Synthesis of a New Allosteric Carrier Containing Three Conformationally Related Subunits

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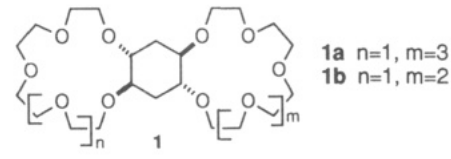
A new allosteric carrier was prepared. This compound consists of three crown ether subunits in which conformational information is transferred through two biphenyl systems. This system is able to complex nonionic species in both external crown ether subunits. The allosteric cooperativity in this system was established and its ability to transport $Hg(CN)_2$ has been studied.

Allosteric cooperativity is the basis of enzyme control and many other vital biological processes such as oxygen binding by hemoglobin. The activity of allosteric systems is regulated by conformational changes induced by the reversible binding of some agents. Several synthetic models which mimic allosteric cooperativity have been described.[1-4] Systems containing two identical macrocyclic subunits like bis(BF-19-C-5) synthesized by Rebek[5] allowed us, by using a circulating system,[3b] to establish that a strong positive allosteric cooperativity in binding does not always provide more effective transport across liquid membranes.

To improve transport efficacy, two different theoretic pathways are possible. One of them consists of using carriers with negative allosteric cooperativity; thus compound 1 is able to transport twice as much Na^+ and K^+ than the two corresponding monocyclic compounds combined.[6] This odd behavior could be explained as a consequence of negative allosteric cooperativity. Such a system is only able to complex cations in one of its two subunits at a time because when one complex is formed, the other subunit has an unsuitable conformation to bind any species. The other useful pathway to increase transport would be to prepare macrocyclic compounds with different subunits and with an association constant relationship adequate not only in complexing the species in the source phase but also in releasing it in the receiver phase.

With this goal, we have undertaken the preparation of compound **2**, in which one of the three subunits, the central one, is different not only in its size but in the characteristic of donor atoms as well. The three crown ethers of this compound are conformationally related through two biphenyl systems. In this way, when a complex is formed in one of the crowns, the conformational information is transferred to the other crown ether subunits and so these parts of the complete system would adopt a more favorable conformation to complex the species to be transported. This structural characteristic is present in bis(BF-19-C-5), but compound **2** has clear differences with the former

Chart 1



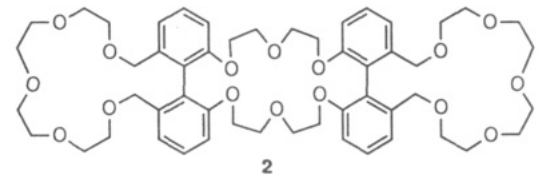
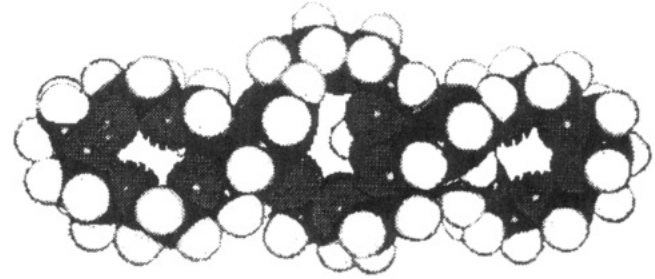
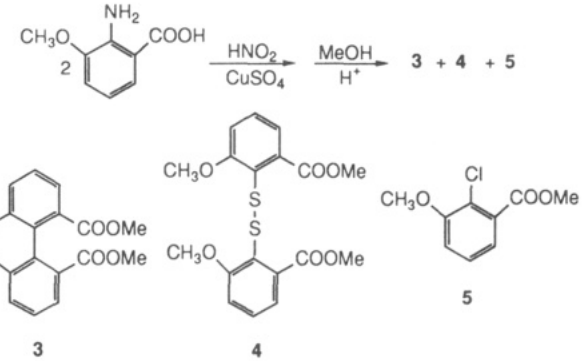


Chart 2



Scheme 1



one. First of all, in **2** there are three cavities (Chart 2) and even more important, the central crown ether has a different size (it is bigger), and four of its oxygens are directly bound to aromatic rings. In this way, two different factors influence the association constant relationship.

Synthesis

The synthesis of compound **2** was achieved from 6,6′-dimethoxy-2,2′-diphenic acid which was prepared from

® Abstract published in *Advance ACS Abstracts*, April 15, 1994.

(1) Tabushi, I. *Pure Appl. Chem.* **1988**, *60*, 581–586.
(2) Traylor, T. G.; Mitchell, M. J.; Ciccone, J. P.; Nelson, S. *J. Am. Chem. Soc.* **1982**, *104*, 4986–4989.
(3) (a) Rebek, J. *Acc. Chem. Res.* **1984**, *17*, 258–264. (b) Gaviña, F.; Luis, S. V.; Costero, A. M.; Burguete, M. I.; Rebek, J. *J. Am. Chem. Soc.* **1988**, *110*, 7140–7143.
(4) Gagnaire, G.; Jeunet, A.; Pierre, J-L. *Tetrahedron Lett.* **1992**, 2021–2024.
(5) Rebek, J., Jr.; Costello, T.; Marshall, L.; Wattley, R.; Gadwood, R. C.; Onan, K. *J. Am. Chem. Soc.* **1985**, *107*, 7481–7487.
(6) (a) Costero, A. M.; Rodriguez, S. *Tetrahedron Lett.* **1992**, 623–626. (b) Costero, A. M.; Rodriguez, S. *Tetrahedron.* **1992**, 6265–6272.

