Bull. Chem. Soc. Jpn., **74**, 883—888 (2001)

Allosteric Binding of K^+ to Crown Ether Macrocycles Appended to a Lanthanum Double Decker System

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(Received August 1, 2000)

A benzocrown-appended lanthanum(III) porphyrin double decker has been synthesized which is capable of binding simple K^+ in a cooperative, allosteric fashion to form a 1:4 complex. Analysis of this system by means of the Hill equation gave an overall binding constant (K_b) of 1.0×10^{14} (mol dm^{-3})$^{-4}$ and a Hill coefficient of 4.0. This same system, however, binds Na^+ in a conventional, linear fashion. These results indicate that the novel allosteric binding occurs as the result of 1:2 sandwich complexes between K^+ and appended crown ether moieties of the two joined porphyrins. The suitability of lanthanum(III) over cerium(IV) for preparing this highly crowded double decker porphyrin is also noted.

We have recently become interested in the use of nonlinear, "allosteric" binding for producing novel binding modes of sugars and other chiral guest molecules.[1] Allosteric systems provide a means of obtaining chemical feedback which is a necessary step towards achieving total control over molecular-scale chemical processes.[2–4] Nonlinear binding, by definition, requires that the initial binding of a guest has a different effect to subsequent host-guest interactions. The simplest mode of allosteric action takes the form of heterotropic allosterism,[5–14] where the binding of one chemical species influences the binding of a second chemical species. Homotropic allosterism, however, is considerably more difficult to achieve because the initial binding of a guest species must have a different effect to that of the subsequent interactions between the same host and guest.[15]

With this goal in mind, we have prepared two systems capable of achieving positive homotropic allosterism utilizing cerium(IV) porphyrin double decker complexes (**1** and **2**) as the central motif.[16,17] In the first case,[16] appended *meso* pyridine units were used to bind allosterically chiral dicarboxylic acids. In the second case,[17] the concept was developed so that the chirality of suitable dicarboxylic acids could be transcribed to the pro-chiral double decker species.

To date, we have focused on organic molecules with discrete binding sites as guest systems. The control of simple electrolytes is also a worthy target, however, stemming from their crucial roles in biological,[18–20] chemical[21–23] and industrial processes.[24,25] In this case, a host demonstrating positive homotropic allosterism can be used to act as a chemical "buffer" for salts, maintaining the concentration of a species within a certain range. We therefore synthesized and studied compounds **3** and **4**, porphyrin double decker complexes with *meso*-benzocrown ether moieties that are capable of trapping metal ions (Chart 1).[26]

In this system, the two porphyrins can rotate relative to each other like two wheels with the central metal ion acting as an "axle".[27–30] The benzo-15-crown-5 ether moieties at the *meso* positions of the porphyrins are suitable for forming sandwich complexes with large metal ions such as K^+.[31] The large entropy penalty associated with hindering the rotational movements of the porphyrin planes and phenyl moieties, however, prevents the facile binding of a single metal cation (Fig. 1). The cooperative action of more than one cation, however, can compensate for this entropy penalty and so the double decker can form complexes with a sufficient excess of metal ions. The binding behavior of the first metal ion is expected to be differentiated from that of the subsequent metal ions as a result of the significant decrease in entropy that occurs only after complexation of the first ion.

Results and Discussion

Synthesis of the Double Decker Porphyrins. The synthesis of **3** and **4** is illustrated in Scheme 1. The tetrakis(benzocrown)porphyrin **5** has been synthesized previously[32] and the progress up to this point proved to be relatively facile. It is known that porphyrin double decker complexes can be formed from a number of different metals.[33,34] We chose to study both a Ce(IV) sandwiched system and a La(III) sandwiched system for this study because of subtle differences in their properties.[33] Previous double decker systems synthesised by this

