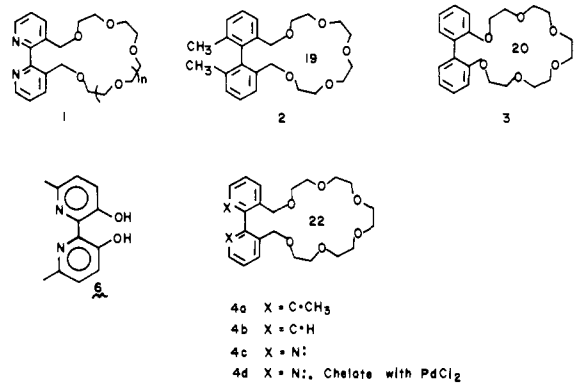
Allosteric Effects: An On-Off Switch

J. Rebek, Jr.,* and L. Marshall

Contribution from the Department of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania 15260. *Received June 21, 1982*

Abstract: A model for allosteric effects is described in which conformational changes induced by binding at one site alter the receptivity of a remote binding site. The system involves a 2,2′-bipyridyl function attached at the 3,3′-positions to a macrocyclic polyether. The rate of release of $Hg(CF_3)_2$ by the 22-membered polyether ring is shown to be slowed by 7 orders of magnitude through binding of $PdCl_2$ at the bipyridyl function. The magnitude and predictability of this allosteric effect are attributed to the ability of a 22-membered ring to permit the rapid passage of a CF_3 group while smaller rings permit only slower passage.

We have recently introduced systems **1** as model compounds



for allosteric effects. These structures incorporate two remote but interdependent binding sites, and we were able to show that the transport of alkali metal ions by the crown ether site was subject to modest control by binding of a transition metal at the bipyridyl site.[1] Since ion transport rates are not easily related to changes in cavity sizes, or even to association constants,[2] the allosteric effect in this case can be rationalized but not predicted. Here we show that both the predictability and the magnitude of allosteric effects involving models can be quite large, provided that highly specific interactions are involved.

Results and Discussion

Rates of Complex Formation. We have found that the interaction of $Hg(CF_3)_2$ with crown ethers is highly specific with respect to macroring size. This specificity is most apparent in the *kinetics* of complexation. For example, no binding could be detected to either 18-crown-6 or the 19-membered **2**, but with the 20-membered **3**, slow complexation occurs in a process that takes days to reach equilibrium. With 21-crown-7 in MeOH, a complex is rapidly precipitated. Release of the metal from this complex in toluene takes several weeks; indeed, the crown ether is best recovered from the complex by $LiAlH_4$ reduction rather than by the usual extraction methods.

The sensitivity of these rates to ring size (rather than, say, to the number of oxygen atoms) suggests a rotaxane structure for the complex (Figure 1) in which a CF_3 group must penetrate the macroring before the ethereal oxygens can converge on the Hg atom. A crystallographic study, to be published elsewhere, substantiates this type of structure. In addition, ample precedent exists in the rotaxane structures shown by Me_2Ti binding to 18-crown-6 derivatives.[3]

The binding rates of $Hg(CF_3)_2$ to crown ethers are amenable to study by NMR with 22-membered rings, but even within this constant ring size complexation dynamics are a function of quite subtle effects. As the size of the seemingly remote group in the 6,6′-position decreases (**4a** → **4c**), the rate of dissociation of the complex increases. The table in Figure 2 records the free energies of activation, $\Delta G^{\ddagger}_c$, for dissociation of $Hg(CF_3)_2$ from its complexes with **4**. These were calculated at the coalescence temperature by using the approximation formula and the ^1H or ^{19}F NMR spectra in two solvent systems.

A likely interpretation may be visualized in Figure 3. Passage of a trifluoromethyl group through the 22-membered ring is difficult unless the dihedral angle, θ, is large. The maximum value for this angle (the effective size of the macrocycle) is determined by buttressing effects involving the 6,6′-substituents and the benzyl hydrogens at the 2,2′-positions. Thus, fastest dissociation is observed with the smallest (lone pair) group.

