Preparation and Characterization of Glycerol-Based Cleavable Surfactants and Derived Vesicles

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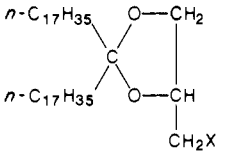
Abstract: Four cleavable double-chain surfactants were synthesized: [(2,2-diheptadecyl-1,3-dioxolan-4-yl)methyl]trimethylammonium methanesulfonate (**1a**), the analogous bromide (**1b**), [(2,2-diheptadecyl-1,3-dioxolan-4-yl)methyl]dimethyl(3-sulfopropyl)ammonium hydroxide inner salt (**1c**), and sodium 3-[(2,2-diheptadecyl-1,3-dioxolan-4-yl)methoxy]-1-propanesulfonate (**1d**). Vesicles of **1a**, **1b**, and **1d** prepared by sonication were characterized by ^{1}H NMR line width narrowing, dynamic laser light scattering, differential scanning calorimetry, and dye entrapment and leakage studies. In vesicular form, the hydrolytic lability of **1d** was greater than that of **1a**/**1b**, due to a combination of electrostatic effects resulting from the different subtituents on the dioxolane ring. Neutral organic compounds can be readily isolated from vesicular solutions of **1d** after its hydrolysis. Thus **1d** is appropriate for the application of vesicular media to preparative chemistry.

Vesicles are closed bilayer structures formed by double-chain surfactants in water.[2] Numerous organic reactions have been performed in vesicular media,[2] and in several instances impressive selectivity has been realized that is unobtainable in conventional solvents.[3] In general, such selectivity derives from the ability of vesicles to solubilize, orient, and compartmentalize reactants. Before vesicular media can be used on a practical level in preparative chemistry, however, a major obstacle must be overcome, namely, the difficulty inherent in the isolation of products from surfactant-based solutions. As presented herein, we have addressed this problem by the preparation and characterization of cleavable double-chain surfactants **1** and their derived vesicles. After their use in vesicular form, **1** can be converted to nonsurfactant compounds to facilitate product isolation by standard procedures. Several other cleavable double-chain surfactants,[4] as well as a variety of single-chain analogues,[5] have been described recently.

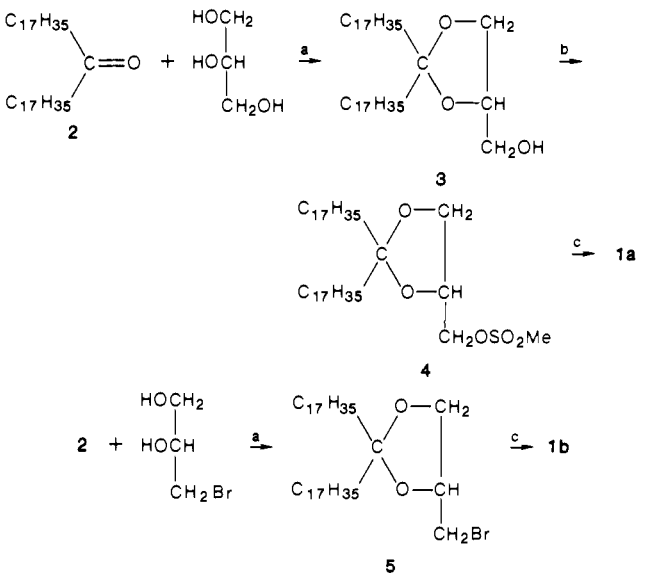


1a, X = $N^+Me_3MeSO_3^-$
b, X = $N^+Me_3Br^-$
c, X = $N^+Me_2(CH_2)_3SO_3^-$
d, X = $O(CH_2)_3SO_3^-Na^+$

Results

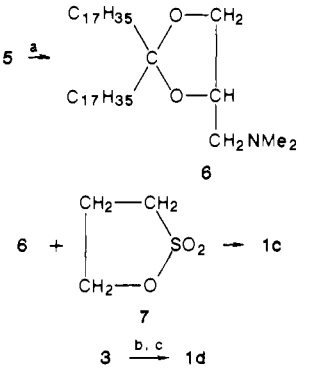
Syntheses. The preparations of **1a** and **1b** are outlined in Scheme I. Ketalization of glycerol with ketone **2** gave hydroxy ketal **3**, which was converted to methanesulfonate **4**. The reaction of **4** with Me_3N in MeOH at 75 °C yielded **1a**. Ketalization of 3-bromo-1,2-propanediol with **2** gave **5**, which was converted to **1b** with the procedure for **1a**. Alternatively, **5** was prepared from the reaction of **4** with LiBr in 3-pentanone.

Scheme I[a]



[a] (a) p-$MeC_6H_4SO_3H$, C_6H_6; (b) $MeSO_2Cl$, Et_3N, CH_2Cl_2; (c) Me_3N, MeOH.

Scheme II[a]



[a] (a) Me_3N, MeOH; (b) NaH, C_6H_6; (c) **7**.

(1) On leave from Jagiellonian University, Kraków, Poland.
(2) Fendler, J. H. *Membrane Mimetic Chemistry*; Wiley-Interscience: New York, 1982; Chapters 6 and 12.
(3) For example, see: (a) Porter, N. A.; Ok, D.; Huff, J. B.; Adams, C. M.; McPhail, A. T.; Kim, K. *J. Am. Chem. Soc.* **1988**, *110*, 1896. (b) Ueoka, R.; Matsumoto, Y.; Moss, R. A.; Swarup, S.; Sugii, A.; Harada, K.; Kikuchi, J.; Murakami, Y. *J. Am. Chem. Soc.* **1988**, *110*, 1588. (c) Groves, J. T.; Newmann, R. *J. Org. Chem.* **1988**, *53*, 3891. (d) Mitzutani, T.; Whitten, D. G. *J. Am. Chem. Soc.* **1985**, *107*, 3621 and references therein.
(4) (a) Jaeger, D. A.; Chou, P. K.; Bolikal, D.; Ok, D.; Kim, K. Y.; Huff, J. B.; Yi, E.; Porter, N. A. *J. Am. Chem. Soc.* **1988**, *110*, 5123. (b) Jaeger, D. A.; Golich, T. G. *J. Am. Oil Chem. Soc.* **1987**, *64*, 1550. (c) Nuhn, P.; Dobner, B.; Kertscher, P.; Weissflog, W.; Brezesinski, G. *Pharmazie* **1981**, *36*, 537. (d) Eibl, H.; Nicksch, A. *Chem. Phys. Lipids* **1978**, *22*, 1.
(5) (a) Jaeger, D. A.; Ward, M. D.; Dutta, A. K. *J. Org. Chem.* **1988**, *53*, 1557. (b) Jaeger, D. A.; Finley, C. T.; Walter, M. R.; Martin, C. A. *J. Org. Chem.* **1986**, *51*, 3956. (c) Jaeger, D. A.; Martin, C. A.; Golich, T. G. *J. Org. Chem.* **1984**, *49*, 4545. (d) Jaeger, D. A.; Frey, M. R. *J. Org. Chem.* **1982**, *47*, 311. (e) Cuomo, J.; Merrifield, J. H.; Keana, J. F. W. *J. Org. Chem.* **1980**, *45*, 4216. (f) Epstein, W. W.; Jones, D. S.; Bruenger, D.; Rilling, H. C. *Anal. Biochem.* **1982**, *119*, 304. (g) Hayashi, Y.; Shirai, F.; Shimizu, T.; Nagano, Y.; Teramura, K. *J. Am. Oil Chem. Soc.* **1985**, *62*, 555.

