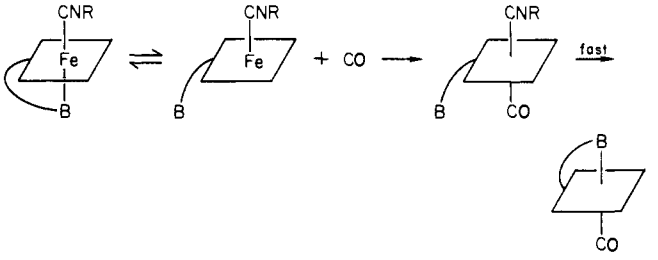
Table II. Comparison of the Equilibrium Constants (I and L) and Kinetic Constants for Association (i' and l') and Dissociation (i and l) in the Binding of n-BuNC and CO to Hemes

heme	i', M^{-1} s^{-1}	i, s^{-1}	I, M^{-1}	l', M^{-1} s^{-1}	l, s^{-1}	L, M^{-1}
chelated protoheme (1)[a]	2.2×10^8	0.5[b]	4.4×10^8	1.1×10^7	0.025[c]	4×10^8
	(1.7×10^8)[d,e]	(0.023)[d]	(7×10^9)[d]			
Mb[f]	5.8×10^4	1.0	5.7×10^4	5×10^5	0.017	3×10^7
	(2.3×10^2)[d]	(0.01)[d]	(2.5×10^4)[d]			
Hb(R) α[g]	3.5×10^4	0.32	1.1×10^5	6×10^6	0.009	7×10^8
Hb(R) β[g]	2.4×10^5	4.0	6×10^4			

[a] In benzene, 20 °C. [b] Calculated from I and i'. [c] Reference 8. [d] For (p-toluenesulfonyl)methyl isocyanide. [e] Calculated from I and i. [f] Reference 10. [g] References 2 and 11.

$\times 10^{-3}$ M) gave rates independent of [BuNC], $k = 0.6$ s^{-1}. This indicates that CO and BuNC do not compete for **1**, but the system returns to equilibrium via a "base-off" path.[3]



Direct determination of the dissociation rate for TMIC was obtained by mixing **1**-TMIC with chelated mesoheme[8] and following the formation of mesoheme–TMIC at 418 nm or the disappearance of **1**-TMIC at 428 nm. This method prevents reaction via the base-off pathway. From $i_{TMIC} = 0.023$ s^{-1} and I_{TMIC}, the on rate for TMIC is calculated $i' = 1.7 \times 10^8$ M^{-1} s^{-1}. The electronic differences between TMIC and BuNC appear only in the off rates.

Data for TMIC binding to purified sperm whale myoglobin[9] were obtained in phosphate buffer, pH 7.3. The dissociation rate was obtained by CO displacement. Spectral data for Mb–TMIC [λ_{max} = 431, 529, 559 nm ($\epsilon \times 10^3$ = 161, 13.2, 15.3, respectively)] are similar to those reported for other isocyanide derivatives of Mb.[7]

The equilibrium and kinetic differences between **1** and Mb or Hb are summarized in Table II. BuNC binding to Hb shows chain heterogeneity which is sensitive to pH and phosphates.[11] The 10^4 times difference between **1** and the proteins is seen to arise almost exclusively from association differences. The most remarkable example is the binding of TMIC, which suffers a 10^6 times decrease in association rate and a twofold decrease in dissociation rate in going from **1** to Mb! Large variations in CNR binding to proteins with increasing steric bulk of R also arise almost exclusively from association-rate differences.[10] These facts are most consistent with a conformationally flexible heme pocket where most of the pocket rearrangement occurs prior to the transition state for ligand addition. The lower association rate for CO addition to Mb suggests some distal steric hindrance is present.[3] The similarity between CO association rates for **1** and Hb(R) suggests little or no steric effects in the R state.

