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Molecular Recognition of Amino Sugars by a Porphyrin-based Receptor in Aqueous Media

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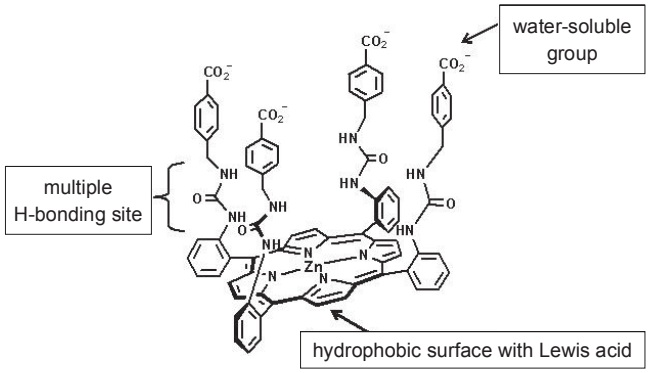
The molecular recognition of neutral sugars in a biological system involves hydrogen bonding, CH-π interactions, metal coordination, and van der Waals interactions.[1] Furthermore, the binding of ionic sugars often relies on ion pairing and ionic hydrogen bonding, as shown in the biological recognition process of sialic acids.[2] The molecular recognition of carbohydrates by synthetic receptors has attracted the interest of many chemists in the last few decades.[3] Among the large variety of carbohydrate receptors reported previously, intensive efforts have been focused on developing a system that utilizes hydrogen-bonding interactions in a hydrophobic environment[4] − a well-known motif of saccharide recognition discovered in sugar binding proteins such as concanavalin A and L-arabinose-binding protein.[5] Although many artificial receptors mimicking these binding events were reported to be operative in aprotic non-polar solvents, they are less effective in more polar solvents or aqueous media. This limitation arises because carbohydrates are highly solvated by water molecules − a thermodynamic barrier which may hamper receptor-saccharide interactions based on hydrogen bonding.



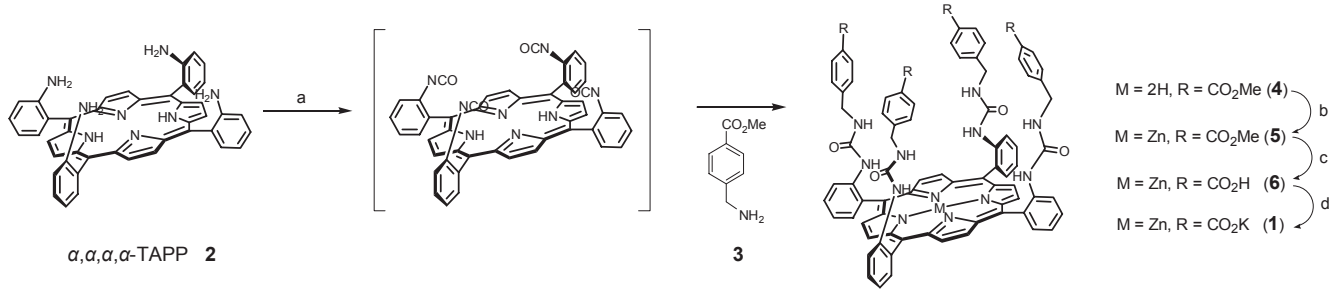
Scheme 1. Designed receptor **1** for recognition of amino sugars in aqueous media

In previous works, urea-appended porphyrins have been developed for efficient carbohydrate recognition in organic media.[6] In particular, aspartate-urea-appended porphyrin showed fairly high affinity for *n*-octyl-β-D-glucoside in chloroform ($K_a = 2.0 \times 10^7$ M^{-1}) and recognized the same guest with an affinity of 340 M^{-1} in $CDCl_3/CD_3OD$ (v/v = 10:1), a more polar solvent.[6a] These results indicated a significant potential for extending the research on carbohydrate recognition by urea-appended porphyrins to aqueous media.[7]

Herein, we present the synthesis of a porphyrin-based receptor that is soluble in aqueous media, and its binding properties for amino sugars.

