First Successful Molecular Design of an Artificial Lewis Oligosaccharide Binding System Utilizing Positive Homotropic Allosterism

Atsushi Sugasaki, Kazunori Sugiyasu, Masato Ikeda, Masayuki Takeuchi, and Seiji Shinkai*

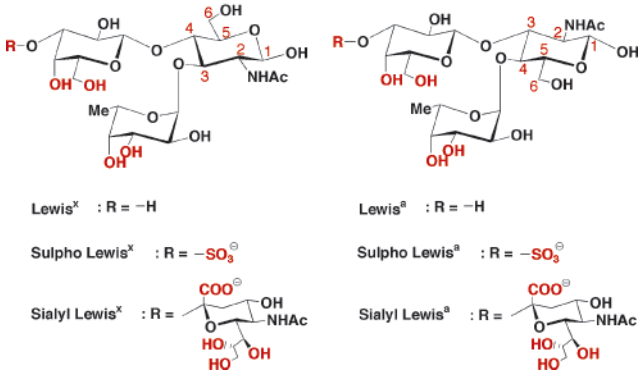
Contribution from the Department of Chemistry and Biochemistry, Graduate School of Engineering, Kyushu University, Fukuoka 812-8581, Japan

Received March 28, 2001. Revised Manuscript Received August 14, 2001

Abstract: We have designed phenylboronic acid group appended Ce(IV) bis(porphyrinate) double decker **1** and meso−meso linked porphyrin **2**, useful for the allosteric binding of biologically important saccharides, Lewis oligosaccharides. Compound **1** binds Lewis oligosaccharides in aqueous media because of the boronic acid−diol interaction, but the complexation event can occur only above the critical concentrations because of the sigmoidal [oligosaccharide] versus [complex] isotherm. Compound **1** has a sufficiently high affinity with Lewis oligosaccharides ($K = 10^5-10^6$ M^{-2}) with Hill coefficients n of 1.8−2.0, and Lewis^X series and Lewis^a series give opposite, symmetrical CD spectra. This is the first example of efficient binding of Lewis oligosaccharides to the artificial receptor, which has become possible by positive homotropic allosterism.

Introduction

It is known that oligosaccharides play crucial roles in molecular recognition events in biological systems. Particularly noteworthy are Lewis oligosaccharides, such as Lewis^X (Le^X) and Lewis^a (Le^a), which are involved in the adhesion of leukocytes and neutrophils to vesicular endothelical cells during normal and pathogenic inflammatory responses.[1,2] Appearance of Lewis oligosaccharides on the cell surface and in the blood serum above the critical concentrations is often a sign associated with tumor progression and malignancy. For example, overexpression of sialyl Lewis^X (sLe^X) containing mucins has been found in the sera of gastrointestinal, pancreatic, and breast cancer patients.[3] Thus, it is undoubted that sensitive detection of Lewis oligosaccharides is one of the most urgent subjects to be developed in the chemical therapeutic field. So far, however, the investigation has been limited to the approach from the biochemical field;[4,5] the typical examples are (i) the structural analysis of Lewis oligosaccharides/E-, P-, and L-selectin complexes as a model of cell adhesion and (ii) the synthetic approach to these oligosaccharides to develop inhibitors for selectins.



Our research purpose is, in contrast to these preceding biochemical approaches, to develop rationally designed artificial synthetic receptors for these oligosaccharides, which will lead to generation of small molecular antagonists and design of specific sensory systems. It is undoubted that the development of such artificial receptors is very useful for the sensitive and convenient detection of these oligosaccharide antigens in the blood serum.[6]

Then, what is the potential strategy for the molecular design? There are two basic requirements: that is, (i) to sensitively detect Lewis oligosaccharides, the receptors must have the large

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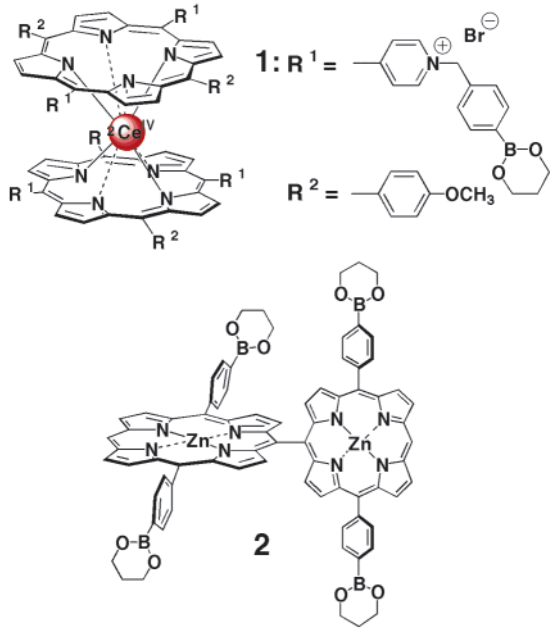
10.1021/ja010806e CCC: $20.00
Published on Web 09/29/2001

association constants and (ii) because they are expressed even in normal tissues and sera,[7] the receptor's function must change from a tense (T) state to a rest (R) state only above the critical concentrations as alllosteric proteins seen in nature.