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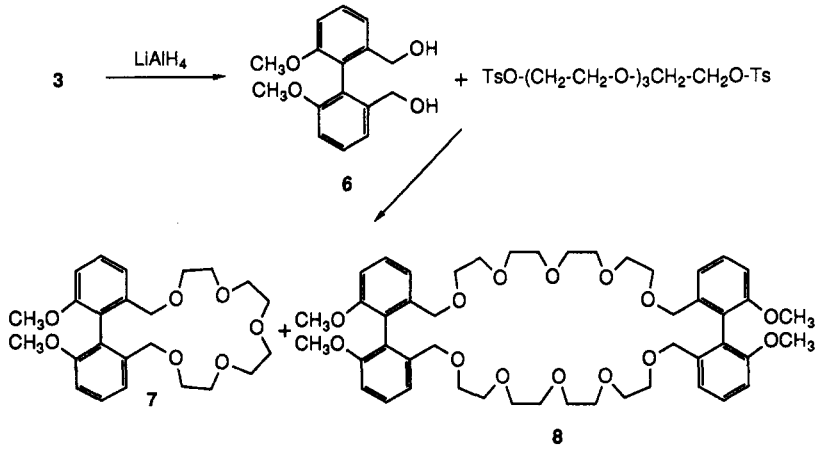
Scheme 2



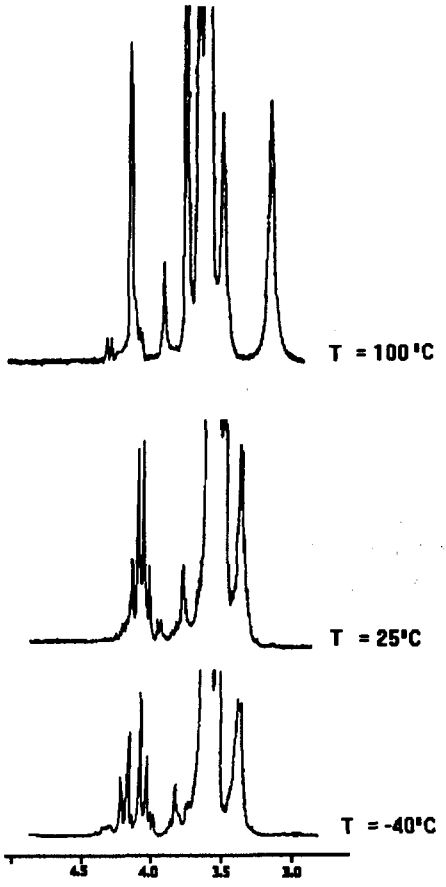
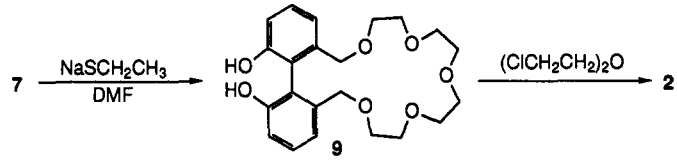


Figure 1. Benzylic signals in variable-temperature ^1H NMR spectra of compound 8.

2-amino-3-methoxybenzoic acid through a Gatterman dimerization.[7] After esterification 6,6′-dimethoxy-2,2′-diphenic acid dimethyl ester (**3**) was isolated in a 65% yield. As byproducts of this reaction bis[2-(methoxycarbonyl)-6-methoxyphenyl]disulfide (**4**) and 2-chloro-3-methoxybenzoic acid methyl ester (**5**) were separated in a proportion of 6.4 and 19.7%, respectively; the mechanism of this reaction probably involves free radicals, and a disulfide group could be introduced from the sodium metabisulfite present in the reaction medium. In order to improve the yield of the major product, the reaction was repeated in absence of metabisulfite[8] obtaining compound **3** in an 81.3% yield and **5** in an 11.5% yield.

Scheme 3



Reduction of **3** with $LiAlH_4$ gave **6** and from it compound **7** was prepared by cyclization with tetraethylene glycol ditosylate[5] (35.8%).

In this reaction, compound **8** was also obtained in a 24% yield. This compound, which had an $R_f = 0$, showed similar ^1H and ^{13}C NMR spectra to **7**; only the benzylic hydrogen signals, that in **7** were a clear double doublet, in **8** had a poorer resolution. In the mass spectrum, a peak at higher m/e than the molecular peak of **7** was detected. These facts are consistent with the proposed structure which was confirmed through elemental analysis. ^1H NMR experiments at variable temperature allowed us to explain the broad signals of benzylic hydrogens (Figure 1).