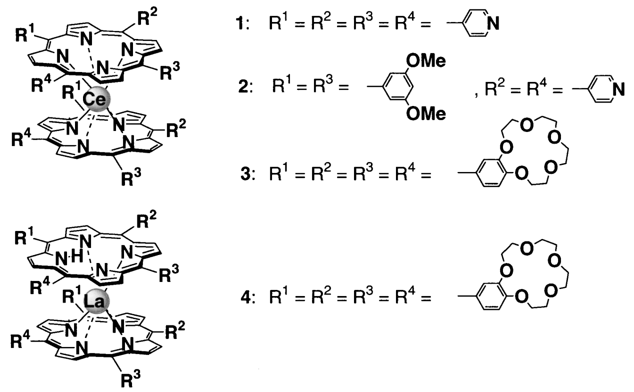


Chart 1.

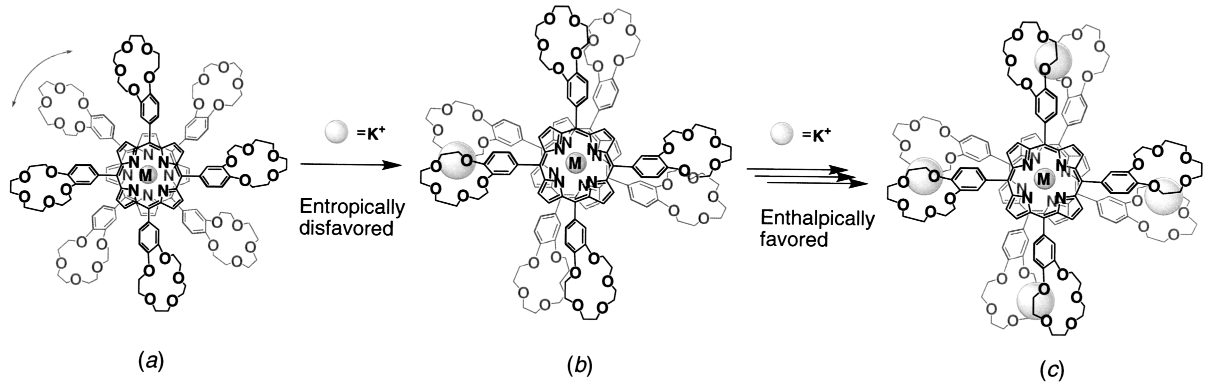
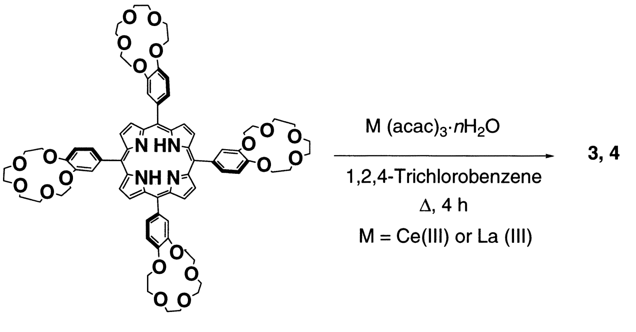


Fig. 1. Schematic representation of the action of **4**. In (*a*), rotatational oscillations hinder complexation of the first K^+ in a crown ether sandwich complex. Once the first K^+ ion has reduced these rotational movements (*b*), however, binding of subsequent K^+ becomes progressively easier until saturation (*c*).



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Scheme 1.

group have used Ce(IV) for the central metal ion as the 4+ charge makes it an ideal complement to the fully deprotonated porphyrins whereas the larger and softer La(III) has better properties for filling the central cavity in the double decker.

