The main features of $[Cp_2Ru_2(C_8H_8)]^{2+}$ (**4**) are as follows (Figure 1). The eight-carbon ring of the neutral precursor has opened, giving a chain whose termini now bridge the newly formed Ru–Ru bond. The metals have inserted into a C–C bond of the original cot ring and two new Ru–C σ bonds have formed. Each metal is bonded to five carbons of the chain (by two σ and three π bonds) and to the other metal (Ru–Ru distance 2.7291 (4) Å). The central structure is basically that of a ten-membered dimetallacyclic ring; the Cp rings adopt a cis configuration.

The flyover dication **4** shows CV behavior complementary to that of **3**. Thus, it reduces at −0.28 V and has a coupled anodic wave at +0.02 V. Coulometric reduction of **4** in acetone consumes two electrons and gives solutions with the same electrochemical behavior as **3**. Extraction of the reduced solutions with benzene allowed isolation of **3**, confirming that reduction of **4** results in re-formation of the cyclooctatetraene ring. Thus, it is clear that **3** and **4** constitute a chemically reversible redox couple involving an overall two-electron transfer.[10]

$$\underset{\mathbf{3}}{Cp_2Ru_2(cot)} \rightleftarrows \underset{\mathbf{4}}{Cp_2Ru_2(C_8H_8)^{2+}} + 2e^-$$

Dimetallacycles have been viewed as key intermediates in the formation of cyclooctatetraenes from alkynes,[11] accounting in part for the interest in flyover-type metallacycles.[8,11-13] Up to the present, however, no examples of reversible zipping and unzipping of the final C–C link of the *n*-carbon chain appear to have been reported.[14] The present data show that electron-transfer processes may initiate such intramolecular coupling and uncoupling reactions. This observation should spur investigations of the redox reactions of other metallacyclic complexes.

Acknowledgment. This work was generously supported at Vermont by the National Science Foundations (CHE 86-03728) and at Zürich by the Swiss National Science Foundation (2.846-0.85). A gift of cyclooctatetraene from BASF is gratefully acknowledged. We thank U. Piantini and W. von Philipsborn (University of Zürich) for recording and simulating the NMR spectra of **4**.

Supplementary Material Available: Tables of atomic coordinates, isotropic and anisotropic parameters, bond distances, and bond angles (6 pages); listing of structure factors (33 pages). Ordering information is given on any current masthead page.

(9) For $[C_{18}H_{18}Ru_2][PF_6]_2 \cdot 0.5C_6H_6$: triclinic, $P\bar{1}$, a = 9.237 (3) Å, b = 9.234 (3) Å, c = 16.151 (5) Å, α = 80.31 (3)°, β = 74.09 (2)°, γ = 68.09 (2)°, V = 1225.8 (7) Å^3, Z = 2, D(calcd) = 2.074 g cm^{-3}, μ(Mo Kα) = 14.4 cm^{-1}, T = 293 K, and crystal dimensions = 0.24 × 0.26 × 0.30 mm. A deep red crystal mounted on a glass fiber was found to have no symmetry higher than triclinic. Of 5891 reflections collected ($4° \le 2\theta \le 55°$, Nicolet R3m/μ), 5605 were independent [R(int) = 1.60%] and 4558 with $F_o \ge 3\sigma(F_o)$ were considered observed. The two metal atoms were located by direct methods; subsequent difference Fourier syntheses located all atoms. With all non-hydrogen atoms anisotropic and all hydrogen atoms isotropic: $R(F)$ = 3.89% [all data R(F) = 4.86%], $R(wF)$ = 4.57%, GOF = 1.44, Δ/σ = 0.07, $\Delta(\rho)$ = 0.77 e Å^{-3} (in PF_6^- ion), N_o/N_v = 11.2. SHELXTL (5.1) software (Nicolet Corp., Madison, WI) was used for all computations.

(10) Low-temperature CV measurements suggest that the oxidation consists of two one-electron processes with very similar $E°$ values. The mechanism is under scrutiny.

