Synthesis and Hydrolytic Stability of Cyclic Phosphatidic Acids: Implications for Synthetic- and Proto- cell Studies.

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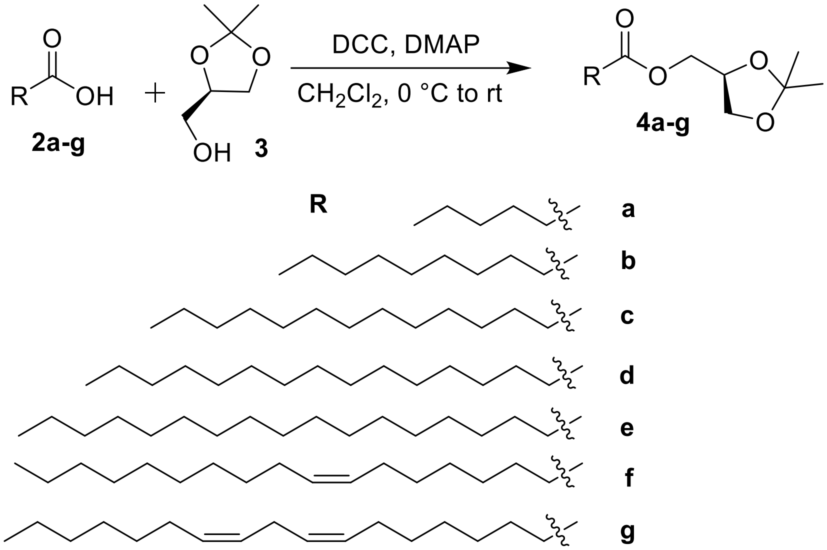
General Methods

All chemicals and solvents were purchased from Acros Organics, Sigma Aldrich, or Alfa Aesar VWR International, Fischer Scientific, TCI Chemicals, Spectrum and Corning, and was used as is. DL-2-amino-n-octanoic acid (>98%) was purchased from TCI Chemicals, 2-hydroxyoctanoic acid (>98%) and 16-hydroxyhexadecanoic acid (97%, contains an impurity at 2.3 ppm that persists throughout the synthesis of **1k**) were purchased from Alfa Aesar, 10-hydroxydecanoic acid (>95%, contains an impurity at 2.3 ppm that persists throughout the synthesis of **1j**) was purchased from Matrix Scientific. Flash Chromatography was performed on Biotage Isolera 1 instrument using Silica gel (SiliaFlash® P60 40-63µm). Thin layer chromatography (TLC) was performed with silica gel w/UV254 from Sorbent Technologies and visualized by UV lamp and/or a stain solution of phosphomolybdic acid (PMA) in ethanol. All cyclic phosphatidic acids (cPAs) used in the stability studies were synthesized. pH was measured using a Accumet Research AR25 pH meter. NMR was recorded at 298 K using AV-600 instrument (600 MHz, 500 MHz for ^{1}H and 150 MHz, 125 MHz for ^{13}C). ^{31}P-NMR spectra were acquired using a Bruker DPX-400 instrument; chemical shifts (δ) are reported in parts per million (ppm) and coupling constants (*J*) in Hertz (Hz). High–resolution mass spectra (HRMS) were obtained on a LCMS TOF mass spectrometer (Agilent ESI-TOF) using electrospray ionization time-of-flight reflectron technique. Microwave reactions were performed using a Microwave synthesizer (Initiator Classic, Biotage), in 2-5 mL reaction vials. Lyophilization of samples with freeze drying was performed on a Sentry 2.0. Lyophilizer from SP Scientific. For cyclic phosphatidic stability studies, % hydrolysis was calculated based on the relative ^{31}P (^{1}H-decoupled) NMR integration of the peaks for glycerol cyclic phosphate, **12** versus corresponding cPAs **1a-f** or ^{1}H NMR integration of the peaks for **5a**, versus those for hexanoic acid, **2a.**

Esterification reaction between fatty acids and *R*-(-)-Solketal



General Procedure: Fatty acids (**2a-g**, 12 mmol) were dissolved in 15 mL of dry CH_2Cl_2 and dimethylaminopyridine (20 mol%) was directly added into the solution. To this stirred solution, *R*-(-)-Solketal (12 mmol) dissolved in 3 ml of dry CH_2Cl_2 was slowly added. The reaction mixture was cooled to 0 °C using an ice bath after which dicyclohexylcarbodiimide (12 mmol) was added as a solid. After overnight stirring at room temperature the dicyclohexylurea produced precipitated out of the solution and was filtered through fritted glass and washed with CH_2Cl_2. More CH_2Cl_2 (40 mL) was added to the filtrate, and the combined organic layers was washed with saturated aqueous solution of $NaHCO_3$ (2 x 10 mL). The organic layer was dried over 30 g of Na_2SO_4, concentrated *in vacuo* by rotovap and purified by flash chromatography using a 50 g silica gel column, eluted with mixtures of Hexanes:EtOAc to afford esters **4a-g (**based on the *Rf* values programmed into the Isolera): **4a** (*Rf* = 0.4, Hexanes:EtOAc 80:20), **4b** (*Rf* = 0.5, Hexanes:EtOAc 80:20), **4c** (*Rf* = 0.6, Hexanes:EtOAc 80:20), **4d** (*Rf* = 0.4, Hexanes:EtOAc 90:10), **4e** (*Rf* = 0.6, Hexanes:EtOAc 95:5), **4f** (*Rf* = 0.5, Hexanes:EtOAc 95:5), **4g** (*Rf* = 0.6, Hexanes:EtOAc 95:5). All fractions were monitored by TLC and visualized using a PMA stain solution and the product containing fractions were combined and concentrated to dryness *in vacuo* to afford the products **4a-g**.

(2,2-dimethyl-1,3-dioxolan-4-yl)methyl hexanoate (**4a**).[1] *Rf* = 0.4, Hexanes:EtOAc 80:20; colorless liquid (88%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 4.20 (m, 1H), 4.05 (dd, *J* = 11.58, 4.92 Hz, 1H), 3.99 (dd, *J* = 11.58, 5.94 Hz, 1H), 3.96 (dd, *J* = 8.46, 6.54 Hz, 1H), 3.63 (dd, *J* = 8.46, 6.18 Hz, 1H), 2.23 (t, *J* = 7.60 Hz, 2H), 1.52 (quint, *J* = 7.60 Hz, 2H), 1.32 (s, 3H), 1.25 (s, 3H), 1.24–1.17 (m, 4H), 0.79 (t, *J* = 7.10 Hz, 3H). **^{13}C NMR** (150 MHz, $CDCl_3$): δ 173.4, 109.6, 73.6, 66.3, 64.4, 33.9, 31.2, 26.5, 25.3, 24.5, 22.1, 13.8.

(2,2-dimethyl-1,3-dioxolan-4-yl)methyl decanoate (**4b**):[2] *Rf* = 0.5, Hexanes:EtOAc 80:20; colorless liquid (86%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 4.28 (m, 1H), 4.12 (dd, *J* = 11.52, 3.16 Hz, 1H), 4.06 (dd, *J* = 12.06, 5.94 Hz, 1H), 4.04 (dd, *J* = 8.46, 6.42 Hz, 1H), 3.71 (dd, *J* = 8.46, 6.30 Hz, 1H), 2.31 (t, *J* = 7.44 Hz, 2H), 1.59 (quint, *J* = 7.20 Hz, 2H), 1.40 (s, 3H), 1.34 (s, 3H), 1.31–1.15 (m, 12H), 0.85 (t, *J* = 7.08 Hz, 3H). **^{13}C NMR** (150 MHz, $CDCl_3$): δ 173.7, 109.9, 73.8, 66.4, 65.0, 34.2, 31.9, 29.5, 29.3, 29.2, 26.8, 25.5, 25.0, 22.7, 14.2.

(2,2-dimethyl-1,3-dioxolan-4-yl)methyl tetradecanoate (**4c**):[2] *Rf* = 0.6, Hexanes:Hexanes:EtOAc 80:20; white solid (91%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 4.29 (m, 1H), 4.14 (dd, *J* = 13.38, 5.11 Hz, 1H), 4.09–4.04 (m, 2H), 3.71 (m, 1H), 2.32 (t, *J* = 7.56 Hz, 2H), 1.61 (m, 2H), 1.41 (s, 3H), 1.35 (s, 3H), 1.32–1.16 (m, 20H), 0.86 (t, *J* = 6.78 Hz, 3H). **^{13}C NMR** (150 MHz, $CDCl_3$): δ 173.7, 109.9, 73.8, 66.5, 64.6, 34.2, 32.0, 29.7, 29.60, 29.4, 29.3, 29.2, 26.8, 25.5, 25.0, 22.8, 14.2.

(2,2-dimethyl-1,3-dioxolan-4-yl)methyl palmitate (**4d**):[2] *Rf* = 0.4, Hexanes:EtOAc 90:10; white solid (83%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 4.18 (m, 1H), 4.03 (dd, *J* = 11.46, 4.80 Hz, 1H), 3.98 (dd, *J* = 11.46, 5.82 Hz, 1H), 3.95 (dd, *J* = 8.76, 6.42 Hz, 1H), 3.62 (dd, *J* = 8.52, 6.06 Hz, 1H), 2.22 (t, *J* = 7.56 Hz, 2H), 1.51 (quint, *J* = 7.32 Hz, 2H), 1.31 (s, 3H), 1.24 (s, 3H), 1.23–1.06 (m, 24H), 0.77 (t, *J* = 7.02 Hz, 3H). **^{13}C NMR** (150 MHz, $CDCl_3$): δ 173.2, 109.5, 73.6, 66.3, 64.3, 33.9, 31.9, 29.64, 29.63, 29.61, 29.60, 29.58, 29.54, 29.40, 29.31, 29.20, 29.05, 26.6, 25.3, 24.8, 22.6, 14.0.

(2,2-dimethyl-1,3-dioxolan-4-yl)methyl stearate (**4e**):[2] *Rf* = 0.4, Hexanes:EtOAc 95:5; white solid (84%). **^{1}H NMR** 500 MHz, $CDCl_3$): δ 4.32 (m, 1H), 4.17 (dd, *J* = 11.5, 4.5 Hz, 1H), 4.09 (m, 2H), 3.74 (dd, *J* = 8, 6.5 Hz, 1H), 2.35 (t, *J* = 7.5 Hz, 2H), 1.63 (quint, *J* = 7

Hz, 2H), 1.44 (s, 3H), 1.38 (s, 3H), 1.28 (m, 28H), 0.89 (t, *J* = 7 Hz, 3H). **^{13}C NMR** (125 MHz, $CDCl_3$): δ 173.6, 109.8, 73.7, 66.4, 64.5, 34.1, 31.9, 29.69, 29.67, 29.66, 29.64, 29.59, 29.45, 29.36, 29.25, 29.1, 26.8, 25.4, 24.9, 22.7, 14.1.

(2,2-dimethyl-1,3-dioxolan-4-yl)methyl oleate (**4f**):[2] *Rf* = 0.5, Hexanes:EtOAc 95:5; colorless oil (90%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 5.25 (m, 2H), 4.22 (m, 1H), 4.06 (dd, *J* = 11.58, 4.92 Hz, 1H), 4.02 (dd, *J* = 11.46, 5.82 Hz, 1H), 3.98 (dd, *J* = 8.58, 6.42 Hz, 1H), 3.65 (dd, *J* = 8.64, 6.18 Hz, 1H), 2.26 (t, *J* = 7.62 Hz, 2H), 1.94 (m, 4H), 1.55 (m, 2H), 1.34 (s, 3H), 1.28 (s, 3H), 1.27–1.05 (m, 20H), 0.80 (t, *J* = 6.84 Hz, 3H). **^{13}C NMR** (150 MHz, $CDCl_3$): δ 173.7, 130.1, 129.9, 73.8, 66.5, 64.7, 34.2, 32.0, 29.90, 29.82, 29.66, 29.46, 29.29, 29.26, 29.23, 27.35, 27.30, 26.8, 25.5, 25.0, 22.8, 14.2.

(2,2-dimethyl-1,3-dioxolan-4-yl)methyl (9Z,12Z)-octadeca-9,12-dienoate (**4g**):[3] *Rf* = 0.6, Hexanes:EtOAc 95:5; colorless oil (79%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 5.30 (m, 4H), 4.25 (m, 1H), 4.10 (m, 1H), 4.07–3.99 (m, 2H), 3.63 (m, 1H), 2.72 (m, 2H), 2.28 (t, *J* = 7.68 Hz, 2H), 1.99 (m, 4H), 1.58 (m, 2H), 1.38 (s, 3H), 1.31 (s, 3H), 1.28–1.17 (m, 14H), 0.84 (t, *J* = 7.08 Hz, 3H). **^{13}C NMR** (150 MHz, $CDCl_3$): δ 173.4, 130.1, 130.0, 128.1, 127.9, 73.7, 66.4, 64.5, 34.1, 31.5, 29.60, 29.36, 29.17, 29.10, 27.21, 27.19, 26.7, 25.7, 25.4, 24.9, 22.6, 14.1.

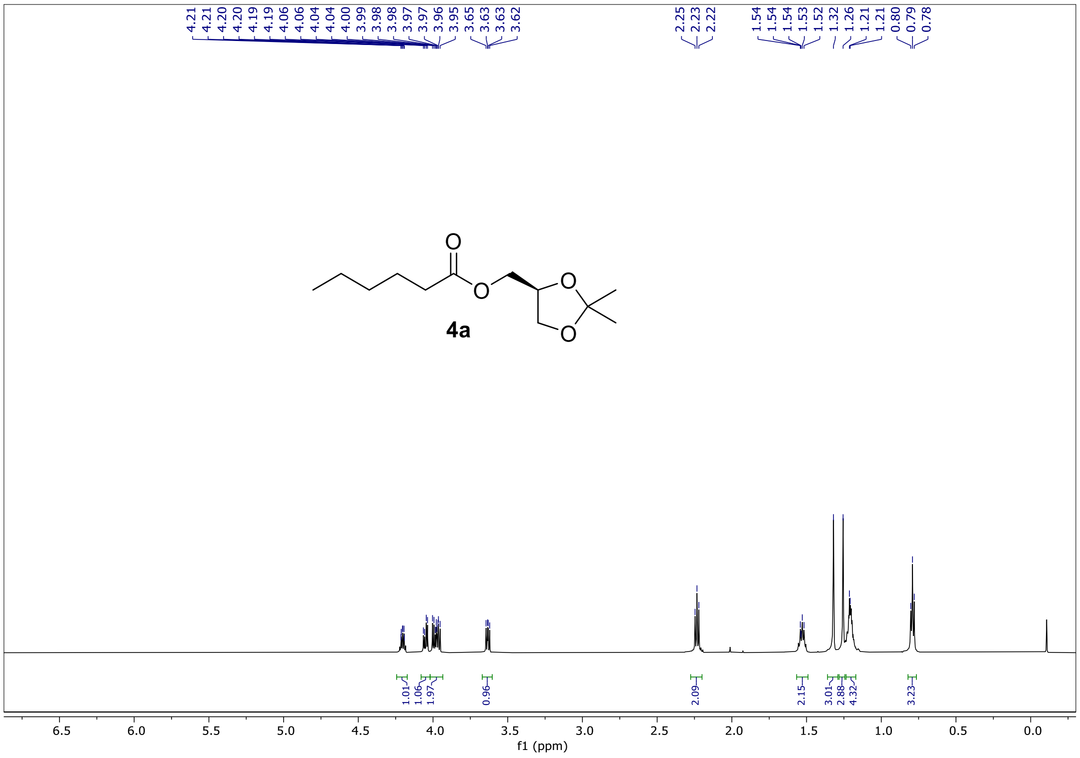


Figure S1. ^{1}H NMR spectrum of **4a** in $CDCl_3$

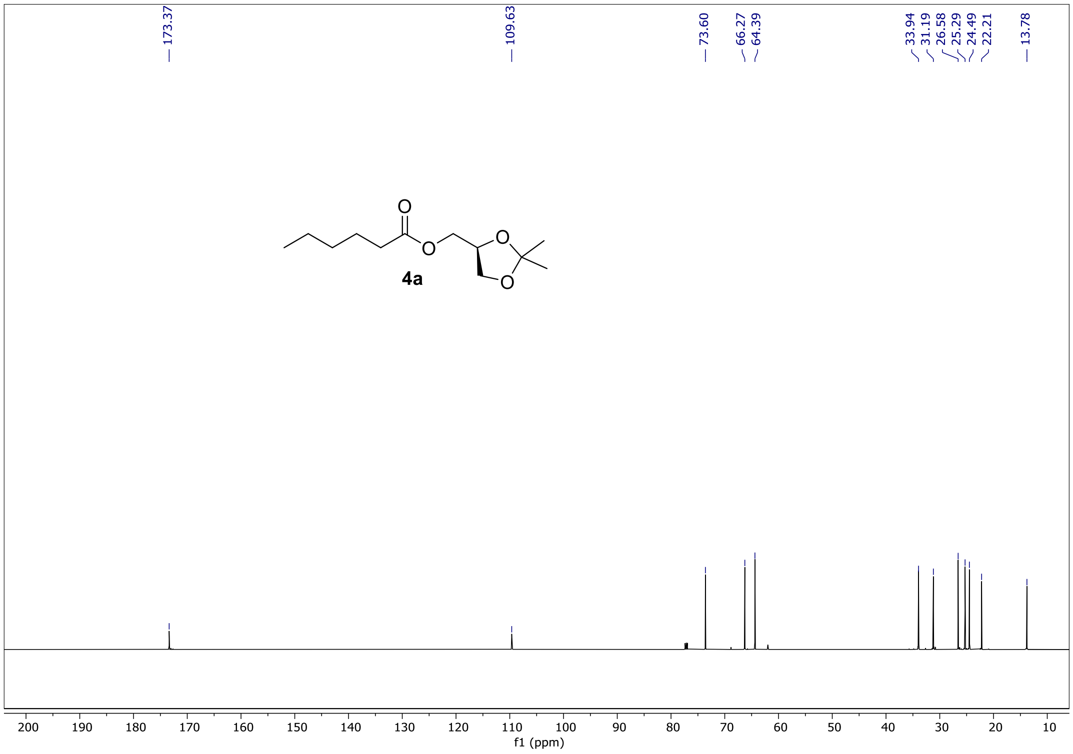


Figure S2. ^{13}C NMR spectrum of **4a** in $CDCl_3$

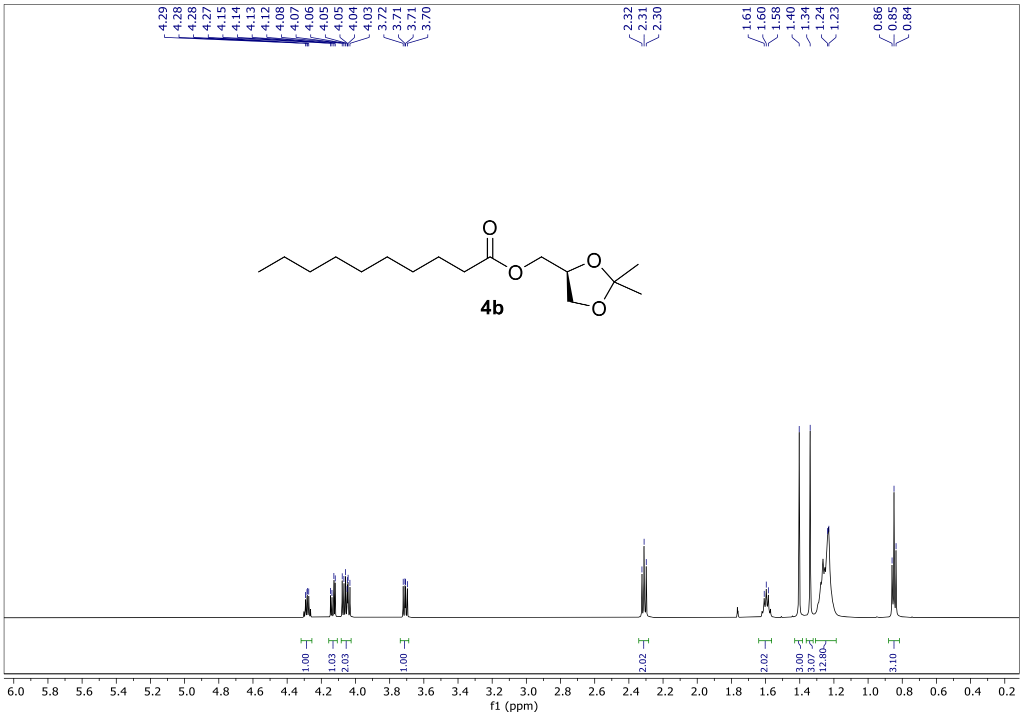


Figure S3. ^{1}H NMR spectrum of **4b** in $CDCl_3$

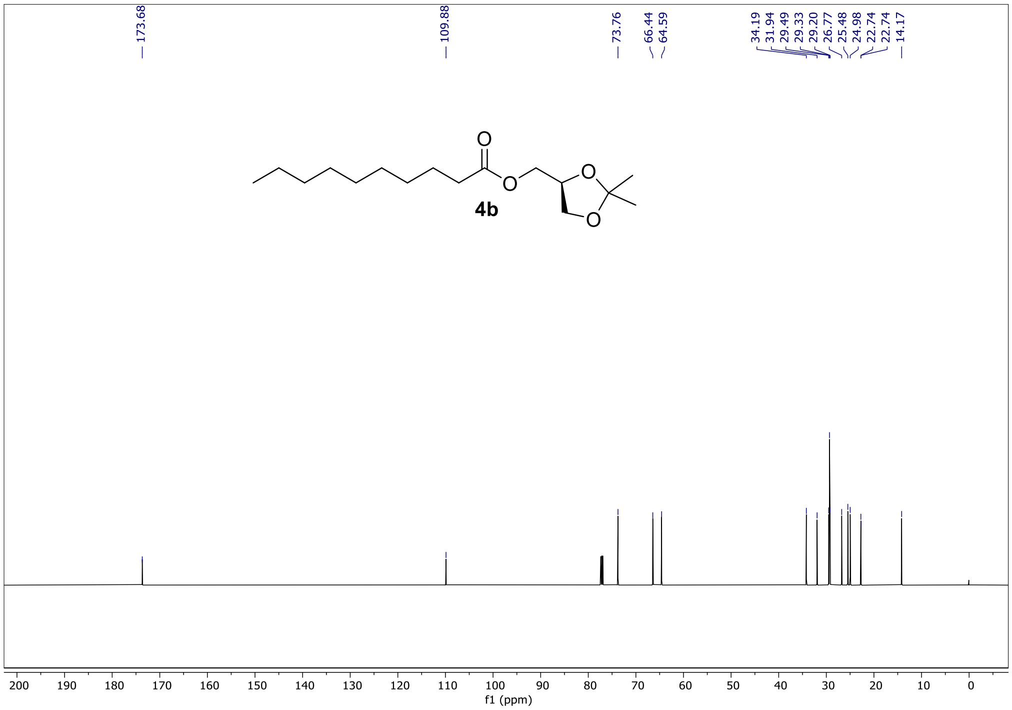


Figure S4. ^{13}C NMR spectrum of **4b** in $CDCl_3$

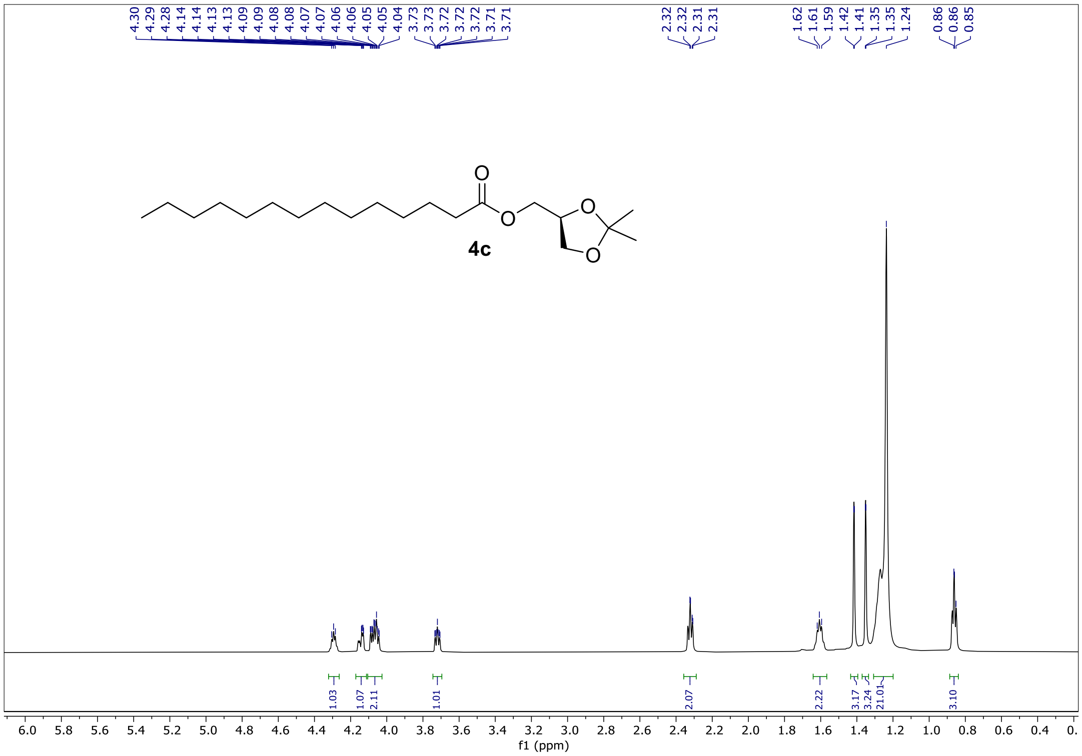


Figure S5. ^{1}H NMR spectrum of **4c** in CDCl$_3$

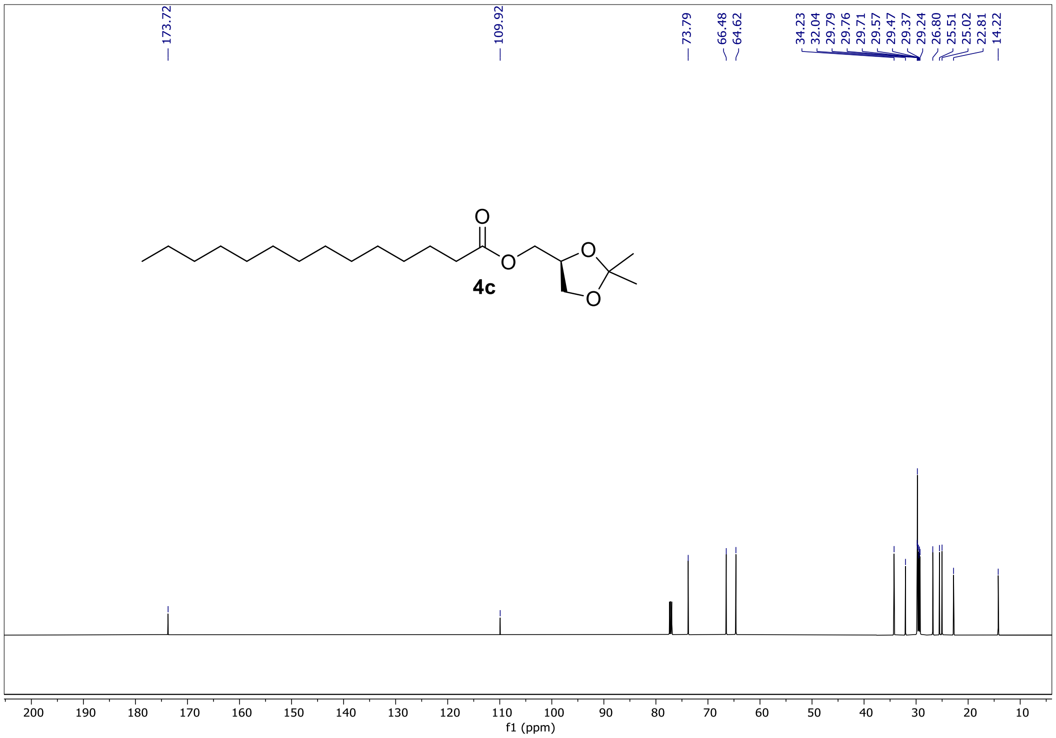


Figure S6. ^{13}C NMR spectrum of **4c** in CDCl$_3$

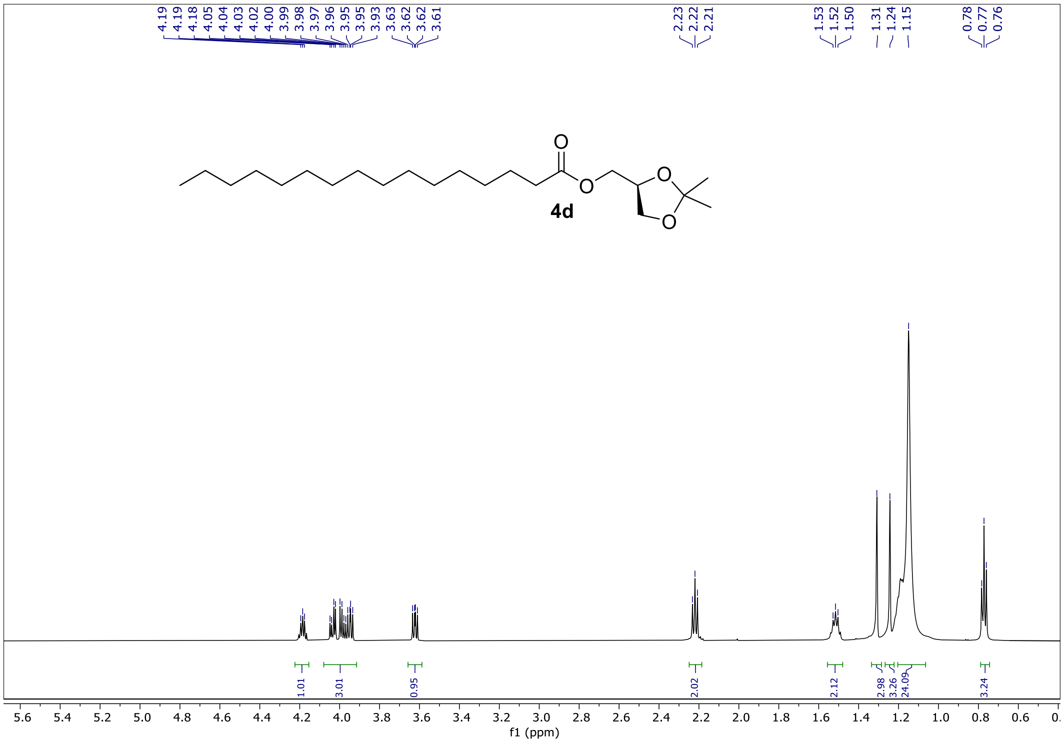


Figure S7. ^{1}H NMR spectrum of **4d** in $CDCl_3$

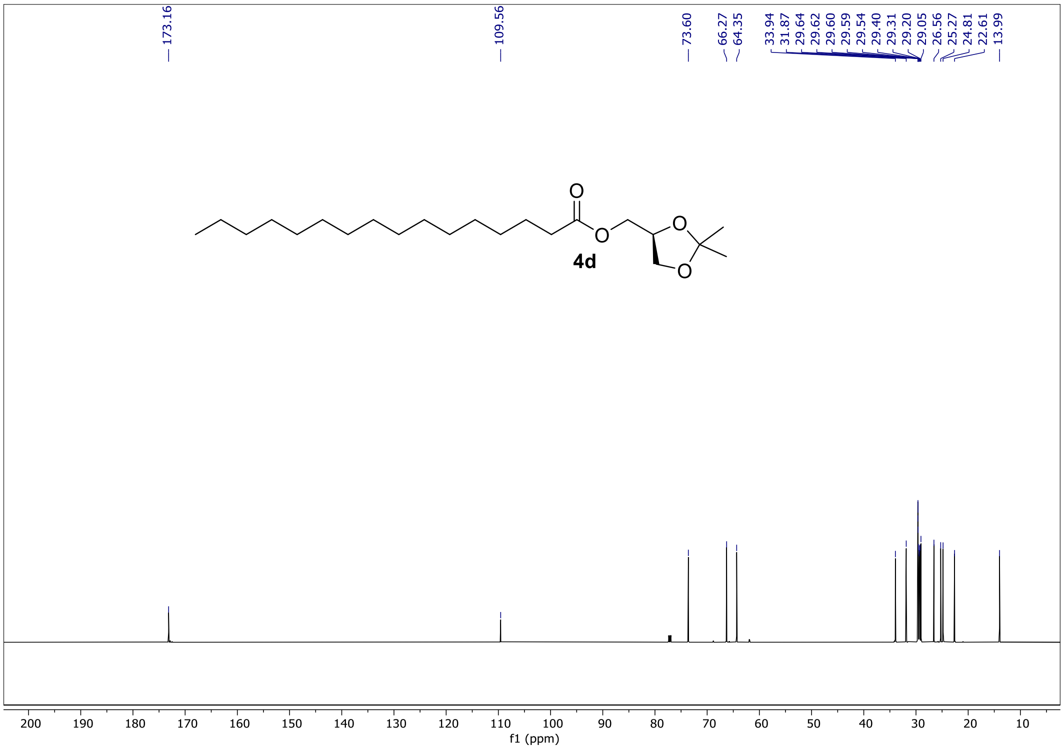


Figure S8. ^{13}C NMR spectrum of **4d** in $CDCl_3$

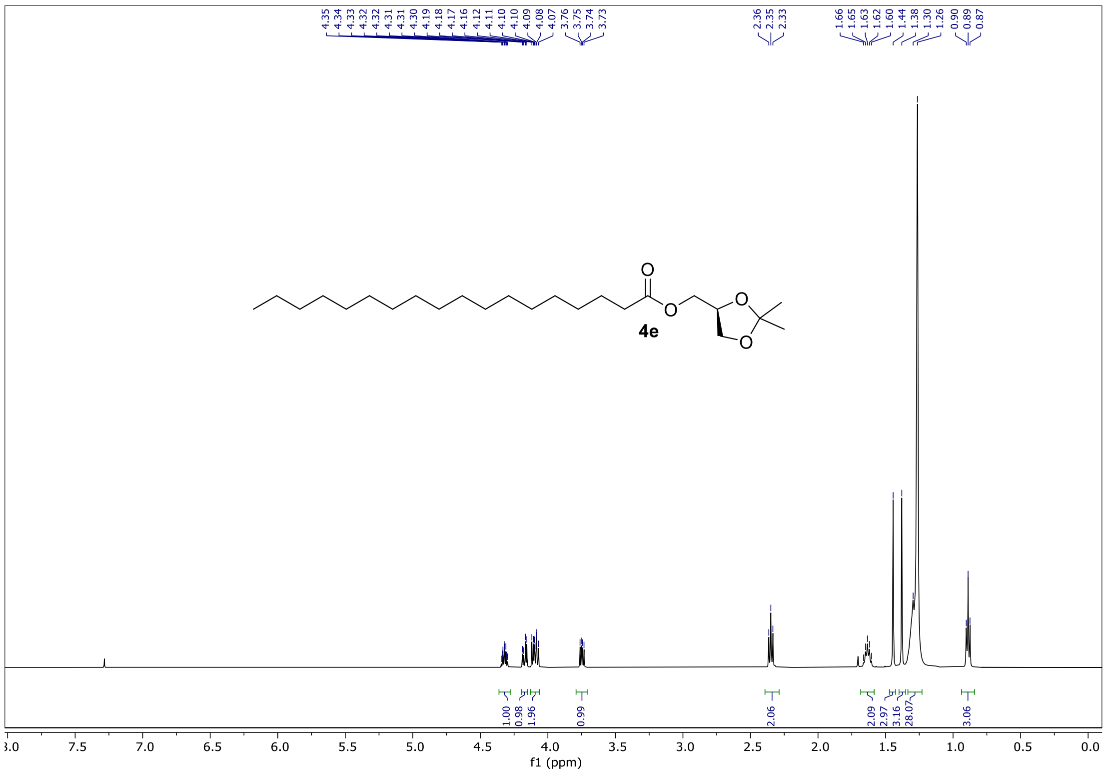


Figure S9. ^{1}H NMR spectrum of **4e** in CDCl$_3$

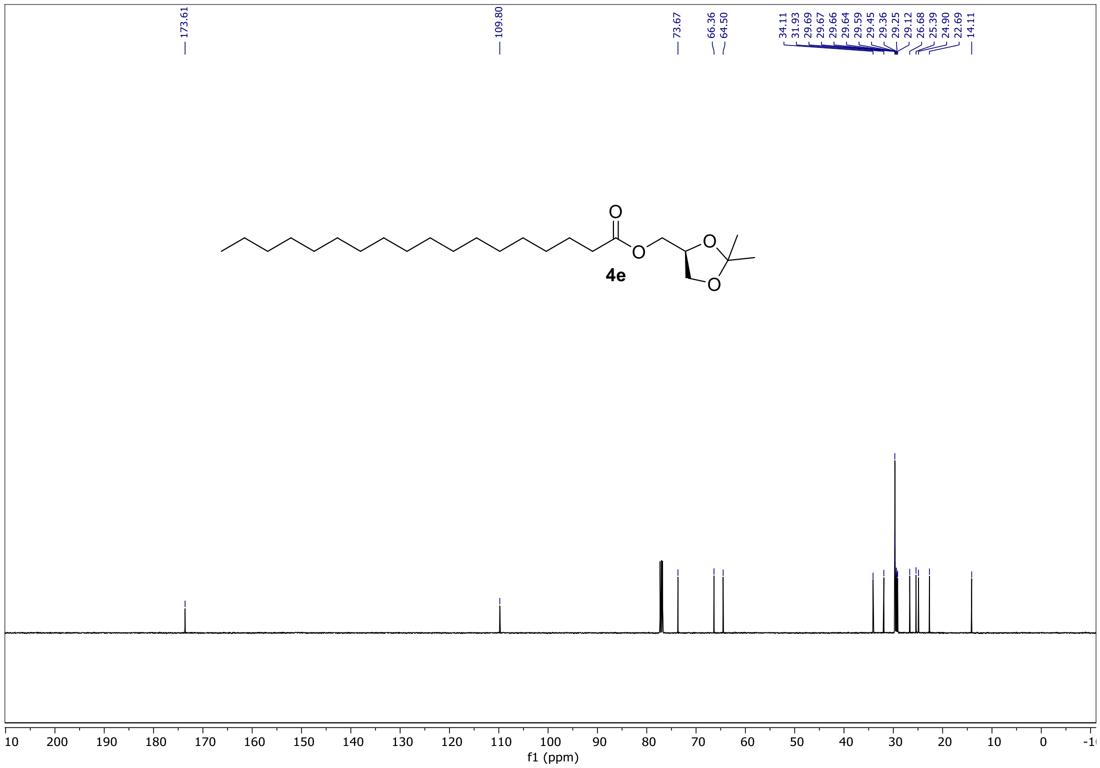


Figure S10. ^{13}C NMR spectrum of **4e** in CDCl$_3$

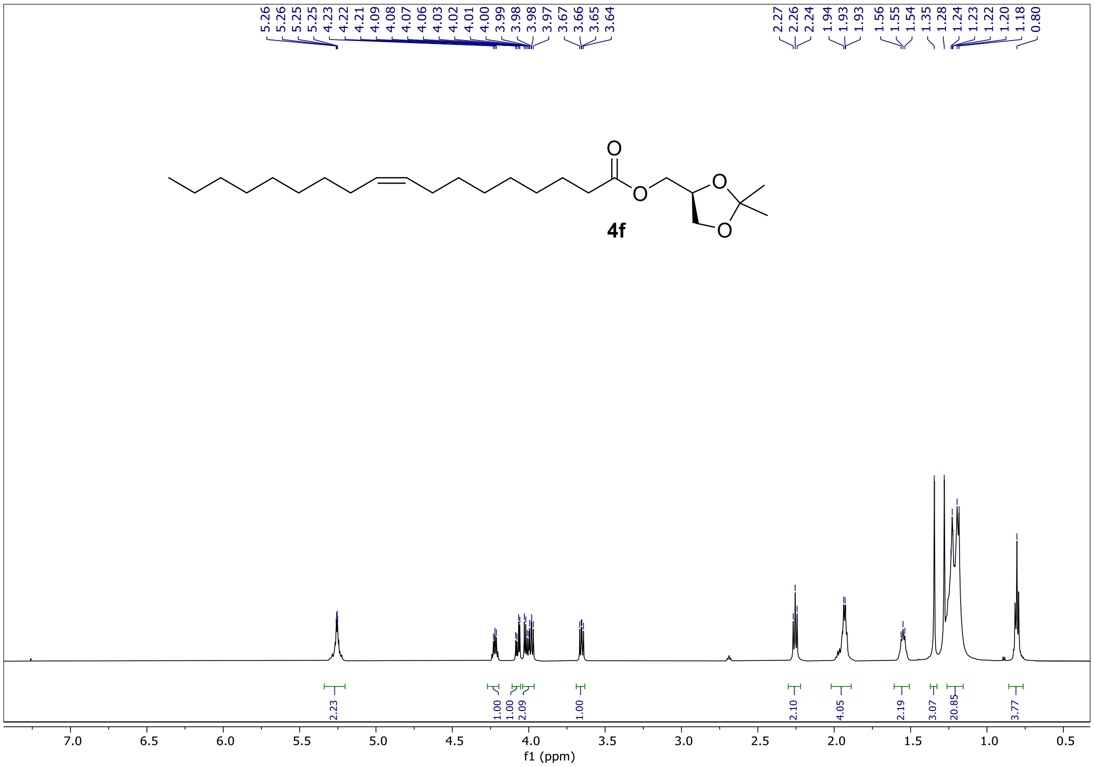


Figure S11. ^{1}H NMR spectrum of **4f** in $CDCl_3$

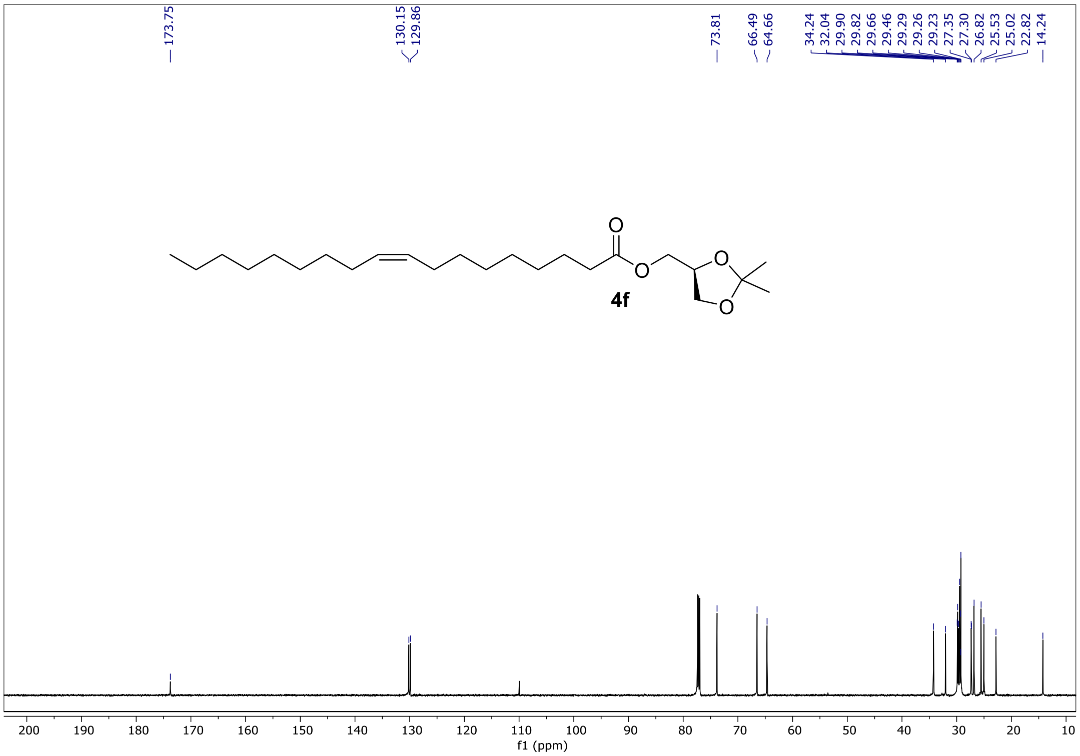


Figure S12. ^{13}C NMR spectrum of **4f** in $CDCl_3$

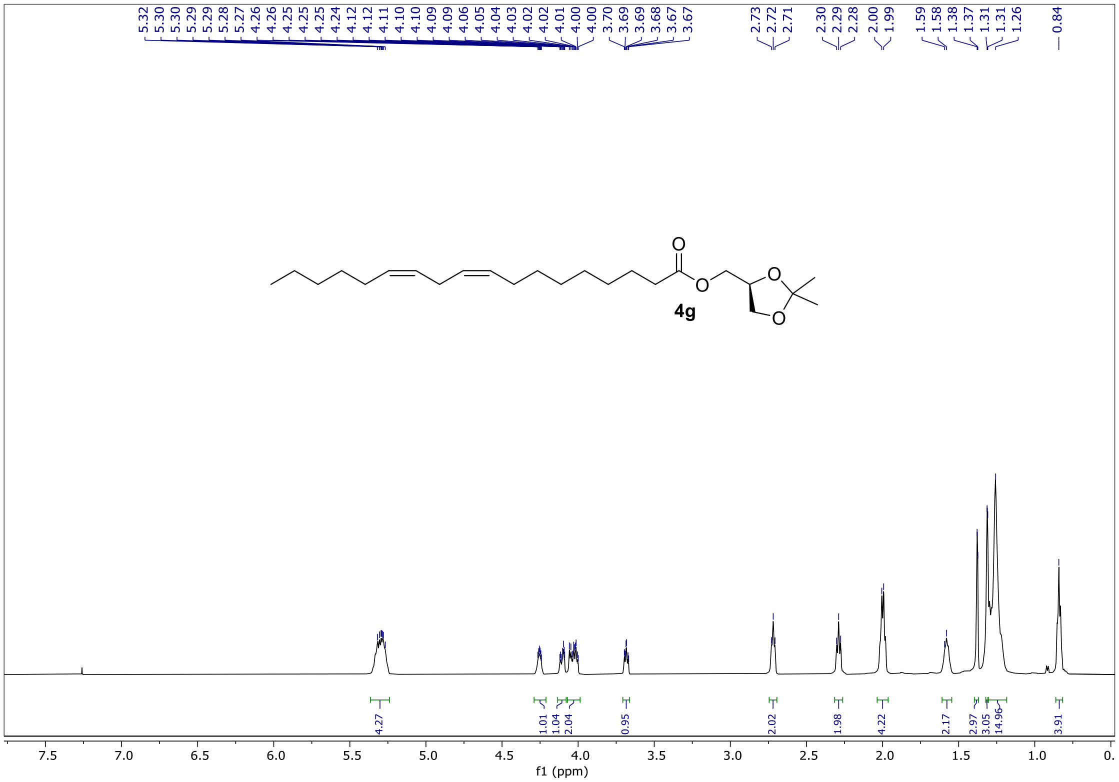


Figure S13. ^{1}H NMR spectrum of **4g** in $CDCl_3$

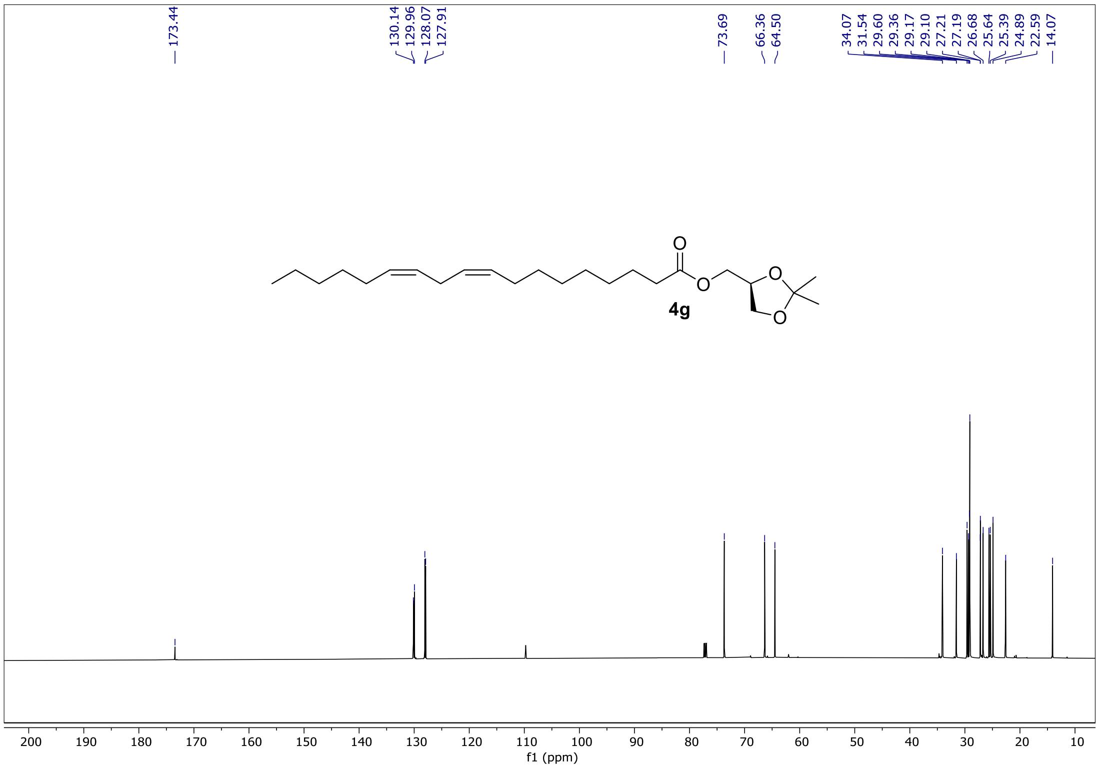
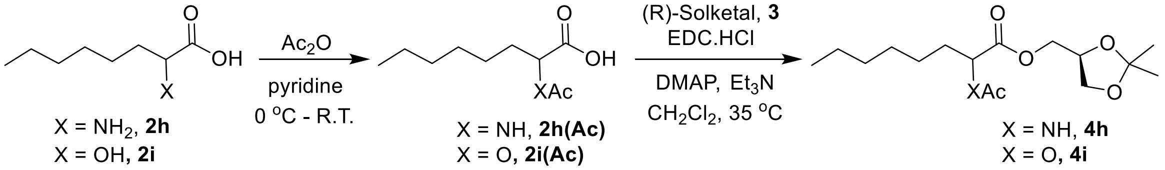
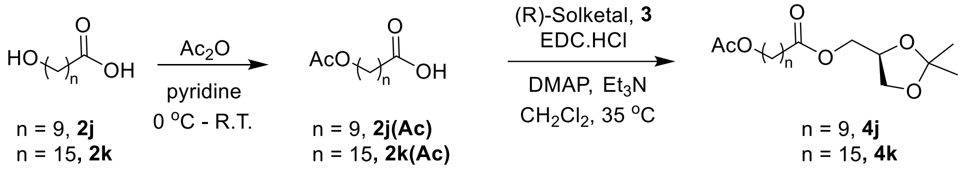


Figure S14. ^{13}C NMR spectrum of **4g** in $CDCl_3$

Esterification reaction between fatty acids (2h-k) and *R*-(-)-Solketal





General procedure for acetylation**:** Fatty acid (**2h-k**, 8.9 mmol) was dissolved in dry pyridine (40 mL, 4.5 mL/mmol) in 100 mL RB flask. After cooled to 0 °C, Ac_2O (1.8 mL, 13.4 mmol, 1.5 equiv.) was added dropwise over 5 min under N_2 atmosphere and the reaction mixture was allowed to stir at room temperature for 6 hrs. After completion of the reaction by TLC, pyridine was removed under rotavap and quenched with 5M HCl solution. The compound was extracted with EtOAc (3 × 40 mL), the combined organic layer was washed with 5M HCl solution (1 × 40 mL) followed by brine (1 × 40 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo* by rotavap to obtain the acetylated product, **2h(Ac)-k(Ac)** in quantitative yields. The crude product was used for the next step without further purification.

