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A new approach to the synthesis of lysophosphatidylcholines and related derivatives

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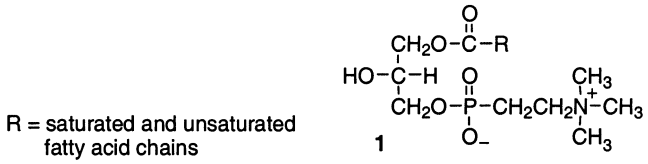
Abstract—A new stereospecific synthesis of lysophosphatidylcholines is reported. The sequence relies on orthogonal protection of hydroxyl groups derived from glyceric acid, using fluorenylmethylcarbonate versus tetrahydropyranyl ether functions, that allow regiospecific introduction of substituents to obtain the target phospholipid compound.

Development of new synthetic methods for the preparation of biologically active phospholipid derivatives is one of the most timely problems in membrane-chemistry today.[1] The compounds are required for structural and dynamic studies of biomembranes[2] and membrane-bound enzymes[3] to establish structure–activity relationships with respect to phospholipid–phospholipid and phospholipid–protein interactions.[4] Specifically, lysophosphatidylcholine **1** belongs to an important



class of phospholipid compounds that are not only substrates and products of phospholipid metabolizing enzymes,[5] but also have a wide range of physiological roles in their own right.[6–9] Lysophospholipids have long been known to function as immunomodulators,[7] and have recently been recognized as highly potent extracellular regulators of cell growth, differentiation, and related activities through G-protein coupled receptors.[8] Elucidation of the mechanistic details involved in the enzymological, cell-biological and membrane-biophysical activities of lysophosphatidylcholines remains to be accomplished, and it greatly depends on availability of efficient synthetic methods for preparation of structurally variable lysophospholipid derivatives.

To date preparation of lysophospholipids has mainly relied on semisynthesis,[10] which imposes significant limitations on the scope and the scale of the compounds that can be prepared. We now describe a new scheme for the synthesis of lysophosphatidylcholines **1**, providing a general method that should be applicable to the preparation of saturated as well as unsaturated compounds, including functionalized derivatives with spectroscopically active reporter groups as structural and mechanistic probes for biophysical and cell-biological studies.

Reduction of 2,3-*O*-isopropylidene-L-methyl glycerate **2** with $LiBH_4$ in ether yielded the corresponding alcohol (95%) which was acylated with palmitic acid/DCC in the presence of catalytic amount of 4-(dimethylamino)pyridine (DMAP) in chloroform at rt overnight. The resulting ester **3** was purified by silica gel chromatography (hexane–ethyl acetate 2:3) and isolated in 95% yield. Acid-catalyzed deprotection of **3** with 0.4N HCl in 90% aq. dioxane at rt for 2 h, followed by freeze-drying and chromatography yielded pure 1-palmitoyl-*sn*-glycerol **4** (84%).

Regiospecific monoacylation of compound **4** was accomplished using twofold molar excess of diol **4** in reaction with FMOC-chloroformate in the presence of 1 equiv. DMAP in methylene chloride at −10°C for 30 min. The product **5** was chromatographed on silica gel (hexane–ethyl acetate 1:3) to afford analytically pure FMOC-carbonate **5** in 58% yield. Absence of the ^1H NMR signal in the δ 5.00–5.09 region clearly indicated that the secondary hydroxyl group of the compound **5**

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remained intact in the isolated product.[11] Tetrahydropyranylation of the *sn*-2-hydroxyl group with pyridinium *p*-toluenesulfonate catalysis gave compound **6**, isolated in 86% yield as a colorless oil. Selective cleavage of the FMOC-carbonate function was achieved using 10% piperidine solution in CH_2Cl_2 at rt for 1 h. Compound **7** was isolated by chromatography, followed by freeze-drying from benzene as a hygroscopic white solid (87%).

