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A β-Mannoside-Selective Pyrrolic Tripodal Receptor

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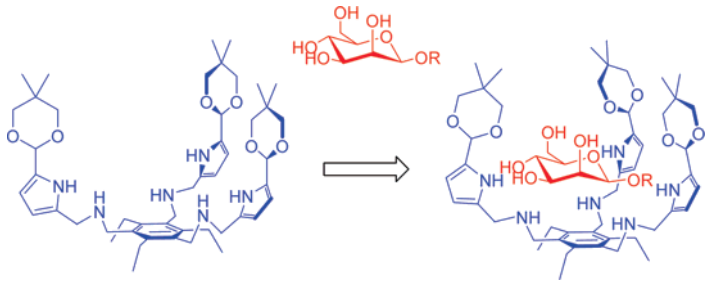
ABSTRACT



Acetalic substituents strategically located in a pyrrolic tripodal structure provide a new synthetic receptor endowed with unprecedented affinity for mannosides and the highest selectivity for β-mannose ever reported for synthetic H-bonding receptors. Binding properties have been determined by NMR, ITC, and ESI-MS techniques, while affinities have been univocally assessed by the BC_{50}^{0} parameter, a general descriptor of binding affinity.

There has been considerable interest in recent years in synthetic receptors for molecular recognition of carbohydrates,[1] fueled by the discovery that many biological processes crucial for living cells rely on recognition of specific saccharides.[2] Among the monosaccharides involved in recognition processes, D-mannose, both as α and β anomers, is ubiquitously found in glycoconjugates and essential for various biological functions. For example, mannose is the terminal sugar of most N-glycans,[2] acting as a marker in recognition processes, and is found in unusual density on the glycan shield of HIV.[3] On the other hand, mannose is specifically recognized by several proteins widespread in living organisms, from viruses and bacteria to plants and animals, such as the lectin concanavalin A (Con A), the mannose binding proteins (MBPs), and the human mannose receptor (hMR).[2]

Despite the prominent role of mannose in glycobiology, most synthetic receptors reported in the literature preferentially bind to glucose,[1] whereas receptors showing some level of preference for mannose are rare,[4] and as far as we are aware of, neutral synthetic H-bonding receptors specifically recognizing mannose have not yet been reported. In the

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present Communication, we wish to describe a new member of a family of tripodal receptors that specifically recognizes mannosides, even in a polar solvent, with unprecedented affinity and, to the best of our knowledge, with the best selectivity reported up to date for the β-mannosyl residue.

We recently described a new generation of cyclic[5] (**1**) and acyclic[6] (**2**) receptors featuring pyrrolic binding sites, which proved to effectively and selectively bind to monosaccharides (Figure 1). In an effort to expand on recognition properties,

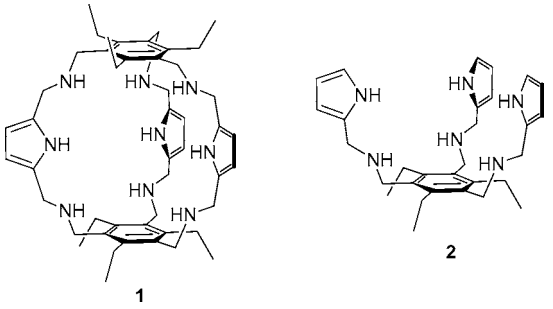
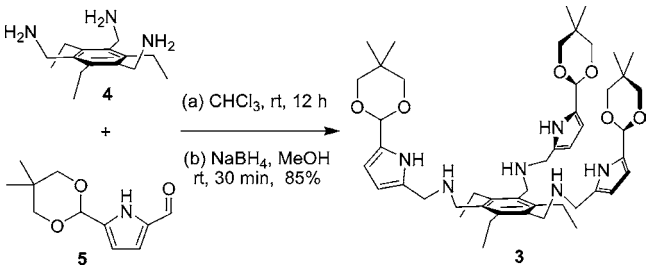


Figure 1. Pyrrolic receptors.

we thought these could be significantly improved by implementing additional H-bonding groups into the scaffold of **2**. Molecular models (see Supporting Information) suggested, although only qualitatively, that acetalic substituents located in the α position of pyrroles may assume a convergent geometry which may be appropriate for binding, acting as H-bonding acceptors.

