Azacrown Ethers as Amphiphile Headgroups: Formation of Stable Aggregates from Two- and Three-Armed Lariat Ethers

Stephen L. De Wall, Keshi Wang, Daniel R. Berger, Shigeru Watanabe, Jeanette C. Hernandez, and George W. Gokel*

Bioorganic Chemistry Program and Department of Molecular Biology & Pharmacology, Washington University School of Medicine, 660 South Euclid Avenue, Campus Box 8103, St. Louis, Missouri 63110

Received May 27, 1997[⊗]

Ten 18-membered ring lariat ether compounds have been prepared as one-, two-, and three-armed derivatives of aza-, diaza-, and triaza-18-crown-6. They include *N*-[[(3-cholestanyloxy)carbonyl]-methyl]aza-18-crown-6, **1**, *N*-tetradecylaza-18-crown-6, **2**, *N*,*N*′-dibutyl-4,13-diaza-18-crown-6, **3**, *N*,*N*′-dinonyl-4,13-diaza-18-crown-6, **4**, *N*,*N*′-didodecyl-4,13-diaza-18-crown-6, **5**, *N*,*N*′-ditetradecyl-4,13-diaza-18-crown-6, **6**, *N*,*N*′-dioctadecyl-4,13-diaza-18-crown-6, **7**, *N*,*N*′-bis[[(3-cholestanyloxy)-carbonyl]methyl]-4,13-diaza-18-crown-6, **8**, *N*,*N*′-bis[[(3-cholestanyloxy)carbonyl]decyl]-4,13-diaza-18-crown-6, **9**, and *N*,*N*′,*N*″-tri-*n*-hexyl-4,10,16-triaza-18-crown-6, **10**. Compounds **2**, **8**, and **9** are previously unreported. Aqueous suspensions of these monomers were sonicated, and the first evidence for stable aggregates formed from diaza and triaza lariat ethers (**3**−**10**) was obtained. The formation of aggregates from **3** or **10** is especially notable since the side chains are butyl or hexyl, respectively. The aggregates were studied by a combination of laser light scattering, electron microscopy, and dye entrapment. All of the amphiphiles proved to form aggregates thought to be vesicles except **2**, which formed micelles. The similarity in sizes of the vesicles, apparently irrespective of side chain, and the general indifference of aggregate size to the presence of cations suggest that headgroup organization determines overall size in this case. Protonation of one or more macroring nitrogen atoms could lead to a hydrogen-bond network that would stabilize the aggregates and have low affinity for added cations.

Introduction

The overwhelming importance of crown ethers[1] lies in their remarkable ability to complex cations, particularly those in the alkali and alkaline earth metal families.[2] Cation binding by these macrocycles has been extensively investigated during the past 3 decades.[3] More recently, efforts have been made to adapt crown ethers as subunits to interact with cations in structures designed to function as cation channels.[4] Examples of this include crown-ether-derived isonitrile channels,[5] crown ethers incorporated as central "relay" units,[6] and crowns incorporated along a helical peptide chain.[7] Our own design for a cation-conducting channel also utilizes crown ethers, but they are positioned to function as headgroups that can stabilize the amphiphilic structure within the bilayer.[8] This design is in accord with our postulate that amino acids such as tryptophan may stabilize the position of transmembrane proteins in natural bilayers.[9]

Simple crown ethers are constructed from alternating ethylene units and heteroatoms, normally ether oxygens. In this respect, they are similar to poly(ethylene glycol)s which possess extended $(CH_2CH_2O)_n$ chains.[10] The poly-(ethylene glycol)s, or PEGs, are well known as surface active agents.[11] It is well established that PEGs can form neutral liposomes,[12] called niosomes.[13] It seemed obvious that crown ethers should serve as headgroups for neutral amphiphilic molecules. Indeed, it was established by work of Okahara and Kuwamura that crown-ether-based amphiphiles could form aggregates, but these were invariably of the micelle type.[14]

* Corresponding author. Tel: 314/362-9297. Fax: 314/362-9298 or 7058. E-mail: ggokel@pharmdec.wustl.edu.

⊗ Abstract published in *Advance ACS Abstracts,* September 15, 1997.

(1) (a) Gokel, G. W. *Crown Ethers and Cryptands*; Royal Society of Chemistry: Letchworth, U.K., 1991. (b) Gokel, G. W. Crown Ethers and Cryptands. In *Large Ring Molecules*; Semlyen, J. A., Ed.; John Wiley: New York, 1996; pp 263−307.

(2) Inoue, Y.; Gokel, G. W. *Cation Binding by Macrocycles*; Marcel Dekker, Inc.: New York, 1990.

(3) Gokel, G. W., Ed. *Comprehensive Supramolecular Chemistry*; Elsevier Science: Oxford, 1996; Vol. 1.

(4) Gokel, G. W.; Murillo, O. *Acc. Chem. Res.* **1996**, *29*, 425−432.

(5) (a) Neevel, J. G.; Nolte, R. *Tetrahedron Lett.* **1984**, *25* (21), 2263−2266. (b) Nolte, R. J. M.; Beijnen, A. J. M.; Neevel, J. G.; Zwikker, J. W.; Verkley, A. J.; Drenth, W. *Isr. J. Chem.* **1984**, *24*, 297−301. (c) Kragten, U. F.; Roks, M. F. M.; Nolte, R. J. M. *J. Chem. Soc., Chem. Commun.* **1985**, 1275−1276. (d) Roks, M. F. M.; Nolte, R. J. M. *Macromolecules* **1992**, *25*, 5398.

(6) (a) Jullien, L.; Lehn, J. *Tetrahedron Lett.* **1988**, *29*, 3803−3806. (b) Jullien, L.; Lehn, J. M. *J. Inclusion Phenom.* **1992**, *12*, 55−74. (c) Carmichel, V. E.; Dutton, P.; Fyles, T.; James, T.; Swan, J.; Zojaji, M. *J. Am. Chem. Soc.* **1989**, *111*, 767−769. (d) Fyles, T.; James, T.; Kaye, K. *Can. J. Chem.* **1990,** *68*, 976−978. (e) Fyles, T.; Kaye, K.; James, T.; Smiley, D. *Tetrahedron Lett.* **1990**, 1233. (f) Kaye, K.; Fyles, T. *J. Am. Chem. Soc.* **1993**, *115*, 12315−12321. (g) Fyles, T.; James, T.; Pryhitka, A.; Zojaji, M. *J. Org. Chem.* **1993**, *58*, 7456−7468.

(7) Voyer, N.; Robitaille, M. *J. Am. Chem. Soc.* **1995**, *117*, 6599−6600.

(8) (a) Nakano, A.; Xie, Q.; Mallen, J.; Echegoyen, L.; Gokel, G. W. *J. Am. Chem. Soc.* **1990**, *112*, 1287. (b) Murillo, O.; Watanabe, S.; Nakano, A.; Gokel, G. W. *J. Am. Chem. Soc.* **1995**, *117*, 7665−7679.

(9) Abel, E.; Fedders, M. F.; Gokel, G. W. *J. Am. Chem. Soc.* **1995**, *117*, 1265−1270. See also: Schiffer, M.; Chang, C.-H.; Stevens, F. J. *Protein Eng.* **1992**, *5*, 213−214.