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(13) (a) Geibel, W.; Wilke, G.; Goddard, R.; Kruger, C.; Mynott, R. *J. Organomet. Chem.* **1978**, *160*, 139. (b) Goddard, R.; Kruger, C. In *Electron Distributions and the Chemical Bond*; Coppens, P., Hall, G., Eds.; Plenum: New York, 1982; p 297. (c) Wilke, G. In *Fundamental Research in Homogeneous Catalysis*; Tsutsui, M., Ed.; Plenum: New York, 1978; Vol. 3, p 1.

(14) The flyover complex $Cp_2Cr_2(C_8H_8)$,[13a] structurally similar to 4 but with two fewer valence electrons, was originally believed to reversibly rearrange to a complex with a closed cot ring[11] an interpretation which was later discarded.[13a] Unusual line-broadening in its NMR spectra are now established as due to temperature-dependent paramagnetism (Dr. J. Heck, personal communication to A.S., 1987). Non-reversible thermal rearrangement to the closed-chain isomer has been observed.[13b,c] Arewgoda et al.[15] have identified hexakis(trifluoromethyl)benzene in the decomposition of the flyover radical anion $Co_2(CO)_4[\mu\text{-}C_6(CF_3)_6]^-$.

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Rigid Molecular Tweezers: Synthesis, Characterization, and Complexation Chemistry of a Diacridine

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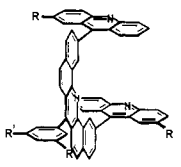
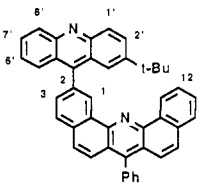
Synthetic receptors containing two aromatic (complexing) chromophores connected by a single spacer have been referred to as *molecular tweezers*.[1] These nonmacrocyclic receptors can have distinct advantages over their cyclic relatives (cyclophanes[2]) in terms of the size and *topology* of the guest which can be complexed.[3] Thus, a molecular tweezer with an ca. 7-Å interchromophore distance can complex guests of unrestricted length and width provided they have the thickness of a single aromatic ring. With respect to topology it is not surprising that both natural and synthetic DNA bis intercalators possess the molecular tweezer structure type.[4-6]

Little is known about the structural and electronic requirements for optimum complexation by a molecular tweezer. Chen and Whitlock have shown that in aqueous medium the spacer unit should be rigid in order to prevent self-association of the complexing chromophores.[1] Molecular tweezers studied thus far have contained spacers of varying degrees of rigidity but none have *preorganized* the cavity for complexation since they are conformationally mobile.[1,5,6] The importance of preorganization in the complexation of metal ions by crown ethers is now well appreciated as a result of Cram's studies of spherands.[7]

Herein we describe the synthesis of the first molecular tweezer **1** in which a rigid spacer enforces a syn-cofacial orientation of the two complexing (acridine) chromophores. The acridine moieties in **1** show remarkable cooperativity in complexation while



1a: R, R' = H
1b: R = t-Bu, R' = Me
1c: R, R' = t-Bu



2

a flexible, yet noncollapsing diacridine **7** complexes very weakly. The dibenz[*c*,*h*]acridine spacer was chosen since its C-2 to C-12 distance is 7.24 Å and chromophores attached at these positions appeared likely to lie in parallel planes.[8,9] The synthesis began

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(2) Wilcox, C. S.; Cowart, M. D. *Tetrahedron Lett.* **1986**, 5563. Shepodd, T. J.; Petti, M. A.; Dougherty, D. A. *J. Am. Chem. Soc.* **1986**, *108*, 6085. Diederich, F.; Dick, K. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 715. Jarvi, E. T.; Whitlock, H. W. *J. Am. Chem. Soc.* **1982**, *104*, 7196. Odashima, K.; Itai, A.; Iitaka, Y.; Koga, K. *J. Am. Chem. Soc.* **1980**, *102*, 2504. Tabushi, I.; Sasaki, H.; Kuroda, Y. *J. Am. Chem. Soc.* **1976**, *98*, 5727.

