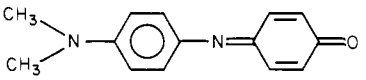
interface is impaired by the difficulty of placing above the water phase *both* the hydrocarbon portion of the tails and the aromatic "cap". Hexapus and fatty acids are similar, on the other hand, in that they aggregate in aqueous solutions as shown by light scattering experiments.[17] A plot of Rayleigh ratios for a 90° scattering vs. hexapus concentration ($4.1 \times 10^{-4} - 1.0 \times 10^{-2}$ M at pH 9.5) curves downward and corresponds (when coupled with differential refractometry data) to an aggregation number of 9 ± 1. Since there are 6 chains per hexapus, each aggregate contains 54 chains which equals, perhaps coincidentally, a typical aggregation number for a single-chained surfactant. The Rayleigh plot shows no break indicative of a CMC; thus if hexapus has a CMC, it must be smaller than 4×10^{-4} M. This point was investigated further by using pinacyanol chloride as a spectrophotometric probe for aggregation; the dye is known to change from pink to blue in the presence of anionic mielles. We found that the absorbance of 5.5×10^{-6} M dye at 610 nm remains essentially constant from 1.6×10^{-5} to 1.0×10^{-2} M hexapus at pH 9.5. Below 1.0×10^{-5} M hexapus, where its concentration approximates that of the probe, the absorbance decreases precipitously. It can be concluded that the CMC of hexapus, if one exists, must be less than 1×10^{-5} M (compared to 1×10^{-2} M for a 12-carbon surfactant).

Addition of 0.010 M hexapus to an aqueous solution of 4.2×10^{-5} M phenol blue (pH 9.50) induces a 67% hyperchromic shift in the visible spectrum as well as a peak narrowing. From



absorptivity vs. [hexapus] data plus the Ketelaar equation,[18] we calculated an association constant of 1.0×10^4 M^{-1} between phenol blue and hexapus. Since phenol blue has a solvent-sensitive λ_{max} (e.g., 575, 605, and 658 in benzene, ethanol, and water),[19] we could also assess the environment of the dye when fully bound to hexapus (or hexapus aggregates). This environment is highly polar (λ_{max} = 657 mm in 0.010 M hexapus), suggesting that water is plentiful at the host-guest binding sites.

An aqueous hexapus solution (0.011 M, pH 9.50) was sonicated with excess naphthalene at 55 °C, cooled, filtered through UF sintered glass, and spectrophotometrically assayed for naphthalene (λ = 312 nm, ϵ 136). It was found that hexapus solubilizes 1 naphthalene for every 2.5 hexapus molecules. Almost 40% ethanol is required to achieve an equivalent concentration of naphthalene in water without hexapus. A similar experiment showed that hexapus binds *p*-nitroaniline with 1:1 stoichiometry. Hexapus (0.010 M) also enhances the solubility of cholesterol in water although to a relatively small extent (3×10^{-4} M). Finally, hexapus binds *p*-nitrophenyl butyrate and inhibits its base-catalyzed hydrolysis. For example, the observed rate constant decreases from 20.1×10^{-3} to 1.86×10^{-3} min^{-1} when 0.0106 M hexapus is added to a borate buffer (pH 9.50, 25.0 °C). Inclusion of the ester among the carboxylate-terminated chains protects the ester from hydroxide attack. Rate data from several hexapus concentrations (treated by means a scheme commonly applied to micellar kinetics of simple surfactants[20]) yielded an association constant of 9.9×10^3 M^{-1} for the ester. This compares with, for example, a K_{assoc} = 2.9×10^2 M^{-1} for complexation of *m*-chlorophenyl acetate with β-cyclodextrin[2].

In summary, hexapus was found to be an effective new complexing agent for a variety of organic molecules. The nonspecificity no doubt relates to the flexible nature of the six chains. In the future we plan to convert the terminal carboxylates into catalytically active groups and thus create an enzyme simulant; the anticipated universal binding will be both an advantage and disadvantage. Future work will also center on locating the binding sites of hexapus. At present we do not know whether guests associate near the carboxylates or near the aromatic cap; nor do we know whether the binding takes place inside the hexapus cavity or external to the cavity but within the aggregate. Nonetheless, the hexapus system seems in concert with Lord Todd's recent dictum that "the organic chemist must turn his attention seriously to the study of large molecules where conformations or, if you will, tertiary structures can be adopted which permit the specific inclusion of other smaller molecules which can then react with one another".[21]

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(17) We thank Professor E. W. Anacker of the Montana State University for his hospitality and guidance during a summer in which one of us (F.M.M.) learned light scattering methods.

