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A General Approach to Anionic Acid-Labile Surfactants with Tunable Properties

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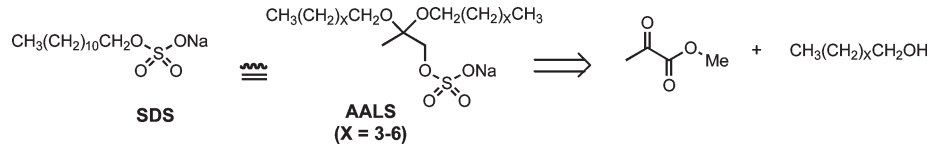

A general approach to the synthesis of a new series of unique sulfate anionic acid-labile surfactants (AALS) was developed. In this approach, the ketal was derived from methyl pyruvate, and the sulfate motif was introduced via sulfitylation of the alcohol, oxidation, and finally conversion of the sulfate diester to the desired sodium salt. The physicochemical properties in aqueous solution of this novel series of surfactants, such as CMCs, solubility, acid lability, and stability were studied.

Introduction

Sodium dodecyl sulfate (SDS) is an anionic surfactant or detergent that is widely used in proteomics for polyacrylamide gel electrophoresis (PAGE) separations, as well as for facilitation of the solubilization and denaturation of proteins. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is the most common preparative and analytical technique for the separation of macromolecules such as proteins.[1] SDS forms a complex with proteins in solution to both increase their solubility and impart an overall net negative charge to the protein−surfactant complex that is used to drive electrophoretic migration of the protein under the influence of an applied electric field. However, SDS and other common surfactants, such as TRITON X, TWEEN, CHAPS and CHAPSO, that are used for sample preparation in proteomic experiments can interfere with mass-spectrometry-based proteomic techniques used for the analysis, identification, and characterization of proteins.[2]

Recently, cleavable surfactants have been used in biological applications as replacement for common biological detergents.[3] For example, PPS Silent Surfactant (zwitterionic surfactant),[3c] Rapigest (anionic surfactant) (**1**),[4] and ProteaseMAX (anionic surfactant) (**4**)[5] (Scheme 1) are commercially available acid-labile surfactants that were created to address these issues in biological sample preparation and protein analysis. However, the zwitterionic nature of PPS Silent Surfactant and its chemical properties make it less than ideal for use in one-dimensional PAGE electrophoretic separations, as its net neutral charge state does not induce movement of bound proteins in an electric field. Although anionic in nature, Rapigest (**1**) forms a film on the sample surface upon cleavage, which is the result of the poor aqueous solubility of the 13-carbon ketone hydrolysis product **2**. Similar problems have been observed from the acid labile furan alcohol product **5** that results from ProteaseMax (**4**) hydrolysis. Consequently, an additional sample preparation step is required to remove these films, which can lead to additional sample losses or contamination due to increased sample handling and to an overall increase in the sample prep time. All three of these commercially available acid labile surfactants, the PPS Silent Surfactant, Rapigest, and ProteaseMAX, suffer from a slow rate

