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Steroidal Aza-Lariat Ethers: Syntheses and Aggregation Behavior

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Ten steroid-substituted lariat ether compounds have been prepared including one- and two—armed derivatives of aza-15-crown-5 and aza- and diaza-18-crown-6. They include, *N*-(3-cholesteryloxycarbonylmethyl)-aza-15-crown-5, **1**, *N*-(3-dihydrocholesteryloxycarbonylmethyl)-aza-15-crown-5, **2**, *N*-(3-cholesteryloxycarbonylmethyl)-aza-18-crown-6, **3**, *N*-(3-dihydrocholesteryloxycarbonylmethyl)-aza-18-crown-6, **4**, *N*-(3-dihydrocholesteryloxycarbonylpropyl)-aza-15-crown-5, **5**, *N*-(3-dihydrocholesteryloxycarbonylpentyl)-aza-15-crown-5, **6**, *N*-(3-dihydrocholesteryloxycarbonylheptyl)-aza-15-crown-5, **7**, *N*-(3-dihydrocholesteryloxycarbonyldecyl)-aza-15-crown-5, **8**, *N,N'*-bis(3-cholestanyloxycarbonylmethyl)-4,13-diaza-18-crown-6, **9**, and *N,N'*-bis(3-cholestanyloxycarbonyldecyl)-4,13-diaza-18-crown-6, **10**. Aqueous suspensions of these monomers were sonicated. Laser light scattering experiments showed that stable, vesicular aggregates formed. Dye entrapment experiments confirmed the vesicular nature of these aggregates in selected cases. Likewise, the effect of added salts (selected from among NaCl, KCl, $BaCl_2$, 0–25 equivalents) on aggregate size was assessed. The results were found to agree with a theoretical model developed by Israelachvilli, Ninham, Evans and their coworkers.

INTRODUCTION

During the past several years, we[1] and others[2] have undertaken development of various model systems[3] intended to mimic the cation-conducting activity of natural protein channels.[4] One of the challenges in the design of such a system is to decide how the channels will be anchored within the headgroup. How this is accomplished in transmembrane protein channels remains unclear but one possibility is that certain amino acid sidechains near the water/lipid boundary may be able to serve as "headgroups" which stabilize the overall assembly. A candidate for such an amino acid is tryptophan which possesses an indole sidechain.[5] Indeed, synthetic amphiphilic monomers having indole as a headgroup proved to form stable vesicles when the sidechain was sufficiently hydrophobic.[6]

Our strategy for the design of a synthetic cation-channel model involves the use of crown ether headgroups. In our design, they derive from lariat ethers[7] because the latter are versatile

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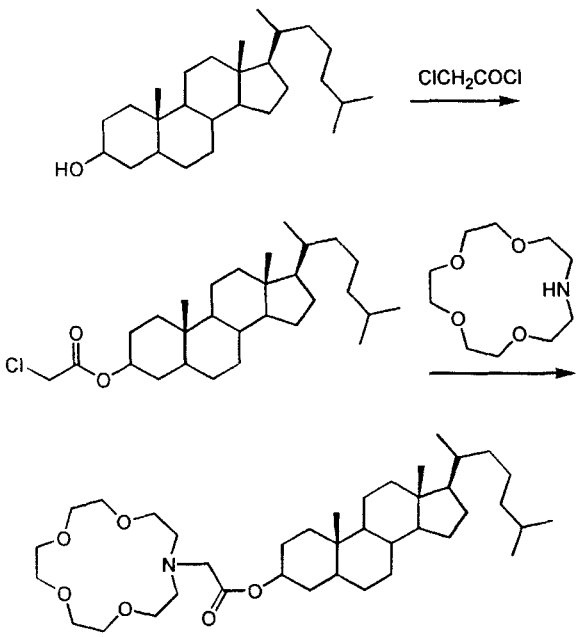
intermediates and because they provide the essential elements of polar, cation binder and flexible mechanical linkage. It was already established from the work of Okahara[8] and Kuwamura[9] that crown ether residues could serve as headgroups for the formation of organized assemblies, namely micelles. We expected to use steroidal residues as stabilizing "sidearms" in channel mimics so it was of interest to see if steroidal lariat ethers[10] would exhibit aggregation behavior the same as or different from that apparent in the hydrocarbon-tailed azacrowns studied by Okahara and Kuwamura. Such structures are also of obvious interest as monomers for liposomes of potential utility in drug delivery.[11] We have reported in preliminary studies that the steroids do, indeed, form aggregates but they are vesicular rather than micellar.[12] In the present report, we describe the syntheses of several novel steroidal lariat ethers and describe the results of aggregation studies which show that diaza-18-crown-6 derivatives behave differently from their monoaza relatives.



