Neopentyl 3-Triflyloxypropanesulfonate. A Reactive Sulfopropylation Reagent for the Preparation of Chemiluminescent Labels

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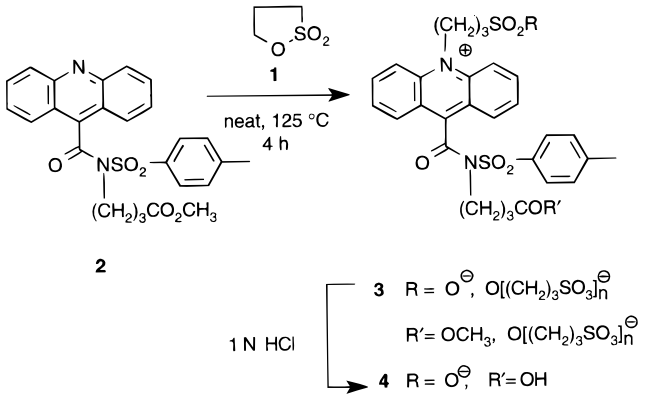
Chemiluminescent acridinium salts by virtue of their high quantum efficiency can be detected at the attomole (10^{-18} mol) level and below[1] and thus have recently been used as labeling reagents for ultrasensitive clinical assays.[2] The N^{10}-methyl derivatives have generally been employed and are efficiently prepared by quarternization of the acridine precursor with methyl fluorosulfate or methyl triflate; poor conversion is seen with alkylating reagents with nucleophilic leaving groups (I^-, Br^-, Cl^-). The aqueous solubility of N^{10}-methylacridinium salts is not optimal under the conditions of the assay, and we initially sought to improve this aspect of the chemiluminescent label by introducing a solubilizing substituent at the N^{10}-position in place of the N^{10}-methyl group.

The sulfopropyl group has been used extensively to improve the aqueous solubility and otherwise enhance the hydrophilicity of a variety of surfactants,[3,4] dyes,[5] nucleosides,[5,6] proteins[7,8] and polymers[9,10] and was therefore considered for modifying chemiluminescent acridinium salts. Recently, a method to introduce the sulfopropyl group has been described in which an allyl group present in the substrate subsequently reacts with bisulfite via radical initiation.[3,9] Alkylation with sodium 3-chloropropylsulfonate[11] has also been reported. Both methods were unsuitable for introducing the sulfopropyl group onto the chemiluminescent acridinium label, since acridinium salts are reactive in radical-mediated reactions and alkyl halide alkylating reagents exhibit poor conversion to the quarternized product. For the most part, the sulfopropyl group has been introduced by reaction of nucleophiles with commercially available and inexpensive, 1,3-propane sultone (**1**).[12] Primary amines[13] and sulfhydryl groups[7,8] react readily at ambient temperature, but heterocyclic aromatic amines (pyridine, quinoline, acridine, etc.), amides, carboxylic acids, and alkoxides[13] require more drastic conditions, even heating the substrate at >100 °C in neat 1,3-propane sultone.

Scheme 1



Such drastic conditions were necessary for the synthesis of the sulfopropylated chemiluminescent acridinium label **4**[12−17] (Scheme 1). Sulfopropylation of acridine **2** proceeded efficiently on heating in neat 1,3-propane sultone at 125 °C for 4 h. Under these vigorous reaction conditions, though, a mixture was produced consisting the desired methyl ester (**3**, R = O^-, R′ = OCH_3) and the sulfopropyl esters (**3**, R = O^-, $O[(CH_2)_3SO_3]_n$, R′ = $O[(CH_2)_3SO_3]_n$). Rather than isolating each compound, the mixture was separated by flash chromatography from the starting acridine and then hydrolyzed in refluxing aqueous hydrochloric acid to give the single compound **4** in high yield. The zwitterionic compound **4** displayed better solubility than the corresponding positively charged N^{10}-methyl derivative and has subsequently been used in the development of clinical immunoassays for a variety of analytes.[18−26]

