A New Tripodal Receptor for Molecular Recognition of Monosaccharides. A Paradigm for Assessing Glycoside Binding Affinities and Selectivities by 1H NMR Spectroscopy

Alberto Vacca,[†] Cristina Nativi,[‡] Martina Cacciarini,[‡] Roberto Pergoli,[‡] and Stefano Roelens*,[§]

Contribution from the Dipartimento di Chimica, Dipartimento di Chimica Organica, and CNR, Istituto di Chimica dei Composti Organometallici, Dipartimento di Chimica Organica, Università di Firenze, Polo Scientifico, I-50019 Sesto Fiorentino, Firenze, Italy

Received July 12, 2004; E-mail: stefano.roelens@unifi.it

Abstract: A new tripodal receptor for the recognition of monosaccharides is described. The prototypical host **1** features a 1,3,5-substituted 2,4,6-triethylbenzene scaffold bearing three convergent H-bonding units. The binding ability of the *t*-octyl derivative **1a** toward a set of octylglycosides of biologically relevant monosaccharides, including Glc, Gal, Man, and GlcNAc, was investigated by 1H NMR in CDCl$_3$. A protocol for the correct evaluation of binding affinities was established, which can be generally applied for the recognition of monosaccharides by 1H NMR spectroscopy. A three-constant equilibrium model, including 1:1 and 2:1 host–guest association and dimerization of the receptor, was ascertained for the interaction of **1a** with all the investigated glycosides. An affinity index, which we defined *median binding concentration* BC$_{50}$ in analogy to the IC$_{50}$ parameter, intended to address the general issue of comparing dimensionally heterogeneous binding data, and a limiting BC$_{50}^0$ quantity describing *intrinsic* binding affinities were developed for evaluating the results. BC$_{50}^0$ values for **1a** range from 1 to 6 mM, indicating an *intrinsic* binding affinity in the millimolar range and a selectivity factor of 5 toward the investigated glycosides. The treatment has been extended to include any generic host–guest system involved in single or multiple binding equilibria.

Introduction

Molecular recognition of carbohydrates is an actively investigated topic in bioorganic chemistry.[1] A strong impulse is given by the number of biological processes relying on molecular recognition of carbohydrates, such as cell adhesion, cell infection, and immune response.[2] In addition, molecular recognition events are involved in carbohydrate metabolism and transport and in regulation of enzyme activity.[2] The poor understanding of the principles governing molecular recognition of carbohydrates at the molecular level has stimulated an intense

research largely pursued through synthetic receptors, which could be appropriately designed and modified to selectively recognize specific saccharides and unravel the factors involved and the criteria required for effective recognition.[1a,e,f] Given the complexity of recognition of glycoconjugates, the attention has been mainly focused on monosaccharides or short oligosaccharides. This crude simplification has been based on the evidence that in biological events, even for complex polysaccharides, only the terminal mono- or oligosaccharides are usually deputed to recognition processes.[2]

Although molecular recognition of carbohydrates is relevant in water, most studies with synthetic receptors have been conducted in lipophilic solvents, often in CDCl$_3$, for several reasons: binding properties can be conveniently detected and quantitatively measured by NMR spectroscopy, the investigated receptors are often soluble in organic solvents but not in water, and most important, H-bonding interactions are enhanced, often dramatically, compared to water and other competitive polar media. A noncompetitive organic solvent represents therefore a convenient environment for the screening of candidate receptors, even though intramolecular H-bonding of saccharides must be overcome for binding to occur. Saccharides are, however, insoluble in CDCl$_3$ and other lipophilic solvents. This issue has been addressed by making use of mono- and

(1) (a) Striegler, S. *Curr. Org. Chem.* **2003**, *7*, 81−102. (b) Dam, T. K.; Brewer, C. F. *Chem. Rev.* **2002**, *102*, 387−429. (c) Mellet, C. O.; Defaye, J.; García Fernández, J. M. *Chem.−Eur. J.* **2002**, *8*, 1982−1990. (d) Host−Guest Chemistry. Mimetic Approaches to Study Carbohydrate Recognition. In *Topics in Current Chemistry*; Penadés, S., Ed.; Springer-Verlag: Heidelberg, 2002; Vol. 218. (e) Davis, A. P.; Wareham, R. S. *Angew. Chem., Int. Ed.* **1999**, *38*, 2978−2996. (f) James, T. D.; Sandanayake, K. R. A. S.; Shinkai, S. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1910−1922. (g) Lee, Y. C.; Lee, R. T. *Acc. Chem. Res.* **1995**, *28*, 321−327.
(2) (a) Ernst, B.; Hart, W.; Sinay, P. *Carbohydrates in Chemistry and Biology*; Wiley−VCH: Weinheim, 2000; Part II, Vol. 4. (b) Lindhorst, T. K. *Essentials of Carbohydrate Chemistry and Biochemistry*; Wiley−VCH: Weinheim, 2000. (c) *Essentials of Glycobiology*; Varki, A., Cummings, R., Esko, J., Freeze, H., Hart, G., Marth, J., Eds.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1999.

oligosaccharides glycosylated with lipophilic groups, such as aromatics and alkyl chains, which are more or less freely soluble in organic solvents and allow a direct evaluation of receptor's binding capabilities. A quite large number of studies on synthetic receptors and their binding properties toward glycosidic guests in organic solvents has thus become available in the past few years.[3−17] Although some interesting results have been published, the comprehension of the principles underlying recognition and the search for efficient, selective receptors are yet challenging goals.

Among the different artificial receptors reported up to date, benzene-based tripodal receptors have been extensively exploited for binding cations and anions[18] but are still largely unexplored for the recognition of saccharides.[8,12] In this context, we designed a new prototypical tripodal receptor for the selective recognition of monosaccharides, featuring a triethylbenzene scaffold bearing three convergent H-bonding units (**1**, see Scheme 1). We thought that the adaptive nature of this architecture, together with the capability of establishing both H-bonding and hydrophobic interactions with the carbohydrate moiety,[19] could be particularly useful to gather information on

Scheme 1. Synthesis of Receptor 1
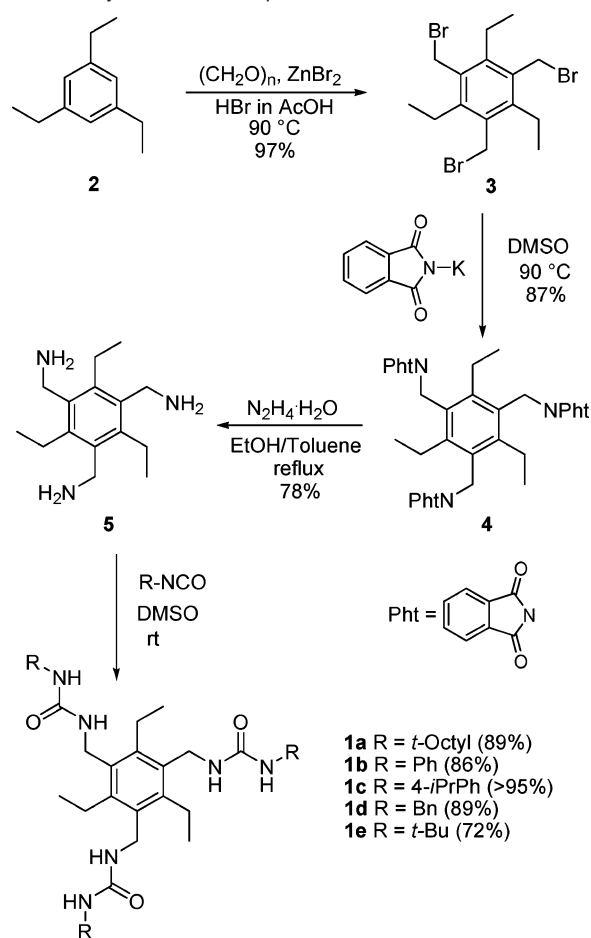
1a R = *t*-Octyl (89%)
1b R = Ph (86%)
1c R = 4-*i*PrPh (>95%)
1d R = Bn (89%)
1e R = *t*-Bu (72%)

