Organometallic Amphiphiles: Oxidized Ferrocene as Headgroup for Redox-Switched Bilayer and Monolayer Membranes

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Abstract: A family of 15 ferrocene derivatives has been prepared, most of which are reported for the first time. This includes $FcCH_2O$-3-cholestanyl, **1**; $FcCH_2O(CH_2)_{13}CH_3$, **2**; $FcCH_2O(CH_2)_{15}CH_3$, **3**; $FcCH_2O(CH_2)_{17}CH_3$, **4**; $FcCH_2N[(CH_2)_{17}CH_3]_2$, **5**; $FcCH_2O(CH_2)_8OCH_2Fc$, **6**; $FcCH_2O(CH_2)_{12}OCH_2Fc$, **7**; $FcCH_2O(CH_2)_{16}OCH_2Fc$, **8**; $Fc(CH_2)_{22}Fc$, **9**; $FcCH_2$-3,17-β-estradioxy-CH_2Fc, **10**; Fc-1,1′-$[COO(CH_2)_{16}CH_3]$, **11**; $FcCONH(CH_2)_{17}CH_3$, **12**; Fc-1,1′-$\{CON[(CH_2)_{17}CH_3]_2\}_2$, **13**; Fc-1,1′-(COO-3-dihydrocholesteryl), **14**; and Fc-1,1′-(COO-3-cholesteryl), **15**. Redox potentials for **1**−**15** have been determined and are in the range 400−450 mV for **2**−**6** (*vs* SSCE) and 509 mV for **1**, 972 mV for **7**, 806 mV for **8**, 711 mV for **9**, 941 mV for **10**, and 945 mV for **11** (*vs* Ag/AgCl). Upon oxidation with Ce(IV), aqueous suspensions of compounds **1**−**5** and **7**−**10** formed stable vesicles after sonication. The charged monomers that formed vesicles afforded aggregates in the 2000−3000 Å range that were characterized by laser light scattering and negative stain electron microscopy. In the absence of an oxidizing agent, vesicles failed to form from any of the 15 monomers even after prolonged sonication. Addition of 500 μM aqueous $Na_2S_2O_4$ solution collapsed the vesicles formed from **1**−**5** and **7**−**10**, and the original amphiphile monomers were detected afterward by thin layer chromatography. It was concluded from cyclic voltammetry that both ferrocene residues in **8** were oxidized. Vesicles formed from **7**−**10** represent the first examples of a redox-switched bolaamphiphile.

Introduction

The membranes that define the boundaries of organelles and cells are formed from amphiphilic molecules.[1] These natural barriers are normally phospholipids derived from glycerol, fatty acids, and other components. The membranes often contain "additives" specific to their function. Special types of membranes often exhibit unusual structural elements.[2] For example, the special responsiveness of the cardiac membrane is due in part to the presence of vinyl ethers in amphiphiles known as plasmalogens.[3] It seems reasonable to assume that non-naturally occurring, amphiphilic molecules could impart special properties to membranes or that chemically-switchable membranes could be prepared *de novo* from specially designed monomers. This principle has been demonstrated recently by the construction of amphiphiles that insert into phospholipid vesicles and engender proton-,[4] sodium-,[5] or potassium-selective flux.[6]

The organization of amphiphiles into vesicles or liposomes is likewise a much studied process. Vesicular lysis is a well-established process, but the controlled collapse of vesicles is less well studied. There is a dearth of knowledge on control or switchability of vesicular aggregates. Clearly, strong acid or strong base can collapse existing vesicles as can concentrated salt solutions. We sought more selective environmental controls and attempted to develop systems that could be collapsed once formed or formed only when "pro-amphiphilic" monomers were chemically altered by such mild switches into amphiphiles. Redox-switched aggregates were reported by Grätzel and coworkers[7] who studied amphiphilic copper cyclam complexes. Saji studied amphiphilic ferrocenes[8] but, like Grätzel, obtained only micellar structures.

In our first effort, we chose ferrocene as a redox-switchable headgroup for a steroidal derivative. Ferrocene can be converted from its relatively nonpolar neutral state (Fe is formally 2+) to the ferrocenium cation [Fe(III)].[9] It should be noted that ferrocene has been used as a building block in switchable complexing agents by Hall,[10] by Beer,[11] and also in previous studies from our own group.[12] We also demonstrated that amphiphilic nickel phenanthroline complexes and bis(hexadecylamine)silver complexes could both form stable liposomes

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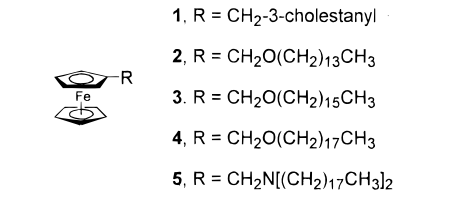
upon sonication. The phenanthroline-based organometallic vesicles were collapsed by exposure to 100 μM aqueous $Na_2S_2O_4$. The silver complexes were collapsed by exposure to dilute formalin solution.[13] In a separate study, either dilute aqueous $Na_2S_2O_4$ or H_2NOH readily disassembled bis(hexadecylamine)copper(II) salts.[14]

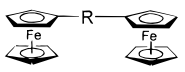
In the present paper, we describe a family of redox-switched, amphiphilic ferrocene derivatives that aggregate to form vesicles as well as the first example of reversible liposome formation from an organometallic bolaamphiphile[15] which uses ferrocene as both switchable headgroups. We also present evidence that, for the latter case, liposomes form only when both ferrocene residues are oxidized and that these aggregates collapse when the ferrocenium ion is reduced.

Results and Discussion

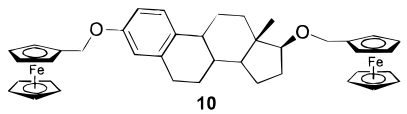
Fifteen compounds were prepared for the present study. They are shown as **1**−**15**. The first structure is ferrocenylmethyl 3-cholestanyl ether. This compound was the first example of an organometallic, redox-switched monomer that formed stable, vesicular aggregates.[9] Compounds **2**−**4** are ferrocenylmethyl



1, R = CH_2-3-cholestanyl
2, R = $CH_2O(CH_2)_{13}CH_3$
3. R = $CH_2O(CH_2)_{15}CH_3$
4, R = $CH_2O(CH_2)_{17}CH_3$
5, R = $CH_2N[(CH_2)_{17}CH_3]_2$



6, R = $CH_2O(CH_2)_8OCH_2$
7, R = $CH_2O(CH_2)_{12}OCH_2$
8, R = $CH_2O(CH_2)_{16}OCH_2$
9, R = $(CH_2)_{22}$

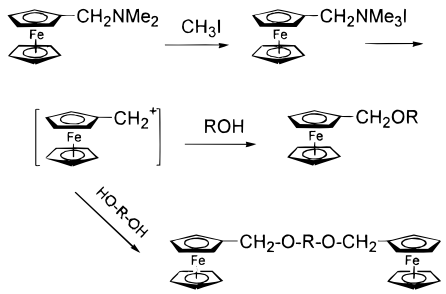


10

11, CO-O$(CH_2)_{16}CH_3$
12, CO-NH$(CH_2)_{17}CH_3$
13, CO-N$[(CH_2)_{17}CH_3]_2$
14, CO-O-dihydrocholesteryl
15, CO-O-cholesteryl

alkyl ethers in which the alkyl groups range from tetradecyl to octadecyl. Two octadecyl chains are present in tertiary ferrocenyl amine **5**. Compounds **6**−**10** are bolaamphiphiles in which two ferrocenyl residues are attached to opposite (α, ω) ends of a spacer chain. In compounds **6**−**8**, the spacer chains are of the form $CH_2O(CH_2)_nOCH_2$ where "*n*" varies from 8 to 16. The spacer chain is a simple 22-methylene hydrocarbon in **9**, and **10** uses the rigid, 3,17-β-estradiol unit as a rigid spacer. Compounds **11**−**15** are 1,1-disubstituted ferrocenyl ester and amide derivatives which are amphiphiles rather than bolaamphiphiles.

Scheme 1



Compound Syntheses. Compounds **1**−**4** were prepared by a Williamson reaction[16] that takes advantage of the extraordinary stability of the α-ferrocenylmethyl carbocation.[17] Commercially available [(*N*,*N*′-dimethylamino)methyl]ferrocene (Me_2NCH_2-Fc) was treated with CH_3I and the appropriate nucleophile, *e.g.*, ROH, in the presence of K_2CO_3. The intermediate tetraalkylammonium salt loses trimethylamine, and $FcCH_2^+$ is trapped by ROH to give $FcCH_2OR$.

In our original report of compound **1**,[9] we obtained only a 7% yield of the product. By working in a solvent mixture consisting of equal volumes of CH_3CN and $CH_3CH_2CH_2CN$, yields of 36%, 39%, and 81% were obtained for **2**, **3**, and **4**, respectively. The latter compound was prepared last in this series, and its higher yield may reflect increased operator skill; no other difference was obvious. Compounds **2**−**4** were all low melting solids (mp 40−50 °C; see the Experimental Section). The approach is shown in Scheme 1. Although this is not a traditional method for the synthesis of unsymmetrical ethers, it uses a readily available tertiary amine and the reaction conditions are mild.

