Carbohydrate−Carbohydrate Interactions in Water with Glycophanes as Model Systems

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The synthesis and conformational properties of glycophanes **2** and **3** (cyclodextrin−cyclophane hybrid receptors containing two maltose units linked by (4-hydroxymethyl) benzoic acid spacer) are described. The binding properties in water of these receptors with a series of 4-nitrophenyl glycosides with α- and *β*-configurations at the anomeric center have been studied using ^{1}H NMR spectroscopy and molecular mechanics calculations. A comparison of these properties with those of glycophane **1** (an α,α-trehalose containing glycophane) and α-cyclodextrin (αCD) using the same glycosides shows the existence of a stabilizing contribution to the free energy of binding in the case of of glycophanes but not in the case of the αCD system. This contribution is due to carbohydrate−carbohydrate interactions between both host and guest lipophilic sugar surfaces. Glycophanes **1**, **2**, and **3** show similar α/*β* selectivity on binding the ligands, despite the great flexibility of **3** related to **1** and **2**. Parallels are drawn between the thermodynamic behavior of these model systems and that proposed for sugar−protein interactions.

Introduction

There exists strong evidence that, in addition to the well-established carbohydrate−protein interactions,[1] cells use carbohydrate−carbohydrate (CARB−CARB) interactions as molecular mechanisms for adhesion.[2] The molecules involved in this adhesion are glycolipids[2] (Le^x, GM_3, Gg_3, etc.) and proteoglycans as a proteoglycan[2b] isolated from a marine sponge. A characteristic feature of this interaction is its extremely low affinity that has to be compensated for by aggregation and subsequent multivalency and its dependence on bivalent cations (Ca^{2+}, Mg^{2+}, and Mn^{2+}).[2]

Hakomori has proposed complementarity between hydrophobic surfaces and subsequent Ca^{2+} chelation to some oxygen atoms of the carbohydrate[2a] to reinforce the interaction between glycolipids; however, structural data about this interaction or absolute values of association energies have not yet been determined.

Some years ago we began to use model systems to understand how carbohydrates interact with each other in water. We designed and synthesized a novel type of receptors named glycophanes. These receptors are composed of disaccharides and aromatic segments and may be considered as cyclodextrin−cyclophane hybrids.[3] The question we addressed with these receptors was whether it was possible to show CARB−CARB interactions in water between the constituent sugars of these receptors and simple saccharides, even though calorimetric evidence suggests that this interaction is an unfavorable process.[4] With the system formed by one of these receptors, glycophane **1**, and a series of 4-nitrophenyl glycosides (PNPGly) (Chart 1), we have shown the existence of CARB−CARB interactions in water between lipophilic patches of both host and guest carbohydrate moieties.[5] The contribution of this interaction to the free energy of binding (Δ*G*) was up to 1.8 kcal·mol^{-1}.

In this paper we report a detailed account of the synthesis and properties of two new glycophanes **2** and **3**, and we present the similarities and differences in saccharide binding among these receptors and glycophane **1** toward 4-nitrophenyl glycosides (PNPGly).

One of the requirements of synthetic hosts is to be relatively rigid so that loss of conformational entropy upon binding of a guest will be minimized. On the contrary, a more flexible receptor can allow better complementarity between host and guest surfaces by an "induced fit" mechanism. The different flexibilities of **2** and **3** and their reference partner the α-cyclodextrin (αCD) may allow us to address this question. The binding ability of αCD and glycophanes **1**, **2**, and **3** is also compared in terms of differential contributions of the CARB−CARB interaction to the free energy of binding.

Results and Discussion

Synthesis and Interconversion of Glycophanes 2 and 3. The first glycophane we reported, compound **1**, was designed on the basis of a conformationally restricted and highly symmetric disaccharide, α,α-trehalose, and an electron-rich aromatic segment, 2,7-dihydroxynaph-

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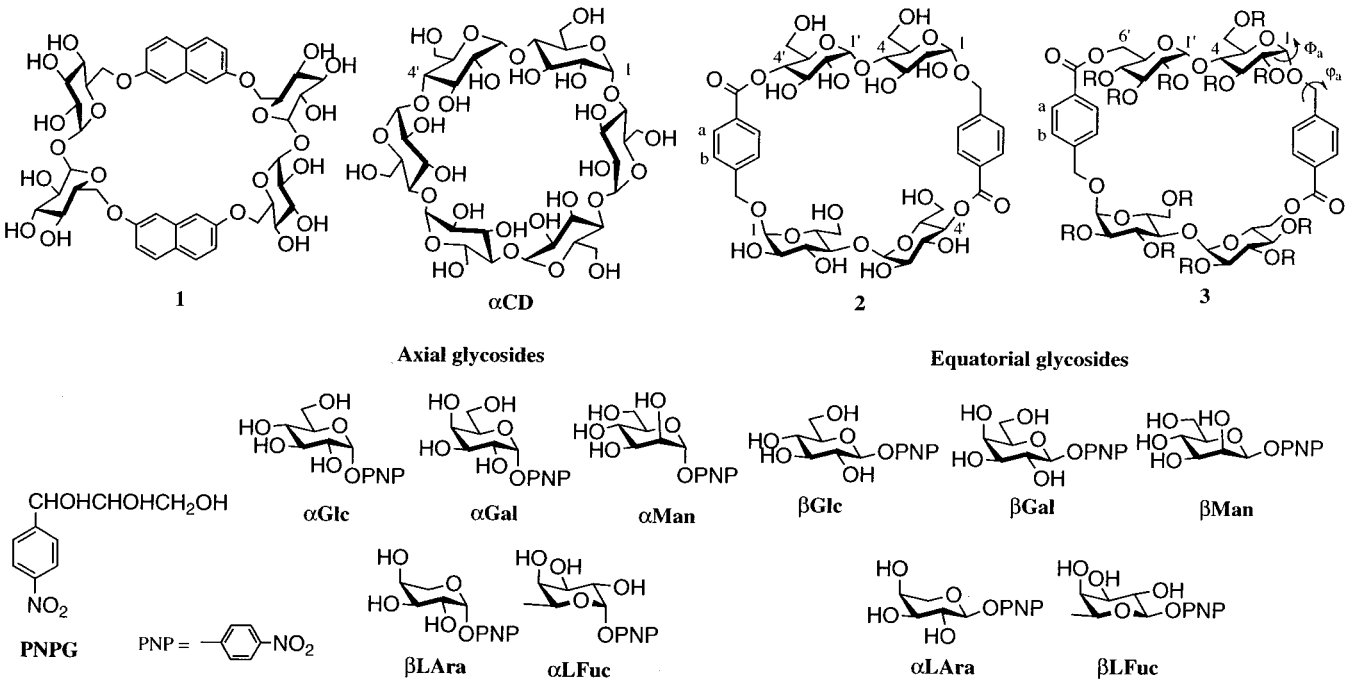
Chart 1. Structure of Glycophanes 1, 2, 3, and αCD and the Axial and Equatorial 4-Nitrophenyl Glycosides



thalene[3,5] (Chart 1). For the new glycophanes **2** and **3** we have used the disaccharide maltose [4-*O*-(α-D-glucopyranosyl)-D-glucopyranose] and (4-hydroxymethyl)-benzoic acid as the aromatic segment (Chart 1). These cyclodextrin−cyclophane hybrid receptors are the result of the substitution of two glucose units (1 and 4) in α-cyclodextrin by an aromatic ring. It was thought that in the new glycophanes **2** and **3** the maltose moiety will expose more surface to the solvent than in the case of the torus-shaped cyclodextrins. The hydroxymethyl group of the aromatic ring is linked to the anomeric position of the maltose unit, while the carboxyl group is linked to either position 4′ (glycophane **2**) or position 6′ (glycophane **3**) of the other maltose molecule. This confers on the receptors different flexibilities and topologies, so that the maltose moieties present different regions of their surfaces to interact with the ligands.

Glycophane **2** was synthesized starting from maltose, and glycophane **3** was obtained by transacylation of **2** in water in almost quantitative yield. A preliminary communication on the synthesis of **2** has already been reported.[6] The synthetic route is summarized in Scheme 1, and the synthetic details are given in the Experimental Section. For the synthesis of glycophane **3**, a route similar to that for **2** starting from compound **8** could be envisaged (Scheme 1). However, the nearly quantitative transacylation in water of the ester group from position 4′ to 6′ in **2** permitted ready access to glycophane **3**.

In an aqueous solution, glycophane **2** undergoes a spontaneous double transacylation by transposition of the ester group from the 4′ to the 6′ position of the maltose moiety to give the new isomeric glycophane **3**. The reaction was followed by HPLC in a reverse-phase column, and after 2 days at 37 °C, the transesterification was complete. During this process a new product, in addition to **2** and **3**, was detected, probably the asymmetric glycophane that results from the transposition of only one of the ester groups. The transacylation rate of **2** depends on temperature, solvent, and concentration. At 50 °C, more than 80% of **3** was formed after 24 h. In methanol or in a micellar aqueous solution of sodium dodecyl sulfate, the rate is much slower, and in DMSO the transposition is not observed at all. Furthermore, as the concentration of **2** increases, the transposition rate decreases dramatically. The same is observed when glycophane **2** is dissolved in the presence of some aromatic guests (picric acid; 2,3-disulfonic naphthalene; or 1,3,5-trihydroxybenzene) that interact with the receptor. The inclusion of these guests in the cavity of **2** probably changes its geometry so that the carbonyl group is placed further away from the hydroxyl group at position 6′. A similar effect could explain the decrease of the transposition rate when the concentration of **2** increases. The amphiphilic nature of **2** makes possible self-association by including an aromatic ring of one of the molecules in a second molecule.

Conformational Studies of Glycophanes 2 and 3. The solution conformation of the isomeric glycophanes **2** and **3** has been analyzed by NMR spectroscopy and molecular mechanics and dynamics simulations, and the results have already been published.[7] The comparison of the calculated minima for αCD, **2**, and **3** given in Figure 1 shows the structural differences between these receptors. From these differences a differential binding behavior toward the same ligands is to be expected.

