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Molecular Design of Artificial Molecular and Ion Recognition Systems with Allosteric Guest Responses

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ABSTRACT

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 biomimetic design of such allosteric systems is of great significance in order to regulate the complexation ability or the catalytic activity of artificial receptors according to an allosteric manner. 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. Allosteric systems are classified into four different categories: positive heterotropic, negative heterotropic, positive homotropic, and negative homotropic. In this Account, we account for our artificial allosteric systems and discuss the basic concept for molecular design of such allosteric systems and what kinds of new functions come out of such dynamic systems.

Introduction

Allosteric interaction between subunits is known as one of the elegant strategies for precisely regulating and controlling the functions in biological systems.¹ In allosteric systems, the binding event and information of the effector are efficiently transmitted across the other remote binding or catalytic sites by some appropriate conforma-

tional change (for example, a transition from T- (tense) to R- (relaxed) state) *at the right time and in the right place*.¹

The biomimetic design of allosteric systems^{2–19} is of great significance, because they are readily applicable to the efficient regulation of capture and release of analytes, catalytic reactions, and information transduction to the remote site that are frequently seen in biological systems. From the viewpoint of constructing a molecular sensory system, the methodology is very useful to amplify, integrate, and convert the weak chemical or physical signals into other signals so that we can readily read out and record. Allosteric complexation could be also used to transcribe “digital” behaviors in the world of molecules, because, ultimately, their behaviors may be switched “on” or “off” only at the specific threshold conditions regulated by, for example, the effector concentration. Allosteric systems provide a means of obtaining chemical feedback that is a necessary step toward achieving total control over molecular-scale chemical processes. An additional characteristic feature of allosterism is nonlinear binding which, by definition, requires that the initial binding of a guest has a different effect from subsequent host–guest interactions and avoids an information randomization.^{5a,19d}

Basically, the allosteric systems are classified into four different categories:^{1,2a} (a) negative heterotropic, (b) positive heterotropic, (c) negative homotropic, and (d) positive homotropic. The simplest mode of allosteric action takes the form of heterotropic allosterism, where the binding of one chemical species influences the binding of a second chemical species. Homotropic allosterism, which is more important for the efficient regulation of equilibria and catalyses, however, is considerably more difficult to achieve in artificial systems, because the initial binding of a guest species must have a different effect from that of the subsequent interactions between the same host and guest molecules.

There are several successful examples in which slight structural, conformational, or configurational changes induced by ion or molecule binding are efficiently transferred to changes in the subsequent events.^{2–19} The earliest successful efforts to design artificial allosteric systems were reported independently by Rebek,² Traylor,³ and Tabushi.⁴ It is noteworthy to mention that they succeeded independently in reproducing the most difficult positive homotropic allosterism in a small molecular system. Rebek et al. utilized a HgCN₂ binding-induced conformational change in crown ether appended biaryls.² Traylor et al. showed how allosterism is produced through ligand-assisted subunit aggregation utilizing pyridine-appended porphyrinatoiron complexes.³ In Tabushi's system, a cobalt complex of a gable porphyrin binds dioxygen allosterically via simultaneous breaking and bridging of the axial ligand.⁴

In this Account, we introduce the concept of molecular design for several dynamic molecular or ion recognition systems exhibiting four different allosteric responses,

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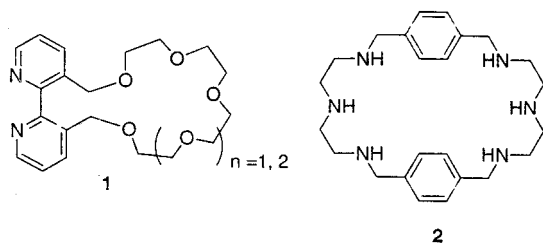
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Seiji Shinkai was born in Fukuoka, Japan, in 1944 and received his Ph. D. in 1972 from Kyushu University, where he became a lecturer soon afterward. After postdoctoral work at the University of California, Santa Barbara, with Thomas C. Bruice, he joined Kyushu University in 1974 and became full professor there in 1988. He also worked as the director of the Shinkai Chemirecognics Project (a government-owned project under the Japanese Research Development Corporation (JRDC)) (1990–1995). Currently, he is also the director of the Chemotransfiguration Project (JST). His interests focus on host–guest chemistry, molecular recognition, liquid crystals, and enzyme models.

particularly focusing on our own recent research achievements related to sugar sensing systems.⁵

