Supporting Information

Synthesis and Cellular Labeling of Caged Phosphatidylinositol Derivatives

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Supplementary Figures

Figure S1

A.

Figure S1. Cell entry. A) Images of HeLa cells incubated with 1b for 30 sec, 5.5 and 30.5 min as well as after uncaging with 405 nm light for 20 sec (30.8 min). Note the increase of fluorescence after uncaging due to increased quantum yield of the released coumarin. B) Mean fluorescence intensities from whole surface area of single cells were measured following background subtraction from images of HeLa cells incubated with 1b for 30 sec, 5.5 min, 10.5 min, 15.5 min, 20.5 min, 25.5, 30.5 min and after uncaging (30.8 min, 35.8 min, 40.8 min). The fluorescence intensities at each time point were normalized to the maximum fluorescence intensity measured right after uncaging at 30.8 min. Error bars represent standard deviation. Statistical significance was evaluated using unpaired, two-tailed Student’s t-test (n=12 cells from 3 independent dishes, **: p<0.01, n.s.: not significant).
Figure S2. TLC of caged and uncaged PI derivatives on HPTLC silica 60. A) 10 mM stock solution of 1b in MeOH (first lane) and the same after illumination with >400 nm (second lane); 10 mM stock of coumarin 11 (third lane); 10 mM stock solution of 1a in MeOH (fourth lane) and the same after illumination with >400 nm (fifth lane). The plate was sequentially developed in chloroform:methanol:water:acetic acid 65:25:4:1 for 6 cm and cyclohexane:ethylacetate 1:1 for 9 cm.
Assay Methodology

Cell Culture
HeLa Kyoto cells were routinely cultured in DMEM low glucose supplemented with 10% (v/v) FBS at 37°C, 5% (v/v) CO₂. Prior to compound incubations, cells were washed twice in a HEPES-buffered physiological saline (20 mM HEPES pH 7.4, 140 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl₂, 1.0 mM MgCl₂) supplemented with 1.0 g/L D-glucose and 2 mM L-glutamine. Lipid derivatives were dissolved in DMSO and pre-mixed in a 2:1 ratio with 20% (w/v) Pluronic F-127 in DMSO prior to addition to cells. Lipid incubations were performed on the microscopy stage in HEPES-buffered physiological saline at 37°C and atmospheric CO₂.

Lipid Extraction & TLC Analysis
To determine the metabolic fate of trifunctional PI 1a, cells were loaded with the lipid derivative (10 uM) for 5 min. After three washes, cells were illuminated with >400 nm light using a 1000 W high-pressure mercury lamp (Newport) for 2 min (+UV) on ice or kept in the dark on ice for 2 min (-UV), washed, and then incubated further in the absence of the compound for the indicated periods of time. At the end of the incubations, cells were washed and scraped in 300 µL of ice-cold D-PBS. The scraped cells were transferred into 2 mL tubes, to which 600 µL of methanol was added at room temperature (RT). Samples were vortexed for 10 s. Then, 150 µL of chloroform was added and samples were vortexed for 10 s again. Samples were centrifuged at 20.000 x g for 5 min at RT to pellet down all macromolecules such as proteins, nucleic acids, etc. The supernatants were transferred to fresh tubes, to which 300 µL of chloroform and 600 µL of 0.1% (v.v) aqueous acetic acid were successively added. Following thorough vortex mixing, samples were centrifuged at 20.000 x g at RT for 2 min. The lower phases were transferred into fresh tubes. The collected lower phases were dried down in a cooled CentriVap (Labconco, Kansas City, MO, USA) at RT with a carefully set vacuum control to prevent solvent bumping. Lipids were labeled with 3-azido-7-hydroxycoumarin via copper-catalyzed click reaction prior to TLC analysis. The dried lipid extracts were re-dissolved in 8 µL of chloroform, to which 30 µL was added from a copper-click master mix containing 5 µL of 10 mM 3-azido-7-hydroxycoumarin in acetonitrile, 100 µL of 10 mM tetrakis(acetonitrile)copper(I) tetrafluoroborate in acetonitrile, and 400 µL of ethanol. Samples were briefly vortexed and spun down. Samples were placed in a thermoblock at 37°C for 3 to 4 hours until all the liquid
condensed under the lid of the tubes. At the end of the reaction, the tubes were briefly spun down and dried again in the CentriVap (Labconco) at RT.
The extracted and click-labeled lipids were re-dissolved in water-saturated chloroform and spotted onto non-fluorescent HPTLC silica gel 60 glass plates (Merck). The plates were sequentially developed in chloroform:methanol:water:acetic acid 65:25:4:1 for 6 cm and cyclohexane:ethylacetate 1:1 for 9 cm. The plates were gently desiccated in between the two developments. The plates were imaged using the SYBR Green channel of a BioRad Chemidoc Touch Imaging System.

**Live Cell Imaging of Compound Entry**

HeLa Kyoto cells were imaged on an Olympus Fluoview 1200 confocal microscope 30 sec after addition of compounds 1a or 1b (10 uM), respectively, with 5 min intervals. Images were taken with a 1% laser power (405nm) to limit undesired uncaging. Following the image at 30.5 min, caged compounds were illuminated for 20 s with 100% laser power. An image was taken immediately after uncaging at 30.8 min.

For fluorescence quantification, each frame was subjected to background subtraction. A further background subtraction was applied using the background-subtracted auto-fluorescence of the cells in an image that was taken prior to compound addition.

**Imaging of Photo-crosslinked Lipid-Protein Complexes in Fixed cells**

Live HeLa Kyoto cells were incubated with lipid derivatives followed by successive applications of light for uncaging (>400 nm light) and photo-crosslinking (>345 nm light) on ice using a 1000 W high-pressure mercury lamp (Newport). Following the illuminations, cells were fixed in 100% methanol at -20°C for 10 min. Non-crosslinked lipids and cleaved coumarin were extracted at RT by four acidic extractions (1 min each) with methanol:chloroform:acetic acid 55:10:0.75. Cells were rehydrated in D-PBS for 15 min. Then, cells were blocked in 3% (w/v) BSA in D-PBS for 15 min. The crosslinked lipids were then labeled with Alexa 488 – picolyl azide via a copper-catalyzed clicked reaction. The final reaction conditions were 100 mM sodium phosphate pH 7.0, 1 µM Alexa 488 – picolyl azide, 2 mM CuSO₄, 10 mM BTTAA (2-(4-((bis((1-(tert-butyl)-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)-1H-1,2,3-triazol-1-yl)acetic acid), and 100 mM sodium ascorbate. The reaction was allowed to continue for 1 hour at RT in the dark. The reaction solution was removed and cells were washed twice in 3% (w/v) BSA in D-PBS and once in D-PBS. Finally, cells were mounted in SlowFade Diamond antifade mountant prior to imaging. Cells
were imaged for Alexa 488 fluorescence on a Olympus Fluoview 1200 or a Zeiss LSM 780 confocal microscope.

**Proteomic Analysis of Photo-crosslinked Proteins**

HeLa Kyoto cells were loaded with 5 µM trifunctional PI 1a for 5 min. Following three washes to remove the excess compound, cells were first illuminated at >400 nm for 2 min and then at >345 nm for 2 min (+UV sample) or only illuminated at >400 nm for 4 min (-UV sample) on ice using a 1000 W high-pressure mercury lamp (Newport). Following the illuminations, cells were lysed on ice for 15 min in 200 mM HEPES pH 8.0, 9 M urea, 4.5% (w/v) CHAPS, 1 M NaCl. Lysates were diluted 1:1 with deionized water. Nucleic acids were digested at RT for 30 min by the addition of benzonase (0.5 U/µL) and 1 mM MgCl$_2$. Lysates were centrifuged at 20,000 x g for 15 min. The supernatants were filtered through 0.22 µm low-protein binding PVFDF membranes. The filtered lysates were mixed picolyl azide-coated beads (Click Chemistry Tools) together with copper-click reagents. The final reaction conditions were 100 mM HEPES pH 8.0, 4 M urea, 2% (w/v) CHAPS, 0.5 M NaCl, 1 mM CuSO$_4$, 5 mM BTTAA, 10 mM sodium ascorbate. The reactions continued for 18 hours on a sample rotator at RT. The beads were then washed twice in deionized water. The clicked protein-lipid conjugates were reduced with DTT (10 mM) for 15 min at 70 ℃ in an SDS buffer (100 mM Tris pH 8.0, 2% (w/v) SDS, 250 mM NaCl, 5 mM EDTA). Subsequently, the proteins were alkylated with iodoacetamide (40 mM) in the SDS buffer in the dark for 30 min at RT. The beads were sequentially washed five times with the SDS buffer, 10 times with a urea buffer (100 mM Tris pH 8.0, 8 M urea), and 10 times with 20% (v/v) acetonitrile in water at RT. The proteins on the beads were released through peptidic digestion by trypsin in 100 mM Tris pH 8.0, 1 mM CaCl$_2$, 10% (v/v) acetonitrile for 18 hours at 37 ℃. The peptides were then desalted on “trifunctional” C18 columns (Sep-Pak tC18 1 cc Vac Cartridge, 100 mg Sorbent per Cartridge, 37-55 µm Particle Size) and then dried in a cooled CentriVap (Labconco) prior to mass spectrometry analysis. All MS/MS samples were analyzed using Mascot (Matrix Science, London, UK; version 2.5.1). Mascot was set up to search the human reference proteome UniProtKB Homo sapiens (UP000005640). Scaffold (version 4.8.9, Proteome Software Inc., Portland, OR) was used to validate MS/MS based peptide and protein identifications. Protein identifications were accepted if they contained at least 2 uniquely identified peptides. Quantitative values were obtained from normalized total spectra in Scaffold.
Chemical Syntheses

General Procedures

All chemicals were obtained from commercial sources (Acros, Sigma, Alfa Aesar, TCI or Merck) and were used without further purification. Solvents for flash chromatography were from VWR and dry solvents were from Sigma or Acros. Deuterated solvents were obtained from Deutero GmbH, Karlsruhe, Germany. Enantiopure 3,6-di-O-butyryl-1,2:4,5-di-O-isopropylidene-myoinositol was obtained from SiChem GmbH, Bremen, Germany. From this compound 2 was prepared as described by Mentel et al.[1] All reactions were carried out using dry solvents under inert atmosphere unless stated otherwise in the respective experimental procedure.

