Efficient Stereospecific Synthesis of Diamide Analogs of Phosphatidylcholine Starting from 1-(4′-Methoxyphenyl)-*sn*-glycerol

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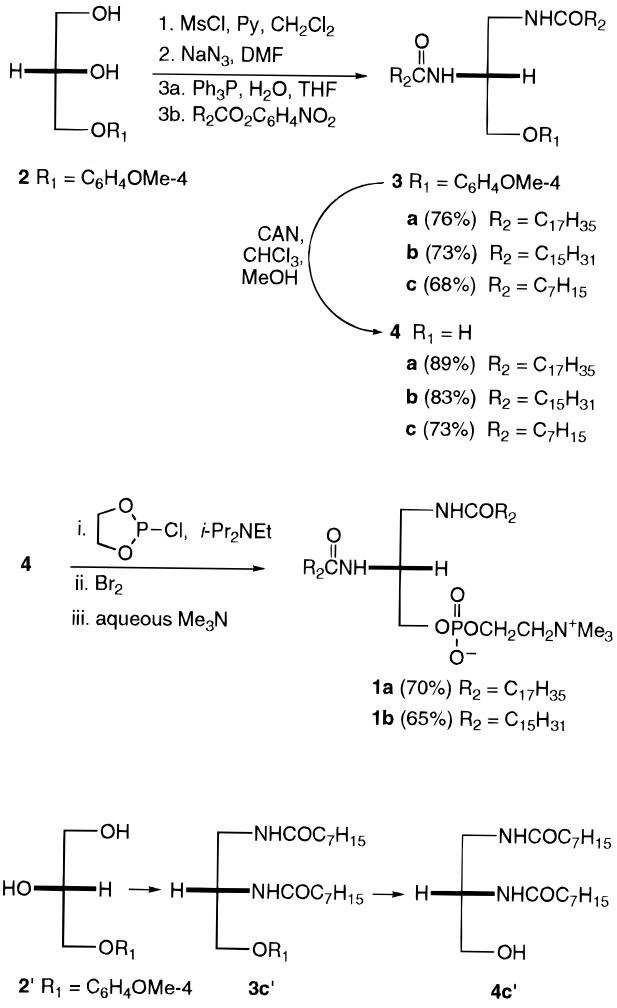
Amide analogs of diacylglycerophosphocholines are of interest as inhibitors of phospholipase A_2[1] and as potential anti-HIV[2] and antitumor agents.[3] Synthetic amide-linked lipids have been used to investigate the influence of lipid structure on lipid movement in bilayer membranes.[4,5] Low-yielding achiral syntheses of 1,2-bis-(acylamino)-1,2-dideoxy-*sn*-glycerophosphocholine (**1**) have been reported starting from 2,3-dibromopropanol,[2] 2,3-dibromopropionic acid,[5,6] and L-asparagine.[6] We report here an efficient chiral synthesis of **1** starting from readily available 1-(4′-methoxyphenyl)-*sn*-glycerol (**2**);[7] **2′**, the enantiomer of **2**, afforded 2,3-diamido-1-propanol (**4c′**), which can be converted into the enantiomer of **1**.

Results and Discussion

Introduction of Two Amide Groups into *sn*-Glycerol Derivative 2. In order to synthesize optically active diamide analogs of phosphocholine we chose 1-(4′-methoxyphenyl)-*sn*-glycerol (**2**) as the starting material. The protected glycerol **2** was prepared by asymmetric dihydroxylation[7a] of allyl 4-methoxyphenyl ether using AD-mix supplemented with potassium persulfate.[7b] Glycerol **2** was converted to the dimesylate by using methanesulfonyl chloride in the presence of pyridine (Scheme 1). The dimesylate was subjected to a S_N2 reaction in a suspension of sodium azide in DMF. The diazide was converted to the long-chain diamide by the reduction of azide groups with triphenylphosphine−water[8] followed by in-situ acylation of the resulting diamine with an excess of 4-nitrophenyl stearate, 4-nitrophenyl palmitate, or 4-nitrophenyl caprylate in THF to give diamide **3**. Recrystallization of **3** in THF−acetone provided pure diamidopropyl aryl ether **3** in 68−76% overall yield from glycerol **2**.

