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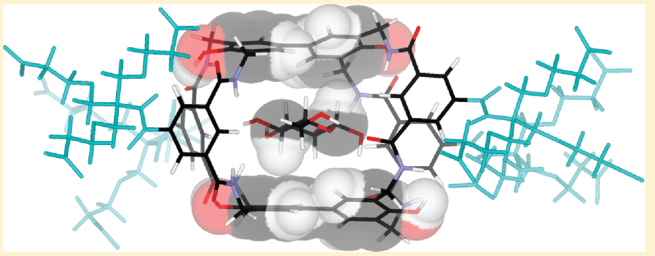
Substituent Effects in Synthetic Lectins - Exploring the Role of CH−π Interactions in Carbohydrate Recognition

Nicholas P. Barwell and Anthony P. Davis*

School of Chemistry, University of Bristol, Cantock's Close, Bristol, U.K. BS8 1TS

Supporting Information

ABSTRACT: Contacts between aromatic surfaces and saccharide CH groups are common motifs in natural carbohydrate recognition. These CH−π interactions are modeled in "synthetic lectins" which employ oligophenyl units as apolar surfaces. Here we report the synthesis and study of new synthetic lectins with fluoro- and hydroxy-substituted biphenyl units, designed to explore the role of π-electron density in carbohydrate CH−π interactions. We find evidence that recognition can be moderated through electronic effects but that other factors such as cavity hydration are also important and sometimes predominant in determining binding strengths.



INTRODUCTION

The binding of carbohydrates in water by proteins such as lectins is one of the less understood aspects of biomolecular recognition.[1] It is generally accepted that persuading saccharides to displace water molecules from a binding site is intrinsically difficult. The hydroxyl groups on carbohydrates have similar binding properties to those of the solvent, and exchanging one for the other is unlikely to yield much energetic benefit. Binding free energy must be garnered from subtle differences in hydrogen bonding patterns, the release of confined water molecules (raising entropy), and noncovalent bonding to groups other than hydroxyl (e.g., the CH groups). The CH−π interaction,[2] in particular, is thought to play an important role.[3] Many saccharide units possess patches of codirected CH groups which together can make contact with a flat surface. Proteins often provide aromatic surfaces to complement these patches, a well-known example being the *E. coli* glucose/galactose chemoreceptor protein (Figure 1).[4] Such contacts should lead to the release of high-energy water molecules, and thus hydrophobic binding, but there is an additional more specific effect. Both theory and experiment suggest that the electron-poor CH hydrogen atom should interact favorably with the electron-rich surface of the π-system. The attraction should increase with π-electron density,[5] and it is notable that the electron-rich tryptophan indole seems especially widely used in nature.

Model studies can be useful in clarifying the role of specific noncovalent interactions in biological processes. In the case of carbohydrate CH−π interactions, a number of investigations have implied that increased π-electron density does indeed lead to stronger binding.[6] However, these have not included fully synthetic lectin analogues in which the geometry of substrate binding is well-established. We have recently developed a family of receptors capable of binding carbohydrates in water with good

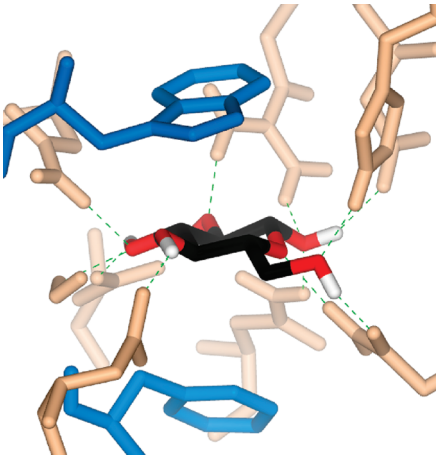


Figure 1. Binding site of the *E. coli* glucose/galactose chemoreceptor protein, with glucose substrate.[4] Aromatic tryptophan and phenylalanine residues are shown in blue, and polar residues in gold.

affinities and excellent selectivities.[7,8] As illustrated in Figure 2, the general design employs oligophenyl units, to define the roof and floor of the binding cavity, and isophthalamide pillars, to hold them apart. The cavities are complementary to "all-equatorial" carbohydrates such as β-linked glucose or *N*-acetylglucosamine (GlcNAc) **1**, or oligomers such as cellobiose **4**. The roof and floor are positioned to contact the axial CH groups in the substrates, contributing hydrophobic and CH−π interactions to binding. The isophthalamide spacers can bind to substrate OH or

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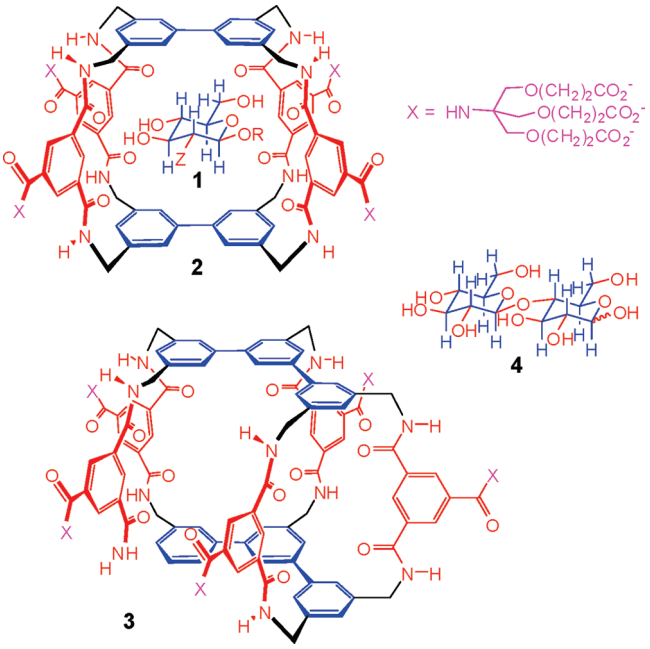


Figure 2. Synthetic lectins for all-equatorial carbohydrates. Monosaccharide receptor **2** is shown binding β-glucosyl **1** (Z = OH) or β-GlcNAc **1** (Z = NHAc). Disaccharide receptor **3** is similarly complementary to cellobiose **4**.

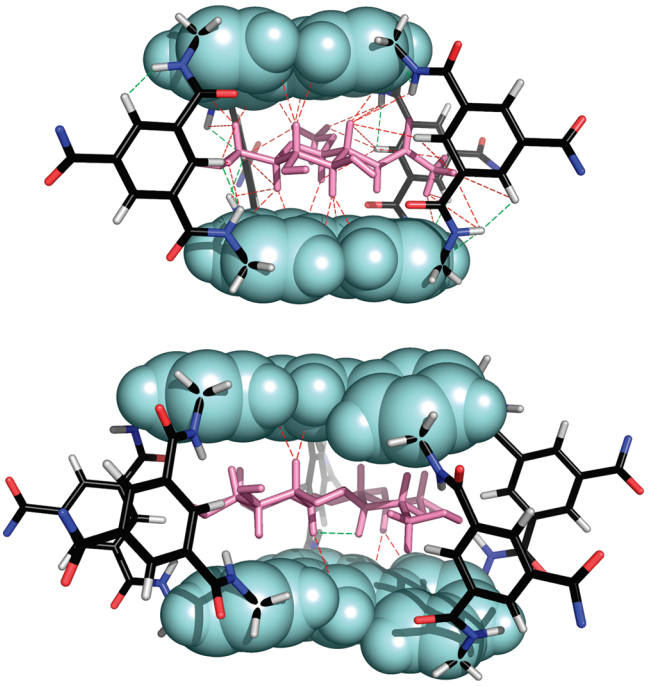
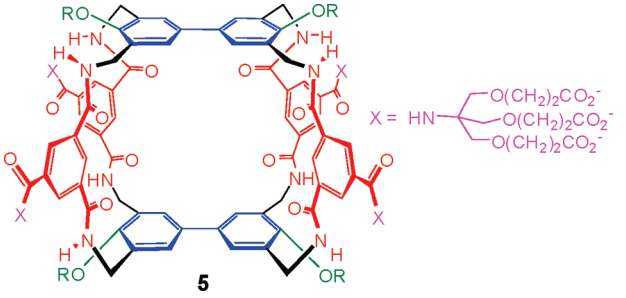


Figure 3. NMR/molecular modeling structures for the complexes between (top) **2** and GlcNAcβ-OMe **1** (Z = NHAc, R = Me),[8d] and (bottom) **3** and cellobiose **4** (β anomer).[8c] The bi- and terphenyl units are highlighted in pale cyan (space-filling mode), and the carbohydrates are shown as pink. Intermolecular NOE contacts are shown as red broken lines. The water-solubilizing tricarboxylate groups are omitted. The structure of **2.1** (Z = NHAc, R = Me) is supported by 51 NOE contacts and is essentially unambiguous. The structure of **3.4** is supported by fewer cross-peaks, but the positioning of the substrate is nonetheless certain.