N-Acetyl-2-aminooctanoic acid (**2h(Ac)**): *Rf* = 0.3, Hexanes:EtOAc 0:100; Brownish semisolid (crude, quantitative). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 6.44 (s, 1H), 2.08 (s, 4H), 1.99 – 1.62 (m, 3H), 1.28 (s, 18H), 0.89 (s, 7H). **^{13}C NMR** (150 MHz, $CDCl_3$): δ 175.7, 171.2, 52.5, 32.1, 31.6, 29.7, 28.9, 25.2, 22.5, 14.0. **HRMS (ESI)** m/z calcd for $C_{10}H_{19}NO_3$ $[M+H]^+$ 202.1438, found 202.1431. Note: Broadness of the ^{1}H NMR spectra was observed in $CDCl_3$.

O-Acetyl-2-hydroxyoctanoic acid (**2i(Ac)**): *Rf* = 0.5, Hexanes:EtOAc 40:60; Colorless liquid (crude, quantitative). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.1 – 7.5 (brs, 1H), 5.03 (dd, *J* = 7.1, 5.6 Hz, 1H), 2.17 (s, 3H), 1.89 (qd, *J* = 7.1, 3.9 Hz, 2H), 1.51 – 1.24 (m, 8H), 0.90 (t, *J* = 6.9 Hz, 3H). **^{13}C NMR** (150 MHz, $CDCl_3$): δ 175.8, 170.7, 71.9, 31.5, 30.9, 28.7, 25.0, 22.5, 20.6, 14.0. **HRMS (ESI)** m/z calcd for $C_{10}H_{18}O_4$ [M-H] 201.1132, found 201.1134.

10-Acetoxydecanoic acid (**2j(Ac)**)[4]: *Rf* = 0.34, Hexanes:EtOAc 80:20; colorless oil (crude contains ~20% of impurity from the starting material [triplet-CH_2 peak at 2.3 ppm]). ^1H and ^{13}C NMR data was matched with the reported data.[4] **HRMS (ESI)** m/z calcd for $C_{12}H_{23}O_4$ $[M+H]^+$ 231.1591, found 231.1589.

16-Acetoxyhexadecanoic acid (**2k(Ac)**): *Rf* = 0.47, Hexanes:EtOAc 80:20; off-white solid (crude contains ~15% of impurity from the starting material [triplet-CH_2 peak at 2.3 ppm]).). **^{1}H NMR** (600 MHz, CDC l_3) δ 4.60 (t, *J* =7.2 Hz, 2H), 2.49–2.32 (m, 2H), 2.05 (s, 3H), 1.62 (m, 4H), 1.34–1.22 (m, 22H).**^{13}C NMR** (150 MHz, $CDCl_3$) δ 179.8, 171.3, 64.7, 35.28, 34.0, 29.6, 29.56, 29.51, 29.42, 29.40, 29.2, 29.0, 28.9, 28.60, 25.90, 24.6, 24.1, 22.2, 21.0 **HRMS (ESI)** m/z calcd for $C_{18}H_{35}O_4$ $[M+H]^+$ 315.2530, found 315.2525.

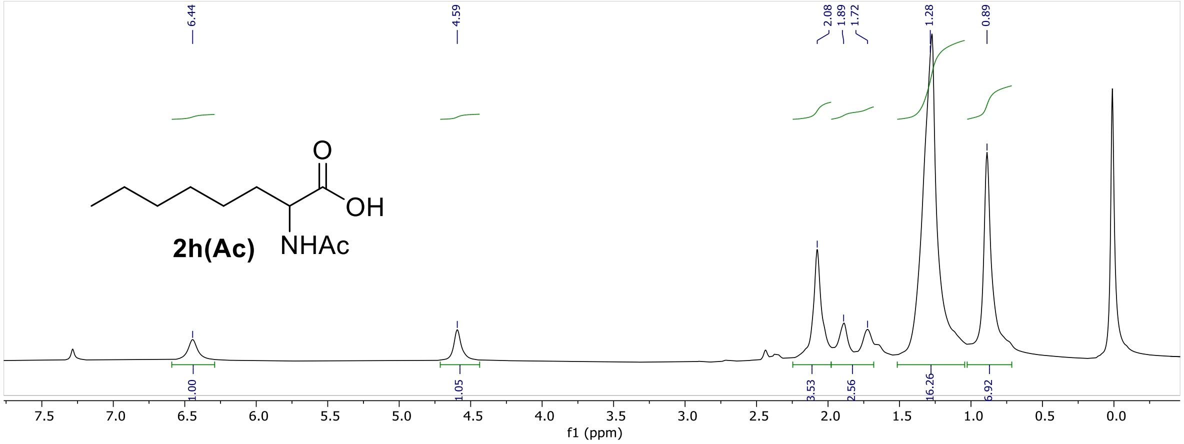


Figure S15. ^1H NMR spectrum of **2h(Ac)** in $CDCl_3$

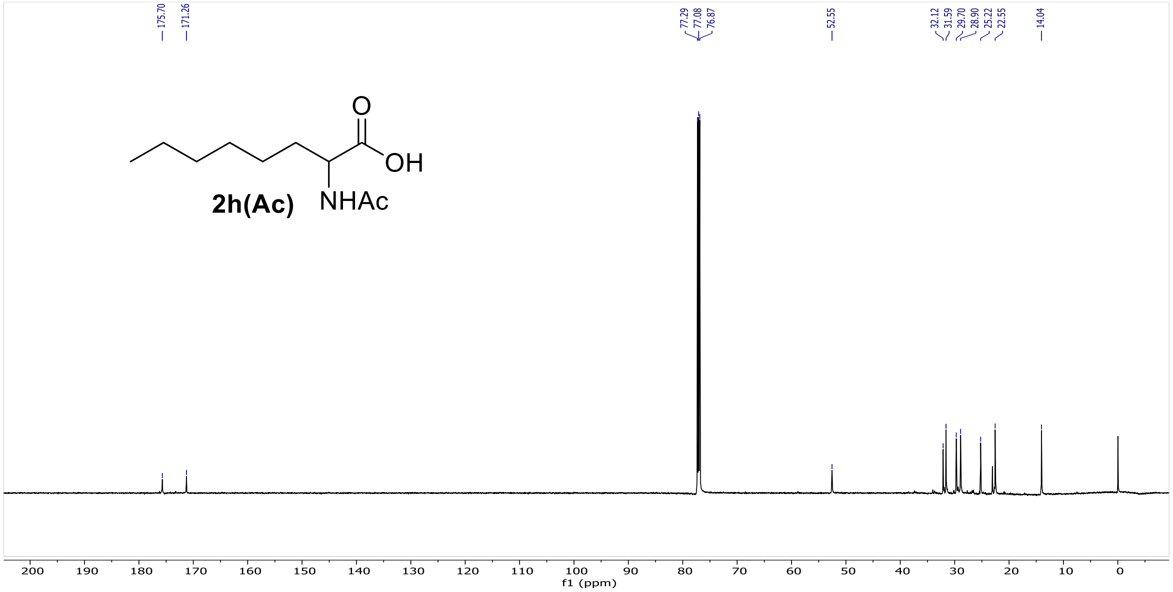


Figure S16. ^{13}C NMR spectrum of **2h(Ac)** in $CDCl_3$

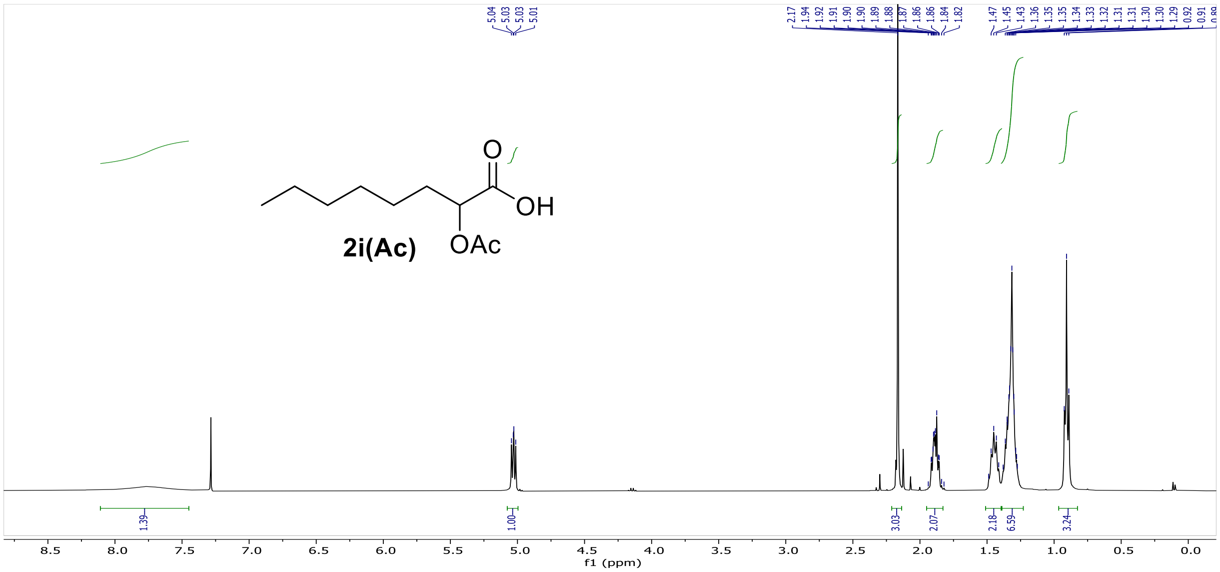


Figure S17. ^{1}H NMR spectrum of **2i(Ac)** in $CDCl_3$

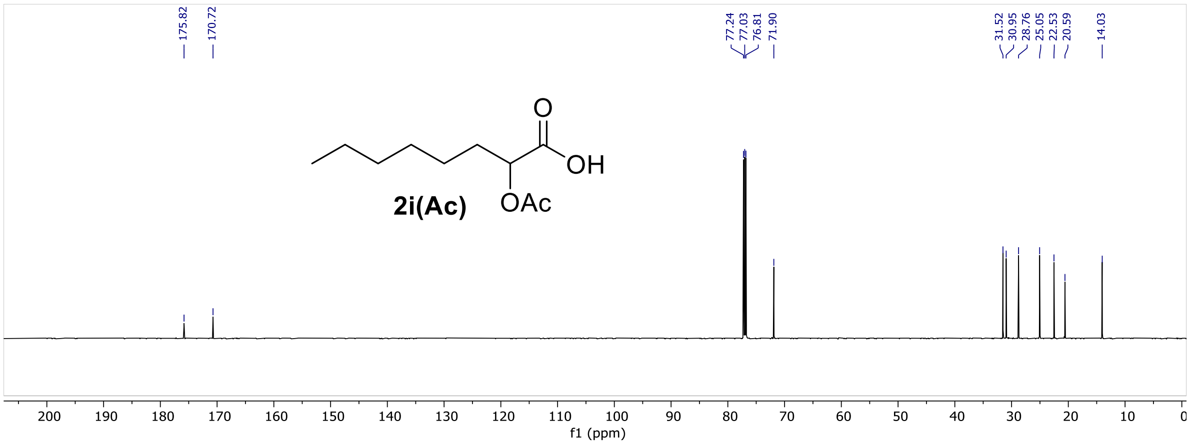


Figure S18. ^{13}C NMR spectrum of **2i(Ac)** in $CDCl_3$

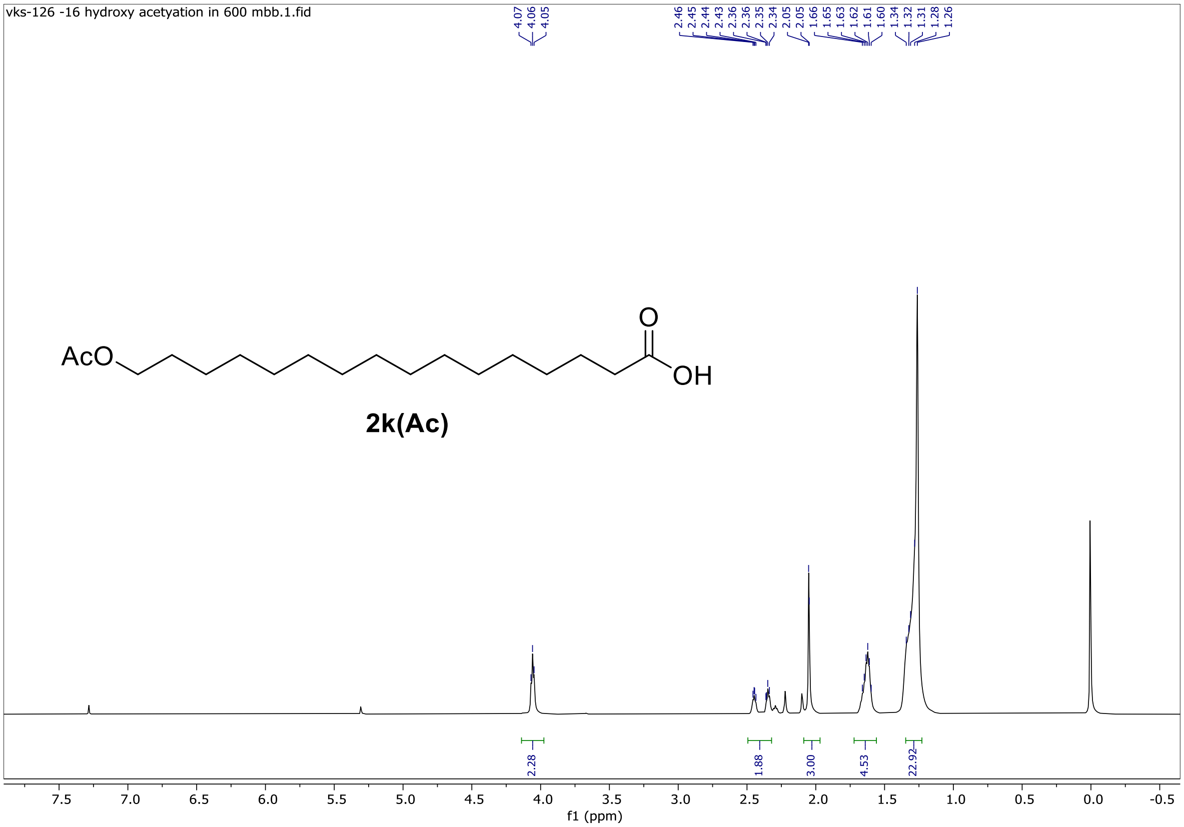


Figure S19. ^{1}H NMR spectrum of **2k(Ac)** in $CDCl_3$

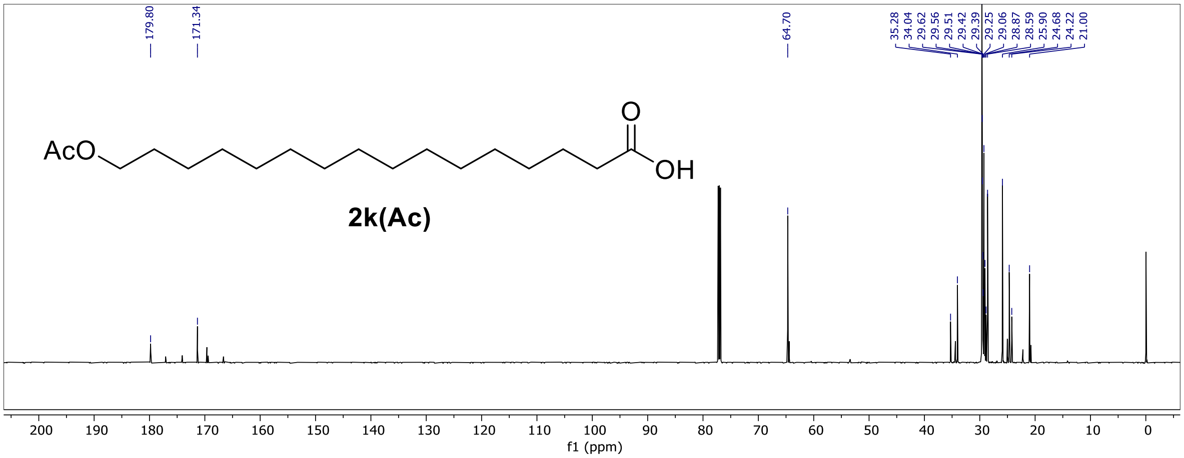


Figure S20. ^{13}C NMR spectrum of **2k(Ac)** in $CDCl_3$.

Alternative general procedure for esterification of **2h(Ac)-2k(Ac):** The crude product (2h(Ac)-k(Ac), 2 mmol), DMAP (50 mg, 0.4 mmol, 0.2 equiv.) and EDC-HCl (380 mg, 2 mmol, 1 equiv.) was dissolved in CH_2Cl_2 (10 mL) at room temperature under nitrogen atmosphere. To this, Et_3N (700 uL, 5 mmol, 2.5 equiv.) was added dropwise, followed by the addition of a solution of R-Solketal (400 mg, 3 mmol, 1.5 equiv.) in CH_2Cl_2 (3 mL) at room temperature under nitrogen atmosphere. The reaction mixture was stirred at 35 °C for overnight for the completion of the reaction. After completion of the reaction by TLC, the reaction mixture was diluted with CH_2Cl_2 and washed with $NaHCO_3$ solution (1 × 40 mL) followed by brine solution (1 × 40 mL). The organic layer was dried over Na_2SO_4 and the solvents were removed under rotavap and purified by column chromatography. The products, **4h** and **4i** were obtained as a 1:1 diastereomeric mixture in 31% and 30% respectively and the other products, **4j** and **4k** were obtained as a single isomer in 28% and 20% respectively.

(2,2-dimethyl-1,3-dioxolan-4-yl)methyl-2-acetamidooctanoate (**4h**): *Rf* = 0.55, Hexanes:EtOAc 70:30; Colorless liquid (31% in 2 steps, 1:1 diastereomeric mixture). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 5.99 (d, *J* = 6.3 Hz, 1H), 4.64 (tdd, *J* = 7.8, 5.3, 2.5 Hz, 1H), 4.34 (p, *J* = 5.5 Hz, 1H), 4.25–4.17 (m, 2H), 4.09 (ddd, *J* = 8.3, 6.4, 1.7 Hz, 1H), 3.77 (ddd, *J* = 11.9, 8.5, 5.8 Hz, 1H), 2.04 (s, 3H), 1.45 (d, *J* = 2.0 Hz, 3H), 1.41 – 1.31 (m, 9H), 1.37 – 1.17 (m, 8H), 0.89 (t, *J* = 6.8 Hz, 4H). **^{13}C NMR** (150 MHz, $CDCl_3$): δ 172.60, 172.59, 109.9, 109.85, 73.36, 73.24, 66.2, 65.3, 65.2, 52.28, 52.23, 32.54, 32.50, 31.38, 31.57, 28.9, 26.72, 26.70, 25.31, 25.29, 25.16, 25.13, 23.2, 22.5, 14.0. **HRMS (ESI)** m/z calcd for $C_{16}H_{29}NNaO_5$ $[M+Na]^+$ 338.1943, found 338.1929.

(2,2-dimethyl-1,3-dioxolan-4-yl)methyl-2-acetaoxyoctanoate (**4i**): *Rf* = 0.65, Hexanes:EtOAc 70:30; Colorless liquid (30% in 2 steps, 1:1 diastereomeric mixture). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 5.00 (ddt, *J* = 6.2, 3.4, 1.8 Hz, 1H), 4.33 (h, *J* = 5.3 Hz, 1H), 4.22 (d, *J* = 5.1 Hz, 1H), 4.09 (ddt, *J* = 8.7, 6.5, 1.3 Hz, 1H), 3.77 (dddd, *J* = 9.7, 8.5, 5.9, 1.3 Hz, 1H), 2.16 (s, 1H), 1.94 – 1.77 (m, 1H), 1.45 (s, 1H), 1.38 (s, 1H), 1.37–1.27 (m, 7H). **^{13}C NMR** (150 MHz, $CDCl_3$): δ 170.66, 170.62, 170.29, 170.27, 109.86, 109.79, 73.36, 73.30, 72.39, 72.33, 66.2, 65.1, 64.9, 31.5, 31.07, 28.8, 26.7, 26.6, 25.36, 25.3, 25.0, 22.5, 20.6, 14.0. **HRMS (ESI)** m/z calcd for $C_{16}H_{28}NaO_6$ $[M+Na]^+$ 339.1784, found 339.1774.

(S)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl-10-acetoxydecanoate (**4j**): *Rf* = 0.62, Hexanes:EtOAc 80:20; Colorless oil (28 % in 2 steps, contains 5% of the impurity, which was carried over from the previous step) **^{1}H NMR** (600 MHz, $CDCl_3$) δ 4.31 (m, 1H), 4.16

(m, 1H), 4.11–4.01 (m, 4H), 3.73 (m, 1H), 2.34 (m, 2H), 2.0 (m, 3H), 1.61 (m, 4H), 1.43 (s, 3H), 1.36 (s, 3H), 1.32–1.27 (m, 9H). **^{13}C NMR** (150 MHz, $CDCl_3$) δ 173.6, 171.2, 109.8, 73.7, 66.3, 64.6, 64.52, 29.2, 29.15, 29.12, 29.05, 28.5, 26.67, 25.8, 25.4, 24.83, 20.9. **HRMS (ESI)** m/z calcd for $C_{18}H_{33}O_6$ $[M+H]^+$ 345.2272, found 345.2267.

(S)-(2,2-dimethyl-1,3-dioxolan-4-yl)methyl 16-acetoxyhexadecanoate (**4k**): *Rf* = 0.72, Hexanes:EtOAc 80:20; off-white solid (20 % in 2 steps, contains 4% of the impurity, which was carried over from the previous step)). **^{1}H NMR** (600 MHz, $CDCl_3$) δ 4.33 (qd, *J* = 4.8, 6.6 Hz, 1H), 4.17 (dd, *J* = 4.8, 11.4 Hz, 1H), 4.12- 4.05 (m, 4H) , 3.75 (dd, *J* = 6.6, 8.4 Hz, 1H), 2.36 (t, *J* = 6.5Hz, 2H), 2.0 (s, 3H), 1.63 (m, 4H), 1.45 (s, 3H), 1.39 (s, 3H), 1.31-1.25 (m, 22H) **^{13}C NMR** (150 MHz, $CDCl_3$) δ 173.6, 171.2, 109.8, 73.7, 66.4, 64.7, 64.5, 29.64, 29.63, 29.59, 29.57, 29.52, 29.45, 29.26, 29.12, 28.61, 26.7, 25.9, 25.4, 24.9, 21.0. **HRMS (ESI)** m/z calcd for $C_{24}H_{44}NaO_6$ $[M+Na]^+$ 451.3030, found 451.3032.

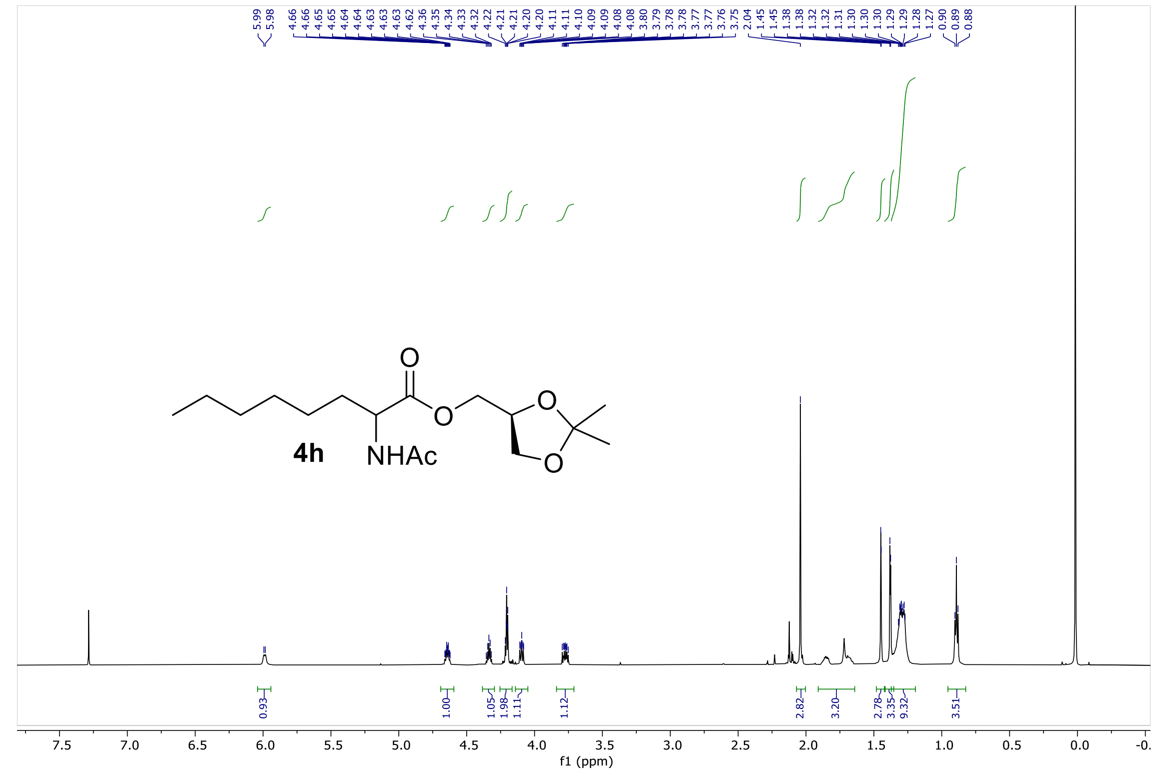


Figure S21. ^{1}H NMR spectrum of **4h** in $CDCl_3$

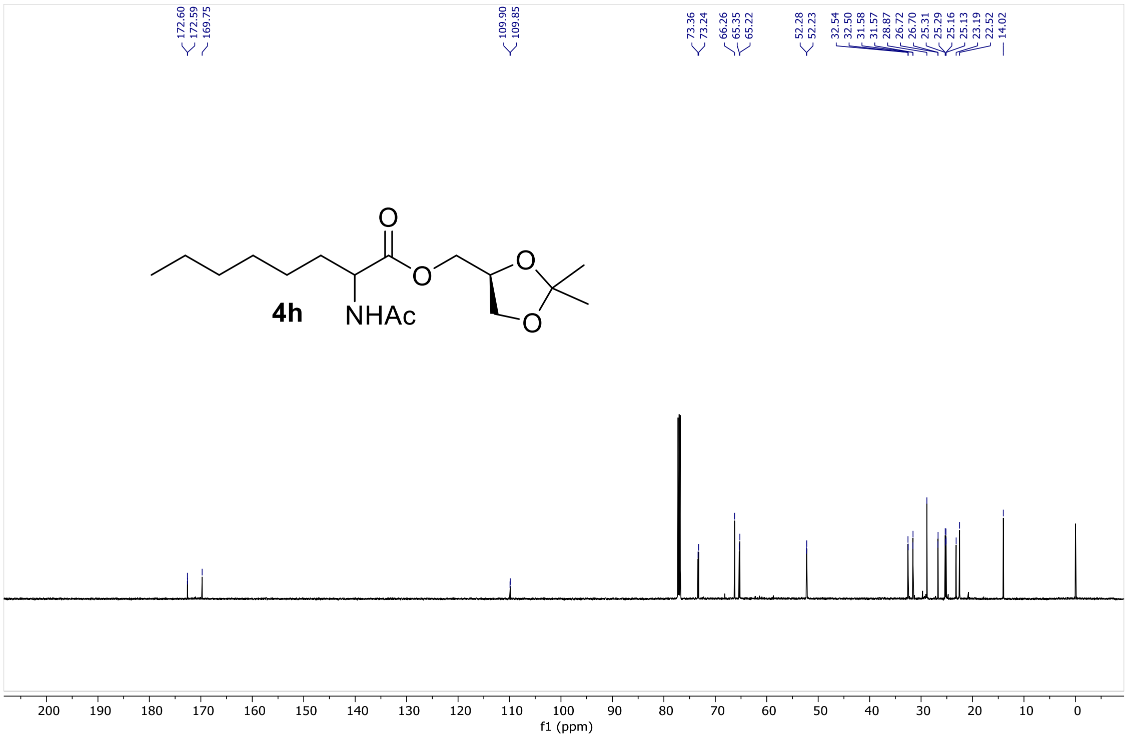


Figure S22. ^{13}C NMR spectrum of **4h** in $CDCl_3$

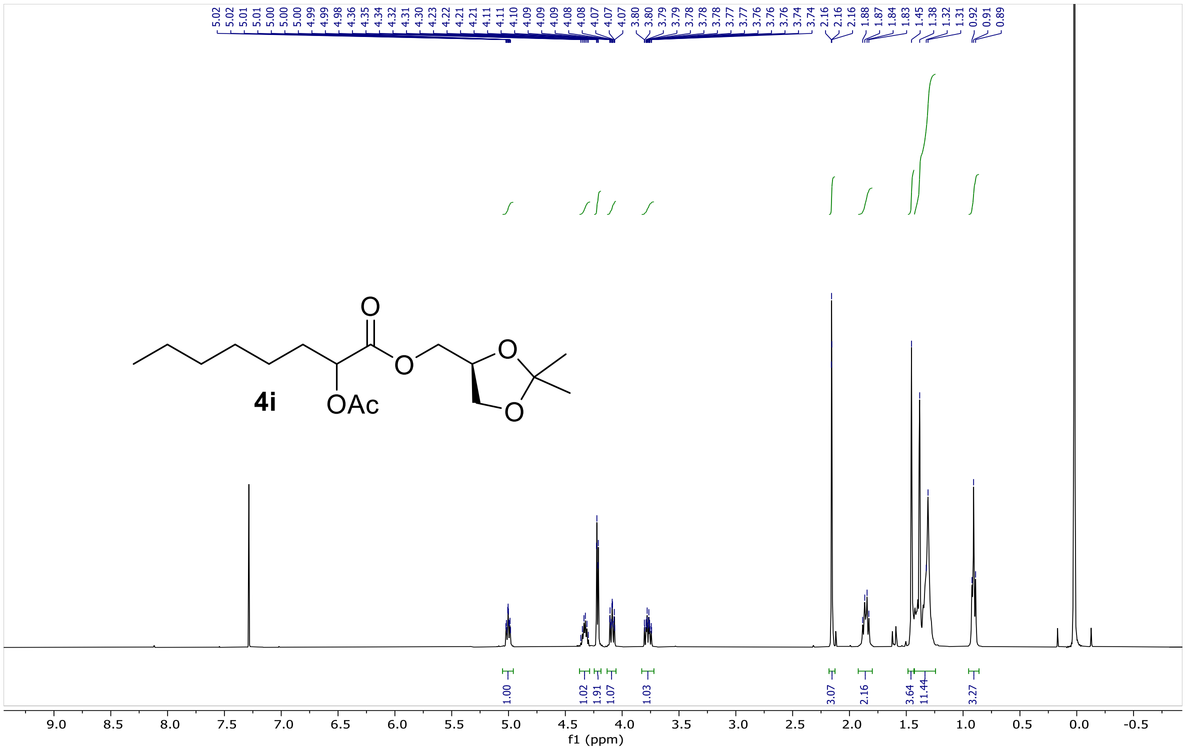


Figure S23. ^{1}H NMR spectrum of **4i** in $CDCl_3$

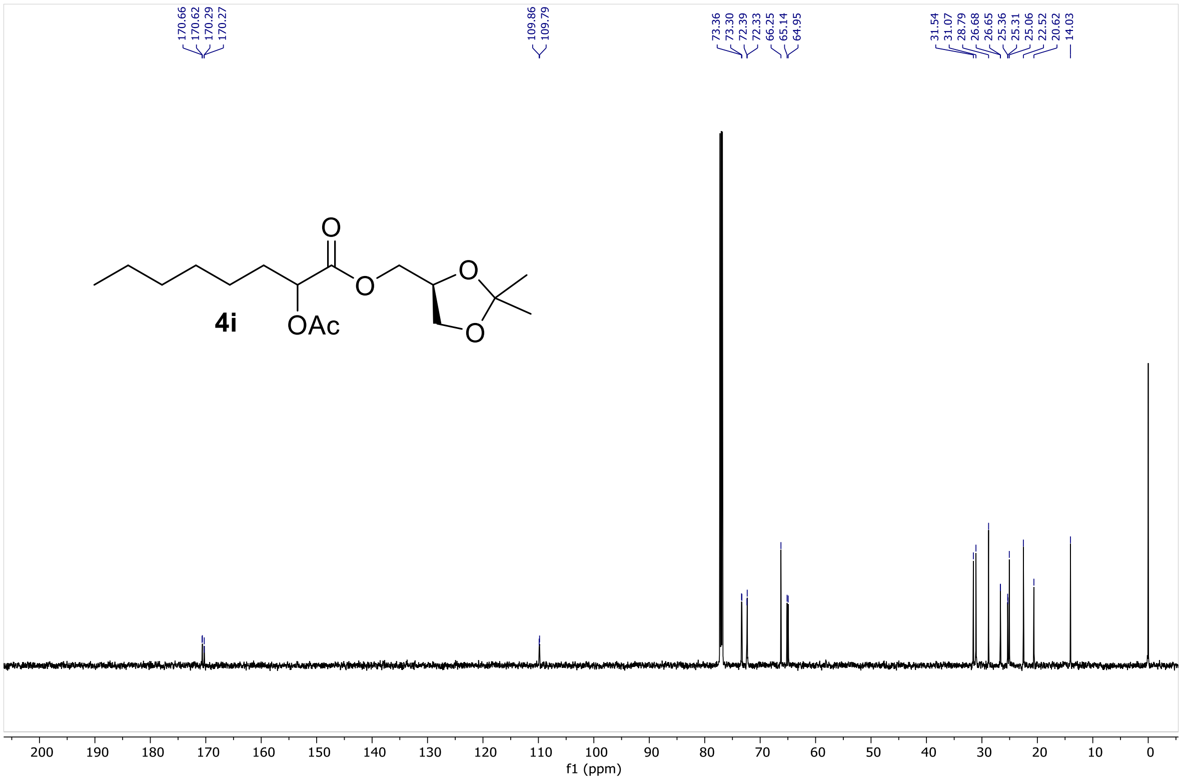


Figure S24. ^{13}C NMR spectrum of **4i** in $CDCl_3$

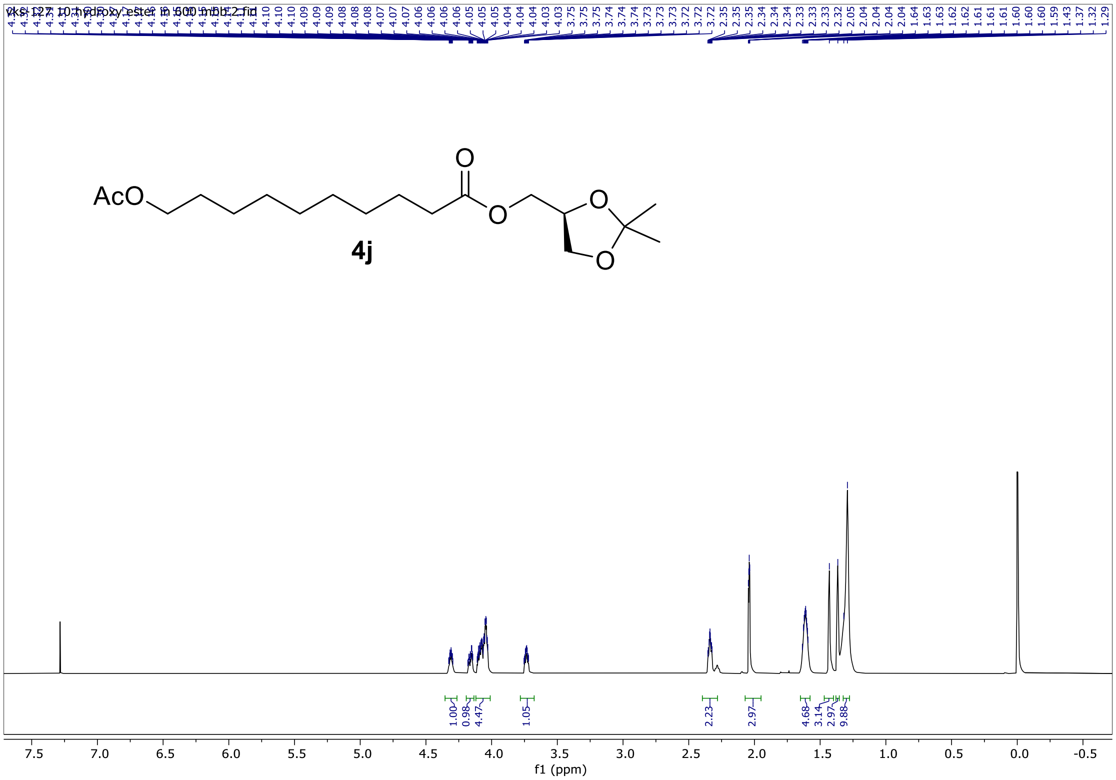


Figure S25. ^{1}H NMR spectrum of **4j** in $CDCl_3$

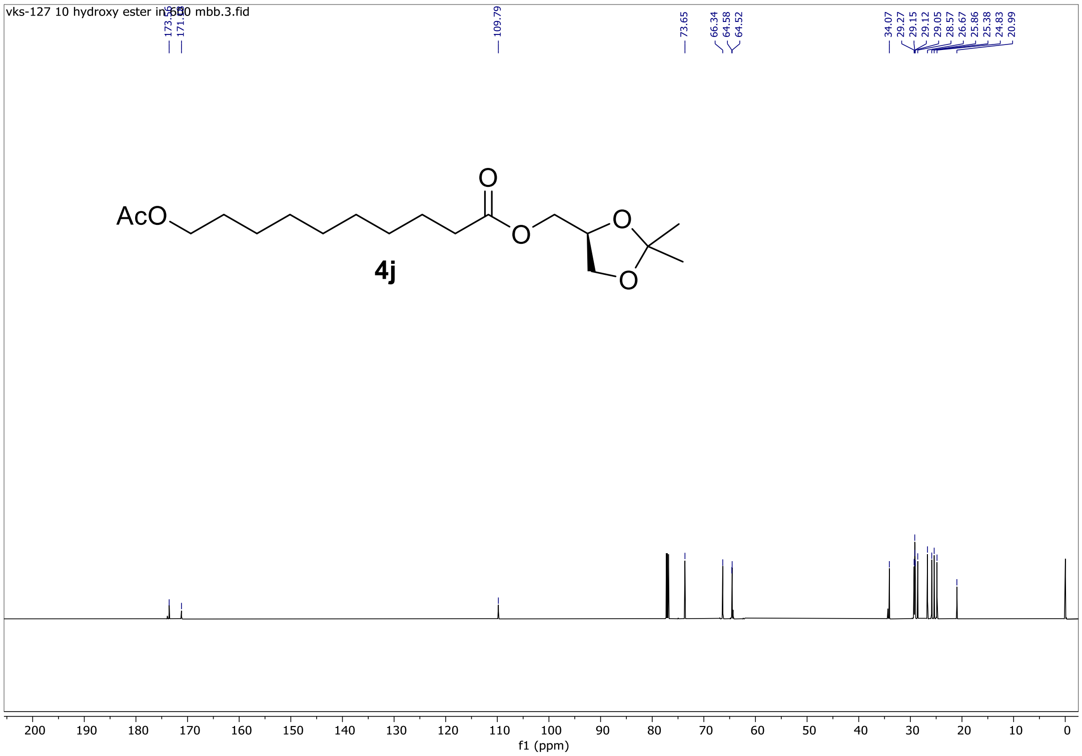


Figure S26. ^{13}C NMR spectrum of **4j** in $CDCl_3$

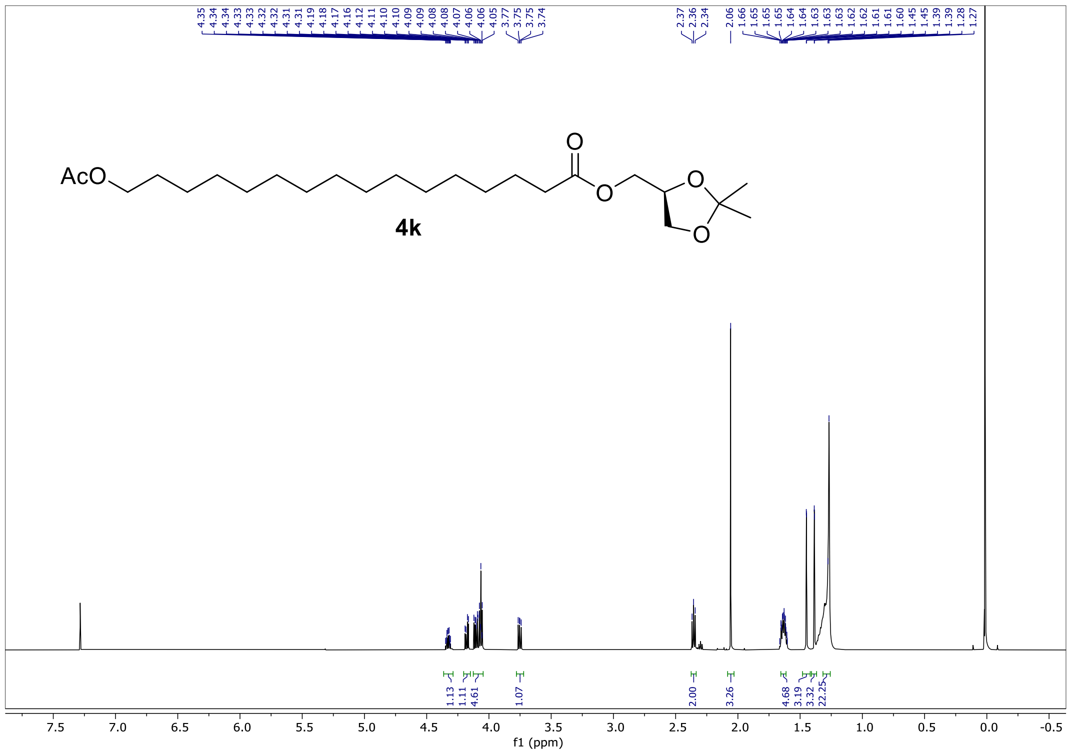


Figure S27. ^{1}H NMR spectrum of **4k** in $CDCl_3$

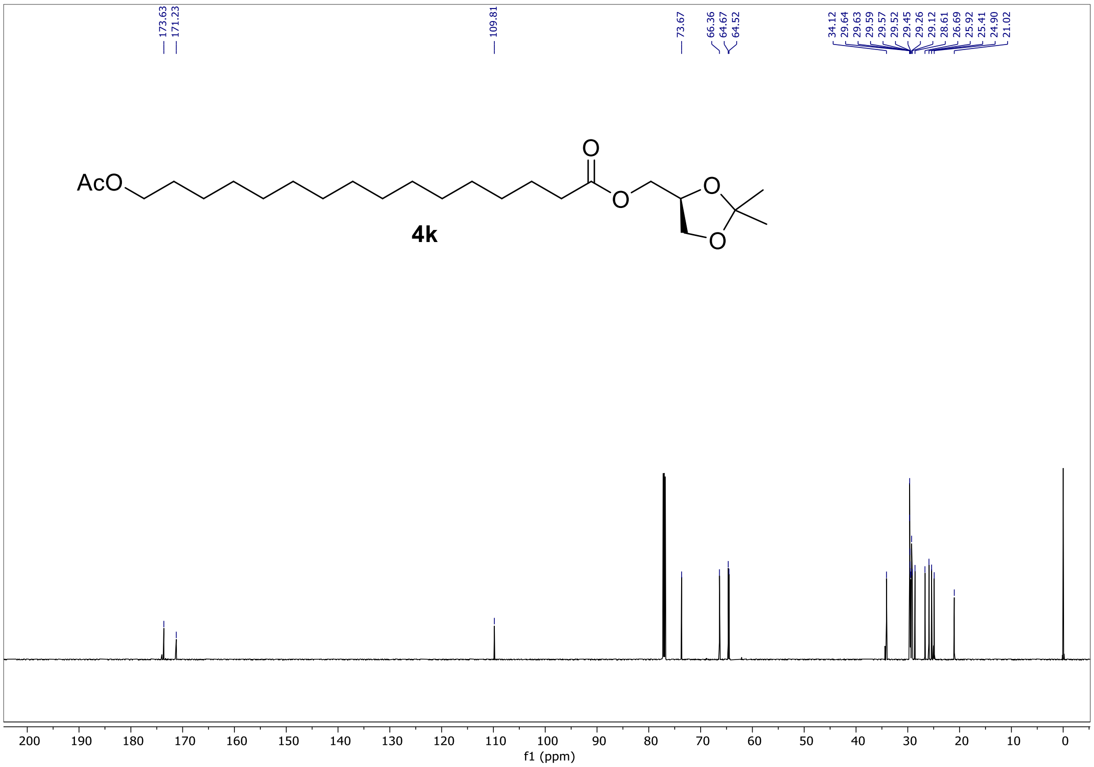
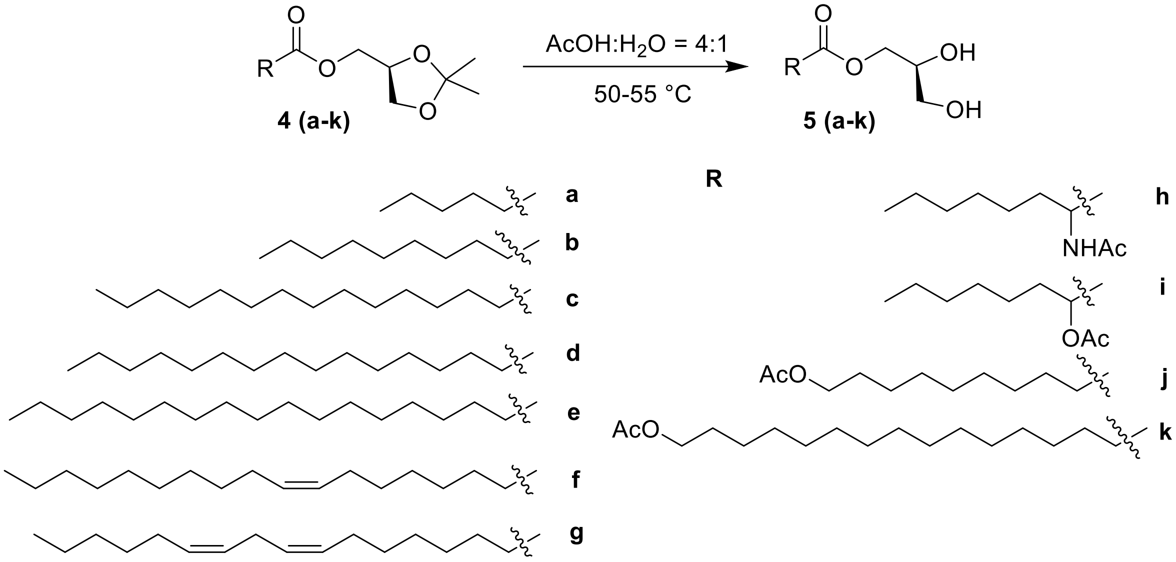


Figure S28. ^{13}C NMR spectrum of **4k** in $CDCl_3$

Hydrolysis of diacetal glyceride 4a-k



General procedure for the preparation of **5a-k**: A solution of diacetal glyceride **4 (a-k)** (1 g, 1 equiv.) in AcOH /H_2O (4:1, 30-40 ml)[Π] was stirred at 50-55 °C. After 4 hours, complete consumption of the starting material was observed over TLC. The heating bath was removed, the reaction mixture was transferred into a beaker followed by the addition of saturated aqueous $NaHCO_3$ (250 mL) and EtOAc (250 mL). Stirred the mixture slowly for 15 minutes to neutralize the acetic acid present in the reaction mixture and transferred the entire material into a separating funnel to extract the organic layer. The aqueous layer was extracted with EtOAc (1 × 100 mL) and the combined organic layer was washed again with saturated aqueous $NaHCO_3$ (250 mL). Finally, the organic layer was washed with brine (200 mL) and dried over anhydrous Na_2SO_4, and concentrated. The residue obtained was kept under a high vacuum for 12 hours to afford glyceride **5** (**a-k**).