A new porphyrin receptor **1** was designed for carbohydrate recognition in an aqueous solvent (Scheme 1). Receptor **1** consists of three functional moieties, i.e., urea, a zinc-inserted porphyrin plane, and a carboxylate group. Four urea groups are arranged in a convergent and equatorial manner with respect to the porphyrin plane to engage in multiple hydrogen bonding interactions with carbohydrates. The zinc-inserted porphyrin plane is expected to serve as a Lewis acid-base interaction site as well as a hydrophobic interaction site in aqueous media. Finally, carboxylate is introduced to improve solubility in polar solvents; moreover, this group can also participate in hydrogen bonding as well as electrostatic interactions with cationic species.

Receptor **1** was synthesized following the procedure presented in Scheme 2. The free-base porphyrin methyl ester **4** was prepared from the reaction between 4-aminomethyl benzoic acid methyl ester **3** and α,α,α,α-tetrakis(*o*-isocyanatophenyl) porphyrin, which was generated *in situ* from α,α,α,α-tetrakis



Scheme 2. Synthesis of receptor **1**. (a) $COCl_2$, TEA, THF; (b) $Zn(OAc)_2$, MeOH-$CHCl_3$; (c) KOH, MeOH-THF, then H_3O^+; (d) KOH

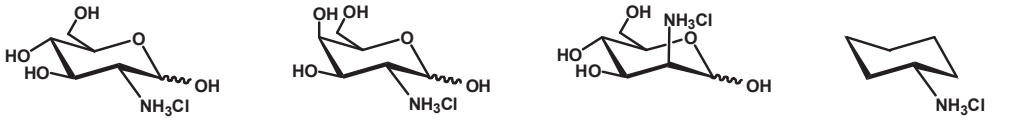


Figure 1. Selected guests for binding study.

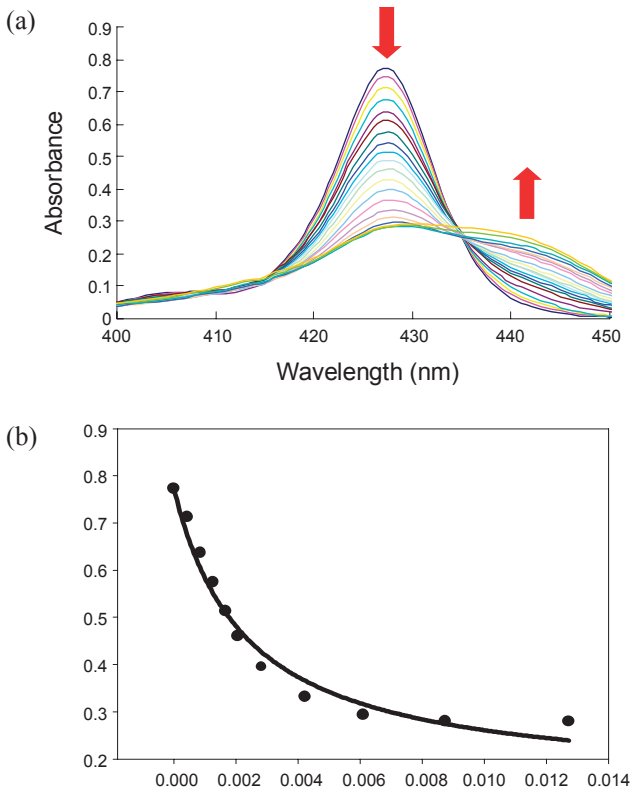


Figure 2. (a) UV-visible absorption spectra of receptor **1** (2.5 μM) after the addition of 0 - 6500 equiv of D-mannosamine·HCl. (b) Titration curve from the change of absorbance at 427 nm. All the experiments were conducted in MeOH-H_2O (v/v = 1:3).