(10) (a) Simon, W.; Morf, W. E.; Meier, P. C. *Struct. Bonding* **1973**, *16*, 113. (b) Vögtle, G.; Weber, E. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 753.

(11) Schick, M. J. *Nonionic Surfactants*; Marcel Dekker: New York, 1967.

(12) Lasic, D. D.; Papahadjopoulos, D. *Science* **1995**, *267*, 1275.

(13) (a) Ribier, A.; Handjani-Vila, R. M.; Bardez, E.; Valeur, B. *Colloids Surf.* **1984**, *10*, 155. (b) Azmin, M. N.; Florence, A. T.; Handjani-Vila, R. M.; Stuart, J. F. B.; Vanlerberghe, G.; Whittaker, J. S. *J. Pharm. Pharmacol.* **1985**, *37*, 237. (c) Baillie, A. J.; Florence, A. T.; Hume, L. R.; Muirhead, G. T.; Rogerson, A. *J. Pharm. Pharmacol.* **1985**, *37*, 863.

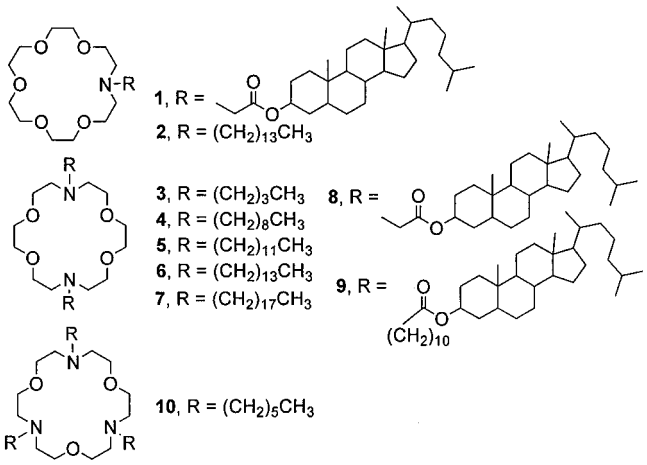
(14) (a) Kuwamura, T.; Kawachi, T. *Yukagaku* **1979**, *28*, 195; *Chem. Abstr.* 90:206248d. (b) Kuwamura, T.; Yoshida, S. *Nippon Kagaku Kaishi* **1980**, 427; *Chem. Abstr.* 93:28168e. (c) Okahara, M.; Kuo, P. L.; Yamamura, S.; Ikeda, I. *J. Chem. Soc., Chem. Commun.* **1980**, 586. (d) Kuo, P.; Tsuchiya, K.; Ikeda, I.; Okahara, M. *J. Colloid Interface Sci.* **1983**, *92*, 463.

S0022-3263(97)00929-8 CCC: $14.00

Our first effort to develop vesicle-forming crown amphiphiles used cholesterol as a side arm since sterols are known to stabilize membranes *in vivo*.[15] As anticipated, 15-membered ring lariat ethers having either cholestanyl or cholesteryl side chains formed, upon mild sonication in water, neutral vesicles that had average diameters of 300−400 Å.[16] Similar treatment of the 18-membered ring lariat ether having a cholesteryl side arm gave small aggregates (∼150 Å). In this size range, the aggregates may correspond either to vesicles or to micelles. In the presence of KCl, aggregates were observed by laser light scattering to have sizes ranging from ∼230 (1 equiv of KCl) to ∼330 Å (20 equiv of KCl) which we presume are vesicles.[17] The twin goals of the present project were to assess the ability of azacrown ethers generally to function as amphiphile headgroups and to determine how, if at all, the aggregation state was influenced by the presence of alkali metal cations such as those transported by natural channels.

Results

Compounds Studied. Ten compounds, including three previously unreported structures, were evaluated as part of the present effort. These include two aza-18-crown-6 derivatives (**1**, **2**), seven diaza-18-crown-6 derivatives (**3**−**9**), and *N*,*N*′,*N*″-tri-*n*-hexyltriaza-18-crown-6 **10**. Their structures are shown below. Compounds **2**−**7** and **10** have simple alkyl side arms ranging in length from *n*-butyl to *n*-octadecyl. In addition, compounds **1**, **8**, and **9** have (cholestanyloxy)carbonyl groups attached to nitrogen by either a methylene (**1**, **8**) or a decyl (**9**) chain.



The preparations of the single-armed compounds were accomplished either by alkylation or by acylation followed by reduction. The diaza-18-crown-6 derivatives were prepared by one of three methods. These include single-step cyclization from the appropriate amine,[18] alkylation, or acylation followed by reduction.[19]

Table 1. Particle Diameters Determined by Laser Light Scattering Data for Aggregates Formed from Crown Ether Derivatives[a]

	apparent diameter of aggregates (Å)		
		cumulant distribution	
compd	unimodal	by intensity	by weight
1[b]	180 ± 60	188 ± 60(97%)	147 ± 50(100%)
		8220 ± 960(3%)	8220 ± 960(0.005%)
1 + K^+[b]	238 ± 60	221 ± 60	189 ± 50
2[d]	119[c]	129 ± 50	81 ± 40
3[d]	2350 ± 860	1050 ± 280(18%)	927 ± 230(56%)
		2860 ± 800(82%)	2920 ± 850(44%)
4	4550 ± 1300	4860 ± 1000	5020 ± 970
4[b]	3260 ± 1100	3830 ± 1500	4200 ± 1600
5	2970 ± 710	2800 ± 730	2930 ± 720
5 + K^+	2930[e]	2810 ± 730	2940 ± 720
6	2510 ± 800	2270 ± 340(79%)	2250 ± 440(61%)
		5870 ± 2100(21%)	6380 ± 2300(39%)
6 + K^+	2450 ± 790	2100 ± 400(63%)	1970 ± 480(47%)
		5310 ± 620(37%)	5320 ± 620(53%)
7	2200[c]	582 ± 220(14%)	420 ± 170(89%)
		3340 ± 1100(86%)	3590 ± 1200(11%)
7 + K^+	2140[c]	429 ± 190(14%)	257 ± 130(97%)
		3100 ± 670(86%)	3200 ± 600(3%)
8	2460 ± 710	2820 ± 1400	2960 ± 1700
8 + Ba^{2+}	2660 ± 790	2300 ± 300(89%)	2320 ± 370(70%)
		8220 ± 960(11%)	8220 ± 960(30%)
9[f]	2080 ± 770	2540 ± 1400	1790 ± 1600
9 + K^+[f]	1920 ± 660	2200 ± 430	2110 ± 550
10	2850 ± 690	2750 ± 720	2880 ± 730

[a] Aggregates were prepared by using a tip sonicator and concentrations of suspensions were 1 mM, except where otherwise specified. When a salt was added, the molar ratio of the salt to the amphiphile was 10:1. [b] A bath sonicator was used to prepare aggregates at 45 °C. [c] Standard deviation was broad. [d] The suspension concentration was 15 mM. At lower concentrations, no aggregation could be detected by dynamic light scattering. [e] The standard deviation was narrow. [f] The suspension concentration was 0.5 mM.