The conformational behavior of **2** and **3** is rather different. Glycophane **2** presents in solution only a family of conformers with geometry similar to that of αCD (Figure 1A,B). In the global minimum, the interglycosidic angles ϕ (H1′−C1′−O4−C4) and φ (C1′−O4−C4−H4) of the maltose units are −4° and −30°, respectively, which are similar to those of the minimum B (ϕ/φ

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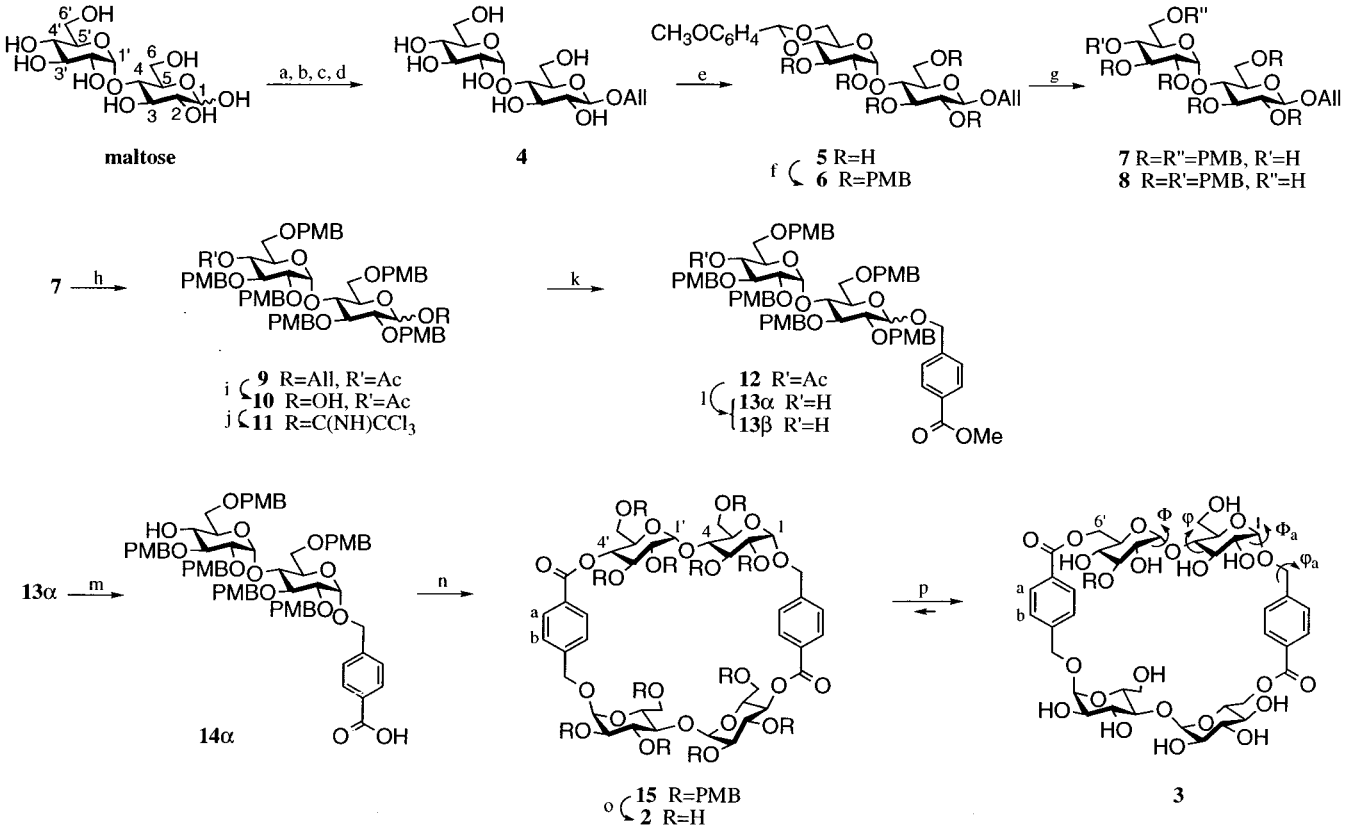
Scheme 1[a] **Synthesis of Glycophanes 2 and 3**



[a] Reagents and conditions: (a) $NaOAc/Ac_2O$; (b) HBr 33%/AcOH/CH_2Cl_2, 25 °C, 45 min; (c) AllOH/$Hg(OAc)_2$, 25 °C, 4 h; (d) NaOMe/MeOH, 25 °C, 10−12 h, 44% overall; (e) *p*-$MeOC_6H_4CH(OMe)_2$/*p*-TsOH/DMF, 60 °C, 2 h, 62%; (f) NaH/*p*-$MeOC_6H_5CH_2Cl$ (PMBCl)/DMF, 25 °C, 16 h, 89%; (g) $NaCNBH_3/CF_3COOH$/DMF, 25 °C, 16 h, **7**: 74%, **8**: 11%; (h) Ac_2O/py, 25 °C, 15 h, 98%; (i) $RhCl(PPh_3)_3$/10%EtOH/To, 85 °C, 16 h, then HgO/$HgCl_2$/acetone/H_2O, 100 °C, 2.5 h, 81%; (j) $K_2CO_3/Cl_3CCN/CH_2Cl_2$, 25 °C, 15 h; (k) TMSOTf/$HOCH_2PhCOOMe/CH_2Cl_2$, −78 °C, 20 min, 80% (α/β 1.5:1); (l) MeONa/MeOH, 25 °C, 18 h, **13α**: 56%, **13β**: 39%; (m) KOHaq/MeOH/THF, 25 °C, 20 h, 100%; (n) DCC/DMAP/DMAP·HCl/$CHCl_3$, reflux, 6 h, 29%; (o) CF_3COOH 5%/CH_2Cl_2, 25 °C, 5 h, 95%; (p) H_2O, 50 °C, 3 days, 98%.

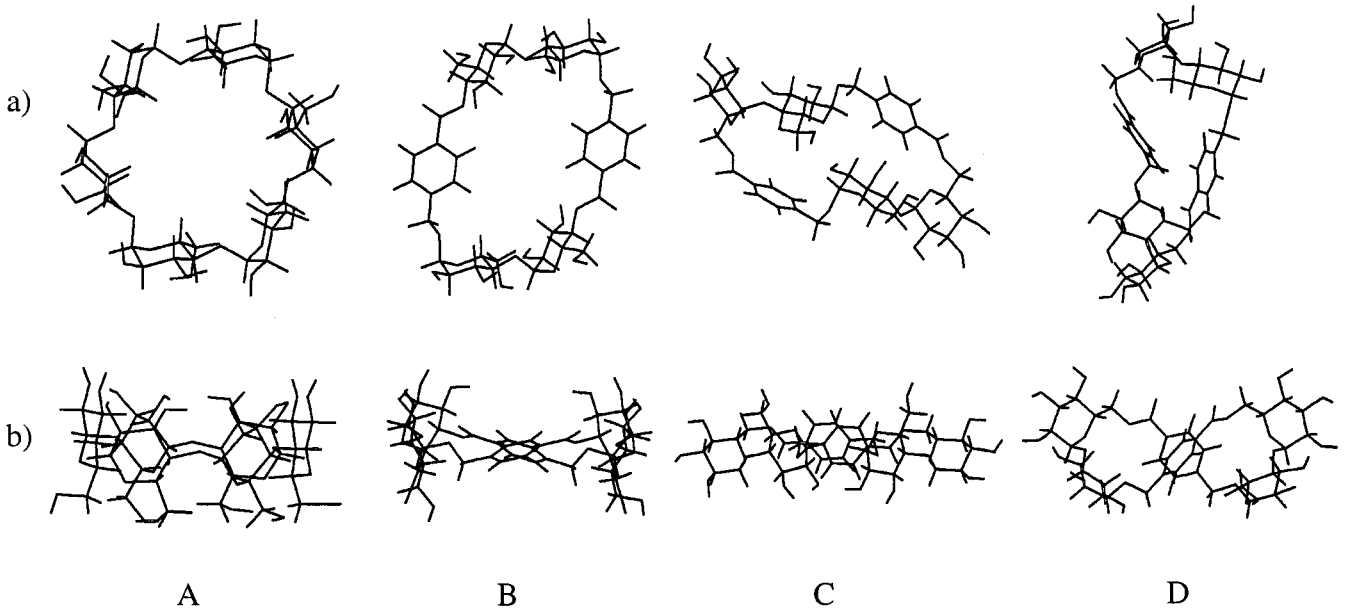


Figure 1. Top view (a) and front view (b) of the calculated global minima for αCD (A); glycophane **2** (B), and glycophane **3** (C and D).

−3°/−30°) found for maltose in solution.[8] While glycophane **2** is fairly rigid, glycophane **3** is rather flexible and shows, in aqueous solution, an equilibrium between three different conformers (the third conformer is not shown in Figure 1).[7] In the global minimum (Figure 1C), the cavity is collapsed by a double sugar aromatic stacking between the aromatic ring and the β-face of the glucose

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Table 1. Association Constants (K_a, M^{-1}) and Free Energy of Binding ($-\Delta G$, kcal·mol^{-1}) of the Complexes Formed between α-Cyclodextrin or Glycophanes 1, 2, and 3 and 4-Nitrophenyl Glycosides or 1-(4-Nitrophenyl)glycerol (PNPG)

	α−CD			**1**		**2**			**3**		
	$-\Delta G^a$	$-\Delta G^b$	$-\Delta\Delta G^c$	$-\Delta G^a$	$-\Delta\Delta G^c$	$K_a{}^d$	$-\Delta G$	$-\Delta\Delta G^c$	$K_a{}^d$	$-\Delta G$	$-\Delta\Delta G^c$
αGlc	2.9 ± 0.1	2.8 ± 0.01	0	2.8 ± 0.1	1.0	78	2.6 ± 0.1	0.7	22	1.9 ± 0.1	0.2
αGal	2.8 ± 0.1	2.7 ± 0.01	−0.1	2.6 ± 0.1	0.8	37	2.2 ± 0.1	0.3	20	1.8 ± 0.1	0.1
αMan	3.0 ± 0.1	2.9 ± 0.01	0.1	3.0 ± 0.1	1.2	100	2.8 ± 0.1	0.9	57	2.4 ± 0.1	0.7
βLAra	3.0 ± 0.1	2.9 ± 0.01	0.1	3.0 ± 0.1	1.2	92	2.7 ± 0.1	0.8	42	2.2 ± 0.1	0.5
αLFuc	2.9 ± 0.1	2.9 ± 0.01	0	3.3 ± 0.1	1.5	101	2.8 ± 0.1	0.9	40	2.2 ± 0.1	0.5
βGlc	2.6 ± 0.1	2.7 ± 0.01	−0.3	2.7 ± 0.1	0.9	32	2.1 ± 0.1	0.2	19	1.8 ± 0.1	0.1
βGal	2.9 ± 0.1	2.8 ± 0.01	0	2.7 ± 0.1	0.9	36	2.2 ± 0.1	0.3	23	1.9 ± 0.1	0.2
βMann	2.8 ± 0.1	2.8 ± 0.01	−0.1	2.6 ± 0.1	0.8	55	2.4 ± 0.1	0.5	22	1.9 ± 0.1	0.2
αLAra	2.8 ± 0.1	2.6 ± 0.01	−0.1	2.6 ± 0.1	0.8	60	2.5 ± 0.1	0.6	26	2.0 ± 0.1	0.3
βLFuc	2.8 ± 0.1	2.8 ± 0.01	−0.1	2.8 ± 0.1	1.0	64	2.5 ± 0.1	0.6	18	1.7 ± 0.1	0.0
PNPG	2.9 ± 0.1	*e*		1.8 ± 0.1		23	1.9 ± 0.1		16	1.7 ± 0.1	

[a] From NMR data in D_2O at 303 K.[5] [b] From calorimetry data in H_2O at 298 K.[13] [c] ΔG(PNP−glycosides) − ΔG(PNPG) in kcal·mol^{-1}. [d] From NMR data in D_2O at 303 K; maximum percent of estimated error was 20%. [e] Not measured.

Table 2. Induced Chemical Shifts (ICS)[a] Observed in ^{1}H NMR for Glycophane 2 upon the Formation of the Complexes with 4-Nitrophenyl Glycosides

	axial glycosides					equatorial glycosides				
	2−αGlc	2−αGal	2−αMan	2−βLAra	2−αLFuc	2−βGlc	2−βGal	2−βMan	2−αLAra	2−βLFuc
Ha	−0.188	−0.360	−0.361	−0.211	−0.123	−0.287	−0.299	−0.127	−0.091	−0.187
Hb	−0.080	−0.161	−0.148	−0.088	−0.048	−0.117	−0.122	−0.051	−0.035	−0.073
H4′	−0.021	−0.046	−0.039	−0.019	−0.041	−0.057	−0.056	−0.017	*b*	−0.041
H3′	−0.027	*b*	−0.055	*b*	*b*	−0.074	−0.088	−0.035	−0.024	−0.052
H5′	−0.014	*b*	*b*	*b*	*b*	−0.056	*b*	−0.025	*b*	*b*
H3	+0.040	*b*	+0.079	*b*	+0.021	+0.065	+0.068	+0.031	+0.023	+0.042

[a] For ICS: +, downfield shifts; −, upfield shifts. [b] It could not be observed.

units. This interaction forces glycophane **3** to adopt a folded geometry which places the maltose molecule in a conformation (ϕ/ψ −55°/−60°, ϕ_a/ψ_a 69°/70°) different from that found in glycophane **2** (ϕ/ψ −4°/−30°, ϕ_a/ψ_a 68°/−17°). The second minimum (Figure 1D) has interglycosidic angles ϕ and ψ (11°/15°) similar to those found for maltose in the solid state.[9] The aromatic rings show a face-to-edge $\pi-\pi$ interaction, which closes the cavity. The equilibrium between both conformers is influenced by temperature and solvent[7] as well as by the presence of ligands in the aqueous solution (see below). The different conformational presentation of the maltose moiety in **2**, **3**, and αCD makes these molecules good models to study the influence of oligosaccharide flexibility[7,10] and surface presentation[11] in the molecular recognition of carbohydrates.

Binding Studies. The new glycophanes **2** and **3** have electron-deficient aromatic walls and, as is to be expected from the nature of arene−arene interactions,[12] will interact favorably with the electron-deficient 4-nitrophenyl glycosides. The electron-rich benzyl β-D-glucopyranoside derivative interacts unfavorably with glycophanes **2** and **3**.

Thus, the association constants, K_a in water between **2** and **3** and a series of 4-nitrophenyl glycosides with axial α-D-gluco- (αGlc), α-D-galacto- (αGal), α-D-manno- (αMan), β-L-arabino- (βLAra), and α-L-fucopyranoside (αLFuc) and equatorial β-D-gluco- (βGlc), β-D-galacto- (βGal), β-D-manno- (βMan), α-L-arabino- (αLAra), and β-L-fucopyranoside (βLFuc) configuration at the anomeric center (Chart 1) have been determined by ^{1}H NMR spectroscopy. To evaluate the contribution of the pyranose ring, the K_a of the 1-(4-nitrophenyl) glycerol (PNPG), a lineal-chain saccharide, was also determined as reference ligand. The association constant of the other reference compound, the 4-nitrophenol, could not be determined in this case because of solubility problems. Table 1 summarizes the association constants and the corresponding free energies of binding (ΔG kcal·mol^{-1}) at 30 °C for all complexes. For comparative reasons, the ΔG values previously determined for αCD[5,13] and glycophane **1**[5] complexes are also given in Table 1.

The binding experiments were carried out at constant concentration of the host and by the addition of increasing concentrations of guests, as described in the Experimental Section. Different induced chemical shift (ICS) patterns for **2** and **3** were obtained upon addition of the guests (Tables 2 and 3).

In the case of glycophane **2** (Table 2), upfield shifts for the aromatic protons (Ha and Hb) and of H3′, H5′, and H4′ and downfield shifts for H3 were observed. This pattern was the same in all cases, indicating a common geometry for all complexes. These shifts are consistent with a geometry where the aromatic ring of the ligand is parallel to the aromatic walls of the host, placing the H3′ and H5′ protons in the shielding cone of the 4-nitrophenyl ring and the H3 and, probably, H5 (its signal was overlapped) outside the cone. The upfield shift observed for the H4′ proton, which is outside of the cavity, can be explained by changes in the position of the adjacent carbonyl group upon complexation, as confirmed by molecular mechanics simulations (see below).

For glycophane **3**, upfield shifts for H1′, H4′, H5′, H6′R, H6′S, and aromatic protons, and downfield shifts for H2, H3, and H4, could be observed upon addition of the

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Table 3. Induced Chemical Shifts (ICS)[a] Observed in ^{1}H NMR for Glycophane 3 upon the Formation of the Complexes with 4-Nitrophenyl Glycosides

	axial glycosides					equatorial glycosides				
	3−αGlc	3−αGal	3−αMan	3−βLAra	3−αLFuc	3−βGlc	3−βGal	3−βMan	3−αLAra	3−βLFuc
Ha	−0.080	−0.085	−0.078	−0.071	−0.112	−0.122	−0.102	−0.125	−0.060	−0.071
Hb	−0.023	−0.026	+0.008	−0.018	−0.038	−0.048	−0.038	−0.040	−0.018	−0.024
H1′	−0.073	−0.082	−0.123	−0.062	−0.075	−0.089	−0.075	−0.098	−0.050	−0.071
H6′R	−0.107	−0.120	−0.269	−0.091	−0.069	−0.096	−0.075	−0.125	−0.064	−0.058
H6′S	−0.067	−0.084	−0.155	−0.060	−0.078	−0.104	−0.085	−0.121	−0.054	−0.061
H2[b]	+0.110	+0.118	+0.162	+0.102	+0.145	+0.129	+0.122	+0.159	+0.084	+0.096
H3	*c*	*c*	+0.028	+0.039	+0.034	+0.041	*c*	*c*	+0.021	+0.020
H5′	−0.024	−0.017	−0.089	−0.033	−0.002	−0.052	*c*	*c*	*c*	−0.015
H4′	−0.026	−0.033	−0.062	−0.020	−0.006	−0.026	−0.020	−0.033	−0.016	−0.040
H4[b]	+0.062	+0.059	+0.086	+0.059	+0.077	+0.026	+0.074	+0.090	+0.043	+0.067

[a] For ICS: +, downfield shifts; −, upfield shifts. [b] ICS caused by the conformational changes, from folded to unfolded forms. [c] It could not be observed.

ligands (Table 3). In a first attempt it is difficult to establish a geometry for the complexes of **3** that satisfies all the observed ICS. However, it should be remembered that the major conformer of **3** is in a folded conformation (Figure 1C), which does not possess a cavity for binding, so that the equilibrium has to be shifted to an open conformation to accept the guest in a clear "induced-fit" mechanism. The ICS observed for **3** can be explained as the result of two effects: (a) the folded conformation being shifted to an open one, as indicated by the downfield shift of the H1, H2, H4, H6R, and H6S protons (H1, H6R, and H6S are not shown in Table 3); and (b) the effect of binding of the aromatic guest into the opened cavity, shown by the upfield shift of the aromatic and the H5′, H6′R, and H6′S protons and the downfield shift of the H3 proton. Thus, the interaction of glycophane **3** with the ligands implies both a strong conformational change upon binding and a reduction of the conformational space of **3**, which may result in unfavorable entropy effects. This fact may be the cause of the lower association constants observed for glycophane **3** compared to those observed for the more rigid receptors **1**, **2**, and αCD (Table 1).

We have tried to assess the tridimensional structure of the complexes by intermolecular NOEs. For the **2** and **3** complexes, monodimensional NOEs and ROESY experiments were carried out. Intermolecular NOEs between the aromatic protons of the host and guests were observed. However, the intermolecular NOEs observed between the sugar residues were too small to draw an unambiguous conclusion about the geometry of the complexes.

Molecular Modeling. The interactions of **2** and **3** with 4-nitrophenyl α- and β-D-gluco- and -mannopyranosides were examined using the MACROMODEL v4.5 package. The guests were docked into the glycophane hosts as described in the Experimental Section. The global minimum previously found for the free glycophane **2** (Figure 1B) was used as the starting geometry to dock the different ligands. In the case of **3**, the global minimum is a folded conformation without a cavity. Therefore, the nonfolded conformation (Figure 1D) was taken as the starting geometry for the docking. The structures of the free hosts present two different faces. Calculations for the two modes of ligand entry into the host cavity were performed. For glycophane **2**, the energies obtained for the two types of complexes were similar. However, for **3**, the complexes where the ligands enter at the secondary hydroxyl side are more stable than complexes obtained by entry of the ligands at the primary hydroxyl side.

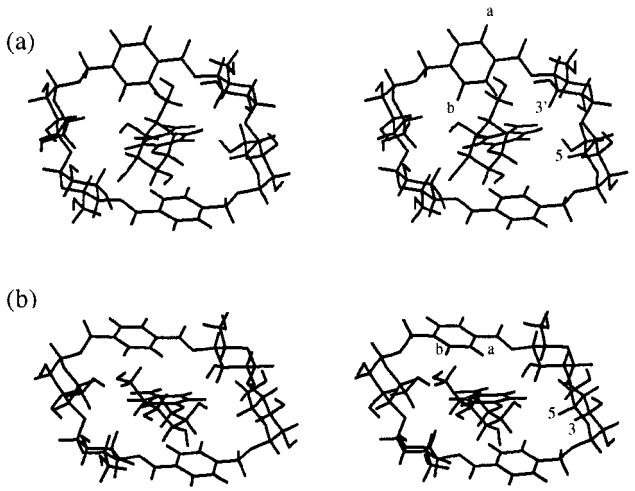

Figure 2. Stereoview of the calculated minima for the complexes **2**−αMan (a) and **2**−βMan (b).