The cavity in compound **8** is wide enough to permit biphenyl subunits to pass through it, but this conversion is slow at room temperature in the resonance time. Therefore the spectrum showed the characteristic aspect of a mixture of two different conformers. Moreover, when these conformational changes are possible, both benzylic hydrogens are identical and only a singlet is shown at high temperatures; at a lower temperature (−40 °C) one conformer is strongly favorable and the double doublet corresponding to two diastereotopic hydrogens can be observed. The other limit conformation is present in a very small concentration and its signals can barely be detected in the ^1H NMR spectrum.

All the attempts to demethylate compound **7** by using Lewis acids failed and only nucleophilic conditions ($NaSCH_2CH_3$/DMF)[9] allowed us to obtain **9** that was isolated in an 80% yield. Cyclization of this compound with bis(2-chloroethyl) ether[10] permitted us to obtain tricyclic ether **2**.

This compound possesses two chiral axes and it could exist in two different diastereoisomeric compounds, **2a** and **2b**. One of them is a *dl* pair and the other a meso form. ^1H and ^{13}C NMR demonstrated that only one of

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(8) Leffler, J. E.; Graybill, B. M. *J. Phys. Chem.* **1959**, *53*, 1457–1460.
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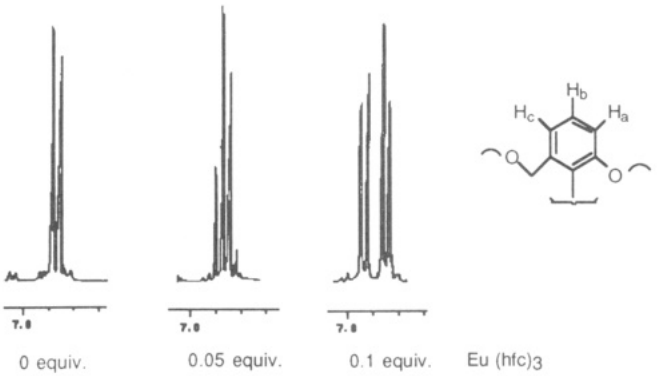
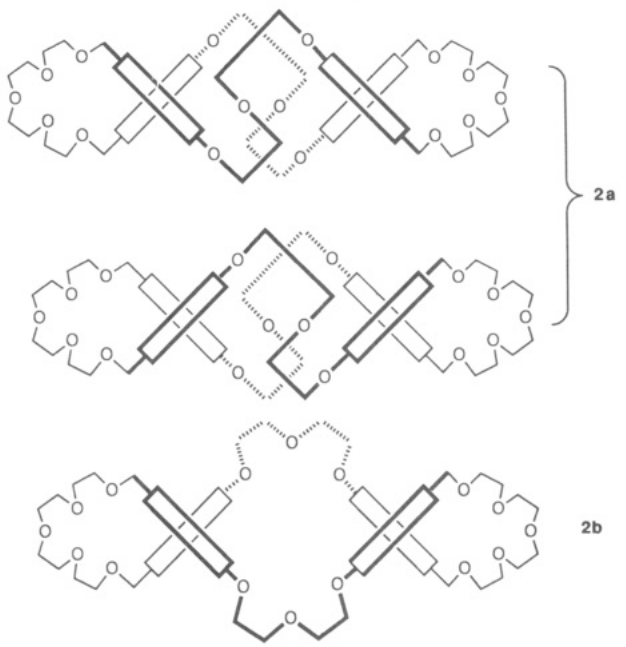


Figure 2. H_a Resonances at 300 MHz of **2** in the presence of different concentrations of $Eu(hfc)_3$.

Scheme 4



the diastereoisomers had been obtained, and studies using $Eu(hfc)_3$ as a shifting reagent permitted us to propose that the product was the *dl* pair (Figure 2).

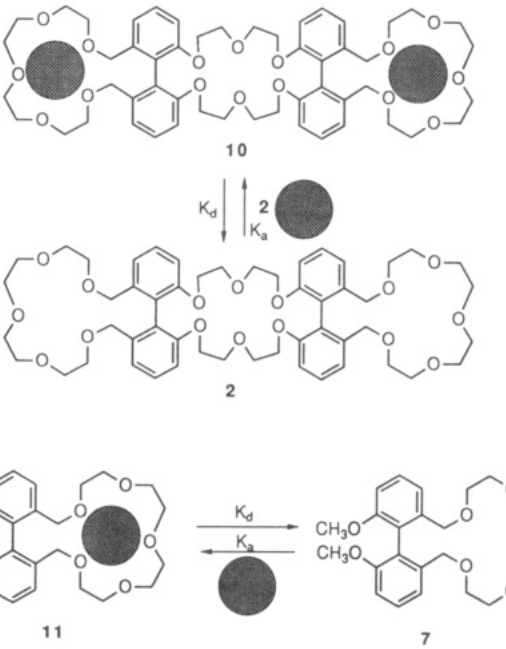
The fact that only one diastereoisomer had been obtained seems to support a strong staking interaction between both biphenyl subunits. On the other hand, smaller steric repulsions are observed in the *dl* pair than in the meso form.