 (3) (a) Kikuchi, Y.; Tanaka, Y.; Sutarto, S.; Kobayashi, K.; Toi, H.; Aoyama, Y. *J. Am. Chem. Soc.* **1992**, *114*, 10302−10306. (b) Aoyama, Y.; Tanaka, Y.; Sugahara, S. *J. Am. Chem. Soc.* **1989**, *111*, 5397−5404.
 (4) (a) Bonar-Law, R. P.; Ladomenou, K. *Chem. Commun.* **2002**, 2108−2109. (b) Bonar-Law, R. P.; Sanders, J. K. M. *J. Am. Chem. Soc.* **1995**, *117*, 259−271.
 (5) (a) Velasco, T.; Lecollinet, G.; Ryan, T.; Davis, A. P. *Org. Biomol. Chem.* **2004**, *2*, 645−647. (b) Lecollinet, G.; Dominey, A. P.; Velasco, T.; Davis, A. P. *Angew. Chem., Int. Ed.* **2002**, *41*, 4093−4096. (c) Davis, A. P.; Wareham, R. S. *Angew. Chem., Int. Ed.* **1998**, *37*, 2270−2273. (d) Bonar-Law, R. P.; Davis, A. P.; Murray, B. A. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1407−1408.
 (6) (a) Welti, R.; Diederich, F. *Helv. Chim. Acta* **2003**, *86*, 494−503. (b) Droz, A. S.; Neidlein, U.; Anderson, S.; Seiler, P.; Diederich, F. *Helv. Chim. Acta* **2001**, *84*, 2243−2289. (c) Bähr, A.; Felber, B.; Schneider, K.; Diederich, F. *Helv. Chim. Acta* **2000**, *83*, 1346−1376. (d) Smith, D. K.; Diederich, F. *Chem. Commun.* **1998**, 2501−2502. (e) Bähr, A.; Droz, A. S.; Püntener, M.; Neidlein, U.; Anderson, S.; Seiler, P.; Diederich, F. *Helv. Chim. Acta* **1998**, *81*, 1931−1963. (f) Anderson, S.; Neidlein, U.; Gramlich, V.; Diederich, F. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1596−1600.
 (7) (a) Das, G.; Hamilton, A. D. *Tetrahedron Lett.* **1997**, *38*, 3675−3678. (b) Das, G.; Hamilton, A. D. *J. Am. Chem. Soc.* **1994**, *116*, 11139−11140.
 (8) (a) Kim, Y.-H.; Hong, J.-I. *Angew. Chem., Int. Ed.* **2002**, *41*, 2947−2950. (b) Kim, H.-J.; Kim, Y.-H.; Hong, J.-I. *Tetrahedron Lett.* **2001**, *42*, 5049−5052.
 (9) Bitta, J.; Kubik, S. *Org. Lett.* **2001**, *3*, 2637−2640.
(10) (a) Rusin, O.; Lang, K.; Kral, V. *Chem.−Eur. J.* **2002**, *8*, 655−663. (b) Kral, V.; Rusin, O.; Charvatova, J.; Anzenbacher, P., Jr.; Fogl, J. *Tetrahedron Lett.* **2000**, *41*, 10147−10151. (c) Rusin, O.; Kral, V. *Chem. Commun.* **1999**, 2367−2368.
(11) (a) Inouye, M.; Takahashi, K.; Nakazumi, H. *J. Am. Chem. Soc.* **1999**, *121*, 341−345. (b) Inouye, M.; Chiba, J.; Nakazumi, H. *J. Org. Chem.* **1999**, *64*, 8170−8176.
(12) (a) Mazik, M.; Sicking, W. *Tetrahedron Lett.* **2004**, *45*, 3117−3121. (b) Mazik, M.; Radunz, W.; Sicking, W. *Org. Lett.* **2002**, *4*, 4579−4582. (c) Mazik, M.; Sicking, W. *Chem.−Eur. J.* **2001**, *7*, 664−670. (d) Mazik, M.; Bandmann, H.; Sicking, W. *Angew. Chem., Int. Ed.* **2000**, *39*, 551−554.
(13) (a) Wada, K.; Mizutani, T.; Kitagawa, S. *J. Org. Chem.* **2003**, *68*, 5123−5131. (b) Mizutani, T.; Kurahashi, T.; Murakami, T.; Matsumi, N.; Ogoshi, H. *J. Am. Chem. Soc.* **1997**, *119*, 8991−9001.
(14) Ishi-I, T.; Mateos-Timoneda, M. A.; Timmerman, P.; Crego-Calama, M.; Reinhoudt, D. N.; Shinkai, S. *Angew. Chem., Int. Ed.* **2003**, *42*, 2300−2305.
(15) (a) Tamaru, S−i.; Shinkai, S.; Khasanov, A. B.; Bell, T. W. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 4972−4976. (b) Tamaru, S.-i.; Yamamoto, M.; Shinkai, S.; Khasanov, A. B.; Bell, T. W. *Chem.−Eur. J.* **2001**, *7*, 5270−5276.
(16) Liu, R.; Still, W. C. *Tetrahedron Lett.* **1993**, *34*, 2573−2576.
(17) (a) Segura, M.; Bricoli, B.; Casnati, A.; Munoz, E. M.; Sansone, F.; Ungaro, R.; Vicent, C. *J. Org. Chem.* **2003**, *68*, 6296−6303. (b) Sansone, F.; Chierici, E.; Casnati, A.; Ungaro, R. *Org. Biomol. Chem.* **2003**, *1*, 1802−1809.
(18) For a recent review on benzene-based tripodal receptors, see: Hennrich, G.; Anslyn, E. V. *Chem.−Eur. J.* **2002**, *8*, 2218−2224.
(19) (a) Morales, J. C.; Zurita, D.; Penadés, S. *J. Org. Chem.* **1998**, *63*, 9212−9222. (b) Junquera, E.; Laynez, J.; Menéndez, M.; Sharma, S.; Penadés, S. *J. Org. Chem.* **1996**, *61*, 6790−6798. (c) Jimenez-Barbero, J.; Junquera, E.; Martin-Pastor, M.; Sharma, S.; Vicent, C.; Penadés, S. *J. Am. Chem. Soc.* **1995**, *117*, 11198−11204.

the steric, geometric, and functional group requirements for effective recognition. The binding affinity and selectivity of the tripodal receptor were tested vs a set of representative α- and β-octyl glycosides, selected among the most relevant to biological recognition processes, by measuring the corresponding association constants through ^{1}H NMR spectroscopy in CDCl$_3$. Since this unexpectedly turned out to be a nontrivial and largely underestimated task, we wish to report here, together with the synthesis of the tripodal receptor and the assessment of its binding ability, a detailed analysis of the binding data and a description of the pitfalls encountered, which allowed us to establish a paradigm for the correct evaluation of binding affinities that can be generally applied for the recognition of monosaccharides by ^{1}H NMR spectroscopy. We also wish to describe a new quantity, which we propose as a generalized index of binding efficacy, intended to address for the first time the general issue of comparing heterogeneous binding data for host−guest systems exhibiting different association patterns, coming from different literature sources or measured by different techniques.

Results and Discussion

Synthesis of the Tripodal Receptor. The design of the tripodal receptor was based on the 1,3,5-substituted 2,4,6-triethylbenzene scaffold, on account of the marked preference for the alternate substituents pattern exhibited by this structure, which directs the three binding arms toward the same side of the aromatic ring.[18] Although the architecture is not rigidly

Scheme 2
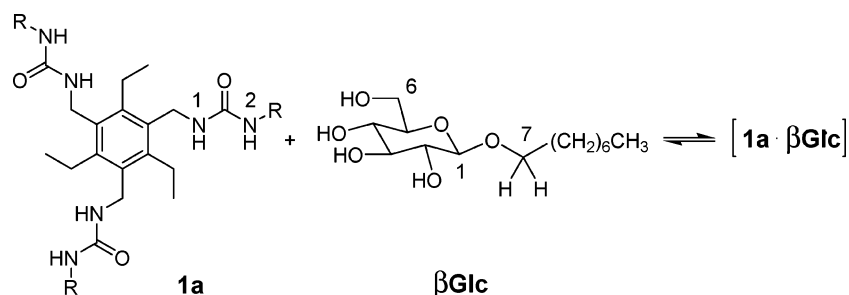
preorganized, the conformational bias for directing binding groups on the same side of the scaffold has been shown to be worth $10-15$ kJ mol^{-1} [20] and can therefore provide the necessary prerequisites of convergence and adaptivity required for a putative host for monosaccharides. In our design, three convergent ureidic groups located on the sidearms would provide H-bonding interactions with the polar groups of the saccharide, while the aromatic ring would establish van der Waals contacts with the aliphatic backbone of the guest. Size and shape complementarity was checked by molecular modeling calculations, although docking requirements were quite loose because of the flexibility of the structure. The choice of the ureidic groups as the appropriate ligands was made on account of the double H-bonding donor−acceptor nature of this functionality, which could concertedly be exploited to achieve functional complementarity depending on the best fitting requirements of the six polar groups of the monosaccharide, namely, the four hydroxyl and the two ether groups. The synthetic pathway followed is shown in Scheme 1.

The ureidic groups were connected to the aromatic scaffold by methylene bridges, which proved to be the appropriate spacers for achieving the correct binding geometry. Eventually, the lipophilic substituents of the prototypical receptor **1** were selected in order to impart an acceptable solubility to the tris-ureas in chloroform; in addition, bulky substituents were privileged to minimize the tendency of ureas to self-aggregate and to build a hydrophobic shell around the hydrophilic moiety of the monosaccharide. Unfortunately, only the tris-*t*-octylurea **1a** displayed a solubility range useful for titration experiments, and therefore binding data could be obtained only for this receptor.