Aggregate Preparation. Aggregates were prepared by the lipid hydration method.[20] In this technique, the amphiphile (10^{-5} mol) was dissolved in CH_2Cl_2 (∼2 mL) and then dried under vacuum to form a thin layer on the bottom of the test tube. After 10 mL of deionized water was added (to reach a concentration of 1 mM), the suspension was sonicated to form aggregates by using either a bath or tip sonicator. Most of the data shown in Table 1 were obtained by using a tip sonicator. The aggregate suspensions were analyzed using a dynamic laser light scattering instrument (Coulter N4MD). The data obtained are recorded in Table 1.

Discussion

Formation of Aggregates. The first observation of importance is that aggregates form from a variety of substituted diaza-18-crown-6 derivatives and the triaza-18-crown-6 derivative. The aggregation behavior of these systems has not previously been established. Furthermore, in the case of compounds **3** and **10**, stable aggregates form from amphiphiles having remarkably short tails (*n*-butyl, *n*-hexyl). The stability of aggregates formed from **3** and **10** is somewhat less than those formed from the longer-chained monomers studied here, but

(15) (a) Papahadjopoulos, D. *Annu. Rev. Biophys. Bioeng.* **1980**, *9*, 467. (b) Bloch, K. Cholesterol: Evolution of Structure and Function. In *Biochemistry of Lipids and Membranes*; Vance, D. E., Vance, J. E., Eds.; Benjamin/Cummings Publishing Co., Inc.: Reading, MA, **1985**; p 1. (c) Finegold, L., Ed. *Cholesterol in Membrane Models*; CRC Press: Boca Raton, FL, 1993.

(16) Echegoyen, L. E.; Hernandez, J. C.; Kaifer, A.; Gokel, G. W.; Echegoyen, L. *J. Chem. Soc., Chem. Commun.* **1988**, 836−837.

(17) Echegoyen, L. E.; Portugal, L.; Miller, S. R.; Hernandez, J. C.; Echegoyen, L.; Gokel, G. W. *Tetrahedron Lett.* **1988**, *29*, 4065−4068.

(18) Gatto, V. J.; Gokel, G. W. *J. Am. Chem. Soc.* **1984**, *106*, 8240−8244.

(19) Gatto, V. J.; Arnold, K. A.; Viscariello, A. M.; Miller, S. R.; Morgan, C. R.; Gokel, G. W. *J. Org. Chem.* **1986**, *51*, 5373−5384.

(20) Saunders, L.; Perrin, J.; Gammock, D. B. *J. Pharm. Pharmacol.* **1962**, *14*, 567.

aggregates formed from **10**, for example, remained intact for several days.

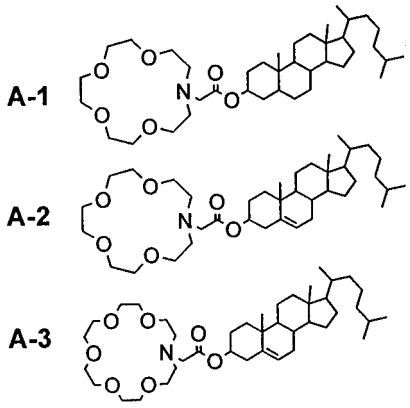
Whether or not aggregates form from each of these monomers has been determined by three different methods. The most common experimental method is laser light scattering. Each suspension was analyzed, and the data were reduced using internal instrument software. The values reported are for average diameters of particles that are assumed by the program to be spherical. Calculations are done for a simple unimodal distribution, and the cumulant distribution is calculated by intensity and weight. Representative data are recorded in the first row of Table 1 for compound **1**. The unimodal distribution is calculated to be 180 ± 60 Å. This 33% distribution range is typical of the data obtained for these compounds. The second-order calculations give (intensity) 188 ± 60 Å and (weight) 147 ± 50 Å. These values are within experimental error of each other. The data found on the second line for **1** suggest the presence of much larger particles having sizes of >8000 Å, which are present only to the extent of 0.005−3%. The exact chemical composition of such particles is unknown, but very large aggregates are possible as is the presence of unfiltered dust.

Particle size was also assessed by use of electron microscopy. The electron-microscopic technique used here involves the so-called negative stain protocol. In this case, the membrane suspension is applied to Butvar/carbon-coated Cu^0 mesh grids. After removal of excess fluid, a 1% uranyl acetate stain solution is applied. Removal of the stain and air-drying gives a sample that may be observed by electron microscopy and photographed. This technique gave the photomicrographs presented here (see below).

Third, dye entrapment was used to demonstrate that the aggregates constituted closed-shell structures. Dynamic micelles and unstructured "clumps" should not entrap an organic dye. In the present case, methylene blue was used because it had previously been applied in the study of a steroidal aza-15-crown-5 derivative.[16] Thus, an aqueous suspension (pH = 6.3) of ditetradecyldiaza-18-crown-6 **6** was sonicated in the presence of methylene blue (λ_{max} = 665 nm). The solution was dialyzed to remove dye present in the bulk medium. The vesicles were then lysed, and the amount of dye present was evaluated quantitatively by using visible spectroscopy. Three repetitions gave an average dye entrapment of ∼13 ± 4%. This compares to a value of ∼4% previously obtained for the single-side-armed, steroidal aza-15-crown-5 derivatives.[16] All of the aggregate suspensions were studied by laser light scattering. Electron microscopy and dye entrapment were conducted on selected amphiphiles, and in all cases, the results comport with the aggregation states suggested by dynamic turbidimetry.

Characteristics of Aggregates Formed. The compounds studied may be grouped in various ways to answer different questions. First, all of the compounds may be collected to compare 18-membered ring derivatives having one (**1**, **2**), two (**3**−**9**), or three (**10**) side arms. Second, we may group for comparison the one-armed (**1**) and two-armed (**8**, **9**) steroidal derivatives. The latter two compounds also differ by the length of the extender chain between the macrocyclic ring and the steroid. Finally, we may compare compounds that have either two (**4**) or three (**10**) tails but which possess the same number of carbon atoms. Additionally, for each of these comparisons, the question of cation influence on the aggregation behavior can be posed.

Steroid-Side-Armed Lariat Ethers Having 15- and 18-Membered Rings. Three close relatives of compound **1** have previously been prepared.[21] They are shown below as **A-1**−**A-3** (for analogs 1−3). Compound **A-1** is the 15-membered ring analog of **1**. Structure **A-2** is identical except that a double bond is present at $\Delta^{5,6}$ of the B-ring, *i.e.*, **A-2** is a cholesterol, rather than dihydrocholesterol, derivative. The final compound in this group of three is the unsaturated (cholesteryl) analog of **1** (**A-3**).



In previous work, we found that compounds **A-1** and **A-2** formed vesicles of about 300−350 Å diameter whether size was assessed by laser light scattering or electron microscopy.[9] The presence or absence of the steroid double bond made little difference in the type or size of aggregates formed by sonication. In this case, incremental addition of up to 25 equiv of NaCl or up to 50 equiv of KCl appeared to slightly increase the average vesicle diameter, but the values were not beyond the experimental boundaries previously observed.

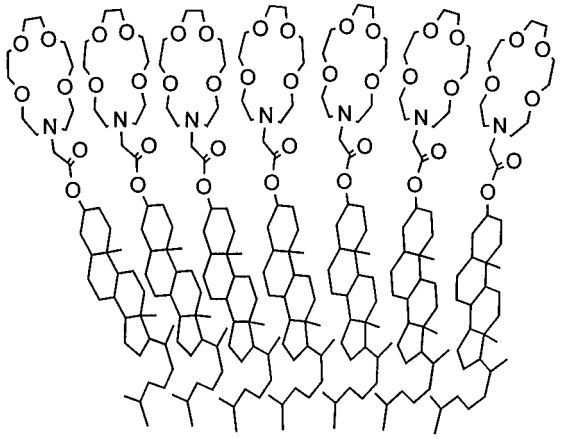
The 18-membered ring counterpart (**1**) of **A-1** formed smaller aggregates (180 *vs* 340 Å) than its 15-membered relative, but addition of KCl had as little effect as previously reported for **A-1**.[16] The analog of **1** (**A-3**) which has an 18-membered ring but is unsaturated in the steroid's B-ring behaves rather differently from the saturated counterpart. Aggregates formed from **A-3** were found to have average diameters (determined by laser light scattering) of 155 ± 40 Å. Addition of 1, 5, 10, and 20 equiv of KCl increased the average size to ∼360 Å.[17] Enlargement of the aggregates is surprising since certain poly(ethylene glycol)-based vesicles undergo osmotic shrinkage under similar circumstances.[13b]

Aggregation of Single-Hydrocarbon-Armed Lariat Ethers. Okahara, Kuwamura, and their co-workers[14] showed early on that crown ether compounds having single-alkyl chains formed micelles. In the present study, sonication of *N*-tetradecylaza-18-crown-6 (**2**) afforded aggregates having an average diameter of 80−130 Å determined by laser light scattering. It seems likely that these small aggregates are micellar.

It is interesting to consider why the 15- and 18-membered ring steroidal compounds appear to form vesicles, albeit relatively small ones, when other known single-chained lariat ethers form micelles. The answer

(21) Gokel, G. W.; Hernandez, J. C.; Viscariello, A. M.; Arnold, K. A.; Campana, C. F.; Echegoyen, L.; Fronczek, F. R.; Gandour, R. D.; Morgan, C. R.; Trafton, J. E.; Minganti, C.; Eiband, D.; Schultz, R. A.; Tamminen, M. *J. Org. Chem.* **1987**, *52*, 2963−2968.

Chart 1. Schematic Representation of Steroid-Induced Organization of A-1 (Partial Structure)



must lie in the organizing ability of the steroidal side chain. Not only are steroids capable of stacking one upon another, but cholestanol is considerably bulkier than a simple alkyl chain. The aggregation of **1** or **A-1** thus appears to be controlled by the steroid packing and not by interactions among the crown headgroups. The formation of any hydrogen-bonded network involving the headgroups in these structures would enhance stability and make access to and complexation of alkali metal cations poor. If the side chains are the dominant organizational force, then cation complexation could occur more readily and repulsion between cation-complexed headgroups might slightly enlarge the vesicles. Indeed, among the 10 compounds reported here, **1** is the only compound whose aggregates alter on addition of cations, and enlargement rather than contraction is apparent. The organizational arrangement suggested by CPK molecular models is shown schematically in Chart 1.

Aggregation of Two-Armed Diaza-18-crown-6 Derivatives. In the series of compounds **3**−**7**, there exists the opportunity to determine how systematic lengthening of two identical side arms affects aggregate size. Using the unimodal data analysis of laser light scattering, we note that the aggregate sizes are **3**, 2350 Å; **4**, 4550 Å; **5**, 2970 Å; **6**, 2510 Å; and **7**, 2200 Å. As can be seen in Table 1, the data ranges are 30−40%. Thus the calculated diameter of $\mathbf{4}_n$ vesicles appears larger than the others, but all of the size values lie within the experimental range. The difference between aggregate sizes for **2** and **6**, which have side arms of identical length, is quite striking. Two conclusions can be drawn from these observations. First, it is headgroup size and interaction, rather than side chain ordering, that is determining vesicle size in the case of **6**. Second, since **2** and **6** have the same ring size and side arm length, the headgroup orientation in aggregates of **2** must be much different from those in **6**, reinforcing the conclusion expressed above that the aggregates that form from **2** are micellar.

Sonication of aqueous suspensions of **5**−**7** containing 10 equiv of KCl gave aggregates that were similar in size to each other and to the sizes of aggregates observed in the absence of salt. Our choice of K^+ rather than Na^+ for study in this case was based upon both binding and steric considerations. Unlike the all-oxygen crowns,[22] diaza-15-crown-5 binds Na^+ more strongly in polar solvents than it complexes K^+. On the other hand, the larger diaza-18-crown-6 derivatives sterically fit K^+ and bind it more strongly than Na^+. Thus, representative Na^+ and K^+ binding constants ($\log_{10} K_S$ in methanol at 25 °C) are as follows: **3**, 2.84 (Na^+), 3.82 (K^+); **4**, 2.95 (Na^+), 3.70 (K^+); and **5**, 2.99 (Na^+), 3.80 (K^+).[23]

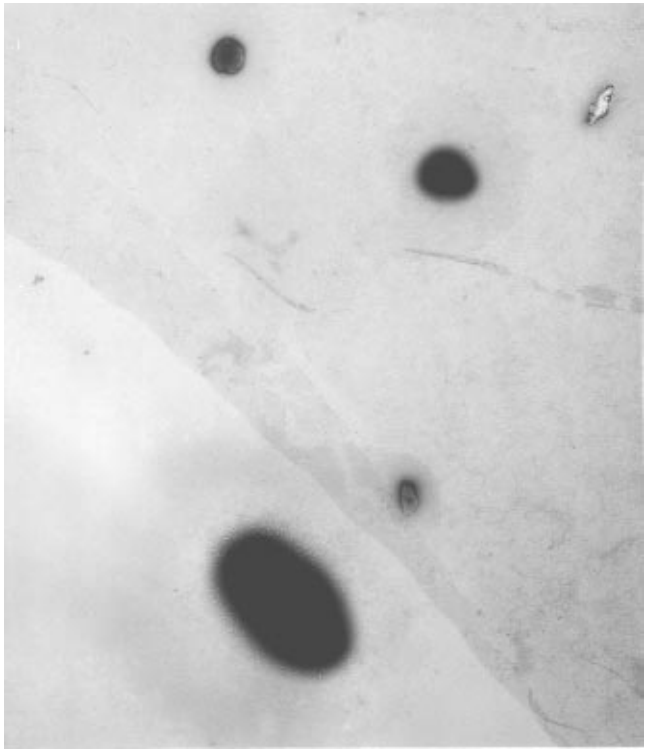


Figure 1. Negative stain electron micrograph of vesicles formed from **3**, magnification ×25000 (reproduced at 45% of original).

Aggregates formed from **6** were further evaluated by use of negative stain electron microscopy. It is apparent from the dark, nearly circular structures that well-defined aggregates have formed from *N*,*N*′-ditetradecyl-4,13-diaza-18-crown-6, **6** (see Figure 1). The dark outline apparent in the upper left structure is presumably the boundary bilayer. Some size variation is obvious in this figure but this is normal. The particle in the lower center of the photograph is clearly larger than the ∼2500 Å size found by light scattering for the majority of the aggregates. Note, however, that light scattering found 20−40% of significantly larger particles (∼6000 Å) as well. Thus the two methods nicely complement each other and provide similar information about aggregation.

Analysis by laser light scattering of aggregates formed from **6** in the presence of 10 equiv of KCl suggests that similarly sized particles are present although the fraction of larger aggregates has increased. The negative stain electromicrograph shown as Figure 2 confirms that size distribution and shows that the particles remain essentially spherical in the presence of a large excess of salt. In this case, the boundary bilayers are generally apparent.

For *N*,*N*′-di-*n*-octadecyl-4,13-diaza-18-crown-6, **7**, a distribution of particle sizes is observed in the absence and presence of KCl. When no salt is present, the unimodal analysis gives an average particle size of about

(22) Gokel, G. W.; Goli, D. M.; Minganti, C.; Echegoyen, L. *J. Am. Chem. Soc.* **1983**, *105*, 6786.

(23) Arnold, K. A.; Hernandez, J. C.; Li, C.; Mallen, J. V.; Nakano, A.; Schall, O. F.; Trafton, J. E.; Tsesarskaja, M.; White, B. D.; Gokel, G. W. *Supramol. Chem.* **1995**, *5*, 45.

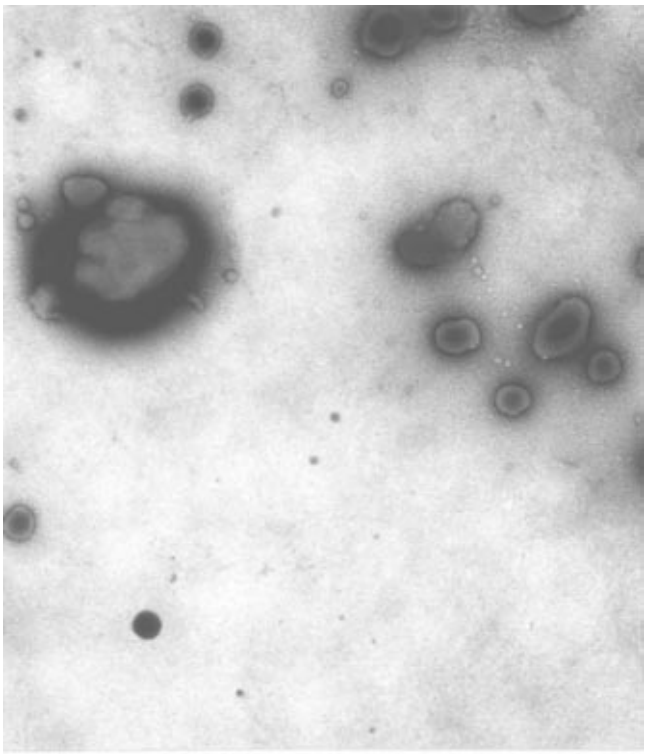
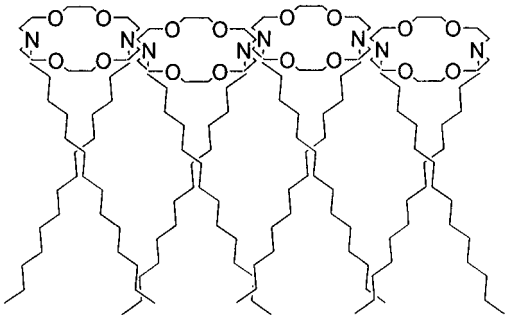


Figure 2. Negative stain electron micrograph of vesicles formed from **3** in the presence of 10 equiv of KCl, magnification ×75000 (reproduced at 45% of original).

Chart 2. Schematic Representation of Headgroup-Induced Organization of 8 (Partial Structure)



2200 Å. The apparent size diminishes only slightly (within experimental error) to 2140 Å when 10 equiv of KCl are present during sonication. The cumulant distributions suggest a somewhat more varied situation, however. Although the average particle sizes for aggregates of **6** and **7** are about the same, the cumulant distribution suggests that some of the particles are in the 400−600 Å range and a significant fraction are larger (3300−3600 Å). When 10 equiv of KCl are added in this case, some aggregate shrinkage is observed but the percentage of particle distributions remains constant. Despite some differences, the trends are similar in the two cases.

A consideration of (i) the remarkable uniformity of aggregates formed from compounds **3**−**7**, (ii) the steric requirements of the identical headgroups, and (iii) the indifference of aggregate size to chain length suggests that the headgroups rather than the side chains are the primary determinant of aggregate size. The macrocycles may align as shown in Chart 2. This arrangement was suggested by an examination of CPK models (see below), permits good contact among hydrocarbon chains, and maximizes the possibility of bridging between protonated nitrogens and oxygen or nonprotonated acceptors. If a significant number of nitrogen atoms are protonated (see below), this should enhance overall vesicle stability, organize the headgroups irrespective of chain length, and impede complexation of the macrorings to alkali metal cations.

Compounds **1** and **8** have identical side chains and identical ring sizes. Of course, two side chains rather than one are present in **8**. The dramatic difference in aggregate size (∼200 *vs* ∼2500 Å) can be accounted for as above by organization due to the two-nitrogen ring of the latter rather than the side chain of the former. In order to test this postulate, we prepared **9** which is identical to **8** except that the steroidal side chain is present at the end of a decyl, rather than a methylene, chain. In the 15-membered ring case, the presence of the steroid had an important effect on aggregation: generally vesicles rather than micelles were formed. In the absence of any cation, **8** and **9** form aggregates that are identical in size despite the fact that the steroids are very differently positioned with respect to the headgroups.

Since **1** showed a small apparent change in aggregate size when excess KCl was added, its analog (**8**) was studied in the presence of similarly sized but much more charge-dense $BaCl_2$. Little alteration in aggregate size could be detected. Likewise, longer-chained steroid **9** formed aggregates of similar size in the presence or absence of excess KCl.

A final test of the "ring-defined" aggregate concept was undertaken with the preparation of triaza-18-crown-6 derivative **10**. As noted above, this is the first aggregation behavior study of any triaza-18-crown-6 derivative. If the macrocyclic rings lie approximately perpendicular to the bilayer plane as illustrated in Chart 2 for the diazacrowns, aggregate size should also be similar to that observed for the diazacrowns. A comparison of **10** with **3**, which has approximately equal chain length, or with **4**, which has the same number of non-hydrogen atoms, reveals that all three compounds form aggregates of similar size.

In this connection, it is remarkable that **3** forms aggregates at all. Note that (excepting **2**) they form the least stable aggregates that were observed in this series (see below). The alkyl chain length is only four carbons, and aggregation would not normally be observed for a compound of the type *HG*-butyl whether *HG* is cationic or anionic. This observation further reinforces the concept of headgroup organization in these novel macrocycles.