[Π] For compound **1e**, 24 mL/mmol was used to overcome solubility issues.

2,3-dihydroxypropyl hexanoate (**5a**):[5] *Rf* = 0.5; Hexanes:EtOAc 20:80; colorless oil (87%), **^{1}H NMR** (500 MHz, CD_3OD): δ 4.16 (dd, *J* = 11.5, 4.5 Hz, 1H), 4.08 (dd, *J* = 11.5, 6.5 Hz, 1H), 3.84 (quint, *J* = 5.5 Hz, 1H), 3.57 (m, 2H), 2.37 (t, *J* = 7 Hz, 2H), 1.64 (quint, *J* = 7.5 Hz, 2H), 1.35 (m, 4H), 0.93 (t, *J* = 6.5 Hz, 3H). **^{13}C NMR** (125 MHz, CD_3OD): δ 174.1, 69.8, 65.1, 62.7, 33.5, 31.0, 24.3, 22.0, 12.9.

2,3-dihydroxypropyl decanoate (**5b**):[2] *Rf* = 0.55; Hexanes:EtOAc 20:80; white solid (quantitative). **^{1}H NMR** (500 MHz, $CDCl_3$): δ 4.22 (dd, *J* = 11.5, 4.5 Hz, 1H), δ 4.16 (dd, *J* = 11.5, 6 Hz, 1H), 3.90 (quint, *J* = 5, 1H), 3.71 (dd, *J* = 11.5, 4 Hz, 1H), 361 (dd, *J* = 11.5, 6 Hz, 1H), 2.37 (t, *J* = 6.5 Hz, 2H), 1.64 (quint, *J* = 7.5 Hz, 2H), 1.29 (m, 12H), 0.90 (t, *J* = 6.5 Hz, 3H). **^{13}C NMR** (125 MHz, $CDCl_3$): δ 174.4, 70.3, 65.2, 63.4, 34.2, 31.9, 29.4, 29.3, 29.1, 24.9, 22.7, 14.1.

2,3-dihydroxypropyl tetradecanoate (**5c**):[2] *Rf* = 0.45, Hexanes:EtOAc 40:60; white solid (quantitative). **^{1}H NMR** (500 MHz, $CDCl_3$): δ 4.16 (m, 2H), 3.93 (quint, *J* = 5.5 Hz, 1H), 3.70 (dd, *J* = 11.5, 4 Hz, 1H), 3.59 (dd, *J* = 11.5, 3 Hz, 1H), 2.91 (brs, 2H), 2.35 (t, *J* = 8Hz, 2H), 1.63 (m, 2H), 1.28 (m, 20H), 0.89 (t, *J* = 6.5 Hz, 3H). **^{13}C NMR** (125 MHz, $CDCl_3$): δ 174.4, 70.3, 65.1, 63.4, 34.2, 31.9, 29.68, 29.65, 29.61, 29.5, 29.4, 29.3, 29.1, 24.9, 22.7, 14.1.

2,3-dihydroxypropyl palmitate (**5d**):[2] Rf = 0.4, Hexanes:EtOAc 40:60; white solid (quantitative). ^{1}**H NMR** (500 MHz, $CDCl_3$): δ 4.19 (m, 2H), 3.94 (quint, *J* = 5.5 Hz, 1H), 3.71 (dd, *J* = 11.5, 4 Hz, 1H), 3.61 (dd, *J* = 11.5, 6 Hz, 1H), 2.34 (t, *J* = 7.5 Hz, 2H), 1.64 (quint, *J* = 7.5 Hz, 2H), 1.32–1.26 (m, 24H), 0.90 (t, *J* = 6.5 Hz, 3H). **^{13}C NMR** (120 MHz, $CDCl_3$): δ 174.4, 70.3, 65.2, 63.3, 34.2, 31.9, 29.7, 29.68, 29.66, 29.60, 29.5, 29.4, 29.3, 29.1, 24.9, 22.7,14.1.

2,3-dihydroxypropyl stearate (**5e**):[2] *Rf* = 0.4, Hexanes:EtOAc 40:60; white solid (98%). **^{1}H NMR** (500 MHz, $CDCl_3$): δ 4.20 (m, 2H), 3.95 (m, 1H), 3.71 (dd, *J* = 11.5, 4 Hz, 1H), 3.62 (dd, *J* = 11.5, 6 Hz, 1H), 2.37 (t, *J* = 7.5Hz, 2H), 1.65 (quint, *J* = 7.5 Hz, 2H), 1.28 (m, 28H), 0.90 (t, *J* = 6.5 Hz, 3H). **^{13}C NMR** (MHz, $CDCl_3$): δ 174.4, 70.3, 65.2, 63.3, 34.2, 31.9, 29.71, 29.68, 29.67, 29.65, 29.61, 29.5, 29.4, 29.3, 29.1, 24.9, 22.7, 14.1.

2,3-dihydroxypropyl oleate (**5f**):[2] *Rf* = 0.45, Hexanes:EtOAc 40:60; colorless oil (Quantitative). **^{1}H NMR** (500 MHz, $CDCl_3$): δ 5.35 (m, 2H), 4.18 (m, 2H), 3.94 (m, 1H), 3.70 (m, 1H) 3.60 (m, 1H), 2.60 (brs, 1H), 2.36 (t, *J* = 7.5 Hz, 2H), 2.02 (m, 4H), 1.64 (m, 2H), 1.30 (m, 20H), 0.89 (t, *J* = 7 Hz, 3H). **^{13}C NMR** (125 MHz, $CDCl_3$): δ 174.4, 130.0, 129.7, 70.3, 65.16, 65.14, 63.4, 34.2, 31.9, 29.8, 29.7, 29.5, 29.3, 29.2, 29.1, 27.2, 27.1, 24.9, 22.7, 14.1.

2,3-dihydroxypropyl (9Z,12Z)-octadeca-9,12-dienoate (**5g**):[3] *Rf* = 0.5, Hexanes:EtOAc 40:60; colorless oil (quantitative). **^{1}H NMR** (500 MHz, CD_3OD): δ 5.422–5.30 (m, 4H), 4.16 (dd, *J* = 11.5, 4.5 Hz, 1H), 4.08 (dd, *J* = 11.5, 6.5 Hz, 1H), 3.84 (quint, *J* = 5.5 Hz, 1H), 3.62–3.52 (m, 2H), 3.33 (s, OH), 2.80 (t, *J* = 7 Hz, 2H), 2.37 (t, *J* = 7.5 Hz, 2H), 2.09 (m, 4H), 1.64 (m, 2H), 1.41–1.33 (m, 14H), 0.93 (t, *J* = 6.5 Hz, 3H). **^{13}C NMR** (120 MHz, CD_3OD): δ 174.0, 129.58, 129.50, 127.7, 127.6, 69.8, 65.1, 62.7, 33.6, 31.3, 29.4, 29.1, 28.9, 28.87, 28.85, 25.2, 24.6, 22.3, 13.1.

2,3-dihydroxypropyl 2-acetamidooctanoate (**5h**): *Rf* = 0.3, Hexanes:EtOAc 0:100; Colorless liquid (quantitative, 1:1 diastereomeric mixture). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 6.13 (d, *J* = 7.4 Hz, 1H), 4.50 (tdd, *J* = 7.8, 5.6, 2.4 Hz, 1H), 4.34 (dt, *J* = 11.6, 5.9 Hz, 1H), 4.21 – 4.13 (m, 1H), 4.01 – 3.93 (m, 1H), 3.81 – 3.49 (m, 2H), 2.05 (s, 3H), 1.84 (q, *J* = 9.2 Hz, 1H), 1.68 (dt, *J* = 15.1, 8.0 Hz, 1H), 1.38 – 1.26 (m, 12H), 0.90 (t, *J* = 6.8 Hz, 4H). **^{13}C NMR** (150 MHz, $CDCl_3$): δ 172.86, 172.85, 170.7, 170.6, 69.90, 69.85, 66.2, 66.0, 63.21, 63.20, 52.85, 52.82, 32.0, 31.5, 28.8, 25.4, 23.0, 22.5, 14.0. **HRMS (ESI)** m/z calcd for $C_{13}H_{25}NNaO_5$ $[M+Na]^+$ 298.1630, found 298.1615.

2,3-dihydroxypropyl 2-acetaoxyoctanoate (**5i**): *Rf* = 0.3, Hexanes:EtOAc 20:80; Colorless liquid (quantitative, 1:1 diastereomeric mixture). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 4.94 (t, *J* = 6.5 Hz, 1H), 4.40 – 4.15 (m, 2H), 3.96 (p, *J* = 5.4 Hz, 1H), 3.79 – 3.58 (m, 2H), 2.16 (s, 2H), 1.85 (dd, *J* = 10.8, 4.9 Hz, 3H), 1.55–1.18 (m, 12H), 0.91 (t, *J* = 6.7 Hz, 4H). **^{13}C NMR** (150 MHz, $CDCl_3$): δ 171.22, 171.17, 170.86, 170.81, 72.80, 72.78, 69.92, 69.90, 65.9, 65.8, 63.13, 63.1, 31.5, 31.0, 28.8, 25.1, 22.5, 20.6, 14.0. **HRMS (ESI)** m/z calcd for $C_{13}H_{24}NaO_6$ $[M+Na]^+$ 299.1471, found 229.1461.

(S)-2,3-dihydroxypropyl-10-acetoxydecanoate (**5j**): *Rf* = 0.2, Hexanes:EtOAc 40:60; Colorless oil (Crude contains ~20% of the impurity which was carried over from the previous step). **^{1}H NMR** (600 MHz, $CDCl_3$) δ 4.18 (dd, *J* = 4.8, 11.4 Hz, 2H), 4.06 (td, *J* = 1.2, 7.2 Hz, 2H), 3.94 (m, 1H), 3.71 (dd, *J* = 4.2, 11.4 Hz, 1H), 3.61 (dd, *J* = 6, 11.4 Hz, 1H), 2.36 (t, *J* = 7.2 Hz, 2H), 2.06 (s, 3H), 1.63 (m, 4H), 1.34-1.28 (m, 10H) **^{13}C NMR** (150 MHz, $CDCl_3$) δ 174.3, 171.4, 70.3, 65.2, 64.64, 63.36, 34.1, 29.24, 29.10, 29.0, 28.55, 25.83, 24.86, 21.0. **HRMS (ESI)** m/z calcd for $C_{15}H_{28}NaO_6$ $[M+Na]^+$ 327.1778, found 327.1773.

(S)-2,3-dihydroxypropyl-16-acetoxyhexadecanoate (**5k**): *Rf* = 0.3, Hexanes:EtOAc 40:60; off-white solid (Crude contains ~16% of the impurity, which was carried over from the previous step). **^{1}H NMR** (600 MHz, $CDCl_3$) δ 4.242–4.12 (qd, *J* = 4.8, 11.4 Hz, 2H), 4.06(t, *J* = 7.2Hz, 2H), 3.94 (m, 1H), 3.70 (dd, *J* = 4.2, 12 Hz, 1H), 3.61 (dd, *J* = 6, 12 Hz, 1H), 2.77 (brs, 1H), 2.36 (t, *J* = 7.8Hz, 2H), 2.06 (s, 3H), 1.63 (m, 4H), 1.30–1.24 (m, 22H) **^{13}C NMR** (150 MHz, $CDCl_3$) δ 174.34, 171.4, 70.3, 65.2, 64.7, 63.3, 34.2, 29.65, 29.63, 29.61, 29.57, 29.55, 29.53, 29.50, 29.48, 29.44, 29.24, 29.12, 28.6, 25.9, 24.9, 21.0. **HRMS (ESI)** m/z calcd for $C_{21}H_{40}NaO_6$ $[M+Na]^+$ 411.2717, found 411.2719.

