A Hydrolytic Reporter of Cu(II) Availability in Artificial Liposomes

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The dioctadecyl ammonium surfactant **1**, functionalized with a *p*-nitrophenyl ester of picolinic acid, has been synthesized and its hydrolysis in covesicular blends of nonfunctional surfactants dihexadecyldimethylammonium bromide, **3**, dioctadecyldimethylammonium bromide, **4**, and racemic 1,2-bis(palmitoyloxy)-3-(trimethylammonium)propyl bromide, **5**, studied after addition of Cu(II) ions at pH = 5.0 and different temperatures. The cleavage of **1** gives the *p*-nitrophenoxide surfactant **2**, which shows a strong absorption band at 400 nm. Clear biphasic kinetics were observed for all vesicular systems at a temperature below the gel-to-liquid crystal phase transition temperature (T_c) of the membrane: the first, faster process (≈60% of the ester cleaved) was associated with the Cu(II)-catalyzed hydrolysis of the exovesicular ester; the slower one (the remaining ≈40% of the ester cleaved) was associated with the uncatalyzed hydrolysis of the endovesicular ester. Above T_c only a monoexponential process was observed. Variable temperature experiments allowed one to conclude that the cationic vesicles studied are totally impermeable to Cu(II) ions either below or above their T_c which controls the rate of the transbilayer movements of the lipids.

The report by Kunitake[1] in 1977 that simple long-chain dialkylammonium surfactants may form vesicular aggregates has spurred an increasing interest in the use of synthetic surfactants for the creation of artificial liposomes. The fine tuning of the structure of the surfactant may allow one to control the transbilayer movement of the lipids,[2] permeation[3] of protons and group IA or IIA cations and, eventually, chemical differentiation[2d,4] of the two leaflets of the bilayer. Little is known about the kinetic phenomena involving transition metal ions[5] such as Cu(II),[6] Co(II),[7] and Ni(II). It is commonly accepted that the free ions are unable to permeate across natural liposomes.[8] There is evidence that the transport of copper[9] in living organisms is performed by proteins acting as carriers.

Recently Moss and his associates used cleavable, functional surfactants to monitor proton permeation and flip-flop phenomena in artificial liposomes.[2] We thought that such an approach could be also used for the study of permeation of transition metal ions, Cu(II) in particular, across the bilayer of cationic vesicles.

Following this idea and on the basis of the well-known hydrolytic lability of activated esters of picolinic acid[10] in the presence of Cu(II) and other transition metal ions we have designed functional surfactant **1**. We have focused our study on cationic aggregates because they do not interact with positively charged transition metal ions as do anionic or zwitterionic amphiphiles (counterion effect[11]). Our cleavable molecule has been designed in such a way as to release the metal ion binding subunit after hydrolysis so that there is no worry about the possibility of the cleaved surfactant to act as a carrier. Transport of Cu(II) across cationic vesicles by ligand surfactants has been recently reported by us.[12] It is worth mentioning, in this context, that "sensors" for metal ions, i.e. molecules forming spectroscopically detectable complexes, may also act as potential carriers, in this way hampering the interpretation of the data. We emphasize that the focus of the present investigation is not on the creation of a new catalytic system, rather on how to use a known process (accelerated hydrolysis of picolinate esters by Cu(II)) to understand kinetic phenomena associated with Cu(II) permeation and surfactant mobility in vesicular aggregates.

Results and Discussion

Surfactant **1**, a *p*-nitrophenyl picolinate bearing two octadecyl hydrocarbon chains at the ammonium group has been synthesized as outlined in Scheme I. This molecule gives, after hydrolysis, picolinic acid and the *p*-nitrophenol surfactant **2**[13] (Chart I) which is no longer a ligand since the chelating moiety (picolinic acid) has been hydrolytically removed. This latter process can be

(1) Kunitake, T.; Okahata, Y. *J. Am. Chem. Soc.* **1977**, *99*, 3860.

(2) (a) Moss, R. A.; Ganguli, S.; Okumura, Y.; Fujita, T. *J. Am. Chem. Soc.* **1990**, *112*, 6391. (b) Moss, R. A.; Fujita, T.; Ganguli, S. *Langmuir* **1990**, *6*, 1197. (c) Fujita, T.; Moss, R. A. *Chem. Lett.* **1991**, 795. (d) Moss, R. A.; Okumura, Y. *J. Am. Chem. Soc.* **1992**, *114*, 1750.

