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The First Example of Positive Allosterism in an Aqueous Saccharide-Binding System Designed on a Ce(IV) Bis(porphyrinate) Double Decker Scaffold

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Abstract—The first example of a positive, homotropic system in aqueous saccharide-binding has been achieved using a Ce(IV) bis(porphyrinate) double decker scaffold bearing two pairs of boronic acid groups (compound **2**). In this system, the binding of the first guest (1:2 saccharide/boronic acid complex) suppresses the rotational freedom of the two porphyrin planes, which facilitates the binding of the second guest. As a result, two pairs of boronic acid groups in **2** can autoacceleratively bind these guests and yield CD-active species. The analyses of CD intensity–guest concentration plots according to the Hill equation resulted in $K=3.7\times10^4\ M^{-2}$ and $n=2.0$ for D-fructose and $K=9.6\times10^5\ M^{-2}$ and $n=1.6$ for D-glucose. The present system which is in action even in aqueous media is widely applicable to allosteric control of drug release, catalytic reaction, information transduction, etc. of saccharide-containing guest molecules.

Introduction

Positive or negative allosterisms are ubiquitously seen in nature where the biological events must be efficiently regulated in response to chemical or physical signals from the outside world. The typical examples are observed for a cooperative dioxygen binding to hemoglobin,[1] hexamerization of arginine repressor,[2] a cooperative effect with respect to the concentration of arachidonate-containing phospholipids in cytosolic phospholipase A_2,[3,4] etc. Furthermore, the methodology is very useful to amplify and convert weak chemical or physical signals into other signals which are convenient for us to read out and record. The biomimetic design of such allosteric systems is of great significance because they are readily applicable to the efficient regulation of drug release, catalytic reactions, information transduction, etc. It is not so difficult to reproduce a heterotropic allosterism in artificial systems,[5–12] whilst design of a homotropic allosterism is more important but more difficult.[13,14] In particular, the successful examples for a positive, homotropic allosterism are very limited. To the best of our knowledge, there is only one precedent for a positive, homotropic allosterism which features cooperative extraction of saccharides with a resorcinol cyclic tetramer host into organic media.[13]

It is undoubted, however, that the allosteric saccharide-binding which can take place even in aqueous media is more practical and more applicable to many related systems: for example, many water-soluble drugs such as vancomycin, ramoplanin, and teicoplanin have a saccharide moiety and the allosteric capture and release of these drugs are of great significance. Very recently, we found that a porphyrin-based Ce(IV) bis(porphyrinate) double decker **1** shows a unique positive, homotropic allosterism for certain dicarboxylic acid guests.[15] In this system, the binding event of the first dicarboxylic acid guest to a pair of pyridyl groups by the hydrogen-bonding interaction can suppress the rotation of the two porphyrin planes without inducing a plane inclination; the subsequent binding of the three dicarboxylic acid guests to the remaining three pairs of pyridyl groups can then occur autoacceleratively.[15] This double decker architecture can be applied as a scaffold to design an allosteric saccharide-binding system by introducing boronic acid groups which are known to act as excellent saccharide receptors in aqueous media.[16] Taking these lines of expectation into consideration, we newly designed compound **2** bearing two pairs of boronic acid groups. We have found that **2** can bind saccharides according to a positive, homotropic allosterism in aqueous media to form 1:2 **2**·saccharide complexes. To the best of our knowledge, this is the first positive allosteric system for the saccharide-binding useful in aqueous media.

Keywords: allosterism; double decker porphyrin; saccharide; boronic acid; molecular recognition.