Complexation Studies

Attempts to bind alkali metals carried out by Laszlo[11] and Rebek[5] had demonstrated that coulombic interactions make binding of a second ion more difficult than the first. Therefore we used the nonionic Hg derivative, $Hg(CN)_2$, as a guest in our complexation experiments. When a solution of **2** and $Hg(CN)_2$, both in acetone, were mixed, a solid complex, **10**, was isolated. Nitrogen analysis indicated a 1:2 stoichiometry; this complex was always the only compound isolated, not only when a ratio ether: $Hg(CN)_2$ (1:2), was used, but also when the concentration in the mercury salt was lower or higher than that necessary to have a 1:1 or 1:2 stoichiometry, respectively.

(11) Bouquant, J.; Deville, A.; Grandjezn, J.; Laszlo, P. *J. Am. Chem. Soc.* **1982**, *104*, 686–689.

Scheme 5



NMR studies showed that the complex was a symmetric molecule, meaning that the Hg atoms are located in the two external subunits of **2**. These results were surprising because it had been established that binding of $Hg(CN)_2$ to 6-oxygen macrocycles was greater than to 5-oxygen sites. However in **2**, that has both size subunits, complexation by the 5-oxygen crown ethers is more favorable. This fact could be explained if the subunit conformations in **2** were fixed to some extent in such a way that complexation by the 5-oxygen subunits was easier than by the central one.

The existence of this conformation would be responsible for the formation of 1:1 complex, but the 1:2 complex could only be due to the allosteric cooperativity, taking into account that the 1:2 complex is formed even in an excess of the crown ether. When the 1:2 complex was dissolved and studied by NMR, the presence of the 1:1 complex was never detected.

The allosteric cooperativity of **2** is according to the slope of the Hill plot; the data over the range of 30 to 70% saturation allowed us to determine a slope close to 1.2. The product $K_1^iK_2^i = K_1K_2 = 3300\ M^{-2}$ was determined by dissolving the complex in $(CD_3)_2CO:C_6D_6$ (1:1) and registering the NMR spectrum in which the signals of both species could be integrated. For all these data it is possible

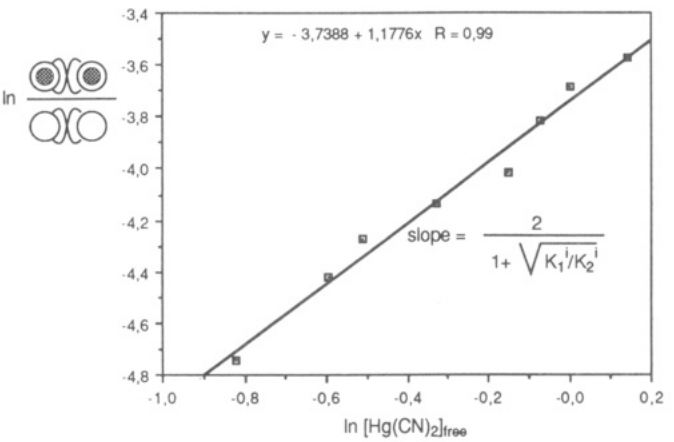


Figure 3. Hill plot.

Table 1. Molar Concentration of Species Transported/mol of Carrier

carrier	Na^+	$Hg(CN)_2$
2	0.050	127.7
7	0.135	100.2

to establish that K_1^i = 40 M^{-1} and K_2^i = 82 M^{-1}. For comparison, a similar study of the monocyclic counterpart 7 was undertaken. Complex 11 showed K = 15 M^{-1}, a value lower than that observed for K_1^i but similar to that reported for 6,6′-dimethyl-BF-19-C-5 (K = 13 M^{-1}). Therefore something unusual is involved in the initial binding to macrotricycle 2, probably a more-suitable conformation, although an alternative explanation could be based on entropic effects as has been proposed by Cooper.[12]

Transport Experiments

Transport of $Hg(CN)_2$ through a $CHCl_3$ liquid membrane with 2 and a control experiment with 7 were examined within a U-tube. An aqueous solution of $Hg(CN)_2$ was placed in the left arm of the tube and atomic absorption analysis was used to determine the transport to the right arm. Control experiments established that $CHCl_3$ showed very little tendency to transport mercury in the absence of carriers. When the experiments were carried out under the same conditions, transport efficiency of 2 was slightly higher than that of 7 when molar concentrations were considered (Table 1). In absence of allosteric cooperativity (two independent cavities), the $Hg(CN)_2$ amount transported by 2 should have been twice than that transported by 7. As this result has not been observed, the behavior of 2 could be understood as that of a system in which the complexation at the first subunit activates the complexation at the second one, and only this subunit is involved in transport. As a consequence of this activation one of the two complexing subunits of 2 is more efficient than the sole site in 7.