The consequences of binding a transition metal ion at the bipyridiyl function as in **4d** are predictable. Chelation restricts θ to small values and hinders the passage of the CF_3 group in and out of the macrocycle. The observed increased barrier to dissociation, ca. 10 kcal, is consistent with this anticipation and corresponds to a factor of $>10^7$ in rate. The remote bipyridyl group acts here as an on-off switch[4] for the uptake and release of $Hg(CF_3)_2$ by the crown ether.

Equilibria. The considerations described above lead to comparable effects on the rate that $Hg(CF_3)_2$ moves in *and* out of the ethereal cavities of **4a-d**. That is, constrictions that slow the rate of CF_3 passage *into* the macrocycle will also slow its rate *out*. As a result, the association constants for **5a-d** might all be identical. On the other hand, if there exists an optimum θ for binding the Hg, then the association constants could vary with the ability of the crowns to reach it.

The equilibrium constant K_a for **4a** was obtained by integrating the free and bound ^{19}F resonances for solutions of known concentrations of **4a** and $Hg(CF_3)_2$. Some 40 such determinations were made since the values for **4b-d** were to be related to that for **4a**. The values obtained are plotted in Figure 4 as a function of temperature in two different solvents. The least-squares slope in toluene-d_8 gives $\Delta H = -15.1 \pm 0.4$ kcal/mol, $\Delta S = -32.4 \pm 1.2$ eu, and, at $T = 295$ °C, $K_a = 12000 \pm 900$ M^{-1}. For the acetone-d_6/(50%) benzene-d_6 solvent mixture, the corresponding values are $\Delta H = -10.6 \pm 0.4$ kcal/mol, $\Delta S = -25.8 \pm 1.6$ eu, and $K_a = 150 \pm 10$ M^{-1}.

Direct, pairwise competition of **4a** and ethers **4b-d** for a limited amount of $Hg(CF_3)_2$ gave the equilibrium constants shown in Figure 3. All of these are seen to be within a factor of 5 and indicate that the optimum geometry for complexing the Hg atom is accessible to any of the ethers. The crystallographic study[5] on

(1) Rebek, J.; Wattley, R. V. *J. Am. Chem. Soc.* **1980**, *102*, 4853-4854.
(2) For recent progress on this issue, see: Lamb, J. D.; Christensen, J. J.; Oscarson, J. L.; Nielsen, B. L.; Asay, B. W.; Izatt, R. M. *J. Am. Chem. Soc.* **1980**, *102*, 6820-6824.
(3) Hendrick, K.; Matthews, R. W.; Podejma, B. L.; Tasker, P. A. *J. Chem. Soc., Chem. Commun.* **1982**, 118; Hughes, D.; Truter, M. *Ibid.* **1982**, 727.
(4) A similar switch, using photoresponsive crown ethers, has recently been developed: Shinkai, S. Minami, T.; Kusano, Y.; Manabe, O. *J. Am. Chem. Soc.* **1983**, *105*, 1851.
(5) Performed by Professor K. Onan of Northeastern University.

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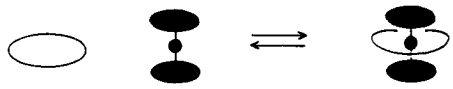
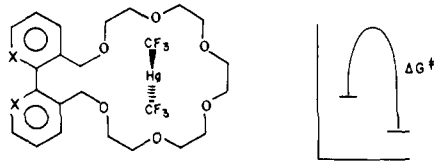


Figure 1.



	Dissociation Barrier $\Delta G_c^{\ddagger}$ (kcal/mole)		Equilibria K_{eq} (295° K)	
X =	C_7D_8	$(CD_3)_2CO/C_6D_6$	C_7D_8	$(CD_3)_2CO/C_6D_6$
C·CH_3	16.5	15.1	1.2×10^4	150
C-H	13.4	13.4	2.0×10^4	450
N:	11.3	12.3	4.1×10^4	700
N: $PdCl_2$	—	23	—	280
N: Pd_2X_4	—	—	—	310

Figure 2.

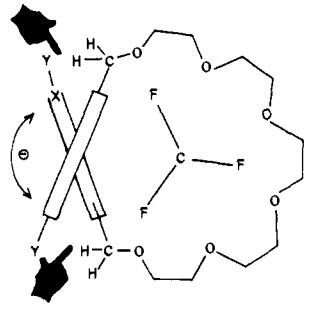


Figure 3.