The approach noted above was inappropriate for the synthesis of tertiary amine **5**. In this case, ferrocenylmethanol was converted to (chloromethyl)ferrocene by reaction with PCl_3 and pyridine in THF. A solution of dioctadecylamine in benzene and K_2CO_3 were added to the above, and $FcCH_2(NC_{18}H_{37})_2$, **5**, was obtained as a stable, yellow solid.

Bolaamphiphiles **6**−**8** were prepared in essentially the fashion described for **1**−**4** except that 2 equiv of $FcCH_2NMe_2$ was allowed to react with methyl iodide followed by treatment with the appropriate diol. The bolaamphiphiles were isolated as yellow solids with melting points in the 85−100 °C range. β-Estradiol derivative **10** was transformed into the corresponding 3,17-substituted bolyte in a similar fashion. Its melting point of 141−143 °C suggests that it is, as anticipated, more rigid than bolaamphiphile ethers **6**−**8**.

Compound **9** is a non-ether bolaamphiphile. It was constructed from docosanedioic acid. The diacid was converted into the diacid dichloride using $SOCl_2$, and then allowed to react at room temperature with ferrocene in the presence of $AlCl_3$. The resulting diketone $FcCO(CH_2)_{20}COFc$ was reduced using Clemmensen conditions to the α,ω-diferrocenylhydrocarbon (Scheme 2).

Preparation of compounds **11**−**15** involved either esterification or amidation of commercially available 1,1′-dicarboxyferrocene using oxalyl chloride to convert the diacid into the diacid chloride. After addition of either the alcohol or amide, the

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Scheme 2

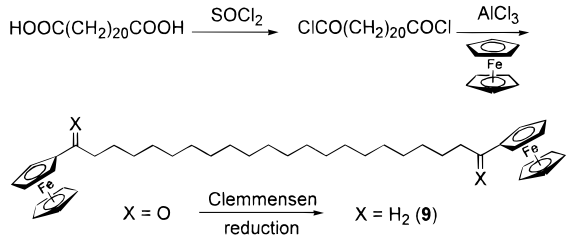

Table 1. Redox Potentials for Substituted Ferrocenes

compd no.	substituent on ferrocene	potential (mV)	solvent
1	CH_2O-3-cholestanyl	509[a]	CH_2Cl_2
2	$CH_2O(CH_2)_{13}CH_3$	410[b]	CH_3CN
3	$CH_2O(CH_2)_{15}CH_3$	405[b]	CH_3CN
4	$CH_2O(CH_2)_{17}CH_3$	410[b]	CH_3CN
5	$CH_2N[(CH_2)_{17}CH_3]_2$	443[b]	CH_2Cl_2
6	$CH_2O(CH_2)_8OCH_2Fc$	418[b]	CH_3CN
7	$CH_2O(CH_2)_{12}OCH_2Fc$	420[b]	CH_3CN
8	$CH_2O(CH_2)_{16}OCH_2Fc$	425[b]	CH_3CN
9	$(CH_2)_{22}Fc$	460[b]	DMF
10	CH_2-3,17-β-estradioxy-CH_2Fc	442[b]	CH_3CN
11	1,1′-$COO(CH_2)_{16}CH_3$	972[a]	CH_2Cl_2
12	1,1′-$CONH(CH_2)_{17}CH_3$	806[a]	CH_2Cl_2
13	1,1′-$CON[(CH_2)_{17}CH_3]_2$	711[a]	CH_2Cl_2
14	1,1′-COO-dihydrocholesteryl	941[a]	CH_2Cl_2
15	1,1S-COO-cholesteryl	945[a]	CH_2Cl_2

[a] Determined *vs* Ag/AgCl. [b] Determined *vs* Hg/Hg_2Cl_2.

reaction mixture was worked up and the product obtained by chromatography, crystallization, or both.

Determination of Redox Potentials. The redox potential of ferrocene is known to be ~400 mV depending on solvent. This is a readily accessible potential and is the basis of ferrocene's importance in electrochemical studies generally. Redox potentials for the compounds reported here were determined by cyclic voltammetry. In each case, the electroactive species (1 mM) was present in dry CH_2Cl_2 or CH_3CN along with 0.1 M tetrabutylammonium hexafluorophosphate. Glassy carbon was used as the working electrode, Pt° was the counter electrode, and either Ag/AgCl or sodium saturated calomel (SSCE) was the reference. Scan rates were 100, 200, and 500 mV/s, and sensitivities were 2−10 μA/cm. The redox potentials for the 15 compounds reported in this study are recorded in Table 1.

In compounds **1**−**10**, a saturated (sp^3-hybridized) carbon is attached to the ferrocene cyclopentadiene ring. Even when the second atom is electronegative oxygen or nitrogen, the redox potential is similar to that for ferrocene. When the side chain is attached as an ester or amide, the connecting atom is sp^2-hybridized. The electron-withdrawing effect of the carbonyl group is to substantially alter the redox potential of the monomer, rendering the charged state less accessible and less stable.

Vesicle Formation. Vesicle formation was attempted with each of the neutral monomers using a modified lipid hydration method.[18] In no case could any aggregate be detected by laser light scattering (Coulter N4SD).[19] Oxidation of **1**−**8** and **10** by treatment with $Ce(NH_4)_2(NO_3)_6$ gave the amphiphilic alkylferrocenium cations which were sonicated while the suspension was continuously protected from the air. In all cases, aggregate formation was clearly indicated by dynamic light scattering

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(19) Neutral compounds **6**, **11**, and **13** formed aggregates upon sonication in water. (Oxidized **6** is water soluble.) No further study of these vesicle systems was undertaken.

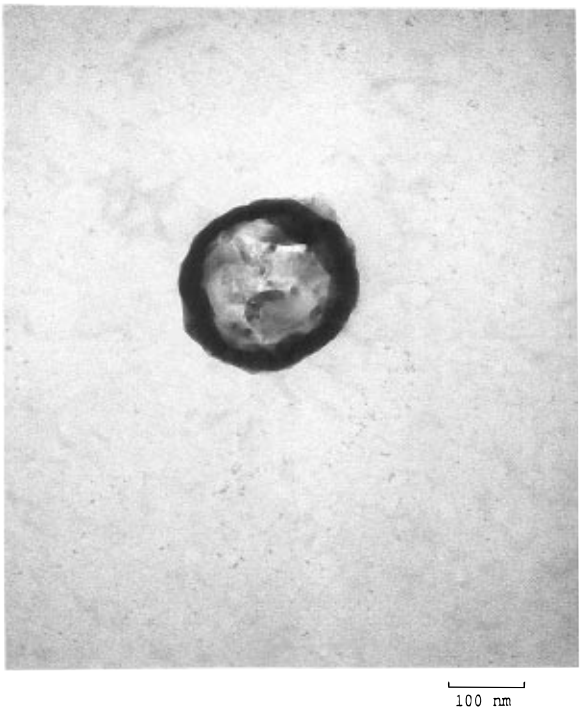

Figure 1. Negative stain electron micrograph of $\mathbf{4}^+$, magnification 250000× (reproduced at 40% of original size).

methods (Table 2). Relatively large (~2000 Å) vesicles of similar sizes were obtained for compounds **1**−**4** and **7**−**10**. The vesicles formed from oxidized **5** or **7** were slightly less uniform than the other cases. The former represents an interesting case because, to our knowledge, no example of a ferrocenylamine has ever been reported to undergo redox switching of the type described here.

The most remarkable finding of this study is that the two-headed ferrocene derivatives **7**−**10** can be oxidized to a ferrocenium amphiphile that aggregates into stable vesicles. This is the first example of a redox-switchable, organometallic bolaamphiphile. The formation of liposomes was confirmed, as noted, by laser light scattering and also by negative stain electron microscopy. The laser light scattering data are recorded in Table 2.

Diferrocene bolaamphiphile **6** possesses a rather short hydrocarbon chain. It can readily be oxidized, but as expected, the dipositive bolaamphiphile is water soluble and fails to aggregate.

Electron Microscopy. Negative stain electron microscopy was used to further characterize the vesicles formed from the ferrocenyl monomers. One of the vesicles resulting from sonication of $[FcCH_2O(CH_2)_{17}CH_3]^+$, $\mathbf{4}^+$, is shown in Figure 1. Several features should be noted. First, the aggregate is essentially spherical in shape. Second, the size is 2400 Å when measured directly from the photograph. This agrees very well with the laser light scattering data. Third, the dark membrane is visible on the exterior of the vesicle. The membrane appears larger than might be expected, perhaps because the negative stain method gives a more three-dimensional view than observed using the freeze fracture technique.

Like the vesicles derived from $\mathbf{4}^+$, aggregates formed from doubly-oxidized bolaamphiphile **8** $[FcCH_2O(CH_2)_{16}OCH_2Fc]$ show clearly defined spherical aggregates. In this case, a wider range of aggregate sizes is observed, but the membrane boundaries are again clearly defined. A number of circular forms are apparent which have a size of ~2000 Å. In addition, however, there are numerous smaller, although generally well-defined, structures that have sizes closer to 1000 Å. The electron micrograph is shown in Figure 2.

