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Registry No. 3, 123380-94-9; 4, 123380-95-0; 5, 3189-13-7; 6, 62467-65-6; 7, 123380-86-9; 8, 23084-35-7; 9, 103986-22-7; 10, 123380-87-0; 11, 123380-90-5; 12, 123380-90-5; 12 acid chloride, 123380-91-6; 13, 123380-92-7; 14, 123380-93-8; CC-1065, 69866-21-3; TCBOC-Cl, 66270-36-8; methyl 6-methoxy-2,3-dihydro-1*H*-indole-3-acetate, 123380-88-1.

Aggregate Structure and Ligand Location Strongly Influence Cu^{2+} Binding Ability of Cationic Metallosurfactants

Paolo Scrimin,* Paolo Tecilla, Umberto Tonellato,* and Tiziano Vendrame

Dipartimento di Chimica Organica and Centro CNR Meccanismi di Reazioni Organiche, Università di Padova, Via Marzolo 1, 35131 Padova, Italy

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Transition-metal metallosurfactants have recently been reported as being effective agents for electron storage,[1] dioxygen complexation,[2] amplification of chemical signals,[3] and catalysis in the cleavage of phosphoric[4] or carboxylic acid[5] esters. On the other hand, aggregates of synthetic surfactants (micelles and, particularly, vesicles) have been investigated with increasing regularity as models of biological membranes.[6] Transition-metal cations permeation across biological membranes is of primary importance, since these ions are involved in several catalytic processes.[7] In this respect, it appears highly relevant to investigate the effect of the aggregate structure and ligand location on the ability of ligand surfactants to bind transition-metal ions. This issue, in particular for Cu^{2+} cations, is addressed in the present paper.

Results and Discussion

For the purposes of this study, we investigated the properties of ligand surfactants with a moderate binding constant for Cu^{2+} ions, and whose complex formation could be easily detected. Accordingly, ligands **1**–**4** were synthesized. They contain the same ligand moiety and cationic headgroup, two thioethereal functions that allow easy spectroscopic monitoring of Cu^{2+} complexation,[8] and only differ in the length of the hydrocarbon chains. The synthetic strategy is outlined in Scheme I.

Ligand **1** is rather soluble in water, where it does not form aggregates, and was synthesized as a reference model. Compound **2** forms micelles[9] (cmc = 7.5×10^{-5} M) and surfactants **3** and **4** form, upon sonication, vesicles[10] (average size as determined by dynamic light scattering measurements and gel-to-liquid crystal phase transition temperature, T_c, were 510 Å, 28 °C and 625 Å, 40 °C, respectively). Inspection of CPK models suggests that, *in the aggregates*, the ligand moiety of the surfactants is located in a hydrophobic region of the organized assembly. The Cu^{2+} binding constants, K_b, of the different ligands have been determined by following the increase in the absorbance of the complex (310–390 nm) upon addition of the ligand to a $Cu(NO_3)_2$ solution (see Experimental Section). The K_b values have been evaluated in two different environments, namely, in CH_3OH, where no aggregates are formed, and in aqueous buffer (4-morpholinoethanesulfonic acid buffer, MES, pH = 6.3) where ligands **2**–**4** form aggregates. The K_b measurements

Scheme I

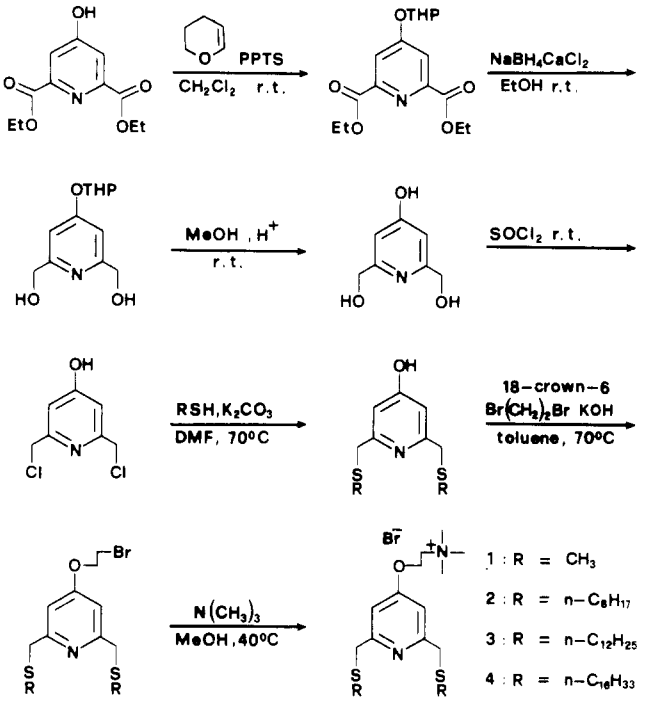


Table I. $Cu(NO_3)_2$ Binding Constants[a] of Surfactants 1–6 in CH_3OH[b] and H_2O (0.05 M MES Buffer, pH = 6.3)

