Vesicles derived from **2** were prepared by (a) coating the lipid onto the walls of a round-bottomed flask (chloroform evaporation), (b) dispersing the lipid into distilled water (vortex mixing), and (c) irradiating the dispersion with ultrasound at 50 °C under a nitrogen atmosphere to constant turbidity (Heat Systems Model W-375 R bath-type sonicator operating at 275 W). Thin-layer chromatography of the resulting dispersion indicated that no lipid decomposition occurred during the sonication process (R_f = 0.22).[14]

Vesicle polymerization (disulfide formation) was carried out either by direct UV irradiation at 254 nm (30–60 min) or by oxidation with excess H_2O_2 at 40 °C (20 equiv, 3 h).[17-19] In the former case, thin-layer chromatrography indicated the complete disappearance of **2** and a single lipid spot at the origin ($R_f = 0$). Quantitative analysis for thiol groups using 5,5′-dithiobis(2-nitrobenzoic acid) (Ellman's Reagent) revealed a 95% loss after 0.5 h of irradiation.[20] Electron micrographs recorded on a Philips 400 TEM microscope, using 2% uranyl acetate as a staining agent, confirmed the presence of closed vesicles having diameters ranging between 200 and 800 Å (Figure 1). The estimated thickness of the vesicle membrane is 40–50 Å, which is consistent with the bilayer thickness found in other unilamellar liposomes.[21] With hydrogen peroxide oxidation (pH 7), the starting material was apparently converted to an oligomerized vesicle, whose components had an R_f equaling 0.08 (broad spot); thiol analysis of the product showed that the extent of oxidiation was ca. 55%. Similar hydrogen peroxide mediated oxidation carried out at pH 8.5 produced polymerized vesicles having an R_f equaling 0 and showing a 95% decrease in thiol content.

In contrast to their nonpolymerized analogues, which precipitate on standing within 48 h, photopolymerized vesicles of **2** showed no detectable change in turbidity after 10 days. Improved stability of these polymerized vesicles was further demonstrated by their response to exposure to strong ionic detergent. Thus, addition of 0.16% sodium dodecylsulfate to nonpolymerized and photopolymerized (1 h) dispersions of **2** resulted in an 82% and 12% loss in turbidity, respectively. By using procedures similar to those previously described,[3] photopolymerized vesicles of **2** entrapped ca. 0.02% of [^{14}C]sucrose and retained 75% and 60% of the marker when subjected to dialysis for 2 and 4 h, respectively. Nonpolymerized vesicles of **2** had similar entrapment and retained 54% of the sucrose after 2 h and 46% after 4 h.

Preliminary evidence for substantial reversibility of polymerized **2** has been obtained via reductive regeneration of the lipid monomer. Thus, treatment of freeze-dried, UV or H_2O_2 (pH 8.5) polymerized dispersions of **2** with 40 equiv of tri-*n*-butylphosphine in $C_2H_5OH-H_2O$ (5/1) for 18 h at 40 °C followed by quantitative TLC (phosphorus analysis) indicated at 90% regeneration of **2** (R_f 0.22). Direct reduction of the *aqueous vesicle dispersion* of photopolymerized **2** with 200 equiv of dithiothreitol (50 °C, 3 h) liberated 25% of **2** (quantitative TLC). The turbidity of the resulting dispersion remained unchanged under these conditions, and electron microscopy confirmed the retention of the vesicle structure. Similar reduction of the H_2O_2-polymerized dispersion of **2** regenerated ca. 70% of the monomer; the turbidity of the dispersion was unaltered.

Efforts now underway are aimed at (1) optimizing vesicle oxidation and reduction using chemical, photochemical, and electrochemical means, (2) determining the degree of polymerization within the membrane, (3) synthesizing cross-linked polymerized phosphatidylcholine vesicles based on disulfide formation, and (4) examining the potential utility of this new class of vesicles as membrane models and as drug carriers. Complete details of these studies will be reported in due course.

