Results and Discussion

Pl-4-Ps as substrates for SopB

<table>
<thead>
<tr>
<th>IP$_2$ presentation</th>
<th>[IP$_2$] (µM)</th>
<th>ΔOD$_{285}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous solution</td>
<td>10</td>
<td>0.02 (±0.002)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.01 (±0.006)</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.01 (±0.003)</td>
</tr>
<tr>
<td>Micelles</td>
<td>10</td>
<td>0.09 (±0.004)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.09 (±0.012)</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.09 (±0.006)</td>
</tr>
</tbody>
</table>

Supplementary Table 1. Attempted dephosphorylation of IP$_2$ by SopB phosphatase in aqueous and detergent-based solution, measured colorimetrically (malachite green endpoint assay, 10 µg/well, 20 min). No significant reaction was observed.

Experimental

Calculation of $K_M$ and $V_{max}$

Kinetic parameters, $K_M$ and Activity (relative $V_{max}$), expressed as a percentage in Table 1, were calculated using Grafit software (courtesy of Professor Robin Leatherbarrow). An example screen shot of a graph fitted from one set of results for one lipid is shown in Supplementary Graph 1.

Supplementary Graph 1. An example of the fitting of colorimetric data from malachite green endpoint assays of Salmonella phosphatase SopB, with PI-4-P using Michaelis-Menten kinetics. OD = optical density (625 nm), DSPIP = sn-1,2-Distearoylphosphatidylinositol 4-phosphate.

Additional procedures

S-Acetyl mandelic acid, 8a. S-Mandelic acid (5.00 g, 32.9 mmol) was stirred in acetyl chloride (10 mL) for 1 h, during which time the solid dissolved. The volatile components were removed in vacuo and the residual white solid dissolved in water (50 mL) with gentle heating. This was diluted further with water (400 mL) and cooled in an ice-water bath to afford the product as needle-shaped, colourless crystals. Upon standing, a second crop of crystals formed (combined yield 6.06 g, 95%). $[α]_D^{25} +154.0^o$ (c 2.0, acetone, lit. +153.0°); $\delta_H$ (400 MHz, CDCl$_3$) 7.48-7.47 (2H, m), 7.49-7.39 (3H, m), 5.93 (1H, s), 2.19 (3H, s); $\delta_C$ (125 MHz, CDCl$_3$) 173.9, 170.6 (2 × C=O), 133.2 (Ph C), 129.4, 128.9 (2C), 127.7 (CH) (5 × Ph CH), 74.2 (CH), 20.6 (CH$_2$); HRMS (EI+) $m/z$ found [M$^+$] = 194.0581, C$_{10}$H$_{10}$O$_4$ requires 194.0579.

sn-3-O-Benzyl-1,2-O-isopropylidene glycerol. S- (+)-1,2-O-isopropylidene glycerol (1.00 g, 7.57 mmol) was dissolved in DMF (20 mL), cooled in an ice-water bath, and sodium hydride (60% dispersion in mineral oil, 334 mg, 8.32 mmol, 1.05 eq.) added. Once the effervescence had subsided, benzyl chloride (871 μg, 7.57 mmol) was added in one portion. After stirring for 36 h, water (4 mL) and diethyl ether (100 mL) were added. The organic solution was washed with water (2 × 800 mL), dried (MgSO$_4$), and the solvent removed in vacuo to afford the title compound as a pale yellow oil (1.55 g, 100%) requiring no further purification. $R_f$ (EtOAc-pet. spirit, 1:1) 0.80; $\delta_H$ (400 MHz, CDCl$_3$ with 0.01 M triethylamine) 7.38-7.28 (5H, m, 5 × Ph CH), 4.65-4.55 (2H, m, PhCH$_2$O), 4.33 (1H, quin., J 6.1, Gly 2-CH$_2$), 4.11-4.07 (2H, m, Gly 1-CH$_2$), 3.58 (1H, dd, J 9.7, 6.1), 3.50 (1H, dd, J 9.8, 5.6) (Gly 3-CH$_2$), 1.44 (3H, s, CH$_3$), 1.39 (3H, s, CH$_3$); MS (CI+, NH$_4^+$) m/z found [M$^+$] = 222.1, C$_{13}$H$_{21}$O$_5$$^+$ requires 222.1, also observed [M$^+$+NH$_4^+$]$^+$ = 240. These data agree with those reported for this compound$^1$.