Heterotropic Allosteric Systems

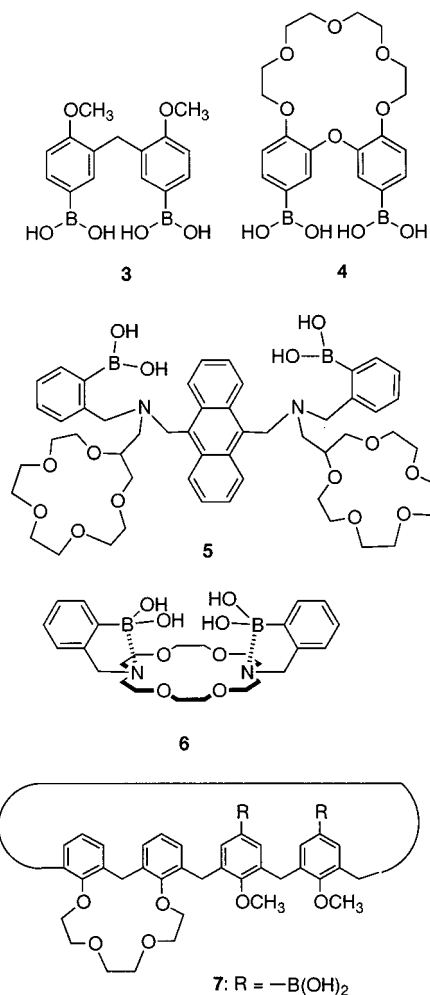
In the design of a heterotropic allosteric system, a guest binding at one site must cause a negative or positive effect on a different guest binding at the remote second site. To design a negative heterotropic system, the first guest binding must induce a major conformational change so as to make the second guest binding less favorable; whereas, to design a positive heterotropic system, the first guest binding must make the remote site more suitable for the second guest binding. There are many examples of artificial heterotropic allosteric systems. Most of these systems have used crown ether derivatives⁶ and/or transition-metal-binding moieties (bipyridine,⁷ β -diketone,⁸ salen,⁹ ethylenediamine,¹⁰ and aminodicarboxylate¹¹ derivatives) as effector binding sites that are known to cause predictable conformational changes. Heterotropic allosteric regulation of hydrogen-bonding receptors,^{12–15} a cyclopeptide receptor,¹⁶ and a reaction^{17,18} has been also reported. As a pioneering work, Rebek et al. reproduced an artificial heterotropic allostereism with compound **1** where a crown ether moiety (alkali-metal-binding site) and a bipyridine moiety (transition-metal-binding site) are allosterically coupled.^{6a} Schneider and Balde demonstrated that a metal cation binding to ethylenediamine moieties of **2** results in construction of hollow space where a lipophilic guest molecule is allosterically recognized.^{10c}



Negative Heterotropic Allosteric Systems

We have currently been interested in sugar recognition and reading out of the recognition processes in aqueous solution.²⁰ The binding of a saccharide immobilizes and orients two phenyl planes of diphenylmethane-3,3'-diboronic acid, **3**, in a chiral manner.²¹ This asymmetric immobilization process can be readily "read out" as a change in circular dichroism (CD) of the benzene chromophores. If a crown ether is combined with the basic molecular skeleton of **3**, then the ideal starting structure **3** has the methylene bridge of **3** replaced with an oxygen. With these objects in mind, we presented the first saccharide-binding allosteric system in which a saccharide-binding site and a metal-binding site are coupled.²² Conformational reorganization of the host (**4**) concomitant with metal ion complexation causes a reduction in the amount of 1:1 saccharide–diboronic acid complex, which is easily monitored by the decrease in the CD intensity.

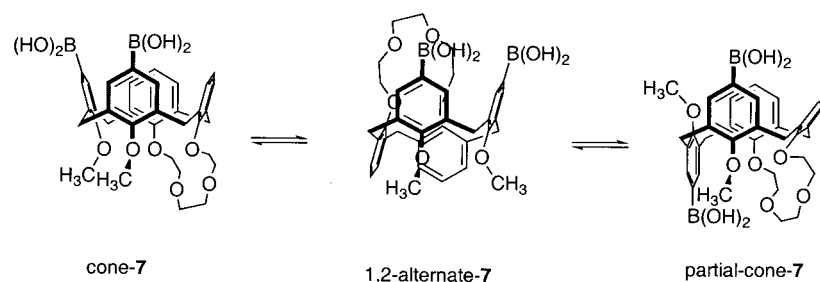
Presumably, the reorganization produces a disposition of the boronic acids which is unsuitable for 1:1 binding with saccharides.