Table 2. Laser Light Scattering Data for Aggregates Formed from Oxidized Ferrocene Derivatives[a]

compd no.	R on ferrocene	unimodal diam (Å)	cumulant distribution (Å)	
			by intensity	by weight
1	CH_2O-3-cholestanyl	2030 ± 720	2730 ± 1000	2780 ± 1200
2	$CH_2O(CH_2)_{13}CH_3$	1900 ± 610	2180 ± 620	2060 ± 730
3	$CH_2O(CH_2)_{15}CH_3$	1990 ± 620	2230 ± 1100	1620 ± 1100
4	$CH_2O(CH_2)_{17}CH_3$	1730 ± 540	1840 ± 940	1360 ± 810
5	$CH_2N[(CH_2)_{17}CH_3]_2$	2390 ± 800	818 ± 140 (12%) 2980 ± 250 (88%)	772 ± 120 (62%) 2980 ± 250 (38%)
6	$CH_2O(CH_2)_8OCH_2Fc$	sol[b]	sol	sol
7	$CH_2O(CH_2)_{12}OCH_2Fc$	1980 ± 740	1020 ± 90 (31%) 3260 ± 370 (69%)	1010 ± 80 (69%) 3290 ± 410 (31%)
8	$CH_2O(CH_2)_{16}OCH_2Fc$	2250 ± 630	2220 ± 260	2180 ± 260
9	$(CH_2)_{22}Fc$	2260 ± 660	2740 ± 1100	2740 ± 1300
10	CH_2O-3,17-estradioxy-OCH_2Fc	2950 ± 850	2880 ± 580	2960 ± 600
11	1,1′-$COO(CH_2)_{16}CH_3$	u[c]	u	u
12	1,1′-$CONH(CH_2)_{17}CH_3$	u	u	u
13	1,1′-$CON[(CH)_{17}CH_3]_2$	u	u	u
14	1,1′-COO-dihydrocholesteryl	u	u	u
15	1,1′-COO-cholesteryl	u	u	u

[a] Determined on a Coulter N4SD submicrometer particle analyzer. [b] "sol" means that the oxidized monomer was water soluble; any aggregates present were not detected by light scattering. nd means "none detected". [c] "u" means that the oxidized monomers were unstable.

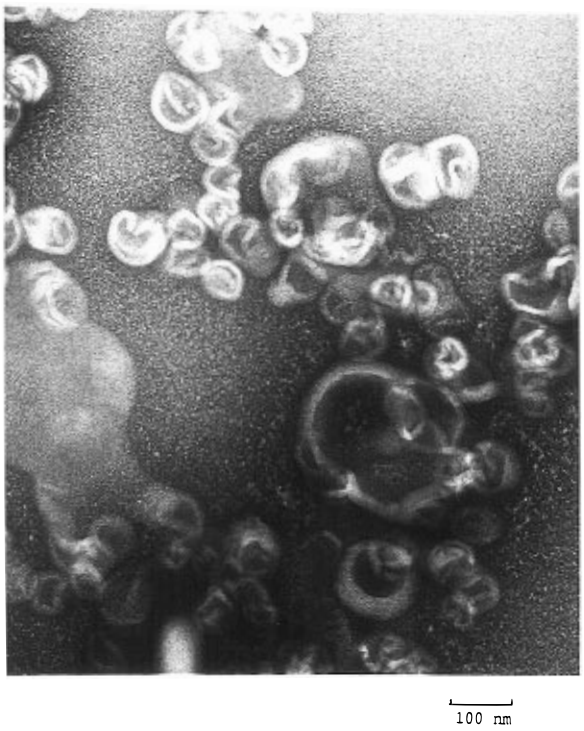

Figure 2. Negative stain electron micrograph of $\mathbf{8}^{2+}$, magnification 200000× (reproduced at 40% of original size).

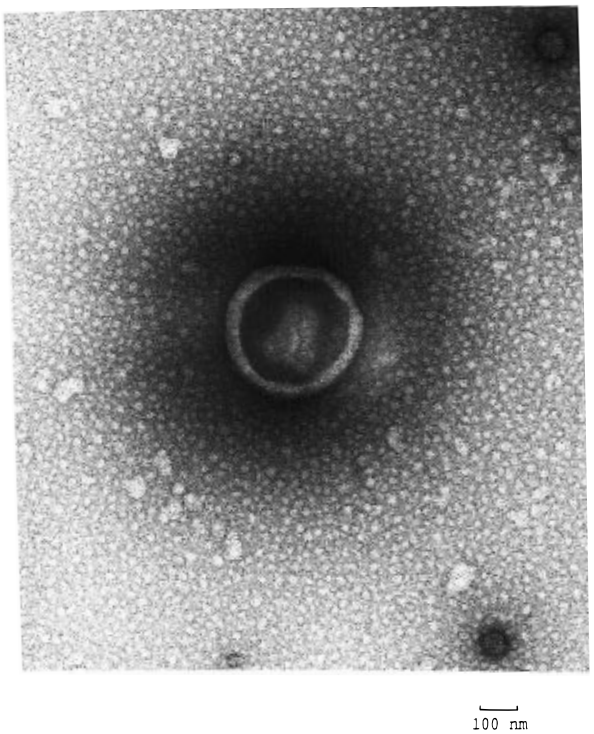

Figure 3. Negative stain electron micrograph of $\mathbf{7}^{2+}$, magnification 125000× (reproduced at 40% of original size).

Using a somewhat different staining method, we were able to obtain better resolved electron micrographs of **7** [$FcCH_2O(CH_2)_{12}OCH_2Fc$] (Figure 3), the analog of **8** that has a spacer chain four carbons shorter. The external membrane boundary is clearly visible, and the size measured from the photograph agrees well with the distribution obtained by dynamic turbidimetry. Again, size, shape, and boundary margins confirm vesicle formation.

Headgroup Identity in the Bolaamphiphile-Based Aggregates. An important question is whether only one or both of the ferrocene "headgroups" is oxidized. The fact that none of the alkyl-substituted ferrocenes forms stable liposomes when neutral suggests that both iron atoms are oxidized to the 3+ state in aggregates of bolyte, **7**−**10**. Even so, it is possible that only one of the ferrocenes is oxidized and that monolayer vesicles are formed in which one of the membrane surfaces is not oxidized. Another possibility is that half of the charges of each singly-charged bolaamphiphile reside on the outer surface of the membrane while the remainder are on the vesicle's interior. In such a case, there might be an interaction between the iron atoms which are alternately Fe(II) and Fe(III). This possibility is based upon the known ability of ferrocenyl iron to serve as a donor for other metal cations.[20] These two structural arrangements are illustrated in Figure 4, panels A and B. It is also possible, however unlikely, that bilayers can form from the arrangement shown in Figure 4C. In this case, the external surface of the bilayer would consist of ferrocenium cations and the neutral ferrocenes would interdigitate within the bilayer.

We have addressed the question of single *vs* double oxidation in the following way. We note that sonication is not undertaken in our experimental protocol until monomer **8** is exposed to oxidizing agent. Thus, it is not obvious how formation of

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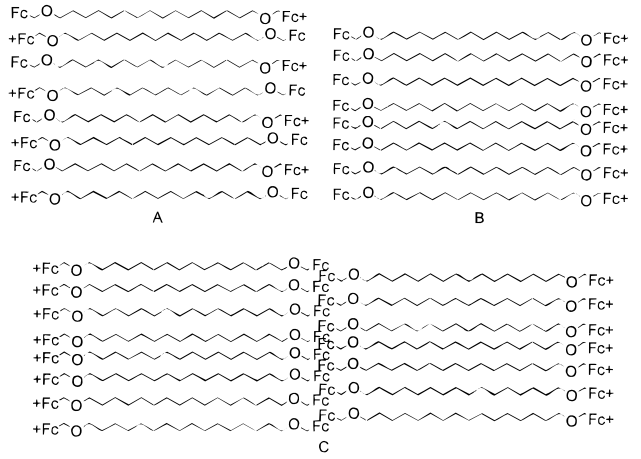

Figure 4. Possible amphiphile orientations in membranes formed from $\mathbf{6}^{2+}$.

partially oxidized aggregates could physically protect the monomers from further reaction. Moreover, the two ferrocene residues of **8** are separated by 20 nonconjugated atoms. There is no reason to assume that oxidation of one ferrocene residue will affect the other by some type of electronic communication through the saturated chain.

The anodic voltammetric behavior of compounds **1**−**10** is characterized as expected by the reversible monoelectronic oxidation of the ferrocene moiety. Cyclic voltammetry of **8** reveals only *one* anodic redox couple at a half-wave potential ($E_{1/2}$) of 432 mV *vs* SSCE. This behavior is characteristic of molecules containing two (or more) identical, but fully independent redox sites.[21] The single wave resulting from the merging of the individual waves—corresponding to each one of the redox sites—is located close to the half-wave potentials.[22] By comparison, compound **2** exhibits one voltammetric wave at 425 mV *vs* SSCE. The currents associated with this wave are smaller (*ca*. half) than those associated with the voltammetric wave of compound **8**, indicating the two-electron character of the latter. The cyclic voltammogram is shown in Figure 5.

These voltammetric findings are consistent with approximately 20 Å maximum separation of the ferrocenes and the lack of unsaturation in the bridge of compound **8**. Communication between the two ferrocene headgroups should be minimal, and indeed, no evidence for it is observed. Therefore, as both ferrocene groups in **8** exhibit very similar oxidation potentials and the compound is treated with a substantial excess of oxidizing agent prior to the formation of vesicles, we conclude that the effective bolaamphiphilic monomer for vesicle formation is the corresponding dication $\mathbf{8}^{2+}$. In this case, the ferrocenium (oxidized ferrocene) residues serve as the polar headgroups for both the interior and exterior surfaces of the vesicle.