Preliminary Binding Studies. In the generally adopted procedure, association constants are determined by ^{1}H NMR titrations. As complexation is usually under a fast exchange regime on the NMR time scale, the chemical shifts of the time-averaged signals for the free and complexed species of the analyte are monitored for increasing amounts of titrating agent. Fit of complexation induced shifts of the *individual* signals to the 1:1 binding isotherm by nonlinear regression yields the relevant parameters, i.e., the shifts of the free and the complexed species and the association constant. Commercially available 1-*O*-octyl-β-D-glucopyranoside (βGlc) is the most frequently used glycoside to test a newly synthesized receptor and, due to its large solubility in most organic solvents, is usually employed as the titrating agent, while following the shift of the receptor signals upon complexation. To compare the results with literature reports, we evaluated the association of **1a** with βGlc

Table 1. Association Constants K_a (M^{-1}) and Limiting Shift Values $\Delta\delta_\infty$ (ppm) for binding of **1a** to βGlc, Calculated by Fitting the Shifts of Individual Receptor's and Glycoside's Signals to a 1:1 Association Model, When Titrating with βGlc (Entries 1−5) and **1a** (Entries 6−17), Respectively[a]

entry	signal	K_a[b] (SE)	$\Delta\delta_\infty$
1	NH-1	1098 (30)	+1.104
2[c]		1095 (33)	+1.103
3	NH-2	1354 (56)	+0.383
4[c]		1440 (43)	+0.382
5	CH$_2$N	1265 (102)	−0.108
6	OH-2	298 (3)	+1.449
7[c]		309 (14)	+1.444
8	OH-3	789 (24)	+2.037
9	OH-4	269 (6)	+2.633
10	OH-6	345 (23)	+2.698
11	CH-1	306 (9)	−0.364
12	CH-2	506 (9)	−1.497
13	CH-3	387 (9)	−0.577
14	CH-4	441 (8)	−1.065
15	CH-5	325 (11)	−0.641
16	CH-6	302 (11)	−0.332
17	CH-6′	299 (14)	−0.390

[a] Measured by ^{1}H NMR (300/700 MHz) at $T = 296$ K in CDCl$_3$ on $0.8-1.2$ mM solutions of analyte using titrant concentrations up to $13-15$ mM. [b] Standard error of the nonlinear least-squares fit. [c] Data from duplicate experiments.

in CDCl$_3$ at $T = 296$ K according to the above procedure (Scheme 2). Fitting of data to the 1:1 binding isotherm gave the results reported in Table 1, entries 1−5, which show that the binding ability of **1a** compares well with most synthetic receptors reported to date.[3−17] However, although the reproducibility of data and the quality of the fit appeared very good, featuring an average standard error of 3%, the discrepancy of K_a values obtained from different signals was outside the experimental error. As a check, the association was measured by reversing the roles of the analyte and the titrating agent, i.e., by titrating the glycoside with the receptor and determining K_a values by fitting the shifts of the glucose signals (Table 1, entries 6−17). The quality of the fit was again good, but the discrepancy of values was even more marked, both within the set of glucose signals, with K_a ranging from 269 to 789 M^{-1}, and in comparison with those obtained from the receptor signals, which were significantly larger. Altogether, results unequivocally revealed the occurrence of multiple equilibria. Indeed, the inconsistent K_a values obtained from different signals indicate that a simple 1:1 association model cannot hold,[21] whereas inconsistencies observed when reversing the analyte with the

(20) Wallace, K. J.; Belcher, W. J.; Turner, D. R.; Syed, K. F.; Steed, J. W. *J. Am. Chem. Soc.* **2003**, *125*, 9699−9715.

(21) Due to the limited solubility of the receptor, the continuous variation method (Job's plot) could not provide a reliable assessment of adduct's stoichiometry. It has been pointed out that the continuous variation method can be seriously misleading when applied outside the required conditions. See: Huang, C. Y.; Zhou, R.; Yang, D. C. H.; Chock, P. B. *Biophys. Chem.* **2003**, *100*, 143−149.
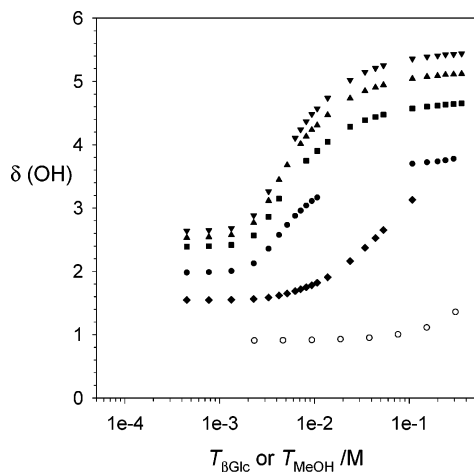
Figure 1. Semilogarithmic plot of the chemical shifts of the OH resonances of βGlc [OH-2 (■), OH-3 (▼), OH-4 (▲), OH-6 (●)], internal water (◆), and methanol (○) vs total concentration of βGlc or MeOH from dilution experiments at 300 MHz and $T = 296$ K in CDCl₃. Methanol shifts are from an independent experiment.

titrant are a clear indication that additional association phenomena are superposed to the host−guest process.

Glycoside's Self-Association. Self-aggregation of glycosides in CDCl₃ has been noted by several authors and investigated in more detail in some cases;[3a,4b] yet, its relevance to titration experiments in the evaluation of binding affinities[22] has been too often underestimated: commercial availability and (apparent) large solubility invariably made βGlc and analogous glycosides the reagents of choice for testing synthetic receptors without consideration of self-association phenomena. To take into account the contribution of the latter, self-association of βGlc was investigated through dilution experiments by ¹H NMR under our conditions. In Figure 1 the shifts of the OH signals of the glycoside and of the water present in solution are reported vs the concentration of βGlc. The shift of the OH signal of methanol from an independent dilution experiment is also reported for comparison. Strong variations of glycoside's signals are evident above 1 mM, whereas for methanol the variation is negligible up to 0.1 M. Furthermore, in contrast to the profile exhibited by the water signal, the βGlc signals show a sigmoidal-like profile that indicates some kind of cooperativity in the self-association process, to which water does not seem to take part. Indeed, the shift profile exhibited by βGlc is typical for the formation of micellar aggregates,[23] giving an estimated critical micellar concentration of 2−3 mM.[24] Thus, the apparent solubility of βGlc is deceptive: use of βGlc above 1 mM would strongly affect the determination of binding constants, not only because the glucoside signals report an unseparable combination of contributions but also because micellar aggregates may themselves behave as competitive receptors. In addition, the unpredictable, increasingly smaller concentration of active monomeric species with respect to its analytical concentration will artificially enhance the apparent binding constant, challenging a correct determination in concentration ranges where aggregates are formed.

(22) Hamelin, B.; Jullien, L. *J. Chem. Soc., Faraday Trans.* **1997**, *93*, 2153-2160.
(23) Evans, D. F.; Wennerström, H. *The Colloidal Domain*; VCH: New York, NY, 1994; p 144.
(24) βGlc is a widespread surfactant in water; it is not surprising that it may form reverse micelles in chloroform.
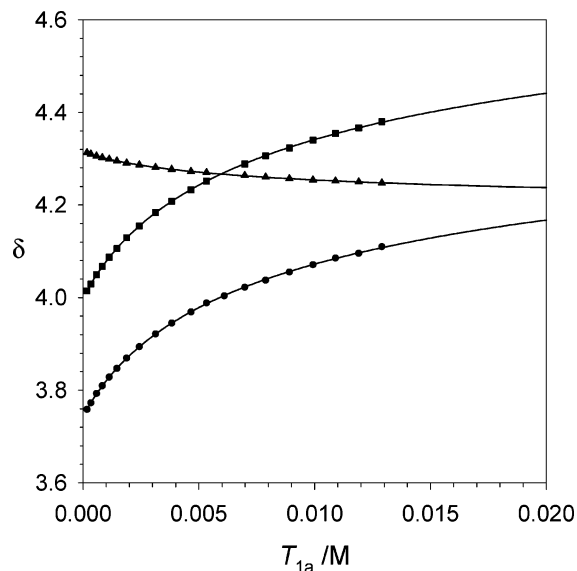
Figure 2. Plot of the chemical shifts of the NH [NH-1 (●), NH-2 (■)] and CH₂N (▲) resonances of **1a** vs total concentration of **1a** from dilution experiments at 300 MHz and $T = 296$ K in CDCl₃. Symbols are experimental data points; solid lines are best fit curves obtained by simultaneous nonlinear regression of all data giving $K_{dim} = 54 \pm 1$ M⁻¹ (log $K_{dim} = 1.732 \pm 0.009$).