TLC was performed on pre-coated plates of silica gel (Merck, 60 F254) using UV light (254 or 366 nm) or a solution of phosphomolybdic acid in EtOH (10 g phosphomolybdic acid in 100 mL EtOH) for visualization. Preparative column chromatography was performed using silica gel 60 (grain size 0.04-0.063 mm) from Macherey-Nagel GmbH, Germany with a pressure of 1-1.5 bar. For RP flash column chromatography LiChroprep® RP-18 material (Merck, grain size 0.040-0.063 mm) or LiChroprep® C18 (Macherey-Nagel, grain size 0.060-0.080 mm) was employed.

HPLC analysis was performed on a Knauer Smartline pump 1000 using a Knauer Smartline UV Detector 2500 with a LiChroCART® 250-5 mm cartridge (LiChrospher 100 RP18 (10 µm, Merck)), or a Shimadzu instrument with Nucleodur column (Machery-Nagel).

Preparative HPLC was performed using a Knauer K-1800 preparative pump with a K-2501 UV detector and a Merck Prepbar steel column (250 x 50 mm) filled with RP18 material (Merck, LiChrospher®, 220 g, 12 µm). For all HPLC experiments, the eluents were methanol-water mixtures unless stated otherwise; compositions are given in % methanol.

$^1$H-, $^{13}$C-, and $^{31}$P-NMR spectra were obtained on a 400 MHz Bruker UltraShield™ spectrometer. Chemical shifts of $^1$H- and $^{13}$C-NMR spectra are referenced to solvent resonances, $^{31}$P-NMR spectra are referenced to 85% phosphoric acid. J values are given in Hz and chemical shifts in ppm. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; p, pentet; m, multiplet; b, broad. $^{13}$C- and $^{31}$P-NMR spectra were broadband hydrogen decoupled. Mass spectra (ESI) were recorded using a Waters Micromass ZQ mass spectrometer or a HP Esquire-LC mass spectrometer. High-resolution mass spectra were recorded at the University of Heidelberg (ICR Apex-Qe instrument, ESI).

Melting points were determined on a Buechi B-540 and are uncorrected.
Synthetic procedures and analytical data

Synthesis of head group 7

6-O-Butyryl-3-O-(9H-fluorene-9-ylmethoxycarbonyl)-1,2:4,5-di-O-iso-propylidene-myoinositol (3)

A solution of 6-O-butyryl-1,2:4,5-di-O-iso-propylidene-myoinositol 2 [1] (540 mg, 1.6 mmol) in DCM (20 mL) and MeCN (20 mL) was evaporated under reduced pressure and dried at 24 °C/0.012 mbar. Under argon, anhydrous DCM (15 mL) and dry pyridine (372 µL, 4.6 mmol) were added via syringe. The resulting solution was cooled in an ice bath and Fmoc-Cl (864 mg, 3.3 mmol) was added with stirring. The cooling bath was removed and stirring continued for 2 h. The slightly colored reaction was diluted with DCM (25 mL), washed three times with phosphate buffer (3 x 40 mL), brine (40 mL) and evaporated under reduced pressure to obtain the crude compound as a white solid that was purified by chromatography on silica with DCM:n-pentane:MeOH 9:1:0 → 9:0:1.

Yield: 878 mg (97%) white solid, mp. 219-219.5 °C, t<sub>R</sub> 100% MeOH = 2.4 min; R<sub>f</sub> DCM = 0.56.

OR: [α]<sub>D</sub><sup>20</sup> = -1.5 (c = 2.1, CHCl<sub>3</sub>).

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ = 7.80 (d, <i>J</i>=7.5, 2H, Fm), 7.68 (d, <i>J</i>=7.4, 2H, Fm), 7.44 (t, <i>J</i>=7.4, 2H, Fm), 7.35 (dt, <i>J</i>=6.4, 3.2, 2H, Fm), 5.37 (dd, <i>J</i>=11.1, 6.8, 1H, ins-H), 5.06 (dd, <i>J</i>=10.6, 4.4, 1H, ins-H), 4.68 (t, <i>J</i>=4.6, 1H, ins-H), 4.52 (dd, <i>J</i>=10.3, 7.6, 1H, Fm), 4.42 (dd, <i>J</i>=10.3, 7.4, 1H, Fm), 4.34 (t, <i>J</i>=7.5, 1H, 9-H), 4.29 – 4.23 (m, 1H, ins-H), 4.19 (dd, <i>J</i>=6.7, 4.8, 1H, ins-H), 3.54 (dd, <i>J</i>=11.1, 9.5, 1H, ins-H), 2.41 (t, <i>J</i>=7.4, 2H, α-CH<sub>2</sub>), 1.72 (dd, <i>J</i>=14.9, 7.4, 2H, β-CH<sub>2</sub>), 1.65 (s, 3H, CH<sub>3</sub>-ketal), 1.52 (s, 3H, CH<sub>3</sub>-ketal), 1.47 (s, 3H, CH<sub>3</sub>-ketal), 1.37 (s, 3H, CH<sub>3</sub>-ketal), 1.00 (t, <i>J</i>=7.4, 3H, γ-CH<sub>3</sub>).

<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ = 172.46 (s, 1C, CO<sub>2</sub>), 154.41, 143.28, 143.19, 141.32, 141.28, 127.95, 127.22, 127.17, 125.38, 125.28, 120.09, 120.07, 113.14 (s, 1C, O-C-O), 110.87 (s, 1C, O-C-O), 79.57, 77.39, 77.07, 76.75, 76.29, 74.96, 74.79, 74.03, 73.52, 70.48 (s, 1C, CH<sub>2</sub>-Fm), 46.57, 36.13, 27.65 (s, 1C, CH<sub>3</sub>-ketal), 26.91 (s, 1C, CH<sub>3</sub>-ketal), 26.85 (s, 1C, CH<sub>3</sub>-ketal), 26.05 (s, 1C, CH<sub>3</sub>-ketal), 18.32 (s, 1C, β-CH<sub>2</sub>), 13.52 (s, 1C, γ-CH<sub>3</sub>).

HRMS: m/z found 575.2258, calculated for C<sub>31</sub>H<sub>36</sub>NaO<sub>9</sub> 575.2252 [M+Na]<sup>+</sup>; m/z found 591.2000, calculated for C<sub>31</sub>H<sub>36</sub>K<sub>0</sub>O<sub>5</sub> 591.1991 [M+K]<sup>+</sup>.

6-O-Butyryl-3-O-(9H-fluorene-9-ylmethoxycarbonyl)-1,2-O-iso-propylidene-myoinositol (4)
3 (934 mg, <1.6 mmol) was dissolved in DCM (34 mL) and formic acid (10 mL, 265 mmol) was added with stirring. After 3 h the reaction was diluted with DCM (70 mL), washed with 1N K$_2$HPO$_4$ (200 mL), phosphate buffer (100 mL, pH 7.4), brine (100 mL), dried Na$_2$SO$_4$, filtered, and evaporated under reduced pressure to afford crude 4 as a semi-solid.

Chromatography on silica with DCM:EtOAc 3:1 afforded the pure compound.

Yield: 320 mg (38%), white solid (from EtOAc, MeCN), $t_R$ 95% MeOH = 2.2 min.

Mp. 116.5-118 °C (from MeCN).

[α]$^\text{D}_{20}$ = +0.7 (c = 2.8, chloroform).

$^1$H NMR (400 MHz, CDCl$_3$) δ = 7.79 (d, J=7.5, 2H, Fm), 7.66 (d, J=7.5, 2H, Fm), 7.43 (t, J=7.4, 2H, Fm), 7.33 (td, J=7.1, 2.4, 2H, Fm), 5.17 (dd, J=10.2, 7.6, 1H, ins-H), 4.93 (dd, J=10.1, 4.0, 1H, ins-H), 4.56 (t, J=4.4, 1H, ins-H), 4.49 (d, J=7.5, 2H, Fm), 4.32 (t, J=7.4, 1H, Fm), 4.18 (dd, J=7.6, 4.9, 1H, ins-H), 4.12 (t, J=9.7, 1H, ins-H), 3.47 (t, J=9.8, 2H, ins-H, OH), 3.19 (bs, 1H, OH), 2.39 (t, J=7.4, 2H, α-CH$_2$), 1.79 – 1.64 (m, 2H, β-CH$_2$), 1.62 (s, 3H, ketal), 1.37 (s, 3H, ketal), 0.97 (t, J=7.4, 3H, γ-CH$_3$).

$^{13}$C NMR (101 MHz, CDCl$_3$) δ = 173.91 (s, 1C, CO$_2$), 154.90 (s, 1C, CO$_2$), 143.27, 143.22, 141.33, 141.29, 127.95, 127.24, 127.18, 125.27, 125.24, 120.09, 110.95 (C$_\text{q}$ ketal), 77.40, 77.08, 76.77, 76.49 (ins-CH), 75.26 (ins-CH), 75.00 (ins-CH), 73.56 (ins-CH), 72.32 (ins-CH), 70.87 (ins-CH), 70.45 (Fm-CH$_2$), 46.63, 36.14 (α-CH$_2$), 27.78 (CH$_3$ ketal), 26.12 (CH$_3$ ketal), 18.39 (β-CH$_2$), 13.54 (γ-CH$_3$).

HRMS: m/z found 535.1945, calculated for C$_{28}$H$_{32}$NaO$_9$ 535.1939 [M+Na]$^+$; m/z found 551.1685, calculated for C$_{28}$H$_{32}$K$_2$O$_9$ 551.1678 [M+K]$^+$.

6-O-Butyryl-3,4,5-tri-O-(9H-fluorene-9-ylmethoxycarbonyl)-1,2-O-iso-propylidene-myoinositol (5)

A solution of 4 (370 mg, 0.72 mmol) in MeCN (10 mL) was evaporated under reduced pressure. The solid residue was further dried at 24 °C/0.012 mbar, dissolved in anhydrous DCM (10 mL) under argon and cooled in an ice bath. Fmoc-Cl (686 mg, 2.7 mmol) and dry pyridine (300 µL, 3.7 mmol) were added with stirring. The cooling bath was removed and stirring continued for 2 h. The slightly colored reaction was diluted with DCM (25 mL), washed three times with phosphate buffer (3 x 40 mL), brine (40 mL) and evaporated under reduced pressure to obtain the crude compound as a semi-solid that was purified by preparative HPLC.
Yield: 675 mg (98%) white solid (from 95% MeOH), colorless foam (from DCM), mp. 103-105 °C, \( t_{R} \) 100% MeOH = 3.8 min, 95% MeOH = 11.3 min.

OR: \([\alpha]_{D}^{20} = +1.4 \) (c = 3.4, CHCl₃).