The second example for the negative heterotropic system utilizes the metal-binding properties characteristic of crown ethers, which are that they tend to form 1:1 complexes when the metal size fits the crown cavity size, whereas they tend to form 1:2 sandwich complexes when the metal size is larger than the crown cavity size.²³ With this work, we took a step toward controlling the saccharide selectivity of our fluorescent saccharide cleft **5** by external stimuli.²³ The decrease in CD intensity at 258 nm caused by added metal ion was proportional to the change in the fluorescence intensity at 0.1 M metal ion, and a particularly large decrease was observed for potassium, strontium, and barium, which tend to form 1:2 sandwich complexes.²³ Clearly, decomposition of the cyclic 1:1 complex with D-glucose is the cause of the decrease in the CD and fluorescence intensities. In conclusion, this is a novel negative heterotropic system which can mimic the action of the Na⁺/D-glucose cotransport protein in nature.

The third example features more direct coupling between a diazacrown ether and a diboronic acid function: that is, a novel diaza-18-crown-6-based sugar receptor (**6**) bearing two boronic acid groups has been developed.²⁴

Scheme 1



One can expect for **6** that boronic acids in the side arms and a metal cation bound to the crown cavity competitively interact as Lewis acids with two basic nitrogens in diaza-18-crown-6. Compound **6** forms a 1:1 complex with D-fructose or D-glucose with the aid of the intramolecular B \cdots N interaction. Added Ca²⁺ competes with the boronic acids for the nitrogens; that is, the B \cdots N interaction is weakened by the Ca²⁺ \cdots N interaction. Without the aid of the B \cdots N interaction, the diboronic acid cleft loses the saccharide-binding ability and releases the saccharide in the neutral pH region. In this system, metal- and saccharide-binding sites interact not only through a conformational change but also through a competitive interaction with nitrogens in diaza-18-crown-6 ring.²⁴

The concept of negative heterotropic allosterism is very important in dynamic molecular or ion recognition systems, for example, to release guests only in the presence of some certain threshold concentration of effectors, to regenerate guest-selective electrodes, to control the membrane transport rate of a specific guest by an effector, and so forth.

Positive Heterotropic Allosteric Systems

Calix[4]arenes are composed of repeating 3,3'-diphenylmethane units which are useful to design a boronic-acid-based glucose receptor. Hence, if two boronic acids are introduced into the *para* positions of the proximal phenyl units, the compound includes the basic structure of **3** within the calix[4]arene skeleton. It is known that calix[4]arenes change their conformation in response to metal binding.²⁵ This suggests that two boronic acids situated in the upper rim acting as a saccharide-binding site may "communicate" with the metal-binding site situated on the lower rim: that is, the metal-binding event can change the calix[4]arene conformation, which eventually leads to a change in the relative spatial position of the two boronic acids (Scheme 1). With these objects in mind, we designed compound **7**; a crown strap was used to hold two proximal phenyl units in a syn conformation and to make the resultant ¹H NMR spectral analysis easier.²⁶ Although free cone-**7** can form a CD-active 1:1 complex with D-glucose, cone-**7**·metal complexes cannot bind D-glucose because of the significant flattening of the phenyl groups (i.e., the distance between two boronic acid groups is lengthened). On the other hand, addition of "soft" metal cations such as K⁺, Rb⁺, and Cs⁺ increased the 1,2-alternate conformer. The distance between two boronic acids in 1,2-alternate-**7**

is suitable for D-glucose binding to yield a CD-active 1:1 complex.²⁶ We tested whether the saccharide-binding site on the upper rim can "communicate" with the metal-binding site on the lower rim through the calix[4]arene cavity. The CD band was weakened with increasing Na⁺, Mg²⁺, or Ca²⁺ concentration: this response is classified as negative heterotropic allosterism. On the other hand, when K⁺, Rb⁺, or Cs⁺ was added, the CD intensities increased, and the spectral shape was changed. We consider that this band is attributable to the ternary complexes, 1,2-alternate-**7**·D-glucose·metal: this response is classified as positive heterotropic allosterism. Therefore, the present system is a unique example that shows both negative and positive allosteric interactions between metal ions and saccharides in a calix[4]arene host.²⁶

