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Cost-effective and Large-scale synthesis of 16:0 Lysophosphatidic Acid

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Abstract

Lysophosphatidic acid (LPA) is a bioactive compound that has gained attention due to its role in neoplastic diseases. Popularity of the compound has necessitated the use of large quantities of the phospholipid for *in vivo* and *in vitro* testing but methods for generating LPA require the use costly procedures, namely phosphoramidite coupling reagents. Additionally there has been no reported large-scale synthesis of LPA. In the present study we report the cost-effective and large-scale synthesis of 16:0 LPA.

Lysophosphatidic acid (LPA) (Figure 1) is a naturally occurring lysophospholipid implicated in a number of biological processes including cell proliferation and cancer progression¹. Soluble enzyme Autotaxin (ATX) catalyzes the hydrolysis of lysophosphatidylcholine (LPC) to yield lysophosphatidic acid (LPA) which signals cells via a set of five G-protein coupled receptors (GPCRs). LPA receptor stimulation is ultimately responsible for the downstream biological effects of LPA. As a result, LPA and its analogues have become exceedingly useful in probing the biology of neoplastic diseases.

Many syntheses of LPA and its analogues have been reported^{ii,iii,iv,v,vi}. These routes have not been attempted on a large scale and typically employ the use of expensive phosphoramidite reagents to install the phosphate head group. The present method provides a route to LPA from cheap, commercially available starting materials with large-scale yields comparable to previous syntheses.

Chlorophosphates provide an economical alternative to phosphoramidites, especially on large scale (**2**) (Scheme 1). DOWEX-50 acidic resin is utilized to deprotect the isopropylidene group (**3**). The acyl tail is regioselectively attached through an adapted method^{vii,viii} by the addition of pamitoyl chloride, 2,4,6-collidine to the 1,2 diol using cryogenic conditions (**4**). Subsequent TMSBr deprotection of the methyl esters yielded (**5**) in high yield overall.

Because of the widespread availability of fatty acid derived acyl chloride reagents this methodology lends itself to diversification of tail groups in medicinal chemistry applications. Using this procedure from the racemic glycerol, each LPA isomer can be made from each of the commercially available isopropylideneglycerols.

Experimental

All nonaqueous reactions were carried out in oven or flame-dried glassware under an argon or nitrogen atmosphere with dry solvents and magnetic stirring. The argon and nitrogen

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were dried by passing through a tube of Drierite. Anhydrous diethyl ether (Et₂O), toluene, dichloromethane (CH₂Cl₂), methanol (MeOH), tetrahydrofuran (THF), and N,N-dimethylformamide (DMF) were purchased from Aldrich or VMR Chemicals and used as received. THF was dried over activated molecular sieves (4 Å) prior to use. All other reagents were purchased from Acros chemicals and Aldrich chemicals. Except as indicated otherwise, reactions were monitored by thin layer chromatography (TLC) using 0.25 mm Whatman precoated silica gel plates. Flash chromatography was performed with the indicated solvents and Dynamic Adsorbents silica gel (particle size 0.023-0.040 mm). Proton (1H) NMR spectra were recorded on a Varian UnityInova 500/51 or Varian UnityInova 300/54 at 300 K. Chemical shifts are reported in ppm (δ) values relative to the solvent as follows: CDCl₃ (δ 7.24 for proton NMR), DMSO-*d*₆ (δ 2.50 for proton NMR), CD₃OD (δ 3.31 for proton NMR). All high-resolution mass spectrometry was carried out by the High-Resolution Mass Spectrometry Facility in the School of Chemical Sciences at the University of Illinois Urbana-Champaign (Urbana, IL).