(3) (a) Menger, F. M. In *Frontier in Supramolecular Organic Chemistry and Photochemistry*; Schneider, H.-J., Dürr, H., Eds., VCH: Weinheim, 1991; p 193. (b) Fyles, T. M.; Kaye, K. C.; James, T. D.; Smiley, D. W. M. *Tetrahedron Lett.* **1990**, *31*, 1233. (c) Menger, F. M.; Aikens, P. *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 898.

(4) (a) Moss, R. A.; Bhattacharya, S.; Chatterjee, S. *J. Am. Chem. Soc.* **1989**, *111*, 3680 and references therein. (b) Moss, R. A.; Bhattacharya, S.; Scrimin, P.; Swarup, S. *J. Am. Chem. Soc.* **1987**, *109*, 5740. (c) Moss, R. A.; Swarup, S. *J. Am. Chem. Soc.* **1986**, *108*, 5341.

(5) Several studies focused on paramagnetic ions like lanthanides because of the possibility of using NMR as an investigative tool (see: Ting, D. Z.; Hagan, P. S.; Chan, S. I.; Dall, J. D.; Springer, C. S. *Biophys. J.* **1981**, *34*, 189). Cu(II) appears not suitable for the NMR investigation because line broadening prevails on chemical shift shifting.

(6) (a) Fuhrhop, J.-H.; Koesling, V.; Schönberger, G. *Liebigs Ann. Chem.* **1984**, 1634. (b) Fuhrhop, J.-H.; Lehmann, T. *Liebigs Ann. Chem.* **1984**, 1057.

(7) Morris, S. J.; Bradley, D.; Blumenthal, R. *Biochim. Biophys. Acta* **1985**, *818*, 365.

(8) Deamer, D. W.; Bramhall, J. *Chem. Phys. Lip.* **1986**, *40*, 167. However temperature may play a key role in controlling the permeation process; see for instance: Lavaczeck, R.; Blackman, R.; Kainasho, M. *Biochim. Biophys. Acta* **1977**, *468*, 411.

(9) (a) Sarkar, B. In *Metal Ions in Biological Systems*; Sigel, H., Ed.; Dekker: New York, 1981; Vol. 12, Chapter 6. (b) Sarkar, B.; Laussac, J.-P. *Biochemistry* **1984**, *23*, 2832.

(10) Fife, H. T.; Przystas, T. *J. Am. Chem. Soc.* **1985**, *107*, 1041.

(11) Bunton, C. A.; Nome, F.; Quina, F. H.; Romsted, L. S. *Acc. Chem. Res.* **1991**, *24*, 357.

(12) Scrimin, P.; Tecilla, P.; Tonellato, U. *J. Am. Chem. Soc.* **1992**, *114*, 5086.

(13) Compound **2** has been already described, see: Moss, R. A.; Fujita, T. *Tetrahedron Lett.* **1990**, *31*, 2377. This same paper reports a pK_a = 4.5 for **2** in cationic aggregates.

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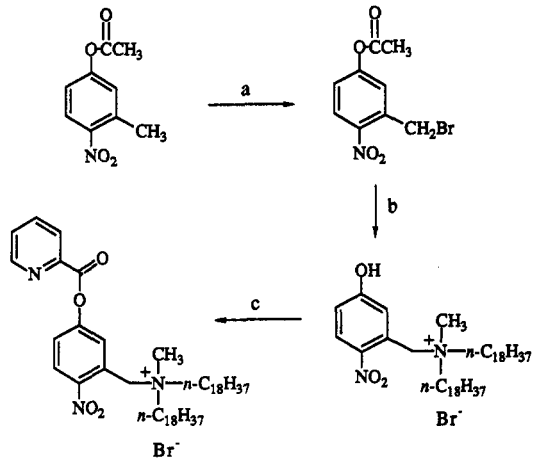
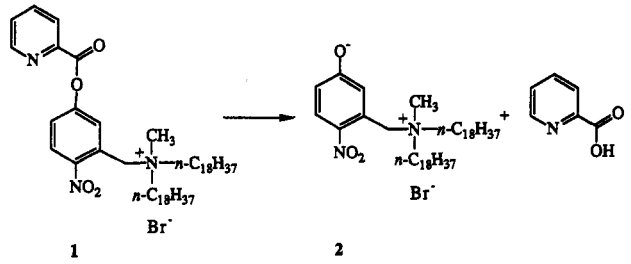
Scheme I [a]



