that rotation of the molecule places the 4-benzyl substituent into the Trp[8] binding pocket of SRIF-14 (i.e., the benzene ring acts as an indole surrogate; Figure 2b). Subsequently, we reported structure−activity relationships that confirmed the above proposition beyond reasonable doubt.[7] We later termed this phenomenon radial symmetry.[8]

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Calculations of electrostatic potentials of the aromatic substituents next allowed us to explain why a benzene moiety can replace the C1 indole of glycoside-based SRIF-14 peptidomimetics in the Trp[8] binding pocket and why pyridine and pyrazinyl rings cannot (Figure 2c).[3] The electrostatic potential is defined as the energy of interaction of a point positive charge with the nuclei and electrons of the molecule of interest.[9] Thus, the electron distributions of the heterocyclic aromatics at C4 in (+)-**8a**−**c** and (+)-**9** lack a significant negative potential in the region of the π-cloud and thereby fail to bind the aromatic Trp[8] binding pocket (Figures 2 and 3).

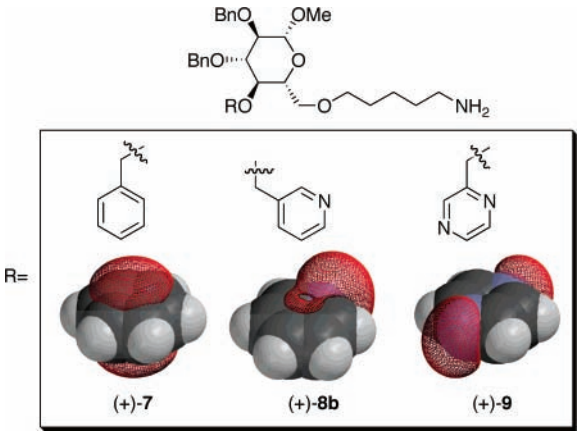

Figure 3. Congeners of desindole (+)-**7** and their corresponding EP calculations. Spartan 6-311G**MP2 MO analysis of aromatic electrostatic potentials that involve interactions of a positive charge not only with the π-cloud but also with all other electrons and nuclei of the molecule. The mesh surfaces depicted are the surfaces upon which the electrostatic potential (i.e., the attraction of the molecule for a positive point charge) equals −10 kcal mol^{-1}. Structures are arranged in decreasing order of the measured distance from the electrostatic potential surface to the molecular plane.[9]

Electrostatic potential is a versatile property that can be computed accurately because it does not require modeling of binding sites or specific interactions. We choose to present EP surfaces because they dramatically show the way in which significant attraction for a positive charge extends well beyond the van der Waals space-filling molecular surface. These surfaces give an easily visualized comparison of the relative ability of aromatic π-clouds to give effective π-donor interactions. Although all aromatic rings show significant π-clouds, the EP surfaces of pyridine and pyrazine differ dramatically from those of indole and benzene.

Herein, we report extension of such EP calculations that correctly predict that analogues based on (+)-**2** can indeed provide the π-cloud interactions required for significant binding at hSST4. We computed the electrostatic potentials of the relevant aromatic substituents at C1 of the glycosides (+)-**10**−(+)-**13** (Figure 4), using Spartan 6-311G**MP2 molecular orbital analysis.[9]

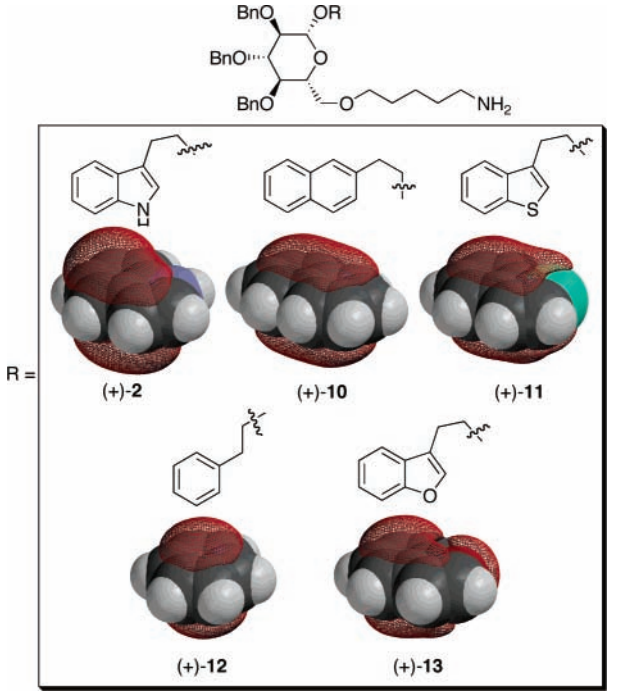

Figure 4. Analogues of (+)-**2** in conjunction with EP calculations. Structures are arranged in decreasing order of the measured distance from the electrostatic potential surface to the molecular plane.[9]

