Facile and Efficient Synthesis of Bolaamphiphilic Tetraether Phosphocholines

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A facile synthesis of ether-linked bolaform phospholipids in good yields has been developed. Triflic acid catalyzed oxirane ring opening of benzyl-protected *rac*-glycidyl with long-chain 1,*ω*-alkanediols ($n = 16, 20$) produced 1,1′-diglycerol diethers in 80−90% yield. Double alkylation of the secondary hydroxy groups with 1-bromooctane or 1-bromodecane gave the corresponding benzyl-protected tetraethers in 66% yield. Hydrogenolysis of the benzyl groups in the presence of Pd/C (55−66% yield) followed by phosphorylation with 2 equiv of 2-chloro-2-oxo-1,3,2-dioxaphospholane and amination with excess trimethylamine produced the tetraether bolaform bisphosphocholines as white powders in ∼75% yield. This approach provides a reliable and efficient method for preparing a wide variety of symmetrical bolaform phospholipids on a multigram scale.

Introduction

Tetraether bolaamphiphiles have been the focus of several ongoing investigations in this laboratory.[1−7] Our interest in these materials stems from their ability to form stable membrane vesicles with lamellar thicknesses on the order of 2.4 nm,[3,5] a dimension that is ideally suited for studies of coupled electron and ion transport phenomena across vesicle membranes using vectorially inserted gramicidin-based diads and triads.[1,2] Large quantities of bisphosphocholine bolaamphiphiles are required for these studies because they are used as the host membrane matrix. Synthetic pathways for bisphosphoric[6] and bisphosphocholine[7] bolaamphiphiles **8** and **9**, as well as other bolaform derivatives, have been described;[8−19] however, the overall yields of purified materials are generally quite low. We sought a new synthesis that would utilize readily available starting materials that could be converted in good yields to phosphocholine-type bolaamphiphiles in a minimum number of steps. Previously, we reported the reaction of solketal with long-chain 1,*ω*-dibromoalkanes, which required the synthesis of 1,16-dibromohexadecane or 1,-20-dibromoeicosane from the corresponding diol or diacid, respectively.[6] This pathway produced 1,1′-polymethylene diglycerols with four hydroxy groups that required selective protection prior to installation of the secondary ether substituents. Efficient and selective protection of the primary hydroxy groups, however, proved to be difficult. Protection with triphenylmethyl chloride gave the desired product in only 30% yield, with the triply protected diglycerol recovered as the main product. Unfortunately, replacement of trityl chloride with *tert*-butyldiphenylchlorosilane (TBDPS-Cl) did not improve the yield; about 50% of the silyl ether was hydrolyzed to the silanol, even when the reactions employed lyophilized 1,1′-polymethylene diglycerol precursors in the presence of molecular sieves,[20] thus limiting the yield of the doubly TBDPS protected diglycerol to 20−30%. Reactions employing an excess of silyl chloride resulted in an increased production of the triply protected diglycerol.

Our previously reported synthesis of **8** and **9** required six steps starting from oxiranemethanol 3-nitrobenzenesulfonate (NBS) and long-chain 1,*ω*-alkanediols.[7] The primary limitations of this pathway are (i) the second alkylation step which uses alkyl triflates in the presence of 1,8-bis(dimethylamino)naphthalene, (ii) the cleavage of the NBS protecting group under conditions that do not generate either elimination or oligomeric products, and (iii) the relatively high costs of chiral glycidyl sulfonates

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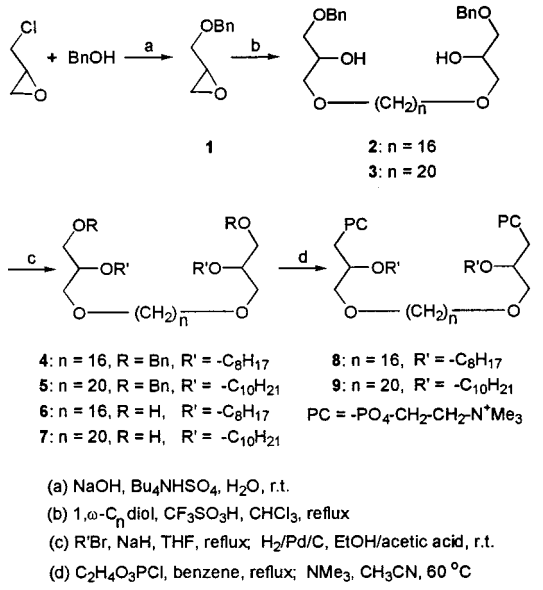

Figure 1. Synthesis pathway to bolaamphiphilic tetraether phosphocholines.

and alkyl triflates. The tedious chromatographic purifications required after each step and the low overall yields observed also made this synthesis impractical for large scale preparations.

