

Phase transfer of monosaccharides through noncovalent interactions: Selective extraction of glucose by a lipophilic cage receptor

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We have previously shown that macrotricyclic host **1a** is a powerful receptor for glucopyranosyl units in the nonpolar medium of chloroform. However, the solubility properties of **1a** did not permit studies of the extraction of carbohydrates from aqueous solution. This paper describes the synthesis of the new variant **1b**, furnished with a highly lipophilic exterior array of 12 benzyloxy substituents. In homogeneous solution, **1b** behaves much as **1a**, binding *n*-octyl β -D-glucoside with $K_a = 720 \text{ M}^{-1}$ in $\text{CD}_3\text{OH}/\text{CDCl}_3$ (8:92). In two-phase experiments, the improved solubility of **1b** allows carbohydrate extraction to be observed. Three hexoses (glucose, galactose, and mannose), two pentoses (ribose and xylose), and the two methyl glucosides are all extracted substantially into chloroform from 1 M aqueous solutions. Among the hexoses, **1b** shows notable affinity and selectivity for glucose, extracting detectable amounts even from 0.1 M aqueous solutions.

Carbohydrate recognition is an active area of supramolecular chemistry, motivated by the biological importance of saccharides and also by the unusual challenge represented by these complex substrates (1–13). On the one hand, carbohydrates must be recognized and processed during metabolism, whereas saccharide motifs are known to mediate cell–cell recognition, the infection of cells by pathogens, and many aspects of the immune response (14–17). On the other, carbohydrates possess structures that are fairly large, irregular, and multivalent, while being quite similar to clusters of water molecules. Even in nonpolar solvents, selective carbohydrate receptors require extended, sophisticated architectures (1, 3–6). In the presence of liquid water, the problem becomes yet more difficult. Recognition by means of boronate formation has been relatively successful (2, 7–10), but there are few effective systems for binding saccharides from water by using noncovalent bonds (1, 11–13).

A receptor can be shown to discriminate between saccharide and water in one of two ways. Firstly (and most obviously), it can be studied in homogeneous aqueous solution. Secondly, it can be designed for solubility in nonpolar media and studied in a two-phase system, demonstrating the ability to extract or transport the carbohydrate (18–24). This latter approach is directly relevant to the transport of carbohydrates across biological membranes. Boron-based systems have again proved quite effective (22–24). However, for those receptors operating through noncovalent interactions, success has been limited thus far. Positive results have been obtained with the less hydrophilic saccharides at high aqueous concentrations (18, 20), but not in general with the more hydrophilic substrates such as glucose.[§]

Recently we described the tricyclic tetraester **1a** (Fig. 1), a novel receptor that showed an exceptional affinity for *n*-octyl β -D-glucoside, remarkable β vs. α selectivity, and the unusual ability to dissolve solid glucose in CHCl_3 (25). Although we hoped to demonstrate the extraction of glucose from aqueous solution by using **1a**, our attempts were frustrated by solubility

problems. Although soluble in chloroform under normal circumstances, **1a** appeared to precipitate on the CHCl_3 – H_2O interface during extraction experiments. The design of **1** allows for the tuning of solubility properties by variation of the externally directed group X. We now report the synthesis and properties of a new variant **1b**, in which the exterior is rendered highly lipophilic by the incorporation of 12 benzyl substituents. We have used this compound to confirm that the tricyclic core in **1** can indeed extract glucose into chloroform from aqueous solution. The synthesis of **1b** (described in detail in *Supporting Text*, which is published as supporting information on the PNAS web site, www.pnas.org) includes improvements on previously published methodology that enhance the prospects for further applications of this system.

Materials and Methods

Chemical Synthesis. Preparative details for receptor **1b** and control compound **21** are provided in *Supporting Text*.