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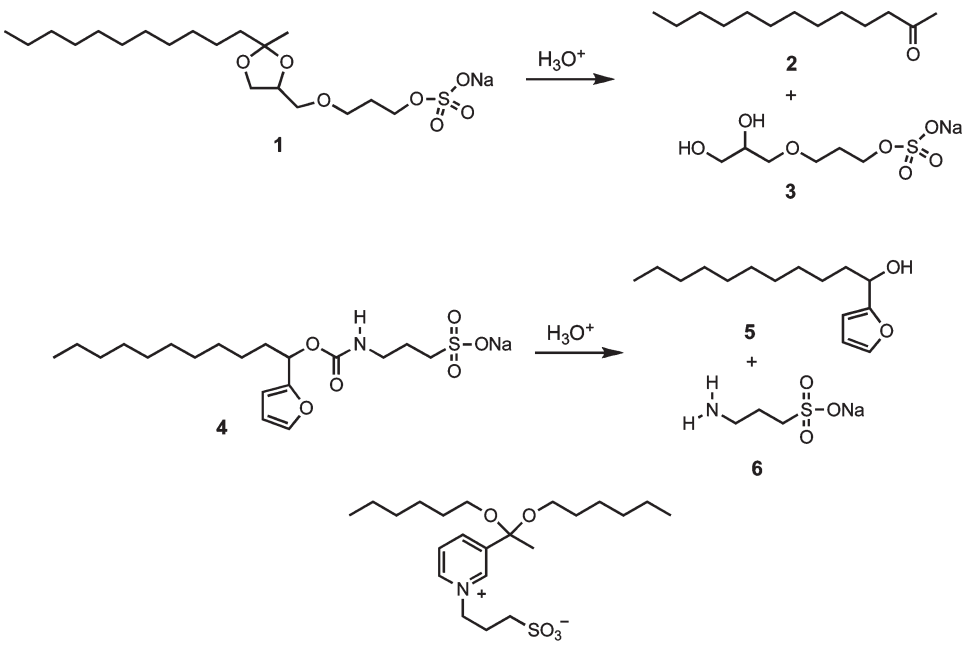
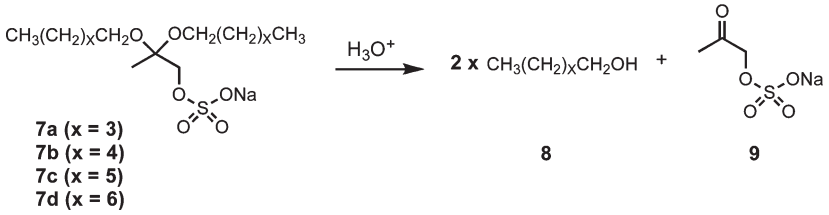
SCHEME 1. Commercially Available Anionic Acid-Labile Surfactants and Their Hydrolysis Products



SCHEME 2. Proposed New and Improved Anionic Acid-Labile Surfactants



of acid hydrolysis and can require long incubation times (about 2 h) under acidic conditions (e.g., pH < 2).

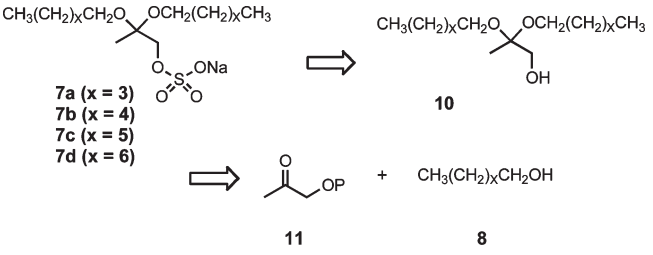
Therefore, a need exists for an acid-cleavable anionic surfactant that has all the surfactant properties for proteomic analytical techniques yet exhibits a greater rate of acid hydrolysis and breaks down to simpler molecular fragments. For our purposes, we desired new anionic acid-labile surfactants (AALS) with improved acid lability and tunable surfactant properties (e.g., critical micelle concentration/CMC), which decompose to intermediates with minimal lipid characteristics.

To meet these needs we decided to prepare sulfate surfactants that are described by structure **7** and that mimic the structure of SDS (Scheme 2). We chose the ketal as the acid labile functional group because of its high acid lability and straightforward synthetic accessibility. We envisioned that ketal made from linear alcohols ranging from C-5 to C-8 would have our desired surfactant properties. In turn, ketals of this type would degrade (**7** to **8** and **9**) into products of shorter carbon length with increased aqueous solubility, thus preventing formation of the troublesome hydrophobic film.

Results and Discussion

Synthesis. Retrosynthetically, we envisioned the surfactants **7** as being derived from the direct sulfation of primary alcohol **10** (Scheme 3). The ketal of **10**, in turn, could be installed by an acid-catalyzed ketalization of a protected

SCHEME 3. Initial Retrosynthetic Analysis



hydroxy acetone **11** and the required primary alcohol **8**. This approach suffered from the difficulty in finding a suitable acid stable protecting group for hydroxyl acetone and the careful chromatography required for the purification of **7** from the direct sulfation reaction (vide infra).