\(^1\)H NMR (400 MHz, CDCl₃) \( \delta = 7.82 – 7.14 \) (m, 24H, Fm aryl), 5.64 (dd, \( J = 10.1, 8.2, 1H, \) ins-H), 5.50 (dd, \( J = 8.3, 6.2, 1H, \) ins-H), 5.29 (dd, \( J = 10.1, 3.7, 1H, \) ins-H), 5.09 (t, \( J = 8.2, 1H, \) ins-H), 4.71 (dd, \( J = 5.5, 3.8, 1H, \) ins-H), 4.45 – 4.07 (m, 9H, \( t_{R} \) = 2.8 min, 3 x Fm CH₂, 3 x Fm 9-H), 4.36 (t, \( J = 6.0, 1H, \) H-Ins), 2.43 – 2.26 (m, 2H, \( t_{R} \) = 2.8 min, \( \alpha\)-CH₂), 1.73 (s, 3H, CH₃-ketal), 1.71 – 1.61 (m, 2H, \( \beta\)-CH₂), 1.43 (s, 3H, CH₃-ketal), 0.92 (t, \( J = 7.4, 3H, \gamma\)-CH₃).

\(^{13}\)C NMR (101 MHz, CDCl₃) \( \delta = 171.89 \) (CO₂), 154.35 (CO₃), 154.34 (CO₃), 154.17 (CO₃), 143.18, 143.08, 143.05, 143.04, 142.92, 141.29, 141.22, 141.20, 141.17, 127.95, 127.90, 127.87, 127.81, 127.22, 127.17, 125.28, 125.25, 125.23, 125.19, 125.15, 120.09, 120.04, 120.00, 111.56 (C₉ ketal), 77.42, 77.10, 76.79, 75.87 (ins CH), 75.33 (ins CH), 73.04 (ins CH), 72.76 (ins CH), 71.38 (ins CH), 70.71 (2 x Fm CH₂), 70.67 (Fm CH₂), 46.45 (Fm CH), 46.39 (Fm CH), 46.37 (Fm CH), 36.01 (\( \alpha\)-CH₂), 25.54 (CH₃ ketal), 18.38 (\( \beta\)-CH₂), 13.54 (\( \gamma\)-CH₃).

HRMS: m/z found 979.3310, calculated for C₅₈H₅₂NaO₁₃⁺ 979.3300 [M+Na]+; m/z found 995.3056, calculated for C₅₈H₅₂NaO₁₃⁺ 995.30395 [M+K]+.

6-O-Butyryl-3,4,5-tri-O-(9H-fluorene-9-ylmethoxycarbonyl)-myo-inositol (6)

\( 5 \) (675 mg, 705 \( \mu \)mol) was dissolved in chloroform (15 mL) and trifluoroacetic acid (3 mL, 39.2 mmol) was added with stirring. The progress of the reaction was monitored by HPLC-MS (100% MeOH, \( t_{R} \) = 3.8 min, \( t_{R} \) = 2.8 min, m/z 940 [M+Na]+). After 1 h the reaction was diluted with EtOAc (85 mL), washed with phosphate buffer (100 mL, containing 7 g, 39 mmol, Na₂HPO₄, resulting pH = 5-6), phosphate buffer (100 mL, pH 7.4, resulting pH = 7), brine (100 mL) and evaporated under reduced pressure to afford \( 6 \) as a semi-solid.

Yield: 608 mg (94%), white foam (from EtOAc), glassy solid (from MeCN), insoluble in MeOH, \( t_{R} \) 100% MeOH = 2.8 min.

\([\alpha]_{D}^{20} = -3.0 \) (c = 4.1, chloroform).

\(^1\)H NMR (400 MHz, CDCl₃) \( \delta = 7.78 – 7.15 \) (m, 24H, Fm aryl), 5.67 (t, \( J = 10.1, 1H, \) ins-H), 5.59 (t, \( J = 9.9, 1H, \) ins-H), 5.20 (t, \( J = 9.9, 1H, \) ins-H), 5.01 (dd, \( J = 10.4, 2.5, 1H, \) ins-H), 4.45 – 4.34 (m, 2H, ins-H, Fm), 4.34 – 4.23 (m, 2H), 4.23 – 4.00 (m, 6H), 3.81 (s, 1H, ins-H), 3.34 (d, \( J = 2.0, 1H, \) OH), 3.21 (d, \( J = 8.4, 1H, \) OH), 2.41 – 2.24 (m, 2H, \( \alpha\)-CH₂), 1.69 – 1.56 (m, 2H, \( \beta\)-CH₂), 0.89 (t, \( J = 7.4, 3H, \gamma\)-CH₃).

\(^{13}\)C NMR (101 MHz, CDCl₃) \( \delta = 173.98 \) (CO₂), 154.45 (CO₃), 154.28 (CO₃), 154.14 (CO₃), 143.09, 143.05, 142.94, 142.92, 142.90, 141.24, 141.20, 141.14, 141.09, 141.07, 127.95, 127.89, 127.87, 127.80, 127.76, 127.72, 127.19, 127.15, 127.09, 125.21, 125.18, 125.16,
125.11, 125.02, 120.10, 120.02, 119.96, 119.94, 77.40, 77.08, 76.77, 74.89 (ins-CH), 74.59 (ins-CH), 73.88 (ins-CH), 72.09 (ins-CH), 70.69 (Fm-CH<sub>2</sub>), 70.62 (Fm-CH<sub>2</sub>), 70.52 (Fm-CH<sub>2</sub>), 70.49 (ins-CH), 46.50 (Fm-CH), 46.27 (Fm-CH), 36.14 (α-CH<sub>2</sub>), 18.41 (β-CH<sub>2</sub>), 13.48 (γ-CH<sub>3</sub>).

HRMS: m/z found 939.2997, calculated for C<sub>55</sub>H<sub>48</sub>NaO<sub>13</sub> <sup>+</sup> 939.2987 [M+Na]<sup>+</sup>; m/z found 955.2742, calculated for C<sub>55</sub>H<sub>48</sub>KO<sub>13</sub> <sup>+</sup> 955.2727 [M+K]<sup>+</sup>.

2,6-Di-O-butyryl-3,4,5-tri-O-(9H-fluorene-9-ylmethoxycarbonyl)-myo-inositol (7)

1. Preparation of the catalyst
Poly(4-vinylpyridine) (VP, 2 g) was swirled with DCM (30 mL) and TFA (7 mL). The resin was filtered off, washed with DCM:MeOH 25:25, DCM (50 mL) and dried at 24°C/0.1 mbar for 2 h.

2. Reaction
Crude 6 (600 mg, 654 µmol) was evaporated from a toluene solution (30 mL) under reduced pressure and dried at 23 °C/0.04 mbar for 2 h. Under an argon atmosphere, PVP/TFA, activated MS 4Å (2 g), anhydrous DCM (20 mL) and trimethyl orthobutyrate (5 mL, 31 mmol) were subsequently added. The mixture was agitated on an orbital shaker at 23°C. After 20 h analytical HPLC indicated complete conversion (100% MeOH, <i>t</i><sub>R</sub> 6 = 2.8 min, <i>t</i><sub>R</sub> cyclic intermediate = 4.0 min, m/z 1024 [M+Na]<sup>+</sup>). The reaction was diluted with DCM (200 mL), solids removed by filtration, washed with DCM (2 x 100 mL) and the filtrate evaporated under reduced pressure. The colorless syrup obtained was dissolved in THF (100 mL) and freshly prepared DOWEX 50WX8 (H<sup>+</sup>, 30 mL), water (75 mL) and 37% HCl (1 mL) were added. The mixture was shaken on an orbital shaker for 2 h. The resin was filtered off, washed with MeCN (2 x 100 mL) and the colorless filtrate evaporated under reduced pressure. The crude was purified by preparative HPLC.

Yield: 0.6 g (93%) white solid, <i>R</i><sub>f</sub> DCM:EtOAc 19:1 = 0.14, 9:1 = 0.75; <i>t</i><sub>R</sub> 100% MeOH = 3.3 min, 95% = 9.4 min.

[α]<sup>20</sup><sub>D</sub> = -11.3 (c = 4.9, chloroform).

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ = 7.89 – 7.06 (m, 24H, 3 x Fm aryl), 5.86 (t, <i>J</i>=2.8, 1H, ins-H), 5.56 (t, <i>J</i>=10.2, 1H, ins-H), 5.55 (t, <i>J</i>=10.1, 1H, ins-H), 5.25 (t, <i>J</i>=9.9, 1H, ins-H), 5.08 (dd, <i>J</i>=10.4, 2.8, 1H, ins-H), 4.55 – 4.46 (m, 1H, Fm), 4.35 – 4.18 (m, 4H, Fm), 4.18 – 4.06 (m, 4H, Fm), 4.05 – 3.96 (m, 1H, H-1), 2.72 (d, <i>J</i>=7.4, 1H, OH), 2.64 – 2.49 (m, 2H, β-CH<sub>2</sub>), 2.41 – 2.22 (m, 2H, β-CH<sub>2</sub>), 1.88 – 1.76 (m, 2H, β-CH<sub>2</sub>), 1.62 – 1.55 (m, 2H, β-CH<sub>2</sub>), 1.09 (t, <i>J</i>=7.4, 3H, CH<sub>3</sub>), 0.89 (t, <i>J</i>=7.4, 3H, CH<sub>3</sub>).

<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ = 173.90 (CO<sub>2</sub>), 172.98 (CO<sub>3</sub>), 154.38 (CO<sub>3</sub>), 154.22 (CO<sub>3</sub>), 153.96 (CO<sub>3</sub>), 143.28, 143.04, 143.02, 143.00, 142.86, 142.84, 141.26, 141.22, 141.20, 141.10, 141.07, 127.91, 127.85, 127.77, 127.17, 127.13, 125.26, 125.21, 125.15, 125.09, 125.04, 120.05, 120.00, 77.40, 77.08, 76.77, 74.57 (ins-CH), 73.82 (ins-CH), 72.96 (ins-CH), 71.89 (ins-CH), 70.78 (Fm-CH<sub>2</sub>), 70.72 (2 x Fm-CH<sub>2</sub>), 69.91 (ins-CH), 68.98 (ins-CH), 46.48
(9-CH), 46.25 (9-CH), 46.22 (9-CH), 36.11 (α-CH₂), 36.01 (α-CH₂), 18.67 (β-CH₂), 18.36 (β-CH₂), 13.61 (CH₃), 13.45 (CH₃).