UV−**Vis Study.** The concept of redox switching as it applies here involves the conversion of an organic-soluble (lipophilic) entity into a charged amphiphile. Application of the switch is intended to alter the solubility and thus the aggregation properties of the species in question. Any attempt to analyze this system must take account of the deliberate alteration in solubility properties.

Analysis by cyclic voltammetry showed that two ferrocene subunits present in the same bolaamphiphilic molecule could be simultaneously oxidized in organic media (see above). From this, it was logically inferred that oxidation in water, the reaction medium for the present system, was also simultaneous. The oxidation in water was confirmed as follows. Known $FcCH_2OCH_3$ was oxidized using Ce(IV) in water at a concentration of 2 mM. The absorption of Ce(IV) at ∼440 nm obscures the UV spectrum of the neutral compound, but when the compound is oxidized, λ_{max} is 628 nm (water). Unlike **8**, oxidized bolaamphiphile **6** is water-soluble. If complete oxidation of both ferrocenes occurs in water, the UV−vis spectrum for a 2 mM aqueous solution of $FcCH_2OCH_3$ should be essentially indistinguishable from that of a 1 mM solution of $\mathbf{6}^{2+}$. The two spectra are essentially indistinguishable ($[FcCH_2OCH_3^+] = 2$ mM, $\lambda_{max} = 628$ nm, $A = 0.756$; $[\mathbf{6}^{2+}] = 1$ mM, $\lambda_{max} = 628$, $A = 0.770$).

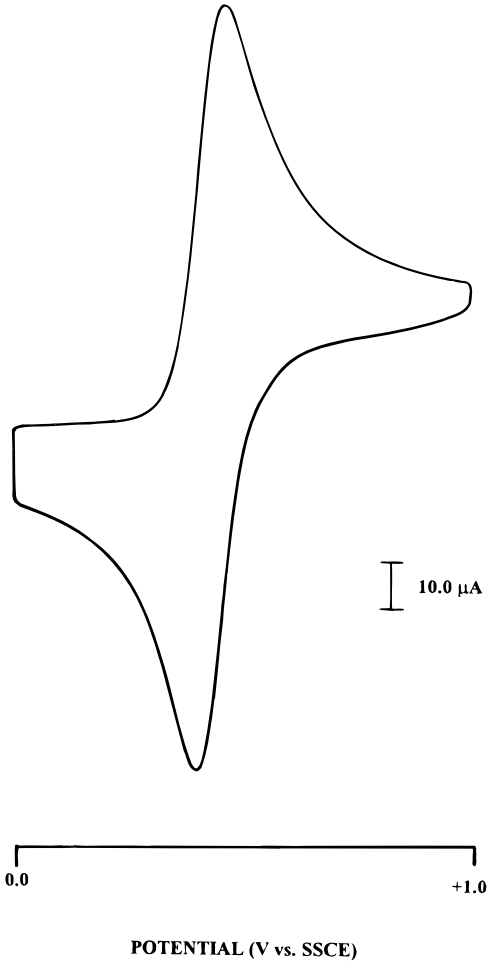

Figure 5. Anodic voltammetric response of 1.0 mM **6** in CH_3CN/0.1 M $TBAPF_6$ at 25 °C. Working electrode: glassy carbon (0.08 cm^2). Scan rate: 0.5 V/s.

A quantitative assessment of oxidation of $FcCH_2OC_{18}H_{37}$, **4**, was done by treating with Ce(IV), sonicating, and then extracting (CH_2Cl_2). Evaporation of the organic solvent followed by chromatography afforded unreacted **4** that amounted to ∼10% (determined by UV analysis) of the original quantity. Coupled with the experiment described above, this suggests that ∼90% of **4** oxidized in water. The remainder (∼10%) of material was presumably nonoxidized, did not aggregate, and was lost during centrifugation and/or filtration. Since the two ferrocene residues of **7**−**10** are electronically uncoupled, we infer that both are oxidized to the ferrocenium cation in high yield.

Rigidifying the Spacer Chain. Even when both ferrocenes are rapidly and simultaneously oxidized, it is still possible that a C-shaped, two-headed monomer could contribute to bilayer formation. In this case, two charged headgroups would be

(21) (a) Polcyn, D. S.; Shain, I. *Anal. Chem.* **1966**, *38*, 370. (b) Flanagan, J. B.; Margel, S.; Bard, A. J.; Anson, F. C. *J. Am. Chem. Soc.* **1978**, *100*, 4248.

(22) For two fully independent (non-communicating) redox sites, theory predicts a difference of 35.6 mV (at 25 °C) between the two potentials: $2 \times 35.6 = 71.2$.

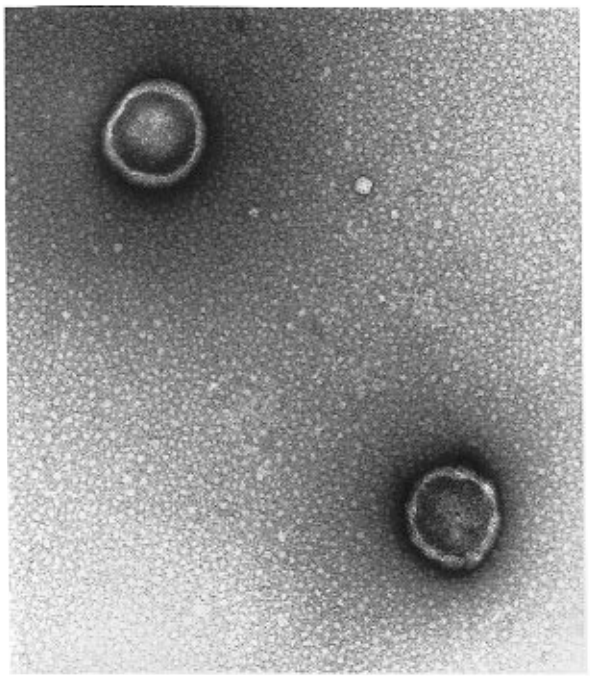

Figure 6. Negative stain electron micrograph of 10^{2+}, magnification 100000× (reproduced at 40% of original size).

associated with a double-stranded tail resulting from spacer chain folding. β-Estradiol was chosen as a spacer chain because is it rigid, it is largely saturated, and its stereochemistry is well known. The bis(ferrocenylmethyl) diether was prepared as described above and oxidized and the suspension sonicated to form vesicles. Laser light scattering showed that vesicles of ~3000 Å diameter were formed. Vesicle formation and size were both confirmed by negative stain electron microscopy (see Figure 6). No qualitative difference between **10** and **6**−**8** was observed during oxidation or sonication, suggesting identical behavior. Since the estradiol framework cannot fold, we infer that the spacer chains of **6**−**8** are likewise extended.

Liposome Stability and Switching. The liposomes that formed were stable for a minimum of two weeks, and most were intact after two months of standing at room temperature in the dark. Qualitatively, the survival times of the single-ferrocene-based aggregates appeared to increase with alkyl chain length.

Dynamic light scattering analysis of the vesicle suspension prepared from oxidized **4** indicated a unimodal diameter at 2450 ± 840 Å. The hydrodynamic sizes judged by intensity and by weight were 2570 ± 830 Å and 2600 ± 980 Å, respectively. A sample of this suspension was diluted 10-fold with water and again subjected to light scattering analysis. The vesicle sizes (unimodal 2340 ± 730 Å; 2250 ± 260 Å by intensity; 2250 ± 260 Å by weight) were the same within experimental error. This is interesting since the concentration of oxidizing agent within the vesicles must exceed that outside by 10-fold.

In the presence of 1mM external NaCl, the vesicle sizes also remained the same (unimodal diameter 2440 ± 820 Å; 2220 ± 330 Å by intensity; 2160 ± 430 Å by weight) within experimental error. In contrast to the effect of NaCl, above, the presence of 500 μM sodium dithionite under anaerobic conditions caused the vesicles to collapse. In this case, the vesicles fused together within 5 min to become large aggregates (mean unimodal diameter ~5.6 × 10^5 Å). Over time, the aggregates enlarged even further, and precipitation became apparent to the unaided eye. After incubation for 3−4 h, an amorphous, cottony-yellow solid separated. The precipitate was extracted with ether. Thin layer chromatographic analysis showed a single spot, identical to starting material. The effects of salt addition on the system are summarized in Table 3.

Table 3. Redox Switching and Salt Effect on Aggregates from Oxidized Ferrocenylmethyl Octadecyl Ether, **4**, by Dynamic Light Scattering Data[a]

	light scattering results		
		cumulant distribution (Å)	
external salt	unimodal diam (Å)	by intensity	by weight
none[c]	2340 ± 730	2250 ± 260	2250 ± 260
none[b]	2450 ± 840	2570 ± 830	2600 ± 980
NaCl	2440 ± 820	2220 ± 330	2160 ± 430
$Na_2S_2O_4$[d]	56100[e]	29000 ± 6500[f]	23100 ± 15000[f]
$Na_2S_2O_4$[g]	N/A	N/A	N/A

[a] All the analytes were 50 μM surfactant except specified cases. The external NaCl concentration was 1 mM. The external $Na_2S_2O_4$ concentration was 0.5 mM. Please see the Experimental Section for the preparation procedure. [b] Original vesicle solution (0.5 mM surfactant). [c] Diluted vesicle solution (50 μM surfactant), and no other reagent was added. [d] 5 min incubation after adding $Na_2S_2O_4$. [e] The distribution is broad. [f] Data here can only be used as reference since some large aggregates (dust) were formed. [g] After 4 h of incubation, cottony-yellow, solid precipitates were observed.

