Supplemental information to:

The Phospholipase A₁ Activity of Lysophospholipase A-I Links Platelet Activation to LPA Production During Blood Coagulation

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Synthesis of 2-Oleyl-sn-glycero-3-phosphocholine

Reagents used in the synthesis of 2-Oleyl-sn-glycero-3-phosphocholine were purchased from Acros (Morris Plains, NJ), Sigma-Aldrich, AK Scientific, Inc. (Union City, CA), or Alfa Aesar Inc. (Ward Hill, MA) and used as received. Dry solvents were obtained from a Pure-Solv solvent delivery system purchased from Innovative Technology, Inc. (Newburyport, MA). 2-Oleyl-sn-glycero-3-phosphocholine (1) was synthesized as shown in Scheme 1. The synthetic scheme began with 3-O-(4-Methoxybenzyl)-sn-glycerol (2), which contains the appropriate stereochemistry for the subsequent target product and was synthesized as previously described (1, 2). First, the primary alcohol of diol 2 was selectively protected through reaction with tert-butyldiphenyl chlorosilane to produce compound 3. Next, ether bond formation using oleyl triflate was performed to afford fully protected glycerol 4, followed by deprotection of the p-methoxybenzyl (PMB) group to provide the free alcohol of 5. Phosphoramidite chemistry was then used to install the choline phosphodiester of 7, at which time it was found that the cyanoethyl protecting group on the phosphate was deprotected in situ. When followed by mass spectrometry, this was found to happen during the process of purifying compound 7. The yield for the 2-step production of 7 is somewhat low, likely due to the challenges associated with forming mixed phosphodiesters and the purification of the lyso-lipid product. Finally, the removal of the silyl protecting group of 7 yielded LPC analog 1. It is worth mentioning that a similar approach was also pursued that involved a glycerol precursor with opposite stereochemistry, initial removal of the silyl group, and deprotection of the PMB in the final step. However, this alternative route led to significant challenges in purifying the final product, and was thus abandoned in
favor of the route described above. LPC analog 1 has previously been reported in enantiomerically pure (3) as well as racemic form (4) using different synthetic routes.

3-O-(4-Methoxybenzyl)-1-O-(tert-Butyldiphenylsilyl)-sn-glycerol (3) Diol 2 was synthesized from S-glycerol acetonide (purchased from AK Scientific, Inc.) according to a known procedure (1, 2). Diol 2 (0.430g, 2.026mmol) was then dissolved in dry N,N-dimethylformamide (20mL), to which was added tert-butyldiphenyl chlorosilane (0.525mL, 2.026mmol), followed by imidazole (0.359g, 5.270mmol). The reaction mixture was next allowed to stir at room temperature overnight. The solvent was then concentrated under reduced pressure and the resulting residue was dissolved in chloroform (100mL) and washed with water (2 x 50mL). The organic layer was then dried with magnesium sulfate and the solvent evaporated to yield the crude product. Purification via column chromatography with silica gel and gradient elution with 15–35% ethyl acetate/hexanes yielded 3 as a colorless oil (0.73g, 80%). The product matched previous characterizations (5). \(^{1}\)H NMR (300 MHz, CDCl\(_3\)): \(\delta 7.62–7.65\) (d, \(J=9.0\) Hz, 4H), 7.34–7.42 (m, 6H), 7.20-7.25 (m, 2H), 6.84-6.87 (d, \(J=9.0\) Hz, 2H), 4.45 (s, 2H), 3.88–3.92 (m, 1H), 3.79 (s, 3H), 3.69–3.71 (d, \(J=6.0\) Hz, 2H), 3.50-3.54 (m, 2H), 2.47 (d, \(J=6.0\) Hz, 1H), 1.06 (s, 9H); MALDI-HRMS [M+Na]\(^{+}\) calcd: 473.2119, found: 473.2093.

1-O-(tert-Butyldiphenylsilyl)-2-oleyl-3-O-(4-Methoxybenzyl)-sn-glycerol (4) The procedure for synthesis of 4 was modified from similar ether-tail forming reactions (6). Compound 3 (0.660g, 1.465mmol) was dissolved in dry dichloromethane (40mL), to which was added 1,8-Bis(dimethylamino)naphthalene (proton sponge, 1.10g, 5.15mmol) and oleyl triflate (2.900g, 7.240mmol), which was prepared from a known
procedure (7). The solution was next heated to reflux and allowed to stir overnight. The solvent was then removed under reduced pressure to yield the crude product 4, which was purified by column chromatography with silica gel and gradient elution with 5–10% acetone/hexanes to yield 4 as a yellowish oil (0.714 g, 71%). $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 7.66–7.75 (m, 4H), 7.32–7.40 (m, 6H), 7.22–7.30 (m, 2H), 6.83-6.91 (m, 2H), 5.35-5.41 (m, 2H), 4.47-4.52 (m, 2H), 3.79 (s, 3H), 3.38–3.70 (m, 7H), 2.04 (m, 4H), 1.58 (m, 2H), 1.30-1.36 (m, 22H), 1.03-1.06 (m, 9H), 0.90-0.94 (m, 3H); $^{13}$C NMR (100.6 MHz, CDCl$_3$): $\delta$ 159.14, 135.99, 135.64, 133.57, 129.90, 129.48, 129.21, 129.13, 127.66, 127.43, 113.69, 113.59, 79.48, 73.02, 71.38, 69.71, 63.50, 55.16, 32.70, 31.99, 30.18, 29.78, 29.72, 29.58, 29.33, 26.20, 22.76, 19.25, 14.21; MALDI-HRMS [M+Na]$^+$ calcd: 723.4779, found: 723.4759.