Results and Discussion

The synthesis described here (Figure 1) utilizes benzyl glycidyl ether (**1**), formed by coupling benzyl alcohol with epichlorohydrin as the glycerol precursor in >90% yield.[21,22] Triflic acid catalyzed oxirane ring opening with long-chain 1,ω-alkanediols gave the benzyl-protected diether diols **2** and **3** in 80−90% yield.[23] It proved important to use a 3:1 epoxide/alkanediol ratio in this step because lower ratios (e.g., 2:1) reduced the yields to 30−40% and left some unreacted alkanediol which was difficult to chromatographically separate from the products **2** and **3**. Also, very small catalytic amounts of triflic acid (∼2 mol %) were required, as higher concentrations of catalyst were found to decrease the yield of the diether diols **2** and **3** via addition of a third equivalent of **1**. Subsequent alkylation of the secondary hydroxy groups, carried out via Williamson synthesis using sodium hydride and the appropriate 1-bromoalkane,[24,25] gave the benzyl-protected tetraethers **4** and **5** in 66% yield.[26] Hydrogenolysis of the benzyl groups was conducted in the presence of palladium on carbon and gave the tetraether diols **6** and **7** in moderate yield (55−66%).[27] The purified diols **6** and **7** were then phosphorylated and aminated via the established 2-chloro-2-oxo-1,3,2-dioxaphospholane/trimethylamine route,[27−29] to give the bolaform tetraether phosphocholines **8** and **9** as white powders in ∼75% isolated yield. The structures of the phosphocholine bolaamphiphiles **8** and **9** were confirmed by ^{1}H and ^{13}C NMR spectroscopy (Figure 2).

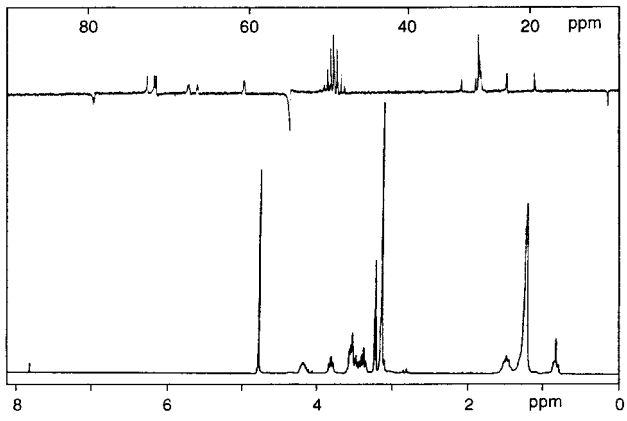

Figure 2. ^{1}H and ^{13}C APT (attached proton test) NMR spectra of bolaform phosphocholine **8**.

Conclusions

A facile synthesis of ether-linked bolaform phospholipids has been developed. The approach is efficient and utilizes readily purified intermediates, making it amenable to the large scale production of these bolaamphiphiles. Application of enantiomerically pure glyceryl synthons, in principle, should also enable the synthesis of chiral bolaform phospholipids.

Experimental Section

All yields reported are for isolated products (>95% pure). Melting points were determined with a Perkin-Elmer DSC-7/intracooler.[6] All products are characterized by ^{1}H and ^{13}C NMR; satisfactory mass spectra and elemental analyses were obtained where appropriate. NMR chemical shifts are reported in ppm with tetramethylsilane and solvent peaks as internal standards for the proton and carbon spectra, respectively.

Materials. All reagents were used as received. Solvents were purified by distillation from an appropriate desiccant.

1-*O*-Benzyl-*rac*-glycidol (1). A mixture of aqueous sodium hydroxide (20 mL, 50% w/w), racemic epichlorohydrin (14.2 g, 153.4 mmol), and tetrabutylammonium hydrogen sulfate (0.5 g, 1.47 mmol) was vigorously stirred at room temperature. Benzyl alcohol (3.3 g, 30.9 mmol) was then added so that the temperature did not exceed 25 °C and the mixture stirred at room temperature for 20 h. Cold water (80 mL) was added, the aqueous phase extracted (2 × 100 mL) with ether, and the extract washed (50 mL) with a brine solution. The ether phase was dried over magnesium sulfate and the solvent removed by rotary evaporation. Filtration of the remaining oil through a 3-cm plug of silica gel (6-cm diameter) using 5:1 hexane/ether as eluent gave **1** in 92% yield (4.65 g, 28.3 mmol). TLC: R_f = 0.25, 5:1 hexane/ether. ^{1}H NMR ($CDCl_3$): 2.63 (dd, J_{AM} = 2.6 Hz, J_{AB} = 5.0 Hz, 1H), 2.81 (dd, J_{BM} = 4.4 Hz, J_{AB} = 5.0 Hz, 1H), 3.20 (m, 1H), 3.44 (dd, $J_{A'M}$ = 5.8 Hz, $J_{A'B'}$ = 11.4 Hz, 1H), 3.78 (dd, $J_{B'M}$ = 3.0 Hz, $J_{A'B'}$ = 11.4 Hz, 1H), 4.60 (d, 2H), 7.36 (m, 5H).