Receptor's Self-Association. Analogous dilution experiments of **1a** in CDCl₃ showed a strong downfield shift of the NH signals with increasing concentration, as expected for self-association through H-bonding, together with a weak upfield shift of the benzylic methylene signal (Figure 2). Fortunately, a simple dimerization model could fit with excellent agreement the experimental data in the concentration range allowed by the receptor solubility. A nonlinear least-squares analysis of the shifts from the three individual signals gave consistently the same dimerization constant within the experimental error, in good agreement with the value of $K_{dim} = 54 \pm 1$ M⁻¹ (log $K_{dim} = 1.732 \pm 0.009$) obtained by simultaneous fit of all data; the excellent standard deviation and the random distribution of residuals assured that the receptor's self-association equilibria were correctly accounted for by a dimerization model. Simultaneous fit to models including aggregates of higher stoichiometry was also attempted, but convergence was not attained in any case. The dimerization constant and the shift values of monomer and dimer obtained for **1a**[25] were included in the analysis of the host−guest binding equilibria.

Binding of Glycosides. An evaluation of the binding affinity of **1a** toward the set of octyl glycosides reported in Chart 1 was undertaken. Glucose (Glc), Galactose (Gal), Mannose (Man), and *N*-acetyl glucosamine (GlcNAc) 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.[2] Calculation and refinement of cumulative binding constants β_i from the set of binding isotherms were achieved by simultaneous fit of all the available signals to the general expression for the observed chemical shift δ of a nucleus under fast exchange

(25) See Supporting Information.

Chart 1
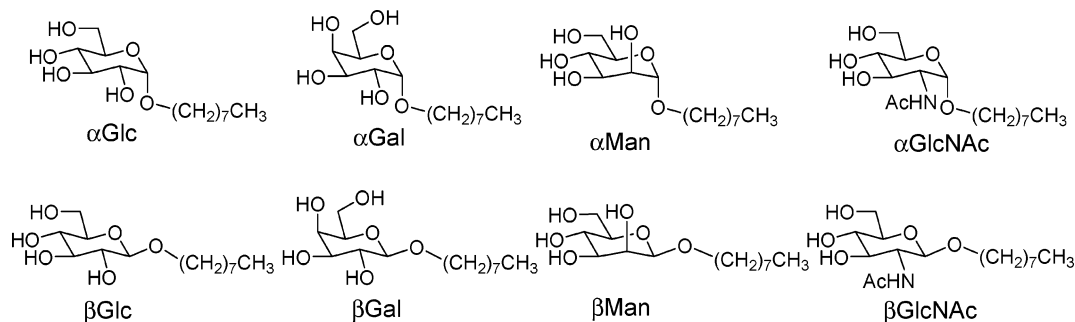
conditions, which is the weighted average of the shifts δ_i of the nucleus in all the species present at equilibrium:

$$\delta = \sum_i f_i \delta_i$$

where

$$f_i = \frac{x_i C_i}{T_X}$$

with T_X standing for the total concentration of the reagent containing the nucleus, x_i standing for the stoichiometric coefficient of the reagent in the i-th species, and C_i standing for its equilibrium concentration. The mass balance equation for each reagent, expressed as a function of cumulative binding constants β_i, provides a system

$$T_A = [A] + \sum_i a_i C_i = [A] + \sum_i a_i \beta_i [A]^{a_i} [B]^{b_i}...$$

$$T_B = [B] + \sum_i b_i C_i = [B] + \sum_i b_i \beta_i [A]^{a_i}[B]^{b_i}...$$

$$.....$$

of n equations which can be solved for the n unknown reagent concentrations. Numerical fit of the experimental data through nonlinear least-squares regression gives best values for the β_i and δ_i parameters. Based on the described findings, association with the receptor was evaluated by keeping the concentration of the glycosidic analyte near or below 1 mM and varying the concentration of **1a** through its solubilty range (up to 15 mM). In this range, complexation induced shifts in the ^{1}H NMR spectra were observed for all the protons of the monosaccharidic moiety and for the protons of the glycosidic methylene, while significant shifts on the receptor's side were observed for the NH protons and for the adjacent methylene signals (see Scheme 2 for numbering scheme). The observed shifts indicated that the monosaccharide is bound within the cleft organized by the three ureidic arms of the receptor, whereas the octyl chain resides outside the cleft. Two steps were mandatory for a correct assessment of binding affinity, namely, finding the correct binding model and selecting the informative signals.

Finding the Model. A 1 mM solution of βGlc in CDCl$_3$ was titrated with increasing amounts of **1a** under the described conditions and the shifts of the βGlc signals were simultaneously fitted to the simplest compatible model including a 1:1 association and the dimerization of the receptor. The agreement of the nonlinear regression was, however, unsatisfactory and the reason appeared more clearly from the titrations of the other glycosides

of the set when considering *all* the available resonances, including signals from the glycoside *and* the titrating agent. Indeed, the shift of the receptor's NH-1 signal showed, upon complexation, a trend necessarily caused by the contribution of more than two species, therefore implying a higher stoichiometry than just 1:1. This feature is clearly appreciated from the plot of receptor's signals in the titration of βGal, reported in Figure 3, when compared to the same signals in the absence of the glycoside (Figure 2). It is evident that relevant information is contained not only in the shifts of the analyte but also in those of the titrating agent, which are usually neglected. To find a more adequate model, we thought that dimeric **1a** may itself bind the glycosides. Thus, the simplest model would include 1:1 and 2:1 adducts and the dimerization of the receptor, as depicted in Scheme 3, where R and G are the receptor and the glycoside, respectively, and β_{mn} are the corresponding cumulative binding constants. The shifts of all the signals that could
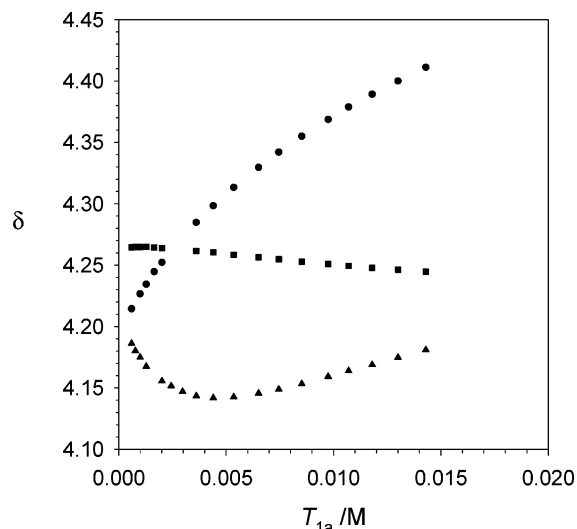

Figure 3. Complexation induced shifts of the NH [NH-1 (▲), NH-2 (●)] and CH$_2$N (■) resonances of **1a** vs total concentration of **1a** in the titration of βGal 1.0 mM at 400 MHz and $T = 296$ K in CDCl$_3$.

Scheme 3. Association Model of **1a** (R) with Glycosides (G)

$$R + G \underset{}{\overset{\beta_{11}}{\rightleftharpoons}} RG \qquad \beta_{11} = \frac{[RG]}{[R][G]}$$

$$2R + G \underset{}{\overset{\beta_{21}}{\rightleftharpoons}} R_2G \qquad \beta_{21} = \frac{[R_2G]}{[R]^2[G]}$$

$$2R \underset{}{\overset{\beta_{dim}}{\rightleftharpoons}} R_2 \qquad \beta_{dim} = \frac{[R_2]}{[R]^2}$$
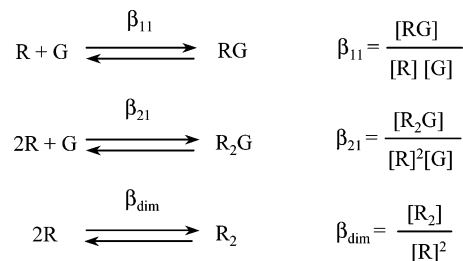
be followed in the spectra along the titration experiments were then simultaneously fitted to the model of Scheme 3, and gratifyingly, the nonlinear regression gave an excellent agreement for all the investigated glycosides, showing global standard deviation values of the fit that leave no doubt on the adequacy of the model.[25,26] In Figure 4, the plot of the fit obtained for the titration of αGal with **1a**, in which up to 16 signals could be followed at 800 MHz, is reported as an illustrative example, showing that even the "anomalous" NH-1 and CH-6 signals were closely accounted for. Attempts to fit experimental data to higher stoichiometry models were unsuccessful; failure to attain convergence in all cases gave further support to the chosen model. On the other hand, the reliability of results was ensured by obtaining for αGal and αGlc at 800 MHz and for αMan at 400 MHz the same association constant values, within the experimental error, as those obtained from duplicate titration experiments at 400 MHz.

