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REDOX-SWITCHED AMPHIPHILES: OXIDIZED FERROCENE DERIVATIVES FORM STABLE VESICLES WHEN EITHER ONE OR TWO ALKYL TAILS ARE PRESENT

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Thirty ferrocene derivatives were prepared and their ability to form vesicles in aqueous solution when oxidized was assessed. The compounds included alkyl ferrocenylmethyl ether derivatives of the form $C_{10}H_9FeCH_2OR$ in which R = octyl, dodecyl, tetradecyl, hexadecyl, octadecyl and eicosanyl. One single-tailed amine derivative, $C_{10}H_9FeCH_2NR_2$, R = octadecyl, was studied. Alkylferrocene derivatives had the form $C_{10}H_9FeR$ in which R = butyl, decyl, tetradecyl, hexadecyl, octadecyl, eicosanyl and docosanyl. Sixteen symmetrical 1,1′-disubstituted ferrocenes were also studied. Three ethers were of the form $C_{10}H_8Fe\text{-}1,1'\text{-}(CH_2OR)_2$, R = tetradecyl, hexadecyl and octadecyl. Four corresponding dialkyl derivatives of the form $C_{10}H_8Fe\text{-}1,1'\text{-}R_2$, R = decyl, tetradecyl, hexadecyl and octadecyl, were assessed. Finally, a range of 1,1′-disubstituted ferrocene derivatives were analyzed. These all had the form $C_{10}H_8Fe\text{-}1,1'\text{-}(COR)_2$, for which R has the following identities: octyl, tridecyl, pentadecyl and heptadecyl (ketones); heptadecyloxy, 3-cholesteryl and 3-cholestanyl (esters); and two amides, $R = NHC_{18}H_{37}$ and $N(C_{18}H_{37})_2$. The alkyl and ether derivatives could be readily oxidized and formed vesicular aggregates upon sonication. The ketones, esters and amides could be oxidized but the ferricenium derivatives did not form stable aggregates. An interesting observation is that the aggregates formed were vesicular whether the ferrocene derivative had one or two alkyl tails. © 1997 by John Wiley & Sons, Ltd.

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INTRODUCTION

All cells and organelles are bounded by membranes. The formation of these natural cellular barriers is one of the most ubiquitous examples of molecular recognition. Biomembranes are generally formed from phospholipids and include proteins and other amphiphilic molecules.[1] Natural phospholipids are derived from glycerol and fatty acids, and are usually terminated in an alcohol such as ethanolamine, choline or inositol. Substantial variations are known, however.[2] For example, the excitable cardiac membrane contains vinyl ethers in amphiphiles known as plasmalogens.[3] A bewildering array of membranes is possible by mixing and matching fatty acids at positions 1 and 2 of glycerol along with variations in the phosphoryl ester at position 3. All of these variations and mixtures of the variations make possible an immense variety of membrane properties, although not all desired properties have yet been achieved.

Synthetic amphiphiles can also be produced in a remarkable variety. The properties of aggregates formed from these diverse monomers will certainly reflect the chemical structures of the amphiphiles but such properties are currently impossible to predict with any accuracy. An important potential application for vesicles is as drug delivery capsules in which a therapeutic agent may be a carrier into the bloodstream. An alternative to trying to develop liposomes that might have the correct balance of properties to be effective in such an application was to develop mechanisms by which the aggregation behavior could be altered by a chemical switch.

In previous work, we have demonstrated that a variety of organometallic residues can serve as headgroups for amphiphiles. These include the ferricenium cation (from **1**), nickel phenanthroline (**3**) and silver and copper amine complexes (**4**–**7**). The ferricenium cation differs from the other two species in that alkylferrocenes are not normally[4] amphiphilic. Thus, sonication of most of the alkylferrocenes described here led to no evidence of aggregation. The nickel phenanthroline and copper amine complexes readily formed

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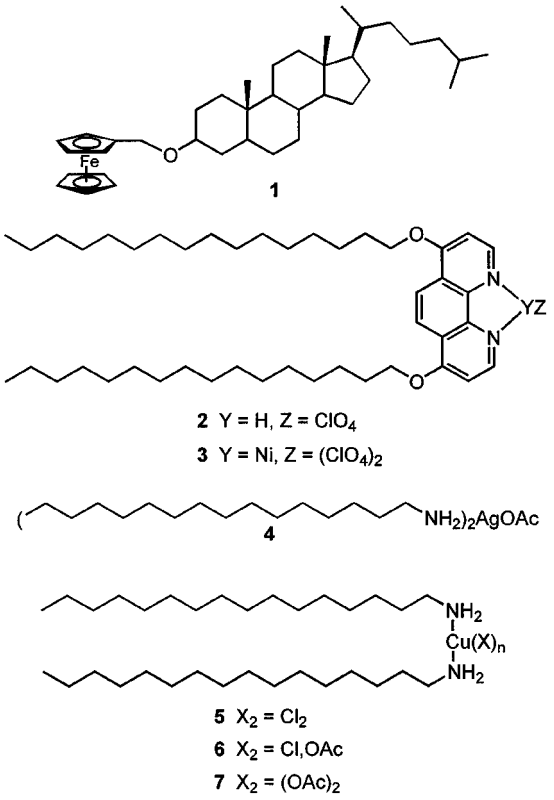
vesicles. We may consider an alkylferrocene to be a 'pro-amphiphile' which can be converted by a simple chemical switch into an amphiphilic monomer. Thus, oxidation of an alkylferrocene (Ce^{IV} or electrochemically) leads to an alkylferricenium salt that aggregates into stable vesicles. An example of switching in aggregates was reported by Monserrat *et al*.,[5] who demonstrated photoinduced processes in copper(II)–crown ether surfactant micelles. In this case, a redox reaction occurred in the micelle, but the aggregates themselves were not redox-switchable.

We present here a survey of 30 ferrocene compounds, most of which form stable aggregates in water after oxidation and sonication. This group of structures represents a systematic survey of several families of compounds that can afford ferrocenyl amphiphiles upon oxidation. This range of compounds is especially interesting because vesicles were formed after oxidation from this group of alkylferricenium derivatives whether one or two alkyl tails are present. To our knowledge, no example of this has previously been reported.