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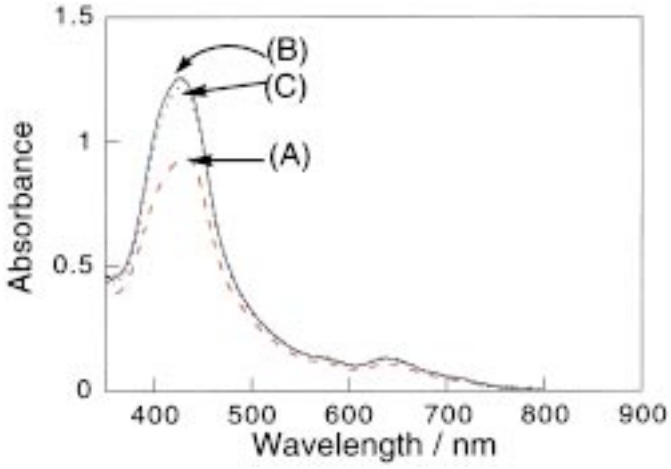
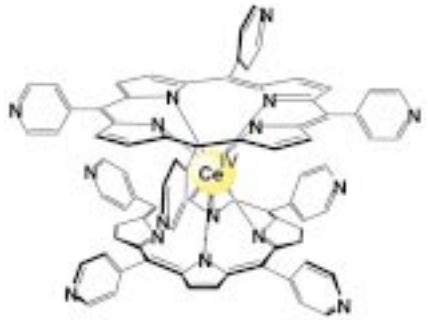


Figure 1. Typical absorption spectra of **2** (1.00×10^{-5} M) in (A) water, (B) water/MeOH=1:2 (v/v), and (C) MeOH at 25°C.



1

2

Results and Discussion

Bis[5,15-bis(4-methoxyphenyl)-10,20-di(4-pyridyl)porphyrinato]cerium(IV) (**5**) was synthesized from 5,15-bis(4-methoxyphenyl)-10,20-di(4-pyridyl)porphyrin in 27% yield in a manner similar to the method of Buchler and Nawra.[17] Treatment of **5** with 2-(4-bromomethylphenyl)-1,3-dioxa-2-borinan in DMF gave 1,3-propanediol-protected **2** in 54% yield. The product was identified by IR and ^{1}H NMR spectral evidence and elemental analysis. This product was used for the further spectral measurements without deprotection treatment.[18]

Firstly, absorption spectra of **2** were measured in water/MeOH mixed solvents at 25°C (Fig. 1). The significant spectral change was induced with increasing water concentration at water/MeOH>40 vol%. From examination of Lambert–Beer's plots, we found that **2** aggregates in these water-rich solvents. On the other hand, the absorption spectra were scarcely changed by the water concentration at water/MeOH<40 vol% and obeyed the Lambert–Beer's rule. We thus chose a water (pH 10.5 with 50 mM carbonate buffer)/MeOH=1:2 (v/v) mixture into which **2** was solubilized discretely.[19] The absorption spectra (λ_{max} 409 nm) were scarcely affected by saccharide addition. In contrast, CD spectra which have the spectral pattern inherent to each saccharide appeared upon addition of saccharides (Fig. 2). In particular, D-fructose gave a very strong CD spectrum. As L-isomers such as L-fructose and L-glucose result in the CD spectra symmetrical to the corresponding D-isomers and the two spectra cross at the θ=0 line, one can assign these spectra to exciton-coupling bands. In general, when a saccharide guest is bound to a boronic acid group in a host, the resultant complex becomes optically active. It has been established, however, that the complex can yield a strongly CD-active species only when the saccharide is bound intramolecularly to two boronic acid groups to form a macrocyclic structure.[16] The results indicate that two diol moieties in saccharides are bound to two boronic acid groups in **2** to bridge two porphyrin planes. This view is