It thus occurred to us that the concept of "positive homotropic allosterism"[8,9] might be successfully applied to the molecular design of a desired Lewis oligosaccharide binding system, because (i) the binding isotherm is characterized by a sigmoid-shaped curve with a steep threshold region and (ii) the association constants and binding signal are amplified by the cooperative action in a positive homotropic allosteric binding process.[9h,i] Provided that positive homotropic allosterism operates, as expected, in the Lewis oligosaccharide binding process, it would become possible to detect these essential oligosaccharides selectively and sensitively only above the critical concentrations.

Recently, we designed phenylboronic acid group appended cerium(IV) bis(porphyrinate) double decker **1**[10] and meso−meso linked porphyrin dimer **2**[11] as artificial oligosaccharide receptors.[12] In these studies, we have demonstrated that (i) compounds **1** and **2** can bind malto- or laminari-oligosaccharides cooperatively and effectively in aqueous solution, (ii) the positive homotropic allosterism is indispensable for highly efficient oligosaccharide binding, and (iii) the binding signal is amplified according to a sigmoidal isotherm through positive homotropic allosterism. These findings clearly show that these compounds would act as candidates for Lewis oligosaccharide binding receptors, which are expected to work even in aqueous solution.

In this contribution, we report that **1** can bind Lewis oligosaccharides because of positive homotropic allosterism in aqueous media to form 1:2 **1**/Lewis oligosaccharide complexes with Hill coefficients 1.8−2.0. To the best of our knowledge,



this is the first totally synthetic artificial receptor which can touch Lewis oligosaccharides in aqueous media *sensitively only above the critical concentrations*. The rational amalgamation of artificial saccharide binding moieties, "boronic acids,"[13] and the concept of "allosterism" now paves the way to construct a biologically important oligosaccharide sensing system useful in aqueous solution.

Results and Discussion

Molecular Design. Diboronic acid derivatives, which can react with four of the five OH groups of saccharide to form intramolecular 1:1 complexes, show a different stability order, which is related to the specific spatial position of two boronic acid groups.[13] This implies that one can recognize a specific saccharide guest by appropriate manipulation of two boronic acids in a same host molecule. The present research aim is to extend this concept to the selective binding of biologically important Lewis oligosaccharides. This idea has been tested with a few diboronic acid systems bearing a "long" and "rigid" spacer: for example, diphenyl-3,3′-diboronic acid, stilbene-3,3′-diboronic acid,[14] and *cis*-5,15-bis[2-(dihydroxyboronyl)phenyl]-10,20-diphenylporphirin[15] show some selectivity for certain disaccharides, but the selectivity and the affinity observed so far are not so high. To improve the affinity and the selectivity toward oligosaccharides, one should look for a new conceptual design scheme by which one might be able to finely tune the distance between two boronic acid groups. From recent research on artificial cooperative recognition systems, it has been suggested that positive homotropic allosterism can be utilized as a new concept to achieve both high guest selectivity and high guest affinity which cannot be attained by the conventional 1:1-type guest binding.[9] The scaffolds that show positive

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(10) Sugasaki, A.; Ikeda, M.; Takeuchi, M.; Shinkai, S. *Angew. Chem., Int. Ed.* **2000**, *39*, 3839. The phenyl boronic acid group appended Ce(IV) bis(porphyrinate) double decker **1** can bind maltooligosaccharides effectively through positive homotropic allosterism to form only the 1:2 complexes. In this system, the binding of the first oligosaccharide to a pair of boronic acid groups, although very weak, can suppress the rotation of the two porphyrin planes; as a result, the subsequent binding of the oligosaccharide to the residual pair of aligned boronic acid groups can occur cooperatively.