Our second-generation approach to sodium sulfate **7** began with the protected sulfate diester **12** (Scheme 4). Sulfate diester **12** could be prepared by a two-step sulfation of alcohol **10** (sulfitylation/oxidation).[6] Finally, primary alcohol **10** could be derived from methyl pyruvate and the desired alcohol **8**, via a ketalization and reduction sequence.[7]

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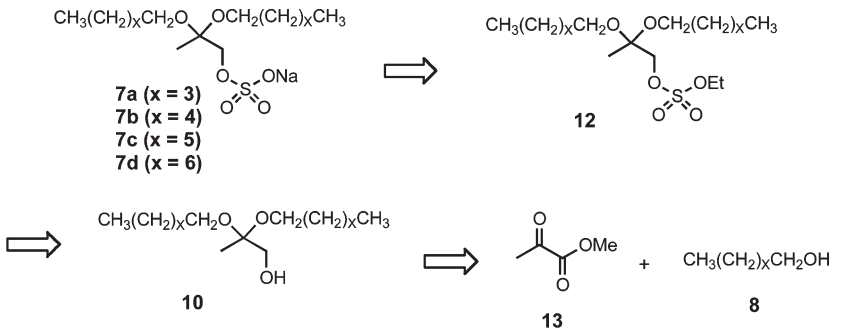
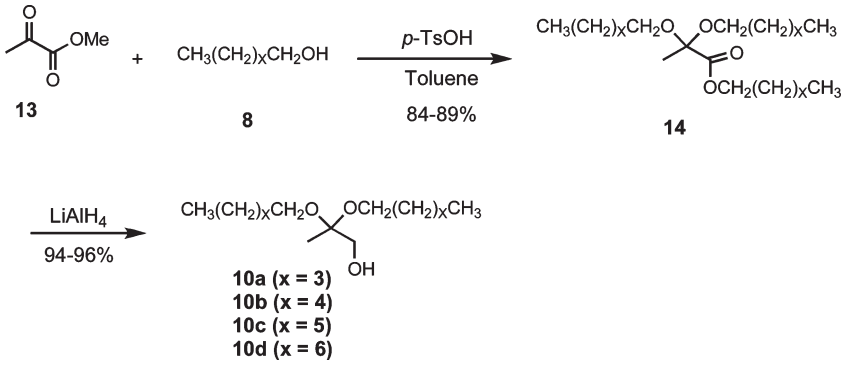
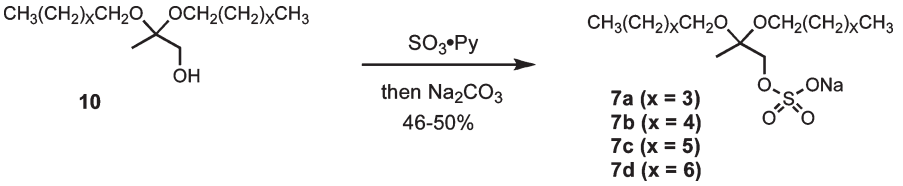
SCHEME 4. Revised Retrosynthetic Analysis



SCHEME 5. Synthesis of Ketal Alcohols



SCHEME 6. Synthesis of Surfactants by Direct Sulfation of Ketal Alcohol



The ketal esters **14a**−**d** were obtained by condensation of methyl pyruvate with various linear alcohols (C-5 through C-8) under acid condition.[7] The ketal esters **14a**−**d** were then reduced with $LiAlH_4$ to give desired ketal alcohols **10a**−**d** in good yields (94−96%) (Scheme 5). We found these ketals to be quite sensitive to acid hydrolysis during silica gel chromatography. To our delight, the simple addition of Et_3N (~1%) to the eluent solved this problem.