Structures 1–7

RESULTS AND DISCUSSION

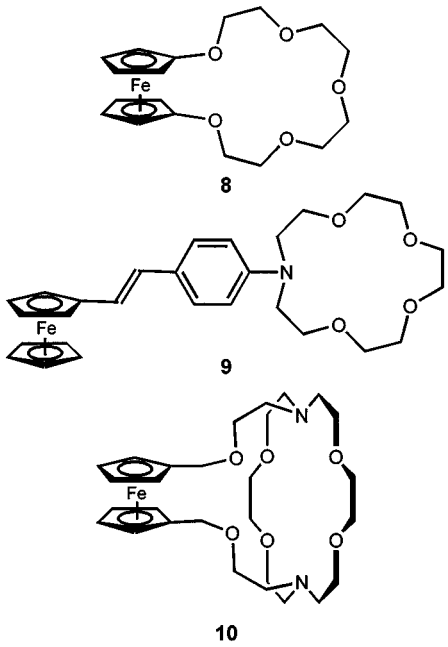
Choice of ferrocene as the redox switch

Ferrocene is attractive as a potential redox switch since it contains an integral metal, formally in the Fe^{II} state, it dissolves readily in hydrocarbon solvents such as hexanes, and is relatively non-polar. When it is oxidized at modest potentials, the blue, relatively stable ferricenium cation is formed. An alkylferrocene may thus be oxidized to an amphiphilic structure.

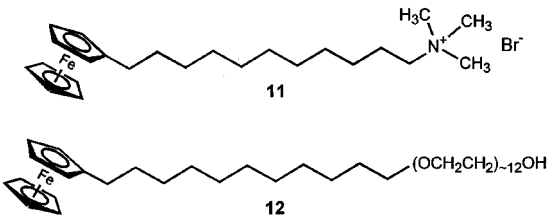
Ferrocene has been used previously in switching applications. A crown ether containing a ferrocene unit, pentaoxa[13]ferrocenophane, **8**, was reported by Saji and Kinoshita.[6] Electrochemical studies showed an abrupt decrease in its cation binding constants by electrochemical oxidation of ferrocene. This could be explained by the electrostatic repulsion between the positive charge on the ferrocene moiety and the metal cation. Beer *et al*.[7] reported the preparation of redox-responsive azacrown ethers conjugated to a ferrocene moiety. Complexation of cations by **9** shifted ferrocene's oxidation wave to more positive potentials. A ferrocenylcryptand, **10**, devised by our group permitted redox-switchable complexation of a variety of cations including micromolar Ag^+ in water.[8]

Redox-switchable molecular aggregates

Several groups have reported redox reactions of ferrocene inside membranes but not redox-switchable membranes.[9] Saji *et al*.[10] reported that *N,N,N*-(trimethyl)-11-aminounde-



Structures 8–10



Structures 11 and 12

cylferrocenyl bromide, **11**, formed micelles in aqueous Li_2SO_4 solution. Oxidation of micelles formed from **11** disrupted organization. It was found that **11** dissolved a water-insoluble dye but, when oxidized, $\mathbf{11}^+$ had a higher diffusion coefficient and failed to dissolve the dye, both of which indicate a lack of aggregation. Compound **12** was prepared by the same group[11] but studied further by Takeoka *et al.*,[12] who demonstrated electrochemical control of drug release from its micelles. Perylene, a model hydrophobic drug, dissolved to the extent of 2 mM in the presence of **12** (micellar form) but not when oxidized (monomer).

Compounds studied

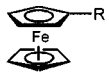
Thirty compounds were prepared for the present study. Their structures are shown as **13**–**42**.

Compound syntheses

Compounds **13**–**18** are ferrocenylmethyl alkyl ethers in which the alkyl groups range from octyl to eicosanyl. Two octadecyl chains are present in the tertiary ferrocenylamine **19**. Compounds **20**–**26** are monosubstituted alkylferrocenes in which the alkyl groups range from butyl to docosanyl. Compounds **27**–**29** are 1,1′bis(2-oxyalkyl)ferrocenes and compounds **30**–**33** are 1,1′-disubstituted alkylferrocene derivatives. Compounds **34**–**42** are 1,1′-disubstituted ferrocenyl ketone, ester and amide derivatives.

Compounds **13**–**18** were prepared by a Williamson reaction[13] that takes advantage of the extraordinary stability of the α-ferrocenylmethyl carbocation.[14] Commercially available *N*,*N*′-dimethylaminomethylferrocene ($FcCH_2NMe_2$) 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 Fc-CH_2^+ is trapped by ROH to give $FcCH_2OR$.

In our original report on compound **1**,[15] 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 30–80% were obtained for **13**–**19**. Except for **13**, which was a liquid at room temperature, compounds **14**–**18** were all low-melting solids (m.p. 30–55 °C; see Experimental). 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 starting material and the reaction conditions are mild.



13, R = $CH_2O(CH_2)_7CH_3$
14, R = $CH_2O(CH_2)_{11}CH_3$
15, R = $CH_2O(CH_2)_{13}CH_3$
16, R = $CH_2O(CH_2)_{15}CH_3$
17, R = $CH_2O(CH_2)_{17}CH_3$
18, R = $CH_2O(CH_2)_{19}CH_3$
19, R = $CH_2N[(CH_2)_{17}CH_3]_2$

20, R = $(CH_2)_3CH_3$
21, R = $(CH_2)_9CH_3$
22, R = $(CH_2)_{13}CH_3$
23, R = $(CH_2)_{15}CH_3$
24, R = $(CH_2)_{17}CH_3$
25, R = $(CH_2)_{19}CH_3$
26, R = $(CH_2)_{21}CH_3$

27, R = $CH_2O(CH_2)_{13}CH_3$
28, R = $CH_2O(CH_2)_{15}CH_3$
29, R = $CH_2O(CH_2)_{17}CH_3$
30, R = $(CH_2)_9CH_3$
31, R = $(CH_2)_{13}CH_3$
32, R = $(CH_2)_{15}CH_3$
33, R = $(CH_2)_{17}CH_3$

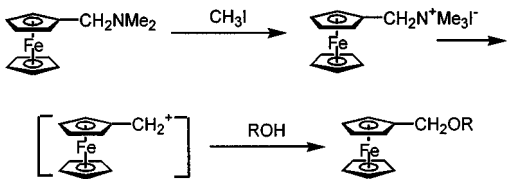
34, R = $CO(CH_2)_7CH_3$
35, R = $CO(CH_2)_{12}CH_3$
36, R = $CO(CH_2)_{14}CH_3$
37, R = $CO(CH_2)_{16}CH_3$
38, R = $COO(CH_2)_{16}CH_3$
39, R = $CONH(CH_2)_{17}CH_3$
40, R = $CON[(CH_2)_{17}CH_3]_2$
41, R = COO-cholestanyl
42, R = COO-cholesteryl

Structures 13–42

The approach noted above was inappropriate for the synthesis of the tertiary amine **19**. In this case, ferrocenylmethanol was converted into chloromethylferrocene 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 $FcCH_2(NC_{18}H_{37})_2$, **19**, was obtained as a stable, yellow solid.

Compounds **20**–**26** are monosubstituted alkylferrocenes. 1-*n*-Butylferrocene, **20**, is commercially available. Alkylferrocenes **21**–**26** were synthesized by Clemmensen reduction of the corresponding monosubstituted acylferrocenes, which were prepared by the reaction of the appropriate acid chloride (1 equiv.) with aluminum chloride (1 equiv.) and ferrocene (1 equiv.).