When Na^+ transport experiments were carried out by using 2 and 7, transport with both carriers was around 10^{-3}–10^{-4} lower than when $Hg(CN)_2$ was transported. As was expected, taking into account electrostatic repulsions in Na^+, transport with carrier 2 is even less efficient than with 7.

Conclusions

We have prepared a new allosteric carrier, 2, in seven steps from 3-methoxy-2-aminobenzoic acid with an 8% overall yield. This compound, obtained just as a diastereoisomer, binds $Hg(CN)_2$ always giving 10 even when a deficiency of the mercury salts were used. This complex is a symmetric molecule with the mercury atoms in the external subunits. These results allow us to affirm that 2 presents positive allosteric cooperativity between the two external subunits. The transmission of conformational information between these two crown-ether subunits is produced by the rigidity of the dihedral angles of both biaryl systems. This rigidity is fastened by the central subunit that links both biphenyls. This central crown ether has a conformation which is not suitable for complex $Hg(CN)_2$. When carrier 2 is used to transport $Hg(CN)_2$ only one of its sites is involved in transport and the other external subunit acts as an activator homotropic center.

(12) Copper, A.; Dryden, D. T. F. *Eur. Biophys. J.* **1984**, *11*, 103–109.

On the other hand, obtaining 8 with a moderate yield even under high-dilution conditions showed us that the intermolecular cyclization is able to compete with the intramolecular one to give compound 7.

Experimental Section

General Methods. The products whose syntheses are not described were purchased from Aldrich Chemical Co. and used without further purification. IR spectra were in KBr pellets. TLC analyses were carried out on 0.2-mm Merck PC 60 F 245 silica gel plates. Column chromatographies were carried out on Merck 60 A-CC silica gel. Microanalyses were determined by Servicio de Microanálisis (C.S.I.C.).

Dimethyl 6,6′-dimethoxy-2,2′-diphenate (3). Method A. 2-Amino-3-methoxybenzoic acid (3 g, 0.018 mol) was ground in a mortar with HCl (5 mL) and water (10 mL). The suspension was cooled to 0–5 °C, and a solution of $NaNO_2$ (1.25 g, 0.018 mol in 20 mL of water) was added to it for 15–20 min. The resulting diazonium salt solution was filtered if necessary and kept below 5 °C until used. Three different solutions were necessary. The first (solution 1) was prepared by dissolving copper sulfate pentahydrate (6.75 g) in water (25 mL) and ammonium hydroxide (5.5 mL); the second solution (solution 2) contained sodium metabisulfite (10 g) and ammonium hydroxide (10 mL). Solution 3 was prepared by dissolving anhydrous ferric chloride (6.5 g) in water (10 mL) and concentrated hydrochloric acid (33 mL). Solutions 1 and 2 were mixed and cooled at 30 °C, after which, the diazonium salt solution was added with rapid stirring followed by solution 3. The reaction was kept at room temperature and after a short time 6,6′-dimethoxy-2,2′-diphenic acid was separated through filtration. This acid was purified by dissolution in sodium hydrogen carbonate solution and reprecipitation (67.7%); from the filtrate another solid was separated after 24 h. This second solid was a mixture of two compounds. To carry out their separation they were esterified by heating with methyl alcohol (15 mL) and concentrated sulfuric acid (1.5 mL) for 6 h under reflux. 4 (6.4%) and 5 (19.7%) were isolated through silica gel column chromatography (CCl_4–Et_2O 3/1). 4: mp = 96 °C; 300-MHz ^1H NMR (CD_3OD) δ 7.39 (1 H, t, J = 7.9 Hz), 7.12 (1 H, d, J = 7.9 Hz), 7.07 (1 H, d, J = 7.9 Hz), 3.69 (3 H, s), 3.67 (3 H, s); 75-MHz ^{13}C NMR ($CDCl_3$) δ 167.9 (s), 160.0 (s), 137.9 (s), 130.0 (d), 124.7 (s), 120.3 (d), 113.3 (d), 56.0 (q), 52.2 (q); MS m/e 394 (M^+), 197 ($M^+/2$), 165 ($M^+/2$ – S). Anal. Calcd for $C_{18}H_{18}O_6S_2$: C, 54.8%; H, 4.56%; S, 16.24%. Found: C, 54.6%; H, 4.55%; S, 16.14%. 5: mp = 37–38 °C; 300-MHz ^1H NMR (CD_3OD) δ 7.33 (1 H, t, J = 7.8 Hz), 7.26 (1 H, dd, J = 7.8 Hz, J = 1.5 Hz), 7.21 (1 H, dd, J = 7.8 Hz, J = 1.5 Hz), 3.9 (3 H, s), 3.8 (3 H, s); 75-MHz ^{13}C NMR (CD_3OD) δ 168.0 (s), 156.9 (s), 133.6 (s), 128.4 (d), 122.7 (d), 115.7 (d), 56.8 (q), 52.7 (q); MS m/e 200.5 (M^+), 169 ($M^+ - CH_3O$), 154 ($M^+ - C_2H_6O$). Anal. Calcd for $C_9H_9O_3Cl$: C, 53.85%; H, 4.48%; Cl, 17.60%. Found: C, 53.90%; H, 4.50%; Cl, 17.57%.