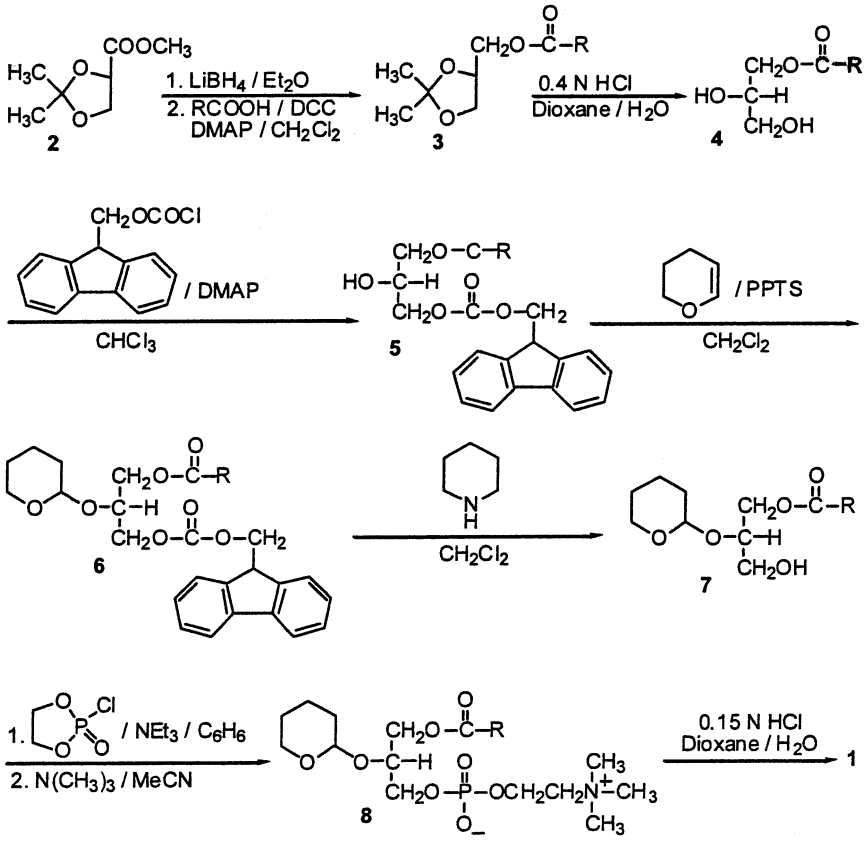
Phosphorylation of the functionalized glycerol **7** at the *sn*-3 position was carried out with 2-chloro-2-oxo-1,3,2-dioxaphospholane/triethylamine in dry benzene.[1b] The phosphorylated intermediate was treated with anhydrous trimethylamine in acetonitrile at 65°C (in a pressure bottle) for 24 h to afford the crude phospholipid **8** that separated from the reaction mixture on cooling. The product **8** was purified by silica gel chromatography (chloroform–methanol–water 65:25:4) and obtained in 61% yield.

Compound **8** was subjected to acid hydrolysis in 0.15N HCl in dioxane–water (1:1), freeze-dried, and chromatographed on Sephadex LH-20 using chloroform–methanol (1:1) to give the target compound 1-palmitoyl-*sn*-glycerophosphocholine **1′** in 98% yield. Evidence that no acid-catalyzed acyl migration occurred during deprotection step came from both the ^1H NMR and ^{31}P NMR spectra of the product **1′**. Specifically, the proton NMR spectrum of compound **1′** shows base-line absorption in the δ 5.00–5.09 range,[11] and the phosphorus NMR shows a single peak at δ 0.12 while no second peak is apparent in the spectrum.[5]

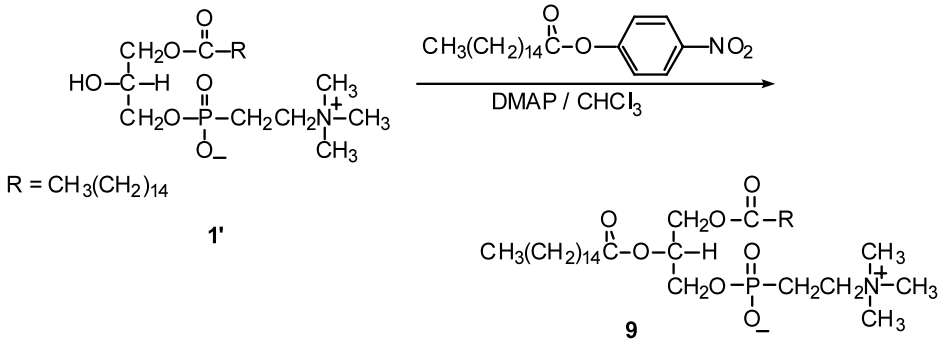
Since lysophosphatidylcholines also serve as synthetic precursors of diacyl glycerophosphocholines we used the product **1′** that became available from Scheme 1 to prepare the corresponding phosphatidylcholine via DMAP-catalyzed acyl transfer reaction from a *p*-nitrophenyl ester to the *sn*-2-hydroxyl group of compound **1′** (Eq. (1)).

Thus, reaction of 1-palmitoyl-*sn*-glycerophosphocholine **1′** with fourfold excess of *p*-nitrophenyl ester/DMAP in anhydrous $CHCl_3$, sonicated at 40°C for 48 h, afforded the corresponding diacyl compound **9** as a single phospholipid. The product **9** was purified by first passing it through a Bio-Rad AG 50-X (H^+) cation exchange resin (to remove the catalyst), followed by silica gel chromatography (chloroform–methanol–water 65:25:4) to give pure **9** in 62% yield.