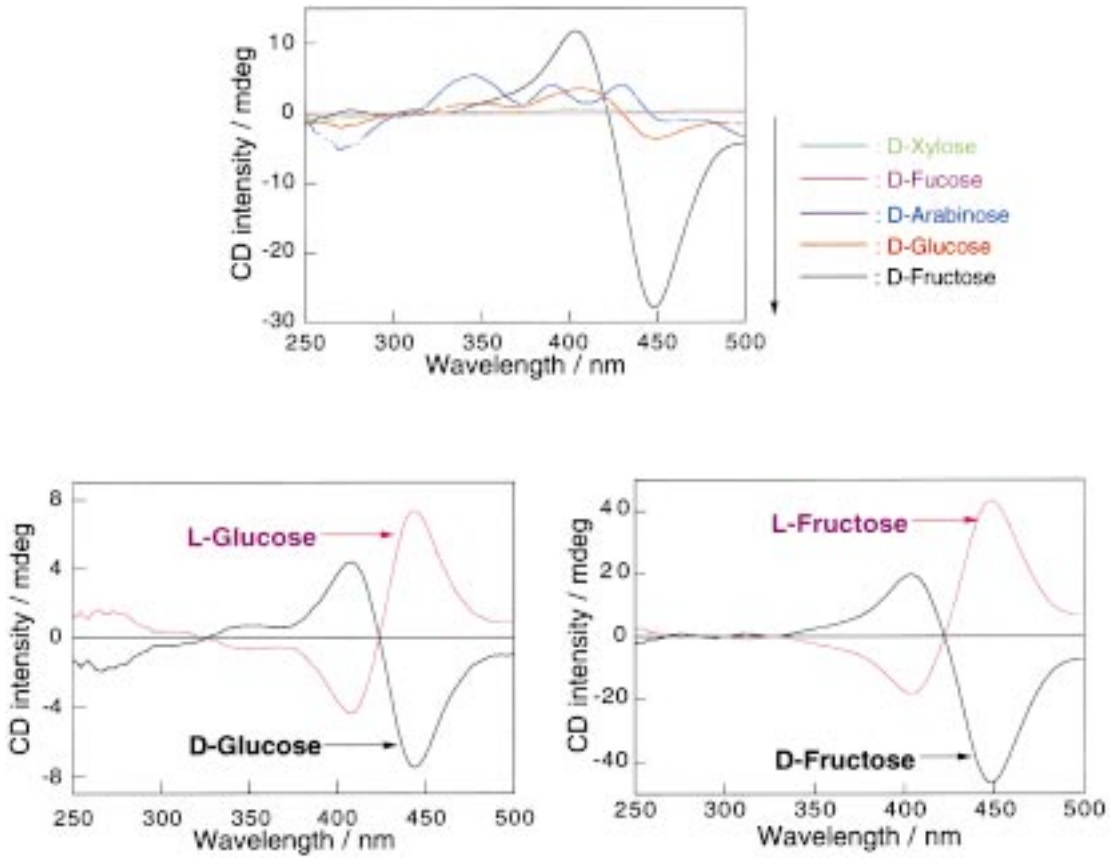


Figure 2. CD spectra of **2** (1.00×10^{-5} M) in the presence of various saccharides (0.100 M) at 25°C

further supported by the fact that 4,6-*O*-ethylidene-α-D-glucose and D-glucose-6-phosphate dipotassium salt which have only one useful diol moiety cannot yield the perceptible CD band. This bridging effect suppresses the rotation of the two porphyrin planes and is regarded to be the origin of the strong CD band appearance.

To obtain further insights into the binding manner, detailed spectral studies were conducted for D-fructose and D-glucose. The CD spectra measured as a function of saccharide concentration provided several isosbestic points, indicating that the reaction consists of only two species under one equilibrium. Fig. 3 shows plots of CD intensity at 405 nm vs. [saccharide]. The sigmoidal curvature observed for both D-fructose and D-glucose indicates that the binding of the guests to **2** is 'autoaccelerative'.