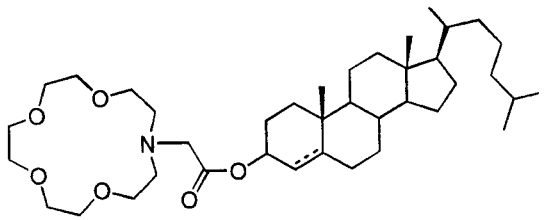
SCHEME 1

RESULTS AND DISCUSSION

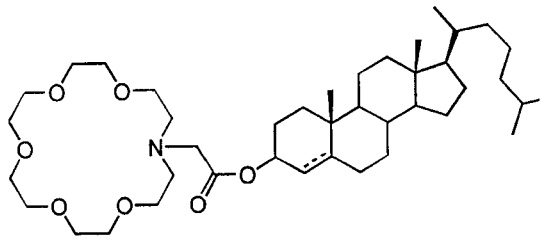
Synthesis of Steroidal Lariat Ethers

The syntheses of the steroidal lariat ethers were accomplished by a two-step procedure. For compounds of the form crown-CH_2COO-steroid, commercially available $ClCH_2COCl$ was used as the starting material. Reaction with either cholesterol or dihydrocholesterol (cholestanol) in the presence of Et_3N led rapidly to the formation of $ClCH_2COO$-steroid. The acylation is straightforward and the resulting ester is activated to alkylation. The cholestanyl chloroacetate was then treated with the appropriate azacrown in the presence of a base such as Na_2CO_3 to afford the steroidal lariat ether product. The approach is shown for the aza-15-crown-5 cholestanyl derivative in scheme 1.

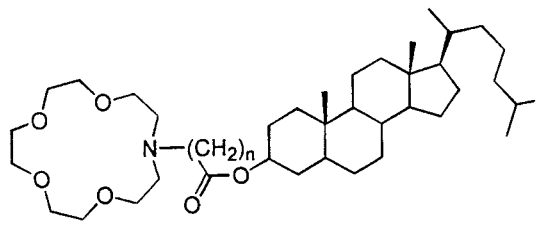
For the preparation of lariat ethers having longer linkers between the steroid and the macrocycle, commercially available ω-haloacid chlorides such as 4-chlorobutryl chloride and 6-bromohexanoyl chloride were purchased and used without further purification. When the appropriate ω-haloacyl halide was unavailable, the corresponding haloacid was treated with thionyl chloride to afford the desired acyl halide in essentially quantitative yield. In the latter case, the purity of the product was assessed by infrared spectroscopy and then used without further purification. Synthesis of the desired lariat ether was accomplished by heating the steroidal ω-halocarboxylates with the azacrown, Na_2CO_3, and a catalytic amount of KI in $CH_3CH_2CH_2CN$ for ~5 d. Purification was achieved by chromatography. The compounds formed are shown as structures **1**–**8**, below. The diaza-18-crown-6 derivatives were prepared by one of three methods. These include single-step cyclization from the appropriate amine,[13] alkylation, or alkylation followed by reduction.[14] The two-armed steroid derivatives are shown as **9** and **10**.



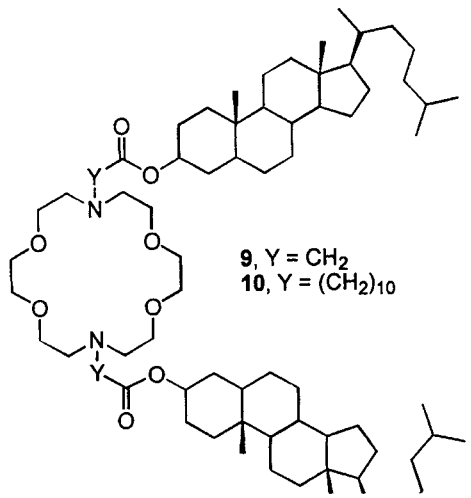
1, cholesteryl
3, dihydrocholesteryl



2, cholesteryl
4, dihydrocholesteryl



5, n = 3
6, n = 5
7, n = 7
8, n = 10



9, Y = CH_2
10, Y = $(CH_2)_{10}$

Aggregate Preparation

Aggregates were prepared by the lipid hydration method.[15] Thus, the amphiphile (10^{-5} mol) was dissolved in CH_2Cl_2 (~2 mL) and then dried *in vacuo* to form a thin layer on the bottom of the test tube. After adding 10 mL of deionized water (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 I 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 below in Table I.