Extraction Experiments (Typical Procedure). A solution of the dodecabenzyl macrotricyclic **1b** (4.0 mg, $1.46 \mu\text{mol}$) in chloroform (5.0 ml; thus $[\text{Host}] = 0.00029 \text{ M}$) and a solution of D-glucose (900 mg, 5 mmol) in distilled water (5.0 ml; thus $[\text{Guest}] = 1 \text{ M}$) were placed in a 50-ml round-bottomed flask and allowed 15 min to warm to 30°C by immersion in a water bath. The flask was shaken vigorously for 1 min before the mixture was poured into a separating funnel and allowed 15 min to separate into two phases. The organic phase was then filtered through a hydrophobic (silicone-treated) filter paper. The filtrate was evaporated *in vacuo* to a white solid that was then dissolved in $700 \mu\text{l}$ of $[\text{D}_6]\text{DMSO}$. A ^1H NMR spectrum (256 scans) was obtained and the integrations were determined as described in *Results and Discussion*.

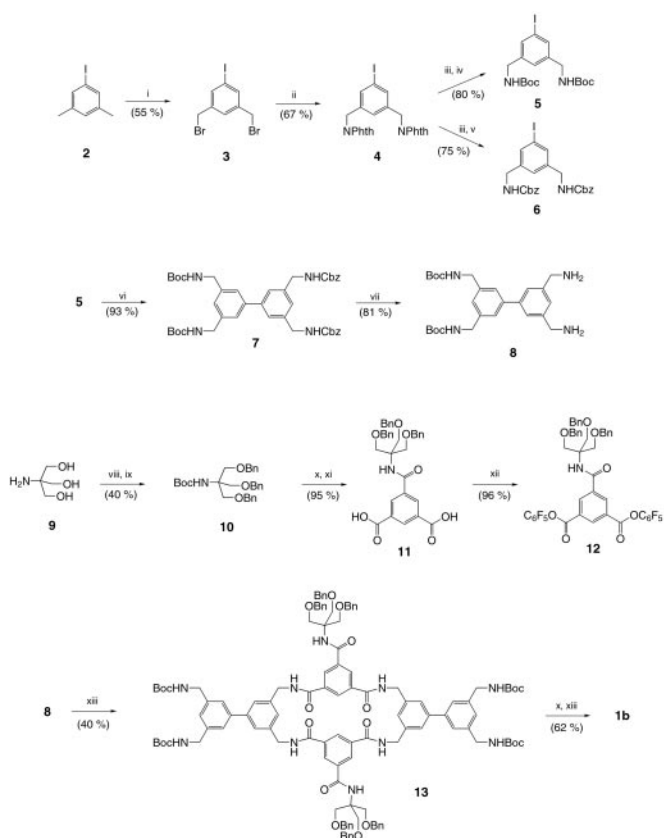
Results and Discussion

Synthesis of Receptor 1b. The synthesis of **1b** is summarized in Scheme 1. The central framework was constructed although two $[2 + 2]$ macrocyclizations, the first to yield **13** and the second to yield the final product. These reactions could be accomplished in acceptable yield at high dilution, because the resulting 24-membered rings are the smallest possible without unreasonable strain (25). Key intermediates were the biphenyl component **7** (25), and the new isophthaloyl spacer unit **12**. To enable the sequential cyclizations, orthogonal protecting groups were used at either end of **7**. In our previous synthesis of **7**, the biaryl unit

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[§]The “unimolecular micelles” described in ref. 19 represent an exception. However, these highly flexible polyols seem unlikely to provide structured, receptor-like environments for their guests.



Scheme 1. (i) *N*-bromo-succinimide, 1,1'-azobis(cyclohexanecarbonitrile), HCOOMe, *hν*; (ii) potassium phthalimide, DMF; (iii) (1) $\text{NH}_2\text{NH}_2\cdot\text{H}_2\text{O}$, MeOH/DCM (4:1), (2) HCl aq.; (iv) $(\text{Boc})_2\text{O}$, THF, $i\text{Pr}_2\text{NEt}$; (v) CbzCl, THF, $i\text{Pr}_2\text{NEt}$; (vi) (1) bis(pinacolato)diboron, $\text{PdCl}_2(\text{dppf})$, KOAc, DMSO, 80°C , (2) $\text{PdCl}_2(\text{dppf})$, Na_2CO_3 (2M), 6; (vii) H_2 , 10% Pd/C, MeOH/DCM (1:1); (viii) $(\text{Boc})_2\text{O}$, MeOH/*t*BuOH (1:1); (ix) BnBr, KOH, DMF; (x) TFA/DCM (1:1); (xi) 1,3,5-benzenetricarbonyl trichloride, $i\text{Pr}_2\text{NEt}$, THF; (xii) $\text{C}_6\text{F}_5\text{OH}$, *N,N'*-dicyclohexylcarbodiimide, THF; (xiii) 12, $i\text{Pr}_2\text{NEt}$, THF-DMF, high dilution. Bn, benzyl; Boc, *tert*-butoxycarbonyl; NPhth, phthalimidyl; Cbz, benzyloxycarbonyl; dppf, diphenyl phosphinoferrocene.

was formed from two aryl bromides by using the organotin methodology of Stille (26). Unfortunately the coupling proceeded in just 15% overall yield. For the present work we made two modifications. Firstly we used aryl iodides, which generally show greater reactivity in Pd-catalyzed couplings; and secondly we used organoboron methodology based on the recently commercialized "B-B" reagent bis(pinacolato)diboron (27). Starting from 5-iodo-*m*-xylene **2**, benzylic bromination followed by Gabriel synthesis gave bis-phthalimide **4**. Cleavage of the phthalimide groups in **4** led to the corresponding diamine, which was converted into the bis-Boc and bis-Cbz protected amines **5** and **6**, respectively. Reaction of **5** with bis(pinacolato)diboron then **6**, following the single-pot procedure of Giroux *et al.* (28), gave biphenyl **7** in up to 93% yield. Hydrogenolysis of the two Cbz groups proceeded well to give the unsymmetrical bis amine **8**.

The synthesis of spacer precursor **12** proceeded from Tris(hydroxymethyl)aminomethane (Tris) **9**. Following the procedure of Ungaro and coworkers (29), **9** was *N*-protected and *O*-benzylated to give **10**. *N* deprotection, slow addition to excess mesityl chloride, then aqueous work-up, gave diacid **11** in excellent yield. Treatment of **11** with pentafluorophenol and *N,N'*-dicyclohexylcarbodiimide gave active ester **12**. Macrocyclization of **8** + **12** under high dilution conditions gave **13** in 40% yield. The latter was converted into the corresponding tetra trifluoroacetic acid (TFA) salt then subjected to the final

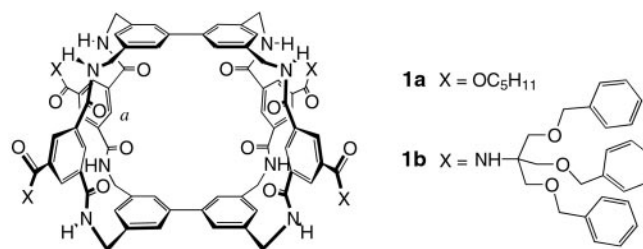


Fig. 1. Structures of the macrotricyclic receptors **1a** and **1b**.

cyclization. The targeted dodecabenzyl macrotricyclic **1b** was isolated in 62% yield and fully characterized. The synthesis conveniently gave **1b** in batches of ≈ 300 mg, a significant improvement on our earlier preparation of **1a**.

Binding Properties of Receptor 1b in Homogeneous Solution. To confirm that the externally directed substituents made little difference to the binding properties of **1**, macrotricyclic **1b** was studied as a receptor for *n*-octyl- β -D-glucopyranoside **14a** (Fig. 2) in homogeneous solution. Tetraester **1a** had previously been found by ^1H NMR to bind **14a** with $K_a = 920\text{ M}^{-1}$ in $\text{CD}_3\text{OH}/\text{CDCl}_3$ (8:92). A ^1H NMR titration of **1b** vs. **14a** produced similar changes to those observed for **1a**. In particular, the signal due to the inward-directed aromatic CH (position *a*; see Fig. 1) moved downfield by about 0.2 ppm. The motions of the latter were consistent with 1:1 binding, and could be analyzed to yield an association constant of 720 M^{-1} , similar to that obtained for receptor **1a**.

Because **1b** gave a well resolved ^1H NMR spectrum in CDCl_3 , which had not been the case for **1a**, it was decided to attempt a binding study in this solvent. After the addition of 0.1 equivalents of **14a**, the signal due to *CHa*, at δ 7.78, shifted downfield by ≈ 0.025 ppm and broadened significantly. The benzylic amide proton peak also moved downfield by ≈ 0.042 ppm and became

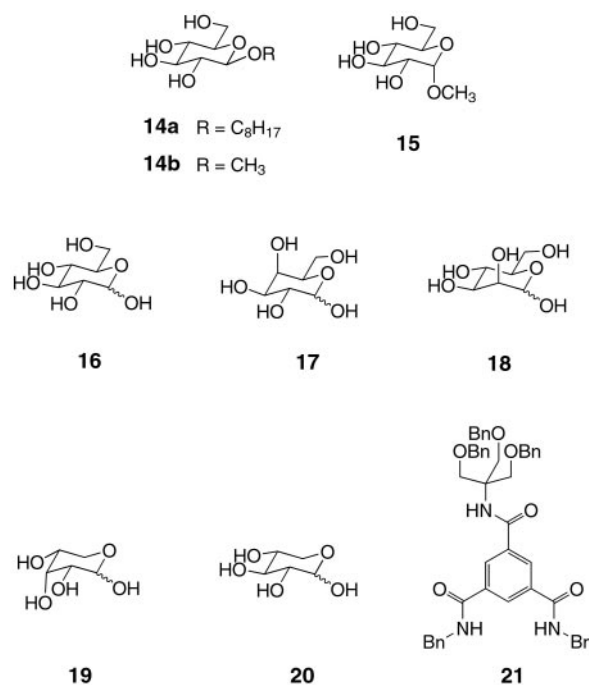


Fig. 2. Structures of monosaccharide substrates **14-20** and control "receptor" **21**.

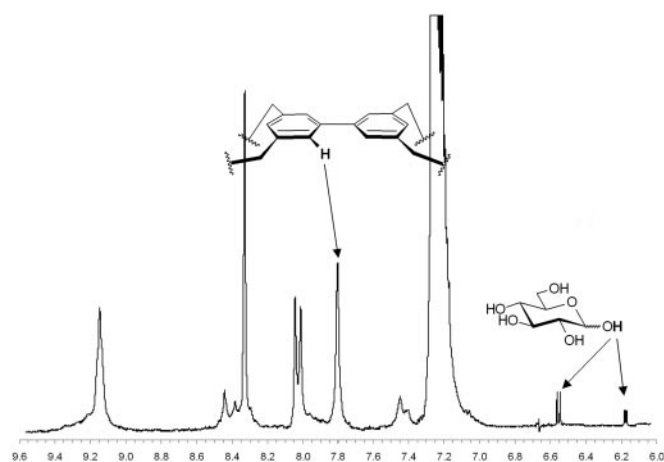


Fig. 3. ^1H spectrum (400 MHz, $[\text{D}_6]\text{DMSO}$) showing the macrotricyclic aromatic protons and the two anomeric hydroxyl protons after extraction of a 1 M D-glucose aqueous solution by a chloroform solution of macrotricyclic **1b**.

broad on the first addition. However, after 0.2 equivalents had been added, all aromatic CH signals broadened significantly, whereas CHa , as well as the signal due to NH , became almost indistinguishable from baseline and therefore impossible to follow for binding constant calculations. It is clear that a strong interaction was occurring between the macrocycle and the sugar, but quantification was not possible by ^1H NMR.

