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A Tricatecholic Receptor for Carbohydrate Recognition: Synthesis and Binding Studies

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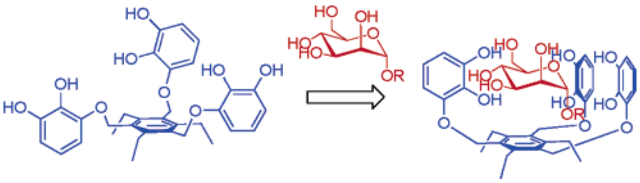
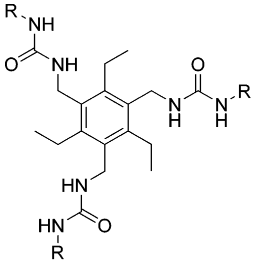


A new tripodal receptor bearing three catechol subunits on a benzene platform has been synthesized in four steps from 1,3,5-triethylbenzene and pyrogallol. The binding ability of the tricatecholic receptor was investigated toward several monosaccharides in $CDCl_3$, where multiple equilibria were detected, and compared to that of a previously reported trisureidic receptor of analogous structure. Association constants were measured by ^1H NMR titrations, and the corresponding affinities were assessed through the BC_{50} parameter, a binding descriptor univocally defining the affinity of a host for a guest in multi-equilibrium systems. Results show that the tripodal catecholic receptor binds the octyl glycosides with affinities ranging from 0.87 to 5.2 mM and with a 6-fold selectivity factor for the α-mannoside over the *β*-glucoside. Although the affinity for glycosides was not appreciably improved with respect to the ureidic receptor, a significant change in selectivity was obtained by the H-bonding group replacement.

Molecular recognition of carbohydrates has been an actively investigated area of research in the past few years[1] because selective recognition of saccharides plays a crucial role in various biological processes, such as cell adhesion, regulation, and growth.[2] Structurally diverse synthetic receptors have been employed to tackle the challenging task of understanding the molecular basis of the recognition processes occurring in Nature, among which neutral hosts designed to interact with mono- or oligosaccharides through H-bonding have been by far the most studied.[3] In this context, we recently reported a new tripodal receptor **1** featuring ureidic H-bonding groups for the recognition of saccharides, which showed affinities in the millimolar range and moderate selectivities toward the octyl glycosides of a set of monosaccharides in $CDCl_3$.[4]



1 R = *tert*-octyl

In search for new receptors of improved recognition ability, we thought that the ureidic groups on the side arms of **1** could be conveniently replaced by catecholic ligands, in the belief that acidic hydroxyls in a chelating arrangement would advantageously substitute the ureidic NH functions as H-bonding partners for glycosidic hydroxyls, still retaining the flexibility of the tripodal architecture and the binding geometry of the bidentate ligands, which was shown to provide the correct geometry for binding monosaccharides.[4] From a literature search, it appeared that catechol ligands, widely found in cation complexes and in natural and synthetic ionophores,[5] are yet unexplored as neutral H-bonding ligands for carbohydrates; thus, tripodal catecholic receptors may be interesting new candidates.[6] We wish to report the synthesis and carbohydrate binding properties of a new tripodal benzene-based catecholic receptor, showing preference for α-mannoside among octyl glycosides of biologically relevant monosaccharides.

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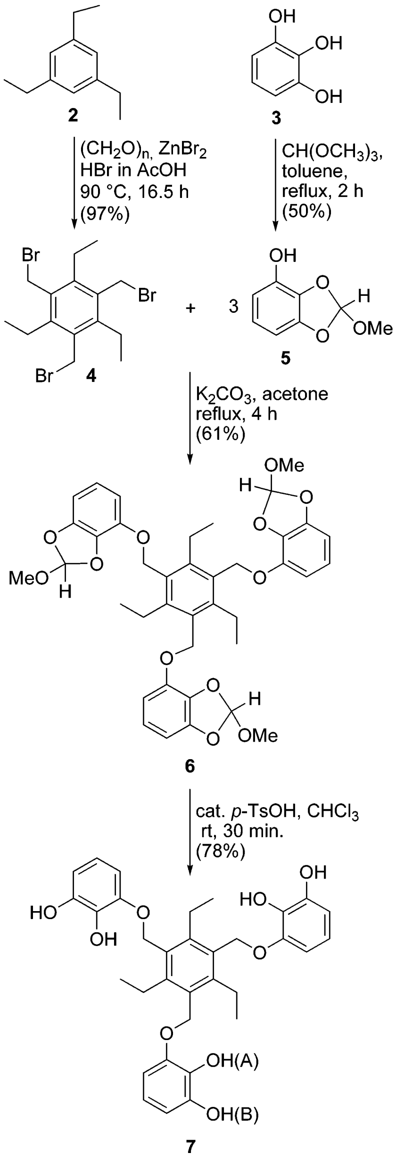
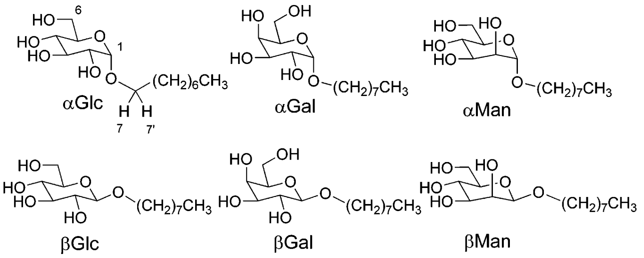
SCHEME 1. Synthesis of the Tripodal Catecholic Receptor 7