Choosing the Signals. From Figure 4 it can be noted that some signals exhibit strong shifts upon complexation (upfield, CH-1 and CH-2; downfield, all OH), whereas much weaker shifts are exhibited by the remaining CH signals of the glycoside and those of the receptor. This feature, which is related to the geometry of the complex, is caused by a combination of contributions from H-bonding (downfield) and aromatic shielding (upfield) and appeared to be general for the tested glycosides. It may be thought that signals showing larger shifts would be more informative and that data sampling could therefore be limited to those signals. Comparison of the results obtained by fitting different combinations of signals for each titration (OH protons only, CH protons only, strongly shifting signals only) with those obtained using all the available resonances showed that although the system is essentially defined by the strongly shifting signals, weakly shifting signals contribute significantly to the overall standard deviation of the fit. For example, for βGal, $\sigma = 0.0008$ ppm was obtained by fitting CH and receptor's signals (8 resonances), whereas fitting OH and receptor's signals (7 resonances) gave a 6-fold larger value ($\sigma = 0.0048$ ppm); using all signals (12 resonances), $\sigma = 0.0038$ ppm was obtained for the overall fit. OH Signals are generally broader than CH signals and much more sensitive to the influence of external factors, such as impurities, traces of acids, adventitious water, etc.; instead, CH signals are usually sharper and remarkably insensitive to perturbing effects. On the other hand, the system is much less defined when neglecting signals from the OH protons, and β values, although more precise, tend to depart from those obtained from all signals.[27] In the above example, $\log \beta_1 = 2.79$ and $\log \beta_2 = 4.88$ were obtained from CH signals, whereas OH signals gave $\log \beta_1 = 2.93$ and $\log \beta_2 = 5.02$, values much closer to those obtained using all signals ($\log \beta_1 = 2.92$ and $\log \beta_2 = 5.01$). From the described systematic testing it was concluded that titrations can indeed be performed by detecting the strongly shifting signals exclu-
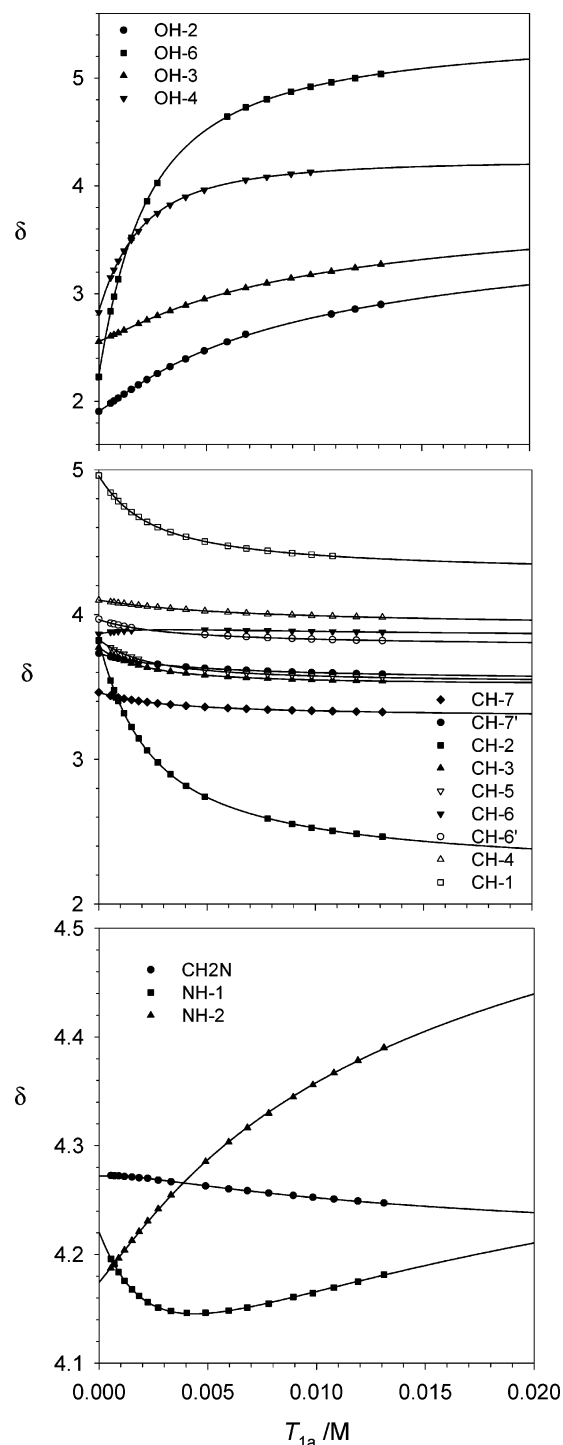

Figure 4. Plot of complexation induced shifts vs total concentration of **1a** in the titration of αGal 0.96 mM with **1a** in CDCl₃ at 800 MHz and $T = 296$ K. Top: glycoside OH signals. Middle: glycoside CH signals. Bottom: receptor signals. Symbols are experimental data points; solid lines are best fit curves obtained through nonlinear regression by simultaneous fit of all data to the model of Scheme 3.

(26) Examples of tripodal receptors bearing ureidic groups that self-assemble into dimeric capsules are reported in recent literature. For example, see: (a) Alajarín, M.; Pastor, A.; Orenes, R.-A.; Steed, J. W.; Arakawa, R. *Chem.—Eur. J.* **2004**, *10*, 1383−1397. (b) Alajarín, M.; Pastor, A.; Orenes, R.-A.; Steed, J. W. *J. Org. Chem.* **2002**, *67*, 7091−7095. Although the model including the adduct of a dimeric receptor with one glycoside molecule unambiguously fits the experimental data, it must be emphasized that it does not constitute a proof of the capsular structure of the dimer nor do we have any independent evidence of it.

(27) To compensate for the lower precision of OH and of receptor's NH signals, a weighting pattern proportional to the observed line width has been applied, but the results were only occasionally improved.

sively, but the precision and the reliability of results, which can be crucial for a safe assessment of the model, are markedly enhanced when including all the available resonances. In addition, it must be remarked that receptor's signals contain essential information and should not be neglected and that simultaneous fit of all signals is crucial to define the system and reveal inconsistencies. In general, relying on one or few

Table 2. Cumulative Association Constants (log β_n) and Standard Free Energies of Binding $-\Delta G°$ (kJ mol^{-1}) with Standard Deviations (σ) for 1:1 and 2:1 Complexes of **1a** with Octyl Glycosides[a]

glycoside	log β_1	$-\Delta G°$ (1:1)	log β_2	$-\Delta G°$ (2:1)
βGal	2.92 ± 0.01	16.53 ± 0.08	5.01 ± 0.03	28.40 ± 0.16
αGal	2.82 ± 0.01	15.99 ± 0.04	4.82 ± 0.02	27.30 ± 0.10
αMan	2.82 ± 0.05	15.99 ± 0.27	4.60 ± 0.11	26.06 ± 0.60
αGlc	2.77 ± 0.01	15.67 ± 0.05	4.80 ± 0.02	27.21 ± 0.09
βGlc	2.67 ± 0.04	15.15 ± 0.22	4.88 ± 0.06	27.67 ± 0.32
αGlcNAc	2.75 ± 0.04	15.56 ± 0.24	4.31 ± 0.13	24.41 ± 0.73
βGlcNAc	2.62 ± 0.03	14.83 ± 0.19	4.50 ± 0.06	25.52 ± 0.36
βMan	2.31 ± 0.18	13.07 ± 1.01	3.98 ± 0.29	22.56 ± 1.62

[a] Measured by ^{1}H NMR (400/800 MHz) from titration experiments at T = 296 K in CDCl$_3$ on 0.6–1.2 mM solutions of glycoside using receptor concentrations up to 13–15 mM. Binding constants were calculated by simultaneous fit of the shifts of all the available signals. The receptor's dimerization constant was set invariant to the independently measured value of log β_{dim} = 1.732 in the nonlinear regression analysis.

individually fitted analyte's signals only may compromise a correct evaluation of glycoside binding.

Glycoside Binding Affinity and Selectivity. The results obtained for the association of **1a** with the set of glycosides of Chart 1 are reported in Table 2 as cumulative log β values for the formation of the 1:1 and 2:1 adducts. Corresponding binding free energies $-\Delta G°$ are quite substantial, ranging from 13.1 to 16.5 and from 22.6 to 28.4 kJ mol^{-1}, respectively.

Although it may be tempting to use the results of Table 2 for a straightforward comparative analysis, it is clear that in this form data are of little use for the assessment of the actual binding ability and selectivity of the receptor toward the glycosides: binding free energies for the two complexation steps do not assess the *overall* binding ability; furthermore, direct comparison of binding constants of different order is unfeasible. To address this issue, we developed a treatment intended to assign to the receptor a descriptor that would unambiguously define its binding ability, which could be extended to a generalized comparison of binding data for host–guest systems.

