graphic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-144465. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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Novel Oligosaccharide Binding to the Cerium(IV) Bis(porphyrinate) Double Decker: Effective Amplification of a Binding Signal through Positive Homotropic Allosterism

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The biomimetic design of allosteric systems is of great significance because they are readily applicable to the efficient regulation of drug release, catalytic reactions, and information transduction.[1] In particular, the positive homotropic allosteric system is useful as a unique tool for amplifying and transforming weak chemical or physical signals into other forms and for constructing novel sensory systems with higher affinity and/or greater selectivity towards analytes. Although there are several examples of artificial heterotropic allosterism in which a substrate and an effector communicate (either positively or negatively) with each other,[1, 2] successful examples of artificial positive homotropic allosterism, however, are very limited.[3–6]

Undoubtedly, the allosteric binding of saccharides that can take place even in aqueous media is essential as a research target in molecular recognition and influential in 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. Previously, we demonstrated that the cerium(IV) bis[tetrakis(4-pyridyl)porphyrinate] double decker (**1**) binds certain dicarboxylic acids in a positive allosteric

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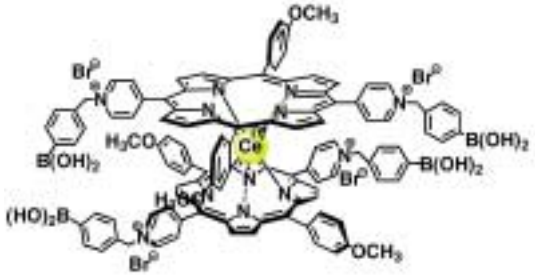

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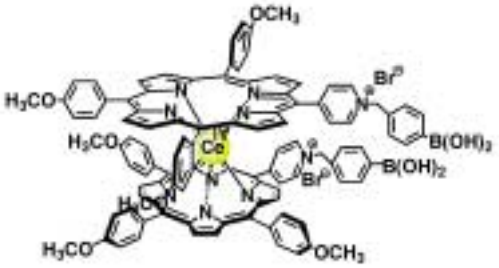

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manner (Hill coefficient 4.0) through hydrogen-bonding interactions to form only the 1:4 complex.[5] In this system the binding of the first dicarboxylic acid to a pair of pyridyl groups through the hydrogen-bonding interaction, although very weak, can suppress the rotation of the two porphyrin planes; as a result, the subsequent binding of the three dicarboxylic acids to the three pairs of aligned pyridyl groups can occur cooperatively. This characteristic double-decker architecture can be used as a scaffold in a system showing positive allosteric binding[6a–c] of saccharides by introducing boronic acid groups, which are known to act as excellent saccharide receptors in aqueous media.[7, 8] By taking these factors into consideration, we designed compound **2** which bears two pairs

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Supporting information for this article is available on the WWW under http://www.wiley-vch.de/home/angewandte/ or from the author.

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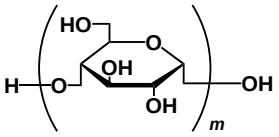
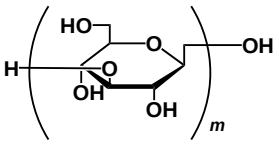
of boronic acid groups. Very surprisingly, we found that **2** can bind oligosaccharides (maltooligosaccharides and laminarioligosaccharides) with the large association constants ($K = 10^5 - 10^6\,\text{M}^{-2}$) typical for a positive homotropic allosterism in aqueous media to form 1:2 **2**:saccharide complexes. This positive homotropic allosterism is indispensable for the highly efficient binding of oligosaccharides which had until now not been achieved,[9] and the amplification of the chemical or physical signal by host–guest interactions.