Our attempts to use Ce(IV) in this system, however, produced **3** in only a very low yield that was almost impossible to purify satisfactorily. The synthesis of double decker systems is generally straightforward and can be achieved by mixing the porphyrin with the 2,4-pentanedione salt of the appropriate metal at high temperature, typically in refluxing 1,2,4-trichlorobenzene.[33] In this case, however, the yields were very low and mass spectra suggested that the product was contaminated by Na^+ which was very difficult to remove by extraction. When another pathway was used to prepare **5** without using any Na^+ in the preceding steps,[35] no double decker was detectable at all after synthesis attempts. It was subsequently discovered that washing a chloroform solution of the tetrakis(benzocrown)porphyrin with aqueous $NaPF_6$ could produce the improved yields of **3**, indicating that the primary problem was caused by complexation of the small, hard Ce(IV) ions by the crown ethers. Unfortunately, removal of the excess Na^+ after synthesis of the double decker was not possible without also dissociating the double decker complex itself. Washing chloroform solutions of the double decker either by use of a separating funnel or by continuous extraction inevitably resulted in the loss of the double decker complex.

In contrast, synthesis of **4** was straightforward and complexation of the La(III) by crown ethers did not appear to be a problem. The compound was stable to being washed with water and column chromatography and was therefore chosen for subsequent analysis by spectroscopy and conductiometric measurements.

Spectroscopic Properties of 4. The complex **4** showed a tendency to form aggregates even at low concentrations (> 0.4 mmol dm^{-3} in 1:1 $CHCl_3$/MeCN) which rendered detailed analyses of this compound rather difficult. MALDI-TOF mass spectrometry provided satisfactory mass spectra, but ^1H NMR spectroscopy of non-complexed **4** proved to be impossible as a result of severe line broadening, presumably caused by the stacking behavior mentioned above. Evidence that intermolecular interactions are responsible for the line broadening was obtained by taking a spectrum of a more dilute solution of **4** (0.4 mmol dm^{-3} in 1:1 $CDCl_3$/MeCN-d_3). In this case, sharper lines could be observed but a very poor signal to noise ratio prevented the acquisition of meaningful data. Stacking would also be expected to cause a change in the UV-vis spectrum. Unfortunately, the extinction coefficient of **4** is so high that UV-vis spectra cannot be reliably obtained at concentrations higher than around 1×10^{-4} mol dm^{-3}. A simple visual inspection shows a change from a predominantly green color at low concentration to a dark brown coloration at higher concentrations.

Additional evidence for the intermolecular interaction hypothesis was gained by observing the ^1H NMR spectrum in the presence of an excess of K^+. In the presence of a large excess of K^+, the ^1H NMR spectrum became sharp and clearly defined even at a concentration of around 1 mmol dm^{-3}. In this case, the crown ether signals were clearly resolved as well as the signals for the aromatic benzocrown protons. These latter signals could be clearly attributed to *endo* and *exo* forms according to whether the respective protons were turned towards the middle of the complex or outwards. Variable temperature experiments did not show any major change in the nature of the spectrum. From these results it can be seen that the effect of K^+ is to slow or immobilize the rotational oscillations.

The form of the UV spectrum for **4** is substantially the same as for the precursor porphyrin **5**,[32] though there is a hypsochromic shift of the Soret band from 423 to 415 nm and a weakening of the Q bands.

As stated above, the apparent stacking behavior of **4** prevented analysis of complexation behaviors by ^{1}H NMR spectroscopy. Addition of KPF_6 to dilute solutions (1.0×10^{-4} mol dm^{-3}) of **4** produced a small change in the Soret band (Fig. 2) but this difference was not sufficiently large to be of use for following the complexation behavior of this compound. Following the change of conductivity of solutions of **4** upon adding Na^+ or K^+ ions, however, was able to provide a clear picture of the interesting properties of this system. We thus decided to adopt a conductivity titration method for observation of this system at a concentration of 1×10^{-4} mol dm^{-3} where stacking was not found to present a problem. In fact, we confirmed that the Lineweaver–Burk plot, which is frequently cited as evidence for discrete dissolution of the substrate, shows a good linearity up to 1×10^{-4} mol dm^{-3} in 1:1 $CHCl_3$/MeCN (used as a standard solvent in the subsequent conductivity titration).