These results and previous kinetic studies afford a kinetic method for distinguishing among the various effects in hemoprotein ligation. Electronic effects in the ligand or proximal base[8] are reflected predominantly in the dissociation rates. Distal side steric effects appear in the association rates.[12] Proximal base steric strain has an approximately equal effect on association and dissociation rates.[2]

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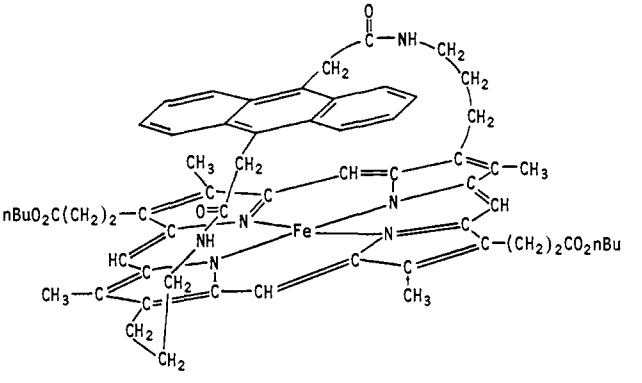
Cyclophane Hemes. 3. Magnitudes of Distal Side Steric Effects in Hemes and Hemoproteins

Sir:

The occurrence and magnitude of the distal side steric effects in hemoproteins remain a subject of great interest.[1-6] On the basis of the similar affinities of simple model compounds and hemoglobin for CO and O_2, we proposed[2] that R-state hemoglobin reacts with CO without distal side steric effects whereas myoglobin, having a lower CO affinity, is subject to this effect. Others[3,4] have suggested that all hemoproteins are subject to distal side steric effects on CO, reducing their affinities relative to model compounds.

We report a comparative study of CO and isocyanide binding to the hindered model compound[5] anthracene-6,6-cyclophane, **1**, which establishes the relative steric effects on ligands of various



1

Anthracene Heme Cyclophane

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Table I. Isocyanide Binding Data of 1, Mb, and Hb

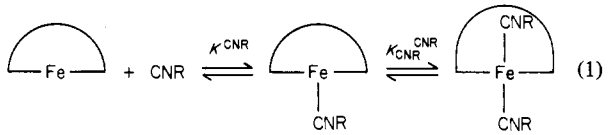
	K_{CNR}^{CNR}, M^{-1}	Q^a	I_1,[b] M^{-1}	I_{Mb},[c] M^{-1}	I_{Hb}, M^{-1}
n-BuNC	900	11	5.5×10^4	5.75×10^4	8×10^4 [d]
$C_6H_{11}NC$	170	45	1.3×10^4	1.9×10^4	4×10^3 [c,e]
t-BuNC	2	4000[f]	1.5×10^2 [f]	8×10^2	1×10^2 [c]
TMIC	220	0.9	7×10^5	2.5×10^4 [g]	

[a] In benzene at 25 °C, estimated error ±10%, for *t*-BuNC ±30%. Symbols are those in common usage.[7] [b] Calculated by using CO binding constant for 1 of 6×10^5 M^{-1}. [c] Reference 8. [d] Average value for α and β chains in R state from ref 9. [e] Data for isopropyl isocyanide. [f] Estimated from K_{CNR}^{CNR}: Q cannot be obtained directly since *t*-BuNC competes with CH_3Im outside at concentrations required for binding inside. [g] Reference 11.

sizes and provides a measure of the magnitude of such steric effects in hemoproteins.

In benzene, the cyclophane **1** binds 1-methylimidazole with an equilibrium constant ($K^B = 3 \times 10^3$ M^{-1}) very close to that (4×10^3 M^{-1}) of deuteroheme dimethyl ester.[7] A second imidazole binds to **1** with an equilibrium constant $K_B^B \sim 4$ M^{-1}, compared to 7×10^4 M^{-1} for deuteroheme dimethyl ester.[7] The distal side hindrance is therefore achieved without the introduction of appreciable strain in the heme itself, allowing a direct evaluation of steric effects by comparing ligand affinities of the hindered and unhindered hemes.