Theoretical Models. It is an interesting general question why certain amphiphiles form vesicles and others form micellar aggregates. This question has been posed by others,[24] and an empirical model based upon the relative sizes of headgroup and side chains has been developed by Israelachvilli,[25] Ninham,[26] Evans,[27] and their co-workers. In this work, the surfactant parameter $V/(LA)$ is used to assess aggregate formation. According

(24) Israelachvilli, J. N. *Intermolecular and Surface Forces*, 2nd ed.; Academic Press: New York, 1992.
(25) Israelachvilli, J. N.; Mitchell, D. J.; Ninham, B. W. *J. Chem. Soc. Faraday Trans.* **1976**, *72*, 1525.
(26) Mitchell, D. J.; Ninham, B. W. *J. Chem. Soc. Faraday Trans. 2* **1981**, *77*, 601.
(27) Evans, D. F.; Ninham, B. W. *J. Phys. Chem.* **1983**, *87*, 5025.

Table 2. Surfactant Parameters and Associated Values for Compounds 1−10[a]

Compd	V_m (Å^3)[b]	V_c (Å^3)[b]	L_m (Å)[b]	L_c (Å)[b]	V_m (LA_m)[b,c]
1[e]	735.0		21.0		0.45
2	327.4	409.0	18.6	19.2	0.22
3	239.4	270.0	6.8	6.6	0.45
4	443.5	539.0	12.6	12.9	0.45
5	570.2	700.0	16.2	16.7	0.45
6	654.8	808.0	18.6	19.2	0.45
7	830.7	1023.2	23.6	24.3	0.45
8[d]	1470.0		21.0		0.89
9[d]	2240.0		32.0		0.89
10	464.6	566.4	8.8	9.1	0.67

[a] Formulas used for calculating V_c and L_c for the alkyl chain: $V_c = (27.4 + 26.9n)$ Å^3 and $L_c = (1.5 + 1.265n)$ Å per hydrocarbon chain, where V is the volume of the hydrocarbon chain(s), L is the fully extended length, and n is the number of carbons. [b] The superscripts "c" and "m" stand for calculated and measured (see text). [c] A_m is the headgroup area; estimated ("measured") from CPK models for 18−crown−6 is 78.5 Å^2. [d] For compounds **1** and **8**, the chain volume was obtained by considering the steroid to be a rectangular box with dimensions 50 × 70 × 210 Å.

to the model, if $0.5 < V/(AL) < 1$, vesicles are anticipated. The relevant data are in Table 2.

The second and third columns of Table 2 present values for the amphiphile's tail volumes. The subscripts "m" or "c" in the table indicate that the value shown was "measured" or "calculated." Both approaches were used in an effort to reduce ambiguity. The "measured" values were determined from CPK molecular models of the compounds in question. This was done by measuring the scale models and applying standard geometric formulas. The corresponding values for alkyl chain length (L) are shown in the fourth and fifth columns. The values shown as "calculated" we obtained by using the published[25−28] formulas: $V_c = (27.4 + 26.9n)$ Å^3 and $L_c = (1.5 + 1.265n)$ Å, where n is the number of carbon atoms. We estimate from CPK models that the headgroup area of 18-crown-6 (D_{3d} conformation) is 78.5 Å^2. It is this value of A_m that is used in the calculation of the surfactant parameter $V_m/(LA_m)$.

According to the theory, when the calculated value $V/(LA)$ falls between the limiting values 0.5 and 1.0, vesicle formation is predicted. At values between 0 and 0.5, micelle formation is anticipated. Note that for our systems, the surfactant parameter is within or near this value for all cases except **2**, which forms micelles. If one stretches the allowed lower limit of the surfactant parameter slightly from 0.45 to 0.5, then compounds **1** and **3**−**10** are all predicted from the model to form vesicles and **2** is anticipated to form micelles. This agrees with the experimental observations. It should also be emphasized that the presumed vesicles formed from **3**, in which the side chains are *n*-butyl groups, are relatively unstable, losing particle definition within about 24 h. Thus the key issue in the present case is not the formation of vesicles, which may reasonably be anticipated from known models,[28] but why the particle size seems not to be sensitive to side chain length.

Partial Protonation of Headgroup Nitrogen. If the headgroup network is organized primarily by hydrogen-bond and water bridge interactions,[29] then the chain length should not be an important determinant of vesicular size. Previous work bears on this point. In studies of our model transmembrane channel compounds, we had occasion to determine the pK_a values for certain azacrown compounds.[9b] The pK_a values for a few azacrowns have been reported in the literature: aza-18-crown-6, pK_a = 9.40;[30] and diaza-18-crown-6, pK_1 = 8.94, pK_2 = 7.81.[31] We determined the pK_a values for *N*,*N* =di-*n*-butyl-diaza-18-crown-6 (pK_1 = 9.40, pK_2 7.97) and *N*,*N* = di-*n*-benzyl-diaza-18-crown-6 (pK_1 = 7.5, pK_2 = 6.83). The exact pH experienced by the crown amphiphiles is not known, but in neutral water, a dialkyl crown will be protonated to the extent of about 10% if the pK_a values are as found for the di-*n*-butyl crown.

It has also been found that bola amphiphiles formed from two aza-15-crown-5 or two aza-18-crown-6 headgroups separated by 12-carbon spacers formed stable lipid monolayers.[32] Light scattering revealed that their aggregate sizes were 730 and 1200 Å, respectively, despite an identity in chain length. Of course, monolayer lipid membranes cannot be compared directly with the bilayers discussed here, but the structures and the aggregates they form are clearly relevant to the present work.

Examination of Molecular Models. If one accepts that the headgroups in these systems organize the amphiphiles into similarly sized aggregates, the question remains of how this is accomplished at the molecular level. Molecular models (CPK) were constructed for *N*,*N*′-di-*n*-dodecyl-4,13-diaza-18-crown-6. The macrocyclic ring was adjusted to the D_{3d} conformation as it is presumed that water is in the crown cavity. This assumption is based upon the affinity of 18-membered crown ethers for water[33] and the known solid state structure for protonated, hydrated aza-18-crown-6.[34] The dodecyl side chains were adjusted to an *all-anti* conformation. When these various conformational adjustments were made, it appeared that the dodecyl chains favored a "crossed" arrangement as shown in Chart 2 (above). When molecular models of seven copies of this amphiphile were arranged in various ways, it appeared that the chains could pack rather closely and nearly fill the void below the crowns. Moreover, interdigitation of the chains seemed to be quite effective when a monomer was inserted from the opposite direction into the model membrane.

Conclusions

In this report, we present evidence for stable aggregate formation by sonication of aqueous suspensions of dialkyl- and trialkyl-substituted diaza- and triaza-18-crown-6 derivatives. The aggregates that formed proved generally to be indifferent to the addition of either NaCl or KCl and variations in the number of side arms, the type (alkyl *vs* steroidal) of side arm, and the length of side arms attached to the macrocyclic rings. These observations lead us to speculate that in this case the

(28) It should be noted that the extent of protonation of the headgroup nitrogen atoms is unknown. In principle, protonation will affect the headgroup's effective size at least some and could substantially affect the effective charge and, in turn, the solvation shell. These issues are recognized but not dealt with in the model described.