entry	ligand	aggregate structure in H_2O	log K_b	
			H_2O	CH_3OH[b]
1	1	no aggregates	4.7	4.6
2	2	micelles	3.7	4.7
3	2 (1:5 with CTABr)	micelles	2.8	
4	2 (1:10 with CTABr)	micelles	2.7	
5	2 (1:20 with CTABr)	micelles	2.7	
6	3	vesicles	no binding[c]	4.9
7	3 (1:20 with CTABr)	micelles	2.5	
8	4	vesicles	no binding[c]	4.6
9	4 (1:20 with CTABr)	micelles	2.6	
10	5	micelles	3.7	4.7
11	6	vesicles	3.6	

[a] At 25 °C; see the Experimental Section for binding constant determination. [b] With 5% water added. [c] See ref 13.

(1) Humphry-Baker, R.; Moroi, Y.; Grätzel, M.; Pelizzetti, E.; Tundo, P. *J. Am. Chem. Soc.* **1980**, *102*, 3689.
(2) Simon, J.; Le Moigne, J.; Markovitsi, D.; Dayantis, J. *J. Am. Chem. Soc.* **1980**, *102*, 7247.
(3) Shimomura, M.; Kunitake, T. *J. Am. Chem. Soc.* **1982**, *104*, 1757.
(4) (a) Gellman, S. H.; Petter, R.; Breslow, R. *J. Am. Chem. Soc.* **1986**, *108*, 2388. (b) Menger, F. M.; Gan, L. H.; Johnson, E.; Durst, D. H. *J. Am. Chem. Soc.* **1987**, *109*, 2800.
(5) (a) Tagaki, W.; Ogino, K. *Top. Current Chem.* **1985**, *128*, 144. (b) Fornasier, R.; Scrimin, P.; Tonellato, U.; Zanta, N. *J. Chem. Soc., Chem. Commun.* **1988**, 716. (c) Fornasier, R.; Scrimin, P.; Tecilla, P.; Tonellato, U. *J. Am. Chem. Soc.* **1989**, *111*, 224.
(6) Fendler, J. *Membrane Mimetic Chemistry*; Wiley: New York, 1982.
(7) Hay, R. W. *Bio-inorganic Chemistry*; Horwood: Chichester, 1984.
(8) This is due to a $\sigma(S) \rightarrow d_{x^2-y^2}(Cu)$ transition that occurs in the 310–390-nm wavelength region; see: Amundsen, A. R.; Whelan, J.; Bosnich, B. *J. Am. Chem. Soc.* **1977**, *99*, 6730.
(9) Nonspherical aggregates are suggested by dynamic light scattering analysis.
(10) The formation of vesicles from synthetic surfactants made of 3,5-disubstituted pyridinium ions has been also reported: Sudhölter E. J. R.; Engberts, J. B. F. N.; Hoekstra, D. *J. Am. Chem. Soc.* **1980**, *102*, 2467.

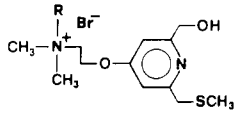
0022-3263/89/1954-5988$01.50/0

in the presence of aggregates, particularly vesicles, are strongly affected by changes in the pH of the aqueous solution due to structural changes of the different membranes.[11] As a consequence, we could not evaluate the binding constants by the proton competition method, as is customary.[12] The data obtained are reported in Table I.

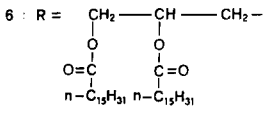
Analysis of Table I allows the following considerations. (a) In methanol, in the absence of aggregates, the binding ability of all ligands 1–4 is substantially similar. (b) In water, the binding constant of micellar **2** is at least 1 order of magnitude smaller than that of model **1** (entries 1 and 2). (c) Comicellization in a cetyltrimethylammonium bromide (CTABr) matrix causes a further decrease (by 1 order of magnitude) in the binding constant, with respect to the homomicellar system (entries 2–5). (d) Vesicular **3** and **4** do not bind Cu(II) ions[13] (entries 6 and 8), even above their T_c. (e) Ligands **3** and **4** do, however, bind Cu(II) ions when their vesicles are destroyed by sonication with excess CTABr[14] (entries 7 and 9).