Registry No. 1, 87050-14-4; **2**, 87050-11-1; **2** homopolymer, 87050-12-2; $C_2H_5SS(CH_2)_{10}C(O)OH$, 87050-15-5; *sn*-glycerol-3-phosphochlorine, 28319-77-9.

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[17] Attempted oxidation by molecular oxygen at room temperature under neutral or basic conditions (pH 8.5) left substantial amounts of unreacted monomer.

[18] Capozzi, G.; Modena G. "Oxidation of Thiols" In "The Chemistry of The Thiol Group", Patai, C. S., Ed.; Wiley: New York, 1974; Part 2, p 785.

[19] Specific procedures used for photopolymerization were similar to those previously described.[3]

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Organometallic Crown Ethers. 1. Metal Acyl Binding to a Crown Ether Held Cation

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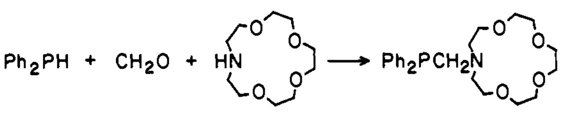
Lewis acids can accelerate alkyl migration to coordinated carbon monoxide by stabilizing the transition state leading to the acyl product.[1] As a means of holding Lewis acidic cations close to transition metals, we have prepared a phosphine functionalized aza crown ether. We now report the binding of a transition-metal acyl ligand to various crown ether held alkali-metal and alkaline-earth cations.[2]

By use of a modification of the Mannich reaction,[5] the combination of diphenylphosphine and monoaza-15-crown-5 with aqueous formaldehyde produces the phosphine aza crown ether **1** as a pure, colorless, air-sensitive oil (reaction 1).[6,7]



(1)

1

1 reacts with $CpFe(CO)_2Me$ (Cp = η^5-C_5H_5) thermally[8] to give the acyl complex **2a** and photochemically[9] to give the methyl

---

[1] (a) Collman, J. P.; Finke, R. G.; Cawse, J. N.; Brauman, J. I. *J. Am. Chem. Soc.* **1978**, *100*, 4766–4772. (b) Butts, S. B.; Strauss, S. H.; Holt, E. M.; Stimson, R. E.; Alcock, N. W.; Shriver, D. F. *Ibid.* **1980**, *102*, 5093–5100. (c) LaCroce, S. J.; Cutler, A. R. *Ibid.* **1982**, *104*, 2312–2314. (d) Berke, H.; Hoffmann, R. *Ibid.* **1978**, *100*, 7224–7236. (e) Nitay, M.; Priester, W.; Rosenblum, M. *Ibid.* **1978**, *100*, 3620–3622. (f) Labinger, J. A.; Miller, J. S. *Ibid.* **1982**, *104*, 6856–6858. (g) Grimmett, D. L.; Labinger, J. A.; Bonfiglio, J. N.; Masuo, S. T.; Shearin, E.; Miller, J. S. *Ibid.* **1982**, *104*, 6858–6859.

[2] The earliest reported organometallic crown ethers[3] showed no evidence of interaction between the crown ether held cations and the transition metals. While our work was in progress, J. Powell et al. reported the syntheses of two aminophosphine ligands similar to ours[4a] and the syntheses of *anionic* metal acyl complexes in which the Li^+ counterion is bound to a chelating phosphinite ligand with crown ether properties.[4b,c]

[3] (a) Hyde, E. M.; Shaw, B. L.; Shepherd, I. *J. Chem. Soc., Dalton Trans.* **1978**, 1696–1705. (b) Shaw, B. L.; Shepherd, I. *Ibid.* **1979**, 1634–1638. (c) Odell, K. J.; Hyde, E. M.; Shaw, B. L.; Shepherd, I. *J. Organomet. Chem.* **1979**, *168*, 103–114.

[4] (a) Powell, J.; May, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 2636–2637. (b) Powell, J.; Kuksis, A.; May, C. J.; Nyburg, S. C.; Smith, S. J. **1981**, *103*, 5941–5943. (c) Powell, J.; Gregg, M.; Kuksis, A., Meindl, P. *Ibid.* **1983**, *105*, 1064–1065.