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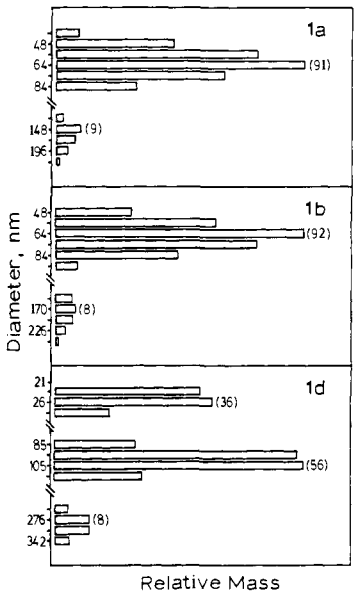
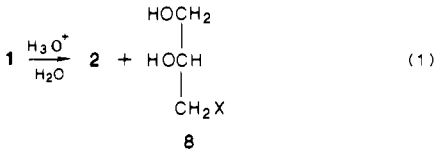


Figure 1. DLLS histograms for vesicular **1a**, **1b**, and **1d**. The percent mass values for the populations are given in parentheses.

The syntheses of **1c** and **1d** are outlined in Scheme II. At 105 °C, **5** and Me_3N in MeOH gave **6**, which was converted to **1c** with sultone **7**. Amine **6** likely derived from its S_N2 displacement by Br^- from an *N*-methyl group of initially formed **1b**. The reaction of the alkoxide ion derived from **3** with **7** gave **1d.**

Vesicle Preparation and Characterization. Vesicles were prepared by sonication (150 W, bath, 50–55 °C) of surfactant in water/buffer solution and were characterized by ^1H NMR line width narrowing, dynamic laser light scattering (DLLS), differential scanning calorimetry (DSC), and dye entrapment and leakage studies. Surfactant **1c** did not readily disperse in water on sonication and therefore was not included in these studies. The labilities of **1a**/**1b** and **1d** in vesicular form with respect to acid-catalyzed hydrolysis to **2** and **8** (eq 1) were also determined. Compounds **8b**, **8c**, and **8d** were independently prepared and characterized (see Experimental Section).



(1)

a, $X = N^+Me_3MeSO_3^-$ **b**, $X = N^+Me_3Br^-$
c, $X = N^+Me_2(CH_2)_3SO_3^-$ **d**, $X = O(CH_2)_3SO_3^-Na^+$

^1H NMR Line Width Narrowing. To a thin film of surfactant formed in an NMR tube D_2O was added. The system was sonicated for a given time (see Experimental Section) and its ^1H NMR spectrum recorded. After an additional sonication period, another ^1H NMR spectrum was obtained. For **1a**, **1b**, and **1d**, the line widths of the signals narrowed and their intensities increased in the second relative to the first spectrum. For example, for **1d** after 4 h the intensity of the methylene envelope at δ 1.3 increased by a factor of 4 and its width at half-height decreased by a factor of 0.7 relative to the spectrum after 15 min of sonication. These observations are consistent with, but do not demand, the formation of vesicles.[6]

DLLS. A thin film of surfactant in a pH 7.4 phosphate buffer was sonicated for 1h, and the vesicular solution was filtered through a 0.45-μm Durapore filter. The filtrate was analyzed at 23 °C by DLLS, and the resultant histograms of relative mass (volume) vs hydrodynamic diameter for **1a**, **1b**, and **1d** are given in Figure 1. For both **1a** and **1b**, two, and for **1d**, three populations of particles were observed.

DSC. The thermotropic properties of **1a**, **1b**, and **1d** were determined by DSC using two methods. In the first,[7] measurements were made on hydrated surfactant pellets resulting from the concentration of pH 7.4 vesicular solutions with a 30000 molecular weight cut-off filter. The phase transition temperatures (T_c) for **1a**, **1b**, and **1d** were 37, 40, and 44 ± 2 °C, respectively. At T_c, a bilayer undergoes a transition from the gel to the less-ordered liquid crystalline state, corresponding to conformational changes of the *n*-alkyl groups.[8]

In the second method, measurements were made on the vesicular solutions themselves, and the resultant thermograms are given in Figure 2. Single transitions were observed for **1a** and **1b** with T_c's of 38.0 and 39.3 ± 0.5 °C and calorimetric enthalpies, ΔH_{cal}, of 43 and 41 kJ/mol, respectively. Several transitions were observed for **1d**: at least two at ca. 29 °C and one at 44.2 ± 0.5 °C. Micelles would not be expected to display phase transitions.

Dye Entrapment and Leakage. Surfactant was sonicated in a pH 7.4 solution containing 0.050 M calcein, a fluorescent dye.[9] At 25 °C, the resultant solution was first filtered through 0.4- and 0.2-μm polycarbonate filters[10] and then subjected to gel filtration chromatography[11] on Sephadex G-25-80 with a pH 7.4 phosphate buffer as eluant. For each surfactant, a portion of the dye eluted at the void volume of the column. As described below, the fluorescence intensity of the eluant at the void volume increased with time, consistent with the incorporation of dye in vesicles, but not micelles. For the latter, the fluorescence intensity would be invariant with time.

The leakage of calcein from vesicles at the void volume was determined by following the fluorescence intensity of the vesicular solution. The fluorescence quantum yield of this dye is concentration dependent and is relatively low at 0.05 M calcein.[9] As calcein escapes from the vesicles into the bulk aqueous phase and is thus diluted, the fluorescence intensity of the solution increases. The course of calcein release from vesicles of **1a**, **1b**, and **1d** at pH 7.4 and 20 °C over an 8-h period is given in Figure 3. At 8 h, the extents of release were 34%, 21%, and 21%, respectively. The enhancement of release at lower pHs will be the subject of future study.

Hydrolytic Lability. Vesicular solutions of **1a**, **1b**, and **1d** in D_2O were prepared by 4 h of sonication and then held at 25 °C. In each case, there was no hydrolysis after 24 h as evidenced by ^1H NMR analysis (absence of **2** and **8**). Also, the line shapes and relative intensities of the signals did not change appreciably over the same period.

A set of vesicular solutions identical with that above was prepared, and after the sonication period, each solution at 25 °C was adjusted to pD 1.4 with DCl–D_2O after its ^1H NMR spectrum was recorded. After 24 h, there were no signs of hydrolysis for **1a** and **1b** by ^1H NMR analysis. However, the signals were substantially broader and less intense than those in the initial spectra. By ^1H NMR analysis, **1d** completely hydrolyzed within 40 min. Complete hydrolysis of **1a** occurred during 2 h at 50 °C in a sonicated 1:1:1 (v/v/v) mixture of 1.0 M HCl–H_2O, $CHCl_3$, and EtOH. The organic solvents served to disrupt the vesicles and thus facilitated hydrolysis.