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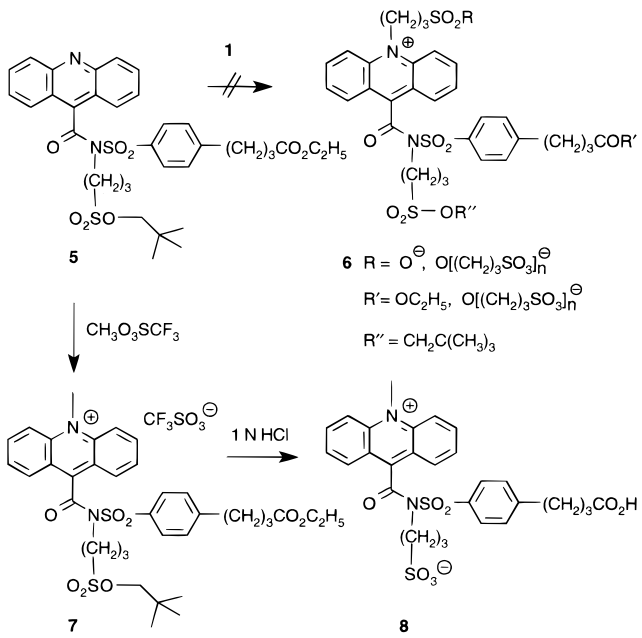
Scheme 2



To address whether further enhancement of the hydrophilicity of the chemiluminescent label would be advantageous, we sought to introduce a second sulfopropyl group on the periphery of the acridinium label. To our surprise, sulfopropylation of the acridine compound **5** (Scheme 2) with 1,3-propane sultone failed to yield any of the desired bis-sulfopropylated acridinium label **6**, even after heating for days at higher temperatures. A possible explanation of this failure may lie with the increased steric demands of **5**. All *N*-sulfonylacridine-9-carboxamide compounds of this type studied to date exist in solution as a mixture of two rotamers.[14] The ^{1}H NMR showed that either the *N*-alkyl sulfonamide side chain or the arylsulfonyl substituent lies in the shielding region above the acridine nucleus. As illustrated in Figure 1, the bulky neopentyl sulfonate and carboethoxypropyl side chains of compound **5** can adopt low-energy conformations in which the acridine N^{10} nitrogen is effectively blocked from reaction with 1,3-propane sultone. The conversion of acridine **5** to acridinium compound **7** with a large excess of the less sterically demanding, more reactive methyl triflate, while slower than reaction with **2** (7 days vs overnight), was efficient. This prompted us to investigate the preparation and use of an alternative triflate-containing sulfopropylation reagent (**11**).

Manipulation of commercially available sodium 3-hydroxypropanesulfonate directly into the desired triflate seemed an unlikely route, being complicated by the insolubility of the sulfonate salt. Rather, we envisioned starting from an alkyl 3-hydroxypropanesulfonate which would issue from the hydroxyethylation of a mesylate anion. Widlanski et al.[29] have shown the utility of mesylate anions in reaction with ketones; however, their reactivity toward epoxides was not explored. The recent work by Roberts et al.[30] and the pioneering work of Truce[29−31] indicated that the neopentyl moiety was suitable for protecting the sulfonic acid group yet easy to remove. As outlined in Scheme 3, neopentyl mesylate **9**, prepared straightforwardly by the reaction of neopentyl alcohol and mesyl chloride, was deprotonated with *n*-butyllithium and subsequently hydroxyethylated with ethylene oxide to give the desired 3-hydroxypropanesulfonate ester **10**. No poly(ethylene glycol) byproducts were observed. Conversion to the triflate using triflic anhydride in pyridine gave **11** as a tan solid in 82% yield after an aqueous workup in greater than 95% purity by ^{1}H NMR. Further purification by flash chromatography yielded **11** (39%) as an analytically pure, white, low-melting solid that was stable for at least 2 weeks in solution at ambient temperatures as judged by ^{1}H NMR. Reactivity of **11** was the same in subsequent reactions regardless of the isolation procedure. The reaction of acridine **2** with 1 equiv of triflate **11** at room temperature for 2 days achieved a 50% conversion to the desired sulfopropylated acridinium compound whereas 1,3-propane sultone is unreactive under these conditions. Initial trials with the more sterically hindered acridine **5** (Scheme 4) showed very little sulfopropylation under the same conditions. Heating the reaction to reflux in dichloromethane to accelerate the reaction offered no advantage. However, when an equivalent of an acid scavenger, 2,6-di-*tert*-butyl-4-methylpyridine, was added along with **11** at room temperature, acridine **5** was slowly sulfopropylated to give the desired acridinium compound **12** in 96% yield based on recovered **5** (34% conversion after 7 days). Under these conditions no ester exchange was seen. Hydrolysis of **12** in aqueous acid removed both