NHAc groups through hydrogen bonding. NOE studies confirm the geometry of binding and can give detailed structures for the complexes (Figure 3).[7b−e] The performance of these systems can be remarkably similar to that of lectins. For example, prototype **2** binds GlcNAc derivative **1** (Z = NHAc, R = Me) with $K_a = 650\ M^{-1}$, comparable to Wheat Germ Agglutinin (the lectin traditionally used for GlcNAc moieties).[7d]

As plausible lectin models, receptors **2** and **3** have previously been employed to study the hydrophobic effect in carbohydrate recognition.[9] The role of CH−π interactions is another issue which should, in principle, be addressable. As discussed above, the binding geometries are clearly established such that there is no doubt that contacts exist between carbohydrate CH groups and the π systems of the oligophenyl units. By introducing substituents into the oligophenyls, it should be possible to vary the electron density in their π-systems. The effects on binding constants would then shed light on the nature of the interactions. Meanwhile affinities would hopefully be raised in some cases, a useful outcome from the viewpoint of applications.

The opportunities for substitution in the oligophenyl units are limited by steric effects; any group added ortho to an Ar−Ar bond will promote a twisted conformation unsuitable for binding, while the positions between CH_2 groups are quite hindered. Nonetheless, small substituents will fit in these latter positions without disruption to the structure. In previous work we prepared a series of receptors **5** with alkoxy groups in the biphenyl 4,4′ positions and were pleased to find that affinities for β-glucosides were raised considerably.[8e] However interpretation of these results in terms of π-electron density was complicated by the hindered nature of the position, which forces the alkoxy group out of plane and disrupts n−π conjugation. We now report a more informative study, featuring substituents which are electronically more diverse and less affected by crowding. The results suggest that π-electron density can indeed be used to modulate binding in carbohydrate receptors but that differences are small and may be masked by other effects.



RESULTS AND DISCUSSION

Receptor Design and Synthesis. For this study we required analogues of **2** in which the biphenyl 4,4′ positions were occupied by small, electronically active substituents. The groups chosen were F, as in **6**, and OH, as in **7**. F was expected to reduce the electron density in the biphenyl π-system, while OH is generally seen as electron-donating (but see later).[10] Moreover deprotonation is possible to give a third species **8**, with greatly increased π-electron density. Unlike the alkoxy groups in **5**, the hydroxyls in **7** can exert their normal effect, being able to lie coplanar to the benzene ring such that the oxygen lone pairs can interact optimally with the π-cloud. Molecular modeling was employed to assess the effects of the F, OH, and O^- substituents on the receptor conformations. Monte Carlo Molecular Mechanics searches[11] on unsubstituted receptor **2** and the new systems **6**−**8** revealed similar

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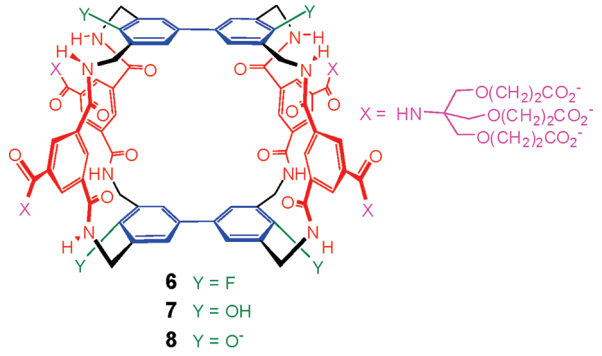
ground state conformations with inward-directed NH groups and twisted biphenyl units.[12] Differences were subtle and seemed unlikely to have major effects on the binding properties.

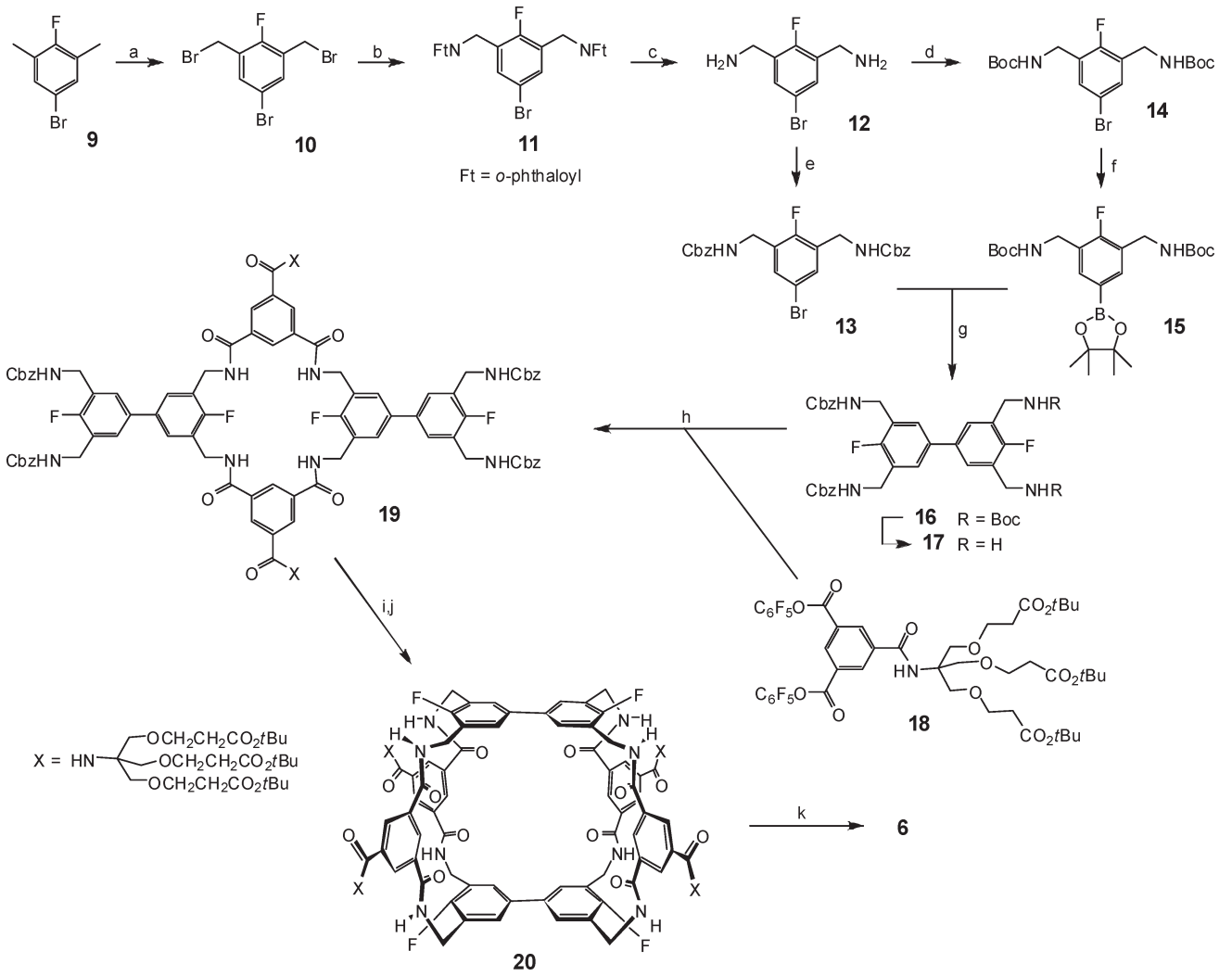


6 Y = F
7 Y = OH
8 Y = O⁻

Tetrafluoro receptor **6** was prepared as shown in Scheme 1.[12] Commercially available 5-bromo-2-fluoro-*m*-xylene **9** was treated with NBS under irradiation with visible light to give tribromide **10**. Conversion to diamine **12** was accomplished via Gabriel synthesis. Two different *N*-protection methods gave **14** and **13**, which were coupled via Suzuki−Miyaura methodology to give biphenyl **16**. The overall yield of **16** from **9** was ∼17%. N-Boc deprotection and macrocyclization with spacer component **18**[8b] under high dilution gave mainly the [2 + 2] product **19**. N-Deprotection, a second macrocylization with **18**, and then O-deprotection gave **6**. The yields for the macrocyclizations were low, at 17% and 25% respectively, but were sufficient to provide material for testing.

Tetrahydroxy receptor **7** was prepared via biphenyl **23**, obtained from the previously described diol **21**[8e] (Scheme 2).[12] The protection scheme in **23** was chosen to allow exposure of two amino groups via *N*-Boc cleavage, unmasking of the second two by reduction of the azides with PPh_3/H_2O, and then deprotection of the phenolic hydroxyls by hydrogenolysis. Cyclizations with **18** after the first and second deprotections gave macrotricycle **26**.