Binding Descriptors. Like in cases involving 1:1 association equilibria,[28] for systems of multiple equilibria the use of the first stoichiometric binding constant has been suggested for comparison of affinities of a receptor for different ligands or of affinities of different receptors for a common ligand.[29] This approximate approach conveniently yields an estimate of binding affinities as long as subsequent stepwise constants follow the same trend or become negligible. A different approach has been adopted by Raymond and co-workers:[30] the concentration of free metal ion in solution, expressed as pM($-$log [M]), has been used for comparing the relative effectiveness of various tris-catecholate ligands toward Fe(III) ions. pM Values obviously depend on total concentrations of reagents and conditions (pH). On the other hand, the IC$_{50}$ value is largely used in biochemistry for comparison purposes. According to a definition useful in our context, the IC$_{50}$ is the median inhibitory concentration (in mol L^{-1}) of an agent (agonist or antagonist), i.e., the concentra-

tion that causes a 50% reduction in the specific binding of a radioligand.[31] Empirical quantitative expressions[32] or rigorous thermodynamic analyses[33] based on the IC$_{50}$ competitive method have been traditionally employed to evaluate affinity constants in the screening of ligands in biochemical processes. In analogy to the biochemical IC$_{50}$, we define the *median binding concentration* BC$_{50}$ as the total concentration (in mol L^{-1}) of a titrating agent (host or guest) that causes a 50% reduction in the concentration of unbound analyte (guest or host) in solution or, equivalently, that complexes 50% of the analyte. Thus, like for IC$_{50}$, the higher the affinity, the lower the BC$_{50}$ value. Although the BC$_{50}$ quantity has the same meaning as IC$_{50}$, it is operationally different. Indeed, displacement of a ligand from a saturated receptor, the competitive way in which IC$_{50}$ is usually measured, is normally unachievable for synthetic receptors showing moderate binding constants; the value of BC$_{50}$ is therefore either directly measured from the unbound analyte concentration, when available, or calculated from the knowledge of the set of binding constants involved. In the present case, where G is the analyte, R is the titrant, and T_G and T_R are the corresponding total concentrations, from the mass balance equation for Scheme 3

$$T_G = [G] + [RG] + [R_2G] \quad (1)$$

For [G] = 50% T_G, i.e., the condition of BC$_{50}$,

$$T_G = 2[G] \quad (2)$$

Substituting into eq 1 and rearranging

$$[G] = [RG] + [R_2G] \quad (3)$$

which can be expressed as a function of the cumulative binding constants β_{11} and β_{21} and rearranged

$$[G] = \beta_{11} [R][G] + \beta_{21} [R]^2[G] \quad (4)$$

$$\beta_{21} [R]^2 + \beta_{11}[R] - 1 = 0 \quad (5)$$

which has an exact solution for [R] in the positive root of eq 6:

$$[R] = [-\beta_{11} \pm (\beta_{11}^2 + 4\beta_{21})^{1/2}]/2\beta_{21} \quad (6)$$

It can be noted that, under the condition of eq 2, [R] depends on β_{11} and β_{21} but not on T_R. From the mass balance equation for T_R we have

$$T_R = [R] + 2[R_2] + [RG] + 2[R_2G] \quad (7)$$

that can be expressed as a function of the cumulative binding constants β_{11} and β_{21}

$$T_R = [R] + 2\beta_{dim}[R]^2 + \beta_{11}[R][G] + 2\beta_{21}[R]^2[G] \quad (8)$$

Under the condition of eq 2, eq 8 becomes

$$BC_{50} = [R] + 2\beta_{dim}[R]^2 + \tfrac{1}{2}T_G\beta_{11}[R] + T_G\beta_{21}[R]^2 \quad (9)$$

(28) "Although it has often been assumed (perhaps too often) that only 1:1 stoichiometry need to be considered, careful investigation sometimes reveals that, even in relatively simple systems, it is necessary to take account of additional complex species". Connors, K. A. *Binding Constants*; Wiley-Interscience: New York, NY, 1987; p 69.
(29) Klotz, I. M. *Ligand–Receptor Energetics: a guide for the perplexed*; Wiley-Interscience: New York, NY, 1997; p 63.
(30) Harris, W. R.; Raymond, K. N.; Weitl, F. L. *J. Am. Chem. Soc.* **1981**, *103*, 2667–2675.

(31) *Oxford Dictionary of Biochemistry and Molecular Biology*, revised edition; Oxford University Press: New York, NY, 2000.
(32) (a) Müller, R. *J. Immunol. Methods* **1980**, *34*, 345–352. (b) Müller, R. *Methods Enzymol.* **1983**, *92*, 589–601.
(33) Winzer, D. J.; Sawyer, W. H. *Anal. Biochem.* **1996**, *241*, 180–185.

which, factorizing T_G, becomes

$$BC_{50} = [R] + 2\beta_{dim}[R]^2 + T_G(^1/_2\beta_{11}[R] + \beta_{21}[R]^2) \quad (10)$$

From eq 10 it can be seen that BC_{50} takes into account *all* the binding constants involved in the system and can therefore be employed to compare binding data.

BC_{50} is clearly a conditional parameter, depending on the total concentration of the analyte T_G at which it is measured (or calculated), which should be therefore specified together with the temperature.[34] However, a very useful property of BC_{50} is that, when the total concentration of the analyte T_G becomes negligible, the value of BC_{50} becomes constant, i.e.,

$$\lim_{T_G \to 0} BC_{50} = const = BC_{50}^0 = [R] + 2\beta_{dim}[R]^2 \quad (11)$$

In contrast to BC_{50} (eq 10), from eq 11 it can be noted that BC_{50}^0, which we may call *intrinsic median binding concentration*, depends on all the equilibrium constants involved in the system but *not* on specific (concentration) conditions. In practice, BC_{50}^0 is measured from (or calculated for) a solution of analyte sufficiently dilute to make BC_{50} invariant. The above treatment, which was derived for our specific case in which the glycoside G is the analyte and the receptor R is the titrant, has been extended in the Appendix to provide a generalized application to all differents models of host−guest systems involving two generic partners A and B, interchangeably usable as analyte and titrant. Specification as analyte and titrant is the only distinction between A and B relevant to BC_{50}, since this index defines the binding ability of the titrant toward the analyte at the specified concentration of the latter. We propose that the BC_{50}^0 quantity is a general and convenient affinity index describing the *intrinsic* binding affinity of a species A for a species B (or vice versa), thus most appropriate to compare binding affinities and selectivities for systems involving multiple equilibria.[35] It should be emphasized that although it is not necessary, BC_{50}^0 can be appropriately employed in cases where a 1:1 association is the only equilibrium involved, allowing a direct comparison of results with those from more complex systems, thus heterogeneous in nature. In this case, it can easily be demonstrated that BC_{50}^0 coincides with the affinity constant $K_d = 1/K_a$, where K_a is the association constant (β_{11}), making the chemical meaning of the *intrinsic median binding concentration* apparent. Indeed, for a 1:1 association eq 1 reduces to eq 1′

$$T_G = [G] + [RG] \quad (1')$$

and under the condition of eq 2, eq 3 becomes eq 3′

$$[G] = [RG] \quad (3')$$

Substituting $K_a[R][G]$ for $[RG]$ from the 1:1 association equilibrium into 3′, eqs 5 and 6 become eqs 5′ and 6′

$$K_a[R] = 1 \quad (5')$$

$$[R] = 1/K_a = K_d \quad (6')$$

Considering now the mass balance equation for T_R, eq 7 becomes eq 7′:

$$T_R = [R] + [RG] = [R] + K_a[R][G] \quad (7')$$

Substituting eq 6′ into 7′, eq 8′ is obtained

$$T_R = K_d + [G] \quad (8')$$

Under the condition of eq 2, eqs 9′ and 11′ are obtained

$$BC_{50} = T_R = K_d + T_G/2 \quad (9')$$

$$\lim_{T_G \to 0} BC_{50} = const = BC_{50}^0 = K_d \quad (11')$$

Thus, for 1:1 associations, comparing BC_{50}^0 values is equivalent to comparing affinity constants. Unfortunately, BC_{50}^0 is insensitive to the formation of complex species multinuclear in the analyte. This property can be inferred from the generalized treatment of BC_{50}^0 reported in the Appendix, from which it is clearly seen that when T_B becomes negligible, terms in B (the analyte) with coefficient larger than 1 will disappear from the expression of BC_{50}^0. Although the presence of complex species multinuclear in B is unfavored, considering that B is the component in defect, the BC_{50}^0 parameter can rigorously be used to assess *intrinsic* affinities whenever complex species multinuclear in B are absent. Should this not be the case, BC_{50} needs to be used instead, at specified conditions and at T_B of the same order of magnitude as BC_{50}^0.[36]

Analysis of Data. BC_{50}^0 Values calculated from β_{11}, β_{21}, and β_{dim} are reported in Table 3, where the glycosides are ranked in decreasing order of relative affinity. Results show that BC_{50}^0 values range from 1 to 6 mM, which indicates a binding affinity in the millimolar range and a selectivity factor of 5 toward the investigated glycosides. An affinity evaluation obtained using the values of the first binding constant K_1 (β_{11}), as proposed by Klotz,[29] is also reported for comparison. Although a general agreement between the two methods is apparent, an inversion of selectivity caused by the contribution from β_{21} occurs for βGlc and αGlcNAc; furthermore, an overall selectivity range attenuated by 20% results from neglecting β_{21}, as can be appreciated from the relative affinity values RA based on BC_{50}^0 and on K_1. The very similar outcome obtained from the two methods is due to the shallow selectivity profile exhibited by the receptor and to the smaller contribution of the second binding constant to BC_{50}^0 compared to the first.[37] This may not always be the case: for example, in the presence of cooperative effects boosting the second (or higher) binding constant(s), the results from the two methods may diverge considerably; in such a case, the only reliable estimate of binding ability would be provided by BC_{50}^0 (or BC_{50}).