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(21) Messel Medal address (Cambridge, April 3, 1981). See *Chem. Ind. (London)*, 317 (1981).

Chemical Model Studies for the Mechanism of Vitamin K Epoxide Reductase

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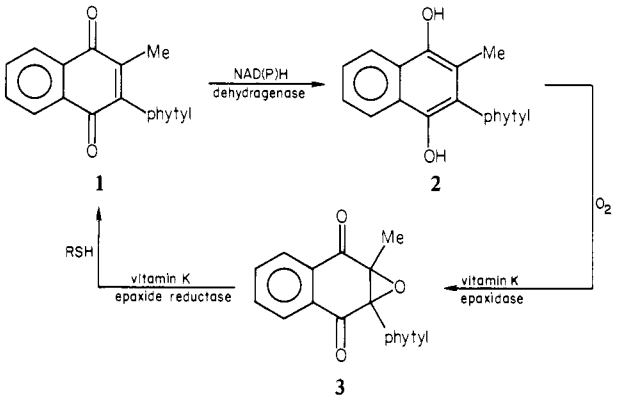
Vitamin K (**1**), which is essential for blood coagulation,[1] is converted into vitamin K 2,3-epoxide (**3**) by a coupled two-enzyme system. NADPH dehydrogenase catalyzes the reduction of vitamin K to its hydroquinone (**2**),[2] and vitamin K epoxidase catalyzes the monooxygenation of **2** to give **3**.[3] The epoxide of



vitamin K is not active in coagulation but is readily converted back to vitamin K by the enzyme vitamin K epoxide reductase[4] in a reaction which has been shown to require exogenous thiols in vitro.[5] It has been found that NADPH cannot be substituted for thiol.[5] Compounds that inhibit this reductase possess anticoagulant activity.[6] Recently I proposed[7] a molecular mechanism for this enzyme which is shown in Scheme I. Results of chemical model studies supporting that mechanistic proposal are communicated here.

The mechanism shown in Scheme I depicts *simultaneous* acid and base catalysis. While such a process is reasonable for an

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(2) Sadowski, J. A.; Esmon, C. T.; Suttie, J. W. *J. Biol. Chem.* **1976**, *251*, 2770.
(3) Matschiner, J. T.; Bell, R. G.; Amelotti, J. M.; Knauer, T. E. *Biochim. Biophys. Acta* **1970**, *201*, 309.
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(5) Siegfried, C. M. *Biochem. Biophys. Res. Commun.* **1978**, *83*, 1488.
(6) (a) Ren, P.; Stark, P. Y.; Johnson, R. L.; Bell, R. G. *J. Pharmacol. Exp. Ther.* **1977**, *201*, 541. (b) Whitlon, D. S.; Sadowski, J. A.; Suttie, J. W. *Biochemistry* **1978**, *17*, 1371.
(7) (a) Silverman, R. B. *J. Am. Chem. Soc.* **1981**, *103*, 3910. (b) *Ibid.* **1980**, *102*, 5421.