Conductivity Measurements. The behavior of **4** when interacting with Na^+ ions was expected to be significantly different to that when interacting with K^+ ions. Na^+ ions are small enough to fit within the cavity of an individual benzo-15-crown-5 ether, whereas K^+ ions can only form sandwich complexes between two of these macrocycles (Fig. 3).[33] The conductance profile for Na^+, therefore, was expected to follow a conventional, linear saturation upward curve as the Na^+ occupied successive crown ether cavities until saturation. With K^+, however, the profile was expected to follow a linear saturation increase at the first stage, where the K^+ cannot easily form a sandwich complex, followed by a "flat spot", where there is enough K^+ for allosteric uptake, which is followed by a second linear increase after saturation of the K^+ binding sites. This was indeed found to be the case.

After confirming that adding $NaPF_6$ or KPF_6 to a simple solvent mixture produced a linear increase in conductance, we proceeded to convert the conductance plot into a concentration plot for the complexed porphyrin double decker complex. Assuming that all complexes are involved at saturation, the conductance data could be converted, by subtracting the background conductance at each point, into the concentration profile of complexed **4**, as shown in Fig. 4 (see Experimental).[36] This graph shows the expected concentration profile for allosteric uptake of K^+; In section (*a*), formation of the 1:4 complex is slow as a result of the low concentration of K^+ and the lack of stability of the 1:1 complex. In section (*b*), however, the concentration of K^+ has increased to the point where 1:4 complexes can be formed readily and there is a sudden increase in the slope of the curve. In section (*c*), **4** is saturated with K^+ and no further uptake can be observed. It is notable that saturation occurs soon after the addition of 4 equivalents of K^+, indicating a strong overall K_b binding constant. Thereafter, a slight decrease in concentration can be observed. While there is a possibility that this decrease arises from the break-up of discrete complexes into less defined stoichiometries as a result of interference from excess K^+, this decrease is not yet significant in terms of experimental error.

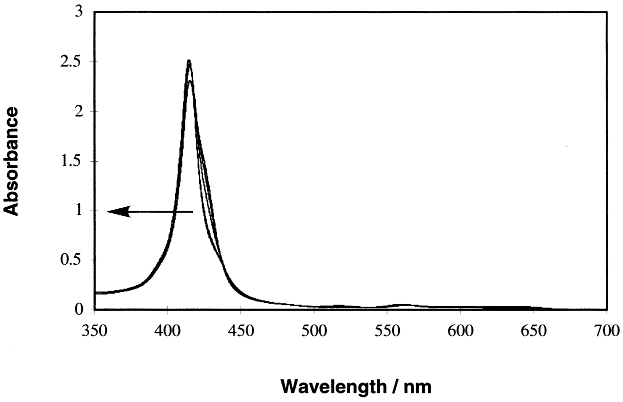

Fig. 2. Change in the UV-vis spectrum of **4** (1:1 $CHCl_3$/MeCN, 1 mm cell, 1.0×10^{-4} mol dm^{-3}) upon adding KPF_6. No further change was observed after adding two equivalents of KPF_6.

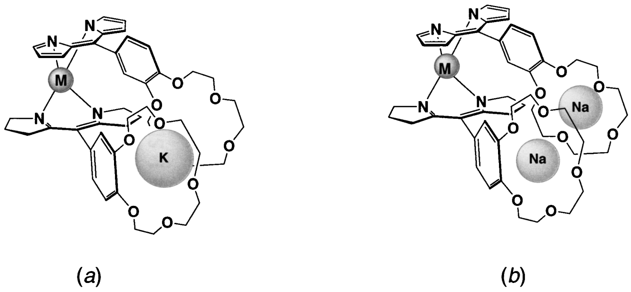

Fig. 3. Schematic representation of the difference between the binding mode of K^+ (*a*) and Na^+ (*b*). In the case of K^+, a single ion spans two crown ethers from separate porphyrin moieties which aligns the remaining crown ethers and leads to nonlinear complexation. The smaller Na^+ ions, however, can fit within individual crown cavities independently of each other. This behavior leads to linear complexation.