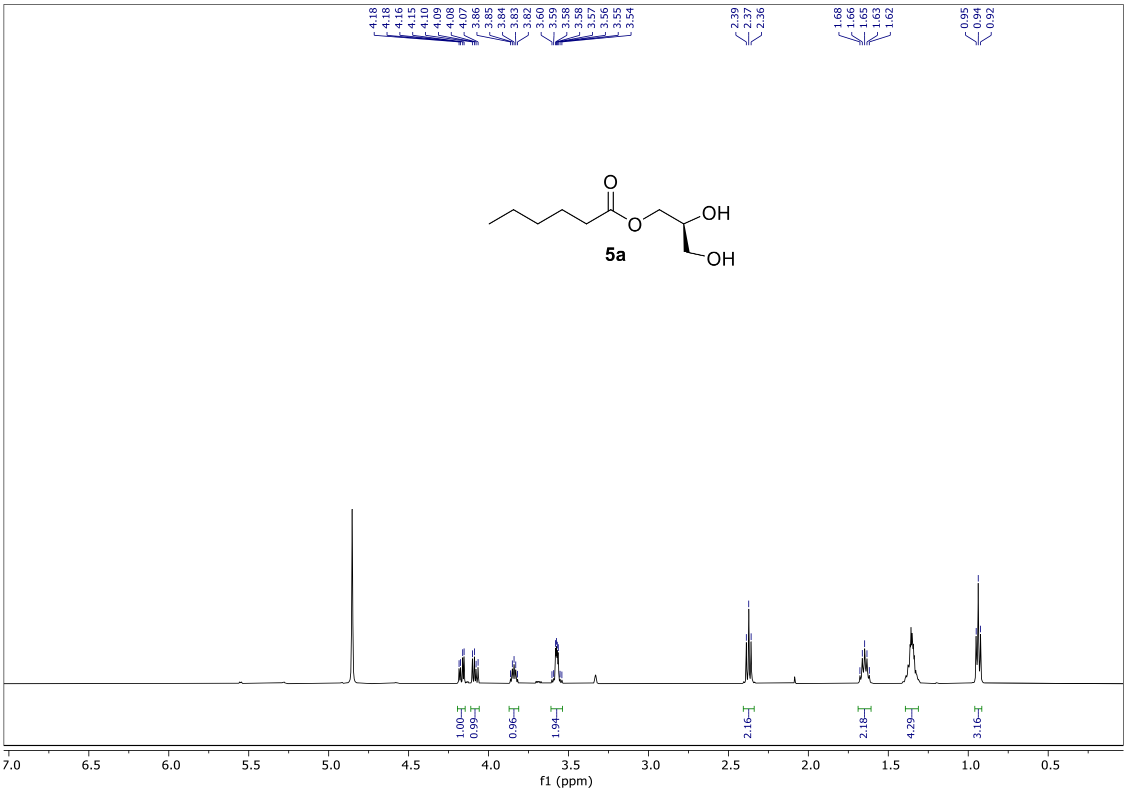


Figure S29.^{1}H NMR spectrum of **5a** in CD_3OD

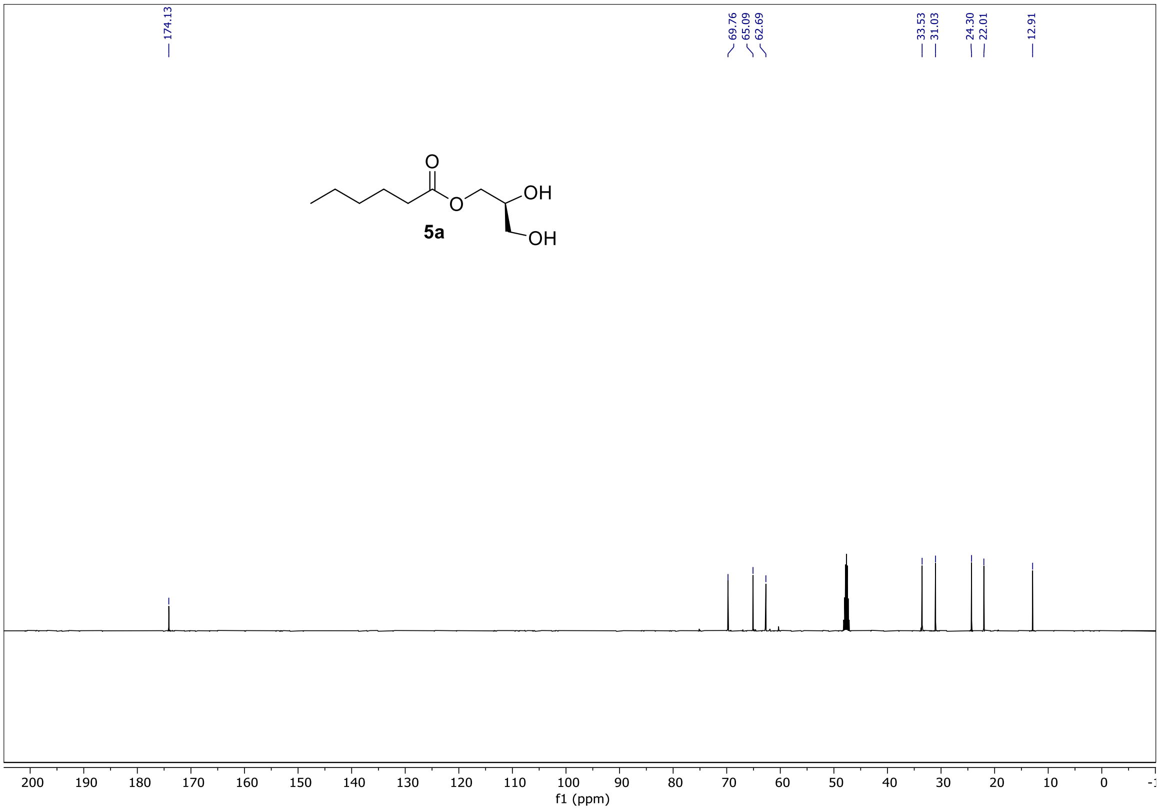


Figure S30.^{13}C NMR spectrum of **5a** in CD_3OD

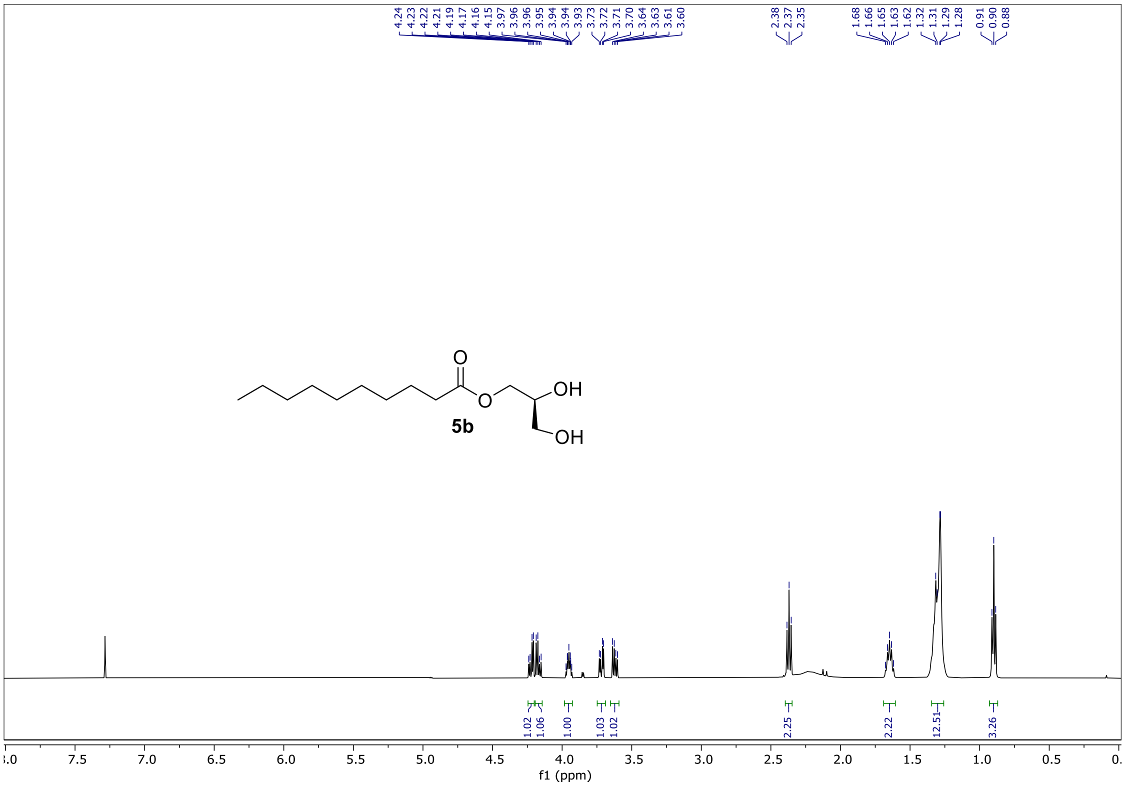


Figure S31.^{1}H NMR spectrum of **5b** in $CDCl_3$

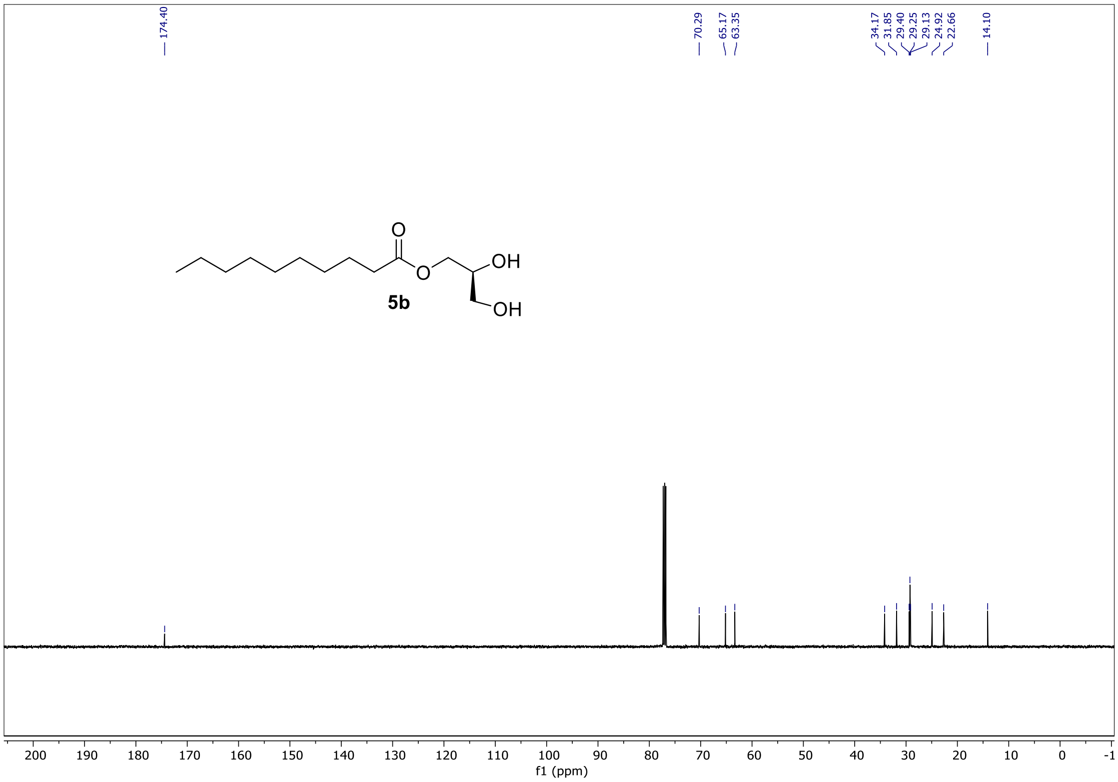


Figure S32.^{13}C NMR spectrum of **5b** in $CDCl_3$

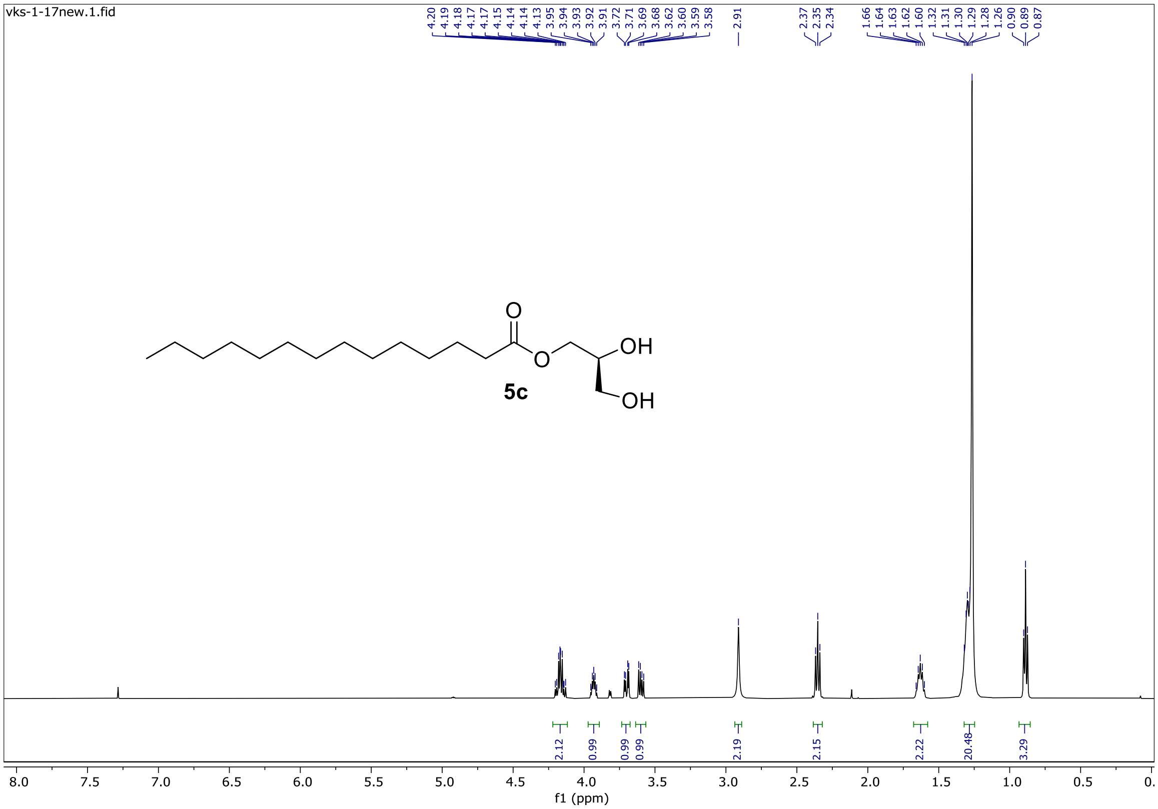


Figure S33.^{1}H NMR spectrum of **5c** in CDCl$_3$

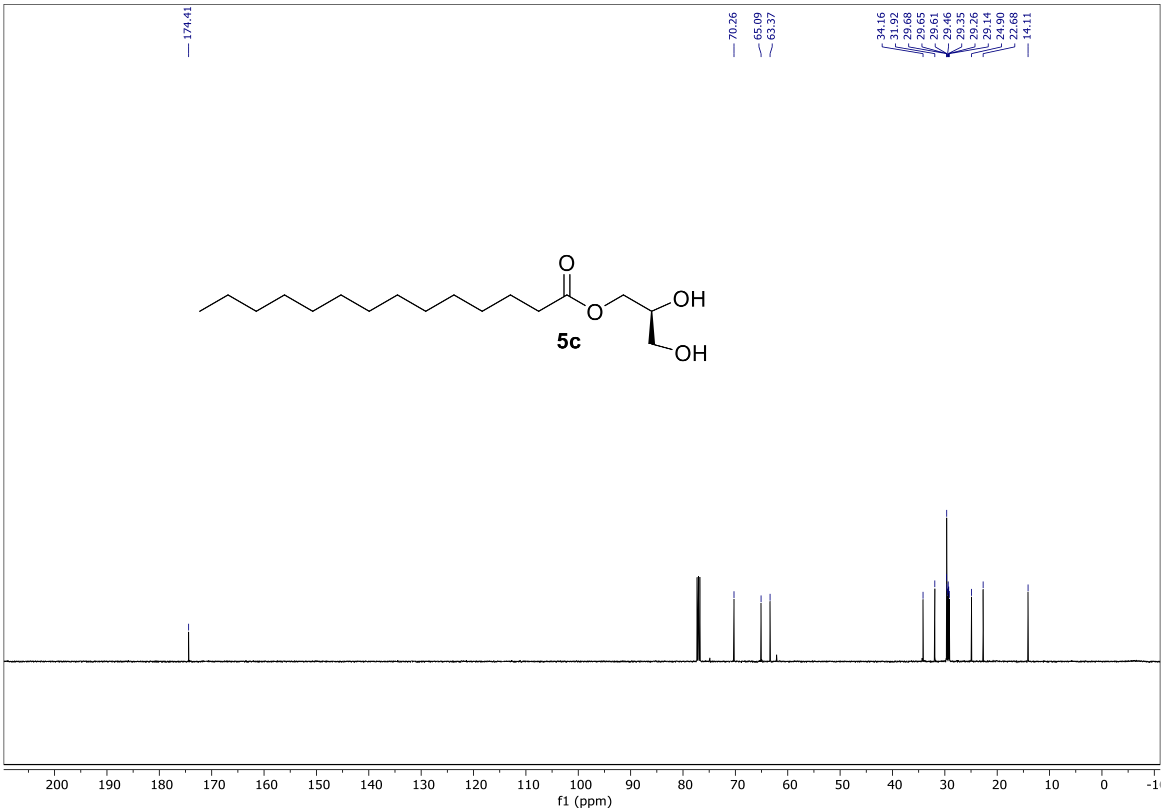


Figure S34.^{13}C NMR spectrum of **5c** in CDCl$_3$

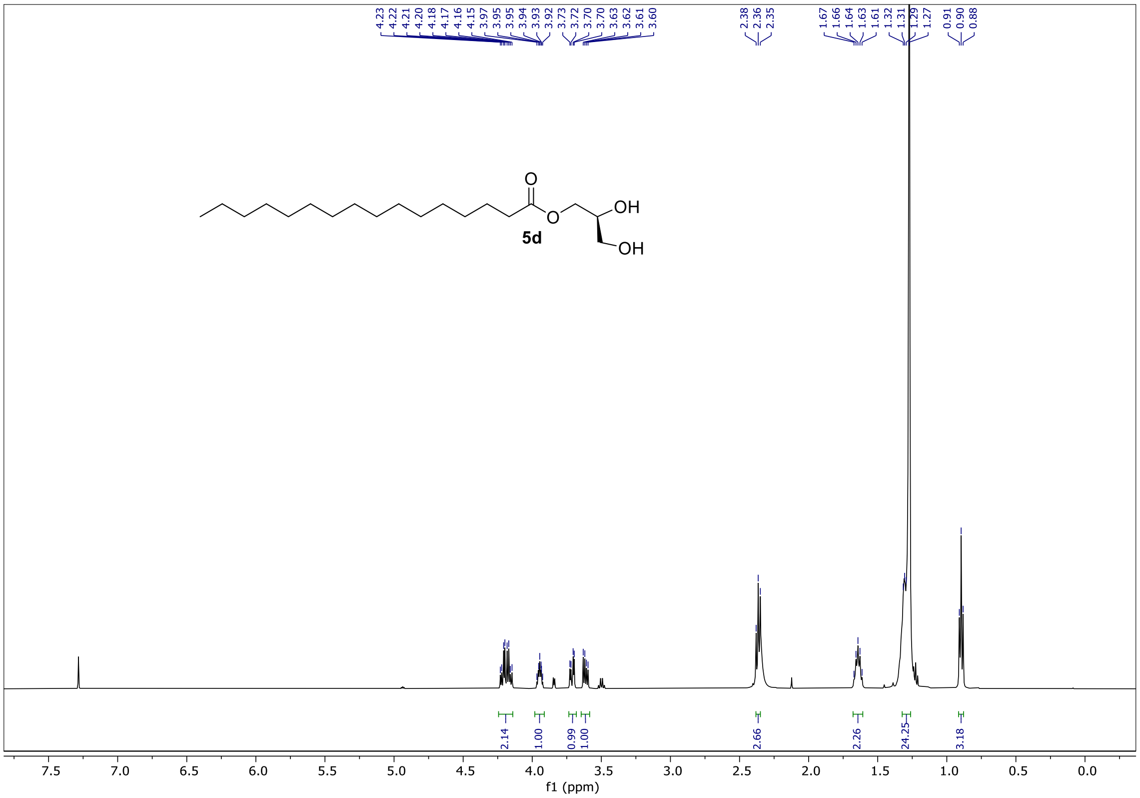


Figure S35.^{1}H NMR spectrum of **5d** in $CDCl_3$

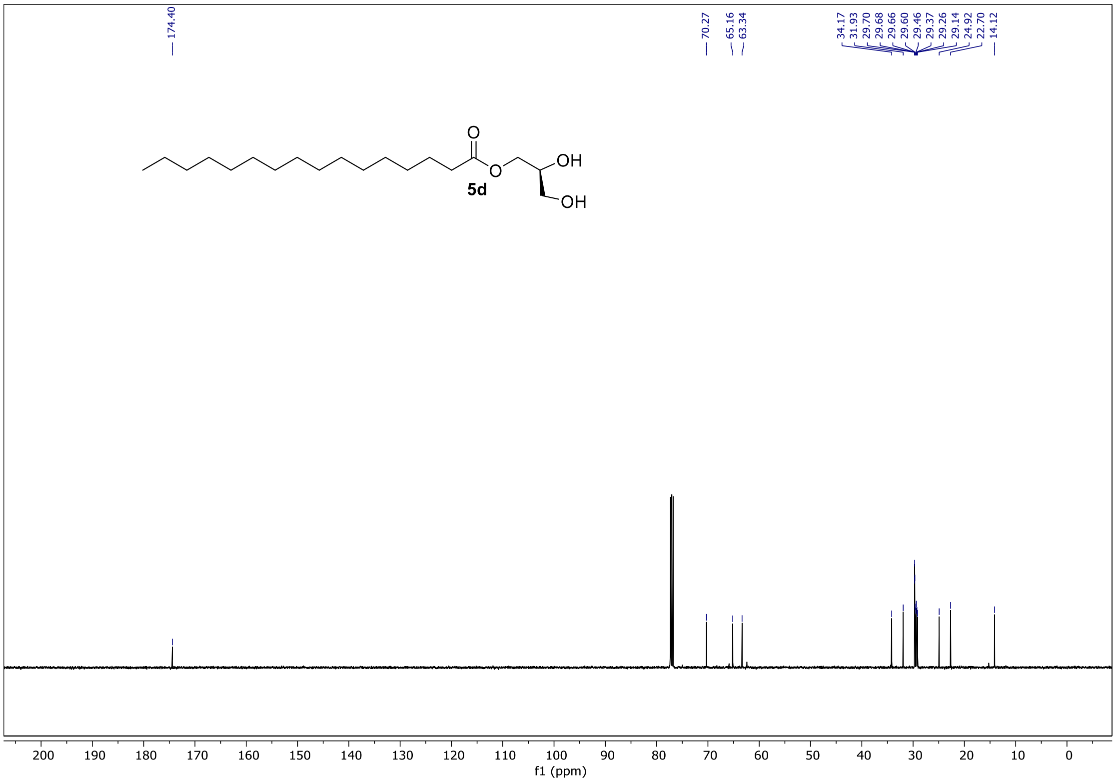


Figure S36.^{13}C NMR spectrum of **5d** in $CDCl_3$

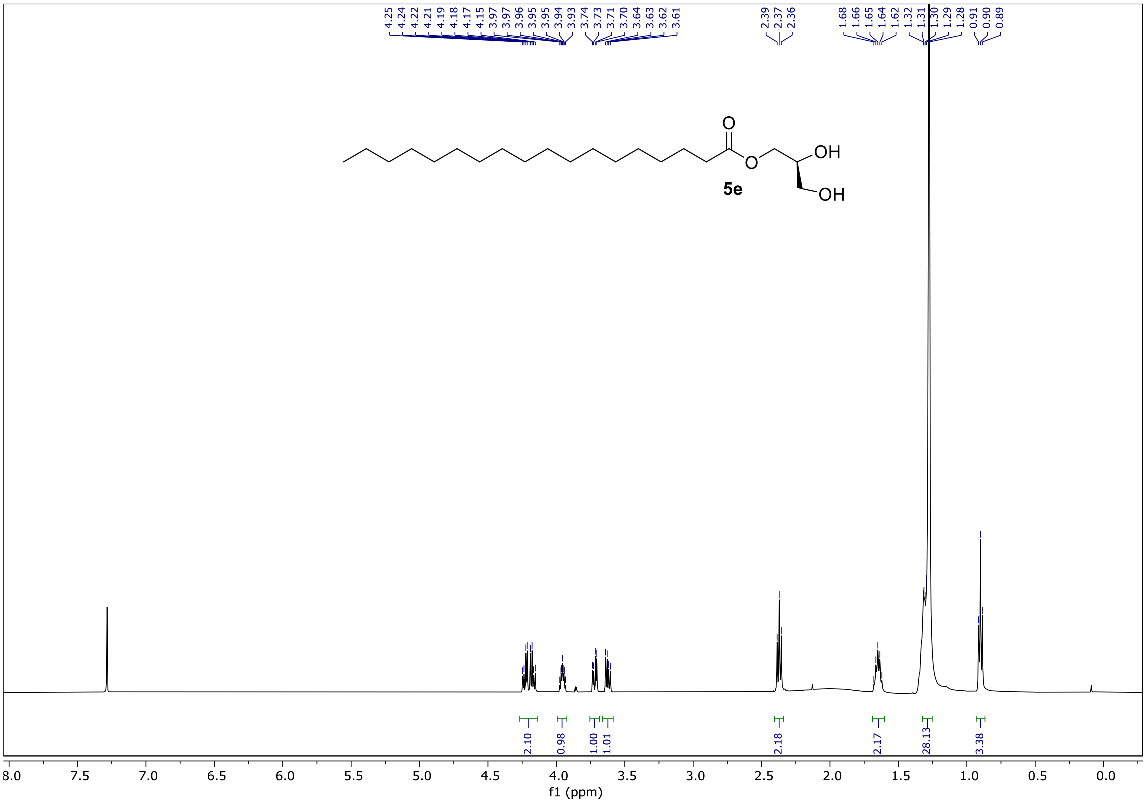


Figure S37. ^{31}H NMR spectrum of **5e** in CDCl$_3$

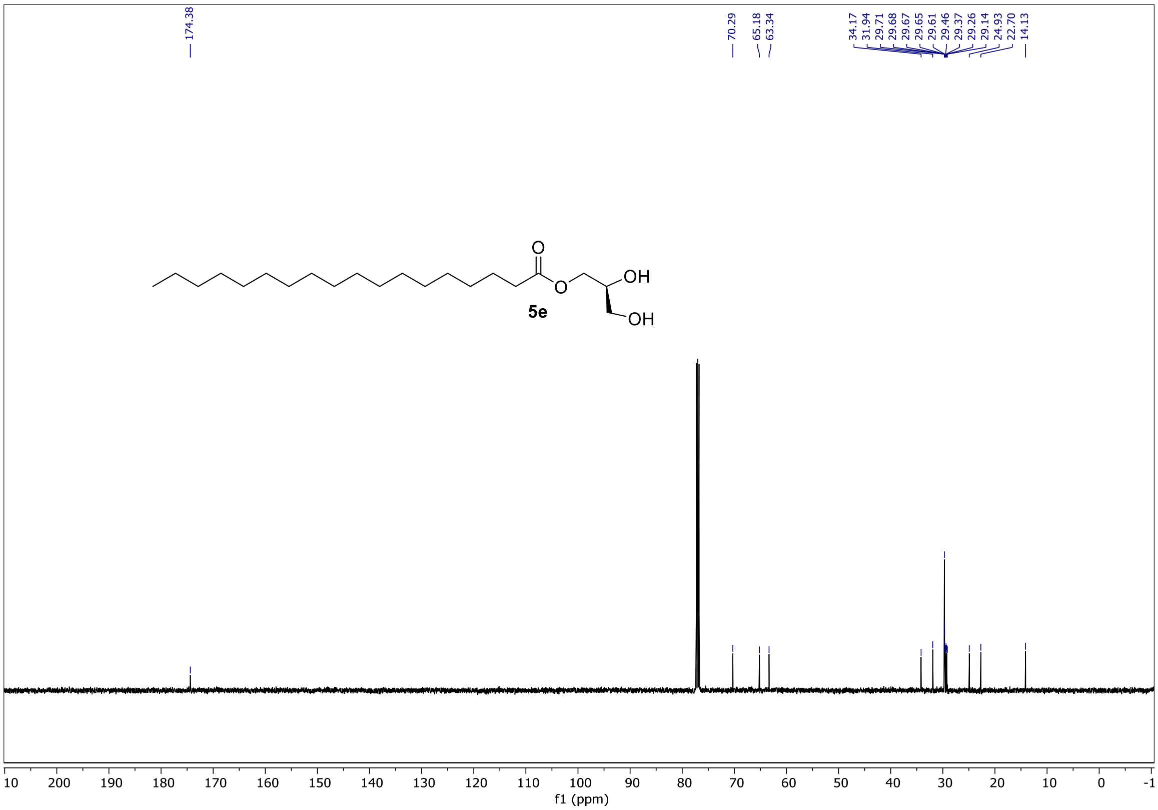


Figure S38.^{13}C NMR spectrum of **5e** in CDCl$_3$

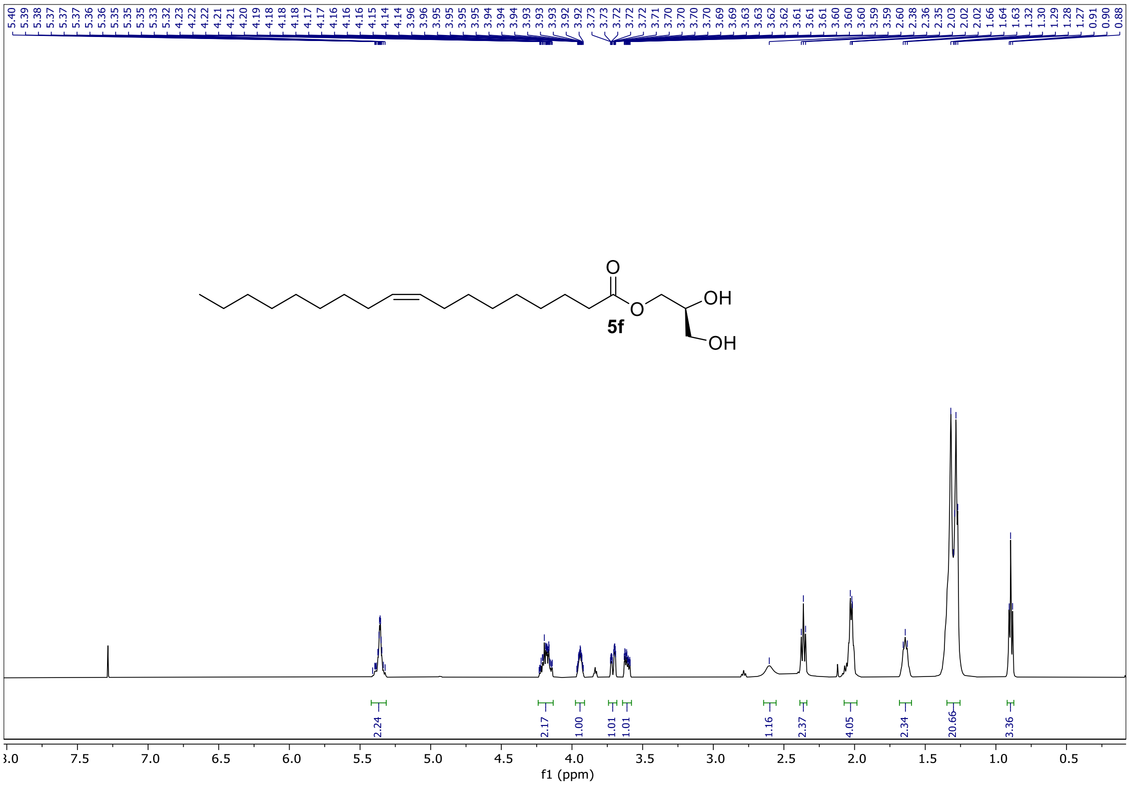


Figure S39.^{1}H NMR spectrum of **5f** in $CDCl_3$

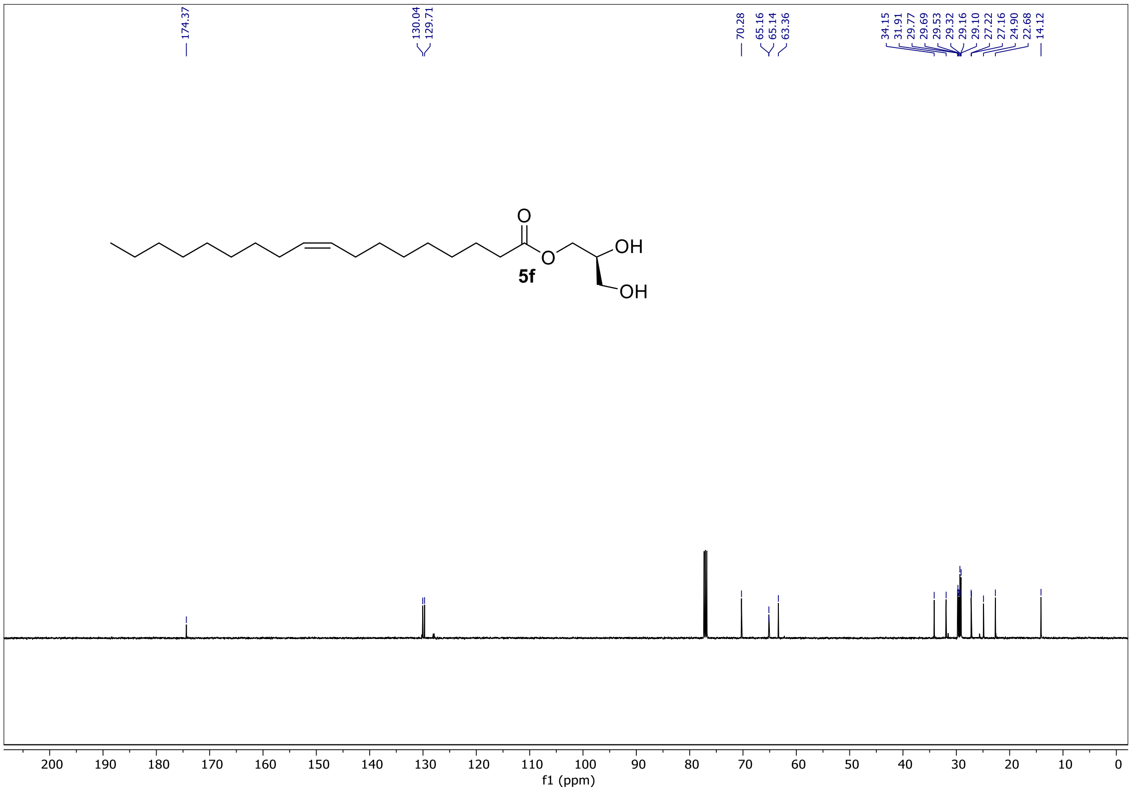


Figure S40.^{13}C NMR spectrum of **5f** in $CDCl_3$

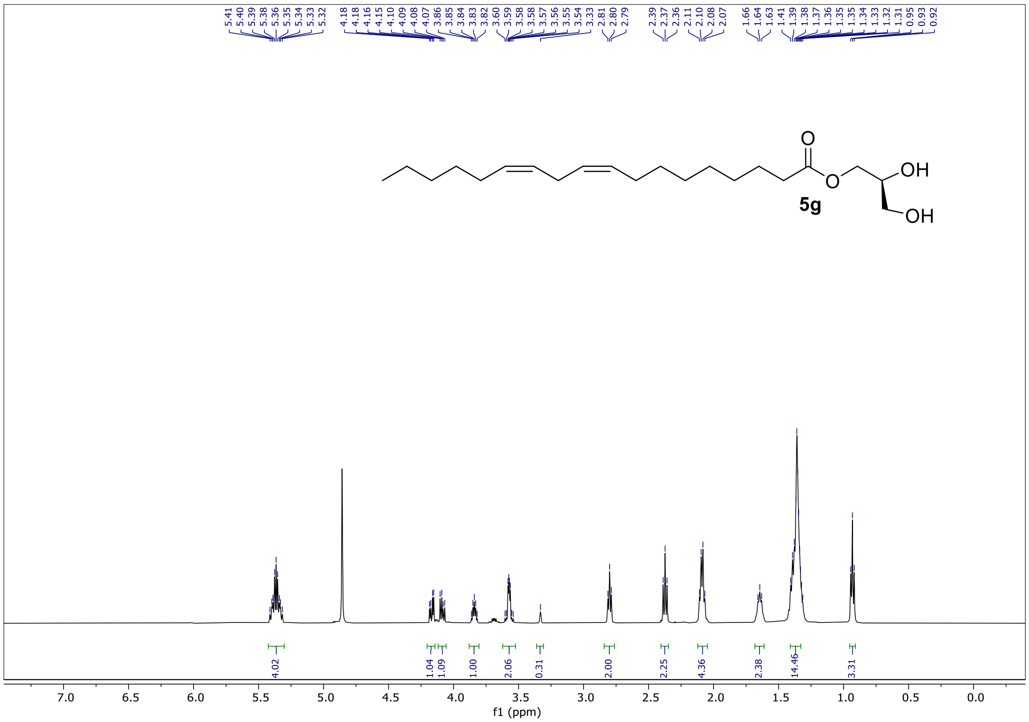


Figure S41. ^{1}H NMR spectrum of **5g** in CD_3OD

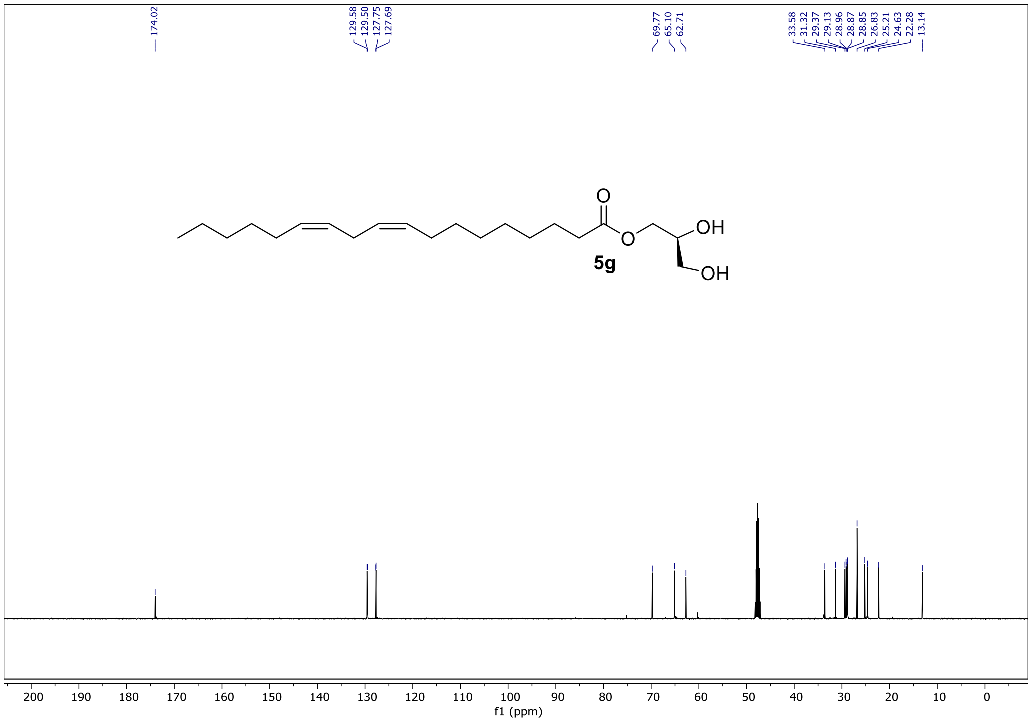


Figure S42.^{13}C NMR spectrum of **5g** in CD_3OD

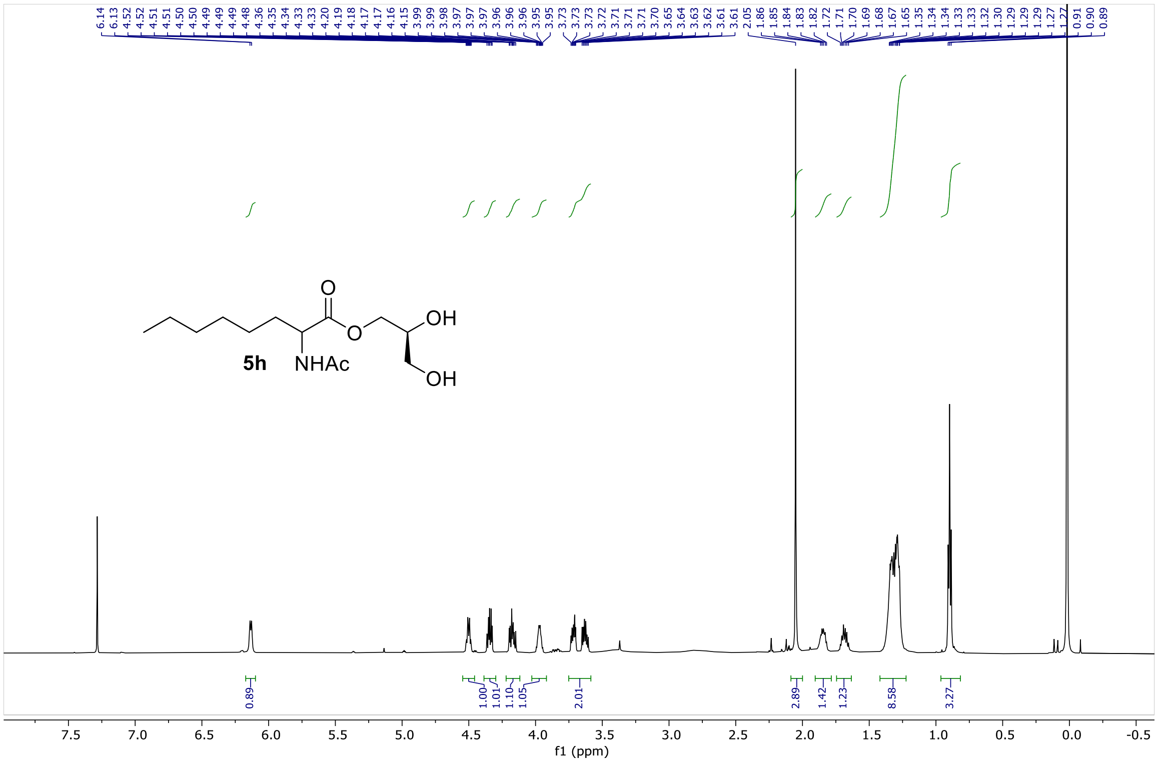


Figure S43. ^{1}H NMR spectrum of **5h** in $CDCl_3$

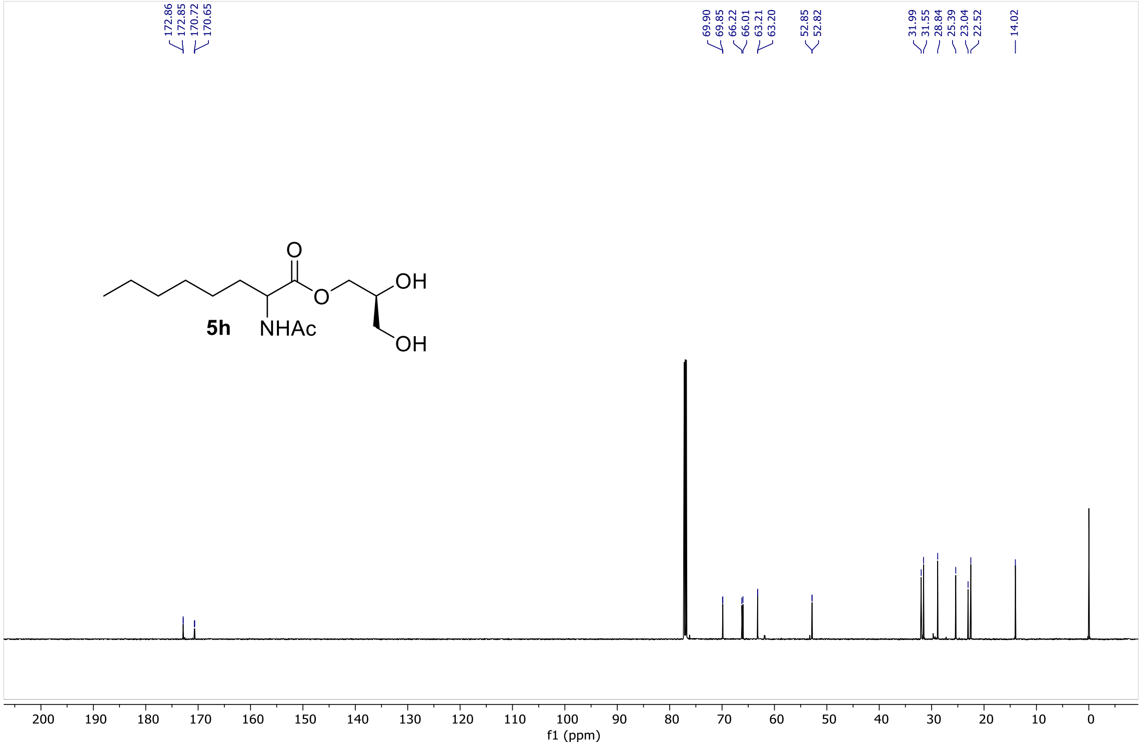


Figure S44. ^{13}C NMR spectrum of **5h** in $CDCl_3$

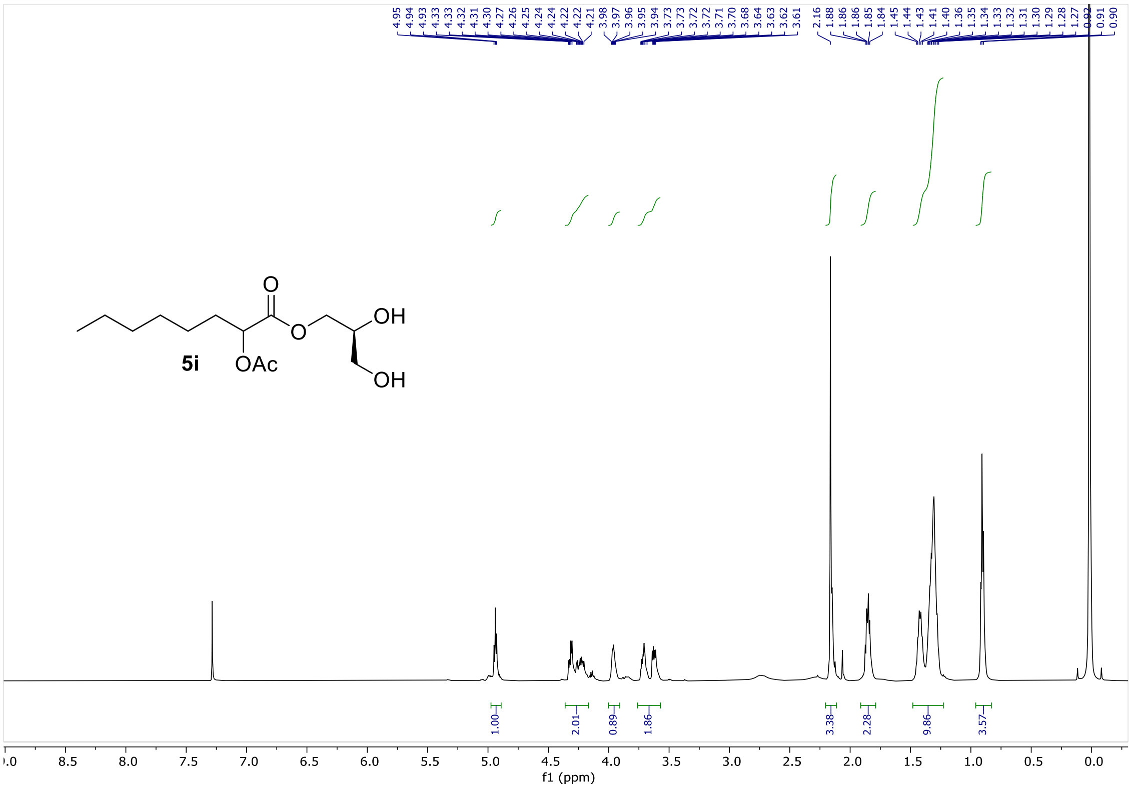


Figure S45. ^{1}H NMR spectrum of **5i** in $CDCl_3$

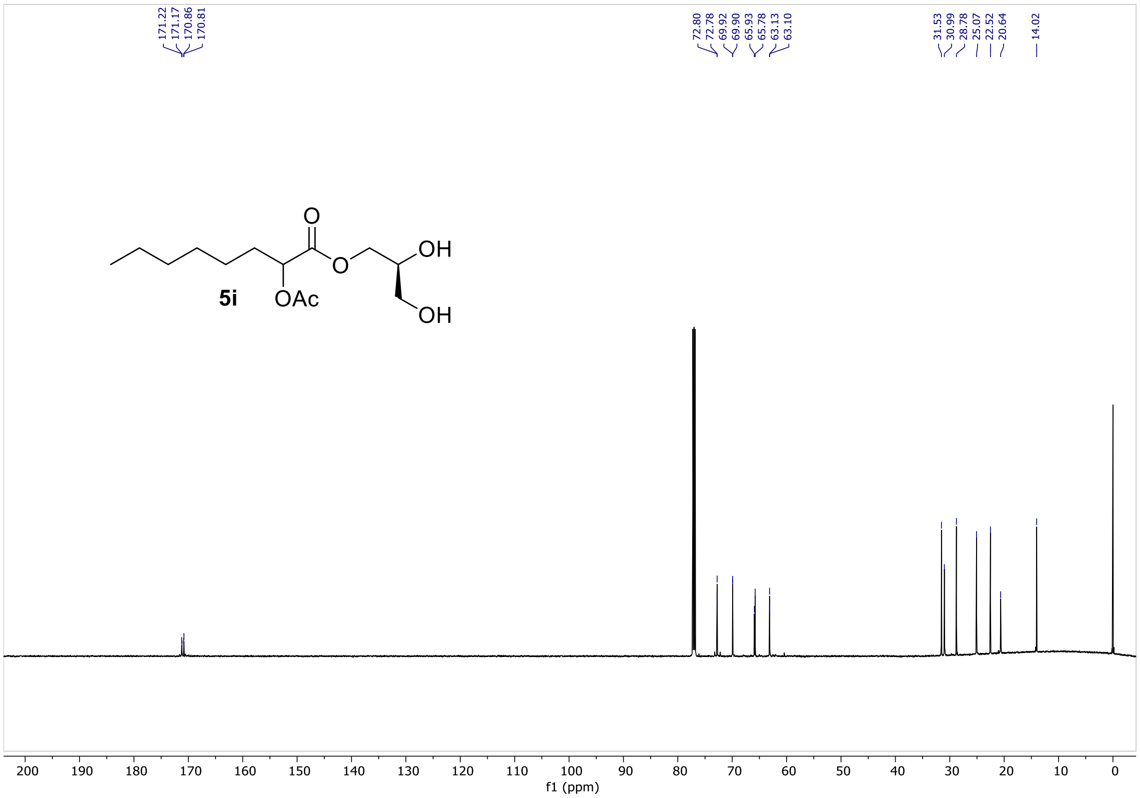


Figure S46. ^{13}C NMR spectrum of **5i** in $CDCl_3$

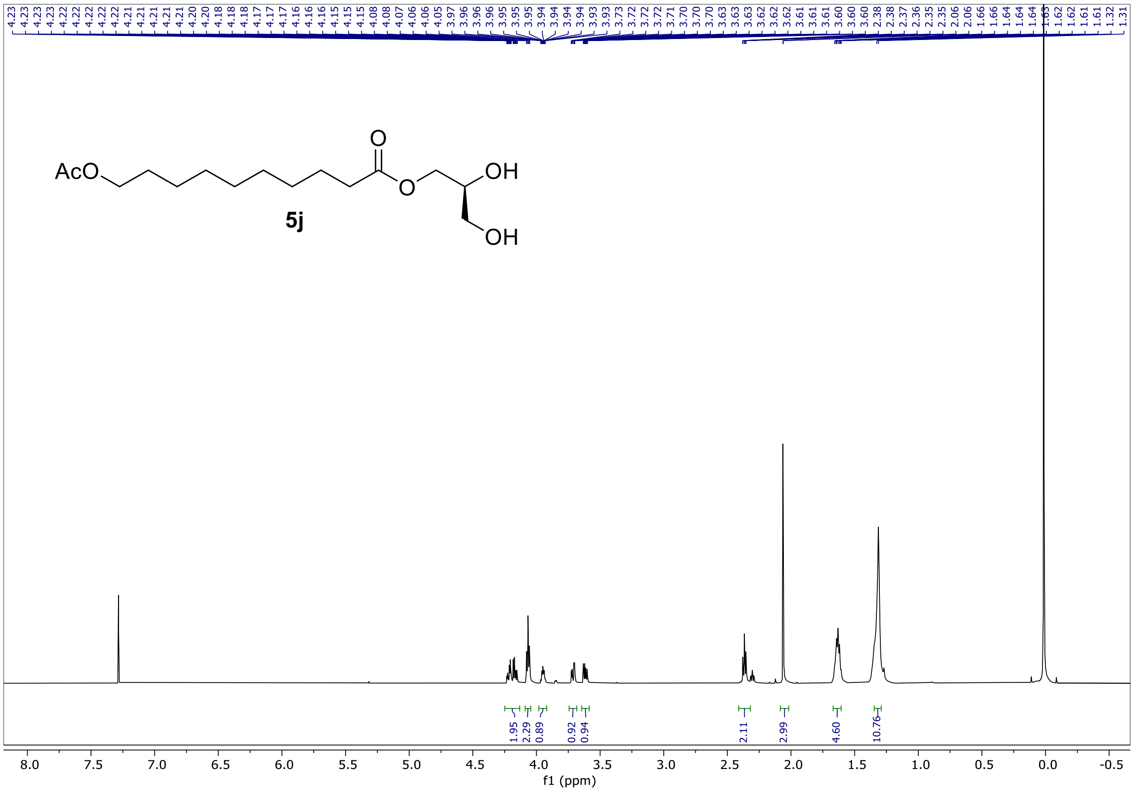


Figure S47. ^{1}H NMR spectrum of **5j** in $CDCl_3$

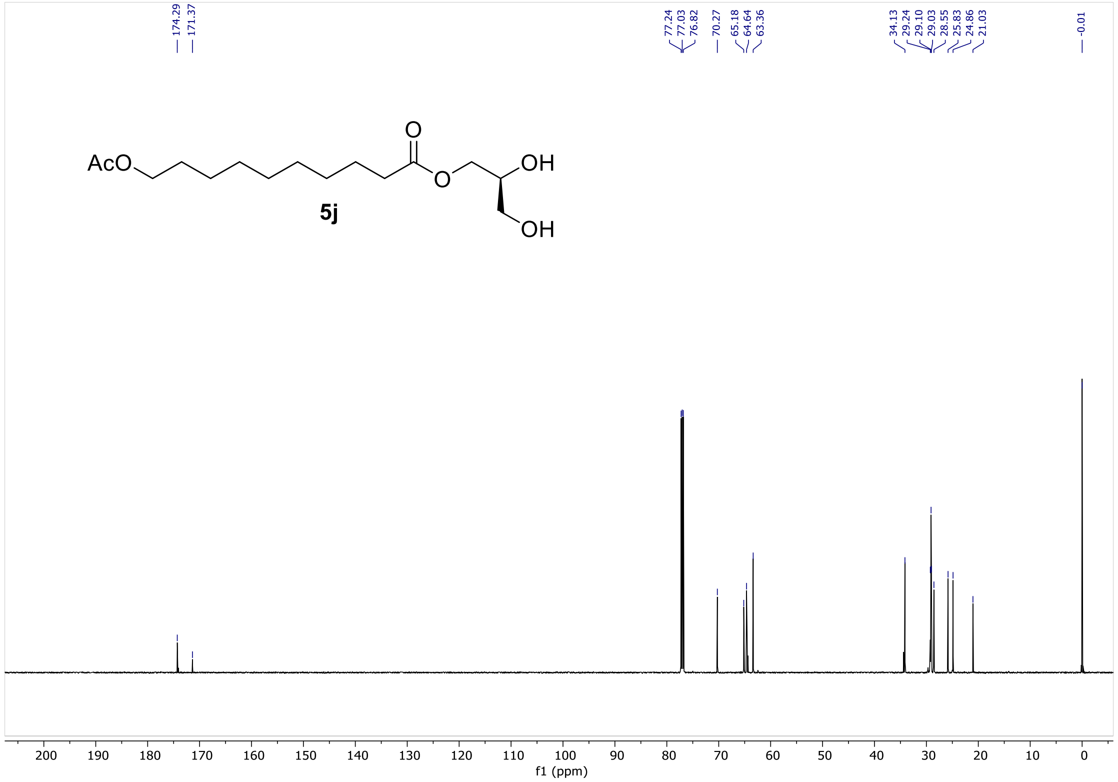


Figure S48. ^{13}C NMR spectrum of **5j** in $CDCl_3$

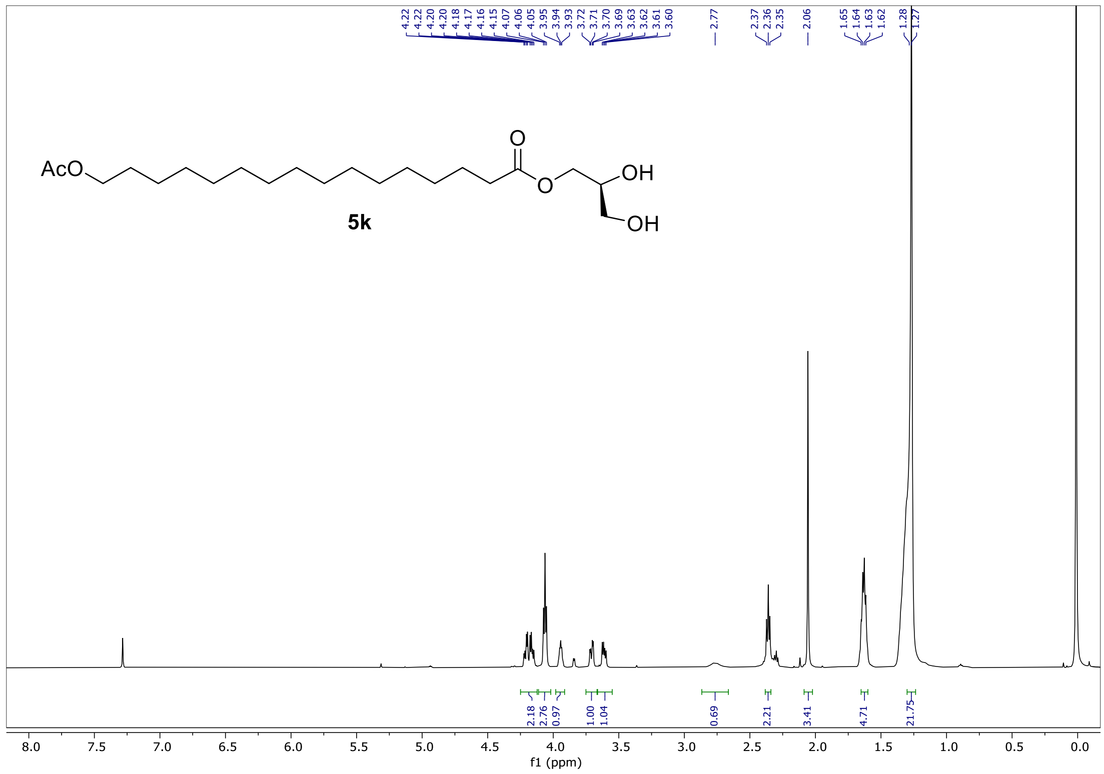


Figure S49. ^{1}H NMR spectrum of **5k** in $CDCl_3$

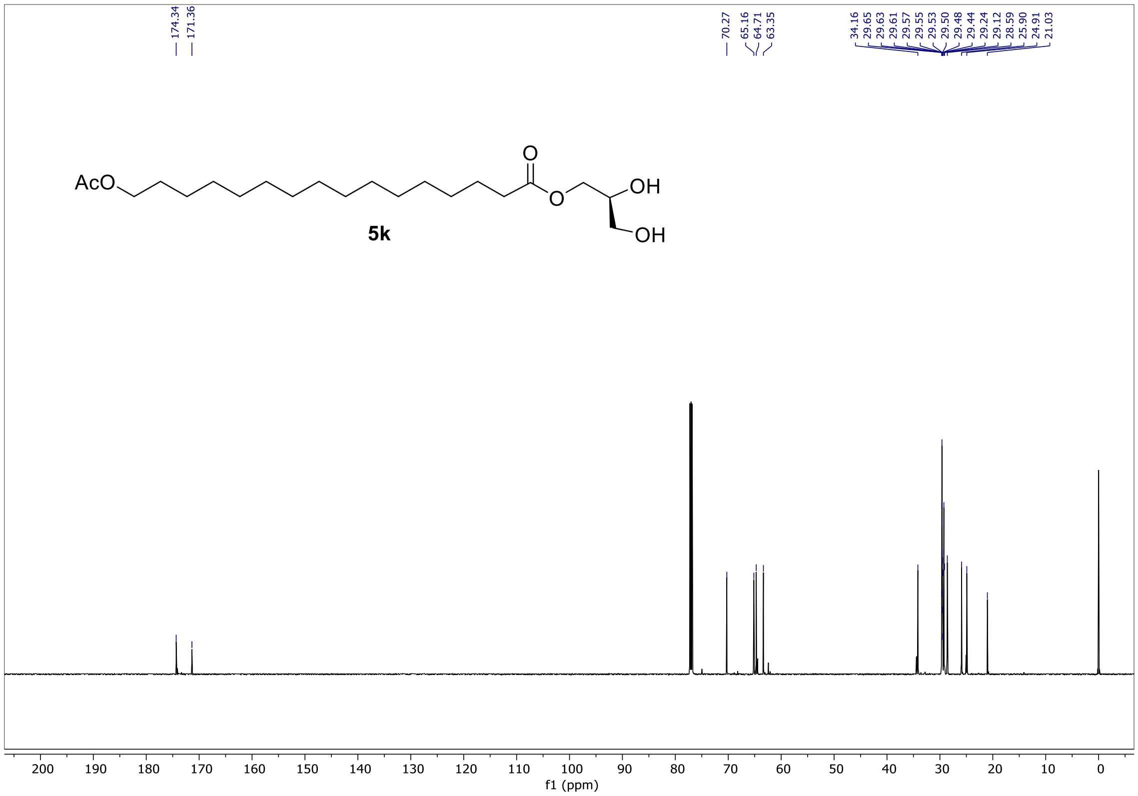
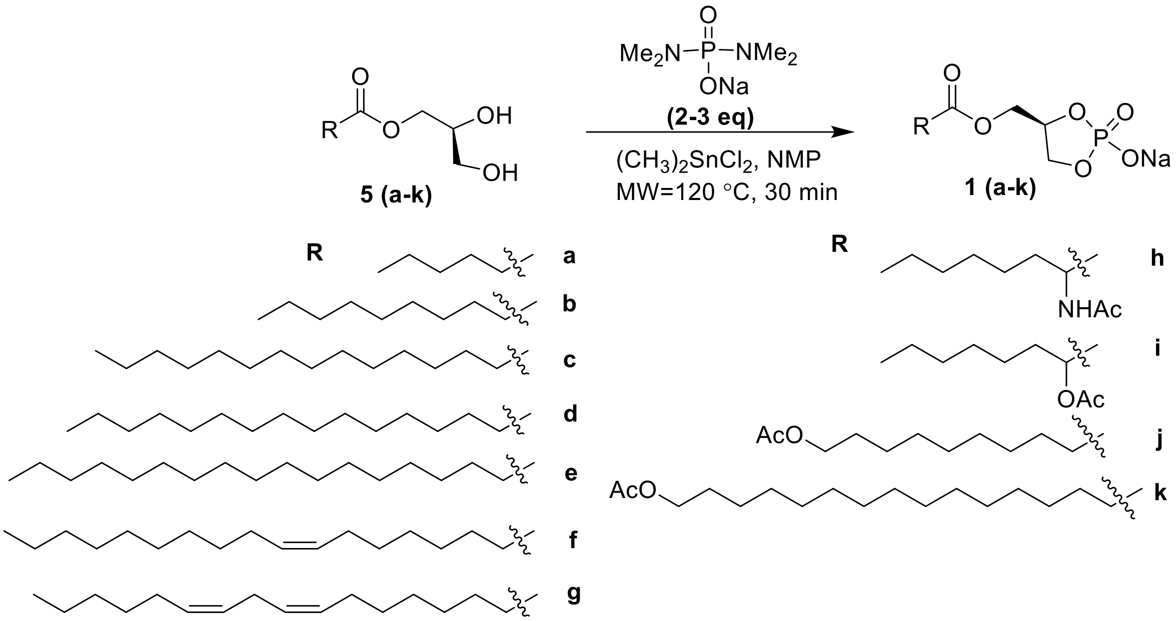


Figure S50. ^{13}C NMR spectrum of **5k** in $CDCl_3$

Cyclophosphorylation reaction



General procedure***:*** To a solution of diols (**5a-k**) (1.0 mmol) in NMP (2 mL) were added 2.0 mmol (**5a-e**) or 3 mmol (**5f-k**) of DMDAP and 0.2 mmol (**5a-e**) or .03 mmol (**5f-k**) of $C_2H_6Cl_2Sn$. The reaction was subjected to microwave (120 °C, normal power) for 30 minutes. A small aliquot of the crude was dissolved in CD_3OD to acquire ^{13}C NMR spectra to monitor the reactions. The reactions were stopped when quantitative conversion was observed. The reaction mixture was subject to different methods of isolation and purification based on the chain length of the fatty acids described in the next section.

Purification of Cyclic Phosphatidic Acids (Method A)

Purification of short alkyl chain Cyclic Phosphatidic Acids (**1a** and **1b**):

The crude reaction mixture was dissolved in MeOH and evaporated to near dryness in a rotavap (60 °C). Remaining NMP (boiling point = 202 °C) was eliminated by high vacuum evaporation (100-300 mTorr) at room temperature for 48 hours. The residue was dissolved in EtOAc and passed through a 50 g silica gel column eluted with EtOAc/MeOH using Flash Chromatography (Isolera) as follows:

	Start (% of MeOH)	**End (% of MeOH)**	**Time (minutes)**
Equilibrate	0	0	1:25
Gradient	0	5	4:00
Gradient	5	100	6:43
Gradient	100	100	4:00

Fractions containing products were identified by ^{31}P NMR, combined, and dried in vacuo to afford the cyclic phosphatidic acids **1a** and **1b**.

Purification of middle and long alkyl chain Cyclic Phosphatidic Acids (**1c-g**):

The crude reaction containing **1c-g** was dissolved in MeOH and evaporated to near dryness in a rotavap at 60 °C (for **1c-e**) and room temperature[a] (for **1f** and **1g**). Remaining NMP (boiling point = 202 °C) was eliminated by high vacuum evaporation (100-300 mTorr) at room temperature for 48 hours. The residue was dissolved in the required amount of water in such a way that the final concentration of cPAs in the solution is at 40 mM (above the critical aggregate concentration).[b] Under these conditions, cPAs were extracted three times from the aqueous layer using 2 mL of n-butanol.[c] The combined n-butanol layer was evaporated to dryness in a rotavap at 60 °C for **1c-g** and room temperature for **1f** and **1g** and again dissolved in the required amount of water but this time in such a way that the final concentration of cPAs in the solution be maintained at 6 mM (below the critical aggregate concentration).[d] At this stage, the remaining non-polar impurities[e] were eliminated by washing the aqueous layer with n-butanol (1mL). Aqueous layers containing cPAs **1c-g** were lyophilized to get the pure materials as powders.

[a] It is important to evaporate with no heating at this stage, in order to avoid cyclophosphate hydrolysis in cPAs **1f** and **1g**.

[b] At this concentration some turbidity was observed indicating the formation of aggregate

[c] n-butanol miscibility in water is less than 1%.

[d] At this concentration cPAs do not form aggregates increasing their solubility in water as ‘soluble monomers’.

[e] Extracted along with the desired phospholipids from the aqueous phase in the first step of n-butanol extraction.

An alternative procedure for the purification of Cyclic Phosphatidic Acids (1b, 1f, 1h-k) (Method B)

The crude reaction containing **1b, 1f** and **1h-k** was dissolved in MeOH and evaporated to near dryness in a rotavap at 50°C -60 °C. Remaining NMP (boiling point = 202 °C) was eliminated by high vacuum at room temperature for 24 hours. The residue was dissolved in the required amount of water in such a way that the final concentration of cPAs in the solution is at 40 mM for **1f**, and **1k**; 100 mM for **1b,** and **1h-j** (above the critical aggregate concentration). Under these conditions, cPAs were extracted more than 5 times from the aqueous layer using 3 mL of n-butanol. The combined n-butanol layer was evaporated to dryness in a rotavap at 50-60 °C and kept under high vacuum for 12 hours. The residue was suspended in diethyl ether (30 mL), then sonicated for ~ 2 min. and diethyl ether were removed after centrifugation. This diethy ether washings were repeated for 2 more times to get the pure material as powder.

Sodium 4-((hexanoyloxy)methyl)-1,3,2-dioxaphospholan-2-olate 2-oxide (**1a**): white solid (85%). **^{1}H NMR** (600 MHz, D_2O): δ 4.76–4.71 (m, 1H), 4.41–4.32 (m, 2H), 4.24 (dd, *J* = 12.18, 6.00 Hz, 1H), 4.12–4.06 (m, 1H), 2.46 (t, *J* = 7.44 Hz, 2H), 1.64 (quint, *J* = 7.44 Hz, 2H), 1.37–1.27 (m, 4H), 0.89 (t, *J* = 7.02 Hz, 3H). **^{13}C NMR** (150 MHz, D_2O): δ 176.3, 73.5, 65.2, 63.7, 33.2, 29.4, 23.4, 20.7, 13.9. {H-decoupled} **^{31}P-NMR** (162 MHz, D_2O) δ 18.56. **HRMS (ESI)** m/z calcd for $[C_9H_{16}O_6P]^-$ 251.0690, found 251.0731.

Sodium 4-((decanoyloxy)methyl)-1,3,2-dioxaphospholan2-olate 2-oxide (**1b**): white solid (89%). **^{1}H NMR** (600 MHz, D_2O): δ 4.74–4.67 (m, 1H), 4.44–4.34 (m, 1H), 4.33–4.21 (m, 2H), 4.19–4.03 (m, 1H), 2.44 (t, *J* = 7.68 Hz, 2H), 1.70–1.57 (m, 2H), 1.43–1.24 (m, 12H), 0.89 (t, *J* = 7.14 Hz, 3H). **^{13}C NMR** (150 MHz, D_2O): δ 175.5, 72.1, 65.4, 62.4, 33.2, 31.0, 29.0, 28.9, 27.8, 24.1, 22.1, 13.3. {H-decoupled} **^{31}P-NMR** (162 MHz, D_2O) δ 18.12. **HRMS (ESI)** m/z calcd for $[C_{13}H_{24}O_6P]^-$ 307.1316, found 307.1347.

Sodium 4-((tetradecanoyloxy)methyl)-1,3,2-dioxaphospholan-2-olate 2-oxide (**1c**): white solid (91%). **^{1}H NMR** (600 MHz, CD_3OD) δ 4.60–4.54 (m, 1H), 4.29–4.16 (m, 3H), 3.99–3.92 (m, 1H), 2.36 (t, *J* = 7.44 Hz, 2H), 2.37–1.57 (m, 2H), 1.34–1.23 (m, 20H), 0.90 (t, *J* = 6.90 Hz, 3H). **^{13}C NMR** (150 MHz, CD_3OD) δ 175.1, 74.7, 66.8, 65.4, 34.8, 33.1, 30.8, 30.8, 30.7, 30.6, 30.5, 30.4, 30.2, 25.4, 24.1, 14.4. {H-decoupled} **^{31}P-NMR** (162 MHz, CD_3OD) δ 18.47. **HRMS (ESI)** m/z calcd for $[C_{17}H_{32}O_6P]^-$ 363.1942, found 363.1987.

Sodium 4-((palmitoyloxy)methyl)-1,3,2-dioxaphospholan-2-olate 2-oxide (**1d**): white solid (80%). **^{1}H NMR** (600 MHz, CD_3OD): δ 4.60–4.52 (m, 1H), 4.30–4.14 (m, 3H), 3.98–3.91 (m, 1H), 2.36 (t, *J* = 7.56 Hz, 2H), 1.67–1.55 (m, 2H), 1.36–1.25 (m, 24H), 0.90 (t, *J* = 6.96 Hz, 3H). **^{13}C NMR** (150 MHz, CD_3OD): δ 178.1, 75.9, 66.8, 64.9, 34.8, 33.1, 30.8, 30.77, 30.75, 30.71, 30.6, 30.46, 30.40, 30.2, 25.9, 24.4, 14.43 {H-decoupled} **^{31}P-NMR** (162 MHz, CD_3OD) δ 19.47. **HRMS (ESI)** m/z calcd for $[C_{19}H_{36}O_6P]^-$ 391.2255, found 391.2302.

Sodium 4-((stearoyloxy)methyl-1,3,2-dioxaphospholan-2-olate 2-oxide) (**1e**): white solid (77%). **^{1}H NMR** (600 MHz, CD_3OD): δ 4.60–4.52 (m, 1H), 4.30–4.12 (m, 3H), 3.98–3.90 (m, 1H), 2.36 (t, *J* = 7.44 Hz, 2H), 1.68–1.56 (m, 2H), 1.44–1.09 (m, 28H), 0.90 (t, *J* = 6.96

Hz, 3H). **^{13}C NMR** (150 MHz, CD_3OD): δ 175.1, 77.5, 66.8, 64.3, 34.8, 33.1, 30.78, 30.76, 30.72, 30.6, 30.5, 30.4, 30.2, 25.9, 23.7, 14.4. {H-decoupled} **^{31}P-NMR** (162 MHz, CD_3OD): δ 19.47. **HRMS (ESI)** m/z calcd for $[C_{21}H_{40}O_6P]^-$ 419.2568, found 419.2619.

Sodium 4-((oleoylloxy)methyl-1,3,2-dioxaphospholan-2-olate 2-oxide) (**1f**): pale yellow solid (93%). **^{1}H NMR** (600 MHz, CD_3OD): δ 5.41–5.27 (m, 2H), 4.62–4.55 (m, 1H), 4.31–4.13 (m, 3H), 4.00–3.93 (m, 1H), 2.36 (t, *J* = 7.44 Hz, 2H), 2.12–1.97 (m, 4H), 1.67–1.57 (m, 2H), 1.51–1.20 (m, 20H), 0.90 (t, *J* = 6.90 Hz, 3H). **^{13}C NMR** (150 MHz, CD_3OD): δ 174.0, 130.9, 130.8, 74.8, 66.9, 65.3, 34.8, 33.1, 30.84, 30.80, 30.6, 30.4, 30.3, 30.28, 30.18, 28.1, 25.9, 23.8, 14.4. {H-decoupled} **^{31}P-NMR** (162 MHz, CD_3OD): δ 19.33. **HRMS (ESI)** m/z calcd for $[C_{21}H_{38}O_6P]^-$ 417.2411, found 417.2463.

Sodium 4-((((9Z,12Z)-octadeca-9,12-dienoyl)oxy)methyl)-1,3,2-dioxaphospholan-2-olate 2-oxide (**1g**): yellow solid (87%). **^{1}H NMR** (600 MHz, CD_3OD): δ 5.50–5.20 (m, 4H), 4.60–4.53 (m, 1H), 4.28–4.16 (m, 3H), 3.99–3.91 (m, 1H), 2.78 (t, *J* = 6.30 Hz, 2H), 2.36 (t, *J* = 7.62 Hz, 2H), 2.12–2.01 (m, 4H), 1.65–1.59 (m, 2H), 1.39–1.29 (m, 14H), 0.91 (t, *J* = 6.90 Hz, 3H). **^{13}C NMR** (150 MHz, CD_3OD): δ 174.4, 130.92, 130.86, 129.08, 129.03, 75.4, 66.8, 65.4, 34.7, 32.6, 28.1, 26.5, 25.9, 23.6, 14.4. {H-decoupled} **^{31}P-NMR** (162 MHz, CD_3OD): δ 19.47. **HRMS (ESI)** m/z calcd for $[C_{21}H_{36}O_6P]^-$ 415.2255, found 415.2309.

Sodium 4-(((2-acetamidooctanoyl)oxy)methyl)-1,3,2-dioxaphospholan-2-olate 2-oxide (**1h**): Off-white solid (89%, 1:1 diastereomeric mixture); ^{1}H NMR (600 MHz, MeOD) δ 4.64–4.55 (m, 1H), 4.40–4.21 (m, 3H), 4.08 – 3.94 (m, 1H), 2.01 (d, *J* = 4.9 Hz, 6H), 1.76–1.62 (m, 2H), 1.34 (ddt, *J* = 14.6, 8.7, 4.5 Hz, 13H), 0.92 (t, *J* = 6.8 Hz, 6H). **^{13}C NMR** (150 MHz, CD_3OD): δ 172.0, 73.0, 65.2, 64.6, 52.35, 31.4, 31.1, 28.5, 25.4, 22.2, 20.9, 13.0. **^{31}P NMR** (162 MHz, CD_3OD): δ 18.5, 18.4. **HRMS (ESI)** m/z calcd for $[C_{13}H_{24}NNaO_7P]^+$ 360.1188, found 360.1184.