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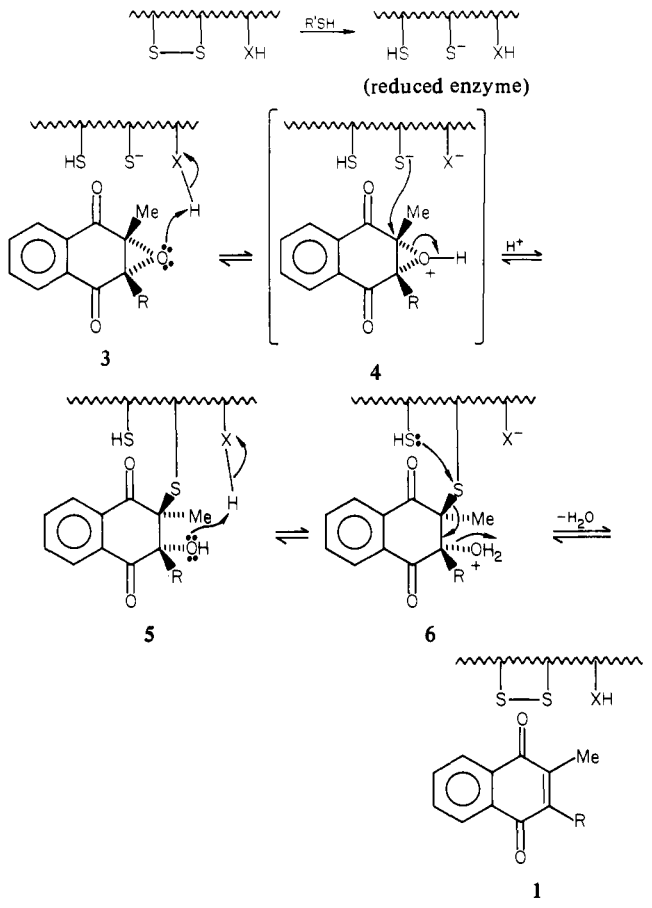
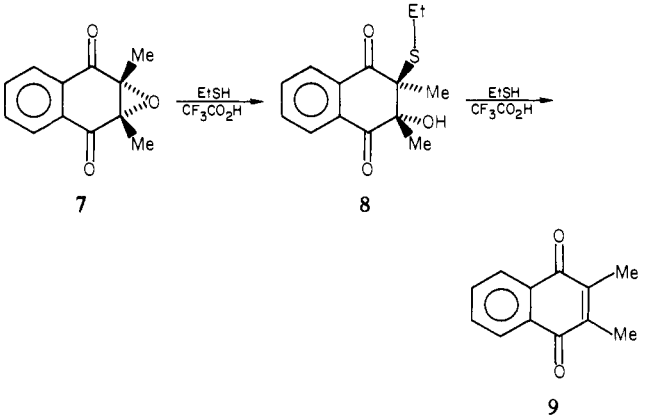
Scheme I. Proposed Mechanism of Action of Vitamin K Epoxide Reductase (R = Phytyl)

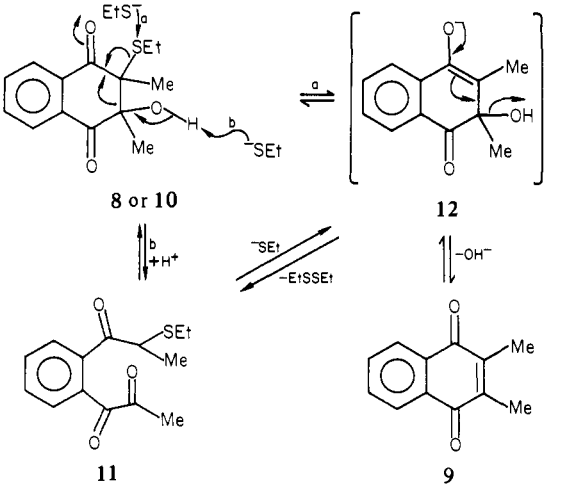


enzyme system, concurrent acid and base catalysis is generally not feasible in model studies. Consequently, these catalytic reactions were studied separately. As a model for vitamin K 2,3-epoxide (2-methyl-3-phytyl-1,4-naphthoquinone 2,3-epoxide; **3**), 2,3-dimethyl-1,4-naphthoquinone 2,3-epoxide (**7**) was prepared.[8] Treatment of **7** at room temperature for 35 min with 2 equiv of ethanethiol in trifluoroacetic acid (a model for the conversion **3** → **5** in Scheme I) gave 2,3-dimethyl-2-(ethylthio)-3-hydroxy-2,3-dihydro-1,4-naphthoquinone (**8**)[9] in a 66% yield after preparative layer silica gel chromatography. Compound **8**, which is



analogous to **5** in the proposed enzymatic scheme, was identified by its elemental analysis,[10] proton[11] and decoupled carbon[12] NMR