3-O-Benzyl-sn-glycerol. sn-3-O-Benzyl-1,2-O-isopropylidene glycerol (2.0 g, 9.0 mmol) was dissolved in DCM (50 mL) to which water (5 mL) and trifluoroacetic acid (10 mL) were added. After stirring vigorously for 16 h, sodium carbonate (10 g, 1.4 eq.) and EtOAc (300 mL) were added. The organic solution was washed with water (2 × 200 mL), dried (MgSO$_4$), and the solvents removed in vacuo. The translucent yellow oil was adsorbed onto silica and fractionated through a column of flash silica using a gradient CH$_2$Cl$_2$-methanol (1.0 → 3.17) to afford the title compound (1.08 g, 66%). $R_f$ (pet. spirit-EtOAc 1:1) 0.20; (CH$_2$Cl$_2$-methanol 9:1) 0.50; $\delta_H$ (400 MHz, CDCl$_3$) 7.41-7.30 (5H, m, 5 × Ph CH), 4.57 (2H, s, PhCH$_2$O), 3.95 (1H, dt, J 5.1, 4.0, Gly 2-CH$_2$), 3.76-3.64 (2H, m, Gly 1-CH$_2$), 3.57 (2H, dd, J 9.8, 5.1, Gly 3-CH$_2$), 2.96 (2H, bs, 2 × OH); HRMS (ESI+) m/z found [M$^+$+H]$^+$ = 182.0942, C$_{15}$H$_{26}$O$_5$ requires 182.0943. These data agree with those reported for this compound$^1$.

3-O-Benzyl-sn-1,2-O-distearoyl glycerol. sn-3-O-Benzyl-glycerol (811 mg, 4.45 mmol) was evaporated from acetonitrile (3 × 3 mL) and dissolved in CH$_2$Cl$_2$ (20 mL). Stearic acid (1.00 g, 9.12 mmol, 2.05 eq.), N-methyl imidazole (3.55 mL, 44.5 mmol, 10.0 eq.), and finally DebcCl (1.59 mL, 11.2 mmol, 2.5 eq.) were added and the mixture stirred for 16 h. After this water (10 mL) was added, the mixture diluted with diethyl ether (300 mL), the organic layer dried over MgSO$_4$, and the solvent removed in vacuo affording the title compound as a white solid (3.00 g, 94%) requiring no further purification. $R_f$ (CH$_2$Cl$_2$) 0.80; $\delta_H$ (400 MHz, CDCl$_3$) 7.40-7.30 (5H, m, 5 × Ph CH), 5.29-5.24 (1H, m, Gly 2-CH$_2$), 4.62-4.54 (2H, d, J 182.0942, C$_{15}$H$_{26}$O$_5$ requires 182.0943. These data agree with those reported for this compound$^1$.

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sn-1,2-O-Distearyloyl glycerol, 19a. sn-3-O-Benzyl-1,2-O-distearyloyl glycerol (3-0 g, 4.19 mmol) was dissolved in reagent grade EtOAc (undried, 40 mL), palladium charcoal (10 mol%, 200 mg) was added and the suspension placed under a nitrogen atmosphere. After evacuating briefly and letting down to nitrogen five times, the final time the flask was opened to hydrogen. After stirring briskly for 48 h, the reaction flask was evacuated and let down to nitrogen three times, the suspension was dried (MgSO₄), filtered through Celite filter aid, washed with liberally with CHCl₃, and the solvent removed in vacuo to give the title compound (2.59 g, 99%) as a low melting point (<35 °C) white solid. Rf (CH₃CN-pet. spirit, 3:1) 0.17; δH (400 MHz, CDCl₃) 5-15-5.90 (1H, m, Gly 1-CH₂), 2.34-2.45 (2H, m, Gly 2-CH₂), 2.92, 3.02 (2H, d, J = 7 Hz, 2 × CH₂COOR), 3.60-3.80 (6H, m, 3 × CH₂COOR), 4.60-4.80 (2H, t, J = 7 Hz, 2 × CH₂OH), 7.32-7.35 (2H, d, 2 × C₆H₅). 