We believe that the active ester/DMAP method for acylation of lysophospholipids provides an improved alternative to the traditional acylation methods used to introduce the *sn*-2-substituent to obtain structurally well-defined phosphatidylcholines. Specifically, in contrast to the widely used fatty acid/DCC/DMAP or fatty acid anhydride/DMAP methods,[10] the use of an active ester acyl donor generates *p*-nitrophenolate as byproduct, which is several orders of magnitude less basic than the corresponding carboxylate ion formed in the reaction mixture.[12] Since base-catalyzed acyl migration and racemization have been reported to be significant concerns in the reaction,[10] a weaker base present in the reaction mixture may improve both regio- and stereospecificity in obtaining the products.



Scheme 1.



(1)

In conclusion, the synthesis here reported provides a rapid and efficient method for preparation of lysophosphatidylcholines and their related diacyl phospholipid derivatives. The strength of the synthesis is in its flexibility with respect to the substituents that can be introduced,[13] and its applicability to the development of new phospholipid analogues with desired target structures for biological and physicochemical studies.[14]

Acknowledgements

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13. We have obtained preliminary evidence that the same series of reactions can also be carried out using a series of substituted fatty acid derivatives. Along these lines, carboxylic acids functionalized at the chain terminal position with fluorescent reporter groups such as coumarin-343, and metal binding tetraazacyclododecyl groups have been incorporated into the synthetic phospholipid compounds.
14. All new compounds were characterized by IR, ^{1}H, ^{13}C NMR, HR MS and elemental analysis. Selected data: **5**: IR ($CHCl_3$): 2928, 2856, 1748, 1732 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 0.89 (br t, 3H, $J=6.7$ Hz), 1.27 (br s, 24H), 1.61–1.65 (m, 2H,), 2.33–2.40 (t, 2H, $J=7.7$ Hz), 4.08–4.18 (m, 2H), 4.19–4.31 (m, 4H), 4.47 (dd, 2H, $J=7.6$, 0.9 Hz), 7.29–7.47 (m, 4H), 7.62 (m, 2H), 7.79 (m, 2H); ^{13}C NMR ($CDCl_3$) δ: 14.08, 22.66, 24.86, 29.10, 29.22, 29.33, 29.43, 29.57, 29.62, 29.65, 31.89, 34.07, 46.70, 64.76, 68.14, 68.53, 70.12, 120.06, 125.07, 127.17, 127.91, 141.29, 143.20, 155.17, 173.89; R_f (EtOAc/hexane 1:3) = 0.35; Anal. calcd for $C_{34}H_{48}O_6$: C 73.88, H 8.75. Found: C 74.18, H 8.66; $[\alpha]_D^{25}$ −3.01 (*c* 1.03, $CHCl_3$:MeOH 4:1). **8**: IR ($CHCl_3$): 3386, 2926, 2855, 1732 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 0.78 (br t, 3H, $J=6.8$ Hz), 1.15 (br s, 24H), 1.27–1.30 (m, 4H), 1.41–1.44 (m, 4H), 2.22 (dt, 2H, $J=7.5$, 2.6 Hz), 3.14 (s, 9H), 3.55 (m, 2H), 3.80–3.95 (m, 4H), 4.12–4.38 (m, 4H), 4.62 (m, 1H), 4.74 (m, 1H); ^{13}C NMR ($CDCl_3$) δ: 13.88, 19.85, 22.64, 24.78, 25.14, 28.98, 29.18, 29.22, 29.32, 29.41, 29.56, 29.51, 30.70, 31.75, 34.04, 48.32, 62.12, 63.62, 68.20, 69.40, 67.41, 64.79, 98.11 174.15; R_f ($CHCl_3$/MeOH/H_2O 65:25:4) = 0.29. FAB MS calcd. for MH^+ $C_{29}H_{58}NO_8P$ 580.3986, found 580.3970. **1'**: R_f ($CHCl_3$/MeOH/H_2O 65:25:4) = 0.13; ^{31}P NMR (D_2O) δ: single peak at 0.12, the external standard pyrophosphate peak is at −5.46 ppm.[5] **9**: $[\alpha]_D^{25}$ +6.25 (*c* 1.02, $CHCl_3$/MeOH 4:1), standard (Avanti): $[\alpha]_D^{25}$ +6.03 (*c* 1.05, $CHCl_3$:MeOH 4:1); complete hydrolysis by bee-venom phospholipase A_2.[15]
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