All minima found for the **2** complexes belong to one major conformation in which the maltose moieties show the same interglycosidic angles, ϕ and φ, as in the free host. A stereoview of the global minima conformers for the **2**−αMan and **2**−βMan complexes are given in Figure 2.

The conformational change observed in the free host upon complexation is only the relative orientation between the aromatic rings, which in the complexes maximizes the interactions with the 4-nitrophenyl group. This change places the carbonyl group at position 4′, further away from the OH6′, which would explain both the upfield shift observed for the 4′ proton (Table 2) and the decrease of the transacylation rate of **2** in the presence of ligand. Besides the expected aromatic−aromatic interactions, van der Waals contacts between lipophilic patches of both host and guest carbohydrate moieties are also present. This CARB−CARB interaction can account for the additional stabilization found in these complexes related to the **2**−PNPG complex (−ΔΔ*G* in Table 1). The calculated geometry for these complexes agrees reasonably with the experimental ICS observed in **2** upon addition of the guests (Figure 2, Table 2).

The docking of the 4-nitrophenyl glycosides into **3** results in two different structural families of complexes, A and B, with similar steric energies due to the big cavity and great flexibility of glycophane **3**. Stereoviews of the

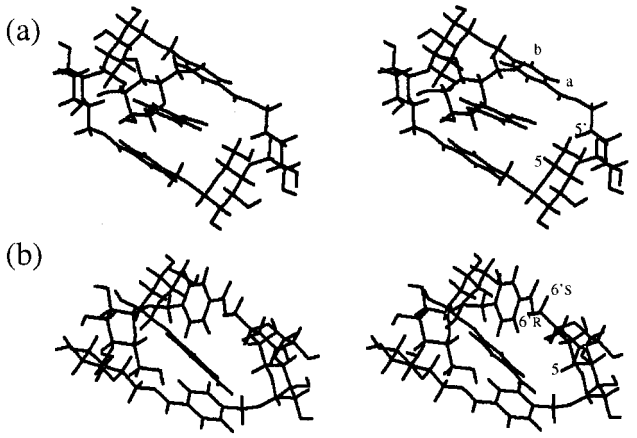

Figure 3. Stereoview of the calculated minima for the complex **3**−αMan: (a) minimum A; (b) minimum B.

minimum energy conformers of A and B structures are given in Figure 3 for the complex formed between **3** and the 4-nitrophenyl α-D-mannopyranoside. In both geometries, the receptor shows a cavity to accept the ligand so that its conformation is different from that of the free receptor. In the calculated structure A (Figure 3a), the sugar residue shows van der Waals contacts with the sugar moiety of the receptor, and a hydrogen bond between the OH3 of the manno residue and the OH4′ of the receptor is also possible. The whole geometry resembles the calculated geometry for the complexes with compound **2**. In the structure B (Figure 3b), one of the maltose units changes the interglycosidic ϕ and ψ angles to 0° and 3°, respectively. In the calculated geometry for the **3**−αMan complex (Figure 3b), contacts between the α face of the mannosyl residue and the aromatic residue of the receptor are favored. Sugar−arene stacking is one of the most frequent binding motifs found in the crystal structure of sugar−protein complexes.[1] However, its contribution to the energetic of binding has only been determined in the case of a maltose-binding protein.[14]

The molecular mechanics calculations for the **3**−PNPGly complexes are consistent with the idea of an induced-fit mechanism for binding, as proposed on the basis of the experimental ICS observed for glycophane **3** upon addition of the guests (see above and Table 3), which implies two processes: (a) a conformational change in the free host **3** (downfield shifts for the H1, H2, and H4 protons) and, (b) the binding of the aromatic guests into the cavity (upfield shift of the aromatic and H5′, H6′R, and H6′S protons and downfield shift of the H3 and H5 protons).

Although the calculated geometry might be different from the real situation in solution, the results presented here agree reasonably with the experimental data observed for these interactions.

Comparison of the Binding Properties of 1, 2, 3, and αCD. The results presented here, together with our previous results, constitute a useful data set on the interaction in water of four different model systems (Figure 4). These systems, constituted by either α-cyclodextrin or glycophanes **1**, **2**, and **3** as receptors and a series of 4-nitrophenyl glycosides of varied stereochemistry as ligands, have been proven to be appropriate

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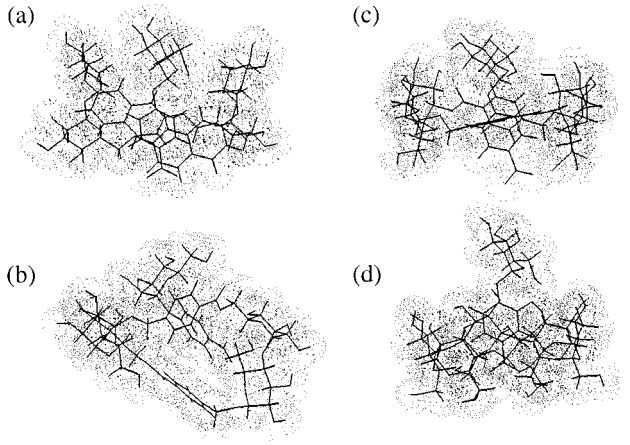

Figure 4. Calculated minima for the complexes **1**−αMan (a); **2**−αMan (b); **3**−αMan (c); and αCD−αMan (d) showing the van der Waals radii.

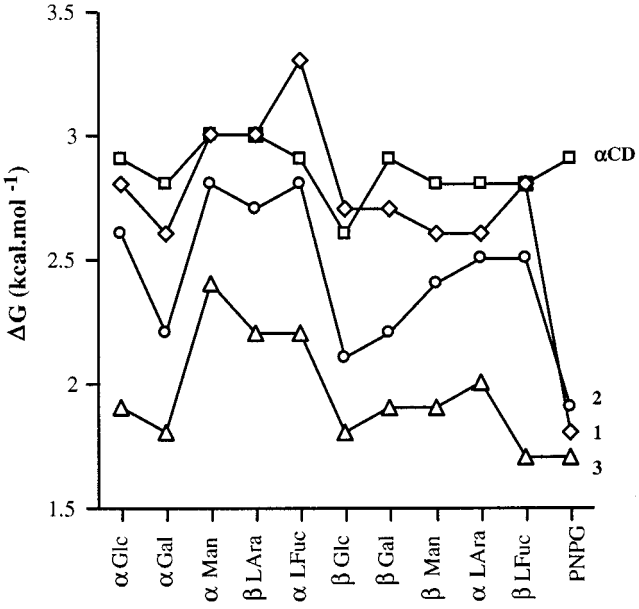

Figure 5. Comparative graphic of the free energy of binding (ΔG/kcal·mol^{-1}) of αCD or glycophanes **1**, **2**, and **3** and axial and equatorial PNPGly and PNPG.

models for studying the nature of the apolar binding between neutral sugars in aqueous solution.

The free energies of binding ($-\Delta G$, Table 1) have been obtained by ^{1}H NMR spectroscopy, and additionally, for the αCD system, accurate values of enthalpy and entropy could be determined by titration calorimetry.[13] Although glycophanes **2** and **3** have little structural homology with glycophane **1**, they clearly show common *tendencies* in their energetic behavior, as the results in Table 1 and Figure 5 reveal: (a) The free energies of binding for the axial glycosides are usually higher than those for the equatorial glycosides. (b) In the equatorial complexes, ΔG values do not change with the stereochemistry of the ligands, while in the axial glycosides, these values increase, depending on the stereochemistry, in the order αGal < αGlc < βLAra < αMan < αLFuc. This influence is not observed either in the axial or in the equatorial complexes with αCD. (c) The free energy of binding decreases in the order **1** > **2** > **3** for all complexes. This decrease is proportional to the increase in conformational flexibility on going from **1** to **3**. (d) In all complexes, a

stabilizing contribution to the binding ($-\Delta\Delta G$, Table 1) was observed due to the presence of the pyranose ring. This stabilization could be evaluated by comparing the ΔG values to those obtained for the complexes that are formed between the PNPG and the glycophanes. The highest contribution was observed for the **1**−αLFuc complex with 1.5 kcal·mol^{-1} stabilization related to the glycerol derivative. The lowest contribution corresponds to the complexes with the most flexible glycophane **3**, in which a small stabilization was found only for the **3**−αMan, **3**−βLAra, and **3**−αLFuc complexes. This contribution was not found in the αCD system. We have attributed this stabilization to CARB−CARB interactions between both host and guest pyranose moieties, present in the glycophane systems but absent in the cyclodextrin system (Figure 4). This interaction may also be the origin of the binding selectivity observed in glycophane systems related to the cyclodextrin system.

The differences among ΔG values for all the systems are too small to draw conclusions about the differential thermodynamic behavior of glycophanes related to the αCD system (Figure 5). These differences should be more evident in the enthalpy and entropy terms. Unfortunately, accurate enthalpy and entropy values could be determined only for the αCD system.[13]

For glycophanes **1** and **3**, we have determined, by van't Hoff analysis, the enthalpies for the **1**−αGlc, **1**−βGlc, **3**−αGlc, and **3**−αMan complexes. ^{1}H NMR spectra were recorded at 283, 293, 303, and 313 K, which resulted in $\Delta H = -6.6$ kcal·mol^{-1} and $T\Delta S = -4.2$ kcal·mol^{-1} for **3**−αGlc complex and $\Delta H = -4.1$ kcal·mol^{-1} and $T\Delta S = -1.7$ kcal·mol^{-1} for the **3**−αMan complex at 298 K. For the **1**−αGlc and **1**−βGlc complexes, these values are $\Delta H = -4.8$ and -4.1 kcal·mol^{-1} and $T\Delta S = -1.9$ and -1.4 kcal·mol^{-1}, respectively, as previously determined.[5] The values found by calorimetry for the αCD complexes[13] at this temperature are $\Delta H = -5.4$ kcal·mol^{-1} and $T\Delta S = -2.6$ for αCD−αGlc, $\Delta H = -4.5$ kcal·mol^{-1} and $T\Delta S = -1.8$ kcal·mol^{-1} for the αCD−βGlc, and $\Delta H = -5.8$ kcal·mol^{-1} and $T\Delta S = -2.9$ kcal·mol^{-1} for the αCD−αMan complexes. These results indicate that all complexes are exothermic and enthalpy driven near room temperature with an unfavorable entropy term. The contribution provided by the CARB−CARB interaction to the enthalpy of binding seems to be the only difference between glycophanes and cyclodextrin.