The failure of vesicular **3** and **4** to bind Cu(II) ions was confirmed by fluorescence experiments using 1,8-anilinonaphthalenesulfonic acid (ANS). The ANS fluorescence is effectively quenched by Cu(II) ions[15] only in the presence of micellar **2**. No effect whatever is observed in the presence of vesicular **3** or **4**. (See Figure S1 of the supplementary material.) Moreover, when a methanolic solution of the Cu(II) complex of either surfactant **3** or **4** is injected into a water solution, *metal-free* vesicular aggregates[16] are obtained. This result indicates that *metal ions are squeezed out from the artificial membranes as soon as the latter are formed.*

Table I also shows the binding constants observed[17] for ligands **5** and **6**, which form micelles and vesicles, respectively. They differ from surfactants **2–4** in the location of the ligand moiety. Within the aggregate, the ligand is likely to be oriented toward the bulk water solution. Interestingly, vesicles of **6** *do bind* Cu(II) ions, with a K_b rather similar to that of micellar **2** or **5** (entry 10 in Table I).



5: R = n-$C_{16}H_{33}$



These results bring out interesting differences in the structural behavior of micelles and vesicles.

Micelles. A decrease of the binding constant is observed with respect to the nonmicellar model **1**, regardless of the location of the ligand moiety (i.e., both in the case of **2** and **5**). This effect is probably due to the electrostatic repulsion between cations forced into the small volume of the aggregate.[18] In a cationic CTABr matrix such an effect is more relevant.

Vesicles. There is a dramatic difference in behavior, depending on the location of the ligand subunit. When the ligand is positioned outside the vesicular membrane, as in **6**, the K_b is similar to those determined for the micellar aggregates. When it is located inside the membrane, as in **3** and **4**, no binding is observed.[13] This failure to complex Cu^{2+} ions is not related to the structure of the single surfactant: as a matter of fact, disruption of the vesicles by addition of excess CTABr restores the binding ability of **3** and **4**, and in this matrix (as well as in methanol) they behave in a manner analogous to their sibling **2**.

Among the arguments that may be invoked to explain the behavior of the vesicular aggregate, two seem most relevant.[19] First, the possible failure of Cu(II) ions to permeate the membrane, which may be related to the highly hydrophobic environment;[20] second, the high packing order of the aggregate may preclude the modifications required for binding. The latter point is revealed by an inspection of CPK models of **4**: the pyridine ring and the two paraffinic chains must be forced out of coplanarity to accommodate a Cu(II) ion, and this adjustment is inhibited by the immobility of the long hydrophobic tails in the vesicular bilayer. The observation that vesicles made of a preformed Cu^{2+} complex either of **3** or **4** extrude the metal ion lends further support to the latter arguments, though it is conceivable that both factors contribute to the observed behavior. Micelles are much more hydrated than vesicles[20] and, being loosely organized, the single surfactant is free to assume the proper geometry for Cu^{2+} complexation.

Assuming that electrostatic and other factors affect the binding constant to the same extent in micelles and vesicles containing **3** or **4**, the free energy required to disrupt the packing of the vesicular membrane to allow Cu^{2+} complexation must be larger than 5 kcal/mol, i.e., the energy involved in the binding process. This is a rough estimate, requiring further experiments, which are now in progress in this laboratory.

Experimental Section

General Methods. ^1H NMR spectra were recorded on a 200-MHz Bruker WP-200 SY spectrometer. UV–vis spectra were recorded on a Perkin-Elmer Lambda 5 spectrophotometer equipped with a thermostatted cell holder. Surface tension measurements to evaluate the cmc of micellar solutions were made on a Krüss Type 8451 tensiometer. Vesicle sizes were determined by dynamic light scattering using a Nicomp 370 autocorrelator equipped with a Spectra-Physics 2016 argon laser. Microanalyses were performed by the Laboratorio di Microanalisi of this department.

(11) Jain, M. *Introduction to Biological Membranes*, 2nd ed.; Wiley: New York, 1988.

(12) See, for instance: Damu, K. V.; Shikjee, M. S.; Michael, J. P.; Howard, A. S.; Hancock, R. D. *Inorg. Chem.* **1986**, *25*, 3879.

(13) We see no changes in the UV–vis spectrum attributable to the formation of a complex. Accordingly, under the conditions used, the value of the binding constant was estimated to be lower than 5 M^{-1}.

(14) Particularly in the case of surfactant **4**, simple swirling in the presence of the CTABr solution *is not enough* in order to ensure the formation of comicellar aggregates. In fact, dynamic light scattering revealed the presence of vesicular **4** even after prolonged stirring. Unimodal comicellar aggregates obtained after sonication give bimodal distributions (micellar and vesicular systems) on standing for a few days.