Aggregate Formation

The formation of vesicles was confirmed by using both laser light scattering and electron microscopy. The values reported in the present work are for average diameters of particles that are presumed by the light scattering instrument's software to be spherical. The standard deviations of 25–40% are common for such studies.

The aza-15-crown-5 lariats in which the steroid is attached to the macrocycle by a glycinyl residue give aggregates of a similar size irrespective of whether the tail contains cholesterol (**1**) or cholestanol (**3**). As the spacer chain is extended from one carbon to three (acetyl to butyryl, **3** → **5**) larger aggregates (~1300 Å) form although about a quarter of the particles have a mean size near that observed for **3**. The remaining 15-membered ring derivatives also form aggregates in the 1000–1500 Å range as well. There is an increase in the average aggregate size (2000–2500 Å) when the cholestanyl residues are attached to 18-membered, diaza-18-crown-6 rings, whether the spacers are short (**9**) or long (**10**).

Addition of 0–25 equivalents of either NaCl or KCl to suspensions containing aggregates formed from **3** was assessed by laser light scattering. In all cases, the aggregate size was observed to fall in the range 350–410 Å. These values are all within experimental error of the size observed in the absence of any salt. It is interest-

TABLE I Particle diameters determined by laser light scattering data for aggregates formed from crown ether derivatives[a]

Cpd. No.	Ring size	Side arm[b]	Aggregate size	Salt added	Equiv	Aggregate size
1	15	CH_2CO	350 ± 110 Å[d] (98%)	—	0	—
2	18	CH_2CO	155 ± 40 Å[d]	KCl	1	230 ± 70 Å
					5	240 ± 90 Å
					10	360 ± 90 Å
					20	330 ± 130 Å
3	15	CH_2CO	340 ± 130 Å[d] (97%)	NaCl	0–25	all cases: 350–410 Å
				KCl	0–50	
4	18	CH_2CO	ND	—	0	—
5	15	$(CH_2)_3CO$	298 ± 70 Å (22%) 1290 ± 460 Å (78%)	—	0	—
6	15	$(CH_2)_5CO$	1150 ± 480 Å (95%)	—	0	—
7	15	$(CH_2)_7CO$	1030 ± 390 Å (97%)	—	0	—
8	15	$(CH_2)_{10}CO$	1500 ± 570 Å (82%)	NaCl	10	2450 ± 830 (88%) 560 ± 80 Å (12%)
			340 ± 120 Å (18%)	KCl	10	1480 ± 600 Å
9	18	CH_2CO	2460 ± 710 Å	$BaCl_2$	10	2660 ± 790 Å
10	18	$(CH_2)_{10}CO$	2080 ± 770 Å	KCl	10	1920 ± 660 Å

[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]Sidearm between macrocycle and steroid, attached at the dihydrocholesterol 3-position.
[c]The steroid was cholesterol rather than cholestanol.
[d]A bath sonicator was used to prepare aggregates at 45°C.

ing to note that osmotic shrinkage has been observed in the polyethylene glycol-based cases.[16] The single-steroid structures do not exhibit this behavior even though they form neutral liposomes (niosomes) as well. When the chain linking the steroid to the macrocycle was increased in length to 11 carbons (compound **8**), larger vesicles (~1500 Å) were formed but addition of NaCl or KCl failed to alter vesicular size beyond the experimental margin (approximately ±30%).

A similar resistance to osmotic shrinkage was observed in the cases of two-armed steroidal BiBLEs, **9** and **10**. In the absence of any added salt, the vesicular diameters were approximately 2000 Å and 2500 Å respectively. Addition of $BaCl_2$ to niosomes formed from **9** or KCl to niosomes formed from **10** gave no variation (beyond the experimental boundaries) in aggregate size that could be detected by use of dynamic turbidimetry.

Particle size was also assessed by use of electron microscopy. The so-called negative stain protocol was used in the present studies. In this case, the membrane suspension is applied to Butvar/carbon-coated Cu° 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. The photomicrographs shown were all obtained by using the negative stain technique. Photomicrographs are presented below for compound **9** after sonication (Figure 1) and in the presence of $BaCl_2$ (Figure 2). In both cases, the sizes ob-