These thermodynamic patterns are similar to those found for monosaccharides binding to lectins;[15] the bulk of the binding energy is enthalpic with a small entropy term which opposes binding. Toone[15c] has called attention to the similarity of this energetic pattern with that of the binding, in aqueous solution, of apolar ligands to their corresponding receptors.[16] Since the only similarity among these systems is the solvent water, it has been assumed that the net observed thermodynamic properties are controlled by the behavior of water surrounding both host and guest complementary surfaces.[17] This can be the case in our systems. The common *tendencies* observed and the similar thermodynamics in the interaction of glycophanes **1**, **2**, and **3** with 4-nitrophenyl glycosides might indicate that the small differences in the energetics of binding observed in these systems can only be due to differential dehydration process related to the ability of sugars to structure water molecules in a stereospecific way. However, to confirm this hypothesis, accurate values of enthalpy and entropy will be necessary.

In conclusion, we have shown with our glycophane systems that CARB−CARB interactions in water between small sugars (our hosts and guests may be considered as small glycoconjugates) are possible and contribute to the affinity of binding. The aromatic rings are necessary to bring both host and guest carbohydrate moieties together. Clearly, the presence of carbohydrate residues increases the affinity. Divalent cations were not present, and nonpolar interactions between sugar surfaces seem to be involved in stabilizing binding. The interaction shows α/β selectivity similar to that of lectins.[15a,d,e] Flexibility does not seem to oppose selectivity in glycophanes, and despite the great flexibility of **3**, which results in lower association constants, the α/β selectivity observed in this receptor is of the same order of magnitude as that in the more rigid receptors **1** and **2**.

Establishing the structural origin of affinity and selectivity as well as the connection between structure and thermodynamics in carbohydrate interactions remains a major challenge. The results presented here show that our small systems may be considered good models to mimic carbohydrate interactions in water. They reproduce the nonpolar interactions between lipophilic surfaces found in sugars binding to proteins. They show some selectivity as a function of the stereochemical diversity of these molecules, and additionally, their thermodynamic behavior resembles that of biological systems.

The proposal that nonpolar interactions are involved in CARB−CARB recognition may be quite unexpected for structures as polar as carbohydrates. However, as proposed by Hakomori,[2a] "complementarity of two interacting carbohydrates could be based on hydrophobic interaction between the respective hydrophobic surfaces...".

Experimental Section

General Techniques. Chemicals, including 4-nitrophenyl glycosides, were obtained from commercial sources (Sigma, Aldrich, or Fluka). Solvents were dried as recommended in the literature. Thin-layer chromatography (TLC) was performed on aluminum sheets coated with silica gel 60 F254 (Merck). Column chromatography was carried out on silica gel 60 (Merck, 230−400 mesh). HPLC was performed on a C_{18} reverse-phase column using UV detection. NMR spectra were recorded on 300 or 500 MHz spectrometers with the solvent reference as internal standards. Deuterated solvents used were CD_3OD (99.8% purity, Scharlau), D_2O (99.8% purity, Merck), and DMSO (99.96% purity, Aldrich). The steady-state NOEs were obtained at 500 MHz through the interleaved differential technique using a saturation delay of 5−10 s. The 2D rotating-frame NOE (ROESY, CAMELSPIN) experiments

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were performed in the phase-sensitive mode at 500 MHz. The spin-locking period consists of a train of 30° pulses (2.5 μs) separated by delays of 50 μs, and total mixing time was set to 350 ms. The radio frequency carrier was set at $\delta = 6$ ppm to minimize Hartmann−Hahn effects.

Binding Studies. Titration experiments were performed using NMR spectroscopy at a constant temperature of 303 K. Chemical shifts in D_2O were referenced to external C_6D_6 (7.15 ppm) in a coaxial tube. Solutions of glycophanes **2** and **3** (0.2−0.9 mM) were freshly prepared for every new experiment. Solutions for the guests (10−100 mM) were prepared from the host solution in order to have a constant concentration of the host during the titration. Binding constants were determined by adding in portions, via microsyringe, a solution of guest to a solution of host. The ^{1}H NMR spectrum of each solution was recorded, and the chemical shifts of different protons obtained at 10−12 different host:guest concentration ratios were used in an iterative least-squares-fitting procedure, assuming formation of a 1:1 complex.[18] Each titration experiment was performed two or three times, and the association constants given in Table 1 are the weighted averages of different protons when the observed ICS was at least 0.08 ppm. Saturation reached 30−60%, depending on association constants. The maximum percent of estimated error was 20%.

Computation Procedure. All calculations were performed in a Silicon Graphics workstation with the MM3* force field as integrated in MACROMODEL v4.5. The initial structures for glycophanes **2** and **3** were obtained from an MM3* minimization using the MACROMODEL program and the GB/SA solvation model described by Still and co-workers.[19] The starting geometries for **2** and **3** were built from glucose units in their stable $^4C_1$ *chair* conformation, and the interglycosidic torsional angles of the maltose unit, ϕ and ψ, defined as H1′C1′O4C4 and C1′O4C4H4, were fixed at −3° and −30°, one of the typical minima found for maltose (minimum B). The glycosidic angles of reducing glucose, ϕ_a and ψ_a defined as H1C1O1C(CH_2) and C1O1C(CH_2)C(Ph), were established at $\phi_a = -60°$ following the exo-anomeric effect, and $\psi_a = -180°$ to allow closure of the macrocycle. The conformation of the hydroxymethyl groups for glycophane **2** was set to trans−gauche (*tg*). This position is the least favored in glucose derivatives;[20] therefore, the hydroxymethyl groups will proceed to *gg* or *gt* during the minimization. In the case of glycophane **3**, the hydroxymethyl group of the reducing glucose was set to *tg* as for **2**, and the hydroxymethyl group linked to the aromatic ring was set to *gt*. This is the preferred orientation in solution for **3** as indicated by the coupling constants $J_{5',6'S} = 10.2$ Hz, $J_{5',6'R} < 1$ Hz, and $J_{6'S,6'R} = 11.8$ Hz.

A Monte Carlo (MC) approach was used because of the elevated number of torsional angles in both hosts. One of the bonds between the carbonyl group and oxygen (O4′ for **2**, or O6′ for **3**) was chosen as the ring closure bond. The GB/SA solvation model was used to simulate the water during the conformational search. A global search using 1000 MC steps was performed for each glycophane. A number of torsions between 2 and 13 were randomly selected for modification at each MC step. After each MC step, the resultant geometry was minimized using 5000 gradient conjugate steps. Structures were tested for duplication according to a least-squares criterion (0.25 Å) and the Numbering System Rotation implemented in MACROMODEL for highly symmetric molecules. An energy window of 50 kJ·mol^{-1} was used as the criterion to accept a given conformation. The total number of conformers provided in the search was 310 for glycophane **2** and 222 for glycophane **3**. A criterion of 12 kJ·mol^{-1} over the global minimum was established before comparing the structures obtained in order to select the final set of conformers. Because of the higher flexibility of glycophane **3**, a criterion of 25 kJ·mol^{-1} over the global minimum was chosen. Finally, 25 conformers for **2** and 20 conformers for **3** were selected. The global minimum was extensively minimized, and then its conformational stability was checked using 500 ps of molecular dynamic simulations (MD) by using the MM3* force field. The Shake option[21] to fix C−H bonds was employed during the simulation, and the temperature was kept fixed at 300 K by coupling to a temperature bath.[22] Trajectory frames were saved every 1 ps of simulation. Additionally, 900 ps of MD simulations were performed at 500 K to check the stability of the "folded" and "nonfolded" minima of **3**.

The docking studies were performed from the global minima found in the previous analysis for the isolated glycophanes **2** and **3**. The "nonfolded" conformation of **3** was taken as starting geometry for **3**. The 4-nitrophenyl glycosides were minimized as previously described.[5] The protocol followed was as follows: First, every substrate was docked manually into the cavity and the complex was extensively minimized. Since the cavities of the hosts present two different faces, calculation for the two modes of substrate entry into the cavity was performed. Second, 100 MC steps with random translation and rotation of the guest around the cavity of the host were carried out. Limits between 0 and 2 Å and between 0° and 15° were used for rotation and translation, respectively. In each step, 5000 iterative minimizations using the PR conjugate gradient algorithms were performed. An energy window of 50 kJ·mol^{-1} was used as the criterion to accept a given conformation. In all cases, those complexes with a steric energy difference of 12 kJ·mol^{-1} over the corresponding global minimum were not considered for subsequent analysis.

Allyl 4-*O*-α-D-Glucopyranosyl-*β*-D-glucopyranose (4). Maltose was converted into the octa-*O*-acetyl-*β*-D-maltoside as described.[23] The octa-*O*-acetyl-*β*-D-maltoside (50 g, 74 mmol) in CH_2Cl_2 (300 mL) was treated with a solution of 33% hydrogen bromide in AcOH (100 mL) and stirred at rt for 45 min. The mixture was then diluted with CH_2Cl_2 (500 mL) before being washed successively with ice (ca. 500 g), a saturated solution of $NaHCO_3$ (300 mL), and water (300 mL). The organic extracts were dried (Na_2SO_4) and concentrated to yield a white solid (50 g, 97%), which was identified as hepta-*O*-acetyl-α-D-maltosyl bromide.[24] This compound (12.38 g, 17.5 mmol) was dissolved in allylic alcohol (40 mL), mercuric acetate (4.78 g, 0.85 equiv) was then added, and the reaction mixture was stirred at rt for 4 h. The solvents were evaporated in vacuo, and the residue was dissolved in CH_2Cl_2 (ca. 150 mL) and washed with a 10% aqueous solution of KI (5 × 50 mL). The organic phase was dried (Na_2SO_4) and concentrated, and the residue was purified by column chromatography (AcOEt/hexane 1:2 to AcOEt) to obtain a white solid, which could be crystallized in isopropyl ether/AcOEt (7:1) to yield the allyl hepta-*O*-acetyl-*β*-D-maltoside. The product was suspended in MeOH, and NaOMe/MeOH (1 M, 1 mL) was added. The mixture was stirred overnight, and then the solution was neutralized with Amberlite-H^+ IR-120, filtered, and concentrated to dryness, to obtain the allyl-*β*-D-maltoside (**4**).[25] Yield: 44% starting from maltose.