HRMS: m/z found 1009.3413, calculated for C₅₉H₅₄NaO₁₄⁺ 1009.3406 [M+Na]⁺; m/z found 1025.3158, calculated for C₅₉H₅₄KO₁₄⁺ 1025.31451 [M+K]⁺.

(7-Diethylamino-2-oxo-2H-chromen-4-ylmethyl)-N,N',N'-tetraisopropylphosphoramidite 12

A stirring mixture of bis(diisopropylamino)chlorophosphine (5.0 g, 18.7 mmol) and dry THF (20 mL) was cooled in an ice bath and dry NEt₃ (4.0 mL, 28.7 mmol) and a solution of 11 [2, 3] (4.0 g, 16.2 mmol) in anhydrous THF (60 mL) was added via a syringe under argon. After 30 min, ³¹P NMR indicated complete consumption of the chlorophosphine. After 1 h the suspension was diluted with cyclohexane:NEt₃ (99:1, 100 mL) and solids were removed by filtration, washed with cyclohexane (2 x 20 mL) and the dark filtrate was concentrated under reduced pressure. The crude was purified by chromatography on deactivated (cyclohexane:NEt₃ 9:1) silica with n-pentane:DCM:NEt₃ 75:24:1.

Yield: 5.14 g (67%) white solid, Rf cyclohexane:DCM:NEt₃ 75:24:1 = 0.52.

¹H NMR (400 MHz, CDCl₃) δ = 7.30 (d, J=9.0, 1H, H-5), 6.57 (dd, J=8.9, 2.5, 1H, H-6), 6.52 (d, J=2.5, 1H, H-8), 6.35 (s, 1H, H-3), 4.74 (d, J=6.9, 2H, CH₂O), 3.70 – 3.52 (m, 4H, 4 x CH), 3.42 (q, J=7.1, 4H, N(CH₂CH₃)₂), 1.34 – 1.08 (m, 30H, N(CH₂CH₃)₂, 8 x CH₃).

³¹P NMR (162 MHz, CDCl₃) δ = 122.53 (s, 1P).

¹³C NMR (101 MHz, CDCl₃) δ = 162.57, 156.02, 154.02, 153.93, 150.32, 124.17, 108.40, 106.52, 105.82, 97.75, 77.37, 77.05, 76.73, 62.02 (d, JCP=24.3, CH₂O), 44.73 (d, JCP=12.7, 4 x CH), 44.70 (s, 2 x CH₂CH₃), 24.70, 24.62, 24.00, 23.95, 12.44 (s, 2 x CH₂CH₃).

HRMS: m/z found 476.3057, calculated for C₂₆H₄₃N₃O₃P⁻ 476.3048 [M-H]⁻.

Synthesis of phosphoramidites 13-16

3-O-(7-Diethylamino-2-oxo-2H-chromen-4-ylmethyl) 2-O-arachidonyl-1-O-(3’(4’”-pentyn-1’”-yl)-H-diazirine-3’-octanoyl)-sn-glycero-N,N-diisopropylphosphoramidite P-diastereomeric mixture (13)
A solution of 2-\textit{O}-arachidonoyl-1-\textit{O}-stearoyl-\textit{sn}-glycerol [4] (158 mg, 253 µmol) in toluene (15 mL) and 1\textit{H}-tetrazole solution in MeCN (~0.45 M, 560 µL, 252 µmol) was evaporated under reduced pressure and dried at 23 °C/0.04 mbar. Under argon atmosphere and cooling in an ice bath a solution of phosphoramidite 12 (121 mg, 253 µmol) in anhydrous DCM (3 mL) was added. The cooling bath was removed and stirring was continued at 23 °C. After 1.5 h the mixture was diluted with cyclohexane (10 mL, containing 200 µL, 1.43 mmol NEt\textsubscript{3}) and concentrated under reduced pressure. The residue was purified by chromatography on deactivated (n-heptane:NEt\textsubscript{3} 9:1) silica with n-heptane:EtOAc:NEt\textsubscript{3} 90:9:1 \(\rightarrow\) 80:19:1.

Yield: 168 mg (66%) yellow oil, R\(\text{f}\) heptane:EtOAc:NEt\textsubscript{3} 92:7:1 = 0.02.

\(\text{\textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) } \delta = 7.36 – 7.31 (m, 1H, H-5), 6.57 (dd, J=8.8, 2.8, 1H, H-6), 6.53 (d, J=2.5, 1H, H-8), 6.26 (s, 1H, H-3), 5.37 (d, J=5.2, 8H), 5.24 (s, 1H), 4.78 (d, J=12.2, 2H), 4.44 – 4.32 (m, 1H), 4.26 – 4.14 (m, 1H), 3.90 – 3.59 (m, 4H), 3.43 (q, J=7.1, 4H), 2.90 - 2.76 (m, 6H, 3 x CH\textsubscript{2}(CH=CH)\textsubscript{2}), 2.41 – 2.27 (m, 4H), 1.97 (t, J=6.9, 3H).

\(\text{\textsuperscript{31}P NMR (162 MHz, CDCl\textsubscript{3}) } \delta = 149.98 (s, 0.54 P, dia-1), 149.81 (s, 0.46 P, dia-2).

\(\text{\textsuperscript{13}C NMR (101 MHz, CDCl\textsubscript{3}) } \delta = 173.26 (C=O), 172.71 (C=O), 172.69, 156.13, 152.74, 152.69, 152.66, 152.62, 150.42, 130.45, 128.88, 128.83, 128.57, 128.24, 128.10, 127.83, 127.52, 124.35, 106.34, 106.14, 97.76, 83.40, 77.40, 77.08, 76.76, 70.81, 70.73, 68.88, 62.30, 62.24, 61.83, 61.67, 61.60, 61.54, 61.50, 61.44, 61.36, 61.31, 44.69, 43.33, 43.32, 43.21, 43.20, 34.02, 33.68, 32.81, 31.80, 31.49, 29.30, 29.17, 29.10, 29.09, 29.01, 28.35, 28.29, 27.19, 26.51, 25.62, 25.60, 24.80, 24.77, 24.71, 24.65, 24.58, 23.77, 22.73, 22.55, 17.93, 14.05, 12.44.

HRMS: m/z found 1023.6340, calculated for C\textsubscript{58}H\textsubscript{89}N\textsubscript{4}NaO\textsubscript{8}P\textsuperscript{+} 1023.63102 [M+Na]\textsuperscript{+}.

A solution of 2-\textit{O}-arachidonoyl-1-\textit{O}-stearoyl-\textit{sn}-glycerol [4-6] (417 mg, 646 µmol) in toluene (15 mL) and 1\textit{H}-tetrazole solution in MeCN (~0.45 M, 1.3 mL, 585 µmol) was evaporated...
under reduced pressure and dried at 23°C/0.04 mbar. Under argon atmosphere and cooling in an ice bath, a solution of phosphoramidite \(\text{12} \) (350 mg, 733 µmol) in anhydrous DCM (5 mL) was added. The cooling bath was removed and stirring was continued at 23°C. After 1.5 h the mixture was diluted with cyclohexane (10 mL, containing 200 µL, 1.43 mmol NEt\(_3\)) and concentrated under reduced pressure. The residue was purified by chromatography on deactivated (cyclohexane:NEt\(_3\) 9:1) silica with cyclohexane:EtOAc:NEt\(_3\) 92:7:1.

Yield: 606 mg (92%) yellow oil, \(R_f\) cyclohexane:EtOAc:NEt\(_3\) 92:7:1 = 0.13.

Product contains 12.5 mol% \(\text{12} \) (\(^{31}\)P NMR).

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta = 7.36 – 7.30 \text{ ppm} \) (m, 1H, H-5), 6.58 (dd, \(J = 9.0, 2.5, 1H, H-6\)), 6.52 (d, \(J = 2.5, 1H, H-8\)), 6.26 (s, 1H, H-3), 5.49 – 5.29 (m, 8H, 4 x CH=CH), 5.28 – 5.19 (m, 1H), 4.87 – 4.68 (m, 2H), 4.44 – 4.31 (m, 1H), 4.26 – 4.09 (m, 1H), 3.89 – 3.79 (m, 3H), 3.42 (q, \(J = 7.1\), 4H, (CH\(_2\)CH\(_3\))\(_2\)), 2.96 – 2.67 (m, 6H, 3 x CH\(_2\)(CH=CH)\(_2\)), 2.42 – 2.25 (m, 4H), 2.18 – 1.99 (m, 4H), 1.78 – 1.66 (m, 2H), 1.66 – 1.55 (m, 2H), 1.42 – 1.18 (m, 52H), 0.94 – 0.83 (m, 6H).

\(^{31}\)P NMR (162 MHz, CDCl\(_3\)) \(\delta = 149.95 \) (s, 0.5 P, dia-1), 149.79 (s, 0.5 P, dia-2).

\(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta = 173.35, 172.73, 162.24, 156.13, 150.41, 130.46, 128.84, 128.57, 128.24, 128.11, 127.83, 124.35, 108.43, 106.14, 97.76, 77.37, 77.05, 76.74, 44.70, 34.09, 33.69, 31.92, 31.51, 29.70, 29.49, 29.36, 29.32, 29.14, 27.21, 26.91, 26.52, 25.60, 24.90, 24.77, 24.65, 22.69, 22.57, 14.11, 14.06, 12.43.

HRMS: m/z found 1021.7396, calculated for C\(_{61}\)H\(_{102}\)N\(_2\)O\(_8\)P\(^+\) 1021.7368 [M+H]\(^+\); m/z found 1043.7206, calculated for C\(_{61}\)H\(_{101}\)N\(_2\)NaO\(_8\)P\(^+\) 1043.7188 [M+Na]\(^+\).

2-\(\text{O}-\text{Arachidonoyl-1-O-stearoyl-sn-glycero-3-(9H-fluorene-9-ylmethyl diisopropylphosphoramidite) P-diastereomeric mixture (15)}\)

2-\(\text{O}-\text{Arachidonoyl-1-O-stearoyl-sn-glycero-3-(9H-fluorene-9-ylmethyl diisopropylphosphoramidite) P-diastereomeric mixture (15)}\)

\(2-O-\text{Arachidonoyl-1-O-stearoyl-sn-glycerol [4-6]} \) (415 mg, 0.64 mmol) in toluene (10 mL) was evaporated under reduced pressure. \(1H\)-tetrazole solution in MeCN (0.45 M, 1.43 mL, 0.64 mmol) was added and the mixture was evaporated at 0.4 mbar for 1 h. Under an argon atmosphere, a solution of 9\(H\)-fluorene-9-ylmethyl \(N,N,N',N'-\text{tetraisopropylphosphoramidite [7, 8]} \) (275 mg, 0.64 mmol) in anhydrous DCM (5 mL) was added. The suspension was cooled in an ice bath. After 30 min the cooling bath was removed and stirring was continued at 20 °C. After 2 h the mixture was diluted with cyclohexane (10 mL) and concentrated under reduced pressure. The residue was purified by chromatography on deactivated (eluent:NEt\(_3\) 9:1) silica with cyclohexane:EtOAc:NEt\(_3\) 92:7:1.

Yield: 584 mg (94%) colorless oil, \(R_f\) cyclohexane:EtOAc:NEt\(_3\) 92:7:1 = 0.88.

Purity: ~96% (NMR).
$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ = 7.80 – 7.59 (m, 4H), 7.45 – 7.24 (m, 4H), 5.46 – 5.26 (m, 8H, 4 x CH=CH), 5.24 – 5.15 (m, 1H), 4.40 – 4.31 (m, 1H), 4.22 – 4.12 (m, 2H), 4.05 – 3.97 (m, 1H), 3.87 – 3.50 (m, 5H), 2.90 – 2.74 (m, 6H, 3 x CH$_2$(CH=CH)$_2$), 2.36 – 2.22 (m, 4H), 2.14 – 2.00 (m, 4H), 1.75 – 1.52 (m, 4H), 1.40 – 1.20 (m, 34H), 1.20 – 1.10 (m, 12H, 2 x CH(CH$_3$)$_2$), 0.93 – 0.83 (m, 6H).

$^{31}$P NMR (162 MHz, CDCl$_3$) $\delta$ = 148.25 (s, 0.5 P, dia-1), 148.17 (s, 0.5 P, dia-2).

$^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ = 173.40, 172.75, 144.88, 144.87, 144.51, 144.50, 141.37, 141.27, 130.50, 128.89, 128.87, 128.74, 128.60, 128.26, 128.15, 127.87, 127.73, 127.55, 127.46, 127.42, 127.36, 127.05, 126.97, 126.90, 126.89, 126.84, 125.44, 125.20, 125.16, 119.98, 119.85, 119.79, 77.37, 77.05, 76.74, 70.96, 70.90, 70.88, 66.24, 66.18, 66.07, 66.01, 62.48, 62.44, 61.68, 61.51, 61.48, 61.31, 49.22, 49.16, 49.09, 43.12, 43.00, 34.14, 33.73, 31.95, 31.54, 29.73, 29.69, 29.67, 29.51, 29.39, 29.35, 29.32, 29.17, 27.24, 26.93, 26.53, 25.65, 25.63, 25.62, 24.92, 24.88, 24.79, 24.70, 24.63, 24.59, 24.52, 22.72, 22.60, 14.15, 14.10.

HRMS: m/z found 1008.68592, calculated for C$_{61}$H$_{96}$KNO$_6$P + 1008.66126 [M+K]$^+$; m/z found 1008.68592, calculated for C$_{61}$H$_{96}$NNaO$_7$P + 1008.68224 [M+O+Na]$^+$.

$2$-O-Arachidonoyl-$1$-$O$-stearoyl-$sn$-glycerol $[4$-$6$] (390 mg, 605 µmol) in toluene (10 mL) and EtOAc (10 mL) was evaporated under reduced pressure. $1H$-tetrazole solution in MeCN (~0.45 M, 1.2 mL, 540 µmol) was added and the mixture was evaporated at 0.4 mbar for 1 h. Under argon atmosphere a solution of 4-acetoxybenzyl $N$,$N$,$N'$,$N'$-tetraisopropylphosphoramidite $[9, 10]$ (240 mg, 605 µmol) in anhydrous DCM (5 mL) was added and the suspension was stirred at 24 °C. After 2 h the mixture was diluted with n-heptane (10 mL containing 1% NEt$_3$) and applied on top of a column of deactivated (eluents:NEt$_3$ 9:1) silica. The compound was eluted with n-heptane:EtOAc:NEt$_3$ 92:7:1.

Yield: 500 mg (88%) colorless oil.

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ = 7.37 (d, $J$=8.4, 2H), 7.13 – 7.02 (m, 2H), 5.49 – 5.29 (m, 8H, 4 x CH=CH), 5.27 – 5.16 (m, 1H), 4.80 – 4.58 (m, 2H), 4.43 – 4.29 (m, 1H), 4.24 – 4.16 (m, 1H), 3.87 – 3.70 (m, 2H), 3.70 – 3.55 (m, 2H), 2.94 – 2.73 (m, 6H, 3 x CH$_2$(CH=CH)$_2$), 2.40 – 2.26 (m, 4H), 2.31 (s, 3H, CH$_3$CO), 2.19 – 2.02 (m, 4H), 1.79 – 1.67 (m, 2H), 1.67 – 1.55 (m, 2H), 1.49 – 1.24 (m, 34H), 1.24 – 1.11 (m, 12H, 2 x CH(CH$_3$)$_2$), 0.95 (m, 6H).

$^{31}$P NMR (162 MHz, CDCl$_3$) $\delta$ = 148.93 (s, 0.5 P, dia-1), 148.75 (s, 0.5 P, dia-2).
$^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ = 173.38 (C=O), 172.74 (C=O), 169.47 (C=O), 149.85, 137.00, 136.97, 136.92, 136.89, 130.48, 128.89, 128.86, 128.58, 128.24, 128.14, 127.96, 127.85, 127.54, 121.38, 77.36, 77.04, 76.73, 70.94, 70.90, 70.87, 70.82, 64.90, 64.86, 64.72, 64.68, 62.46, 62.42, 61.76, 61.59, 61.42, 60.38, 43.14, 43.02, 34.12, 33.72, 31.93, 31.52, 29.71, 29.67, 29.65, 29.50, 29.37, 29.33, 29.31, 29.15, 27.22, 26.53, 25.64, 25.61, 25.60, 24.90, 24.78, 24.68, 24.61, 24.54, 24.52, 22.70, 22.58, 21.12, 21.04, 14.20, 14.12, 14.08.

HRMS: m/z found 940.6788, calculated for C$_{56}$H$_{95}$NO$_8$P$^+$ 940.67898 [M+H]$^+$; m/z found 962.6607, calculated for C$_{56}$H$_{94}$NaO$_8$P$^+$ 962.66093 [M+Na]$^+$. 
Synthesis of protected phosphoinositides 17a-d

1-O-(2-O-Arachidonyl-1-O-(3′(4′-pentyn-1′-yl)-H-diazirine-3′-octanoyl)-sn-glycero)phosphoryl-2,6-di-O-butyryl-3,4,5-tri-O-(9H-flourene-9-ylmethoxycarbonyl) myo-inositol-(7-diethylamino-2-oxo-2H-chromen-4-ylmethyl) ester P-diastereomeric mixture (17a)

A solution of 7 (86 mg, 86 µmol) in toluene (15 mL) and 1H-tetrazole solution in MeCN (~0.45 M, 1.5 mL, 675 µmol) was evaporated under reduced pressure and dried at 23°C/0.01 mbar. Under argon atmosphere a solution of phosphoramidite 13 (90 mg, 90 µmol) in anhydrous DCM (3.2 mL) was added with stirring. After 2 h the mixture was diluted with DCM (10 mL) and cooled in a dry ice/acetone bath. Peracetic acid solution (11.2 µL, 90 µmol) was added and the cooling bath removed. After 1 h volatiles were removed under reduced pressure. The crude was purified by preparative HPLC (MeOH:EtOAc 4:1).

Yield: 154 mg (93%) yellow oil, \( t_R \) MeOH:EtOAc 4:1 = 6.0 min.

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta = 7.80 – 7.57 \) (m, 6H), 7.57 – 7.08 (m, 19H, H-5), 6.67 – 6.55 (m, 1H, H-6), 6.55 – 6.48 (m, 1H, H-8), 6.25 – 6.16 (m, 1H, H-3), 6.05 (t, \( J=2.8 \), 0.4H), 6.03 (t, \( J=2.7 \), 0.6H), 5.76 (t, \( J=10.0 \), 1H), 5.63 – 5.54 (m, 1H), 5.47 – 5.05 (m, 4 x CH=CH), 4.88 – 4.72 (m, 1H), 4.52 – 4.02 (m, 13H), 3.48 – 3.29 (m, 4H, N(CH\(_2\)CH\(_3\))), 2.94 – 2.71 (m, 3 x CH\(_2\)(CHCH)), 2.64 – 2.44 (m, 2H), 2.43 – 2.28 (m, 5H), 2.26 – 2.02 (m, 7H), 1.97 (t, \( J=2.6 \), 1H, CCH), 1.86 – 1.43 (m, 10H), 1.44 – 1.14 (m, 24H), 1.13 – 0.98 (m, 5H), 0.95 – 0.73 (m, 6H).

\(^{31}\)P NMR (162 MHz, CDCl\(_3\)) \( \delta = -1.72 \) (s, 0.34 P, dia-1), -1.82 (s, 0.66 P, dia-2).

\(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \( \delta = 173.22, 173.17, 172.61, 172.56, 172.41, 172.38, 172.25, 172.16, 161.52, 161.42, 156.34, 156.32, 154.13, 153.75, 153.73, 150.79, 150.75, 148.53, 148.39, 148.30, 143.18, 143.14, 142.96, 142.93, 142.81, 141.24, 141.16, 141.08, 141.05, 130.50, 128.96, 128.92, 128.80, 128.61, 128.29, 128.13, 127.87, 127.82, 127.75, 127.56, 127.18, 127.10, 125.24, 125.22, 125.18, 125.10, 125.01, 124.36, 124.32, 120.01, 119.96, 119.90, 108.74, 106.75, 106.58, 105.43, 105.42, 97.83, 83.47, 77.40, 77.28, 77.21, 77.08, 76.76, 74.46, 73.64, 73.57, 73.55, 72.17, 70.84, 70.81, 70.77, 69.31, 69.24, 68.93, 68.19, 66.39, 66.33, 65.42, 65.37, 61.38, 61.34, 46.41, 46.22, 46.17, 44.75, 44.72, 36.05, 36.00, 35.87, 35.84, 33.88, 33.49, 32.83, 31.81, 31.52, 29.33, 29.22, 29.14, 29.12, 29.04, 28.42, 27.22, 26.47, 25.64, 25.62, 24.73, 24.67, 23.81, 22.76, 22.59, 18.73, 18.67, 18.25, 18.14, 17.96, 14.22, 14.10, 13.57, 13.55, 13.51, 13.45, 12.43.