[a] (a) NBS, CCl_4, reflux, hν; (b) $CH_3N(C_{18}H_{37})_2$, CH_2Cl_2, rt, and then Al_2O_3 chromatography and HBr; (c) picolinoyl chloride, CH_2Cl_2, reflux.

Chart I



monitored by following the formation of *p*-nitrophenol or *p*-nitrophenoxide absorption bands at 317 or 400 nm, respectively.[14] Surfactant **1**, incorporated in unilamellar vesicles, is hydrolyzed faster in the presence of Cu(II) ions, thus acting as a reporter of their availability.

Covesicles made of surfactant **1** and nonfunctional matrices of dihexadecyldimethylammonium bromide, **3**, dioctadecyldimethylammonium bromide, **4**, or racemic 1,2-bis(palmitoyloxy)-3-(trimethylammonium)propyl bromide, **5**, were obtained by sonication of CH_2Cl_2-cast films of the three different blends (1:10 composition functional/nonfunctional lipid) in 0.05 M 2-morpholinoethane sulfonate (MES) buffer at pH = 5.0. The hydrodynamic diameters, determined by dynamic light scattering, and the gel-to-liquid crystal phase transition temperatures (T_c), determined from temperature-dependent discontinuities in the fluorescence polarization of covesicallized 1,6-diphenyl-1,3,5-hexatriene,[15] are reported in footnote *c* to Table I for the three aggregates. The dimensions of the aggregates indicate formation of unilamellar structures.

Figure S1 of the supplementary material reports the fluorescence and light-scattering experiments performed on aggregates loaded with ethidium bromide (EB) after their permeation through Sephadex. It clearly shows that trapping of EB does occur in all cases: this strongly supports formation of closed vesicles.

In the absence of Cu(II) ions and at pH = 5.0, 25 °C, covesicalized **1** hydrolyzes with an observed rate constant, $k_\psi = 1.4 \times 10^{-4}$ s^{-1} with small differences between the three coaggregates (see Table I, runs 1, 4, 7). Upon addition

(14) At the pH used for the present investigation (5.0), surfactant **2** was largely in its zwitterionic form (see also ref 13).

(15) (a) Andrich, M. P.; Vanderkooi, J. M. *Biochemistry* **1976**, *15*, 1257. (b) Lentz, B. R. *Chem. Phys. Lip.* **1989**, *50*, 171.

Table I. Observed Rate Constants,[a] k_ψ(s^{-1}), for the Hydrolysis of Surfactant 1 at pH = 5.0[b] Using Different Covesicular Blends and Conditions

run	covesicle[c]	T, °C	10^4[Cu(II)], M	10^3k_f, s^{-1}	10^4k_s, s^{-1}	% fast/% slow
1	1/3	25	0	–	1.2	*d*
2	1/3	25	5.3	2.7	*e*	100/0
3	1/3	10	5.3	0.74	0.8	69/31
4	1/4	25	0	–	1.3	*d*
5	1/4	25	5.3	3.0	1.8	62/38
6	1/4	42	3.2	14.1	*e*	100/0
7	1/5	25	0	–	1.6	*d*
8	1/5	25	5.3	4.7	2.6	65/35

[a] Each value is the average of at least three independent measurements. [b] 0.05 M MES buffer. [c] Hydrodynamic diameters (Å) and phase transition temperatures (°C) for the different blends are as follows: 1/3 349, 23; 1/4 404, 35; 1/5 779, 47. [d] "Regular" monophasic kinetic behavior. [e] No detectable slow reaction; the final absorbance was the same, within the limits of the experimental error, as was the hydrolysis without added Cu(II).