(15) Cu^{2+} is known to be an effective quencher of fluorescence probes, see: Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Plenum Press: New York, 1983; Chapter 9. For other recent examples of fluorescence quenching in vesicular aggregates by Cu^{2+} and other transition-metal cations, see: (a) Fuhrhop, J.-H.; Lehmann, T. *Liebigs Ann. Chem.* **1984**, 1057. (b) Morris, S. J.; Bradley, D.; Blumenthal, R. *Biochim. Biophys. Acta* **1985**, *818*, 365.

(16) With this procedure large vesicles are formed of average size 1690 Å (**3**) and 1530 Å (**4**). For another example, see: Moss, R. A.; Kim, K. Y. *Israel J. Chem.* **1985**, *25*, 11.

(17) Scrimin, P.; Tecilla, P.; Tonellato, U., unpublished results.

(18) Le Moigne, J.; Simon, J. *J. Phys. Chem.* **1980**, *84*, 170.

(19) As suggested by a referee, a third rationale may be added to explain the phenomenon: since avarage head-to-head distances in micelles are much longer than those in smooth planar vesicle bilayers, electrostatics tend to drive the metal out in the latter.

(20) Menger, F. M.; Aikens, P.; Wood, M., Jr. *J. Chem. Soc., Chem. Commun.* **1988**, 180.

Materials. The diethyl ester of chelidamic acid was synthesized from chelidamic acid (Janssen) according to a reported procedure.[21] Diethyl 4-((tetrahydro-2-pyranyl)oxy)-2,6-pyridinedicarboxylate was synthesized as described.[21] $Cu(NO_3)_2$ was analytical grade commercial product. The Cu^{2+} stock solution was titrated against EDTA as previously reported.[5c] 2-(*N*-Morpholino)ethanesulfonic acid (MES) used as the buffer was an Aldrich product, used as received.

4-Hydroxy-2,6-bis(chloromethyl)pyridine. To 100 mL of EtOH were added 3 g (9.3 mmol) of diethyl 4-((tetrahydro-2-pyranyl)oxy)-2,6-pyridinedicarboxylate and 1 g (28 mmol) of $NaBH_4$. After H_2 evolution ceased, $CaCl_2$ (3.1 g, 28 mmol) was cautiously added in portions. The reaction mixture was vigorously stirred for 2.5 h at room temperature. Excess hydride was then quenched by adding 100 mL of water. Ethanol was removed under reduced pressure and the solution was added to 100 mL of brine and extracted with $CHCl_3$ (4 × 200 mL). Evaporation of the dried (Na_2SO_4) chloroform solution afforded 2.1 g (94% yield) of the protected dihydroxy derivative as an oil, which crystallized on standing: NMR δ_{CDCl_3} 1.43–2.01 (m, 6 H, 3 CH_2 THP), 3.0 (br s, 2 H, 2 OH), 3.60–3.85 (m, 2 H, OCH_2 THP), 4.75 (s, 4 H, 2 OCH_2), 5.55 (m, 1 H, CH THP), 6.80 (s, 2 H, Py H3 and H5).

The product was deprotected by dissolving the material in 100 mL of CH_3OH containing 0.5 mL of concentrated HCl. After 0.5 h of stirring, the solvent was rotary evaporated to leave 4-hydroxy-2,6-bis(hydroxymethyl)pyridine as a colorless solid (1.3 g, 95% yield): NMR $\delta_{CDCl_3/CD_3OD\ 10:1}$ 4.80 (s, 4 H, 2 CH_2), 7.10 (s, 2 H, Py H3 and H5).

This material was dissolved in 70 mL of $SOCl_2$ and stirred at room temperature for 4 days, protected from moisture ($CaCl_2$) and light. Evaporation of $SOCl_2$ afforded a brownish solid, which was dissolved in toluene, and the solvent was removed to eliminate residues of the chlorinating reagent. The slightly dark solid obtained (2.2 g, 95% yield) was the HCl salt of the dichloride: NMR $\delta_{CDCl_3/CD_3OD\ 1:1}$ 4.85 (s, 4 H, 2 CH_2), 7.62 (s, 2 H, Py H3 and H5). This material was used without any further purification.

General Procedure for the Synthesis of Ligands 2–4. To the above dichloride (720 mg, 3.1 mmol) dissolved in 20 mL of dry DMF was added finely powdered K_2CO_3 (1.2 g), and the solution was kept under a nitrogen atmosphere. The proper thiol (15 mmol) was then added and the slurry heated to 60 °C under vigorous stirring. After 3 h the reaction mixture was stripped of the DMF and 100 mL of water (which was then neutralized to pH = 6 with HCl) was added to the residue. Extraction with $CHCl_3$ (5 × 70 mL) and evaporation of the dried (Na_2SO_4) organic solvent afforded a solid, which was purified by column chromatography (SiO_2, $CHCl_3$/MeOH 20:1). The following compounds were obtained from the different thiols.