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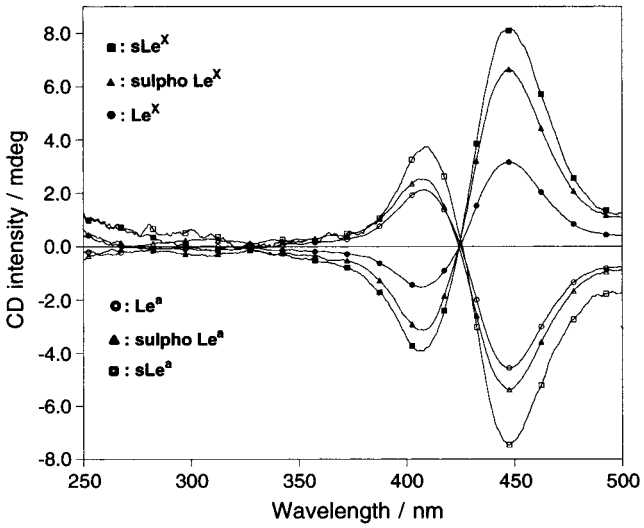

Figure 1. CD spectra of **1** (1.00×10^{-4} M) in the presence of Lewis oligosaccharides (2.10×10^{-3} M) at 25 °C.

homotropic allosterism are mostly with dynamic motion and are skillfully combined with the molecular recognition systems so that the subsequent guest binding can occur more favorably than the first guest binding. We thus amalgamate two strategies, diboronic acid receptor and positive homotoropic allosterism, in designing compounds **1** and **2** that are expected to work as a robust oligosaccharide binding system. Compounds **1** and **2** are a phenylboronic acid appended bis[porphyrinato]cerium(IV) double decker porphyrin and a meso−meso linked porphyrin, respectively. In these compounds, two porphyrins can rotate (or oscillate) relative to each other like two wheels with the central metal ion or bridging C−C bond acting as an axle.[16] One can thus expect a cooperative allosteric binding mode to form 1:2 host−guest complexes.

Compounds **1** and **2** were identified by IR and ^{1}H NMR spectral evidence and elemental analyses. These products were used for further spectral measurements without removal of 1,3-propandiol groups. ^{1}H NMR spectral measurements have established that the protecting groups are readily eliminated in aqueous media. In fact, addition of 1,3-propanediol ($\sim 10^{-3}$ M) scarcely affected the CD intensity versus [saccharide] plot. Hence, one can use this compound without deprotection treatment.

Lewis Oligosaccharide Binding Studies. Binding affinities of **1** and **2** toward Lewis oligosaccharides were evaluated by UV−vis and CD spectroscopic methods. Addition of Lewis oligosaccharide to a solution of **1** (1.00×10^{-5} M) or **2** (1.00×10^{-5} M) adjusted with a mixture of 50 mM carbonate buffer/MeOH to pH 10.5 resulted in virtually no change in the absorption and fluorescent spectra. In contrast, exciton-coupling-type CD bands, which have a spectral pattern inherent to each oligosaccharide structures, were clearly observed upon addition of oligosaccharides only when we used compound **1** (Figure 1), whereas CD spectral measurements for **2** with Lewis oligosaccharides did not yield any perceptible CD bands. In general, when a saccharide guest is bound to a boronic acid group in a host molecule, the resultant complex becomes optically CD-active.[13] 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.[17] The results indicate that two diol moieties in saccharides are bound to two boronic acid groups in **1** to bridge two porphyrin planes chirally. It is seen from Figure 1 that the CD sign (positive exciton-coupling bands) obtained from the **1**/Le^X family system is opposite to that (negative exciton-coupling bands) obtained from the **1**/Le^a family system. Judging from the fact that the boronic acid group can interact only with the *cis*-1,2-diol or 1,3-diol moiety in saccharides,[13] it is undoubted that phenylboronic acid groups in **1** interact with the 4,6-diol group of the galactose moiety and the 3,4-diol group of the fucose moiety in the Le^X (Galβ1,4-[Fucα1,3]GlcNAc) or Le^a (Galβ1,3[Fucα1,4]GlcNAc) backbone. In fact, when **1** (0.50 mM) and Le^X (2.0 mM) were allowed to react in CD_3OD, the 6-methyl protons adjacent to the 3,4-diol group of the fucose moiety shifted from 1.17 to 1.71 ppm ($\Delta\delta = 0.54$ ppm) owing to the deshielding effect of the complexed phenylboronic acid group.[18] Meanwhile, we found that compound **2** acts as an excellent maltotetraose (tetrasaccharide) receptor.[11] The distance between two boronic acids in **2** is estimated to be about 15.8 Å, which is too long to bridge between the galactose moiety and the fucose moiety in Lewis oligosaccharides. Furthermore, the rotation of porphyrin rings around the bridging C−C bond, by which the distance between two boronic acid groups becomes variable, is relatively limited compared with that of **1**. These should be the possible reasons why compound **2** cannot bind any Lewis oligosccharides.