(29) Whitesides, G. A.; Simanek, E. E.; Mathias, J. P.; Seto, C. T.; Chin, D. N.; Mammen, M.; Gordon, D. A. *Acc. Chem. Res.* **1995**, *28*, 37−44.
(30) Kataky, R.; Matthes, K.; Nicholson, P.; Parker, D.; Buschmann, H. *J. Chem. Soc. Perkin Trans. 2* **1990**, 1425.
(31) Suh, M.; Eom, T.; Kim, S. *Bull. Korean Chem. Soc.* **1983**, *4*, 231.
(32) Muñoz, S.; Mallén, J.; Nakano, A.; Chen, Z.; Gay, I.; Echegoyen, L.; Gokel, G. W. *J. Am. Chem. Soc.* **1993**, *115*, 1705.
(33) (a) Goldberg, A. *Acta Crystallogr.* ***1978***, *B34*, 2538. (b) Helgeson, R. C.; Tarnowski, T. L.; Cram, D. J. *J. Org. Chem.* **1979**, *44*, 2538.
(34) Gokel, G. W.; Garcia, B. J. *Tetrahedron Lett.* **1977**, 317.

amphiphile headgroup is the critical determinant of aggregate size and structure. Single-armed lariat ether amphiphiles form small aggregates that adopt micellar or vesicular structures depending upon whether the alkyl chain is polymethylenic or steroidal. It is conjectured that the major interaction in this case is between the tails. The expected strong interaction of the steroids causes formation of (small) vesicles, whereas alkyl tails of similar overall length afford micelles. When two or three alkyl chains are present, the headgroups appear to dominate aggregate organization, probably by forming a hydrogen-bond network that controls vesicle size. Reasonably stable aggregate formation was observed from amphiphiles having remarkably short side chains, a novel finding and another indication that the headgroup may be the prime determinant of aggregate type from azacrown amphiphiles.

Experimental Section

^{1}H-NMR spectra were recorded at 300, 500, or 600 MHz in $CDCl_3$ solvents and are reported in ppm (δ) downfield from internal $(CH_3)_4Si$. ^{13}C-NMR spectra were recorded at proportional frequencies as noted above. Infrared spectra were recorded on a Perkin-Elmer 1310 infrared spectrophotometer and were calibrated against the 1601 cm^{-1} band of polystyrene. Melting points were determined on a Thomas Hoover apparatus in open capillaries and are uncorrected. Thin layer chromatographic (TLC) analyses were performed on aluminum oxide 60 F-254 neutral (type E) with a 0.2 mm layer thickness or on silica gel 60 F-254 with a 0.2 mm layer thickness. Preparative chromatography columns were packed with activated aluminum oxide (MCB 80−325 mesh, chromatographic grade, AX 611) or with Kieselgel 60 (70−230 mesh). Chromatotron chromatography was performed on a Harrison Research Model 7924 chromatotron with 2 mm thick circular plates prepared from Kieselgel 60 PF-254.

All reactions were conducted under dry N_2 unless otherwise stated. All reagents were the best (non-LC) grade commercially available and were distilled, recrystallized, or used without further purification, as appropriate. Molecular distillation temperatures refer to the oven temperature of a Kugelrohr apparatus. Combustion analyses were performed by Atlantic Microlab, Inc., Atlanta, GA, and are reported as percents.

Dynamic light scattering was performed on a Coulter Model N4MD submicron particle analyzer. Sonication was performed using either a Branson 2210 bath sonicator or a Branson Sonifier 450 tip sonicator.

Preparation of Steroidal Side Arm Precursors. To a solution of chloroacetyl chloride (8.72 g, 77.2 mmol) and 4-(*N*,*N*-dimethylamino)pyridine (50 mg, 0.4 mmol) in benzene (480 mL) was added a solution of dihydrocholesterol (25 g, 64.3 mmol) and Et_3N (6.85 g, 67.7 mmol). The temperature was maintained at 0−5 °C during the addition. The reaction mixture was then stirred at ambient temperature for 24 h. The mixture was filtered, and the filtrate was concentrated *in vacuo*. The crude product was crystallized from EtOH to give 2-(chloroacetyl)dihydrocholesterol ester (23.3 g, 78%) as a white crystalline solid (mp 184−185 °C). ^{1}H-NMR: 0.621−1.979 (multiple peaks, 46H, steroid), 4.015 (s, 2H, Cl-CH_2-COO), 4.784 (m, 1H, COO-C*H*-R_2).

Dihydrocholesteryl 11-Bromoundecanoate. The procedure described above for 2-(chloroacetyl)dihydrocholesterol ester was followed to afford the desired compound in 50% yield as white crystals (mp 66−67 °C) after recrystallization from absolute ethanol. ^{1}H-NMR (R-600): 0.47−2.08 (m, 62H, steroid, $BrCH_2(CH_2)_8CH_2$), 2.17 (t, 2H, CH_2CO), 3.31 (t, 2H, $BrCH_2$) 4.52 (m, 1H, C_3-steroidal). IR (KBr): 2940 (s), 2860 (s), 1740 (m) cm^{-1}.

***N*-[[(3-Cholestanyloxy)carbonyl]methyl]-aza-18-crown-6, 1.** A solution of 2-(chloroacetyl)dihydrocholesterol ester (see above; 698 mg, 1.5 mmol), aza-18-crown-6 (421 mg, 1.6 mmol), and K_2CO_3 (210 mg, 1.5 mmol) was refluxed for 6 h. The reaction mixture was cooled and filtered, and the filtrate was concentrated *in vacuo*. The filtrate was then chromatographed over Al_2O_3 (10% 2-propanol/hexane). The first fraction, following recrystallization, gave *N*-[[(3-cholestanyloxy)carbonyl]-methyl]aza-18-crown-6 (750 mg, 72%) as a white crystalline solid (mp 57−59 °C). ^{1}H-NMR: 0.647−1.982 (multiple peaks, 46H, steroid), 2.955 (t, 4H, O-CH_2-CH_2-N), 3.482 (s, 2H, N-CH_2-COO), 3.651 (m, 20H, CH_2-O-CH_2 within crown), 4.720 (m, 1H, COO-C*H*-R_2). IR (CCl_4): 2985 (s), 1750 (s) cm^{-1}.

***N*-Tetradecylaza-18-crown-6, 2.** 1-Bromotetradecane (555 mg, 2 mmol), aza-18-crown-6 (553 mg, 2.1 mmol), K_2CO_3 (2 g, excess), and KI (50 mg, catalyst) were suspended in 30 mL of butyronitrile. The mixture was then set to reflux for 5 h. After the reaction mixture cooled, it was filtered, and the filtrate was concentrated. The crude product was chromatographed over Al_2O_3 (10% 2-propanol/hexanes) to afford **2** (470 mg, 51%) as a light yellow oil. ^{1}H-NMR ($CDCl_3$): 0.88 (t, 3H), 1.25 (s, 22H), 1.44 (m, 2H), 2.50 (t, 2H), 2.78 (t, 4H), 3.66 (m, 20H). Anal. Calcd for $C_{26}H_{53}NO_5$: C, 67.93; H, 11.62; N, 3.05. Found: C, 67.78; H, 11.61; N, 2.99.