2,6-Bis[(*n*-octylthio)methyl]-4-hydroxypyridine: 700 mg (54% yield), mp 124–126 °C; NMR $\delta_{CDCl_3/CD_3OD\ 1:1}$ 0.87 (br t, 6 H, 2 CH_3), 1.27 (m, 20 H, 2 $(CH_2)_5$), 1.56 (m, 4 H, 2 CH_2CH_2S), 2.54 (br t, 4 H, 2 SCH_2CH_2), 3.92 (s, 4 H, 2 SCH_2Py), 6.95 (s, 2 H, Py H3 and H5).

2,6-Bis[(*n*-dodecylthio)methyl]-4-hydroxypyridine: 1.1 g (65% yield), mp 135–136 °C; NMR $\delta_{CDCl_3/CD_3OD\ 10:1}$ 0.88 (br t, 6 H, 2 CH_3), 1.25 (m, 36 H, 2 $(CH_2)_9$), 1.56 (m, 4 H, 2 CH_2CH_2S), 2.55 (br, t, 4 H, 2 SCH_2CH_2), 3.97 (s, 4 H, 2 SCH_2Py), 7.05 (s, 2 H, Py H3 and H5).

2,6-Bis[(*n*-hexadecylthio)methyl]-4-hydroxypyridine: 1.8 g (91% yield), mp 134–135 °C; NMR $\delta_{CDCl_3/CD_3OD\ 10:1}$ 0.90 (br t, 6 H, 2 CH_3), 1.27 (m, 52 H, 2$(CH_2)_{13}$), 1.60 (m, 4 H, 2 CH_2CH_2S), 2.55 (br t, 4 H, 2 SCH_2CH_2), 3.85 (s, 4 H, 2 SCH_2Py), 6.97 (s, 2 H, Py H3 and H5).

The dithioether derivative (2 mmol) was added to a toluene solution (100 mL) containing 12 mL (13.9 mmol) of 1,2-dibromoethane and 100 mg (0.38 mmol) of 18-crown-6. Two grams of finely powdered KOH were then added and the vigorously stirred slurry was kept at 70 °C for 1 h. To the cooled reaction mixture was added $CHCl_3$ (150 mL), and the organic solution was washed with water (4 × 250 mL). The dried organic layer (Na_2SO_4) was evaporated under reduced pressure to leave the crude bromide. To this material, dissolved in the minimum amount of ethanol, was added 30 mL of a 30% solution of $(CH_3)_3N$ in ethanol, and the solution was kept at 50 °C with stirring for 15 h in a screw-top pressure tube. The solvent was then evaporated to dryness and $CHCl_3$ (150 mL) added. The chloroform solution was washed with water (4 × 150 mL) and the dried (Na_2SO_4) organic layer evaporated to leave a solid material, which was purified by crystallization. The following compounds were obtained.

2,6-Bis[(*n*-octylthio)methyl]-4-[[2-(trimethylammonio)ethyl]oxy]pyridine bromide (2): 750 mg (65% yield), mp ~145 °C (gel), ~195 °C (liquid crystal) from $CHCl_3/Et_2O$; NMR $\delta_{CDCl_3/CD_3OD\ 1:1}$ 0.88 (br t, 6 H, 2 CH_3), 1.26 (m, 20 H, 2 $(CH_2)_5$, 1.58 (m, 4 H, 2 SCH_2CH_2), 2.52 (br t, 4 H, 2 SCH_2CH_2), 3.33 (s, 9 H, $N(CH_3)_3$), 3.82 (s, 4 H, 2 SCH_2Py), 4.03 (br t, 2 H, CH_2N), 4.62 (br t, 2 H, OCH_2), 7.01 (s, 2 H, Py H3 and H5).

Anal. Calcd for $C_{28}H_{53}BrN_2OS_2$: C, 58.21; H, 9.25; N, 4.85. Found: C, 58.12; H, 9.49; N, 4.97.