$1$-O-(tert-Butyldiphenylsilyl)-2-oleyl-sn-glycerol (5) Compound 4 (0.325g, 0.463mmol) was combined with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ, 0.315g, 1.389mmol) in dichloromethane (10mL) and water (1mL). The reaction mixture was then stirred at rt. After 10h, saturated sodium bicarbonate (100mL) was added to quench the reaction, and the resulting solution was then extracted twice with chloroform (2 x 100mL). The organic layers were then combined and dried with magnesium sulfate and the solvent removed by rotary evaporation to yield the crude product, which was carried on to the next step without further purification.

$1$-O-(tert-Butyldiphenylsilyl)-2-oleoyl-sn-glycero-3-phosphocholine (7) Crude compound 5 was combined with bis-(N,N-diisopropylamino) cyanoethyl phosphine (6, 0.205g, 0.606mmol), and $1H$-tetrazole (0.740mL of a 0.45M solution in acetonitrile, 0.333mmol) in dichloromethane (10mL), and the solution was allowed to stir at rt for 1h. To this
stirred solution, choline tosylate (0.334g, 1.212mmol), and 1H-tetrazole (2.690mL of a 0.45M solution in acetonitrile, 1.212mmol) were added, and the solution was allowed to stir at room temperature for another 12h, after which tert-butylhydroperoxide (0.470mL, 4.848mmol) was added. After 1h, the reaction was quenched by adding 50mL of saturated sodium thiosulfate aqueous solution. Next, the resulting solution was extracted with methanol/methylene chloride (v/v 1:4, 2 x 80mL), and the organic layers were combined and dried with magnesium sulfate. The solvent was then removed under reduced pressure, and the resulting residue was purified by column chromatography with silica gel through gradient elution of 5–30% methanol/dichloromethane to yield 7 as a colorless oil (0.060 g, 17 % yield over 2 steps). 1H NMR (300 MHz, CDCl₃): δ 7.65–7.69 (m, 4H), 7.26–7.38 (m, 6H), 5.32-5.35 (m, 2H), 4.14-4.18 (m, 2H), 3.89-3.91 (m, 2H), 3.49-3.74 (m, 5H), 3.28-3.30 (m, 2H), 3.22 (s, 9H), 1.99-2.01 (m, 2H), 1.47-1.49 (m, 2H), 1.19-1.23 (m, 22H), 1.02 (s, 9H), 0.86 (t, J=6.0 Hz, 3H); 31P NMR: δ −0.59; MALDI-HRMS [M-H]+ calcd: 746.4939, found: 746.4946.

2-Oleyl-sn-glycero-3-phosphocholine (1) Compound 7 (0.060g, 0.080mmol) and tetrabutylammonium fluoride trihydrate (TBAF, 0.126g, 0.40mmol) were dissolved in tetrahydrofuran (10mL), and the reaction was allowed to stir at room temperature overnight, at which point the solvent was removed under reduced pressure. Purification of the resulting residue by column chromatography with 3 g of silica gel and a gradient eluant of 10–50% methanol/chloroform yielded 7, but also contained TBAF as a contaminant. This mixture was then dissolved in water and stirred with Chelex-100 resin, sodium form, for 3h. The solution was then loaded directly onto a C18 reverse phase column and eluted with a gradient of water/methanol mixtures to yield 7 as an off-
white paste (0.025g, 62%). The product matched previous characterizations. (3, 4) $^1$H NMR (300 MHz, CD$_3$OD/CDCl$_3$, v/v 1:2): δ 5.33-5.36 (m, 2H), 4.4-4.28 (m, 2H), 4.14 (m, 1H), 3.96 (t, J=6.0 Hz, 2H), 3.49-3.74 (m, 5H), 3.34-3.36 (m, 2H), 3.21 (s, 9H), 1.99-2.04 (m, 4H), 1.52-1.59 (m, 2H), 1.25-1.32 (m, 22H), 0.86 (t, J=6.0 Hz, 3H); $^{31}$P NMR: δ 4.01; MALDI-HRMS [M-H]$^+$ calcd: 508.3762, found: 508.3768.

Column chromatography was performed using 230–400 mesh silica gel purchased from Sorbent Technologies (Atlanta, GA). NMR spectra were obtained using a Varian Mercury 300 spectrometer. Mass spectra were obtained with a Voyager DE MALDI-TOF spectrometer (PerSeptive Biosystemes, Framingham,MA).

References:


