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An indolocarbazole-bridged macrocyclic porphyrin dimer having homotropic allosterism with inhibitory control†

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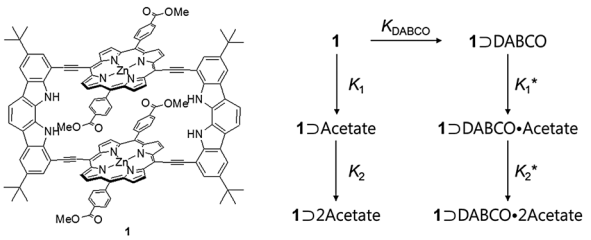
A macrocyclic host, a pair of zinc porphyrins bridged by two anion-acceptable indolocarbazole moieties, has shown strong positive homotropic allosterism upon anionic guest bindings with an inhibitory control mechanism by DABCO addition.

Designing synthetic anion-binding receptors is very important because many anions play critical roles in chemical and biological processes.[1] Recently, several indole-based anion receptors have been reported, in which the NH protons in the indole moieties bind strongly to several anions *via* hydrogen bonds.[2] On the other hand, allosterism is a common but a very important regulation mechanism in biological events.[3,4] Many proteins possess multiple guest-binding sites that show allosteric guest binding phenomena, in which the first guest binding causes a structural alteration of the host protein and influences additional guest-binding affinity. Designing such artificial allosteric systems is of great interest because not only they play crucial roles in biosystems but also it would be helpful to design them for the functional nano-devices. Many scientists have tried to synthesize artificial receptors with multiple guest binding sites to mimic biosystems, but only a limited number of synthetic receptors have shown positive allosterism.[5] In this regard, we have recently reported a new type of molecular tweezers composed of biindole moieties and porphyrins, demonstrating strong positive allosteric bindings between 1,4-diazabicyclo[2,2,2]-octane (DABCO) and chloride.[6] As a continuation of this research, we report herein a new type of macrocyclic host, and wish to highlight positive homotropic allosterism of anionic guests and the control of allostericity. A macrocyclic host compound (**1**) (Scheme 1), a pair of zinc porphyrins bridged by two anion-acceptable indolocarbazole moieties, was synthesized and characterized by ^{1}H, ^{13}C NMR and MALDI-TOF-MS analyses. In the ^{1}H NMR study, addition of either DABCO or acetate to **1** resulted in the chemical shift changes. As shown in Fig. 1, the signal of NH protons in indolocarbazole moieties is strongly downfield-shifted by the addition of acetate ions, indicating that the NH protons participate in hydrogen bonding. In contrast, the signal of NH protons is slightly upfield-shifted by the addition of DABCO,



Scheme 1 Structure of **1** and expression of binding constants for each guest binding processes.

indicating that DABCO and anionic guests have different binding modes. With the addition of DABCO, a major change in the ^{1}H NMR spectrum occurs in the phenyl protons of porphyrin, whereas the pyrrolic protons as well as protons in indolocarbazole units are shifted by acetate addition, indicating that the structural alternation of **1** is induced by acetate bindings. To determine the binding affinity of **1**, several anionic guests and DABCO were titrated with **1** in THF at 25 °C, and spectroscopic changes in Soret and Q bands were monitored. The absorption spectrum of **1** was changed by the addition of anionic guests with clear isosbestic points (see ESI†). The continuous variation method (Job's plot)[7a] together with the spectroscopic titration demonstrated that **1** and all anionic guests form a 1 : 2 host–guest complex. Interestingly, based on the spectroscopic titration of various anionic guests, it has been found that the anionic guest bindings occur in a positive allosteric manner. As shown in Fig. 2, the

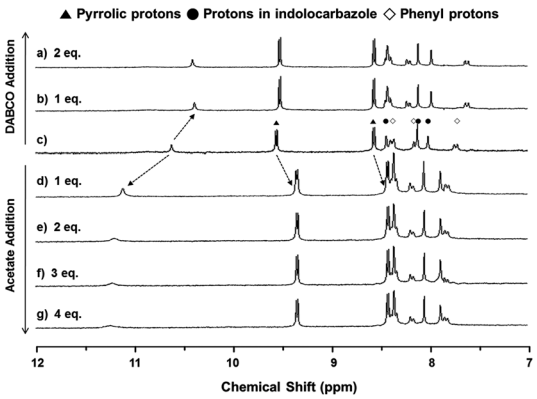


Fig. 1 Partial ^{1}H NMR spectra (250 MHz, THF-d_8, 25 °C) of (c) **1** (1 mM); containing (a–b) DABCO and (d–g) acetate.