To obtain further insights into the **1**·Le^X and **1**·Le^a complex structures, the most stable conformations were evaluated by computational methods (Discover 3/Insight II).[19] In the initial structures, the boronic acid complexation sites are the 4,6-diol group of the galactose moiety and the 3,4-diol group of the fucose moiety. The resultant structures are illustrated in Figure 2. It is seen from Figure 2 that the **1**·Le^X complex has a right-handed helical twist, whereas the **1**·Le^a complex has a left-handed helical twist. These results clearly show that the structural difference in C-3 and C-4 of the *N*-acetylglucosamine residue between Le^X and Le^a is the origin of the opposite CD signs observed for the two families in Figure 1.

The CD spectral changes induced by Lewis oligosaccharides were examined more in detail. The CD spectra measured as a function of the saccharide concentration provided an isosbestic point, indicating that the reaction consists of only two species

(16) It is known that the rate of the porphyrin ring rotation in the Ce• bis(porphyrinate) double decker is comparable with or slower than the NMR time scale. However, the allosteric behavior is basically observable for the present "static" equilibrium system as long as porphyrin rings are able to rotate. See references: (a) Takeuchi, M.; Imada, T.; Ikeda, M.; Shinkai, S. *Tetrahedron Lett.* **1998**, *39*, 7897. (b) Ikeda, M.; Takeuchi, M.; Shinkai, S.; Tani, F.; Naruta, Y. *Bull. Chem. Soc. Jpn.* **2001**, *74*, 739. (c) Tashiro, K.; Konishi, K.; Aida, T. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 856. (d) Tashiro, K.; Fujiwara, K.; Konishi, K.; Aida, T. *Chem. Commun.* **1998**, 1121. (e) Tashiro, K.; Konishi, K.; Aida, T. *J. Am. Chem. Soc.* **2000**, *122*, 7921.

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(18) The ^{1}H NMR spectrum (600 MHz, −40 °C, CD_3OD) of the **1**·Le^a complex was so broadened in aqueous solution and at room temperature that the signal assignment was nearly impossible.

(19) Conformations with low potential energy encountered during 100 ps MD simulation at 500 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 the ESFF. In our calculation system, C_2 symmetrical structures were selected as the initial structures. The resultant energy-minimized structures feature D_2 symmetry, and two fucose rings exist at opposite sides of the porphyrin plane. When the calculation was started from C_S symmetrical structures, the resultant structures again feature D_2 symmetry, although two fucose rings existing at opposite sides of the porphyrin plane are a little closer to the pophyrin plane.