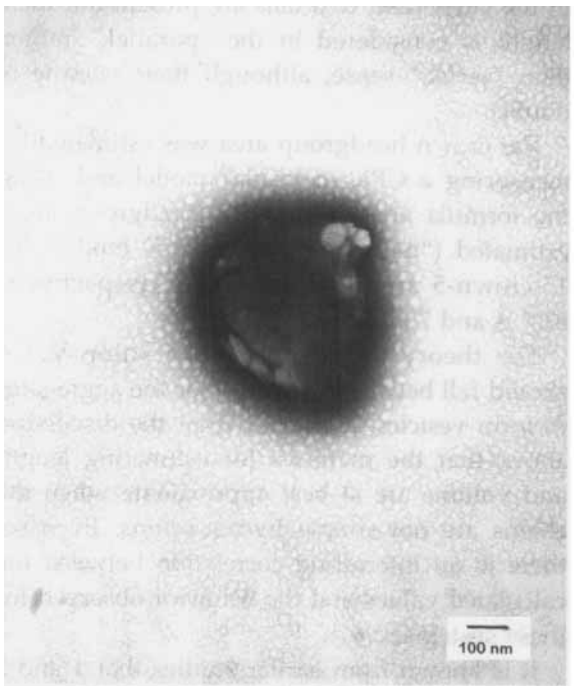


FIGURE 1 Electron micrograph of **9** in the absence of added salt, magnification ×125,000.

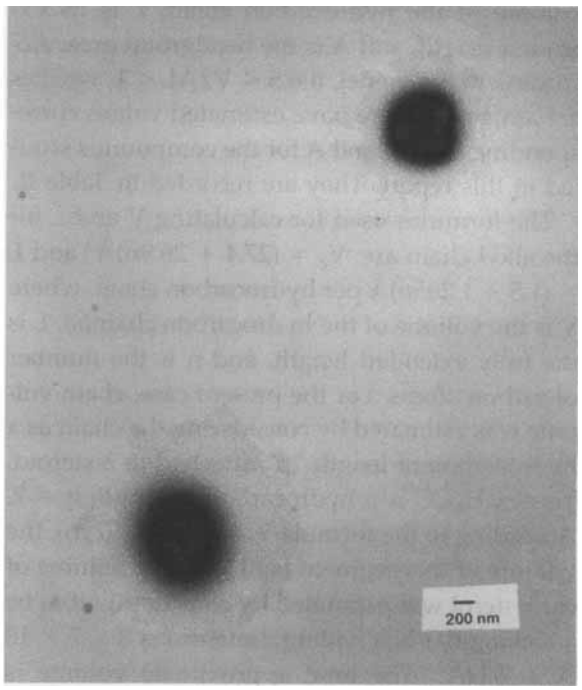


FIGURE 2 Electron micrograph of **9** in the presence of $BaCl_2$, magnification ×25000.

served for these aggregates are larger than estimated by laser light scattering but the presence of regular, spherical aggregates is obvious.

Unlike the all-oxygen crowns,[17] 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^+. Aza-15-crown-5 derivatives **1** and **2** show identical cation binding strengths (in CH_3OH at 25 ± 0.1°C) as follows: log K_s Na^+ = 4.1; K^+ = 4.0.[18] We have not measured binding constants for the other derivatives but data are available for certain close relatives. For example, <15N>$CH_2COOC_{12}H_{25}$ is analogous to **1**. Its Na^+ and K^+ binding constants are 4.07 and 3.95, respectively. These values are within experimental error of those noted above. Assuming a similar correlation for the 18-membered ring structures, we note that <18N>$CH_2COOC_{18}H_{37}$ has the following binding constants: log K_s Na^+ = 4.6; K^+ = 5.8. 4,13-Diaza-18-crown-6 having a CH_2COOEt substituent on each nitrogen binds Na^+ and K^+ with constants (log K_s)[18] of 5.51 and 5.78, respectively.

Aggregates of **3** were characterized by determining volume entrapment using the dye methylene blue. The observed volume entrapment of ~4% agrees well with values published for unilamellar liposomes.[19] Compounds **9** and **10** proved less tractable when attempts were made to determine volume entrapment.

Theoretical Models

The question of why certain amphiphiles form vesicles and others form micellar aggregates has been posed[20] and an empirical model based upon the relative sizes of headgroups and sidechains has been developed by Israelachvilli,[21] Ninham,[22] Evans[23] and their coworkers. The results of this work suggest that a "surfactant parameter," V/(LA), may be used to assess aggregate formation. In this equation, V is the

volume of the hydrocarbon chain, L is its extended length, and A is the headgroup area. According to the model, if $0.5 < V/AL < 1$, vesicles are anticipated. We have estimated values corresponding to V, L, and A for the compounds studied in this report. They are recorded in Table II.