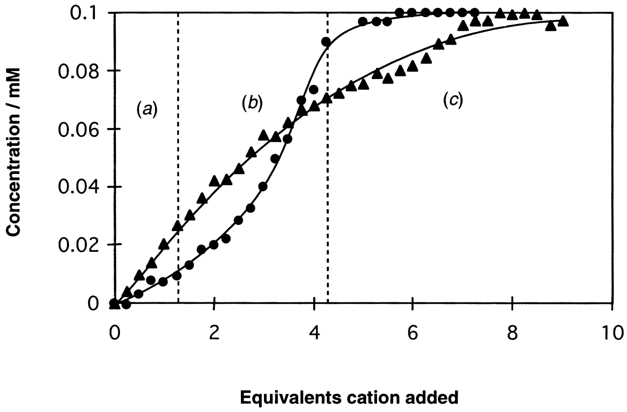

Fig. 4. Concentration profile of cation complexed double decker porphyrin upon titrating **4** (1:1 $CHCl_3$/MeCN, 1.0×10^{-4} mol dm^{-3}) with $NaPF_6$ (triangles) or KPF_6 (circles).

There is, however, a significant difference between the behavior of **4** in the presence of K^+ and that in the presence of Na^+. Titration of a 1.0×10^{-4} mol dm^{-3} solution of **4** with $NaPF_6$ demonstrated the slight upward curve in conductivity that is to be expected from a linear uptake of Na^+ until saturation. The conductance plot was converted into a concentration profile using the same method as that used for the K^+ experiment. It is noticeable that saturation is achieved at around eight equivalents of Na^+, which corresponds to single occupancy of each crown ether. Most importantly, the plot shows the conventional linear saturation curve of the small Na^+ cations, which is essentially different from the nonlinear allosteric complexation of the larger K^+ cations.

The peculiar behavior of **4** in the K^+-binding is also shown by comparison with that of free base porphyrin **5**. The conductance titration of **5** + K^+ and **5** + Na^+ systems both featured a small, linear uptake of these metal ions over the same concentration range. The results imply that the non-linear allosteric uptake of K^+ is inherent to the structure of double decker porphyrin **4**.

At this stage, it was necessary to provide additional evidence for the stoichiometry of these complexes by means of a Job plot.[37] As mentioned above, ^{1}H NMR spectroscopy was not applicable to the analysis of this system. UV-vis spectroscopy was also unsuitable, as a result of the small change in the UV-vis spectrum. Even a Job plot based on conductance measurements was unable to give a clear picture, as a result of the relatively small change in conductance brought about by complexation compared to the change in conductance brought about by changing the ratio of the K^+ in the solution. We have therefore been unable to obtain a Job plot for this system but, given that the binding constants for the cation crown ether interactions are likely to be high in these organic solvents, the saturation at 4 equivalents of K^+ or 8 equivalents of Na+ is strongly indicative of maximum occupancy for the respective binding modes. The 1:4 stoichiometry of the K^+ complex implies that 1:2 K^+/crown ether sandwich complexes are formed which bridge the two porphyrin planes, whereas the 1:8 stoichiometry of the Na^+ complexes suggests that 1:1 cation/crown ether complexes are formed in this case.

A more direct determination of the stoichiometry was permitted by means of the Hill equation, which is defined as: $\log (y/(1-y)) = n \log [\text{guest}] + \log K_b$, where $y = K_b/([\text{guest}]^{-n} + K_b)$, n is the Hill coefficient, K_b is the overall binding constant and y is the extent of complexation.[38] By analyzing the area of maximum change in the concentration profile ((*b*) in Fig. 4) according to this equation, the Hill coefficient was determined to be 4.0 (correlation coefficient: $R = 0.98$) (Fig. 5). This value corresponds directly to the stoichiometry of the guest species in allosteric interactions. Furthermore, the overall K_b binding constant can be estimated from this plot to be around 1.0×10^{14} (mol dm^{-3})$^{-4}$. The fact that positive allosterism is achieved only by titration with K^+, which tends to form sandwich complexes with benzo-15-crown-5 moieties, clearly indicates that the cooperative K^+ binding to **4** stems from the suppression of porphyrin ring rotation by the first K^+ binding, which preorganizes the residual three pairs of benzo-15-crown-5 moietites (Fig. 1).