Scheme 1[a]



[a] Reagents and conditions: (a) NBS, methyl acetate, *hν*, 62%; (b) potassium phthalimide, DMF, 100%; (c) hydrazine, chloroform/methanol (1:4), reflux, 96%; (d) Boc_2O, DIPEA, THF, 88%; (e) Benzyl chloroformate, DIPEA, THF, 52%; (f) bispinacolato diboron, Pd(dppf)Cl_2 (3 mol %), KOAc (4 equiv), DMF, 80 °C, 37%; (g) Pd(dppf)Cl_2 (5 mol %), Na_2CO_3 (aq., 2 M), DMSO, 60 °C, 86%; (h) TFA, DCM, 0 °C then **18**, DIPEA, THF, 17%; (i) Pd/C, H_2, THF/methanol/NH_3; (j) **18**, DIPEA, THF, 25%; (k) TFA, DCM, 0 °C, 95%.

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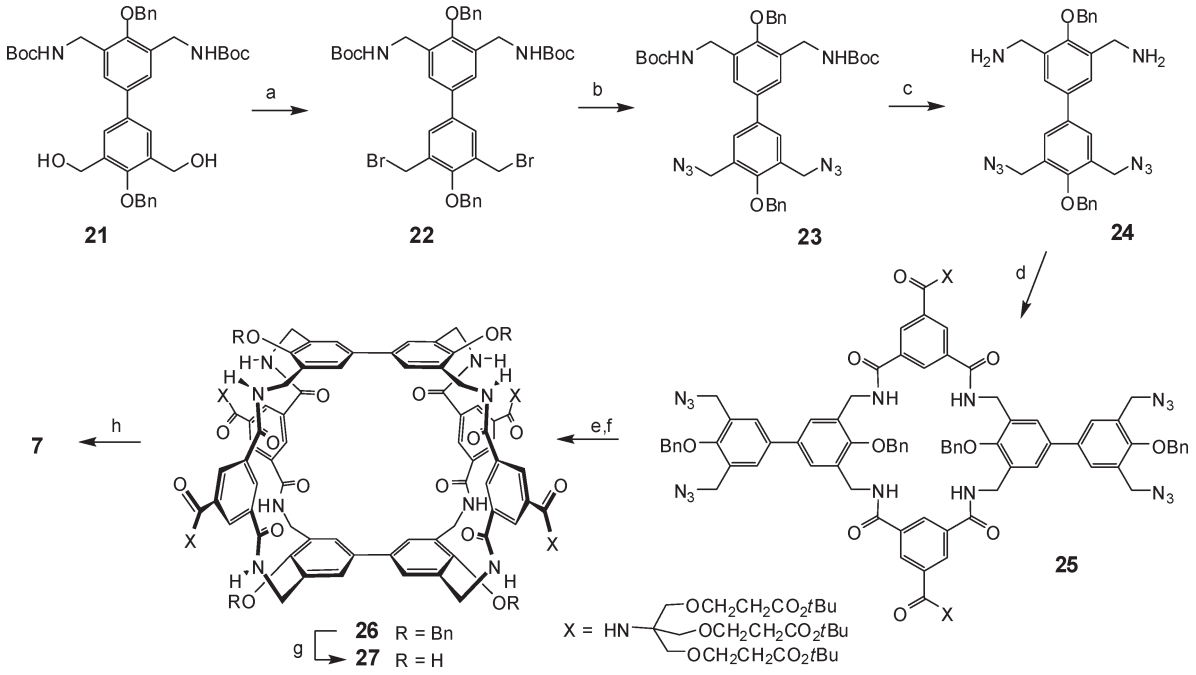
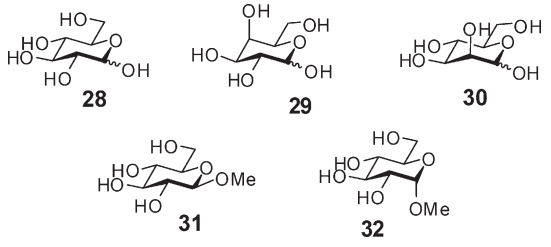
Scheme 2[a]



[a] Reagents and conditions: (a) CBr_4, PPh_3, DCM, 73%; (b) NaN_3, DMF, 60 °C, 100%; (c) TFA, DCM, 0 °C; (d) **18**, DIPEA, THF, 29%; (e) PPh_3, THF, 60 °C then H_2O, 60 °C, 94%; (f) **18**, DIPEA, THF, 28%; (g) Pd/C, H_2, THF/methanol/NH_3, 91%; (h) TFA, DCM, 0 °C, 96%.

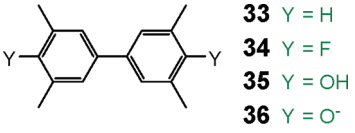
The hydrogenolysis of the benzyl groups proceeded cleanly in 91% yield. Finally, cleavage of the side-chain *tert*-butyl esters gave **7**. The deprotonation of the phenolic hydroxyls in **7** was investigated by UV spectroscopy. Between pH = 6.5 and 8.5 the UV spectrum remained constant, implying full protonation. As the pH was raised further the spectrum changed, stabilizing at pH = 11.5.[12] We therefore presumed that the hydroxyl groups would be completely deprotonated at this and higher pH values. This is consistent with the behavior of 4,4′-biphenol where the pK_a of both hydroxyl groups is around 10.[13]

Measurement and Analysis of Binding Properties. The binding constants of **6**−**8** to glucose **28**, galactose **29**, mannose **30**, methyl β-D-glucoside **31**, and methyl α-D-glucoside **32** were measured by ^{1}H NMR titrations in D_2O.[12] For the titrations on **7** the pH was adjusted to 7.5 by addition of sodium hydroxide. For



the titrations on **8**, further sodium hydroxide was added to bring the pH to 12. As previously observed for **2** and **5**, addition of the carbohydrates caused movements of signals due to receptor aromatic protons. The motions were consistent with 1:1 binding and were analyzed by nonlinear curve-fitting to obtain association constants K_a. In the case of **6** and **7**, the signals due to the internally directed isophthalamide protons moved the greatest amounts and were used to determine K_a values. In the case of **8** the biphenyl protons also moved substantially and could be readily analyzed. Where two signals were analyzed, an average value was calculated unless one seemed obviously less accurate. A typical set of spectra with the corresponding analysis plot are shown in Figure 4. In the great majority of cases, errors were estimated at ±5% or better. For favorable substrates such as glucose and methyl β-D-glucoside, the limiting spectra were remarkably similar to each other and to those previously observed for **2** and **5**. We therefore infer that the binding geometries for all these tricyclic receptors are essentially similar, involving the carbohydrate entering the cavity as depicted in Figure 3.[14]

To assist in the interpretation of the binding data, the surface charge distributions were calculated for tetramethyl biphenyls **33**−**36**, models for the biaryl units in **2** and **6**−**8** respectively.



Surface charge is expected to correlate quite well with the relative strengths of CH−π interactions.[15] The results for **33**−**35** are depicted in Figure 5; the surface of **36** is far more negative than the others and cannot be usefully represented on the same color scale. Some aspects of the charge distributions may seem unexpected. Although hydroxyl groups are normally considered electron-donating, the π-surface of dihydroxybiphenyl **35** is very similar to that of the parent **33**. While hydroxyl groups promote electrophilic attack through π-donation, their effect on the ground state charge distribution is almost neutral (due to opposing σ and π effects). Nonetheless, the series covers a good range of electron densities. Difluoride **34** is significantly