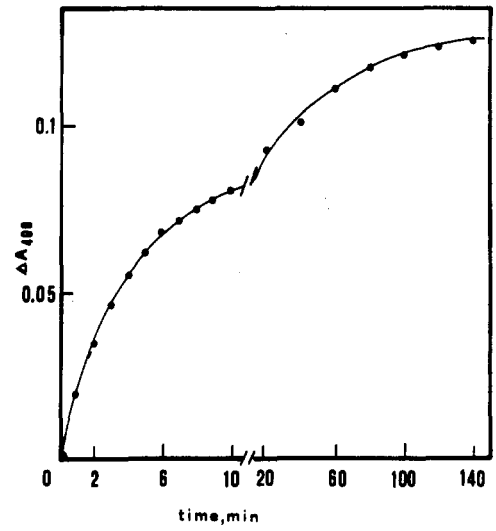


Figure 1. Time course of the absorbance change at 400 nm for the vesicular blend 1/4 at 25 °C after addition of $Cu(NO_3)_2$. For conditions see Table I, run 5. (Note the change of time scale).

of Cu(II) ions *after the creation of the aggregates*, the observed kinetic behavior depends on the vesicular blend and the temperature at which the experiments are performed. At 25 °C covesicles made of 1/3 show a fast monoexponential process accounting for the release of all the *p*-nitrophenoxide (run 2). On the contrary, the two blends 1/4 and 1/5 clearly show two distinct kinetic phenomena: the first, with the same rate constant as the Cu(II) accelerated process, accounts for slightly more than 60% of the total *p*-nitrophenyl picolinate; the second has the same rate constant as the uncatalyzed process and accounts for the remaining 40% of the ester (runs 5 and 8). The time course of run 5, reported in Figure 1, illustrates such a biphasic kinetic profile.

Biphasic behavior is also observed in the case of covesicles made of 1/3 when the experiment is performed at 10 °C (run 3), well below the phase transition temperature of this aggregate. On the other hand, a single monophasic release of *p*-nitrophenol is observed with covesicles made of 1/4 when the experiment is run at 42 °C, above the T_c of the system (run 6).[16] Furthermore, for all systems showing a biphasic behavior the fast process is strongly dependent on the Cu(II) concentration: as the concentration of the metal ion increases so does the rate constant. The slow process, on the contrary, shows no

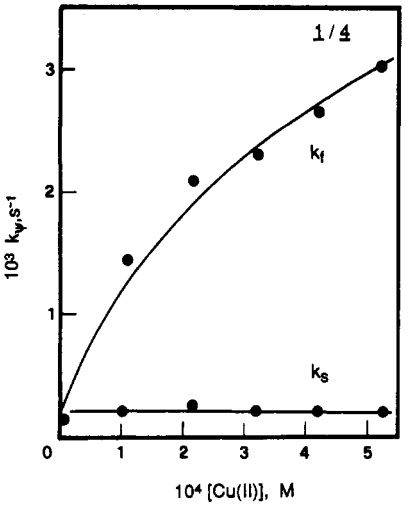


Figure 2. Observed rate constants, k_ψ (s^{-1}), for the cleavage of ester 1 in covesicles made of 1 and 4 as a function of Cu(II) concentration at 25 °C and pH = 5.0. k_f represents the observed rate constant for the fast process while k_s represents that of the slow one.

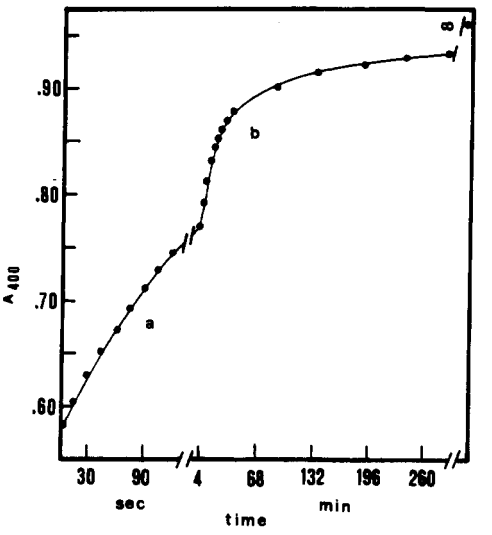


Figure 3. Time course for the release of 2 in the presence of 5.3 × 10^{-4} M Cu(II) and at pH = 5.0 for vesicular blend 1/3; curve a represents the kinetic process at 25 °C and curve b the same process after cooling to 10 °C. The solid lines are computer calculated fits of the absorbance vs time data. Time count has been started with Cu(II) addition; however, at this time part of 1 had already hydrolyzed either during sonication or the set up of the experiment: this accounts for the absorbance value observed at t = 0. (Note also the change of time scale).