With the ketal alcohols **10a**−**d** in hand, we turned our attention to the sulfation of the alcohols. We initially tried to directly convert the primary alcohol in **10a**−**d** to the corresponding sodium sulfate. This was accomplished by reaction of **10a**−**d** with sulfur trioxide pyridine complex (Scheme 6). Unfortunately, this reaction suffered from low reaction yields (~50%) and difficulties with obtaining the surfactants **7a**−**d** in the desired purity (>95%). Therefore, we turned to an alternative sulfation approach developed by Delft.[6]

In this three-step procedure, the ketal alcohols **10a**−**d** were reacted with ethyl chlorosulfite to give sulfite diester **15a**−**d** (85−90%), which were then oxidized with catalytic RuO_4 to the corresponding sulfate diester **12a**−**d**. Finally, deprotection of the sulfate diester **12a**−**d** with NaI provided the desired products **7a**−**d** in high yield (74−82%) and high purity (>95%) (Scheme 7). This scalable 3-step procedure provided a range of desired surfactants for further study.

Physical-Chemical Characterization. 1. Determination of CMC. To evaluate our new sulfates **7a**−**d** for their surfactant properties, we decided to use a variable concentation/^{1}H NMR shift method to measure their critical micellar concentration (CMC).[8] The ^{1}H NMR spectra for surfactants **7a**−**d** were measured in D_2O solutions, and the shifts were plotted against concentration according Harwell's procedure.[8] The aqueous solubility of surfactant **7d** was limited, and therefore no CMC could be determined for it. The resulting CMCs are given in Table 1 together with the CMC of SDS as a comparison. To our delight, our new surfactants had the desired variable surfactant properties in the range of SDS. Thus, the C-6 carbon ketal surfactant (**7b**) had the CMC (7.7 mM) closest to that of SDS (9.7 mM). As we hoped, the C-5 carbon variant (**7a**) had a slightly higher CMC (23.5 mM) and the C-7 carbon variant (**7c**) had a slightly lower CMC (1.9 mM) compared to that of SDS.

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SCHEME 7. Synthesis of Surfactants by Stepwise Sulfation of Ketal Alcohol

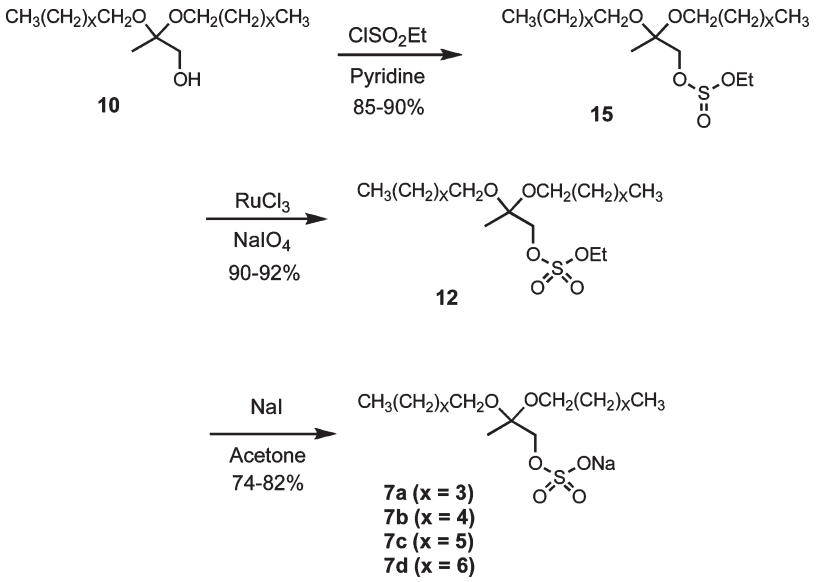

TABLE 1. CMC for Surfactants 7a–c and SDS

entry	surfactants	CMC (mM)
1	**7a**	23.5
2	**7b**	7.7
3	**7c**	1.9
4	SDS	9.7

2. Solubility in Water. The surfactants were tested for their maximal solubility in water to form a clear solution. We found the solubility of surfactants dropped significantly as the carbon chain increased. The C-5 surfactant **7a** was very soluble in water up to 10% or 299 mM. The C-6 surfactant (**7b**) was less soluble in water at about 5% or 138 mM, whereas the C-7 surfactant (**7c**) was slightly soluble at about 1% or 25.6 mM. Using ^{1}H NMR, we were unable to detect the C-8 surfactant (**7d**) in water.