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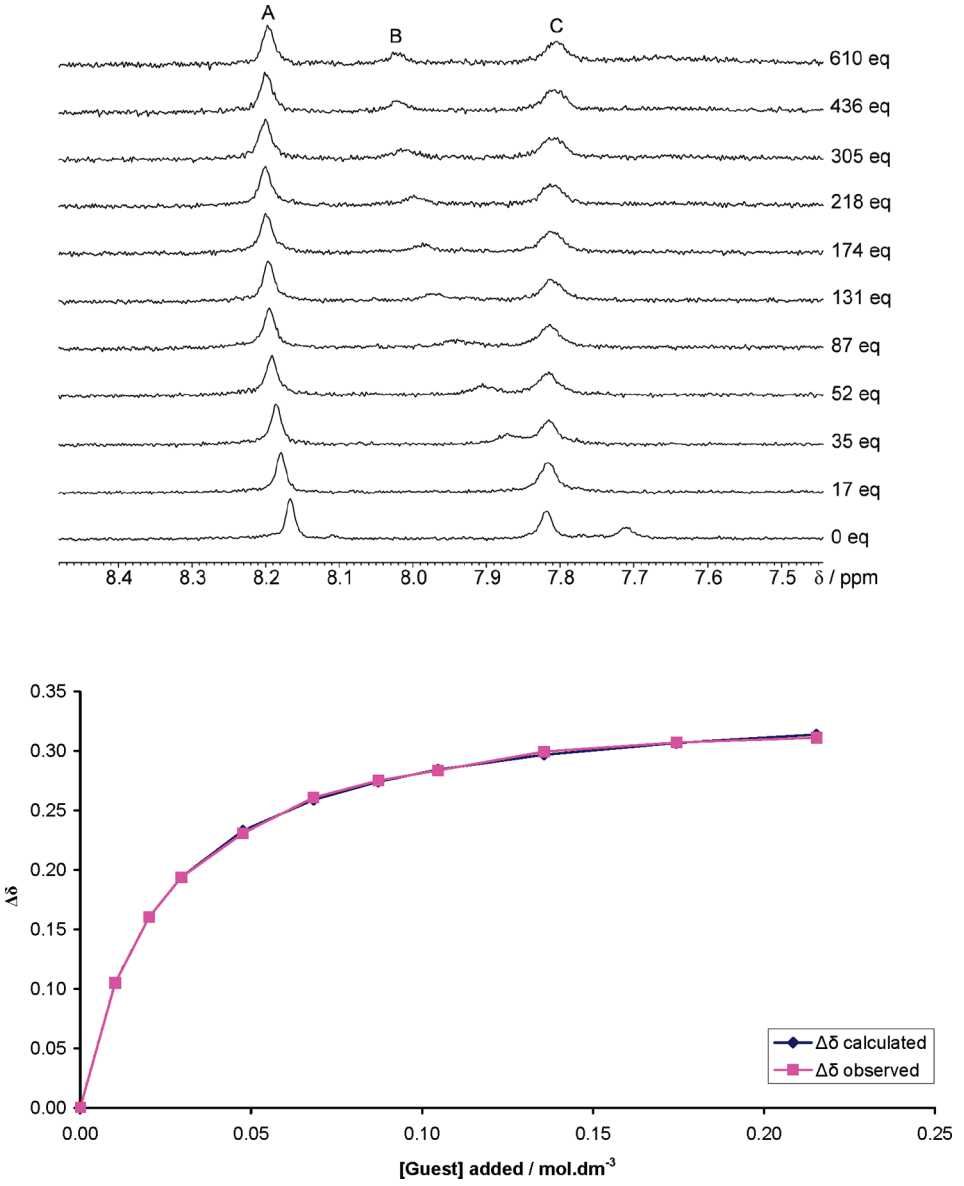

Figure 4. (Top) Partial ^{1}H NMR spectra from the addition of **28** to **7** in D_2O (pH = 7.5). Peak B is the internally directed isophthalamide proton; for other assignments see Figure S1. (Bottom) Binding analysis based on the movements of peak B, assuming 1:1 complex formation. Experimental and calculated values are shown. K_a = 43 M^{-1}. Limiting $\Delta\delta$ = 0.348 ppm.

more electron-poor than **33** or **35**, while **36** is much more electron-rich. The strengths of CH$-\pi$ interactions involving **2** and **6**$-$**8** should therefore increase in the order **6** < **2** ≈ **7** < **8**.

The binding constants measured for the receptors to carbohydrates **28**$-$**32** are listed in Table 1. All showed the expected selectivity for all-equatorial substrates, binding glucose **28**[16] in preference to galactose **29** or mannose **30**, and methyl β-D-glucoside **31** more strongly than α anomer **32**. Binding strengths varied significantly, by factors of 5 and 6 for substrates **28** and **31** respectively. The sequence, however, was not as expected on the basis of CH$-\pi$ effects alone. Thus, all the new receptors, including tetrafluoride **6**, proved stronger than prototype **2**. Although unanticipated this result is potentially useful. Indeed, tetraol **7** is among the most powerful monosaccharide receptors we have studied. On the other hand the exceptional affinities expected for **8** did not materialize. Overall the order of binding strengths for preferred substrates **28** and **31** may be summarized as **2** < **6** ≈ **8** < **7** (i.e., H < F ≈ O^- < OH).

The data can be rationalized by assuming that the CH$-\pi$ effect is operative and can be controlled by varying the electron density, but that differences are small and easily overwhelmed by other factors. Thus F and OH are sterically similar but electronically different, providing arguably the best test for the role of π-electron density. As expected, tetrafluoride **6** is significantly weaker than tetraol **7**. The difference is modest (a factor of ∼2), but this is not too surprising. Modeling of the complex between β-glucose and **7** suggests that only two CH groups may be well-positioned to contact the aromatic π-surfaces (the remainder are closer to aromatic hydrogens; see Figure 6). Calculations imply that the CH$-\pi$ interactions to Ar$-$O units should be ∼1.5 kJmol^{-1} stronger than those to Ar$-$F units.[5a]

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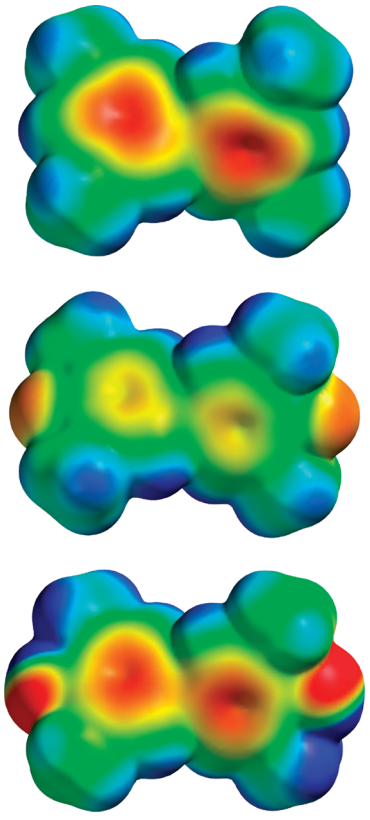

Figure 5. Calculated surface potentials for **33** (top), **34** (middle), and **35** (bottom). Computations were performed using Spartan, Hartree−Fock method, 6-31G* basis set. The colors represent the energy required to bring a point positive charge to the surface of each molecule and are plotted on the same scale: red ≤ −110 kJ mol^{-1} (i.e., negatively charged surface), blue ≥ 68 kJ mol^{-1} (i.e., positively charged surface).

Table 1. Binding Constants of Monosaccharides 28−32 to Receptors 2[a] and 6−8, As Measured by ^{1}H NMR Titration in D_2O

	K_a/M^{-1}			
Carbohydrate	−H **2**[a]	−F **6**	−OH[b] **7**	−O$^-$ [c] **8**
D-Glucose **28**	9	20	43	17
D-Galactose **29**	2	9	8	4
D-Mannose **30**	v.s.[d]	2	3	5
Methyl β-D-glucoside **31**	27	66	155	90
Methyl α-D-glucoside **32**	7	16	26	15

[a] Data from ref 8a. [b] pH = 7.5. [c] pH = 12. [d] Very small. Minor signal movements, almost linear with substrate concentration.

Two such interactions would therefore result in a difference of 3 kJ mol^{-1} in the gas phase, equivalent to an ∼3-fold ratio of binding constants, quite similar to that observed.

On the other hand both **2** and **8** are weaker than expected on the basis of CH−π effects, and this may relate to the ease of hydration of their cavities. In the case of **8**, the basic phenoxide centers should act as strong H-bond acceptors and could nucleate networks of H-bonded water molecules in the vicinity of the cavity. Indeed, modeling shows that $(H_2O)_2$ bridges can form across either end of the cavity and that these can be linked by a chain of three water molecules running through the binding site.[12] Displacement of these solvent molecules could be relatively difficult and might thus contribute little to (or even inhibit) binding.[17] In the case of **2** the biphenyl units are slightly smaller than those of **6** and **7** and may be less hydrophobic. Certainly it is expected that hydrophobicity should increase between **2** and **6**, as substituting H with F is known to have this effect.[18]

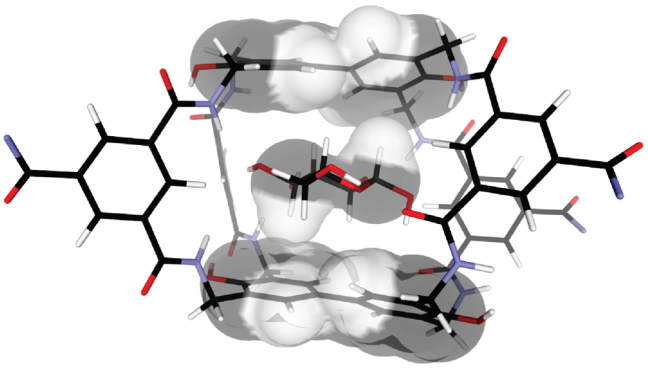

Figure 6. Molecular modeling of β-D-glucose in the binding site of **7**. This structure is the global minimum from a Monte Carlo Molecular Mechanics calculation in which random variations were made to the carbohydrate's position and conformation.[12] The conformation of the receptor was taken from the NOE study of the complex between **2** and MeO-β-D-GlcNAc.[8d] Transparent surfaces highlight the two unambiguous CH−π interactions within the complex.

CONCLUSIONS

Contact between aromatic rings and saccharide CH groups clearly plays an important role in carbohydrate recognition. With this research we provide further evidence that this CH−π effect can be moderated by changing the electron density of the π-system. However, the work also highlights the fact that the changes in binding energy tend to be small. Enhancing the π-electron density can be a useful strategy in optimizing synthetic lectins, but all consequences of a design change, especially effects on binding site hydration, must be taken into account.