The formulas used for calculating V and L for the alkyl chain are: $V_c = (27.4 + 26.9n)Å^3$ and $L = (1.5 + 1.265n)Å$ 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 carbon atoms. For the present case, chain volume was estimated by considering the chain as a hydrocarbon of length "n" attached to a steroid. Thus, CH_2CO is a hydrocarbon of length n = 2. According to the formula V = (27.4 + 26.9n), the volume of this segment is $81.2Å^3$ The volume of cholesterol was estimated by considering it to be a rectangular box having dimensions 5 × 7 × 18 Å = $630Å^3$. The total approximate volume is therefore $711Å^3$, the value shown for compounds **1–4** in column 5 of Table II. Double this value ($1422Å^3$) is entered for two-tailed compound **9**. Other volumes were calculated accordingly. The extended length parameter was estimated similarly by adding 18Å (the overall length of the steroid estimated from CPK models) to the value obtained from the formula: $L = (1.5 + 1.265n)Å$. Thus, for compound **1**, the connector chain had n = 2 and L = 4. Added to the 18 Å length of the steroid, the value of L is 22Å. This value is entered for compounds **1–4** and **9**. In the latter case, two tails are present but their length is considered in the "parallel," rather than "serial," sense, although their volume is double.

The crown headgroup area was estimated by measuring a CPK molecular model and using the formula area = πr^2. The headgroup areas estimated ("measured") from CPK models for 15-crown-5 and 18-crown-6 are, respectively, 67.7 Å and 78.5 $Å^2$.

The theory suggests that the value V/LA should fall between 0.5 and 1 for the aggregates to form vesicles. It is clear from the discussion above that the methods for estimating length and volume are at best approximate when the chains are not simple hydrocarbons. Even so, there is an interesting correlation between the calculated values and the behavior observed for these systems.

It is known from earlier studies that **1** and **3** form stable vesicles. These have been characterized by dynamic turbidimetry, dye entrapment, and electron microscopy. Still, the calculated value for V/LA is only 0.48 suggesting some ambiguity about the form of their aggregates. Because the theory is good but not perfect and because the methods for calculating V, L, and A are approximate, it is not difficult to imagine that even $0.41 \approx 0.5$.

Compound **2** was studied previously and gave somewhat unusual behavior.[24] The calculated value for compound **2** is 0.41. Laser light

TABLE II Surfactant parameters and associated values for compounds 1–10.[a]

Cpd. No.	ring size	spacer	cholesteryl/cholestanyl	V($Å^3$)	L(Å)	A($Å^2$)	V/LA
1	15	CH_2CO	cholesteryl	711	22	67.7	0.48
2	18	CH_2CO	cholesteryl	711	22	78.5	0.41
3	15	CH_2CO	cholestanyl	711	22	67.7	0.48
4	18	CH_2CO	cholestanyl	711	22	78.5	0.41
5	15	$(CH_2)_3CO$	cholestanyl	765	25	67.7	0.45
6	15	$(CH_2)_5CO$	cholestanyl	819	27	67.7	0.45
7	15	$(CH_2)_7CO$	cholestanyl	873	30	67.7	0.43
8	15	$(CH_2)_{10}CO$	cholestanyl	953	33	67.7	0.43
9	18	CH_2CO	cholestanyl	1422	22	78.5	0.82
10	18	$(CH_2)_{10}CO$	cholestanyl	1904	33	78.5	0.73

[a]The formulas used for calculating V, L, and A are found in the text.

scattering shows that aggregates are about 155 Å in size, a value that was interpreted to mean micelles, rather than vesicles, formed. In all other cases reported here, larger aggregates are formed which give sizes consistent with vesicle formation. It should be noted that for compound **8**, a 10% difference in calculated headgroup area would move the surfactant parameter to 0.5 from the value shown in Table II of 0.43.

CONCLUSIONS

A family of steroidal lariat ether compounds has been prepared and shown to form aggregates. The single-chained derivative having an 18-membered ring (**2**) forms micelles but 18-membered ring derivatives having two sidechains form vesicles. The 15-crown-5 derivatives all form vesicles when steroidal tails are present. Unlike niosomes formed from monomers having polyethylene glycol headgroups, the vesicles are relatively insensitive to the presence of salts.

EXPERIMENTAL SECTION

^{1}H-NMR 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 were recorded at proportional frequencies as noted above. Infrared spectra were recorded on a Perkin-Elmer 1710 Fourier Transform Infrared Spectrophotometer and were calibrated against the 1601 cm-1 band of polystyrene. Optical rotations were measured on a Perkin-Elmer Model 241 Polarimeter in a glass microcell (100 mm path length, 1 mL volume) with a Na gas discharge lamp as the light source. 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.