The triacetalic receptor **3** was readily available in 85% yield by condensation of the triamine **4** with the appropriate aldehyde **5**[7] through a previously described procedure (Scheme 1).[6]

Scheme 1. Synthesis of Receptor **3**



The recognition properties of **3** were tested versus the set of octyl glycosides of the monosaccharides in Figure 2, most

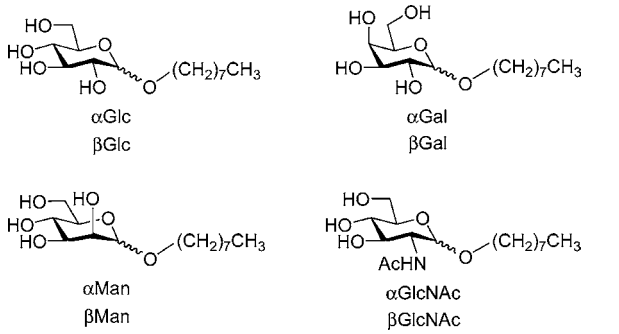


Figure 2. Monosaccharides tested in recognition experiments.

often found as epitopes in recognition events. Association constants were measured by ^{1}H NMR titrations in $CDCl_3$ at $T = 298$ K, following a previously established protocol;[8] results are reported in Table 1 as cumulative binding

Table 1. Cumulative Association Constants (log β_n) for 1:1 and 2:1 Complexes of Receptor **3** and Octyl Glycosides and Corresponding BC_{50}^0 (μM) Values for **3** and **2** in $CDCl_3$[a]

glycoside	log β_{11}	log β_{21}	BC_{50}^0 (**3**)	BC_{50}^0 (**2**)[b]
αGlc	3.23 ± 0.01	4.98 ± 0.16	570 ± 20	570 ± 20
βGlc	4.40 ± 0.04	7.30 ± 0.09	39 ± 3	24 ± 2
αGal	2.651 ± 0.005	n.d.[c]	2250 ± 20	790 ± 20
βGal	3.730 ± 0.002	5.04 ± 0.05	185 ± 1	70 ± 1
αMan	5.54 ± 0.12	9.71 ± 0.18	2.8 ± 0.7	43 ± 1
βMan	*d*	*d*	<1	37 ± 1
αGlcNAc	5.18 ± 0.02	8.94 ± 0.04	6.4 ± 0.3	72 ± 7
βGlcNAc	5.14 ± 0.03	9.08 ± 0.04	6.9 ± 0.5	18 ± 1

[a] Measured by ^{1}H NMR (400, 900 MHz) from titration experiments at $T = 298$ K on 0.2−1.1 mM stock solutions of glycoside for receptor concentrations up to 20 mM. For **3**, log $\beta_{dim} = 0.075 \pm 0.017$. BC_{50}^0 calculated using the "BC_{50} Calculator" (see Supporting Information). [b] Data from ref 6. [c] Nondetectable. [d] Too large to be measured.

constants (see Supporting Information). Since 1:1 and 2:1 host-to-guest adducts were detected, in addition to dimerization of the receptor, affinities were assessed by the BC_{50}^0 parameter,[6,8] a generalized affinity descriptor univocally defining the binding ability of a receptor in chemical systems involving multiple equilibria. 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; thus, the lower BC_{50}^0, the higher the affinity. The BC_{50}^0 values calculated from cumulative binding constants are reported in Table 1, together with those previously obtained for the parent receptor **2**[6] for direct comparison. It is clearly

