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Phenyl Glycopyranoside Recognition in Water Using Stoddart's Cyclobis(paraquat-*p*-phenylene) Receptor

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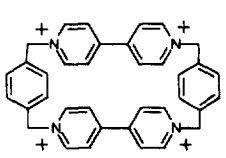
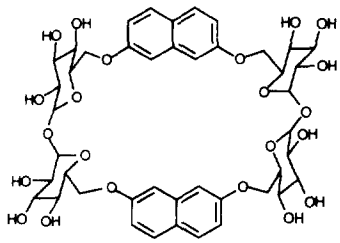
Key Words: Molecular recognition; Cyclophane; Phenyl glycopyranoside; Association constants; Anomeric center.

Abstract: *The cyclobis(paraquat-p-phenylene) receptor associates with various phenoxy-substituted guest compounds in water, including phenyl glycopyranosides. The receptor was found to bind phenyl β-D-glucopyranoside 0.6 kcal/mol more strongly than phenyl α-D-glucopyranoside. In addition, there was a general trend of increased affinity for anionic guests compared to structurally related neutral compounds.*

Stoddart and coworkers have shown that cyclobis(paraquat-*p*-phenylene), $\mathbf{1}^{4+}$, is capable of binding electron-rich aromatic residues in organic and aqueous solution.[1] In particular, Stoddart and Kaifer have observed high associations with electron-rich aromatic amino acids (*e.g.*, tyrosine, tryptophane),[2] and neurotranmitters (*e.g.*, catecholamines)[3] in aqueous solution. Cyclophane $\mathbf{1}^{4+}$ has a number of structural attributes that simplify the determination and interpretation of its binding ability:[1] (i) Association with an electron-rich aromatic residue produces a colored, charge-transfer complex. Binding constants are readily determined by monitoring the appearance of this complex using standard spectrometric methods. (ii) The internal cavity of cyclophane $\mathbf{1}^{4+}$ has a shape (10.3 x 6.8 Å) and hydrophobicity that make it a selective inclusion receptor for aromatic residues. (iii) Cyclophane $\mathbf{1}^{4+}$ has a rigid structure that hardly changes upon inclusion of an aromatic guest, thus differences in guest association constants can be attributed directly to differences in guest structures. (iv) The solubility of tetracation $\mathbf{1}^{4+}$ is strongly dependent on the identity of its counter-ions. For example, the tetrachloride salt is water-soluble, whereas the tetrakis(hexafluorophosphate) salt is soluble in acetonitrile. This allows binding affinities to be determined in solvents with substantially different properties. A consideration of the above features suggests cyclophane $\mathbf{1}^{4+}$ can be employed as a binding probe for studies in molecular recognition.



$\mathbf{1}^{4+}$



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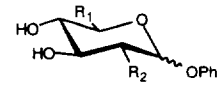
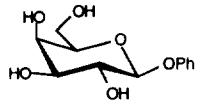
Our interest lies in saccharide recognition in aqueous solution.[4] The past few years have seen an increasing number of reports of small synthetic receptors designed to bind sugars using non-covalent

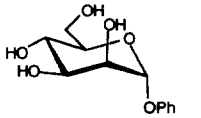
interactions.[5-7] Most have been hydrogen bonding systems operating in non-polar solvents. Of particular relevance to the work described here is glycophane **2**, described by Penadés and coworkers.[6] Host **2** was found to bind *p*-nitrophenyl glycopyranosides in water using a combination of hydrophobic forces, aromatic donor-acceptor interactions, and hydrogen bonding. Association constants were less than 300 M^{-1}, with a general binding preference for the α-anomer. Here we report on the ability of $\mathbf{1}^{4+}$ (tetrachloride salt) to bind various phenoxy-substituted organic compounds in water, including a range of phenyl D-glycopyranosides. In short, we find that host $\mathbf{1}^{4+}$ binds phenyl β-D-glycopyranosides more strongly than phenyl α-D-glycopyranosides.



phenyl D-glucoside
(R_1 = CH_2OH, R_2 = OH)
phenyl N-acetyl-D-glucosamidine
(R_1 = CH_2OH, R_2 = $NHCOCH_3$)
phenyl D-glucuronide
(R_1 = COOH, R_2 = OH)



phenyl β-D-galactoside



phenyl α-D-mannoside

Association constants, K_{assn}, and molar absorptivities, ε_{obs}, were determined from spectrometric titration studies at wavelength, λ_{obs}, using the method described by Stoddart.[3,8] The values reported in Table 1 are an average of at least five independent trials, each using freshly made solutions.