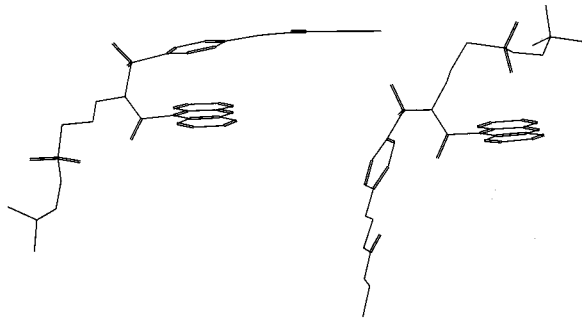
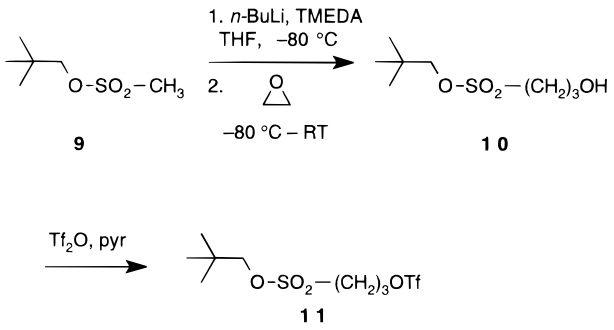


Figure 1. Low-energy rotamer conformations of acridine **5**.

Scheme 3



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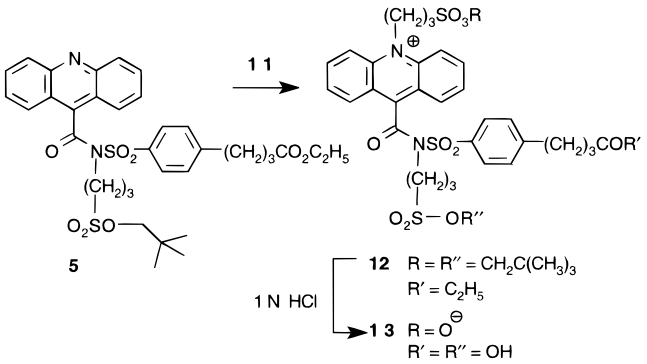
Scheme 4



the neopentyl sulfonate protecting groups and the ethyl ester to afford **13** quantitatively. Whereas acridinium compound **4** precipitated out of the hydrolysis mixture, bis-sulfopropylated **13** was completely soluble. Evaluation of chemiluminescent bis-sulfopropylated acridinium salt **13** as a label in an immunoassay format is ongoing.