Scheme II. Possible Routes for the Conversion of 8 or 10 to 9



spectra, and IR[13] spectrum. The stereochemistry drawn for **8** was derived from the known reaction of thiols with epoxides to yield exclusive *anti*-β-hydroxy sulfides[14] (and from the isolation of the same compound with base catalysis as described below). When the reaction was allowed to proceed for 6 h, a 52% yield of **8** and a 40% yield of 2,3-dimethyl-1,4-naphthoquinone (**9**), identified by comparison of its NMR and IR spectra with those of an authentic sample of **9**,[15] were isolated. No reaction was observed after 16 h when CH_3COOH was substitued for CF_3COOH as expected from the weak basicity of epoxides.[16] The reaction of **8** at room temperature for 26 h with ethanethiol in trifluoroacetic acid (a model for the conversion of **5** → vitamin K, Scheme I) produced naphthoquinone **9** in a 79% yield. Again, no reaction occurred at room temperature in 26 h when CH_3COOH was substituted for CF_3COOH. These experiments support the proposed mechanism and demonstrate the rate enhancement suggested as a result of proton donation to the epoxide in the first step of the mechanism (**3** → **4**) and to the hydroxyl group in the third step (**5** → **6**).

Similar results were obtained under basic conditions. Treatment of **7** with ethanethiol and triethylamine (a model for the reaction of reduced vitamin K epoxide reductase with **3**) in acetonitrile for 4 h[17] gave **8** in a quantitative yield. Treatment of **8** with additional ethanethiol and triethylamine in acetonitrile at room temperature gave no reaction after 17 h; however, **8** rapidly reacted with sodium ethylthiolate in ethanol. After 15 min at room temperature, 73% of the starting material had been consumed and two new aromatic compounds were isolated by preparative layer silica gel chromatography. The major product (60%) was identified as 2,3-dimethyl-1,4-naphthoquinone (**9**). This reaction is analogous to the formation of vitamin K by the proposed reductive elimination of **6** (**6** → vitamin K, Scheme I). The elemental analysis,[18] proton[19] and decoupled carbon[20] NMR spectra, and IR[21] spectrum suggest that the minor product (40%) is the erythro

(8) Fieser, L. F.; Campbell, W. P.; Fry, E. M.; Gates, M. D., Jr. *J. Am. Chem. Soc.* **1939**, *61*, 3216.

(9) Although some of the structures are drawn as optically active molecules in order to indicate the stereochemical relationship of the substituents, the racemic mixtures actually were obtained.

(10) Anal. ($C_{14}H_{16}O_3S$) C, H, S; mp 93–93.5 °C (*n*-hexane).

(11) ^1H NMR ($CDCl_3$) δ 1.08 (t, 3 H), 1.63 (s, 3 H), 1.72 (s, 3 H), 2.40 (m, 2 H), 3.11 (s, 1 H), 7.45–8.15 (m, 4 H).

(12) ^1H NMR ($CDCl_3$) δ 13.84, 16.55, 18.87, 23.81, 61.04, 80.32, 126.89, 127.21, 132.20, 132.73, 133.85, 134.16, 192.82, 195.28.

(13) IR (KBr) 3460 (s), 1700 (s), 1690 (s), 1593 (m), 1450 (m), 1265 (s) cm^{-1}.

(14) Wohl, R. A. *Chimia* **1974**, *28*, 1.

(15) Arnold, R. T.; Larson, R. *J. Org. Chem.* **1940**, *5*, 250.

(16) No reaction was observed after 51 h when ethanethiol was omitted, suggesting that **8** is not derived from a prior acid-catalyzed rearrangement of **7**.

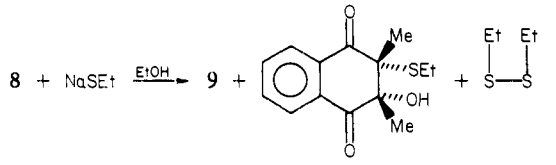
(17) When benzyl or phenyl mercaptan was used as the nucleophile, the reaction at room temperature was complete within a few minutes possibly as a result of increased thiolate concentration because of the lower pK_a of these thiols.

(18) Anal. ($C_{14}H_{16}O_3S$) C, H, S. Kugelrohr distilled [bp 111–115 °C, (0.05 mm)].