In terms of overall selectivity, it can be noted that most of the investigated glycosides cluster within a narrow range of

(34) It must be stressed that binding abilities are themselves conditional, in that hosts may show different affinities or different selectivity patterns for different concentrations of guests; the BC_{50} quantity will reflect these variations varying correspondingly.

(35) To expedite the calculation of BC_{50} and BC_{50}^0, a utility program based on the general treatment described in the Appendix (see Supporting Information) has been developed and made available for free upon request at the authors' e-mail addresses.

(36) While this paper was submitted, a paper appeared in which the thermodynamic stability of two hydrogen bonded assemblies of different molecularity was compared by means of a C_{50} parameter, defined as the concentration at which 50% of the components are incorporated in the assembly. See: ten Cate, M. G. J.; Huskens, J.; Crego-Calama, M.; Reinhoudt, D. N. *Chem.−Eur. J.* **2004**, *10*, 3632−3639.

(37) The contribution from A_aB species to the value of BC_{50}^0 of reagent A complexing B decreases with the stoichiometric coefficient a. See Appendix in the Supporting Information.

Table 3. Intrinsic Median Binding Concentration BC_{50}^0 (mM), First Stoichiometric Binding Constant K_1 (M^{-1}), and Corresponding Relative Affinity Values (RA) for Adducts of **1a** with Glycosides[a]

glycoside	$BC_{50}^{0,b}$ (mM)	RA (BC_{50}^0)	K_1 (M^{-1})	RA (K_1)
βGal	1.191	5.02	827	4.09
αGal	1.523	3.93	663	3.28
αMan	1.600	3.74	664	3.29
αGlc	1.715	3.49	582	2.88
βGlc	1.969	3.04	472	2.34
αGlcNAc	1.998	2.99	557	2.76
βGlcNAc	2.552	2.34	414	2.05
βMan	5.981	1.00	202	1.00

[a] Calculated from the β values reported in Table 2. [b] Calculated by eqs 6 and 11.

values, showing little selectivity except for βMan and βGal; indeed, **1a** binds to βGal 5-fold better than to βMan, a non-negligible factor for monosaccharides of very similar structure. α/β Selectivity is not generally significant among this set of glycosides: selectivity ratios ranging from 0.78 to 1.28 are observed, except for the mannoside, which exhibits a respectable α/β ratio of 3.74. These results indicate that binding of βMan is disfavored compared to the other glycosides. Apparently, the interaction between receptor **1a** and a monosaccharide is disfavored by an axial hydroxyl group in the 2 position but favored when this is in the 4 position. Considering that axial protons on the β face sistematically experience the largest (upfield) shifts, particularly the one in the 2 position, the hypothesis may be put forward that, in the 1:1 adduct, glycosides are bound inside the cleft, with their β face lying upon the aromatic ring of the receptor. An axial hydroxyl in the 2 position would thus hamper the approach to the receptor, while in the 4 position it may more favorably H-bond to the ureidic groups. The proposed geometry is supported by the upfield shift experienced by the NH-1 protons of the receptor in the 1:1 adduct, which indicates a reorientation toward the interior of the cleft upon complexation. The NMR information does not allow an unambiguous assignment of the structure of the adduct; however the steric and geometric requirements for binding do not appear strict enough to impart a strong selectivity to the receptor.

Experimental Section

General. Reagents were purchased from commercial suppliers and used without purification. Unless otherwise stated, all air and moisture sensitive reactions were performed under inert atmosphere. ^{1}H NMR spectra were obtained at 200–800 MHz. Chemical shifts are reported in parts per million (δ) relative to TMS, using the solvent line as secondary internal reference [CHCl$_3$ (7.26 ppm) for spectra run in CDCl$_3$ and DMSO (2.54 ppm) for spectra run in DMSO-d_6]. ^{13}C NMR spectra were obtained at 50 MHz. Chemical shifts are reported in δ relative to TMS, using the solvent line as secondary internal reference [CHCl$_3$ (77.0 ppm) and DMSO (40.45 ppm)]. Melting points are uncorrected.

Titrations and Data Analysis. Titrations were performed in 5 mm NMR tubes using Hamilton microsyringes, following a previously described technique.[38] To avoid interference of traces of acid in solution, CDCl$_3$ was additionally treated by eluting through a short column of alumina right before use. Mathematical analysis of data and graphic presentation of results were performed using the program Hyp-NMR2004, an upgraded release of HYPNMR[39] handling general host–

guest association equilibria. The program performs a Gauss–Newton–Marquardt least-squares fitting of the experimental data by minimizing the error square sum U,

$$U = \sum_i w_i (\delta_i^{\text{obsd}} - \delta_i^{\text{calcd}})^2$$

where w_i represents the statistical weight assigned to each observed point, and δ_i^{obsd} and δ_i^{calcd} are the i-th observed and calculated chemical shifts, respectively. The program performs simultaneous fit of multiple signals to models involving multiple equilibria. The refinement process yields best-fit values for equilibrium constants and individual chemical shifts of each nucleus in each chemical species. HypNMR2004 is available at: http://www.chim1.unifi.it/group/vacsab/hypnmr.htm. The utility program for computing BC_{50} and BC_{50}^0 is available for free at the authors' e-mail addresses.

Materials. αGlc, βGlc, and αGal were commercial samples. The other octyl glycosides were known compounds[40] and were prepared according to a literature method.[41] Spectral assignments, confirmed through 2D NMR spectra, were in agreement with literature data.

1,3,5-Tris(bromomethyl)-2,4,6-triethylbenzene (3). To a mixture of paraformaldehyde (16.7 g, 556.3 mmol) and triethylbenzene **2** (10 mL, 53.1 mmol) in 100 mL of HBr/AcOH (30 wt %) zinc bromide (19.7 g, 87.5 mmol) was slowly added at room temperature. The mixture was heated to 90 °C for 16.5 h, during which time white crystals were formed. The reaction was cooled to room temperature, and the white solid was filtered off, washed with water, and dried under vacuum overnight to give 22.79 g (51.7 mmol, 97%) of **3** as a white solid. Mp 169–170 °C; ^{1}H NMR (0.1 M in CDCl$_3$, 200 MHz) δ 4.58 (s, 6H), 2.94 (q, $J = 7.7$ Hz, 6H), 1.34 (t, $J = 7.7$ Hz, 9H); ^{13}C NMR (CDCl$_3$, 50 MHz) δ 145.0, 132.6, 28.5, 22.7, 15.6; MS m/z (%) 442 (2), 441 (1), 439 (2), 437 (1), 361 (38), 360 (34), 358 (42), 201 (21), 199 (27), 185 (26), 170 (95), 157 (29), 155 (26), 142 (100), 128 (85), 91 (12), 89 (20), 85 (30), 75 (16). Anal. Calcd for C$_{15}$H$_{21}$Br$_3$: C, 40.85; H, 4.80. Found: C, 41.05; H, 4.69.