[5] Grim, S. O.; Matienzo, L. J. *Tetrahedron Lett.* **1973**, 2951–2953.

[6] **1**: ^{1}H NMR ($CDCl_3$, 80 MHz) δ 7.4 (m, Ph), 3.63 (s, t, J = 6 Hz, OCH_2CH_2O and NCH_2CH_2O), 3.47 (d, J_{PH} = 4.4 Hz, PCH_2N), 2.97 (t, J = 6 Hz, NCH_2CH_2O); mass spectrum, 232.1551 (parent ion − PPh_2).

[7] Complete spectroscopic characterizations are contained in the supplementary material.

[8] Bibler, J. P.; Wojcicki, A. *Inorg. Chem.* **1966**, *5*, 889–892.

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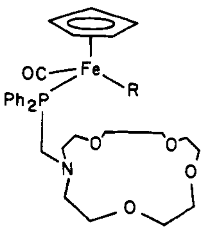
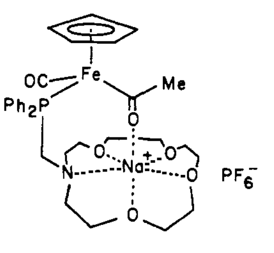
complex **2b**. Following workup by column chromatography[10] and



2a R = COMe
2b R = Me



2a · NaPF$_6$

low-temperature recrystallization, both compounds are obtained as pure crystalline solids.[7,11]

Methylene chloride solutions of **2a** or **2b** react rapidly with alkali-metal or alkaline-earth salts to produce homogeneous solutions. Changes in the infrared spectra (Table I), particularly the ν_{CO} (ether) region,[12] are consistent with the formation of crown ether–cation complexes. The dramatic changes in ν_{CO} (terminal carbonyl, acyl) of **2a** upon complex formation suggest intramolecular coordination of the acyl ligand to the crown ether held cation (e.g., **2a**·NaPF$_6$).[13] The terminal carbonyl frequencies increase and the acyl frequencies decrease, as expected for acyl coordination to a Lewis acid.[1b] The more highly acidic alkaline-earth cations cause the largest shifts. Salts with the potassium ion, which is too large to fit into the 15-crown-5 ring,[14] do not form stable complexes. The NaPF$_6$ adducts **2a**·NaPF$_6$ and **2b**·NaPF$_6$ can be isolated by filtering the methylene chloride solutions to remove excess NaPF$_6$ and adding pentane to induce crystallization.[7,15]

Room-temperature NMR spectra of mixtures containing the crown ether and its NaPF$_6$ adduct show a single average set of resonances due to the rapid exchange of the NaPF$_6$ from one crown ether to the other (reactions 2 and 3). At 3 °C, line

$$\mathbf{2a} + \mathbf{2a}^{*}\cdot\mathrm{NaPF_6} \rightleftarrows \mathbf{2a}\cdot\mathrm{NaPF_6} + \mathbf{2a}^{*} \tag{2}$$

$$\mathbf{2b} + \mathbf{2b}^{*}\cdot\mathrm{NaPF_6} \rightleftarrows \mathbf{2b}\cdot\mathrm{NaPF_6} + \mathbf{2b}^{*} \tag{3}$$

broadening and/or peak separation can be observed by ^{31}P NMR (phosphine resonances). A simple two-site line-shape analysis[16] provides the exchange rate constants. The observed rate constants depend upon the concentration of cation-free **2** (equation 4). The

$$k_{\mathrm{obsd}}(\mathbf{2}\cdot\mathrm{NaPF_6}) = k_1 + k_2[\mathbf{2}] \tag{4}$$

values of k_1 and k_2 determined by a weighted linear least-squares fit of [**2**] vs. k_{obsd} (Table II and Figure 1) reveal that for both **2a** and **2b** the bimolecular term $k_2[\mathbf{2}]$ predominates over the unimolecular term k_1. A bimolecular exchange mechanism, which probably proceeds through the associated 2:1 intermediate ($\mathbf{2}\cdot\mathrm{Na^+}\cdot\mathbf{2})\mathrm{PF_6}^-$,[17] avoids dissociating "naked" NaPF$_6$ into a poor donor solvent.[18] The bimolecular exchange rate of **2a**·NaPF$_6$ is approximately 10 times slower than **2b**·NaPF$_6$. *This rate difference reflects the contribution of the acyl–cation bond to the kinetic stability of* ***2a****·NaPF$_6$.*