1H NMR (400 MHz, CDCl₃) \(\delta = 2.35-2.40 \times 2 \times C\)H₂COOR, 3.60-3.80 \(\times 6 \times H\), 4.60-4.80 \(\times 2 \times C\)H₂OH, 7.32-7.35 \(\times 2 \times C\)H₅. 

After stirring for 2 min, 0.2 M sodium acetate buffer (5 mL) was added and the mixture was diluted with CH₂Cl₂ (20 mL). The organic layer was washed with water (20 mL), dried (Na₂SO₄), and the solvent removed in vacuo. The pale yellow oil was triturated with pet. spirit-acetic acid (99:1, 10 mL) and the solids discarded. The supernatant was evaporated and the residual colourless oil fractionated through a column of flash silica; the silica was pre-acidified by flushing with EtOAc-EtOH-AcOH (190:20:1, v/v/v), then eluted with a gradient of diethyl ether-pet. spirit (0:1→1:3) to afford the title compound as a colourless oil (547 mg, 75%). Rf (EtOAc-pet. spirit, 1:4) 0.34; δδ (400 MHz, CDCl₃) 5-10-5.30 (6H, m, \(3 \times C\)=CH=CH₂), 5-11 (1H, m, 2-CH₂), 4.34-4.39 (1H, dd, J = 13.9, 4.5), 4.26 (1H, dd, J = 12.0, 5.6) (1-CH₂), 3.80-3.73 (2H, m, 3-CH₂), 2.83-2.80 (4H, m, 2 × CH₂=CH=CH₂), 2.39-2.70 (2H, t, J = 7.6), 2.35 (2H, t, J = 7.7) (2 × CH₂COOH), 2.1-2.0 (4H, m, 2 × CH₂=CH=CH₂), 1.73-1.60 (4H, m, 2 × CH₂COCH₂), 1.47-1.20 (36H, m, 18 × CH₃), 0.91 (6H, t, J = 6.7, 2 × C₂H₅); δC (125 MHz, CDCl₃), 173.8, 173.2 (2 × C=O), 130.5, 129.5, 128.5, 128.4, 128.0, 127.6 (3 × C=CH), 172-2 (2 × C=O), 151.3, 151.2, 148.7 (3 × C), 130.0, 129.7, 129.5, 129.3, 129.2, 129.7 (2C), 126.6 (2C), 126-3, 126-3, 123-5, 123-5, 122-7, 112-6, 116-4 (2C), [(13 × P)CH + (CH=CH₂) + (2 × P)C], 75-6 (P)CH₂COCH₂, 70-1 (Gly 2-CH₂), 62-7, 61-7 (Gly 1-CH₂ + Gly 3-CH₂), 34-17, 34-10 (2 × CH₂COOR), 31-9, 31-5, 29-7, 29-5 (8C), 29-3 (3C), 29-1, 27-2, 26-9, 25-6, 24-8, 24-5, 22-70 (2C), 22-58 (2C) (2 × CH₂), 14-1 (2 × CH₃); HRMS (ESI+) \(m/z\) found [M+Na]⁺ = 987-6018, CₘHₙOₙNa requires 987-6009.
sn-1-O-Stearoyl-2-O-oleoyl glycerol, 19b. sn-1-O-Stearoyl-2-O-oleoyl-3-O-(9-phenylxanthen-9-yl) glycerol (970 mg, 1.10 mmol) was used instead of sn-1-O-stearoyl-2-O-γ-linolenoyl-3-O-(9-phenylxanthen-9-yl) glycerol (above) with pyrrole (763 µL, 11.0 mmol, 10 equiv.) and dichloroacetic acid (182 µL, 2.20 mmol, 2.0 equiv.). This reaction afforded title compound as a colourless oil (634 mg, 93%). \(R_t\) (diethyl ether-pet. spirit, 3:17) 0.45; \(\delta H\) (400 MHz, CDCl₃) 5.40-5.30 (2H, m, \(\text{H} = \text{CH}\)), 5.13-5.07 (1H, m, Gly-2-CH₂), 4.35-4.27 (2H, m, Gly-1-CH₃), 3.76 (2H, dd, J 1, 4-6, Gly-3-CH₂), 3.28 (2H, t, J 7.5, CH₂COOR), 2.36 (2H, t, J 7.5, CH₂COOR), 2.08-1.99 (4H, m, CH₃CH=CHCH₃), 1.70-1.60 (4H, m, 2 × CH₂CH₂COOR), 1.40-1.20 (48H, m, 24 × CH₂), 0.90 (6H, t, J 6.8, 2 × CH₃); \(\delta C\) (125 MHz, CDCl₃) 173-9, 173-5 (2 × C=O), 130-0, 129-7 (H=CH₂), 72-1 (Gly-2-CH₂), 62-0, 61-6 (2 × Gly CH₂), 34-3, 34-0 (2 × CH₂COORs), 31-9 (2C), 31-6, 29-76, 29-69 (7C), 29-52, 29-47, 29-32 (3C), 29-27, 29-17, 29-11 (2C), 29-06, 27-22 (2C), 27-16, 24-89 (2C), 22-68 (2C) (28 × CH₃), 14-11 (2C, 2 × CH₃); HRMS (ESI+) \(m/z\) [M+H]+ = 623-5623, C₄₉H₇₃O₂₈S requires 623-5610.