Compound **2**[6c, 10] has previously been synthesized by treatment of bis[5,15-bis(4-methoxyphenyl)-10,20-di(4-pyridyl)porphyrinato]cerium(IV) with 2-(4-bromomethylphenyl)-1,3-dioxaborane in DMF. 1,3-Propanediol-protected **2** was characterized by IR and ^{1}H NMR spectroscopy and elemental analysis. This product was used for further spectral measurements without deprotection of the propanediol groups.[11]

We evaluated the binding affinities of **2** toward oligosaccharides such as maltooligosaccharides ($\mathbf{M}_m$: α-1,4-linked oligomers of D-glucose) and laminarioligosaccharides ($\mathbf{L}_m$: β-1,3-linked oligomers of D-glucose). The addition of oligosaccharide to a solution of **2** (1.00×10^{-5} M) in a mixture of 50 mM carbonate buffer and MeOH (1:1, v:v) at pH 10.5, resulted in



m = 1: $\mathbf{M_1}$
= 2: $\mathbf{M_2}$
= 3: $\mathbf{M_3}$
= 4: $\mathbf{M_4}$
= 5: $\mathbf{M_5}$
= 6: $\mathbf{M_6}$
= 7: $\mathbf{M_7}$



m = 2: $\mathbf{L_2}$
= 3: $\mathbf{L_3}$
= 4: $\mathbf{L_4}$
= 5: $\mathbf{L_5}$
= 6: $\mathbf{L_6}$
= 7: $\mathbf{L_7}$

virtually no change in the absorption spectra ($\lambda_{max} = 409$ nm) of **2**. In contrast, exciton-coupling-type CD bands, which have a spectral pattern inherent to each oligosaccharide, were clearly observed upon addition of oligosaccharides (Figure 1). The saturated CD_{max} values at 405 nm plotted against ***m*** reveal that a) in the $\mathbf{M}_m$ series, maltose ($\mathbf{M_2}$) and maltopentaose ($\mathbf{M_5}$) give a particularly strong peak at 405 nm whereas D-glucose ($\mathbf{M_1}$) and the other maltooligosaccharides give a relatively weak peak at 405 nm, b) the complexes with $\mathbf{M_1}$ and $\mathbf{M_5}-\mathbf{M_7}$ give a positive peak at 405 nm whereas $\mathbf{M_2}-\mathbf{M_4}$ all give a negative peak at 405 nm, and c) in the $\mathbf{L}_m$ series, $\mathbf{L_1}-\mathbf{L_7}$ all give a positive peak at 405 nm (see Supporting Information). The results suggest that 1) $\mathbf{M_2}$ and $\mathbf{M_5}$ can form stable complexes with **2**, 2) two porphyrin planes are oriented into opposite

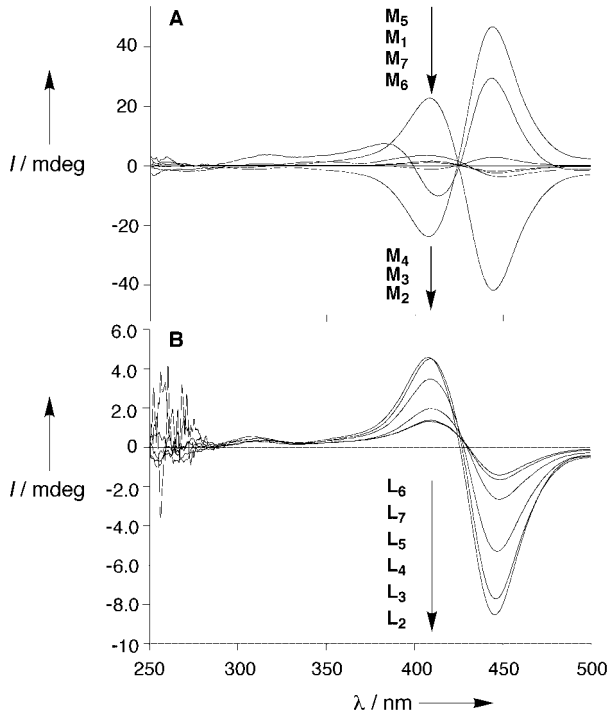


Figure 1. CD spectra of **2** (1.00×10^{-5} M) in the presence of $\mathbf{M}_m$ (A) or $\mathbf{L}_m$ (B); I denotes the CD intensity; $[\mathbf{M}_m] = 3.00 \times 10^{-3}$ M; $[\mathbf{L}_m] = 2.00 \times 10^{-2}$ M) at 25 °C in a mixture of 50 mM carbonate buffer and MeOH (1:1, v:v) at pH 10.5.