Ethers **27**–**29** were synthesized by alkylation of commercially available 1,1′-ferrocenedimethanol with the



Scheme 1

appropriate 1-bromoalkane in the presence of an excess of powdered KOH in DMSO. The products were yellow solids and 45–65% yields were obtained.

1,1′-Disubstituted ferrocene derivatives **30**–**33** were synthesized by following the same synthetic pathway as that used for **21**–**26**. Ketones **34**–**37** were prepared by the reaction of the appropriate acid chloride (2 equiv.) with aluminum chloride (2 equiv.) and ferrocene (1 equiv.). Clemmensen reduction of **34**–**37** afforded **30** as a red–brown liquid and **31**–**33** as yellow solids.

Redox potentials

The redox potential of ferrocene is known to be *ca* 400 mV depending on the solvent. This is a readily accessible potential and is the basis of ferrocene's importance in electrochemical studies generally. The redox potentials (vs Ag/AgCl) of the simple alkyl derivatives were similar to ferrocene itself but higher (700–900 mV) when a carbonyl group was attached directly to ferrocene.[16]

Vesicle formation

Vesicle formation was attempted with each of the neutral monomers using a modification of the lipid hydration method.[17] In no case could any aggregate be detected by laser light scattering (Coulter N4SD). (Neutral compounds **38** and **40** formed aggregates upon sonication in water and no further study of these vesicle systems was undertaken.) Oxidation of these monomers by treatment with $Ce(NH_4)_2(NO_3)_6$ gave the corresponding ferricenium cation derivatives, which were sonicated in water while the suspension was continuously protected from the air. In all cases except **34**–**42**, which are 1,1′-disubstituted ferrocenyl ketone, ester and amide derivatives, and **20**, which is soluble in water after oxidization, aggregate formation was clearly indicated by dynamic light scattering methods (Table 1). Relatively large (*ca* 2000 Å) vesicles of similar sizes were obtained, although some of them were slightly less uniform (a bimodal distribution was observed) than others.

The vesicle formation from oxidized **19** 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.

It is an intriguing property of the ferrocenyl amphiphile derivatives having either one (**13**–**18** and **21**–**26**) or two aliphatic chains (**27**–**33**) that they form stable vesicles after oxidation. To our knowledge, no other example of vesicle formation from redox-switchable amphiphiles having the same headgroup bearing either a single aliphatic chain or twin aliphatic chains has been reported.

Electron microscopic characterization of vesicle structures

Negative stain electron microscopy was used to characterize further the vesicles formed from the ferrocenyl monomers. Negative stain electron micrographs are shown for $Fc(CH_2)_{17}CH_3$, **24** (Figure 1), and 1,1′-$Fc[(CH_2)_{15}CH_3]_2$, **28** (Figure 2). In both cases, the approximately spherical outline of the vesicles is apparent along with the distinct membrane boundary. The side-chains of these two compounds are nearly the same length and in both cases, the vesicles are *ca* 2000 Å in size.

Aggregates from oxidized single- and twin-tailed ferrocene derivatives

It is an empirical rule that two-aliphatic-chained amphiphiles aggregate into vesicles and single-chained amphiphiles aggregate into micelles. As with all 'rules of thumb,' exceptions are known. Several research groups[18,19] have reported that single-aliphatic-chained amphiphiles aggregate into vesicles rather than micelles. However, to the best of our knowledge, no case has yet been reported in which both single- and double-tailed, redox-switchable amphiphiles form vesicles by using the same tail(s) and head group(s).

Israelachvilli and co-workers[20] proposed to use the surfactant parameter $V/(LA)$ to assess aggregate formation. According to the model, if $0 < V/(LA) < 0{\cdot}5$, micelles will be formed and if $0{\cdot}5 < V/(LA) < 1$, vesicles are anticipated. In most cases hitherto reported, micelles form from single-tailed amphiphiles because their surfactant parameter is in the range $1/3 < V/(LA) < 1/2$. All things being equal, the corresponding twin-tailed monomers will have $V/LA > 2/3$ and form vesicles rather than micelles.

The ferrocene system permits ready access to amphiphiles which possess the same headgroup but either one or two hydrophobic chains. Of course, the geometries of the two systems may differ. In the single-tail case, the ferrocenyl axis could be tilted with respect to the bilayer surface [Figure 3 (bottom)]. The headgroup is shown between the two extreme cases: perpendicular and parallel to the surface of the bilayer. We estimated the head group area (oxidized ferrocene) by using CPK molecular models. Thus, $A = 43{\cdot}5$ Å^2 ($6{\cdot}40 \times 6{\cdot}80$ Å^2) if the oxidized ferrocene axis is parallel to the surface of the bilayer and $A = 32{\cdot}2$ Å^2 [$\pi r^2 = 3{\cdot}14 \times (3{\cdot}20)^2$ Å^2] if the oxidized ferrocene axis is perpendicular to the surface of the bilayer. V/L was calculated based on reported formulas:[20] $V/L = 21$ for a single alkyl tail and $V/L = 42$ for two alkyl tails (the ether oxygen was calculated as if it were a methylene group). In the two-tailed case, the ferrocenyl axes might be parallel to the surface of the bilayer. Therefore, the double-tailed ferrocene derivatives have $V/(LA) = 0{\cdot}965$. According to Israelachvilli's model and as observed experimentally, vesicles should be formed from oxidized twin-tailed ferrocene derivatives.

In the single-tailed case, we estimated the headgroup area to be less than $43{\cdot}5$ Å^2 but larger than $32{\cdot}2$ Å^2 depending upon its orientation with respect to the bilayer. This means that the value of $V/(LA)$ would lie between $0{\cdot}48$ and $0{\cdot}65$.

Several copies of CPK models of monomers were prepared and examined. The 'measured' headgroup area for the single-tailed oxidized ferrocene derivatives is 35·8 Å^2 (5·60×6·40 Å^2), assuming that the ferrocene derivatives are compactly stacked with *ca* 130° angle included between the ferrocene axis and alkyl chain axis (Figure 3). Thus, single-tailed oxidized ferrocene derivatives would have *V/(LA)*=0·59 and vesicles, rather than micelles, would be expected to form.

To our knowledge, this is the first report of an amphiphilic system in which both single-tailed and double-tailed redox-switchable amphiphiles bearing the same head and tail(s) form vesicles. These results comport with the theoretical model presented above.