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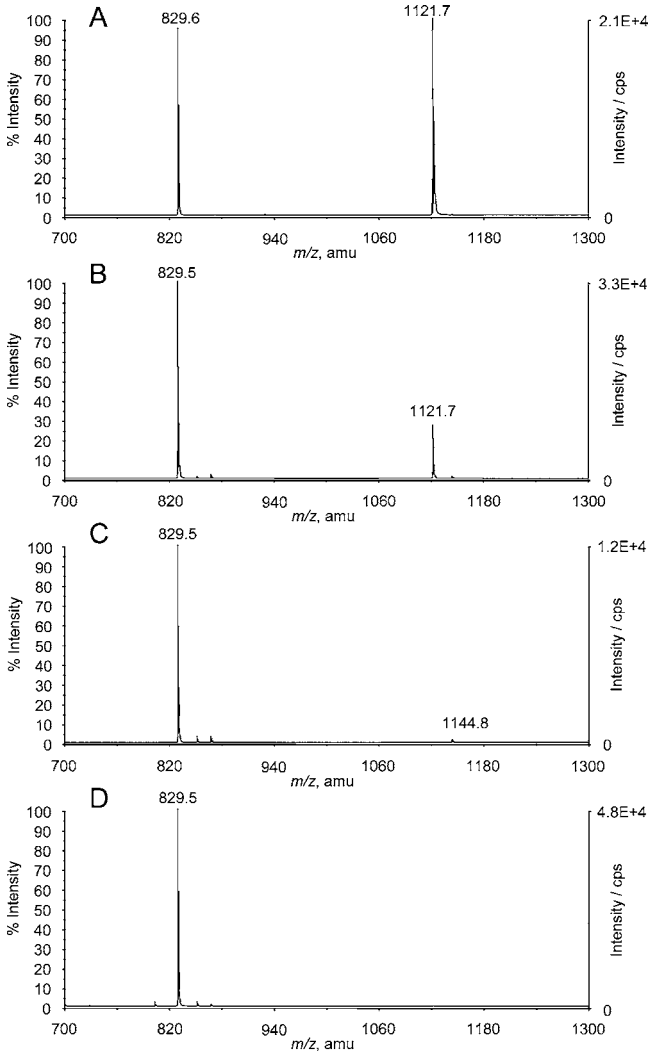

Figure 3. (+) ESI-MS spectra of: (A) **3** + βMan, 0.2 mM each; (B) **3** + αMan, 0.2 mM each; (C) **3** + αGal, 0.2 mM each; (D) **3** + βGlc pentaacetate, 0.2 mM each; *m/z* 829.5, [**3** + H]$^+$; *m/z* 1121.7, [**3**•glycoside + H]$^+$; *m/z* 1144.8, (impurity). Solvent = CH_3CN; ESI voltage = 6 kV; orifice = 46 V.