3-(Trimethylsilyloxy)propionitrile, 12. 3-Hydroxypropionitrile (40.3 mL, 590 mmol) and triethylamine (84-5 mL, 610 mmol, 1.03 equiv.) were dissolved in diethyl ether (100 mL) and cooled to 0 °C. A solution of trimethylsilyl chloride (76-6 mL, 600 mmol, 1.02 equiv.) in diethyl ether (200 mL) was added dropwise to the briskly stirred solution. After 16 h, the reaction mixture was filtered under a nitrogen gas, washing with diethyl ether. The solvent was then removed in vacuo and the resulting brown oil was distilled (20 mmHg, 75-85 °C) to afford the title compound as a colourless oil (78-0 g, 92%). \(\delta H\) (400 MHz, CDCl₃) 3-77 (2H, t, J 6.3, SiOCCH₂), 2-52 (2H, t, J 6.3, CH₂CN), 0-01 (9H, s, 3 × CH₃); \(\delta C\) (101-3 MHz, CDCl₃) 165-18, 139-2, 8-1 ppm. These data are consistent other studies that use this compound.⁴-³⁴

1-O-[(Cycanoethyloxy)(sn-1-O-stearoyl-2-oleoylglycerol) phosphonyl]-2,3:5,6-O-dicyclohexylidene-my o-inositol, 23a. To 1-O-(dicyanoethyloxyphosphoryl)-2,3:5,6-O-dicyclohexylidene-my o-inositol (11, 253 mg, 0.44 mmol) with EtOAc (1 mL) in CH₂Cl₂ (2 mL), MeCN (1 mL). The residual salt, plus sn-1-O-stearoyl-2-O-oleoyl glycerol (19b, 551 mg, 0.88 mmol, 2.0 equiv.) and 3-nitrotriazole (22, 402 mg, 3.52 mmol, 8.0 equiv.) in CH₂Cl₂-MeCN-pyridine (2:2:1, 5 mL) were then reacted with mesitylene sulfonyl chloride (385 mg, 1.76 mmol, 4 equiv.) in pyridine (1 mL), added dropwise, to afford the title compound (356 mg, 75%). \(R_t\) (EtOAc) 0-90; \(\delta H\) (400 MHz, CDCl₃) 5-40-5.37 (2H, m, \(\text{H} = \text{CH}\)), 5-37-5.31 (1H, m, Gly-2-CH₂), 4-82-4.76 (1H, m, Ins-1-CH₃), 4-62 (1H, dd, J 4-4, 8-8, Ins-2-CH₂), 4-42-4.15 (6H, m, POCH₂+ Gly 3-CH₂+ Gly 1-CH₂), 4-10 (1H, m, J 5-4, Ins-3-CH₃), 4-03 (1H, m, J 3-9, 9-9, Ins-6-CH₂), 3-92 (1H, m, J 6-5, 10-7, Ins-4-CH₂), 3-40 (1H, m, J 10-2, Ins-5-CH₂), 2-85 (2H, m, J 6-5, CH₂CN), 2-40-2.30 (4H, m, J 7-7, 2 × CH₂COOR), 2-10-2.00 (4H, m, CH₃CH=CHCH₃), 1-80-1-10 (68H, m, 34 × CH₂), 0-90 (6H, t, J 6-8, 2 × CH₂) ppm; \(\delta C\) (162 MHz, CDCl₃) -2.81 (0.5P), -2.94 (0.5P) ppm; HRMS (ESI+) \(m/z\) found [M+H]+ = 1,078-7323, C₆₀H₁₀₀N₁₀O₃P requires 1,078-7324.