A study of the kinetics of the hydrolysis of **1d** at 25 °C and pH 3.0 in HCl–H_2O gave a one-point, pseudo-first-order rate constant, k_ψ, of 0.0123 ± 0.0005 min^{-1} over the first 30% of hydrolysis. This value corresponds to a half-life of 56 min. Since the nature of the system changed over the course of the hydrolysis, if for no other reason than the formation of hydrolysis products

(6) (a) Finer, E. G.; Flook, A. G.; Hauser, H. *Biochim. Biophys. Acta* **1972**, *260*, 49. (b) Finer, E. G.; Flook, A. G.; Hauser, H. *Biochim. Biophys. Acta* **1972**, *260*, 59. (c) Browning, J. L. In *Liposomes: From Physical Structure to Therapeutic Applications*; Knight, C. G., Ed.; Elsevier/North Holland: New York, 1981; p 189.

(7) Jacobson, K.; Papahadjopoulos, D. *Biochemistry* **1975**, *14*, 152.

(8) Wilkinson, D. A.; Nagle, J. F. In ref 6c; p 273.

(9) Allen, T. M. In *Liposome Technology*; Gregoriadis, G., Ed.; CRC Press: Boca Raton, FL, 1984; Vol. 3, p 177.

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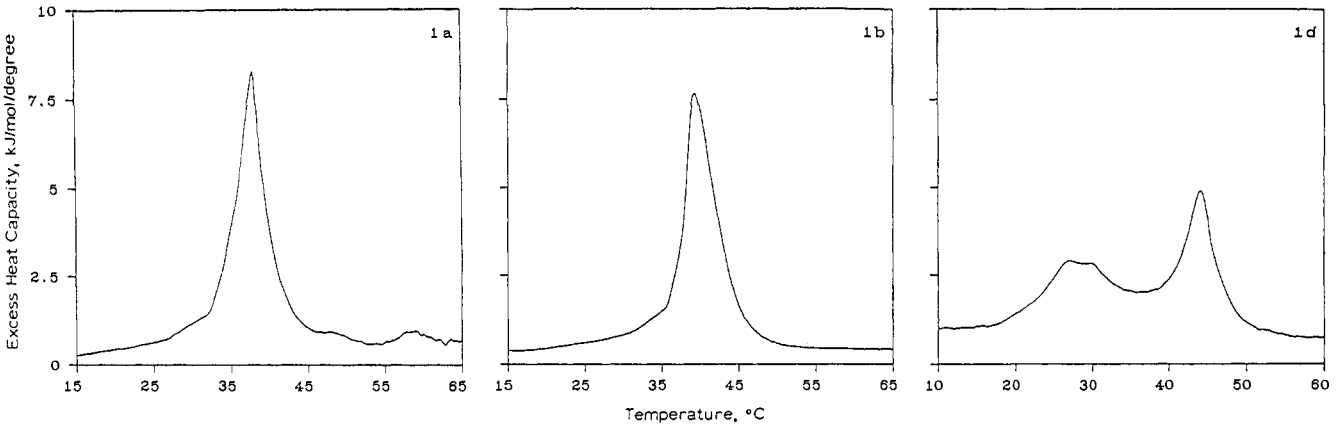


Figure 2. DSC thermograms for vesicular **1a**, **1b**, and **1d**.

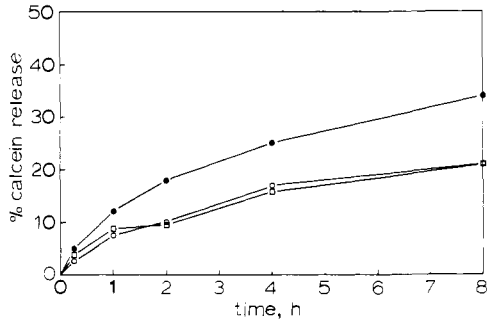


Figure 3. Calcein release from vesicular **1a** (●), **1b** (□), and **1d** (○).

2 and **8d**, k_ψ represents an average value.

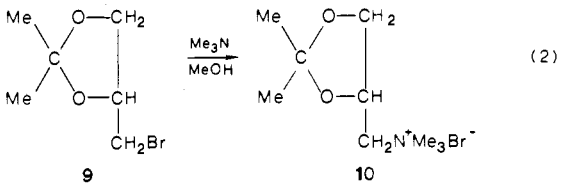
The hydrolytic labilities of the surfactants were also investigated by two FT-IR methods. In the first, a vesicular solution was concentrated as above, and the resultant surfactant pellet was analyzed by the attenuated total reflectance (ATR) method. Specifically, vesicular solutions of **1a** and **1b** in D_2O were adjusted to pD 1.4 with DCl–D_2O at 25 °C. After 24 h, they were concentrated and analyzed. In each case, no absorption was detected in the 1700-cm^{-1} region, indicating the absence of **2** and thus the lack of hydrolysis, consistent with the ^1H NMR results above. In a control, a mixture of **1b** and 13 mol % **2** in D_2O was sonicated for 4 h, followed by concentration and FT-IR analysis. Two absorption bands corresponding to **2** were clearly evident at 1698 and 1704 cm^{-1}. The observation of two bands suggests that the carbonyl group of **2** resided in two different environments, which is not unreasonable given the heterogeneous nature of the sample. In the second FT-IR method, vesicular solutions identical with those above were analyzed without concentration using a cell with CaF_2 windows. However, this method was abandoned since ketone **2** could not be detected in control samples prepared from 5 mol % **2** and **1a** or **1b**, presumably due to its insolubility. Indeed, by visual inspection **2** did not dissolve during sonication of the control samples.

A striking example of the potential for straightforward isolation of neutral organic products from vesicular **1d** was obtained in a study of the regiochemistry and relative rates of monohalogenation of *n*-alkyl phenyl ethers in vesicular media.[12] In a control, 1.00 mL of 0.020 M vesicular **1d** containing 1.1×10^{-4} M pentyl phenyl ether was subjected to a workup procedure involving the acid-catalyzed hydrolysis of **1d** to **2** and **8d**. By HPLC analysis [25 cm × 4.6 mm i.d. column of 10-μm Econosil C18 (Alltech); MeCN–H_2O elution; UV detection (210 nm)] of the resultant MeCN solution of pentyl phenyl ether (devoid of **2**) with nonyl phenyl ether as an internal standard, the recovery of the former was 100%. Thus 0.018 mg of pentyl phenyl ether was isolated from a solution containing 14.5 mg of **1d**. In general, such an isolation from a vesicular solution based on a conventional non-cleavable surfactant would be extremely difficult if not impossible.

(12) Jaeger, D. A.; Clennan, M. W., to be published.