We were stimulated to design new artificial receptors in which an "open" form active to guest binding is generated from an intramolecularly hydrogen-bonded "closed" form only when it perceives a stimulus.²⁷ It is already known that in calix[4]aryl esters and amides the four carbonyl groups are turned outward to reduce electrostatic repulsion among carbonyl oxygens, whereas bound Na⁺ mechanically changes from the *exo*-annulus carbonyls to the *endo*-annulus carbonyls for the carbonyl oxygens to trap a Na⁺ ion acting as an effector.^{28,29} We thus considered that the metal-induced structural change can be used to generate an "open" form from a "closed" form in an allosteric manner. In chloroform:acetonitrile = 9:1 v/v, compound **8a** exists as a "closed" form because of the formation of intramolecular hydrogen bonds and cannot bind its complementary guests (e.g., lactams).^{27a} On the other hand, Na⁺ bound to the ionophoric cavity cleaves the intramolecular hydrogen bonds, and the exposed receptor sites can bind the guests through intermolecular hydrogen bonds (Scheme 2).^{27a} In this system, one may regard Na⁺ to be a positive effector that can activate the molecular recognition event. In compound **8b** which bears two pyridine-2,6-diamido units, a "closed" form can be converted to an "open" form by Na⁺ binding.^{27b} Thus, the "open" form binds guests having a complementary recognition site of CO–NH–CO. The particular interest is the binding of flavins (VB₂ family) which can be readily detected by the fluorescence decrease.^{27b} Another interesting feature is the application of **8b** to metal-induced "monomer–polymer interconversion".^{27c} Barbituric acid derivatives (BAD) have two CO–NH–CO recognition sites within a molecule. One may thus expect that BAD and **8b** in the "closed" form exist

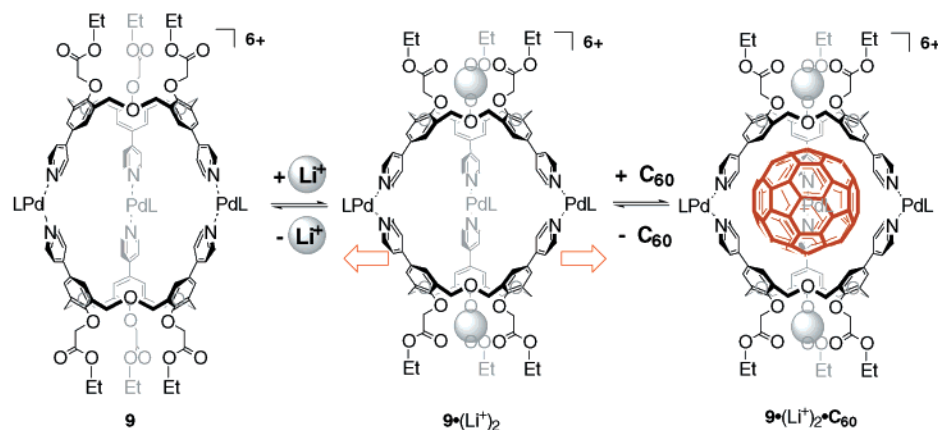
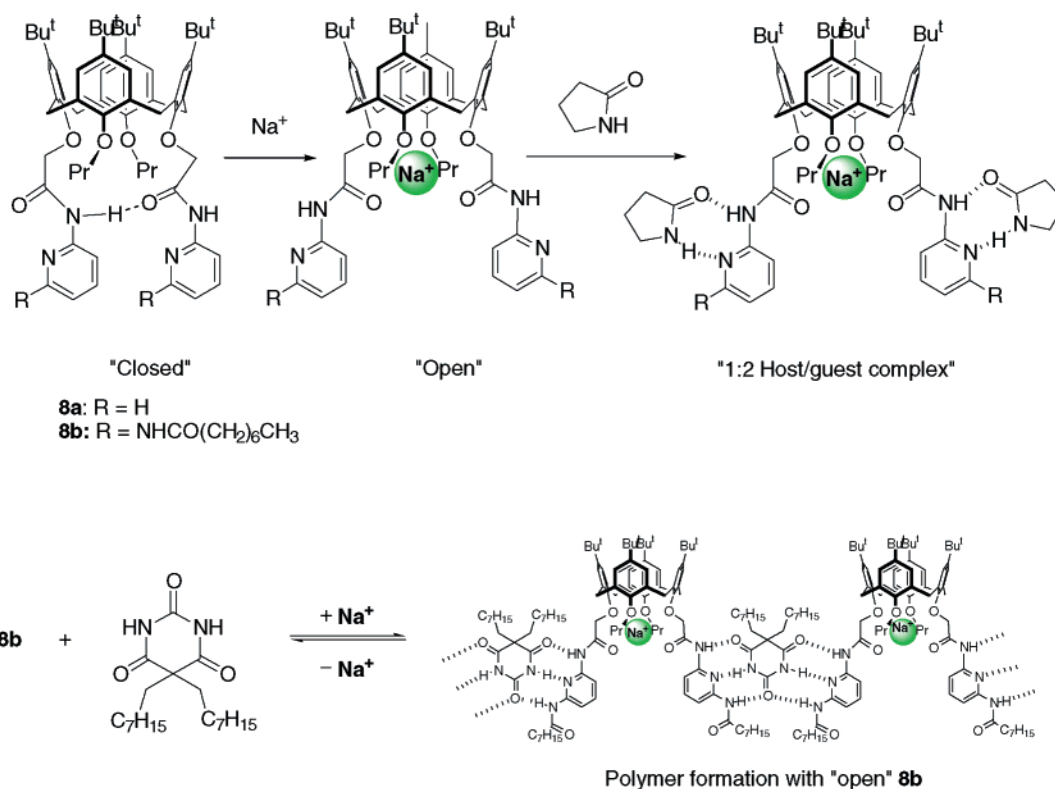