Table I. Infrared Data for **2** + Excess Salt in CH$_2$Cl$_2$ (ν_{CO} regions, cm^{-1})

complex	salt	terminal carbonyl	acyl	ether
2a	none	1917	1596	1126
	LiClO$_4$	1934	1588	1095
	NaPF$_6$	1928	1565	1119
	MgI$_2$	1958	1521	1091
	CaI$_2$	1952	1517	1088
2b	none	1900		1126
	LiClO$_4$	1882		1102
	NaPF$_6$	1896		1114
	MgI$_2$	1897		1089
	CaI$_2$	1895		1090

Table II. Derived Values of k_1 and k_2 (eq 4)[a]

complex	$10^{-1}k_1$, s^{-1}	$10^{-4}k_2$, M^{-1} s^{-1}
2a·NaPF$_6$	-5.3 ± 4.1	3.85 ± 0.36
2b·NaPF$_6$	31 ± 29	37.2 ± 2.6

[a] Errors represent one standard deviation.

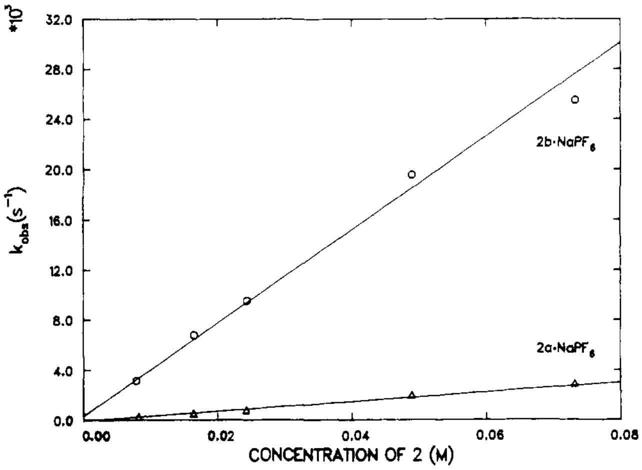


Figure 1.

The equilibrium constant for the exchange of NaPF$_6$ between **2a** and **2b** can be calculated from room-temperature ^{1}H NMR spectra of equilibrium mixtures (reaction 5). These spectra show

$$\mathbf{2a} + \mathbf{2b}\cdot\mathrm{NaPF_6} \xrightleftharpoons{K_{\mathrm{eq}} = 4.7 \pm 0.8} \mathbf{2a}\cdot\mathrm{NaPF_6} + \mathbf{2b} \tag{5}$$

only two sets of resonances, one the result of **2a** + **2a**·NaPF$_6$ (reaction 2), and the other the result of **2b** + **2b**·NaPF$_6$ (reaction 3). The ratios of free to complexed crown ether in the equilibrium mixture (**2a**/**2a**·NaPF$_6$ and **2b**/**2b**·NaPF$_6$) were determined from the observed chemical shifts, and K_{eq} was calculated directly.[19] *The enhanced NaPF$_6$ binding ability of* ***2a*** *relative to* ***2b*** *reflects the contribution of the acyl–cation bond to the thermodynamic stability of* ***2a****·NaPF$_6$.*

By comparing the cation-binding properties of two complexes that differ only by an acyl CO group, we have provided both qualitative and quantitative evidence for the metal acyl–cation bond. We are currently investigating the effect of crown ether held cations on the kinetics of the alkyl migration reaction.

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(10) Activity I alumina, 0.5–5% CH$_3$OH in CH$_3$OC(CH$_3$)$_3$.