In summary, neopentyl 3-triflyloxypropanesulfonate **11** was easily prepared and was shown to be much more reactive than 1,3-propane sultone in the preparation of sterically hindered sulfopropylated acridinium chemiluminescent labels. Whereas 1,3-propane sultone failed to react with the acridine **5** under forcing conditions (>125 °C, neat), triflate **11** was sufficiently reactive at room temperature to quarternize the acridine at the N^{10} nitrogen, yielding gram quantities of a chemiluminescent acridinium labeling reagent with the most desirable solubility characteristics yet described. Since this reagent worked so well under these "worst case" conditions, it is expected that this reactive sulfopropylating reagent will find use in the functionalization of a wide range of heterocylic bases, and other substrates that react poorly with 1,3-propane sultone, and do so under much milder conditions.

Experimental Section

Reagents were obtained from Aldrich Chemical Co. (Milwaukee, WI) unless otherwise noted. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded at on a Varian Gemini 2300 spectrometer (Palo Alto, CA). Chemical shifts are reported in ppm (δ) using tetramethylsilane (TMS) as the internal reference; coupling constants (J) are in hertz. Elemental analyses were performed by Robertson Microlit Laboratories, Inc. (Madison, NJ). Melting points were performed on an Electrothermal model 9100 digital melting point apparatus (Gillette, NJ) and are uncorrected. Electrospray ionization mass spectrometry ESI/MS was carried out on a Perkin-Elmer (Norwalk, CT) Sciex API 100 Benchtop system employing the Turbo IonSpray ion source.

9-[[(4-Methoxy-4-oxobutyl)[(4-methylphenyl)sulfonyl]-amino]carbonyl]-10-(3-sulfopropyl)acridinium Inner Salt (3). A mixture of compound **2** (9.0 g, 18.9 mmol) and 1,3-propane sultone (25.0 g, 205 mmol) in a 250 mL round-bottomed flask covered with aluminum foil was heated at 125 °C in an oil bath for 5 h under nitrogen. The reaction mixture was then cooled to room temperature, dissolved in a small amount of methanol, added to silica gel (100 g), and evaporated in vacuo. The compound adsorbed on silica gel was loaded onto a flash column (600 g silica gel). The column was eluted with dichloromethane (500 mL), 5% methanol in dichloromethane (3.5 L), and 15% methanol in dichloromethane (6 L). The fractions which showed strong fluorescent yellow spots on TLC (silica gel, 15% methanol in dichloromethane) were combined and evaporated in vacuo to afford the mixture **3** (15.2 g). It was used directly in the hydrolysis reaction without further characterization.

9-[[(3-Carboxypropyl)[(4-methylphenyl)sulfonyl]amino]-carbonyl]-10-(3-sulfopropyl)acridinium Inner Salt (4). The mixture of the acridinium compounds (**3**) and 1 N HCl (500 mL) was heated to reflux for 4.25 h under nitrogen and then cooled at room temperature for 16 h. During this time a yellow precipitate formed. The mixture was cooled in ice for 45 min, and the precipitate was collected by filtration, washed with acetonitrile (40 mL), and dried in vacuo to afford pure **4** (8.56 g, 78%): ^{1}H NMR (CD_3OD) δ 8.93 (2 H, d, J = 9), 8.50−8.40 (2 H, m), 8.21 (2 H, d, J = 8), 8.10−7.78 (4 H, m), 7.63 (1 H, d, J = 8), 7.25−7.14 (2 H, m), 5.80−5.65 (2 H, m), 4.29 (1 H, t, J = 7), 3.54−3.46 (1 H, m), 3.27−3.14 (1 H, m), 2.70−2.52 (3 H, m), 2.38 (3 H, s), 2.32−2.20 (1 H, m), 1.92 (1 H, t, J = 6), 1.60−1.50 (1 H, m); ESI/MS m/z 585.5 $(M + H)^+$. Anal. Calcd for $C_{28}H_{28}N_2O_8S_2$: C, 57.52; H, 4.83; N, 4.79; S, 10.97. Found: C, 57.38; H, 4.89; N, 4.74; S, 10.91.

