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A Chiral Pyrrolic Tripodal Receptor Enantioselectively Recognizes β-Mannose and β-Mannosides

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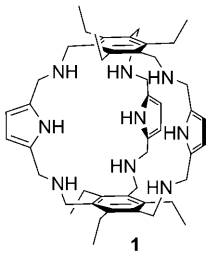
Biomimetic receptors for carbohydrates have been widely used to investigate the recognition phenomena occurring in biological systems.[1] Among the plethora of synthetic receptors for carbohydrates reported in the literature, only a limited number are chiral. In addition, of the several papers dealing with recognition of carbohydrates by chiral synthetic receptors,[2–20] only a few are concerned with the effect of receptor's chirality on the enantioselective recognition of sugars.[2–8] This is somewhat surprising because selective recognition can be expected when enantiomerically pure natural saccharides bind to opposite enantiomers of a chiral receptor. Indeed, apart from the extensive work of Diederich and co-workers on dendritic clefts and cyclophanic and macropolycyclic receptors,[2] following the first report by Davis and co-workers,[3] very few investigations were specifically focused on the enantioselective recognition of monosaccharides by chiral receptors.[4–8] In particular, to our knowledge, the enantioselective recognition of mannosides has only been reported in two cases, in which 1-octyl-α-D-mannopyranoside binds to the enantiomers of the receptor with little or no discrimination.[2b,6] We have recently reported a family of pyrrolic tripodal receptors possessing distinguished recognition properties towards monosaccharides.[21] We describe herein the first member of a new generation of chiral tripodal receptors, showing unprecedented affinities towards mannosides even in polar solvents, and marked selectivities with respect to other monosaccharides; most remarkably, the all-*S* enantiomer of the receptor selectively binds to β-mannose and β-mannosides with an outstanding enantiomeric discrimination with respect to the all-*R* enantiomer, proving that chirality of the receptor is a key feature in the selective recognition of mannose.



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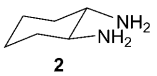

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In a previous paper, we described a pyrrolic cage receptor (**1**) that specifically recognizes the β-glucosyl residue.[22] Although both β-D-glucose and methyl-β-D-glucoside could be dissolved in apolar solvents by the receptor, the measured affinity for octyl-β-D-glucoside in $CDCl_3$ was no larger than 20 μM. We attributed the cause of this relatively modest affinity to the size of the cavity, which appeared to be slightly too tight for the glucosyl residue. In an effort to achieve a better fit, we tried to design a cage endowed with a some-

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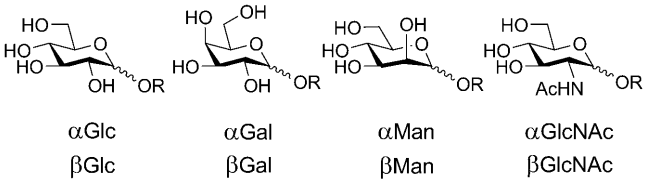
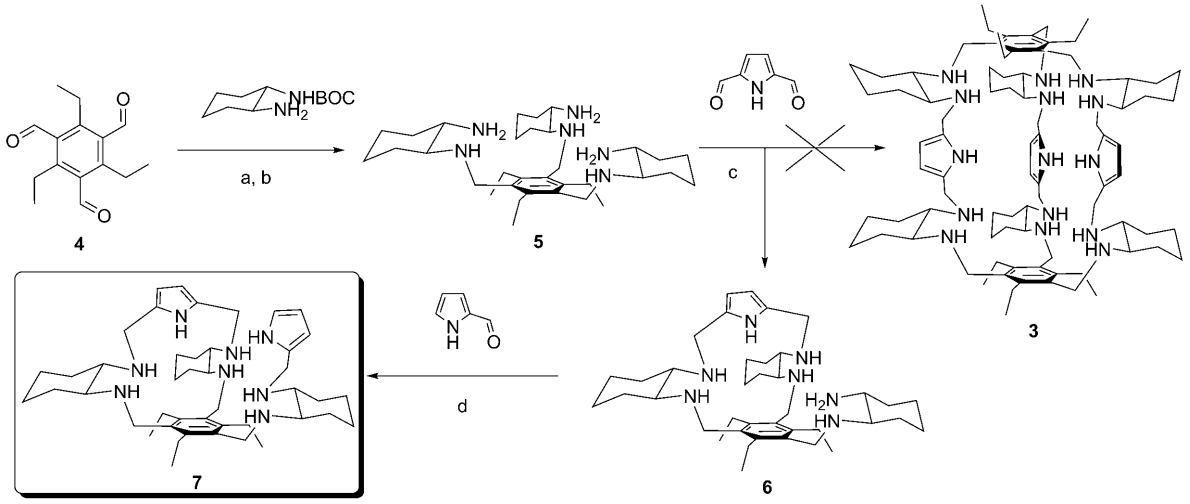
what enlarged cavity, in the belief that fine-tuning of the cage size would lead to a significantly improved affinity even in more polar media. Replacement of *trans*-1,2-diaminocyclohexane (**2**) for the amino groups in the structure of **1**, leading to the corresponding dodecaamino cage **3**, appeared to be the appropriate modification. Since **2** has been shown to recognize the *trans*-1,2 arrangement of a number of diols through a well-matched hydrogen-bonding network,[23] it could be anticipated to recognize the *trans*-1,2 diol arrangement in monosaccharides and, in addition, both the enantiomerically pure (*R*,*R*)- and (*S*,*S*)-diamines were readily available to investigate the enantioselective recognition of monosaccarides. The synthesis of **3** was thus attempted according to Scheme 1.