Cholesteryl Chloroacetate

A solution of cholesterol (1.93 g, 5 mmol) and Et_3N (0.51 g, 5 mmol) in C_6H_6 (25 mL) was added dropwise to an ~10°C solution of chloroacetyl chloride (0.57 g, 5 mmol) in C_6H_6 (20 mL). The solution was heated under reflux at 80°C for 24 h, cooled to room temperature, and worked up. Crystallization (abs. EtOH) afforded the title compound (1.53 g, 66%) as a white solid, mp 160–161°C.

Cholestanyl Chloroacetate

A solution of dihydrocholesterol (1.94 g, 5 mmol) and Et_3N (0.51 g, 5 mmol) in toluene (25 mL) was added dropwise to an ~10°C solution of chloroacetyl chloride (0.57 g, 5 mmol) in toluene (20 mL). The solution was heated under reflux at 80°C for 24 h, cooled to room temperature, filtered, and concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 (50 mL), washed with 3N HCl (4 × 25 mL), 5% Na_2CO_3 (2 × 25

mL), dried, concentrated *in vacuo*, and then crystallized (abs. EtOH) to afford the title compound (1.5 g, 65%) as a white solid, mp 180–181°C.

Cholesteryl (4-aza-15-crown-5)acetate, 1, was prepared as previously reported.[9] The product was obtained in 68% yield as a white solid (mp 85–86°C) having $[\alpha]_D^{25} = -23.85°$ (c 2, $CHCl_3$).

Cholesteryl (4-aza-18-crown-6)acetate, 2, was prepared as previously reported.[9] The product was obtained in 63% yield as a waxy, white solid (mp 66–67°C) having $[\alpha]_D^{25} = -20.6°$ (c 2, $CHCl_3$).

Cholestanyl (4-aza-15-crown-5)acetate, 3, was prepared as previously reported.[9] The product was obtained in 67% yield as a white solid (mp 60–61°C) having $[\alpha]_D^{25} = 12.1°$ (c 2, $CHCl_3$).

Cholestanyl (4-aza-18-crown-6)acetate, 4, was prepared as previously reported.[9] The product was obtained in 65% yield as a waxy, white solid (mp 55–56°C) having $[\alpha]_D^{25} = 9.4°$ (c 2, $CHCl_3$).

Preparation of Cholestanyl Esters of ω-halo Carboxylic Acids

The title compounds were synthesized by the reaction between ω-halo carboxlic acid halides and dihydrocholesterol in the presence of triethylamine. 4-Chlorobutyryl chloride and 6-bromohexanoyl chloride were purchased from Aldrich Chemical Co. and used without purification. 8-Bromooctanoyl chloride and 11-bromoundecanoyl chloride were prepared from the corresponding acid by the reaction with thionyl chloride in the usual fashion and used without further purification.

Cholestanyl 4-chlorobutanoate

The procedure described for the synthesis of cholestanyl 6-bromohexanoate was followed and the title compound was obtained (1.36 g, 55%) as flakes, mp 97.5–98.0°C. ^{1}H-NMR: 0.47–2.17 (m, 48H, steroid and $ClCH_2CH_2CH_2$), 2.45 (t, 2H, CH_2CO), 3.57 (t, 2H, $ClCH_2$), 4.68 (brs, 1H, CHO); IR (KBr disk): 2950, 2885 and 2860 cm^{-1} (ν_{CH}), 1740 cm^{-1} ($\nu_{C=O}$, ester). Anal. calcd for $C_{31}H_{53}O_2Cl$: C, 75.49, H, 10.83%. Found: C, 75.50; H, 10.90%.

Cholestanyl ω-(4-aza-15-crown-5)butanoate, 5

The procedure described above for the synthesis of cholestanyl 8-(4-aza-15-crown-5) octanoate was followed to afford the title compound (350 mg, 32%) as a colorless oil. ^{1}H-NMR: 0.64 (s, 3H, steroid C-18 CH_3), 0.81 (s, 3H, steroid C-19 CH_3), 0.85 and 0.86 (6H, d of d, steroid C-26 and C-27 CH_3), 0.88 (3H, d, steroid C-21 CH_3), 0.92–1.98 (m, 33H, steroid and $CH_2CH_2CH_2CO$), 2.28 (t, 3H, CH_2CO), 2.25 (t, 2H, NCH_2), 2.74 (t, 4H, $(CH_2CH_2)_2N$), 3.60–3.70 (m, 16H, $OCH_2CH_2OCH_2CH_2N$), 4.67 (m, 1H, CHO); IR (Neat) 2950 and 2880 cm^{-1} (ν_{CH}), 1745 cm^{-1} ($\nu_{C=O}$, ester). $[\alpha]_D^{25} = 9.46°$ (c 2.05, $CHCl_3$). Anal. calcd for $C_{41}H_{73}O_6N$: C, 72.84; H, 10.88; N, 2.07%. Found: C, 72.72; H, 10.89; N, 2.03%.