Several points concerning the data in Table 3 are worth noting. First, the vesicles formed from oxidized **4** were surprisingly stable. This was so even after 10-fold dilution, in which case the oxidizing reagent, ammonium cerium(IV) nitrate, was 10-fold more concentrated within the vesicles than outside. In this case, the osmotic pressure was apparently not sufficient to collapse the vesicles. Second, external NaCl (1 mM) did not induce vesicular lysis or electrolyte-induced flocculation of the vesicles. Third, and most important, the reducing agent $Na_2S_2O_4$ can collapse the vesicles at a concentration in water of 500 μM. This is the same sodium concentration present when 1 mM NaCl was used, but the vesicles were stable in the latter case.

Recycling the Monomers. The family of compounds **1**−**5** and **7**−**10** can all be oxidized and sonicated to form stable liposomes. The liposomes have been characterized as described above. In each case, the aggregates have been treated with excess aqueous $Na_2S_2O_4$ and the monomers recovered. The monomers thus obtained can be recycled and oxidized and aggregates formed again from them. The process can be exemplified by using compound **4**. Thus, 10 mL of the aqueous suspension containing **4** was treated with 1 mL of 100 mM aqueous $Na_2S_2O_4$ solution. An immediate color change from blue to yellow was observed, and simultaneously, precipitation commenced. After 10 min, the suspension was centrifuged and washed once with deionized water. After oxidation and sonication as described above, the precipitated monomers again formed vesicles. The vesicles obtained from "recycled" monomers (unimodal diameter at 1440 ± 530 Å; 1660 ± 450 Å by intensity; 1460 ± 440 Å by weight) were slightly smaller than the original vesicular aggregates (unimodal diameter at 2450 ± 840 Å; 2570 ± 830 Å by intensity; 2600 ± 980 Å by weight), but all of the values are within the experimental margin. The color, stability, redox switchability, etc. of these systems did not seem to have been altered by prior use.

Conclusion

We report here the first example of a nonpolar compound that can be converted by oxidation into a bolaamphiphile. Sonication of the amphiphile affords stable liposomes that have been characterized by laser light scattering and negative stain electron microscopy. Contact of the vesicle suspension by a dilute aqueous solution of reducing agent collapses the vesicles and restores the nonpolar, amphiphilic monomers. The process

may be repeated without significant variation in either monomer purity or aggregate structure.

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 $(CH_3)_4Si$ unless otherwise noted. ^{13}C-NMR spectra were recorded at corresponding frequencies. Infrared spectra were recorded on a Perkin-Elmer 1710 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 noted. All reagents were the best 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.

Ferrocenylmethyl Cholestanyl Ether, 1. To [(dimethylamino)-methyl]ferrocene (749 mg, 3 mmol) in acetone (30 mL) was added 1.16 g of dihydrocholesterol followed by addition of methyl iodide (426 mg, 3 mmol). The reaction was heated under reflux for 72 h. The solvent was evaporated, the reaction mixture was diluted with water and extracted 3× with CH_2Cl_2, the organic layers were combined and dried with brine and $MgSO_4$, the solvent was evaporated, and the crude product was chromatographed over silica gel (eluant 1:20 EtOAc/hexanes). The product (119 mg, 7%) was crystallized from acetone to afford a yellow solid. Mp 134−136 °C. $[\alpha]_D^{25} = +11.8°$ (c = 1, CH_2Cl_2). ^{1}H-NMR: 0.64 (s), 0.77 (s), 0.86 (s), 0.87 (s), 0.89 (d, J = 6.4 Hz), 0.45−2.2 (steroidal, 46 H), 3.28 (m, 1 H), 4.14 (s, 5 H), 4.25 (s, 2 H), 4.27 (s, 2 H). IR (KBr): 2950 (d), 2900 (d), 1460 (d), 1100 (m) cm^{-1}. Anal. Calcd for $C_{38}H_{58}OFe$: C, 77.79; H, 9.96. Found: C, 77.89; H, 10.02. ^{13}C-NMR: 84.499, 77.641, 77.216, 69.327, 69.327, 68.409, 68.409, 68.409, 68.409, 68.409, 68.295, 68.295, 65.982, 56.560, 56.340, 54.482, 44.969, 42.633, 40.099, 39.537, 37.095, 36.200, 35.805, 35.540, 34.910, 32.179, 28.925, 28.356, 28.265, 28.022, 24.237, 23.850, 22.818, 22.568, 21.256, 18.692, 12.312, 12.092. DCI mass spectrum: m/z (relative intensity) 587 (<1, M + H$^+$), 586 (<1, M$^+$), 371 (8), 199 (100). $E_{1/2}$ 509 mV (Ag/AgCl, CH_2Cl_2).

Ferrocenylmethyl Tetradecyl Ether, 2. [(Dimethylamino)methyl]-ferrocene (1.46 g, 6 mmol), 1-tetradecanol (1.29 g, 6 mmol), iodomethane (0.85 g, 6 mmol), and K_2CO_3 (3 g, excess) were heated in the mixed solvent of dry CH_3CN (15 mL) and $CH_3CH_3CH_2CN$ (10 mL) at reflux for 24 h. The mixture was cooled to room temperature and concentrated *in vacuo*. The crude mixture was then diluted with H_2O (30 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The organic layers were combined, washed with brine, dried ($MgSO_4$), and concentrated *in vacuo*. Column chromatography (silica gel, EtOAc/hexanes, 1:20 v/v) afforded **2** (0.89 g, 36%) as a yellow solid. Mp 40.5−41.5 °C. ^{1}H-NMR: 0.88 (t, 3 H), 1.27 (s, 22 H), 1.55 (m, 2 H), 3.39 (t, 2 H), 4.12−4.25 (three peaks, 11 H, −OCH_2-ferrocene). Anal. Calcd for $C_{25}H_{40}OFe$: C, 72.80; H, 9.78. Found: C, 72.91; H, 9.80. $E_{1/2}$ 410 mV (Hg/Hg_2Cl_2, CH_3CN).

Ferrocenylmethyl Hexadecyl Ether, 3. [(Dimethylamino)methyl]-ferrocene (1.46 g, 6 mmol), 1-hexadecanol (1.45 g, 6 mmol), iodomethane (0.85 g, 6 mmol), and K_2CO_3 (3 g, excess) were heated in the mixed solvent of dry CH_3CN (15 mL) and $CH_3CH_2CH_2CN$ (10 mL) at reflux for 24 h. The mixture was cooled to room temperature and concentrated *in vacuo*. The crude mixture was then diluted with H_2O (30 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The organic layers were combined, washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. Column chromatography (silica gel, EtOAc/hexanes, 1:20 v/v) afforded **3** (1.01 g, 39%) as a yellow solid. Mp 43−44 °C. ^{1}H-NMR: 0.88 (t, 3 H), 1.26 (s, 26 H), 1.57 (m, 2 H), 3.39 (t, 2 H), 4.12−4.25 (three peaks 11 H, −OCH_2-ferrocene). Anal. Calcd for $C_{27}H_{44}OFe$: C, 73.62; H, 10.07. Found: C, 73.66; H, 10.13. $E_{1/2}$ 405 mV (Hg/Hg_2Cl_2, CH_3CN).

Ferrocenylmethyl Octadecyl Ether, 4. [(Dimethylamino)methyl]-ferrocene (1.46 g, 6 mmol), 1-hexadecanol (1.89 g, 7 mmol), iodomethane (0.85 g, 6 mmol), and K_2CO_3 (3 g, excess) were heated in the mixed solvent of dry CH_3CN (15 mL) and $CH_3(CH_2)_2CN$ (10 mL) at reflux for 48 h. The mixture was cooled to ambient temperature and concentrated *in vacuo*. The crude mixture was then diluted with H_2O (30 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The organic layers were combined, washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. Column chromatography (silica gel, EtOAc/hexanes 1:20 v/v) afforded pure **4** (2.28 g, 81%) as a yellow solid. Mp 51−53 °C. ^{1}H-NMR: 0.88 (t, 3 H), 1.26 (s, 30 H), 1.57 (m, 2 H), 3.39 (t, 2 H), 4.12−4.25 (three peaks 11 H, −OCH_2-ferrocene). Anal. Calcd for $C_{29}H_{48}OFe$: C, 74.34; H, 10.33. Found: C, 74.14, H, 10.41. $E_{1/2}$ 410 mV (Hg/Hg_2Cl_2, CH_3CN).

(Ferrocenylmethyl)di-*n*-octadecylamine, 5. To ferrocenylmethanol (1 g, 4.6 mmol) in THF (25 mL) was added freshly distilled PCl_3 (0.18 mL, 2.06 mmol) followed by addition of pyridine (0.37 mL, 4.6 mmol). The reaction was allowed to stir at ambient temperature for 8 h. The reaction mixture was filtered, and the resulting mixture was used directly in the next step.

