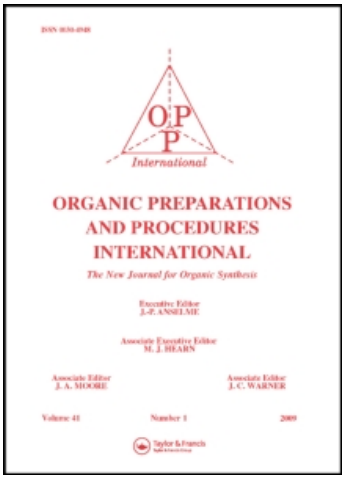

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LIPOSOMES PREPARED FROM 2-[*bis*-(2-HEXACOSA-10, 12-DIYNOLOXYETHYL)AMINO]ETHANESULFONIC ACID

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LIPOSOMES PREPARED FROM 2-[*bis*-(2-HEXACOSA-10,12-DIYNOLOXYETHYL)AMINO]ETHANESULFONIC ACID

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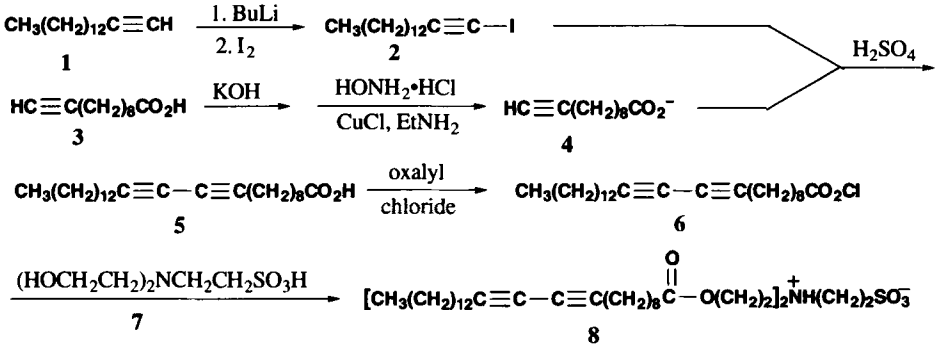
Diacetylene-containing amphiphatic[1] molecules may be formed into liposomes and stabilized by crosslinking with ultraviolet light. Applications range from fundamental studies of membrane-bound enzymes[2] to electronics.[3] One such well-studied polymerizable amphiphatic molecule is the sulfolipid 2-[*bis*-(2-hexacosa-10,12-diynoyloxyethyl)amino] ethanesulfonic acid (**8**).[4] We describe improvements in the procedures and report in detail the terse descriptions of the literature.[5,6] The preparation of 1-iodopentadecyne-1 (**2**) has been improved from 76% to 100% by the use of *n-butyllithium*[7] instead of the Grignard reagent.[6]



EXPERIMENTAL SECTION

All reactions were performed under a dry argon atmosphere to protect from oxygen and moisture. All glassware were dried overnight at 104°. The sonicator was Model 300 by Fisher Scientific (Springfield, NJ). The ultraviolet sunlamp, Model RSK6 by General Electric (Cleveland, OH), was available at a local department store and proved superior to ultraviolet lamps from laboratory suppliers. Liposome diameters were measured using a Coulter Multisizer with 70-μm aperture. ^{1}H NMR spectra were obtained on a Varian XL-200e spectrometer operating at 200 MHz. All spectra were run in 5-mm sample tubes using $CDCl_3$ as solvent. In the following description, the colors of products are indicated as a guide; however, the colors strongly depend on concentration and may vary.