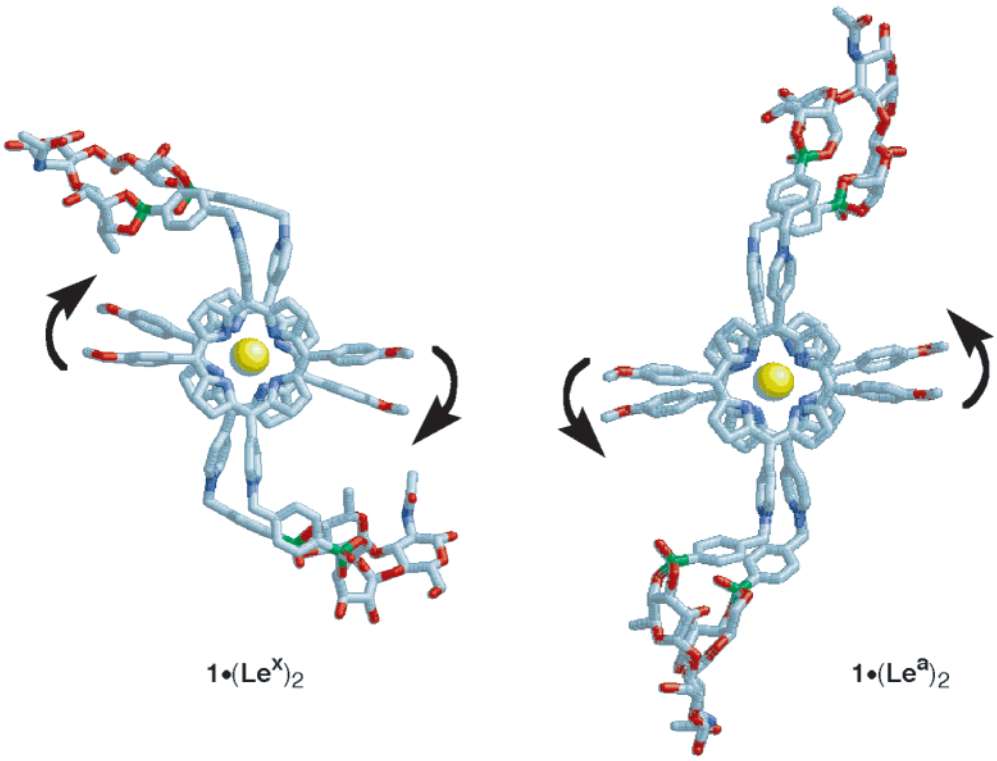

Figure 2. Energy-minimized structures of the **1**·Le^X (left) and **1**·Le^a (right) complexes.

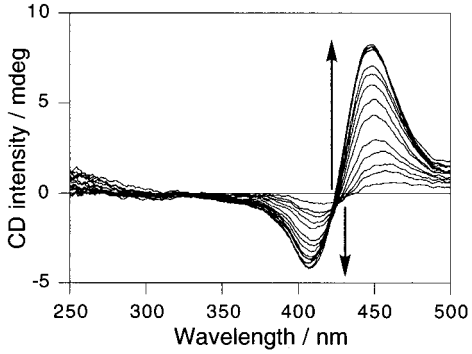

Figure 3. CD spectral changes of **1** (1.00×10^{-4} M) with an increase in Lewis oligosaccharide concentration ([sLe^x] = 0.00 to ~2.10×10^{-3} M) at 25 °C in a mixture of water (pH 10.5 with 50 mM carbonate buffer)/MeOH = 1:1 (v/v).

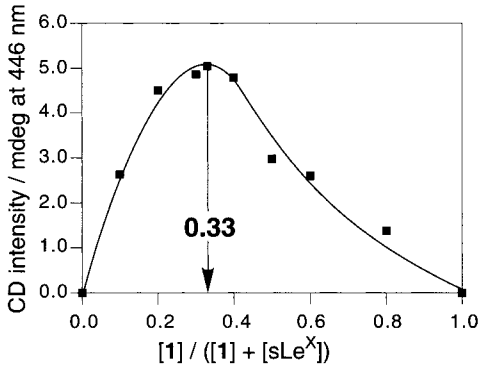

Figure 4. Job plot for **1**·sLe^X complex formation.

in a single equilibrium (Figures 3 and S1 in the Supporting Information). The stoichiometry of the CD-active complexes was further confirmed by a Job plot.[20] The plot of the CD intensity at 447 nm against [**1**]/([**1**] + [saccharide]) has a maximum at 0.33. A typical example for the **1**/sLe^x system is shown in Figure 4. This finding supports the view that the complex consists of one host (**1**) and two Lewis oligosaccharides. Plots of the CD intensity at 447 nm versus [saccharide] are shown in Figure 5. Compound **1** shows a sigmoidal binding isotherm for Lewis oligosaccharides, indicating that the binding of two equivalents of Lewis oligosaccharides to **1** occurs "cooperatively". This cooperative saccharides-binding profile can be analyzed with the Hill equation: $\log(y/(1-y)) = n \log[\text{saccharide}] + \log K$, where n, K, and y are the Hill coefficient, the association constant, and the extent of complexation, respectively, and $y = K/([\text{saccharide}]^{-n} + K)$[21] (Table 1). It is seen from Table 1 that (i) **1** has a sufficiently high affinity with Lewis oligosaccharides ($K = 10^5-10^6$ M^{-2}), (ii) Hill coefficients n of 1.8−2.0 for Lewis oligosaccharides are consistent with a highly cooperative binding mechanism forming the 1:2 complexes, (iii) the saturated CD$_{max}$ values increase with an increase in the K values in each Lewis oligosaccharide family, and (iv) selectivity among Lewis saccharides is not so high. In Scatchard plots,[22] the positive and negative allosterisms are expressed by the upward and downward curvatures, respectively. The maximum values (y_m) are correlated with Hill coefficients (n) with $n = 1/(1 - y_m)$.[22] Scatchard plots of the Lewis oligosaccharides always result in the upward curvature (see Figure S2), which indicates the operation of the positive allosterism in the binding to **1**.