To the filtered solution (above) were added K_2CO_3 (3 g, excess), dioctadecylamine (2.4 g, 4.6 mmol), and distilled benzene (15 mL). The reaction mixture was heated overnight under reflux. The mixture was cooled and filtered, and the solvent was evaporated *in vacuo*. The crude product was shaken with CH_2Cl_2 (40 mL) and H_2O (30 mL), dried ($MgSO_4$), and then chromatographed over a column of silica gel (eluant EtOAc/hexanes, 1:40 v/v) to afford **5** (1.24 g, 37%) as a waxy, yellow solid. Mp 41.5−42.5 °C. ^{1}H-NMR: 0.88 (t, 6 H), 1.26 (s, 60 H), 1.42 (br, 4 H), 2.29 (t, 4 H), 3.48 (s, 2 H), 4.08 (s, 2H), 4.09 (s, 5 H), 4.11 (s, 2H). Anal. Calcd for $C_{47}H_{85}NFe$: C, 78.40; H, 11.90; N, 1.95. Found: C, 78.28, H; 11.90; N, 1.93. $E_{1/2}$ 443 mV (Hg/Hg_2Cl_2, CH_2Cl_2).

1,8-Bis(1-ferrocenylmethoxy)octane, 6. [(Dimethylamino)methyl]-ferrocene (1.17 g, 4.8 mmol), 1,8-octanediol (293 mg, 2 mmol), iodomethane (0.30 mL, 4.8 mmol), and K_2CO_3 (2 g, excess) were heated in $CH_3(CH_2)_2CN$ (40 mL) at reflux under N_2 for 48 h. The mixture was cooled to room temperature and filtered through a short silica gel bed (EtOAc/hexanes, 1:5 v/v). The fractions containing product were collected and concentrated. The crude product obtained was dissolved and purified by flash chromatography (silica gel, EtOAc/hexanes, 1:10 v/v) to afforded a yellow solid. Recrystallization from EtOAc and MeOH gave **6** (760 mg, 70%). Mp 92−95 °C. ^{1}H NMR: 1.26 (s, 8 H), 1.53 (m, 4 H), 3.39 (t, 4 H), 4.13−4.25 (three peaks, 22 H, −OCH_2-ferrocene). Anal. Calcd for $C_{30}H_{38}O_2Fe_2$: C, 66.44; H, 7.06. Found: C, 66.56; H, 6.90. $E_{1/2}$ 418 mV (Hg/Hg_2Cl_2, CH_3CN).

1,12-Bis(1-ferrocenylmethoxy)dodecane, 7. [(Dimethylamino)-methyl]ferrocene (1.17 g, 4.8 mmol), 1,12-dodecanediol (405 mg, 2 mmol), iodomethane (0.30 mL, 4.8 mmol), and K_2CO_3 (2 g, excess) were heated in $CH_3(CH_2)_2CN$ (40 mL) at reflux for 48 h. The mixture was cooled to room temperature and filtered through a short silica gel bed (EtOAc/hexanes, 1:5 v/v). The fractions containing product were collected and concentrated. The crude product obtained was purified by flash chromatography (silica gel, EtOAc/hexanes, 1:10 v/v) to afford a yellow solid. Recrystallization from EtOAc and MeOH gave **10** (860 mg, 72%). Mp 96−98 °C. ^{1}H-NMR: 1.24 (s, 16 H), 1.54 (m, 4 H), 3.39 (t, 4 H), 4.13−4.26 (three peaks, 22 H, −OCH_2-ferrocene). Anal. Calcd for $C_{34}H_{46}O_2Fe_2$: C, 68.24; H, 7.75. Found: C, 68.37; H, 7.70. $E_{1/2}$ 420 mV (Hg/Hg_2Cl_2, CH_3CN).

1,16-Bis(ferrocenylmethyl) Hexadecyl Diether, 8. [(Dimethylamino)methyl]ferrocene (1.3 g, 5.33 mmol), 1,16-hexadecandiol (0.92 g, 3.55 mmol), iodomethane (0.76 g, 5.33 mmol), and K_2CO_3 (3 g, excess) were heated in dry CH_3CN (15 mL) and $CH_3(CH_2)_2CN$ (10 mL) at reflux for 48 h. The mixture was cooled to room temperature and concentrated *in vacuo*. The crude product was then diluted with H_2O (30 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The organic layers were combined, washed with brine, dried ($MgSO_4$), and concentrated *in vacuo*. Column chromatography (silica gel, EtOAc/

hexanes, 1:5 v/v) afforded **6** (0.53 g, 23%) as a yellow solid. Mp 85−87 °C. ^{1}H-NMR: 1.23 (s, 24 H), 1.54 (m, 4 H), 3.38 (t, 4 H), 4.17−4.27 (three peaks, 22 H, $-OCH_2$-ferrocene). Anal. Calcd for $C_{25}H_{40}OFe$: C, 72.80; H, 9.78. Found: C, 72.91, H; 9.80. $E_{1/2}$ 425 mV (Hg/Hg_2Cl_2, CH_2Cl_2).

A byproduct presumed to be $FcCH_2O(CH_2)_{16}OH$ was detected by TLC but was not isolated.

1,22-Docosanedioyldiferrocene. A solution of docosanedioic acid (2.22 g, 6 mmol) in $SOCl_2$ (25 mL) was heated at reflux overnight. Excess $SOCl_2$was evaporated to afford a light yellow solid, docosanedioyl dichloride, which was used without further purification.

The docosanedioyl dichloride (above) was dissolved in CH_2Cl_2 (15 mL) and slowly transferred to a suspension of $AlCl_3$ (933 mg, 7 mmol) in CH_2Cl_2 (15 mL). The solution thus obtained was added dropwise (2 h) to a solution of ferrocene (4.46 g, 24 mmol) in CH_2Cl_2 (30 mL) with stirring. After 12 h, the reaction mixture was poured onto ice (100 g). The organic phase was washed (water) until neutral, dried ($MgSO_4$), and then chromatographed (flash, silica gel, EtOAc/$CHCl_3$/hexanes, 1:2:10 to 1:2:5 v/v/v) to afford an orange solid. Crystallization from EtOAc and MeOH gave 1,22-bis(ferrocenylcarbonyl)docosane (530 mg, 13%). Mp 88.5−90.5 °C. ^{1}H-NMR: 1.26 (m, 32 H), 1.70 (m, 4 H), 2.70 (t, 4 H), 4.20 (s, 10 H, −ferrocene), 4.49 (t, 4 H, −ferrocene), 4.78 (t, 4 H, −ferrocene). Anal. Calcd for $C_{42}H_{58}O_2Fe_2$: C, 71.39; H, 8.27. Found: C, 71.30; H, 8.31.

1,22-Diferrocenyldocosane, 9. Zinc (850 mg, 13mmol) was amalgamated by stirring with mercuric chloride (65 mg, 0.24 mmol), H_2O (1.3 mL) and concentrated HCl (0.05 mL) for 5 min. The aqueous phase was removed and then H_2O (1.5 mL), concentrated HCl (3 mL), toluene (15 mL), and 1,22-bis(ferrocenylcarbonyl)docosane (106 mg, 0.15 mmol) were added quickly in that order. The mixture was heated at reflux for 4 h. The amalgam was removed by filtration and washed with ether. The combined organic phase was washed with H_2O until neutral and dried over $MgSO_4$. The crude product was purified by flash chromatography (silica gel, hexanes) to afford a yellow solid. Crystallization from EtOAc and MeOH gave **9** (30 mg, 29%). Mp 100−102 °C. ^{1}H-NMR: 1.25 (s, 36 H), 1.48 (m, 4 H), 2.27 (t, 4 H), 4.09−4.14 (three peaks, 18 H, −ferrocene). Anal. Calcd for $C_{42}H_{62}Fe_2$: C, 74.33; H, 9.21. Found: C, 74.30; H, 9.22. $E_{1/2}$ 460 mV (Hg/Hg_2Cl_2, DMF).

Bis(Ferrocenylmethyl)-β-Estradiol Diether, 10. [(Dimethylamino)methyl]ferrocene (1.17 g, 4.8 mmol), β-estradiol (545 mg, 2 mmol), iodomethane (0.30 mL, 4.8 mmol), and K_2CO_3 (2 g, excess) were heated in $CH_3(CH_2)_2CN$ (40 mL) at reflux for 48 h. The mixture was cooled to room temperature and filtered through a short silica gel bed (EtOAc/hexanes, 1:5 v/v). The fractions containing product were collected and concentrated. The crude product obtained was dissolved in CH_2Cl_2 and purified by flash chromatography (silica gel, twice, EtOAc/hexanes, 1:10 v/v) to afford a yellow solid. Recrystallization from EtOAc and MeOH gave **10** (230 mg, 17%). Mp 141−143 °C. ^{1}H-NMR: 0.81 (s, 3 H, CH_3 on C-13), 1.15−2.32 (m, 13 H), 2.83 (br m, 2 H, CH_2-16), 3.47 (t, 1 H, CH-17), 4.14−4.31 (four peaks, 20 H, $-OCH_2$-ferrocene on C-17 and −ferrocene of -OCH_2ferrocene on C-3), 4.75 (s, 2 H, CH_2 of $-OCH_2$-ferrocene on C-3), 6.68 (d, 1 H, CH-3), 6.77 (dd, 1 H, CH_2), 7.19 (d, 1 H, CH-1). Anal. Calcd for $C_{40}H_{44}O_2Fe_2$: C, 71.87; H, 6.63. Found: C, 71.88; H, 6.63. $E_{1/2}$ 442 mV (Hg/Hg_2Cl_2, CH_3CN).