Sodium 4-(((2-acetoxyoctanoyl)oxy)methyl)-1,3,2-dioxaphospholan-2-olate 2-oxide (**1i**): Off-white solid (92%, diastereomeric mixtures); ^{1}H NMR (600 MHz, CD_3OD) δ 4.98 (d, *J*

= 5.0 Hz, 1H), 4.61 – 4.55 (m, 1H), 4.34 – 4.18 (m, 4H), 4.07 (t, *J* = 6.9 Hz, 1H), 3.95 (dq, *J* = 16.5, 8.7 Hz, 1H), 2.12 (s, 3H), 2.04 (s, 1H), 1.85 (dt, *J* = 14.6, 7.5 Hz, 2H), 1.64 (d, *J* = 6.8 Hz, 2H), 1.44 (s, 2H), 1.35 (s, 18H), 0.98 (s, 1H), 0.93 (t, *J* = 6.6 Hz, 3H).). ^{13}C NMR (150 MHz, CD_3OD): δ 173.7, 171.0, 170.9, 170.3, 73.42, 73.40, 73.12, 73.10, 73.06, 73.04, 72.33, 65.4, 65.3, 65.2, 64.9, 64.8, 64.73, 64.68, 64.3, 64.02, 63.98, 33.4, 31.3, 30.65, 30.63, 29.0, 28.9, 28.7, 28.5, 28.3, 25.6, 24.5, 22.2, 19.09, 19.08, 13.0. **^{31}P NMR** (162 MHz, CD_3OD): δ 18.2, 18.1. **HRMS (ESI)** m/z calcd for $[C_{13}H_{23}NaO_8P]^+$ 361.1028, found 361.1025. Note: This compound was found to be unstable with respect to time (in 3 days in MeOD solution, decomposition was observed by ^{1}H NMR).

Sodium (R)-4-(((10-acetoxydecanoyl)oxy)methyl)-1,3,2-dioxaphospholan-2-olate 2-oxide (**1j**): off-white solid (84%, contains ~19% of the impurity, which was carried over from the previous step). **^{1}H NMR** (600 MHz, CD_3OD) δ 4.58 (m, 1H), 4.30 – 4.18 (m, 3H), 4.09 – 4.06 (t, *J* = 6.6 Hz, 2H), 3.96 (td, *J* = 6.6, 9 Hz, 1H), 2.38 (t, *J* = 7.8 Hz, 2H), 2.04 (s, 3H), 1.63 (m, 4H), 1.42-1.31 (m, 10H) **^{13}C NMR** (150 MHz, CD_3OD) δ173.6, 171.7, 73.3, 73.2, 65.4, 64.3, 64.05, 64.01, 33.9, 33.4, 29.04, 28.9, 28.4, 25.5, 24.5, 19.4 {H-decoupled} **^{31}P-NMR** (162 MHz, CD_3OD) δ 18.3. **HRMS (ESI)** m/z calcd for $C_{15}H_{27}NaO_8P$ $[M+Na]^+$ 389.1336, found 389.1336.

Sodium (R)-4-(((16-acetoxyhexadecanoyl)oxy)methyl)-1,3,2-dioxaphospholan-2-olate 2-oxide (**1k**): off-white solid (77%, contains ~11% of the impurity, which was carried over from the previous step). **^{1}H NMR** (600 MHz, CD_3OD) δ 4.58 (m, 1H), 4.29 – 4.18 (m, 3H), 4.07 (t, *J* = 6.6 Hz, 2H), 3.96 (td, *J* = 7.2, 9 Hz, 2H), 2.38 (t, *J* = 7.8 Hz, 2H), 2.04 (s, 3H), 1.63 (m, 4H), 1.42 – 1.27 (m, 22 H) **^{13}C NMR** (150 MHz, CD_3OD) δ 173.7, 171.7, 73.29, 73.27, 65.4, 64.3, 64.05, 64.01, 33.9, 33.4, 29.34, 29.33, 29.31, 29.27, 29.23, 29.19, 29.0, 28.9, 28.8, 28.3, 25.6, 24.5, 19.4 {H-decoupled} **^{31}P-NMR** (162 MHz, CD_3OD) δ 18.3. **HRMS (ESI)** m/z calcd for $C_{21}H_{39}NaO_8P$ $[M+Na]^+$ 473.2275, found 473.2268.

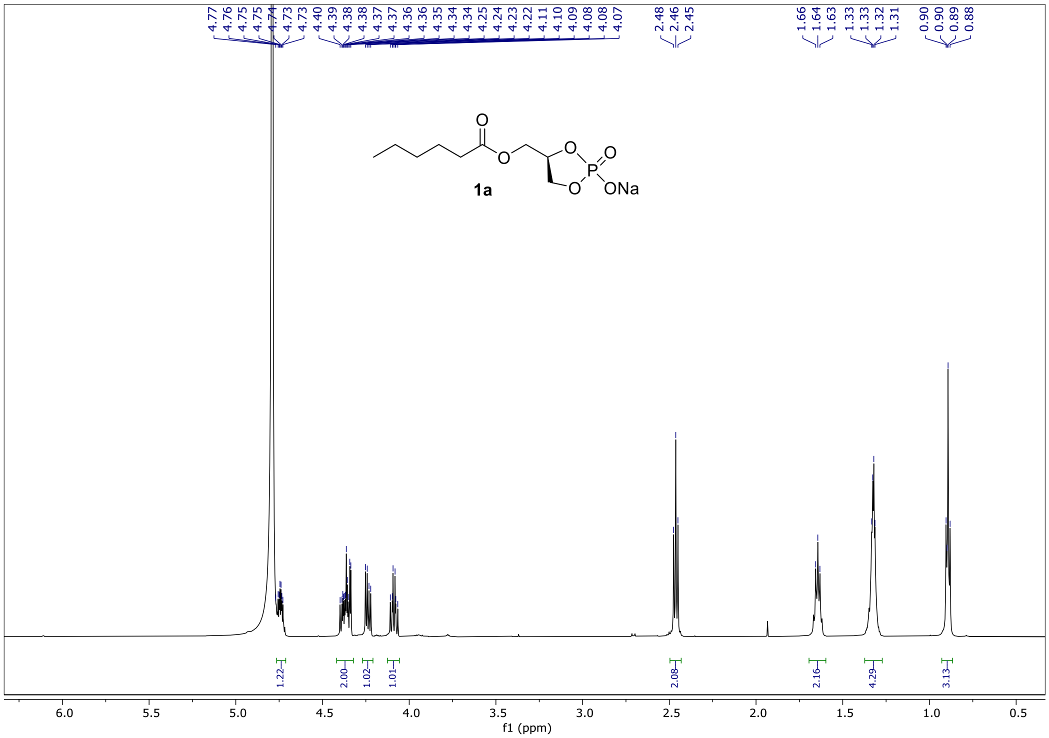


Figure S51. ^{1}H NMR spectrum of **1a** in D_2O

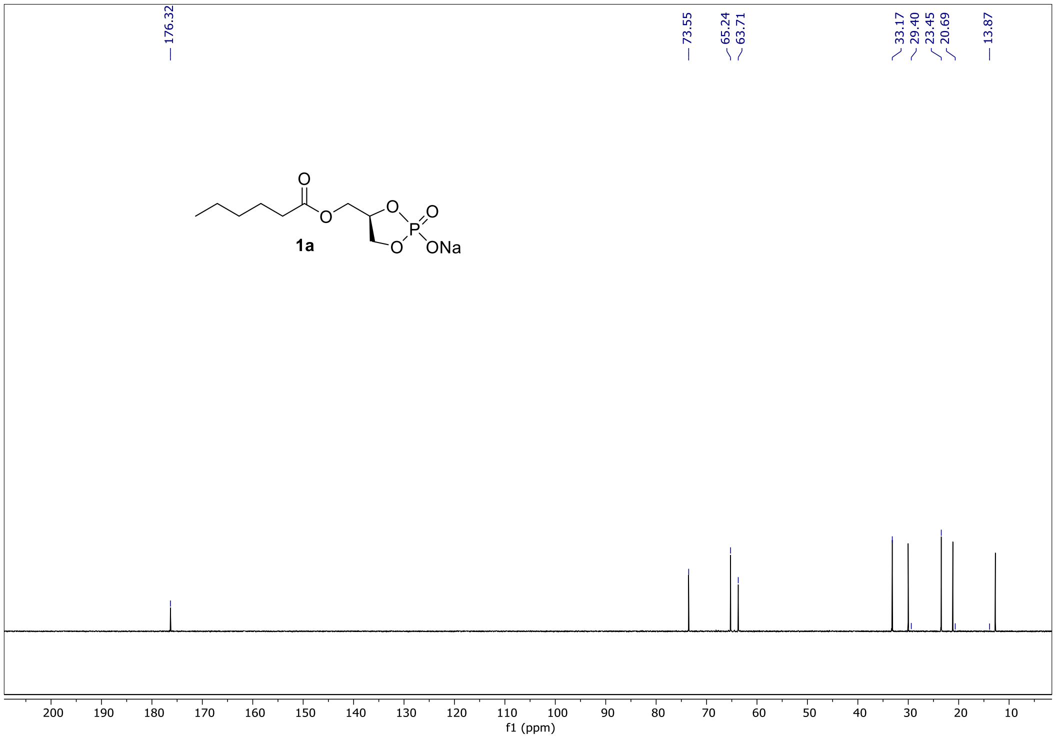


Figure S52. ^{13}C NMR spectrum of **1a** in D_2O

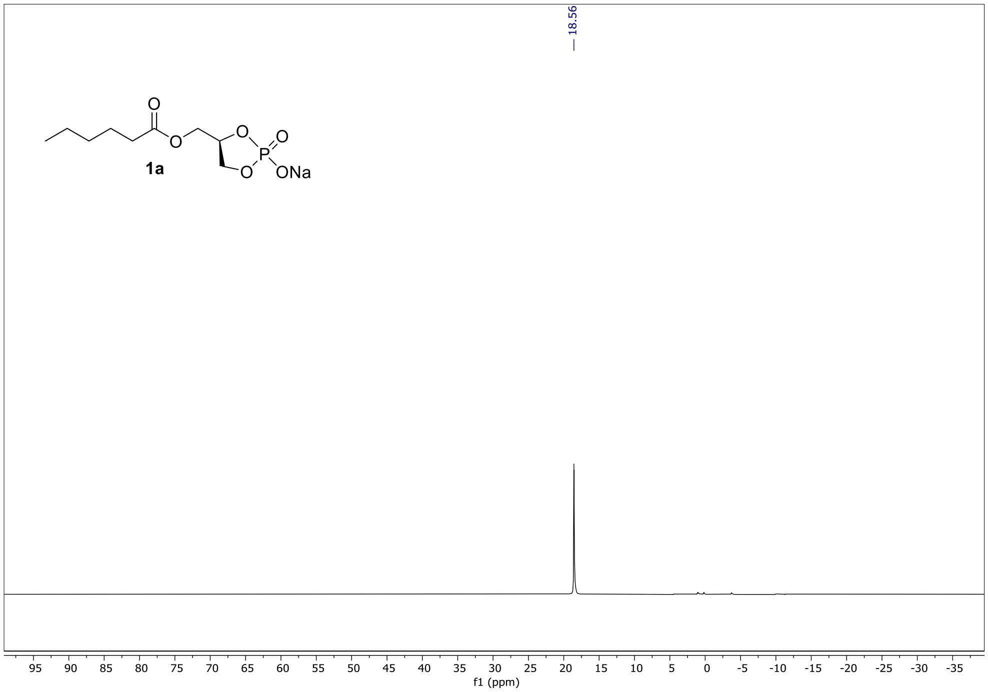


Figure S53. {H-decoupled} ^{31}P NMR spectrum of **1a** in D_2O

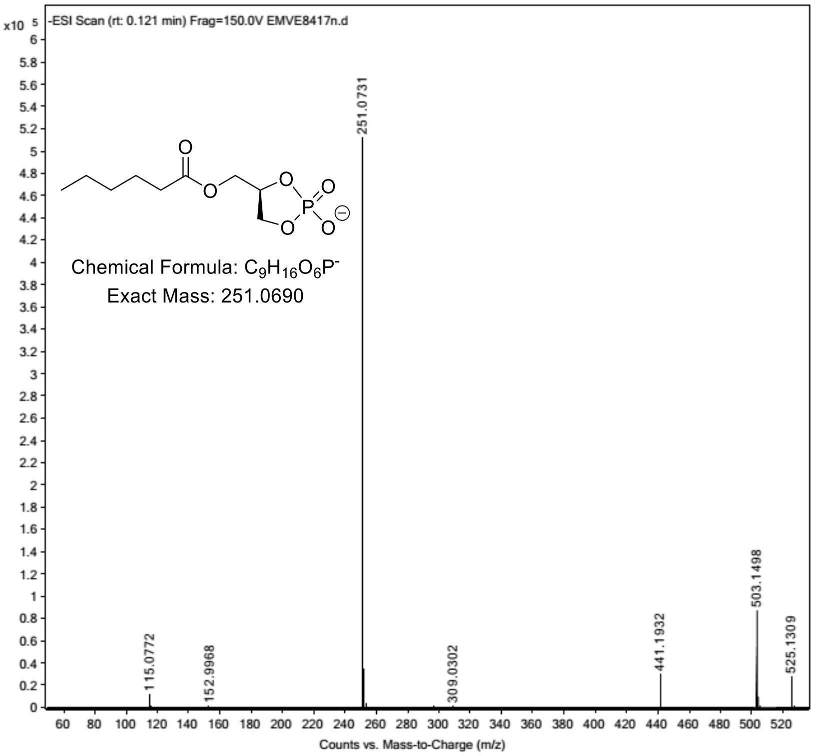


Figure S54. HRMS (ESI) of sodium **1a**, m/z calcd for $[C_9H_{16}O_6P]^-$ 251.0690, found 251.0731.

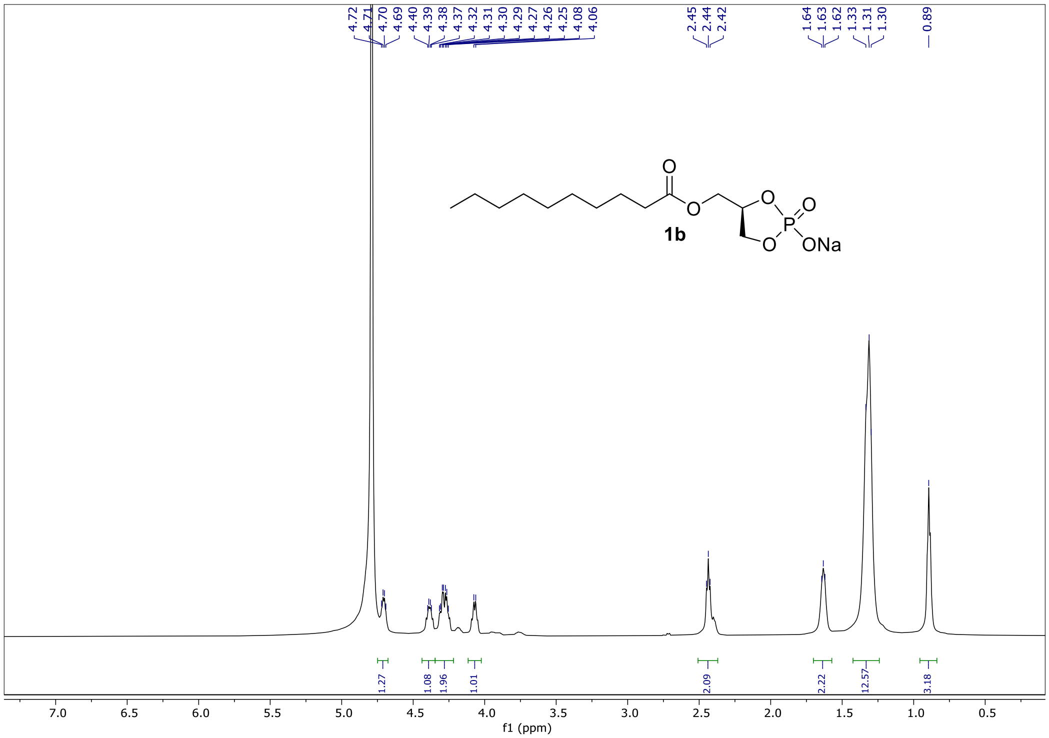


Figure S55. ^{1}H NMR spectrum of **1b** in D_2O

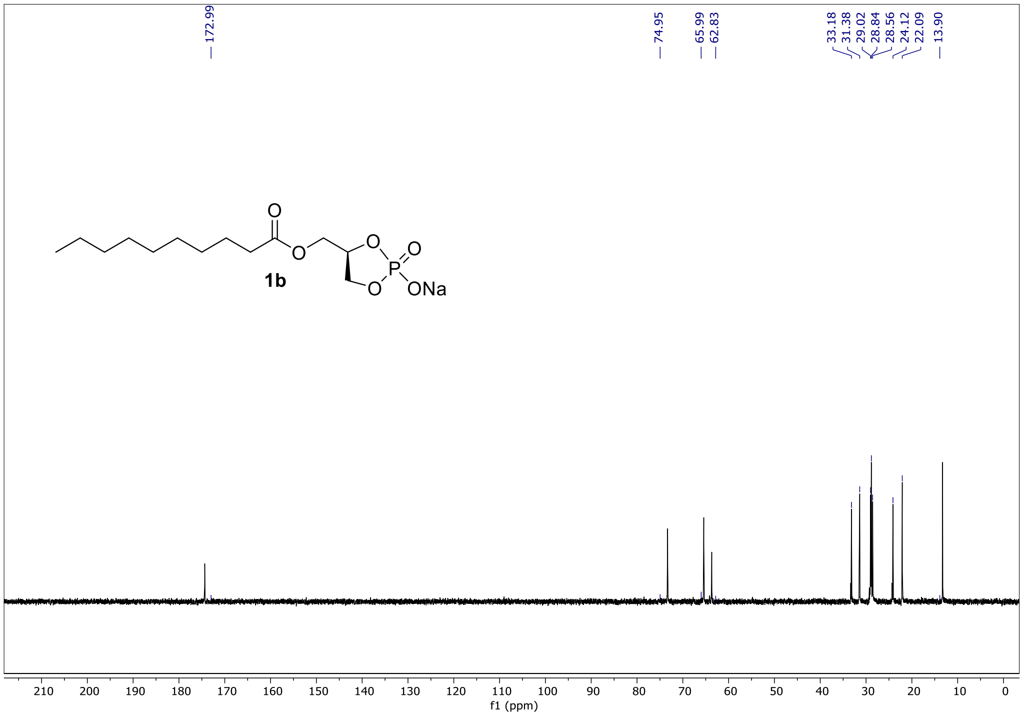


Figure S56. ^{13}C NMR spectrum of **1b** in D_2O

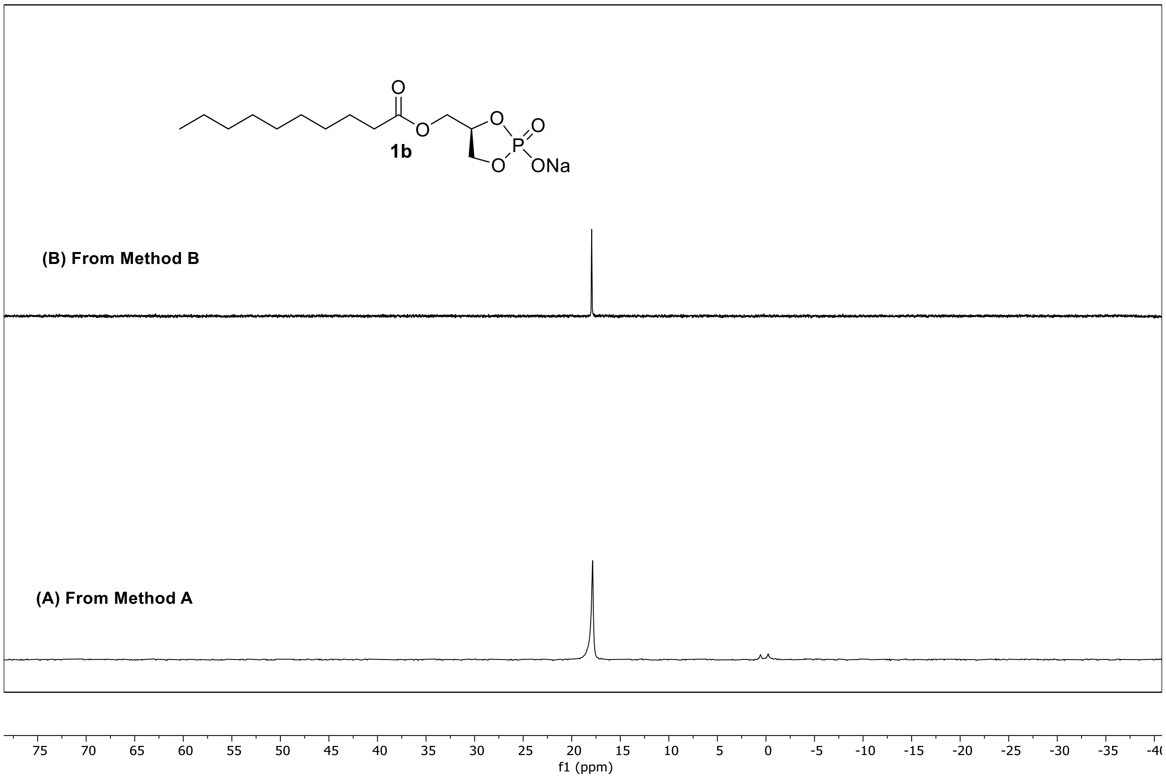


Figure S57. ^{31}P {H-decoupled} NMR spectrum of **1b** in D_2O (A) Purified by Method A. (B) Purified by Method B.

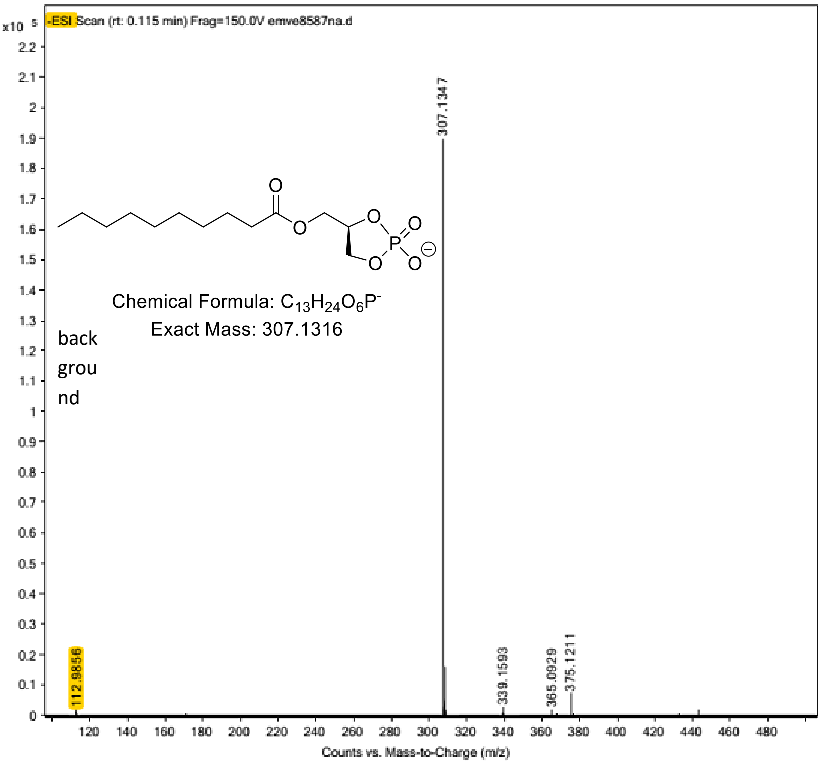


Figure S58. HRMS (ESI) of **1b**, m/z calcd for $[C_{13}H_{24}O_6P]^-$ 307.1316, found 307.1347.

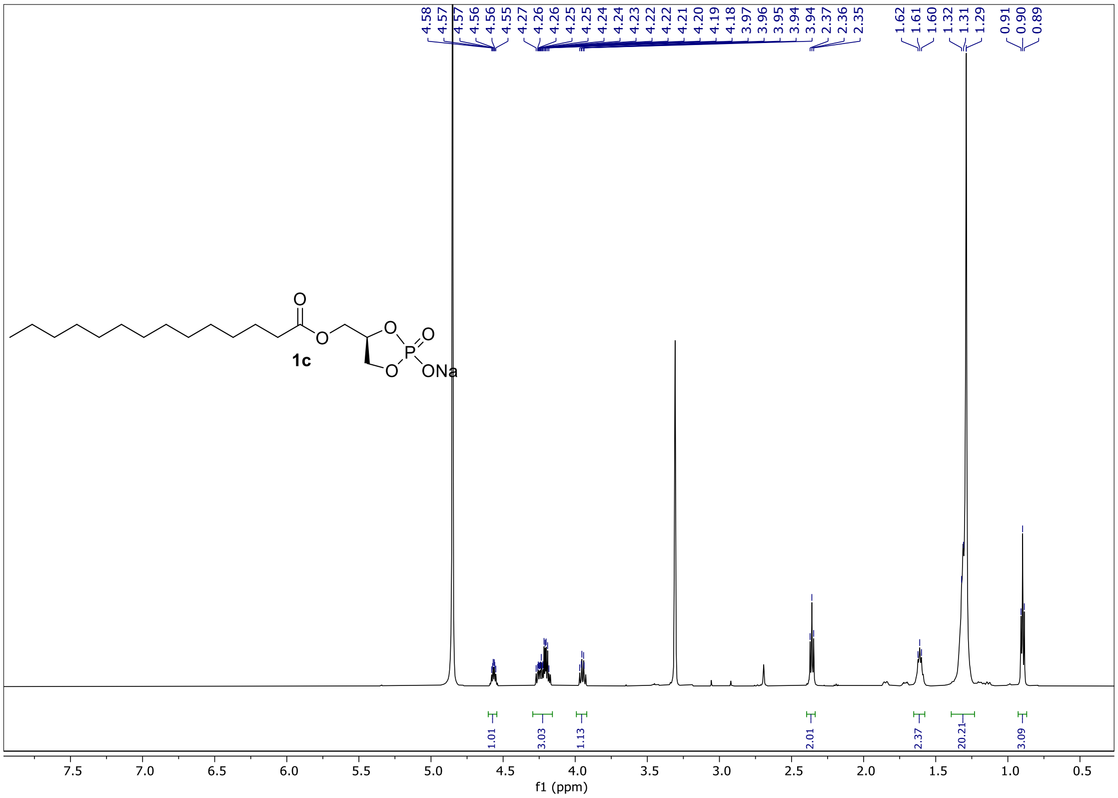


Figure S59. ^{1}H NMR spectrum of **1c** in CD_3OD

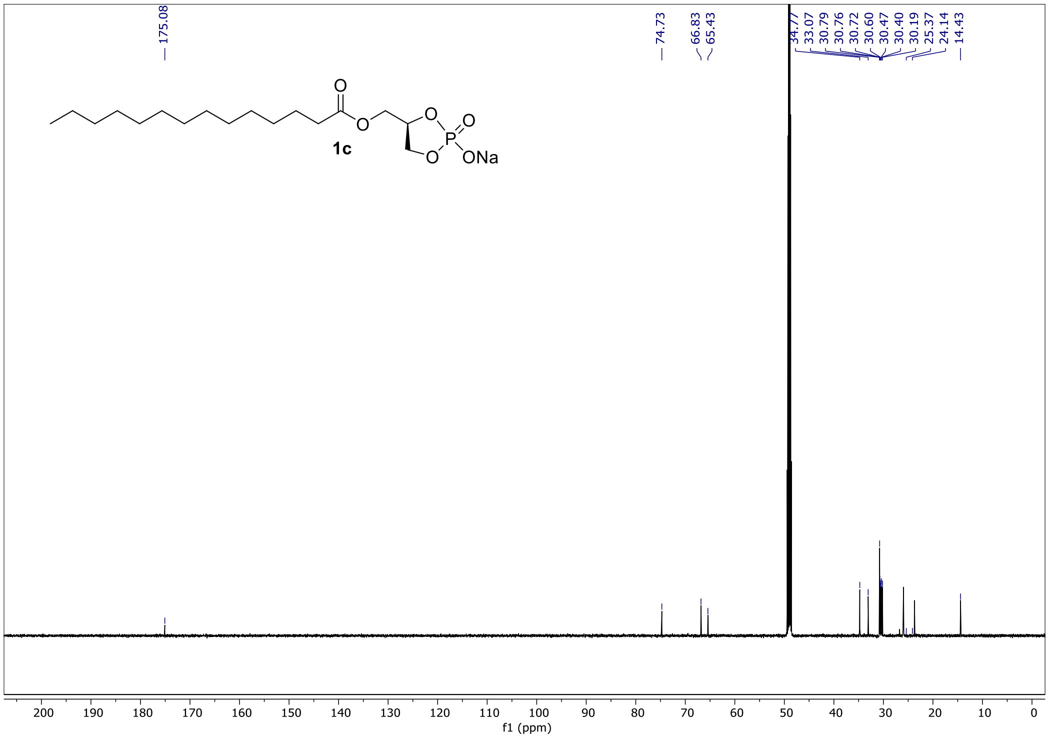


Figure S60. ^{13}C NMR spectrum of **1c** in CD_3OD

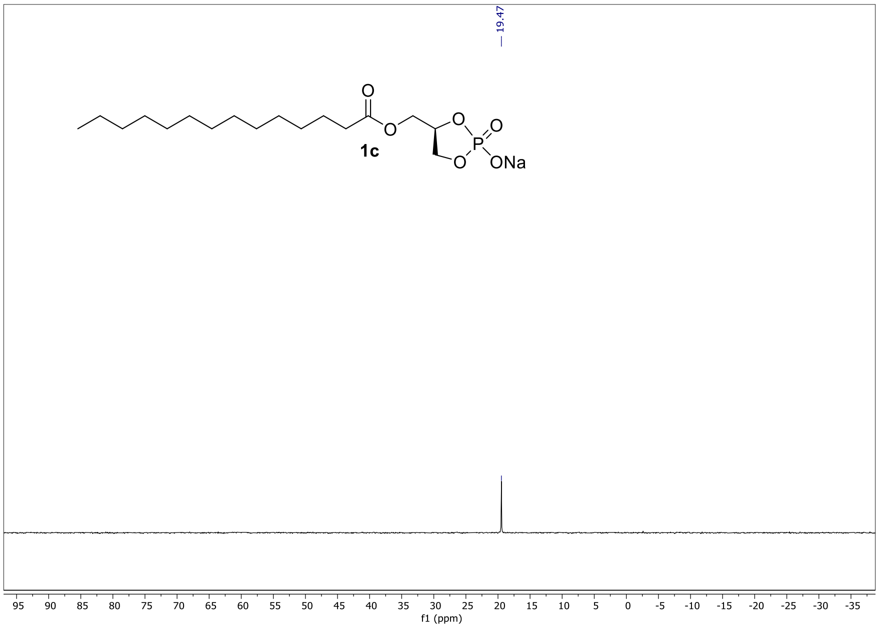


Figure S61. ^{31}P {H-decoupled} NMR spectrum of **1c** in CD_3OD.

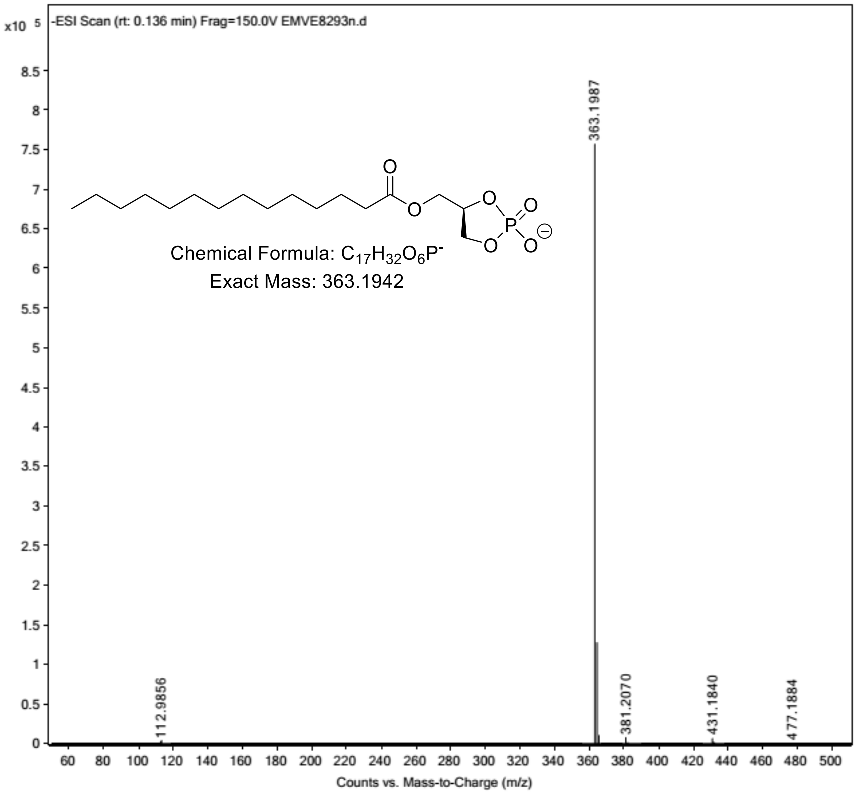


Figure S62. HRMS (ESI) of **1c**, m/z calcd for $[C_{17}H_{32}O_6P]^-$ 363.1942, found 363.1987.

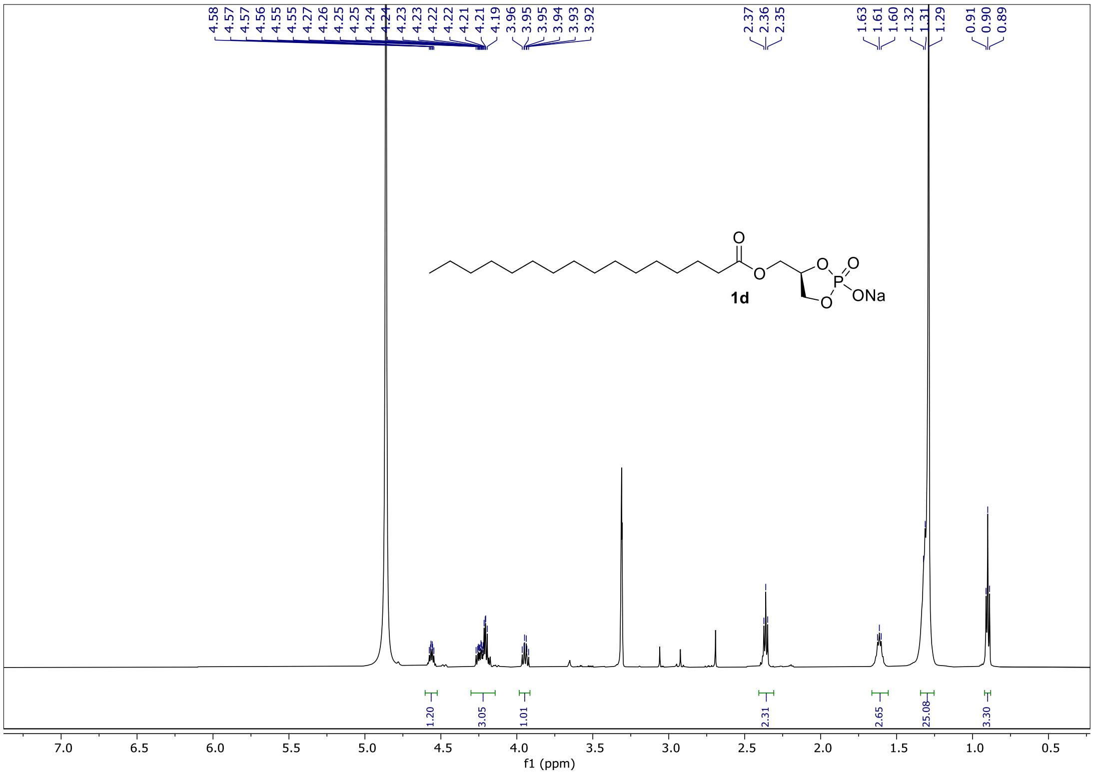


Figure S63. ^{1}H NMR spectrum of **1d** in CD_3OD

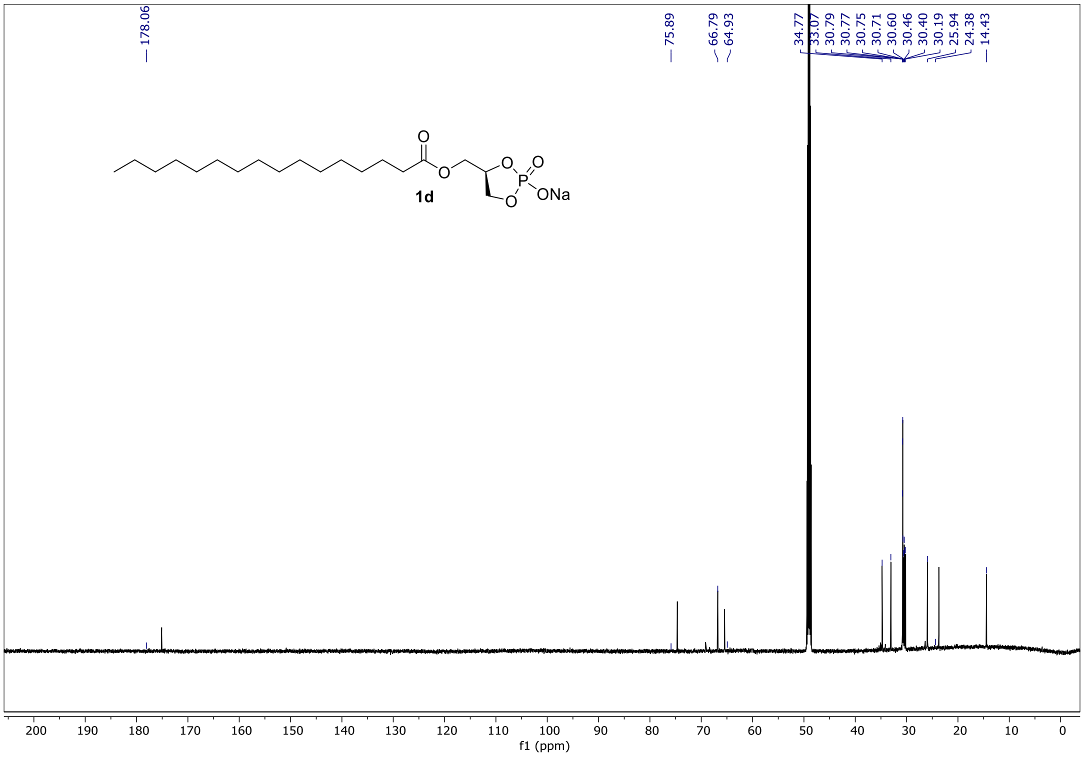


Figure S64. ^{13}C NMR spectrum of **1d** in CD_3OD

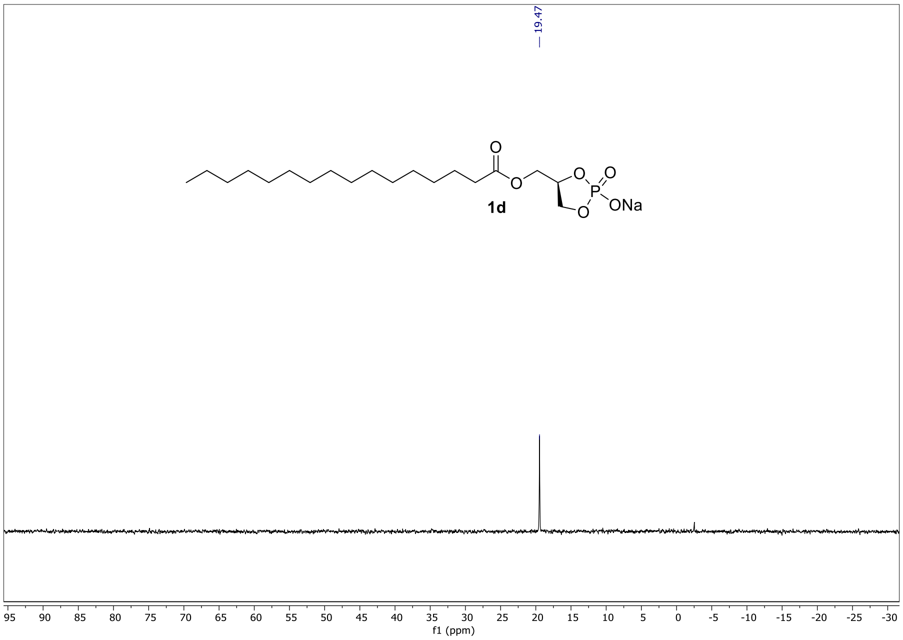


Figure S65. ^{31}P {H-decoupled} NMR spectrum **1d** in CD_3OD

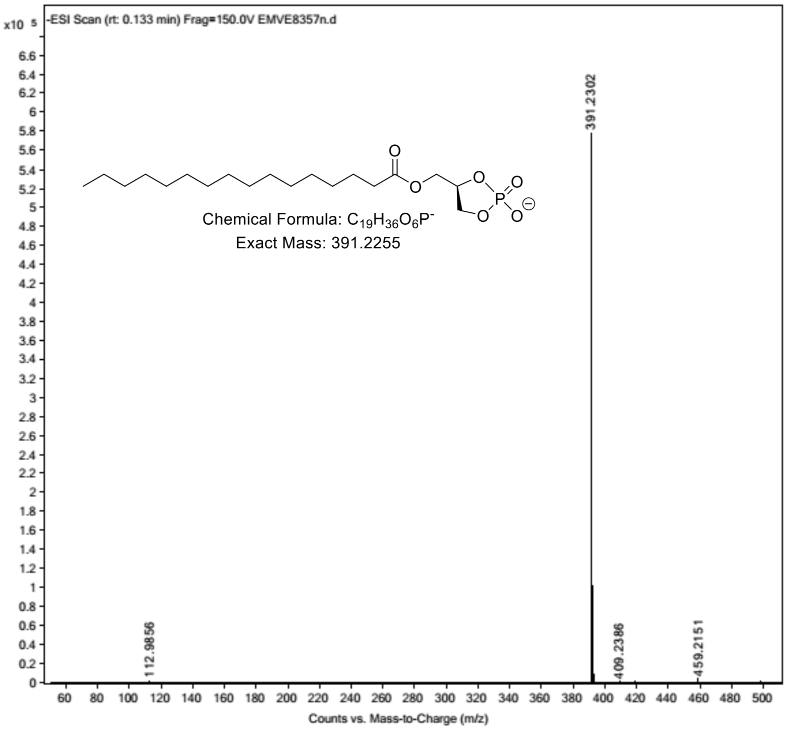


Figure S66. HRMS (ESI) of **1d**, m/z calcd for $[C_{19}H_{36}O_6P]^-$ 391.2255, found 391.2302.

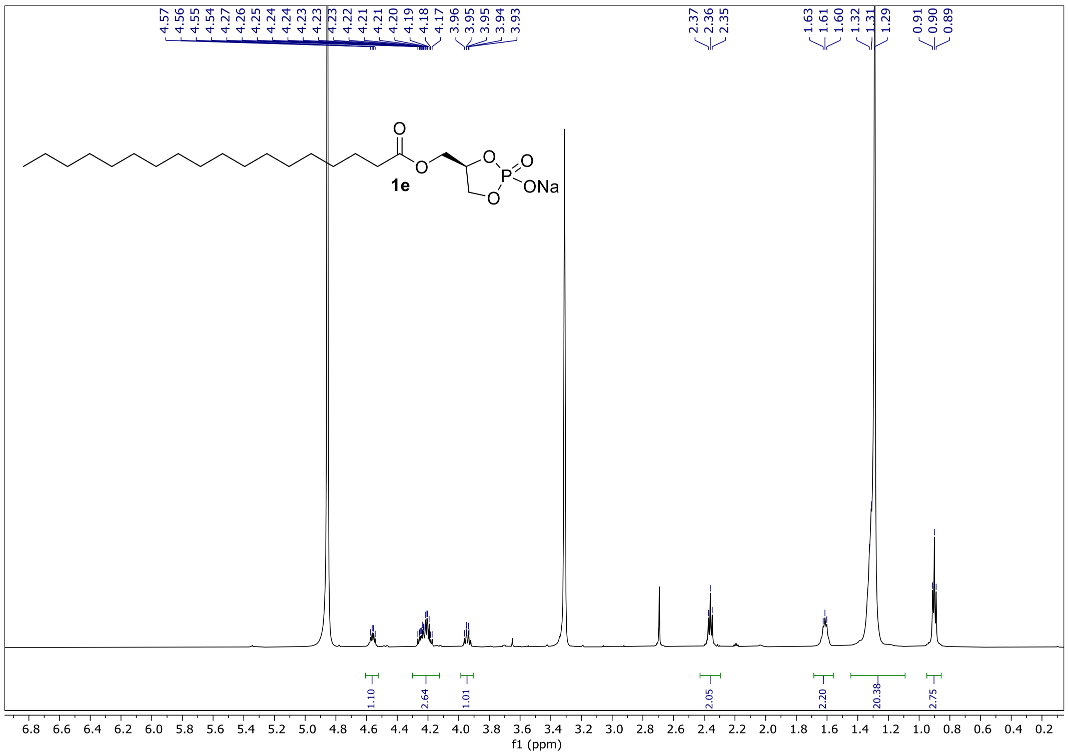


Figure S67. ^{1}H NMR spectrum of **1e** in CD_3OD

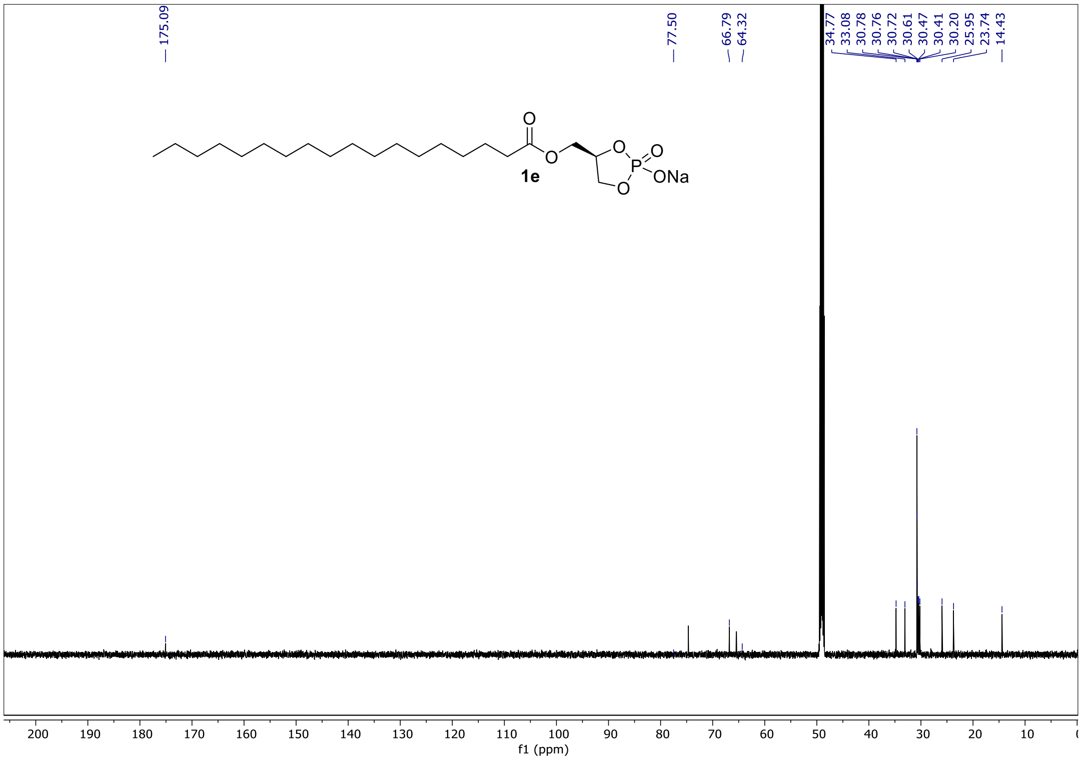


Figure S68. ^{13}C NMR spectrum of **1e** in CD_3OD

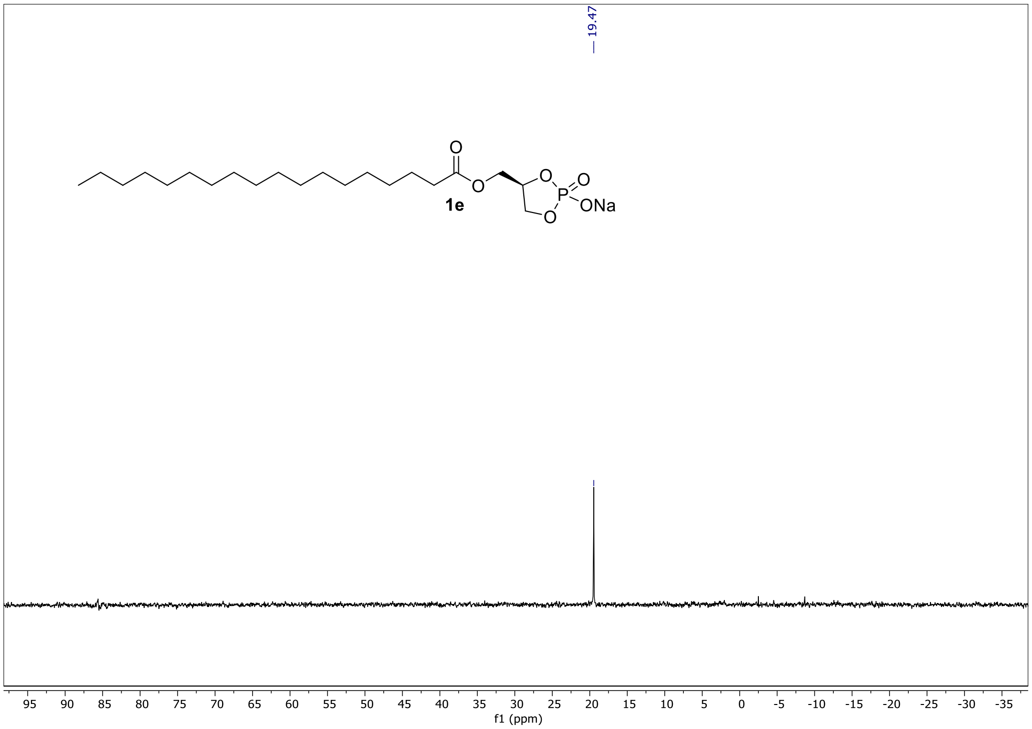


Figure S69. ^{31}P {H-decoupled} NMR spectrum of **1e** in CD_3OD

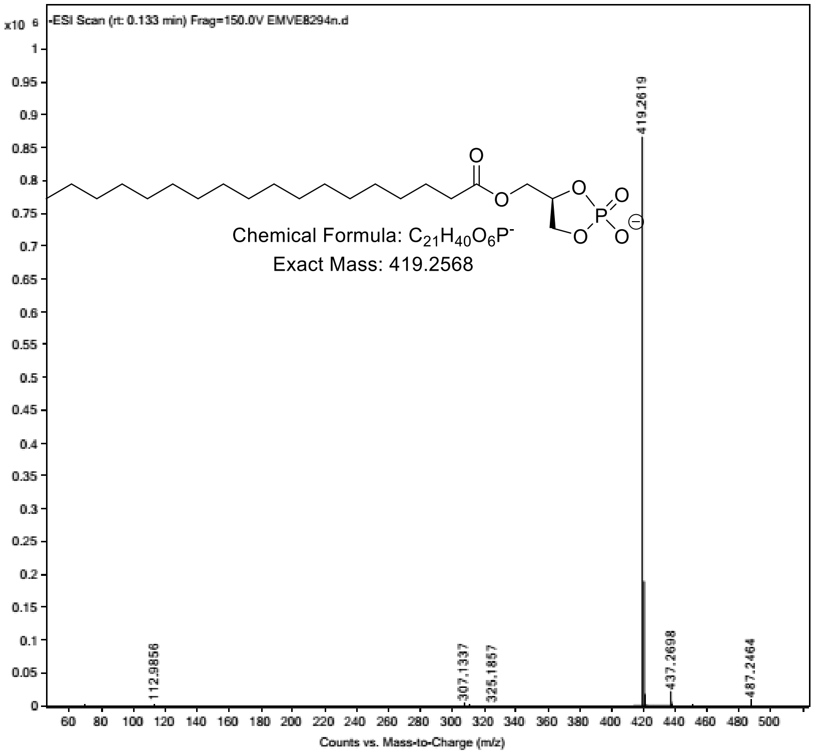


Figure S70. HRMS (ESI) of **1e**, m/z calcd for $[C_{21}H_{40}O_6P]^-$ 419.2568, found 419.2619.