(19) ^1H NMR ($CDCl_3$) δ 1.04 (t, 3 H), 1.37 (s, 3 H), 1.75 (s 3 H), 2.32 (m, 2 H), 4.22 (br s, 1 H), 7.6–8.25 (m, 4 H).

(20) ^1H NMR ($CDCl_3$) δ 13.73, 15.27, 23.81, 24.79, 62.52, 80.08, 126.77, 127.53, 131.10, 133.17, 134.15, 134.89, 191.53, 198.92.

isomer of **8** (**10**). None of **10** was observed in the acid-catalyzed



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reactions either in the presence or absence of ethanethiol. Another product in the reaction was identified by gas chromatography as diethyl disulfide and represents a model for the formation of the oxidized enzyme (**6** → vitamin K) in Scheme I. When compound **10** was treated with sodium ethylthiolate in ethanol for 15 min, 71% of the starting material reacted, and two new aromatic compounds were isolated. These two compounds were identified as **9** (62%) and **8** (38%) by comparison of their IR spectra with those of the authentic compounds. This indicates that the two isomers, **8** and **10**, are interconvertible under basic conditions,[22] and both produce naphthoquinone **9**. The mechanism for the interconversion of **8** and **10** was determined[23] to involve a retro-aldol–aldol condensation via **11** (pathway b, Scheme II) rather than thiol reduction to **12** (pathway a, Scheme II) followed by attack of **12** on the newly formed disulfide. However, the conversion of **8** or **10** to **9** under basic conditions could proceed either directly to **12** or via **11**. Oki et al.[24] have shown that β-keto sulfides (such as **6** or **11**) are reduced by thiols to ketones, presumably via the corresponding enolate. Since **8** also is converted to **9** in trifluoroacetic acid (vide supra) and no **10** is produced,[25] both routes (via **11** or directly to **12**) may be feasible depending upon conditions. Although Scheme I depicts direct sulfide reduction and elimination (**6** → vitamin K), the pathway which seems to be favored in acid, the ring cleavage pathway via **11**, which is favored in base,[23] also is a possibility. If this is the case, rather than enzyme-catalyzed proton donation to the hydroxyl group (**5** → **6**) an enzyme-catalyzed deprotonation of the hydroxyl would be required. The interconversion of **8** and **10** is probably not relevant to the enzyme model since the sulfide linkage formed would be to the enzyme and isomerization would be sterically difficult.

The observation that **8** does not undergo reaction at room temperature with ethanethiol and triethylamine in acetonitrile but rapidly reacts with sodium ethylthiolate in ethanol to give **9** can be rationalized on the basis of a difference in the nucleophilicity (and basicity[23]) of the anions[26] and as a solvent effect.[27] In accordance with the enhanced nucleophile (base) rationalization,[26] when **8** was treated with ethanethiol and triethylamine in ethanol at room temperature, no reaction took place in 5.5 h. Since **8** and **10** are rapidly converted to naphthoquinone **9** by sodium ethylthiolate in ethanol, the sodium salt must be a more powerful nucleophile than the triethylammonium salt.[26] Furthermore, the reaction of **8** or **10** with sodium ethylthiolate in acetonitrile leads to no reductive desulfuration[23] unlike the rapid reaction to give **9** in ethanol. Formation of enolate **12** and elimination of hydroxide apparently is favored in the hydroxylic solvent.[27]

A second reasonable enzymatic mechanism can be excluded on the basis of the results described here. Sulfhydryl attack could occur at one of the vitamin K epoxide carbonyl groups to give a hemithioketal. Attack of this α-hydroxy sulfide by thiolate with concomitant epoxide ring opening followed by enol tautomerization and hydroxide elimination also would give the naphthoquinone. However, the intermediate that was isolated contains *two* carbonyl groups;[12,13] only one carbonyl would be observed if hemithioketal formation were important. The model studies in this communication therefore provide chemical support for the mechanism of vitamin K epoxide reductase in Scheme I.

(21) IR (film) 3480 (s), 1698 (s), 1683 (s), 1592 (m), 1443 (m), 1265 (s) cm^{-1}.

(22) No evidence for this interconversion has been found under acidic conditions starting from either isomer.

(23) Silverman, R. B. *J. Org. Chem.* **1981**, *46*, in press.

(24) Oki, M.; Funakoshi, W.; Nakamura, A. *Bull. Chem. Soc. Jpn.* **1971**, *44*, 828.