Discussion

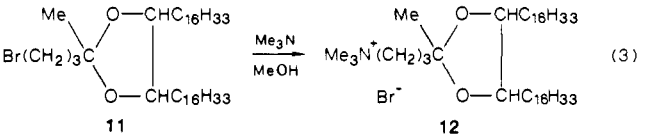
Two features of the above syntheses are noteworthy. First, the total lack of reaction at 25 °C of **4**/**5** with Me_3N in MeOH to give **1a**/**1b** was surprising, since **9** can be converted to **10** under the same conditions (eq 2).[13] The limited solubility of **4**/**5** cannot



(2)

9 **10**

be the sole reason for its lesser reactivity because **11**, which should have a comparable solubility, yielded **12** in Me_3N–MeOH at 25 °C (eq 3).[4a] Perhaps the heptadecyl chains are coiled/aggregated



(3)

11 **12**

in MeOH with consequent steric hindrance of Me_3N's attack at the primary carbon atom of **4**/**5**. Rate retardations due to the coiling/aggregation of long-chain alkyl groups have been reported previously for other systems.[14] Second, the demethylation of *N*-methyl quaternary ammonium compounds by S_N2 displacement with Br^-/I^- in an alcohol solvent generally requires more drastic conditions than used to prepare **6** from **5**.[15] The proposed displacement of **6** from an *N*-methyl group of **1b** was perhaps facilitated by the latter's aggregation. Such aggregation would concentrate Br^- and the quaternary ammonium head groups at the aggregate–MeOH interface, with a resultant greater S_N2 reactivity than expected for monomeric **1b**. However, it is generally believed that surfactants do not aggregate in MeOH.[16] But the aggregation of n-$C_nH_{2n+1}N^+Me_3Br^-$ (n = 10, 12, 14, 16, and 18) in MeOH containing >2 wt % C_6H_6 has been reported.[17]

In the DLLS histograms of Figure 1, the smaller particles grouped at 64 nm for **1a** and **1b** are assigned to small unilamellar vesicles (SUV's). For **1d**, the population grouped at 26 nm is assigned to SUV's, and that at 105 nm to multilamellar vesicles (MLV's). These assignments are generally consistent with the ranges of sizes for SUV's and MLV's.[2] Micelles are not reasonable

(13) Jaeger, D. A.; Mohebalian, J., to be published.
(14) For example, see: Fan, W.-Q.; Jiang, X.-K. *J. Am. Chem. Soc.* **1985**, *107*, 7680 and references therein.
(15) For example, see: (a) House, H. O.; Müller, H. C.; Pitt, C. G.; Wickham, P. P. *J. Org. Chem.* **1963**, *28*, 2407. (b) Saito, S.; May, E. L. *J. Org. Chem.* **1962**, *26*, 4536.
(16) Magid, L. In *Solution Chemistry of Surfactants*; Mittal, K. L., Ed.; Plenum Press: New York, 1979; Vol. 1, p 427.
(17) Chattopadhyay, A. K.; Drifford, M.; Treiner, C. *J. Phys. Chem.* **1985**, *89*, 1537.

possibilities for these particles because they would likely have hydrodynamic diameters <20 nm.[18] No physical significance can be ascribed to the larger/largest populations for **1a**, **1b**, and **1d**. They probably correspond to a few large particles that are grouped into parts of the bimodal/trimodal distributions by the mathematical analysis of the light scattering data.

The DSC thermograms of Figure 2 are consistent with the above DLLS assignments. The single transitions observed for **1a** and **1b** correspond to the SUV's detected by DLLS. The transitions at ca. 29 and 44.2 °C for **1d** likely correspond to the SUV's and MLV's, respectively. Others have reported data in accord with a variation of T_c with surfactant aggregate morphology.[19] For example, Kunitake and co-workers[19a] found a single transition at T_c = 54 °C and two transitions at T_c = 39 and 45 °C for aggregates of $(n\text{-}C_{18}H_{37})_2N^+Me_2Br^-$ prepared by dispersion and sonication, respectively.

It is interesting to note that **1a** and **1b**, which differ only in counterion, display different T_c's and calcein release profiles, even though their DLLS histograms are essentially the same. Furthermore, the disparities are expressed at [**1a**(**b**)] = 0.01 M in a medium containing 0.10 M Cl^- and lesser amounts of HPO_4^{2-} and $H_2PO_4^-$. Clearly, the affinities of Br^- and/or $MeSO_3^-$ for the vesicular quaternary ammonium head groups are greater than that of Cl^-. It is known that the affinity of Br^- toward micellar quaternary ammonium head groups is greater than that of Cl^-.[20]

In general, double-chain surfactants, including compounds such as **1a**, **1b**, and **1d**, are expected to form vesicles on sonication in H_2O.[2] However, the formation of micelles alone, or micelles plus vesicles, cannot be dismissed a priori.[18,21] But taken as a whole, and as noted above, the results from the ^1H NMR line width narrowing, DLLS, DSC, and dye entrapment and release studies are consistent with the formation of only vesicles from **1a**, **1b**, and **1d**. The DLLS results in particular are inconsistent with the presence of micelles.

The difference in the lability/stability characteristics of **1a**/**1b** and **1d** with respect to acid-catalyzed hydrolysis (eq 1), which probably involves protonation of the dioxolane ring,[22] must derive from their dissimilar substituents. On an intramolecular basis, the cationic substituent of **1a**/**1b** electrostatically hinders, whereas the anionic substituent of **1d** facilitates protonation. On an aggregate basis, electrostatic depletion and accumulation of H_3O^+ exist at the bilayer–water interfaces of vesicular **1a**/**1b** and **1d**, respectively, relative to the bulk aqueous phase.[20,23] Overall, the greater reactivity of **1d** results from both of these related effects.

In the practical application of **1** to vesicular-mediated reactions of organic substrates, the desired products must be separated from **2** and **8** after hydrolysis of the surfactant. Water-soluble **8** can be easily separated from a neutral product by simple extraction, and **2** can be removed by any one of a number of standard methods. In many instances as above,[12] the separation of **2** will be facilitated by its insolubility in solvents such as MeCN, Et_2O, Me_2CO, and hexane.

In summary, cleavable double-chain surfactants **1** have been synthesized, and vesicles derived from **1a**, **1b**, and **1d** have been characterized. With varying ease, **1a**/**1b** and **1d** undergo acid-catalyzed hydrolysis to give nonsurfactant compounds **2** and **8**. Thus these surfactants can be used for vesicular-mediated reactions of organic substrates, followed by their hydrolysis and straightforward isolation of the desired reaction products.

(18) For example, micelles of 70 mM diheptanoylphosphatidylcholine at 20 °C have a mean hydrodynamic diameter of 12 nm (Burns, R. A., Jr.; Donovan, J. M.; Roberts, M. F. *Biochemistry* **1983**, *22*, 964).

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(22) March, J. *Advanced Organic Chemistry*, 3rd ed.; Wiley: New York, 1985; p 329.

(23) Bunton, C. A.; Ohmenzetter, K.; Sepulveda, L. *J. Phys. Chem.* **1977**, *81*, 2000.