Deprotection of the 4-Methoxyphenyl Group. After the desired diamido groups had been introduced into the glycerol backbone of **3**, the 4-methoxyphenyl group was removed by using CAN.[7b,9] The removal of the 4-methoxyphenyl group by CAN is usually carried out in CH_3CN-H_2O solution.[7b,9] Due to the poor solubility of diamidopropyl aryl ether **3** in this solvent system, it was necessary to carry out the deprotection in another solvent. We found that $CHCl_3$−MeOH 1:1 was a suitable solvent system for this purpose in the presence of 3 equiv of CAN. The removal of the 4-methoxyphenyl group provided 2,3-diamidopropanol **4** in 73−89% yield.

Scheme 1. Synthesis of Diamide Analogs of Phosphatidylcholine (1)



Insertion of the Phosphocholine Moiety into Diamidopropanol 4. It has been reported that phosphorylation reactions of *rac*-diamidopropanol **4** by using *β*-bromoethyl phosphodichloridate or 2-chloro-1,3,2-dioxaphospholane followed by introduction of the quaternary N^+Me_3 salt provide *rac*-1,2-bis(acylamino)-1,2-dideoxy-glycerophosphocholines **1** in only about 12%[6] and 40%[2] yields, respectively. Instead, we chose to phosphitylate **3** using commercially available ethylene chlorophosphite.[10] Our procedure, in which the phosphocholine moiety is inserted into diamidopropanol **4** by use of trivalent phosphorus, provided a higher yield (65−70% overall yield from **4**) than those obtained with pentavalent phosphorus.

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Experimental Section

General Methods. Melting points are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ at 400 and 100 MHz, respectively. FAB HRMS were obtained at Michigan State University. Optical rotations were measured in a cell of 1-dm pathlength. TLC was carried out with silica gel GF (250 μm) glass plates from Analtech (Newark, DE) and silica gel 60 aluminum sheets from EM Separations (Gibbstown, NJ). The compounds were visualized by heating with phosphorus spray, iodine chamber, and/or short-wavelength UV light. For flash chromatography, silica gel 60 (230−400 ASTM mesh) was used (purchased from Aldrich). Solvents were dried as follows: CH_2Cl_2 was distilled from CaH_2; C_6H_6 was washed with concentrated H_2SO_4 and water, dried over $CaCl_2$, and then distilled over sodium metal; and pyridine and DMF were dried by refluxing over BaO followed by distillation just before use. 1- and 3-(4′-methoxyphenyl)-*sn*-glycerol (**2** and **2′**)[7b] and 4-nitrophenyl esters[11] were prepared by the methods described earlier.

(*R*)-2,3-Dioctadecanamidopropyl 4′-Methoxyphenyl Ether (3a). To a solution of 1-(4′-methoxyphenyl)-*sn*-glycerol (**2**, 1.0 g, 5.0 mmol) and pyridine (2.0 mL, 25 mmol) in 20 mL of CH_2Cl_2 was added methanesulfonyl chloride (0.80 mL, 10.4 mmol) at −10 °C. After being stirred for 4 h at 0 °C, the mixture was diluted with EtOAc and washed with water, 10% aqueous $NaHSO_4$ solution, saturated aqueous $NaHCO_3$ solution, and water. The solvents were removed in vacuo to give the crude dimesylate as a white solid. The crude dimesylate was dissolved in 30 mL of DMF, and sodium azide (3.25 g, 50.0 mmol) was added. The reaction mixture was stirred at 90 °C for 24 h. The product was extracted with EtOAc and concentrated to give a crude diazide. The colored impurity was removed by dissolving the crude diazide in 20 mL of hexane−EtOAc 10:1 and filtering the solution through a pad of silica gel, which was rinsed with 100 mL of hexane−EtOAc 10:1. The filtrate was concentrated under reduced pressure to give the diazide as a colorless oil. To the solution of the diazide in wet THF were added triphenylphosphine (2.63 g, 10 mmol) and 4-nitrophenyl stearate (3.78 g, 10 mmol) at the same time. After the reaction mixture was stirred for 24 h at rt and was refluxed for 2 h, it was diluted with acetone and cooled to −20 °C. The product was collected by filtration and purified by recrystallization in THF−acetone three times to give 2.77 g (76%) of diamidopropyl aryl ether **3a** as a white solid: mp 108−110 °C; $[\alpha]^{25}_D$ +15.82° (*c* 1.4, $CHCl_3$); IR (KBr) 3460, 1623 cm^{-1}; ^{1}H NMR δ 6.88−6.80 (m, 4H), 6.71 (d, 1H, J = 6.1 Hz), 6.28 (bs, 1H), 4.30 (m, 1H), 4.09−4.06 (dd, 1H, J = 3.8, 9.6 Hz), 3.88−3.85 (dd, 1H, J = 6.4, 9.6 Hz), 3.78 (s, 3H), 3.69−3.61 (m, 1H), 3.41−3.46 (m, 1H), 2.36 (t, 4H, J = 7.3 Hz), 1.63−1.77 (m, 4H), 1.25 (s, 56H), 0.88 (t, 6H, J = 5.9 Hz); ^{13}C NMR δ 175.26, 174.42, 154.23, 152.30, 115.42, 114.73, 67.69, 55.69, 49.90, 41.62, 36.73, 36.59, 32.11, 31.94, 31.77, 30.32, 30.08, 29.73, 29.70, 29.68, 29.55, 29.39, 29.33, 29.20, 26.31, 25.80, 25.70, 22.87, 22.71, 14.15; HRMS (FAB, MH$^+$) m/z calcd for $C_{46}H_{85}N_2O_4$ 729.6509, found 729.6512.