9-[[[[4-(4-Ethoxy-4-oxobutyl)phenyl]sulfonyl][4-(2,2-dimethylpropoxy)-4-sulfobutyl]amino]carbonyl]-10-methylacridinium Triflate Salt (7). To the stirred solution of compound **5** (11.0 g, 16.45 mmol) in dry dichloromethane (165 mL) was added freshly opened methyl triflate (18.6 mL, 164.4 mmol) in one portion. The reaction mixture was stirred at room temperature for 7 days. Solvents were removed under reduced pressure on a rotary evaporator. The remaining material was redissolved in a small amount of dichloromethane and applied to a gravity column (1 kg silica gel). The column was eluted with 50% ethyl acetate in hexanes (2 L), 60% ethyl acetate in hexanes (2 L), 80% ethyl acetate in hexanes (2 L), 100% ethyl acetate (6 L), and 5% methanol in dichloromethane. A small amount of unreacted starting material **5** (~0.45 g) was recovered from the ethyl acetate/hexanes fractions. All fractions containing fluorescent material were collected, combined, and evaporated in vacuo to afford the desired compound **7** (11.8 g, 14.2 mmol, 87%) as a mixture of rotamers: ESI/Ms m/z 683.8 (M^+); ^{1}H NMR (CD_3OD) δ 8.80−6.80 (12 H, m), 4.91 and 4.83 (3 H, s, 1/1 ratio, N-methyl), 4.12 (2 H, m, ethyl ester), 1.24 (3 H, m, ethyl ester), 1.03 and 0.78 (s, methyl groups of neopentyl sulfonate); HPLC [3.9 × 300 mm μBondapak C-18, 65:35:0.1 acetonitrile/water/trifluoroacetic acid, 1 mL/min, 254 nm] t_R, 4.5 min. The mixture was used in the next step without further purification.

9-[[[[4-(3-Carboxypropyl)phenyl]sulfonyl][3-sulfopropyl]amino]carbonyl]-10-methylacridinium Inner Salt (8). The acridinium neopentyl sulfonate **7** (11.8 g) was heated to reflux with 1 N HCl (400 mL) for 4.25 h. The hot yellow solution was decanted away from the insoluble tar material and allowed to cool to room temperature. A precipitate quickly formed upon cooling. The precipitate was collected by filtration, washed with a small amount of distilled water, and dried over phosphorus pentoxide in vacuo to afford pure product **8** (8.38 g, 14.2 mmol, 87%): ESI/MS m/z 585.6 $(M + H)^+$; ^{1}H NMR (pyridine-d_5) δ 8.77 (2 H, d, J = 9), 8.55 (2 H, d, J = 8), 8.26 (2 H, t, J = 8), 8.04 (2 H, d, J = 8), 7.82 (2 H, t, J = 7), 7.64 (2 H, d, J = 8), 4.92 (3 H, s), 4.32 (2 H, t, J = 8), 2.86 (2 H, t, J = 8), 2.58 (2 H, m), 2.16 (4 H, m), 2.04 (2 H, m). Anal. Calcd for $C_{28}H_{28}N_2O_8S_2(H_2O)$: C, 55.80; H, 5.02; N, 4.64; S, 10.64. Found: C, 55.65; H, 4.93; N, 4.42; S, 10.48.

Neopentyl Mesylate (9).[33] To the cooled (0 °C), stirred solution of neopentyl alcohol (25.1 g, 0.285 mol) and methanesulfonyl chloride (20.5 mL, 0.265 mol) in dichloromethane (500 mL) was added dropwise triethylamine (112 mL, 0.804 mol). The internal reaction temperature was kept below 10 °C (immersed thermometer). Addition was complete in 2 h. The reaction mixture was stirred at 0 °C for an additional 2.5 h and then washed with saturated sodium bicarbonate (500 mL), dried over anhydrous magnesium sulfate, filtered, and evaporated in vacuo. The crude oil was purified by bulb-to-bulb distillation using a Kugelrohr apparatus under diminished pressure [~100 °C (air

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bath temperature), 0.5 mmHg] to afford the mesylate (37.3 g, 85%) as a clear oil: ^{1}H NMR ($CDCl_3$) δ 3.88 (2 H, s), 3.01 (3 H, s), 1.00 (9 H, s); ^{13}C NMR ($CDCl_3$) δ 79.0 (1 C), 36.9 (1 C), 31.6 (1 C), 25.9 (3 C); ESI/MS *m*/*z* 184.0 $(M + NH_4)^+$.