FIGURE 1. Association of [60]fullerene with $9 \cdot (\text{Li}^+)_2$ in which the phenyl groups are flattened by Li^+ binding to the lower rim.

Scheme 2

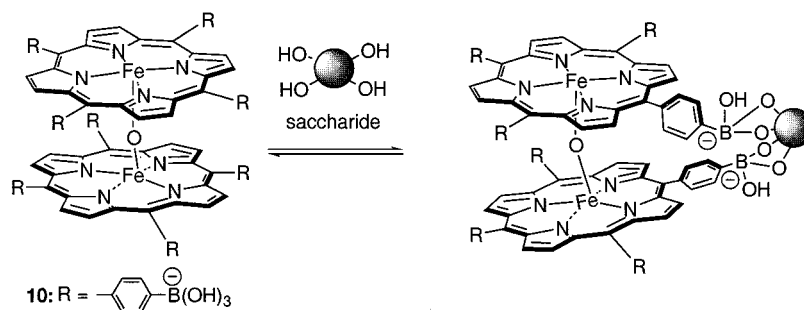


discretely in solution, whereas they can result in a $\cdots\mathbf{8b}\cdots\text{BAD}\cdots\mathbf{8b}\cdots\text{BAD}\cdots$ hydrogen-bond-dependent polymer with the "open" form (Scheme 2). It was confirmed on the basis of ^1H NMR spectroscopy and dynamic light scattering that such a self-assembled polymer is formed only in the presence of Na^+ .^{27c}

Positive heterotropic allostery is also seen for molecular recognition events of self-assembled molecular capsules. Capsulelike cage molecule **9** for the inclusion of [60]fullerene was constructed by dimerization of pyridine-containing homooxacalix[3]aryl esters utilizing a Pd(II)–pyridine interaction.³⁰ It was found that capsule **9** can include [60]fullerene but not [70]fullerene at all, and the exchange rate between the complexed and uncomplexed [60]fullerene in the inner cavity is slower than the ^1H and ^{13}C NMR time scales, giving rise to new separate NMR

peaks assignable to the capsule·[60]fullerene complex. From the integral intensity ratio, the association constant (K) was estimated to be 39 M^{-1} at 303 K.³⁰ Interestingly, the addition of Li^+ cation, which is bound to the ionophoric lower rim, changed the capsular molecule into a conformationally more flattened arrangement which showed higher [60]fullerene inclusion ability. Li^+ cation could enhance the K up to 2100 M^{-1} at 303 K, whereas Na^+ cation totally inhibited inclusion complex formation (Figure 1).³⁰ This system is a rare example which shows both negative and positive heterotropic allosterisms between alkali metal cations and [60]fullerene. The drastic effects of Li^+ as a positive effector and Na^+ as a negative effector indicate that in a capsular host, a large change in the inclusion ability can be induced by a small change in the capsular structure.

Scheme 3



Homotropic Allosteric System

We have recently become interested in the use of non-linear, allosteric binding for producing novel binding modes of guest molecules.³¹ In artificial systems, homotropic allostery is more difficult to reproduce, and examples are accordingly rare,^{2–5,19} because the initial binding of a guest species must have a different effect from that of the subsequent interactions between the same host and guest. Especially in the positive homotropic one, the guest binding information in a subunit should be passed to other all subunits *in unison*. When we started this project, to our best knowledge, there was only one precedent reported by Aoyama et al. for a positive homotropic system with a large Hill coefficient (n); this system features cooperative binding of saccharides to a resorcinol cyclic tetramer host ($n = 4$).^{19c}

How can we design and reproduce “positive homotropic allostery” in an artificial molecular system with large n ? In other words, how can we switch a molecule’s function from the state with low activity (T-state) to that with high activity (R-state) when it successively recognizes guests? The contrivance lies in the biological system. Some allosteric proteins are supposed to have a contrivance of the allosteric transition occurring via *subunit(s) rotation about its own symmetry axis* without dissociation of the oligo subunits.^{32,33a} With this scheme in mind, we have designed two systems potentially capable of achieving positive homotropic allostery utilizing face-to-face type dimeric porphyrins, namely μ -oxo-bis[porphyrinatoiron(III)] and bis[porphyrinato]cerium(IV) double decker complexes.³⁴ In this scheme, the two porphyrins can rotate relative to each other like two wheels with the central metal ion or bridging oxygen acting as an “axle”. Furthermore, one can easily introduce the binding sites on the meso positions and pyrrole β -positions in a porphyrin platform.³⁴

Negative Homotropic Allosteric Systems

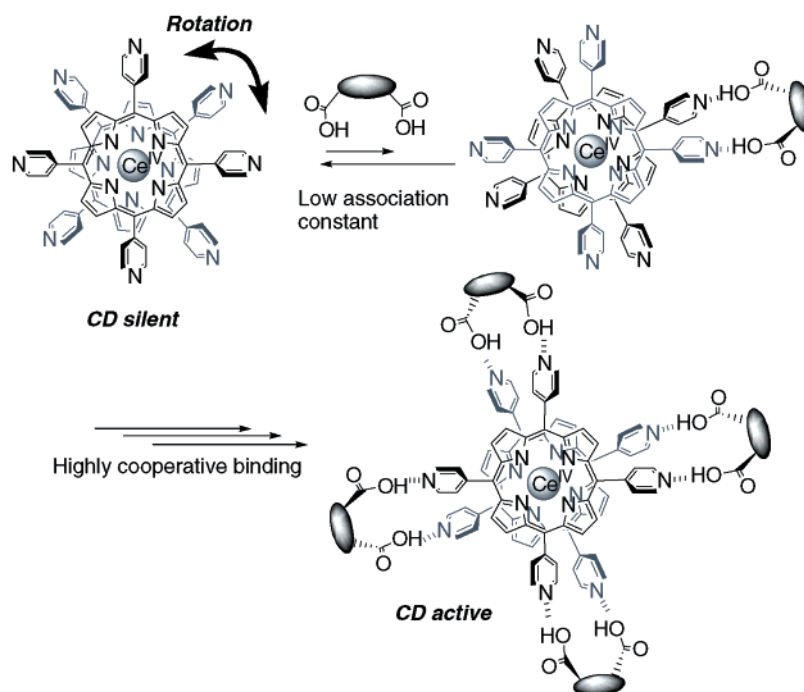
To arrange two boronic-acid-appended porphyrins in an appropriate spatial position, a μ -oxo dimer of porphyrinatoiron(III), **10**,³⁵ would have great potential: the μ -oxo dimer is formed stably in basic aqueous solution where the boronic acid–saccharide complex is also formed stably. Examination using CD spectroscopy established that only glucose and galactose can give the strong CD band in the Soret band region among many mono-

saccharides tested.³⁵ To obtain quantitative insights into the binding mode, we estimated the stoichiometry of the complexes by a continuous variation plot of CD intensity. Very surprisingly, a sharp maximum in the plots of CD intensity versus $[\mathbf{10}]/([\mathbf{10}] + [\text{D-glucose}])$ appeared at 0.5. The results indicate that even though **10** has eight boronic acids, only one pair of boronic acids is used to form the 1:1 **10**–saccharide complexes. Examination of CPK molecular models reveals that when two boronic acids react with four saccharide OH groups in these saccharides, they must get close to each other and the Fe–O–Fe bond angle is tilted to 150° from the regular 180° bond angle. As a result, the distance between two boronic acids in the remaining three pairs becomes too long to complex saccharides intramolecularly (Scheme 3). Hence, this binding mode is classified as negative homotropic allostery (or non-cooperativity). From plots of CD intensity (θ at 380 nm) versus [saccharide], we estimated the association constants (K) to be $1.51 \times 10^5 \text{ M}^{-1}$ for glucose and $2.43 \times 10^4 \text{ M}^{-1}$ for galactose.³⁵ These values are the largest for artificial saccharide receptors working in aqueous solution and 1–2 orders of magnitude greater than those achieved so far.