2,6-Bis[(*n*-dodecylthio)methyl]-4-[[2-(trimethylammonio)ethyl]oxy]pyridine bromide (3): 1.1 g (80% yield), mp ~134 °C (gel), ~200 °C dec (liquid crystal), from $EtOH/Et_2O$; NMR $\delta_{CDCl_3/CD_3OD\ 10:1}$ 0.88 (br t, 6 H, 2 CH_3), 1.25 (m, 36 H, 2 $(CH_2)_9$), 1.58 (m, 4 H, 2 SCH_2CH_2), 2.53 (t, 4 H, 2 SCH_2CH_2), 3.44 (s, 9 H, $N(CH_3)_3$), 3.86 (s, 4 H, 2 SCH_2Py), 4.20 (br t, 2 H, CH_2N), 4.65 (br t, 2 H, OCH_2), 7.03 (s, 2 H, Py H3 and H5).

Anal. Calcd for $C_{36}H_{69}BrN_2OS_2$: C, 62.67; H, 10.08; N, 4.06. Found: C, 62.33; H, 10.28; N, 3.85.

2,6-Bis[(*n*-hexadecylthio)methyl]-4-[[2-(trimethylammonio)ethyl]oxy]pyridine bromide (4): 1.2 g (79% yield) mp ~140 °C (gel), ~190 °C (liquid crystal) from $CHCl_3/Et_2O$; NMR $\delta_{CDCl_3/CD_3OD\ 1:1}$ 0.89 (br t, 6 H, 2 CH_3), 1.27 (m, 52 H, 2 $(CH_2)_{13}$), 1.58 (m, 4 H, 2 SCH_2CH_2), 2.52 (t, 4 H, 2 SCH_2CH_2), 3.32 (s, 9 H, $N(CH_3)_3$), 3.82 (s, 4 H, SCH_2Py), 3.96 (br t, 2 H, CH_2N), 4.63 (br t, 2 H, OCH_2), 7.05 (s, 2 H, Py H3 and H5).

Anal. Calcd for $C_{44}H_{85}BrN_2OS_2$: C, 65.88; H, 10.68; N, 3.49. Found: C, 65.93; H, 11.05; N, 3.55.

A slightly different procedure has been followed for the synthesis of **2,6-bis[(methylthio)methyl]-4-[[2-(trimethylammonio)ethyl]oxy]pyridine bromide (1)**. The dichloride (260 mg, 1.1 mmol) and sodium thiomethoxide (474 mg, 6.8 mmol) were dissolved in 50 mL of dry ethanol. The reaction mixture was heated to 70 °C under a N_2 atmosphere. Evaporation of the solvent afforded a solid material, which was purified by column chromatography (SiO_2, $CHCl_3$/MeOH 10:1) to give 2,6-bis-[(methylthio)methyl]-4-hydroxypyridine (220 mg, 92% yield): NMR $\delta_{CDCl_3/CD_3OD\ 1:1}$ 2.07 (s, 6 H, 2 CH_3), 3.60 (s, 4 H, 2 CH_2), 6.35 (s, 2 H, Py H3 and H5).

For the subsequent steps we followed the same procedure as for the synthesis of compounds **2-4**. Compound 1 was obtained as a sticky solid after purification by chromatography on Al_2O_3 ($CHCl_3$/MeOH 4:1) (360 mg, 86% yield): NMR δ_{CD_3OD} 2.04 (s, 6 H, 2 CH_3S), 3.30 (s, 9 H, $N(CH_3)_3$), 3.74 (s, 4 H, 2 CH_2S), 3.94 (br t, 2 H, CH_2N), 4.63 (br t, 2 H, OCH_2), 7.02 (s, 2 H, Py H3 and H5).

No satisfactory elemental analysis could be obtained due to the high hygroscopicity of this compound.

Vesicle Preparation and Characterization. All vesicle solutions were prepared from suspensions of the proper surfactant in 0.05 M MES buffer, pH = 6.3, by sonication (Artek Sonic Model M150 sonicator, immersion probe, 60% power output, 20 min) at 50 °C. The vesicle solutions were allowed to cool slowly to 25 °C and then filtered through 0.45-μm Millipore filters before use. In the case of **3** and **4**, vesicles were also obtained by fast injection of 0.5 mL of a methanolic solution of the surfactant containing a 3-fold excess of $Cu(NO_3)_2$ into 10 mL of the buffer solution kept at 50 °C under vigorous stirring. Gel-to-liquid crystal phase transition temperatures, T_c, were determined from fluorescence polarization[22] studies using covesicallized 1,6-diphenyl-1,3,5-hexatriene (DPH) as a probe under the following conditions: [DPH] = 2.5×10^{-6} M, [surfactant] = 2.5×10^{-4} M.