EXPERIMENTAL SECTION

General Methods. Proton and carbon NMR spectra were recorded at 400 MHz or at 500 MHz. Chemical shifts are reported in ppm downfield from tetramethylsilane, for proton and carbon. Solvents for synthesis were dried by passing through a modified Grubbs system.[19] Routine monitoring of reactions was performed using precoated silica gel TLC plates. Spots were visualized by UV light, ethanolic solution of phosphomolybdic acid, potassium permanganate, or ninhydrin. Flash column chromatography[20] was performed using silica gel (particle size 35−70 μm) as the absorbent.

5-Bromo-1,3-bis-(bromomethyl)-2-fluorobenzene 10. 5-Bromo-2-fluoro-*m*-xylene (5.0 g, 24.6 mmol) was dissolved in methyl acetate (25 mL), and *N*-bromosuccinimide (9.2 g, 52 mmol) was added under nitrogen. The reaction mixture was then refluxed for 3.5 h using a 100 W lamp, causing the mixture to change from a colorless suspension to a deep red solution. The solvent was evaporated, and the residue obtained was washed with boiling hexane. The solvent was evaporated, and the product was recrystallized from hexane to yield **10** as white needle-shaped crystals (5.43 g, 15.2 mmol, 62%). R_f = 0.27 (hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.45 (d, ^4J(H,F) = 1.2 Hz, 4H), 7.50

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(d, ^4J(H,F) = 6.3 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.0 (d, ^3J(C,F) = 4.6 Hz), 116.6 (d, ^4J(C,F) = 3.8 Hz), 127.7 (d, ^2J(C,F) = 16.2 Hz), 134.2 (d, ^3J(C,F) = 3.8 Hz), 157.5 (d, ^1J(C,F) = 254 Hz); ^{19}F NMR (283 MHz, $CDCl_3$) δ −122.72 (t, ^4J(H,F) = 6.4 Hz, 1F); HRMS (EI): m/z calculated for $C_8H_6Br_3F^+$ $[M]^+$, 357.8004; found, 357.8016.

1-Bromo-4-fluoro-3,5-bis-(phthalimido-methyl)benzene 11. Dibromide **10** (1.67 g, 4.6 mmol) and potassium phthalimide (1.90 g, 10.7 mmol) were suspended in DMF (20 mL) and stirred for 3 h. H_2O (100 mL) was added, and the reaction mixture was then filtered. The solid obtained was dissolved in chloroform (100 mL) and washed with water (3 × 100 mL), dried over Na_2CO_3, and evaporated to yield the bis-phthalimide **11** as a white solid (2.30 g, 4.67 mmol, 100%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.90 (s, 4H), 7.35 (d, ^4J(H,F) = 6.3 Hz, 2H), 7.76 (m, 4H), 7.89 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 34.8, 116.7 (d, ^4J(C,F) = 3.8 Hz), 123.6, 125.6 (d, ^2J(C,F) = 16.2 Hz), 131.9, 132.1 (d, ^3J(C,F) = 3.9 Hz), 134.2, 157.4 (d, ^1J(C,F) = 251 Hz), 167.6; ^{19}F NMR (283 MHz, $CDCl_3$) δ −123.30 (t, ^4J(H,F) = 6.0 Hz); HRMS (ESI): m/z calculated for $C_{24}H_{14}BrFN_2O_4Na^+$ $[M + Na]^+$, 515.0013; found, 515.0019.

1,3-Bis-(amino-methyl)-5-bromo-2-fluorobenzene 12. Bis-phthalimide **11** (2.30 g, 4.67 mmol) was suspended in chloroform/methanol (1:4, 50 mL) under nitrogen. Hydrazine (1.17 mL, 23.3 mmol, 5 equiv) was added, and the reaction mixture was heated at reflux overnight. The reaction mixture was allowed to cool to room temperature, and water (20 mL) was added. The organic solvents were evaporated, and concentrated HCl (20 mL) was added. The aqueous solution was stirred for 45 min forming a white precipitate which was removed by filtration. The pH of the solution obtained was adjusted to pH 14 by the addition of NaOH pellets and was extracted with DCM (3 × 50 mL). The organic phases were combined, dried over Na_2CO_3, and evaporated to yield **12** as a white solid (0.85 g, 3.66 mmol, 78%). R_f = 0.69 (10% MeOH sat. NH_3/DCM); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.88 (s, 4H), 7.38 (d, ^4J(H,F) = 6.1 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.1 (d, ^3J(C,F) = 4.8 Hz), 116.7 (d, ^4J(C,F) = 3.8 Hz), 130.4 (d, ^3J(C,F) = 4.6 Hz), 132.3 (d, ^2J(C,F) = 16.9 Hz), 157.9 (d, ^1J(C,F) = 244 Hz); ^{19}F NMR (283 MHz, $CDCl_3$) δ −128.8 (t, ^4J(H,F) = 6.1 Hz); HRMS (EI): m/z calculated for $C_8H_{10}BrFN_2^+$ $[M]^+$, 232.0011; found, 232.0002.

1,3-Bis-(benzyloxycarbonylamino-methyl)-5-bromo-2-fluorobenzene 13. Diamine **12** (695 mg, 2.98 mmol) and DIPEA (1.17 mL, 6.70 mmol) were dissolved in THF (50 mL) under nitrogen and cooled to 0 °C, after which benzyl chloroformate (0.95 mL, 6.70 mmol) was added. The reaction mixture was allowed to warm to room temperature and stirred overnight before the solvent was evaporated, and the oil obtained was dissolved in EtOAc (50 mL). This solution was washed with saturated aqueous NH_4Cl (50 mL), water (50 mL), saturated aqueous $NaHCO_3$ (50 mL), and brine (50 mL). The organic phase was dried over Na_2CO_3 and evaporated. The white solid obtained was then recrystallized from hexane/EtOAc (1:1, 30 mL) to yield **13** as white needle-shaped crystals (0.77 g, 1.5 mmol, 52%). R_f = 0.43 (3:2, hexane/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.38 (d, ^3J(H,H) = 6.1 Hz, 4HN), 5.13 (s, 4H), 5.22 (bs, 2H), 7.30−7.42 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 38.6, 67.1, 116.7, 127.8 (d, ^2J(C,F) = 16.1 Hz), 128.1, 128.2, 128.5, 131.6 (d, ^3J(C,F) = 3.8 Hz), 136.2, 156.3, 157.8 (d, ^1J(C,F) = 246 Hz); ^{19}F NMR (283 MHz, $CDCl_3$) δ −126. 93 (t, ^4J(H,F) = 6.0 Hz); HRMS (ESI): m/z calculated for $C_{24}H_{22}BrFN_2O_4Na^+$ $[M + Na]^+$, 523.0639; found, 523.0648.

1,3-Bis-(*tert*-butoxycarbonylamino-methyl)-5-bromo-2-fluorobenzene 14. Diamine **12** (538 mg, 2.31 mmol) and DIPEA (0.90 mL, 5.16 mmol) were dissolved in THF (50 mL) under nitrogen and cooled to 0 °C, after which di-*tert*-butyl carbonate (1.13 g, 5.16 mmol) was added. The reaction mixture was allowed to warm to room temperature and stirred overnight before the solvent was evaporated, and the oil obtained was dissolved in EtOAc (50 mL). This solution was washed with saturated aqueous NH_4Cl (50 mL), water (50 mL), saturated aqueous $NaHCO_3$ (50 mL), and brine (50 mL). The organic phase was dried over Na_2CO_3 and evaporated. The white solid obtained was then recrystallized from hexane/EtOAc (24:1, 50 mL) to yield **14** as white needle-shaped crystals (876 mg, 2.02 mmol, 88%). R_f = 0.57 (3:2, hexane/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (s, 18H), 4.33 (d, ^3J(H,H) = 5.8 Hz, 4H), 4.90 (bs, 2H), 7.37 (d, ^4J(H,F) = 6.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.3, 38.2, 80.0, 116.7 (d, ^4J(C,F) = 3.0 Hz), 128.2 (d, ^2J(C,F) = 16.2 Hz), 131.2 (d, ^3J(C,F) = 3.9 Hz), 155.7, 157.8 (d, ^1J(C,F) = 245 Hz); ^{19}F NMR (283 MHz, $CDCl_3$) δ −127.20 (s); HRMS (ESI): m/z calculated for $C_{18}H_{26}BrFN_2O_4Na^+$ $[M + Na]^+$, 455.0952; found, 455.0949.

1,3-Bis-(*tert*-butoxycarbonylamino-methyl)-2-fluoro-5-(4′,4′,5′,5′-tetramethyl-1,3,2-dioxaborolan-2-yl)-benzene 15. Bis-carbamate **14** (734 mg, 1.69 mmol), bis-(pinacolato) diboron (450 mg, 1.78 mmol), and KOAc (580 mg, 5.93 mmol) were dried under vacuum for 25 min before being dissolved in DMF (30 mL). Pd(dppf)Cl_2 (41 mg, 0.05 mmol) was added under nitrogen. The reaction mixture was heated at 60 °C overnight before the solvent was evaporated. The residue obtained was dissolved in water (100 mL) and extracted with ethyl acetate (3 × 70 mL), dried over sodium sulfate, filtered, and evaporated to yield a black oil. The oil was purified by flash chromatography (eluent DCM to DCM/EtOAc 17:3) producing the arylboronate **15** as a white solid (300 mg, 0.62 mmol, 37%). R_f = 0.59 (3:2, hexane/ethyl acetate); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33 (s, 12H), 1.46 (s, 18H), 4.37 (d, ^3J(H,H) = 5.1 Hz, 4H), 4.85 (bs, 2H), 7.69 (d, ^4J(H,F) = 7.3 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.8, 28.4, 38.7, 79.6, 84.0, 125.2 (d, ^2J(C,F) = 14.6 Hz), 135.7, 155.7, 161.4 (d, ^1J(C,F) = 253 Hz); ^{19}F NMR (283 MHz, $CDCl_3$) δ −120.33 (s); HRMS (ESI): m/z calculated for $C_{24}H_{38}BFN_2O_6Na^+$ $[M + Na]^+$, 503.2699; found, 503.2702.

3,5-Bis-(benzyloxycarbonylamino-methyl)-3′,5′-bis-(*tert*-butoxycarbonylamino-methyl)-4,4′-difluorobiphenyl 16. Bis-carbamate **13** (303 mg, 0.60 mmol) and arylboronate **15** (290 mg, 0.60 mmol) were dissolved in DMSO (20 mL) and degassed, after which $PdCl_2$(dppf) (15 mg, 0.02 mmol) and Na_2CO_3 aq. (2 M, 0.60 mL, 1.20 mmol) were added and the reaction mixture was heated at 60 °C overnight. The solvent was then removed, and residue obtained was dissolved in EtOAc (25 mL) and washed with H_2O (2 × 25 mL) and brine (25 mL), dried over sodium sulfate, filtered, and evaporated to yield a black oil. The oil was purified by flash chromatography (eluent DCM/EtOAc 9:1 to 7:3) producing **16** as a white solid (403 mg, 0.52 mmol, 86%). R_f = 0.31 (3:2, hexane/ethyl acetate); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (s, 18H), 4.35 (d, ^3J(H,H) = 5.6 Hz, 4HNHBoc), 4.42 (d, ^3J(H,H) = 5.9 Hz, 4HNHCbz), 5.12 (s, 4HPh), 5.34 (bs, 2H CH_2NHBoc), 5.68 (bs, 2H CH_2NHCbz), 7.15−7.38 (m, 14H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.4, 38.6, 39.0, 67.0, 79.8, 125.9 (d, ^2J(C,F) = 15.4 Hz), 126.3 (d, ^2J(C,F) = 14.7 Hz), 127.3, 127.5, 128.1, 128.2, 128.4, 135.7, 136.0, 136.2, 155.8, 156.4, 158.6 (d, ^1J(C,F) = 248.3 Hz); ^{19}F NMR (283 MHz, $CDCl_3$) δ 131.49 (m); HRMS (ESI): m/z calculated for $C_{42}H_{48}F_2N_4O_8Na^+$ $[M + Na]^+$, 797.3332; found, 797.3352.

3,5-Bis-(amino-methyl)-3′,5′-bis-(benzyloxycarbonylamino-methyl) 4,4′-difluorobiphenyl 17. Biaryl **16** (336 mg, 0.43 mmol) was dissolved in dry DCM (40 mL) under nitrogen and cooled to 0 °C, and TFA (12 mL) was added slowly. This solution was stirred for 45 min after which the solvent was evaporated. The residue obtained was dissolved in DCM (30 mL) and washed with aqueous sodium hydroxide (20 mL, 2 M). The aqueous solution was extracted with DCM (3 × 30 mL), and the organic fractions were combined, dried over Na_2SO_4, and reduced under reduced pressure producing the diamine **17** as a white solid (248 mg, 0.43 mmol, 100%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.52 (bs, 4H), 3.93 (s, 4HNH_2), 4.46 (d, ^3J(H,H) = 5.9 Hz, 4HNHCbz), 5.11 (s, 4H), 5.39 (bs, 2H), 7.20−7.37 (m, 12H), 7.44 (d, ^4J(H,F) = 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 39.2, 40.6, 66.9, 126.0 (d, ^2J(C,F) = 15.3 Hz), 130.6 (d, ^2J(C,F) = 16.1 Hz), 126.5 (d, ^3J(C,F) = 4.6 Hz), 127.9 (d, ^2J(C,F) = 2.3 Hz), 128.1, 128.1, 128.5, 135.9 (d, ^4J(C,F) = 3.1 Hz),

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136.3, 136.7 (d, ^4J(C,F) = 3.1 Hz), 156.3, 158.7 (d, ^1J(C,F) = 245.2 Hz), 158.8 (d, ^1J(C,F) = 246.7 Hz); ^{19}F NMR (283 MHz, $CDCl_3$) δ −127.40 (s, 1F), −128.94 (s, 1F); HRMS (ESI): *m*/*z* calculated for $C_{32}H_{33}F_2N_4O_4^+$ $[M + H]^+$, 575.2464; found, 575.2484.

Tetra(benzyloxycarbonyl) Protected Macrocycle 19. Diamine **17** (246 mg, 0.43 mmol) and DIPEA (450 μL, 2.58 mmol, 6 equiv) were dissolved in dry THF (500 mL) under nitrogen, and a solution of bis-pentafluorophenyl ester **18**[7b] (441 mg, 0.43 mmol) in dry THF (10 mL) was added dropwise over a 50 h period. The reaction mixture was stirred for a further 24 h before the solvent was evaporated. The oil obtained was dissolved in DCM (30 mL) and washed with saturated aqueous NH_4Cl (25 mL), H_2O (25 mL), saturated aqueous $NaHCO_3$ (25 mL), and brine (25 mL) before being dried over Na_2SO_4 and evaporated. The residue was purified by flash chromatography (eluent DCM to DCM/EtOAc 1:1) resulting in a white solid which was further purified by preparative high performance liquid chromatography (Hichrom Kromasil column, 150 mm × 21.2 mm, 5 μm, eluent: methanol/water 80:20 to 95:5 in 14 min, then to 100:0 after 50 min, flow rate 15 mL·min^{-1}). The macrocycle **19** (89 mg, 0.04 mmol, 17%) was isolated as a white solid. Retention time = 19 min; ^{1}H NMR (400 MHz, 92:8 $CDCl_3/CD_3OD$) δ 1.29 (s, 54H), 2.37 (t, ^3J(H,H) = 6.2 Hz, 12H), 3.59 (t, ^3J(H,H) = 6.2 Hz, 12H), 3.73 (s, 12H), 4.33 (d, ^3J(H,H) = 5.4 Hz, 8H), 4.54 (s, ^3J(H,H) = 4.7 Hz, 8H), 4.98 (s, 8H), 6.16 (bs, 4H), 6.84 (bs, 2H), 7.10−7.25 (m, 20H, Cbz-ArCH), 7.33 (d, ^4J(H,F) = 6.3 Hz, 4H), 7.45 (d, ^4J(H,F) = 5.9 Hz, 4H), 8.10 (s, 2H), 8.24 (bs, 4H), 8.26 (s, 4H); ^{13}C NMR (100 MHz, 92:8 $CDCl_3/CD_3OD$) δ 27.7, 36.1, 38.2, 38.7, 60.4, 66.6, 68.7 ($C(CH_2O)_3$), 80.6, 125.4 (d, ^2J(C,F) = 12.6 Hz), 125.9 (d, ^2J(C,F) = 12.7 Hz), 127.5, 127.8, 127.9, 128.3, 129.0, 129.6, 134.5, 135.7, 136.1, 136.2, 156.6, 159.2 (d, ^1J(C,F) = 249.1 Hz), 166.3, 166.6, 171.2; ^{19}F NMR (283 MHz, 92:8 $CDCl_3/CD_3OD$) δ −126.48 (s, 1F), −126.86 (s, 1F); HRMS (ESI): *m*/*z* calculated for $C_{132}H_{158}F_4N_{10}O_{32}Na^+$ $[M + Na]^+$, 2494.0872; found, 2494.0907.