(*o*-aminophenyl)porphyrin **2**. A typical zinc insertion method yielded zinc porphyrin **5**, and subsequent hydrolysis resulted in the final product **1**.[8]

UV-visible spectroscopy was used to investigate the binding properties of receptor **1** in aqueous media. The absorbance at the Soret band (427 nm) is linearly proportional to the concentration of **1** in the range of 1.6 μM to 2.5 μM in MeOH-H_2O (v/v = 1:3), R = 1.0 (data not shown). It could be inferred that receptor **1** does not aggregate but exists as a monomeric species in this condition.

We first tested neutral carbohydrates such as glucose, galactose, mannose, ribose, maltose, and maltotriose. Unfortunately, these guests did not cause any spectral changes in receptor **1**. On the other hand, the absorbance at the Soret band significantly changed with the addition of amino sugars. Therefore, we performed UV-visible titration of receptor **1** with various amino sugars (Figure 1).

UV-visible titration of receptor **1** with the guests revealed spectral changes at the Soret band of porphyrin as amino sugars were bound, implying that Lewis acid-base interactions occurred at the porphyrin core. In addition, the clearly observed isosbestic point suggests that there are only two states through which the 1:1 complex is formed (Figure 2).

Table 1. Binding constants (K_a, [M^{-1}]) and free energy changes (ΔG^o, [kcal·mol^{-1}]) from UV-visible titrations of **1** with guests in MeOH-H_2O (v/v = 1:3) at 298 K[a]

Guest	K_a [M^{-1}]	ΔG^o [kcal·mol^{-1}]
D-glucosamine·HCl	3.8 (±0.47[b]) × 10	2.2
D-galactosamine·HCl	1.7 (±0.16) × 10^2	3.0
D-mannosamine·HCl	4.3 (±0.68) × 10^2	3.6
cyclohexylamine·HCl	ND[c]	ND

[a]Experimental conditions; [**1**] = 2.5 μM for all the guests, [Guest] = 7.3 - 66 mM for D-glucosamine·HCl, 0.24 - 21 mM for D-galactosamine·HCl, 0.22 - 16 mM for D-mannosamine·HCl; [b]Standard deviations; [c]ND = not determined due to weak interaction.

The binding constants for the formation of complexes between receptors and guests were calculated by fitting the absorbance curve at λ_{max} (427 nm) as a function of carbohydrate concentration to a 1:1 binding isotherm (Table 1). It is notable that receptor **1** can bind amino sugars even in the aqueous solvent through non-covalent interactions. The apparent binding constants for the guests are ordered as follows; D-mannosamine·HCl > D-galactosamine·HCl > D-glucosamine·HCl. The higher affinity for D-mannosamine·HCl indicates that the amino group at the axial position is favorable for the binding interaction. In addition, receptor **1** does not appear to bind cyclohexylamine·HCl, which implies that the hydroxyl groups of a guest play an important role in the binding event.

Depending on the role of the amino group, two binding modes could be hypothesized for the formation of complexes between receptor **1** and its guests. One involves the ammonium group interacting with the carboxylate of receptor **1** through electrostatic interaction or charged hydrogen bonding, with the remaining hydroxyl groups of the guest hydrogen-bonding to urea NHs and (/or) one oxygen coordinating to the zinc inserted in the porphyrin core. The other involves the amino group coordinating to the zinc atom. In this case, the remaining hydroxyl groups would still interact with receptor **1** through hydrogen bonding.

To investigate the role of the amino group, we conducted UV-visible titration of receptor **1** with D-mannosamine·HCl in different buffered solutions. The effect of pH on the binding properties of receptor **1** was compared in three solvent systems (Table 2). The highest affinity was obtained in distilled water; K_a = 430 M^{-1}. When a 100 mM HEPES (4-(2-hydroxyethyl)-

Table 2. Binding constants (K_a, [M^{-1}]) and free energy changes (ΔG^o, [kcal·mol^{-1}]) from UV-visible titrations of **1** with D-mannosamine·HCl at 298 K[a]

Buffered solution	K_a [M^{-1}]	ΔG^o [kcal·mol^{-1}]
distilled water	430	3.6
HEPES (pH 7.4)	51	2.3
CHES (pH 10)	ND[b]	ND

[a]MeOH-H_2O (v/v = 1:3), HEPES = 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid, CHES = 2-(*N*-cyclohexylamino)ethanesulfonic acid; [b]ND = not determined due to little change in absorbance.