***N*,*N*′-Dibutyl-4,13-diaza-18-crown-6, 3, *N*,*N*′-Dinonyl-4,13-diaza-18-crown-6, 4, *N*,*N*′-Didodecyl-4,13-diaza-18-crown-6, 5, *N*,*N*′-Ditetradecyl-4,13-diaza-18-crown-6, 6, and *N*,*N*′-Dioctadecyl -4,13-diaza-18-crown-6, 7.** Compounds **3**−**7** were prepared as described in the literature.[35]

***N*,*N*′-Bis[[(3-cholestanyloxy)carbonyl]methyl]-4,13-diaza-18-crown-6, 8.** A solution of 2-(chloroacetyl)dihydrocholesterol ester (see above; 3.37 g, 7.24 mmol), diaza-18-crown-6 (2.02 g, 7.68 mmol), Na_2CO_3 (8.66 g, 82 mmol), and KI (50 mg, 0.3 mmol) in PrCN (200 mL) was refluxed for 15 h. The reaction mixture was cooled and filtered, and the filtrate was concentrated *in vacuo*. The filtrate was then chromatographed over a short column of Al_2O_3 (3% MeOH/CH_2Cl_2). The first fraction, following recrystallization, gave the disubstituted product (1.68 g, 20%) as a white crystalline solid (mp 120−121 °C). ^{1}H-NMR: 0.645−1.970 (multiple peaks, 92H, steroid), 2.956 (t, 8H, O-CH_2-CH_2-N), 3.467 (s, 4H, N-CH_2-COO), 3.611 (m, 16H, CH_2-O-CH_2 within crown), 4.728 (m, 2H, COO-C*H*-R_2). Anal. Calcd for $C_{70}H_{122}N_2O_8$: C, 75.09; H, 10.98; N, 2.50. Found: C, 74.96; H, 10.92; N, 2.48.

***N*,*N*′-Bis[[(3-cholestanyloxy)carbonyl]decyl]-4,13-diaza-18-crown-6, 9.** The title compound was obtained as a white waxy solid (mp 66−67 °C) in 33% yield. ^{1}H-NMR: 0.64 (s, 3H, C_{18}-steroid), 0.77−1.97 (m, 118H, steroid, $NCH_2(CH_2)_8$), 2.25 (t, 4H, J = 7.5 Hz, CH_2CO), 2.47 (t, 4H, J = 7.7 Hz, $NCH_2(CH_2)_8$), 2.77 (t, 8H, J = 5.5 Hz, $(O\text{-}CCH_2)_2N$), 3.57 (m, 16H, $(OCH_2\text{-}C)_2N$), 4.69 (m, HH, C_3-steroid). IR (CCl_4): 2940 (m), 2880 (m), 1725 (br) cm^{-1}. $[\alpha]^{25}_D$ +8.6 (c = 2). Anal. Calcd for $C_{88}H_{158}N_2O_8$: C, 77.03; H, 11.61. Found: C, 76.82; H, 11.58. DCI mass spectrum: 1373 (10, M^+), 749 (20), 264 (100).

***N*,*N*′,*N*″-Tri-*n*-hexyltriaza-18-crown-6, 10.** Compound **10** was prepared as described previously.[36]

Vesicle Preparation. The amphiphile (10^{-5} mol) was placed in a 15 mL test tube and dissolved in ~2 mL of CH_2Cl_2. The solvent was evaporated by purging with dry N_2. The test tube was then evacuated (1−2 Torr) for 1 h. Deionized H_2O (10 mL) was added. For bath sonication, the suspension was sonicated for 30 min at the desired temperature. For tip sonication, the suspension was sonicated at 30 W with a tip sonicator in an ice bath for 30 min. The suspension was centrifuged for 15 min at 3200 rpm and then filtered through a 1.0 μm nucleopore polycarbonate membrane. The suspension was characterized by particle analyzer at 20 °C and a 90° angle for 200 s.

Vesicle Preparation in the Presence of Salt. The vesicle preparation was the same as above except that the crown:cation (1:10) complex, rather than the free crown, was used.

(35) Gatto, V. J.; Arnold, K. A.; Viscariello, A. M.; Miller, S. R.; Morgan, C. R.; Gokel, G. W. *J. Org. Chem.* **1986**, *51*, 5373−5384.

(36) (a) Miller, S. R.; Cleary, T. P.; Trafton, J. E.; Smeraglia, C.; Fronczek, F. R.; Gokel, G. W. *J. Chem. Soc., Chem. Commun.* **1989**, 806−808. (b) Tsesarskaja, M.; Cleary, T. P.; Miller, S. R.; Trafton, J. E.; Bott, S.; Atwood, J. L.; Gokel, G. W. *J. Inclusion Phenom.*, **1992**, *12*, 187−197.

Electron Microscopy: Negative Stain Protocol. Membrane vesicles were attached to Butvar/carbon-coated, 400 mesh copper grids by applying 10−15:l drops of the vesicle suspensions onto the grids and allowing them to remain for 1−5 min. Excess fluid was wicked off the grids by touching their edges to filter paper, and 12 μL drops of 1% uranyl acetate were applied for 15−30 s. The stain was wicked off with filter paper, and the grids were air-dried. The specimens were viewed in a Hitachi H-600 transmission electron microscope, operated at 75 kV.

Determination of Percent Dye Encapsulation. A liposome suspension of **6** was prepared as described above; however, the sample was prepared in 10.0 mL of 0.1 mM methylene blue (MB) (λ_{max} = 664.7 nm), and the final concentration of the sample was 5.0 mM (pH 6.3), instead of 1.0 mM.

A 3.0 mL aliquot of the resulting vesicle suspension was added to a dialysis membrane (Spectra/Por 6 CE membrane, molecular weight cutoff: 3500 Da). Additionally, a "control" dialysis membrane was prepared using 3.0 mL of 0.1 mM MB to correct for the dye that did not clear the membrane within 48 h. Each sample was then dialyzed for 48 h against four 4 L changes of deionized water (pH 6.3) to remove the extravesicular dye.

After completing the dialysis, particle diameter for the preparation was determined using dynamic light scattering. Results from the unimodal analysis show that the average diameter of the vesicle suspension of **6** is 2890 Å (narrow standard deviation). This result is consistent with the result shown in Table 1 for **6**.

Next, 1.75 mL aliquots of the particle suspensions pre- and postdialysis and the control were added to separate vials and then lysed by adding 16 μL of Triton X-100 followed by sonication for a short period of time. Upon lysis compound **6** precipitated, so 1.0 mL aliquots of both pre- and postdialysis solutions containing **6** were centrifuged (3200 rpm) for 1 h. The supernatant solutions were collected, and the absorbances for them and for the "control" were determined at 665 nm. All absorbance measurements were done against a blank that was prepared by dissolving 16 μL of Triton X-100 in 1.75 mL of deionized water.

The percent of dye encapsulated by the liposomes was determined by taking the ratio of the absorbance of the dialyzed solutions and the absorbance of the undialyzed solutions. The control in most cases showed minimal absorbance above the blank, but this value was subtracted from the absorbance of the dialyzed samples. By this method the vesicles prepared from **6** showed an entrapment of 0.1297 ± 0.0436 (∼13%).

Acknowledgment. We thank the NIH for a grant (GM-36262) that supported this work.

JO970929A