Table 1. Laser light scattering data for aggregates formed from oxidized ferrocene derivatives[a]

Compound No.	R on ferrocene	Unimodal diameter (Å)	Cumulant distribution (Å)	
			By intensity	by weight
1	CH_2O-3-cholestanyl	2030±720	2730±1000	2780±1200
13	$CH_2O(CH_2)_7CH_3$	1400±440	1440±530	1100±420
14	$CH_2O(CH_2)_{11}CH_3$	1470[c]	1720±810 (77%)	1130±710 (85%)
			5710±2200 (23%)	6290±240 (15%)
15	$CH_2O(CH_2)_{13}CH_3$	1900±610	2180±620	2060±730
16	$CH_2O(CH_2)_{15}CH_3$	1990±620	2230±1100	1620±1100
17	$CH_2O(CH_2)_{17}CH_3$	1730±540	1840±940	1360±810
18	$CH_2O(CH_2)_{19}CH_3$	1780±610	2270±1000	2040±1200
19	$CH_2N[(CH_2)_{17}CH_3]_2$	2390±800	818±140 (12%)	772±120 (62%)
			2980±250 (88%)	2980± (38%)
20	$(CH_2)_3CH_3$	Sol[b]	Sol	Sol
21	$(CH_2)_9CH_3$	1140[c]	404±100 (14%)	355±90 (93%)
			1740±4100 (86%)	1560±470 (7%)
22	$(CH_2)_{13}CH_3$	1020[c]	361±100 (17%)	317±80 (95%)
			1700±490 (83%)	1480±490 (5%)
23	$(CH_2)_{15}CH_3$	1610±580	1860±640	1580±680
24	$(CH_2)_{17}CH_3$	1880±610	2220±1000	1770±1100
25	$(CH_2)_{19}CH_3$	3030[c]	487±130 (3%)	419±120 (62%)
			3550±890 (97%)	3700±850 (38%)
26	$(CH_2)_{21}CH_3$	2520±900	2920±1200	3040±1500
27	1,1′-$CH_2O(CH_2)_{13}CH_3$	1730[c]	366±150 (5%)	271±100 (86%)
			2730±1600 (95%)	2130±1800 (14%)
28	1,1′-$CH_2O(CH_2)_{15}CH_3$	1900±700	2440±1300	2100±1500
29	1,1′-$CH_2O(CH_2)_{17}CH_3$	1740±580	2160±830	1910±960
30	1,1′-$(CH_2)_9CH_3$	1470±550	1130±480 (46%)	779±330 (87%)
			2870±680 (54%)	2890±66 (13%)
31	1,1′-$(CH_2)_{13}CH_3$	855[c]	460±150 (44%)	365±120 (99%)
			2110±710 (56%)	2130±730 (1%)
32	1,1′-$(CH_2)_{15}CH_3$	1740±600	2220±980	1940±1100
33	1,1′-$(CH_2)_{17}CH_3$	2790[d]	3000±530	3010±510
34	1,1′-$CO(CH_2)_7CH_3$	n[e]	n	n
35	1,1′-$CO(CH_2)_{12}CH_3$	n	n	n
36	1,1′-$CO(CH_2)_{14}CH_3$	n	n	n
37	1,1′-$CO(CH_2)_{16}CH_3$	n	n	n
38	1,1′-$COO(CH_2)_{16}CH_3$	u[f]	u	u
39	1,1′-$CONH(CH_2)_{17}CH_3$	u	u	u
40	1,1′-$CON[(CH_2)_{17}CH_3]_2$	u	u	u
41	1,1′-COO-cholestanyl	u	u	u
42	1,1′-COO-cholesteryl	u	u	u

[a] Determined on a Coulter N4SD submicron particle analyzer.
[b] Sol means that the oxidized monomer was water soluble; any aggregates present were not detected by light scattering.
[c] Distribution was broad.
[d] Distribution was narrow.
[e] n means that the monomer could not be oxidized by ammonium cerium(IV) nitrtate aqueous solution.
[f] u means that the oxidized monomers were unstable.

Redox switching and recycling

We have recently demonstrated for bola-amphiphilic relatives of the compounds reported here that the 'pro-amphiphiles' can be oxidized (Ce^{IV}), the aggregates reduced ($Na_2S_2O_4$) and the monomers recovered.[16] The process is efficient but some decomposition is apparent by TLC analysis. Reused monomers can be oxidized and afford aggregates whose sizes are within experimental error of those obtained originally.

CONCLUSION

We report here a broad family of 'pro-amphiphilic' one- and two-tailed ferrocene derivatives that can be oxidized and sonicated to form aggregates. Exposure of the aggregate to a reducing agent, converts the amphiphile to a non-polar ferrocene derivative and the aggregates collapse. The monomers undergo a slight decomposition during this process but may be reused to give identical aggregates when the cycle is repeated. The stable liposomes have been characterized by laser light scattering and negative stain electron microscopy. In the absence of an oxidizing agent, vesicles failed to form from these compounds.

Both oxidized single- and twin-tailed ferrocene derivatives formed stable vesicles. These systems constitute the first examples of redox-switchable amphiphiles having the same headgroup that form vesicles whether the hydrophobic part of the molecule is composed of one or two tails.

EXPERIMENTAL

^{1}H NMR spectra were recorded at 300, 500 or 600 MHz in $CDCl_3$ solvent 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 Model 1710 Fourier transform 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-

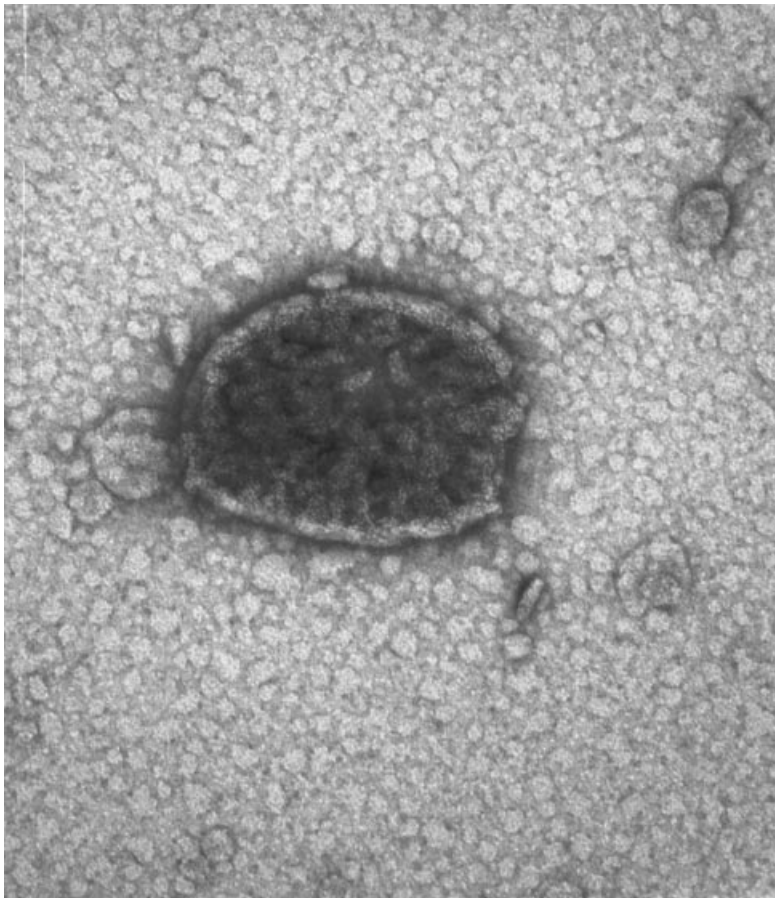


Figure 1. Negative stain electron micrograph of aggregates formed by oxidation and sonication of **24**

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 chromatographic 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 stated otherwise. All reagents were of 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 (Atlanta, GA, USA) and are reported as percentages.

Ferrocenylmethyl cholestanyl ether, 1. Compound **1** was prepared as recently described.[16]

General procedure for syntheses of compounds 13–18. Dimethylaminomethylferrocene (1·46 g, 6 mmol), ROH (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 ($MgSO_4$) and concentrated *in vacuo*. Column chromatography (silica gel, EtOAc–hexanes, 1:20, v/v) afforded the desired compound.

Ferrocenylmethyl octyl ether, 13. The title compound was prepared on a 4 mmol scale as described in the general procedure (above). The product (673 mg, 51%) was obtained as a yellow oil. ^{1}H NMR: 0·88 (t, 3H), 1·27 (s, 10H), 1·55 (m, 2 H), 3·39 (t, 2 H), 4·12–4·25 (three peaks, 11 H, CH_2 ferrocene). *Anal.* calcd for $C_{19}H_{28}OFe$: C, 69·52; H, 8·60%. Found: C, 69·60; H, 8·60%.

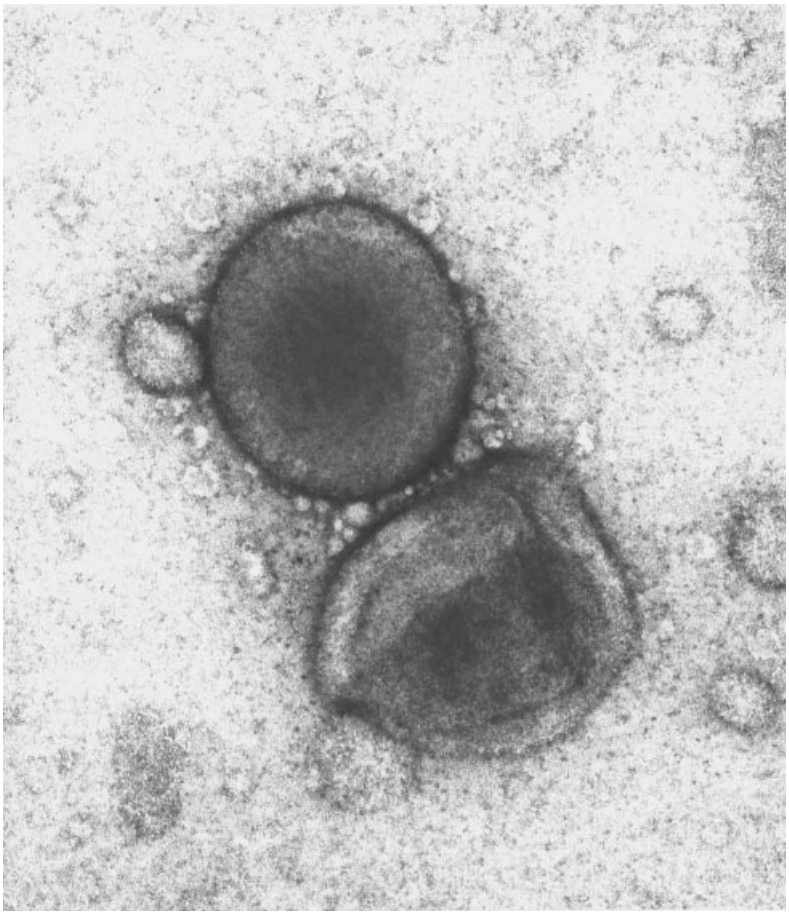


Figure 2. Negative stain electron micrograph of aggregates formed by oxidation and sonication of **28**

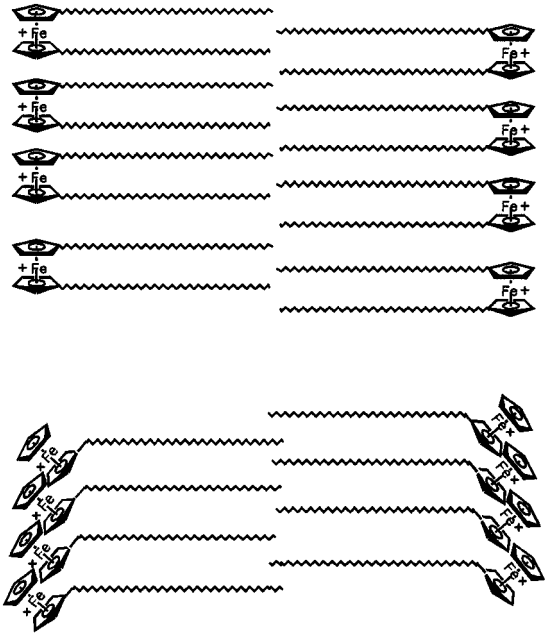


Figure 3. Possible amphiphile orientation in membranes formed from oxidized single-tailed (bottom) and double-tailed (right) ferrocene derivatives.

Ferrocenylmethyl dodecyl ether, 14. The title compound was prepared on a 4 mmol scale as described in the general procedure reported above. The product (436 mg, 28%) was obtained as a red–brown solid, m.p. 31–33 °C. ^{1}H NMR: 0·88 (t, 3 H), 1·27 (s, 18 H), 1·55 (m, 2 H), 3·39 (t, 2 H), 4·12–4·25 (three peaks, 11 H, CH_2ferrocene). *Anal.* calcd for $C_{23}H_{36}OFe$: C, 71·87; H, 9·44%. Found: C, 71·74; H, 9·45%.

Ferrocenylmethyl tetradecyl ether, 15, ferrocenylmethyl hexadecyl ether, 16, ferrocenylmethyl octadecyl ether, 17, ferrocenylmethyl eicosanyl ether, 18 and ferrocenylmethyldi-*n*-octadecylamine, 19. These compounds were prepared as recently described.[16]

1-*n*-Butylferrocene, 20. The title compound was purchased from Sigma–Aldrich and used without further purification.

General procedure for syntheses of compounds 21–26. The syntheses of compounds **21**–**26** were accomplished in two steps. First, monosubstituted acylferrocenes were prepared by Friedel–Crafts acylation. The compounds thus obtained were then subjected to Clemmensen reduction. The general synthetic procedures are recorded below.

Preparation of acid chlorides. If not commercially available, the appropriate acid chloride was prepared. A solution of the appropriate acid (5 mmol) in $SOCl_2$ (25 ml) was heated at reflux overnight. Excess $SOCl_2$ was evaporated to afford the acid chloride as a light yellow solid, which was used without further purification.

Preparation of ferrocenyl alkyl ketones. A solution of the acid chloride (2 mmol., see above) and $AlCl_3$ in 10 ml of CH_2Cl_2 was added dropwise (2 h) to a solution of ferrocene (2 mmol) in CH_2Cl_2 (10 ml) while stirring at room temperature. After 1 h, the reaction mixture was poured on to ice (20 g). The organic phase was washed (water) until neutral and dried over $MgSO_4$. After filtration, the solvent was evaporated. The crude product was either crystallized from methanol or chromatographed over silica using EtOAc–hexanes (1:10, v/v) to afford the desired ketone.

Preparation of 1-n-alkylferrocenes. Granular zinc (1·7 g, 26 mmol, 20 mesh) was amalgamated by stirring for 5 min with $HgCl_2$ (125 mg, 0·46 mmol), H_2O (2·5 ml) and concentrated HCl (*ca* 37%, v/v, 0·1 ml). The aqueous phase was decanted and replaced by H_2O (3 ml), concentrated HCl (6 ml) and the ketone (1 mmol, see above), which was dissolved in benzene (4 ml). The mixture was heated at reflux for 4 h. At intervals during the reaction, concentrated HCl (0·5 ml) was added. The mixture was cooled to room temperature. The amalgam was picked out and washed with diethyl ether. The combined organic phase was washed with water until neutral and then dried over $MgSO_4$. The crude product was purified either by crystallization from methanol or propan-2-ol or by flash chromatography (silica gel, hexanes) to afford the desired product.

1-*n*-Decylferrocene, 21. *Preparation of ferrocenyl nonyl ketone.* Ferrocenyl nonyl ketone was prepared on a 20 mmol scale according to the general procedure described above. The ketone (5·0 g, 74%) was obtained as a red solid, m.p. 32–34 °C. ^{1}H NMR: 0·88 (t, 3 H), 1·27 (s, 12 H), 1·71 (m, 2 H), 2·67 (t, 2 H), 4·20 (s, 5 H, ferrocene), 4·49 (t, 2 H, ferrocene) 4·78 (t, 2 H, ferrocene). *Anal.* calcd for $C_{20}H_{28}OFe$: C, 70·59; H, 8·29%. Found: C, 70·63, H, 8·27%.

*Preparation of **21***. The ketone (above) was reduced with Zn(Hg) on a 5 mmol scale as described in the general procedure (above). The product (1·2 g, 74%) was obtained after crystallization (CH_3OH) as a yellow solid, m.p. 31–32 °C (lit.[21] 35–36 °C). ^{1}H NMR: 0·88 (t, 3 H), 1·27 (s, 14 H), 1·49 (m, 2 H), 2·30 (t, 2 H), 4·05–4·10 (three peaks, 9 H, ferrocene). *Anal.* calcd for $C_{20}H_{30}Fe$: C, 73·62; H, 9·27%. Found: C, 73·57, H, 9·24%.

1-*n*-Tetradecylferrocene, 22. *Preparation of ferrocenyl tridecyl ketone.* Ferrocenyl tridecyl ketone was prepared on a 5 mmol scale according to the general procedure described above. The ketone (1·38 g, 70%) was obtained as a yellow solid, m.p. 52–54 °C. ^{1}H NMR: 0·88 (t, 3 H), 1·26 (s, 20 H), 1·71 (m, 2 H), 2·6/9 (t, 2 H), 4·20 (s, 5 H, ferrocene), 4·49 (t, 2 H, ferrocene) 4·78 (t, 2 H, ferrocene). *Anal.* calcd for $C_{24}H_{36}OFe$: C, 72·72; H, 9·15%. Found: C,

72·60, H, 9·10%.

*Preparation of **22***. The ketone (above) was reduced with Zn(Hg) on a 1 mmol scale as described in the general procedure (above). The product (320 mg, 84%) was obtained after crystallization (CH_3OH) as a yellow solid, m.p. 47–49 °C. ^{1}H NMR: 0·88 (t, 3 H), 1·26 (s, 22 H), 1·49 (m, 2 H), 2·31 (t, 2 H), 4·04–4·09 (three peaks, 9 H, ferrocene). *Anal.* calcd for $C_{24}H_{38}Fe$: C, 75·38; H, 10·02%. Found: C, 75·40, H, 9·95%.

1-*n*-Hexadecylferrocene, 23. *Preparation of ferrocenyl pentadecyl ketone.* Ferrocenyl pentadecyl ketone was prepared on a 5 mmol scale according to the general procedure described above. The ketone (1·71 g, 80%) was obtained as a yellow solid, m.p. 60·5–62 °C (lit.[22] 59–60 °C). ^{1}H NMR: 0·88 (t, 3 H), 1·26 (s, 24 H), 1·71 (m, 2 H), 2·69 (t, 2 H), 4·20 (s, 5 H, ferrocene), 4·49 (t, 2 H, ferrocene) 4·78 (t, 2 H, ferrocene).

*Preparation of **24***. The ketone (above) was reduced with Zn(Hg) on a 1 mmol scale as described in the general procedure (above). The product (331 mg, 81%) was obtained as a yellow solid after crystallization from methanol, m.p. 54–55 °C (lit.[22] 55–56 °C). ^{1}H NMR: 0·88 (t, 3 H), 1·26 (s, 26 H), 1·49 (m, 2 H), 2·31 (t, 2 H), 4·04–4·09 (three peaks, 9 H, ferrocene). *Anal.* calcd for $C_{26}H_{42}Fe$: C, 76·08; H, 10·31%. Found: C, 75·95, H, 10·24%.

1-*n*-Octadecylferrocene, 24. *Preparation of ferrocenyl heptadecyl ketone.* Ferrocenyl heptadecyl ketone was prepared on a 2 mmol scale according to the general procedure described above. The ketone (750 mg, 83%) was obtained as a yellow solid, m.p. 64–65 °C. ^{1}H NMR: 0·88 (t, 3 H), 1·26 (s, 28 H), 1·71 (m, 2 H), 2·69 (t, 2 H), 4·20 (s, 5 H, ferrocene), 4·49 (t, 2 H, ferrocene) 4·78 (t, 2 H, ferrocene). *Anal.* calcd for $C_{28}H_{44}OFe$: C, 74·32; H, 9·80%. Found: C, 74·44, H, 9·85%.

*Preparation of **24***. The ketone (above) was reduced with Zn(Hg) on a 0·82 mmol scale as described in the general procedure (above). The product (190 mg, 56%) was obtained as a yellow solid after crystallization from methanol, m.p. 58–60 °C. ^{1}H NMR: 0·88 (t, 3 H), 1·26 (s, 30 H), 1·49 (m, 2 H), 2·31 (t, 2 H), 4·04–4·09 (three peaks, 9 H, ferrocene). *Anal.* calcd for $C_{28}H_{46}Fe$: C, 76·69; H, 10·57%. Found: C, 76·77, H, 10·57%.