Neopentyl 3-Hydroxy-1-propanesulfonate (10). A solution of *n*-butyllithium (44 mL, 110 mmol, 2.5 M) in hexanes was added dropwise to the cooled (−80 °C, diethyl ether/dry ice bath), stirred solution of neopentyl mesylate **9** (16.6 g, 100 mmol) and tetramethylethylenediamine (30 mL, 200 mmol) in dry THF (220 mL) over 20 min under argon. Stirring was continued for an additional 30 min; then a solution of ethylene oxide (22 mL, 110 mmol, 5.14 M) in dry THF was added dropwise over 15 min. The reaction mixture was stirred at room temperature for 16 h, diluted with diethyl ether (300 mL), and washed with a saturated sodium chloride solution (600 mL). The organic layer was separated, dried over anhydrous magnesium sulfate, filtered, and evaporated in vacuo. The resulting oil was purified by bulb-to-bulb distillation under diminished pressure [Kugelrohr apparatus, ∼100 °C (air bath temperature), 0.1 mmHg] to afford pure product **10** (16.0 g, 76%): ^{1}H NMR ($CDCl_3$) δ 3.89 (2 H, s), 3.81 (2 H, t, $J = 6$), 3.28 (2 H, m), 2.11 (2 H, m), 1.80 (1 H, broad s), 1.00 (9 H, s); ^{13}C NMR ($CDCl_3$) δ 78.7 (1 C), 60.1 (1 C), 46.8 (1 C), 31.6 (1 C), 26.4 (1 C), 25.9 (3 C). Anal. Calcd for $C_8H_{18}O_4S$: C, 45.69; H, 8.62; S, 15.25. Found: C, 45.46; H, 8.58; S, 15.13.

Neopentyl 3-Triflyloxy-1-propanesulfonate (11). Triflic anhydride (10 mL, 0.060 mol) was added dropwise over 15 min to the stirred, cooled (0 °C) solution of **10** (12.54 g, 0.060 mol) and pyridine (5.4 mL, 0.066 mol) in dry dichloromethane (360 mL) under argon. After the addition was complete, the reaction mixture was stirred at 0 °C for 2 h. The reaction mixture was then washed with ice cold 5% aqueous sodium bisulfate (2 × 250 mL), 10% aqueous sodium bicarbonate (2 × 250 mL), and saturated aqueous sodium chloride (500 mL). The organic phase was dried over anhydrous sodium sulfate, filtered, and then evaporated under reduced pressure to give **11** (15 g, 0.049 mol, 82%) as a tan solid. This material was used in subsequent reactions without further purification. In a separate run, analytically pure material was obtained after flash column chromatography (500 silica gel, 20% ethyl acetate in hexanes) to afford **11** (8.0 g, 39%) as a white crystalline solid: mp 44−45 °C; ^{1}H NMR ($CDCl_3$) δ 4.70 (2 H, t, $J = 6$), 3.91 (2 H, s), 3.26 (2 H, t, $J = 7$), 2.40 (2 H, m), 0.99 (9 H, s); ^{13}C NMR ($CDCl_3$) δ 79.3 (1 C), 74.1 (1 C), 45.7 (1 C), 31.7 (1 C), 25.9 (3 C), 24.0 (1 C). Anal. Calcd for $C_9H_{17}F_3O_6S_2$: C, 31.58; H, 5.00; F, 16.65; S, 18.73. Found: C, 31.73; H, 5.11; F, 16.73; S, 18.56.