Cholestanyl 6-bromohexanoate (General Procedure)

A solution of dihydrocholesterol (1.93 g, 5 mmol) and triethylamine (0.51 g, 5 mmol) in benzene (25 mL) was added dropwise to a 6–9°C solution of 6-bromohexanoyl chloride (1.07 g, 5.5 mmol) in benzene (20 mL). The solution was stirring overnight at room temperature, then refluxed for 4 hrs, and worked up. Crystallization (abs. EtOH) afforded the title compound (1.63 g, 58%) as white, leaf-like crystals, mp 74.5–75.5°C. ^{1}H-NMR: 0.47–2.10 (m, 52H, steroid and $BrCH_2(CH_2)_3CH_2$), 2.25 (t, 2H, CH_2CO), 3.36 (t, 2H, $BrCH_2$), 4.64 (brs, 1H, CHO); IR (KBr disk) 2950, 2880 and 2860 cm^{-1} (ν_{CH}), 1750 cm^{-1} ($\nu_{C=O}$, ester). Anal. calcd for $C_{33}H_{57}O_2Br$: C, 70.06, H, 10.16%. Found: C, 70.11; H, 10.19%.

Cholestanyl 6-(4-aza-15-crown-5)hexanoate, 6

The procedure described above for the synthesis of cholestanyl 8-(4-aza-15-crown-6) octanoate was followed to afford the title compound (310 mg, 29%). ^{1}H-NMR: 0.64 (s, 3H, C-28 steroid CH_3), 0.81 (s, 3H, C_{19} steroid CH_3), 0.85 and 0.86 (6H, d of d, C-26 and C-27 steroid CH_3), 0.89 (d, 3H, C_{21}steroid CH_3), 0.92–1.98 (m, 37H, steroid and $CH_2(CH_2)_3$ CH_2CO), 2.25 (t, 3H, CH_2CO), 2.51 (t, 2H, NCH_2), 2.75 (t, 4H, $(OCH_2CH_2)_2N$), 3.60–3.70 (m, 16H, $OCH_2CH_2OCH_2CH_2N$), 4.68 (m, 1H, CHO); IR (Neat) 2960 and 2880 cm^{-1} (ν_{CH}) and 1750 cm^{-1} ($\nu_{C=O}$ ester). $[\alpha]_D^{25}$ = 9.15° (c 2.46, $CHCl_3$). Anal. calcd for $C_{43}H_{77}O_6N$: C, 73.35; H, 11.02; N, 1.99%. Found: C, 73.08; H, 11.01; N, 2.05%.

Cholestanyl 8-bromooctanoate

The procedure described above for the synthesis of cholestanyl 6-bromohexanoate was followed to afford title compound (2.87 g, 47%) as leaves, mp 62.5–64.0°C. ^{1}H-NMR: 0.47–2.18 (m, 56H, steroid and $BrCH_2(CH_2)_5CH_2$), 2.36 (t, 2H, CH_2CO), 3.38 (t, 2H, $BrCH_2$), 4.62 (brs, 1H, CHO); IR (KBr disk): 2940 and 2870 cm^{-1} (ν_{CH}), 1745 cm^{-1} ($\nu_{C=O}$, ester). Anal. calcd for $C_{35}H_{61}O_2Br$: C, 70.80, H, 10.35%. Found: C, 70.85; H, 10.41%.