dependence on the concentration of Cu(II). This is clearly illustrated in Figure 2 for the blend 1/4 at 25 °C. Analogous plots for the blends 1/5 at 25 °C and 1/3 at 10 °C are reported in Figures S2 and S3 of the supplementary material.

Two explanations hold equally well for the observed phenomena:[17] (a) Fluidity of the membrane controls Cu(II) ion permeation;[8] above T_c this is fast and the transition metal ions catalyze the hydrolysis of the ester residing on both the internal and external layers of the vesicle. Below the phase transition temperature, permeation is negligible and only cleavage of the exovesicular ester is Cu(II) catalyzed. This is clearly supported by the insensitivity of the slow kinetic process to the concentration of Cu(II) ions (see Figures 2, S2, and S3); (b) the metal ion does not permeate the membrane in any case. Rather, the transbilayer movement (flip-flop) of the ester surfactant is affected by the fluidity of the bilayer being slow in the gel phase and fast in the liquid-crystal phase. If this is the case, the Cu(II) ions, although confined in the bulk water, can catalyze the hydrolysis of all ester molecules as they move rapidly from the interior to the exterior of the vesicular membrane (and vice versa). The control of the flip-flop rate by the fluidity of the membrane has been recently demonstrated by Moss and his associates.[2]

A crucial experiment, reported in Figure 3, clearly shows that explanation b is correct. In this experiment covesicles made of 1/3 were thermostatted at 25 °C in a glass-jacketed cuvette and the kinetic run started by addition of the proper amount of Cu(II) ions. At this temperature (see Table I, run 2) ester 1 is hydrolyzed via a Cu(II)-catalyzed, monophasic process. The time course of the increase of absorbance (curve a of Figure 3) was fitted by a monoexponential (first order) equation with k_ψ = 3.5 × 10^{-3} s^{-1}. After ca. 50% of the ester was cleaved, the temperature was rapidly lowered to 10 °C and the remaining kinetic process followed at this temperature. Biphasic behavior was then observed (curve b of Figure 3) with k_f = 7.9 × 10^{-4} s^{-1} and k_s = 6.0 × 10^{-5} s^{-1} values quite similar to those obtaiend for this system at 10 °C (run 3 of Table I). This clearly indicates that no Cu(II) permeation had occurred at 25 °C. Permeation of Cu(II) ions in fact would lead to the disappearance of the slow process due to the uncatalyzed cleavage of the covesicallized ester. A similar behavior was observed for 1/4 covesicles although in this case the experiment is more difficult due to the higher temperatures of operation.[18]

In conclusion, by using the hydrolytically labile ester-surfactant 1 we have obtained clear evidence that cationic vesicles are totally impermeable to Cu(II) ions either below or above T_c. The latter appears to control the rate of the flip-flop of covesicallized 1: this process is fast above but slow below the phase transition temperature (within the time scale of our hydrolytic experiment). The results of this study are in general good agreement with those published by Moss and co-workers[2a,b,d] as far as the dependence of the flip-flop rate on the different matrices studied is concerned. Clearly, regardless of the structure of the host surfactant, the fluidity of the matrix plays a key role in influencing the rate of flip-flop. This is particularly clear for the 1/4 and 1/5 systems. For the system 1/3 (made up of a C_{18} surfactant reporter in a C_{16} matrix) a comparison with that investigated by the above authors is less straightforward. We observe a biphasic behavior at 10 °C with k_s = 8 × 10^{-5} s^{-1} (see run 3 of Table I): this set the upper limit of the rate constant for the flip-flop process of 1 under the conditions explored. For the C_{16} surfactant investigated by Moss, at 15 °C the k_s value is ca. 1 × 10^{-2} s^{-1}, i.e. more than 2 orders of magnitude

(16) A similar experiment with 1/5 covesicles was also performed at 50 °C; however, at this temperature these aggregates are unstable, developing some turbidity which hampers a clear analysis of the kinetics. Within this limitation, this vesicular blend shows a biphasic kinetic behavior even at 50 °C. Possibly, this temperature is too close to T_c.