1-Iodopentadecyne-1 (2).- A 1-L, three-neck, round-bottom flask fitted with a magnetic stirring bar and 125-mL dropping funnel was submerged in an ice bath. For about 30 min, the empty glassware was purged with argon. With stirring and continued argon purge, 27.6 mL (21.8 g, 0.105 mol)

of 1-pentadecyne (**1**) (Lancaster Synthesis, Ltd., Windham, NH) was added to 600 mL anhydrous petroleum ether (distilled from P_2O_5). Then, 66.0 mL (0.105 mol) of 1.6-M *n*-butyllithium in hexanes (Aldrich, Milwaukee, WI) was added dropwise. The resulting gel was stirred with a glass rod and 5-mL aliquots of anhydrous petroleum ether were repeatedly added until the white clumps fully dissolved. The mixture was stirred for 1 hr to ensure complete deprotonation of pentadecyne. The solution turned light peach in color. While the argon purge was maintained, 29.4 g of solid iodine (0.115 mol) was added rapidly. Stirring was continued for 40 min to complete the reaction.

The mixture was then transferred to a 500-mL separatory funnel and washed successively with 100 mL distilled water, 2 x 50 mL saturated aqueous $NaHSO_3$, 50 mL distilled water, and 50 mL saturated aqueous NaCl. The yellow organic layer was dried over Na_2SO_4 and evaporated on a rotary evaporator (water aspirator) at 30° to give 35.1 g (100%) pentadecynyl iodide (**2**) as an amber liquid. It was stored in a freezer and wrapped with aluminum foil to protect it from light. ^1H NMR ($CDCl_3$): δ 0.89 (t, J = 7 Hz), 1.26 (s, overlapping methylenes), 1.40-1.60 (m), 2.35 (t, J = 8 Hz).

Carboxylate of Undecynoic Acid (4).- To the same glassware used to prepare **2**, but without the ice bath, 17.9 g (0.0984 mol) 10-undecynoic acid (**3**, Wiley Organics, Columbus, OH) and 79.0 mL of 10% KOH (0.14 mol) were added while stirring and purging with argon. To this basic solution (pH > 10), 0.53 g (0.0076 mol) hydroxylamine hydrochloride and a solution of 2.63 g (0.027 mol) CuCl dissolved in 26.3 mL 70% aqueous ethylamine were added and stirred for 30 min to yield a solution of **4**.

Hexacosa-10,12-diynoic Acid (5).- A solution of 35.1 g (0.105 mol) of **2** in 23.5 mL methanol was added dropwise to the round-bottom flask containing **4** with vigorous stirring for 45 min, purging with argon, and cooling in an ice bath. The resulting heterogeneous, dull yellow mixture was stirred for 1 hr. After warming to room temperature, it was acidified to pH 4 with 2 N H_2SO_4; the color changed to brown and then to pink. The solution was transferred to a 500-mL separatory funnel, and washed with 75 mL diethyl ether and 2 x 50 mL diethyl ether. The organic layers were pooled, washed with 50 mL saturated aqueous NaCl, dried over Na_2SO_4, and filtered to remove solids. Evaporation at 30° in a rotary evaporator (water aspirator) gave 6.84 g (18%) of **5** as a solid. Koch[6] obtained 33%; Hub *et al.*[5] obtained 55%. mp 68-69° ^1H NMR: δ 0.86 (t, J = 8 Hz), 1.24 (s, overlapping methylenes), 1.30 (s, broad), 140-1.64 (m), 2.22 (t, J = 8 Hz), 2.33 (t., J = 9 Hz).

Acyl Chloride (6). - Under an argon purge, 6.84 g (0.0176 mol) of **5** and 8.21 g (0.065 mol) of oxalyl chloride were placed in a 250-mL round-bottom flask with a magnetic stirring bar. The contents of the flask were protected from light by aluminum foil. It was stirred for 3 days at room temperature; the gentle argon purge safely vented the evolved HCl and CO. Then, the round-bottom flask was heated to 70-80° and evacuated (water aspirator) for 30 min. After cooling to room temperature, the contents were transferred to a 500-mL separatory funnel by washing successively with 20 volumes and then 10 volumes of hexane. The pooled hexane solution was washed with 3 x 50 mL ice-cold distilled water and 20 mL saturated NaCl until the organic phase was clear. The yellow organic phase was dried over Na_2SO_4 and evaporated at 65° on a rotary evaporator (water aspirator) to give 8.7 g (117% yield due to impurities; Koch[6] obtained 89%) of **6**.