Extraction of Sugars from Aqueous into Organic Phase. We next proceeded to study the extraction by **1b** of monosaccharides **14b–20** (Fig. 2) from water into the apolar medium of CHCl_3 . Aqueous sugar solutions were equilibrated with **1b** in CHCl_3 at 30°C , the organic phases were separated and evaporated, and the residues redissolved in $[\text{D}_6]\text{DMSO}$ for analysis by ^1H NMR. Fig. 3 shows a part of the spectrum obtained from a typical experiment, the extraction of a 1 M aqueous solution of D-glucose **16**. Because the signals due to sugar CH were obscured by those of the receptor, the two anomeric hydroxyl protons [corresponding to the α and β anomers of D-glucose; $\delta = 6.18$ and 6.56 ppm, respectively (30)] were integrated with respect to the host's protons signal at $\delta = 7.80$ ppm to give the guest:host ratio. Control experiments established that none of the carbohydrates entered the organic phase in the absence of the receptor.

A summary of the extractions performed with **1b** is given in Table 1. The receptor proved capable of taking up a range of substrates from 1 M aqueous solutions and (where determined) from solutions of lower concentration. The positive results for the methyl glucosides **14b** and **15** are not surprising, given that **14b** was extracted efficiently by our earlier "cholaphanes" (20), and also by Aoyama's octahydroxy calixarene (31). D-ribose **19** had also proved a good substrate for the calixarene (18). However, the results for the aldohexoses D-glucose **16**, D-galactose **17**, and D-mannose **18** are exceptional. As far as we are aware, these hydrophilic substrates have not previously been extracted from water by means of noncovalent bonding to a

Table 1. Extractabilities of monosaccharide substrates from water into chloroform by receptor **1b**

Substrate	Concentration of substrate in aqueous phase		
	1.0 M	0.5 M	0.1 M
Methyl β -D-glucoside 14b	1.0		
Methyl α -D-glucoside 15	1.0		
D-Glucose 16	1.0	0.5	<0.1*
D-Galactose 17	0.2	<0.1*	None detectable
D-Mannose 18	<0.1*	<0.1*	None detectable
D-Ribose 19	0.7		
D-Xylose 20	1.1		

Values in mole equivalents with respect to receptor. Errors estimated at $\pm 20\%$. Each experiment was performed at least twice.

*Carbohydrate detectable, but amounts too small for quantification by NMR integration.

structured, preorganized receptor. The selectivity shown by **1b** is also striking. In the case of glucose **16**, a full equivalent is extracted from 1 M aqueous solution, and detectable quantities enter the organic phase even from the 0.1 M solution. The affinities for galactose **17** and mannose **18** are considerably smaller. The order of extractabilities (glucose > galactose > mannose) does not reflect the estimated degrees of hydration (mannose < glucose < galactose) (32, 33), suggesting a stereospecific interaction with the receptor. Indeed, as initially proposed (25), the tricyclic core of **1** may be especially complementary to substrates with equatorial substituents. In this context, it is notable that xylose **20** is also strongly extracted.

Finally, control experiments were performed with triamide **21** and macrocycle **13**, to probe the effect of dissecting the framework of **1b**. Both were tested against D-glucose, at 0.5 M and 1 M aqueous concentrations, respectively. In neither case was carbohydrate detected in the organic phase, suggesting that the full cage structure of **1b** is necessary for extraction to take place.[†]

Conclusion

In summary, we have synthesized a new macrotricyclic carbohydrate receptor **1**, and have demonstrated its ability to extract monosaccharides from water into chloroform. The receptor is especially effective with glucose, succeeding at aqueous substrate concentrations as low as 0.1 M. In future work we will seek to modify the tricyclic core so as to further increase affinity, and also to introduce reporter functionality (so that, for example, the molecule changes color on binding). Progress on these fronts may lead to sensors for glucose at physiological concentrations (≈ 0.005 M), providing robust alternatives to the current systems based on glucose oxidase (34).

[†]See ref. 25 for a related control experiment, involving a monocyclic analogue of **1a**. In this case ^1H NMR titration revealed a much reduced affinity for glucoside **14a** in CDCl_3 .

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