directions in the $\mathbf{L}_m$, $\mathbf{M_1}$, and $\mathbf{M_5}-\mathbf{M_7}$ complexes than in the other complexes. In addition, *n*-dodecyl-β-maltoside and *p*-nitrophenyl-α-maltopentaoside, which have only one available diol moiety for binding to boronic acid, do not yield any perceptible CD bands.[12] These findings consistently support the view that two diol moieties in the two terminal glucose units of the saccharides are bound to two boronic acid groups in **2** and bridge two porphyrin planes. This bridging effect suppresses the rotation of the two porphyrin planes and is regarded as the origin of the strong CD band.[2i]

Detailed spectral studies were carried out on the $\mathbf{M}_m$ series to obtain further insights into the binding mode. The CD spectra measured as a function of the saccharide concentration provided several isosbestic points, which indicated that the reaction consists of only two species in a single equilibrium (see Supporting Information). Figure 2 shows plots of the CD intensity at 405 nm versus $[\mathbf{M}_m]$. Very interestingly, compound **2** shows a sigmoidal binding isotherm for $\mathbf{M_1}-\mathbf{M_5}$, which indicates that the binding of the guests to **2** is "cooperative". 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 $y = K/([\text{guest}]^{-n} + K)$.[13] We obtained K (M^{-2}) and n for maltooligosaccharides from the intercept and the slope of the linear plots, respectively (Table 1 and see Supporting Information). It is seen from Table 1 that a) the association constants for oligosaccharides are greater than that of the monosaccharide $\mathbf{M_1}$ (D-glucose), b) Hill coefficients n of 1.6–2.0 for $\mathbf{M}_m$ are consistent with a highly cooperative binding mechanism, and c) the saturated CD_{max} values increase with an increase in the K values. These

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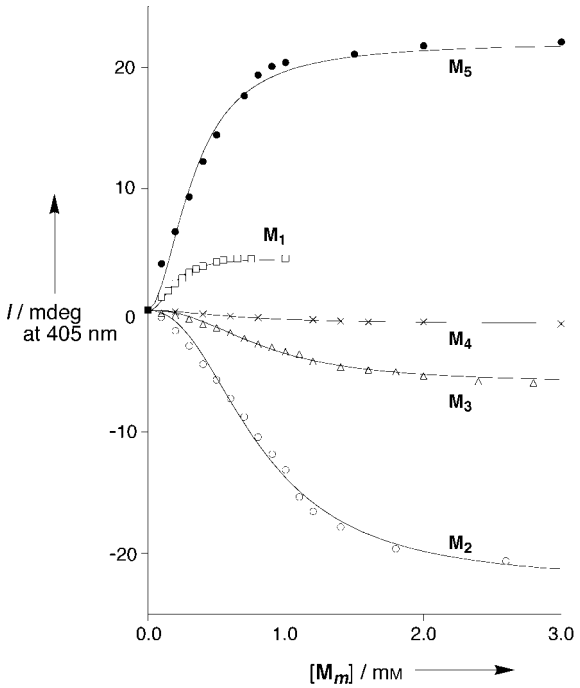


Figure 2. Plots of the CD intensity of **2** (1.00×10^{-5} M) at 405 nm versus $[\mathbf{M}_m]$. The solid lines represent the theoretical curves for the formation of the $[\mathbf{2} \cdot (\mathbf{M}_m)_2]$ complex. The measurement conditions are given in the caption to Figure 1.

Table 1. Binding parameters obtained from the Hill plot and the Job plot.