(*R*)-2,3-Dihexadecanamidopropyl 4′-Methoxyphenyl Ether (3b). The compound was prepared in 73% yield from 1-(4′-methoxyphenyl)-*sn*-glycerol (**2**) and 4-nitrophenyl palmitate by the procedure described above: mp 113−115 °C; $[\alpha]^{25}_D$ +14.78° (*c* 1.4, $CHCl_3$); ^{1}H NMR δ 6.77−6.88 (m, 4H), 6.70 (d, 1H, J = 6.1 Hz), 6.28 (bs, 1H), 4.30 (m, 1H), 4.09−4.06 (dd, 1H, J = 3.8, 9.6 Hz), 3.88−3.85 (dd, 1H, J = 6.4, 9.6 Hz), 3.76 (s, 3H), 3.71−3.64 (m, 1H), 3.43−3.48 (m, 1H), 2.19 (t, 4H, J = 7.6 Hz), 1.63−1.59 (m, 4H), 1.25 (s, 48H), 0.88 (t, 6H, J = 6.6 Hz); ^{13}C NMR δ 175.23, 174.40, 154.22, 152.29, 115.41, 114.72, 67.69, 55.69, 49.90, 41.62, 36.73, 36.59, 32.11, 31.94, 31.77, 30.32, 30.08, 29.73, 29.70, 29.68, 29.55, 29.39, 29.33, 29.29, 26.31, 25.80, 25.70, 22.87, 22.71, 14.14; HRMS (FAB, MH$^+$) m/z calcd for $C_{42}H_{77}N_2O_4$ 673.5883, found 673.5878.

(*R*)-2,3-Dioctanamidopropyl 4′-Methoxyphenyl Ether (3c). The compound was prepared in 68% yield from 1-(4′-methoxyphenyl)-*sn*-glycerol (**2**) and 4-nitrophenyl caprylate by the procedure described above except for the following variation. It was necessary to remove triphenylphosphine oxide after reduction of the diazide by triphenylphosphine in THF−H_2O because the R_f values of **3c** and triphenylphosphine oxide coincide (elution with $CHCl_3$−MeOH 10:1). After the reduction, the reaction mixture was concentrated to give a residue that was dissolved in $CHCl_3$ and filtered through a pad of silica gel (elution with $CHCl_3$) to remove triphenylphosphine oxide. The 1,2-diaminopropyl 4′-methoxyphenyl ether was eluted with $CHCl_3$−CH_3OH−NH_4OH 65:35:8, concentrated, and then reacted with 4-nitrophenyl caprylate. The product was purified by column chromatography on silica gel (elution with $CHCl_3$−MeOH 50:1) to give **3c** as a white solid: mp 97−98 °C; $[\alpha]^{25}_D$ +19.89° (*c* 2.0, $CHCl_3$); ^{1}H NMR δ 6.89 (d, 1H, J = 6.7 Hz), 6.85−6.78 (m, 4H), 6.69 (bs, 1H), 4.30 (bs, 1H), 4.06−4.03 (dd, 1H, J = 3.8, 9.6 Hz), 3.86−3.84 (dd, 1H, J = 6.4, 9.6 Hz), 3.75 (s, 3H), 3.70−3.62 (m, 1H), 3.46−3.42 (m, 1H), 2.19 (t, 4H, J = 7.6 Hz), 1.62−1.60 (m, 4H), 1.25 (s, 16H), 0.88 (t, 6H, J = 6.6 Hz); ^{13}C NMR δ 175.11, 174.22, 154.10, 152.31, 115.38, 114.63, 67.85, 55.62, 49.88, 41.33, 36.65, 36.50, 31.64, 29.73, 29.21, 29.16, 28.97, 28.81, 25.75, 25.64, 22.55, 14.00; HRMS (FAB, MH$^+$) m/z calcd for $C_{26}H_{45}N_2O_4$ 449.3379, found 449.3377.