HRMS: m/z found 1924.8600, calculated for C\(_{111}H_{128}N_{3}NaO_{23}P^{+} \) 1924.8568 [M+Na]\(^+\).
1-O-(2-O-Arachidonyl-1-O-stearoyl-sn-glycero)phosphoryl-2,6-di-O-butyryl-3,4,5-tri-O-(9H-fluorene-9-ylmethoxycarbonyl) myo-inositol-(7-diethylamino-2-oxo-2H-chromen-4-ylmethyl) ester P-diastereomeric mixture (17b)

A solution of 7 (100 mg, 101 µmol) in toluene (15 mL) and 1H-tetrazole solution in MeCN (~0.45 M, 1.3 mL, 585 µmol) was evaporated under reduced pressure and dried at 23°C/0.04 mbar. Under an argon atmosphere, a solution of phosphoramidite 14 (103 mg, 102 µmol) in anhydrous DCM (5 mL) was added with stirring. After 2 h the mixture was diluted with DCM (10 mL) and cooled in a dry ice/acetone bath. Peracetic acid solution (12.7 µL, 102 µmol) was added and the cooling bath removed. After 1 h volatiles were removed under reduced pressure. The crude was purified on a column of LiChroprep RP18 (40-63 µm, 100 g) with MeOH:EtOAc 1:0 → 2:1.

Yield: 159 mg (82%) pale yellow oil, t<sub>R</sub> MeOH:EtOAc 4:1 = ~20 min.

1H NMR (400 MHz, CDCl<sub>3</sub>) δ = 7.78 – 7.15 (m, 25H, H-5), 6.66 – 6.55 (m, 1H, H-6), 6.55 – 6.48 (m, 1H, H-8), 6.25 – 6.18 (m, 1H, H-3), 6.03 (t, J=2.8, 0.5H), 6.01 (t, J=2.8, 0.5H), 5.80 – 5.70 (m, 1H), 5.61 – 5.53 (m, 1H), 5.47 – 5.05 (m, 13H), 4.83 – 4.71 (m, 1H), 4.52 – 4.39 (m, 1H), 4.39 – 4.02 (m, 12H), 3.48 – 3.34 (m, 4H, N(CH<sub>2</sub>CH<sub>3</sub>)<sub>2</sub>), 2.89 – 2.74 (m, 6H, 3 x CH<sub>2</sub>(CHCH)<sub>2</sub>), 2.64 – 2.47 (m, 2H), 2.43 – 2.27 (m, 5H), 2.24 (t, J=7.5, 1H), 2.18 – 2.02 (m, 4H), 1.87 – 1.42 (m, 8H), 1.42 - 1.15 (m, 40H), 1.10 – 0.74 (m, 12H).

31P NMR (162 MHz, CDCl<sub>3</sub>) δ = -1.69 (s, 0.5 P, dia-1), -1.76 (s, 0.5 P, dia-2).

13C NMR (101 MHz, CDCl<sub>3</sub>) δ = 173.28, 173.23, 172.61, 172.57, 172.42, 172.39, 172.27, 172.19, 161.51, 161.41, 156.35, 156.33, 154.15, 153.77, 153.75, 150.78, 150.73, 148.53, 148.44, 148.40, 148.31, 143.19, 143.15, 142.98, 142.96, 142.94, 142.81, 141.25, 141.17, 141.09, 141.06, 130.49, 128.96, 128.92, 128.81, 128.60, 128.28, 128.14, 128.01, 127.88, 127.82, 127.75, 127.57, 127.18, 127.10, 125.25, 125.22, 125.19, 125.01, 124.38, 124.34, 120.01, 119.97, 108.74, 108.72, 106.78, 106.63, 105.45, 105.44, 97.83, 77.43, 57.11, 76.79, 74.47, 73.65, 73.60, 73.54, 72.18, 70.81, 70.78, 69.34, 69.29, 69.27, 68.22, 66.47, 66.41, 66.39, 66.34, 65.42, 65.39, 65.30, 65.27, 61.38, 61.34, 46.42, 46.23, 46.17, 44.75, 44.71, 36.06, 36.01, 35.88, 35.84, 33.95, 33.50, 31.95, 31.53, 29.73, 29.69, 29.54, 29.46, 29.39, 29.34, 29.17, 27.23, 26.48, 25.65, 25.63, 24.82, 24.68, 22.72, 22.60, 18.74, 18.68, 18.26, 18.15, 14.22, 14.16, 14.11, 13.58, 13.52, 13.45, 12.43.

HRMS: m/z found 1944.9484, calculated for C<sub>114</sub>H<sub>140</sub>N<sub>Na</sub>O<sub>5</sub>P<sup>+</sup> 1944.9446 [M+Na]<sup>+</sup>.

1-O-(2-O-Arachidonyl-1-O-stearoyl-sn-glycero)phosphoryl-2,6-di-O-butyryl-3,4,5-tri-O-(9H-fluorene-9-ylmethoxycarbonyl) myo-inositol-(9H-fluorene-9-ylmethyl) ester P-diastereomeric mixture (17c)
A solution of 7 (148 mg, 150 µmol) in toluene (20 mL) and 1H-tetrazole solution in MeCN (~0.45 M, 1.33 mL, 600 µmol) was evaporated under reduced pressure and dried at 24 °C/0.002 mbar. Under an argon atmosphere, a solution of phosphoramidite 15 (180 mg, 185 µmol) in anhydrous DCM (5 mL) was added with stirring. After 2 h the mixture was diluted with DCM (30 mL) and cooled in a dry ice/acetone bath. Peracetic acid solution (25 µL, 185 µmol) was added and the cooling bath was removed. After 1 h volatiles were removed under reduced pressure. 31P NMR indicated complete oxidation. The crude was purified by preparative HPLC (MeOH:EtOAc 9:1 → 4:1).

Yield: 285 mg (99.8%) colorless oil, tR MeOH:EtOAc 4:1 = min.

1H NMR (400 MHz, CDCl3) δ = 8.00 – 7.14 (m, 32H), 5.90 (t, J=2.8, 0.41H), 5.83 (t, J=10.2, 1H), 5.46 – 5.28 (m, 8H, 4 x CH=CH), 5.26 – 5.15 (m, 1H), 5.14 – 4.94 (m, 2H), 4.65- 4.42 (m, 3H), 4.35 – 3.89 (m, 14H), 2.93 – 2.71 (m, 6H, 3 x CH2(CHCH)2), 2.69 – 2.38 (m, 2H), 2.38 – 2.22 (m, 4H), 2.18 – 1.99 (m, 4H), 1.97 – 1.45 (m, 8H), 1.44 – 1.17 (m, 36H), 1.08 – 0.66 (m, 12H).

31P NMR (162 MHz, CDCl3) δ = -1.74 (s, 0.42 P, dia-1), -3.04 (s, 0.58 P, dia-2).

13C NMR (101 MHz, CDCl3) δ = 173.25, 173.21, 172.55, 172.52, 172.29, 172.17, 172.12, 154.22, 154.19, 153.82, 153.81, 143.25, 143.05, 143.02, 142.97, 142.91, 142.90, 142.87, 142.63, 141.82, 141.64, 141.61, 141.28, 141.22, 141.19, 141.11, 141.10, 130.65, 130.62, 130.52, 129.11, 129.00, 128.94, 128.86, 128.82, 128.65, 128.52, 128.51, 128.44, 128.33, 128.21, 128.13, 128.04, 127.91, 127.89, 127.85, 127.79, 127.59, 127.46, 127.41, 127.34, 127.21, 127.15, 127.11, 125.55, 125.47, 125.44, 125.29, 125.23, 125.16, 125.13, 125.07, 124.97, 124.81, 120.38, 120.35, 120.25, 120.21, 120.14, 120.05, 120.00, 119.78, 77.47, 77.35, 77.27, 77.15, 77.00, 76.99, 76.97, 76.83, 74.40, 74.32, 73.72, 73.66, 73.08, 73.03, 72.80, 72.76, 72.25, 72.18, 70.82, 70.64, 70.59, 69.50, 69.38, 69.32, 69.21, 69.18, 69.09, 69.04, 68.99, 68.93, 68.13, 67.97, 66.14, 66.09, 65.92, 65.87, 61.61, 61.53, 60.41, 48.17, 48.10, 48.02, 47.94, 46.46, 46.28, 46.23, 46.16, 46.07, 46.03, 36.03, 35.97, 35.63, 35.29, 34.08, 33.98, 33.52, 33.50, 31.99, 31.81, 31.56, 30.05, 29.97, 29.77, 29.57, 29.43, 29.37, 29.19, 29.10, 27.27, 26.51, 25.85, 25.69, 25.67, 25.49, 25.47, 24.86, 24.72, 24.69, 22.76, 22.63, 21.09, 18.73, 18.64, 18.17, 18.07, 14.25, 14.20, 14.15, 14.03, 13.58, 13.56, 13.51, 13.39.

MS: Calculated m/z for C114H135NaO21P+ 1893.91257, found 1894.9 [M+Na]⁺.

1-O-(2-O-Arachidonyl-1-O-stearoyl-sn-glycero)phosphoryl-2,6-di-O-butyryl-3,4,5-tri-O-(9H-fluorene-9-ylmethoxycarbonyl) myo-inositol-(4-acetoxybenzyl) ester P-diastereomeric mixture (17d)
A solution of 7 (94 mg, 150 µmol) in toluene (20 mL) and 1H-tetrazole solution in MeCN (~0.45 M, 422 µL, 190 µmol) was evaporated under reduced pressure and dried at 24 °C/0.002 mbar. Under argon atmosphere a solution of phosphoramidite 16 (103 mg, 110 µmol) in anhydrous DCM (2 mL) was added with stirring. After 3 h the mixture was diluted with DCM (30 mL) and cooled in a dry ice/acetone bath. Peracetic acid solution (14 µL, 110 µmol) was added and the cooling bath was removed. After 1 h volatiles were removed under reduced pressure. The crude (250 mg) was purified by preparative HPLC (MeOH:EtOAc 4:1 \(\rightarrow\) 7:3).

Yield: 166.0 mg (94%).