6,6′-Dimethoxy-2,2′-diphenic acid was esterified under the same conditions to give 3 (96.6%): mp = 137 °C; 200-MHz ^1H NMR ($CDCl_3$) δ 7.63 (1 H, dd, J = 7.0 Hz, J = 1.5 Hz), 7.40 (1 H, t, J = 7.0 Hz), 7.12 (1 H, dd, J = 7.0 Hz, J = 1.5 Hz), 3.7 (3 H, s), 3.6 (3 H, s); 50-MHz ^{13}C NMR ($CDCl_3$) δ 167.4 (s), 156.4 (s), 131.2 (s), 128.0 (d), 127.6 (s), 121.9 (d), 114.1 (d), 56.0 (q), 51.6 (q).

Method B. After formation of the diazonium salt solution as above, the reducing agent was prepared from two different solutions. Solution 1 was formed by dissolving copper sulfate pentahydrate (15 g) in 50 mL of water and 25 mL of ammonium hydroxide solution (28%). Solution 2 was prepared by dissolving hydroxylamine hydrochloride (4.25 g) in 15 mL of water and 9 mL of 6 N sodium hydroxyde solution. Solution 1 was cooled at 10 °C and treated with a fresh cold solution 2. In an ice bath the diazonium salt solution was introduced below the surface of the reducing solution for about 20 min while stirring. The mixture was kept in the same bath for an additional 10–15 min. The solution was then boiled, while 30 mL of concentrated hydrochloric acid was carefully added. The diphenic acid precipitated during the acid addition was separated by filtration (84.6%). From the filtrate another solid, identified as 2-chloro-3-meth-

oxybenzoic acid, was isolated after about 24 h (4.7%). 6,6'-Dimethoxy-2,2'-diphenic acid was purified by treatment with charcoal in a sodium hydrogen carbonate solution and recrystallized from 95% ethanol. Both compounds were esterified as described in method A giving **3** (81.3%) and **5** (4.5%).

2,2'-Bis(hydroxymethyl)-6,6'-dimethoxybiphenyl (6). A solution of **3** (0.2 g in 20 mL of dry THF) was added to a suspension of $LiAlH_4$ (0.075 g in 25 mL of dry THF) and refluxed for 6 h. An ammonium chloride solution was added to the cool reaction mixture. The suspension obtained was extracted with dichloromethane (2 × 40 mL). The organic phases were dried ($MgSO_4$) and concentrated and gave a white solid (0.170 g). After recrystallization from acetone, **6** (0.159 g, 95.5%) was obtained: mp = 158 °C; 200-MHz ^1H NMR ($CDCl_3$) δ 7.34 (1 H, J = 8 Hz), 7.18 (1 H, d, J = 8 Hz), 6.96 (1 H, d, J = 8 Hz), 4.15 (2 H, m), 3.56 (3 H, s); 50-MHz ^{13}C NMR ($CDCl_3$) δ 157.5 (s), 142.6 (s), 129.0 (d), 124.4 (s), 120.7 (d), 110.3 (d), 62.5 (t), 55.7 (q).

Synthesis of 6,6'-Dimethoxy-2,2'-biphenyl-19-C-5 (7). **6** (0.5 g, 1.82 mmol) was added to an NaH suspension (0.35 g, 14.56 mmol) in dry THF (100 mL) under inert atmosphere and refluxed for 1 day. A solution of tetraethylene glycol ditosylate (0.914 g, 1.82 mmol) in dry THF (50 mL) was added very slowly (4 h) and refluxed for another day. The reaction was stopped by water addtion until gas was not observed. After filtration, additional water was added to the liquid phase and then extracted with dichloromethane. The organic phase was dried ($MgSO_4$) and concentrated to give a brown oil. After silica gel column chromatography (ethyl acetate–hexane 6/4 rising polarity), **7** (0.282 g, 35.8%) was obtained as a white solid: mp = 72–73 °C; 200-MHz ^1H NMR ($CDCl_3$) δ 7.4–7.15 (2 H, m), 6.88 (1 H, d, J = 8.1 Hz), 4.25 (1 H, d, J = 12.3 Hz), 4.13 (1 H, d, J = 12.3 Hz), 3.65 (3 H, s), 3.60–3.25 (8 H, m); 50-MHz ^{13}C NMR ($CDCl_3$) δ 156.6 (s), 139.1 (s), 128.5 (d), 123.9 (s), 120.4 (d), 109.8 (d), 70.9 (t), 70.7 (t), 70.5 (t), 70.0 (t), 55.8 (q); MS m/e 432 (M^+), 238 ($M^+ - C_8H_4O_5$). Anal. Calcd for $C_{24}H_{32}O_7$: C, 66.65%; H, 7.46%. Found: C, 66.8%; H, 7.13%. From the same column, by using methanol as an eluent, **8** was isolated as a yellow wax (0.197 g, 23.6%): 200-MHz ^1H NMR ($CDCl_3$) δ 7.33 (1 H, t, J = 8.0 Hz), 7.19 (1 H, d, J = 8.0 Hz), 6.87 (1 H, d, J = 8.0 Hz), 4.13 (2 H, m, CH_2), 3.55 (11 H, m, CH_2, CH_3). The signal to 4.13 ppm became a single signal at 100 °C and two doublets (J = 12 Hz) at −40 °C. 50-MHz ^{13}C NMR ($CDCl_3$) δ 156.3 (s), 138.6 (s), 128.3 (d), 123.2 (s), 119.4 (d), 109.4 (d), 70.0 (t), 69.3 (t), 55.4 (q). Anal. Calcd for $C_{48}H_{64}O_{14}$: C, 66.66%; H, 7.40%. Found: C, 66.30%; H, 7.60%.