Synthesis. Catecholic substituents were conveniently introduced into the tripodal scaffold by ether linkages through the synthetic steps reported in Scheme 1.

1,3,5-Tris(bromomethyl)-2,4,6-triethylbenzene (**4**), prepared from the commercially available 1,3,5-triethylbenzene **2**,[7] was reacted with the protected pyrogallol derivative **5** to afford the protected tricatecholic derivative **6**. Protection as the orthoester resulted in being the method of choice; the alternative protection of pyrogallol as the acetonide proved not to be a viable alternative, as subsequent deprotection failed under several conditions. Attempts to protect pyrogallol by reacting **3** with di-*tert*-butyldichlorosilane or ethyl propiolate failed, as well. On the other hand, protection of the hydroxyl groups was mandatory, as reacting **4** with unprotected pyrogallol consistently gave untractable mixtures. Deprotection of **6** to the catecholic receptor **7** was eventually accomplished by acidic cleavage.

CHART 1



Binding Studies. The binding affinities of receptor **7** toward the octyl glycosides of monosaccharides depicted in Chart 1 were measured by ^{1}H NMR titrations in $CDCl_3$ at $T = 298$ K. Glucose (Glc), galactose (Gal), and mannose (Man) were selected among the most frequently encountered monosaccharidic epitopes, present as terminal α- or β-glycosides in more complex oligo- or polysaccharides on cell surfaces and in glycoconjugates.

Addition of increasing amounts of **7** to the glycosidic guests induced a significant shift of the signals of both reagents, clearly indicating binding phenomena under fast exchange, which were particularly evident for catecholic and glycosidic hydroxyl protons and for some of the glycosidic CH protons. The upfield shift of the latter protons and the downfield shift of the former protons (except for OH(A), see later), which could be monitored if acidic impurities were carefully removed from the medium, were consistent with the shielding effect of the aromatic ring current and the deshielding effect from H-bonding, respectively, in agreement with the formation of a host−guest complex in which the glycoside is located in the cleft of the receptor. The titration of βGal with **7** is reported in Figure 1 as a typical example.

An in depth investigation of all the aspects involved in the correct determination of binding affinities toward glycosides by NMR titrations (reactant self-association, chemical model, choice of signals, etc.) has been described in a previous paper.[4] In the present study, we closely followed the established titration protocol and the optimized analysis of data, which consisted of diluting the receptor with a stock solution of the glycoside and of the simultaneous fit of all the available signals of both reagents to the appropriate model of chemical equilibria, respectively. As with the ureidic receptor **1**, experimental data were not adequately fitted by a simple 1:1 association. Binding of the receptor to the investigated glycosides required a model including 1:1 and 2:1 association equilibria in all cases, while no evidence of reagent self-association was found from dilution experiments. The excellent agreement between experimental and calculated shifts for all the detected signals can be easily appreciated from the plots in Figure 1. The results obtained with the glycosides of Chart 1 are reported in Table 1 as cumulative log β values for the formation of the 1:1 and 2:1 receptor to glycoside adducts. Because more than one complex species is present in all cases, binding constants cannot be directly used to assess the overall affinity of the receptor for each glycoside. This can be conveniently determined using the BC_{50} parameter,[4] a binding descriptor univocally defining the affinity of a host for a guest in multi-equilibrium systems, which can be calculated from the measured binding constants.[8] The BC_{50} parameter is defined as the total concentration of receptor necessary to bind

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(7) Walsdorff, C.; Saak, W.; Pohe, S. *J. Chem. Res. (M)* **1996**, 1601−1618.

(8) To expedite the calculation of BC_{50} and BC_{50}^0, a utility program based on the general treatment described in ref 4 has been developed and made available for free upon request at the corresponding author's e-mail address.