Experimental Section

General Procedures and Materials. ^1H (270 MHz) and ^{13}C (67.8 MHz) NMR spectra were recorded in $CDCl_3$ and D_2O with Me_4Si and $Me_3SiCD_2CD_2CO_2Na$, respectively, as internal standards. FAB high-resolution mass spectra were obtained by the Midwest Center for Mass Spectrometry, a National Science Foundation Regional Instrumentation Facility. Fluorescence measurements were made on a Perkin-Elmer Model LS-5 fluorescence spectrophotometer. The pD measurements of solutions in 5-mm NMR tubes were obtained with a 3-mm (diameter) Ag/AgCl combination electrode (Sargent-Welch S-30070-05); each value reported is that of the "meter pH reading" + 0.4.[24] All melting points are uncorrected. Elemental analyses were performed by Atlantic Microlab, Atlanta, GA.

The pH 7.4 phosphate buffer (μ = 0.11) contained 3.0 mM Na_2HPO_4, 1.33 mM KH_2PO_4, 0.10 M NaCl, and 0.10 mM EDTA in doubly distilled or HPLC-grade H_2O. Calcein (Kodak) was purified by literature procedures[9,25] and then dissolved in the minimum amount of 1.0 M NaOH, followed by dilution with H_2O to 0.05 M, and adjustment to pH 7.4.

2,2-Diheptadecyl-1,3-dioxolane-4-methanol (3). A solution of 28.2 g (55.7 mmol) of 18-pentatriacontanone (Pfaltz and Bauer), mp 88–89 °C (lit.[26] mp 89–89.5 °C), 5.53 g (60.0 mmol) of glycerol, 100 mg of *p*-$MeC_6H_4SO_3H\cdot H_2O$, and 500 mL of C_6H_6 was refluxed for 45 h under a Dean–Stark trap fitted with a drying tube ($CaCl_2$). The reaction mixture was washed with 500 mL of aqueous 5% $NaHCO_3$, dried (Na_2SO_4), and rotary evaporated. The resultant crude product was recrystallized from Me_2CO (4 °C) to give 25.2 g (78%) of **3**: mp 44–46 °C; ^1H NMR ($CDCl_3$) δ 4.21 (m, 1 H), 4.02 (t, J = 7 Hz, 1 H), 3.73 (m, 2 H), 3.59 (m, 1 H), 1.87 (br t, 1 H, OH), 1.60 (m, 4 H, CH_2CO), 1.25 (s, 60 H, $(CH_2)_{15}$), 0.88 (t, 6 H, CH_3); ^{13}C NMR ($CDCl_3$) δ 112.83, 76.16, 65.86, 62.95, 37.39, 36.82, 31.93, 29.92, 29.70, 29.37, 24.02, 23.75, 22.71, 14.15. Anal. Calcd for $C_{38}H_{76}O_3$: C, 78.55; H, 13.19. Found: C, 78.78; H, 13.19.

(2,2-Diheptadecyl-1,3-dioxolan-4-yl)methyl Methanesulfonate (4). To a stirred solution of 5.00 g (8.61 mmol) of **3** and 1.50 g (14.8 mmol) of Et_3N in 60 mL of CH_2Cl_2 at −5 °C was added 1.25 g (10.9 mmol) of $MeSO_2Cl$ during 15 min. After an additional 30 min at −5 °C, the reaction mixture was washed with ice-cold H_2O, ice-cold 10% hydrochloric acid, saturated aqueous $NaHCO_3$, and saturated aqueous NaCl and then it was dried ($MgSO_4$) and rotary evaporated. The resultant crude product was recrystallized from Me_2CO (4 °C) to give 4.8 g (85%) of **4**: mp 59–60 °C; ^1H NMR ($CDCl_3$) δ 4.36 (m, 1 H), 4.22 (m, 2 H), 4.10 (m, 1 H), 3.74 (m, 1 H), 3.06 (s, 3 H, CH_3SO_3), 1.58 (m, 4 H, CH_2CO), 1.25 (s, 60 H, $(CH_2)_{15}$), 0.88 (t, 6 H, CH_3); ^{13}C NMR ($CDCl_3$) δ 113.78, 73.28, 69.17, 66.15, 37.61, 37.26, 36.61, 31.90, 29.67, 29.35, 23.94, 23.67, 22.69, 14.12; IR (KBr) 2900 (s), 2845 (s), 1460 (m), 1347 (s), 1169 (s), 1085 (m), 960 (s), 840 (m), 718 cm^{-1} (m). Anal. Calcd for $C_{39}H_{78}O_5S$: C, 71.07; H, 11.93. Found: C, 71.28; H, 11.94.

[(2,2-Diheptadecyl-1,3-dioxolan-4-yl)methyl]trimethylammonium Methanesulfonate (1a). A solution of 2.60 g (3.94 mmol) of **4** in 100 mL each of 25% (w/v) Me_3N–MeOH (Kodak) and MeOH was held at 75 °C for 48 h in an autoclave and then rotary evaporated. The resultant crude product was dried at 80 °C (20 mmHg) and recrystallized from Me_2CO (4 °C) to give 2.1 g (74%) of **1a**: mp 88 → 103 °C; ^1H NMR ($CDCl_3$) δ 4.62 (m, 1 H), 4.32 (m, 2 H), 3.62 (t, J = 8 Hz, 1 H), 3.42 (s, 9 H, $(CH_3)_3N$), 3.20 (m, 1 H), 2.74 (s, 3 H, $CH_3SO_3^-$), 1.58 (m, 4 H, CH_2CO), 1.25 (s, 60 H, $(CH_2)_{15}$), 0.88 (t, 6 H, CH_3); IR (KBr) 2920 (s), 2858 (m), 1465 (m), 1190 (m), 1090 (w), 1040 (w), 965 (w), 930 (w), 770 (w), 715 cm^{-1} (w). Anal. Calcd for $C_{42}H_{87}NO_5S$: C, 70.24; H, 12.21. Found: C, 70.36; H, 12.32.

(2,2-Diheptadecyl-1,3-dioxolan-4-yl)methyl Bromide (5). With the procedure used for **3**, 10.0 g (19.7 mmol) of 18-pentatriacontanone, 6.10 g (39.4 mmol) of 3-bromo-1,2-propanediol (Aldrich), and 60 mg of *p*-$MeC_6H_4SO_3H\cdot H_2O$ in 125 mL of C_6H_6 yielded crude product that was recrystallized from Me_2CO (4 °C) to give 10.6 g (84%) of **5**: mp 55–56 °C; ^1H NMR ($CDCl_3$) δ 4.31 (m, 1 H, OC*H*CH_2Br), 4.14 (m, 1 H, OC$H_a$$H_b$), 3.78 (m, 1 H, OC$H_a$$H_b$), 3.45 (m, 1 H, C$H_a$$H_b$Br), 3.29 (m, 1 H, C$H_a$$H_b$Br), 1.58 (4 H, CH_2CO), 1.25 (s, 60 H, $(CH_2)_{15}$), 0.88 (t, 6 H, CH_3); ^{13}C NMR ($CDCl_3$) δ 113.87, 75.29, 68.87, 37.58, 36.90, 32.67, 31.93, 29.70, 29.59, 29.37, 23.91, 23.72, 22.71, 14.12. Anal. Calcd for $C_{38}H_{75}BrO_2$: C, 70.88; H, 11.74. Found: C, 70.96; H, 11.75.