***tert*-Butyl Protected Tetrafluoro Receptor 20.** Pd on C (100 mg) was activated by heating at 200 °C under vacuum for 4 h after which a solution of macrocycle **19** (86 mg, 0.04 mmol) in THF and MeOH saturated with NH_3 (1:1, 100 mL) was added. The flask was evacuated and filled with hydrogen (1 atm), and the reaction mixture was stirred for 1.5 h. The reaction mixture was then filtered over Celite and washed with ethyl acetate before the filtrate was evaporated to yield the tetraamine as a white solid (64 mg) which was then dissolved in dry THF (150 mL) containing DIPEA (23 μL, 0.13 mmol). A solution of bis-pentafluorophenyl ester **18**[7b] (48 mg, 0.05 mmol) in dry THF (10 mL) was added dropwise over 50 h to the tetraamine and DIPEA solution under a nitrogen environment at room temperature. After the addition the reaction mixture was allowed to stir for a further 24 h before the solvent was evaporated. The oil obtained was dissolved in DCM (30 mL) and washed with saturated aqueous NH_4Cl (25 mL), H_2O (25 mL), saturated aqueous $NaHCO_3$ (25 mL), and brine (25 mL) before being dried over Na_2SO_4 and evaporated. This was purified by preparative high performance liquid chromatography (Hichrom Kromasil column, 150 mm × 21.2 mm, 5 μm, eluent: methanol/water 80:20 to 95:5 in 14 min, then to 100:0 after 50 min, flow rate 15 mL·min^{-1}). The pure tricycle **20** (18 mg, 5.5 μmol, 25%) was isolated as a white solid. Retention time = 21 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.40 (s, 108H), 2.46 (t, ^3J(H,H) = 6.2 Hz, 24H), 3.69 (t, ^3J(H,H) = 6.2 Hz, 24H), 3.83 (s, 24H), 4.46 (dd, ^2J(H,H) = 13.8 Hz, ^3J(H,H) = 4.8 Hz, 8H), 4.74 (dd, ^2J(H,H) = 13.8 Hz, ^3J(H,H) = 4.8 Hz, 8H), 6.75 (s, 4H), 7.22 (bs, 8H), 7.78 (d, ^4J(H,F) = 6.3 Hz, 8H), 8.06 (s, 4H), 8.30 (s, 8H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.0, 36.4, 39.5, 60.4, 67.1, 69.0, 80.6, 125.5 (d, ^2J(C,F) = 15.5 Hz), 128.7, 128.9, 129.0 (d, ^3J(C,F) = 3.1 Hz), 133.5 (d, ^4J(C,F) = 2.5 Hz), 135.0, 136.5, 160.0 (d, ^1J(C,F) = 251.1 Hz), 166.3, 166.5, 171.2; ^{19}F NMR (283 MHz, $CDCl_3$) δ −124.77 (s); HRMS (ESI): *m*/*z* calcd for $C_{168}H_{228}F_4N_{12}O_{48}Na_2^{2+}$ $[M + 2Na]^+$, 1651.7745; found, 1651.7794.

Tetrafluoro Receptor 6. Tricycle **20** (18 mg, 5.52 μmol) was dissolved in DCM (10 mL) under a nitrogen environment and cooled in ice water. TFA (2 mL) was added dropwise, and the reaction mixture was stirred for 5 h at which point the solvent was removed in vacuo. The white solid obtained was dissolved in methanol/water (6:4, 10 mL), and the pH of the solution was adjusted to pH 7 with aqueous sodium hydroxide (0.5 M) which was monitored with a pH meter. The solvent was removed to yield the salt **6** as a white solid (15 mg, 5.27 μmol, 95%). ^{1}H NMR (500 MHz, D_2O) δ = 2.45 (t, ^3J(H,H) = 6.4 Hz, 24H), 3.75 (t, ^3J(H,H) = 6.4 Hz, 24H), 3.85 (s, 24H), 4.34 (d, ^2J(H,H) = 15.2 Hz, 8H), 4.85 (d, ^2J(H,H) = 15.2 Hz, 8H), 7.77 (d, ^4J(H,F) = 6.1 Hz, 8H), 7.84 (s, 4H), 8.22 (s, 8H); HRMS (ESI): *m*/*z* calcd for $C_{120}H_{132}F_4N_{12}O_{48}Na_2^{2+}$ $[M + 2Na]^{2+}$, 1315.3989; found, 1315.4028.

3,5-Bis-[(*tert*-butyloxycarbonyl)aminomethyl]-3′,5′-bis-(bromomethyl)-4,4′-bis-(benzyloxy)biphenyl 22. Diol **21** (400 mg, 0.49 mmol) and PPh_3 (388 mg, 1.48 mmol, 3 equiv) were dissolved in dry THF (40 mL) under nitrogen and cooled to 0 °C, after which CBr_4 (491 mL, 1.48 mmol, 3 equiv) was added. The reaction was warmed to room temperature after 10 min, and the reaction mixture was then left to stir overnight, after which the solvent was evaporated. The residue obtained was purified by flash chromatography (eluent DCM to DCM/ethyl acetate 9:1) producing **22** as a white solid (219 mg, 0.27 mmol, 79%). R_f = 0.68 (3:2, hexane/ethyl acetate); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.48 (s, 18H), 4.42 (d, ^3J(H,H) = 5.6 Hz, 4H), 4.59 (s, 4H), 4.91 (bs, 2H), 4.92 (s, 2H), 5.25 (s, 2H), 7.38−7.49 (m, 8H), 7.45 (s, 2H), 7.57−7.60 (m, 2H), 7.58 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.7, 28.4, 39.9, 76.3, 76.3, 79.7, 127.1, 128.1, 128.3, 128.5, 128.6, 128.7, 128.8, 130.7, 132.6, 133.0, 136.0, 136.4, 136.6, 137.5, 154.5, 154.5, 155.9; HRMS (ESI): *m*/*z* calculated for $C_{40}H_{46}Br_2N_2O_6Na^+$ $[M + Na]^+$, 831.1615; found, 831.1599.

3,5-Bis-[(*tert*-Butyloxycarbonyl)aminomethyl]-3′,5′-bis-(azidomethyl)-4,4′-bis-(benzyloxy)biphenyl 23. Dibromide **22** (219 mg, 0.26 mmol) was dissolved in DMF (15 mL). NaN_3 (85 mg, 1.30 mmol, 5 equiv) was added. The reaction mixture was heated at 60 °C under nitrogen for 30 h before the solvent was removed by evaporation. The solid obtained was dissolved in ethyl acetate (30 mL) and washed with water (3 × 30 mL) and brine (30 mL). The organic phase was dried over Na_2SO_4 and evaporated under reduced pressure to yield **23** as a white solid (199 mg, 0.26 mmol, 100%). R_f = 0.36 (3:2, hexane/ethyl acetate); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (s, 18H), 4.43 (m, 8H), 4.88 (bs, 2H), 4.92, 4.99, 7.36−7.49 (m, 12H), 7.53 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.4, 39.9, 49.8, 76.3, 77.2, 79.6, 127.2, 128.0, 128.3, 128.5, 128.6, 128.7, 129.1, 130.1, 132.9, 136.3, 136.4, 136.5, 137.2, 154.5, 154.8, 155.9; HRMS (ESI): *m*/*z* calculated for $C_{40}H_{46}N_8O_6Na^+$ $[M + Na]^+$, 757.3433; found, 757.3430.

3,5-Bis-(aminomethyl)-3′,5′-bis-(azidomethyl)-4,4′-bis-(benzyloxy)biphenyl 24. Diazide **23** (199 mg, 0.26 mmol) was dissolved in dry DCM (10 mL) under nitrogen and cooled to 0 °C. TFA (2 mL) was added slowly. This solution was stirred for 2 h after which the solvent was evaporated, and the residue obtained was dissolved in ethyl acetate (30 mL) and washed with a sodium hydroxide solution (20 mL, 2 M). The aqueous solution was extracted with DCM (3 × 30 mL), and the organic fractions were combined, dried over Na_2SO_4, and evaporated under reduced pressure. The oil obtained was purified by flash chromatography (eluent DCM to DCM/NH_3-saturated methanol, 49:1) producing **24** as a white solid (139 mg, 0.26 mmol, 100%). R_f = 0.81 (9:1, DCM/NH_3-saturated methanol); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.54 (bs, 4H), 3.99 (s, 4H_2), 4.46 (s, 4H), 4.97, 5.01, 7.36−7.51 (m, 10H), 7.50 (s, 2H), 7.59 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 42.0, 49.8, 76.3, 77.4, 126.4, 127.9, 128.0, 128.4, 128.6, 128.7, 128.8, 129.2, 130.0, 136.3, 136.4, 137.0, 137.3, 137.7, 154.6, 154.7; HRMS (ESI): *m*/*z* calculated for $C_{30}H_{31}N_8O_2^+$ $[M + H]^+$, 535.2564; found, 535.2584.