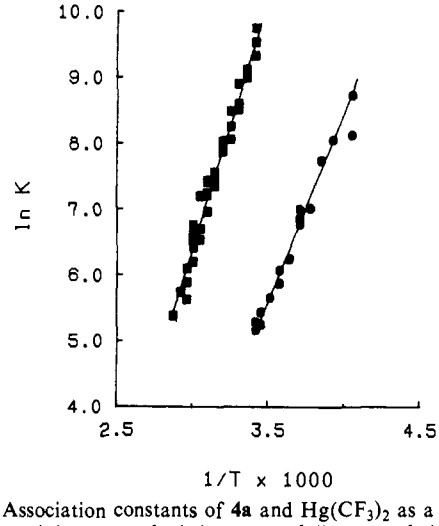


Figure 4. Association constants of **4a** and $Hg(CF_3)_2$ as a function of temperature: (■) toluene-d_8; (●) acetone-d_6/benzene-d_6 (1/1 v/v).

4a reveals that only one of the two benzyl oxygens is involved in binding. This would suggest that there is no optimal θ for the complexes.

Mechanism. What can be said about the mechanism by which $Hg(CF_3)_2$ binds to the Pd/crown complex **4d**? One possibility is prior *dissociation* of the $PdCl_2$ from the crown as the slow step, followed by rapid complexation of $Hg(CF_3)_2$ and **4c** and then rebinding of the Pd (Scheme I, path A). This sequence was excluded by observing that (1) the rate of binding with **4d** was

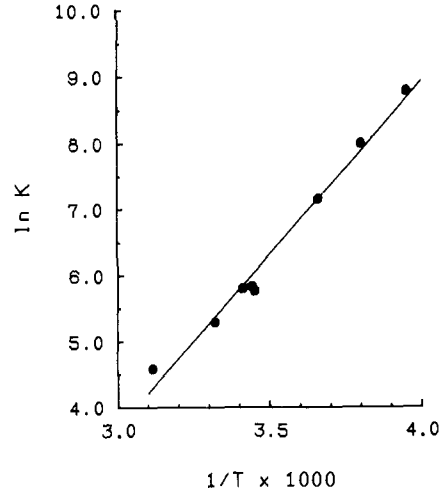
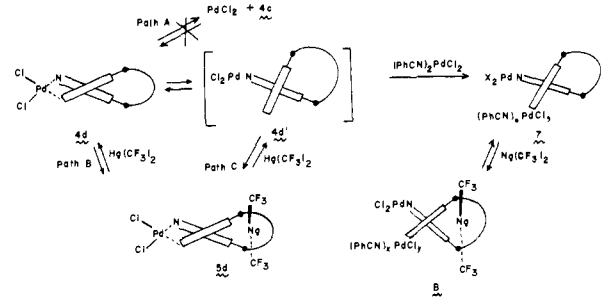


Figure 5. Association constant for the Pd_2/crown as a function of temperature in acetone-d_6/benzene-d_6 (1/1 v/v).

Scheme I



dependent on the concentration of $Hg(CF_3)_2$ and (2) no "free" $PdCl_2$ was generated. The latter was established through control experiments, which showed that **6**[7] rapidly precipitated "free Pd" [as its $(PhCN)_2PdCl_2$ complex] from solutions at comparable concentrations. However, when Hg was added to a solution of **4d** and **6**, no precipitate formed, and the rate of complexation to **4d** was unaffected.

Another possibility involves direct insertion of the CF_3 group into the reduced cavity of **4d** (path B). This cannot be excluded. A third possibility involves *dechelation* of **4d** to **4d'** (path C) followed by binding of $Hg(CF_3)_2$ and then rechelation to **5d**. This possibility is attractive since rapid access to **4d'** can be established. For example, the addition of $(PhCN)_2PdCl_2$ to **4d** under these conditions results in the rapid formation of a 2/1 complex. The ^1H NMR of this shows a loss of symmetry in the aromatic and benzyl regions, in accord with two different Pd's chelated at the bipyridyl. Addition of $Hg(CF_3)_2$ results in a new complex, **8**, showing different, broadened, ^{19}F resonances for its CF_3 groups, again resulting from the loss of symmetry (δ −37.2 to −37.3). The rates and equilibria involved in the formation of **8** are comparable to those involved in **4a**. Variable temperature experiments with the Pd_2 complex and $Hg(CF_3)_2$ in acetone/50% benzene afforded the equilibrium constants plotted in Figure 5. The least-squares slope gives $\Delta H = -10.4 \pm 0.5$ kcal/mol, $\Delta S = -23.8 \pm 2.0$ eu, and, at $T = 295$ °C, $K_a = 310 \pm 50$ M^{-1}. The low solubility of the complex, however, precluded an accurate measurement of the dissociation barrier.