In another preparation, a solution of 0.33 g (0.50 mmol) of **4** and 0.090 g (1.0 mmol) of LiBr in 25 mL of 3-pentanone was refluxed for

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24 h, filtered, and rotary evaporated. The crude product was recrystallized from Me_2CO (4 °C) to give 0.30 g (94%) of **4**: mp 55–56 °C.

[(2,2-Diheptadecyl-1,3-dioxolan-4-yl)methyl]trimethylammonium Bromide (1b). A solution of 2.58 g (4.01 mmol) of **5** in 100 mL each of 25% (w/v) Me_3N–MeOH and MeOH was held at 60 °C in an autoclave for 7 days and then rotary evaporated. The residue was dried at 80 °C (20 mmHg) and recrystallized from Me_2CO (4 °C) with Norit decolorization. The resultant material was treated with MeOH (25 °C), and unreacted **5** was removed by filtration. After rotary evaporation, the crude product was recrystallized from Me_2CO (4 °C) to give 2.1 g (75%) of **1b**: mp 179–180 °C; ^1H NMR ($CDCl_3$) δ 4.62 (m, 2 H), 4.30 (m, 1 H), 3.56 (s + m, 10 H, CH and $(CH_3)_3N$), 3.21 (m, 1 H), 1.59 (m, 4 H, CH_2CO), 1.25 (s, 60 H, $(CH_2)_{30}$), 0.88 (t, 6 H, CH_3); ^{13}C NMR ($CDCl_3$) δ 115.03, 69.99, 68.46, 67.38, 54.68, 37.20, 36.28, 31.82, 29.62, 29.27, 23.91, 22.58, 14.02. Anal. Calcd for $C_{41}H_{84}BrNO_2$: C, 70.05; H, 12.04. Found: C, 70.09; H, 12.05.

[(2,2-Diheptadecyl-1,3-dioxolan-4-yl)methyl]dimethylamine (6). A solution of 2.58 g (4.01 mmol) of **5** in 150 mL of 25% (w/v) Me_3N–MeOH and 50 mL of MeOH was held at 40 °C for 1 day and then at 105 °C for 3 days in an autoclave. The precipitated product was dried at 25 °C (20 mmHg) and recrystallized from Me_2CO (4 °C) to give 2.1 g (86%) of **6**: mp 45–46 °C; ^1H NMR ($CDCl_3$) δ 4.21 (m, 1 H, $OCHCH_2N$), 4.08 (m, 1 H, OCH_aCH_b), 3.52 (m, 1 H, OCH_aCH_b), 2.49 (m, 1 H, CH_aCH_bN), 2.34 (m, 1 H, CH_aCH_bN), 2.28 (s, 6 H, $(CH_3)_2N$), 1.59 (m, 4 H, CH_2CO), 1.25 (s, 60 H, $(CH_2)_{30}$), 0.88 (t, 6 H, CH_3); ^{13}C NMR ($CDCl_3$) 112.66, 74.25, 68.95, 62.59, 46.20, 37.69, 37.37, 31.93, 29.95, 29.89, 29.70, 29.35, 23.97, 23.64, 22.69, 14.10. Anal. Calcd for $C_{40}H_{81}NO_2$: C, 79.01; H, 13.43. Found: C, 79.15; H, 13.45.

[(2,2-Diheptadecyl-1,3-dioxolan-4-yl)methyl]dimethyl(3-sulfopropyl)-ammonium Hydroxide Inner Salt (1c). A solution of 0.40 g (0.66 mmol) of **6** and 0.16 g (1.3 mmol) of 1,3-propanesultone (Aldrich) in 50 mL of Me_2CO was refluxed for 24 h. The precipitated product was dried at 80 °C (20 mmHg) and recrystallized from MeOH to give 0.30 g (63%) of **1c**: mp 209–210 °C; ^1H NMR ($CDCl_3$) δ 4.59 (m, 1 H), 4.31 (m, 1 H), 4.10 (d, J = 13 Hz, 1 H), 3.83 (m, 2 H, NCH_2CH_2), 3.62 (m, 1 H), 3.30 (s, 3 H, CH_3N), 3.25 (s, 3 H, CH_3N), 3.14 (m, 1 H), 2.91 (m, 2 H, CH_2SO_3), 2.29 (m, 2 H, NCH_2CH_2), 1.58 (m, 4 H, CH_2CO), 1.26 (s, 60 H, $(CH_2)_{15}$), 0.88 (t, 6 H, CH_3); ^{13}C NMR ($CDCl_3$) δ 115.03, 69.91, 67.65, 66.32, 65.99, 52.40, 50.85, 47.23, 37.28, 36.36, 31.90, 29.70, 29.35, 23.99, 22.66, 19.51, 14.12. Anal. Calcd for $C_{43}H_{87}NO_5S$: C, 70.73; H, 12.01. Found: C, 70.51; H, 12.08.

Sodium 3-[(2,2-Diheptadecyl-1,3-dioxolan-4-yl)methoxy]-1-propanesulfonate (1d). A solution of 5.00 g (8.61 mmol) of **3** and 0.29 g (12 mmol) of NaH in 180 mL of C_6H_6 (distilled from $LiAlH_4$) was refluxed under N_2 for 24 h before and for 24 h after the addition of 1.25 g (10.2 mmol) of 1,3-propanesultone. After rotary evaporation, the residue was recrystallized twice from 95% EtOH (25 °C) to give 4.49 g (72%) of **1d**: mp 181–182 °C; ^1H NMR ($CDCl_3$) δ 4.25 (m, 1 H), 4.03 (m, 1 H), 3.55 (m, 4 H), 3.37 (m, 1 H), 2.98 (m, 2 H, CH_2SO_3), 2.03 (m, 2 H, $CH_2CH_2SO_3$), 1.55 (m, 4 H, CH_2CO), 1.25 (s, 60 H, $(CH_2)_{15}$), 0.88 (t, 6 H, CH_3); ^{13}C NMR ($CDCl_3$) δ 112.94, 74.44, 72.32, 69.91, 67.40, 47.53, 37.39, 36.80, 31.96, 29.81, 29.40, 24.78, 24.10, 23.83, 22.71, 14.12. Anal. Calcd for $C_{41}H_{81}O_6SNa$: C, 67.91; H, 11.26. Found: C, 67.90; H, 11.30.