1-O-[(Cycanoethyloxy)(sn-1-O-stearoyl-2-O-γ-linolenoyl glycerol) phosphonyl]-2,3:5,6-O-dicyclohexylidene-my o-inositol, 23c. This compound was prepared by the same procedure as for 23a, first treating 1-O-(dicyanoethyloxyphosphoryl)-2,3:5,6-O-dicyclohexylidene-my o-inositol (11, 198 mg, 0.376 mmol) with EtOAc (1 mL) in CH₂Cl₂ (2 mL), MeCN (1 mL). The residual salt, plus sn-1-O-stearoyl-2-O-γ-linolenoyl glycerol (19b, 547 mg, 0.88 mmol, 2.0 equiv.) and 3-nitrotriazole (23, 343 mg, 3.01 mmol, 8.0 equiv.) in CH₂Cl₂-MeCN-pyridine (2:2:1, 5 mL) were then reacted with mesitylene sulfonyl chloride (329 mg, 1.50 mmol, 4 equiv.) in pyridine (1 mL), added dropwise 80 µL/2 min, to afford the title compound as an off-white solid (380 mg, 94%). \(R_t\) (EtOAc-pet. spirit, 1:1) 0-40; \(\delta H\) (400 MHz, CDCl₃) 5-47-5.25 [7H, m, (3 × \(\text{H} = \text{CH}\)) + Gly 2-CH₂], 4-83-4.72 (1H, m, Ins-1-CH₃), 4-61 (1H, m, J 4-5, Ins-2-CH₂), 4-40-4.25 (5H, m, OCH₂CH₂CN + Gly 3-CH₂), 4-19 (1H, dd, Gly 3-CH₂), 4-10 (1H, m, Ins-3-CH₃), 4-04 (0.5H, m, J 10-0), 4-02 (0.5H, m, J 9-9 Hz) (Ins-6-CH₂), 3-90 (1H, dd, J 10-7, 6-5, Ins-4-CH₂), 3-40 (1H, m, J 9-8 Hz, Ins-5-CH₂), 2-87-2.80 (6H, m, CH₂CN)
1-O-[(Cyanothioxy)(sn-1,2-O-distearylglycerol)phosphoryl]-4-O-(dicyanothioxyphosphoryl)-2,3:5,6-O-dicyclohexylenedimy-o-inositol, 24a. 1-O-[(Cyanothioxy)(sn-1,2-O-distearylglycerol)phosphoryl]-2,3:5,6-O-dicyclohexylenedimy-o-inositol (23a, 250 mg, 0·231 mmol) was evaporated from pyridine (3 × 2 mL) and then dissolved in CHCl₃-pyridine (3:2, 2·5 mL) to which N-methyl imidazole (222 µL, 2·78 mmol, 12·0 eq.) then dicyanothioxyphosphorochloridite in CHCl₃ (15, 0·1g/mL stock, 2·5 mL, 12·0 eq.) was added. After 16 h cyanoothanol (157 µL, 2·57 mmol, 11·1 eq.) was added and the mixture stirred for 30 min. The solution was then cooled to 0 °C and 5 M tert-butyl hydroperoxide in decanes (249 µL, 1·25 mmol, 5·0 eq.) was added. After 12 h water (5 mL) was added and the solution was concentrated in vacuo. The resulting mixture was suspended in MeCN-water (1:9, 100 mL) and fractionated through a column of silanised silica, eluting with a gradient of MeCN-water (1:4→7:3), and flushed with EtOAc. The appropriate fractions were combined, dried (MgSO₄), and adsorbed onto silica.