(R)-(2,2-dimethyl-1,3-dioxolan-4-yl)methyl dimethyl phosphate (2)

A flame dried, 250 mL, single-necked, round-bottomed flask equipped with a egg-shaped magnetic stir bar (25 mm × 15 mm), capped with a rubber septum and connected to a nitrogen line is charged with 114 mL (0.5 M) of anhydrous DCM and 7.03 mL (56.7 mmol, 1 equiv.) of (*S*)-(+)-1,2-Isopropylidenedeglycerol. The reaction is cooled to 0 °C via ice water bath and vigorously stirred. To the reaction is added 5.8 g (85.1 mmol, 1.5 equiv.) of solid imidazole in one portion. After addition of imidazole, 9.2 mL of dimethyl chlorophosphate is added in 1 mL portions by syringe over a period of 10 minutes. The reaction is brought immediately to room temperature and the reaction is allowed to stir vigorously for 3.5 hours. Reaction is monitored by TLC analysis on silica gel with 100% EtOAc as eluent and visualization with Henessian's stain. The starting alcohol has R_f = 0.78 (blue) and the phosphate product has R_f = 0.51 (blue). After completion by TLC the reaction is diluted with 200 mL of DCM in one portion at 25 °C and transferred to a 500 mL separatory funnel. The organic layer is washed with brine (3 × 100 mL). The combined aqueous layers are then extracted with DCM (2 × 50 mL). The combined organic layers are dried with adding sodium sulfate (75 g) and swirling for 5 minutes at room temperature. Drying agent is then filtered through a 150 mL coarse frit into a 1000 mL round-bottomed flask. The dried organic layers are concentrated on a rotary evaporator (40 °C, 10 mmHg) to yield a crude, clear oil.

The crude oil is charged on a column (18 in. × 2 in.) of 320 g of silica gel and eluted with 700 mL of 100% ethyl acetate. At that point, fraction collection (25-mL fractions) is begun, and elution is continued with 675 mL of 100% ethyl acetate and then 1825 mL of 5% methanol/ethyl acetate (1:19 MeOH:EtOAc). The desired product is obtained in fractions 15-100, which are concentrated by rotary evaporation (40 °C, 10 mmHg) and dried overnight under vacuum (5 × 10⁻³ mmHg) to afford 11.9 – 12.3 g (49.8 – 52.7 mmol, 88 – 93 % yield) of product as a clear viscous oil.

R_f = 0.51 (100% EtOAc); ³¹P NMR (121 MHz, CDCl₃) (Phosphorus NMR signals are referenced to 85% H₃PO₄ (δ = 0.00) using a triphenylphosphate external standard (δ = -16.58)) δ 1.75; ¹H NMR (300 MHz, CDCl₃) δ 4.18 (dt, J = 5.6, 10.9, 1H), 3.91 (apparent tdd, J = 1.4, 6.0, 6.9, 3H), 3.69 (m, 1H), 3.66 (s, 3H), 3.62 (s, 3H), 1.28 (s, 3H), 1.21 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 109.1, 73.8 (J_{CP} = 6.8 Hz), 67.2 (J_{CP} = 5.3 Hz) 65.3, 53.7, 53.6, 26.1, 24.6. HRMS (FAB) m/z calc'd for C₈H₁₈O₆PNa: [M]⁺: 263.0659, found 263.0660. Anal. calcd. for C₈H₁₇O₆P: C, 40.00; H, 7.13; found: C, 39.65; H, 7.17; [α]_D +5.21 (c 1.19, CH₂Cl₂).

(R)-2,3-dihydroxypropyl dimethyl phosphate (3)

A flame dried, 250 mL, single-necked, round-bottomed flask equipped with a egg-shaped magnetic stir bar (25 mm × 15 mm), capped with a rubber septum and connected to a nitrogen line is charged with 53 mL (1.0 M) of methanol and 12.3 g (52.9 mmol, 1 equiv.) of (R)-(2,2-dimethyl-1,3-dioxolan-4-yl)methyl dimethyl phosphate. DOWEX-50W (31.6 g, 79.35 mmol, 1.5 equiv.) is then added to the reaction in one portion. The reaction is allowed to stir at room temperature for 1.5 hours and monitored by TLC analysis on silica gel with 10% MeOH/DCM as eluent and visualization with Henessian's stain. The starting isopropylidene has R_f = 0.66 (blue) and the diol product has R_f = 0.48 (blue). After completion by TLC the reaction is filtered through a 150 mL coarse frit into a 500 mL round-bottomed flask to remove the spent resin. The eluent is concentrated on a rotary evaporator (40 °C, 10 mmHg) to yield a crude, pale yellow oil.