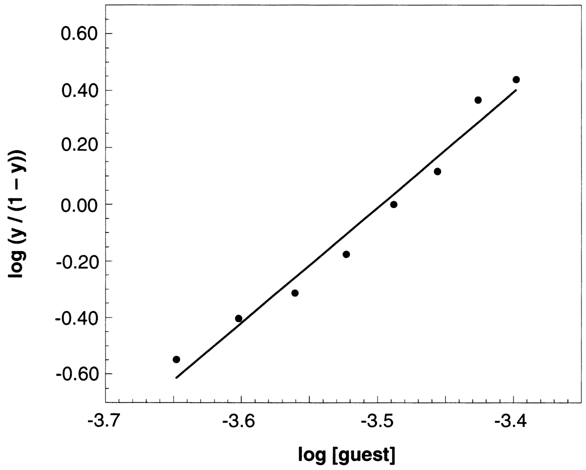

Fig. 5. Line of best fit according to the Hill equation (see text). The concentration data were taken from the section marked (*b*) on the concentration profile (Fig. 5).

Conclusion

This study shows that a crown ether appended porphyrin, capable of binding K^+ in a linear fashion can be converted to a system capable of nonlinear binding by conversion to a lanthanide double decker complex. An interplay between an entropic penalty and an enthalpic benefit is expected to be the cause of this allosteric binding. The greater suitability of the La(III) ion *versus* the Ce(IV) ion for synthesising this double decker complex, where crown ether cavities apparently interfere with the reaction with Ce(IV), has also been established. It is expected that systems similar to this one may be used as "cation buffers" to keep the concentration of a particular cation within predefined limits. Allosteric complexation could also be used to transcribe "digital" behavior to the world of molecules as, ultimately, their behavior may be switched "on" or "off" only at certain threshold conditions of concentration.

Experimental

General. All starting materials and solvents were purchased from Tokyo Kasei Organic Chemicals or Wako Organic Chemicals and were used as supplied. Basic alumina was obtained from Merck (Basic Alumina 90, 0.063–0.2 mm particle size) and GPC performed on Biobeads S-X8 200–400 mesh as supplied by Bio-Rad. Conductance measurements were performed at 25 °C using a TOA CM-30G EC meter. UV-vis spectra were performed on a Shimadzu UV-2500 PC spectrometer and NMR spectra were obtained using a Bruker DX600 spectrometer.

Conductivity Measurements.[36] The conversion was performed by plotting the difference between the observed conductance and the theoretical conductance that would be achieved by simply adding K^+. The maximum deviation was then taken as corresponding to saturation of the double decker complexes by K^+. All concentrations were then scaled according to the maximum concentration being 1×10^{-4} mol dm^{-3} and the minimum being zero.

Materials. *meso*-Tetrakis(benzo-15-crown-5)porphyrin (**5**) was synthesized according to literature procedures.[32]

Bis[*meso*-tetrakis(benzo-15-crown-5)porphyrinato]cerium(IV) (3). It was not possible to purify **3** satisfactorily for analysis (see Results and Discussion, above) and so this experimental result is provided for comparison purposes only. Prior to reaction, **5** was dissolved in chloroform and washed with an aqueous solution of $NaPF_6$. The chloroform layer was separated, the solvent removed and the residue (Na^+ complexed **5**) (0.6 g, 0.4