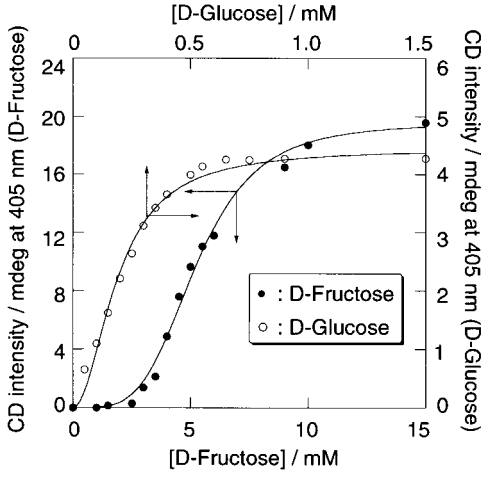


Figure 3. Plots of CD intensity for **2** (1.00×10^{-5} M) vs. the concentration of saccharide guests.

This cooperative guest binding profile can be analyzed with the Hill equation: $\log(y/(1-y))=n\log[\text{guest}]+\log K$, where K and n are the association constant and Hill coefficient, respectively, and $y=K/([\text{guest}]^{-n}+K)$.[20] From the slope and the intercept of the linear plots we obtained $K=3.7\times10^4\ M^{-2}$ and $n=2.0$ for D-fructose and $K=9.6\times10^5\ M^{-2}$ and $n=1.6$ for D-glucose. D-Fructose has the cooperativity (shown with n) larger than D-glucose, but the K value for D-fructose is smaller by 26-fold than that for D-glucose. Presumably, the binding of the first D-fructose guest strongly inhibits the rotation of the two porphyrin planes, which leads to the high cooperativity and the appearance of the particularly strong CD spectral band. As a result, the complex structure becomes so rigid that the K value cannot be so large as that for D-glucose.

The 1:2 **2**·saccharide stoichiometry of the CD-active complexes was further corroborated by a Job plot.[21] As shown in Fig. 4, a plot of CD intensity at 447 nm against [**2**]/([**2**]+[D-glucose]) has a maximum at 0.33. This supports the view that the complex consists of one **2** host and two D-glucose guests. In a Scatchard plot,[22] the positive and negative allosterisms are expressed by the upward and

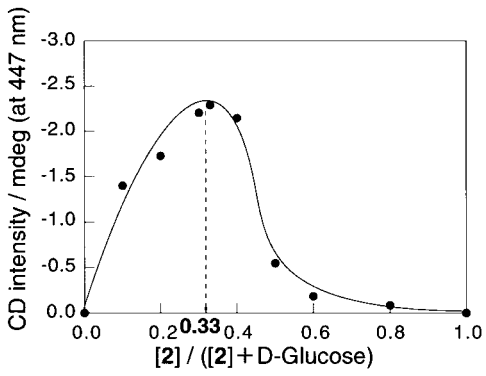


Figure 4. Job plot: the sum [**2**]+[D-glucose] was kept constant (1.00×10^{-4} M). The downward curvature in [**2**]/([**2**]+[D-glucose])=0.5–0.8 region is due to allostericity in the D-glucose binding.

downward curvatures, respectively. As shown in Fig. 5, both D-fructose and D-glucose result in the upward curvature supporting the positive allosterism. The maximum values (y_m) are correlated with the Hill coefficients (n) with $n = 1/(1 - y_m)$.[22] The computation according to this relationship predicts that the y_m values for D-fructose and D-glucose should appear at 0.50 and 0.37, respectively. It is seen from Fig. 5 that this requirement is almost satisfied.

The foregoing findings consistently indicate that the binding of the first saccharide guest suppressed the rotational freedom of the two porphyrin planes, which facilitates the binding of the second saccharide guest. As a result, two pairs of boronic acid groups in **2** can cooperatively bind these saccharide guest molecules and yield CD-active species. This novel saccharide-binding manner is schematically shown in Fig. 6.