The crude oil is charged on a column (18 in. × 2 in.) of 150 g of silica gel and eluted with 300 mL of 10% MeOH/DCM. At that point, fraction collection (25-mL fractions) is begun, and elution is continued with 1250 mL of 10% MeOH/DCM. The desired product is obtained in fractions 12-47, which are concentrated by rotary evaporation (40 °C, 10 mmHg) and dried overnight under vacuum (5×10^{-3} mmHg) to afford 9.8 – 9.9 g (49.2 – 49.5 mmol, 93 – 94 % yield) of product as a pale yellow viscous oil.

R_f = 0.48 (10% MeOH/DCM); ^{31}P NMR (121 MHz, CDCl_3) δ 2.02; ^1H NMR (300 MHz, DMSO) δ 4.98 (d, J = 5.3, 1H), 4.65 (t, J = 5.6, 1H), 3.95 (m, 1H), 3.80 (m, 1H), 3.67 (s, 3H), 3.63 (s, 3H), 3.34 (m, 3H). ^{13}C NMR (75 MHz, CDCl_3) δ 70.5 (J_{CP} = 6.8 Hz), 68.7 (J_{CP} = 6.0 Hz), 62.6, 54.6, 54.5. HRMS (FAB) m/z calc'd for $\text{C}_5\text{H}_{13}\text{O}_6\text{PNa}$: $[\text{M}]^+$: 223.0345, found 223.0347. Anal. calcd. for $\text{C}_5\text{H}_{13}\text{O}_6\text{P}$: C, 30.01; H, 6.55; found: C, 29.57; H, 6.70; $[\alpha]_{\text{D}} +1.21$ (c 1.26, MeOH).

(R)-3-((dimethoxyphosphoryl)oxy)-2-hydroxypropyl palmitate (4)

A flame dried, 500 mL, single-necked, round-bottomed flask equipped with a egg-shaped magnetic stir bar (25 mm × 15 mm), capped with a rubber septum and connected to a nitrogen line is charged with 247 mL (0.1 M) of DCM and 9.9 g (49.5 mmol, 1 equiv.) of (R)-2,3-dihydroxypropyl dimethyl phosphate. The solution is cooled to -78 °C via dry ice/acetone bath to which 2,4,6-trimethylpyridine (12.9 mL, 99.0 mmol, 2 equiv.) is added dropwise by syringe over a period of 10 minutes. Palmitoyl chloride (Note 14) (15.1 mL, 49.9, 1.05 equiv.) is then added dropwise by syringe to the solution over 15 minutes. The reaction is allowed to stir at -78 °C for exactly 2 hours and then immediately concentrated on a rotary evaporator (40 °C, 10 mmHg). Crude solid is dissolved in 500 mL EtOAc and placed into a 1000 mL separatory funnel. The organic layer is washed 3 × 100 mL 1 N HCl and resulting combined aqueous components are extracted with 2 × 100 mL EtOAc. The combined organics are dried with the addition of 100 g of sodium sulfate and swirling for 5 minutes at room temperature. Drying agent is then filtered through a 150 mL coarse frit into a 1000 mL round-bottomed flask. The dried organic layers are concentrated on a rotary evaporator (40 °C, 10 mmHg) to yield a crude white solid. The solid is taken up in 150 mL chloroform and, ensuring that all crude material is dissolved, 22 g of silica gel is added to form a slurry. Resulting slurry is concentrated on rotary evaporator (25 °C, 10 mmHg) and then placed on vacuum (5×10^{-3} mmHg) for 2 hours.

Silica gel containing crude solid is charged on a column (18 in. × 2 in.) of 320 g of silica gel and eluted with 550 mL of 25% EtOAc/Hexanes (1:3 EtOAc/Hexanes). At that point, fraction collection (25-mL fractions) is begun, and elution is continued with 1650 mL of 25% EtOAc/Hexanes then 2 L of 5% MeOH/EtOAc (1:19 MeOH/EtOAc). The desired product is obtained in fractions 65-128, which are concentrated by rotary evaporation (40 °C, 10

mmHg) and dried overnight under vacuum (5×10^{-3} mmHg) to afford 9.9 – 11.7 g (22.0 – 25.9 mmol, 45 – 52 % yield) of product as a white solid.