(*S*)-2,3-Dioctanamidopropyl 4-Methoxyphenyl Ether (3c′). The compound was prepared in 66% yield from 3-(4′-methoxyphenyl)-*sn*-glycerol (**2′**) by the procedure described above: mp 99−100 °C; $[\alpha]^{25}_D$ −20.98° (*c* 2.0, $CHCl_3$).

(*R*)-2,3-Dioctadecanamido-1-propanol (4a). To a solution of diamidopropyl ether **3a** (2.19 g, 3.0 mmol) in 300 mL of $CHCl_3$−CH_3OH 1:1 was added CAN (5.48 g, 10.0 mmol), and the mixture was stirred overnight at rt. The reaction mixture was diluted with $CHCl_3$ and was washed with aqueous 10% Na_2SO_3 solution and brine. The product was purified by recrystallization in MeOH twice, followed by column chromatography on silica gel (elution with $CHCl_3$−MeOH 50:1), to give 1.66 g (89%) of diamidopropanol **4a**[12] as a white solid (elution with $CHCl_3$−CH_3OH 50:1): mp 115−117 °C; IR (KBr) 3436, 3295, 1637 cm^{-1}; ^{1}H NMR δ 6.72 (d, 1H, J = 6.1 Hz), 6.30 (bs, 1H), 4.31 (bs, 1H), 4.11−4.18 (m, 1H), 3.42−3.76 (m, 4H), 2.18 (t, 4H, J = 7.3 Hz), 1.63−1.77 (m, 4H), 1.25 (s, 56H), 0.88 (t, 6H, J = 5.9 Hz); ^{13}C NMR δ 175.24, 174.39, 55.69, 49.90, 41.62, 36.73, 36.59, 32.11, 31.94, 31.77, 30.32, 30.08, 29.73, 29.70, 29.68, 29.55, 29.39, 29.33, 29.29, 26.31, 25.80, 25.70, 22.87, 22.71, 14.14; HRMS (FAB, MH$^+$) m/z calcd for $C_{39}H_{79}N_2O_3$ 623.6090, found 623.6109.

(*R*)-2,3-Dihexadecanamido-1-propanol (4b). The compound was prepared in 83% yield from diamidopropyl ether **3b**[12] by the procedure described above: mp 112−114 °C (*rac*-**4b** lit.[2] mp 110−112.5 °C); ^{1}H NMR δ 6.89 (d, 1H, J = 6.2 Hz), 6.71 (bs, 1H), 4.31 (bs, 1H), 4.03−4.09 (m, 1H), 3.43−3.81 (m, 4H), 2.18 (t, 4H, J = 7.3 Hz), 1.63−1.77 (m, 4H), 1.25 (s, 48H), 0.88 (t, 6H, J = 5.9 Hz); ^{13}C NMR δ 175.23, 174.40, 55.69, 49.90, 41.62, 36.73, 36.59, 32.11, 31.94, 31.77, 30.32, 30.08, 29.73, 29.70, 29.68, 29.55, 29.39, 29.33, 29.29, 26.31, 25.80, 25.70, 22.87, 22.71, 14.14; HRMS (FAB, MH$^+$) m/z calcd for $C_{27}H_{71}N_2O_3$ 567.5465, found 567.5455.

(*R*)-2,3-Dioctanamido-1-propanol (4c). The compound was prepared in 73% yield from diamidopropyl ether **3c** by the procedure described above: mp 102−103 °C; $[\alpha]^{25}_D$ +3.06° (*c* 5.0, $CHCl_3$); ^{1}H NMR δ 6.98 (bs, 1H, D_2O exchangeable), 6.76 (d, 1H, J = 6.7 Hz, D_2O exchangeable), 3.87 (bs, 1H), 3.69−3.65 (m, 1H), 3.58−3.47 (m, 2H), 3.30−3.24 (m, 1H), 3.14 (bs, 1H, D_2O exchangeable), 2.23 and 2.19 (t, 4H, J = 7.3 Hz), 1.63−1.77 (m, 4H), 1.25 (s, 16H), 0.88 (t, 6H, J = 5.9 Hz); ^{13}C NMR δ 176.02, 174.28, 61.35, 51.56, 39.57, 36.61, 36.89, 31.68, 29.23, 29.19, 28.99, 25.75, 25.61, 14.07; HRMS (FAB, MH$^+$) m/z calcd for $C_{19}H_{39}N_2O_3$ 343.2961, found 343.2961.

(*S*)-2,3-Dioctanamido-1-propanol (4c′). The compound was prepared in 70% yield from diamido ether **3c′** by the procedure described above: mp 101−102 °C; $[\alpha]^{25}_D$ −2.63° (*c* 5.0, $CHCl_3$).

(*R*)-1,2-Bis(stearoylamino)-1,2-deoxyphosphatidylcholine (1a). To a solution of diamidopropanol **4a** (623 mg, 1.0 mmol) and *N*-ethyl-*N*,*N*-diisopropylamine (0.50 mL, 3.59 mmol) in 50 mL of CH_2Cl_2 was added ethylene chlorophosphite (0.6 mL, 3.44 mmol) at 0 °C. After the mixture was stirred for 24 h, bromine (6.72 mmol of a stock solution in CCl_4) was added at 0 °C. After 10 min, the solvent was removed under reduced pressure. The residue was dissolved in 39 mL of CH_3CN/2-PrOH/$CHCl_3$ (5:5:3), and 21 mL of 45% aqueous trimethylamine was added at rt. After 24 h, the solvents were removed, the residue was dissolved in THF−H_2O 9:1, and the solution was

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(12) The solubility of the compounds in $CHCl_3$ was poor; therefore, the optical rotations could not be measured.

passed through a TMD-8 ion exchange column. The fractions containing the product were pooled and concentrated, giving crude diamidophospholipid **1a**. The product was purified by column chromatography on silica gel (elution with $CHCl_3-CH_3OH-H_2O$ 65:25:4). Removal of silica gel by filtration through a Metricel filter followed by lyophilization from C_6H_6 provided 575 mg (73%) of pure 1,2-diamido-1,2-deoxyphosphatidylcholine **1a** as a white solid; IR (KBr) 3436, 1637, 1243 cm^{-1}; $[\alpha]^{25}_D$ +1.06° (*c* 2.5, $CHCl_3$−MeOH 1:1); ^1H NMR ($CDCl_3-CD_3OD$) δ 7.34 (bs), 6.85 (d, J = 6.8 Hz), 4.15−3.25 (m, 9H), 3.24 (s, 9H), 2.28−2.15 (m, 4H), 1.63−1.77 (m, 4H), 1.25 (s, 56H), 0.88 (t, 6H, J = 5.9 Hz); ^{13}C NMR δ 176.08, 175.35, 64.50, 58.17, 54.44, 50.38, 40.26, 36.70, 36.65, 32.16, 29.93, 29.80, 29.66, 29.59, 29.39, 29.33, 29.29, 25.91, 25.70, 22.90, 22.71, 14.19; HRMS (FAB, MH^+) m/z calcd for $C_{44}H_{91}N_3O_6P$ 788.6645, found 788.6696.

(*R*)-1,2-Bis(palmitoylamido)-1,2-deoxyphosphatidylcholine (1b). The compound was prepared in 65% yield from diamidopropanol **4b** by the procedure described above: $[\alpha]^{25}_D$ +2.14° (*c* 2.5, $CHCl_3$−MeOH 1:1); ^1H NMR δ 7.32 (bs), 6.84 (d, J = 6.8 Hz), 4.16−3.20 (m, 9H), 3.25 (s, 9H), 2.29−2.16 (m, 4H), 1.64−1.78 (m, 4H), 1.25 (s, 48H), 0.88 (t, 6H, J = 5.9 Hz); ^{13}C NMR δ 175.49, 174.71, 64.00, 58.95, 53.91, 39.70, 36.14, 35.00, 31.57, 29.43, 29.21, 29.06, 29.00, 25.91, 25.51, 25.44, 22.32, 13.69; HRMS (FAB, MH^+) m/z calcd for $C_{40}H_{83}N_3O_6P$ 732.6020, found 732.6016.

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Supporting Information Available: ^1H- and ^{13}C-NMR spectra (18 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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