Table 1: Binding of Guests With Host $\mathbf{1}^{4+}$ in Aqueous Buffer, pH 7.4, at 295 K.[a]

Entry	Guest	K_{assn} 10^3 (M^{-1})[b]	ΔG_{assn} (kcal mol^{-1})	ε_{obs} 10^2 (M^{-1} cm^{-1})[a]	λ_{obs} (nm)	^{13}C δ for phenoxy C4 (ppm)[c]
1	phenyl β–D-glucoside	4.1 ± 0.5	4.9	2.3 ± 0.6	326	121.7
2	phenyl α-D-glucoside	1.4 ± 0.3	4.3	2.2 ± 0.3	327	121.8
3	phenyl β-D-galactoside	5.3 ± 0.7	5.1	2.0 ± 0.1	326	121.7
4	phenyl α-D-mannoside	1.5 ± 0.1	4.3	2.2 ± 0.2	327	121.9
5	phenyl N-acetyl-β-D-glucosaminide	1.0 ± 0.1	4.1	1.8 ± 0.1	327	122.0
6	phenyl N-acetyl-α-D-glucosaminide	0.2 ± 0.04	3.3	1.8 ± 0.3	327	122.2
7	phenyl β-D-glucuronide	12.1 ± 0.5	5.6	2.6 ± 0.1	325	-
8	phenoxyacetic acid, **3**	4.9 ± 0.2	5.0	2.1 ± 0.1	327	-
9	3-phenoxypropionic acid, **4**	12.5 ± 0.1	5.6	2.0 ± 0.1	327	-
10	4-methoxyphenylacetic acid, **5**	7.0 ± 0.4	5.2	2.1 ± 0.1	328	-
11	phenoxymethylpenicillinic acid, **6**	1.1 ± 0.3	4.1	2.5 ± 0.1	324	-
12	phenoxyacetamide, **7**	1.2 ± 0.1	4.2	2.1 ± 0.1	325	121.1
13	3-phenoxy-1,2-propanediol, **8**	2.8 ± 0.1	4.7	2.1 ± 0.1	328	120.5

[a]See reference 8 for further details. [b]Averages and stanadard deviations calculated from at least five independent runs. [c]In DMSO-d_6

As shown in Table 1, host $\mathbf{1}^{4+}$ bound phenyl β-D-glucopyranoside 0.6 kcal/mol more strongly than the α-anomer (entries 1 and 2). The trend also held for phenyl β-D-galactopyranoside and phenyl α-D-mannopyranoside (entries 3 and 4), as well as the anomers of phenyl N-acetyl-glucosaminide (entries 5 and 6), although in the latter case both binding affinities were reduced. In general, 1:1 binding stoichiometries for the $\mathbf{1}^{4+}$:phenyl glycopyranoside complexes were confirmed from Job plots.[9] Strong evidence that the guest phenyl ring was binding inside the host cavity was gained from ^1H NMR titration experiments. Incremental addition of host $\mathbf{1}^{4+}$ to the phenyl glycopyranoside guests produced a strong upfield movement of the guest phenyl

resonances with concomitant signal broadening.[10] After the addition of about five molar equivalents of **1**$^{4+}$, the guest phenyl signals had disappeared into the baseline. The signals for the hydrogens on the pyranoside ring, on the other hand, remained essentially unchanged. These observations are in general agreement with previous binding studies using host **1**$^{4+}$.[2,3]

To better understand the factors that affect host / guest binding, association constants were determined for a series of control compounds (Table 1). The strong binding observed with phenyl glucuronide (entry 7) suggested that coulombic attractions between tetracationic host **1**$^{4+}$, and an anionic guest can increase K_{assn}. In agreement with this suggestion is the trend of increased binding for phenoxy-substituted carboxylic acids **3** - **5** (entries 8 - 10) compared to the neutral controls **7** and **8** (entries 12 and 13). The proximity and orientation of the anionic carboxylate relative to the phenoxy group appears to be important. In particular, if the guest structure rigidly fixes the two groups with a large separation, no increase in K_{assn} is observed (compare entries 11 and 12).