(11) **2a** (73% yield): Anal. (C$_{31}$H$_{40}$FeNO$_6$P) C, H, Fe, N, P. **2b** (75% yield): Anal. (C$_{30}$H$_{40}$FeNO$_5$P) C, H, Fe, N, P.

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(15) **2a**(CH$_2$Cl$_2$)·NaPF$_6$ (87% yield): Anal. (C$_{32}$H$_{42}$Cl$_2$F$_6$FeNNaO$_6$P$_2$) C, H, F, Fe, N, P. **2b·NaPF$_6$** (89% yield): Anal. (C$_{30}$H$_{40}$F$_6$FeNNaO$_5$P$_2$) C, H, F, Fe, N, P.

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(18) A bimolecular mechanism has been proposed in certain cryptand–cation exchange reactions: Cox, B. G.; Garcia-Rosas, J.; Schneider, H. *J. Am. Chem. Soc.* **1982**, *104*, 2434–2437.

(19) Separate NMR experiments with reactions 2 and 3 established a linear relationship between the observed chemical shift and the fraction of NaPF$_6$ complex in the mixture. For another example of this method, see: Reinhoudt, D. N.; Gray, R. T.; DeJong, F., Smit, C. J. *Tetrahedron* **1977**, *33*, 563–571.

Acknowledgment. The capable technical assistance of D. Rothfuss is greatly appreciated.

Registry No. 1, 87101-78-8; **2a**, 87101-79-9; **2a**·$NaPF_6$, 87101-81-3; **2b**, 87114-18-9; **2b**·$NaPF_6$, 87101-83-5.

Supplementary Material Available: NMR (^1H, ^{13}C, and ^{31}P) characterization of **1**, **2a**, **2a**·$NaPF_6$, **2b**, and **2b**·$NaPF_6$ (3 pages). Ordering information is given on any current masthead page.

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^{17}O NMR as a Probe of Nucleic Acid Dynamics

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Given the importance of structural flexibility of polynucleotides, considerable effort has been directed toward providing a detailed characterization of nucleic acid dynamics. NMR spin-relaxation techniques provide a convenient measure of molecular motion, and a variety of nuclei (^1H, ^{13}C, ^{15}N, and ^{31}P) have been used in studies of polynucleotides.[2-9] The relaxation times for various nuclei are sensitive to different types of internal motion by virtue of the different processes dominating the relaxation; in principle, the analysis of results obtained with several probe nuclei should lead to a detailed description of nucleic acid dynamics. A complete analysis of this type would be facilitated by a nuclear probe sensitive only to the reorientation of the phosphate groups and uncomplicated by spin interactions with sites on adjacent ribose rings. Specific enrichment of the nonesterified phosphoryl oxygens with ^{17}O would provide the desired spin probe. ^{17}O NMR spin relaxation is dominated by the interaction of the nuclear quadrupole moment with the electric field gradient defined by the bonding electrons; this relaxation mechanism provides a well-defined monitor of P–O bond reorientation. In this communication we demonstrate that useful ^{17}O NMR spin-relaxation data can be obtained for a simple polynucleotide, polyadenylic acid (poly A). While a complete analysis of the spin relaxation in this spin $^5/_2$ system is not presented, a qualitative analysis of the data leads to useful conclusions regarding internal motions.

The ^{17}O-labeled poly A was the generous gift of Professor Philip H. Bolton of Wesleyan University. This material was prepared by the action of polynucleotide phosphorylase on [α-$^{17}O_2$]ADP synthesized in this laboratory and having a ^{17}O enrichment of 50% in each labeled position.[10] Spectra were obtained on a Bruker CXP-200 spectrometer with a probe of our own design.

The spectra in Figure 1 demonstrate the temperature dependence of the poly A ^{17}O line shape. The sharp resonance defining 0 ppm in each spectrum is from the residual ^{17}O in ^{17}O-depleted H_2O (0.008%). The broad resonance centered at ~80 ppm is from the labeled nonesterified phosphoryl oxygens in the poly A. At 90 °C the poly A resonance is well represented as a single Lorentzian peak with a full width at half-height of 1400 Hz. The relative areas of the poly A and H_2O resonances are consistent with the relative concentrations of ^{17}O in each, indicating the single Lorentzian represents all of the labeled sites in poly A. At lower temperatures it is apparent that the resonance is no longer a single Lorentzian. This is particularly true at 50 °C. Below 40 °C it was not possible to discern a poly A resonance, indicating the resonance width to be substantially in excess of the response width of the probe, 10 KHz.