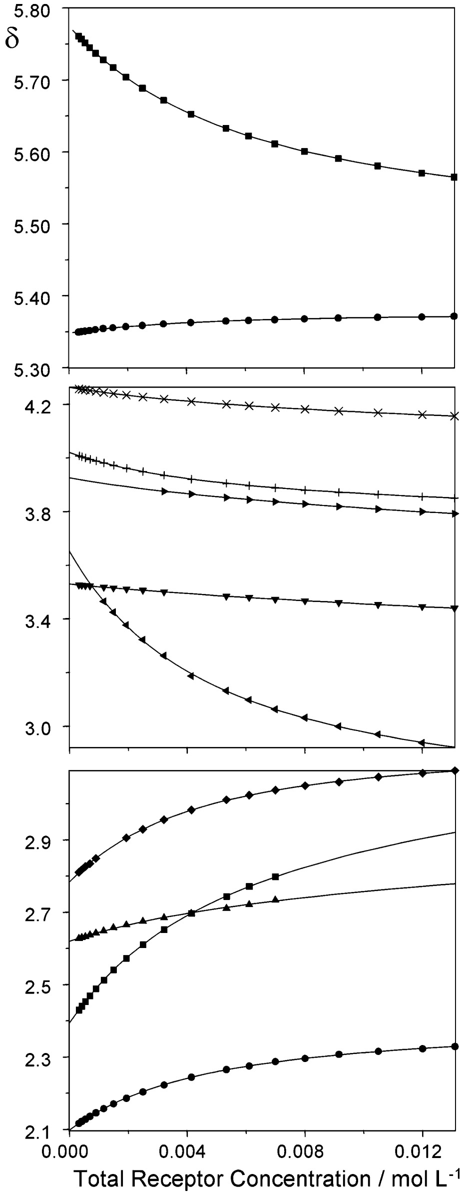

FIGURE 1. Plot of complexation induced shifts versus receptor's total concentration in the titration of βGal 1.1 mM with **7** in $CDCl_3$ at 400 MHz and $T = 298$ K. Bottom: galactose OH signals (● OH-6; ■ OH-2; ▲ OH-3; ♦ OH-4). Middle: galactose CH signals (← CH-2; ▼ CH-7; → CH-7′; + CH-4; × CH-1). Top: receptor's catechol OH signals (● OH−B; ■ OH-A). Symbols are experimental data points; solid lines are best fit curves obtained through nonlinear regression by simultaneous fit of all data.

TABLE 1. Cumulative Association Constants (log β_n) with Standard Deviations (σ) for 1:1 and 2:1 Adducts, Intrinsic Median Binding Concentration (B C$^0_{50}$), and Relative Affinities (RA) for Complexes of Receptor 7 with Octyl Glycosides[a]

glycoside	log β_{11}	log β_{21}	BC$^0_{50}$ (mM)	RA
αMan	3.03 ± 0.04	5.04 ± 0.04	0.87 ± 0.07	6.0
βMan	2.59 ± 0.07	4.42 ± 0.13	2.2 ± 0.3	2.4
βGal	2.46 ± 0.03	3.85 ± 0.21	3.2 ± 0.2	1.6
αGlc	2.39 ± 0.06	3.94 ± 0.16	3.6 ± 0.4	1.4
αGal	2.33 ± 0.04	3.98 ± 0.08	4.0 ± 0.3	1.3
βGlc	2.25 ± 0.05	3.48 ± 0.26	5.2 ± 0.6	1.0

[a] Measured by ^{1}H NMR (400 MHz) from titration experiments at $T = 298$ K in $CDCl_3$ on 0.8−1.2 mM stock solutions of glycoside using receptor concentrations up to 15 mM. Binding constants β_{11} (M^{-1}) and β_{21} (M^{-2}) were calculated by simultaneous nonlinear least-square fit of all the available signals shifts. BC$^0_{50}$values were calculated from log β_i values using the BC$_{50}$ Calculator program; see ref 8.

50% of the ligand and is most conveniently employed as its limiting value for dilute solutions, the *intrinsic median binding concentration* BC$^0_{50}$, which is independent of the concentration of the ligand. BC$^0_{50}$ values calculated from the binding constants are reported in Table 1 for each glycoside, together with the corresponding relative affinity derived from normalized B C$^0_{50}$ values.