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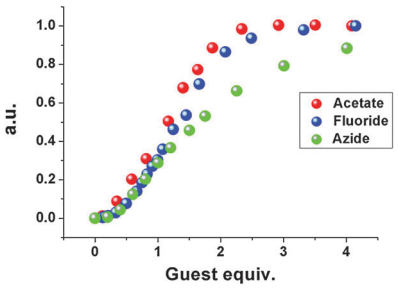
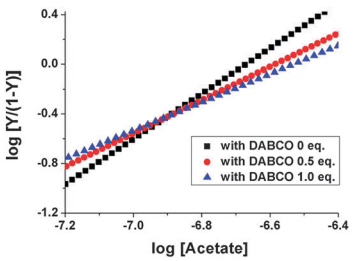


Fig. 2 Titration binding isotherms of acetate, azide, and fluoride ions to **1**.

titration binding isotherms of acetate, azide, and fluoride are obviously sigmoidal shape curves. These guest-binding profiles were analysed with the nonlinear curve fitting method and the Hill equation: $\log(y/(1-y)) = n\log[\text{guest}] + \log K$, where y, K and n are the fractional saturation of the host, the association constant, and the Hill coefficient, respectively.[7] The maximum n value is equal to the number of binding sites of a host molecule. If the Hill coefficient is 2.0, it implies that a host–guest complex shows perfectly cooperative two-site binding. From the slope and the intercept of the linear plot, we obtained log K and n values for various anionic guests. As shown in Table 1, the values of the Hill coefficients for acetate, azide, and fluoride bindings are 1.83, 1.88, and 1.72, respectively. From the spectroscopic titration data, the primary and secondary association constants, K_1 and K_2, respectively, were also determined by the nonlinear curve fitting method using the commercially available Hypspec software. The ratios of K_2/K_1 are 24.5, 35.3, and 3.4 for acetate, azide, and fluoride bindings to **1**, respectively. Compared with $K_2/K_1 = 0.25$ for the non-cooperative system, these K_2/K_1 values are very large, reinforcing the positive allosterism for anion bindings to **1**. Considering the structural rigidity of porphyrin and indolocarbazole moieties in **1**, the only rotatable bond is ethynyl linkages between porphyrin and indolocarbazole units. When the first anionic guest binds to **1**, the angle between porphyrin and indolocarbazole units would be changed. In other words, the first anionic guest binding onto the indolocarbazole unit induces conformational alternation of **1**, which facilitates additional guest binding. When the DABCO was added to **1**, both Soret and Q bands were also drastically changed with clear isosbestic points. Job's plot demonstrated that DABCO and **1** had a 1 : 1 stoichiometry when forming a host–guest complex, where the association constant (K_{DABCO}) was determined to be 1.06×10^6 M^{-1}. The absorption spectra of **1**⊃DABCO were changed with clear isosbestic points by the addition of anions, indicating that **1**⊃DABCO can simultaneously accommodate anions. The stoichiometry of **1**⊃DABCO to anions, determined by Job's plot, was still maintained as 1 : 2. Interestingly, however, the Hill coefficient and log K of anion bindings to **1**⊃DABCO were greatly decreased, suggesting that DABCO binding to **1** decreases the allostericity of anionic guest binding. To know how the DABCO binding influences the allosterism of acetate binding to **1**, acetate was titrated to **1** by changing the equivalence of DABCO addition. As shown in Fig. 3, the slope of the Hill plot was decreased by an increment of DABCO addition, illustrating that the allostericity of acetate binding to **1** can be controlled by DABCO addition. The Hill coefficient n and log K are also summarized in Fig. 3. Using the nonlinear curve fitting method, the association constants K_1* and K_2* of anions to **1**⊃DABCO were again estimated (Table 1). Compared to the case of **1** without DABCO, **1**⊃DABCO showed increased association constant K_1*, representing heterotopic allosterism between DABCO and anions. The binding of DABCO to **1** may create an excellent cavity for anionic guest binding, thereby the free energy change (ΔG) needed for the anionic guest-induced conformational alternation of **1** might be reduced. On the other hand, the secondary association constant K_2* for anion binding was decreased by the accommodation of DABCO, possibly because of the steric repulsion between DABCO and anionic guests. The first anion binding process to **1**⊃DABCO may include two different effects, one is conformational optimization of **1** induced by DABCO binding and the other is steric repulsion between DABCO and the anionic guest. On the other hand, the second anion binding process includes only steric repulsion effect. As a result of DABCO binding to **1**, the secondary association constant K_2* became smaller than the primary association constant K_1*, and **1** lost positive allosterism of anionic guest binding. In other words, DABCO works as an excellent modulator for the homotropic allostericbinding of anionic guests, because the accommodation of DABCO can fix the conformational flexibility of **1**. In this regard, we have calculated and compared ΔG values for each process (Table 2). The loss of ΔG by the steric repulsion can be calculated from the difference between ΔG_2 and ΔG_2*. The smallest fluoride



DABCO	*n*	log *K*
0 eq.	1.83	12.19
0.5 eq	1.35	8.86
1.0 eq.	1.14	7.44

Fig. 3 Hill plots for acetate binding to **1** in the presence of DABCO. Hill coefficient n and log K are summarized in Table 1.