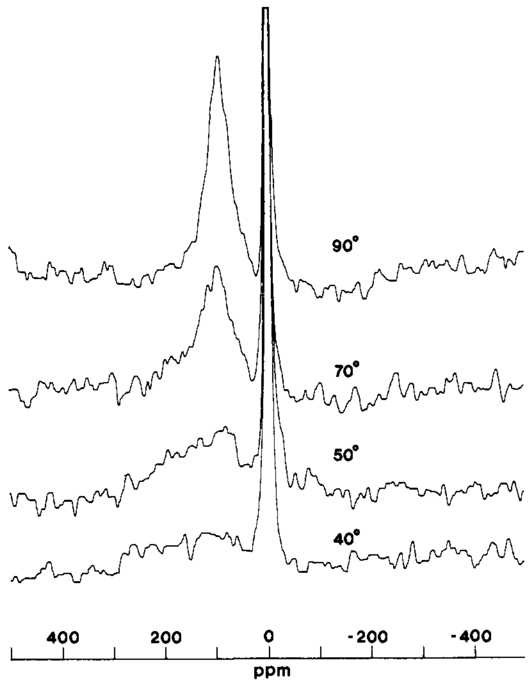

Figure 1. ^{17}O NMR spectra at 27.1 MHz of [^{17}O]poly A as a function of temperature. Labeled poly A (4 mM in monomeric units) was dissolved in 0.1 M sodium cacodylate buffer containing 0.1 M NaCl and 2 mM EDTA; ^{17}O-depleted water (0.008% ^{17}O) was used as the solvent.

Table I. ^{17}O Spin Relaxation Parameters for Poly A

temp, °C	line width, Hz	T_2,[a] ms	T_1,[b] ms	$\bar{l}$[c] (bases)	α[c]
90	1400	0.23		1.6	0.12
80	1500 ± 100	0.21 ± 0.01	0.45	1.8	0.17
70	1750	0.18 ± 0.02		2.0	0.24
65	2130 ± 300	0.15 ± 0.02	0.25	2.1	0.28
60	NL[d]			2.3	0.32
50	NL[d]		0.4 ± 0.1	2.8	0.43
40	NL[d]			3.7	0.54
30	NR[d]			5.2	0.67

[a] Calculated from the full width at half-maximum; $T_2 = (\pi \times \text{line width})^{-1}$. [b] Estimates from the least-squares fits of ln $([A(\infty) - A(t)]/A(\infty))$ vs. delay. [c] $\bar{l}$ = average number of contiguously stacked bases; α = fraction of bases in the stacked state. Both calculated using $\Delta H° = -9.6$ kcal/mol stack, $\Delta S° = -30.3$ cal K^{-1}/mol stack, $\sigma = 1$.[14] [d] NL = non-Lorentzian; NR = no observed resonance.

Table I contains estimates of the transverse relaxation times (T_2) calculated from line widths at those temperatures where the resonance appears to be Lorentzian and undiminished in area. For spin $^5/_2$ nuclei deviation from a Lorentzian line shape occurs outside of the fast motion limit.[11-13] For ^{17}O at 27.1 MHz (1.7 × 10^8 s^{-1}), non-Lorentzian resonances should be observed for effective correlation times on the order of a few nanoseconds.[12] The deviation from Lorentzian line shape near 40 °C is then consistent with ^{31}P studies of poly A in which the isotropic tumbling responsible for relaxation was found to be on the order of nanoseconds at this temperature.[7] The temperature dependence of the ^{17}O line shape near 40 °C is sharp and parallels the degree of base stacking, α, or number of contiguously stacked bases, l

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