The energy-minimized structure for the 1:2 **2**·D-glucose complex has been estimated by a computational method using Insight II/Discover 3 (Molecular Simulations Inc.)[23]. The results are shown in Fig. 7. As the D-glucose complex is formed in an aqueous medium containing carbonate buffer, one may adopt the furanose form for the bound guest molecule according to a proposal by Norrild and Eggert (see Fig. 8C).[24] The energy-minimized structure thus obtained has been compared with the crystal structure determined for **3** (symmetrical double decker as in **2**) and **4** (asymmetrical double decker).[25,26] The data are summarized in Tables 1 and 2.

Firstly, the Ce–N distance is ca. 2.48 Å for **3** and **4**, which shows a very good agreement with that of energy-minimized **2** (2.48 Å: Table 1). Secondly, the X-ray structures of **3** and **4** feature warped, dome-like porphyrin planes which are induced by steric and/or electrostatic repulsion. This warping magnitude can be evaluated by angle A which is defined as an angle between the least-squares plane of four nitrogens and that of a pyrrole plane (Fig. 8A). Compounds **3** and **4** have 15.5 and 13.9°, respectively,[25,26] while energy-minimized **2** has 19.6° (Table 2). Although angle A for energy-minimized **2** is somewhat

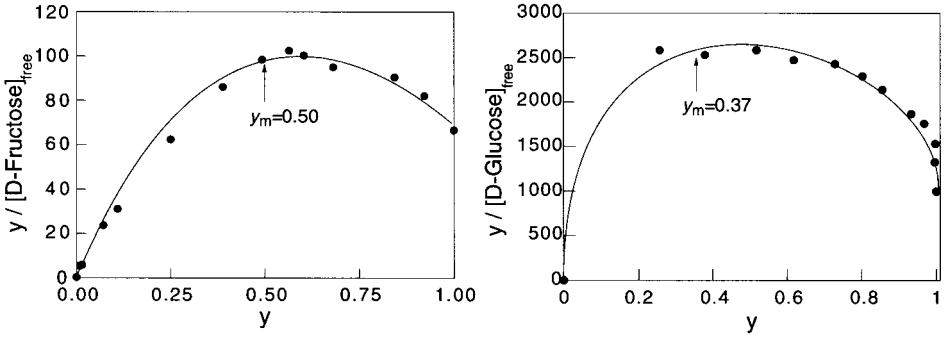


Figure 5. Scatchard plots.

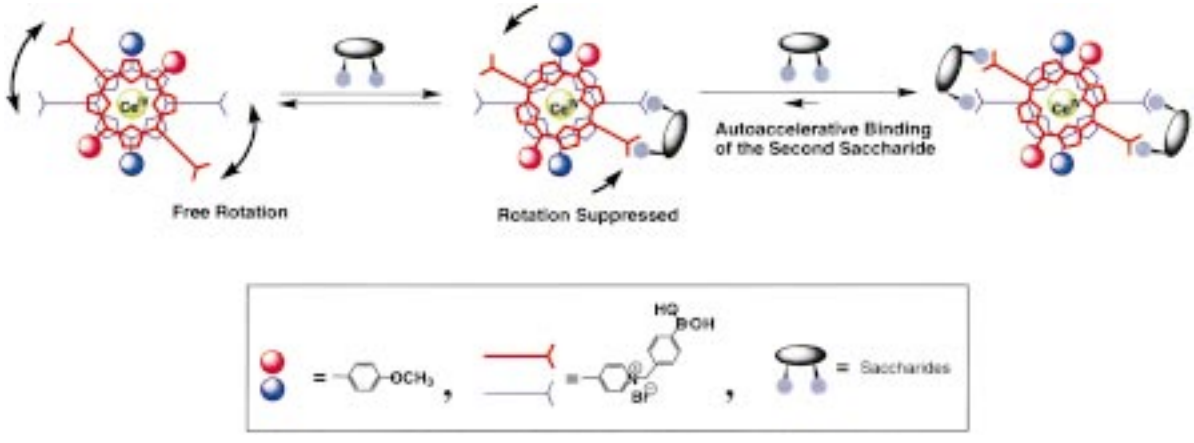


Figure 6. Schematic representation of positive, homotropic saccharide-binding to **2**.