3. Acid Lability in Aqueous Solution. In addition to the appropriate CMC properties, these new surfactants **7a–d** also displayed the required acid lability. To test the surfactants' acid lability, a 1% solution of the surfactant **7a–d** in D_2O was treated with a solution of 1% TFA in D_2O until the pH reached 2. The reaction was monitored by ^{1}H NMR, which showed that all of surfactants **7a–d** completely hydrolyzed in 5 min to a 2:1 ratio of alcohol **8** and ketone **9**. When 1% solution of the surfactant **7a–d** in D_2O was treated with an acetate buffer at pH = 3, all of surfactants **7a–d** were completely hydrolyzed in 30 min (see Supporting Information).

4. Stability in Water. Finally, the surfactants also displayed excellent stability as a storable solid and adequate stability in aqueous solution. To study the stabilities of the surfactants in aqueous solution, surfactants **7a–c** were dissolved in D_2O to give 1% solution. The solutions were monitored weekly by ^{1}H NMR upon storage at room temperature and at 4 °C. The decomposition rates were determined by integrations of the corresponding hydrolysis product peaks (alcohol **8** and ketone **9**) and comparison to the peaks of the remaining starting material. A typical plot of the stability rate is shown in Figure 1 for the C-6 surfactant (**7b**) in aqueous solution.

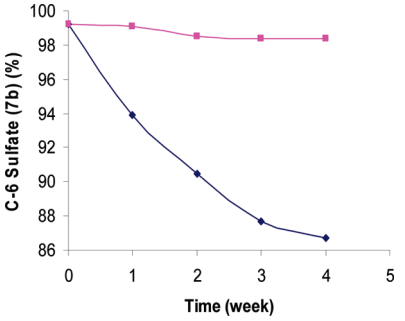

FIGURE 1. Percentage of C-6 sulfate (**7b**) in D_2O versus time (week) plot at 23 °C (rt) (♦) and 4 °C (refrigerated) (■).

Not surprisingly, the surfactant stability was much greater at lower temperature (4 °C) with less than a 1% increase in the amount of hydrolysis products in 1 month. Thus aqueous solutions of surfactants can be used after ~1 month storage at lower temperature. The other surfactants **7a** and **7c** showed similar stabilities in aqueous solutions.

In summary, a general approach to sulfate anionic acid-labile surfactants has been developed. These anionic acid labile surfactants have tunable physical properties (e.g., CMC) and cleanly hydrolyze under mild aqueous acid condition (pH = 2) in 5 min to give simple cleavage products with minimal surfactant properties. The hydrolysis products can be washed away using standard reversed phase sample cleanup and/or solid phase extraction techniques. The application of these anionic acid-labile surfactants will be reported in due course.

Experimental Section

Hexyl 2,2-Bis(hexyloxy)propanoate (14b). To a solution of methyl pyruvate **13** (10.0 g, 98.0 mmol) in toluene (100 mL) were added 1-hexanol **8b** (40.1 g, 392 mmol) and *p*-TsOH (186 mg, 0.98 mmol). The mixture was heated to reflux for 10 h with azeotropic removal of water from the reaction mixture. The reaction was quenched with saturated $NaHCO_3$ (100 mL), and the reaction mixture was extracted with ethyl acetate (2×100 mL).