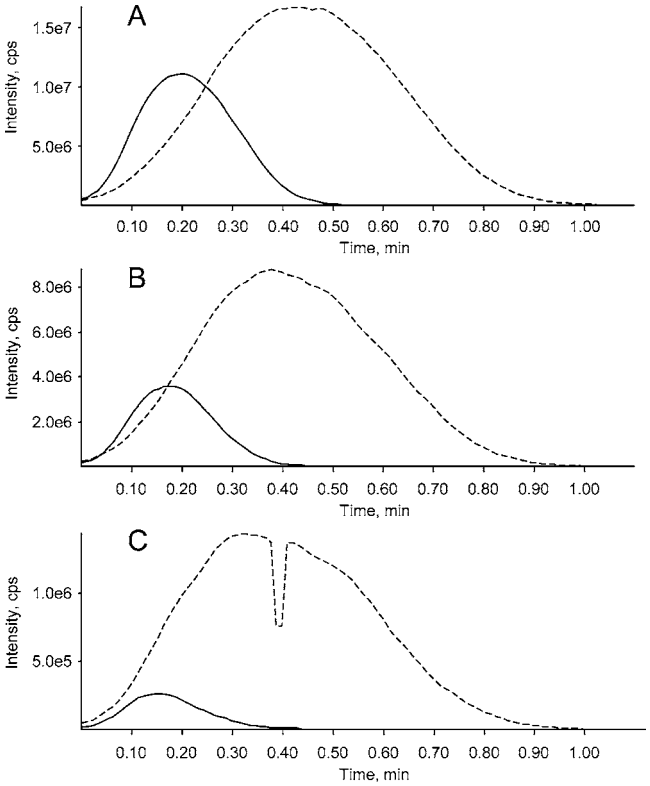

Figure 4. CID MS/MS analysis of the complex detected as the [M + H]$^+$ ion at *m/z* 1121.7. Products: 1121.7 ([M + H]$^+$, solid line), 829.5 ([**3** + H]$^+$, dotted line). Solvent = CH_3CN; ESI voltage = 6 kV; sampling cone potential = 46 V; signal acquisition = 1.11 min, 109 scans over collision energies from 6 to 60 eV in 0.5 eV steps; collision gas pressure (*P*) = 3.0×10^{-5} Torr. (A): **3** + βMan, 0.2 mM each; crossing point = 19.0 eV. (B): **3** + αMan, 0.2 mM each; crossing point = 14.5 eV. (C): **3** + αGal, 0.2 mM each.

apparent that, compared to **2**, affinities are lower for Gal but higher for GlcNAc and even more for Man, spanning a selectivity range exceeding 3 orders of magnitude. However, the most striking result is the affinity exhibited for βMan, which is estimated in the nanomolar range. Indeed, binding constants were too large to be measured by ^{1}H NMR, but an upper limit of 1 μM for BC_{50}^0 could be inferred by comparison with the titration data of αMan. As far as we are aware, this is the largest affinity ever reported for a synthetic receptor for mannose. Selectivity versus other glycosides is also noteworthy, with an outstanding factor of more than 800 between αMan and αGal, and expectedly much larger between βMan and αGal. In contrast, poorer discrimination is observed between αMan and α- and βGlcNAc, although selectivity may be anticipated to be significant for βMan. We believe this affinity pattern has to be ascribed to the additional H-bonding interactions that both the amidic NH of GlcNAc and the axial hydroxyl of Man, in contrast to other glycosides, can establish with the acetal group. Support for this interpretation is provided by the strong downfield shift of the amidic NH proton observed upon binding for both anomers of GlcNAc, indicative of H-bond formation and absent with a lack of acetalic substituents, concomitant to the corresponding upfield shift of the acetyl protons, due to aromatic ring current shielding, both pointing to an orientation of the acetylamino moiety orthogonal to the pyranose ring plane, with the NH bond directed toward the acetalic groups in a fashion similar to that of the axial hydroxyl of mannose (see Supporting Information).[9]

To assess the affinity of **3** for βMan, rather than evaluating binding constants by a different technique, we thought it would be more informative and significant to measure affinities in a more competitive solvent. Association constants measured in CD_3CN are reported in Table 2, together with the corresponding BC_{50}^0 values.

(9) Unfortunately, direct evidence of H-bonding to the axial hydroxyl of Man cannot be obtained, as glycosidic hydroxyl protons, receptor's aminic protons, and water's protons collapse into an average signal.

Table 2. Cumulative Association Constants (log β_n) for 1:1 and 2:1 Complexes of **3** with Octyl Glycosides in CD_3CN, Corresponding BC_{50}^0 (μM) Values, and Affinity Ratios (AR) between BC_{50}^0 Values in CD_3CN and $CDCl_3$[a]

glycoside	log β_{11}	log β_{21}	BC_{50}^0	AR
αGlc	1.592 ± 0.008	n.d.[b]	25600 ± 500	45
βGlc	2.100 ± 0.003	n.d.[b]	7940 ± 50	204
αGal	1.55 ± 0.01	n.d.[b]	28300 ± 800	13
βGal	1.988 ± 0.002	n.d.[b]	10290 ± 50	56
αMan	2.233 ± 0.003	n.d.[b]	5850 ± 40	2090
βMan	3.12 ± 0.02	5.40 ± 0.08	680 ± 30	>680
αGlcNAc	2.231 ± 0.002	n.d.[b]	5880 ± 20	919
βGlcNAc	2.155 ± 0.003	n.d.[b]	6990 ± 50	1013