Tetra(azido) Tetrabenzyloxy Macrocycle 25. Diamine **24** (139 mg, 0.26 mmol) and DIPEA (283 μL, 1.63 mmol, 6 equiv) were dissolved in dry THF (300 mL) under nitrogen. A solution of

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bis-pentafluorophenyl ester **18**[7b] (279 mg, 0.27 mmol) in dry THF (50 mL) was added dropwise over a 30 h period. The reaction mixture was stirred for a further 10 h before the solvent was evaporated under reduced pressure. The oil obtained was purified to by flash chromatography (eluent DCM to ethyl acetate) resulting in a white solid which was further purified by preparative high performance liquid chromatography (Hichrom Kromasil column, 150 mm × 21.2 mm, 5 μm, eluent: methanol/water 80:20 to 95:5 in 14 min, then to 100:0 in a further 36 min, flow rate 15 mL·min^{-1}). The macrocycle **25** was isolated as a white solid (90 mg, 0.04 mmol, 29%). Retention time = 27 min; ^{1}H NMR (400 MHz, 92:8 $CDCl_3/CD_3OD$) δ 1.39 (s, 54H), 2.48 (t, ^3J(H,H) = 6.3 Hz, 12H), 3.70 (t, ^3J(H,H) = 6.3 Hz, 12H), 3.84 (s, 12H), 4.38 (s, 8H), 4.62 (s, 8H), 4.94 (s, 4H), 4.99 (s, 4H), 7.28−7.46 (m, 24H), 7.50 (s, 4H), 8.12 (t, ^4J(H,H) = 1.7 Hz, 2H), 8.40 (d, ^4J(H,H) = 1.7 Hz, 4H); ^{13}C NMR (100 MHz, 92:8 $CDCl_3/CD_3OD$) δ 27.8, 36.1, 39.1, 49.5, 60.3, 66.9, 68.8, 77.1, 77.2, 80.6, 127.5, 128.3, 128.9, 129.2, 130.0, 132.2, 134.4, 127.8, 128.1, 128.5, 128.6, 136.1, 136.2, 136.2, 136.5, 136.6, 154.8.2, 154.9, 166.0, 166.6, 171.1; HRMS (ESI): m/z calculated for $C_{128}H_{154}N_{18}O_{28}Na_2^{2+}$ $[M + 2Na]^{2+}$, 1218.5482; found, 1218.5453.

***tert*-Butyl Protected Tetrabenzyloxy Macrotricycle 26.** PPh_3 (491 mg, 1.87 mmol) was added to a solution of tetraazide **25** (224 mg, 0.09 mmol) dissolved in THF, and the reaction mixture was heated at 60 °C under nitrogen overnight. H_2O (2 mL) was added. The reaction mixture was heated at 60 °C for a further 4 h, after which the solvent was evaporated to give a crude tetraamine which was used directly after flash chromatography (eluent DCM to 19:1 DCM/methanol saturated with NH_3) in the subsequent cyclization process. A solution of bis-pentafluorophenyl ester **18**[7b] (208 mg, 0.20 mmol) in dry THF (50 mL) was added dropwise over 30 h to a solution of the foregoing tetraamine (210 mg, 0.09 mmol) and DIPEA (94 μL, 0.54 mmol) in dry THF (250 mL) under nitrogen at room temperature. After the addition the reaction mixture was allowed to stir for a further 24 h, after which the solvent was evaporated. The oil obtained was dissolved in DCM (30 mL) and washed with saturated aqueous NH_4Cl (25 mL) and brine (25 mL) before being dried over Na_2SO_4 and evaporated. The residue was purified by flash chromatography (eluent DCM to ethyl acetate) resulting in a white solid which was subjected to further purification by preparative high performance liquid chromatography (Hichrom Kromasil column, 150 mm × 21.2 mm, 5 μm, eluent: methanol/water 80:20 to 95:5 in 14 min, then to 100:0 in a further 36 min, flow rate 15 mL·min^{-1}). Macrotricycle **26** (86 mg, 0.02 mmol, 26%) was isolated as a white solid. Retention time = 27 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.39 (s, 108H), 2.45 (t, ^3J(H,H) = 6.3 Hz, 24H), 3.70 (t, ^3J(H,H) = 6.3 Hz, 24H), 4.41 (dd, ^2J(H,H) = 13.8 Hz, ^3J(H,H) = 5.3 Hz, 8H), 4.69 (dd, ^2J(H,H) = 13.8 Hz, ^3J(H,H) = 4.9 Hz, 8H), 5.00 (s, 8H), 6.63 (t, ^3J(H,H) = 5.2 Hz, 8H), 6.68 (bs, 4H), 7.34−7.42 (m, 20H, 7.74 (s, 8H), 7.90 (s, 4H), 8.21 (s, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.0, 36.2, 41.1, 60.4, 67.1, 69.0, 76.5, 80.5, 128.1, 128.7, 128.9, 132.1, 134.3, 135.1, 136.4, 136.7, 156.3, 166.1, 166.3, 170.9; HRMS (ESI): m/z calcd for $C_{196}H_{256}N_{12}O_{52}Na_2^{2+}$ $[M + 2Na]^{2+}$, 1827.8771; found, 1827.8829.

***tert*-Butyl Protected Tetrahydroxy Receptor 27.** Pd on C (40 mg) was activated by heating at 140 °C under vacuum overnight, after which a solution of tricycle **26** (45 mg, 12.5 μmol) in THF and MeOH saturated with NH_3 (1:1, 10 mL) was added. The flask was evacuated and filled with hydrogen (1 atm), and the reaction mixture was stirred for 3 h. The reaction mixture was then filtered over Celite and washed with ethyl acetate, after which the filtrate was evaporated to yield **27** as a white solid (37 mg, 11.3 μmol, 91%). Retention time = 26 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.41 (s, 108H), 2.48 (t, ^3J(H,H) = 6.3 Hz, 24H), 3.69 (t, ^3J(H,H) = 6.3 Hz, 24H), 3.84 (s, 24H), 4.29 (dd, ^2J(H,H) = 13.8 Hz, ^3J(H,H) = 6.0 Hz, 8H), 4.71 (dd, ^2J(H,H) = 13.8 Hz, ^3J(H,H) = 5.3 Hz, 8H), 6.80 (s, 4H), 7.16 (bs, 8H), 7.52 (s, 8H), 7.88 (s, 4H), 8.23 (s, 8H), 9.26 (s, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.1, 36.4, 41.2, 60.5, 67.2, 69.1, 80.7, 125.6, 127.5, 128.4, 129.6, 135.6, 136.5, 153.9, 166.2, 168.3, 171.2; MS (ESI): m/z calcd for $C_{168}H_{232}N_{12}O_{52}Na_2^{2+}$ $[M + 2Na]^{2+}$, 1647.7832; found, 1647.7872.

Tetrahydroxy Receptor 7. Macrotricycle **27** (8 mg, 2.5 μmol) was dissolved in DCM (5 mL) under nitrogen and cooled to 0 °C in an ice bath. TFA (1 mL) was added dropwise, and the reaction mixture was stirred for 5 h at which point the solvent was evaporated. The white solid obtained was dissolved in methanol/water (6:4, 10 mL). The pH of the solution was adjusted to pH 7 by the addition of a sodium hydroxide solution (0.5 M); this was monitored using a pH meter. The solvent was removed by lyophilization to yield the salt **7** as a white powder (7 mg, 2.4 μmol, 96%). ^{1}H NMR (500 MHz, D_2O, pH = 7.5) δ 2.46 (t, ^3J(H,H) = 6.3 Hz, 24H), 3.75 (t, ^3J(H,H) = 6.3 Hz, 24H), 3.85 (s, 24H), 4.36 (d, ^2J(H,H) = 13.8 Hz, 8H), 7.71 (s, 8H), 7.82 (s, 4H), 8.17 (s, 8H); ^{1}H NMR (500 MHz, D_2O, pH = 7.5) δ 2.46 (t, ^3J(H,H) = 6.8 Hz, 24H), 3.76 (t, ^3J(H,H) = 6.8 Hz, 24H), 3.87 (s, 24H), 4.19 (d, ^2J(H,H) = 13.6 Hz, 8H), 7.42 (s, 8H), 8.07 (s, 4H), 8.28 (s, 8H); MS (ESI): m/z calcd for $C_{120}H_{136}N_{12}O_{52}Na_2^{2+}$ $[M + 2Na]^{2+}$, 1311.4075; found, 1311.4101.

■ ASSOCIATED CONTENT

Supporting Information. NMR spectra for characterization, details of binding experiments including spectra and analysis plots, UV spectra of **7** at different pH values, details of molecular modeling. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
*E-mail: Anthony.Davis@bristol.ac.uk

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(16) Note that the binding constants reported to reducing sugars are averaged values, with contributions from both α and β anomers. For further discussion of this issue see ref 7e.

(17) Affinities to **8** might also be lowered by ionization of the carbohydrate substrates, giving alkoxide species which cannot bind to the tetra-anionic cavity. The pK_a of glucose in water has been measured as 12.45 (see El Rassi, Z.; Nashabeh, W. In *Carbohydrate analysis - high performance liquid chromatography and capillary electrophoresis*; El Rassi, Z., Ed.; Elsevier: Amsterdam, 1995; Vol. 58, p 267), so at pH = 12 (as used to study **8**) a substantial minority of glucose molecules should be ionized. By reducing the concentration of neutral glucose molecules, this effect could lower the apparent binding constant by a factor approaching 2. We are grateful to a referee for raising this possibility.

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