The combined organic layers were washed with brine (100 mL) and dried over anhydrous sodium sulfate. The solvent was removed, and the residue was purified by silica gel chromatography (1% to 10% ethyl acetate/hexane) to give hexyl 2,2-bis(hexyloxy)propanoate **14b** (29.5 g, 84%) as a colorless oil: R_f (15% EtOAc/hexane) = 0.53; IR (thin film, cm^{-1}) 2956, 2930, 2860, 1746 (C=O), 1467, 1280, 1137, 1062; ^1H NMR (600 MHz, $CDCl_3$) δ 4.14 (t, J = 7.2 Hz, 2H), 3.48 (ddd, J = 9.0, 7.2, 6.6 Hz, 2H), 3.35 (ddd, J = 9.0, 7.2, 6.6 Hz, 2H), 1.65−1.63 (m, 2H), 1.59−1.54 (m, 4H), 1.49 (s, 3H), 1.35−1.24 (m, 18H), 0.86 (t, J = 7.2 Hz, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.2, 99.5, 65.4, 62.6, 31.7, 31.3, 29.7, 28.5, 25.8, 25.5, 22.6, 22.5, 21.9, 14.0, 13.9; HRMS calcd for $[C_{21}H_{42}O_4Na^+]$ 381.2975, found 381.2973.

2,2-Bis(hexyloxy)propan-1-ol (10b). To a mixture of $LiAlH_4$ (3.44 g, 90.5 mmol) in Et_2O (200 mL) was added a solution of ester **14b** (29.5 g, 82.3 mmol) in Et_2O (100 mL). After addition, the mixture was refluxed for 6 h. The reaction mixture was cooled to 0 °C and quenched with ethyl acetate (20 mL) and H_2O (20 mL). The mixture was added to saturated potassium sodium tartrate (300 mL) and stirred at 23 °C for 12 h. The mixture was extracted with Et_2O (2 × 200 mL), and the combined organic layers were dried over anhydrous sodium sulfate. The solvent was removed, and the residue was purified by silica gel chromatography (1% to 10% ethyl acetate/hexane) to give 2,2-bis(hexyloxy)propan-1-ol **10b** (20.6 g, 94%) as a colorless oil: R_f (15% EtOAc/hexane) = 0.20; IR (thin film, cm^{-1}) 3430, 2955, 2929, 2859, 1467, 1378, 1244, 1155, 1112, 1064, 876; ^1H NMR (600 MHz, C_6D_6) δ 3.58 (d, J = 6.6 Hz, 2H), 3.43 (t, J = 6.6 Hz, 4H), 1.58 (t, J = 6.6 Hz, 1H), 1.53 (m, 4H), 1.36 (s, 3H), 1.34−1.22 (m, 12H), 0.88 (t, J = 7.2 Hz, 6H); ^{13}C NMR (150 MHz, C_6D_6) δ 101.0, 66.2, 61.3, 32.5, 30.9, 26.8, 23.4, 21.3, 14.6; HRMS calcd for $[C_{15}H_{32}O_3Na^+]$ 283.2244, found 283.2244.

2,2-Bis(hexyloxy)propyl Ethyl Sulfite (15b). To a solution of alcohol **10b** (20.6 g, 79.1 mmol) in CH_2Cl_2 (200 mL) were added pyridine (8.12 g, 102.8 mmol) and ethyl chlorosulfite (12.2 g, 94.9 mmol). The reaction mixture was stirred at 23 °C for 10 h. The reaction was quenched with water (100 mL) and extracted with CH_2Cl_2 (2 × 100 mL). The combined organic layers were washed with HCl (1 N, 100 mL), saturated $NaHCO_3$ (100 mL), and brine (100 mL) and dried over anhydrous sodium sulfate. The solvent was removed, and the residue was purified by silica gel chromatography (1% to 10% ethyl acetate/hexane) to give 2,2-bis(hexyloxy)propanyl ethyl sulfite **15b** (24.5 g, 88%) as a colorless oil: R_f (15% EtOAc/hexane) = 0.44; IR (thin film, cm^{-1}) 2932, 2872, 1467, 1379, 1213, 1194, 1176, 1001, 888; ^1H NMR (600 MHz, $CDCl_3$) δ 4.11−4.00 (m, 2H), 3.93 (d, J = 10.8 Hz, 1H), 3.77 (d, J = 10.8 Hz, 1H), 3.42−3.37 (m, 4H), 1.51−1.48 (m, 4H), 1.33 (s, 3H), 1.31−1.24 (m, 15H), 0.85 (t, J = 7.2 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 99.0, 62.9, 60.8, 58.5, 58.3, 31.6, 29.8, 25.9, 22.6, 20.9, 15.3, 14.0; HRMS calcd for $[C_{17}H_{36}O_5SNa^+]$ 375.2176, found 375.2177.