Demethylation of 7 with NaEtS in DMF. A 0.5 M solution of NaEtS in dry DMF was prepared in a flask under argon atmosphere. THen, 7.4 mL (3.7 10^{-3} mol) of this solution was added to 6,6'-dimethoxy-2,2'-BF-19-C-5 (0.4 g, 9.28 10^{-4} mol) and the resulting solution was heated in a graphite bath at 115–120 °C under argon for 24 h. The cooled mixture was then acidified with 10% aqueous HCl and extracted with EtOAc (3 × 10 mL). The combined organic extracts were washed with 10% aqueous NaOH (3 × 5 mL). The chilled basic aqueous washings were acidified with 10% aqueous HCl and extracted with EtOAc (3 × 10 mL). The combined organic extracts were washed with water and dried ($MgSO_4$). Removal of solvent by rotatory evaporation afforded the phenolic product **9** (0.3 g, 80%): mp = 142–143 °C; 300-MHz ^1H NMR ($CDCl_3$) δ 7.32 (1H, t, J = 7 Hz), 7.13 (1H, d, J = 7 Hz), 7.02 (1H, d, J = 7 Hz), 5.4 (1H, s), 4.32 (1H, d, J = 10 Hz), 4.24 (1H, d, J = 10 Hz), 3.18–3.70 (8H, m); 50-MHz ^{13}C NMR ($CDCl_3$) δ 154.0 (s), 139.1 (s), 129.9 (s), 122.2 (d), 120.6 (s), 116.3 (d), 71.0 (t), 70.9 (t), 70.6 (t), 70.4 (t), 69.6 (t). Anal. Calcd for $C_{22}H_{28}O_7$: C, 65.34%; H, 6.93%. Found: C, 65.40%; H, 6.85%.

Synthesis of 2. A dry, 100-mL, three-necked flask was fitted with a reflux condenser, a thermometer, and an inlet pipe connected to a peristaltic pump. An inlet tube at the top of the reflux condenser was used to maintain a static argon atmosphere in the reaction vessel throughout the reaction. The flask was charged with **9** (0.4 g, 9.95 × 10^{-4} mol), and 12 mL of commercial 1-butanol before magnetic stirring was started, and NaOH in pellets (40 mg, 10^{-3} mol) were added. The mixture was heated rapidly to reflux (about 115 °C), and a solution of bis(2-chloroethyl) ether in 10 mL of 1-butanol was added through a thin tube using a precision pump with continuous stirring and heating, as slowly as possible. After the resulting mixture had been refluxed with stirring for an additional 1 h, it was cooled to 90 °C and an additional portion of NaOH in pellets (40 mg, 10^{-3} mol) was added. The mixture was refluxed, with stirring, for 30 min, and a solution of bis(2-chloroethyl) ether in 10 mL of 1-butanol was added under the same conditions as the first step. The final reaction mixture was refluxed, with stirring, for 24 h. The hot reaction mixture was filtered. By removing the solvent in a rotary evaporator (15 mbar, 40 °C), a semisolid compound was obtained. After silica gel column chromatography (hexane/ethyl acetate 1:3, rising polarity), a solid white product (R_f = 0.2) identified as **2** was obtained: mp = 105 °C; 300-MHz ^1H NMR ($CDCl_3$) δ 7.3 (1 H, t, J = 8 Hz), 7.2 (1H, d, J = 8 Hz), 6.88 (1H, d, J = 8 Hz), 4.27 (1H, d, J = 11.8 Hz), 4.16 (1H, d, J = 11.8 Hz), 4.08 (1H, ddd, J_1 = 12.2, J_2 = 5.6, J_3 = 1.8 Hz), 4.24 (1H, ddd, J_1 = 12.2, J_2 = 5.8, J_3 = 2.0 Hz), 3.7–3.3 (10H, m); 75-MHz ^{13}C NMR ($CDCl_3$) δ 155.7 (s), 139.2 (s), 128.1 (d), 125.9 (s), 121.0 (d), 113.5 (d), 71.1 (t), 71.0 (t), 70.77 (t), 70.74 (t), 70.5 (t), 70.2 (t), 69.9 (t). Anal. Calcd for $C_{52}H_{68}O_{16}$: C, 65.80%; H, 7.22%. Found: C, 65.68%; H, 7.33%.