The dry powder was poured onto a column of silica and fractionated, eluting with a gradient of first diethyl ether-pet. spirit (0:1 → 1:0) then methanol-EtOAc (0:1 → 1:1), to afford the title compound as a white greasy solid (248 mg, 85%). R₂ (EtOAc) 0·26; δf (400 MHz, CDCl₃) 5·3-5·27 (1H, m, Gly 2-CH₂), 4·8-4·73 (1H, m, Ins 1-H), 4·7-4·62 (2H, m, 4-Ins + H-2-I), 4·45-4·15 [11H, m, (3 × OC₂H₅CN) + Gly 3-CH₃ + Gly 1-CH₂ + Ins 3-H], 4·15-4·08 (1H, m, Ins 6-H), 3·51 (1H, t, J 10·2, Ins 5-H), 2·86-2·80 (6H, m, 3 × CH₂CN), 2·40-2·30 (4H, m, 2 × CH₂CO₂), 1·85-1·20 (80H, m, 40 × CH₃), 0·90 (6H, t, J 6·8, 2 × CH₃); δf (162 MHz, CDCl₃) -2·31 (1P), -2·78 (0·4P), -2·97 (0·6P); δc (125 MHz, CDCl₃) 173·2, 172·8, (2 × C=O), 116·23, 116·13, 116·10 (3 × C=NN), 114·44, 112·1 (2 × acetal C), 81·0, 79·4, 76·1, 75·8, 75·1, 74·6, 69·1 (6 × Ins-CH + Gly + Gly 2-CH₂), 66·0, 62·2, 61·2, 61·7, 60·4 [(2 × Gly-CH₂) + (3 × POCH₃)], 37·5, 36·2 (2 × CH₂COORS), 35·1 (2C), 34·14 (4C), 33·99 (4C), 31·9 (3C), 29·70 (7C), 29·50 (6C), 29·35 (3C), 29·1 (2C), 24·84, 24·67, 23·95 (2C), 22·7, 21·0 (2C), 19·61, 19·50 [(30 × fatty acid CH₂) + (10 × cyclohexylidene CH₃) + (3 × CH₂CN)], 14·24, 14·11 (2 × CH₃); MS (ESI+) m/z found [M+N⁺] = 1288·7 (62%), C₆H₁₁₁₃NO₃PNa requires 1288·7, also observed [M+Na⁺⁺] = 1283·7 (40%), [M-C₆H₅]⁺ = 1186·6 (78%), [DAG⁺]⁺ = 607·5 (25%).
acid CH₃) + (10 × cyclohexylidene CH₂) + (3 × CH₂CN)], 14-08, 14-03 (2 × CH₃); MS (ESI+) m/z found [M+Na]⁺ = 1,282.6 (48%), C₅₀H₈₀N₂O₁₆P₂Na requires 1,282-6, also observed [M+NH₄]⁺ = 1,277-7 (30%), [M-C₄H₄]⁺ = 1,180.6 (53%), [DAG]⁺ = 601-5 (22%).

