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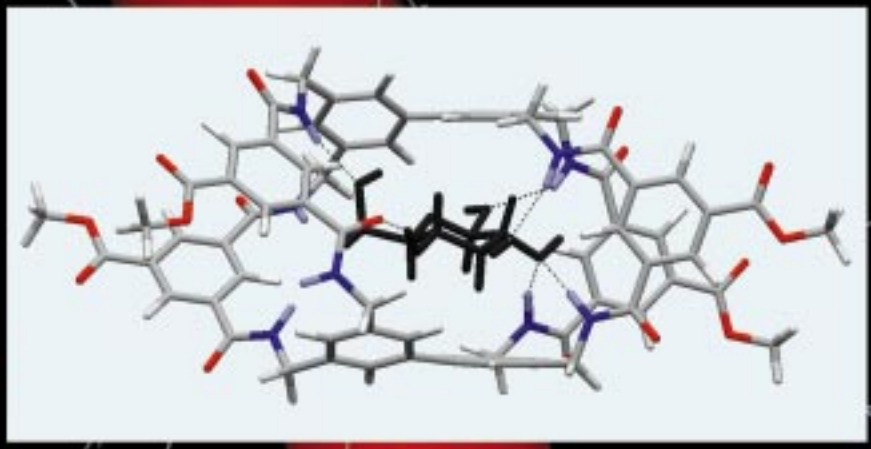
X-ray crystal structure of glucose bound to the *E. coli* galactose chemoreceptor protein (*Science* **1988**, *242*, 1290; see also Figure 1). Polar and apolar residues surrounding the carbohydrate are highlighted in yellow and green, respectively. Hydrogen bonds in the neighborhood of the binding site are shown as broken green lines.



Inset: A synthetic receptor designed to mimic the binding site by providing both polar and apolar interactions.

Carbohydrate Recognition through Noncovalent Interactions: A Challenge for Biomimetic and Supramolecular Chemistry

Anthony P. Davis* and Richard S. Wareham

"Nucleic acids for control, proteins for effect, lipids and carbohydrates as fuel and building materials." As a crude summary of the natural world, this statement would have satisfied most biochemists three decades ago. However, the placing of carbohydrates at the bottom of this hierarchy was undeserved and, with hindsight, unwise. As carriers of information, oligosaccharides have far greater potential than the other two biopolymers. While three different nucleotides or amino acids can be used to create just six distinguishable molecules, over 1000 possibilities may be generated from three monosaccharides. It was unlikely that Nature would ignore this resource, and it is now quite clear that she did not. Carbohydrate recognition is known to mediate cell adhesion (and therefore, to a large extent, the development of multicellular organisms), the infection of cells by pathogens, the distribution and reactivity of proteins within cells, and many aspects of the immune response. "Glycobiologists" studying these effects can justly claim to be working at one of the major frontiers in the biological sciences. To supramolecular chemists, carbohydrate recognition represents a challenge to architectural and synthetic talents, and an opportunity to answer fundamental questions in an important and topical area. On the one hand, carbohydrates present complex three-dimensional arrays of functionality to their surroundings, posing intriguing problems in receptor design. On the other, the principles of saccharide recognition by biomolecules are not well understood. X-ray crystallography reveals the expected polar and hydrophobic interactions in protein – carbohydrate complexes, but does not explain the thermodynamic driving force for desolvation and complex formation. This review charts the progress of supramolecular chemistry towards truly biomimetic carbohydrate recognition. The journey is not yet complete, with the most challenging part still to come. However, the landmarks have been pleasing, often impressive, and perhaps suggestive of future success.

Keywords: bioorganic chemistry · carbohydrates · molecular recognition · receptors · supramolecular chemistry

1. Introduction

The role of oligosaccharides in biological regulation has attracted a great deal of attention in recent years.[1] It has been realized that the information-carrying potential of oligosaccharides is far greater than that of proteins and nucleic acids of equivalent molecular weight, and that their presence on cell surfaces and on many proteins suggests an importance which was previously unrecognized. Oligosaccharides are now known to mediate cell – cell recognition, including the infection of cells by bacteria and viruses, moderate the behavior of enzymes and other proteins, and fulfil various functions in the immune response. The recognition of saccharides is also important for carbohydrate metabolism, and for the transport of these highly polar molecules across cell membranes.

The raised profile of carbohydrates has attracted attention from the synthetic chemical community, where a renaissance in carbohydrate chemistry, especially oligosaccharide synthesis,[2] is clearly apparent. Supramolecular chemists have likewise responded, drawn by the challenge of designing carbohydrate receptors, and also by practical and theoretical considerations. Synthetic carbohydrate receptors could be used as drugs (e.g. anti-infective agents), to target cell types (acting as "synthetic antibodies"), to transport saccharides or related pharmaceuticals across cell membranes, or in carbohydrate sensors. On the theoretical side, carbohydrate recognition by natural systems is still quite poorly understood. The X-ray crystallography of protein – carbohydrate complexes reveals, not surprisingly, that the polar groups are bound by hydrogen-bond donor and acceptor groups, or occasionally to metal ions, while the hydrophobic regions are complemented by nonpolar surfaces.[3] However, complex formation requires

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that host and guest be desolvated. What drives the replacement of water by carbohydrate-OH in a natural binding site? This and other issues are subjects of active debate[4] to which studies on synthetic receptors could make important contributions.

It should be said at this stage that carbohydrate recognition which is both effective and genuinely biomimetic is still a goal for the future. This review, which surveys the progress so far, is divided into two main sections. The first covers receptors which operate in organic solvents and, though effective, are not forced to select between carbohydrates and water molecules. Arguably, these are biomimetic only where they can extract carbohydrates from aqueous solution, and therefore mimic transport across cell membranes. The second covers receptors operating in water. Though biomimetic they are not yet effective, by the standards normally expected in supramolecular chemistry. The only synthetic receptors which are truly effective in water rely on boronate formation. However, these systems are neither biomimetic nor, in the strictest sense, supramolecular (given that recognition occurs through covalent bond formation). They are therefore outside the scope of this article and have, in any case, been subjects of several recent reviews.[5]

2. Carbohydrate Recognition in Organic Media

Carbohydrates in their pyranose and furanose forms are generally characterized by a complex, divergent array of polar functional groups. Most are hydroxy groups, capable of donation and acceptance of hydrogen bonds. Perhaps the most obvious approach to carbohydrate recognition is to surround these groups with a complementary, *convergent* arrangement of H-bond donor and/or acceptor functionality. Nature provides ample encouragement for this strategy, as the X-ray crystal structures of protein–carbohydrate complexes invariably reveal intensive hydrogen bonding between host and guest (see for example Figure 1).[3] Hydrogen bonding is especially effective in nonpolar organic media, where solvent does not compete significantly for binding sites. In these circumstances, the preorganized deployment of polar functionality should be sufficient for success.

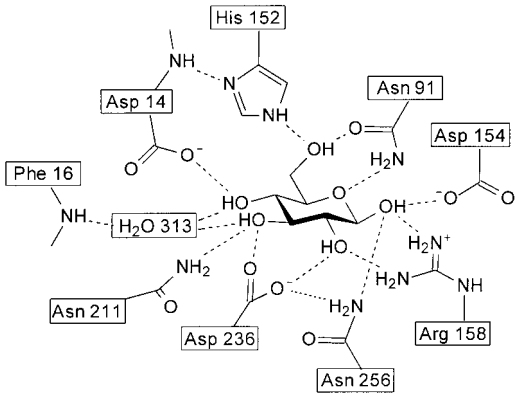

Figure 1. Intermolecular hydrogen bonds in the complex between D-glucose and "D-galactose-binding" bacterial periplasmic protein. (Adapted from reference [3a]; see also frontispiece on p. 2978.)

Although this strategy is readily conceived, its execution is complicated by the size of carbohydrates, the "three-dimensional" arrangement of their functionality (in contrast, for example, to the nucleobases) and the subtle variations in their structures. The frameworks of carbohydrate receptors must be relatively large, so that they can traverse or (ideally) encapsulate their substrates, while maintaining the rigidity necessary for preorganisation of the binding functional groups. Rigidity is also necessary to inhibit "self-quenching" of binding functionality; H-bond donor *and* acceptor sites are present in most (neutral) polar functional groups.

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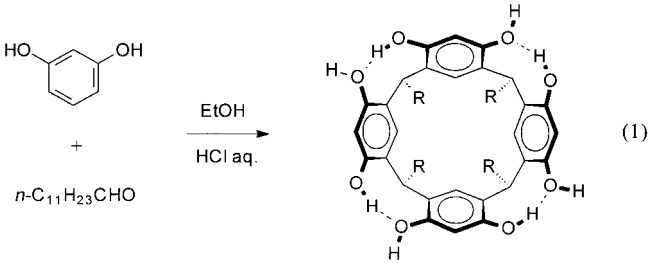
2.1. "Face-to-Face" Recognition

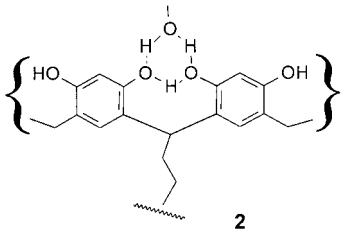
Given the challenge of fully encapsulating a carbohydrate, it is unsurprising that the first successful carbohydrate receptor employed a "face-to-face" binding geometry. The octahydroxy calix[4]arene **1**, first reported by Aoyama et al. in 1988, could be obtained in a single step from resorcinol and dodecanal [Eq. (1)].[6] The bowl-shaped framework of **1**



(1)

1 R = *n*-$C_{11}H_{23}$

cannot encompass a furanose or pyranose, but possesses a preorganized array of binding sites which can span the face of a carbohydrate nucleus. Assuming the illustrated pattern of intramolecular hydrogen bonding, each site can in principle form cooperative hydrogen bonds to a hydroxy group as shown in **2**. Accordingly, **1** was capable of extracting four



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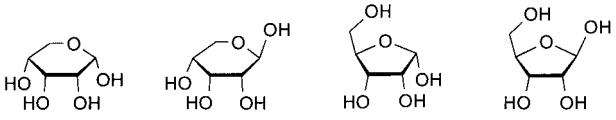
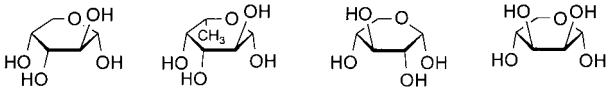
equivalents of water into solution in CCl_4.[6, 7] When the water was replaced by concentrated (1 – 5.5 M) aqueous solutions of certain carbohydrates, these also were extracted. The best studied was D-ribose (**3**), shown to form a complex of stoichiometry $\mathbf{1}\cdot\mathbf{3}\cdot 2H_2O$. The sugar was found to be in its α-pyranose form **3p*α***, despite the predominance of **3p*β*** in aqueous solution (Figure 2). Similarly extracted were D-



3p*α* **3p*β*** **3f*α*** **3f*β***

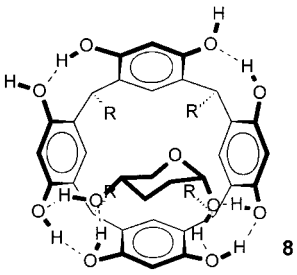
Figure 2. Cyclic forms of D-ribose (**3**). **p** ≡ pyranose, **f** ≡ furanose.

arabinose (**4**) and L-fucose (**5**), but not D-xylose (**6**) or D-lyxose (**7**).[8] These and other experiments[9] suggested that the carbohydrates bound to **1** through a ditopic interaction



4 **5** **6** **7**

involving the C(1) and C(4) hydroxy groups in a *cis* arrangement (see structure **8**), promoted (through an uncertain mechanism) by a third *cis*-hydroxy group at C(3).