(17) We rule out any kinetic control in the binding process of Cu(II) ions (being, for instance, slow at the inner surface) because the slowest rate constant for Cu(II) binding in aggregates is still more than 4 orders of magnitude faster than the hydrolytic process studied here. (See: Tondre, C.; Claude-Montigny, B.; Ismael, M.; Scrimin, P.; Tecilla, P. *Polyhedron* **1991**, *10*, 1791 and refs therein).

(18) This is mainly due to the time required for cooling down the system. During this time a large amount of 1 is cleaved.

larger than that of 1. This k_s is related, by the authors, to the rate of pH equilibration of the membrane so that the flip-flop rate could be much slower than this value. Taking into account these observations and the differences between the two systems, one may still speculate that the transbilayer motion of the C_{18} reporter 1 is slower than that of the C_{16} surfactant within the same matrix. Though this dependence needs to be further substantiated it is again in accord with preliminary data[2b] from Moss's laboratory.

The methodology presented here can be applied to a variety of other transition metal ions and vesicular systems, made either of natural or synthetic lipids so that its scope is not confined to the examples considered here.

Experimental Section

General Methods. Melting points are uncorrected. ^{1}H NMR spectra were recorded on a 200-MHz spectrometer and chemical shifts in ppm are reported relative to internal Me_4Si. UV-vis spectra were recorded on a spectrophotometer equipped with a thermostated cell holder (±0.1 °C temperature control). Vesicle sizes were determined by dynamic light scattering using as a light source an argon laser. Microanalyses were performed by the "Laboratorio di Microanalisi" of this Department.

Materials. Picolinoyl chloride[19] and 3-methyl-4-nitrophenyl acetate[20] were synthesized following literature methods. $Cu(NO_3)_2$ was analytical grade commercial product. The Cu^{2+} stock solution was titrated against EDTA as previously reported.[21] Di-*n*-octadecyldimethylammonium bromide (4) was analytical grade commercial product. Di-*n*-hexadecyldimethylammonium bromide[22] (3), and 1,2-bis(palmitoyloxy)-3-(trimethylammonium)-propyl bromide[23] (5) were prepared as reported. Standard synthetic procedures were followed throughout as for solvents, dehydration, and evaporation.

***N*-Methyl-*N*,*N*-dioctadecylamine.** This compound, already described,[24] was synthesized following the more convenient procedure described here. Stearic acid (5.0 g, 17.6 mmol) was suspended in 30 mL of thionyl chloride and refluxed for 18 h, protected from moisture. After this time, TLC analysis (SiO_2, $CHCl_3/CH_3OH$ 9:1) showed that the reaction was complete. The thionyl chloride was then evaporated, and the crude material solubilized in 100 mL of CH_2Cl_2 and washed (3 × 50 mL) with a saturated solution of $NaHCO_3$. The organic phase was dried and evaporated to afford 5.32 g of octadecanoyl chloride.

To a solution of the above chloride (5.32 g, 17.6 mmol) and triethylamine (1.78 g) in 100 mL of CH_2Cl_2 was added, dropwise, a solution of octadecylamine (4.74 g, 17.6 mmol) in 50 mL of CH_2Cl_2. After the addition was complete, the reaction mixture was stirred at room temperature for 48 h, following the disappearance of the amine as shown by TLC (SiO_2, $CHCl_3$, ninhydrin spot test). The mixture was diluted with 100 mL of $CHCl_3$ and washed first with 250 mL of hot 1 M HCl, then with 250 mL of a saturated solution of $NaHCO_3$, and finally with water. The organic phase was dried and evaporated to afford 9.02 g (92%) of *N*-octadecyloctadecanamide analyzed by ^{1}H-NMR (see supplementary material).