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Scheme I

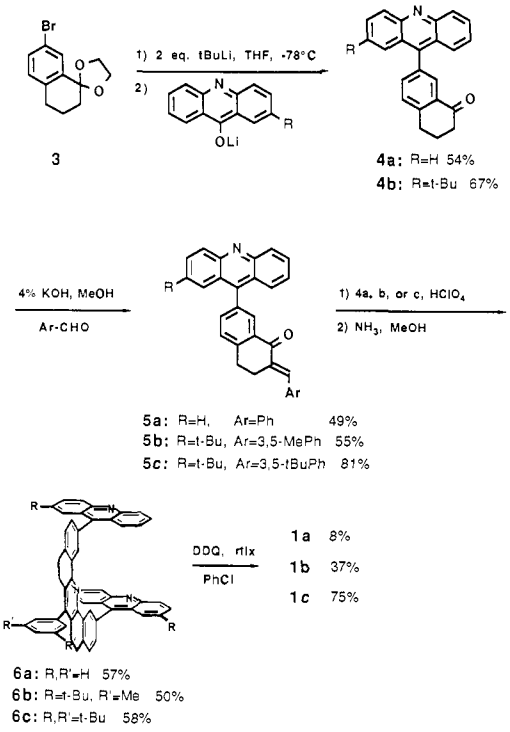


Table I. ^{1}H NMR Shifts of Acridine Moieties in Molecular Tweezers **7**, **6b**, and **1b** and Model Compound **2** Relative to Tetralone **4b**[a]

compd	H-2	H-3	H-4	H-6[b]	*t*-Bu[b]
2	−0.07	−0.12	−0.11	−0.02	0.04
7	0.00	0.00	0.01	0.00	−0.02
6b		0.12	0.11	0.25	0.27
1b		−0.01	−0.01	0.19	0.27

[a] $\Delta\delta$, ppm in $CDCl_3$. Positive values represent upfield shifts. [b] Average of upfield shifts for the atropisomers of **6b** and **1b**.

with 7-bromo-1-tetralone[10] which was converted (TsOH, $PhCH_3$, Dean Stark, 92%) into its ethylene ketal **3** (Scheme I).[11] Metal–halogen exchange (2 equiv of *t*-BuLi, THF, −75 °C) and subsequent addition to the lithium salt of 9-acridone (THF, −75 °C to room temperature) afforded acridinyl-substituted tetralone **4a** in a 54% yield.[12] Condensation of **4a** with benzaldehyde (4% KOH in MeOH) formed benzylidene **5a** (49% yield). Coupling of **5a** with **4a** in perchloric acid, as described by Katritzky,[13] produced a pyrylium salt which was not isolated but treated directly with ammonia to form bisacridine **6a** in a 57% yield (based on **4a**). Dehydrogenation (DDQ, PhCl, reflux) produced molecular tweezer **1a** in a 8% yield. The more highly soluble *tert*-butyl-substituted molecular tweezers **1b** and **1c** were synthesized in an analogous fashion (Scheme I).[14]

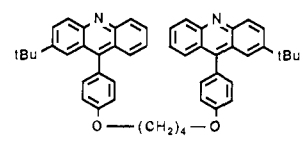
Comparison of the ^{1}H NMR chemical shifts of the acridine proton resonances of molecular tweezers (**7**, **6b**, **1b**) and their monoacridine relatives (**2**, **4b**) reveals the degree of interaction between the acridine moieties (Table I). Remarkably, the flexible diacridine **7** shows negligible shift changes compared with tetralone **4b**, while in rigid molecular tweezers **6b** and **1b** small upfield shifts are experienced by H-3, H-4, H-6, and the *tert*-butyl substituent (compare also with **2**). Either there is no driving force for association of the acridine chromophores of **7** in $CDCl_3$ or it is sterically disfavored. The shifts seen in **6b** and **1b** reflect the proximity of the acridines and are of the same sign and magnitude as those seen in the rigid, noncollapsing naphthalenophanes, which have similar interchromophore distances.[15] These data support the idea that molecular tweezers **6b** and **1b** possess open clefts. It is interesting to note that the more rigid spacer in **1b** appears to be most effective at isolating the acridine moieties.