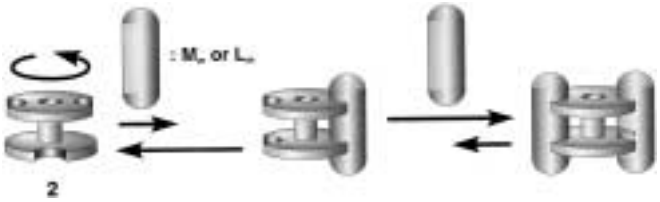
$\mathbf{M}_m$	K [M^{-2}]	n	Stoichiometry
$\mathbf{M}_1$ (D-glucose)	9.6×10^5	1.6	1:2
$\mathbf{M}_2$	2.9×10^6	2.0	1:2
$\mathbf{M}_3$	1.5×10^6	2.0	1:2
$\mathbf{M}_4$	5.7×10^5	1.8	1:2
$\mathbf{M}_5$	2.9×10^6	1.8	1:2
$\mathbf{M}_6$	–[a]	–[a]	–[a]
$\mathbf{M}_7$	–[a]	–[a]	–[a]

[a] The CD spectral changes were too small to determine K and n.

results mean that the saccharide giving the largest CD intensity has the largest affinity to **2**.

The stoichiometry of the CD-active complexes was further confirmed by a Job plot.[14] A plot of the CD intensity at 405 nm against $[\mathbf{2}]/([\mathbf{2}]+[\mathbf{M}_m])$ has a maximum at 0.33 (see Supporting Information). This observation supports the view that the complex consists of one host (**2**) and two guests ($\mathbf{M}_m$). In the Scatchard plots in which Hill coefficients (n) are correlated with the maximum values (y_{max}) by $n = 1/(1 - y_{max})$,[15] the positive and negative allosterisms are expressed by the upward and downward curvatures, respectively. Scatchard plots of the $\mathbf{M}_m$ series result in the upward curvature, which supports the positive allosterism (see Supporting Information).

The findings consistently indicate that the binding of the first saccharide guest suppressed the rotational freedom of the two porphyrin planes and that the remote second binding site is aligned for the highly cooperative binding of the second guest. As a result, two pairs of boronic acid groups in **2** can cooperatively bind the oligosaccharide guest molecules with high association constants and give CD-active species. The binding of the saccharide is conceptually illustrated in Scheme 1. The most stable conformations of the $[\mathbf{2} \cdot (\mathbf{M_2})_2]$ and $[\mathbf{2} \cdot (\mathbf{M_5})_2]$ complexes as calculated by computational



Scheme 1.

methods (Discover 3/Insight II 98.0) are shown in Figure 3.[16] It is seen from Figure 3 that the $[\mathbf{2} \cdot (\mathbf{M_2})_2]$ complex has a right-handed helical twist whereas the $[\mathbf{2} \cdot (\mathbf{M_5})_2]$ complex with the longer oligosaccharide chain length, has a left-handed helical twist. This result undoubtedly shows that the difference in the length of the oligosaccharide is the origin of the opposite CD signs for the two complexes.

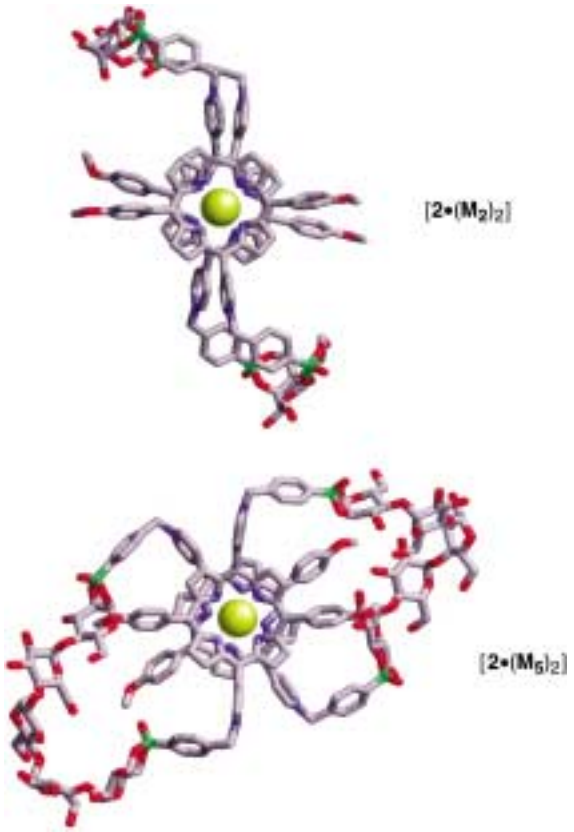


Figure 3. Energy-minimized structures of the $[\mathbf{2} \cdot (\mathbf{M_2})_2]$ (top) and $[\mathbf{2} \cdot (\mathbf{M_5})_2]$ (bottom) complexes.

