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A new approach to the synthesis of lysophospholipids: preparation of lysophosphatidic acid and lysophosphatidylcholine from *p*-nitrophenyl glycerate

Renato Rosseto, Niloufar Bibak and Joseph Hajdu*

Department of Chemistry and Biochemistry, California State University, Northridge, CA 91330-8262, USA

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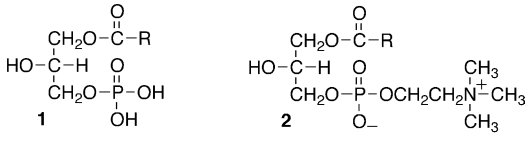
Abstract—A new stereospecific synthesis of lysophosphatidic acid and lysophosphatidylcholine is reported. The sequence relies on *p*-nitrophenyl-D-glycerate as a chiral synthon, including chemoselective reduction of the active ester function without affecting other carboxylic ester groups present in the molecule.

Lysophospholipids have recently become the focus of special attention since it was discovered that in addition to their role in phospholipid metabolism they function as second messengers, exhibiting a broad range of biological activities in their own right.[1–3] Lysophosphatidic acid (**1**), released from activated cells such as platelets, fibroblasts, and leukocytes, stimulates platelet aggregation,[4] promotes smooth muscle contraction,[5] contributes to modulation of blood pressure,[6] and induces cancer cell invasion.[7] Lysophosphatidylcholine (**2**) has been shown to exhibit regulatory activity of signaling enzymes, including activation of p38, AP-1, and JNK kinases[8,9] as well as adenylyl cyclase.[10] It inhibits platelet aggregation,[11,12] and promotes migration of lymphocytes toward the site of inflammatory tissue by inducing secretion of chemotactic agents from macrophages.[13]



R = saturated and unsaturated fatty acid chains

Despite their well-recognized biological importance, elucidation of the mechanistic details involved in the enzymological, cell-biological, and membrane-biophysical activities of lysophospholipids remains to be accomplished, and it depends on availability of efficient synthetic methods for preparation of structurally variable lysophospholipid derivatives.

To date relatively few synthetic methods have been developed for the preparation of lysophospholipids, mainly due to difficulties associated with intramolecular acyl group migration that results in a mixture of 1-acyl and 2-acyl glycerophosphate derivatives.[14,15] Recently we have shown that such acyl migration can be prevented by strategically chosen orthogonal protection of the neighboring hydroxyl groups of the synthetic intermediates.[15] In the present communication we report a new synthesis relying on *p*-nitrophenyl glycerate for the preparation of lysophosphatidic acid and lysophosphatidylcholine. The sequence is based on chemoselective manipulation of the active ester function that provides a rapid and efficient way for elaboration of the desired substituted glycerol skeleton, requiring minimal use of protecting groups for construction of the glycerophospholipid target.

The synthesis starts with preparation of the *p*-nitrophenyl ester of glyceric acid (Scheme 1). Commercially available 2,3-*O*-isopropylidene-D-glyceric acid methyl ester (**3**) was converted to the corresponding carboxylic acid by base catalyzed hydrolysis with NaOH in aqueous dioxane, followed by DCC promoted condensation

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* Corrresponding author. Tel.: +1 818 677 3377; fax: +1 818 677 2912; e-mail: joseph.hajdu@csun.edu

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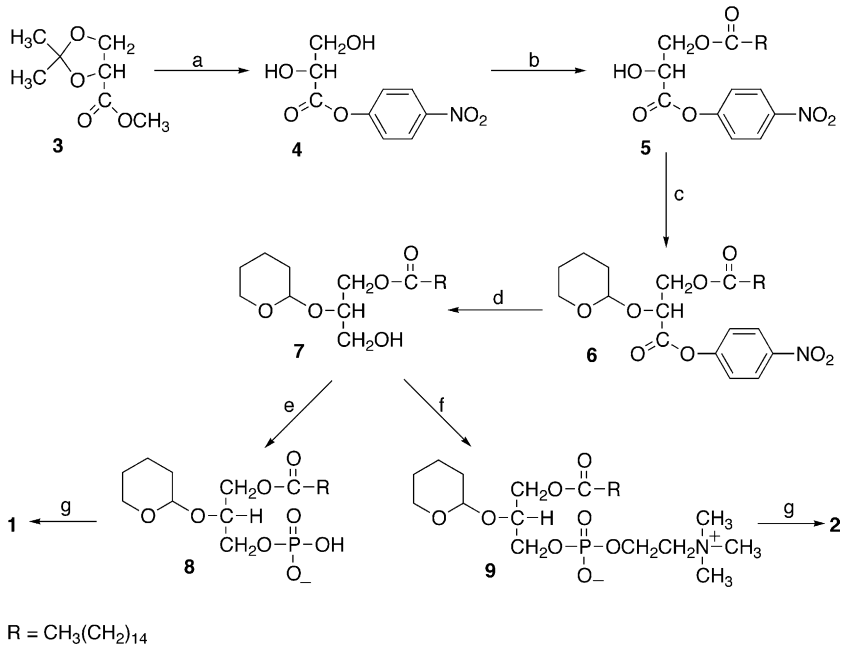