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1,1′-Di-*O*-hexadecamethylene-3,3′-*O*-dibenzyldi-*rac*-glycerol (2). Triflic acid (50 μL) was added to a solution of **1** (4.0 g, 24.4 mmol) and 1,16-hexadecanediol (2.0 g, 7.8 mmol) in 100 mL of dry $CHCl_3$. The mixture was heated at reflux for 48 h and the solvent removed by evaporation. The remaining oil was purified by filtration through a 3-cm plug of silica gel (6-cm diameter) using 2:1 hexane/ethyl acetate as eluent to give the benzyl-protected diether diol **2** in 90% yield (4.12 g, 7.0 mmol). TLC: $R_f = 0.21$, 2:1 hexane/ethyl acetate. ^{1}H NMR ($CDCl_3$): 1.28 (s, 24H), 1.58 (m, 4H), 2.38 (s, 2H), 3.34−3.75 (m, 12H), 3.98 (m, 2H), 4.56 (d, 4H), 7.34 (m, 10H). ^{13}C NMR ($CDCl_3$): 26.1, 29.6, 69.5, 71.7, 73.4, 127.7, 128.4, 138.0. MS (pos. FAB): *m*/*z* 609 (35%, $[M + Na]^+$), 587 (100%, $[M + H]^+$).

1,1′-Di-*O*-eicosamethylene-3,3′-*O*-dibenzyldi-*rac*-glycerol (3). This was prepared as described for **2**, except 1,20-eicosanediol (2.5 g, 8.1 mmol) was used as substrate. Yield: 80% (4.18 g, 6.5 mmol). TLC: $R_f = 0.24$, 2:1 hexane/ethyl acetate. ^{1}H NMR ($CDCl_3$): 1.28 (s, 32H), 1.58 (m, 4H), 2.30 (s, 2H), 3.30−3.75 (m, 12H), 3.98 (m, 2H), 4.56 (d, 4H), 7.34 (m, 10H). MS (pos. FAB): *m*/*z* 643 (100%, $[M + H]^+$).

1,1′-Di-*O*-hexadecamethylene-2,2′-*O*-dioctyl-3,3′-*O*-dibenzyldi-*rac*-glycerol (4). The diether diol **2** (6.0 g, 10.2 mmol), dissolved in 60 mL of dry THF, was added to a suspension of sodium hydride (2.1 g, 52.5 mmol; 60% dispersion in mineral oil) in 90 mL of dry THF. When the gas evolution ceased, 1-bromooctane (19.0 g, 98.4 mmol) was added and the mixture heated at reflux for 3 days. The mixture was filtered through a 2-cm plug of silica gel and the filtrate concentrated by evaporation. The remaining viscous oil was purified by filtration through a 3-cm plug of silica gel (12-cm diameter) using 5:1 hexane/ethyl acetate as eluent to give the benzyl-protected tetraether **4** in 66% yield (5.4 g, 6.7 mmol). TLC: $R_f = 0.58$, 5:1 hexane/ethyl acetate. ^{1}H NMR ($CDCl_3$): 0.88 (t, 6H), 1.28 (s, 44H), 1.58 (m, 8H), 3.30−3.70 (m, 18H), 4.56 (d, 4H), 7.34 (m, 10H).

1,1′-Di-*O*-eicosamethylene-2,2′-*O*-didecyl-3,3′-*O*-dibenzyldi-*rac*-glycerol (5). This was prepared as described for **4**, except 1-bromodecane was used as substrate. Yield: 66%. TLC: $R_f = 0.59$, 5:1 hexane/ethyl acetate. ^{1}H NMR ($CDCl_3$): 0.88 (t, 6H), 1.30 (s, 60H), 1.58 (m, 8H), 3.35−3.75 (m, 18H), 4.56 (d, 4H), 7.34 (m, 10H). ^{13}C NMR ($CDCl_3$): 14.0, 22.6, 25.6, 26.0, 29.2−29.9, 31.8, 32.6, 70.1, 70.6, 71.5, 73.2, 77.8, 127.4, 128.2, 138.2.