1-*n*-Eicosanylferrocene, 25. *Preparation of ferrocenyl nonadecyl ketone.* Ferrocenyl nonadecyl ketone was prepared on a 5 mmol scale according to the general procedure described above. The ketone (1·87 g, 78%) was obtained as a yellow solid, m.p. 71–73 °C. ^{1}H NMR: 0·88 (t, 3 H), 1·26 (s, 32 H), 1·71 (m, 2 H), 2·69 (t, 2 H), 4·20 (s, 5 H, ferrocene), 4·49 (t, 2 H, ferrocene) 4·78 (t, 2 H, ferrocene). *Anal.* calcd for $C_{30}H_{48}OFe$: C, 74·98; H, 10·07%. Found: C, 75·07, H, 10·10%.

*Preparation of **25***. The ketone (above) was reduced with Zn(Hg) on a 1 mmol scale as described in the general procedure (above). The product (340 mg, 73%) was obtained after crystallization from propan-2-ol as a yellow solid, m.p. 65–67 °C. ^{1}H NMR: 0·88 (t, 3 H), 1·26 (s, 34 H), 1·49 (m, 2 H), 2·31 (t, 2 H), 4·04–4·09 (three peaks, 9 H, ferrocene). *Anal.* calcd for $C_{30}H_{50}Fe$: C, 77·23; H, 10·80%. Found: C, 77·33, H, 10·82%.

1-*n*-Docosanylferrocene, 26. *Preparation of ferrocenyl heneicosanyl ketone.* Ferrocenyl heneicosanyl ketone was prepared on a 5 mmol scale according to the general procedure described above. The ketone (1·82 g, 72%) was obtained as a yellow solid, m.p. 73–75 °C. ^{1}H NMR: 0·88 (t, 3 H), 1·26 (s, 36 H), 1·71 (m, 2 H), 2·69 (t, 2 H), 4·20 (s, 5 H, ferrocene), 4·49 (t, 2 H, ferrocene) 4·78 (t, 2 H, ferrocene). *Anal.* calcd for $C_{32}H_{52}OFe$: C, 75·57; H, 10·30%. Found: C, 75·71, H, 10·36%.

*Preparation of **26***. The ketone (above) was reduced with Zn(Hg) on a 1 mmol scale as described in the general procedure (above). The product (360 mg, 73%) was obtained as a yellow solid after crystallization from propan-2-ol, m.p. 69–70·5 °C. ^{1}H NMR: 0·88 (t, 3 H), 1·26 (s, 38 H), 1·49 (m, 2 H), 2·31 (t, 2 H), 4·05–4·09 (three peaks, 9 H, ferrocene). *Anal.* calcd for $C_{32}H_{54}Fe$: C, 77·71; H, 11·00%. Found: C, 77·45, H, 10·95%.

General procedure for the syntheses of compounds 27–29. To DMSO (10 ml) was added powdered KOH (88%, 255 mg, 4 mmol). After stirring for 5 min, commercial 1,1′-ferrocenedimethanol (100 mg, 0·405 mmol) was added, followed immediately by the appropriate 1-bromoalkane (1 mmol, syringe). Stirring was continued for 1 h. The mixture was then poured into H_2O (30 ml) and extracted with 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) afforded the product as a yellow solid.

1,1′Bis(2-oxahexadecyl)ferrocene, 27. The title compound was prepared on a 0·405 mmol scale as described in the general procedure (above). The product was obtained (170 mg, 66%) as a yellow solid, m.p. 37–38 °C. ^{1}H NMR: 0·88 (t, 6 H), 1·25 (s, 44 H), 1·53 (m, 4 H), 3·39 (t, 4 H), 4·12–4·25 [three peaks, 12 H, $(CH_2)_2$ ferrocene]. *Anal.* calcd for $C_{40}H_{70}O_2Fe$: C, 75·21; H, 11·04%. Found: C, 75·29, H, 11·06%.

1,1′-Bis(2-oxaoctadecyl)ferrocene, 28. The title compound was prepared on a 0·405 mmol scale as described in the general procedure (above). The product was obtained (130 mg, 46%) as a yellow solid, m.p. 45·5–47 °C. ^{1}H NMR: 0·88 (t, 6 H), 1·25 (s, 52 H), 1·53 (m, 4 H), 3·39 (t, 4 H), 4·12–4·22 [three peaks, 12 H, $(CH_2)_2$ferrocene]. *Anal.* calcd for $C_{44}H_{78}O_2Fe$: C, 76·05; H, 11·31%. Found: C, 75·98, H, 11·26%.

1,1′-Bis(2-oxaeicosanyl)ferrocene, 29. The title compound was prepared on a 0·405 mmol scale as described in the general procedure (above). The product was obtained (140 mg, 46%) as a yellow solid, m.p. 49–50·5 °C. ^{1}H NMR: 0·88 (t, 6 H), 1·25 (s, 60 H), 1·53 (m, 4 H), 3·39 (t, 4 H), 4·12–4·25 [three peaks, 12 H, —$(OCH_2)_2$ferrocene]. *Anal.* calcd for $C_{48}H_{86}O_2Fe$: C, 76·76; H, 11·54%. Found: C, 76·94, H, 11·67%.

General procedure for syntheses of compounds 30–33. Clemmensen reduction of **34–37** (ketones, see below) afforded **30**–**33** (1,1′-dialkylferrocenes).

Preparation of 1,1′-dialkylferrocenes. Granular zinc (3·4 g, 52 mmol, 20 mesh) was amalgamated by stirring for 5 min with mercury (II) chloride (250 mg, 0·92 mmol), H_2O (5 ml) and concentrated HCl (*ca* 37%, v/v, 0·2 ml). The aqueous phase was decanted and replaced by H_2O (6 ml), concentrated HCl (12 ml), toluene (30 ml) and the appropriate 1,1′-bis(1-oxoalkyl)ferrocene (1 mmol, see below for preparation). The mixture was heated at reflux for 4 h. The amalgam was removed (picked out) and washed with diethyl ether. The combined organic phase was washed with water to neutrality and dried over $MgSO_4$. The crude product was chromatographed (silica, hexanes). Crystallization (if necessary) from EtOAc and MeOH afforded the product.

1,1′-Bis(*n*-decyl)ferrocene, 30. The title compound was prepared on a 0·7 mmol scale as described in the general procedure (above). The product (200 mg, 61%) was obtained as a red–brown liquid. ^{1}H NMR: 0·88 (t, 6 H), 1·27 (s, 24 H), 1·48 (m, 4 H), 2·29 (t, 4 H), 3·97 (bs, 8 H, ferrocene). *Anal.* calcd for $C_{30}H_{50}Fe$: C, 77·23; H, 10·80%. Found: C, 77·35; H, 10·91%.