We next investigated the effect of pH on the CD intensity of **1**/oligosaccharide complexes. First, the CD intensity of **1**·(maltose)$_2$ complex was measured as a function of pH ([**1**] = 5.00×10^{-6} M, [maltose] = 3.00×10^{-3} M), because we have already shown that **1** possesses almost the same affinity for both maltose and sLe^x. The CD intensity of **1**·(maltose)$_2$ complex at 446 nm decreased with lowering medium pH and changed from 28 at pH 10.5 to 12 at pH 9.5 and 1.5 at pH 7.5 (Figure 6).

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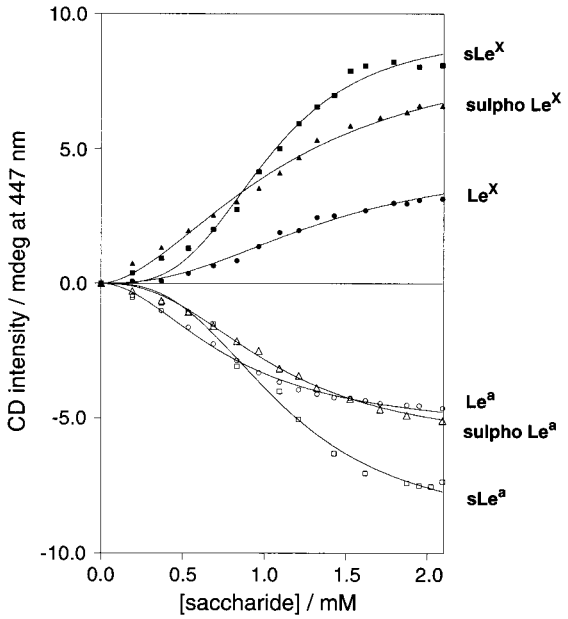

Figure 5. Plots of CD intensity of **1** (1.00×10^{-4} M) versus the concentration of oligosaccharide guests. The solid lines represent the theoretical curves for the formation of the [**1**·saccharide] complexes.

Table 1. Hill Coefficient (n), Association Constant (K/M^{-2}) and Saturated CD Intensity (CD_{446})

Lewis oligosaccharide	n	K/M^{-2}	CD_{446}
Lewis^X	2.0 ± 0.13	9.0×10^5	3.1
sulpho Lewis^X	2.0 ± 0.07	1.1×10^6	6.6
sialyl Lewis^X	2.0 ± 0.19	1.8×10^6	8.1
Lewis^a	1.8 ± 0.05	4.2×10^5	−4.6
sulpho Lewis^a	2.0 ± 0.08	5.7×10^5	−5.1
sialyl Lewis^a	2.0 ± 0.15	1.3×10^6	−7.3

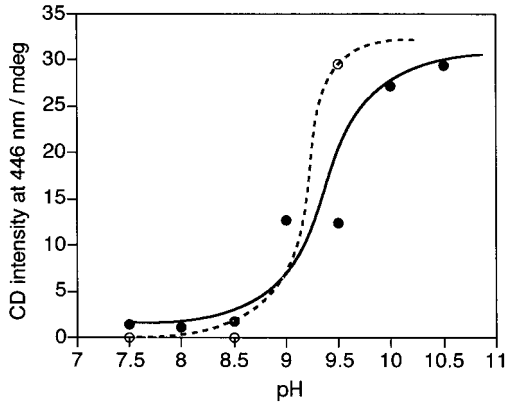

Figure 6. Effect of pH on the CD intensity of **1**/oligosaccharide complex; [**1**] = 5.00×10^{-6} M, [maltose] = 3.00×10^{-3} M (●), [sLe^x] = 2.00×10^{-3} M (○).

This decrease is probably due to the pK_a value of the phenylboronic acid groups in **1**, which is supposed to be ~9.[23] The similar pH dependence was also observed for **1**·(sLe^x)$_2$ complex ([sLe^x] = 2.00×10^{-3} M).

In detecting Lewis saccharides under physiological conditions, one must consider that a lot of chiral compounds would coexist and may interfere with Lewis oligosaccharide binding to **1**. We believe, however, that **1** is useful also under physiological conditions because of the following reasons. First, as mentioned above, the boronic acid group can interact with only *cis*-1,2-diol and 1,3-diol in a saccharide. It is known that Lewis oligosaccharides on the cell or protein surface are mostly connected via 1-OH in GalNac,[4,5] which is not included in the binding event with **1**. Thus, cell- or protein-linked Lewis oligosaccharides should be also bound to **1**. Second, electrostatic interaction should not affect the binding event in aqueous solution. In fact, we previously tested the allosteric binding of tartarate anion to a cationic double decker porphyrin.[9f] The significant binding due to electrostatic interaction was observed only in less polar solvents (THF, methanol, etc.) but not at all in aqueous solution.[9f] Hence, the binding event should not be affected by charges present on the cell or protein surface. Third, we tested whether coexisting D-glucose affects the binding event: D-glucose was selected as a competing guest because its concentration in the blood serum is considerably high (physiological glucose level: 0.3 ~ 1.0 mM) and the complex gives the opposite CD sign.[9e] The CD spectra of the **1**·(sLe^x)$_2$ complex ([**1**] = 5.00×10^{-6} M, [sLe^x] = 2.00×10^{-3} M) were only weakly affected by added D-glucose: for example, the CD intensity at 446 nm decreased 33% at [D-glucose] = 1.00×10^{-3} M and 50% at [D-glucose] = 2.00×10^{-3} M.