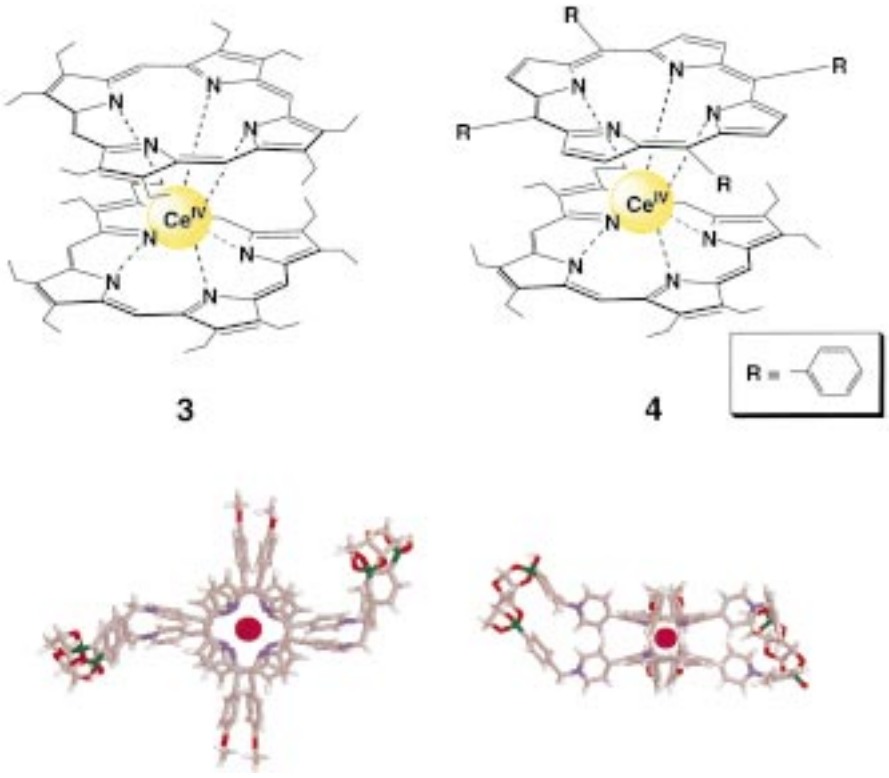


Figure 7. Top view (left) and side view (right) of an energy-minimized 2·D-glucose complex.

larger, one may rationalize this by steric bulkiness of *meso*-aryl substituents in **2**. Thirdly, it is known that two porphyrin planes in double decker porphyrins tend to adopt a 'square antiprism' conformation in order to minimize the steric crowding. As seen in Table 2, angle B for **3** and **4** (41.8 and 44.2°, respectively) exactly satisfied this conformation. In contrast, angle B for **2** is very different: free **2** has a very wide angle (77.5°) whereas **2**·(D-glucose)$_2$