Reaction of the trialdehyde **4** with the mono-BOC-protected diamine **2**, followed by reduction of the resulting Schiff base and subsequent deprotection of the amino groups, yielded the tripodal hexaamine **5**, which was condensed with pyrrole-2,5-dicarbaldehyde under the conditions used for preparing the bicyclic receptor **1**. Contrary to expectations, the monocyclic compound **6** was obtained instead of the bicyclic cage **3**. Although the best yield was obtained for a 1:1 ratio of reactants, compound **6** always constituted the major product isolated from the oligomeric reaction mixture, whereas **3** could not be isolated in any case. Apparently, the described structure modification biased the Schiff base equilibrium toward the formation of a pyrrole-bridged ring, regardless of the ratio of the reactants. While all attempts to prepare the desired cage receptor **3** failed, the monocyclic compound **6** turned out to be a valuable alternative intermediate. Indeed, condensation of **6** with pyrrole-2-carbaldehyde readily afforded the hexaamino dipyrrolic tripodal compound **7**, which from preliminary testing appeared to be a promising receptor for monosaccharides. Receptor **7** was thus prepared in both enantiomerically pure forms, the *R*,*R*,*R*,*R*,*R*,*R* enantiomer ((*R*)-**7**) and the *S*,*S*,*S*,*S*,*S*,*S* enantiomer ((*S*)-**7**), which were submitted to binding tests with the methyl glycosides of a set of monosaccharides, including glucose (Glc), galactose (Gal), mannose (Man), and *N*-acetyl-glucosamine (GlcNAc).



A preliminary screening indicated that, while Glc, Gal, and GlcNAc were moderately bound, strong recognition occurred with mannosides (see Supporting Information, extraction experiments). Moreover, (*S*)-**7** appeared distinctly more effective than (*R*)-**7** toward the β anomer. When an equilibrated mixture of solid mannose, fully insoluble in chloroform and showing a 2:1 α/β anomeric ratio, was stirred with an equimolar solution of (*S*)-**7** in $CDCl_3$, 35% of the solid was dissolved by the receptor in a reversed 1:2 α/β anomeric ratio, showing that βMan was preferentially extracted into solution. βMan was also extracted in benzene, though to a lesser extent (10%), and αMan could not be detected. Even stronger evidence of binding was obtained using the methyl glycosides of mannose (MeαMan and MeβMan: R = Methyl), which are likewise fully insoluble in chloroform. Indeed, when a 3.1 mM solution of (*S*)-**7** in $CDCl_3$ was independently treated with an excess of solid MeαMan and MeβMan, a significantly greater amount of the latter (7.9 mM, 2.5 equiv) than the former (2.6 mM, 0.85 equiv) was found in solution, confirming a strong preference of the receptor for βMan. It is worth noting that more than the stoichiometric amount of MeβMan is extracted by (*S*)-**7** from the solid, suggesting the occurrence of



Scheme 1. Synthesis of receptor **7**. Reagents and conditions: a) *tert*-butoxycarbonyl-*trans*-1,2-diaminocyclohexane, $CH_3OH/CHCl_3$ 1:1, 70°C, 7.5 h, then $NaBH_4$, RT, 1 h, 78%; b) CF_3COOH, CH_2Cl_2, RT, 1.5 h, 91%; c) pyrrole-2,5-dicarbaldehyde, $CHCl_3$, 70°C, 12 h, then $NaBH_4$, CH_3OH, RT, 1 h, 63%; d) $CHCl_3$, RT, 12 h, then $NaBH_4$, CH_3OH, RT, 1 h, 50%.