1,1-Ferrocenediyl Carboxyheptadecanoate, 11. To 620 mg (2 mmol) of 1,1′-bis(chlorocarbonyl)ferrocene in 15 mL of benzene, at ambient temperature, was added a suspension of heptadecanol (1.024 g, 4 mmol) and Et_3N (404 mg, 4 mmol) in benzene (10 mL) followed by addition of 4-(dimethylamino)pyridine (50 mg). The reaction mixture was heated at reflux temperature for 5 h. The solvent was evaporated, and the resulting solid was suspended in CH_2Cl_2 and then chromatographed over silica gel (eluant 1:20 EtOAc/hexanes). The product **11** was isolated and recrystallized from acetone to afford the diester (1.03 g, 69%) as a yellow solid. Mp 65−67 °C. ^{13}C-NMR: 170.509, 76.694, 73.099, 72.803, 72.803, 71.430, 71.430, 64.625, 31.930, 29.708, 29.708, 29.708, 29.708, 29.708, 29.677, 29.677, 29.639, 29.617, 29.374, 29.344, 28.858, 26.059, 22.698, 14.134. ^{1}H-NMR: 0.88 (t, J = 6.4 Hz, 6 H), 1.15−1.60 (m, 56 H), 1.72 (quintet, 4 H), 4.21 (t, J = 6.7 Hz, 4 H), 4.40 (s, 4 H), 4.82 (d, J = 1.6 Hz, 4 H). IR (KBr): 2880 (m), 1715 (br s), 1280 (d), 1160 (d) cm^{-1}. Anal. Calcd for $C_{46}H_{78}O_4Fe$: C, 73.57; H, 10.47. Found: C, 73.52, H, 10.52. DCI mass spectrum: m/z (relative intensity) 752 (100, M + H$^+$), 751 (70, M$^+$), 495 (78), 257 (13), 251 (18), 239 (21). $E_{1/2}$ 972 mV (Ag/AgCl, CH_2Cl_2).

***N,N′*-dioctadecylferrocene-1,1′-dicarboxamide, 12**. This compound was previously synthesized by Fujihara and co-workers.[21] To 620 mg (2 mmol) of 1,1′-bis(chlorocarbonyl)ferrocene in 20 mL of benzene at ambient temperature was added a suspension of *n*-octadecylamine (1.076 g, 4 mmol) and Et_3N (404 mg, 2 mmol) in 10 mL of benzene. The reaction mixture was allowed to stir for 5 h, and then the solvent was evaporated and the crude product chromatographed over silica with 1:20 EtOAc/hexanes. The product was then recrystallized from a mixture of acetone and CH_2Cl_2 to afford **12** (993 mg, 64%) as a yellow solid. Mp 105−107 °C. ^{13}C-NMR: 170.1, 133.301, 127.521, 127.521, 95.706, 70.733, 70.733, 39.911, 31.909, 29.785, 29.686, 29.686, 29.686, 29.686, 29.625, 29.625, 29.383, 29.337, 27.069, 22.662, 14.082. ^{1}H-NMR: 0.87 (t, J = 7.0 Hz, 6 H), 1.12−1.46 (56 H), 1.56−1.70 (m, 8 H), 3.39 (q, J_{ab} = 6.7 Hz, J_{ac} = 6.8 Hz, 4 H), 4.36 (d, J = 1.5 Hz, 4 H), 4.47 (d, J = 1.5 Hz, 4 H), 6.74 (t, J = 5.3 Hz, 2 H). IR (KBr): 3400 (s), 2910 (d), 1650 (s), 1550 (s) cm^{-1}. Anal. Calcd for $C_{48}H_{84}N_2O_2Fe$: C, 74.19; H, 10.90. Found: C, 74.01; H, 10.93. DCI mass spectrum: m/z (relative intensity) 778 (100, M + 2H$^+$), 777 (85, M + H$^+$), 776 (65, M+), 537 (4), 362 (44), 360 (23), 296 (10), 294 (15), 241 (10). $E_{1/2}$ 806 mV (Ag/AgCl, CH_2Cl_2).

1,1′-bis[(Octadecylamino)carbonyl]ferrocene, 13, was prepared by a modification of the procedure reported by Fujihara and co-workers.[23] To 620 mg (2 mmol) of 1,1′-bis(chlorocarbonyl)ferrocene in benzene (20 mL) at ambient temperature was added a suspension of *n*-octadecylamine (1.076 g, 4 mmol) and Et_3N (404 mg, 2 mmol) in benzene (10 mL). The reaction mixture was allowed to stir for 5 h, the solvent was evaporated, and the crude material was chromatographed over silica gel (eluant 1:20 EtOAc/hexanes). The product was crystallized from a mixture of acetone and CH_2Cl_2 to afford the diamide (993 mg, 64%) as a yellow solid. Mp 105−107 °C. ^{13}C-NMR: 170.1, 133.301, 127.521, 127.521, 95.706, 70.733, 70.733, 39.911, 31.909, 29.785, 29.686, 29.686, 29.686, 29.686, 29.625, 29.625, 29.383, 29.337, 27.069, 22.662, 14.082. ^{1}H-NMR: 0.87 (t, J = 7.0 Hz, 6 H), 1.12−1.46 (56 H), 1.56−1.70 (m, 8 H), 3.39 (q, J_{ab} = 6.7 Hz, J_{ac} = 6.8 Hz, 4 H), 4.36 (d, J = 1.5 Hz, 4 H), 4.47 (d, J = 1.5 Hz, 4 H), 6.74 (t, J = 5.3 Hz, 2H). IR (KBr): 3400 (s), 2910 (d), 1650 (s), 1550 (s) cm^{-1}. Anal. Calcd for $C_{48}H_{84}N_2O_2Fe$: C, 74.19; H, 10.90. Found: C, 74.01; H, 10.93. DCI mass spectrum: m/z (relative intensity) 778 (100, M + 2H$^+$), 777 (85, M + H$^+$), 776 (65, M$^+$), 537 (4), 362 (44), 360 (23), 296 (10), 294 (15), 241 (10). $E_{1/2}$ 711 mV (Ag/AgCl, CH_2Cl_2).

1,1′-Ferrocenediyl Carboxydihydrocholesterate, 14. To 620 mg (2 mmol) of 1,1′-bis(chlorocarbonyl)ferrocene in 20 mL of benzene at ambient temperature was added dropwise a solution of dihydrocholesterol (1.55 g, 4 mmol), 50 mg of 4-(dimethylamino)pyridine (DMAP), and (404 mg, 4 mmol) of Et_3N in 10 mL of benzene. The reaction mixture was then allowed to reflux for 48 h. The solvent was then evaporated under reduced pressure and the residue dissolved in CH_2Cl_2 and chromatographed over silica using 1:20 EtOAc/hexanes. Crystallization from CH_2Cl_2/Et_2O afforded **14** (668 mg, 27%). Mp 287−291 °C. $[\alpha]_D^{25}$ = +24.4° (c = 1, CH_2Cl_2). ^{13}C-NMR: 170.028, 127.525, 73.749, 73.377, 72.944, 72.944, 71.473, 71.367, 56.453, 56.309, 54.298, 44.793, 42.616, 40.022, 39.521, 36.843, 36.183, 35.812, 35.546, 35.523, 34.317, 32.034, 28.711, 28.249, 28.006, 27.786, 24.220, 23.856, 22.802, 22.552, 21.247, 18.675, 12.356, 12.098. ^{1}H-NMR: 0.52−2.1 (steroidal, 46 H) 0.67 (s, 6 H), 0.85 (d, J = 1.6 Hz, 12 H), 0.87 (s, 6 H), 0.88 (s, 6 H), 0.91 (d, J = 6.4 Hz, 6 H), 4.36 (d, J = 1.6 Hz, 4 H), 4.79 (d, J = 2 Hz, 4 H), 4.85 (m, 2 H). IR (KBr): 2960 (br s), 2880 (d), 1715 (s) 1470 (br s), 1290 (s), 1160 (s) cm^{-1}. Anal. Calcd for $C_{66}H_{102}O_4Fe$: C, 78.07; H, 10.12. Found: C, 77.99; H, 10.13. DCI mass spectrum: m/z (relative intensity) 1016 (6, M + H$^+$), 1015 (15, M$^+$), 646 (31), 628 (8), 372 (60), 371 (57), 370 (81), 275 (83), 257 (72), 57 (100). $E_{1/2}$ 945 mV (Ag/AgCl, CH_2Cl_2).