Scheme 1. Reagents and conditions: (a) (i) 1.2 M NaOH, dioxane–water (4:1), 0 °C, rt, 45 min; (ii) Dowex 50-X8, H^+, dioxane–water (10:1), rt, 15 min; (iii) *p*-$NO_2C_6H_4OH$, DCC, CH_2Cl_2; (iv) 0.3 N HCl, dioxane, rt, 4 h; (b) palmitoyl chloride, MeCN–benzene (1:1), rt, 72 h; (c) DHP, PPTS, $CHCl_3$; (d) $NaBH_4$, glyme; (e) (i) $iPr_2NP(OCH_2CH_2CN)_2$, tetrazole, $CHCl_3$–MeCN, (3:1); (ii) 30% aq H_2O_2–CH_2Cl_2; (iii) 0.2 M DBU, toluene, 110 °C, 16 h; (f) (i) ethylene chlorophosphate, Et_3N, benzene; (ii) $(CH_3)_3N$, MeCN, 65 °C; (g) 0.15 N HCl, dioxane–water (1:1).

with *p*-nitrophenol, and subsequent acidolytic cleavage of the isopropylidene function to give the active ester of D-glyceric acid (**4**) in an overall yield of 66%. Regiospecific monoacylation of compound **4** at the primary alcohol function was accomplished using two-fold molar excess of palmitoyl chloride in a mixture of MeCN/benzene (1:1) at room temperature for 72 h. The solution was diluted with glacial acetic acid, freeze-dried, and the product **4** was chromatographed on a Sephadex LH-20 column using dichloromethane–ethyl acetate (1:1) to give the pure *sn*-1-palmitoyl ester **5** as a white powder in 62% yield. Absence of the ^1H NMR signal in the δ 5.00–5.09 region clearly indicated that the secondary hydroxyl group remained unaffected in the course of the reaction.[15,16]

Tetrahydropyranylation at the *sn*-2-glycerol position was carried out with 1.2 equiv 3,4-dihydropyran in $CHCl_3$, in the presence of pyridinium *p*-toluenesulfonate as catalyst, at rt for 1 h. Compound **6** was isolated by extraction from a mixture of benzene–water, then freeze-dried from benzene yielding an analytically pure colorless oily product (92%). Selective reduction of the *sn*-3-*p*-nitrophenyl ester function of **6** was achieved with excess $NaBH_4$ in 1,2-dimethoxyethane[17] at rt for 1 h. The resulting mixture was passed through a short plug of silica gel, eluted first with $CHCl_3$ followed by EtOAc to give the crude 1-palmitoyl-2-tetrahydropyranyl-*sn*-glycerol **7**. This product was further treated with a mixture of benzene–water, then freeze-dried from benzene to afford the analytically pure alcohol **7** in 62% yield as a white powder. This glycerol derivative **7** turned out to be a most useful intermediate for the synthesis of lysophospholipids,[15] allowing incorporation of the desired headgroup at the incipient *sn*-3-position. We applied it for the synthesis of **1** and **2** using readily available phosphorylation methods for elaboration of the phosphomonoester and phosphodiester functions, respectively.

For the preparation of lysophosphatidic acid, compound **7** was phosphorylated by treatment with bis(β-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite[18] in the presence of tetrazole in MeCN–$CHCl_3$ (1:3), at rt for 48 h (58% yield), followed by oxidation of the phosphite intermediate in a biphasic mixture of 30% aq H_2O_2/dichloromethane (90%), and subsequent base catalyzed elimination of the cyanoethyl groups with 2.5 equiv DBU in refluxing toluene overnight gave the phosphomonoester **8** in 70% yield. Finally, acid catalyzed cleavage of the *sn*-2-tetrahydropyranyl group was carried out in 0.15 N HCl in dioxane–water (1:1, 86% yield). As has been shown previously, under these conditions no acyl migration from the primary to the secondary alcohol position was detected. Indeed, the ^1H NMR of product **1** shows baseline absorption in the δ 5.00-5.09 range.[16]

For the synthesis of the target lysophosphatidylcholine, compound **7** was allowed to react with 2-chloro-2-oxo-1,3,2-dioxaphospholane/triethylamine in anhydrous benzene,[19] followed by treatment of the phosphorylated intermediate with trimethylamine in acetonitrile at 65 °C (in a pressure bottle) for 24 h. The resulting phosphocholine **9** was then chromatographed on silica gel (chloroform–methanol–water 65:25:4, 62% yield), and subjected to acid hydrolysis to remove the *sn*-2-tetrahydropyranyl protecting group under the same conditions as used for deprotection of the secondary alcohol

function of lysophosphatidic acid to give the lysophosphatidylcholine **2** isolated in 98% yield.