\(^1\)H NMR (400 MHz, CDCl\(_3\)) δ = 7.93 – 7.17 (m, 30H), 7.14 (d, \(J=6.9\), 2H), 6.01 (s, 1H), 5.76 (s, 1H), 5.59 (s, 1H), 5.40 (s, 9H), 5.31 – 4.93 (m, 6H), 4.82 – 4.58 (m, 1H), 4.55 – 4.39 (m, 1H), 4.39 – 3.93 (m, 12H), 2.94 – 2.75 (m, 6H, 3 x CH\(_2\)(CHCH)\(_2\)), 2.64 – 2.44 (m, 2H), 2.44 – 2.25 (m, 8H), 2.20 – 2.02 (m, 5H), 1.86 – 1.67 (m, 4H), 1.67 – 1.19 (m, 38H), 1.12 – 0.74 (m, 12H).

\(^{31}\)P NMR (162 MHz, CDCl\(_3\)) δ = -1.63 (s, 0.5 P, dia-1), -1.92 (s, 0.5 P, dia-2).

\(^{13}\)C NMR (101 MHz, CDCl\(_3\)) δ = 173.26, 173.20, 172.57, 172.53, 172.29, 172.24, 172.21, 169.16, 169.14, 154.16, 153.74, 151.05, 143.21, 143.18, 142.99, 142.84, 141.25, 141.18, 141.08, 141.06, 132.83, 132.74, 132.67, 130.51, 129.38, 129.30, 128.96, 128.92, 128.86, 128.83, 128.62, 128.31, 128.29, 128.14, 128.13, 127.91, 127.87, 127.84, 127.81, 127.74, 127.56, 127.18, 127.10, 125.23, 125.20, 125.17, 125.12, 125.03, 122.04, 121.98, 120.02, 119.95, 77.39, 77.07, 76.75, 74.51, 73.60, 73.53, 73.26, 73.05, 72.28, 72.24, 70.80, 70.76, 69.45, 69.40, 69.30, 68.21, 68.08, 65.98, 65.85, 61.51, 46.43, 46.23, 46.18, 36.03, 35.99, 35.90, 35.77, 33.97, 33.53, 33.50, 31.95, 31.53, 29.73, 29.68, 29.54, 29.39, 29.34, 29.17, 27.23, 26.49, 25.65, 25.63, 24.83, 24.69, 22.72, 22.60, 21.09, 21.08, 18.71, 18.67, 18.22, 18.08, 14.15, 14.10, 13.56, 13.55, 13.53, 13.43.

MS: Calculated m/z for C\(_{109}H\(_{134}\)O\(_{23}\)P\(^+\) 1841.90480, found 1842.9 [M+H]\(^+\); calculated m/z for C\(_{109}H\(_{133}\)NaO\(_{23}\)P\(^+\) 1863.88675, found 1864.9 [M+Na]\(^+\), calculated m/z for C\(_{109}H\(_{133}\)KO\(_{23}\)P\(^+\) 1879.86069, found 1881.1 [M+K]\(^+\).
Synthesis of target compounds 1a-d

1-O-(2-O-Arachidonyl-1-O-(3′(4′′-pentyln-1′′-yl)-H-diazirine-3′-octanoyl)-sn-glycero)phosphoryl-2,6-di-O-butyryl-myoinositol-(7-diethylamino-2-oxo-2H-chromen-4-ylmethyl) ester P-diastereomeric mixture (1a)

To a solution of dry 17a (160 mg, 84 µmol) in MeCN (3 mL) was added dimethylethylamine (1 mL, 9.2 mmol) with stirring at 23°C. After 2 h volatiles were removed under reduced pressure. The crude was purified by preparative HPLC (95% MeOH).

Yield: 68 mg (65%) yellow oil, tR 100% MeOH = 4.3 min, 95% MeOH = 13.5 min.

1H NMR (400 MHz, CDCl3) δ = 7.29 (d, J=9.0, 0.39 H, H-5), 7.25 (d, J=9.0, 0.61H), 6.65 – 6.55 (m, 1H, H-5), 6.55 – 6.47 (m, 1H, H-8), 6.24 – 6.15 (m, 1H, H-3), 5.78 – 5.70 (m, 1H), 5.47 – 5.20 (m, 10H, 4 x CH=CH), 5.20 – 5.01 (m, 2H), 4.65 – 4.50 (m, 1H), 4.43 – 4.30 (m, 1H), 4.30 – 3.50 (m, 6H), 3.42 (q, J=7.0, 4H, N(CH2CH3)2), 2.90 – 2.70 (m, 6H, 3 x CH2(CHCH)2), 2.48 – 2.20 (m, 8H), 2.20 – 2.14 (m, 2H), 2.14 – 2.00 (m, 4H), 1.96 (t, J=2.6, 1H, CCH), 1.76 – 1.52 (m, 8H), 1.52 – 1.44 (m, 2H), 1.42 – 1.13 (m, 2H), 1.30 – 1.01 (m, 2H), 1.00 – 0.78 (m, 9H).

31P NMR (162 MHz, CDCl3) δ = -1.66 (s, 0.57 P, dia-1), -1.98 (s, 0.43 P, dia-2).

13C NMR (101 MHz, CDCl3) δ = 173.47, 173.39, 173.30, 173.25, 173.15, 172.75, 172.58, 162.06, 161.77, 156.24, 156.17, 150.83, 130.49, 128.99, 128.94, 128.76, 128.72, 128.61, 128.30, 128.08, 127.83, 127.52, 124.34, 124.23, 108.90, 108.83, 106.19, 105.81, 105.37, 105.34, 97.79, 97.72, 83.45, 77.35, 77.24, 77.04, 76.86, 76.85, 76.72, 74.87, 74.82, 73.30, 72.70, 72.66, 72.13, 72.10, 72.08, 71.41, 71.34, 69.65, 69.44, 69.35, 69.26, 68.90, 66.20, 66.15, 65.13, 65.08, 61.68, 61.49, 44.75, 36.11, 36.08, 35.99, 35.93, 33.91, 33.86, 33.52, 33.47, 32.82, 31.81, 31.50, 29.31, 29.20, 29.12, 29.10, 29.02, 28.41, 27.20, 26.44, 25.62, 25.60, 24.72, 24.67, 23.79, 22.74, 22.56, 18.55, 18.48, 18.23, 18.13, 17.94, 14.07, 13.61, 13.58, 13.54, 13.51, 12.42.

HRMS: m/z found 1258.6548, calculated for C66H98N3NaO17P+ 1258.6526 [M+Na]+.

1-O-(2-O-Arachidonyl-1-O-stearoyl-sn-glycero)phosphoryl-2,6-di-O-butyryl-myoinositol (7-diethylamino-2-oxo-2H-chromen-4-ylmethyl) ester P-diastereomeric mixture (1b)
A solution of 17b (155 mg, 81 µmol) in MeCN (5 mL) was evaporated at 23 °C/0.04 mbar. Under argon atmosphere the yellow oil was dissolved in MeCN (1.5 mL) and dimethylethylamine (0.5 mL, 4.6 mmol) with stirring. After 2 h the solution was diluted with MeCN (10 mL) and evaporated under reduced pressure. The crude was purified by preparative HPLC (MeOH:EtOAc 5:1).

Yield: 100 mg (99%) yellow oil, t<sub>R</sub> MeOH:EtOAc 4:1 = 5 min.

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ = 7.29 (d, <i>J</i>=9.1, 0.46H, H-5), 7.26 (d, <i>J</i>=9.1, 0.54H, H-5), 6.67 – 6.55 (m, 1H, H-6), 6.55 – 6.47 (m, 1H, H-8), 6.24 – 6.15 (m, 1H, H-3), 5.48 – 5.20 (m, 10H), 5.20 – 5.01 (m, 2H), 4.70 – 4.51 (m, 1H), 4.46 – 4.22 (m, 2H), 4.22 – 4.05 (m, 3H), 4.04 – 3.69 (m, 4H), 3.65 – 3.51 (m, 1H), 3.42 (q, <i>J</i>=7.2, 4H, N(CH<sub>2</sub>CH<sub>3</sub>)<sub>2</sub>), 2.90 – 2.74 (m, 6H, 3 x CH<sub>2</sub>(CHCH)<sub>2</sub>), 2.50 – 2.16 (m, 8H), 2.16 – 1.98 (m, 4H), 1.76 – 1.45 (m, 8H), 1.45 – 1.01 (m, 40H), 1.02 – 0.80 (m, 12H).

<sup>31</sup>P NMR (162 MHz, CDCl<sub>3</sub>) δ = -1.65 (s, 0.56 P, dia-1), -1.94 (s, 0.44 P, dia-2).

<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ = 173.40, 173.35, 173.33, 173.28, 173.26, 173.20, 172.74, 172.55, 162.14, 161.83, 156.21, 156.14, 150.82, 149.40, 149.04, 148.96, 130.46, 128.94, 128.89, 128.76, 128.72, 128.58, 128.27, 128.08, 127.82, 127.52, 124.38, 124.25, 108.92, 108.85, 106.11, 105.76, 105.39, 105.34, 97.76, 97.68, 77.38, 77.06, 76.74, 74.96, 74.91, 73.31, 72.58, 72.54, 72.11, 72.06, 71.56, 71.50, 69.58, 69.49, 69.41, 69.35, 69.28, 66.33, 66.32, 66.27, 66.20, 65.10, 65.05, 64.96, 64.92, 61.68, 61.50, 44.73, 36.09, 36.06, 35.97, 35.90, 33.95, 33.90, 33.51, 33.46, 31.91, 31.49, 29.70, 29.65, 29.51, 29.35, 29.30, 29.14, 27.19, 26.43, 25.61, 25.58, 25.58, 24.79, 24.67, 24.66, 22.68, 22.55, 18.52, 18.46, 18.20, 18.10, 14.10, 14.05, 13.61, 13.57, 13.53, 13.50, 12.41.

HRMS: m/z found 1278.7431, calculated for C<sub>69</sub>H<sub>110</sub>NNaO<sub>17</sub>P<sup>+</sup> 1278.7404 [M+Na]<sup>+</sup>.

1-O-(2-O-Arachidonyl-1-O-stearoyl-sn-glycero)phosphoryl-2,6-di-O-butyryl-my-o-inositol (18)
A solution of 17c (140 mg, 75 µmol) in DCM (0.5 mL), MeCN (0.5 mL) and dimethylethylamine (1.5 mL, 13.8 mmol) is stirred at 24°C for 1 h under argon. Volatiles were removed at 24°C/0.01 mbar. The crude was used in the next step without further purification.

HRMS: m/z found 1025.6332, calculated for C_{55}H_{94}O_{15}P^- 1025.63358 [M-H].