(2,3-Dihydroxypropyl)trimethylammonium Bromide (8b). A solution of 10.0 g (64.5 mmol) of 3-bromo-1,2-propanediol in 80 mL of 25% (w/v) Me_3N–MeOH was allowed to stand at 25 °C for 2 days. After an additional 80 mL of Me_3N–MeOH was added, the solution was refluxed for 6 h under a Me_2CO–dry ice condenser. The precipitated crude product was recrystallized from 1:1 (v/v) Me_2CO–MeOH to give 5.8 g (42%) of **8b**: mp 120–121 °C; ^1H NMR (D_2O) δ 4.31 (m, 1 H), 3.60 (m, 2 H), 3.48 (m, 2 H), 3.25 (s, 9 H, $(CH_3)_3N$); ^{13}C NMR (D_2O) δ 70.60, 68.68, 66.14, 56.79. Anal. Calcd for $C_6H_{16}BrNO_2$: C, 33.66; H, 7.53. Found: C, 33.76; H, 7.55.

(2,3-Dihydroxypropyl)dimethyl(3-sulfopropyl)ammonium Hydroxide Inner Salt (8c). A solution of 0.10 g (0.84 mmol) of 3-(dimethylamino)-1,2-propanediol[27] and 0.10 g (0.82 mmol) of 1,3-propanesultone in 50 mL of Me_2CO was refluxed for 24 h. The precipitated crude product was recrystallized from 2:1 (v/v) MeOH–Me_2CO to give 50 mg (25%) of **8c**: mp 230–232 °C; ^1H NMR (D_2O) δ 4.30 (m, 1 H), 3.45–3.75 (m, 6 H), 3.22 (s, 6 H, CH_3N), 2.99 (t, J = 7 Hz, 2 H, CH_2SO_3), 2.28 (m, 2 H, NCH_2CH_2); IR (Nujol) 3322 (s), 1223 (m), 1166 (m), 1089 (w), 1057 cm^{-1} (m). Anal. Calcd for $C_8H_{19}NO_5S$: C, 39.82; H, 7.94. Found: C, 39.71; H, 7.97.

Sodium 3-[(2,2-Dimethyl-1,3-dioxolan-4-yl)methoxy]-1-propanesulfonate. A solution of 4.40 g (33.3 mmol) of 2,2-dimethyl-1,3-dioxolane-4-methanol (Aldrich) and 0.855 g (35.6 mmol) of NaH (57% oil dispersion) in 100 mL of THF (distilled from $LiAlH_4$) was refluxed under N_2 for 2 h before and for 6 h after the addition of 4.10 g (33.5 mmol) of 1,3-propanesultone. After rotary evaporation, the resultant crude product was slurry-extracted with boiling Me_2CO and then dried at 80 °C (0.1 mmHg) to give 5.0 g (54%) of the hygroscopic title compound: mp >300 °C; ^1H NMR (D_2O) δ 4.42 (m, 1 H), 4.15 (m, 1 H), 3.78 (m, 1 H), 3.54–3.73 (m, 4 H), 3.01 (m, 2 H, CH_2SO_3), 2.02 (m, 2 H, OCH_2CH_2), 1.47 (s, 3 H, CH_3), 1.40 (s, 3 H, CH_3); IR (Nujol) 1201 (s), 1114 (w), 1059 cm^{-1} (s). FAB HRMS calcd for $C_9H_{17}SO_6$ (anion) 253.0745, found 253.0723. Attempts to recrystallize the title compound from MeOH–Me_2CO and other solvents resulted in its partial decomposition to **8d**.

Sodium 3-(2,3-Dihydroxypropoxy)propanesulfonate (8d). A solution of 100 mg (0.395 mmol) of the above acetonide in 5 mL of 5% hydrochloric acid was held at 25 °C for 2 h and rotary evaporated. The crude product was slurry-extracted with Me_2CO (40 °C) and then dried at 25 °C to give 80 mg (95%) of **8d**: mp >300 °C; ^1H NMR (D_2O) δ 3.88 (m, 1 H), 3.42–3.77 (m, 6 H), 2.97 (m, 2 H, CH_2SO_3), 2.01 (m, 2 H, OCH_2CH_2); IR (Nujol) 3415 (m), 1188 (s), 1109 (w), 1058 (s), 793 cm^{-1} (w). FAB HRMS calcd for $C_6H_{13}O_6S$ (anion) 213.0433, found 213.0434.

Vesicular Solutions. Vesicular solutions contained 6.0 mg of surfactant per mL, except those for DLLS, which contained 0.5 mg of surfactant per mL. In all experiments not monitored by ^1H NMR, the surfactant, weighed on a Cahn Model RM-2 electrobalance or a Mettler H20 balance, was added to a round-bottomed flask and dissolved in $CHCl_3$ (stored over K_2CO_3/Na_2CO_3). The solution was rotary evaporated at 40–50 °C, and the resultant thin film of surfactant was dried for 15 min at 25 °C (0.01 mmHg). Then the appropriate solvent was added, and the mixture was sonicated with a Branson Model 3200 ultrasonic cleaner (150 W) at 50–55 °C for the appropriate time (see below). In experiments monitored by ^1H NMR, the thin film of surfactant was formed in a 5-mm NMR tube at 25 °C by evaporation of the $CHCl_3$ by a stream of N_2 during 2 h. Then D_2O was added, and the mixture was sonicated as above for the appropriate time.

^1H NMR Line Width Narrowing. For **1a**, ^1H NMR spectra were recorded at 25 °C after 1 and 4 h of sonication. For **1b** and **1d**, spectra were obtained after 0.25 and 4 h of sonication.

DLLS. DLLS was performed on a Nicomp Model 370 submicron particle sizer (90° scattering angle). Each vesicular solution was prepared in the pH 7.4 phosphate buffer with 1 h of sonication, allowed to sit at 25 °C for 4 h, and then filtered through a Millipore Millex HV_4 filter unit (contains a 0.45-μm Durapore membrane) into a 6 mm × 50 mm culture tube (Kimble 73500-650). Immediately thereafter, the tube was inserted into the particle size at 23 °C, and the run was begun. Data were analyzed by the Nicomp distribution analysis procedure, and the results are summarized in Figure 1. In multiple runs for **1a** and **1b**, the results were the same, and for **1d**, the diameters of the two main populations varied from 24 to 36 and from 80 to 110 nm.

DSC. Two methods were used. In the first,[7] 5.0 mL of vesicular solution, prepared in the pH 7.4 phosphate buffer with 1 h of sonication, was placed into a microconcentrator with a 30 000 molecular weight cut-off filter (Amicon Centricon-30) and centrifuged (Sorvall RC2-B centrifuge) for 1 h at 6000 rpm and 5 °C. The resultant hydrated surfactant pellet gave two samples that were analyzed with a Perkin-Elmer Model DSC-1B differential scanning calorimeter. Each scan was from −25 °C to +80 °C at 1.25 °C/min.