$R_f = 0.26$ (25% EtOAc/Hexanes); Visualized with potassium permanganate solution. Product shows as a yellow spot on a purple background; ^{31}P NMR (121 MHz, CDCl_3) δ 2.57; ^1H NMR (300 MHz, CDCl_3) δ 4.11 (apparent ddd, $J = 6.0, 11.2, 15.9$, 5H), 3.80 (s, 3H), 3.76 (s, 3H), 3.09 (s, 1H), 2.32 (t, $J = 7.6$, 2H), 1.59 (apparent dd, $J = 7.1, 14.3$, 2H), 1.23 (s, 24H), 0.86 (t, $J = 6.7$, 3H). ^{13}C NMR (75 MHz, CDCl_3) δ 174.0, 68.9 ($J_{\text{CP}} = 5.3$ Hz), 68.4 ($J_{\text{CP}} = 6.0$ Hz), 64.6, 54.8, 34.4, 32.3, 30.0, 29.9, 29.8, 29.7, 29.5, 25.2, 23.1, 14.4. Anal. calcd. for $\text{C}_{21}\text{H}_{43}\text{O}_7\text{P}$: C, 57.52; H, 9.88; found: C, 57.31; H, 9.89; $[\alpha]_D -2.3$ (c 1.5, CHCl_3).

(R)-2-hydroxy-3-(phosphonooxy)propyl palmitate (5)

A flame dried, 500 mL, single-necked, round-bottomed flask equipped with a egg-shaped magnetic stir bar (25 mm $\times$ 15 mm), capped with a rubber septum and connected to a nitrogen line is charged with 259 mL (0.1 M) of DCM and 11.7 g (25.9 mmol, 1 equiv.) (R)-3-((dimethoxyphosphoryl)oxy)-2-hydroxypropyl palmitate. The solution is cooled to 0 °C via ice-water bath to which bromotrimethylsilane (27.9 mL, 259.0 mmol, 10 equiv.) is added dropwise by syringe over 10 minutes. The reaction is immediately brought to room temperature and is allowed to react for 4 hours. The reaction is then concentrated on a rotary evaporator (40 °C, 10 mmHg) to a crude oil. The crude oil is dissolved in 150 mL of methanol and allowed to mix for 1 hour. The methanol is concentrated on a rotary evaporator (40 °C, 10 mmHg) to a crude oil.

The crude oil is dissolved in hot acetonitrile (80 °C) and immediately placed in a cold room at 4 °C. After allowing crystallization for 4 hours white crystals form and are filtered through a 150 mL fine frit. The eluent is concentrated to oil (40 °C, 10 mmHg), dissolved in 100 mL of hot acetonitrile, placed in 4 °C cold room and a second batch of crystals are harvested. The combined product is dried overnight under vacuum (5×10^{-3} mmHg) to afford 9.2 – 9.4 g (21.7 – 22.7 mmol, 84-89%) of final product as a white powder.

$R_f = 0.13$ (65:25:4 CHCl_3 :MeOH:H₂O; 69%, 27%, 4% CHCl_3 :MeOH:H₂O). Product stains blue on a yellow background with Seebach's stain. ^{31}P NMR (121 MHz, DMSO) δ -0.21; ^1H NMR (300 MHz, DMSO) δ 5.08 (m, 1H), 3.95 (dd, $J = 4.0, 8.3$, 2H), 3.63 (ddd, $J = 5.4, 11.0, 17.3$, 2H), 2.30 (t, $J = 7.3$, 2H), 1.52 (s, 2H), 1.21 (s, 24H), 0.83 (t, $J = 6.6$, 3H). ^{13}C NMR (75 MHz, DMSO) δ 172.5, 71.3 ($J_{\text{CP}} = 7.5$ Hz), 65.0, 34.1, 32.1, 29.9, 29.5, 29.2, 25.1, 22.8, 14.4; HRMS (ESI+) m/z calc'd for $\text{C}_{19}\text{H}_{39}\text{O}_7\text{P}$: $[\text{M}+\text{Na}]^+$: 433.2331, found 433.2336; $[\alpha]_D +4.9$ (c 0.54, DMSO). Product is stable indefinitely when stored under nitrogen and kept at 0 °C.

Acknowledgments

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Figure 1.
16:0 Lysophosphatidic Acid (16:0 LPA)

Scheme 1.
Synthesis of Lysophosphatidic Acid **5**.