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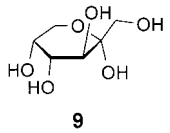
No fully-oxygenated aldohexose was extracted by **1**, presumably because of the hydrophilic terminal CH_2OH group possessed by all these molecules in their pyranose forms. However, it was shown that the ketohexose fructose **9**[8] was successfully extracted by **1** and, to a marginally greater extent, by a trimeric analogue of the receptor.[10] Success was also met with methyl β-D-glucoside (**10**), although the observed stoichiometry of **10**:**1**:1:2 implied a new complex geometry in this case.[11] The glucoside was presumed to be sandwiched between two molecules of **1** (Figure 3). Methyl α-D-glucoside was not extracted, possibly because steric hindrance from the axial methoxy group would prevent association with the second molecule of receptor.



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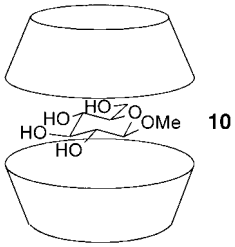

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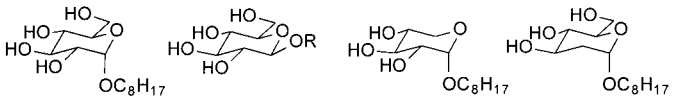
Figure 3. Proposed 2:1 "sandwich" complex between **1** and methyl β-D-glucoside (**10**). Molecules of **1** are represented as truncated cones.

The carbohydrates discussed thus far were necessarily studied by extraction because of their insolubility in nonpolar organic solvents. By contrast, certain long-chain alkyl glycosides are freely soluble in, for example, $CDCl_3$, and are therefore useful for studies in homogeneous solution.[12] Octyl glucosides **11** and **12** were both investigated as substrates for receptor **1**.[11] Complexation could be detected by NMR spectroscopy



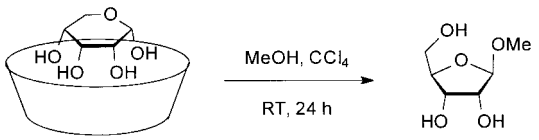
11 **12** R = C_8H_{17} **14** **15**

13 R = $C_{12}H_{25}$

(separate signals for free and bound receptor, indicating slow exchange on the NMR time scale), and also by circular dichroism induced in the achiral receptor by the chiral substrates.[13] Interestingly, the measurements implied substrate:receptor stoichiometries of 4:1, with high degrees of cooperativity. The proposed binding model involved a glucoside at each OH···OH site of the receptor, with reinforcement from inter-saccharide hydrogen bonds. The less oxygenated substrates **14** and **15**, unable to make these latter bonds, were not substantially bound by the receptor.

Receptor **1** was found to have one further property of interest, the ability to control the reactivity of one of its

substrates. The complex between **1** and ribose (**3**) in CCl_4 reacted with methanol to give almost exclusively the β-furanoside **16** [Eq. (2)].[14] This result is somewhat mysterious,

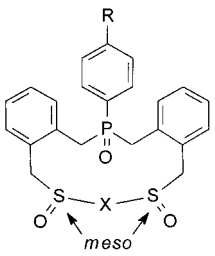


(2)

1·3pα **16**

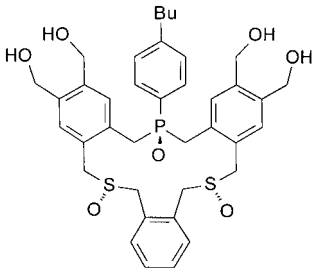
as the ribose is supposed to be bound in the pyranose form **3pα**. However, the selectivity is impressive, as conventional methods generally give a mixture of isomers.

The "face-to-face" binding geometry is also exploited by receptors **17**–**19**, investigated by Gellman and co-workers. Recognition by **17** and **18** was initially studied by HPLC on



17 X = $(CH_2)_3$, R = H

18 X = *o*-xylylene, R = *t*Bu



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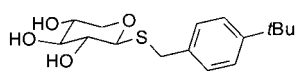
xylose- and ribose-functionalized silica; **17** was more strongly retained by the former, and **18** by the latter. However, NMR binding studies in CD_2Cl_2, with xyloside **20** and riboside **21** as substrates, yielded rather low association constants ($30-40\,M^{-1}$) and could not confirm the selectivity.[15] Having established that the triad of oxygens in **17** and **18** are more suitable for binding alkylammonium ions,[16] the investigators added peripheral hydroxy groups (giving **19**) and studied binding to hexoseammonium salts such as **22**.[17] Despite the use of a hydroxylic medium ($CDCl_3/CD_3OD$, 9:1) their results suggested a significant role for OH···OH interactions within the complexes.



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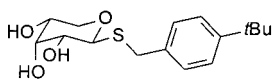

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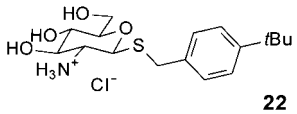

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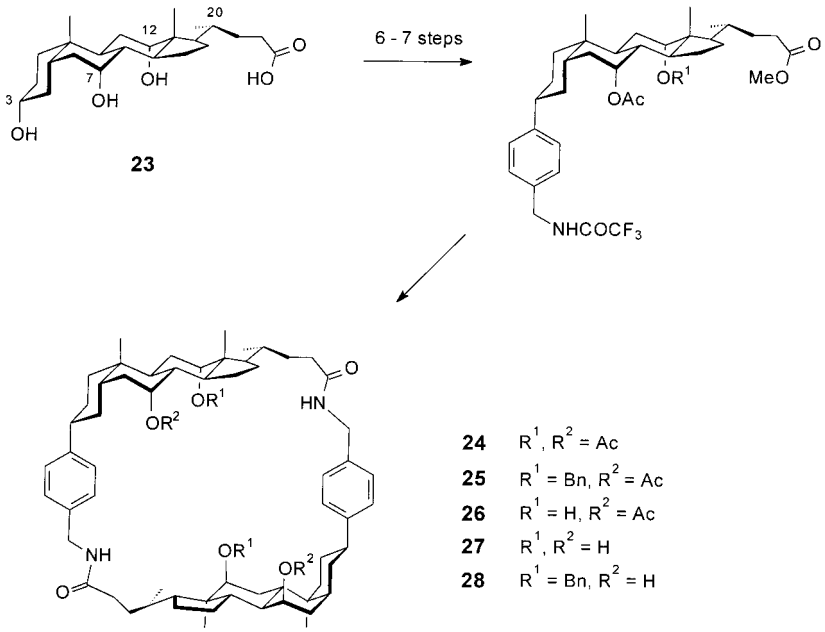
2.2. Encapsulation by Steroid-Based Receptors: The Cholaphanes

Although well-spaced, the binding sites in the above receptors are *co-directed*, rather than *convergent*. While this arrangement is suitable for certain carbohydrates, it predisposes the system to self-association and to the complex stoichiometries observed with certain substrates. It also limits versatility, precluding the simultaneous recognition of all groups in all possible carbohydrates. Progress towards carbohydrate receptors with fully tunable recognition properties required frameworks able to fully encapsulate a monosaccharide nucleus, surrounding it with various patterns of preorganized, inward-directed functionality. Seeking large, rigid and functionalized units as building blocks for such frameworks, several groups have exploited the inexpensive steroid cholic acid (**23**).[18]

The potential of this starting material was demonstrated with the synthesis of the first "cholaphanes", reported in 1989.[19] As shown in Scheme 1, these macrocycles **24**–**28** were prepared from **23** through conversion of the steroidal 3α-OH to a *p*-aminomethylphenyl spacer, followed by cyclodimerization. Two series were synthesized, one in which the steroidal 7- and 12-positions carried the same functional groups (**24**, **27**) and one in which they were differentiated (**25**, **26**, **28**). Although the framework in **24**–**28** is not entirely rigid,[20] it is conformationally restricted by the extended polycyclic/aromatic segments stretching from C20 of the steroids to the benzylic methylene carbon atoms. In open conformations it surrounds a substantial cavity bounded by the annular amides and the inward-directed functional groups at C7 and C12.

In initial tests, the cholaphanes were exposed to dodecyl β-D-glucopyranoside (**13**) in $CDCl_3$.[21] For both **27** and **28**,



23

24 R^1, R^2 = Ac

25 R^1 = Bn, R^2 = Ac

26 R^1 = H, R^2 = Ac

27 R^1, R^2 = H

28 R^1 = Bn, R^2 = H

Scheme 1. Synthesis of "cholaphanes" **24**–**28** from cholic acid (**23**).

complex formation was clearly indicated by ^{1}H NMR spectroscopy. Substantial downfield motions were observed for the amide NH signals, strongly suggesting that they were acting as H-bond donors, and other movements pointed to significant changes in the conformations of the macrocycles. The pattern of shifts was consistent with 1:1 stoichiometry, analysis yielding binding constants (K_a) of 1740 and 700 M^{-1} for **27** and **28**, respectively. Although it could not be proven beyond doubt that the carbohydrate entered the cavities of the macrocycles, the sizes of the binding constants, the number of affected proton signals, and molecular modeling studies (Figure 4) all supported this hypothesis.

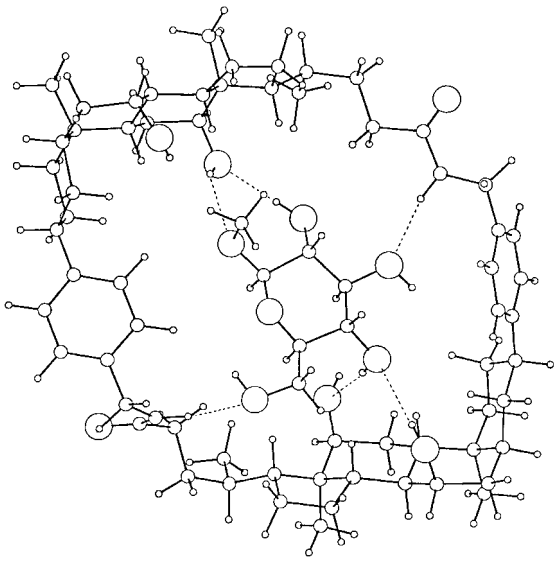
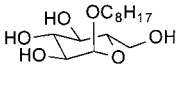


Figure 4. A possible conformation for **27** and methyl β-D-glucoside (employed as a model for **13**) revealed by molecular modeling. Dotted lines represent intermolecular hydrogen bonds, ranging in length from 2.06 to 2.32 Å.

The chirality of the cholaphane framework raised the possibility of enantioselective, as well as diastereoselective, carbohydrate recognition. This was investigated in a series of experiments employing cholaphane **27**, the octyl D-glucosides **11** and **12** and the corresponding L-enantiomers **29** and **30**.[22]



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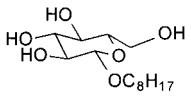

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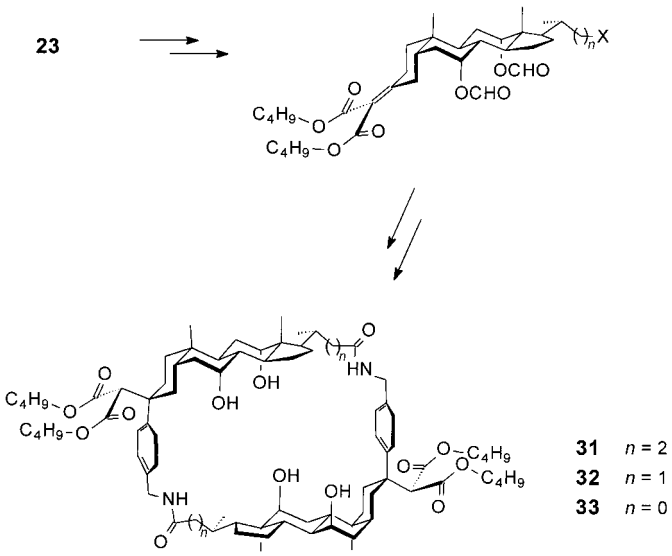
The results, presented in Table 1, revealed significant diastereoselectivity between **11** and **12** (factor of 5.5) and enantioselectivity between **12** and **30** (factor of 3). The enantioselectivity is perhaps especially significant as it cannot be ascribed to a difference in carbohydrate solvation energies, or intramolecular hydrogen bonding patterns (see Section 2.3). All such factors are, of course, identical for the two enantiomers.

Table 1. Association constants K_a in $CDCl_3$ between tetrahydroxycholaphane **27** and the isomeric octyl glucopyranosides **11**, **12**, **29**, and **30**.[a]

Glucoside	K_a [M^{-1}]
11 (α-D)	545 ($\pm$4%)
12 (β-D)	3100 ($\pm$14%)
29 (α-L)	1030 ($\pm$10%)
30 (β-L)	1000 ($\pm$14%)

[a] Determined by ^{1}H NMR titration at 298 K.

For a second generation of cholaphanes, improvements were sought in two respects. First, control of framework flexibility should promote enhanced preorganization of binding groups, greater selectivity, and higher binding constants for well-matched substrates. Second, externally directed alkyl chains would guard against insolubility in nonpolar solvents (the solubility of **27** in $CDCl_3$ had been barely sufficient for the complexation studies). The structural objectives were met with the series of macrocycles **31**–**33** (Scheme 2), in which



Scheme 2. Synthesis of cholaphanes **31**–**33** from cholic acid (**23**). X = protected or masked carboxy group.

sequential shortening of the steroidal side-chain culminates in a receptor (**33**) with very little conformational freedom.[23] Investigations failed to uncover the "well-matched substrate" for **33** (unless it is the cluster of water molecules revealed in the crystal structure; Figure 5), but titrations against glucoside **12** in $CDCl_3$ confirmed that **31**–**33** are all competent as

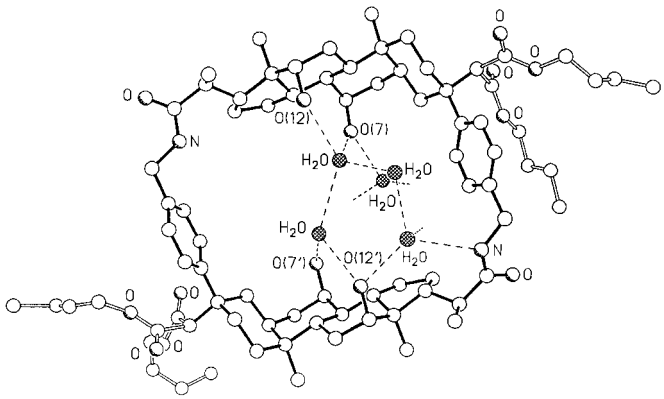
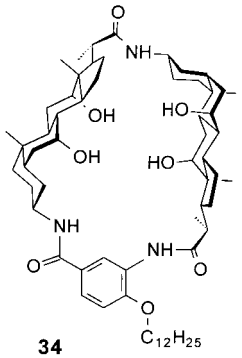


Figure 5. X-ray structure of **33** crystallized from $CHCl_3$/MeOH, showing five included molecules of water per formula unit.

carbohydrate receptors ($K_a = 1305$, 600, and 1560 M^{-1}, respectively). Receptor **33** was able to extract methyl β-D-glucoside (**10**) from aqueous solutions (1–2.5 M) into $CHCl_3$, significantly outperforming **31** and (surprisingly) **27** in this respect.

2.3. Alternative Frameworks Based on Cholic Acid

The "bis-nor" cholic acid unit used in **33** was also employed in the design of "cyclocholamides" such as **34**–**36**.[24] Truncation of the steroidal side chain results again in frameworks with, at most, very limited conformational mobility. A useful feature of this architecture is the tuning of cavity size possible through variation of the third component of the cyclotrimer.



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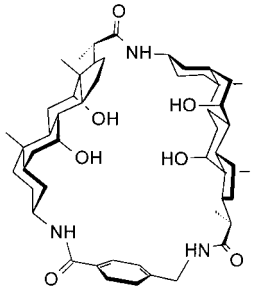

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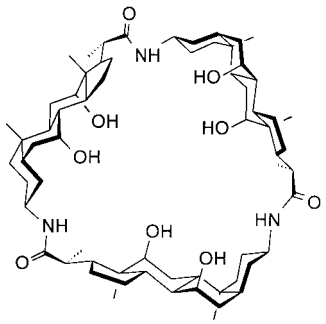

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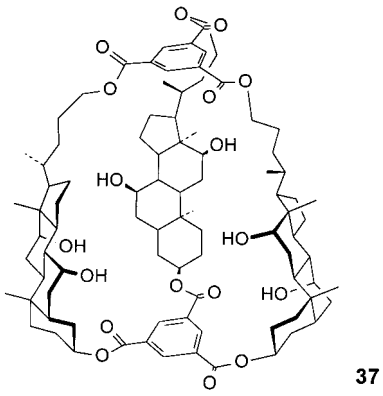
While **35** and **36** both showed evidence of binding to glucoside **12** in $CDCl_3$ (K_a for **12** + **35** = 750 M^{-1}; K_a for **12** + **36** not determined due to multiple stoichiometries[25]) the smaller cavity of **34** proved unable to accept the carbohydrate.

Three molecules of **23** were used, by Kohmoto and coworkers, to construct the "triply bridged cyclophane" **37**.[26] This macrobicyclic hexaol was tested in $CDCl_3$ against glucosides **11** and **12**, yielding significant but modest binding constants of 293 and 245 M^{-1}, respectively. Further work is needed to establish the potential of this novel but rather flexible architecture.

Cholic acid was exploited again in the receptors **38**–**40**, synthesized[27] (Scheme 3) and investigated[28] by Bonar-Law and Sanders. This study was useful in that binding constants were measured for a range of organic-soluble glycosides under several sets of conditions. Some of the results are collected in Table 2. The "cyclocholate" **38**, with just two hydroxy groups, was moderately effective in $CDCl_3$. Addition of the porphyrin spacer (in **39**) produced a general improvement, apparently without direct involvement of the NH groups in binding. Transferring the binding studies to CH_2Cl_2 lowered the binding constants, presumably because of improved solvation of host and/or guest. Introduction of the metal ion (in **40**) caused changes in the geometries of the



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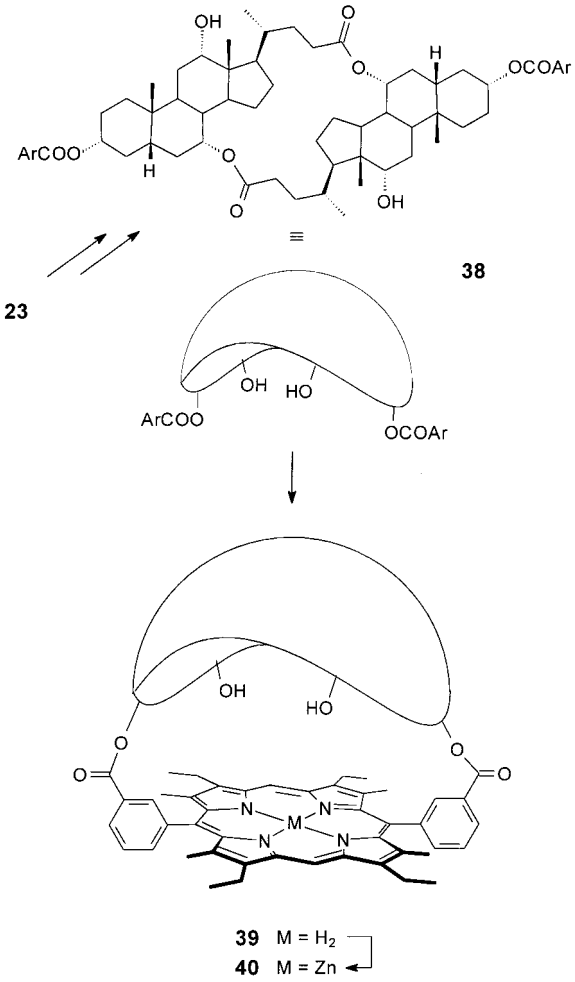
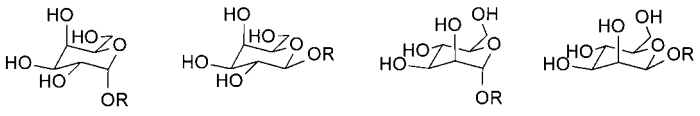


Scheme 3. Preparation of receptors **38**–**40**. Ar = 3-formylphenyl.

complexes, consistent with additional Zn···O interactions, but, rather surprisingly, did not produce substantial increases in K_a. This result was taken to indicate poor complementarity between host and guests, inhibiting the cooperation of all three binding sites. Interestingly, addition of small quantities



41 R = $C_{10}H_{23}$ **43** R = $C_{10}H_{23}$ **45** R = $C_{10}H_{23}$ **47** R = $C_{10}H_{23}$
42 R = C_8H_{17} **44** R = C_8H_{17} **46** R = C_8H_{17} **48** R = C_8H_{17}

Table 2. Association constants K_a [M^{-1}] between receptors **38**–**40** and organic-soluble glycosides (solvents as indicated).[a]

	38	**39**		**40**	
	($CDCl_3$)	($CHCl_3$)	(CH_2Cl_2)	(CH_2Cl_2)	($CH_2Cl_2 + H_2O$)[b]
α-D-galactoside **41**	39			490	
β-D-galactoside **43**				200	
α-D-glucoside **11**	450	1740	370	910	
α-L-glucoside **29**				260	
β-D-glucoside **12**	680	3900	770	1480	6700
α-D-mannoside **45**	2300	30000	7900	7000	12300
β-D-mannoside **47**				5200	18500

[a] Determined by UV and/or ^1H NMR titration at 295 K. [b] Approximately 0.09 mol L^{-1} water added to titration solvent.

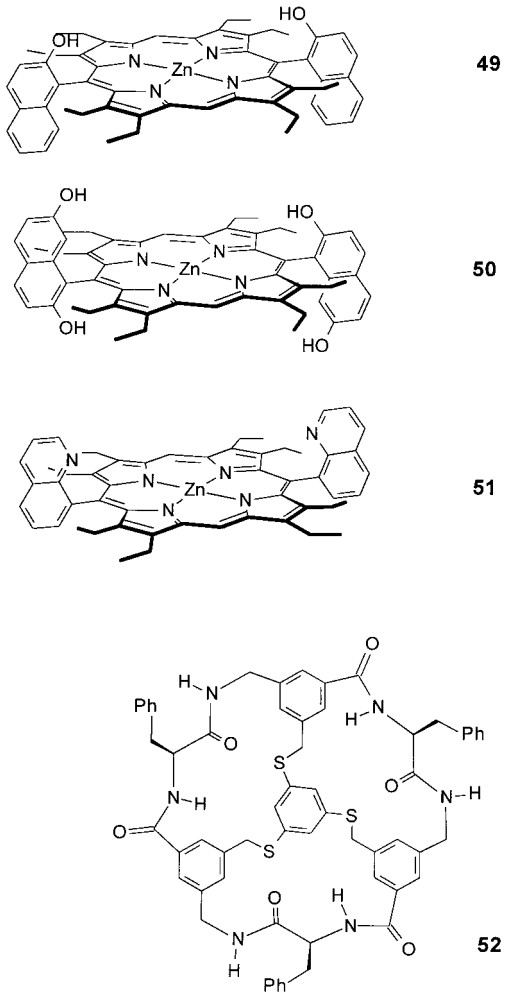
of water or methanol raized the binding constants, presumably by "plugging the gap" between host and guest (a strategy quite widely used by Nature; see Figure 1).

As shown in Table 2, the substrates employed with **38**–**40** included the glucosides **11**, **12**, and **29**, the galactosides **41** and **43**, and the mannosides **45** and **47**. Although the diastereoselectivities are impressive (e.g. a factor of ca. 60 between mannoside and galactoside binding to **38**), the authors point out that the inherent "stickiness" of some carbohydrates may be greater than others. In particular, intramolecular hydrogen bonding might be especially favorable in some cases, reducing the potential for intermolecular H-bond formation. Indeed, control experiments showed the aggregation tendencies of the glycosides to decrease in the order α-mannoside > β-glucoside > α-galactoside; thus the best substrates were also the best able to self-associate. Having said this, the ability of **40** to discriminate between enantiomers **11** and **29** (factor of 3.5) is unquestionably a specific effect.

2.4. Valleys, Bowls, Rings, and Cages: Further Architectures with Convergent Functionality

The Zn-porphyrin unit in **40** may be seen as a model for the metal centers (Ca^{2+}, Mn^{2+} or Mg^{2+}) found in certain carbohydrate binding proteins.[3c] This motif was combined again with H-bonding sites in the "U-shaped" receptors **49**–**51**, by Mizutani, Ogoshi et al. With octyl β-D-glucoside (**12**) in $CHCl_3$, these molecules were able to form complexes with $K_a = 180$, 2090, and 41400 M^{-1}, respectively.[29] Receptor **50** (but not **51**) once more increased in potency on addition of a small quantity of hydroxylic solvent.

The macrotricyclic "bowl" **52**, designed by Still and coworkers, provides another means of surrounding small polar molecules with preorganized hydrogen-bonding functionality. Although used mainly for the recognition of peptides and amino acid derivatives,[30] this receptor is also capable of accepting saccharide substrates.[31] The macrocycle **52** has



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51

52

been tested against a range of glycosides in $CDCl_3$, as illustrated in Table 3.[32] Judgements on the diastereoselectivity must be tempered by the considerations discussed above. Indeed, it is notable that the general pattern of affinities found

Table 3. Association constants K_a measured in $CDCl_3$ between receptor **52** and octyl pyranosides.[a]

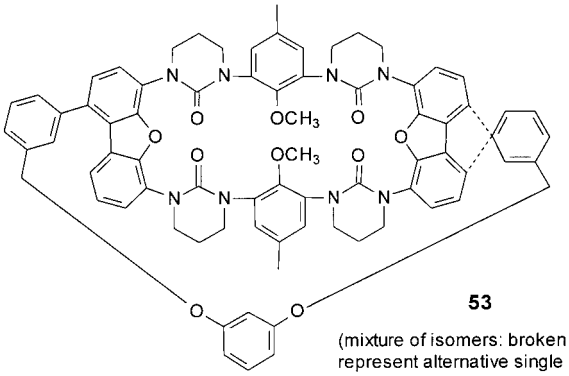
Pyranoside	K_a [M^{-1}]	Pyranoside	K_a [M^{-1}]
α-D-galactoside **42**	270	β-D-galactoside **44**	640
α-D-glucoside **11**	380	α-L-glucoside **29**	160
β-D-glucoside **12**	1770	β-L-glucoside **30**	450
α-D-mannoside **46**	5800	β-D-mannoside **48**	230

[a] Determined by ^1H NMR titration at 296 K.

for **38**–**40** (mannoside > glucoside > galactoside) is repeated with this very different receptor. Nonetheless the enantioselectivity shown for **12** versus **30** (factor of 3.9), and the selectivity between mannoside anomers **46** and **48** (factor of 25) are undoubtedly impressive.

An alternative bowl (or, perhaps more accurately, "basket") was employed in early work by the group of D. J. Cram.[33] Receptor **53** (studied as a mixture) possesses a cavity fringed with potent H-bond acceptor groups. They were unable to extract methyl α- or β-D-glucoside from aqueous solution into $CDCl_3$ (perhaps a reflection of their lack of

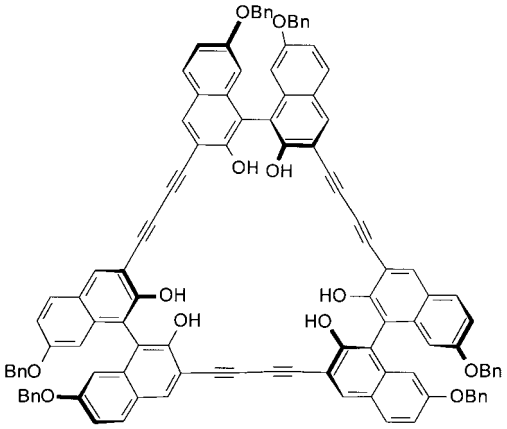
H-bond *donor* capability), but significantly promoted the lipophilization of these substrates from the solid state.



53

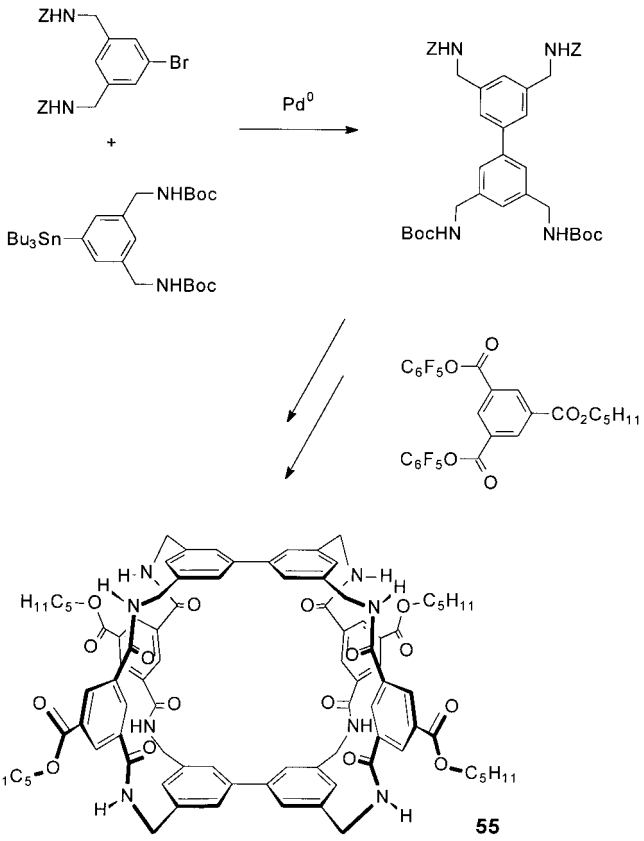
(mixture of isomers: broken lines represent alternative single bonds)

The macrocycle **54** can surround a monosaccharide with six phenolic hydroxy groups.[34] Though competent as a carbohydrate receptor ($K_a = 370$, 195, and 90 M^{-1} in $CDCl_3$ with **12**, **11**, and **29**, respectively), it is somewhat overshadowed by its anionic relatives discussed in Section 2.6.



54

A recent development from the authors' laboratory is the octa-amide **55**.[35] This new architecture, constructed as shown in Scheme 4, was designed to provide both apolar and polar contacts to a monosaccharide such as glucose (Figure 6). As shown in Table 4, it is capable of binding octyl β-D-glucoside (**12**) with $K_a \approx 1000\ M^{-1}$ in the quite competitive medium of $CDCl_3/CD_3OH$ (92:8), and with $K_a = 300000\ M^{-1}$ in pure $CHCl_3$. Also notable are its selectivity for β vs. α glucoside (factor of 50 in the former medium), and its ability to dissolve glucose in $CHCl_3$. The degree of complementarity between host and guest raises the hope that this system might also succeed in water when the esters are hydrolyzed to carboxylates. However, at time of writing this possibility remains to be tested.



Scheme 4. Synthetic route to octaamide **55**. Boc = *tert*-butoxycarbonyl.

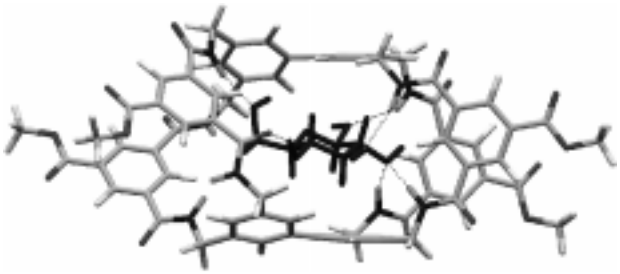


Figure 6. Possible conformation for **55**, modeled as the analogous tetramethyl ester, plus β-D-glucopyranose. Six intermolecular hydrogen bonds are present, shown as broken lines. Hydrogens on carbon atoms 1 – 3 of the carbohydrate are positioned to form close CH-π interactions with the host.

Table 4. Association constants $K_a[M^{-1}]$ between receptor **55** and organic-soluble glycosides (solvents as indicated).

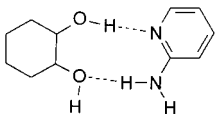
	$CDCl_3/CD_3OH$ (92:8)[a]	$CHCl_3$[b]
β-D-glucoside **12**	980 (±2%)	300000 (±6%)
α-D-glucoside **11**	20 (±23%)	13000 (±8%)
β-D-galactoside **44**	220 (±5%)	110000 (±12%)

[a] Determined by ^1H NMR titration at 303 K. [b] Determined by fluorescence titration.

2.5. Specific Motifs for Carbohydrate Recognition: 2-Aminopyridines and Related Substructures

In all the receptors described thus far, designs have focussed on the *disposition* of binding functionality rather than its precise nature. An alternative approach is to seek recognition motifs likely to be specifically effective for carbohydrates and then, as a second step, incorporate them in more elaborate frameworks. The X-ray crystallography of protein – carbohydrate complexes provides the obvious starting point for such a search. Arguably it is only moderately

helpful, in that most conceivable hydrogen-bonding patterns seem to contribute to natural carbohydrate recognition (see Figure 1).[36] However, several groups have noted the potential of doubly hydrogen-bonding groups for binding vicinal diols, and have discerned a predominance of such groups (primary amides, carboxylates, and guanidiniums) in natural carbohydrate binding sites. Anslyn and co-workers have exploited the 2-aminopyridine unit as a heterocyclic analogue of the asparagine/glutamine primary amide side chain. In principle it can bind a diol as in **56**, and can be incorporated in rigid, preorganized structures such as **57** and **58**.[37] The latter



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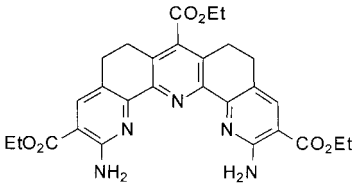

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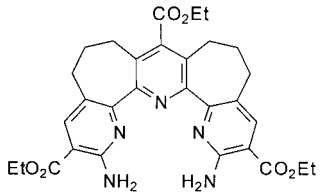

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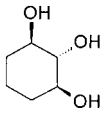
receptors were tested in $CDCl_3$ against *cis*- and *trans*-1,2-cyclohexane diols ($K_a = 5-17\,M^{-1}$), and the triols **59**–**61** ($K_a = 35-110\,M^{-1}$). It is notable that, of the triols, the all-*trans*



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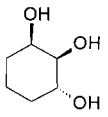

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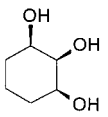
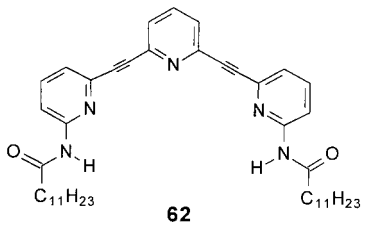


61

isomer **59** was bound more than twice as strongly as **60** and **61**. Given that *trans* 1,2-diols exhibit weaker intramolecular hydrogen bonding than their *cis* isomers,[38] this result provided early evidence for a linkage between strong intramolecular hydrogen bonding and reduced "stickiness" in carbohydrate derivatives (see Section 2.3). The study included just one actual carbohydrate derivative, the glucoside **13**, which was shown to bind to receptor **58** with $K_a = 190\,M^{-1}$.

The pattern of H-bonding "valencies" in **57** and **58** is repeated, with rather broader spacing, in the receptors **62**–**65**. Developed by Inoue et al. to target β-ribofuranosides, they were found to bind the methyl furanoside **16** in $CDCl_3$ with $K_a = 30$, 2400, 5200, and $50\,M^{-1}$, respectively.[39] This sequence reflects the a) value of macrocyclization in preorganising binding functionality, b) the potential for tuning H-bonding power through changes in the basicity (or acidity) of functional groups (in this case by addition of the alkoxy group to the central pyridine ring), and c) the damage which can be caused by intramolecular hydrogen bonding within a receptor (in this case a pair of NH···O bonds in receptor **65**). The binding geometry proposed for the complex between **16** and **63** is illustrated in Figure 7. Macrocycle **64** was able to



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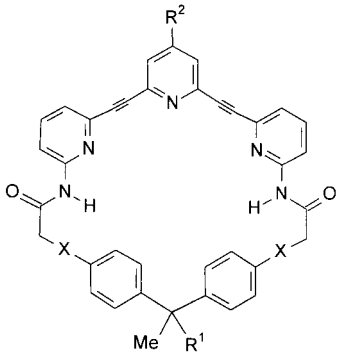


63 R^1 = Me, R^2 = H, X = CH_2

64 R^1 = *i*Bu, R^2 = O*n*Bu, X = CH_2

65 R^1 = Me, R^2 = H, X = O

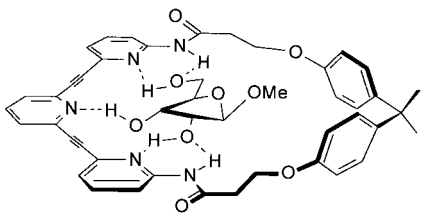
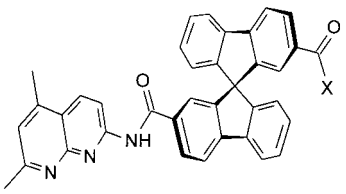


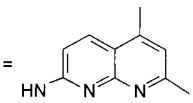
Figure 7. Receptor **63** complexed to ribofuranoside **16** in the geometry hypothesized by Inouye et al.

solubilize the pentoses ribose (**3**), deoxyribose, arabinose (**4**), xylose (**6**), and lyxose (**7**) in $CDCl_3$, as well as the ketohexose fructose (**9**). The aldohexoses glucose, mannose, and galactose were, however, unaffected.

An "extended analogue" of the 2-aminopyridine unit was employed by Diederich and co-workers in receptors **66** and **67**.[40] These "chiral clefts" were prepared in both enantio-



66 X =

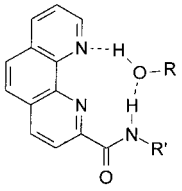


67 X = OCH_3

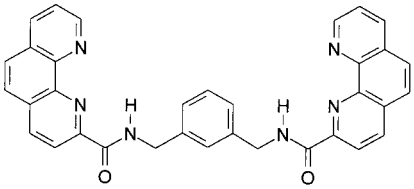
meric forms and shown to bind the octyl glucosides **11** and **12**, and mannoside **46**, in $CDCl_3$. Binding constants ranged between 180 and $1270\,M^{-1}$, in the now-familiar sequence α-glucoside < β-glucoside < α-mannoside (cf. Tables 2 and 3). Perhaps surprisingly receptor **67**, with only one naphthyridine,

proved essentially as potent as **66**. However, while **66** showed significant enantioselectivity (e.g. factor of 2 for the α-glucosides), **67** was ineffective in this respect.

Two-point binding of a single hydroxy group is proposed for phenanthroline carboxamides **68**.[41] This motif has not yet been employed to bind carbohydrates, but has been incorporated in receptors such as **69** targeted at cyclohexanediols. Receptor **69** is complementary to *cis*-1,3-cyclohexanediol,



68·ROH



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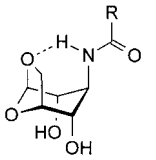

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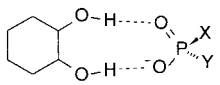
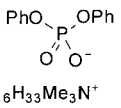
binding this substrate with $K_a = 1150\,\text{M}^{-1}$ in $CDCl_3$ [cf. $13\,\text{M}^{-1}$ for cyclohexanol + **68** ($R' = CH_2Ph$)]. The conformationally restricted diol **70** is also under development as an "interaction unit" for OH recognition.[42]

2.6. Recognition by Anionic Centers: The Phosphate-Diol Motif

Arrays of ionic centers have certain advantages in receptor design. Assuming they are of the same charge, the centers cannot be self-complementary. Rather, they should repel each other and thus help to maintain a well-defined cleft or cavity. Moreover, they often form exceptionally strong hydrogen bonds due to their acidity (if cationic) or basicity (if anionic). Several groups have explored the potential of anionic centers for carbohydrate recognition.[34, 43, 45–47] Particular attention has been paid to phosphates and phosphonates, which can participate in receptor frameworks while providing two H-bond acceptor sites capable, in principle, of binding diol units as in **71**.[44] As illustrated in Table 5, the simple monophosph(on)ate receptors **72**–**75** have been tested against alkyl β-D-glucosides and, in the case of **72** and **73**, several other alcohols and polyols. Phosphonates **73** and **74**, due to Das and Hamilton,[45, 46] are seen to be remarkably potent,



71 X,Y = O, C



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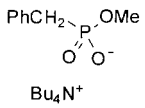

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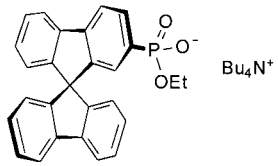

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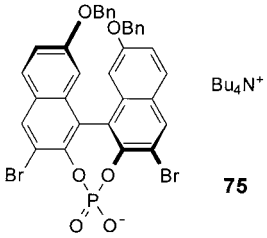
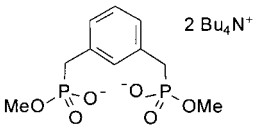


75

especially considering the competitive nature of the CD_3CN solvent. Comparisons between the different substrates reveal a strong enhancement of K_a on moving from alcohol to vicinal diol, and a further substantial increase between diol and carbohydrate.[43, 45]

Receptors **73**–**75** have been "extended" to give the polytopic analogues **76**–**79**. The second phosphonate group in **76** was found to raise binding constants by factors of 4–9



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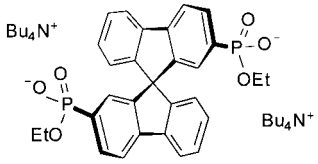


77

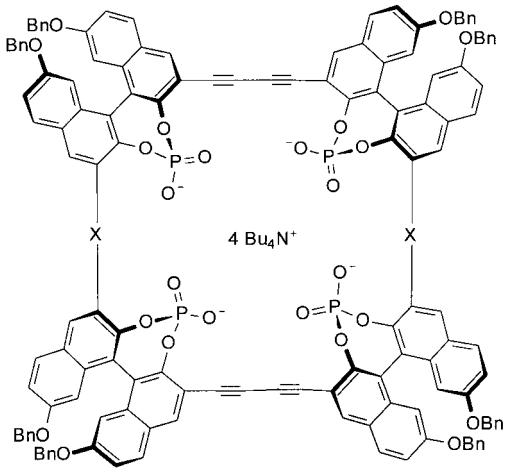
with octyl pyranosides as substrates.[45] Larger increments (factors of 9–17) were observed for the more rigid spirobifluorene framework (**74** vs. **77**).[46] Although the chiral **77** was studied as a racemic mixture, analysis of the data allowed enantioselectivities to be estimated. The ratio of 5.1 for the K_a values with glucoside **12** is the highest yet reported for a biomimetic carbohydrate receptor.

Studies by the Diederich group on tetraphosphate **78** were complicated by higher stoichiometries, but allowed an estimate of $15\,000\,\text{M}^{-1}$ for the binding constant to glucoside **12** in CD_3CN/CD_3OD (98:2).[34] It is notable that monomer **75** showed no detectable binding to **12** after addition of the hydroxylic cosolvent. The larger macrocycle **79** was inves-

Table 5. Association constants K_a between phosph(on)ates **72**–**75** and hydroxy compounds.[a]

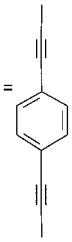
Receptor	Solvent	T [K]	Substrate	K_a [M^{-1}]	Ref.
72	$CDCl_3$[b]	298	cyclohexanol	0.9	[43]
72	$CDCl_3$[b]	298	*trans*-cyclohexane-1,2-diol	13–19[c]	„
72	$CDCl_3$[b]	298	dodecyl β-D-glucoside **13**	470–900[c]	„
73	CD_3CN	293	cyclohexanol	11	[45]
73	CD_3CN	293	*trans*-cyclohexane-1,2-diol	330	„
73	CD_3CN	293	octyl β-D-glucoside **12**	4400	„
74	CD_3CN	293	octyl β-D-glucoside **12**	3220	[46]
75	CD_3CN	300	octyl β-D-glucoside **12**	170	[34]

[a] Determined by ^{1}H NMR titration. [b] Containing water at concentrations of 2.5–3 mM. [c] Estimates of K_a varied depending on choice of NMR signal to be analyzed.

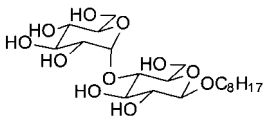


78 X =

79 X =



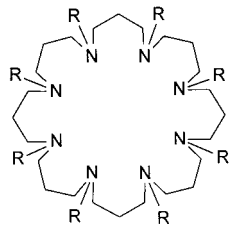
tigated in CD_3CN/CD_3OD (88:12), a still more polar solvent system. Binding to monosaccharide **12** was suppressed in this medium, but disaccharides such as **80** formed 1:1 complexes with K_a values between 10750 and 12500 M^{-1}.[47]



80

2.7. "Recognition" without Preorganization: Receptors Lacking Well-Defined Binding Sites

There remain a few systems which bind carbohydrates in organic media by multiple interactions, but do not provide geometrically defined clefts or cavities for their substrates. For example the flexible octa-amine **81** was found by Aoyama and co-workers to extract ribose, fructose, and glucose from 3 M aqueous solutions into CCl_4.[48] The glucose complex exhib-



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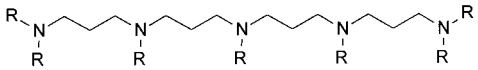
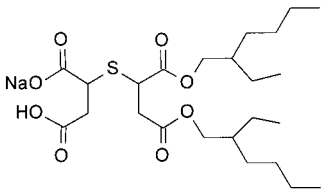


82, R = $CH_2CH(OH)CH(OH)(CH_2)_{10}CH_3$

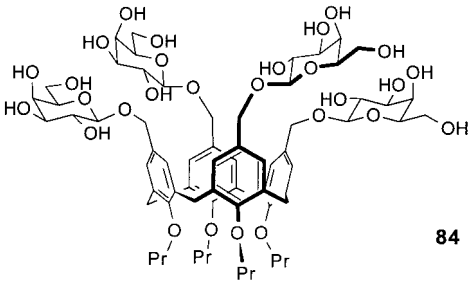
ited 1:1 host–guest stoichiometry, and did not contain significant coextracted water (in the absence of carbohydrate, ca. 40 equivalents of water were solubilized in the organic medium). The acyclic analogue **82** was also effective to some degree. In a study by Greenspoon and Wachtel, reverse micelles composed of surfactant **83** were also shown to solubilize glucose, in this case by dissolving the monohydrate in $CDCl_3$.[49] For the molecular architect, it is sobering to



83

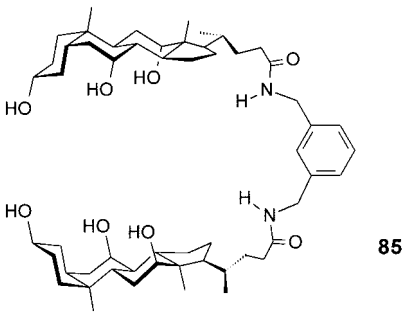
consider that, until the advent of **55**, these were the *only* reports of biomimetic "recognition" of this especially challenging and hydrophilic substrate.

The tetragalactosylcalixarene **84** of Dondoni et al. probably also belongs in this section, possessing a rigid core but rather flexible polar extensions. NMR studies in CD_3OD and



84

$(CD_3)_2SO$ produced no evidence of binding to neutral carbohydrates, but a positive (though unquantified) result for D-glucosamine hydrochloride.[50] Finally, cholic acid reappears in the flexible "tweezer" **85**, from the group of Burrows. Using variable-temperature NMR this molecule was shown to bind pentyl β-D-glucoside in $CDCl_3$,[51] foreshadowing the studies on steroid-derived macrocycles discussed in Section 2.2.



85

3. Carbohydrate Recognition in Aqueous Solution

If carbohydrate recognition is nontrivial in apolar media, it presents an exceptional challenge in water. In aqueous media, a carbohydrate receptor must expel a cluster of water molecules from its binding site before it accepts its substrate.

Yet the typical carbohydrate largely resembles a water cluster, differing only in the precise positioning of hydroxy groups and the presence of small patches of hydrophobic surface. As discussed below, the fact of natural carbohydrate recognition illustrates the effectiveness of preorganization and probably also the subtle, still mysterious nature of water as a solvent. However, with binding constants for protein-monosaccharide complexes apparently limited to about $10^7\,M^{-1}$,[52] even Nature finds carbohydrate recognition difficult.

3.1. The Biological Background; Carbohydrate Recognition in Nature

Natural carbohydrate recognition is mediated by various classes of protein. Leaving aside the enzymes whose function is to both bind and transform carbohydrate substrates, important categories are; a) antibodies, of which over 70% (when induced by whole cells) are normally directed towards oligosaccharide epitopes,[53] b) the lectins, which are used by animals, plants, bacteria and viruses for the recognition of cell-surface oligosaccharides, and c) the bacterial periplasmic proteins which are involved in carbohydrate transport and chemotaxis in Gram-negative organisms. The most intensively studied are the lectins, because of their controlling influence in many biological processes.[1c–e, 3b,c] Lectins were originally isolated from plants, and exploited for their ability to agglutinate certain types of cell (dependent on the cell-surface carbohydrates present) and also to induce mitosis. Well-studied examples are concanavalin A, from the legume *Canavalia ensiformis*, and "GS-IV" from *Griffonia simplicifolia*.[4a,c] Paradoxically, their precise role in plants is still somewhat mysterious.[1d, 3c] However, it has become clear that lectins are ubiquitous in the natural world, and are widely employed for cell recognition and adhesion. Medicinally important examples are the bacterial and viral lectins which initiate the infection of target cells (e.g. influenza haemagglutinin), the collectins, such as "mannose-binding protein", which bind to oligosaccharides on certain invading organisms as part of an antibody-independent immune response,[1e] and the selectins which mediate the recruitment of leukocytes into inflammatory tissue sites.[1c] Although of less general relevance, the bacterial periplasmic proteins are significant because of their high binding constants to monosaccharides (see below), and their suitability for X-ray crystallography.[3a]

Carbohydrate recognition by all three classes of protein has been investigated in the solid state by crystallography, and in solution by various techniques including, in particular, microcalorimetry. Structural studies on the lectins reveal shallow binding sites, consistent with their need to recognize sugar residues protruding from cell surfaces.[3c] Binding to monosaccharides is usually weak (in biological terms), with K_a values typically in the region $10^3-10^4\,M^{-1}$.[1e, 4b] However, extended binding regions lead to much stronger attachment to oligosaccharides in some cases,[54] while in others the provision of multiple binding sites and/or the tendency of lectins to aggregate[55] results in high affinities for multivalent substrates ("glycoside cluster effect").[1e] The limited structural data on antibody-carbohydrate complexes again shows the carbohydrates in clefts at the protein surface.[56] By contrast, the bacterial periplasmic proteins can fully surround their monosaccharide substrates and are correspondingly more potent, with K_a generally between 10^6 and $10^7\,M^{-1}$.[3a]

Protein–carbohydrate recognition is generally established through networks of hydrogen bonds and complementary contacts between nonpolar surfaces. Interestingly, the nonpolar residues on the protein are almost always aromatic, suggesting a role for specific CH-π interactions.[57] For the lectins and periplasmic binding proteins, most of the hydrogen bonds involve planar, multivalent side chain groups (Asn, Asp, Glu, Gln, Arg, His; cf. Figure 1).[3a,c] In contrast, it seems that antibodies make greater use of main chain amides for hydrogen bonding.[56] The most general H-bonding motif appears to be NH···OH···O=C. Although this subsumes many possibilities, it usefully highlights the importance of cooperative hydrogen bonding by the sugar hydroxy groups. While side chain hydroxy groups are rare in carbohydrate binding sites, it is very common to find water molecules in specific bridging positions between protein and substrate. Metal ions such as Ca^{2+} are required for full activity of many lectins (including concanavalin A), but only for the "C-type lectins" (e.g. mannose-binding protein) have direct metal–carbohydrate interactions been observed.

Calorimetric studies have shown that protein–carbohydrate complexation provides an excellent example of "enthalpy–entropy compensation".[4d, 58] In data tabulated by Toone,[4b] binding enthalpies vary from -2.0 to -22 kcal mol^{-1}, while free energies range between -2.8 and -8.5 kcal mol^{-1}. The spread of binding free energies is reduced by entropies which decrease in step with the enthalpies. Thus, a plot of $-\Delta H^\circ$ versus $-T\Delta S^\circ$ reveals points scattered fairly closely about a line of gradient 1.05 (Figure 8). At one end of the scale ($-\Delta H^\circ$ = small), ΔS° is just positive (favouring binding). However, for most of the range ΔS° is negative and opposes complexation.

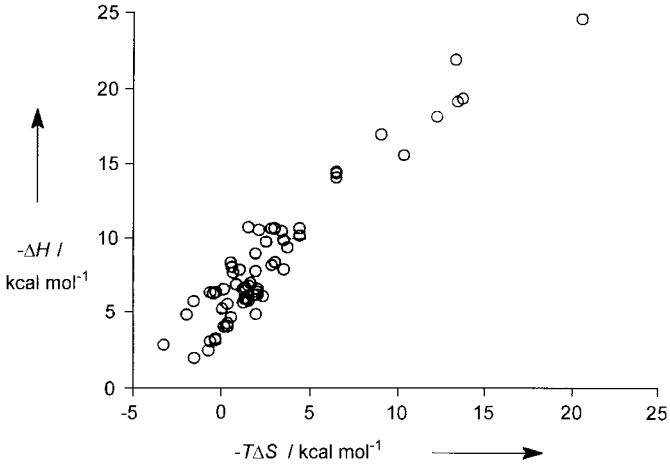


Figure 8. Enthalpy–entropy compensation plot for antibody/lectin–carbohydrate interactions. The slope of the plot is 1.05, with a correlation coefficient of 0.95. (Adapted from ref. [4b].)

3.2. The Driving Force for Natural Carbohydrate Recognition

Despite the wealth of structural data on carbohydrate–protein complexes, the driving force for their formation is still uncertain. The issue relates to wider debates on the nature of the hydrophobic effect, and the effectiveness of hydrogen bonding for molecular recognition in aqueous solution. In somewhat simplistic terms, the central question may be expressed as follows.[59] The formation of a 1:1 complex can be represented by the equilibrium in Equation (3). If all

$$A \cdot solvent + B \cdot solvent \rightleftharpoons A \cdot B + solvent \cdot solvent \quad (3)$$

depicted interactions are similar in strength, equilibrium lies well to the left because of the large excess of solvent. If reaction in the forward direction is to be favored, it must generally be driven *either* by an especially *strong* interaction between A and B (intrinsic binding) *or* by especially *poor* interactions between A and/or B and solvent, compared to the interactions between the solvent molecules themselves (solvophobic binding).

For the recognition of polar molecules in nonpolar solvents, it is usually clear that intrinsic binding is dominant. For the recognition of nonpolar molecules in water, it is equally clear that binding is essentially solvo(hydro)phobic. However, for the recognition of carbohydrates in water it is a) remarkable (at first sight) that Nature succeeds at all, and b) uncertain whether instrinsic or hydrophobic binding is more important. On the one hand, the similarity between the carbohydrate hydroxy groups and the competing water molecules underlay an early suggestion (later modified) that hyrophobic interactions between nonpolar patches on host and guest provided the major driving force.[4c] On the other hand, the H-bond networks observed by crystallography focussed attention on instrinsic binding.[3a] It should be noted, however, that crystallography by itself cannot easily distinguish between the options. Complex formation must inevitably be favoured by complementarity between receptor and substrate, whatever the source of the overall binding free energy.[4b]

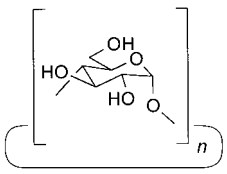
The most prudent assumption must be that both intrinsic and hydrophobic binding contribute significantly to biological carbohydrate recognition. It is undoubtedly credible that the preorganized polar groups within a binding site could make exceptionally favorable contacts with a carbohydrate substrate. Equally, as argued persuasively by Lemieux et al.,[4a,c, 60] the arrangement of polar and nonpolar groups at a "polyamphiphilic surface" (such as a carbohydrate or its binding site) is not necessarily compatible with effective hydration. While polar groups may be strongly bound to water molecules, there may be no efficient way to interconnect the first hydration shell. Displacement of these water molecules could provide an important driving force for complex formation. The negative enthalpies and entropies of formation of most protein–carbohydrate complexes might be thought to preclude dependence on the hydrophobic effect (which is generally understood to promote association through an *increase* in entropy).[61, 62] However studies in supramolecular chemistry and biochemistry have revealed examples of complexation in water which are clearly driven by solvation differences, yet show strongly negative ΔH^{o} and $T\Delta S^{o}$.[63, 59] Moreover, Chervenak and Toone have used calorimetric data for several lectin carbohydrate interactions in H_2O versus D_2O to infer directly that solvent reorganization provides an *enthalpic* driving force for binding.[64]

3.3. Recognition in Model Systems

3.3.1. Pentoses, Hexoses, and Methyl Glycosides

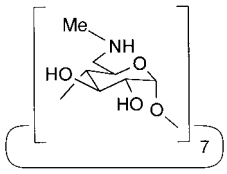
As emphasized above, the biomimetic recognition of carbohydrates presents exceptional challenges which remain to be overcome. To date, most studies in water have taken one of two approaches. On the one hand they have considered substrates for which the challenge is minimized, for example oligosaccharides with extended hydrophobic surfaces, or monosaccharides with hydrophobic appendages. On the other they have employed "difficult" monosaccharide substrates while adopting methods capable of detecting very small binding constants. Work in the latter category is described in this section, while the remainder is covered later.

Several groups have investigated the binding of monosaccharides to β-cyclodextrin (**86**).[65–69] This host is sufficiently large to encapsulate a sugar molecule, and appears to possess the necessary amphiphilicity. Unfortunately its spectroscopic



86 $n = 7$

87 $n = 6$



88

properties give little help to studies of carbohydrate recognition, which have therefore employed calorimetry or indirect methods involving displacement of fluorescent dyes. There are inconsistencies in the reported data,[68] but the majority view is that the binding of hexoses is undetectable[65, 66] or extremely weak[68] (e.g. $K_a = 0.6\,M^{-1}$ for D-glucose),[70] while that of pentoses is slightly stronger (e.g. $K_a = 5.3\,M^{-1}$ for D-ribose,[65] estimates of 1[65] and 17[66] M^{-1} for D-xylose). The hepta(methylamino) analogue **88** was also shown to bind D-ribose, with $K_a = 26\,M^{-1}$.[71]

α-cyclodextrin (**87**) possesses a smaller cavity which can only partially accept a pyranose nucleus. Nonetheless, a calorimetric study on this host, performed by Danil de Namor and co-workers, revealed quite significant binding constants to hexoses as well as pentoses.[66] As shown in Table 6, binding was found to be entropy-driven, with extremely small negative enthalpies (through which complex formation was detected). It is interesting to note a) that the balance between ΔH^{o} and ΔS^{o} concords with the entropy–enthalpy plot in Figure 8, and b) that the general "order of stickiness" mannose > glucose > galactose proposed for organic media

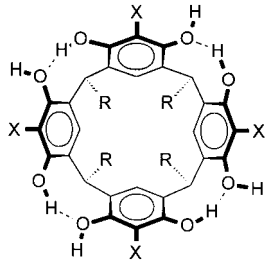
Table 6. Association constants K_a and thermodynamic parameters, determined by microcalorimetry, for binding of monosaccharides to α-cyclodextrin (**87**) at 298.15 K in aqueous solution.[a]

Monosaccharide	K_a [M^{-1}]	ΔG° [kJ mol^{-1}]	ΔH° [kJ mol^{-1}]	ΔS° [J K^{-1} mol^{-1}]
D-glucose	36	−8.9	−0.14	29.4
D-fructose	52	−9.8	−0.05	32.8
D-mannose	59	−10.1	−0.11	33.5
D-galactose	15	−6.8	−0.32	21.7
D-xylose	37	−8.9	−0.09	29.8
L-xylose	117	−11.8	−0.12	39.2

[a] Errors in ΔH° estimated at $\pm 10-20\,\%$. K_a values uncertain by factors of 1.3–2.0.

(see Section 2.3) is repeated here for a study in water. The enantioselectivity recorded for xylose is also of interest. These results are remarkable in that they represent the only examples to date of significant noncovalent binding of the common hexoses to a model system in water. Thus far the measurements have been made by just one technique operating at the limit of its sensitivity. Further studies using other methods could be illuminating, and should be encouraged.

Following their success with **1** in organic media, the group of Aoyama investigated the calixarenes **89**–**91** in water.[72, 73] Though ineffective for most pentoses and hexoses, prototype



89 X = H R = $CH_2CH_2SO_3Na$
90 X = CH_3
91 X = OH

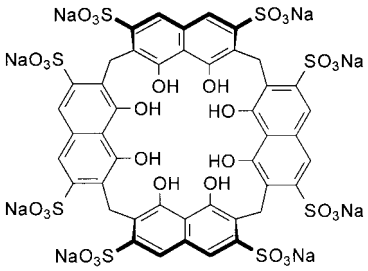
89 showed significant affinities for certain alcohols, and to some extent for the more lipophilic monosaccharides.[72] As shown in Table 7, substitution with methyl or hydroxy groups improved potency,[72] as did the removal of two or four protons.[73] This and other data was used to argue the case for a specific CH-π interaction between host and guest.

"Cyclotetrachromotropylene" (**92**), studied by Poh and Tan, is an interesting variation on the calixarene theme. An extended hydrophobic surface is combined with 16 water-solubilizing functional groups. Its cleft-shaped inner surface

Table 7. Selected association constants K_a for binding of monosaccharides to water-soluble calixarenes **89**–**91**.[a]

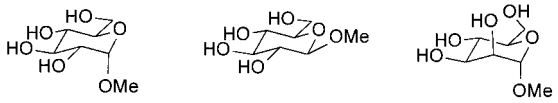
	K_a [M^{-1}]				
	89	**90**	**91**	**89**$^{2-}$	**89**$^{4-}$
D-arabinose	0.85	2.1	2.5		
D-2-deoxyribose	1.2	4.9	3.9		
D-fucose	1.8	6.0	8.4	16	26

[a] Determined by ^{1}H NMR titration at 298 K.



92

appears better suited to linear, as opposed to cyclic, alcohols, as evidenced by K_a values of 3000 M^{-1} to butanol, 2500 M^{-1} to hexanol, and 700 M^{-1} to cyclohexanol. However, while showing no ability to bind xylose, glucose, or mannose, it gave significant binding constants of 28, 6, and 75 M^{-1} to methyl α-D-glucoside (**93**), β-D-glucoside (**10**), and α-D-mannoside (**94**), respectively.[74]

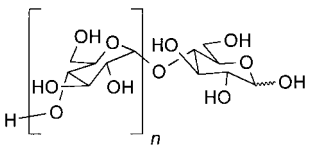


93 **10** **94**

3.3.2. Oligosaccharides

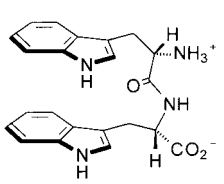
Though structurally complex, oligosaccharides are generally easier to bind than monosaccharides because of their extended surface areas. Indeed, both amyloses[75] and, especially, cycloamyloses (cyclodextrins)[69] associate (as hosts) with a range of molecules in aqueous solution. While all these phenomena are relevant to carbohydrate recognition, we focus here on cases where the oligosaccharides may be seen as "targets".

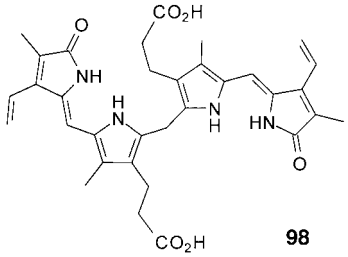
Use of maltose (**95**) and maltotriose (**96**) as substrates allows detectable binding to acyclic, relatively non-preorganized hosts. Both caused fluorescence enhancement in L-tryptophanyl-L-tryptophan (**97**), an interesting (if primitive) model for the apolar motifs found in many natural carbohydrate binding sites. Titration data was consistent with 1:1 binding, but with K_a values of just 1 M^{-1} for **95** and 8 M^{-1} for **96**.[76] A study employing CD spectroscopy revealed that bilirubin (**98**) can also associate with **95** and **96**, as well as other di- and oligo-saccharides (but not glucose).[77]



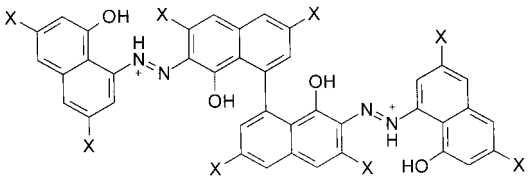
95 $n = 1$
96 $n = 2$

Cyclotetrachromotropylene (**92**) has been found to bind cyclodextrins, with $K_a = 85-140$ M^{-1}.[78] Arguments based both on NMR spectroscopy and molecular models suggest that **92** acts as the host, providing a groove-shaped binding site which can accept the exterior of a cyclodextrin ring. Its linear relative "calcichrome" (**99**) is even more effective ($K_a =$





97 98

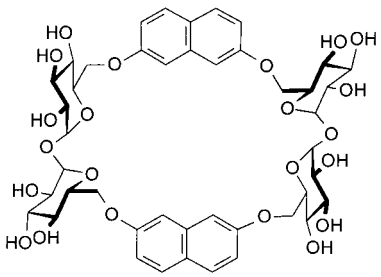


99 X = SO_3^-

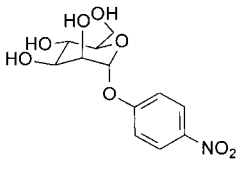
380 – 570 M^{-1}),[79] although in this case the mode of complexation is perhaps less certain.

3.3.3. Weak Interactions Probed through "Enforced Proximity"

Weak interactions, such as those involved in carbohydrate recognition, may be detected as modulations of stronger binding forces. If aryl glycosides are used as substrates, the aryl units may be paired with hydrophobic cavities to give reliable binding motifs which can position the carbohydrate close to receptor functionality. This approach has been adopted, in particular, by the group of Penadés, who have studied the binding of *p*-nitrophenyl glycosides to α-cyclodextrin (**87**) and the "glycophane" **100**.[80] In the case of α-cyclodextrin, the carbohydrate moieties generally lowered complexation relative to *p*-nitrophenol (PNP). By contrast, the PNP glycosides bound more strongly to **102** than did PNP or 1-(*p*-nitrophenyl)glycerol. The additional binding energy (relative to PNP) was in the range 4.6 – 7.5 kJ mol^{-1}, and was greatest for axial glycosides such as α-mannoside **101**.

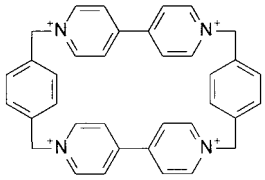


100



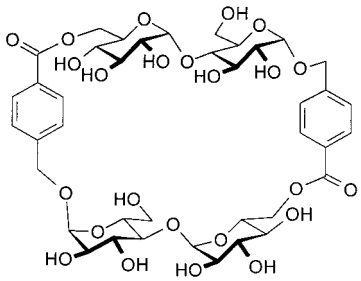
101

Intermolecular NOEs between carbohydrate residues in **100** and **101** supported the notion that carbohydrate – carbohydrate interactions were responsible for the higher affinities. In related work the binding of phenyl glycosides to cyclophane **102** was studied by Smith and Stanley.[81] In this case the equatorial glycosides were more strongly bound, probably for steric reasons.[82]



102

Alternatively, weak interactions can sometimes be studied *within* molecules, taking advantage of covalent bonding to enforce proximity. The glycophane **103** has been thus used, in a demonstration of carbohydrate-arene stacking. NMR spectra in D_2O revealed significant upfield displacements of certain carbohydrate signals, consistent with a closed conformation placing one of the pyranose rings in contact with the aromatic spacer. The shifts were not observed in [D_6]DMSO or CD_3OD, confirming the hydrophobic nature of the interaction.[83]



103

3.4. Carbohydrate Recognition at Surfaces

Finally, it should not be forgotten that natural carbohydrate recognition often takes place at cell surfaces.[54b] It is well-accepted that water molecules behave atypically in the vicinity of an interface, with significant consequences for noncovalent interactions. In particular, it seems that hydrogen bonding may be far stronger at a hydrated surface than in bulk water.[84] Kunitake et al. have studied the binding of carbohydrates from aqueous solution to monolayers of **104** – **106**, as well as Aoyama's calixarene **1** (Figure 9).[85] Their modified electrodes responded to the presence of carbohydrates at remarkably low concentrations (Table 8). Although receptor **1** showed no substantial advantage over **104** and **106**, the differing selectivities strongly suggest a role for specific recognition through non-covalent interactions.

Even more remarkable was the behavior of hydrophobic pockets created on gold surfaces, by the Fuhrhop group, through treatment with a steroidal thiol followed by octadecanethiol (Figure 10).[86] The ion permeabilities of these surfaces were studied by cyclic voltammetry with $[Fe(CN)_6]^{3-}$ as the redox probe. The moderate currents observed initially could be partially blocked by monosaccharides, including

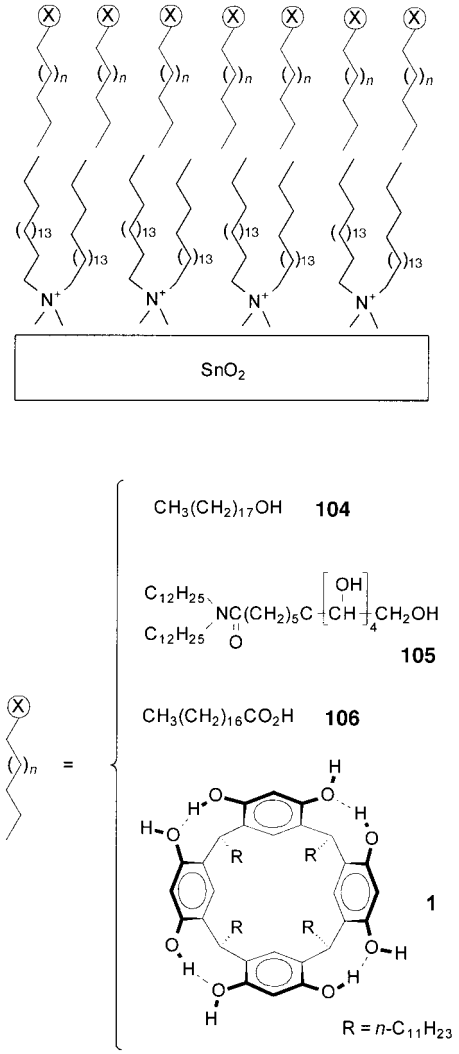


Figure 9. Schematic view of modified electrodes used to detect carbohydrate recognition at monolayer surfaces. Langmuir–Blodgett (LB) deposition is used to coat SnO_2 glass, first with bis(octadecyl)dimethylammonium bromide (to create a hydrophobic surface) and then with amphiphiles **104**–**106** or **1**. The potential of the electrode is monitored against Ag/AgCl in the presence of increasing concentrations of carbohydrates.

Table 8. Threshold concentrations for potentiometric detection of carbohydrates by electrodes modified with **105**–**107** or **1**, as shown in Figure 9.

	Threshold concentrations [10^{-4} M]			
	104	**105**	**106**	**1**
ribose	1.7	4.8	1.0	0.42
xylose	1.8	4.6	0.6	2.2
glucose	8.4	17	0.4	6.0
sucrose	14	25	3.6	8.7

glucose. The effects were slow to build up and difficult to reverse,[87] but did not seem to involve covalent bond formation.

Carbohydrate-binding properties have also been shown for a silica surface under aqueous conditions. In this case the effect relied on "sugar clustering"; the octagalactosyl calixarene **107** was strongly bound while galactose itself showed no affinity.[88]

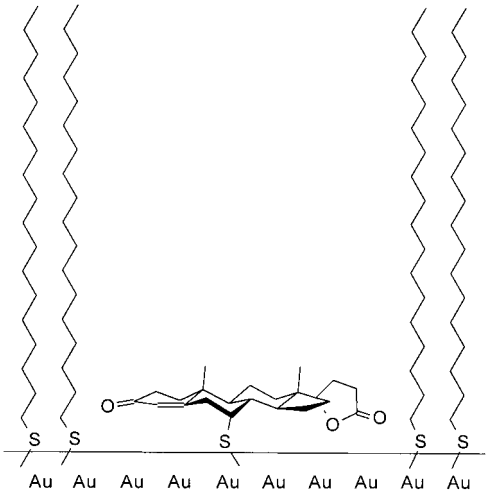
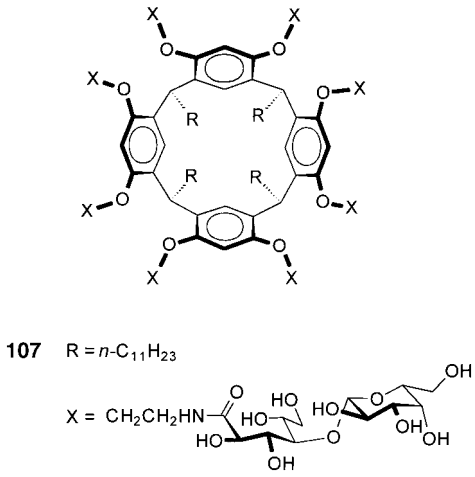


Figure 10. Hydrophobic pocket formed on gold by chemisorption of a steroidal thiol, followed by coverage of the remaining surface with an octadecanethiol self-assembled monolayer.



4. Conclusion

The quest for biomimetic carbohydrate recognition has produced much of interest over the past few years. Strong binding has been achieved in organic media, and apparently also at aqueous interfaces. Studies in aqueous solution have set the stage for future efforts. However, there is still no designed, monomeric receptor which can compete effectively with bulk water for low concentrations of monosaccharide substrates. This applies both in homogeneous media and in two-phase (aqueous-organic) systems, where extractions have invariably employed ≈1M solutions of substrate.[89] The combination of potency with designed specificity is still further away, perhaps inaccessible without radically new approaches. The challenge remains, and should yield insight and enjoyment for some years to come.

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