An NMR sample was kept at room temperature for 13 days. No significant decomposition of the material was observed.

9-[[[[4-(4-Ethoxy-4-oxobutyl)phenyl]sulfonyl][4-(2,2-dimethylpropoxy)-4-sulfobutyl]amino]carbonyl]-10-[4-(2,2-dimethylpropoxy)-4-sulfobutyl]acridinium Triflate Salt (12). To the stirred solution of compound **5** (12.0 g, 18 mmol) in dry dichloromethane (180 mL) were added triflate **11** (8.0 g, 23.4 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (4.8 g, 23.4 mmol). The reaction mixture was stirred at room temperature for 7 days. The solvent was removed under diminished pressure on a rotary evaporator, and the remaining material was purified by gravity column chromatography (1 kg silica gel, 50% ethyl acetate in hexanes to 2% methanol in dichloromethane to 5% methanol in dichloromethane). Starting material (7.5 g, 63%) was recovered from fractions in ethyl acetate/hexanes. The change from ethyl acetate/hexanes to methanol/dichloromethane started after the starting material was completely eluted. All fractions in methanol/dichloromethane containing fluorescent material were collected, combined, and evaporated in vacuo to give the acridinium product **12** (6.28 g, 34%, 96% based on recovered **5**): ESI/Ms *m*/*z* 861.5 (M^+); ^{1}H NMR (300 MHz, CD_3OD + TFA) δ 8.83 (2 H, m), 8.49 (2 H, m), 8.25 (1 H, d, $J = 9$), 8.10 (1 H, br d, $J = 8$), 8.02 (1 H, m), 7.82 (1 H, m), 7.81 (1 H, br d, $J = 9$), 7.67 (1 H, d, $J = 9$), 7.27 (2 H, m), 5.66 (2 H, m), 4.14 (2 H, m, CH_2 of ethyl ester), 4.00 and 3.51 (4 H, m, CH_2 of neopentyl sulfonate), 1.25 (3 H, m, CH_3 of ethyl ester), 1.03, 1.00, 0.99 and 0.77 (18 H, s, CH_3 of neopentyl sulfonate); HPLC [3.9 × 300 mm μBondapak C-18, 65:35:0.1 acetonitrile/water/trifluoroacetic acid, 1 mL/min, 254 nm] t_R, 5.6 min, 94%.

9-[[[[4-(3-Carboxypropyl)phenyl]sulfonyl][3-sulfopropyl]amino]carbonyl]-10-(3-sulfopropyl)acridinium Inner Salt (13). Acridinium compound **12** (4.26 g, 4.2 mmol) was heated to reflux in 1 N HCl (200 mL) for 8 h. After being cooled to room temperature, the yellow solution was decanted and lyophilized to give **13** (2.9 g, 4.2 mmol, 100%) as an amorphous deliquescent solid after drying over P_2O_5: ^{1}H NMR (pyridine-d_5) δ 9.41 (2 H, d, $J = 9$), 8.57 (2 H, d, $J = 8$), 8.35 (2 H, m), 8.02 (2 H, d, $J = 8$), 7.85 (2 H, m), 7.65 (2 H, d, $J = 8$), 6.19 (2 H, m), 4.29 (2 H, m), 3.66 (2 H, m), 2.58 (2 H, m), 3.30 to 2.40 (8 H, m), 2.15 (4 H, m); ESI/MS *m*/*z* 711.4 $(M + NH_4)^+$; HPLC [3.9 × 300 mm μBondapak C-18, 25:75:0.1 acetonitrile/water/formic acid, 1 mL/min, 254 nm] t_R, 6.29 min, 94%.

Supporting Information Available: Preparation and characterization of compounds **2** and **5** are included as Supporting Information (8 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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