Allyl 4-*O*-(4,6-*O*-4-Methoxybenzylidene-α-D-glucopyranosyl)-*β*-D-glucopyranoside (5). To a solution of allyl-*β*-D-maltoside (**4**) (12.44 g, 32.49 mmol) in dry DMF (80 mL) were added 4-methoxybenzaldehyde dimethyl acetal (11.84 g, 64.98 mmol) and *p*-toluenesulfonic acid (1.54 g, 8.12 mmol). The reaction mixture was heated at 60 °C in a rotary evaporator under vacuum for 2 h. Triethylamine (25 mL) was then added, solvent was evaporated in vacuo, and the residue was purified by column chromatography (CH_2Cl_2/MeOH/NEt_3, 14:1:0:1) to

(18) We thank Prof. C. S. Wilcox (Pittsburgh University) and Prof. C. A. Hunter (Sheffield University) for kindly providing the fitting programs.

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obtain **5** (10.00 g, 62%) as a syrup: ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.41 (d, 2H, J = 8.7 Hz), 6.88 (d, 2H, J = 8.7 Hz), 6.05−5.84 (m, 1H), 5.46 (s, 1H), 5.32 (m, 1H, J = 17.2, 1.6 Hz), 5.21 (m, 1H, J = 10.2, 1.2 Hz), 5.13 (d, 1H, J = 3.6 Hz), 4.40−3.29 (m, 15H), 3.79 (s, 3H), 3.07−2.98 (m, 1H; OH), 2.55 (broad s, 1H), 1.80 (broad s, 3H); ^{13}C NMR ($CDCl_3$) δ = 161.9, 135.7, 131.47, 128.82, 117.46, 114.34, 103.42, 103.31, 103.02, 82.46, 81.60, 77.79, 76.54, 74.70, 74.65, 72.12, 71.11, 69.77, 65.01, 62.30, 55.69, 50.27, 50.11, 49.85. Anal. $C_{23}H_{32}O_{12}$: calcd C 55.19, H 6.44; found C 54.93, H 6.26.

Allyl 2,3,6-Tri-*O*-4-methoxybenzyl-4-*O*-(2,3-di-*O*-4-methoxybenzyl-4,6-*O*-4-methoxybenzylidene-α-D-glucopyranosyl)-*β*-D-glucopyranoside (6). NaH (0.165 g, 6.89 mmol) was added to a solution of **5** (0.230 g, 0.459 mmol) in DMF (6 mL), and the mixture was stirred at 0 °C for 20 min. Then 4-methoxybenzyl chloride (0.622 mL, 4.59 mmol) was added dropwise, and stirring was continued at room temperature for 16 h. MeOH was added and the mixture poured into ice. The aqueous phase was extracted with Et_2O (5 × 25 mL), and the combined organic extracts were dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (AcOEt/hexane, 1:4) to obtain **6** (0.450 g, 89%) as a syrup: ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.43 (d, 2H, J = 8.7 Hz), 7.28 (d, 3H, J = 9.2 Hz), 7.20 (d, 3H, J = 9.0 Hz), 7.09 (d, 4H, J = 8.7 Hz), 6.90 (d, 2H, J = 8.8 Hz), 6.81 (d, 6H, J = 8.5 Hz), 6.80 (d, 2H, J = 8.7 Hz), 6.71 (d, 2H, J = 8.7 Hz), 6.18−5.76 (m, 1H), 5.63 (d, 1H, J = 3.9 Hz), 5.49 (s, 1H), 5.34 (m, 1H, J = 17.3, 1.7 Hz), 5.22 (m, 1H, J = 10.3, 1.5 Hz), 4.85 (d, 2H, J = 10.9 Hz), 4.80 (d, 2H, J = 11.5 Hz), 4.68−4.38 (m, 10H), 4.19−3.84 (m, 5H), 3.82 (s, 3H), 3.79 (s, 3H), 3.78 (s, 6H), 3.74 (s, 3H), 3.72 (s, 3H), 3.69−3.43 (m, 6H); ^{13}C NMR ($CDCl_3$) δ = 159.88, 159.18, 159.08, 159.00, 158.77, 134.10, 130.88, 130.29, 130.10, 129.97, 129.79, 129.47, 129.43, 129.18, 128.21, 127.28, 117.08, 113.68, 113.60, 113.44, 102.55, 101.03, 97.09, 84.54, 82.16, 81.90, 78.42, 78.25, 74.78, 74.21, 73.35, 73.02, 71.89, 70.07, 68.84, 68.58, 63.15, 55.13, 55.04. Anal. $C_{63}H_{72}O_{17}$ calcd C 68.71, H 6.58; found C 69.00, H 6.68.

Allyl 2,3,6-Tri-*O*-4-methoxybenzyl-4-*O*-(2,3,6-tri-*O*-4-methoxybenzyl-α-D-glucopyranosyl)-*β*-D-glucopyranoside (7) and Allyl 2,3,6-Tri-*O*-4-methoxybenzyl-4-*O*-(2,3,4-tri-*O*-4-methoxybenzyl-α-D-glucopyranosyl)-*β*-D-glucopyranoside (8). To a suspension of sodium cyanoborohydride (25.00 g, 397.8 mmol) and 3 Å powdered molecular sieves in DMF (130 mL) was added a solution of **6** (25.12 g, 22.81 mmol) in DMF (160 mL) at 0 °C. A solution of trifluoroacetic acid (17.45 mL, 228 mmol) in DMF (90 mL) was then slowly added for 40 min. The mixture was stirred at rt for 16 h, and solvent was evaporated. The residue was extracted with Et_2O (9 × 150 mL), and the combined organic extracts were dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (toluene/AcOEt, 9:2) to obtain **7** (18.7 g, 74%) and **8** (2.79 g, 11%), both as syrups. **Compound 7:** ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.29−7.10 (m, 12H), 6.89−6.72 (m, 12H), 6.04−5.93 (m, 1H), 5.63 (d, 1H, J = 3.6 Hz), 5.36 (m, 1H, J = 17.2, 1.6 Hz), 5.23 (m, 1H, J = 10.4, 1.6 Hz), 4.91−4.33 (m, 15H), 4.22−4.15 (m, 1H), 4.01 (t, 1H, J = 8.9 Hz), 3.80 (s, 6H), 3.79 (s, 9H), 3.75 (s, 3H), 3.74−3.39 (m, 10H); ^{13}C NMR ($CDCl_3$) δ = 159.03, 158.91, 158.64, 137.55, 134.02, 130.81, 130.38, 130.31, 129.92, 129.61, 129.21, 129.04, 128.99, 128.80, 128.14, 128.00, 125.09, 116.89, 113.65, 113.50, 102.42, 96.28, 84.32, 81.61, 80.78, 78.45, 74.61, 74.41, 74.02, 73.19, 72.99, 72.75, 72.45, 71.22, 70.58, 69.89, 69.33, 68.79, 54.94, 21.19. Anal. $C_{63}H_{74}O_{17}$: calcd C 68.58, H 0.76; found C 68.83, H 6.65. **Compound 8:** ^{1}H NMR (300 Mz, $CDCl_3$) δ = 7.27−7.21 (m, 8H), 7.16−7.1 (m, 4H), 6.89−6.81 (m, 10H), 6.78−6.75 (m, 2H), 6.05−5.94 (m, 1H), 5.59 (d, 1H, J = 3.7 Hz; H1′), 5.40 (m, 1H, J = 17.2, 1.6 Hz), 5.35 (m, 1H, J = 10.4, 1.3 Hz), 4.90−4.46 (m, 13H), 4.45 (m, 1H), 4.17 (m, 1H), 4.02 (t, 1H, J = 9.3 Hz), 3.88 (t, 1H, J = 9.3 Hz), 3.83−3.65 (m, 4H), 3.81 (s, 3H), 3.81 (s, 3H), 3.80 (s, 3H), 3.79 (s, 3H), 3.78 (s, 3H), 3.76 (s, 3H), 3.76−3.42 (m, 5H), 3.41 (dd, 1H, J = 9.6, 3.7 Hz; H2′), 1.83 (m, 1H; OH6′); ^{13}C NMR ($CDCl_3$) δ = 159.04, 158.95, 158.85, 158.57, 133.91, 130.72, 130.66, 130.23, 129.90, 129.82, 129.59, 129.38, 129.09, 128.93, 128.74, 128.07, 127.94, 125.03, 116.86, 113.55, 113.46, 113.39, 102.32, 96.07, 84.28, 81.58, 81.33, 78.89, 77.14, 47.85, 74.40, 74.29, 74.00, 73.26, 72.81, 72.67, 72.05, 71.54, 69.83, 68.21, 61.37, 54.88, 54.81. Anal. $C_{63}H_{74}O_{17}$: calcd C 68.58, H 6.76; found C 68.25, H 6.93.

Allyl 2,3,6-Tri-*O*-4-methoxybenzyl-4-*O*-(4-*O*-acetyl-2,3,6-tri-*O*-4-methoxybenzyl-α-D-glucopyranosyl)-*β*-D-glucopyranoside (9). A solution of **7** (18.7 g, 16.95 mmol) in 2:1 pyridine/acetic anhydride was stirred at rt for 15 h. The mixture was concentrated and evaporated with toluene (3 × 100 mL) to obtain **9** (19.00 g, 98%) as a syrup: ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.28−7.07 (m, 12H), 6.92−6.69 (m, 12H), 6.07−5.91 (m, 1H), 5.57 (d, 1H, J = 3.7 Hz; H1′), 5.34 (m, 1H, J = 17.2, 1.6 Hz), 5.22 (m, 1H, J = 10.4, 1.6 Hz), 5.03 (t, 1H, J = 10.1 Hz; H4′), 4.85 (d, 2H, J = 10.4 Hz), 4.75−4.38 (m, 13H), 4.26 (d, 1H, J = 12.5 Hz), 4.15 (m, 1H), 3.97 (t, 1H, J = 10.0 Hz), 3.94−3.71 (m, 3H), 3.78 (s, 9H), 3.77 (s, 6H), 3.72 (s, 3H), 3.57−3.47 (m, 3H), 3.35−3.21 (m, 2H), 1.84 (s, 3H; OAc); ^{13}C NMR ($CDCl_3$) δ = 169.31, 159.05, 158.92, 158.65, 133.97, 130.73, 130.57, 130.33, 130.29, 129.86, 129.79, 129.66, 129.26, 129.08, 128.97, 128.83, 128.18, 128.03, 125.11, 116.98, 113.50, 113.45, 102.42, 96.39, 84.20, 81.66, 78.66, 78.55, 74.42, 74.12, 73.37, 73.04, 72.94, 72.86, 72.81, 70.55, 69.96, 69.27, 69 03, 68.32, 55.00, 54.93, 53.31, 20.73. Anal. $C_{65}H_{76}O_{18}$: calcd C 68.16, H 6.69; found C 68.40, H 6.84.