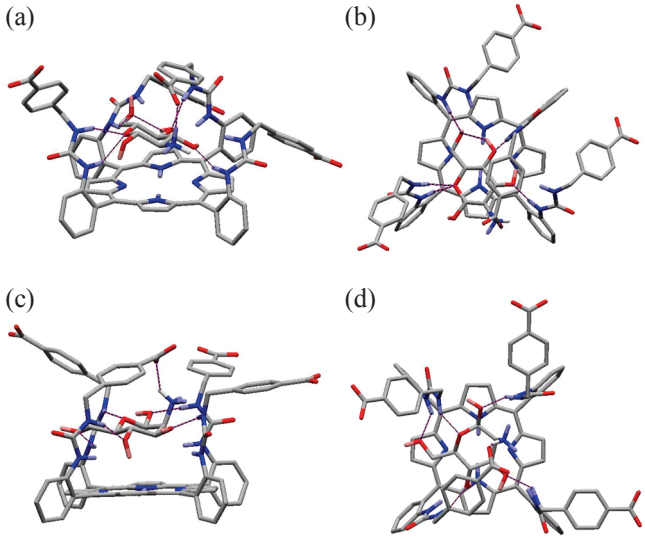


Figure 3. Shown are energy-minimized structures of 1:1 complexes and hydrogen bonds as thin lines (MacroModel 7.0, Amber* force field in water). (a) Side view of **1**·β-D-glucosamine·HCl. (b) Top view of **1**·β-D-glucosamine·HCl. (c) Side view of **1**·β-D-mannosamine·HCl. (d) Top view of **1**·β-D-mannosamine·HCl. For free-base porphyrin of **1**, six intermolecular hydrogen bonds were shown in both cases.

1-piperazineethanesulfonic acid) solution (pH = 7.4) was used, the affinity decreased to $K_a = 51\ M^{-1}$, presumably because many buffer molecules interfere with the binding event. Moreover, no binding interaction was detected in the case of 100 mM CHES (2-(*N*-cyclohexylamino)ethanesulfonic acid) buffered solution (pH = 10). Given that the binding affinity decreased as the pH increased, it could be inferred that the ammonium group interacts with the carboxylate group, consistent with the first hypothesis. Further evidence was obtained from a titration experiment in which no binding was detected when the guest solution was treated with one equivalent of KOH. In summary, these results strongly suggest that the guests are bound to receptor **1** through interactions between ammonium and carboxylate, and that the remaining hydroxyl groups of the guests are involved in other non-covalent interactions such as hydrogen bonding and Lewis acid-base coordination.

Computer-assisted conformational search was conducted to investigate how receptor **1** interacts with amino sugars.[9] Given the lack of an appropriate force-field for zinc porphyrin, the free-base of receptor **1** was chosen for molecular modeling, and D-glucosamine·HCl and D-mannosamine·HCl were selected as the target guests. As shown in Figure 3, the calculated structures revealed two binding interactions; the first an electrostatic or hydrogen bonding between the ammonium of the guest and the carboxylate of the host, and the second multiple hydrogen bonding interactions between OHs and urea NHs. Apparently, both complexes (Figure 1(a), (b) *vs.* 1(c), (d)) showed similar binding interactions with the same number of hydrogen bonds. However, they differ in how the guest is positioned above the porphyrin plane. When β-D-glucosamine is bound to receptor **1**, it is located above the porphyrin plane at a distance within the effective range of hydrogen bonding. On the other hand, β-D-mannosamine is positioned to provide better contact with the surface of porphyrin, indicating that relative to β-D-glucosamine, a greater number of CHs on the ring of β-D-mannosamine could interact with the hydrophobic π surface of receptor **1**. This could be one reason for the selectivity of receptor **1** toward amino sugars.