Table II. Benesi–Hildebrand Analysis of Complexation Data of 2,4,7-Trinitrofluorenone (TNF) by Molecular Tweezers Obtained by ^{1}H NMR in $CDCl_3$[a]

	$\Delta\delta_{max}$[b] (ppm)					
compd	H-1	H-3	H-5	H-6	H-8	K_{assoc}[c] (M^{-1})
7						<5
6c	1.63	0.77	*d*	0.50	*d*	123
1c	*d*	0.71	0.37	0.43	1.59	172

[a] [THF] ≈ 1.5×10^{-3} M, [host] ≈ $5–30 \times 10^{-3}$ M. Data between 20% and 80% of saturation were used. [b] For protons of TNF. Duplicate runs agreed within 6% and were averaged. [c] Duplicate runs agreed within 15% and were averaged as were binding constants obtained from Benesi–Hildebrand analysis of the individual resonances. [d] Peaks became obscured during the titration.

To determine the ability of molecular tweezers **1c** and **6c** to bind aromatic guests, the complexation of 2,4,7-trinitrofluorenone (TNF) was studied by ^{1}H NMR in chloroform-d_1. Addition of increasing amounts of **1c** (**6c**) to a solution of TNF resulted in large upfield shifts of all the TNF proton resonances with leveling indicating saturation (Table II). In contrast, 9-phenylacridine and **2** and **7** induce much smaller shifts in TNF (<0.1 ppm) even at very high concentrations (>0.1 M), and no leveling is observed. We conclude that TNF is sandwiched between the acridine chromophores in **1c** and **6c**. Further support for an inclusion complex comes from the observation that **1c** (**6c**) induces upfield shifts in H-1 and H-8 of TNF which are two times larger in magnitude than those experienced by H-3, H-5, or H-6, while 9-phenylacridine and **2** cause similar shifts in all the protons of TNF. The former observation suggests a highly oriented TNF-**1c** (**6c**) complex in which the TNF carbonyl is directed toward the spacer as in **8**.



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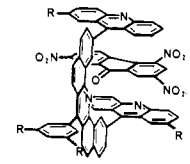

8: R = t-Bu

The ^{1}H NMR titration data was analyzed by using the Benesi–Hildebrand equation (Table II). The association constant for **1c** (K_{assoc} = 172 M^{-1}) is substantially higher than that for **6c** (K_{assoc} = 123 M^{-1}) probably as a result of its greater rigidity. TNF was complexed by 9-phenylacridine, **2**, and **7** very weakly and an accurate binding constant could not be determined. K_{assoc} < 5 M^{-1} was chosen as a conservative upper limit in these cases, and this seems a reasonable estimate since the more electron rich carbazole π-system complexes TNF with an association constant of 6.5 M^{-1} ($CHCl_3$).[16] The dramatic increase in binding constant observed for **1c** and **6c** over the monoacridines is not unexpected, but the poor cooperativity exhibited by the noncollapsing diacridine **7** is notable.

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(11) All new compounds gave correct elemental analysis and/or high resolution mass spectral data and had ^{1}H NMR and IR spectra which were in accord with the assigned structures.
(12) This is a modification of a known procedure using phenyllithium: Lehmstedt, K.; Dostal, F. *Chem. Ber.* **1939**, *72*, 804. Compound **7** was prepared by using this same reaction but starting with 1,4-bis(4-bromophenoxy)butane.
(13) Katritzky, A. R.; Thind, S. S. *J. Chem. Soc., Perkin Trans 1* **1980**, 1895.
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(15) Adams, S. P.; Whitlock, H. W. *J. Am. Chem. Soc.* **1982**, *104*, 1602.
(16) (a) Tazuke, S.; Nagahara, H.; Matsuyama, Y. *Makromol. Chem.* **1980**, *181*, 2199. (b) Although the nature of the binding interaction(s) in our system has not been fully elucidated, there is a clear EDA component: Zimmerman, S. C., unpublished results.

Our work demonstrates the pivotal role played by the spacer in determining the effectiveness of complexation by molecular tweezers. In this regard, optimum binding affinities will be achieved if a suitably sized spacer is both rigid *and* capable of enforcing a syn-cofacial orientation of the complexing chromophores. Further strengthening of the complexation in our system can be expected from improved electron donor-acceptor (EDA) interactions[16b] and involvement of the hydrophobic effect.