sn-1,2-Distearylphosphatidylinositol 4-phosphate, triethylammonium salt, 2a. 1-O-[(Cyanoethyl)sn-1,2-O-stearoyl-glycerol-3-xyloxyphosphoryl]-4-O-(dicyanoethyloxyphosphoryl)-2,3,5,6-O-dicyclohexylidene-myo-inositol (24a, 250 mg, 0-19 mmol) was evaporated from MeCN (3 × 2 mL), and dissolved in CH₃Cl₂-MeCN (1:1, 6 mL). To this was added trimethylsilyle chloride (1 mL) then N,N,N′,N′-tetramethyl-N′-tert-butyl-guanidine (90%, 774 µL, 5-92 mmol, 30-0 eq.). After 16 h, the volatile components were removed in vacuo and the residue was triturated with TmsCl-pet. spirit (1:19) under N₂. The filtrate was re-dissolved in dryness, when ³¹P NMR [-11.2 (1P), -19.5 (1P) ppm] demonstrated complete exchange of cyanoethyl esters. The filtrate was re-dissolved in Et₃N-MeOH (3:2, 5 mL) and stirred for 20 min before again stripping off the solvent in vacuo. The residue was then re-dissolved in AcOH-water (2:1, 6 mL) and after stirring for 48 h the mixture was freeze-dried. The off-white solid was triturated with MeCN, then diethyl ether to afford the title compound as an off-white solid (186 mg, 99%). δ₃¹P (500 MHz CDCl₃-CDOOD) 40 °C) 5-00-5-05 (1H, m, Gly 2-CH₂), 4-40-4-35 (1H, m, Ins 2-CH₂), 4-20-3-95 (5H, m, Gly 3-CH₂ + Gly 1-CH₂ + Ins 4-CH₂), 3-92 (1H, dd, J 9-7, 8-1, 2-6, Ins 1-H), 3-84 (1H, t, J 9-3, Ins 6-H), 3-54 (1H, dd, J 9-6, 2-7, Ins 3-H), 3-20 (1H, t, J 9-0, Ins 5-H), 3-08 (6H, q, J 7-3, 3 × TEA CH₂), 2-32-2-22 (4H, m, 2 × CH₂CH₂COOR), 1-49-1-56 (4H, m, 2 × CH₂CH₂CO₂H), 1-18-1-38 [65H, m, (28 × CH₂) + (3 × TEA CH₂)], 0-80 (6H, m, J 7-0, 2 × CH₂); δ₂ (202 MHz CDCl₃-CDOOD, 1:1, 40 °C) 2-0 (1P), 0-02 (1P); HMRS (ESI-) m/z found [M-H]⁻ = 945-5505, C₅₅H₇₀O₁₆P₂ requires 945-5469.

sn-1-Stearoyl-2-oleoylphosphatidylinositol 4-phosphate, triethylammonium salt, 2b. 1-O-[(Cyanoethyl)sn-1-O-stearoyl-sn-2-oleoyl-glycerol-3-xyloxyphosphoryl]-4-O-(dicyanoethyloxyphosphoryl)-2,3,5,6-O-dicyclohexylidene-myo-inositol (24b, 253 mg, 0-20 mmol) was evaporated from MeCN (3 × 2 mL), and dissolved in CH₃Cl₂-MeCN (1:1, 6 mL). To this was added trimethylsilyle chloride (1 mL) then N,N,N′,N′-tetramethyl-N′-tert-butyl-guanidine (90%, 784 µL, 6-00 mmol, 30-0 eq.). After 16 h, the volatile components were removed in vacuo and the residue was triturated with TmsCl-pet. spirit (1:19) under N₂. The filtrate was re-dissolved in dryness, when ³¹P NMR [-11.2 (1P), -19.5 (1P) ppm] demonstrated complete exchange of cyanoethyl esters. The filtrate was re-dissolved in Et₃N-MeOH (3:2, 5 mL) and stirred for 20 min before again stripping off the solvent in vacuo. The residue was then re-dissolved in AcOH-water (2:1, 6 mL) and after stirring for 48 h the mixture was freeze-dried. The off-white solid was triturated with MeCN, then diethyl ether to afford the title compound as an off-white solid (75 mg, 99%). δ₃¹P (400 MHz CDCl₃-CDOOD [2:1], 25 °C) 5-25-5-12 (6H, m, 3 × HC=CH), 5-08-5-05 (1H, m, Gly 2-CH₂), 4-25-3-85 (7H, m, Ins 4-CH + Ins 1-CH + Ins 2-CH + Gly 3-CH₂ + Gly 1-CH₂), 3-70 (1H, m, Ins 6-H, obscured by CD₂OHO signal), 3-48-3-40 (1H, m, Ins 3-H), 3-28-3-20 (1H, m, Ins 5-H, obscured), 3-18-3-12 (12H, q, J 7-0, TEA CH₂), 3-10 (1H, m, Ins 5-H), 2-20 (4H, m, CH₂CH₂COOR), 1-40 (4H, m, 2 × CH₂CH₂CH=CH₂), 1-45 (4H, m, 2 × CH₂CH₂CH₂CO₂H), 1-00-1-30 [62H, m, (22 × CH₂) + (6 × TEA CH₂)], 0-83 (6H, t, 2 × CH₂); δ₂ (202 MHz CDCl₃-CDOOD [3:2], 50 °C) 1-00 (1P), -1-00 (1P); HMRS (ESI-) m/z found [M-H]⁻ = 945-5505, C₅₅H₇₀O₁₆P₂ requires 945-5474.