Synthesis of [Bis-6,6'-(2,2'-DBF-22-C-6)-19-C-5]·[$Hg(CN)_2$]$_2$ (10). One-third equivalent of $Hg(CN)_2$ (3.98 mg, 0.0158 mmol) in the minimum volume of acetone necessary was added to a solution of 15 mg (0.0158 mmol) of **2** in 0.6 mL of acetone. After a few minutes a white solid precipitated and was filtered and dried. Elemental microanalysis showed that the stoichiometry was 1:2: mp = 198–200 °C; 400-MHz ^1H NMR (C_6D_6/$(CD_3)_2CO$ (1:1)) δ 7.7–7.5 (2H, m), 7.22 (1H, d, J = 7.8 Hz), 4.65 (1H, d, J = 10.6 Hz), 4.54 (1H, d, J = 10.6 Hz), 4.27 (1H, ddd, J_1 = 13.2, J_2 = 8.3, J_3 = 2.2 Hz), 4.13 (1H, ddd, J_1 = 13.2, J_2 = 8.1, J_3 = 2.4 Hz), 3.88–3.40 (10H, m). Anal. Calcd for $C_{56}H_{68}O_{16}N_4Hg_2$: C, 46.14%; H, 4.70%; N, 3.85%. Found: C, 46.20%; H, 4.78%; N, 3.79%.

Synthesis of [6,6'-Dimethoxy-BF-2,2'-19-C-5]·[$Hg(CN)_2$] (11). An amount of 14.1 mg (0.033 mmol) of **7** was dissolved in 0.8 mL of acetone, and 0.033 mmol of $Hg(CN)_2$ in 0.2 mL of acetone was added using a micropipette. The solution was stirred for 4 h. After a slow evaporation of the solvent, white crystals were obtained, which were washed with an additional portion of cool acetone: mp = 190–192 °C. Anal. Calcd for $C_{28}H_{32}O_7N_2Hg$: C, 45.58%; H, 4.70%; N, 4.09%. Found: C, 45.61%; H, 4.67%; N, 4.08%.

Association Constants Determination. General Method. In an NMR tube a perfectly weighed amount of the complex was dissolved in an appropriate and known volume of benzene-d_6:acetone-d_6 (50:50). The reference standard (Me_4Si) was added, and the tube was capped with Teflon tape. The NMR spectra was recorded at 400 MHz and 298 K, and the different signals were identified and integrated. By the integral, ratio (R) between the free form and the complex form could be known and K_a determined.

K_a of 10. The initial concentration of the complex in the NMR tube was 0.01 M. The downfield region of the spectra showed separate signals for the aromatic protons of each of the crown ether species in solution (free 7.11 ppm, doubly bound 7.13 ppm, singly bound were not observed). In this case R was 1.82 and K_a 3.3 × 10^3 M^{-2}.

K_a of 11. The initial concentration of the complex in the NMR tube was 0.029 M. The middle region of the spectra showed separate signals for the benzylic protons of each of the crown ether species in solution (free 4.55 ppm, bound 4.53 ppm). In this case the value of R was 2.96 and K_a 15.4 M^{-1}.

Titration of 2 with $Hg(CN)_2$. A solution of **2** in benzene-d_6:acetone-d_6 (50:50) with a concentration of 0.019 M was prepared. Of this, 0.540 mL were placed in an NMR tube, and the solvent level was marked. A second solution was made in the same solvent with a $Hg(CN)_2$ concentration of 0.028 M. An initial spectrum was recorded, 50 μL of the second solution was added to the NMR tube, and the solvent level was reduced by evaporation to the mark indicated on the tube. The spectrum was then recorded again. This procedure was repeated until the number of equivalents of $Hg(CN)_2$ added was higher than the number of equivalents of the crown ether in the tube. The different spectra showed separate signals for the aromatic protons of the free and complex crown ethers. From the R values, ln(bound sites/free sites) was plotted against $\ln[Hg(CN)_2]_{free}$ (Hill

plot, Figure 3) and the slope value determined (slope = 1.177). Intrinsic association constants were $K^1_1 = 40\ M^{-1}$, $K^1_2 = 82\ M^{-1}$.

Transport Experiments. Transport experiments were carried out in a 13-mm diameter U-tube. The left arm was occupied by 10 mL of an aqueous 0.05 M solution of the corresponding salt (NaCl, $Hg(CN)_2$), and in the right arm, only distilled water (10 mL) was placed. Both solutions were separated through a 1.5×10^{-3} to 4.0×10^{-3} M solution of the carrier in $CHCl_3$. The two necks were closed with a tap and the system was stirred (600 rpm) at 25 °C for 3 days. After that, the salt concentration in the right arm was determined by atomic absorption and the transport efficacy was expressed as molar concentration of the transported species in the right arm/mol of the carrier (see Table 1). Each experiment was repeated at least three times, and the reported results are the average of the determinations. The standard deviation from the mean value among the data in each experiment was lower than 5%.

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