mmol) was dissolved in 1,2,4-trichlorobenzene (5 mL). The solution was brought to reflux and $Ce(acac)_3$ (0.7 g, 1.6 mmol) was added in small portions before the mixture was stirred at reflux for 18 h. After this time, the mixture was allowed to cool before the mixture was filtered and subjected to continuous liquid-liquid extraction with water for three days. Over this time, a large portion of **3** was observed to revert to **5** by tlc and MALDI-TOF mass spectrometry. Finally, the solvent was removed in vacuo and the residue dissolved in $CHCl_3$ was chromatographed on basic alumina to afford a sample of **3**. However, it was still contaminated with Na^+ as a brown solid: Yield 11 mg (2%), In the absence of K^+, **3** also suffered from line broadening, though to a lesser extent than **4**; the following broad signals were observed: ^{1}H NMR (600 MHz, MeCN-d_3) δ 8.87 (16H bs, porphyrin pyrrole), 7.76–7.69 (bm, 8H, phenyl), 7.59–7.48 (bm, 8H, phenyl), 7.18–7.07 (bm, 8H, phenyl), 4.29–3.25 (m, 128H, crown). MALDI-TOF (matrix: dithranol) *m*/*z* (rel intensity) 2953 (M + 2 Na^+, 100) 2965 (M + 4 Na^+, 80).

Bis[*meso*-tetrakis(benzo-15-crown-5)porphyrinato]lanthanum (III) (4). To refluxing 1,2,4-trichlorobenzene and tetrakis(benzocrown)porphyrin **5** (0.16 g, 0.1 mmol) under a N_2 atmosphere, La $(acac)_3$ (0.18 g, 0.4 mmol) was added in small portions. The reaction mixture was refluxed for 4 h before being allowed to cool. The solvent was removed by distillation under vacuum, the residue being dissolved in chloroform and washed with water. The chloroform layer was retained and the chloroform removed under reduced pressure. The residue was then recrystallized from methanol once and the isolated solid was subjected to a thin pad of neutral alumina under reduced pressure using chloroform as eluent. A colored band consisting of **5** and **4** was isolated. This final mixture was subjected to GPC chromatography using benzene as eluent on Bio-beads. Compound **4** eluted first as a green band and **5** eluted second as a red band. The final compound was isolated as a purple solid (though green in dilute solutions). We have tried to confirm the purity of **4** by HR-FABMS using magic bullet, NOPOE, NBA and triglycerol as matrix, but failed. However, a strong peak appeared at 2887 in MALDI-TOFMS measurements which is assignable to $[\mathbf{4}+H]^+$. The 1H NMR spectrum was recorded in pyridine-d_5 with a large excess of KPF_6—see comments in main text for details. From the ^{1}H NMR spectrum, it has been confirmed that compound **4** contains benzene and at least 10 equivalent water molecules within the product solid. Water molecules and benzene in **4** have not been able to be removed using vacuum apparatus at room temperature and some degradation of **4** took place at higher temperature during this treatment: Yield 75 mg (26%), UV-vis (1:1 $CHCl_3$/MeCN) (λ_{max} (log ε) 415 (5.36), 517 (3.70), 559 nm (3.77). ^{1}H NMR (600 MHz, pyridine-d_5, large x.s. KPF_6) δ 8.99 (8 H, pd, *exo*-phenyl), 8.91 (8 H, ps, *exo*-phenyl,), 8.65 (8 H, pd, *exo*-phenyl), 7.10 (8 H, ps, *endo*-phenyl), 6.80–6.82 (8 H, pd, *endo*-phenyl), 4.71–3.57 (128 H, m, crown) (n.b. one set of phenyl protons is obscured by a strong residual pyridine peak, pyrrole protons too broad for observation). MALDI-TOF (matrix: dithranol) *m*/*z* (rel intensity) 2887 (M+H$^+$, 100). mp > 250 °C (decomp.). Found: C 59.9, H 6.88, N 2.81%. $C_{152}H_{168}LaN_8O_{40}\cdot C_6H_6\cdot 10H_2O$ requires C 60.3, H 6.25, N 3.56%.

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, Sports and Culture (#08CE2005). We thank Ms. E. Okasaki of Kyushu University for HRMS measurements.

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