Binding Constant Determination. These were determined by nonlinear regression analysis[23] of the changes in the absorbance in the 310–390-nm region on increasing [Cu(II)] and keeping

(21) Bradshow, J. S.; Colter, M. L.; Nakatsuji, Y.; Spencer, N. O.; Brown, M. F.; Izatt, R. M.; Avena, G.; Tse, P.-K.; Wilson, B. E.; Lamb, J. D.; Dalley, N. K.; Morin, F. G.; Grant, D. M. *J. Org. Chem.* **1985**, *50*, 4865.

(22) Andrich, M. P.; Vanderkooi, J. M. *Biochemistry* **1976**, *14*, 1257.

(23) Using the software package *Enzfitter* by Leatherbarrow, R. J., Elsivier: Amsterdam, 1987.

constant the ligand concentration. In all cases [Cu(II)] was ≳ 8[ligand]. In methanol solution where the extintion coefficient of the complex could be directly determined, binding constants were obtained without interpolation of the data. Under these conditions, the stoichiometry for all complexes was assumed to be 1:1 as confirmed by the spectroscopic (UV–vis) behavior either in water solution or in methanol. Only when the [Cu(II)]/[ligand] ratio is lower than unity are different stoichiometries apparent from the UV–vis analysis.

Fluorescence Quenching Experiments. Solutions of micellar or vesicular aggregates (2.5×10^{-4} M) were prepared in the presence of 1,8-anilinonaphthalenesulfonic acid (4.8×10^{-7} M); their fluorescence emission was determined at 500 nm (excitation wavelength 375 nm) on a Perkin Elmer MPF-66 instrument upon addition of different amounts of $Cu(NO_3)_2$.

Acknowledgment. Skillful technical assistance by E. Castiglione is gratefully acknowledged. P.S. thanks NATO for financial support (Grant for International Collaboration 0315/87).

Registry No. 1, 122899-66-5; 3, 122899-68-7; 4, 122924-10-1; 5, 122899-69-8; 6, 122899-70-1; Cu, 7440-50-8; $Br(CH_2)_2Br$, 106-93-4; $N(CH_3)_3$, 75-50-3; 4-hydroxy-2,6-bis(chloromethyl)pyridine, 122899-73-4; diethyl 4-((tetrahydro-2-pyranyl)oxy)-2,6-pyridinedicarboxylate, 122899-71-2; 4-hydroxy-2,6-bis(hydroxymethyl)pyridine, 122899-72-3; diethyl 4-((tetrahydro-2-pyranyl)oxy)-2,6-bis(hydroxymethyl)pyridine, 98828-63-8; 2,6-bis[(*n*-octylthio)methyl]-4-hydroxypyridine, 122899-74-5; 2,6-bis[(*n*-dodecylthio)methyl]-4-hydroxypyridine, 122899-75-6; 2,6-bis[(*n*-hexadecylthio)methyl]-4-hydroxypyridine, 122899-76-7; 2,6-bis[(methylthio)methyl]-4-hydroxypyridine, 122899-77-8; sodium thiomethoxide, 5188-07-8.

Supplementary Material Available: Figure S1 reporting the ANS fluorescence vs Cu(II) concentration for the different aggregates (1 page). Ordering information is given on any current masthead page.

Change in Conformational Preference between Dithia[3.3](1,4)naphthalenometacyclophanes and the Corresponding [2.2](1,4)Naphthalenometacyclophanes

Yee-Hing Lai* and Teck-Huat Lim

Department of Chemistry, National University of Singapore, Kent Ridge, Republic of Singapore 0511

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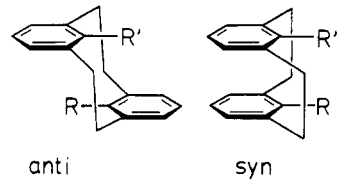
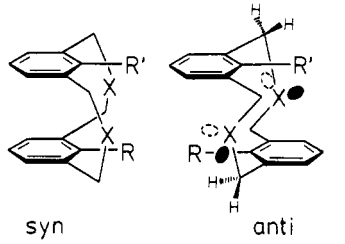
One of the unique properties of [2.2]metacyclophane is its preference for the stepped, anti conformation,[1] *anti*-**1**. Its isomer *syn*-**1** was recently prepared[2] by Mitchell et al. but found to isomerize readily to *anti*-**1** above 0 °C. The lower stability of *syn*-**1** is attributed to the unfavorable π–π interaction between the parallel benzene rings, although another mode of nonbonded interaction involving the intrusion of the H_i protons into the respective π-clouds of the opposite benzene rings is experienced in *anti*-**1**. The corresponding dithia- and diselena[3.3]metacyclophanes, however, were found to adopt preferentially the syn conformation, namely, *syn*-**4**[3] and *syn*-**7**,[4] respectively. The change in preference to the syn conformation is believed to be due to unfavorable torsional strain in the bridges in *anti*-**4** and *anti*-**7** with two bonds and two lone pairs always nearly eclipsed.[5] An interesting observation was that