1-O-(2-O-Arachidonyl-1-O-stearoyl-sn-glycero)phosphoryl-2,6-di-O-butyryl-myoinositol acetoxymethyl ester P-diastereomeric mixture (1c)

To crude 18 was added dry MeCN (0.5 mL), diisopropylethylamine (87 µL, 500 µmol) and bromomethyl acetate (29 µL, 300 µmol) under argon atmosphere. The mixture was stirred at 24°C for 18 h protected from light. Volatiles were removed at 24°C/0.001 mbar. Preparative HPLC (100% MeOH).

Yield: 55 mg (67%) colorless oil, t_R MeOH = 13 min, MeOH:TBME 9:1 = 5.5 min.

^1^H NMR (400 MHz, CDCl_3) δ = 5.73 (s, 1H), 5.55 (s, 2H), 5.51 – 5.30 (m, 11H), 5.30 – 5.09 (m, 1H), 4.60 – 4.42 (m, 1H), 4.42 – 4.28 (m, 1H), 4.15 (s, 3H), 3.78 (s, 2H), 3.72 – 3.51 (m, 1H), 3.08 – 2.88 (m, 1H), 2.88 – 2.79 (m, 7H), 2.53 – 2.24 (m, 9H), 2.22 – 2.02 (m, 11H), 1.71 (dd, J=12.6, 7.4, 6H), 1.61 (s, 2H), 1.50 – 1.14 (m, 37H), 1.07 – 0.94 (m, 6H), 0.94 – 0.75 (m, 7H).

^3^P NMR (162 MHz, CDCl_3) δ = -3.14 (s, 0.5 P, dia-1), -4.16 (s, 0.5 P, dia-2).

^13^C NMR (101 MHz, CDCl_3) δ = 173.58, 173.43, 173.39, 173.31, 173.28, 173.19, 172.97, 172.86, 172.60, 172.54, 172.01, 171.99, 170.86, 169.31, 169.28, 169.00, 130.50, 129.01, 128.96, 128.80, 128.74, 128.61, 128.31, 128.30, 128.09, 128.06, 127.82, 89.83, 82.75, 82.71, 82.61, 82.56, 81.69, 77.35, 77.03, 76.72, 74.97, 74.36, 74.31, 74.25, 74.11, 72.02, 71.97, 71.92, 71.88, 71.85, 71.79, 71.42, 71.35, 71.03, 71.02, 70.89, 69.31, 69.28, 69.23, 69.21, 69.15, 69.08, 68.66, 68.54, 66.09, 66.03, 65.95, 65.92, 65.90, 61.67, 61.53, 36.11, 36.05, 35.98, 35.96, 35.88, 33.97, 33.95, 33.50, 33.47, 31.92, 31.51, 29.70, 29.68, 29.66, 29.55, 29.50, 29.36, 29.32, 29.22, 29.14, 27.21, 26.47, 25.62, 25.60, 24.81, 24.74, 24.66, 22.69, 22.57, 21.13, 20.98, 20.65, 20.61, 18.59, 18.57, 18.26, 18.18, 14.12, 14.07, 13.60, 13.57, 13.54.

MS: Calculated m/z for C_{58}H_{99}NaO_{17}P^+ 1121.65121, found 1122.1 [M+Na]^+. MS analysis showed also the abundance of a species with two AM esters, likely through alkylation of a hydroxyl group on the inositol.

1-O-(2-O-Arachidonyl-1-O-stearoyl-sn-glycero)phosphoryl-2,6-di-O-butyryl-myoinositol 4-acetoxybenzyl ester P-diastereomeric mixture (1d)
To 17d (105 mg, 57 µmol) were subsequently added DCM (0.5 mL), dimethylethylamine (1 mL, 9.2 mmol) and MeCN (0.5 mL) under an argon atmosphere. After stirring for 1 h at 24°C the solution was diluted with EtOAc (2 mL) and MeCN (2 mL) and volatiles were removed at 24°C/0.006 mbar. EtOAc (2 mL) and MeCN were added and evaporated at 24°C/0.01 mbar. The crude was purified by preparative HPLC (MeOH:EtOAc 10:1 → 9:1).

Yield: 56 mg (83.6%) colorless oil, t<sub>R</sub> MeOH:EtOAc 4:1 = 4.5 min, MeOH:EtOAc 9:1 = 6.7 min.

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ = 7.45 (d, <i>J</i>=8.5, 1H), 7.38 (d, <i>J</i>=8.5, 1H), 7.15 – 7.07 (m, 2H), 5.69 (t, <i>J</i>=2.4, 0.5H), 5.52 (t, <i>J</i>=2.4, 0.5H), 5.47 – 5.27 (m, 9H, 4 x CH=CH), 5.27 – 5.14 (m, 1H), 5.10 – 5.02 (m, 1H), 5.02 – 4.94 (m, 1H), 4.57 – 4.25 (m, 2H), 4.21 – 3.98 (m, 3H), 3.94 – 3.20 (m, 6H), 2.92 – 2.73 (m, 6H, 3 x CH(CH<sub>2</sub>)<sub>2</sub>), 2.45 – 2.00 (m, 15H), 1.77 – 1.46 (m, 8H), 1.46 – 1.16 (m, 34H), 1.02 – 0.79 (m, 12H, 4 x ω-CH<sub>3</sub>).

<sup>31</sup>P NMR (162 MHz, CDCl<sub>3</sub>) δ = -1.54 (s, 0.57 P, dia-1), -1.95 (s, 0.43 P, dia-2).

<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ = 173.45, 173.38, 173.28, 173.15, 173.10, 172.76, 172.55, 169.65, 169.26, 150.99, 150.92, 133.12, 133.06, 132.80, 132.72, 130.49, 129.56, 129.20, 128.97, 128.92, 128.80, 128.75, 128.61, 128.29, 128.09, 127.82, 127.52, 121.99, 121.94, 77.36, 77.04, 76.72, 74.43, 74.13, 73.30, 73.22, 72.65, 72.57, 72.05, 71.42, 71.27, 69.64, 69.48, 69.29, 65.75, 61.81, 61.62, 36.09, 36.06, 36.01, 35.87, 33.99, 33.94, 33.54, 33.47, 31.92, 31.51, 29.71, 29.66, 29.52, 29.36, 29.32, 29.15, 27.21, 26.45, 25.62, 25.60, 24.81, 24.68, 24.65, 22.69, 22.57, 21.10, 18.53, 18.49, 18.22, 18.06, 14.12, 14.07, 13.62, 13.57, 13.54, 13.51.

MS: Calculated m/z for C<sub>64</sub>H<sub>103</sub>NaO<sub>17</sub>P<sup>+</sup> 1197.68251, found 1198.3 [M+Na]<sup>+</sup>.
Figure 1: $^1$H NMR spectrum of 3
Figure 2: $^{13}$C NMR spectrum of 3
Figure 3: $^{13}$C APT NMR spectrum of 3
Figure 4: $^1$H NMR spectrum of 4
Figure 5: $^{13}$C NMR spectrum of 4
Figure 6: $^1^3$C APT NMR spectrum of 4
Figure 7: $^1$H NMR spectrum of 5
Figure 8: $^{13}$C NMR spectrum of 5
Figure 9: $^{13}$C APT NMR spectrum of 5
Figure 10: $^1$H NMR spectrum of 6
Figure 11: $^{13}$C NMR spectrum of 6
Figure 12: $^{13}$C APT NMR spectrum of 6
Figure 13: $^1$H NMR spectrum of 7
Figure 14: $^{13}$C NMR spectrum of 7
Figure 15: $^{13}$C APT NMR spectrum of 7
Figure 16: $^1$H NMR spectrum of 12
Figure 17: $^{13}$C NMR spectrum of 12
Figure 18: $^{13}$C APT NMR spectrum of 12
Figure 19: $^{31}$P NMR spectrum of 12
Figure 20: $^1$H NMR spectrum of 13
Figure 21: $^{13}$C NMR spectrum of 13
Figure 22: $^{13}$C APT NMR spectrum of 13
Figure 23: $^{31}$P NMR spectrum of 13
Figure 24: $^1$H NMR spectrum of 14
Figure 25: $^{13}$C NMR spectrum of 14
Figure 26: $^{13}$C APT NMR spectrum of 14
Figure 27: $^{31}$P NMR spectrum of 14
Figure 28: $^1$H NMR spectrum of 15
Figure 29: $^{13}$C APT NMR spectrum of 15
Figure 30: $^{31}$P NMR spectrum of 15
Figure 31: $^1$H NMR spectrum of 16
Figure 32: $^{13}$C NMR spectrum of 16
Figure 33: $^{13}$C APT NMR spectrum of 16
Figure 34: $^{31}$P NMR spectrum of 16
Figure 35: $^1$H NMR spectrum of 17a
Figure 36: $^{13}$C NMR spectrum of 17a
Figure 37: $^{13}$C APT NMR spectrum of 17a
Figure 38: $^{31}$P NMR spectrum of 17a
Figure 39: $^1$H NMR spectrum of 17b
Figure 40: $^{13}$C NMR spectrum of 17b
Figure 41: $^{13}$C APT NMR spectrum of 17b
Figure 42: $^{31}$P NMR spectrum of 17b
Figure 43: $^1$H NMR spectrum of 17c
Figure 44: $^{13}$C NMR spectrum of 17c
Figure 45: $^{13}$C APT NMR spectrum of 17c
Figure 46: $^{31}$P NMR spectrum of 17c
Figure 47: $^1$H NMR spectrum of 17d
Figure 48: $^{13}$C NMR spectrum of 17d
Figure 49: $^{13}$C APT NMR spectrum of 17d
Figure 50: $^{31}\text{P}$ NMR spectrum of 17d
Figure 51: ^1^H NMR spectrum of 1a
Figure 52: $^{13}$C NMR spectrum of 1a
Figure 53: $^{13}$C APT NMR spectrum of 1a
Figure 54: $^{31}$P NMR spectrum of 1a
Figure 55: $^1$H NMR spectrum of 1b
Figure 56: $^{13}$C NMR spectrum of $1b$
Figure 57: $^{13}$C APT NMR spectrum of 1b
Figure 58: $^{31}$P NMR spectrum of 1b
Figure 59: $^1$H NMR spectrum of 1c
Figure 60: $^{13}$C NMR spectrum of 1c
Figure 61: $^{13}$C APT NMR spectrum of 1c
Figure 62: $^{31}$P NMR spectrum of 1c
Figure 62: $^1$H NMR spectrum of 1d
Figure 60: $^{13}$C NMR spectrum of 1d
Figure 61: $^{13}$C APT NMR spectrum of $1d$
Figure 62: $^{31}\text{P}$ NMR spectrum of $1\text{d}$
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