In summary, in order to develop a carbohydrate receptor in aqueous media, we introduced four carboxylate groups into a urea-appended porphyrin previously proven to effectively bind carbohydrates in organic solvents. While receptor **1** does not appear to recognize neutral carbohydrates including monosaccharides, we observed that it effectively binds amino sugars in MeOH-H_2O (v/v = 1:3). Receptor **1** showed moderate affinity for guests in the order: D-mannosamine·HCl > D-galactosamine·HCl > D-glucosamine·HCl. In addition, the highest affinity of $K_a = 430\ M^{-1}$ was obtained for D-mannosamine·HCl, suggesting that the amino group at the axial position is favorable for the binding interaction with the carboxylate of host **1**. Control experiments supported a binding mode in which the amino sugar is bound to receptor **1** through the interaction between ammonium and carboxylate, and the remaining hydroxyl groups of the guest are involved in hydrogen bonding and (/or) Lewis acid-base interactions. Energy- minimized structures revealed that the guests are effectively bound to the binding site of receptor **1** through multiple hydrogen bonding interactions. Unlike previous carbohydrate receptors based on boronic acid moieties, the porphyrin-based receptor **1** recognizes carbohydrates through non-covalent interactions, especially wherein hydrogen bonding plays an important role in the binding event. It is hoped that our system may provide a good model for the development of carbohydrate receptors in aqueous media.

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8. Compound **4**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.66 (s, 2H), 3.74 (s, 12H), 3.90 (d, J = 5.4 Hz, 8H), 6.44 (t, J = 5.9 Hz, 4H), 7.02 (d, J = 8.2 Hz, 8H), 7.28 (s, 4H), 7.37 (t, J = 6.6, 4H), 7.64-7.71 (m, 12H), 7.74 (t, J = 8.5 Hz, 4H), 8.39 (d, J = 8.1 Hz, 4H), 8.77 (s, 8H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 42.28, 51.99, 115.75, 121.44, 121.77, 126.95, 127.96, 129.00, 129.22, 131.06, 135.43, 139.46, 145.38, 155.24, 165.92.
Compound **5**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.74 (s, 12H), 3.80 (d, J = 4.8 Hz, 8H), 6.26 (br, 4H), 6.81 (s, 4H), 6.94 (d, J = 8.1 Hz, 8H), 7.40 (t, J = 7.4 Hz, 4H), 7.62 (d, J = 8.1 Hz, 8H), 7.75 (t, J = 7.6 Hz, 4H), 7.83 (d, J = 7.5 Hz, 4H), 8.36 (d, J = 8.3 Hz, 4H), 8.73 (s, 8H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 42.18, 52.03, 115.59, 121.08, 121.24, 126.82, 127.95, 128.74, 128.98, 131.75, 132.06, 135.15, 139.36, 145.40, 149.67, 155.05, 165.95; ESI-MS [M+1]: found 1504.1.
Compound **6**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.78 (s, 8H), 6.35 (s, 4H), 6.84 (s, 4H), 6.95 (d, J = 7.9 Hz, 8H), 7.37 (t, J = 6.9 Hz, 4H), 7.63 (d, J = 7.9 Hz, 8H), 7.72 (t, J = 7.1 Hz, 4H), 7.81 (d, J = 7.4 Hz, 4H), 8.38 (d, J = 8.2 Hz, 4H), 8.70 (s, 8H), 12.67 (br, 4H).
Compound **1**: Porphyrin **6** was dissolved in methanol. Subsequent addition of 1.0 equivalent of KOH and the successive evaporation of the solvent yielded the final product **1**, which was used without further purification.
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