The most intruiging trend within Table 1 is an apparent 0.7 kcal/mol increase in binding energies for phenyl β-glycopyranosides compared to their α-anomers. Possible explanations for this binding diastereoselectivity include arguments based on sterics, aromatic donor-acceptor interactions, and/or solvation effects. The steric argument focuses on the difference in steric accessibilites between the α- and β-anomeric positions.[11] The β-anomer allows the phenoxy substituent to adopt an equatorial position on the pyranoside ring, whereas, the α-anomer forces the phenoxy group into the sterically more-crowded axial position which may hinder access to the phenoxy-binding host **1**$^{4+}$. One example where a steric effect is clearly operating is the case of the phenyl N-acetyl-glucosaminides which have reduced binding affinities compared to the corresponding anomers of phenyl glucopyranoside (compare entries 5 and 6, with entries 1 and 2 respectively).

An explanation based on aromatic donor-acceptor interactions focuses on the amount of electron density within each glycoside's phenoxy group. Stoddart has established that the highly electron-defficient host **1**$^{4+}$ associates more strongly with electron-rich aromatic guests.[1-3] To see if such a trend exists for the phenyl glycopyranosides in Table 1, ^{13}C NMR was used to determine the relative order of phenoxy group electron densities. We reasoned that the ^{13}C chemical shift for the phenoxy *para* carbon was a measure of relative electron density; the more upfield the chemical shift the more electron-rich the phenyl ring. As indicated in Table 1, there was no significant difference in ^{13}C chemical shifts between glycoside anomers (the spectra were acquired in DMSO so as to eliminate ambiguities due to aqueous solvent effects). Therefore, as judged by this NMR criterion, there was no difference in phenoxy group electron densities.

An argument based on solvation effects would require the change in guest solvation upon association with host **1**$^{4+}$ to be different for each of the phenyl glycopyranoside anomers. Since inclusion of the phenoxy groups into the cavity of host **1**$^{4+}$ is driven, in part, by solvophobic effects in aqueous media, the difference in binding constants for the two anomers may be due to differences in solvophobic binding energies. A test of this explanation is to examine host / guest binding in a solvent where the solvophobic effect is diminished. Thus the association between host **1**$^{4+}$ (as its tetrakis(hexafluorophosphate) salt), and the two anomers of phenyl D-glucopyranoside were determined in acetonitrile. As expected, the associations were found to be substantially weaker (for phenyl β-D-glucopyranoside K_{assn} = 543 M^{-1}; for phenyl α-D-glucopyranoside K_{assn} = 173 M^{-1}), due to the loss of the solvophobic driving force; however, a 0.7 kcal/mol binding preference in favor of the β-anomer was maintained. This result is evidence against a solvation effect as the source of the difference in anomer binding energies.

In conclusion, host $\mathbf{1}^{4+}$ associates quite strongly with phenyl glycopyranosides in aqueous solution. Association is driven by inclusion of the guest phenoxy group inside the cavity of the cyclophane host. Phenyl β-D-glucopyranoside was found to bind 0.6 kcal/mol more strongly than the α-anomer. At present, the experimental evidence does not provide an unambiguous explanation for this binding diastereoselectivity. Chemical intuition suggests that steric differences between the α- and β-anomers may be the controlling factor.[11,12] The very similar amounts of electron density within the glycoside phenyl groups indicate that differences in aromatic donor-acceptor interactions are not likely to be influential. Evidence against a solvation effect is the observation that the aqueous binding diastereoselectivity is maintained in acetonitrile.

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