Nuclear Magnetic Resonance Spectra

The following are the NMR spectra for the compounds:

S-Acetyl Mandelic Acid, 8a.

S-Acetyl mandelate, 8b.

S-Acetyl mandelate, 8b.

S-Acetyl mandelate, 8b.

S-Acetyl mandelate, 8b.

S-Acetyl mandelate, 8b.

S-Acetyl mandelate, 8b.

S-Acetyl mandelate, 8b.

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S-Acetyl mandelate, 8b.

S-Acetyl mandelate, 8b.

S-Acetyl mandelate, 8b.

S-Acetyl mandelate, 8b.

S-Acetyl mandelate, 8b.
1-O-[(Cyanoethyloxy)(sn-1-O-stearoyl-2-O-γ-linolenoylglyceryloxy)phosphoryl]-2,3:5,6-O-dicyclohexylidene-myo-inositol, 23c.
1-O-[(Cyanoethyloxy)(sn-1-O-stearoyl-2-O-arachidonoylglyceryloxy)phosphoryl]-2,3:5,6-O-dicyclohexylidene-myo-inositol, 23d.

Dicyanoethyl phosphorochloridite, 15.
1-O-[(Cyanoethyloxy)(sn-1,2-O-distearoylglyceryloxy)phosphoryl]-4-O-(dicyanoethyloxyphosphoryl)-2,3:5,6-O-dicyclohexylidene-myoinositol, 24a.
1-O-(1-O-stearoyl-sn-2-oleoyl glycer-3-xyloxy)(2-cyanoethyloxy)phosphoryl-4-O-di(2-cyanoethyloxy)phosphoryl-2,3:5,6-O-dicyclohexylidene-myoinositol, 24b.
1-O-[(Cyanoethyloxy)(sn-1-O-stearoyl-2-O-γ-linolenoylglyceryloxy)phosphoryl]-4-O-(dicyanoethyloxyphosphoryl)-2,3:5,6-O-dicyclohexylidene-myoinositol, 24c.
1-O-[(Cyanoethyloxy)(sn-1-O-stearoyl-2-O-arachidonoylglyceryloxy)phosphoryl]-4-O-(dicyanoethyloxyphosphoryl)-2,3:5,6-O-dicyclohexylidene-myoinositol, 24d.

Distearoylphosphatidylinositol 4-Phosphate, triethylammonium salt, 2a.
Stearoyl-oleoyl-phosphatidylinositol-4-phosphate, triethylammonium salt, 2b.
Stearoyl-γ-linolenoyl-phosphatidylinositol-4-phosphate triethylammonium salt, 2b.
Stearoyl-arachidonoyl-phosphatidylinositol-4-phosphate, triethylammonium salt, 2c.

Supplementary References