Isocyanides provide a convenient probe of the steric hindrance provided by the cyclophane cap. Addition of *n*-BuNC to **1** in benzene gives a **1**–*n*-BuNC complex (λ_{max} = 415 nm) (eq 1),



(1)

essentially stoichiometrically $K \geq 10^5$ M^{-1}. A **1**–$(BuNC)_2$ complex (λ_{max} = 425 nm) is formed only at much higher concentrations of *n*-BuNC. Similar titrations with the more bulky cyclohexyl, *tert*-butyl, and *p*-toluenesulfonylmethyl (TMIC) isocyanides show no detectable change in the binding of the first isocyanide to the open side of **1**, but K_{CNR}^{CNR} decreases dramatically with increasing size of CNR. In sharp contrast, titration of simple mesoheme dimethyl ester in benzene shows no detectable mono-*n*-BuNC complex but gives the di-*n*-BuNC complex, stoichiometrically $K^{CNR}K_{CNR}^{CNR} \geq 10^{12}$ M^{-1} and $K_{CNR}^{CNR} > K^{CNR}$.

The binding constants, K_{Im}^{CNR}, for isocyanides with the mono-1-methylimidazole complex of **1** were derived from a competition between CO and CNR as described in the preceding paper (Im = 1-methylimidazole, Hm = **1**, $Q = L/I$) (eq 2–4). Spectral

$$\text{ImHmCNR} + \text{CO} \overset{Q}{\rightleftharpoons} \text{ImHmCO} + \text{CNR} \quad (2)$$

$$\text{ImHm} + \text{CO} \overset{L}{\rightleftharpoons} \text{ImHmCO} \quad (3)$$

$$\text{ImHm} + \text{CNR} \overset{I}{\rightleftharpoons} \text{ImHmCNR} \quad (4)$$

changes consequent upon addition of CO to a benzene solution containing $\sim 5 \times 10^{-6}$ M **1**, 10^{-3} M CNR, and 0.2 M 1-methylimidazole were observed, and I was calculated from Q and the value $L = 6 \times 10^5$ M^{-1} observed by directly titrating **1**-Im with CO. The resulting values of Q, I, and K_{CNR}^{CNR} are shown in Table I along with values of I for hemoglobin and myoglobin. The change in free energy associated with the steric effects in **1** or in hemoproteins can be calculated by subtracting their respective ligation free energies from those of the unhindered but structurally similar model, chelated protoheme, **2** (eq 5). In eq 5, K_P^1 is the

$$\delta\Delta G = -RT \ln (K_H^1/K_P^1) \quad (5)$$

equilibrium constant for binding the ligand (l) to **2** and K_H^1 the corresponding constant for **1**, hemoglobin, or myoglobin. These $\delta\Delta G$ values are shown in Table II.

Table II. Ligation Free-Energy Difference $\delta\Delta G$ (kcal/mol at 298 K) between Chelated Protoheme, **2**,[a,b] and Either Cyclophane 1 or Hemoproteins

	1	Mb	Hb(R)
n-BuNC	5.3	5.3	5.1
t-BuNC	8.2	7.2	8.3
TMIC	5.4	7.5	
CO	3.8	1.6	−0.4[c]

[a] Protoheme monomethyl ester, mono-3-(1-imidazolyl)propylamide.[13a] [b] Binding constants for **2** in benzene taken as 4.4×10^8, 1.7×10^8, 7×10^9, and 4×10^8 M^{-1} for *n*-BuNC, *t*-BuNC, TMIC,[11] and CO.[12] [c] CO binding constants are 3×10^7 M^{-1} for Mb and 7×10^8 M^{-1} for Hb(R).[1,10] This $\delta\Delta G$ value is closer to 0 when the comparison is made with 1 in aqueous cetyltrimethylammonium bromide rather than in benzene,[2]