Conclusion

In summary, we have demonstrated that **1** can effectively bind Lewis oligosaccharides in aqueous media with the aid of positive homotropic allosterism and discriminate between Le^X and Le^a structures as the opposite CD signs. As far as we are aware, this is the first totally synthetic artificial receptor that binds Lewis oligosaccharides in aqueous solution. Our findings obtained here clearly show that the higher affinity of **1** toward Lewis oligosaccharides stems from the action of positive homotropic allosterism: that is, even though the complexation of Lewis oligosaccharides is usually very difficult, the presence of the cooperative action of two pairs of boronic acid groups has enabled us to detect them by a CD spectroscopic method. It is expected, therefore, that the utilization of positive homotropic allosterism in Lewis oligosaccharide recognition represents a new paradigm in biotechnology for selective detection of tumor-associated antigens only above the critical concentrations and promises to provide a general strategy for increasing the affinity of oligosaccharide receptors with their specific substrates.

Experimental Section

General. All starting materials and solvents were purchased form Tokyo Kasei Organic Chemicals or Wako Organic Chemicals and used as supplied. Lewis oligosaccharides were purchased from Funakoshi and were used without further purification. ^{1}H NMR spectra were recorded either on a Bruker AC 250 (250 MHz) or Bruker DRX 600 (600 MHz) spectrometer. Chemical shifts are reported in ppm downfield from tetramethylsilane as an internal standard. Mass spectral data were obtained using a Perseptive Voyager RP MALDI TOF mass spectrometer. UV−vis and CD spectra were recorded with a Shimadzu UV-2500 PC and a JASCO J-720WI spectrophotometer, respectively.

Synthesis of 1. Compound **1** was synthesized by treatment of bis-[5,15-bis(4-methoxyphenyl)-10,20-di(4-pyridyl)porphyrinato]cerium-(IV)[9d] with 2-(4-bromomethylphenyl)-1,3-dioxa-2-borinan[24] in DMF. Detailed synthetic procedure and characterization of **1** were reported previously.[10]

Synthesis of 2. 4-(1,3-dioxaborinan-2-yl)benzaldehyde (115 mg, 0.605 mmol), 2,2′-dipyrromethane (80 mg, 0.55 mmol), and two drops of trifluoroacetic acid were dissolved in 60 mL of dry dichloromethane, and the mixture was stirred for 15 h at room temperature. To this reaction mixture was added chloranil (560 mg, 0.605 mmol). After

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evaporation of the solvent, the red residue was chromatographed (silica gel, $CHCl_3$ to $CHCl_3$−methanol = 100:1) to produce 50 mg of 5,15-bis(4-[1,3,2]-dioxaborinan-2-yl-phenyl)porphyrin. Yield 30%. Mp > 300 °C; ^{1}H NMR (250 MHz, $CDCl_3$−CD_3OD, δ = ppm, J = Hz): 2.25 (m, 4H), 4.37 (m, 8H), 8.21 (d, J = 7.8, 4H), 8.28 (d, J = 7.8, 4H), 9.08 (d, J = 4.6, 4H), 9.40 (d, J = 4.6, 4H), 10.3 (s, 2H). MALDI TOF-MS (CHCA): calcd. (found) for $[M+H]^+$: 631.80 (631.26). Anal. Calcd for $C_{38}H_{32}B_2N_4O_4 \cdot 0.5CHCl_3$: C, 67.01; H, 4.75; N, 8.12%. Found: C, 67.45; H, 4.61; N, 8.89%.