The overall impression from results in Table 1 is that replacement of the catecholic for the ureidic binding groups in the tripodal scaffold did not bring a significant improvement in the binding ability of receptor **1**. Indeed, binding affinities ranging from nearly 1 to 5 mM were observed for **7**, very similar to those obtained for **1** (1−6 mM),[4] indicating that the tripodal receptor is substantially insensitive to the described binding group substitution, although their H-bonding properties were expected to be quite different. In contrast, the selectivity trend exhibited by **7** toward the investigated glycosides is significantly different from that observed for the ureidic progenitor: the most remarkable feature is that, while αMan is the least effectively bound glycoside in the case of **1**, it is the most effectively bound to **7**, showing a 6-fold preference factor over βGlc. Such an inversion of selectivity, although not impressive in size, is certainly noteworthy and is most likely related to a change in binding geometry achieved by the catecholic receptor with respect to the ureidic host. Unfortunately, a convincing interpretation of the complex structure and geometry could not be inferred from the chemical shift variations of the signals of both reagents upon binding. The only clear indication seems to be the systematic upfield shift contribution to the catecholic OH protons, which for the hydroxyl closest to the aromatic platform largely overrides the downfield shift due to H-bonding, indicating that a conformational rearrangement occurs upon binding that orientates the catechol hydroxyl groups toward the interior of the cleft, where they experience the ring current shielding effect. Besides this evidence, it does not appear fortuitous that the axial mannose hydroxyl is the only glycosidic OH signal of the entire investigation shifting upfield upon complexation, and that concomitantly, apart from the H-2 signal, the largest upfield shifts observed are those of the axial H-4 proton of the glycoside and the OH(A) proton of the catechol moiety, suggesting that all four signals reside in the shielding cone of the arene's ring current. This is compatible with a complex's geometry in which the β face of the mannoside is facing the scaffold's arene moiety and in which the inner catecholic hydroxyl lies in close proximity to the axial mannoside hydroxyl and in a correct orientation for H-bonding. Whatever the structural situation, experimental evidence demonstrates that the selectivity of the tripodal receptor can be modulated by appropriately changing the bidentate H-bonding groups, switching preference from βGal to αMan by replacing catecholic for ureidic ligands.

In conclusion, the development of structures bearing different subunits on a benzene-based platform reveals the possibility of modulating the binding selectivity toward different series of monosaccharides depending on the nature of the binding groups. Although a significant improvement in binding ability was not achieved by replacing catecholic for ureidic binding groups in the receptor's side arms, this work demonstrated that the tripodal catecholic receptor **7** is able to bind the set of octyl glycosides in Chart 1 with millimolar affinities in $CDCl_3$ and with a significant selectivity for αMan over *β*Glc.

Experimental Section

1,3,5-Tris(2′-methoxy-1′,3′-benzodioxol-4′-oxymethyl)-2,4,6-triethylbenzene (6): To a solution of **4** (1.81 g, 4.10 mmol) and **5** (2.09 g, 12.4 mmol) in acetone (120 mL) was added K_2CO_3 (5.30 g, 38.4 mmol), and the mixture was heated under reflux for 4 h. The reaction mixture was cooled to room temperature, filtered, and concentrated under vacuum to give **6** (1.74 g, 2.48 mmol, 61%) as a white solid, which was recrystallized from methanol: Mp 92−94 °C; ^1H NMR ($CDCl_3$, 200 MHz) δ 6.96−6.61 (m, 12H), 5.25 (s, 6H), 3.42 (s, 9H), 2.56 (q, $J = 7.4$ Hz, 6H), 1.28 (t, $J = 7.4$ Hz, 9H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 147.5, 146.6, 142.5, 134.5, 130.8, 122.0, 119.2, 109.9, 102.2, 66.1, 49.9, 23.1, 16.6. Anal. Calcd for $C_{39}H_{42}O_{12}$: C, 66.66; H, 6.02. Found: C, 66.52, H, 5.91.

1,3,5-Tris(2′,3′-dihydroxyphenoxymethyl)-2,4,6-triethylbenzene (7): To a solution of **6** (0.360 g, 0.510 mmol) in $CHCl_3$ (5 mL) was added *p*-toluenesulfonic acid (0.15%) as catalyst. The reaction mixture was stirred at room temperature for 30 min, then washed with saturated solution of $NaHCO_3$, and dried over Na_2SO_4. Concentration of the solvent and purification of the crude by flash column chromatography (silica gel, $CHCl_3/CH_3OH$ 9:1) gave **7** (0.230 g, 0.399 mmol, 78%) as a white solid: Mp 178−180 °C; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.86−6.82 (m, 3H), 6.72−6.66 (m, 6H), 5.37 (br s, 3H, OH(A)), 5.30 (br s, 3H, OH(B)), 5.15 (s, 6H), 2.86 (q, $J = 7.6$ Hz, 6H), 1.25 (t, $J = 7.6$ Hz, 9H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 147.0, 146.3, 144.4, 132.7, 130.9, 119.9, 109.4, 103.9, 64.9, 22.9, 16.7; MS-EI *m*/*z* (%) 577(M^+, 17), 448 (23), 326 (23), 199 (26). Anal. Calcd for $C_{33}H_{36}O_9$: C, 68.74; H, 6.29. Found: C, 68.96, H, 6.30.

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Supporting Information Available: General methods and materials. Synthetic procedure for compound **5**. Titration procedure, data analysis, and plots of experimental and calculated shifts for the titration of αMan with **7**. NMR spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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