Summary

The magnitude and the reliability of the allosteric effect in this case is made possible by the initial sensitivity that the CF_3 group shows to effective ring size. The biochemical counterpart is the specificity that enzymes typically show toward their substrates;

(6) In accord with this reasoning, macrobicyclic derivatives of **4** showed no binding cooperativity to $Hg(CF_3)_2$: Rebek, J.; Wattley, R. V.; Costello, T.; Gadwood, R.; Marshall, L. *J. Am. Chem. Soc.* **1980**, *102*, 7398–7400.

(7) Rebek, J.; Trend, J. E.; Wattley, R. V.; Chakravorti, S. *J. Am. Chem. Soc.* **1979**, *101*, 4333–4337; Rebek, J.; Wattley, R. V. *J. Heterocycl. Chem.* **1980**, *17*, 749–751, and ref 6.

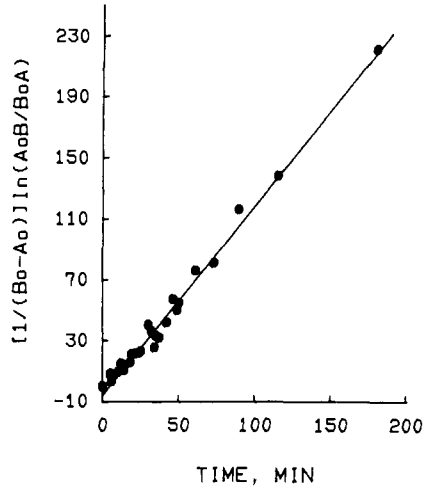


Figure 6. Second-order rate data for complexation of $Hg(CF_3)_2$ by **4d** in acetone-d_6/benzene-d_6 (1/1 v/v): A_0 = initial concentration of **4d**; A = concentration of **4d** at time t; B_0 = initial concentration of $Hg(CF_3)_2$; B = concentration of $Hg(CF_3)_2$ at time t.

conformational changes induced by binding of allosteric effectors should have maximum impact on highly specific enzyme/substrate interactions. In addition, the case at hand demonstrates the possibility of designing complexing agents that are tuned for ligands on a metal center rather than for the metal itself.

Experimental Section

Crown compounds were prepared as previously reported[7] by condenstion of the appropriate poly(ethylene glycol) (or glycol ditosylate) with the corresponding dibromide (or dialcohol). Bis(trifluoromethyl)-mercury, prepared by thermal decarboxylation of mercuric trifluroacetate,[8] was purified by slow sublimation at 60 °C: mp 170 °C dec (lit. mp 170 °C dec);[9] ^{19}F NMR ($CDCl_3$) δ −36.4 with Hg couplings at −34.2 and −38.6 (relative to internal $CFCl_3$). NMR solvents were used without further purification. Acetone-d_6 and benzene-d_6 were purchased from Stohler Isotope Chemicals; toluene-d_8 was from Aldrich.

^1H NMR spectra were obtained on a Bruker 300-MHz instrument equipped with variable temperature control (Me_4Si internal reference). ^{19}F spectra were obtained at 282.2 MHz by using the fluorine probe in the Bruker instrument with the variable temperature control. All ^{19}F shifts are relative to internal $CFCl_3$.

Complex of 21-Crown-7 with $Hg(CF_3)_2$. This complex was prepared by mixing equimolar amounts of the crown and metal in methanol or ether. The precipitated complex can be recrystallized from methanol. The white crystals, mp 179–183 °C, in acetone/50% benzene exhibit a ^1H NMR singlet at δ 3.50 (free 21-Cr-7, δ 3.54) and a ^{19}F NMR singlet at δ −37.1 [free $Hg(CF_3)_2$, δ −36.9]; mass spectrum m/e 579, 535, 397, 340, 308 (no parent at 648). Anal. Calcd for $C_{16}H_{28}O_7F_6Hg$: C, 29.70; H, 4.36; Hg, 31.00. Found: C, 30.01; H, 4.40; Hg, 30.82.