To a solution of 5,15-bis(4-[1,3,2]-dioxaborinan-2-yl-phenyl)porphyrin (40 mg, 0.063 mmol) obtained above in 10 mL of $CHCl_3$ was added 5 mL methanol solution of zinc acetate (680 mg, large excess), and the mixture was stirred for 24 h at room temperature. After evaporation of the solvent, the red residue was chromatographed (silica gel, $CHCl_3$) to yield 39 mg of 5,15-bis(4-dihydroxyboronophenyl)-porphyrinatozinc(II). Yield 86%. Mp (dec) > 300 °C; ^{1}H NMR (250 MHz, DMSO-d_6, δ = ppm, J = Hz): 8.22 (d, 4H), 8.24 (d, 4H), 8.38 (br s, 4H), 8.95 (d, J = 4.2, 4H), 9.51 (d, J = 4.2, 4H), 10.38 (s, 2H). MALDI TOF-MS (CHCA): calcd. (found) for $[M+H]^+$: 614.81 (614.55). Anal. Calcd for $C_{32}H_{22}B_2N_4O_4 \cdot 2H_2O$: C, 59.16; H, 4.03; N, 8.62%. Found: C, 60.68; H, 4.29; N, 8.08%.

5,15-Bis(4-dihydroxyboronophenyl)porphyrinatozinc(II) (170 mg, 0.217 mmol) and propane-1,3-diol (35 μL, 2.2 eq) were dissolved in benzene, and the mixture was refluxed overnight. The residue was washed with water, and the organic layer was dried over anhydrous Na_2SO_4. After evaporation of the solvent, 5,15-bis(4-[1,3,2]-dioxaborinan-2-yl-phenyl)porphyrinatozinc(II) was obtained as a purple solid in 98% isolated yield. This compound was used for the next reaction without further purification.

To a solution of 5,15-bis(4-[1,3,2]-dioxaborinan-2-yl-phenyl)porphyrinatozinc(II) (50 mg, 0.072 mmol) in 50 mL of $CHCl_3$ was added 5 mL of acetonitrile solution of $AgPF_6$ (9 mg, 0.5 equiv), and the mixture was stirred for 5 h at room temperature in the dark.[25] During the reaction, the color of the reaction mixture was changed from purple-red to orange-red. The reaction was quenched by 5 mL of water, and the organic layer was separated. After evaporation of the solvent, the red residue was chromatographed (silica gel, $CHCl_3$−methanol) to produce 2 mg of **2** in 4% isolated yield. Mp (dec) > 300 °C; ^{1}H NMR (250 MHz, $CDCl_3$−CD_3OD, δ = ppm, J = Hz): 2.15 (m, 8H), 4.26 (m, 16H), 8.05−8.06 (m, 12H), 8.22 (d, 8H), 8.59 (m, 4H), 9.06 (m, 4H), 9.41 (m, 4H), 10.27 (s, 2H). MALDI TOF-MS (CHCA): calcd. (found) for $[M+H]^+$: 1386.35 (1386.92).

CD Spectroscopy. To a solution of 1.00×10^{-4} M of **1** or **2** in H_2O (pH 10.5, 50 mM carbonate buffer)/methanol = 1:1 (v/v) was added a stock solution of saccharide prepared in H_2O. The CD spectra from 250 to 500 nm were recorded with JASCO J-720WI at 15 different concentrations of guest saccharides. The measurement temperature was 25 °C, and the cell length is 1 mm.

pH Dependence on CD Intensity. To a solution of 5.00×10^{-6} M of **1** in H_2O/methanol = 1:1 (v/v) (pH 9.0−10.5 adjusted with 10 mM carbonate buffer and pH 7.5−8.5 with 10 mM phosphate buffer) was added a stock solution of saccharide prepared in H_2O. The CD spectra from 250 to 500 nm were recorded with JASCO J-720WI. The measurement temperature was 25 °C, and the cell length is 1 cm.

Competing Binding Experiment. To a mixture of 5.00×10^{-6} M of **1** and 2.00×10^{-3} M of sLe^x in H_2O/methanol = 1:1 (v/v) (pH 9.5 adjusted with 10 mM carbonate) was added a stock solution of D-glucose prepared in H_2O. The CD spectra from 250 to 500 nm were recorded with JASCO J-720WI. The measurement temperature was 25 °C, and the cell length is 1 cm.

Binding Isotherm Analysis. In the analysis of the binding isotherm by Hill plot and Scatchard plot, we have evaluated the concentration of unbound saccharide, [saccharide], by assuming that 100% 1:2 **1**/oligosaccharide complex is formed when the CD intensity is saturated.

Acknowledgment. This work was supported by a Grant-in-Aid for COE Research, "Design and Control of Advanced Molecular Assembly Systems", from the Ministry of Education, Science and Culture, Japan (Grant #08CE2005).

Supporting Information Available: CD data and Scatchard plots (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA010806E

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