1,1′-Ferrocenediyl Carboxycholesterate, 15. To a red, homogeneous solution of 1,1′-bis(chlorocarbonyl)ferrocene (620 mg, 2 mmol) in 10 mL of benzene at ambient temperature was added a solution of 1.54 g (4 mmol) of cholesterol in 10 mL of benzene with 404 mg (4 mmol) of Et_3N and 50 mg of 4-(dimethylamino)pyridine. The reaction

(23) (a) Fujihara, M.; Nishiyama, K.; Yamada, H. *Thin Solid Films* **1985**, *132*, 77; *Chem. Abstr.* **1986**, *105*, 106687f. (b) Nakahara, H.; Katoh, T.; Sato, M.; Fukuda, K. *Thin Solid Films* **1988**, *160*, 153.

mixture was then heated under reflux for 32 h, after which the solvent was evaporated and the residue was suspended in warm CH_2Cl_2 and then chromatographed over silica with 1:20 EtOAc/hexanes. The fractions containing the product were evaporated and recrystallized from CH_2Cl_2/Et_2O to afford **15** (920 mg, 45%) as a yellow solid. Mp 267−269 °C. $[\alpha]_D^{25} = -0.44$ ($c = 4$ CH_2Cl_2). ^{13}C-NMR: 169.863, 139.747, 122.724, 74.045, 73.3552, 72.9077, 72.847, 71.4891, 71.3677, 56.7574, 56.2188, 50.097, 42.3518, 39.7878, 39.5523, 38.3844, 37.0797, 36.6549, 36.2149, 35.8432, 31.944, 31.8909, 28.2649, 28.0753, 28.0297, 24.3051, 23.9182, 22.8258, 22.5755, 21.0811, 19.4274, 18.7371, 11.887. ^{1}H-NMR: 0.69 (s, 6 H), 0.87 (d, $J = 8.0$ Hz, 12 H), 0.92 (d, $J = 6.5$ Hz, 6 H), 1.07 (s, 6 H), 0.95−2.09 (steroidal, 58 H), 2.44 (d, $J = 7.6$ Hz, 4 H), 4.39 (t, $J = 1.6$ Hz, 4 H), 4.75 (m, 2 H), 4.81 (d, $J = 2.0$ Hz, 4 H), 5.41 (m, 2 H). IR (KBr): 2920 (br s), 1715 (br s), 1258 (s), 1149 (s) cm^{-1}. Anal. Calcd for $C_{66}H_{98}O_4Fe$: C, 78.38; H, 9.77. Found: C, 78.28; H, 9.82. DCI Mass spectrum: *m/z* (relative intensity) 1012 (2, M + H$^+$), 1011 (6, M$^+$), 643 (6), 625 (1), 370 (100), 369 (48), 368 (58), 274 (11), 257 (7). $E_{1/2}$ 711 mV (Ag/AgCl, CH_2Cl_2).

Ferrocenylmethyl Methyl Ether. To DMSO (10 mL) was added powered KOH (224 mg, 4 mmol). After stirring for 5 min, commercial ferrocenylmethanol (216 mg, 1mmol) was added, followed immediately by CH_3I (284 mg, 2 mmol). Stirring was continued for 1 h. The mixture was then poured into water (30 mL) and extracted by CH_2Cl_2 (3×30 mL). The organic layers were combined, dried over $MgSO_4$, and concentrated *in vacuo*. Column chromatography (silica, EtOAc/hexanes, 1:10 v/v) afford ferrocenylmethyl methyl ether (218 mg, 95%) as a yellow oil. ^{1}H-NMR: 3.32 (s, 3 H), 4.14−4.23 (m, 11 H, $-OCH_2$-ferrocene).

Determination of Redox Potentials. The electrochemical experiments were performed in CH_3CN (distilled from CaH_2) solution containing 0.1 M tetrabutylammonium hexafluorophosphate (Fluka) as the supporting electrolyte. The single-compartment cell was fitted with a Bioanalytical Systems glassy carbon working electrode (0.08 cm^2), a platinum counter electrode, and a sodium chloride saturated calomel reference electrode (SSCE) built in-house. A Princeton Applied Research Model 175 universal programmer, Model 173 potentiostat, and Model 179 digital coulometer (equipped with positive feedback circuitry for IR compensation) and a Soltex VP-6423S X-Y recorder were used to obtain and record the voltammograms. The analyte concentration was maintained at 1 mM for all experiments. The working electrode was polished immediately before each experiment, and the electrochemical cell was maintained under N_2 gas throughout the experiments. A small amount of activated, neutral alumina was added to the analyte solution to remove traces of moisture.

UV−Vis Study. Ferrocenylmethyl methyl ether (4.6 mg) was oxidized by ammonium cerium(IV) nitrate (55 mg) in water (2 mL) under bath sonication and N_2 for 15 min. The solution was then diluted with water (8 mL) to form 2 mM oxidized ferrocenylmethyl methyl ether solution. The UV−vis absorbance was measured. The same procedure was used to oxidize **6** except that a 1 mM solution was prepared. The UV−vis absorbance was measured for both solutions.

Vesicle Formation for the Neutral Species. Compounds were dissolved in CH_2Cl_2 (∼2 mL) in a sample vial, and the solvent was slowly removed by purging with N_2 gas and drying under high vacuum for 1 h. The sample was then sonicated in deionized water (0.5 mM in concentration) by a Branson cell disruptor (Model 185) at 30 W (90% duty cycle) with a tip sonicator in an ice bath for 30 min. The suspension was centrifuged or filtered through a 1.0 μm nucleopore polycarbonate membrane. The suspension was characterized by a particle analyzer (Coulter Model N4MD) at 20 °C and 90° (angle) for 200 s.

Vesicle Formation for the Oxidized Species. The ferrocene derivative (5 μmol) was suspended in deionized and deoxygenated water (2 mL) in a 15 mL test tube under nitrogen. Ammonium cerium(IV) nitrate (25 μmol) was added to oxidize the ferrocene derivative, and the test tube was immersed in a water bath sonicator (Branson 1200) for 10−30 min. The suspension was diluted (8 mL of deionized and deoxygenated water) and sonicated using a Branson cell disruptor (Model 185) at 30 W (90% duty cycle) with a tip sonicator in an ice bath under nitrogen for 30 min. The suspension was then centrifuged or filtered through a 1.0 μm nucleopore polycarbonate membrane. It was characterized by following the procedure above. Ferrocenylmethyl cholestanyl ether was oxidized in CH_2Cl_2 by using aqueous ammonium cerium(IV) nitrate solution. After drying (N_2 purge), the oxidized compound was further dried under high vacuum (2 h). It was then diluted with 10 mL of deionized and deoxygenated water. Vesicles were then prepared as referenced.

Redox-Switching Ferrocene Derivative Vesicles. A 100 mM aqueous solution of $Na_2S_2O_4$ was prepared in deionized and deoxygenated water. The solution was clarified by filtration through a 0.2 μm nucleopore polycarbonate membrane.

An aliquot (15 μL) of 100 mM $Na_2S_2O_4$ or 200 mM NaCl solution was added to deionized and deoxygenated water (2.7 mL). The solutions were vortexed to ensure the homogeneity. Then, a stock vesicle suspension (300 μL, 0.5 mM) was added to each of the above solutions. After vortexing, the vesicle suspensions were incubated and then analyzed by dynamic laser light scattering.

In order to confirm that monomers obtained from the collapsed vesicles could be reused, a vesicle suspension (10 mL, 0.5 mM surfactant) was prepared in a 15 mL test tube. The suspension was centrifuged, the supernatant was transferred to another 15 mL test tube, and 1 mL of 100 mM aqueous $Na_2S_2O_4$ solution was added. The color of the vesicle suspension immediately began to change from blue to yellow, and precipitation began. After 10 min, the suspension was centrifuged and washed once with deionized water. The suspension was then oxidized and sonicated as done for the original vesicles, and liposomes were obtained again. After centrifuging and filtering, the suspension was analyzed by dynamic light scattering.

Negative Stain Electron Microscopy. Vesicle suspensions were prepared for viewing by transmission electron microscopy using two different methods. These are described below. The important difference between the two methods is as follows. Using method 1, clear membrane boundaries are observed between the internal and external areas of the vesicle, but the efficiency of mechanically adhering aggregates to the EM grids is poor. Adhering aggregates to the grids is more efficient by using the second method, but the definition of membrane boundaries is poorer. In either case, after preparation, the grids were allowed to air dry and observations were then made with a Hitachi H-600 transmission electron microscope operated at 75 kV.

Method 1. Bacitracin solution (0.024%, 10 μL) was added to the vesicle suspension (50 mL), the resulting suspension was mixed, and 10−15 mL of the resulting suspension was applied to Butvar/carbon-coated, 400-mesh copper grids. A period of 1−5 min was allowed to permit attachment of vesicles. Excess fluid was then wicked off the grid by touching their edges to filter paper while leaving a thin film of fluid on the grids. Uranyl acetate (1%, 10−12 mL) was applied to the grid. After 15−30 s, the stain was wicked off with filter paper to leave a thin film of liquid. The grids were then allowed to air dry.

Method 2. Bacitracin solution (0.024%, 10 μL) was added to the vesicle suspension (50 mL). The modified vesicle samples were then mixed with 1% uranyl acetate stain in a ratio of 2 parts of sample to 1 part of stain. The vesicle/stain solutions (10−12 mL) were applied to Butvar/carbon-coated grids, allowed to stand for 1−5 min, and then wicked off with filter paper, leaving a thin film of fluid.

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