1,1′-Di-*O*-hexadecamethylene-2,2′-*O*-dioctyldi-*rac*-glycerol (6). The benzyl protected tetraether **4** (1.3 g, 1.6 mmol) and palladium (1.0 g; 10 wt % on carbon) were added to 80 mL of 5:1 ethanol/acetic acid. Hydrogenolysis was conducted at room temperature at slight positive pressure using hydrogen. The reaction was quenched after 20 h by adding ether (70 mL) to the mixture and filtering it through a 1-cm plug of Celite. The Celite plug was washed (1 × 100 mL) with ether, and the solvent was removed by evaporation. The remaining oil was purified by filtration through a 3-cm plug of silica gel (6-cm diameter) using 2:1 hexane/ethyl acetate as eluent. The tetraether diol **5** was isolated in 66% yield (670 mg, 1.06 mmol) as an oil that slowly solidified at room temperature. TLC: $R_f = 0.33$, 2:1 hexane/ethyl acetate. ^{1}H NMR ($CDCl_3$): 0.88 (t, 6H), 1.28 (s, 44H), 1.58 (m, 8H), 2.38 (br s, 2H), 3.35−3.80 (m, 18H).

1,1′-Di-*O*-eicosamethylene-2,2′-*O*-didecyldi-*rac*-glycerol (7). This was prepared as described for **6**. Yield: 55%. TLC: $R_f = 0.30$, 2:1 hexane/ethyl acetate. Mp 38−40 °C. ^{1}H NMR ($CDCl_3$): 0.90 (t, 6H), 1.30 (s, 60H), 1.58 (m, 8H), 2.16 (s, 2H), 3.35−3.85 (m, 18H). ^{13}C NMR ($CDCl_3$): 14.4, 22.8, 26.4, 29.2−30.4, 32.2, 33.2, 63.2, 70.6, 71.2, 72.2, 78.6.

1,1′-Di-*O*-hexadecamethylene-2,2′-*O*-dioctylbis(*rac*-glycero-3-phosphocholine) (8). 2-Chloro-2-oxo-1,3,2-dioxaphospholane (1.07 g, 7.5 mmol) was added dropwise at 10 °C to a solution of the tetraether diol **6** (1.9 g, 3.0 mmol) and dry triethylamine (871.0 mg, 8.6 mmol) in 30 mL of dry benzene. The mixture was stirred for 10 min. at 10 °C and overnight at room temperature. Crystalline $Et_3N{\cdot}HCl$ was removed by filtration and the solvent evaporated to give the phosphorylated bolaamphiphile as a viscous oil. The residue was transferred to a pressure tube with 25 mL of anhydrous CH_3CN. The pressure tube was then cooled in a dry ice−acetone bath, and cold (0°) anhydrous trimethylamine (4−5 mL) was poured into the tube. Then the tube was sealed and heated in an oil bath for 48 h at 65 °C. A white powder formed during the reaction. The mixture was cooled in an ice bath, the excess trimethylamine gas released, and the solution poured into a round-bottom flask. Volatile components were removed by evaporation and the remaining oil purified by column chromatography on silica gel using $CHCl_3$ with an increasing amount of CH_3OH as eluent. The bolaform phosphocholine **7** was isolated as a white powder in 73% yield (2.1 g, 2.18 mmol). TLC: $R_f = 0.35$, 8:6:4:3 chloroform/acetone/methanol/NH_4OH. ^{1}H NMR (CD_3OD): 0.88 (t, 6H), 1.28 (s, 44H), 1.50 (m, 8H), 3.16 (s, 18H), 3.35−3.65 (m, 18H), 3.82 (t, 4H), 4.18 (br m, 4H). ^{13}C NMR (CD_3OD): 14.6, 23.8, 27.4, 30.2−31.0, 33.2, 54.8, 60.5, 66.4, 67.6, 71.4, 72.8, 79.4. Anal. Calcd for tetrahydrate: C, 55.79; H, 10.73; N, 2.71; P, 6.00. Found: C, 55.7; H, 10.89; N, 3.09; P, 6.46.

1,1′-Di-*O*-eicosamethylene-2,2′-*O*-didecylbis(*rac*-glycero-3-phosphocholine) (9). This was prepared as described for **8**. Yield: 76%. TLC: $R_f = 0.34$, 8:6:4:3 chloroform/acetone/methanol/NH_4OH. ^{1}H NMR (CD_3OD): 0.88 (t, 6H), 1.30 (s, 60H), 1.58 (m, 8H), 3.24 (s, 18H), 3.40−3.75 (m, 18H), 3.88 (t, 4H), 4.28 (br m, 4H). ^{13}C NMR (CD_3OD): 14.6, 23.8, 27.4, 30.2−31.0, 33.2, 54.8, 60.5, 66.4, 67.6, 71.4, 72.8, 79.4. Anal. Calcd for tetrahydrate: C, 58.71; H, 11.09; P, 5.41. Found: C, 58.21; H, 11.06; P, 5.46.

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Supporting Information Available: NMR spectra of compounds **1**−**9** (19 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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