Positive Homotropic Allosteric Systems

The strong negative homotropic allostery mentioned above was attributed to the inclination of two porphyrin planes which was induced by the binding of the first saccharide guest. Here, it occurred to us that if the first guest could suppress the rotation of two porphyrin planes without inclination, the second guest should be bound more efficiently: that is, a positive homotropic allostery should appear in such a system. We thus chose a member of the cerium(IV) bis(porphyrinate) double deckers, namely the mono-, bis-, tris-, and tetrakis(4-pyridyl)porphyrin derivatives (**11a**, **11b_p** and **11b_d**, **11c**, and **11d**, respectively: “p” or “d” denote that the meso substituents are either proximal or distal).³⁶ These molecules exactly satisfy our requirements: (1) a slow rotation of the two porphyrin planes may be allowed at room temperature,^{37,38} in analogy to similar cerium(IV) bis(diaryl-) or bis(tetraaryl)porphyrinates) studied by Aida et al.,³⁸ (2) the inclination of two porphyrin planes is more difficult than that of **10**, and (3) pairs of 4-pyridyl groups should act as allosteric hydrogen-bonding acceptor sites for dicarboxylic acids. Compound **11a** with one pair of pyridyl groups was used as a reference compound.

Scheme 4



First, circular dichroism (CD) spectra of **11d** were recorded in the presence of chiral guest molecules. The exciton-coupling CD bands were clearly observed for **11d** in the presence of dicarboxylic acid guests with a dimethylene spacer [e.g., BOC-L-aspartic acid (**L-12**; BOC = *tert*-butoxycarbonyl) or (1*R*,2*R*)-1,2-cyclohexanedicarboxylic acid ((1*R*,2*R*)-**13**)]. The CD spectra measured as a function of the guest concentration provided several isosbestic points, indicating that the reaction consists of only two species under one equilibrium. Interestingly, the

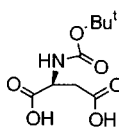
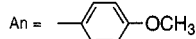
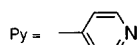
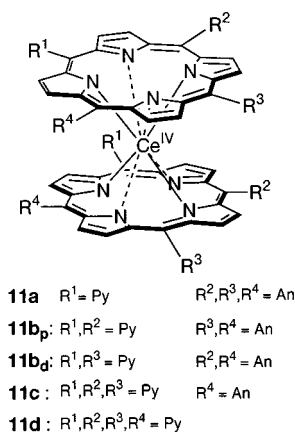
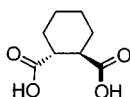
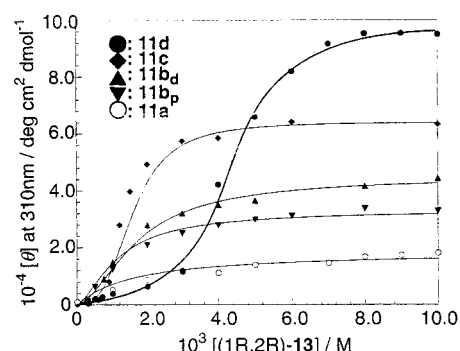
**12****13**

FIGURE 2. Plots of $[\theta]_{\text{max}}$ at 310 nm for **11a**, **11b_p**, **11b_d**, **11c**, and **11d** vs [(1*R*,2*R*)-**13**].

plot of $[\theta]_{\text{max}}$ at 310 nm against the guest concentration results in sigmoidal curvature, clearly indicating that the binding of the guest to **11d** is cooperative (Figure 2).³⁶ This cooperative guest binding profile can be analyzed with the Scatchard plot^{5b,33,39,40} and Hill equation:^{5b,33,39,40} $\log(y/(1-y)) = n \log[G] + \log K$, where $[G]$ is the concentration of the guest, K the association constant, n the Hill coefficient, and $y = K/([G]^{-n} + K)$. In Scatchard plots in which Hill coefficients (n) are correlated with the maximum values (y_{max}) with $n = 1/(1-y_{\text{max}})$, the positive and negative allosterisms are expressed by the upward and downward curvatures, respectively. From the slope and the intercept of Hill plots, we obtained $K = 2.63 \times 10^{11} \text{ M}^{-4}$ and $n = 3.9$ for **L-12** (correlation coefficient: $R = 0.99$) and $K = 2.75 \times 10^9 \text{ M}^{-4}$ and $n = 4.0$ for (1*R*,2*R*)-**13** ($R = 1.00$). The 1:4 composition of the CD-active complexes was further corroborated by continuous variation plots. These findings consistently support the view that four pairs of pyridyl groups in **11d** cooperatively bind these dicarboxylic acids through the hydrogen-bonding interactions and that the two porphyrin planes are immobilized in a chiral