(25) When **10** was treated with ethanethiol in trifluoroacetic acid for 26 h, **9** was produced only in a 2% yield. Since the yield of **9** is 40 times greater under the same conditions starting from **8**, it appears that the ethylthio substituent in **8** is axial to the carbonyl and that either a concerted reductive elimination (**6** → vitamin K) or a reductive E1cb elimination is possible. This also confirms the structure of **8** as the isomer with the sulfide and hydroxyl groups anti, as shown.

(26) The thiolate generated by the reaction of EtSH and Et_3N will be in low concentration because of the greater pK_a of EtSH than Et_3NH^+. Also, the thiolate produced under these conditions probably will be hydrogen bonded to Et_3NH^+ and should have different nucleophilic and basic properties than those of NaSEt.

(27) The enolate formed by thiolate attack on any of the β-hydroxy sulfides (**8**, **10**, **11**) would be stabilized in the hydroxylic solvent as compared with acetonitrile. Similarly, the pK_a of H_2O in a hydroxylic solvent is much lower than in a nonhydroxylic solvent;[28] therefore elimination of hydroxide from **12** to give the naphthoquinone in ethanol would be more favorable than in acetonitrile.

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Chelating Phosphinite Complexes of Group 6 Metal Carbonyls with Crown-Ether-Type Characteristics. Effect of Preferential Cation Binding on the Reactivity of Coordinated Carbon Monoxide

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Coordinated carbon monoxide may be activated with respect to alkyl/aryl migration (i.e., nucleophilic addition) and probably with respect to hydrogenation (methanation and "Fischer-Tropsch-type" synthesis) by formation of an adduct between a Lewis acid and a carbonyl oxygen in $L_nM(R)(CO)$ and/or by stabilization of the acyl product $L_nM(RCO{\rightarrow}A)$ (e.g., A = Li^+, $AlBr_3$, or Cp_2Zr^+).[1-7] We now report that *preferential cation binding by the product molecule* can be utilized to activate coordinated carbon monoxide toward nucleophilic additions.

The series of complexes *cis*-$(M(CO)_4[Ph_2P(OCH_2CH_2)_nOPPh_2])$ (complexes **1**; M = Cr, Mo, W; n = 2, 3, 4, 5) have been prepared in 20–70% yield from the reaction of the appropriate bis(diphenylphosphinite) ligand with $M(CO)_4$(norbornadiene) by using high dilution techniques. Complexes **1**, which have been fully characterized,[8] have structures suggesting potential crown ether reactivity. We have used ^{13}C NMR spectroscopy as a probe of crown-ether-type interactions between **1** (M = Mo) and the alkali metal cations.[9,10] These studies show that **1** (n = 5) will complex Li^+ and Na^+, **1** (n = 4) will complex Li^+ only (see Figure 1), and the ^{13}C chemical shifts of **1** (n = 3 or 2) are unaffected by the presence of group 1A cations (i.e., no or only weak complexation).

It is well-known that the carbonyl complexes $LM(CO)_5$ (L = CO, PR_3) will react with strong nucleophiles, such as MeLi, to

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(7) Butts, S. B.; Richmond, T. G.; Shriver, D. F. *Inorg. Chem.* **1981**, *20*, 278.

(8) For example, Anal. Calcd for **1** (M = Mo), n = 2: C, 56.3; H, 4.1; M_r, 672. Found: C, 56.1; H, 4.0; M_r, 640. Calcd for n = 3: C, 56.2; H, 4.4; M_r, 726. Found: C, 56.0; N, 4.4; M_r, 754. Calcd for n = 4: C, 56.1; H, 4.7; M_r, 770. Found: C, 56.1; H, 4.6; M_r, 735. Calcd for n = 5: C, 56.0; H, 4.9; M_r, 814. Found: C, 55.8; H, 4.7; M_r, 800. Molecular weights determined osmometrically in $CHCl_3$.

(9) Lehn, J. M.; Sonveaux, E.; Willard, A. K. *J. Am. Chem. Soc.* **1978**, *100*, 4914.

(10) Shamsipur, M.; Popov, A. I. *J. Am. Chem. Soc.* **1979**, *101*, 4051.

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