The electrostatic potentials for indole, naphthalene, benzothiophene, benzene, and benzofuran, illustrated in Figure 4, reveal that these surrogates of indole can provide for effective π-donor interactions via, for example, an aromatic edge to face interaction in the Trp[8] binding pocket; a similar interaction is not possible for pyridine and pyrazine surrogates. The latter substituents have electron-deficient π-systems that differ spatially from the relatively electron-rich π-systems of the other aromatic systems mentioned herein.[3]

The EP surfaces have shapes that depend on the structure and architecture of the molecule. To provide a simplified comparison of different molecules, we show in Table 1 the maximum vertical distance of each surface perpendicular to the molecular plane along with the dissociation constants of all analogues. When an aromatic group has some conformational flexibility, it is expected to orient itself so as to place the maximum vertical π-donor interaction as closely

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as possible to the group with which it interacts in the binding pocket. Molecules with larger vertical distances (giving −10 kcal/mol interaction with a point positive charge) are thus expected to have stronger π-donor interactions at any distance from the molecular plane.[10] Consequently, binding abilities are expected to have a direct correlation to the vertical extent of the EP surface.

Table 1. EP-Calculated Vertical Distances and Dissociation Constants of the Designed Congeners of (+)-**2** at hSST4

compound	EP calculation vertical distance,[a] Å	K_i (nM), $n = 3$[b]
(+)-**2**	3.61	1490 ± 749
(+)-**10**	3.12	1415 ± 633
(+)-**11**	3.05	1613 ± 494
(+)-**12**	3.02	1557 ± 200
(+)-**13**	2.96	1671 ± 220

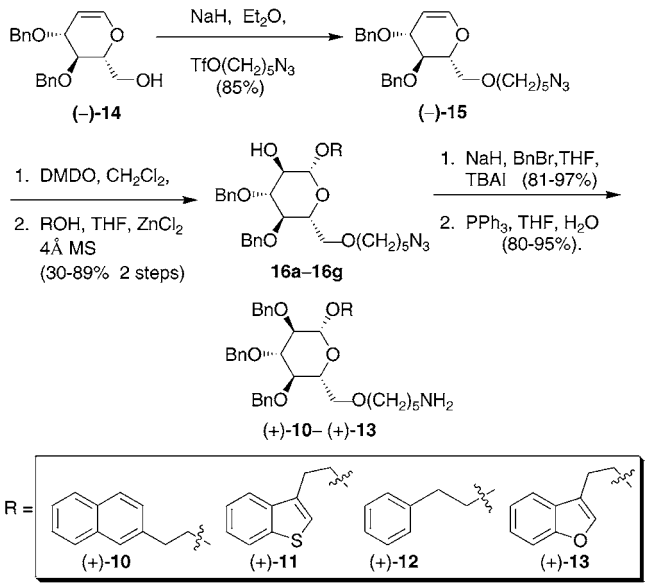
[1] Maximum distance from EP surface to molecular plane. [b] $n = 3$ except for (+)-**2**, where $n = 13$.

Binding assays for (+)-**2** and (+)-**10**−(+)-**13** at the hSST4 receptor (Table 1) were performed using (3-^{125}I-Tyr^{11})-SRIF-14 as the radioligand. The Packard Unifilter assay for SRIF subtype receptor binding was employed in these experiments as described by Birzin and Rohrer.[11] Importantly, the analogues of (+)-**2** do bind the receptor as predicted by the EP calculations. In addition, the identical binding affinities of (+)-**2** and (+)-**12** provide additional validation of our rationalization for the observation that (+)-**7** binds the SRIF receptors.[1a]

The relative dissociation constants for (+)-**2**, (+)-**10**, (+)-**11**, (+)-**12**, and (+)-**13**, however, do not parallel exactly the corresponding electrostatic potentials. We attribute these discrepancies to the large error bars in the K_is that are always seen with relatively weak ligands in the binding assays. It is pleasing, however, that the calculations of the EPs for these ligands correctly predict that they would bind the receptor.

The syntheses of analogues (+)-**10**−(+)-**13** began with the known intermediate alcohol (−)-**14** (Scheme 2),[1a] which was deprotonated using sodium hydride, followed by coupling with the triflate derived from 5-azido-1-pentanol, to install the lysine-mimicking side chain; yields in general were good. Stereospecific epoxidation of the enol ether (−)-**15** with dimethyldioxirane was then followed by treatment with a series of alcohols in the presence of $ZnCl_2$ to generate compounds **16a**−**d**.[1a] Benzylation of the resulting intermediates followed by Staudinger reduction of the azides completed construction of analogues (+)-**10**−(+)-**13** in good yield.

Scheme 2. Synthesis of Congeners of (+)-**2**



Taken together, the data described herein support the previously reported[3] proposition that the electrostatic potentials provide information necessary to predict whether an aromatic ring system possesses the π-cloud required for interaction with the side chains of proteins typified by the Trp[8] receptor binding pocket of SRIF-14.

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Supporting Information Available: Experimental procedures and spectroscopic data for all new compounds and electrostatic potential diagrams. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) This EP surface of −10 kcal/mol for interaction with a point positive charge is also exactly the surface for attraction of a +0.1 partial positive charge at an energy of −1 kcal/mol, expected to be relevant to aromatic−aromatic interactions.

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