complexes of stoichiometry higher than 1:1. This evidence was confirmed by treating a solid mixture of MeαMan and MeβMan with a 2.9 mM solution of (*S*)-**7** in $CDCl_3$ in a competitive experiment featuring a 1:1:1 mole ratio of reactants; 0.17 equivalents of MeαMan and 0.90 equivalents of MeβMan were extracted from the glycoside mixture, showing a nearly twofold enhancement of selectivity with respect to the independent extraction of the glycosides. Altogether, extraction experiments demonstrated that receptor (*S*)-**7** is able to effectively dissolve βMan and its methyl glycosides in lipophylic organic solvents with a significant selectivity over the corresponding α anomers. Eventually, a direct evidence of complexation was obtained by mass spectrometry. The positive mode ESI-MS spectra of mixtures of either MeαMan or MeβMan with (*S*)-**7**, extracted into acetonitrile, unambiguously showed in both cases the peak of the 1:1 complex (see Supporting Information). The presence of higher stoichiometry species could not be demonstrated because of the very dilute conditions in a polar solvent used for the MS experiments.

A quantitative assessment of binding affinities was obtained by ^{1}H NMR titrations of both the (*R*)-**7** and (*S*)-**7** receptors with octyl α and β mannosides (OctαMan and OctβMan: R = Octyl) in which the octyl chain ensured the necessary solubility for the binding measurements. Because the interaction of the receptors with the glycosides was too strong to be measured in $CDCl_3$ by NMR spectroscopy, showing a complex pattern of species in solution, association constants were measured in CD_3CN at $T = 298$ K, for which the occurrence of complex formation was proven by MS spectroscopy. The ESI-MS spectrum of a mixture of (*S*)-**7** and OctβMan in acetonitrile is reported in Figure 1, showing the presence of the 1:1 adduct. Thus, binding interactions were still evident in the markedly more polar medium.

Titrations were performed according to a previously established protocol[24] and the results are reported in Table 1 as cumulative binding constants (see Supporting Information for details).

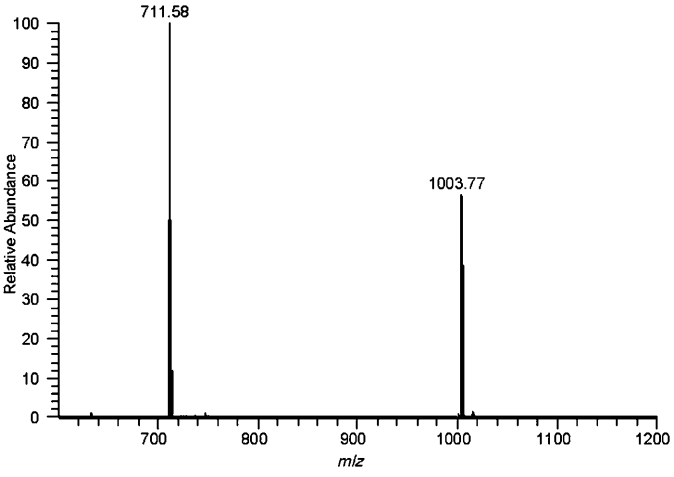


Figure 1. Positive mode ESI-MS spectrum of a mixture of (*S*)-**7** (0.18 mM) and OctβMan (0.90 mM) in CH_3CN. *m/z*: 711.58, $[(S)\text{-}\mathbf{7}+H]^+$;1003.77, $[(S)\text{-}\mathbf{7}\cdot\text{OctβMan}+H]^+$.

Table 1. Intrinsic median binding concentration (BC_{50}^0 [μM])[a] and cumulative association constants ($\log\beta$)[b] for receptor to glycoside (R:G) complexes of **7** with octyl mannosides in CD_3CN.

Receptor	BC_{50}^0	OctαMan $\log\beta$	(R:G)	BC_{50}^0	OctβMan $\log\beta$	(R:G)
		3.49 ± 0.01	(1:1)		2.88 ± 0.02	(1:1)
(*R*)-**7**	299 ± 6			1.222 ± 41	4.30 ± 0.18	(2:1)
		5.94 ± 0.02	(2:1)		7.44 ± 0.08	(3:1)
		3.52 ± 0.01	(1:1)		4.00 ± 0.05	(1:1)
(*S*)-**7**	286 ± 6			83 ± 7	7.04 ± 0.13	(1:2)
		5.87 ± 0.03	(2:1)		10.05 ± 0.15	(1:3)

[a] Calculated from the $\log\beta$ values using the "BC_{50} Calculator" Program (see reference [21]). [b] Measured by ^{1}H NMR (400–900 MHz) from titration experiments at $T = 298$ K. Formation constants were obtained by nonlinear least-square regression analysis of NMR data by simultaneous fit of all the available signals from both reagents.