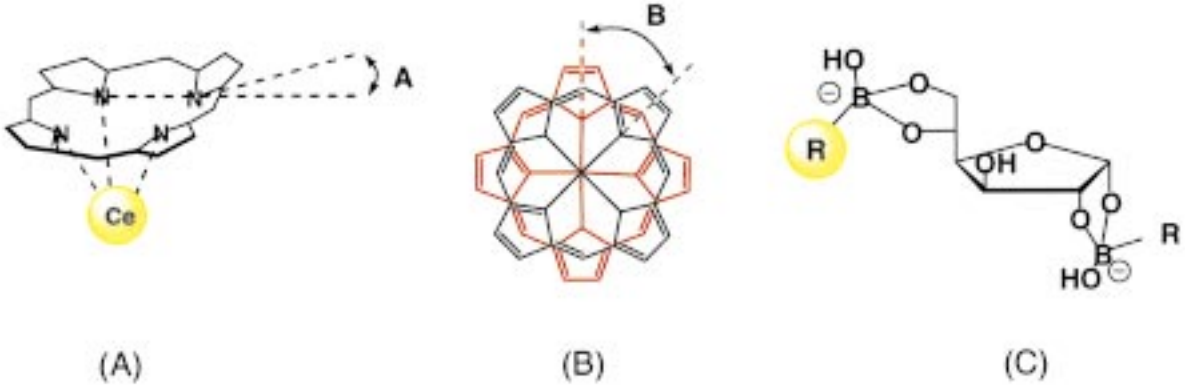


Figure 8. Definition of: (A) angle A; (B) angle B; and (C) complexation mode of D-glucofuranoside with two boronic acids [R-B(OH)$_3^-$].

Table 1. Ce–N bond lengths (Å) in **3**, (OEP) and (TPP) fragments of **4**, energy-minimized **2** and **2**·(D-glucose)$_2$ complex

Bonds (Å)	**3**		**4**		**2**	**2**·(D-glucose)$_2$ complex
			OEP	TPP	Computer calculation	Computer calculation
Ce–N	2.476	2.487	2.465	2.463		
	2.474	2.483	2.465	2.485		
	2.467	2.474	2.481	2.475		
	2.475	2.483	2.473	2.498		
Ce–N			2.471	2.480		
Mean values	2.475		2.476		2.479	2.489

Table 2. Two angles A and B (°) of **3**, (**OEP**) and (**TPP**) fragments of **4**, energy-minimized **2** and **2**·(D-glucose)$_2$ complex

Angles (°)	**3**	**4**	**2** Computer calculation	**2**·(D-glucose)$_2$ complex Computer calculation
A (mean values)	15.5	13.9	19.6	21.1
B (mean values)	41.8	44.2	77.5	17.1

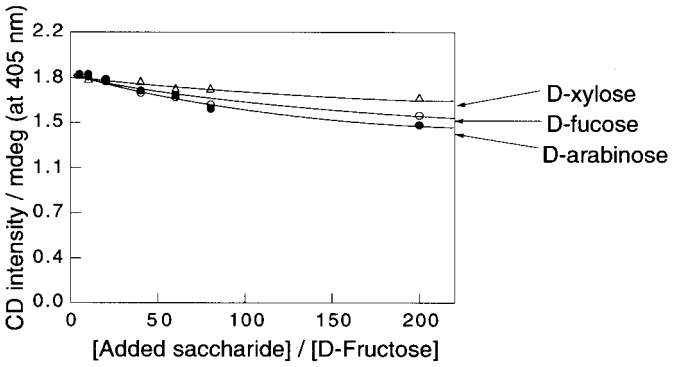


Figure 9. Substitution of D-fructose in ([**2**]=1.0×10^{-6} M, [D-fructose]=1.5×10^{-3} M) with other competitive saccharides.

complex features a very small angle (17.1°) although these angles should be energetically-unfavorable from the viewpoint of steric crowding. The small angle observed for **2**·(D-glucose)$_2$ complex is readily rationalized in terms of bridging of two pairs of boronic acids with D-glucose molecules, but the rationale for the wide angle for free **2** is more difficult. We conceive at present that two pairs of pyridinium groups repulse each other to reduce electrostatic repulsion between two cationic charges.

The foregoing findings indicate that D-fructose gives the complex species with the particularly strong CD band. It is not clear, however, whether this strong intensity is really correlated with the high affinity with D-fructose. To confirm this, we applied the substitution method to the present system: that is, if the affinity with other saccharides is really comparable with that of D-fructose, D-fructose molecules in **2**·(D-fructose)$_2$ complex which is obtained in the plateau region of Fig. 3 can be replaced by added saccharides. Since the CD intensity for other saccharide complexes is incomparably smaller than that for **2**·(D-fructose)$_2$ complex, one can readily estimate this substitution from the decrease in the CD intensity. In Fig. 9, the CD intensity (405 nm) is plotted against added saccharide concentrations. The magnitudes of the CD decrease at added saccharide/D-fructose=200 are 10 % for D-xylose, 18 % for D-fucose and 22 % for D-arabinose. Provided that 2:2 substitution is taking place between D-fructose and added saccharide, it follows that K values are 4.7 M^{-2} for D-xylose, 6.3 M^{-2} for D-fucose and 3.8 M^{-2} for D-arabinose. These values are much smaller than that for D-fructose (3.7×10^4 M^{-2}), indicating that the CD intensity is correlated, although qualitatively, with the affinity.