The crude *N*-octadecyloctadecanamide was dissolved in 50 mL of dry THF and dropped into a suspension of NaH (0.62 g, 60% in oil dispersion) in 50 mL THF. The mixture was then refluxed for 1.5 h under N_2. After cooling to room temperature, methyl iodide (1.23 g, 8.68 mmol) was added and the reaction mixture was further refluxed for 12 h; the reaction was followed by TLC (silica gel, ethyl ether/ethyl acetate 1:1, Feigl reagent). The suspension was percolated through a short pad of silica gel and the organic solution was evaporated to afford 3.30 g (80%) of pure *N*-methyl-*N*-octadecyloctadecanamide as analyzed by ^{1}H-NMR (see supplementary material).

$LiAlH_4$ (7 mL of a 1 M solution in THF) was slowly added with a syringe to a solution of *N*-methyl-*N*-octadecyloctadecanamide (3.30 g, 5.8 mmol) in 200 mL of dry THF. The mixture was stirred at room temperature for 1.5 h and then cooled with an ice bath, and 8 mL of 15% NaOH was added dropwise. The solvent was evaporated, and the crude solid was dissolved in $CHCl_3$ and washed with H_2O. The dried organic layer was distilled to give 2.87 g of crude product, which was recrystallized (ethyl acetate/acetone) to afford 2.46 g (76%) of pure *N*-methyl-*N*,*N*-dioctadecylamine, mp 43–45 °C (lit.[24] mp 40 °C). The ^{1}H-NMR is reported in the supplementary material.

***N*,*N*-Dioctadecyl-*N*-methyl-*N*-[2-nitro-5-(2-picolinoyloxy)benzyl]ammonium Bromide (1).** To a solution of 3-methyl-4-nitrophenyl acetate (1.20 g, 6.20 mmol) and 1.31 g of *N*-bromosuccinimide (7.36 mmol) in 100 mL of CCl_4 was added 200 mg of benzoyl peroxide. The mixture was stirred under reflux while irradiated with an UV lamp (100 W, Pyrex) for 3 h. The hot mixture was then filtered and the filter cake washed several times with hot CCl_4; the combined CCl_4 was distilled off to leave 1.99 g of crude product, which was purified by column chromatography (silica gel, toluene/hexane 7:3). An amount of 0.41 g of 3-(bromomethyl)-4-nitrophenyl acetate was obtained. The ^{1}H-NMR spectrum is reported in the supplementary material. This product was used in the next step without further purification.

The above bromide (405 mg, 1.26 mmol) and *N*-methyl-*N*,*N*-dioctadecylamine (607 mg, 1.41 mmol) were solubilized in 20 mL of CH_2Cl_2 and stirred at room temperature for 3 days. The mixture was then evaporated to afford 900 mg of *N*,*N*-dioctadecyl-*N*-methyl-*N*-[2-nitro-5-(acetyloxy)benzyl]ammonium bromide, slightly contaminated by *N*-methyl-*N*,*N*-dioctadecylamine (^{1}H-NMR analysis). This material was loaded on a column of basic alumina and eluted first with $CHCl_3$ and then with $CHCl_3/CH_3OH$ 9:1. This operation led to the hydrolysis of the acetate so that the phenoxide derivative was obtained. This was converted into the free phenol by washing a $CHCl_3$ solution with 1 M HBr. The organic phase was dried and evaporated to afford a crude solid, which was triturated with ethyl ether to give 690 mg (81%) of pure *N*,*N*-dioctadecyl-*N*-methyl-*N*-(2-nitro-5-oxidobenzyl)-ammonium bromide (2) characterized by a singlet of the quaternary ammonium methyl at δ 3.22 in the ^{1}H-NMR spectrum (for the full spectrum see the supplementary material).