Tables I and II demonstrate the effect of a distal group upon isocyanide binding. Compared to chelated protoheme **2**, the binding constants I of *n*-BuNC and *t*-BuNC are reduced 8000 and 10^6 times, respectively. The steric effects on isocyanides in the cyclophane **1** are remarkably similar to those in hemoproteins as seen either in I or $\delta\Delta G$ (Table II). In contrast, the steric effect on CO is 3.8 kcal/mol in the cyclophane, 1.6 kcal/mol in myoglobin, and essentially zero in Hb(R). These results suggest two kinds of steric effects: (1) a central steric effect caused by distal groups which come in close contact with the first or second atom of the bound ligand by being positioned directly over the iron; (2) a peripheral steric effect encountered by more distant groups in the ligand,

The 6,6-cyclophane shows both kinds of steric effects; the center ring of the anthracene, lying less than 5 Å directly above the iron, provides prohibitively close contact with the second atom of bound CO or CNR. These can be relieved somewhat by a tipping of the anthracene or a bending or tilting of the ligand. The peripheral effect is encountered by contact of one of the outer rings of the anthracene with the $-CH_2R$ or *t*-Bu groups of the isocyanides even when the anthracene is tilted.

Hemoglobin[14] and myoglobin[15] have a distal histidine, His E7, which stands over the iron site in the deoxy form and could provide the central steric effect. However, in the R state of hemoglobin, this histidine has moved clear of this position, resulting in the observed $\delta\Delta G$ = 0 kcal/mol of Table II and no central steric effect.[16] In myoglobin, this shift is smaller, and it accompanies binding, resulting in the $\delta\Delta G$ = 1.6 kcal/mol or a reduction of about 20 in the CO affinity relative to either **2** or Hb(R). The observed structural bending or tilting of the Fe–CO bond in both Hb and Mb may be insignificant if, as others suggest, little energy is required to distort the porphyrin in order to move the ligand off axis while retaining approximate octahedral geometry around the iron.[16,17] The much larger central (CO) steric effect in **1** might be due to its greater rigidity and thus greater difficulty in moving

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the anthracene from its position above the iron.

In addition to His E7, there are in Hb and Mb Phe CD1 and Val E11 flanking the histidine and presenting a peripheral steric effect to the larger isocyanide ligands. These distal groups introduce steric effects on *n*-butyl and *tert*-butyl isocyanides which are rather similar to that found in **1**.

These results indicate that strong CO-binding hemoproteins such as R-state hemoglobin or isolated hemoglobin chains behave like unhindered model compounds with respect to the binding of small ligands such as CO or O_2[2b,13] and thus do *not* possess the distal face steric strain which has been attributed to them.[3,4] Larger ligands encounter steric effects in all hemoproteins, and small ligands encounter steric effects[17] in the more restricted pockets of Mb[13] and peroxidases.

Comparisons of unhindered model compounds with presumably hindered hemoproteins in the preceding paper suggested that distal side steric effects appear only in the association rates. This conclusion is supported by the observations that the 10^4 reduction in TMIC binding to the 6,6-cyclophane **1** is due to a 10^4 reduction in association rates, and the 700-fold reduction in CO binding comes from a 400-fold decrease in association rates. We propose that the conformational flexibility in the anthracene bridge is a unique feature which allows it to model both the static and dynamic aspects of distal side effects in hemoproteins.

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Enantiomerization Trends in 1,8-Bis(trimethylelement)naphthalenes

Sir:

As attested by numerous examples in the literature, close proximity of substituents in the 1,8 (peri) positions of naphthalene often results in out-of-plane deformations, and in a pronounced warping of the molecular skeleton.[1] Particularly striking in this regard are the 1,8-bis(trimethylelement)naphthalenes,[2-10] where repulsion between the peri $(CH_3)_3Z$ (Z = C, Si, Ge, Sn) substituents leads to highly distorted, chiral structures[3,4,9] in which the $(CH_3)_3Z$ groups are displaced to opposite sides of the mean naphthalene plane. However, energy requirements for the interconversion of the enantiomers have thus far remained unknown,