In summary, the synthesis presented here provides a rapid and efficient method for the preparation of lysophosphatidic acids and lysophosphatidylcholines, and it should be applicable to the development of a series of new synthetic lysophospholipid derivatives with the desired target structures for biological and physicochemical studies. In addition, elaboration of the alcohol function by selective sodium borohydride reduction of the *p*-nitrophenyl ester function, in the presence of other carboxylic ester groups, should provide a useful new strategy not only for the synthesis of glycerol derivatives, but also in carbohydrate chemistry, and in the synthesis of natural products where selective manipulation of multiple hydroxyl groups is required. Work toward application of the method for preparation of functionalized lysophospholipids is currently underway in our laboratory.[20]

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20. Selected data: **5**: IR: (cm^{-1}) 3300, 1762, 1714, 1529, 1207, 1126; ^1H NMR ($CDCl_3$, 200 MHz) δ 0.87 (t, 3H, J = 6 Hz), 1.24 (s br, 24H), 1.58 (m br, 2H), 2.36 (t, 2H, J = 7.4 Hz), 4.52–4.69 (m, 3H), 7.32 (d, 2H, J = 9 Hz), 8.29 (d, 2H, 9 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.06, 22.63, 24.82, 29.03, 29.18, 29.30, 29.39, 29.54, 29.60, 29.62, 31.87, 33.93, 64.94, 69.81, 122.16, 125.33, 145.74, 154.61, 169.95, 173.64; R_f ($CHCl_3$/CH_3CN 5:1) = 0.65. Anal. Calcd for $C_{25}H_{39}NO_7$: C 64.49, H 8.44, N 3.01. Found: C 64.85, H 8.49, N 2.88. FAB MS calcd for MNa^+ ($C_{25}H_{39}NO_7Na$) 488.2624, found: 488.2611. **7**: IR ($CHCl_3$): 1732 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 0.86 (t, 3H, J = 6.8 Hz), 1.24 (s, 24H), 1.50–1.58 (m, 6H), 1.59–1.61 (m, 2H), 2.26–2.31 (dt, 2H, J = 7.7, 2.7 Hz), 3.58–3.64 (m, 2H), 3.68–3.70, 4.08–4.12 (m, 2H), 3.88–3.94 (m, 2H), 4.52 (m, 1H), 4.74 (m, 1H); ^{13}C NMR ($CDCl_3$) δ: 14.02, 19.00, 22.58, 24.86, 25.26, 29.08, 29.22, 29.30, 29.42, 29.58, 29.60, 29.62, 30.44, 31.70, 34.17, 58.62, 62.13, 64.19, 69.57, 97.85, 173.41; R_f (*n*-hexane/EtOAc, 3:1) = 0.37; FAB MS calcd for MNH_4^+ ($C_{24}H_{50}NO_5$) 432.3689, found: 432.3698. Anal. Calcd for $C_{24}H_{46}O_5$: C 69.52, H 11.18. Found: C, 69.66, H 11.38. **8**: IR ($CHCl_3$): 1734 cm^{-1} ; ^1H NMR ($CDCl_3$) δ: 0.86 (t, 3H, J = 6.8 Hz), 1.24 (s, 24H), 1.42–1.50 (m, 6H), 1.52–1.56 (m, 2H), 2.28–2.32 (m, 2H), 3.50–3.54 (m, 2H), 3.72 (m, 1H), 3.82–3.40 (m, 2H), 4.00–4.08 (m, 2H), 4.71 (m, 1H); ^{13}C NMR ($CDCl_3$) δ: 14.02, 20.00, 22.61, 24.88, 29.18, 29.30, 29.50, 29.64, 30.85, 31.86, 34.17, 61.98, 66.54, 67.56, 72.62, 98.50, 173.82. FAB MS calcd for MH^+ ($C_{24}H_{46}O_8P^-$) 493.2930, found: 493.2913. **9**: FAB MS calcd for MH^+ ($C_{29}H_{58}NO_8P$) 580.3986, found 580.3970. **1**: FAB MS calcd for M^-($C_{19}H_{38}O_7P^-$) 409.2355, found: 409.2337.