To clarify the role of the positive homotropic allosterism in **2** we studied the binding of maltose ($\mathbf{M_2}$) to **3**, which has only one pair of boronic acids. Analysis of the titration curve for **3** with $\mathbf{M_2}$ gave an association constant of 154 M^{-1} for the formation of the 1:1 complex $[\mathbf{3} \cdot \mathbf{M_2}]$ (see Supporting Information). The saturated CD_{max} value at 446 nm of the $[\mathbf{3} \cdot \mathbf{M_2}]$ complex was 18 times smaller than that of the

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$[\mathbf{2}\cdot(\mathbf{M_2})_2]$ complex. These results clearly support the view that positive homotropic allosterism is indispensable for the highly efficient binding of the oligosaccharide and the amplification of the CD signal from the $[\mathbf{2}\cdot(\mathbf{M}_m)_2]$ complex.

Finally, we measured the ^{1}H NMR chemical shifts of $[\mathbf{2}\cdot(\mathbf{M_2})_2]$ in CD_3OD to obtain structural information (see Supporting Information). The *endo-m*-proton of the 4-methoxyphenyl groups and *endo-m*-proton of the pyridinium groups shifted to higher magnetic fields than in **2**. This observation indicates that the peripheral *meso*-aryl moieties overlap each other in the $[\mathbf{2}\cdot(\mathbf{M_2})_2]$ complex as illustrated in Figure 3.[17]

There are many biologically important oligosaccharides to which this receptor may be applied. As a preliminary experiment, we have found that Sialyl Lewis^X, which is a trigger saccharide for cell adhesion, can be also bound to **2** as a result of positive homotropic allosterism ($K = 3.2\times10^6\,\text{M}^{-2}$, $n = 2.0$: see Supporting Information).

In conclusion, we have demonstrated that **2** is a scaffold for the effective binding of oligosaccharides in aqueous media, and shows positive, homotropic allosterism with Hill coefficients of 1.6–2.0. Significant binding of oligosaccharides is nearly impossible without the aid of positive homotoropic allosterism. With this saccharide receptor it becomes possible for the first time to catch and release various saccharide-containing materials in an allosteric manner. We believe that this system should be widely applicable, for example, to sensing biologically important oligosaccharides, the regulation of the function of saccharide-containing drugs and glycolipid membranes, and the monitoring of enzyme activities.

Experimental Section

CD Spectroscopy: A stock solution of oligosaccharide prepared in water was added to a solution of **2** (1.00×10^{-5} M) in a mixture of carbonate buffer and MeOH (1:1, 50 mM) at pH 10.5. The CD spectra were recorded from 250 to 500 nm with a JASCO J-720WI spectrophotometer at 15 different concentrations of each guest molecule at 25 °C.

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

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[11] ^{1}H NMR spectral measurements have established that the protecting groups are readily eliminated in aqueous media. In fact, the addition of 1,3-propanediol ($\sim10^{-4}$ M) scarcely affected the plots of the CD intensity versus [saccharide] (Figure 2). Hence, this compound can be used without needing any special treatment to deprotect it.

[12] [*n*-dodecyl-β-maltoside] = [*p*-nitrophenyl-α-maltopentaoside] = 2.00×10^{-2} M.

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[16] Conformations with low potential energies located during a 100 ps MD simulation at 300 K were selected. The system was minimized using the conjugate gradient and Newton–Raphson methods until convergence was attained for a gradient of 0.01 kcal mol^{-1} Å^{-1}. The force field used in this study was ESFF.

[17] An alternative possible explanation is that the complex-induced chemical shifts arise from the twist of the *meso*-aryl groups to an outer side (against the Ce^{IV} ion).

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