2,2-Bis(hexyloxy)propyl Ethyl Sulfate (12b). To a solution of sulfite **15b** (24.5 g, 69.5 mmol) in MeCN (200 mL), CH_2Cl_2 (200 mL), and water (300 mL) were added $NaIO_4$ (29.7 g, 139.0 mmol) and $RuCl_3$ (10 mg, 0.05 mmol). The mixture was stirred at 23 °C for 5 h. The mixture was filtered through Celite and extracted with CH_2Cl_2 (2 × 200 mL). The combined organic layers were washed with saturated $NaHCO_3$ (100 mL), brine (100 mL) and dried over anhydrous sodium sulfate. The solvent was removed, and the residue was purified by silica gel chromatography (1% to 10% ethyl acetate/hexane) to give 2,2-bis(hexyloxy)propanyl ethyl sulfate **12b** (23.3 g, 91%) as a colorless oil: R_f (15% EtOAc/hexane) = 0.41; IR (thin film, cm^{-1}) 2931, 2860, 1737, 1467, 1403, 1380, 1196, 1179, 1012, 925, 858; ^1H NMR (600 MHz, $CDCl_3$) δ 4.34 (q, J = 7.2 Hz, 2H), 4.09 (s, 2H), 3.43 (ddd, J = 9.0, 7.2, 6.6 Hz, 2H), 3.38 (ddd, J = 9.0, 7.2, 6.6 Hz, 2H), 1.51 (m, 4H), 1.40 (t, J = 7.2 Hz, 3H), 1.37 (s, 3H), 1.34−1.24 (m, 12H), 0.86 (t, J = 7.2 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 98.2, 72.4, 69.7, 60.9, 31.6, 29.8, 25.9, 22.6, 20.8, 14.5, 14.0; HRMS calcd for $[C_{17}H_{36}O_6SNa^+]$ 391.2125, found 391.2126.

Sodium 2,2-Bis(hexyloxy)propyl Sulfate (7b). To a solution of sulfate diester **12b** (5.6 g, 15.2 mmol) in acetone (15 mL) was added NaI (4.56 g, 30.4 mmol). The solution was stirred at 23 °C for 10 h. The solvent was removed, and the residue was purified by silica gel chromatography (10 to 100% ethyl acetate/hexane) to give sodium 2,2-bis(hexyloxy)propanyl ethyl sulfate **7b** (4.07 g, 74%) as a colorless solid: R_f (20% EtOH/EtOAc) = 0.48; IR (thin film, cm^{-1}) 3506, 2956, 2930, 2056, 1642, 1467, 1380, 1228, 1015, 820; ^1H NMR (600 MHz, $CDCl_3$) δ 3.99 (s, 2H), 3.42 (t, J = 7.2 Hz, 4H), 1.51−1.49 (m, 4H), 1.36 (s, 3H), 1.30−1.24 (m, 12H), 0.86 (t, J = 7.2 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 99.2, 69.8, 60.8, 31.8, 29.8, 25.9, 22.7, 20.8, 14.0; HRMS calcd for $[C_{15}H_{31}O_6S^-]$ 339.1847, found 339.1843.

Supporting Information Available: Experimental procedures, characterization data, and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.