In the second procedure, 0.5-g samples of the vesicular solutions themselves were analyzed with a Hart Scientific Model 7708 differential scanning calorimeter. Scans were made from 5 to 75 °C and from 75 to 5 °C at 1 °C/min. The transitions were reversible as evidenced by the same T_c for successive up-scans. Figure 2 contains the results for **1a**, **1b**, and **1d**, which were the same in duplicate runs for **1a** and **1b**. In a duplicate run for **1d** the ratio of the intensities of the transitions at ca. 29 and 44.2 °C varied slightly. For **1a** and **1b**, the values of ΔH_{cal} were determined by integration of the thermograms.[28]

Dye Entrapment and Leakage. Sephadex G-25-80 was swelled overnight in the pH 7.4 phosphate buffer and then degassed for 8 h at 20 mmHg before its addition to the column (30 cm × 1 cm i.d.). The column outflow was attached to an ISCO Retriever III fraction collector equipped with an ISCO Model UA-5 absorbance monitor (254 nm). The void volume of the column (16.1 mL) was determined by chromatography of 0.20 mL of a 1% (w/w) aqueous solution of blue dextran 2000 (Pharmacia) with the pH 7.4 phosphate buffer as eluant.

A vesicular solution of **1**, prepared with the pH 7.4, 0.05 M calcein solution and 4 h of sonication, was added to an Amicon Model 8010 ultrafiltration cell fitted with two 25-mm (diameter) 0.4-mm polycarbonate filters (Nucleopore 110607). The stirred solution was filtered at 40 psi N_2, and the filtrate was refiltered through two 25-mm 0.2-μm

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polycarbonate filters (110606). Then 0.20 mL of the final filtrate was chromatographed on the above column with the pH 7.4 phosphate buffer as eluant. Each fraction was 4.1 mL (75 drops), and the fifth fraction was used for fluorescence measurements.

The fluorescence intensity of the vesicular solution at 20 °C was monitored over 8 h at 525 nm with excitation at 490 nm. Then, in order to lyse the vesicles, the solution was held at 60 °C for 1 h after the addition of 0.20 mL of 10% (w/w) aqueous Triton X-100.[9,29] The fluorescence intensity of the resultant solution at 20 °C was measured to give a value, after correction for dilution by the Triton X-100 solution, that corresponds to the complete release of calcein by the vesicles. Typically, this value was 10 times greater than the initial fluorescence intensity. The extent of calcein release was obtained from the ratio of fluorescence intensity at a given time to that for complete release. The results for **1a**, **1b**, and **1c** are summarized in Figure 3. At least five runs were made for each surfactant with an average deviation of ≤3% for each point.

Hydrolytic Lability. Vesicular solutions of **1a**, **1b**, and **1d** in D_2O were prepared with 4 h of sonication and held at 25 °C. ^1H NMR spectra of the solutions were recorded immediately after the preparation and after 24 h; no changes were detected. For the studies at pD 1.4, vesicular solutions of **1a**, **1b**, and **1d** in D_2O were prepared and held at 25 °C. Their ^1H NMR spectra were recorded, and the pD was adjusted by the addition of 0.5 M DCl–D_2O. Then the solutions were monitored for surfactant hydrolysis by ^1H NMR analysis.

In one FT-IR method, the vesicular solution was concentrated as above, and the resultant hydrated surfactant pellet was analyzed by the ATR method on an Analect Model FX-6260 FT-IR spectrometer equipped with an Analect Model FXA-525 ATR accessory. The samples were obtained as follows. Vesicular solutions of **1a** and **1b** in D_2O were prepared, held at 25 °C, adjusted to pD 1.4 with 6.0 M DCl–D_2O, and concentrated after 24 h. A vesicular solution of **1b** containing 13 mol % **2** in D_2O was prepared, held at 25 °C, and concentrated after 24 h. In the other FT-IR method, the vesicular solution itself was placed in a Perkin-Elmer Model 481 variable path length cell equipped with CaF_2 windows with a path length of 0.025 mm. Spectra were obtained on a Mattson Cygnus 100 FT-IR spectrometer. This method was abandoned when **2** could not be detected in the control mixtures described in the text.

To a vesicular solution of 30 mg (0.042 mmol) of **1a** in 5.0 mL of H_2O at 25 °C was added 0.46 mL of concentrated hydrochloric acid to give a 1.0 M HCl solution. Then 5.0 mL each of $CHCl_3$ and EtOH was added, and the resultant mixture was sonicated for 2 h at 50–55 °C and lyophilized. To the residue, 2.0 mL of $CDCl_3$ was added, and the mixture was held at 50 °C for 5 min. By ^1H NMR analysis, the hydrolysis of **1a** was complete.

The kinetic study of the hydrolysis of **1d** was performed as follows. A mixture of 6.0 mg (0.0082 mmol) of **1d** and 1.00 mL of HPLC-grade H_2O (boiled to remove CO_2) was sonicated for 1 h at 50–55 °C in a test tube (7 mm × 9 cm) capped with a rubber septum and then allowed to cool to 25 °C. The test tube had been rinsed with aqueous 2 M NaOH, followed by H_2O and HPLC-grade H_2O. The vesicular solution was adjusted to pH 3.0 by the addition of 0.6 M hydrochloric acid, and immediately thereafter, the tube was placed in a H_2O bath thermostated at 25.0 ± 0.1 °C. After 30.0 min the tube was removed, and the reaction mixture was adjusted to pH 8–9 by the addition of aqueous 6 M NaOH. The contents of the tube were transferred to a lypohilizer flask, and the tube was rinsed with two 1-mL portions of H_2O and then 1 mL of $CHCl_3$. The reaction mixture plus washes was lyophilized, and the resultant residue was suspended in 0.75 mL of $CDCl_3$ containing 0.01% Me_4Si (stored over Na_2CO_3) and transferred to a 5-mm NMR tube. The tube was capped and centrifuged upside down to leave undissolved material (**8d** and NaCl) within the cap. The extent of hydrolysis was determined by ^1H NMR analysis of the solution of **1d** and **2**. The calculation was based on the integrals for the terminal Me triplet at δ 0.88 (**1d** and **2**) and the α-CH_2 triplet at δ 2.39 (**2**). The extents of hydrolysis of five runs were 31, 28, 31, 31, and 33%, which gave a one-point, pseudo-first-order rate constant of 0.0123 ± 0.0005 min^{-1}. Control runs with synthetic mixtures of **1d** and **2** verified the accuracy of the analysis procedure.

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Registry No. 1a, 119296-57-0; **1b**, 119296-58-1; **1c**, 119296-60-5; **1d**, 119296-59-2; **2**, 504-53-0; **3**, 80336-92-1; **4**, 119296-53-6; **5**, 119296-54-7; **6**, 119296-55-8; **8b**, 116586-90-4; **8c**, 119296-61-6; **8d**, 20858-17-7; $MeSO_2Cl$, 124-63-0; Me_3N, 75-50-3; glycerol, 56-81-5; 3-bromo-1,2-propanediol, 4704-77-2; 3-(dimethylamino)-1,2-propanediol, 623-57-4; 2,2-dimethyl-1,3-dioxolane-4-methanol, 100-79-8; 1,3-propanesulfone, 1120-71-4; sodium 3-[(2,2-dimethyl-1,3-dioxolan-4-yl)methoxy]-1-propanesulfonate, 119296-62-7; calcein, 1461-15-0.

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