Conclusions

In conclusion, we have demonstrated that **2** shows a novel positive, homotropic allosterism with Hill coefficients of 1.6×2.0 for the saccharide-binding in aqueous media. With this novel saccharide receptor, it becomes possible for the first time to catch and release various saccharide-containing materials according to an allosteric manner. We believe that this system should be widely applicable to the function regulation of saccharide-containing drugs, glycolipid membranes, monitoring of enzyme activities, etc.

Experimental

Material

The synthesis of bis[5,15-bis(4-methoxyphenyl)-10,20-di(4-pyridyl)porphyrinato]cerium(IV) (**5**) will be reported elsewhere.[27] Compound **5** and 2-(4-bromomethylphenyl)-1,3-dioxa-2-borinan (103 mg, 0.403 mmol) were dissolved in dehydrated DMF (10 ml) and the mixture was heated at 55°C under stirring. The progress of the quarternization reaction was monitored by a TLC method (silica gel, *n*-butanol/water/acetic acid=4:1:1 v/v/v). After confirming that the reaction ceased, the solution was evaporated to dryness, the solid residue being washed with ether and then with acetone. The purification by reprecipitation from methanol to ether afforded 1,3-propanediol protected **2** as brownish purple powder: mp>300°C (decomp.), 27 mg (yield 54%). IR (KBr): 1340.0 cm^{-1} (B–O). ^{1}H NMR (600 MHz, methanol-d_4, -60°C; peaks were broadened at room temperature), δ (ppm): 1.70–1.72 (m, 8H), 3.63 (t, 16H, J=6.09 Hz), 4.11 (s, 12H), 6.16 (br. s, 8H), 6.48 (br. s, 4H), 6.97 (br. s, 4H), 7.29 (br. s, 4H), 7.82 (br. s, 4H), 7.89 (br. s, 8H), 8.05 (br. s, 8H), 8.42 (br. s, 8H), 8.68 (br. s, 8H), 9.11 (br. s, 4H), 9.48 (br. s, 4H), 9.89 (br. s, 4H), 10.01 (br. s, 4H). Anal. Calcd for $CeC_{128}H_{123}B_4Br_4N_{12}O_{20}$: C, 57.96; H, 4.67; N, 6.33 %. Found: C, 57.78; H, 4.26; N, 6.48 %.

Miscellaneous

Absorption spectra, CD spectra and ^{1}H NMR spectra were measured with a Shimadzu UV 2500-PC spectrophotometer, a JASCO J-720WI spectrophotometer and a Bruker DMX 600 spectrophotometer, respectively.

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18. ^{1}H NMR spectroscopy showed that the protecting groups are readily eliminated in aqueous media. In fact, addition of 1,3-propanediol ($\sim 10^{-4}$ M) scarcely affected the CD intensity vs. [saccharide] plots (Fig. 3). Hence, one can use this compound without deprotection treatment.
19. Compound **2** decomposed very slowly in this solvent. It was confirmed from an absorption spectroscopic study, however, that **2** is stable at least for 3 h. Hence, all measurements were completed within 1 h.
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23. Conformations with low potential energy encountered during 100 ps MD simulation at 300 K were selected. The system was minimized using the conjugate gradient of 0.01 kcal mol^{-1} Å^{-1}. Force field used in this study was the ESFF.
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