2-[*bis*-(2-Hexacosa-10,12-diynoyloxyethyl)amino]ethanesulfonic Acid (8).- A 100-mL, three-neck, round-bottom flask was equipped with a Friedrichs condenser and a magnetic stirring bar. Under a gentle argon purge, 8.7 g (0.021 mol) of **6** was mixed with 2.3 g (0.011 mol) *N-N-bis*[2-hydroxyethyl]-2-aminoethanesulfonic acid (BES, **7**, Aldrich, Milwaukee, WI) and 21 mL anhydrous chloroform. The BES did not completely dissolve. Anhydrous pyridine (1.5 mL) was added and the mixture was refluxed overnight in a 60° oil bath. The solution was cooled to room temperature and placed in a freezer to form crystals. (Note: If crystals did not appear, some solvent was evaporated at 30° on a rotary evaporator with water aspiration.) If the crystals were reddish-brown rather than off-white, they were washed with chilled methanol. If this failed to remove the color, they were redissolved in a small amount of anhydrous petroleum ether and recrystallized. The mass of **8** was 9.0 g (91%; Koch[6] obtained 89%), mp 105°; 109° according to Tieke[6] ^{1}H NMR: δ 0.88 (t, J = 8 Hz), 1.25 (s, overlapping methylenes), 1.30 (s), 1.40-1.68 (m), 2.24 (t, *J* = 8 Hz), 2.38 (t, *J* = 8 Hz), with broad singlets at 3.26, 3.65, 3.75, and 4.49.

Liposome Formation.- To a 100-mL round-bottom flask were added 60 mL of distilled water and 60 mg of **8**. To produce liposomes, the flask was placed in a 60° oil bath while purging with argon and sonicating at 30, 50, or 65% of full power with a large-tipped probe for 10, 20, or 30 min. The pH was adjusted to 10 with NaOH. The liposomes were exposed to sunlamp light which passed through a water-filled Petri dish to selectively remove infrared radiation. The ultraviolet radiation polymerized the liposomes turning them from colorless to red. To measure the size distribution, 0.5 mL of sample was diluted 50,000 times to 20 μg/L with Coulter Isotonic II solution. The average liposome diameter was 1.6 mm with a range from 1.5 to 6.5 mm. The size distribution was not strongly affected by sonication time or power. The specific surface area was 2.0 to 3.5 m^2/g using 30% power and 20 min time, conditions that produced the largest specific surface area.

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A SUPERIOR SYNTHESIS OF CHOLINERGIC ANABASEINE

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Anabaseine, (3,4,5,6-tetrahydro-2,3'-bipyridine, **1**), a naturally occurring neurotoxin produced by hoplonemertine sea worms[1] competes with the natural neurotransmitter acetylcholine when binding to nicotinic receptor sites. Such compounds are of considerable current interest because it is believed[2] that loss of cholinergic pathways in cell to cell communication contributes to the early loss of memory (Alzheimer's disease). Now underway in many laboratories is a major emphasis on developing novel cholinergic derivatives. Anabaseine is the precursor of 3-benzylidene[3-5] and 3-cinnamylidene[6] derivatives that have novel binding properties to $\alpha_4\beta_2$ and α_7 binding sites in the human brain. Such derivatives command the intense current interest of pharmacologists. We now report a shortened and improved preparation of **1** especially designed for large commercial production because it avoids the use of costly low temperatures and expensive reagents as found in some other preparations.

Three different routes to **1** have been reported. One involves the addition of 3-pyridyl lithium to cyclopentanone followed by a Schmidt reaction with hydrazoic acid.[7] A second employs a rearrangement of 1-nicotinoyl-2-piperidone induced thermally by CaO[8] and a third is based on a mixed Claisen condensation.[9,10] A Claisen route also serves to provide 5'-fluoroanabaseine,[10] N-methyl anabaseine[11] and the tobacco alkaloid myosmine,[12] the 1-pyrroline counterpart of **1**.