[a] Measured by ^{1}H NMR (400, 900 MHz) from titration experiments at T = 298 K on 1.0−1.2 mM stock solutions of glycoside for receptor concentrations up to 35 mM; β_{dim} nondetectable. BC_{50}^0 calculated using the "BC_{50} Calculator" (see Supporting Information). [b] Nondetectable.

It is easily appreciated that, even in a polar solvent, affinities still lie in the low millimolar range, with the notable exception of βMan, which is bound to **3** with an affinity in the micromolar range and with a β/α selectivity factor of nearly an order of magnitude. On the assumption that in acetonitrile affinities of both anomers of mannose are damped to the same extent with respect to chloroform (cf. Figure S5, Supporting Information), we can estimate the affinity of **3** for βMan to be 330 nM in $CDCl_3$, a figure that confirms its unprecedented recognition properties. As a general evidence, affinities are attenuated with respect to $CDCl_3$ to a much greater extent for Man and GlcNAc than for the other glycosides. While supporting the occurrence of an additional H-bonding to the acetal groups, the evidence suggests that this H-bond is probably the most exposed, being the most affected by competition with the polar solvent. Gratifyingly, results were confirmed by ITC measurements in CH_3CN, which gave affinities for αMan and βMan in good agreement with NMR data and evidenced a substantial enthalpic contribution, compensated by an adverse entropic contribution (see Supporting Information).

Some structural evidence of the receptor−glycoside complexes was highly desirable, but unfortunately, all attempts to obtain X-ray quality crystals failed, giving oils or glassy solids from various solvents. Analogously, while 2D NMR experiments in solution did not give any NOE correlations due to fast exchange of partners on the NMR time scale, chemical shift variations did not provide a consistent picture for different glycosides. On the other hand, due to the very adaptive nature of the receptor, molecular mechanics calculation performed on the βMan complex did not give a convincing description of a reliable complex structure. Nevertheless, due to a lack of direct structure information, binding results were independently confirmed in the gas phase by ESI-MS experiments. In the positive ion mode ESI-MS spectra of equimolar mixtures of **3** and βMan, αMan, and αGal, respectively (Figure 3), the peak of the 1:1 complex was observed as the base peak for βMan, with intensity comparable to the peak of the free receptor, but reduced to 30% for αMan, whereas it could only be detected at the noise level for αGal. Instead, βGlc pentaacetate gave no evidence of complexation, showing that, in the absence of H-bonding hydroxyl groups, recognition of a strongly bound glucoside is depleted.

Definitive evidence of complex stability was obtained from the above mixtures by ESI-MS/MS collision-induced dissociation (CID) experiments (Figure 4). CID profiles gave 50% of complex dissociation for collision energies of 19.0 and 14.5 eV for βMan and αMan, respectively, showing a significantly higher stability of the βMan complex, whereas spontaneous dissociation of the adduct was observed for αGal.

In conclusion, we have described a pyrrolic tripodal receptor exhibiting the best recognition properties toward mannosides ever reported for a H-bonding synthetic receptor. Results demonstrate that implementing additional H-bonding substituents, strategically located into the architecture of the pyrrolic tripodal receptor, can dramatically enhance both the affinity for a specific glycoside and selectivity with respect to other monosaccharides.

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Supporting Information Available: General methods, materials, and synthetic procedures; details of titrations and data analysis; plots of ITC and plots of affinities. This material is available free of charge via the Internet at http://pubs.acs.org.

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