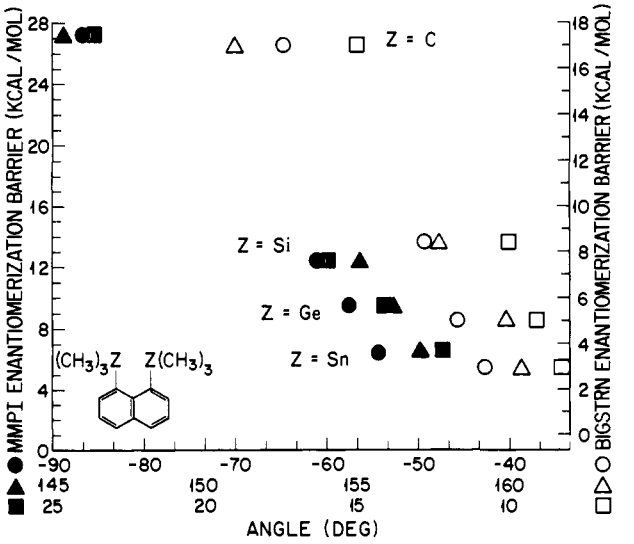
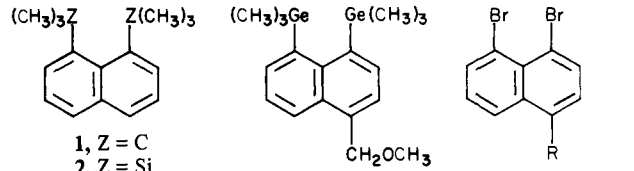


Figure 1. Enantiomerization barriers of 1,8-bis(trimethylelement)-naphthalenes calculated with MMPI (solid symbols, left ordinate) and BIGSTRN (open symbols, right ordinate) as a function of Z1–C1–C8–Z2 (circles), Z1/2–C1/8–C4/5 (triangles), and C1/8–C9–C10–C4/5 (squares).

though NMR evidence suggests a barrier greater than ca. 24 kcal/mol for 5-benzyl-1,3,8-tri-*tert*-butylnaphthalene.[3] We now report the results of empirical force field (EFF) calculations for 1,8-bis(trimethylelement)naphthalenes **1–4**, which predict a



1, Z = C
2, Z = Si
3, Z = Ge
4, Z = Sn

5

6a, R = CH_3
6b, R = CH_2Br
6c, R = CH_2OCH_3

marked dependence of barrier heights on Z, and the experimental determination of an enantiomerization barrier for 1,8-bis(trimethylgermyl)-4-methoxymethylnaphthalene (**5**), which confirms the reliability of values predicted for **3**.

Calculations were performed with the programs BIGSTRN[11] and MMPI,[12] employing previously described approximations.[8,9] Among various reasonable transition state structures explored, the C_s structure in which two of the methyl groups eclipse the naphthalene ring was found to be the lowest in energy for all of the compounds in this series. All structures were optimized with both force fields, the ground states without constraint and the transition states under the constraint of C_s symmetry.

As depicted in Figure 1, the enantiomerization barriers calculated[13] for **1–4** with BIGSTRN and MMPI[14-16] show a direct correlation with the extent of molecular distortion in the ground

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(13) All enantiomerization barriers calculated in this work represent differences in steric energies of ground and transition states.

(14) The activation energy of 18.5 kcal/mol previously calculated[4] with MMPI for the enantiomerization of **1** stands in contrast to the barrier of 27.2 kcal/mol calculated for **1** with MMPI in the present work.[13] The former value was reported[4] as the difference in the enthalpy of formation between ground and transition states. Unfortunately, enthalpies of formation of compounds containing a naphthalene nucleus cannot be calculated by means of the available program,[12] though the procedure for doing so has been outlined in general terms.[15] We are therefore unable to reconcile these two results.[16]

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(16) We are informed that the value of ΔH_f for **1** reported in ref 4 was calculated with a modified version of MMPI (Allinger, N. L.; personal communication).

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