Since, in addition to the 1:1 adducts, formation constants for complex species of higher stoichiometry were measured, affinities were assessed using the BC_{50}^0 parameter, a generalized affinity descriptor univocally defining the binding ability of a receptor in chemical systems involving multiple equilibria.[21,24,25] The BC_{50}^0 descriptor is defined as the total concentration of receptor necessary for binding 50% of the ligand when the fraction of bound receptor is zero, that is, when forming the first complex molecule and, for 1:1 systems, coincides with the dissociation constant K_d. Thus, the BC_{50}^0 parameter can be used for comparing systems fitting different models; the lower BC_{50}^0, the higher the affinity. The BC_{50}^0 values calculated from cumulative binding constants are reported in Table 1, and provide a quantitative evaluation of the binding preference of (*S*)-**7** for OctβMan. From data of Table 1, it is clearly apparent that while no enantiodiscrimination is observed in the binding of OctαMan, a 15:1 enantioselectivity is apparent in the binding of Octβ Man which, to our knowledge, is the highest value reported in the literature. The 83 μM value for the affinity observed in acetonitrile is also worth noting, testifying strong binding even in a markedly polar medium. We had previously described a tripodal achiral receptor featuring selective recognition of OctβMan, which showed an affinity of 680 μM in acetonitrile;[26] the present, new-generation chiral receptor exhibits a near tenfold enhancement in affinity, together with outstanding enantioselectivity, which make (*S*)-**7** the most effective mannoside receptor up to date.

Conclusive evidence of binding was provided by several intermolecular NOE contacts, together with clear and significant changes in both the signals of receptor (*S*)-**7** and of the glycoside, when both entities were combined in acetonitrile (Figure S1 and S2 and Tables S1–S3, in the Supporting Information), which not only demonstrated the occurrence of receptor ligand interactions, but also allowed a definition of the structure of the complex in solution. Indeed, while all attempts to obtain X-ray quality crystals failed, most likely because of the flexible nature of the receptor, the structure of the complex of (*S*)-**7** with OctβMan in solution could be solved by a combination of experimental NMR data and

molecular modeling calculations.[27] Interestingly, although NMR experiments were acquired at such a concentration that the 1:1 complex accounted for nearly 50% of the species in solution, whereas the 1:2 complex was only 5%, a single structure did not satisfy all the experimental data, suggesting the occurrence of two different arrangements of the partners in the dominant 1:1 complex (Figure S3, Supporting Information). The molecular modeling protocol applied (see Supporting Information) gave two families of low-energy conformers for the complex between (*S*)-**7** and OctβMan: type A, with 12 structures within 4.3 kJ mol^{-1} from the global minimum, and type B, with three structures within 9.0 kJ mol^{-1} from the global minimum, both of which were required to fit with good agreement all the experimental NMR evidence simultaneously, including the dramatic upfield shift of the H-4 proton of the mannose moiety, which in all conformations is located at 2.7–2.8 Å from the centroid of the benzene ring of the receptor. The two families of conformers and the corresponding minimum energy structures for each family are depicted in Figures 2 and 3, while the different orientations characterizing the two families of structures can be appreciated from the superimposition of the minimum energy conformers, depicted in Figure S4 (see Supporting Information).