1,3,5-Tris(phthalimidomethyl)-2,4,6-triethylbenzene (4). To a suspension of potassium phthalimide (8.4 g, 45.4 mmol) in dry DMSO (75 mL) 1,3,5-tris(bromomethyl)-2,4,6-triethylbenzene **3** (5.0 g, 11.3 mmol) was added at room temperature, under nitrogen atmosphere. The reaction mixture was heated to 84 °C for 8 h; the solution obtained was cooled to 0 °C, and the formation of a white solid was observed. After 1 h at room temperature, the solid was filtered off, dissolved in water (100 mL), and extracted with CH$_2$Cl$_2$ (2 × 100 mL). The combined organic layers were washed with water (2 × 50 mL), dried over Na$_2$SO$_4$, and concentrated to give **4** (4.88 g, 67%) as white crystals. Then the mother liquor was poured into water (200 mL), and the white precipitate formed was filtered off. The solid was dissolved in CH$_2$Cl$_2$ (100 mL), washed with water (3 × 50 mL), and dried over Na$_2$SO$_4$. Evaporation of the organic solvent gave a crude (2.69 g) which was purified by flash column chromatography on silica gel (hexane/EtOAc 1:1) to afford a second amount of **4** (1.45 g, 20%). Mp 236–238 °C; ^{1}H NMR (0.1 M in CDCl$_3$, 200 MHz) δ 7.83–7.66 (AA'BB' system, 12H), 4.94 (bs, 6H), 3.10 (q, $J = 7.3$ Hz, 6H), 0.96 (t, $J = 7.3$ Hz, 9H). ^{13}C NMR (CDCl$_3$, 50 MHz) δ 168.2, 145.5, 133.8, 132.0, 129.4, 123.2, 37.4, 23.3, 15.7; MS m/z (%) 641 (2), 639 (2), 607 (7), 493 (9), 479 (14), 349 (9), 193 (1), 168 (19), 130 (51), 128 (51), 104 (100). Anal. Calcd for C$_{39}$H$_{33}$N$_3$O$_6$: C, 73.22; H, 5.20; N, 6.57. Found: C, 72.90; H, 5.00; N, 6.39.

1,3,5-Tris(aminomethyl)-2,4,6-triethylbenzene (5). To a suspension of **4** (3.2 g, 5.0 mmol) in 18 mL of EtOH/toluene (2:1) hydrazine hydrate (0.98 mL, 30.8 mmol) was added at room temperature under

(38) Roelens, S.; Torriti, R. *Supramol. Chem.* **1999**, *10*, 225–232.
(39) Frassineti, C.; Ghelli, S.; Gans, P.; Sabatini, A.; Moruzzi, M. S.; Vacca, A. *Anal. Biochem.* **1995**, *231*, 374–382.

(40) (a) αGal, αMan, βMan: see ref 13b. (b) αGlcNAc: Aguilera, B.; Romero-Ramirez, L.; Abad-Rodriguez, J.; Corrales, G.; Nieto-Sampedro, M.; Fernandez-Mayoralas, A. *J. Med. Chem.* **1998**, *41*, 4599–4606. (c) βGlcNAc: Zemlyakov, A. E.; Tsikalov, V. V.; Kalyuzhin, O. V.; Kur'yanov, V. O.; Chirva, V. Y. *Russ. J. Bioorg. Chem.* **2003**, *29*, 286–292.
(41) Adasch, V.; Hoffmann, B.; Milius, W.; Platz, G.; Voss, G. *Carbohydr. Res.* **1998**, *314*, 177–187.

nitrogen atmosphere. The reaction mixture was refluxed for 20 h, and during this time a white solid was formed. The reaction was cooled to room temperature, and the white solid was filtered off, dissolved in a 40% aqueous solution of KOH (120 mL), and extracted with CHCl$_3$ (3 × 150 mL). The combined organic layers were washed with water (3 × 150 mL) and dried over Na$_2$SO$_4$. Evaporation of the organic solvent gave **5** (0.973 g, 78%) as a white solid. Mp 138−140 °C; ^{1}H NMR (0.1 M in CDCl$_3$, 200 MHz) δ 3.87 (bs, 6H), 2.82 (q, J = 7.5 Hz, 6H), 1.26 (bs, 6H), 1.23 (t, J = 7.5 Hz, 9H). ^{13}C NMR (CDCl$_3$, 50 MHz) δ 140.3, 137.4, 39.6, 22.5, 16.8.

1,3,5-Tris(*t*-octylureidomethyl)-2,4,6-triethylbenzene (1a). General Procedure. To an ice-cooled solution of **5** (0.84 g, 3.37 mmol) in dry DMSO (33 mL) under nitrogen atmosphere *t*-octyl-isocyanate (1.83 mL, 10.11 mmol) was slowly added. The reaction mixture was stirred at room temperature for 1.5 h; during this time a white solid was formed. The solid was filtered off, suspended in water (100 mL), and extracted with CHCl$_3$ (3 × 150 mL). The combined organic layers were washed with water (3 × 200 mL), dried over Na$_2$SO$_4$, filtered, and concentrated to give **1a** (1.60 g, 2.24 mmol, 67%) as a white solid. The mother liquor was poured into water (200 mL), and the white precipitate formed was filtered off. The solid so obtained was dissolved in CHCl$_3$ (150 mL), washed with water (2 × 100 mL), and dried over Na$_2$SO$_4$. Evaporation of the solvent gave a crude (0.74 g) which was purified by flash column chromatography on silica gel (CHCl$_3$/EtOAc 2:1) to afford a second amount of **1a** (0.59 g, 0.84 mmol, 22%). Mp > 300 °C; ^{1}H NMR (1 mM in CDCl$_3$, 200 MHz) δ 4.30 (d, J = 4.1 Hz, 6H), 4.06 (s, 3H, NH-2), 3.80 (t, J = 4.1 Hz, 3H, NH-1), 2.71 (q, J = 7.4 Hz, 6H), 1.75 (s, 6H), 1.39 (s, 18H), 1.19 (t, J = 7.4 Hz, 9H), 1.01 (s, 27H). ^{1}H NMR (15 mM in CDCl$_3$, 200 MHz) δ 4.39 (bs, 3H, NH-2), 4.25 (bd, J = 3.5 Hz, 6H), 4.11 (m, 3H, NH-1), 2.68 (q, J = 7.2 Hz, 6H), 1.73 (s, 6H), 1.35 (s, 18H), 1.16 (t, J = 7.2 Hz, 9H), 1.00 (s, 27H). ^{13}C NMR (15 mM in CDCl$_3$, 50 MHz) δ 156.9, 143.5, 132.8, 54.4, 52.0, 38.6, 31.6, 31.5, 30.1, 22.8, 16.7. Anal. Calcd for C$_{42}$H$_{78}$N$_6$O$_3$: C, 70.54; H, 10.99; N, 11.75. Found: C, 70.31; H, 10.99; N, 12.04.

1,3,5-Tris(phenylureidomethyl)-2,4,6-triethylbenzene (1b). Prepared according to the General Procedure. White solid, 207 mg (86%); mp > 300 °C; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 8.30 (s, 3H, NH-2), 7.38−7.17 (m, 4H), 6.92−6.85 (m, 1H), 6.13 (bs, 3H, NH-1), 4.34 (bs, 3H), 2.79 (q, J = 7.7 Hz, 6H), 1.17 (t, J = 7.7 Hz, 9H); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 155.2, 143.2, 140.7, 133.5, 129.2, 121.6, 117.9, 37.7, 22.8, 17.0.

1,3,5-Tris(4-isopropylphenylureidomethyl)-2,4,6-triethylbenzene (1c). Prepared according to the General Procedure. White solid, 223 mg (95%); mp dec > 290 °C; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 8.19 (s, 3H, NH-2), 7.28−7.05 (AA′BB′ system, 12H), 6.07 (bt, 3H, NH-1), 4.33 (bd, 6H), 2.81−2.71 (m, 9H), 1.16−1.13 (m, 9H); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 155.3, 143.2, 141.6, 138.4, 133.6, 126.8, 118.2, 37.7, 33.1, 24.5, 22.8, 16.9.

1,3,5-Tris(benzylureidomethyl)-2,4,6-triethylbenzene (1d). Prepared according to the General Procedure. White solid, 187 mg (89%); mp dec > 280 °C; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 7.34−7.21 (m, 15H), 6.22 (bt, 3H, NH), 5.86 (bt, 3H, NH), 4.24 (bd, 6H), 4.24 (bd, 6H), 2.70 (q, J = 7.2 Hz, 6H), 1.11 (t, J = 7.2 Hz, 9H); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 158.1, 142.3, 141.3, 133.7, 128.7, 127.4, 127.0, 43.4, 38.0, 22.6, 16.9.

1,3,5-Tris(*tert*-butylureidomethyl)-2,4,6-triethylbenzene (1e). Prepared according to the General Procedure. White solid, 126 mg (72%); mp > 300 °C; ^{1}H NMR (DMSO-d_6, 200 MHz): δ 5.62 (s, 3H, NH-2), 5.53 (bt, NH-1), 4.15 (bs, 6H), 2.65 (q, J = 7.2 Hz, 6H), 1.20 (s, 27H), 1.09 (t, J = 7.2 Hz, 9H); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 157.4, 142.7, 133.9, 49.4, 37.4, 29.8, 22.6, 17.0.

Supporting Information Available: Appendix: generalized treatment of BC$_{50}$ and BC$_{50}^0$ for host−guests systems. Data tables, result pages and plots of experimental and calculated shifts from titrations of glycosides of Chart 1 and from self-association experiments (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA045813S