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Alteration of Heme Axial Ligands by Site-Directed Mutagenesis: A Cytochrome Becomes a Catalytic Demethylase

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Cytochrome b_5 is a 17000 dalton protein that serves an electron transport role in the hepatic endoplasmic reticulum, coupling to the fatty acid desaturase complex[1] and cytochrome P-450.[2] A similar cytochrome b_5, lacking a 35 amino acid membrane anchor domain, acts as a soluble reductant of hemoglobin in erythrocytes.[3,4] The redox active center of cytochrome b_5 is iron-protoporphyrin IX ligated to histidine-39 and histidine-63 of the polypeptide chain, and the complete three-dimensional structure of the water-soluble core domain is known from X-ray[5] and NMR[6] investigations.

An important goal of bioinorganic chemistry is elucidating the role of metal ligands in determining the chemical and spectroscopic properties of metalloprotein prosthetic groups. Recently, we reported the total synthesis of a gene coding for rat liver cytochrome b_5 and the high-level expression of this gene in a bacterial environment.[7] With the ability to genetically engineer cytochrome b_5 via in vitro recombinant DNA technology, we now report the replacement of the axial histidine residue of this protein at position 39 with a methionine residue in order to mimic the coordination geometry of cytochromes *c* and b_{562}.[8] The purified methionine-39

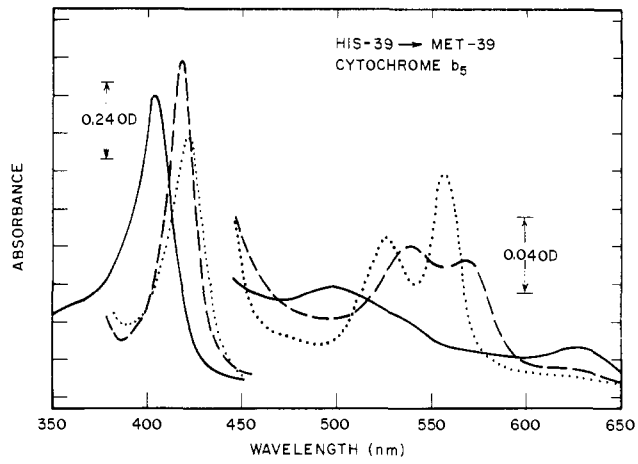

Figure 1. Ferric (solid), ferrous (dotted), and ferrous-carbon monoxide (dashed) optical spectra of H39M-b_5. The low-spin character of the ferrous protein is indicated by the resolved visible bands, and the high-spin nature of the ferric state by the 625-nm charge-transfer transition.

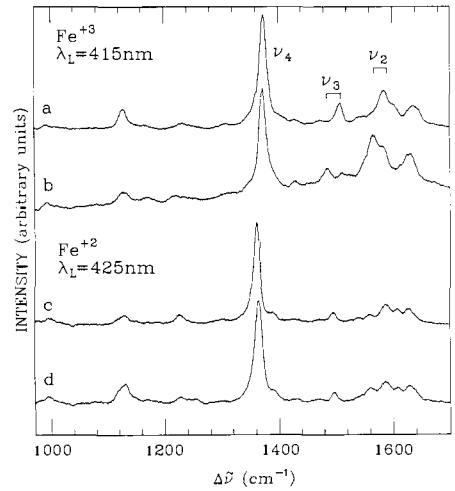

Figure 2. Resonance Raman spectra of native (a,c) and mutant (b,d) cytochrome b_5. The laser excitation wavelengths for oxidized (a,b) and reduced (c,d) proteins are indicated. The spin state and coordination state can be assigned on the basis of the core size marker band ν_3. The oxidation state is assigned based on the marker frequency ν_4 (see Table I).

mutation of cytochrome b_5, hereafter termed H39M, has the optical spectra shown in Figure 1. Electron spin resonance spectra of H39M showed primarily high-spin heme with slight rhombic distortion (g = 6.25, 5.73, 1.99) and a small low-spin component comprising roughly 5% of the total signal with g values of 2.87, 2.28, 1.60. As demonstrated by Figure 1, ferrous H39M-cytochrome b_5 readily binds carbon monoxide to form a stable $Fe^{2+}CO$ state. In Figure 2 we display the Raman spectra of native b_5 and the H39M mutant. The ferric mutant is assigned as six-coordinate high spin; however, a small amount of low spin is present as suggested by the weak band at 1510 cm^{-1} in agreement with the EPR data. At present it is not known whether the methionine-39

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