Table 1 Binding constants for various anionic guest bindings to **1**

	Hill analysis		Nonlinear curve fitting analysis[b]					
Ions	n[a]	Log K	K_1	K_2	K_2/K_1	K_1*	K_2*	K_2*/K_1*
F^-	1.72	10.93	1.60×10^6	5.42×10^6	3.39	1.18×10^8	4.09×10^6	3.47×10^{-2}
Acetate	1.83	12.19	1.06×10^6	2.60×10^7	24.5	1.50×10^7	4.86×10^6	0.324
N_3^-	1.88	10.46	7.11×10^4	2.50×10^6	35.2	6.44×10^5	9.69×10^4	0.150

[a] Hill coefficient, [b] Estimated errors <10%.

Table 2 ΔG changes for each process[a]

Ions	ΔG_1	ΔG_2	ΔG_1*	ΔG_2*	$\Delta G_1 - \Delta G_1$*	$\Delta G_2 - \Delta G_2$	$(\Delta G_1 - \Delta G_1^*) - (\Delta G_2 - \Delta G_2^*)$
F^-	−35.4[b]	−38.4	−46.1	−37.7	−10.7	0.68	11.4
Acetate	−34.5	−42.3	−41.0	−38.1	−6.5	4.2	10.7
N_3^-	−27.7	−36.5	−33.1	−28.5	−5.5	8.0	13.5

[a] Estimated errors <10%, [b] kJ mol^{-1}.

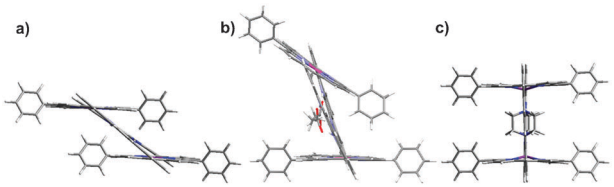


Fig. 4 The optimized structures for (a) **1**, (b) **1**⊃2acetate, and (c) **1**⊃DABCO.

anion exhibited only 0.68 kJ mol^{-1} of energy loss by the accommodation of DABCO, whereas acetate and azide gave 4.2 and 8.0 kJ mol^{-1} of energy loss, respectively. From the difference between ΔG_1 and ΔG_1*, DABCO-induced energy gains in the first anion binding also can be calculated. The smallest fluoride anion exhibited the largest energy gain (10.7 kJ mol^{-1}) by the accommodation of DABCO, which can be explained by the smallest steric repulsion effect. DABCO-induced energy gains for acetate and azide were determined as 6.5 and 5.5 kJ mol^{-1}, respectively. To compensate the steric repulsion effect on the energy gain by the DABCO accommodation, $(\Delta G_1 - \Delta G_1^*) - (\Delta G_2 - \Delta G_2^*)$ were again calculated. As a result, similar values were obtained for all three different anionic guest bindings, indicating that the energy consumed for conformational optimization of **1** by DABCO was 11.8 ± 1.6 kJ mol^{-1}. The structures of **1**, **1**⊃DABCO, and **1**⊃2acetate have been optimized by using DFT at the B3LYP/6-31G level. As shown in Fig. 4, **1** exhibits a parallel alignment of two porphyrin moieties with dihedral angle about 30° against indolocarbazole moieties. The cavity between two porphyrin moieties is too small to fit guest molecules. Therefore, space opening between the two porphyrin moieties should be needed for anionic guest bindings. The optimized structure of **1**⊃2acetate clearly shows structural alternation of host molecules. Nevertheless, when the DABCO binds to **1**, two porphyrin moieties should become perfectly parallel, and indolocarbazole moieties should have a perpendicular alignment against porphyrin moieties. Considering the optimized structure of **1**⊃DABCO, additional anionic guest binding may not induce structural alternation of host molecule. Therefore, after DABCO binding, **1** eventually loses homotropic allosterism upon anionic guest bindings. In conclusion, we have designed a new type macrocyclic host for multiple guest bindings which can simultaneously accommodate anionic guest as well as DABCO. Upon anionic guest binding, the host molecule exhibited strong positive homotropic allosterism. Interestingly, DABCO successfully worked as a heterotopic modulator for the allosteric anion binding to the host. By the accommodation of DABCO to the host molecule, the allosteric city of anion bindings was greatly decreased. The present system is therefore an excellent biomimetic model having homotropic allosterism with an inhibitory control mechanism.

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