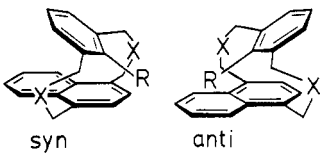


	R	R'
1	H_i	H_i
2	H_i	CH_3
3	CH_3	CH_3

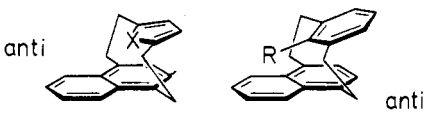


	X	R	R
4	S	H_i	H_i
5	S	H_i	CH_3
6	S	CH_3	CH_3
7	Se	H_i	H_i
8	Se	CH_3	CH_3

either ring contraction[6] or direct desulfurization[7] of *syn*-**4** and its derivatives afforded mainly *anti*-**1** and its corresponding derivatives. Photochemical deselenation[8] of *syn*-**7** also led to the isolation of only *anti*-**1**. The naphthalenometacyclophane **13** could, like **1**, exist in both syn and anti conformers experiencing similarly the respective nonbonded interactions. The related heterophanes **12** apparently adopt mainly the anti conformation;[9] only a very low yield of the syn conformer of **12** (X = S)[9b] was isolated. The dithiacyclophane **9**[10] has, however, been shown to exist, like **4**, in the syn conformation. Photodeselenation of the corresponding diselenacyclophane **10** was recently reported[8] to yield **13**, although there was no mention of the stereochemistry of either **10** or **13**. The above observation prompted us to investigate the photodesulfurization of *syn*-**9**. It would be interesting to determine whether an abrupt change in conformational preference similar to *syn*-**4** → *anti*-**1** is observed going from **9** to **13**.



	X	R
9	S	H_i
10	Se	H_i
11	S	CH_3



12 X = O, S, NH

13 R = H_i

14 R = CH_3

Irradiation[7a] of a solution of *syn*-**9**[10] in trimethyl phosphite with light at 254 nm gave, from TLC studies, only one isomer of **13** (mp 155–156 °C; identical with that reported[8]). Both cyclophanes **15**[11] and **17**[12] are known to be

(1) (a) Wilson, D. J.; Boekelheide, V.; Griffin, R. W. *J. Am. Chem. Soc.* **1960**, *62*, 6302. (b) Vögtle, F.; Neumann, M. v. P. *Angew. Chem., Int. Ed. Engl.* **1973**, *11*, 73. (c) Brown, C. J. *J. Chem. Soc.* **1953**, 3278.

(2) Mitchell, R. H.; Vinod, T. K.; Bushnell, G. W. *J. Am. Chem. Soc.* **1985**, *107*, 3340.

(3) Anker, W.; Bushnell, G. W.; Mitchell, R. H. *Can. J. Chem.* **1979**, *57*, 3080.

(4) (a) Mitchell, R. H. *Can. J. Chem.* **1980**, *58*, 1398. (b) Bushnell, G. W.; Mitchell, R. H. *Can. J. Chem.* **1982**, *60*, 362.

(5) See, for example: Fukazawa, Y.; Aoyagi, M.; Itô, S. *Tetrahedron Lett.* **1979**, 1055.

(6) (a) Mitchell, R. H.; Boekelheide, V. *J. Am. Chem. Soc.* **1974**, *96*, 1547. (b) Otsubo, T.; Boekelheide, V. *Tetrahedron Lett.* **1975**, 3881.

(7) (a) Boekelheide, V.; Reingold, I. D.; Tuttle, M. *J. Chem. Soc., Chem. Commun.* **1973**, 406. (b) Vögtle, F.; Neumann, M. v. P. *Synthesis* **1973**, 85.

(8) Higuchi, H.; Tani, K.; Otsubo, T.; Sakata, Y.; Misumi, S. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 4027.

(9) (a) Wasserman, H. H.; Keehn, P. M. *Tetrahedron Lett.* **1969**, 3227. (b) Mizogami, S.; Osaka, M.; Otsubo, T.; Sakata, Y.; Misumi, S. *Tetrahedron Lett.* **1974**, 799. (c) Rosenfled, S.; Keehn, P. M. *Tetrahedron Lett.* **1973**, 4921. (d) Corson, M.; Foxman, B. M.; Keehn, P. M. *Tetrahedron* **1978**, *34*, 1641.

(10) Lai, Y.-H. *J. Chem. Soc. Perkin Trans 2* **1989**, 643.

0022-3263/89/1954-5991$01.50/0