1,1′-Bis(*n*-tetradecyl)ferrocene, 31. The title compound was prepared on a 0·5 mmol scale as described in the general procedure (above). The product (201 mg, 69%) was obtained after crystallization from EtOAc and MeOH as a yellow solid, m.p. 41·5–43 °C. ^{1}H NMR: 0·88 (t, 6 H), 1·26 (s, 44 H), 1·46 (m, 4 H), 2·24 (t, 4 H), 4·06 (bs, 8 H, ferrocene). *Anal.* calcd for $C_{38}H_{66}Fe$: C, 78·86; H, 11·49%. Found: C, 78·77; H, 11·57%.

1,1′Bis(*n*-hexadecyl)ferrocene, 32. The title compound was prepared on a 1 mmol scale as described in the general procedure (above). The product (380 mg, 60%) was obtained after crystallization from EtOAc and MeOH as a yellow solid, m.p. 49·5–51 °C (lit.[22] 41·2–42·4°C). ^{1}H NMR: 0·88 (t, 6 H), 1·26 (s, 52 H), 1·46 (m, 4 H), 2·21 (t, 4 H), 4·10 (bs, 8 H, ferrocene). *Anal.* calcd for $C_{42}H_{74}Fe$: C, 79·46; H, 11·75%. Found: C, 79·50; H, 11·82%.

1,1′-Bis(*n*-octadecyl)ferrocene, 33. The title compound was prepared on a 0·5 mmol scale as described in the general procedure (above). The product (190 mg, 55%) was obtained after crystallization from EtOAc and MeOH as a yellow solid, m.p. 57·–58 °C. ^{1}H NMR: 0·88 (t, 6 H), 1·26 (s, 60 H), 1·46 (m, 4 H), 2·21 (t, 4 H), 4·10 (bs, 8 H, ferrocene). *Anal.* calcd for $C_{46}H_{82}Fe$: C, 79·96; H, 11·96%. Found: C, 79·97; H, 11·99%.

General procedure for syntheses of compounds 34–37. The appropriate acid chloride (4 mmol) was slowly added to a suspension of $AlCl_3$ (533 mg, 4 mmol) in CH_2Cl_2 (15 ml) while stirring. To the above solution was added dropwise (2 h) a solution of ferrocene (3 mmol) in CH_2Cl_2 (20 ml) while stirring. After 12 h, the reaction mixture was poured on to ice (50 g). After adding $CHCl_3$ (30 ml), the organic phase was washed (water) until neutral, dried over $MgSO_4$ and concentrated *in vacuo*. Column chromatography (silica gel, EtOAc–$CHCl_3$–hexanes, 1:5:10, v/v) afforded the desired compound. The products were further purified by crystallization from EtOAc and MeOH.

1,1′-Bis(1-oxodecyl)ferrocene, 34. The title compound was prepared on a 2 mmol scale as described in the general procedure (above). The product (600 mg, 61%) was obtained after crystallization (MeOH) as an orange solid, m.p. 70–71.5 °C (lit.[20] 68·6–69·8°C). ^{1}H NMR: 0·88 (t, 6 H), 1·26 (s, 24 H), 1·69 (m, 4 H), 2·65 (t, 4 H), 4·48 (t, 4 H, ferrocene), 4·77 (t, 4 H, ferrocene).

1,1′-Bis(1-oxotetradecyl)ferrocene, 35. The title compound was prepared on a 2 mmol scale as described in the general procedure (above). The product (750 mg, 62%) was obtained after crystallization from EtOAc and MeOH as an orange solid, m.p. 83–84 °C. ^{1}H NMR: 0·88 (t, 6 H), 1·26 (s, 40 H), 1·69 (m, 4 H), 2·65 (t, 4 H), 4·48 (t, 4 H, ferrocene), 4·77 (t, 4 H, ferrocene).

1,1′-Bis(1-oxohexadecyl)ferrocene, 36. The title compound was prepared on a 3 mmol scale as described in the general procedure (above). The product (1320 mg, 66%) was obtained after crystallization from EtOAc as an orange solid, m.p. 86·5–87·5°C (lit.[22] 82·4–83·4°C). ^{1}H NMR: 0·88 (t, 6 H), 1·26 (2, 48 H), 1·69 (m, 4 H), 2·65 (t, 4 H), 4·48 (t, 4 H, ferrocene), 4·77 (t, 4 H, ferrocene).

1,1′-Bis(1-oxooctadecyl)ferrocene, 37. The title compound was prepared on a 2 mmol scale as described in the general procedure (above). The product (890 mg, 62%) was obtained after crystallization (EtOAc) as an orange solid, m.p. 90–91·5 °C. ^{1}H NMR: 0·88 (t, 6 H), 1·26 (s, 56 H), 1·69 (m, 4 H), 2·65 (t, 4 H), 4·48 (t, 4 H, ferrocene), 4·77 (t, 4 H, ferrocene).

Ferrocenyl 1,1′-carboxyheptadecanoate, 38, *N,N′*-Bis(octadecyl)ferrocene-1,1′-dicarboxamide, 39, 1,1′-bis[(octadecylamino)carbonyl]ferrocene, 40, ferrocenyl 1,1′-carboxydihydrocholesterate, 41, and ferrocenyl 1,1′-carboxycholesterate, 42. These compounds were prepared as recently described.[16]

Vesicle formation for the neutral species. Compounds were dissolved in CH_2Cl_2 (*ca* 2 ml) in a sample vial and the solvent was slowly removed by purging with N_2 and drying under high vacuum for 1 h. The sample was then sonicated in deionized water (0·5 mM 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 Nuclepore polycarbonate membrane. The suspension was characterized using 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 μM) was added to oxidize the ferrocene derivative and the test-tube was immersed in a water-bath sonicator (Branson Model 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 Nuclepore polycarbonate membrane. It was characterized by following the procedure above. Ferrocenylmethyl cholestanyl ether and all the alkylferrocene derivatives were oxidized in CH_2Cl_2 by using aqueous ammonium cerium(IV) nitrate solution. After drying (N_2 purge), the oxidized compounds were further dried under high vacuum (2 h). The solution 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 deoxygenized and deoxygenated water. The solution was clarified by filtration through a 0·2 μm Nuclepore 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 vortex mixed to ensure homogeneity. Then, stock vesicle suspension (300 μl, 0·5 mM) was added to each of the above solutions. After vortex mixing, 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, vesicle suspension (10 ml, 0·5 mM surfactant) was prepared in a 15 ml test-tube. The suspension was centrifuged, the supernatant was transferred into 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 as 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 elecron microscope operated at 75 kV.

Method 1. Bacitracin solution (0·024%, 10 μl) was added to the vesicle suspension (50 μl), mixed and 10–15 μl 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 μl) 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 sample to 1 part stain. The vesicle–stain solutions (10–12 μl) 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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