The above phenol (0.68 g, 0.89 mmol), solubilized in 50 mL of CH_2Cl_2, was added to a suspension of picolinoyl chloride (1.6 g, 9.03 mmol) in 100 mL of CH_2Cl_2. The resulting slurry was refluxed for 2.5 h under N_2. The hot solution was then filtered twice through a Celite pad and the blue filtrate evaporated to give 1.09 g of a solid that was purified by gel-permeation chromatography (Sephadex LH-20, CH_2Cl_2). The first fractions, collected and evaporated, gave 390 mg of a solid, which was triturated with acetone to afford 300 mg (39%) of pure *N*,*N*-dioctadecyl-*N*-methyl-*N*-[2-nitro-5-(picolinoyloxy)benzyl]ammonium bromide, mp 89 °C (softens) and 118–119 °C (melts, dec): ^{1}H-NMR δ ($CDCl_3$) 0.88 (t, J = 6.71 Hz, 6 H, $N(CH_2)_{17}CH_3$), 1.26 (m, 60 H, $NCH_2CH_2(CH_2)_{15}CH_3$), 1.71 (m, 4 H, $NCH_2CH_2(CH_2)_{15}CH_3$), 2.93 (s, 3 H, NCH_3), 3.05–3.45 (m, 4 H, $NCH_2(CH_2)_{16}CH_3$), 5.03 (s, 2 H, $PhCH_2N$), 7.67–7.88 (m, 3 H, H_6Ph, H_5Py and H_4Ph), 8.07 (t, J = 8.84 Hz, 1 H, H_4Py), 8.26–8.31 (m, 2 H, H_3Ph and H_3Py), 8.86 (d, J = 3.66 Hz, 1 H, H_6Py). Anal. Calcd for $C_{50}H_{86}N_3O_4Br$: C, 68.78; N, 4.81; H, 9.93. Found: C, 70.07; N, 4.82; H, 10.45.

Vesicle Preparation and Characterization. All vesicle solutions were prepared in a 25-mL beaker by dissolving surfactant 1 and the proper nonfunctional additive (3–5) in methylene chloride. The solvent was allowed to evaporate and subsequently the beaker was kept under vacuum for 2 h. The surfactant film was covered with 20 mL of MES buffer 0.05 M, pH 5, and sonicated[25] (Branson Model B15, immersion probe, max power output) for 6 min at 40 °C (surfactant 5), 35 °C (surfactant 4), and 30 °C (surfactant 3). The vesicle solutions were filtered through 0.45-μm Millipore filters before use. Gel-

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to-liquid crystal phase transition temperatures, T_c, were determined from fluorescence polarization[15] studies using covesicallized 1,6-diphenyl-1,3,5-hexatriene (DPH) as a probe under the following conditions: [DPH] = 2.5×10^{-6} M, [1] = 8×10^{-5} M, [additive] = 8×10^{-4} M.

Ethidium Bromide Trapping. Covesicles solutions made up of surfactant 1 (1.0×10^{-4} M), the proper additive (1.0×10^{-3} M), and ethidium bromide (EB, 5.0×10^{-3} M) were prepared in 0.05 M MES buffer, pH 5, and eluted through a Sephadex G-75 column. Sephadex G-75 powder (2.5 g) was allowed to swell overnight in 20 mL of aqueous 0.05 M MES buffer. The resulting slurry was suspended in a 1 × 30 cm glass-jacketed chromatography column. The column was then conditioned by passage of 50 mL of buffer. Solutions of EB-loaded vesicles were poured on the column in 5-mL volumes, allowed to absorb, and then eluted with buffer. Fractions of 2.5 mL were collected and vesicles were found in fractions 5 and 6, as visualized from light scattering. Free EB was held up by the Sephadex and eluted after fraction 10. The fractions containing vesicles were checked for the presence of entrapped EB by using fluorescence spectroscopy (excitation wavelength 480 nm, emission maximum 610 nm). In the case of surfactant 3 the Sephadex column chromatography was performed at 10 °C to prevent the possible leaking of the dye at temperature above T_c.

Kinetic Studies. Solutions were prepared in MES buffer (0.05 M, pH 5). No changes in the pH were observed during the kinetic runs. Kinetics were run under first-order conditions ([1] = $7–8 \times 10^{-5}$ M) and the release of 2 was followed at 400 nm. The rate constants were obtained by nonlinear regression analysis of the absorbance vs time data.[26] The fit error of the constants was always less than 1%.

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Supplementary Material Available: Figures S1–S3 reporting the results of gel-permeation chromatography after EB trapping, plots of the observed rate constants for the hydrolysis of 1 as a function of Cu(II) concentration and of the vesicle's composition, ^{1}H-NMR data for all the intermediates in the synthesis of 1 (4 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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