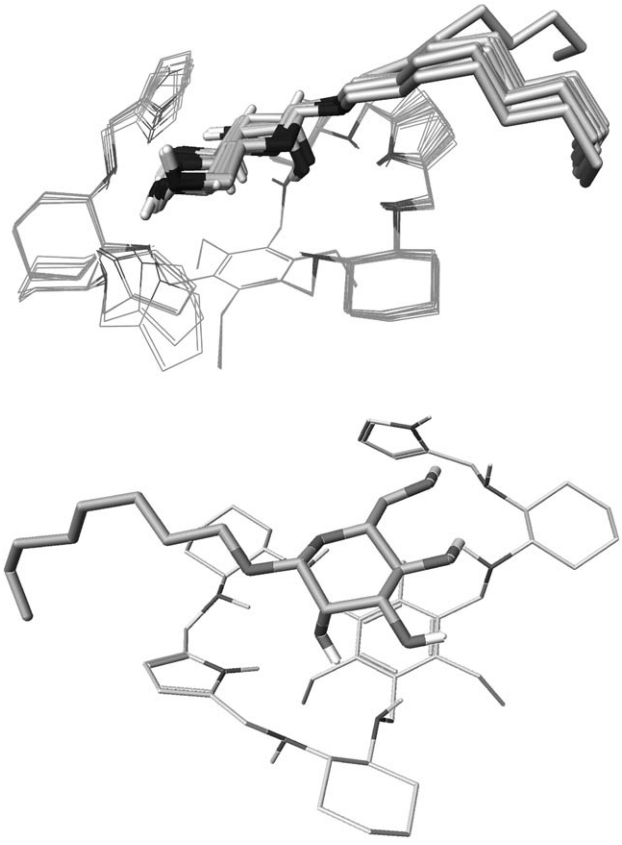


Figure 2. Top: Superimposition of the 12 energy minima structures as obtained from the molecular modeling protocol. The relative orientation (type-A arrangement) of the sugar versus the receptor is depicted. The flexible arm may adopt different orientations (see Supporting Information). Bottom: Structure of the global minimum geometry of the type A family.

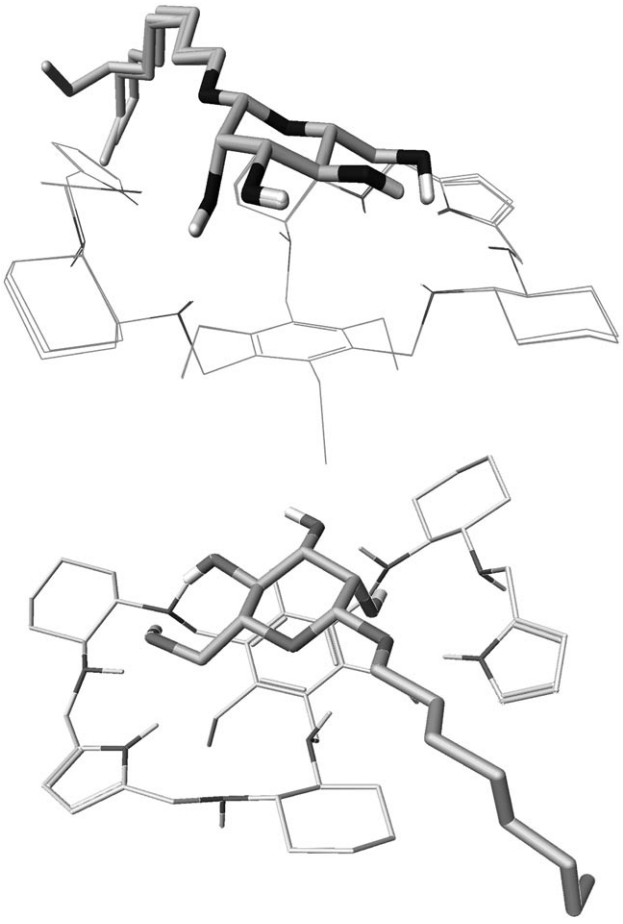


Figure 3. Top: Superimposition of the three energy minima structures as obtained from the molecular modeling protocol. The relative orientation (type-B arrangement) of the sugar versus the receptor is depicted. The flexible arm may adopt different orientations (see Supporting Information). Bottom: Structure of the global minimum geometry of the type-B family.

Remarkably, the hydrogen-bond between a pyrrolic NH and the axial mannosyl OH is the most conserved interaction among the whole set of structures; likewise, in both families the glycosidic chain lies above a pyrrolic ring, in agreement with the unexpected upfield shift experienced by the H-7, H-8, and H-9 protons of the octyl chain. Moreover, in all conformers the β-face of mannose lies on top of the benzene ring of the receptor, in a roughly face-to-face disposition. Such an arrangement suggests that strong hydrogen bonding, particularly to the axial hydroxyl, and additional CH–π interactions may be responsible for the observed affinity for the β anomer of mannosides.

In conclusion, in the present communication we have described the prototype of a new family of chiral receptors based on a tripodal scaffold and featuring pyrrolic binding arms containing the *trans*-1,2-diaminocyclohexane motif, which enantioselectively recognizes β-mannose and β-mannosides with remarkable affinity in a polar medium (aceto-

nitrile), distinct selectivity with respect to the α-anomer, and the highest enantioselectivity of the all-*S* with respect to the all-*R* receptor reported to date. A detailed analysis of the structure in solution from NMR experimental data and molecular modeling calculations revealed strong hydrogen bonding, particularly to the axial hydroxyl of mannose, CH–π interactions between the glycosidic chain and pyrrolic rings, and van der Waals interactions of the β face of the sugar with the benzene ring of the scaffold as the main forces responsible for the observed recognition properties.

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Keywords: carbohydrates · chiral receptors · molecular recognition · NMR spectroscopy · structure elucidation

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