2,3,6-Tri-*O*-4-methoxybenzyl-4-*O*-(4-*O*-acetyl-2,3,6-tri-*O*-4-methoxybenzyl-α-D-glucopyranosyl)-*β*-D-glucopyranose (10). To a solution of **9** (953 mg, 0.832 mmol) and 1,4-diazabicyclo[2.2.2]octane (47 mg, 0.416 mmol) in toluene (4 mL), were added tris(triphenylphosphine)rhodium(I) chloride (308 mg, 0.333 mmol) and 10% aqueous EtOH (35 mL), and the mixture was stirred for 16 h at 85 °C. Then it was concentrated, CH_2Cl_2 was added, and the mixture was washed with water. The aqueous phase was extracted with CH_2Cl_2, and the organic combined extracts were concentrated and dissolved in acetone/water (10:1) (27 mL). Mercury(II) oxide (450 mg, 2.08 mmol) and a solution of mercury(II) chloride (450 mg, 1.66 mmol) in acetone/water (10:1) (10 mL) were added, and the mixture was stirred at 100 °C for 2 h and 20 min. The mixture was cooled at rt and filtered through Celite; the bed was washed with CH_2Cl_2, and the filtrate and washings were concentrated. The residue was diluted with CH_2Cl_2, washed with 10% IK, dried (Na_2SO_4), concentrated, and purified by column chromatography (AcOEt/hexane, 1:2 to 1:1) to obtain **10** (745 mg, 81%) as a syrup: ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.27−7.09 (m, 24H), 6.87−6.72 (m, 24H), 5.55 (d, 1H, J = 3.7 Hz; H1′α or β), 5.53 (d, 1H, J = 3.6 Hz; H1′α or β), 5.17 (m, 1H), 5.05 (t, 1H, J = 10.0 Hz; H4′α or β), 5.02 (t, 1H, J = 10.0 Hz; H4′α or β), 4.85−4.27 (m, 26H), 4.03−3.31 (m, 22H), 3.79 (s, 12H), 3.79 (s, 9H), 3.78 (s, 3H), 3.77 (s, 3H), 3.76 (s, 3H), 3.74 (s, 3H), 3.74 (s, 3H), 1.88 (s, 3H; OAc), 1.87 (s, 3H; OAc); ^{13}C NMR ($CDCl_3$) δ = 169.77, 169.26, 159.04, 158.87, 158.81, 158.53, 138.52, 130.77, 130.55, 130.46, 130.41, 130.21, 129.89, 129.68, 129.49, 129.40, 129.22, 129.11, 129.02, 128.97, 128.90, 128.24, 128.06, 124.02, 123.51, 123.46, 122.98, 113.51, 113.41, 113.34, 113.01, 112.48, 111.97, 111.93, 111.45, 111.42, 97.12, 96.52, 96.32, 90.39, 83.87, 82.43, 80.69, 79.34, 78.52, 78.35, 74.32, 73.85, 73.56, 73.29,73.18, 73.04, 72.83, 72.74, 72.59, 72.17, 70.46, 69.28, 69.14, 68.87, 68.19, 54.89, 54.80, 46.59, 20.61, 12.52. Anal. $C_{62}H_{72}O_{18}$: calcd C 67.37, H 6.56; found C 67.28, H 6.41.

2,3,6-Tri-*O*-4-methoxybenzyl-4-*O*-(4-*O*-acetyl-2,3,6-tri-*O*-4-methoxybenzyl-α-D-glucopyranosyl)-α,*β*-D-glucopyranosyl Trichloroacetimidate (11). A mixture of compound **10** (2.49 g, 2.35 mmol), trichloroacetonitrile (1.88 mL, 18.82 mmol), and flame-dried potassium carbonate (650 mg, 470 mmol) in CH_2Cl_2 (12 mL) was stirred at rt for 15 h. The mixture was filtered through Celite, and the filtrate was evaporated in vacuo to obtain **11** (α/β 1:2, 2.85 g, quantitative) as a syrup. It was used without further purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.71 (s, 1H; C=NH β), 8.62 (s, 1H; C=NH α), 7.28−7.10 (m, 24H), 6.85−6.72 (m, 24H), 6.51 (d, 1H, J = 3.7 Hz; H1α), 5.89 (d, 1H, J = 6.8 Hz; H1β).

4-(Methoxycarbonyl)benzyl 2,3,6-Tri-*O*-4-methoxybenzyl-4-*O*-(4-*O*-acetyl-2,3,6-tri-*O*-4-methoxybenzyl-α-D-glucopyranosyl)-α,*β*-D-glucopyranoside (12). A mixture of **11** (6.04 g, 4.83 mmol), methyl 4-(hydroxymethyl)benzoate (2.00

g, 12.08 mmol) and 3 Å molecular sieves in dry CH_2Cl_2 (120 mL) was stirred at rt for 90 min. Then the temperature was reduced to −78 °C, a solution of trimethylsilyl triflate (0.1 M, 2.4 mL) in dry CH_2Cl_2 was slowly added, and the reaction mixture was stirred for 20 min. NEt_3(5 mL) was added, and the mixture was allowed to rise to rt, filtered through Celite, and washed with water and diluted NaCl solution. The aqueous phase was extracted with CH_2Cl_2, and the combined organic extracts were dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (hexane/acetone, 2:1) to give **12** (α/β 1.5:1, 4.82 g, 80%,). The α/β mixture of compound **12** could be separated by column chromatography (CH_2Cl_2/acetone, 30:1), but it was more convenient to separate them after deacetylation of position 4′ yielding compounds **13**α and **13***β*. **Compound 12α:** ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.11 (d, 2H, J = 8.3 Hz), 7.55 (d, 2H, J = 8.3 Hz), 7.30−7.18 (m, 12H), 6.93−6.78 (m, 12H), 5.68 (d, 1H, J = 3.7 Hz; H1′), 5.12 (t, 1H, J = 9.8 Hz; H4′), 5.02−4.29 (m, 15H), 4.18−3.30 (m, 11H), 3.98 (s, 3H), 3.84 (s, 3H), 3.83 (s, 9H), 3.82 (s, 3H), 3.78 (s, 3H), 1.92 (s, 3H; OAc); ^{13}C NMR (CDC_3) δ = 169.30, 166.65, 159.14, 158.98, 158.89, 158.62, 142.35, 130.86, 130.53, 130.03, 129.84, 129.80, 129.71, 129.48, 129.46, 129.32, 129.11, 128.82, 128.32, 127.67, 113.57, 113.48, 113.44, 113.38, 96.50, 95.55, 81.33, 79.62, 78.59, 74.44, 73.85, 72.91, 72.83, 72.72, 70.52, 69.81, 69.17, 68.59, 68.24, 54.99, 54.91, 51.91, 20.74. **Compound 12*β*:** ^{1}H NMR (300Mz, $CDCl_3$) δ = 8.08 (d, 2H, J = 8.2 Hz), 7.52 (d, 2H, J = 8.2 Hz), 7.32−7.14 (m, 12H), 6.91−6.76 (m, 12H), 5.65 (d, 1H, J = 3.4 Hz; H1′), 5.10−4.36 (m, 16H), 4.14−3.38 (m, 11H), 3.97 (s, 3H), 3.82 (s, 15H), 3.77 (s, 3H), 1.92 (s, 3H; OAc); ^{13}C NMR ($CDCl_3$) δ = 169.36, 166.68, 159.07, 158.95, 158.67, 142.69, 130.64, 130.54, 130.20, 130.15, 129.84, 129.75, 129.56, 129.34, 129.30, 129.13, 128.99, 128.85, 128.35, 128.19, 127.70, 127.22, 113.57, 113.51, 102.46, 96.49, 84.20, 81.67, 78.67, 78.52, 74.54, 74.47, 74.26, 73.49, 72.95, 72.88, 70.50, 70.15, 69.28, 8.91, 68.27, 60.18, 55.03, 54.95, 51.92, 30.70, 20.77, 14.02.

4-(Methoxycarbonyl)benzyl 2,3,6-Tri-*O*-4-methoxybenzyl-4-*O*-(2,3,6-tri-*O*-4-methoxybenzyl-α-D-glucopyranosyl)-α-D-glucopyranoside (13α) and 4-(Methoxycarbonyl)benzyl 2,3,6-Tri-*O*-4-methoxybenzyl-4-*O*-(2,3,6-tri-*O*-4-methoxybenzyl-α-D-glucopyranosyl)-*β*-D-glucopyranoside (13*β*). To a solution of **12** (4.76 g, 3.797 mmol) as an α/β mixture in CH_2Cl_2/MeOH (1:1) (116 mL) was added a solution of NaOMe in MeOH (1M, 2.9 mL). The mixture was stirred at rt for 18 h, neutralized with Amberlite-H^+ IR-120, filtered, and concentrated. The crude was purified by column chromatography (CH_2Cl_2/acetone, 23:1) to obtain 2.54 g of **13**α (56%) and 1.78 g of **13***β* (39%). **Compound 13α:** ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.05 (d, 2H, J = 8.3 Hz), 7.49 (d, 2H, J = 8.3 Hz), 7.30−7.13 (m, 12H), 6.91−6.74 (m, 12H), 5.67 (d, 1H, J = 3.6 Hz; H1′), 5.00−4.31 (m, 15H), 4.17−3.42 (m, 12H), 3.95 (s, 3H; $COOCH_3$), 3.81 (s, 3H), 3.80 (s, 12H), 3.75 (s, 3H); ^{13}C NMR ($CDCl_3$) δ = 166.51, 159.08, 158.91, 158.82, 158.57, 142.35, 130.88, 130.78, 130.06, 129.82, 129.38, 129.30, 129.16, 128.94, 128.78, 128.18, 127.52, 113.54, 113.51, 113.44, 113.40, 96.28, 95.44, 81.39, 80.70, 79.65, 78.41, 74.56, 73.57, 72.88, 72.58, 72.51, 72.41, 72.22, 71.16, 70.50, 69.80, 69.28, 68.46, 66.12, 54.85, 54.80, 53.22, 51.72, 30.45. Anal. $C_{69}H_{78}O_{19}$: calcd C 68.41, H 6.49; found C 68.22, H 6.31. **Compound 13*β*:** ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.03 (d, 2H, J = 8.4 Hz), 7.46 (d, 2H, J = 8.4 Hz), 7.30−7.10 (m, 12H), 6.90−6.72 (m, 12H), 5.61 (d, 1H, J = 3.6 Hz; H1′), 5.05−4.34 (m, 15H), 4.15−3.40 (m, 12H), 3.94 (s, 3H; $COOCH_3$), 3.80 (s, 6H), 3.79 (s, 6H), 3.78 (s, 3H), 3.75 (s, 3H); ^{13}C NMR ($CDCl_3$) δ = 166.68, 158.99, 158.90, 158.64, 142.71, 130.81, 130.70, 130.19, 129.90, 129.47, 129.21, 129.02, 128.95, 128.13, 127.19, 113.63, 113.53, 113.49, 113.45, 102.41, 96.36, 84.29, 81.59, 80.77, 78.43, 74.61, 74.50, 74.08, 73.27, 72.97, 72.73, 72.48, 71.17, 70.61, 70.04, 69.30, 68.72, 54.94, 54.88, 53.26, 51.79. Anal. $C_{69}H_{78}O_{19}$: calcd C 68.41, H 6.49; found C 68.29, H 6.31.