Table 1. Binding Parameters Obtained from Hill's Plot and Benesi–Hildebrand Plot

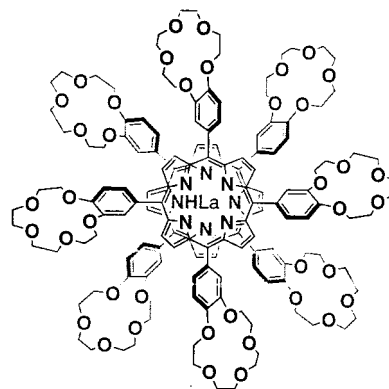
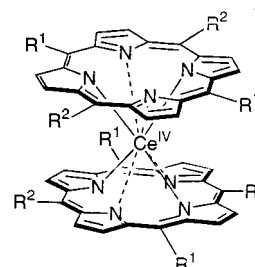
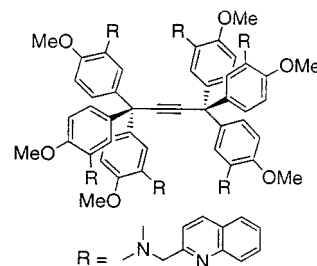
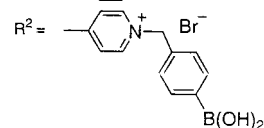
host	log K	n_H	stoichiometry ^a
11a	2.3 ^b	1.0	1:1
11b_p	4.4	1.5	1:2
11b_d	4.7	1.7	1:2
11c	8.4	3.0	nd
11d	9.4	4.0	1:4

^a From continuous variation plot. ^b From Benesi–Hildebrand plot.

conformation to give the CD-active complexes. Hence, this is a rare example of an artificial system with a strong positive allosteric effect and a high n value ($n = 4.0$). In **11d**, the two porphyrin planes can still rotate, but once the rotation is suppressed by the first guest binding, the subsequent binding of the second, third, and fourth guests can occur cooperatively (Scheme 4). This is the origin of the present positive homotropic allosterism.

To obtain further insights into our design scheme, we synthesized **11b_p** and **11b_d** with two pairs of 4-pyridyl groups and **11c** with three pairs of 4-pyridyl groups.^{36b} The CD spectra for **11a**, **11b_p**, **11b_d**, and **11c** measured as a function of (1*R*,2*R*)-**13** concentration provided several isosbestic points. Because the θ versus [double decker] plots for **11b_p**, **11b_d**, and **11c** showed sigmoidal curves similar to those for **11d** (Figure 2), they were analyzed with the Scatchard plot and Hill equation.^{5b,33,39,40} The stoichiometry for these complexes was estimated by a continuous variation plot. It was shown through these spectral studies that **11a** forms a 1:1 complex and **11b_p** and **11b_d** form 1:2 complexes.^{36b} The results are summarized in Table 1. Examination of Table 1 reveals that **11c** gives $n = 3.0$ supporting the view that the complex consists of 1:3 **11c**·(1*R*,2*R*)-**13** and the complexation occurs according to positive homotropic allosterism. Hence, one can regard the K_1 for the binding of the first guest as much smaller than the K_2 and K_3 for the binding of the second and third guests. In contrast, the plots for **11b_p** and **11b_d** give $n = 1.5$ and 1.7, respectively, which are significantly smaller than 2.0. These results suggest that in **11b_p** and **11b_d** K_1 is not sufficiently smaller than K_2 . The plots were analyzed by a nonlinear least-squares method assuming a two-step binding mode with K_1 and K_2 . The K_1 and K_2 values for **11b_p** were 610 M⁻¹ and 750 M⁻¹, respectively, while those for **11b_d** were 400 M⁻¹ and 930 M⁻¹, respectively.

Foregoing results clearly show that all kinds of isomers **11b–d** bind guest dicarboxylic acids cooperatively. We thus applied the basic skeleton of this double decker system to other guest binding systems, where K⁺ ion,⁴¹ silver ion,⁴² and saccharides⁴³ were recognized by compounds **14**, **15**, and **16**, respectively, according to a positive homotropic allosteric manner. Very recently, Glass has demonstrated the interesting allosteric chemical sensory system in which pinwheel receptor **17** can bind silver ions cooperatively and allostery arises via trityl group rotation about the acetylene axle.^{19e} The origin of the positive homotropic allosterism in this system is also elucidated on the basis of our design scheme.

**14****15** R¹, R² = **16** R¹ = **17**

Outlook

In this Account, we have outlined the concept on molecular design of several dynamic molecular or ion recognition systems with four different allosteric responses. As long as ion or molecular recognition is understood only on the basis of static events, it seems to remain an incompetent, closed field. Only when such recognitions are skillfully combined with dynamic events such as allostery, can they show vivid, viable extensions, mimicking the phenomena of regulation, amplification, threshold, phase transition, and so forth, which are indispensable to life processes. Especially, artificial allosteric systems coupled with material science would yield many applications for molecular devices with error filters, materials with buffer functions, highly efficient reaction

systems with switch functions, memory transduction with gate function, and so forth. We believe that this relatively new field will attract many scientists' attention in the years to come.

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