Equilibrium Constants. In toluene-d_8 determination of K_{eq} for the crown **4a** was accomplished by ^{19}F integration of free and bound fluorine resonances (δ −36.2 and −37.3, respectively) for a variety of samples over the temperature range 256–295 K.

Because the crowns **4b** and **4c** showed fast or intermediate exchange rates with $Hg(CF_3)_2$, at 295 °C, competition experiments were run to determine their K_{eq} values. Samples of the dimethyl crown **4a**, $Hg(CF_3)_2$, and crown to be tested were prepared (typical concentration ratio 1/1.5/1) and equilibrated 8–24 h. ^1H NMR integration of the methyl peaks (free, δ 1.86; bound, δ 1.90) or the appropriate benzyl peaks (left-hand side of AB quartet: free, δ 4.26 and 4.30; bound, δ 4.42 and 4.46) affords the fraction of crown **4a** bound as the complex. Due to the magnitude of the K's in toluene, it is then assumed that effectively all remaining metal is bound by the competing crown. In the $(CD_3)_2CO/C_6D_6$ mixture, K_{eq} was calculated for **4a** over the range 247–290 K by ^1H integration of the left-hand portion of the benzyl quartet (free, δ 4.20 and δ 4.24; bound, δ 4.32 and 4.36) and for the Pd_2 complex over the range 253–320 K by ^{19}F integration of free and bound metal. Extrapolation of the linear least-squares plot of ln K vs. $1/T$ afforded the appropriate equilibrium constants. Competition experiments with **4a** were run as in toluene for the crowns **4b** and **4c**. K_{eq} for the $PdCl_2$ crown was directly measured by ^1H integration of the free and bound aromatic doublet (this complex showed a slow exchange rate) and checked by competition experiments with **4a**.

Determination of $\Delta G^{\ddagger}_c$. In acetone/benzene complexes of crowns **4b** and **4c** with 2 equiv of $Hg(CF_3)_2$ showed rapid exchange at ambient temperature. Cooling in the ^{19}F probe afforded the coalescence temperature for **4b** (T_c = 280 °C) and **4c** (T_c = 257 °C) and $\Delta\nu_{max}$ = 112.9 Hz for the free and bound CF_3 resonances. With **4a** and 0.8 equiv of $Hg(CF_3)_2$, heating the sample provided T_c = 307 °C at which temperature equal populations of free and bound metal were present. For the complex **4d**, kinetics of binding to $Hg(CF_3)_2$ were followed by ^{19}F NMR and the second-order rate constant was determined to be 1.22 ± 0.06 M^{-1} min^{-1} at 295 K (Figure 6).

In toluene, ^1H NMR was used to obtain coalescence data from samples of **4a–c** to which 0.5 equiv of $Hg(CF_3)_2$ had been added. For **4a** the methyl resonances were monitored (T_c = 315 K, $\Delta\nu_{max}$ = 14.9). For **4b** the left side of the benzyl signals was observed while irradiating the right side (T_c = 272 K, $\Delta\nu_{max}$ = 42 Hz). The proton at C_4 of the pyridine ring was observed for **4c** while the proton at C_5 was irradiated (T_c = 273 K, $\Delta\nu_{max}$ = 80).

Acknowledgment. We thank the National Institutes of Health and the Alexander von Humboldt Foundation for support, and Professor S. Shinkai's stimulating correspondence and Professor Rich Gandour's helpful suggestions are also warmly acknowledged.

Registry No. 4a, 87174-22-9; **4b**, 87183-20-8; **4c**, 71638-21-6; **4d**, 71636-88-9; 21-crown-7, 33089-36-0; $Hg(CF_3)_2$, 371-76-6.

(8) Connett, J. E.; Deacon, G. B. *J. Chem. Soc. C* **1966**, 1058–1060.
(9) Emeleus, H. J.; Haszeldine, R. *J. Chem. Soc.* **1949**, 2953.