4-(Carboxy)benzyl 2,3,6-Tri-*O*-4-methoxybenzyl-4-*O*-(2,3,6-tri-*O*-4-methoxybenzyl-α-D-glucopyranosyl)-α-D-glucopyranoside (14α). To a solution of **13**α (0.72 g, 0.596 mmol) in THF (22 mL) were added MeOH (22 mL) and aqueous KOH solution (6 N, 22 mL). The mixture was stirred at rt for 20 h and neutralized with 2 N HCl solution. NEt_3 (0.2 mL) was added to keep the pH slightly basic. The phases were separated, the aqueous phase was extracted with CH_2Cl_2 (3 × 100 mL), and the combined organic extracts were dried (Na_2SO_4) and concentrated to yield **14α** (0.71 g, quantitative) as a syrup. This compound was used without further purification: ^{1}H NMR (300 Mz, $CDCl_3$) δ = 8.13 (d, 2H, J = 7.9 Hz), 7.48 (d, 2H, J = 8.0 Hz), 7.31−7.12 (m, 12H), 6.91−6.74 (m, 12H), 5.68 (d, 1H, J = 3.6 Hz; H1′), 5.01−4.32 (m, 15H), 4.19−3.15 (m, 12H), 3.80 (s, 9H), 3.79 (s, 3H), 3.77 (s, 3H), 3.75 (s, 3H); ^{13}C NMR ($CDCl_3$) δ = 170.67, 159.11, 158.99, 158.95, 158.85, 158.59, 141.25, 132.09, 130.97, 130.78, 130.13, 129.85, 129.81, 129.42, 129.28, 129.25, 129.02, 128.84, 128.24, 127.50, 113.61, 113.57, 113.50, 113.43, 96.28, 95.03, 81.46, 80.78, 79.63, 78.46, 74.66, 73.60, 72.95, 72.44, 72.18, 71.14, 70.55, 69.78, 69.26, 54.94, 44.90.

Synthesis of Glycophane 15. A mixture of dicyclohexylcarbodiimide (0.628 g, 3.048 mmol), 4-(dimethylamino)pyridine (0.447 g, 3.658 mmol), and 4-(dimethylamino)pyridine hydrochloride (0.483 g, 3.048 mmol) in ethanol-free $CHCl_3$ (97 mL) was heated to reflux. Then a solution of compound **14**α (1.46 g, 1.219 mmol) in $CHCl_3$ (20 mL) was infused via syringe pump over 90 min (the needle was inserted through the top of the condenser and positioned such that refluxing chloroform washed the drops of the solution). When the addition was completed, the syringe was rinsed with 2 mL of chloroform, and this material was delivered to the reaction vessel by syringe pump over 30 min. The reaction mixture was refluxed for 3 h and 30 min more, cooled to rt, concentrated, and purified by column chromatography twice: hexane/acetone, 10:7, and CH_2Cl_2/acetone, 28:1, affording pure **15** as a white solid (425 mg, 29%): ^{1}H NMR (500Mz, $CDCl_3$) δ = 8.02 (d, 2H, J = 8.3 Hz), 7.49 (d, 2H, J = 8.3 Hz), 7.34 (d, 4H, J = 8.8 Hz), 7.25 (d, 4H, J = 8.5 Hz), 7.23 (d, 4H, J = 8.8 Hz), 7.12 (d, 4H, J = 8.8 Hz), 6.99 (d, 4H, J = 8.5 Hz), 6.98 (d, 4H, J = 8.5 Hz), 6.83 (d, 4H, J = 8.3 Hz), 6.81 (d, 4H, J = 8.6 Hz), 6.79 (d, 4H, J = 8.8 Hz), 6.78 (d, 4H, J = 8.5 Hz), 6.57 (d, 4H, J = 8.8 Hz), 6.56 (d, 4H, J = 8.8 Hz), 5.26 (d, 2H, J = 10.3 Hz; CH_2Ph), 5.18 (t, 2H, J = 10.3 Hz; H4′), 5.00 (d, 2H, J = 3.3 Hz; H1′), 4.82 (d, 2H, J = 3.7 Hz; H1), 4.79−4.72 (m, 8H; CH_2Ph), 4.62 (d, 2H, J = 11.5 Hz; CH_2Ph), 4.56−4.46 (m, 8H; CH_2Ph), 4.34 (s, 4H; CH_2Ph), 4.23−4.17 (AB system, 4H, J = 11.5 Hz; CH_2Ph), 4.12−4.10 (m, 2H; H6), 4.06−4.00 (m, 6H; H3 + H3′ + H5′), 3.96 (t, 2H, J = 9.0 Hz; H4), 3.79 (s, 6H), 3.79 (s, 6H), 3.77 (s, 6H), 3.76 (s, 6H), 3.70 (broad d, 2H; H5), 3.65 (s, 6H), 3.64 (s, 6H), 3.59 (dd, 2H, J = 9.5, 3.64 Hz; H2), 3.54 (dd, 2H, J = 9.8, 3.2 Hz; H2′), 3.39 (broad d, 2H; H6S), 3.37−3.31 (m, 4H; H6′R + H6′S); ^{13}C NMR ($CDCl_3$) δ = 164.84, 159.03, 158.86, 158.78, 158.68, 158.60, 143.46, 131.58, 130.44, 130.11, 130.02, 129.64, 129.53, 129.42, 129.29, 129.09, 129.02, 128.80, 125.89, 113.51, 113.39, 113.23, 99.14, 96.84, 80.89, 79.34, 78.81, 77.19, 75.83, 74.23, 72.92, 72.62, 72.48, 71.98, 70.95, 70.63, 69.37, 68.91, 68.41, 68.35, 54.90, 0.54.74, 29.05; FABMS m/z = 2419.6 [M + guanidinium]$^+$, $C_{136}H_{148}O_{36}$ + guanidiniun ion$^+$ calcd m/z = 2419.6. Anal. $C_{136}H_{148}O_{36}$: calcd C 69.25, H 6.32; found C 68.98, H 6.21.

Synthesis of Glycophane 2. To a solution of **15** (100 mg, 0.042 mmol) in CH_2Cl_2 (5 mL) was added a solution of trifluoroacetic 5% in CH_2Cl_2 (5 mL). The mixture was stirred at rt for 5 h, concentrated, washed with Et_2O (3 × 10 mL), and dried under vacuum for 20 h. It was then purified in water solution by desalting through a C_{18} Sep-pak column. Lyophilization of the filtrate affords **2** as a white solid (37 mg, 95%): ^{1}H NMR (500 MHz, D_2O, 30 °C) δ (to C_6D_6) = 8.12 (d, 4H, J = 8.43 Hz), 7.63 (d, 4H, J = 8.0 Hz), 5.25 (d, 2H, J = 3.7 Hz; H1′), 5.14 (d, 2H, J = 3.3 Hz; H1), 5.12 (t, 2H, J = 9.9 Hz; H4′), 4.87−4.78 (AB system, 4H; CH_2), 4.16 (t, 2H, J = 9.5 Hz; H3′), 4.14 (m, 2H; H5′), 4.07 (t, 2H, J = 9.5 Hz; H3), 3.78 (dd, 2H, J = 9.5, 3.66 Hz; H2′), 3.78 (m, 2H; H5), 3.76 (m, 2H; H6), 3.71−3.61 (m, 8H; H2 + H6′R + H4 + H6′S), 3.56 (m, 2H; H6); ^{13}C NMR (CD_3OD) δ = 167.17, 145.57, 130.95, 130.25, 128.13, 103.94, 100.30, 83.86, 74.77, 74.40, 74.03, 72.91, 70.33, 64.30, 62.48, 61.82; HRFABMS: m/z = 939.2754 [M + Na]$^+$, $C_{40}H_{52}O_{24}Na$ calcd m/z = 939.2746.

Synthesis of Glycophane 3. A solution of **2** (50 mg, 0.054 mmol) in MilliQ quality water (20 mL) was stirred at 50 °C in an orbitalic shaker for 3 days. The mixture was lyophilized to obtain **3** as a white solid (49 mg, 98%): ^{1}H NMR (500 MHz, D_2O, 30 °C) δ (to C_6D_6) = 8.05 (d, 4H, J = 8.5 Hz), 7.50 (d, 4H, J = 8.5 Hz), 5.46 (d, 2H, J = 3.4 Hz; H1′), 4.96 (m, 2H, J = 11.8, 1 Hz; H6′), 4.65 (d, 2H, J = 3.9 Hz; H1), 4.33 (m, 2H, J = 11.8, 10.2 Hz; H6′S), 3.95 (t, 2H, J = 9.3 Hz; H3), 3.93 (m, 2H; H5′), 3.68 (t, 2H, J = 10.2 Hz; H3′), 3.63 (dd, 2H, J = 10.2, 3.4 Hz; H2′), 3.61−3.54 (m, 6H; H5 + H6 + H6S), 3.45 (t, 2H, J = 9.3 Hz; H4), 3.40 (t, 2H, J = 9.2 Hz; H4′), 3.18 (dd, 2H, J = 10.2, 3.4 Hz; H2); ^{13}C NMR (D_2O) δ = 170.29, 145.78, 132.91, 132.75, 131.58, 131.39, 101.88, 99.82, 79.45, 76.07, 75.88, 74.73, 73.97, 73.66, 73.09, 71.60, 67.68, 63.20; HR-FABMS: m/z = 939.2778 $[M + Na]^+$, $C_{40}H_{52}O_{24}Na$ calcd m/z = 939.2746.

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