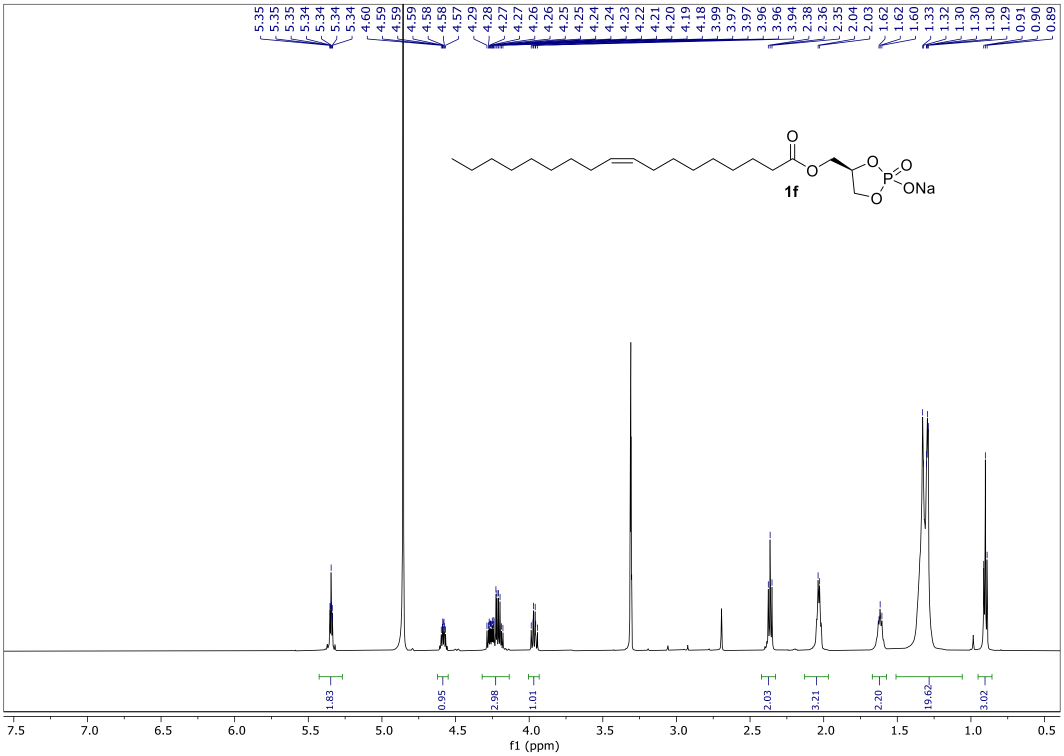


Figure S71. ^{1}H NMR spectrum of **1f** in CD_3OD

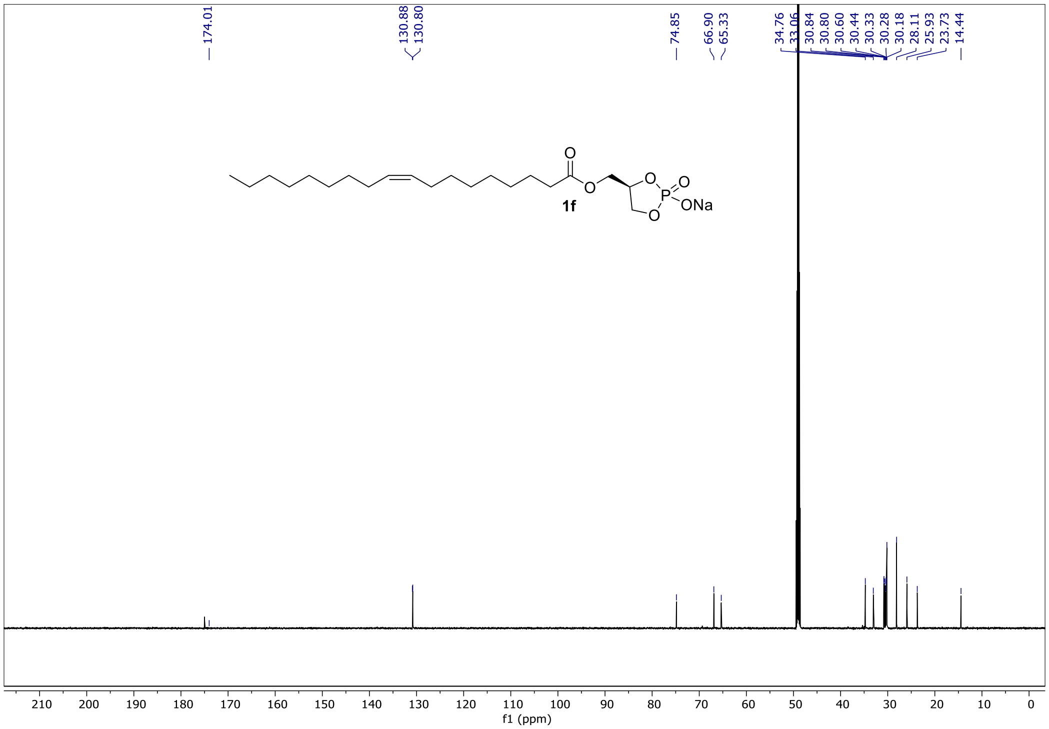


Figure S72. ^{13}C NMR spectrum of **1f** in CD_3OD

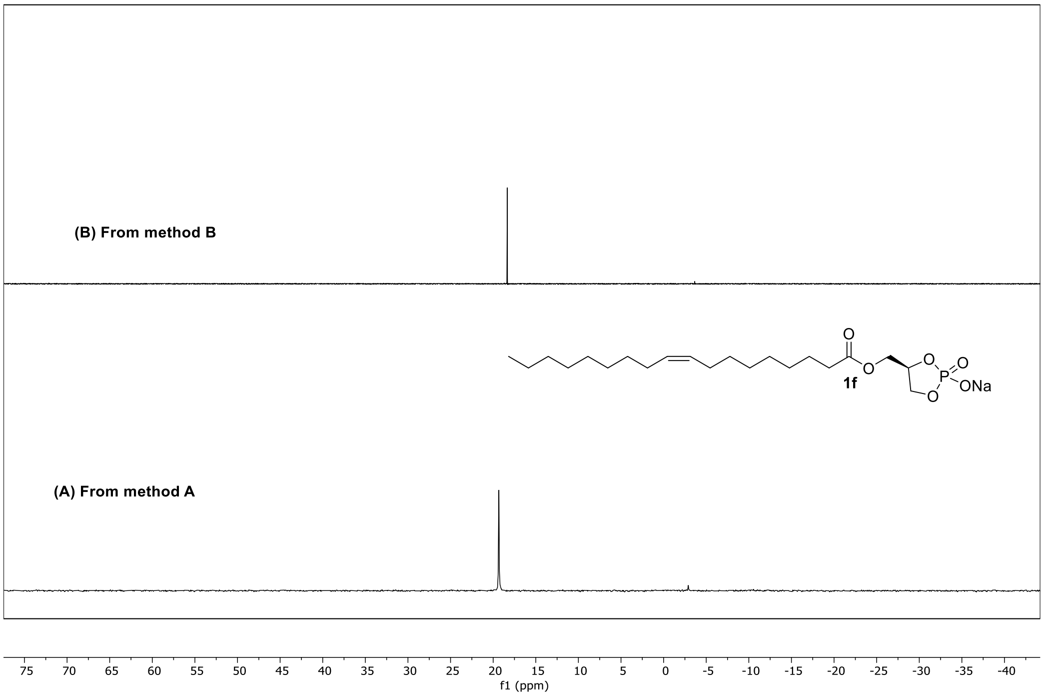


Figure S73. ^{31}P {H-decoupled} NMR spectrum of **1f** in CD_3OD. (A) Purified by method A. (B) Purified by method B.

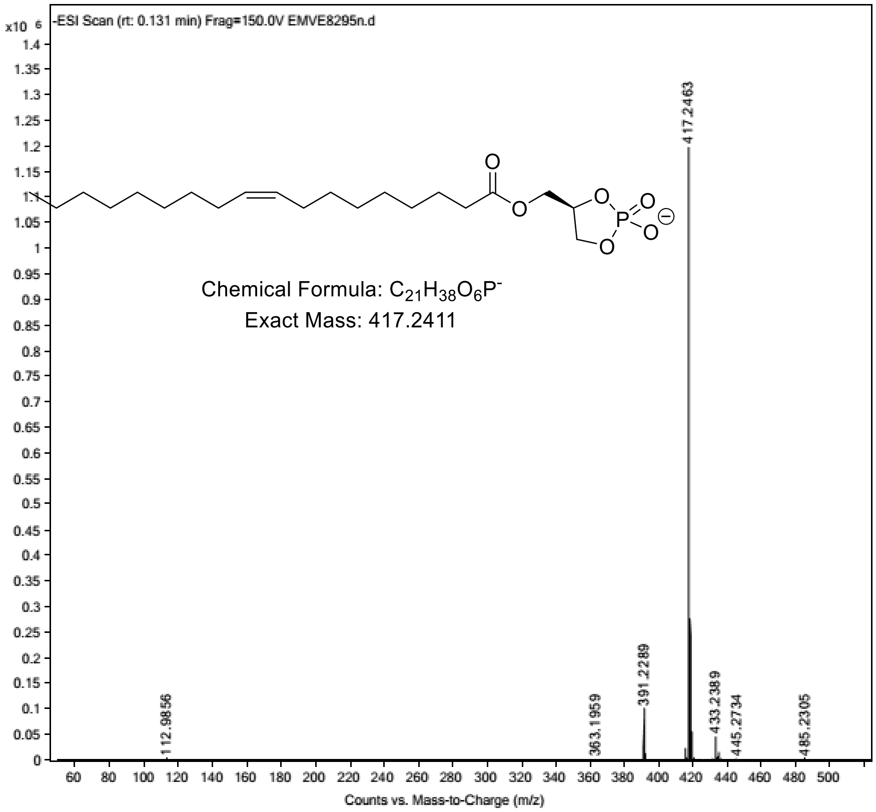


Figure S74. HRMS (ESI) of **1f**, m/z calcd for $[C_{21}H_{38}O_6P]^-$ 417.2411, found 417.2463.

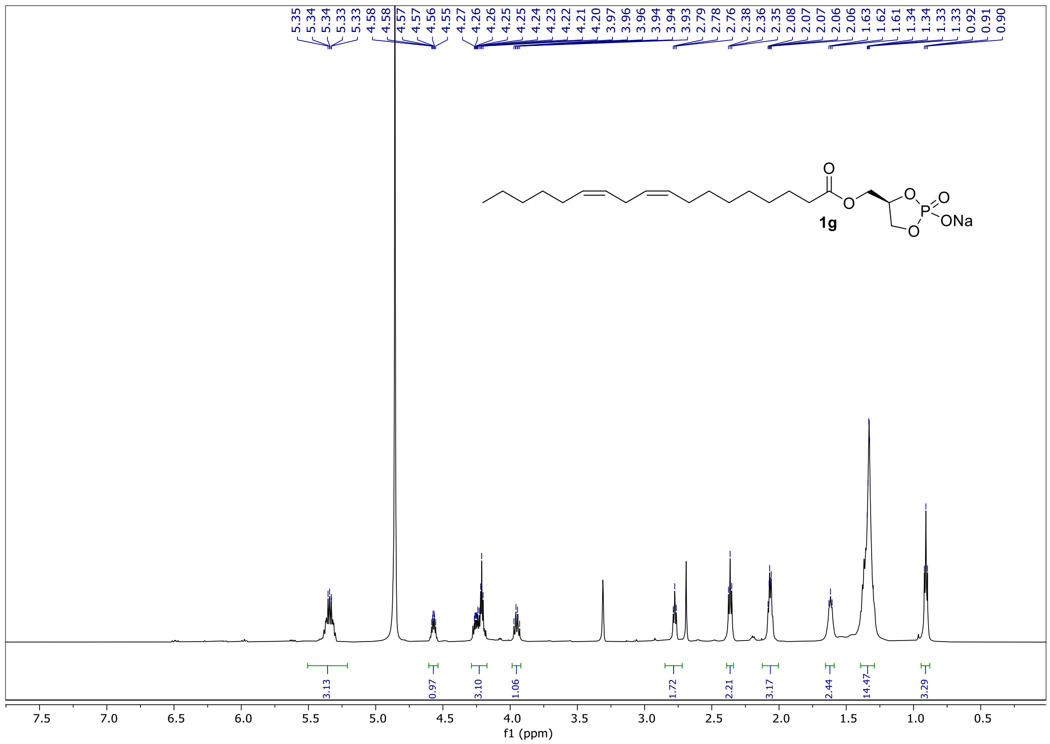


Figure S75. ^{1}H NMR spectrum of **1g** in CD_3OD

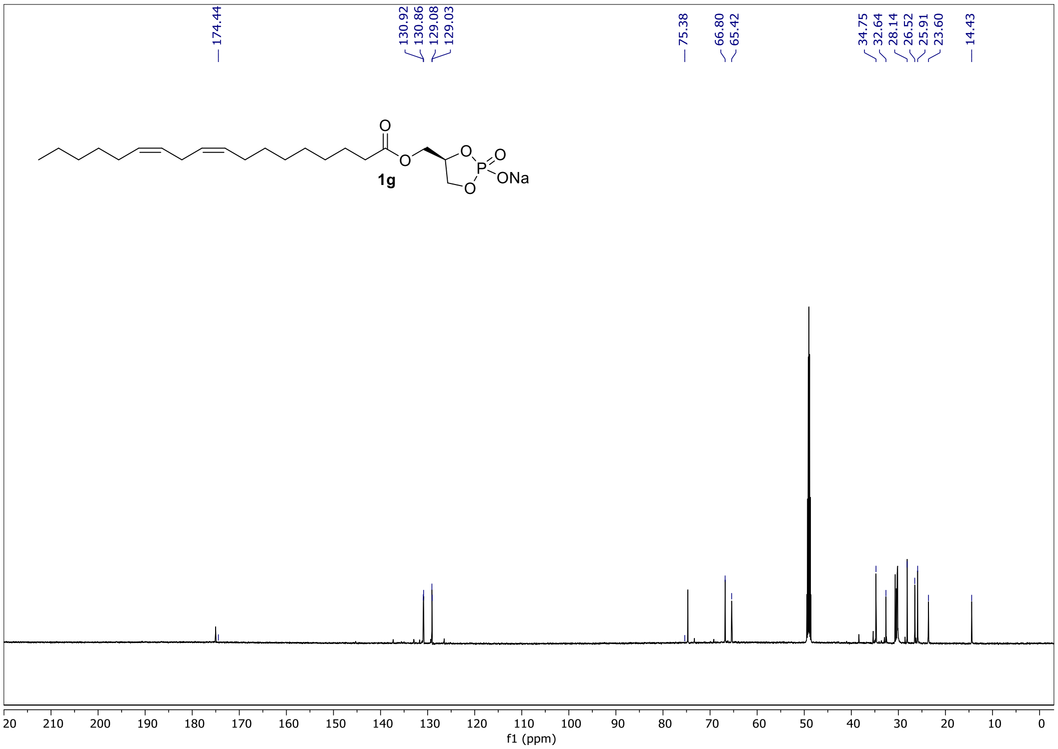


Figure S76. ^{13}C NMR spectrum of **1g** in CD_3OD

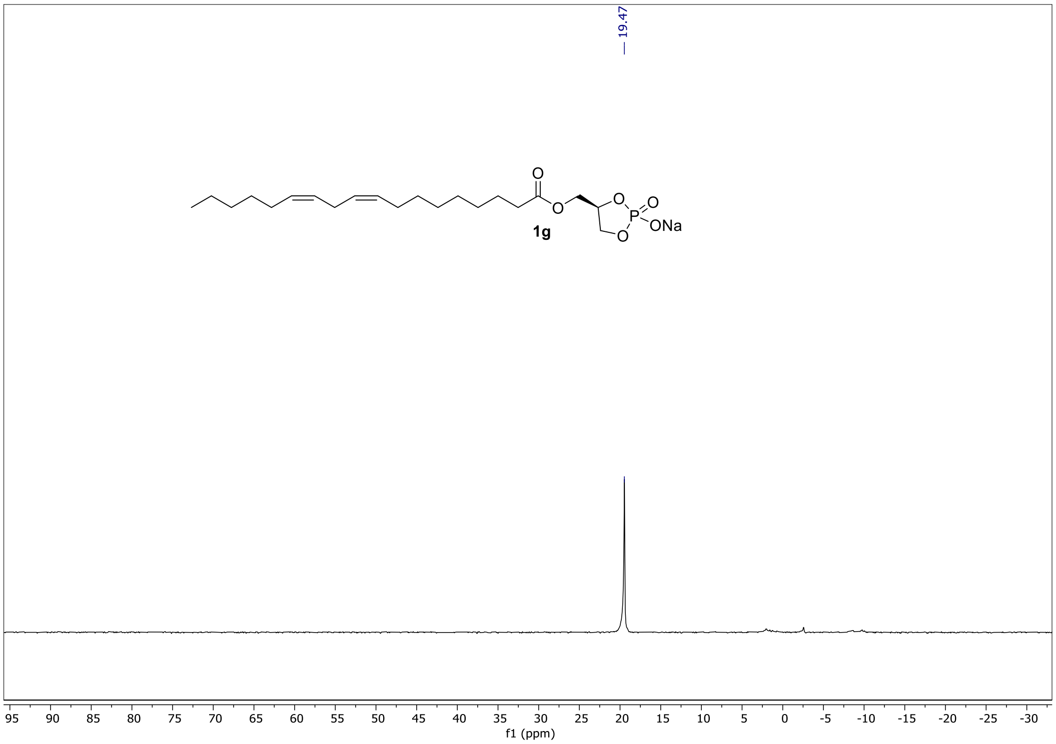


Figure S77. ^{31}P {H-decoupled} NMR spectrum of **1g** in CD_3OD

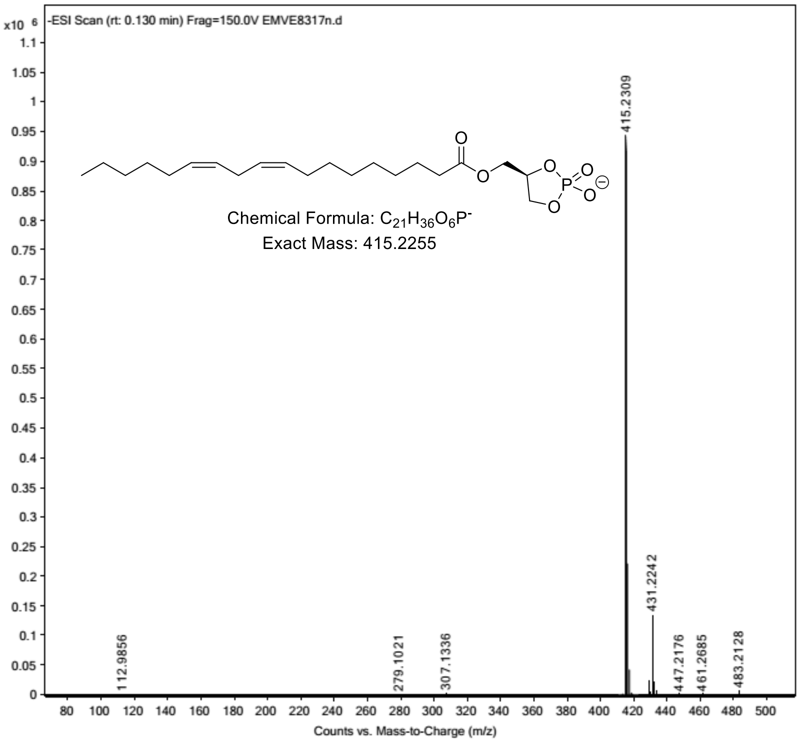


Figure S78. HRMS (ESI) of **1g**, m/z calcd for $[C_{21}H_{36}O_6P]^-$ 415.2255, found 415.2309.

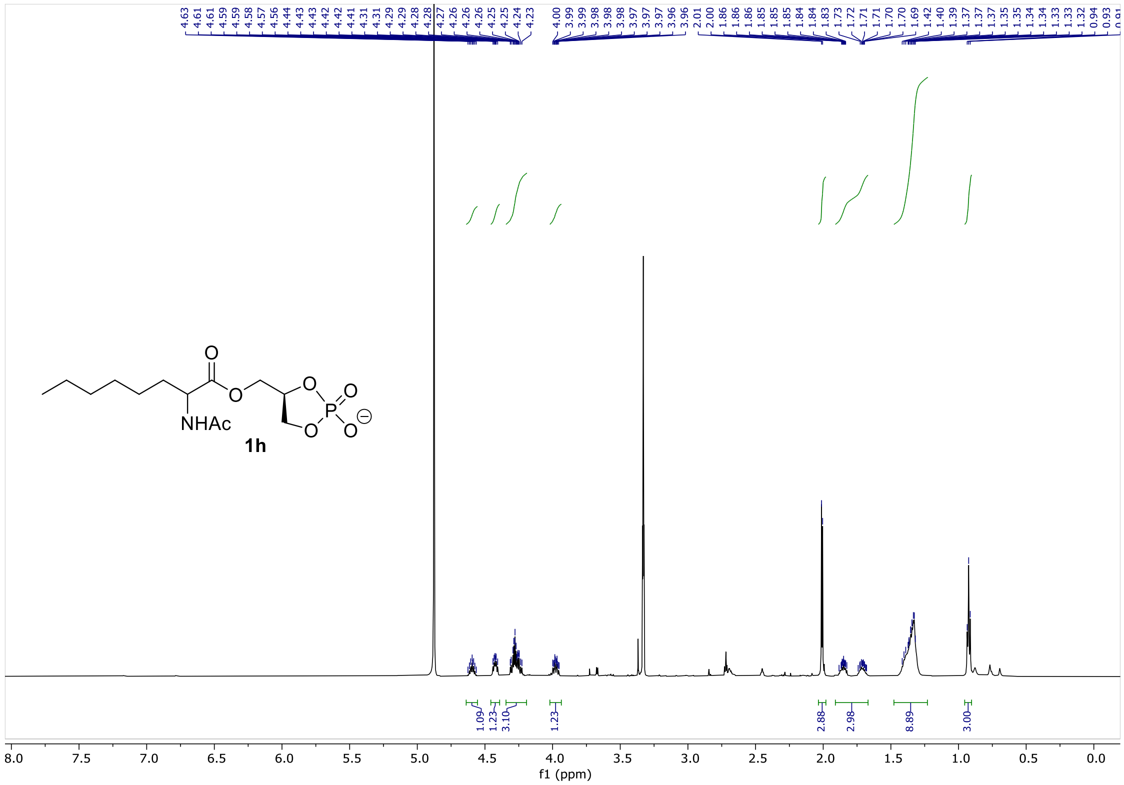


Figure S79. ^{1}H NMR spectrum of **1h** in CD_3OD

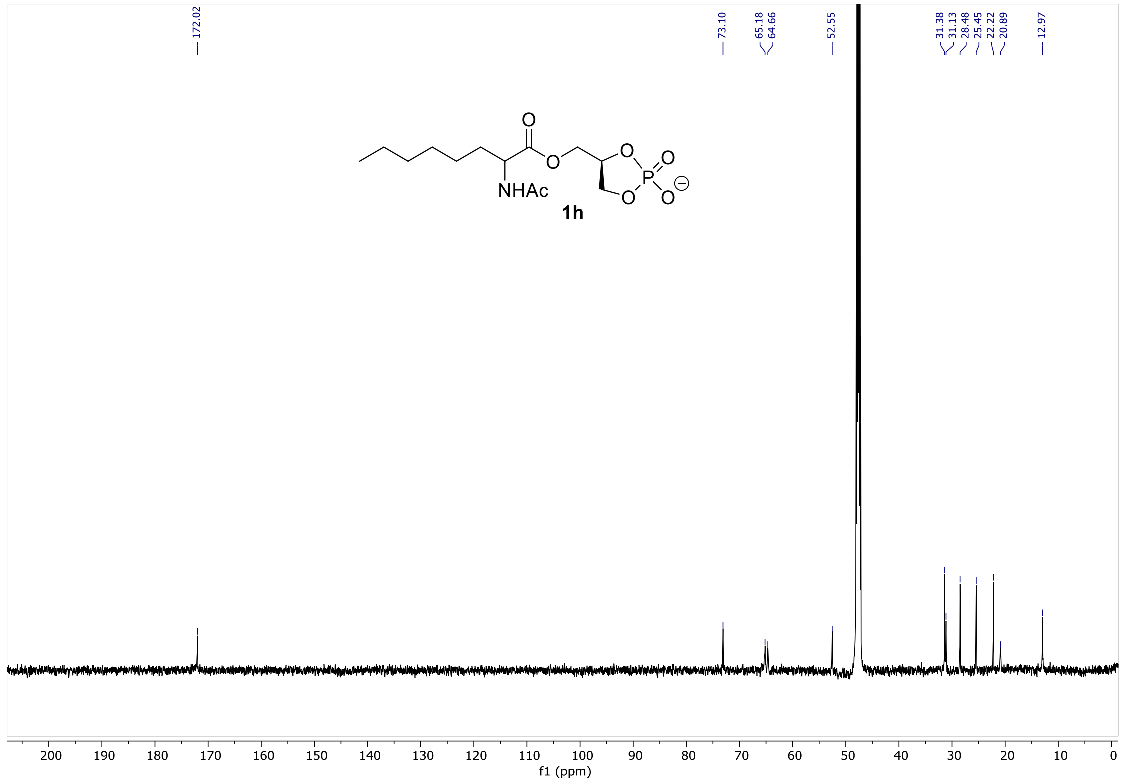


Figure S80. ^{13}C NMR spectrum of **1h** in CD_3OD

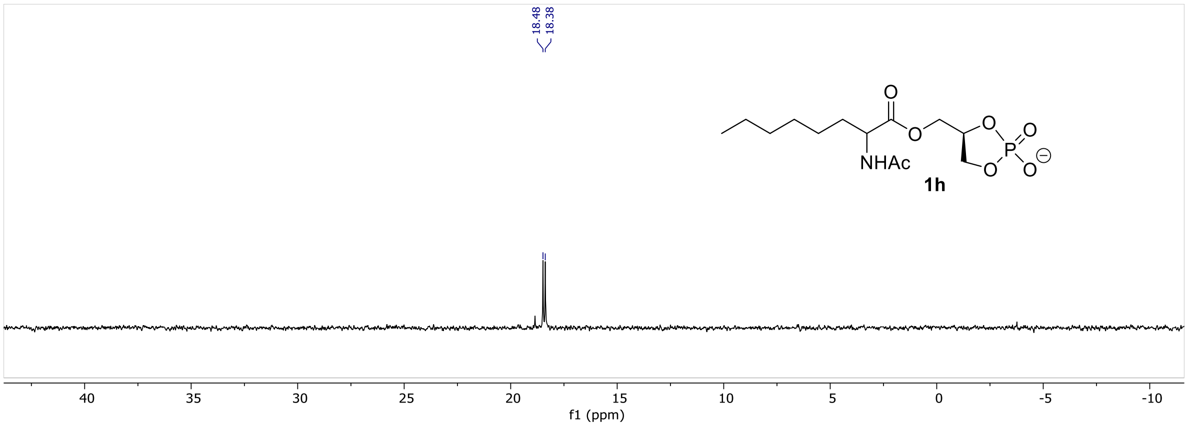


Figure S81. ^{31}P NMR spectrum of **1h** in CD$_3$OD

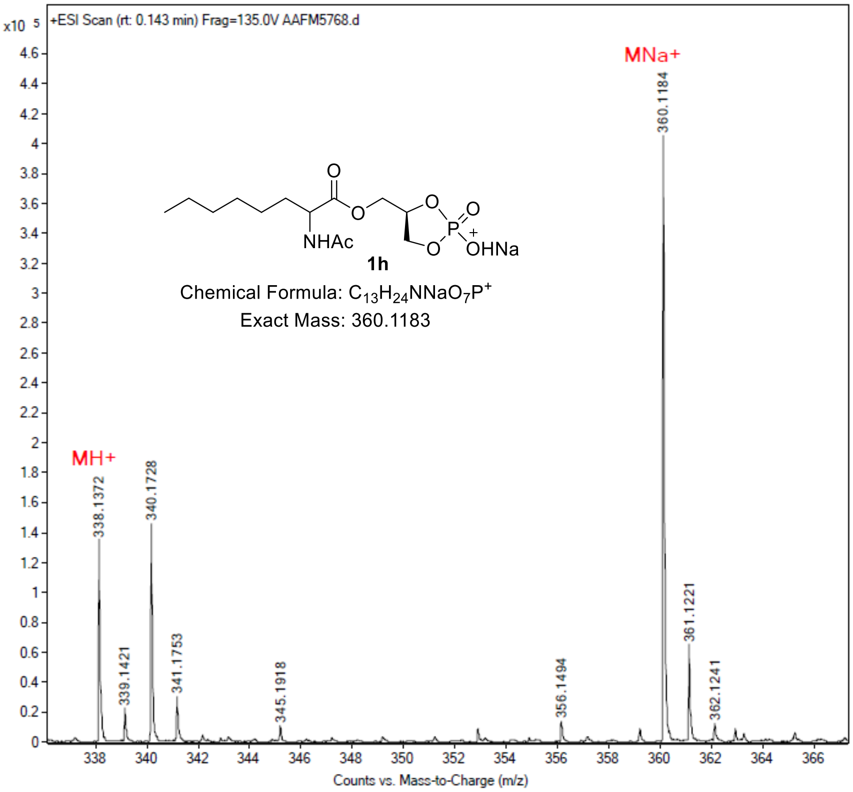


Figure S82. HRMS (ESI) of **1h**. m/z calcd for [$C_{13}H_{24}NNaO_7P$]$^+$ 360.1188, found 360.1184.

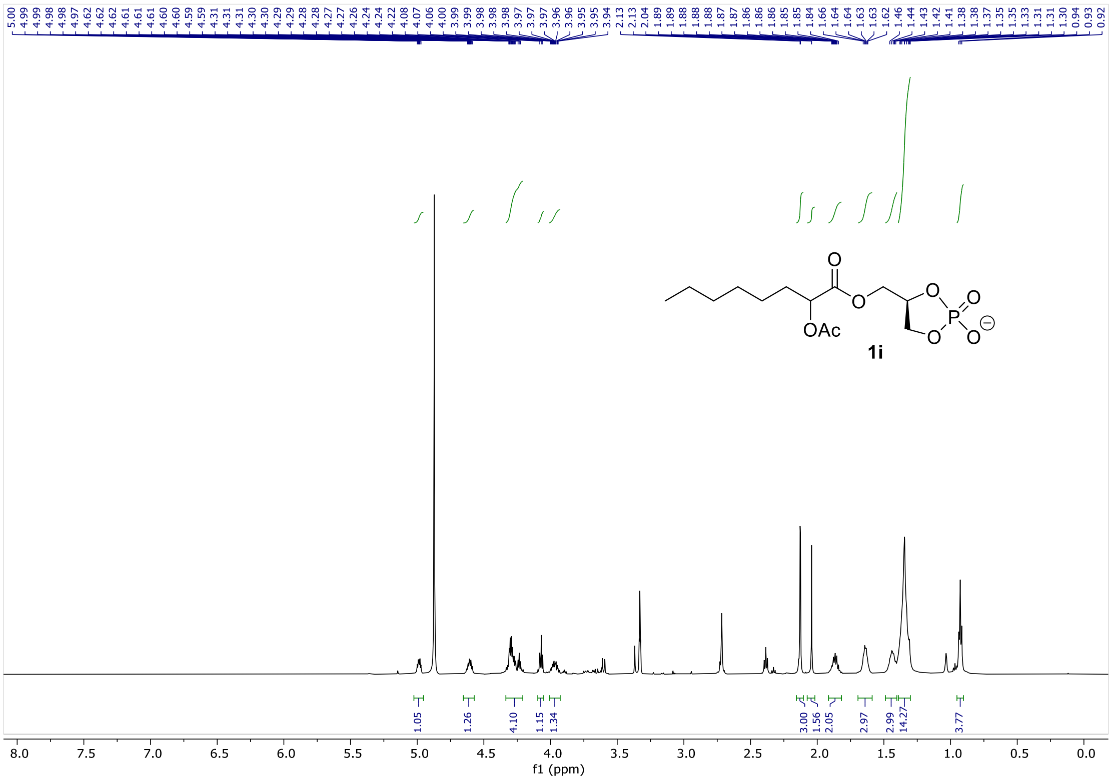


Figure S83. ^{1}H NMR spectrum of **1i** in CD_3OD

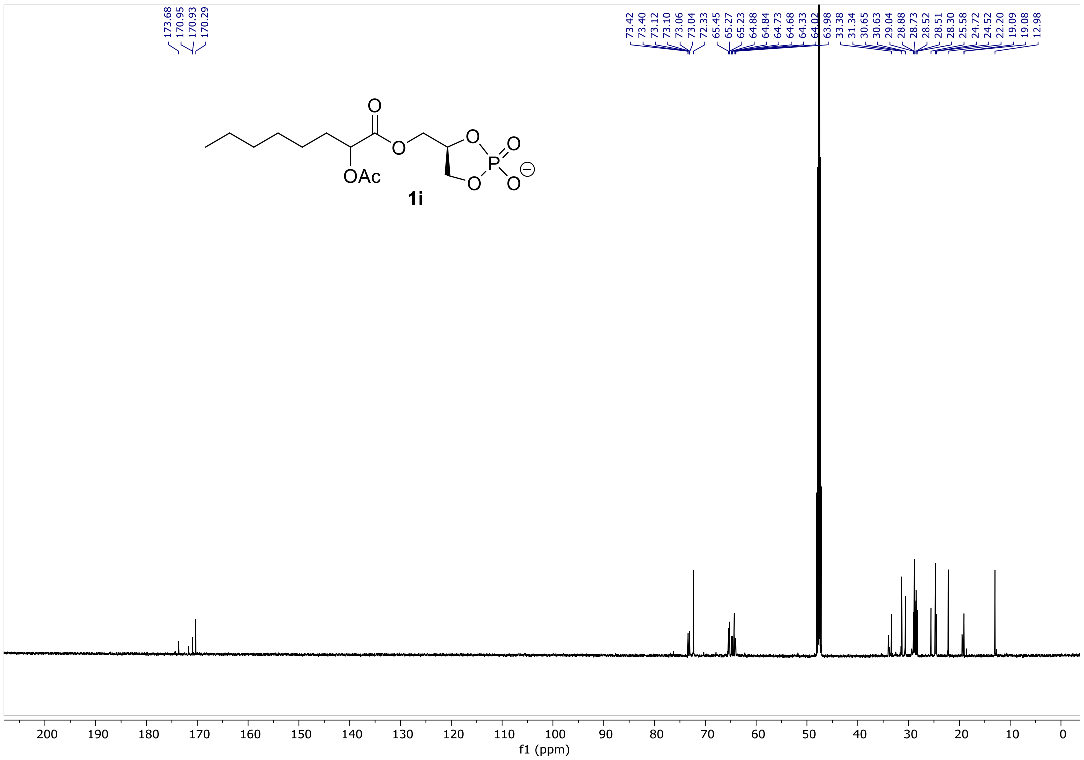


Figure S84. ^{13}C NMR spectrum of **1i** in CD_3OD

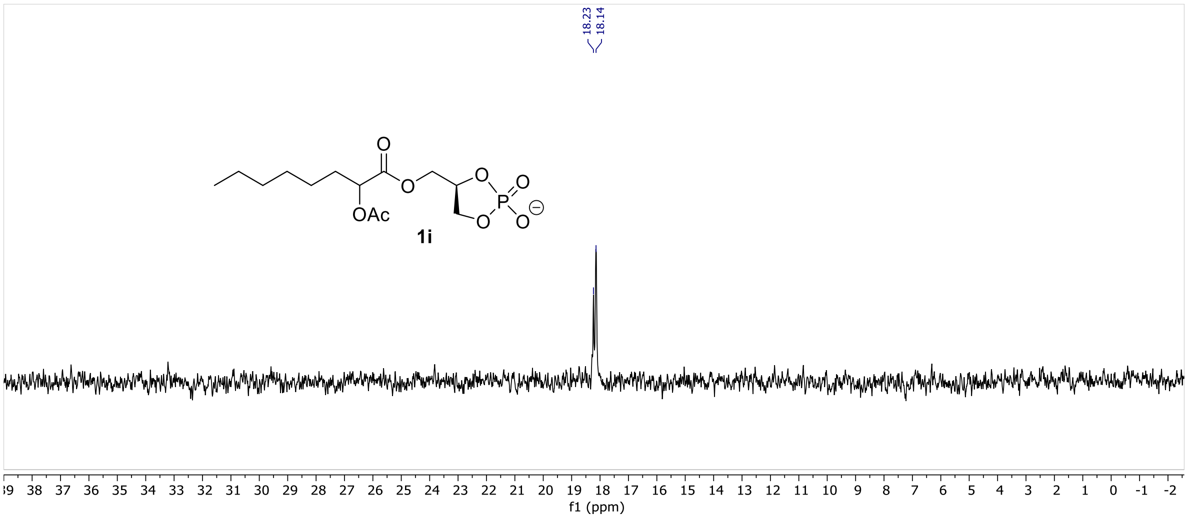


Figure S85. ^{31}P NMR spectrum of **1i** in CD_3OD

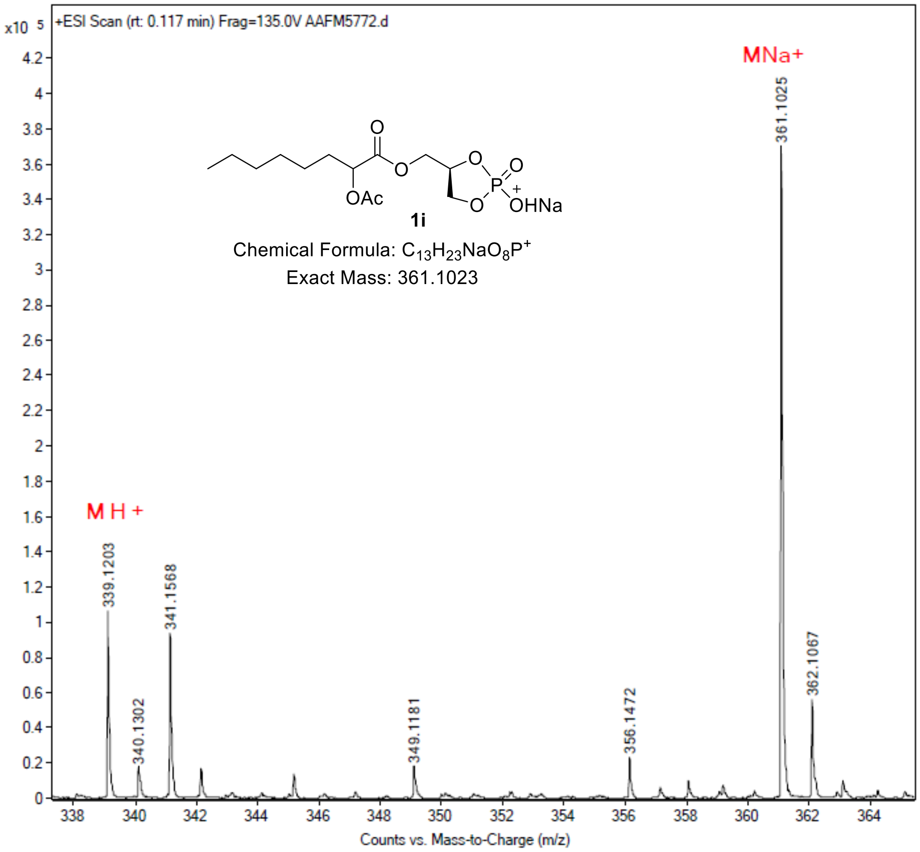


Figure S86. HRMS (ESI) of **1i**. m/z calcd for $[C_{13}H_{23}NaO_8P]^+$ 361.1028, found 361.1025.