Cholestanyl ω-(4-aza-15-crown-5)octanoate, 7

Cholestanyl 8-bromooctanoate (1.18 g, 1.98 mmol), aza-15-crown-5 (392 mg, 1.8 mmol), Na_2CO_3 (2.16 g, 20.3 mmol) and potassium iodide (20 mg) were heated at reflux (115°C) in butyronitrile (40 mL) for 5 days. The mixture was cooled, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography in this sequence (silica gel, 0–50% CH_3OH/CH_2Cl_2; alumina, 0–10% $CH_3OH/CH_3COOC_2H_5$; short alumina, CH_2Cl_2). A colorless oil (665 mg, 51%) was obtained. ^{1}H-NMR: 0.64 (s, 3H, C_{18}-steroid CH_3), 0.81 (3H, C_{19} steroid CH_3), 0.83 and 0.85 (6H, d of d, C_{26} and C_{27} steroid CH_3), 0.89 (3H, d, C_{21} steroid CH_3) 0.92–2.10 (m, 41H, steroid and $CH_2(CH_2)_5CH_2CO$), 2.24 (t, 2H, CH_2CO), 2.47 (t, 2H, $NCH_2(CH_2)9$), 2.73 (t, 4H, OCH_2CH_2N) 3.61–3.67 (s + m, 16H, $OCH_2CH_2OCH_2CH_2N$), 4.69 (m, 1H, CHO); IR (neat): 2955 and 2880 cm^{-1} (ν_{CH}), 1750 cm^{-1} ($\nu_{C=O}$, ester). $[\alpha]_D^{25}$ = 8.17° (c 2.9 $CHCl_3$). Anal. calcd for $C_{45}H_{81}O_6N$: C, 73.82; H, 11.15; N, 1.91%. Found: C, 73.70; H, 11.19; N, 1.99%.

Cholestanyl 11-bromoundecanoate

The procedure described above for the synthesis of cholestanyl 6-bromohexanoate was followed to afford title compound (2.53 g, 40%) as white, leaf-like crystals, mp 67.5–69.0°C. ^{1}H-NMR: 0.47–2.08 (m, 62H, steroid and $BrCH_2(CH_2)_8CH_2$), 2.17 (t, 2H, CH_2CO), 3.31 (t, 2H, $BrCH_2$), 4.52 (brs, 1H, CHO); IR (KBr disk): 2940 and 2860 cm^{-1} (ν_{CH}), 1740 cm^{-1} ($\nu_{C=O}$, ester). Anal. calcd for $C_{38}H_{67}O_2Br$: C, 71.78, H, 10.62%. Found: C, 71.85; H, 10.71%.

Cholestanyl 11-(4-aza-15-crown-5)undecanoate, 8

The procedure described above for the synthesis of cholestanyl 8-(4-aza-15-crown-65)octanoate was followed to afford the title compound (400 mg, 26%). ^{1}H-NMR: 0.64 (s, 3H, steroid C-18 CH_3), 0.82 (s, 3H, steroid C-19 CH_3), 0.86 and 0.87 (d of d, 6H, C_{26} and C_{27} steroid CH_3), 0.91 (d, 3H, steroid C-21 CH_3), 0.93–2.00 (m, 47H, steroid and $CH_2(CH_2)_8CH_2CO$), 2.26 (t, 2H, CH_2CO), 2.49 (t, 2H, NCH_2), 2.75 (t, 4H, $(OCH_2CH_2)_2N$), 3.60–3.70 (m, 16H, $OCH_2CH_2OCH_2CH_2N$), 4.70 (m, 1H, CH)); IR (neat): 2960 and 2885 cm^{-1}(ν_{CH}) 1755 cm^{-1} ($\nu_{C=O}$, ester). $[\alpha]_D^{25}$ = 8.59° (c 1.88, $CHCl_3$).

Anal. calcd for $C_{48}H_{87}O_6N$: C, 74.47; H, 11.33; N, 1.81%. Found: C, 74.35; H, 11.30; N, 1.81%.

N,N'-bis(3-Cholestanyloxycarbonylmethyl)-4,13-diaza-18-crown-6, 9

A solution of (2-chloroacetyl)dihydrocholesterol ester (see above, 3.37g, 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 was cooled, 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-Cholestanyloxycarbonyldecyl)-4,13-diaza-18-crown-6, 10

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 and $NCH_2(CH_2)_8$), 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-$CCH_2)_2$N), 3.57 (m, 16H, (OCH_2-C)$_2$N), 4.69 (m, 2H, C_3-steroid). IR (CCl_4): 2940 (m), 2880 (m), 1725 (br) cm^{-1}. $[\alpha]_D^{25}$ = +8.6 (c 2, $CHCl_3$). 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).

Vesicle Preparation

The amphiphile (0.01 mmol) 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 minutes at the desired temperature. For tip sonication, the suspension was sonicated at 30 Watts with 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 90° angle for 200 sec.

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.

Electron Microscopy: Negative Stain Protocol

Membrane vesicles were attached to Butvar/carbon-coated, 400 mesh copper grids by applying 10–15 μL drops of the vesicles 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 applied for 15–30 sec. The stain was wicked off with filter paper and the grids air dried. The specimens were viewed in a Hitachi H-600 transmission electron microscope, operated at 75 kV.

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