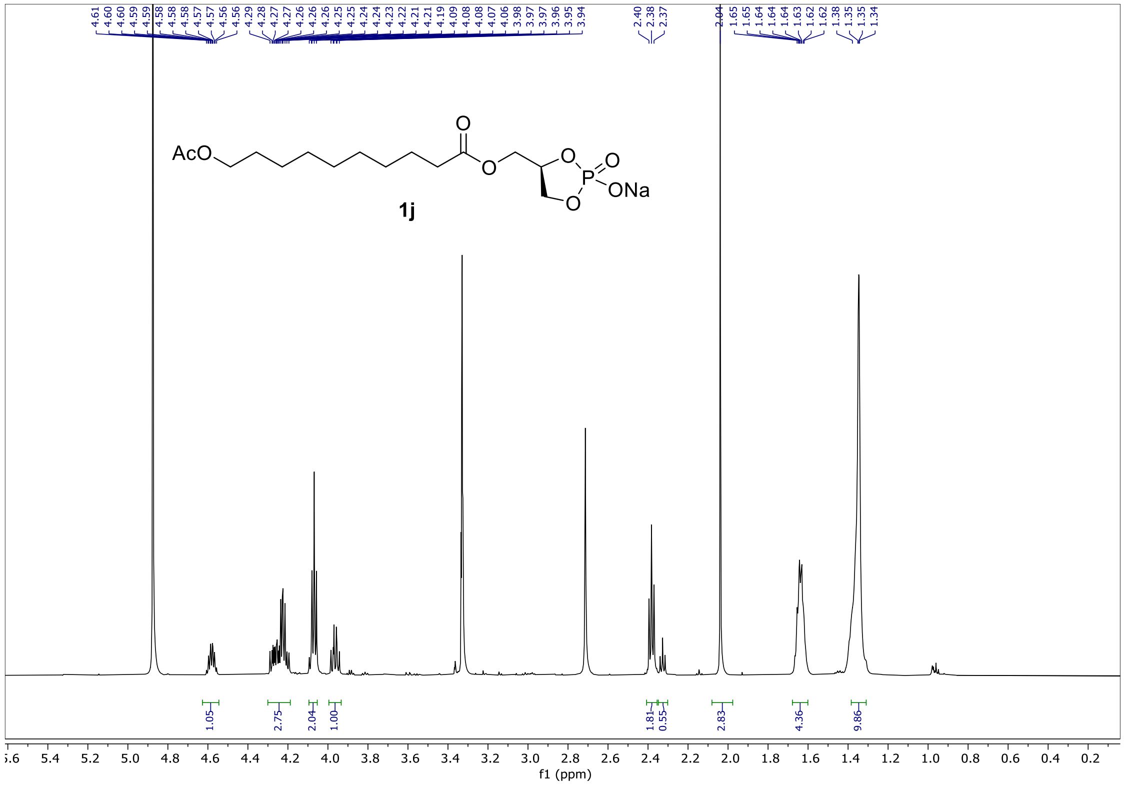


Figure S87. ^{1}H NMR spectrum of **1j** in CD_3OD

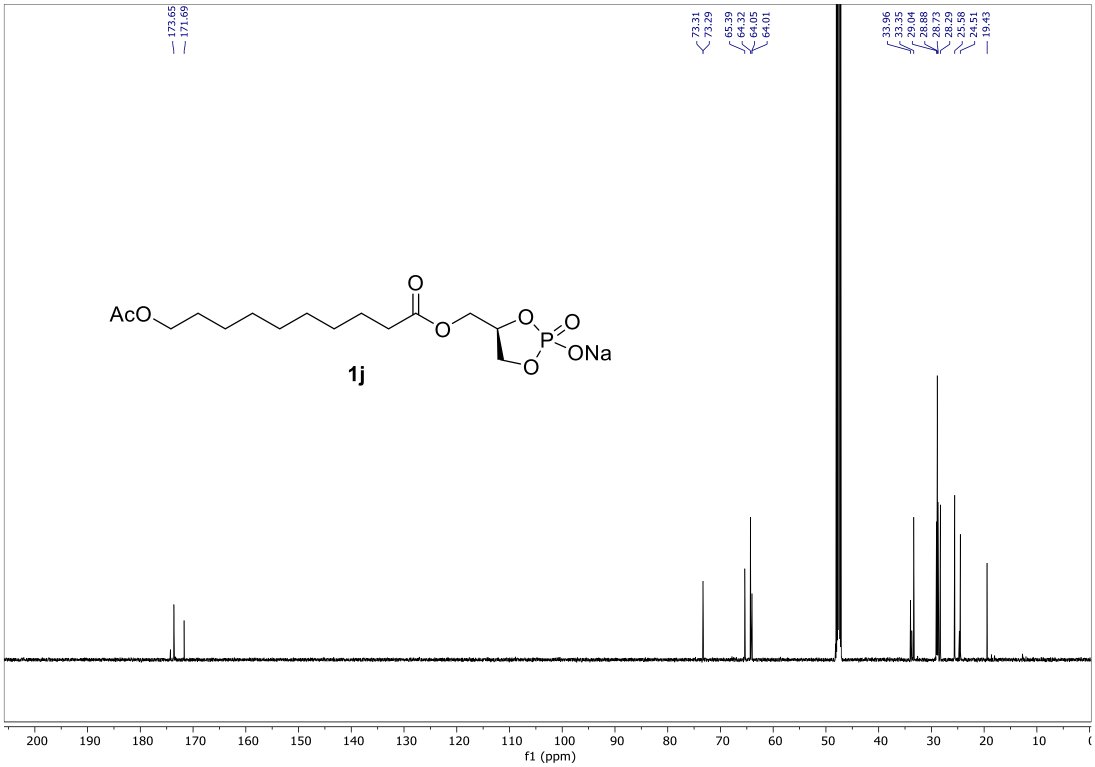


Figure S88. ^{13}C NMR spectrum of **1j** in CD_3OD

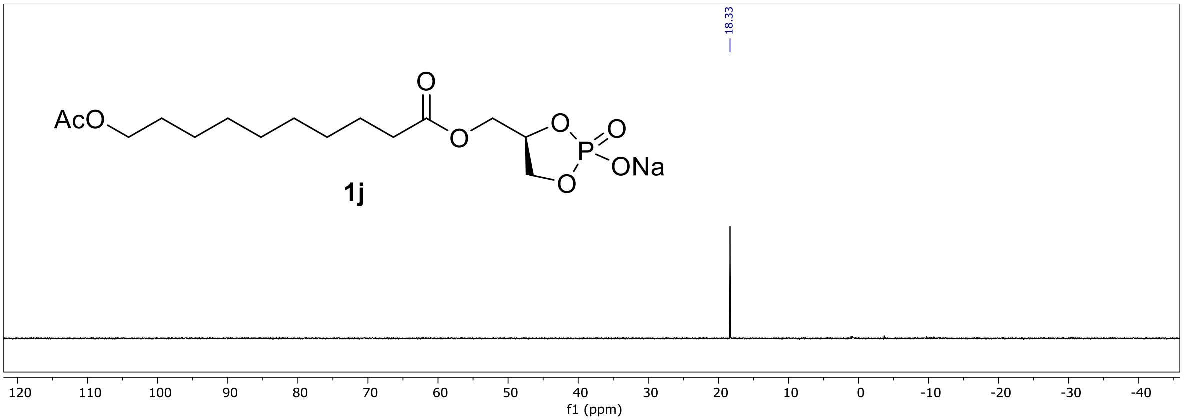


Figure S89. ^{31}P NMR spectrum of **1j** in CD_3OD

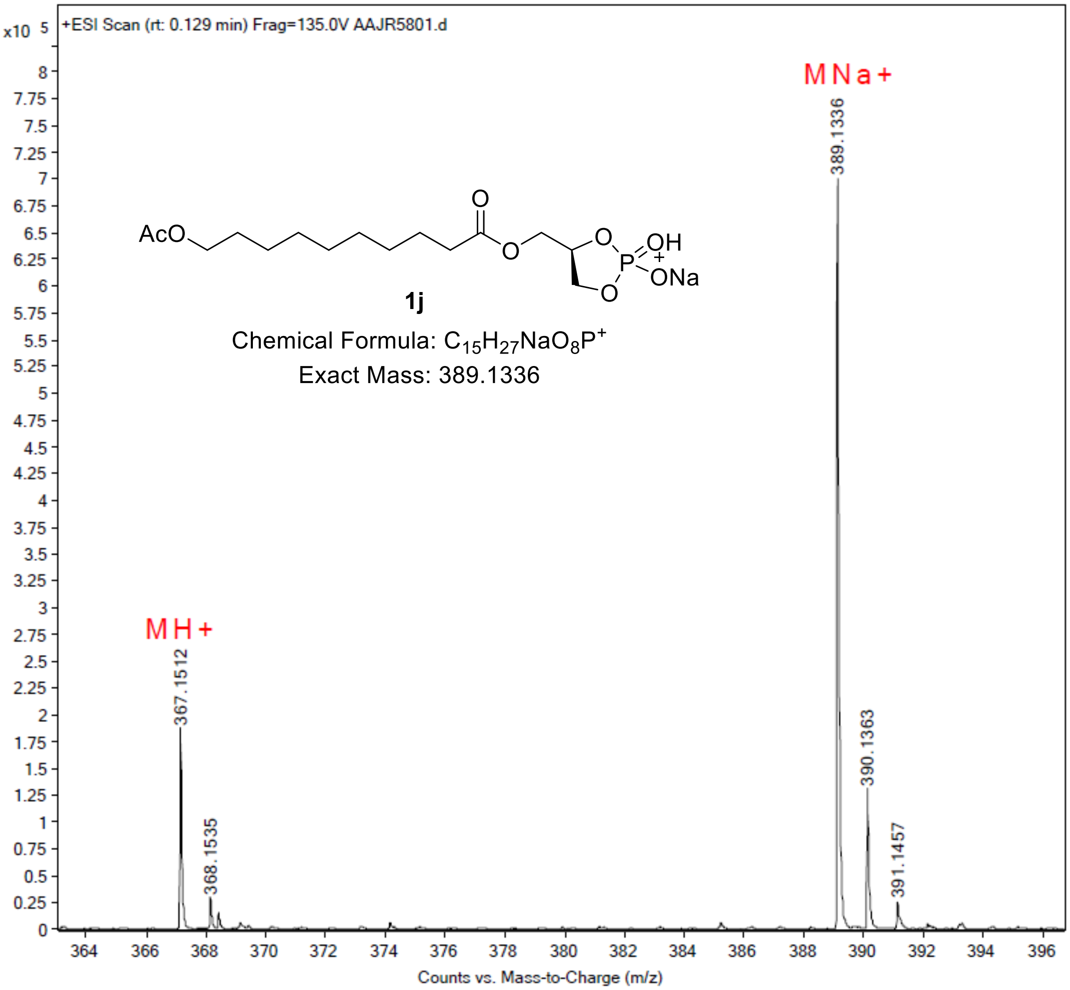


Figure S90. HRMS (ESI) of **1j**. m/z calcd for $[C_{15}H_{27}NaO_8P]^+$ 389.1336, found 389.1336.

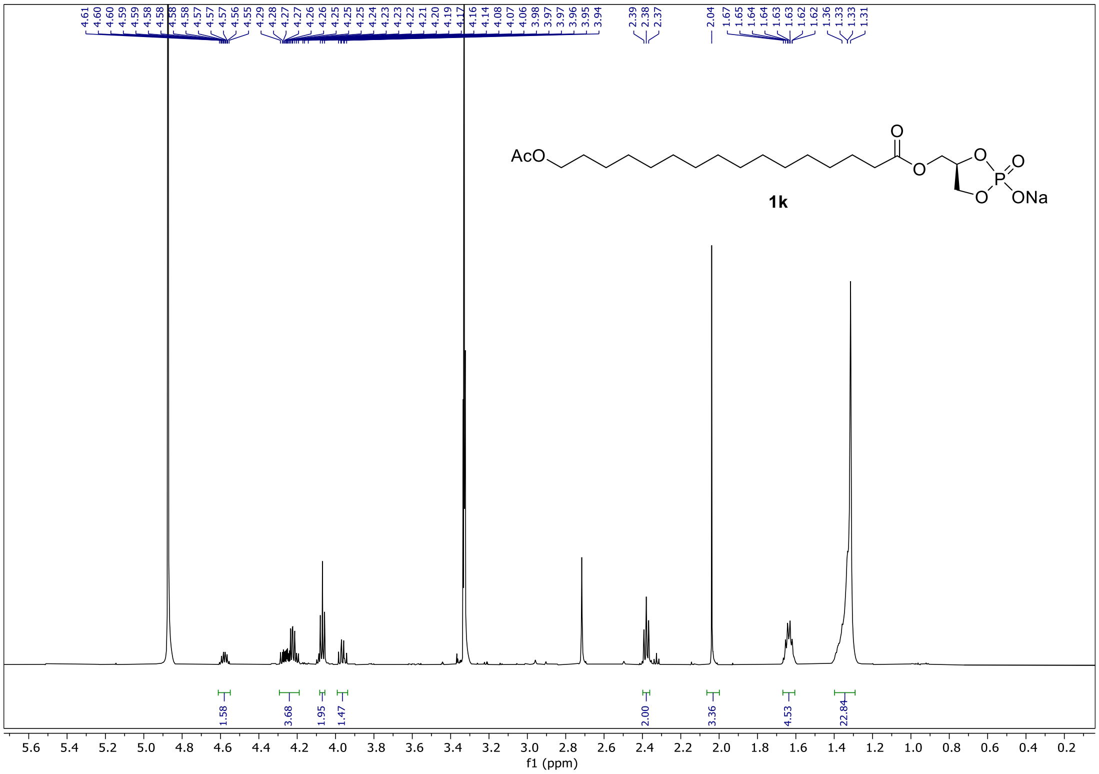


Figure S91. ^{1}H NMR spectrum of **1k** in CD_3OD

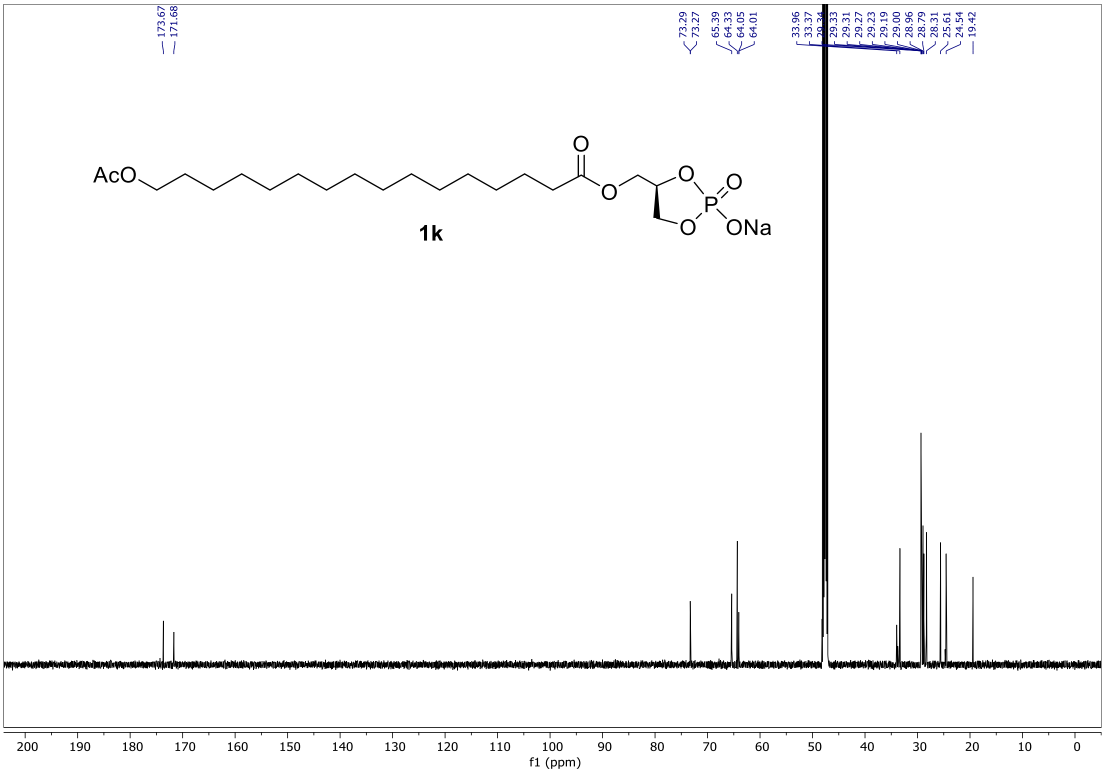


Figure S92. ^{13}C NMR spectrum of **1k** in CD_3OD

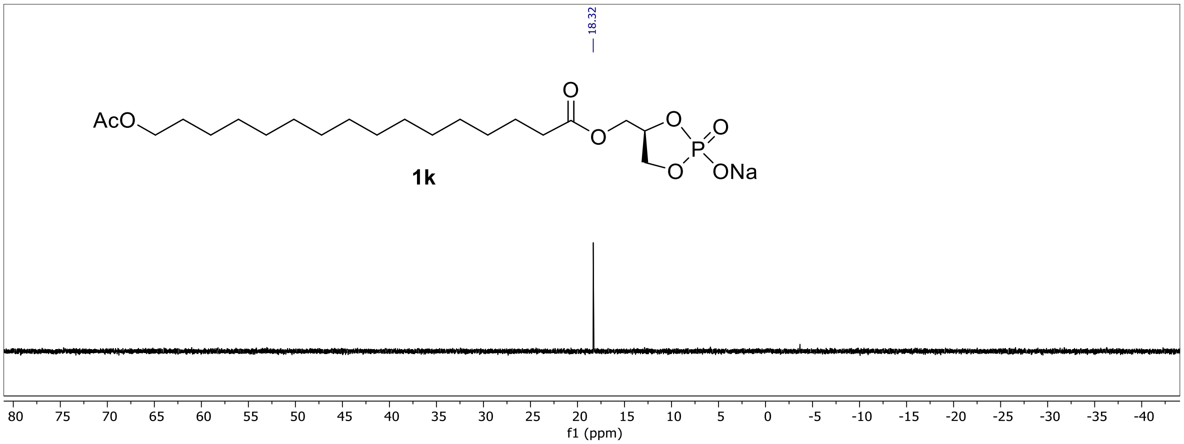


Figure S93. ^{31}P NMR spectrum of **1k** in CD_3OD

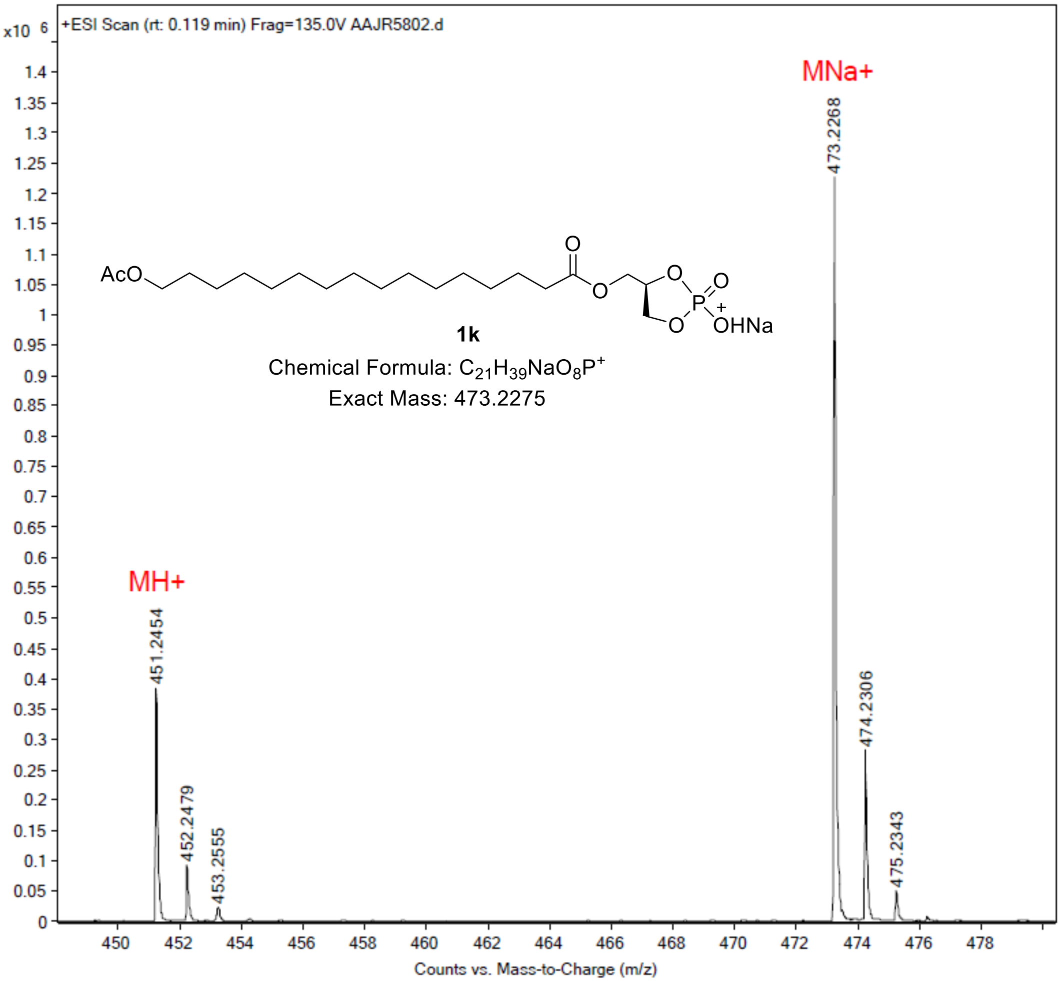


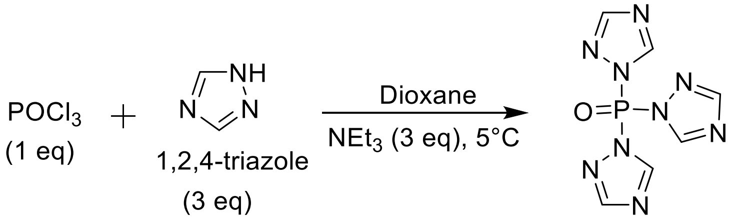
Figure S94. HRMS (ESI) of **1k**. m/z calcd for $[C_{21}H_{39}NaO_8P]^+$ 473.2275, found 473.2268.

Attempts with $POCl_3$ and phosphoryl tris triazole

With diols **5a-g** in hand, we first attempted the most straightforward phosphorylation using $POCl_3$. When the C10 diol **5b** was reacted with $POCl_3$, 10 equivalents of this reagent were necessary to get a total conversion to **1b** (Table S1). Moreover, this reaction proved to be extremely time consuming and erratic; It was hard to pin down the cause of this problem even though all precautions (such as dry solvents, argon atmosphere, new and/or distilled reagents) were taken. And this capricious nature made it impossible to move forward with scaling-up of these reactions, yielding few milligrams of **1b** after difficult work up caused by the phosphate produced from excess $POCl_3$. When the $POCl_3$ condition was applied to **5c** and **5e**, as representatives of the most biological relevant cPAs (having long alkyl lipid chains), insignificant amounts of the corresponding cyclophosphates **1c** and **1e** were observed by ^{31}P NMR.

We then considered the use of another cyclophosphorylating reagent, phosphoryl tris-triazole **7**, which was employed for the synthesis of PHYLPA (Physarum lysophosphatidic acid), a C16-cPA displaying DNA polymerase α inhibitory activity.[6] Treating diols **5e** and **5f** in THF (tetrahydrofuran) with 2 equivalents of freshly prepared **7** (in situ from $POCl_3$ and 1,2,4-triazole)[7,8] produced about 40% of the cyclic phosphate (estimated from the ^{13}C NMR of the reaction mixture). The use of an excess (10 equivalents) of phosphorylating reagent **7** provided near quantitative conversions to the respective cPAs. However, the excess reagent complicated the purification process, resulting in poor isolated yields (**1e**: 28%; **1e**: 23%, Table S1). The phosphorylation chemistries described for the synthesis of cPAs in the literature utilizing reagent **7** were not successful in our hands due to (a) the heterogeneous nature of the phosphoryl tris triazole **7** (Figs. S57-S61); (b) its susceptibility to traces of moisture, and (c) the need for excess of **7** which complicated the work-up and purification processes.

Synthesis and characterization of phosphoryl tris-triazole reagent



7

Synthesis of phosphoryl tris-triazole **7** through the reaction of $POCl_3$ with 1,2,4-triazole and triethyl amine in dry dioxane based on the literature procedure.[7,8]

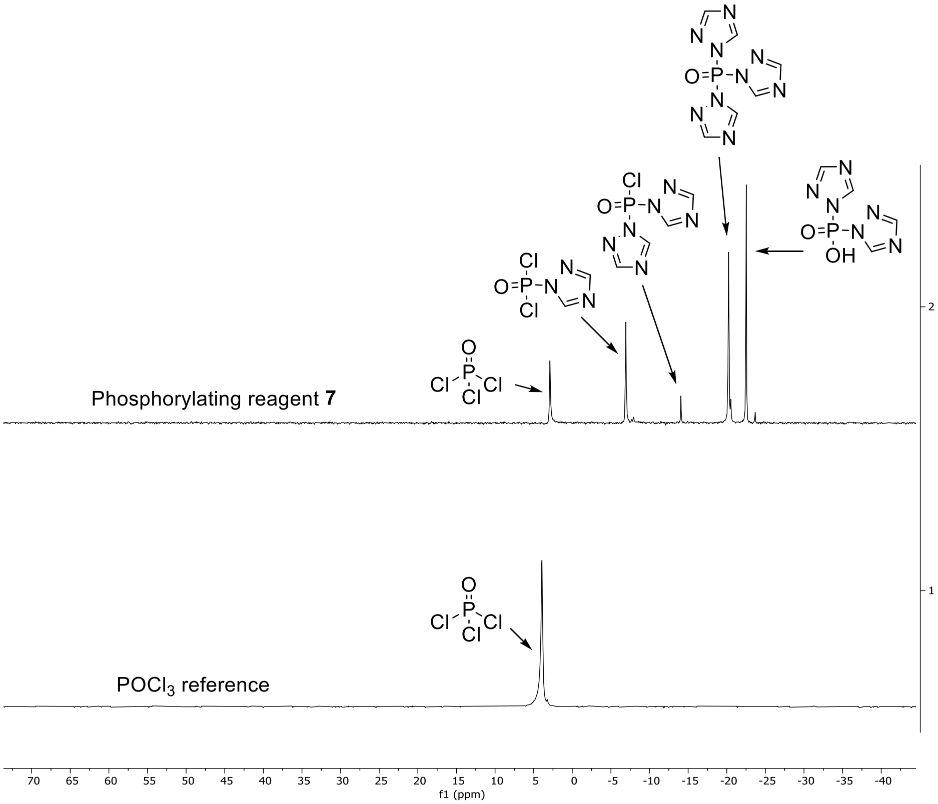


Figure S95. {H-decoupled} ^{31}P NMR spectrum of the phosphorylating reagent **7** (prepared as described in reference 6 through the reaction of 1 eq of $POCl_3$ with 3 eq of 1,2,4-triazole and 3 eq of NEt_3 in dry dioxane) showing a mixture of species including the unreacted $POCl_3$ and its mono, bis and tris adducts with 1,2,4-triazole (all of them, potentially able to react with diols to give the cyclophosphate) along with the hydrolyzed product. These species were identified through experiment shown in Figure S58 and S59. $POCl_3$ spectrum (bottom) is shown for comparison.

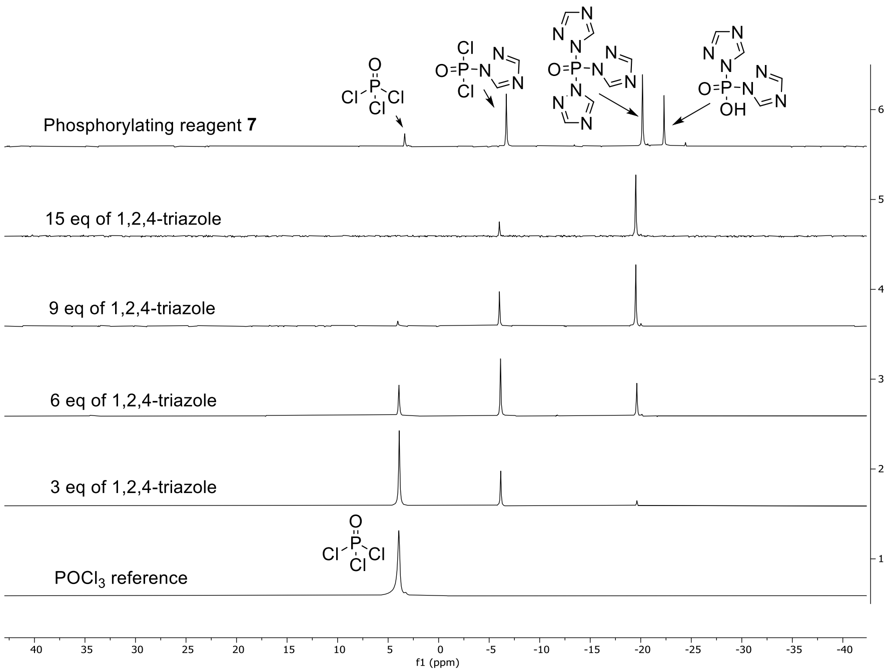


Figure S96. {H-decoupled} ^{31}P NMR spectra of the reaction of $POCl_3$ with increasing equivalents of 1,2,4-triazole (without NEt_3) in dry dioxane. Spectra of phosphorylating reagent **7** (top) and $POCl_3$ (bottom) are shown for comparison.

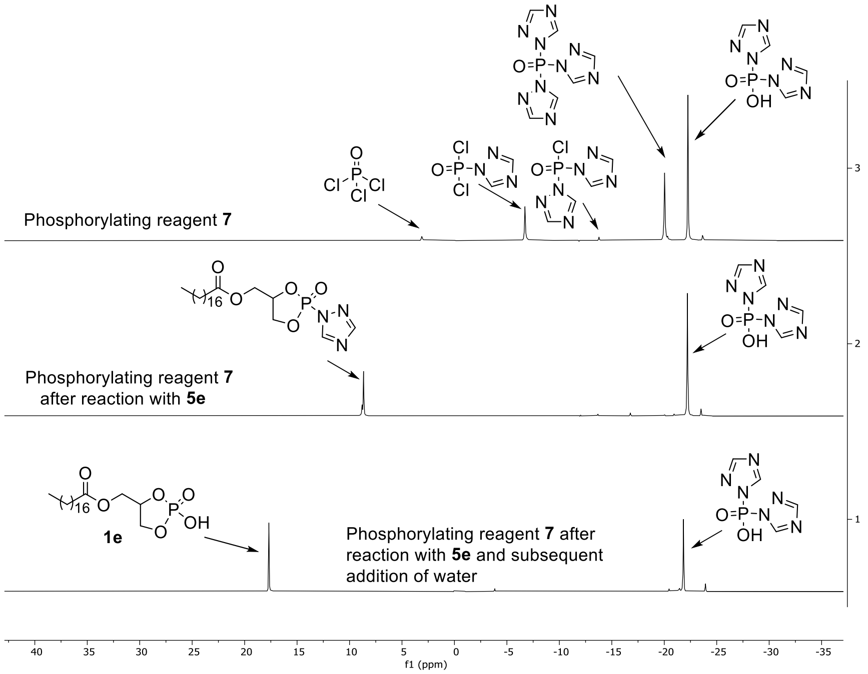


Figure S97. {H-decoupled} ^{31}P NMR spectra of the reaction between the phosphorylating reagent **7** (prepared through the reaction of $POCl_3$ with 1,2,4-triazole) and diol **5e** in dry THF to produce the corresponding cyclophosphate. When the reagent containing a mixture of phosphorus species was reacted with **5e** all the peaks disappeared to produce the cyclic phosphate **1e**, except for the one around -22 ppm, which remained even after water addition, thus was assigned to the hydrolyzed product.

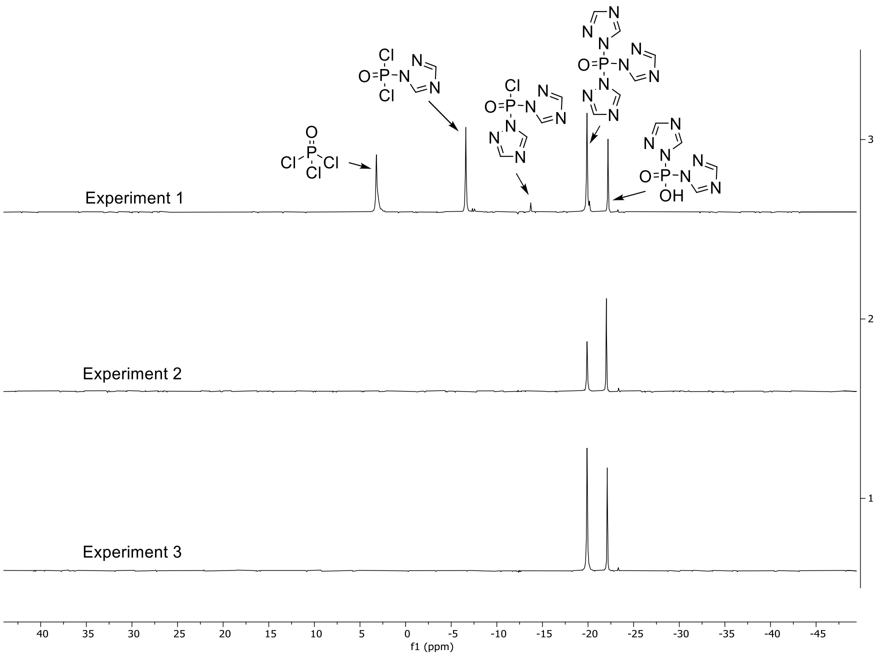


Figure S98. {H-decoupled} ^{31}P NMR spectra of three independent experiments of phosphorylating reagent **7** preparation through reaction of 1 equivalent of $POCl_3$ with 3 equivalents of 1,2,4-triazole and 3 eq of NEt_3 carried out simultaneously using the same reagents in dry THF. Several attempts to produce the pure phosphoryl tris triazole without any interference from the hydrolyzed product was unsuccessful.

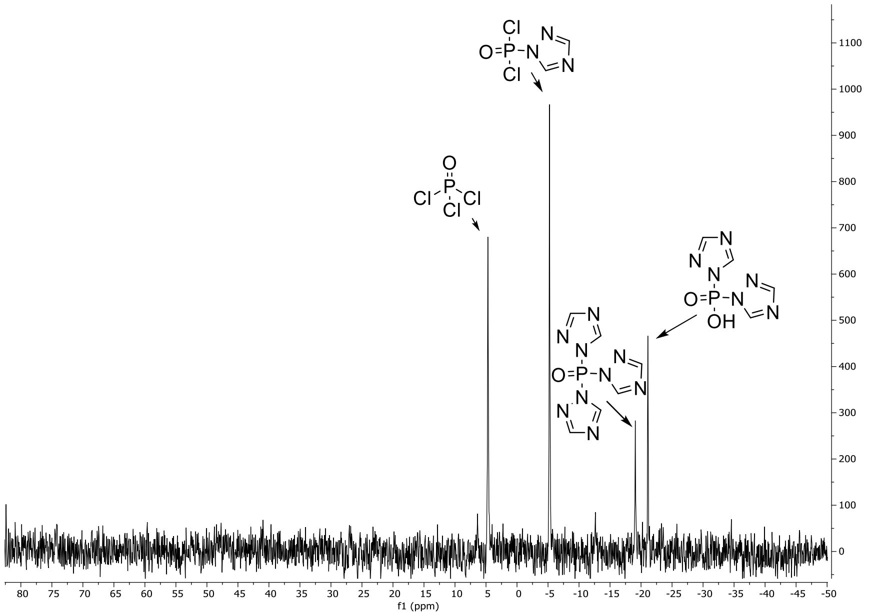


Figure S99. {H-decoupled} ^{31}P NMR spectrum of phosphorylating reagent **7** prepared through reaction of 1 equivalent of $POCl_3$ with 6 equivalents of 1,2,4-triazole (without TEA) in dry acetonitrile. Synthesis of **7** has also been reported through this methodology giving a product with a ^{31}P NMR chemical shift of -1.13 (*d*6 DMSO).[7,8] In our hands this method produced again a mixture of the species as described in Figures S57-S60. Phosphoryl tris-triazole has been poorly characterized in literature,[7,8] probably due difficulties to differentiate between the mono, bis and tris adducts, as all of them are expected to produce similar ^{1}H and ^{13}C NMR data.

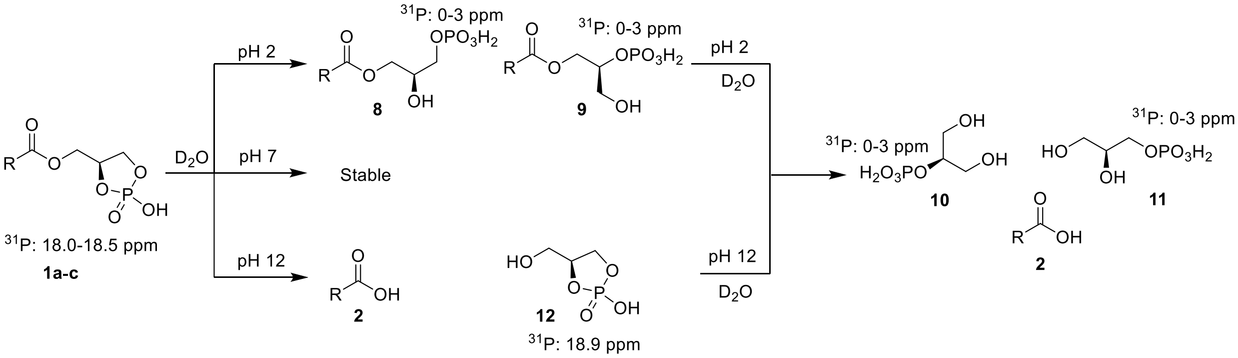
Table S1. Comparison of the optimized yields from the BDMDAP reaction with the % conversion of acylglycerides **5a-g** to cPAs **1a-g** by $POCl_3$ and PTT.

	Cyclophosphorylating Reagent								
	BDMDAP (6)			**$POCl_3$**			**PTT**		
cPA	Equiv. used	Conversion (%)[a]	Isolated yields (%)	Equiv. used	Conversion (%)	Isolated yields (%)	Equiv. used	Conversion (%)	Isolated Yields (%)
1a	2.0	95	85	--	--	--	--	--	--
1b	2.0	95	89	10.0	95[a]	83	--	--	--
1c	2.0	95	91	10.0	>5[b]	--	--	--	--
1d	2.0	95	80	--	--	--	--	--	--
1e	2.0	95	77	10.0	>5[b]	--	10.0	95[a]	28
1f	3.0	95	93	--	--	--	10.0	95[a]	23
1g	3.0	95	87	--	--	--	--	--	--

[a]Estimated by ^{13}C NMR; [b]Estimated by ^{31}P NMR. BDMDAP = bis(dimethylamino)phosphorodiamidate; PTT = phosphoryl tris-triazole. -- not attempted.

Stability studies of cyclic phosphatidic acids (cPAs) over range of pH 2-12 in D_2O

General procedure: cPA (**1a**-**1c**) was taken in D_2O such that the concentration of cPA is 40 mM. The resulting solution was vortexed for ~1 min, followed by sonication for ~1 min for the complete dissolution (cPA **1a** and **1b** were clear solutions, cPA, **1c** was milky solution). pH of the solution was adjusted carefully to desired pH with 1M NaOH/1M HCl solution. ^{31}P NMR of the solution was measured at different time intervals.



Scheme S1: The chemical shifts of the cyclic phosphate group and the phosphate groups as observed by ^{31}P-NMR were used to monitor the hydrolysis over time.

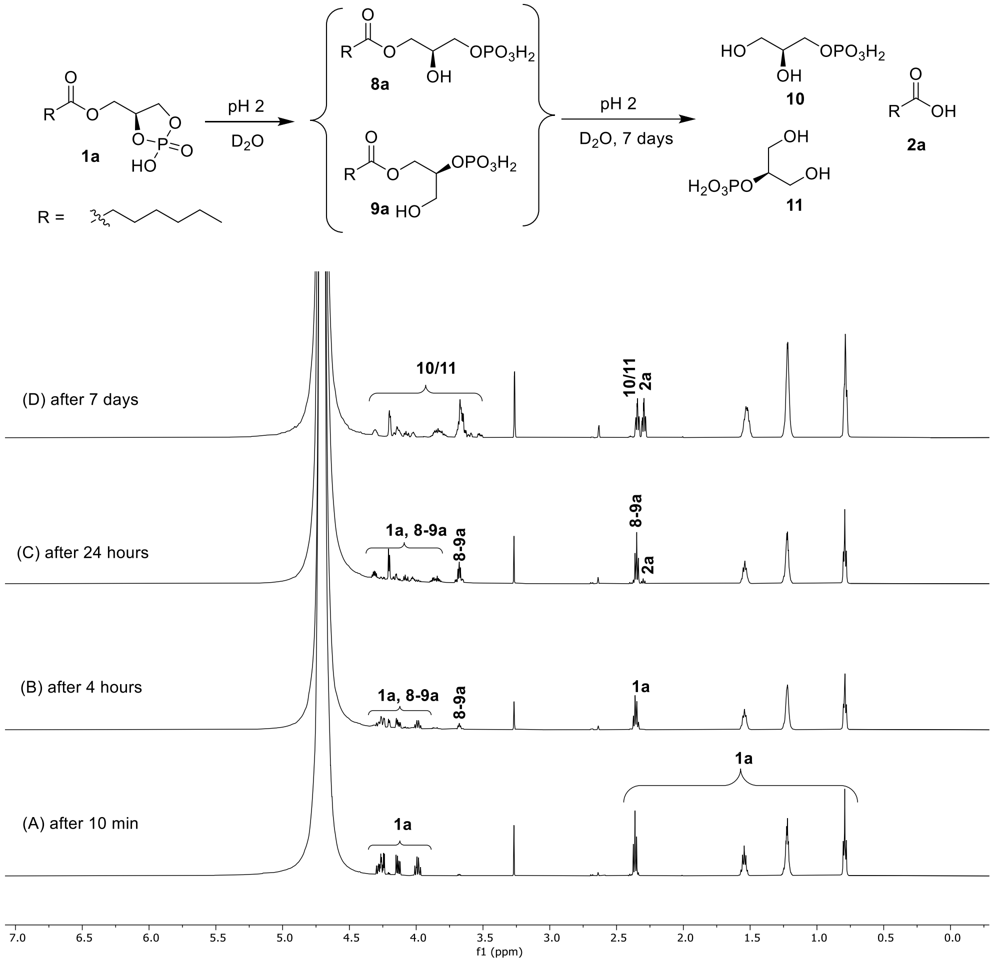


Figure S100. ^{1}H NMR of 40 mM of cPA 6:0 **1a** in D_2O at pH 2.0.

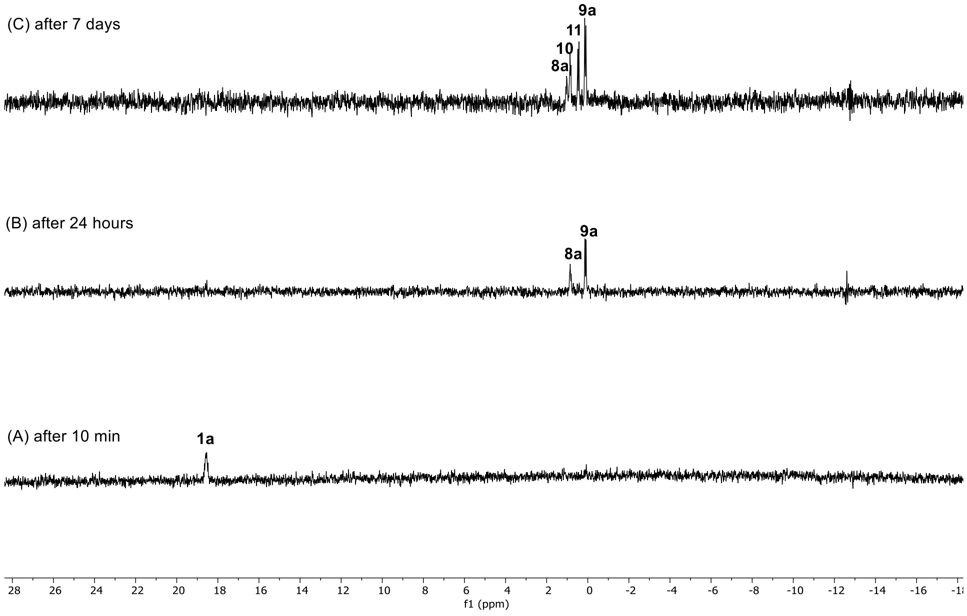
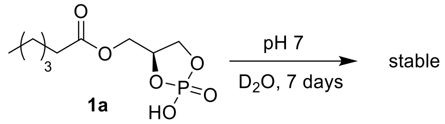


Figure S101. ^{31}P NMR (^{1}H-coupled) of 40 mM of cPA 6:0 **1a** in D_2O at pH 2.0.



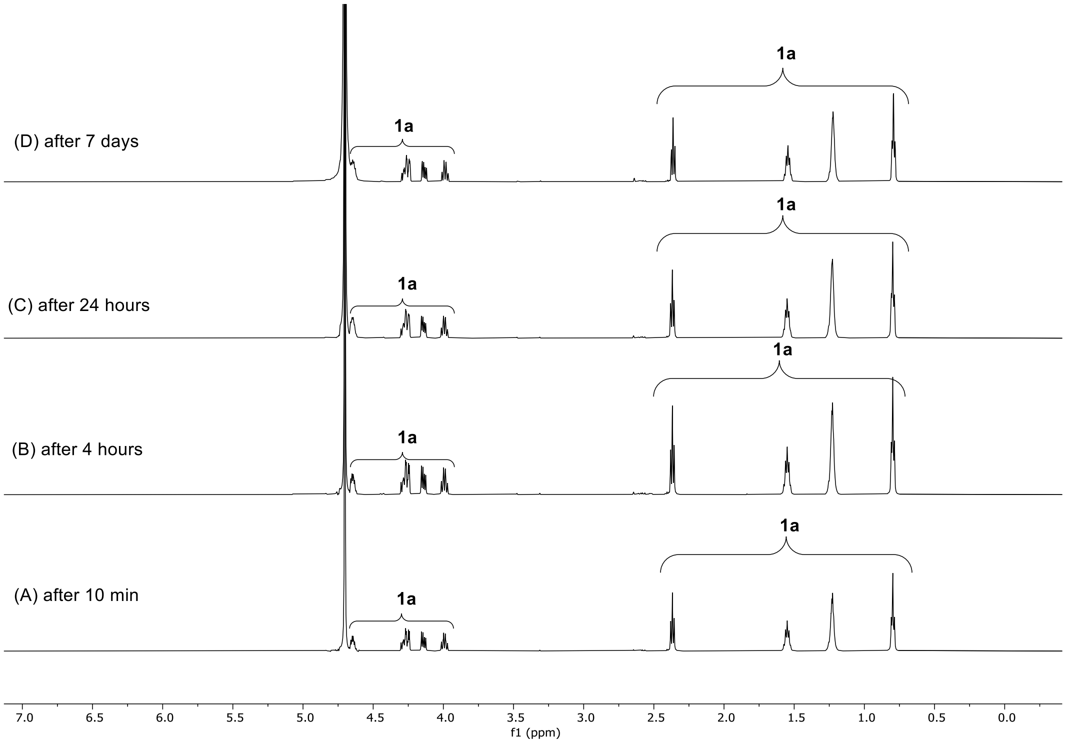


Figure S102. ^{1}H NMR of 40 mM of cPA 6:0 **1a**, in D_2O at pH 7.0.

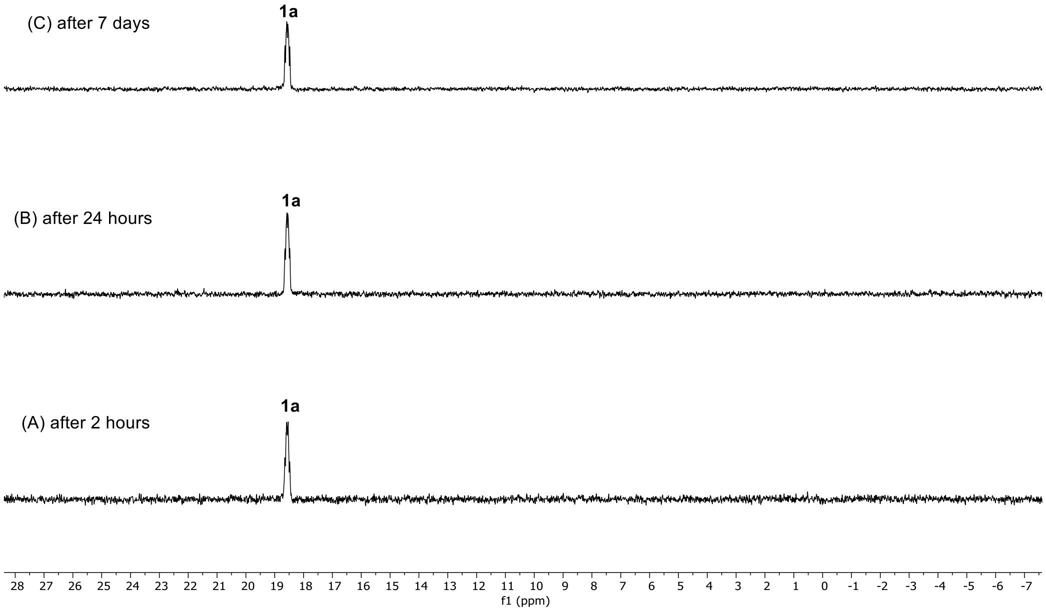
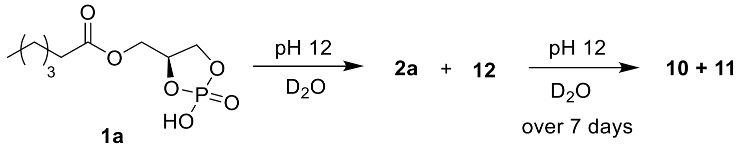


Figure S103. ^{31}P NMR (^{1}H-coupled) of 40 mM of cPA 6:0 **1a**, in D_2O at pH 7.0.



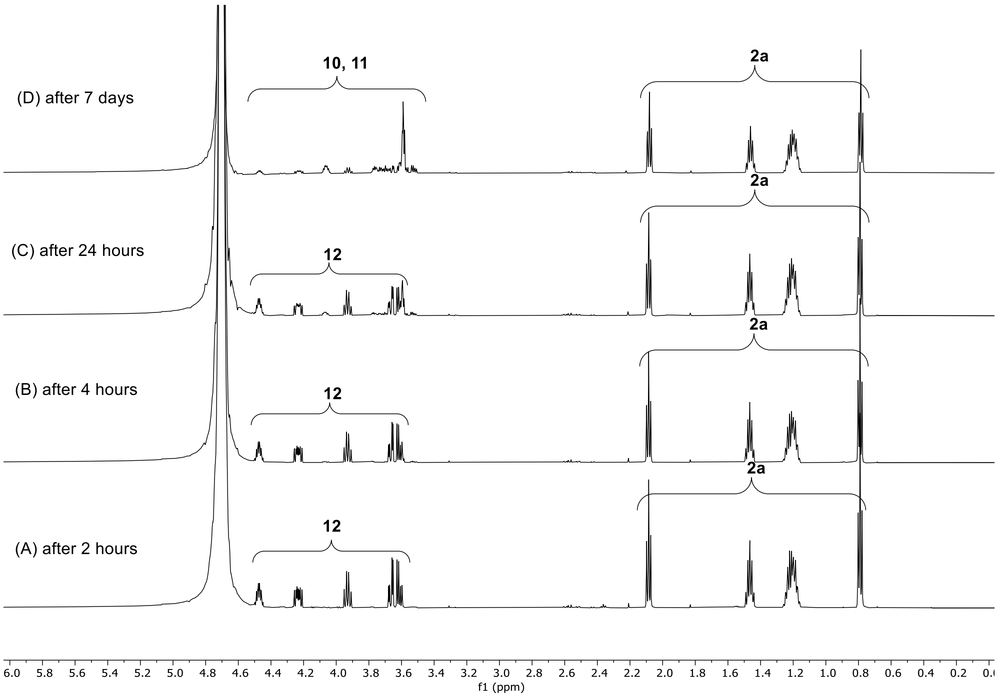


Figure S104. ^{1}H NMR of 40 mM of cPA 6:0 **1a** in D$_2$O at pH 12.0.

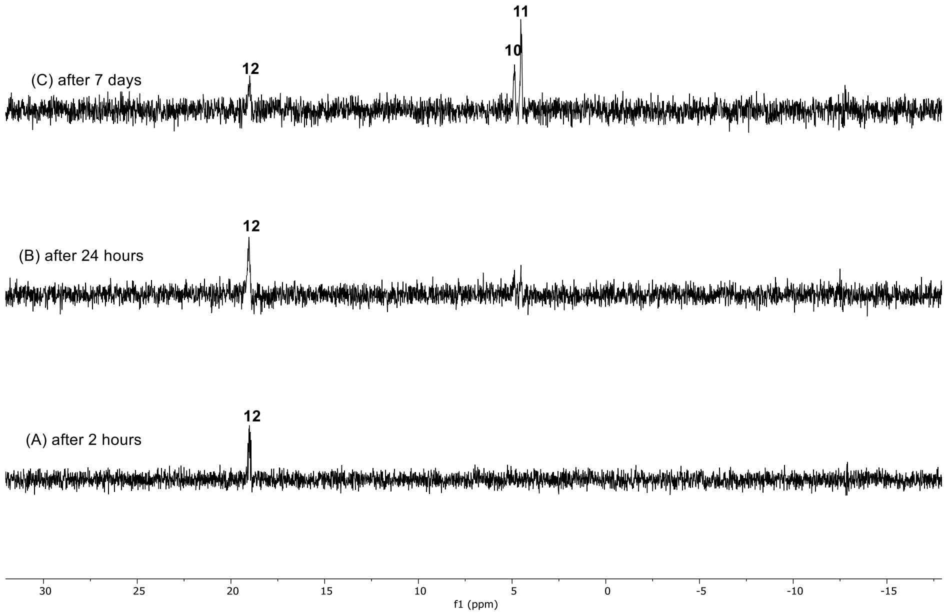


Figure S105. ^{31}P NMR (^{1}H-coupled) of 40 mM of cPA 6:0 **1a**, in D$_2$O at pH 12.0.

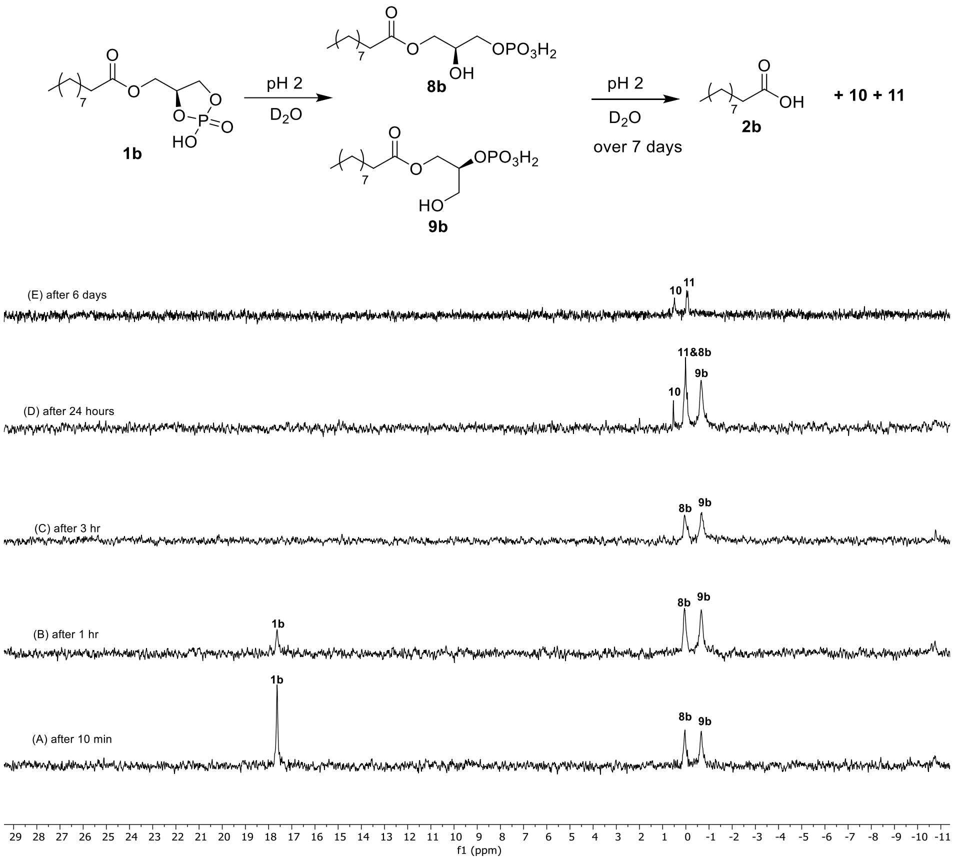


Figure S106. ^{31}P NMR of 40 mM of cPA 10:0 **1b** in D_2O at pH 2.

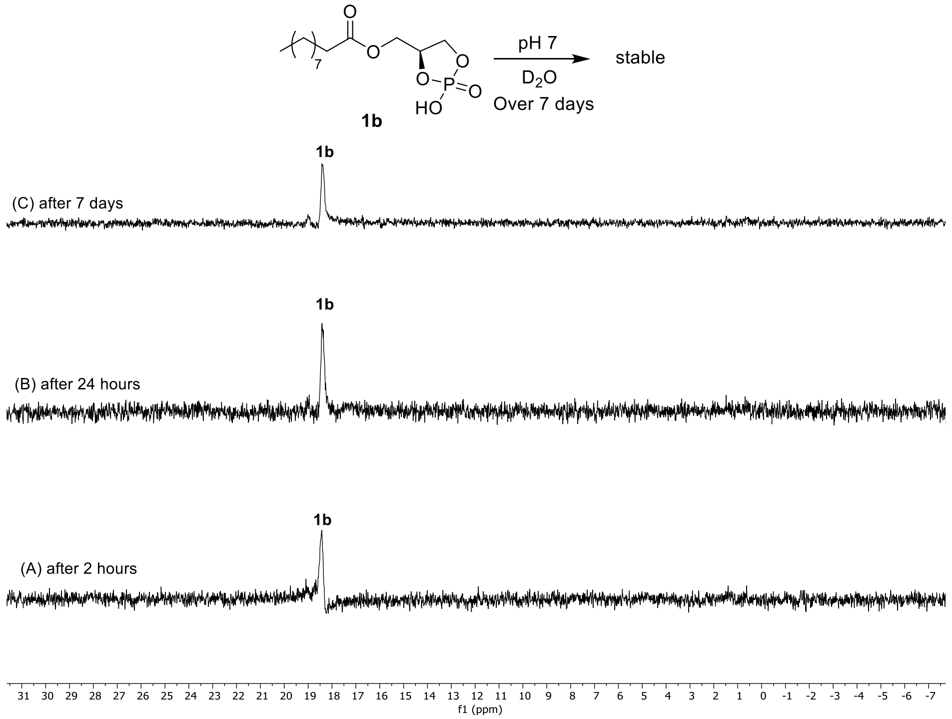


Figure S107. ^{31}P NMR (^1H-coupled) of 40 mM of cPA 10:0 **1b**, in D_2O at pH 7.

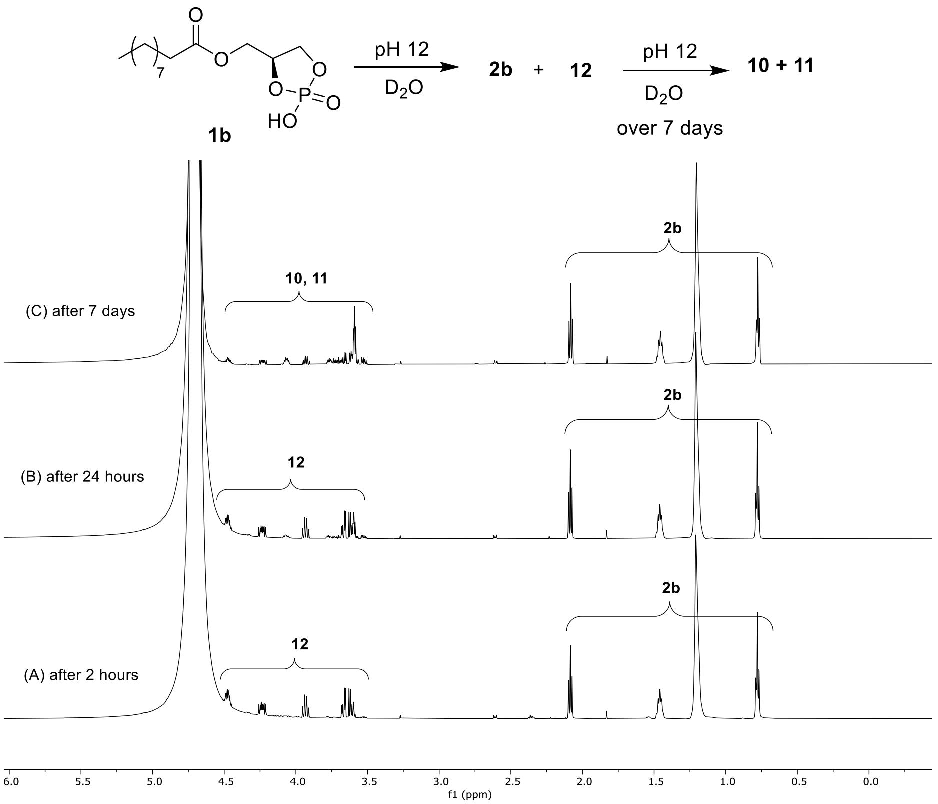


Figure S108. ^{1}H NMR of 40 mM of cPA 10:0 **1b**, in D_2O at pH 12.0. Complete carboxylate ester hydrolysis to **2b** and **12** was observed within 2 hours at pH 12.

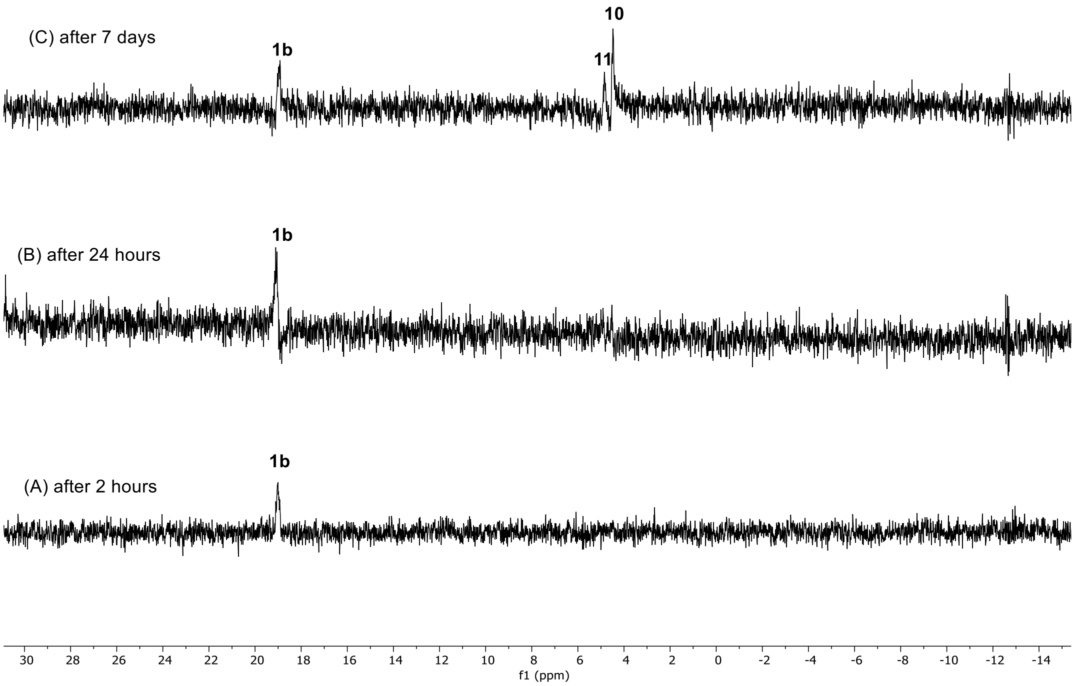


Figure S109. ^{31}P NMR (^{1}H-coupled) of 40 mM of cPA 10:0 **1b**, in D_2O at pH 12.0.

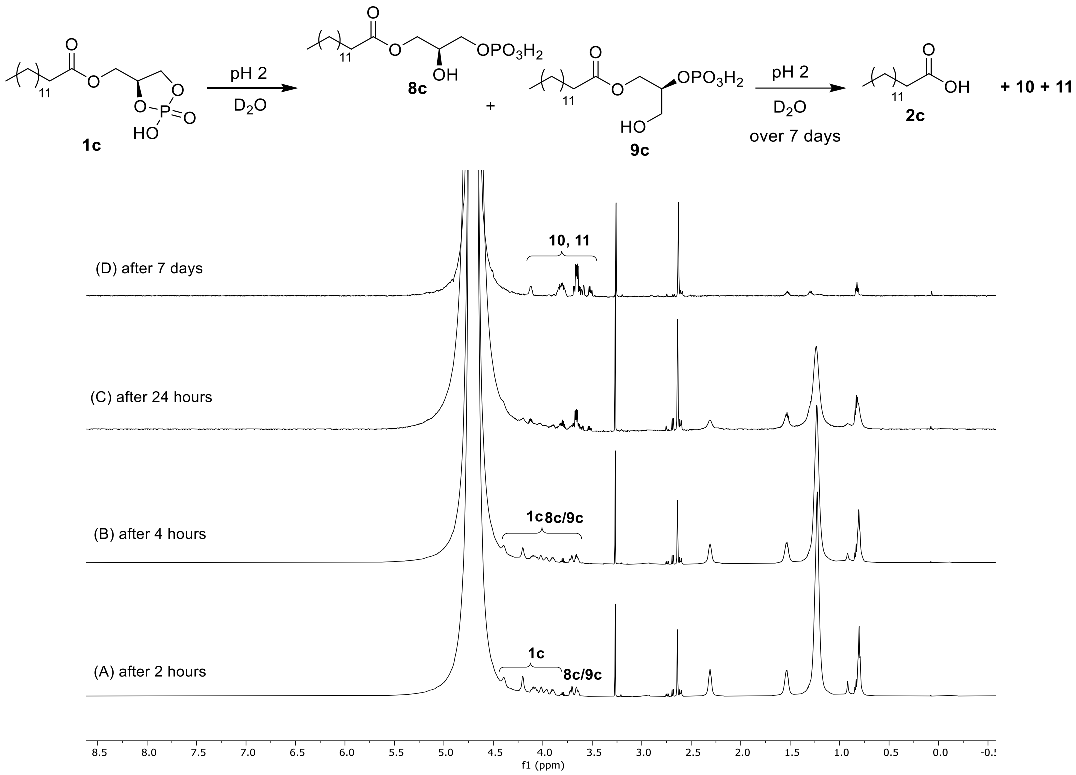


Figure S110. Stability study of 40 mM of cPA 14:0 **1c**, in D_2O at pH 2.0.

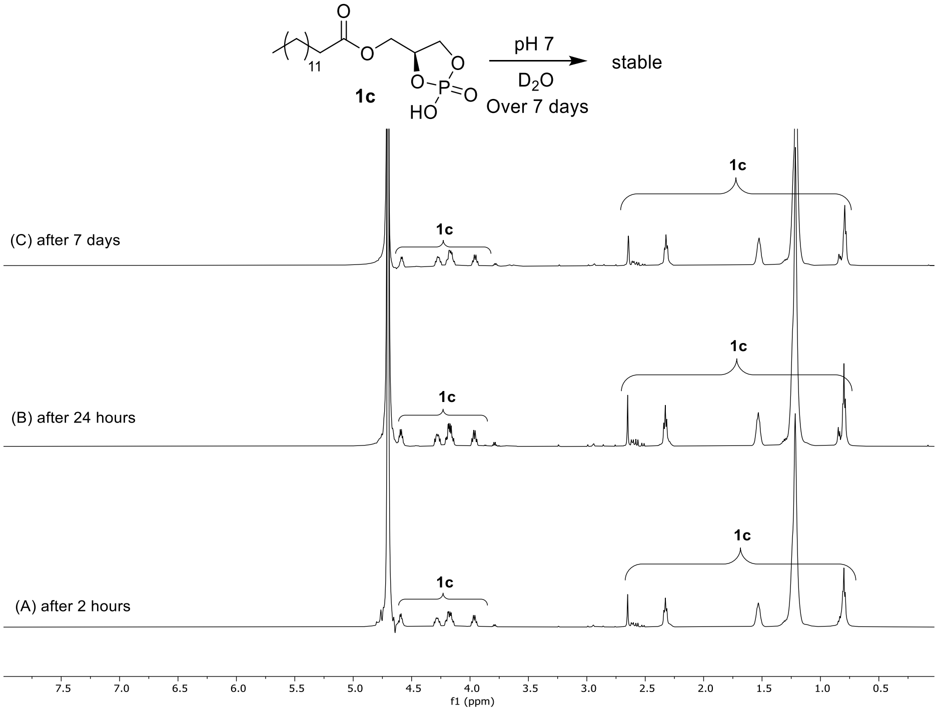


Figure S111. ^1H NMR of 40 mM of cPA 14:0 **1c**, in D_2O at pH 7.0.

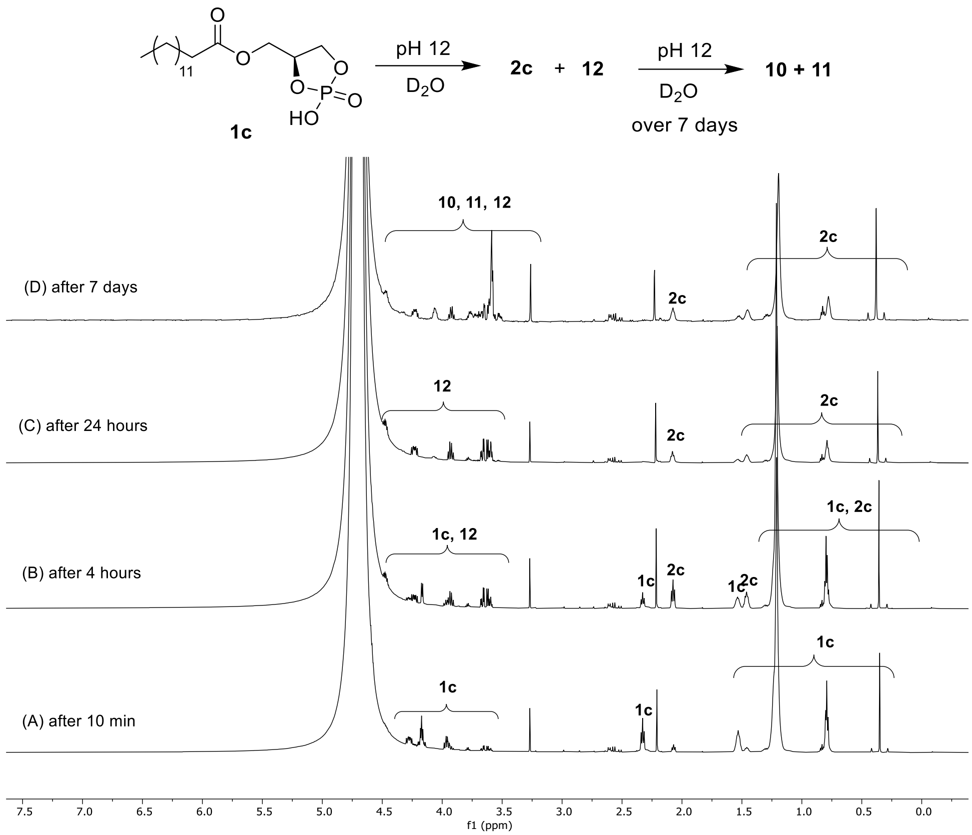


Figure S112. Stability study of 40 mM of cPA 14:0 **1c**, in D_2O at pH 12.0 by ^1H NMR.

Stability studies of 20 mM of CPAs at pH 4-9 in 0.2M buffer

General procedure**:** cPA (**1a-1c, 1f**) was taken in buffer as given below such that the concentration of cPA is 40 mM. The resulting solution was vortexed for ~1 min, followed by sonication for ~1 min for the complete dissolution (cPA **1a, 1b** and **1f** were clear solutions, cPA, **1c** was partially soluble). ^{31}P NMR of the solution was measured at different time intervals.

Table S2.

Buffer	pH	Buffer concentration	CPA concentration
Sodium acetate	4.0	0.2 M	20 mM
Sodium phosphate	6.0	0.2 M	20 mM
Phosphate-Buffered Saline (PBS)	7.2	1X	20 mM
Sodium phosphate	8.0	0.2 M	20 mM
Sodium bicarbonate	9.0	0.2 M	20 mM

% Carboxylate ester hydrolysis was based on the relative ^{31}P (^{1}H-decoupled) NMR integration of **12** versus **1b**.

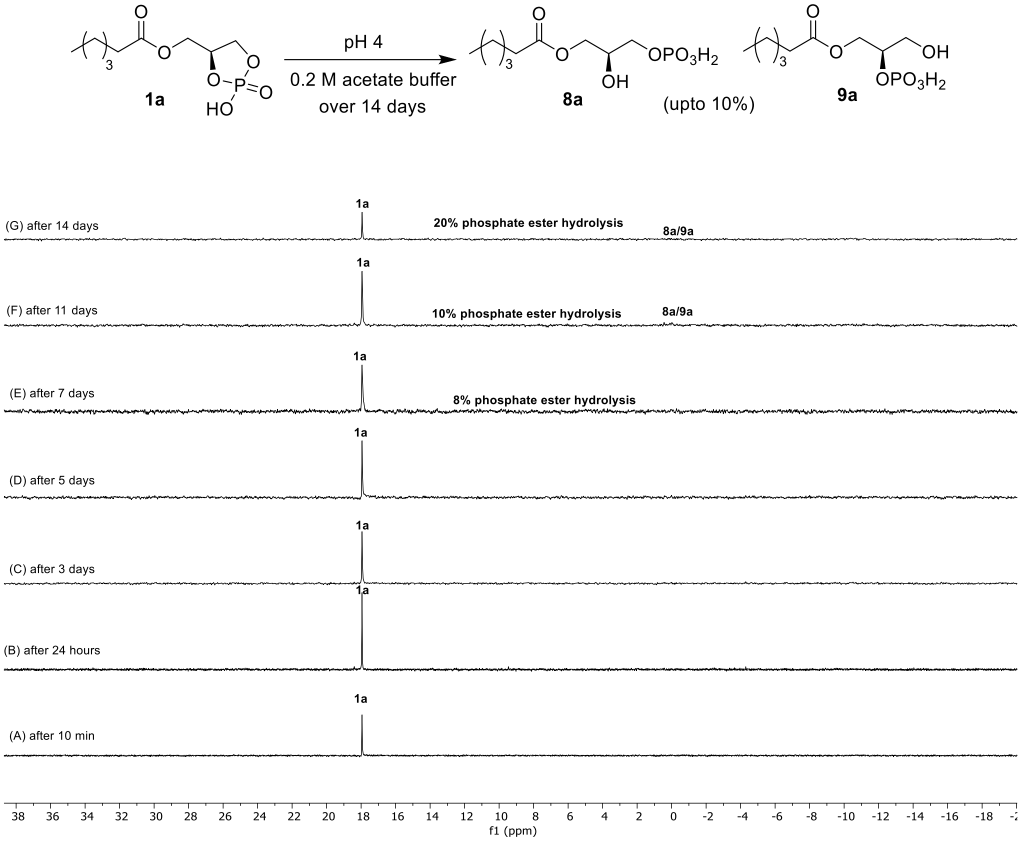


Figure S113. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 20 mM of cPA 6:0 **1a**, in 0.2 M acetate buffer at pH 4.

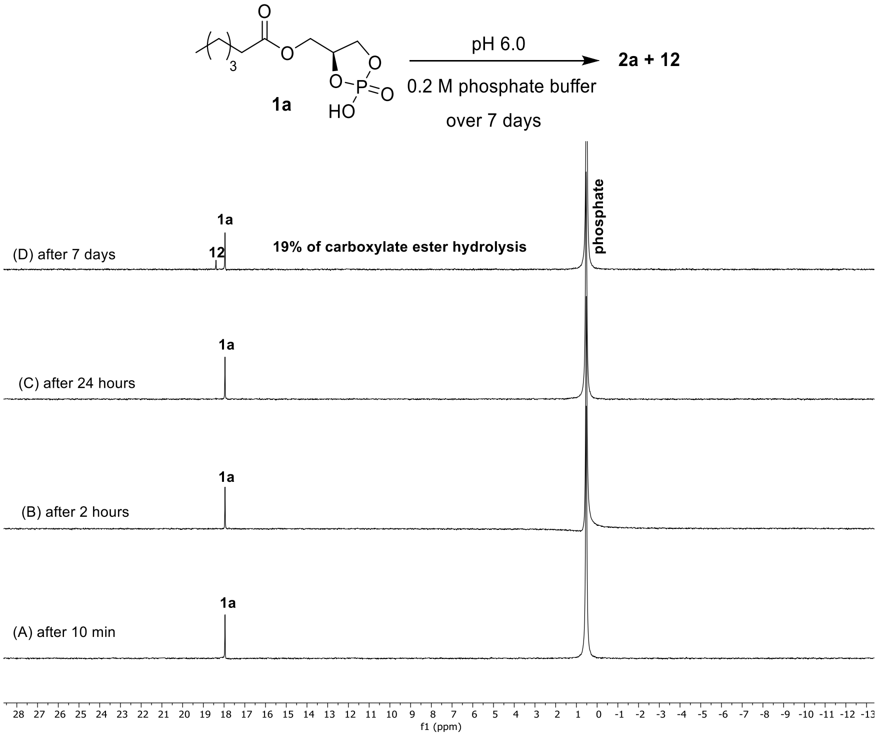


Figure S114. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 20 mM of cPA 6:0 **1a**, in 0.2 M phosphate buffer at pH 6.

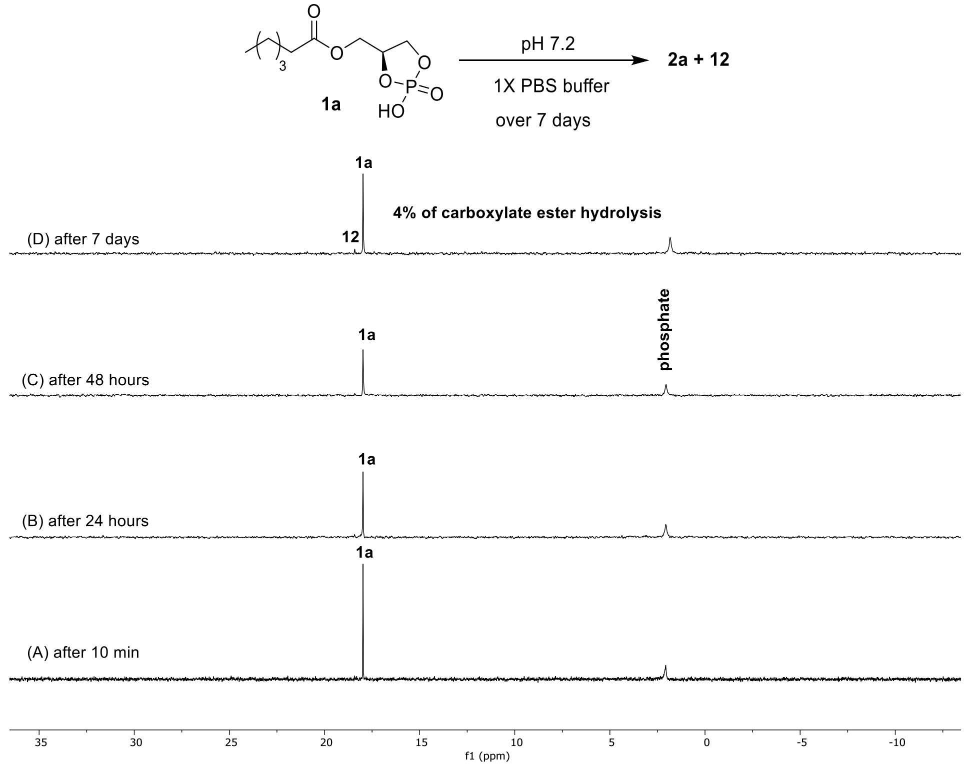


Figure S115. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 20 mM of CPA 6:0 **1a**, in 1X PBS buffer at pH 7.2.

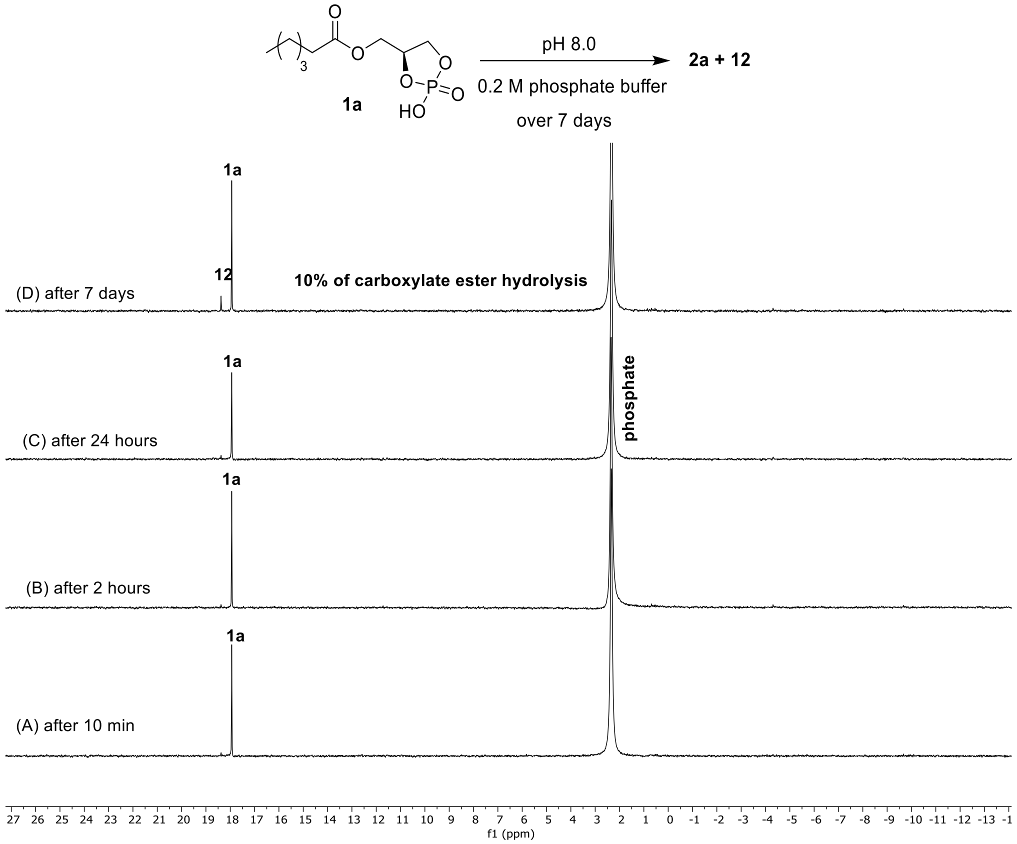
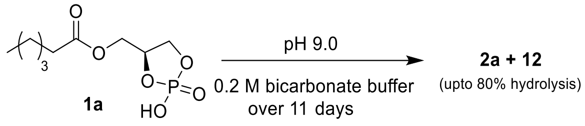


Figure S116. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 20 mM of cPA 6:0 **1a**, in 0.2 M phosphate buffer at pH 8.



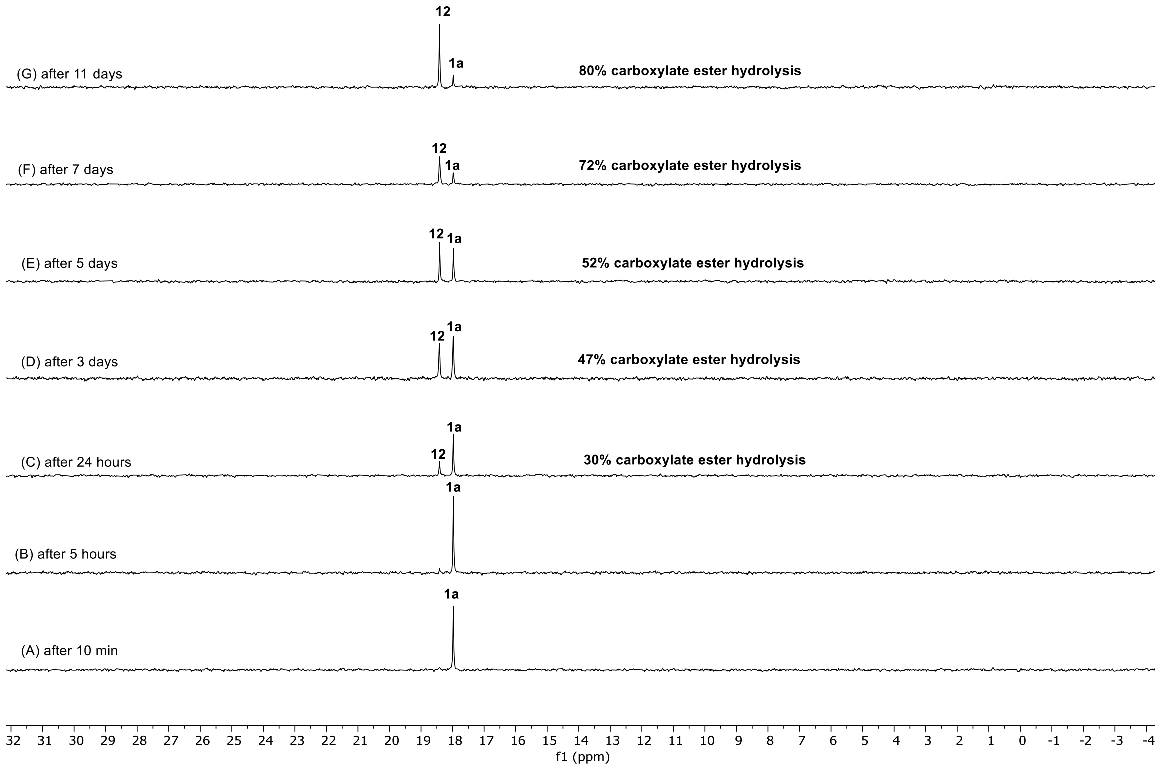
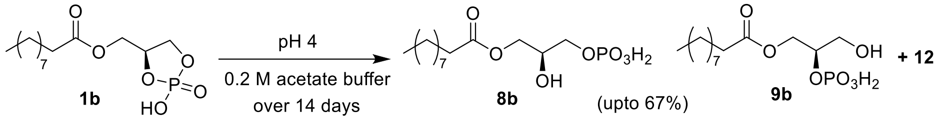


Figure S117. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 20 mM of cPA 6:0 **1a**, in 0.2 M bicarbonate buffer at pH 9.



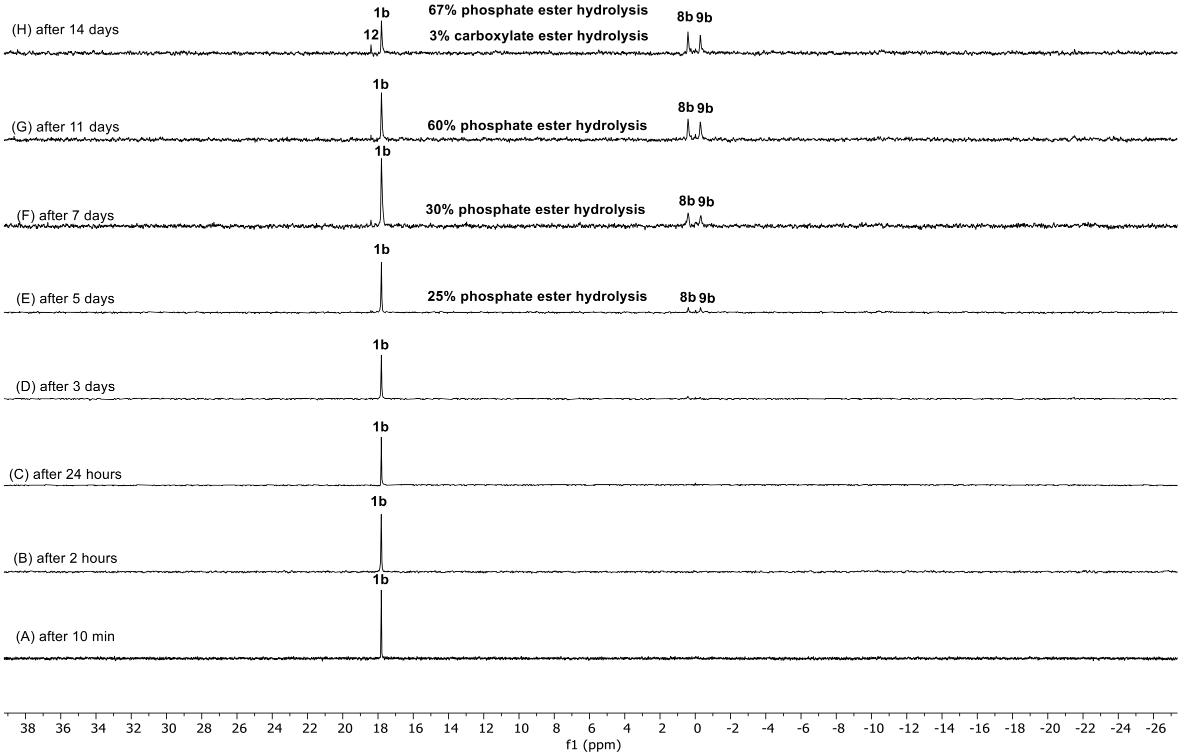


Figure S118. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 20 mM of cPA 10:0 **1b**, in 0.2 M acetate buffer at pH 4.

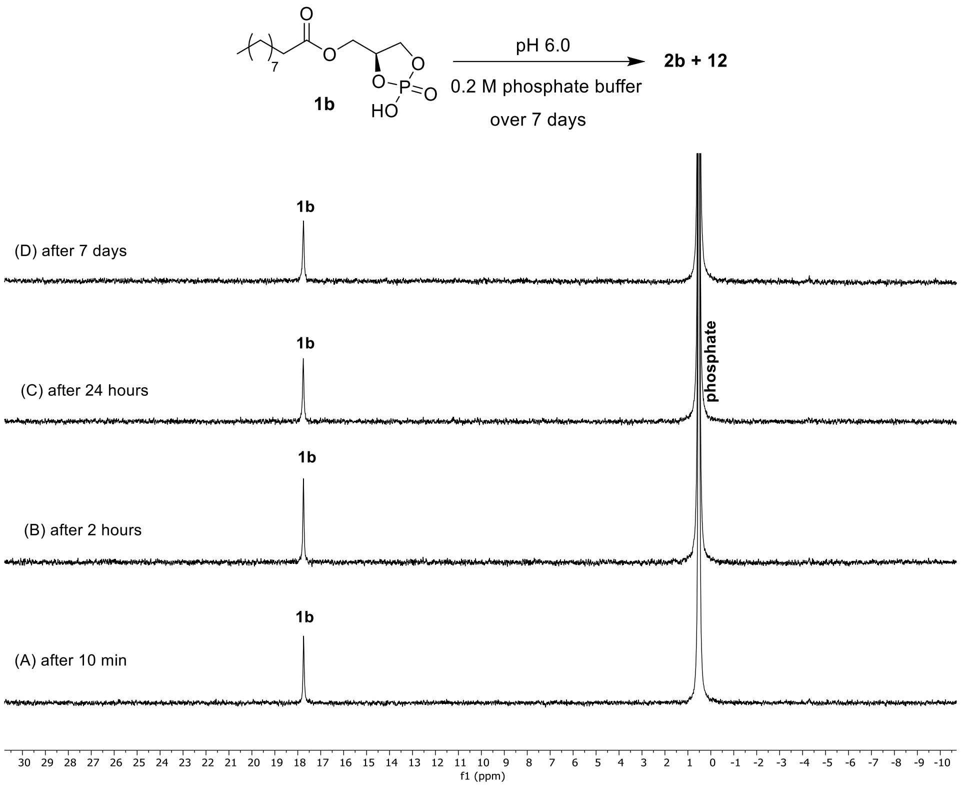


Figure S119. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 20 mM of cPA 10:0 **1b**, in 0.2 M phosphate buffer at pH 6.

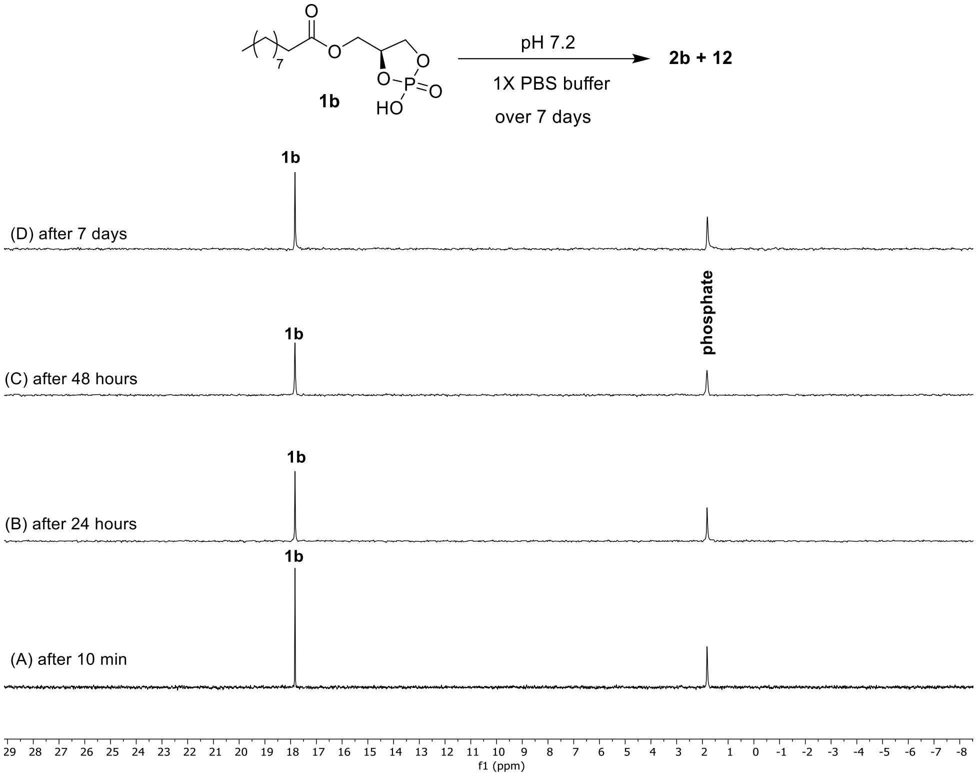


Figure S120. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 20 mM of cPA 10:0 **1b**, in 1X PBS buffer at pH 7.2.

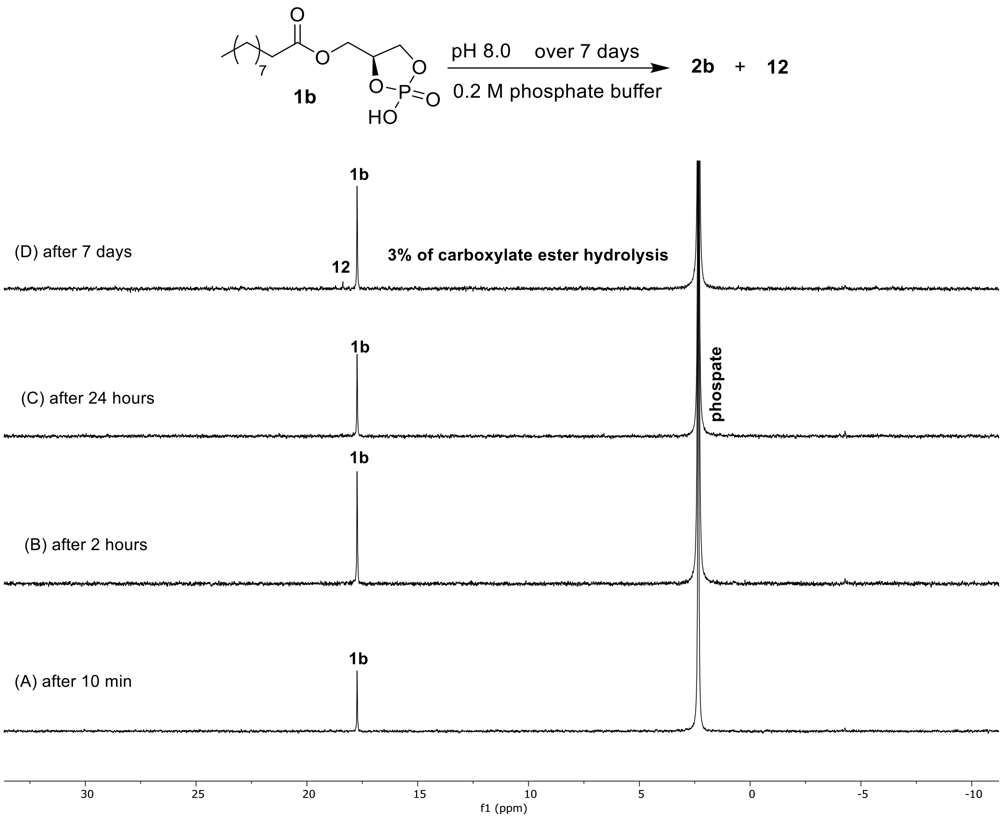
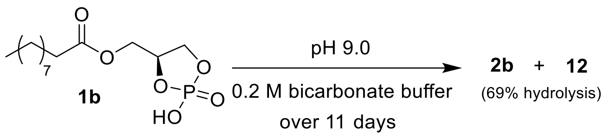


Figure S121. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 20 mM of cPA 10:0 **1b**, in 0.2 M phosphate buffer, pH 8.



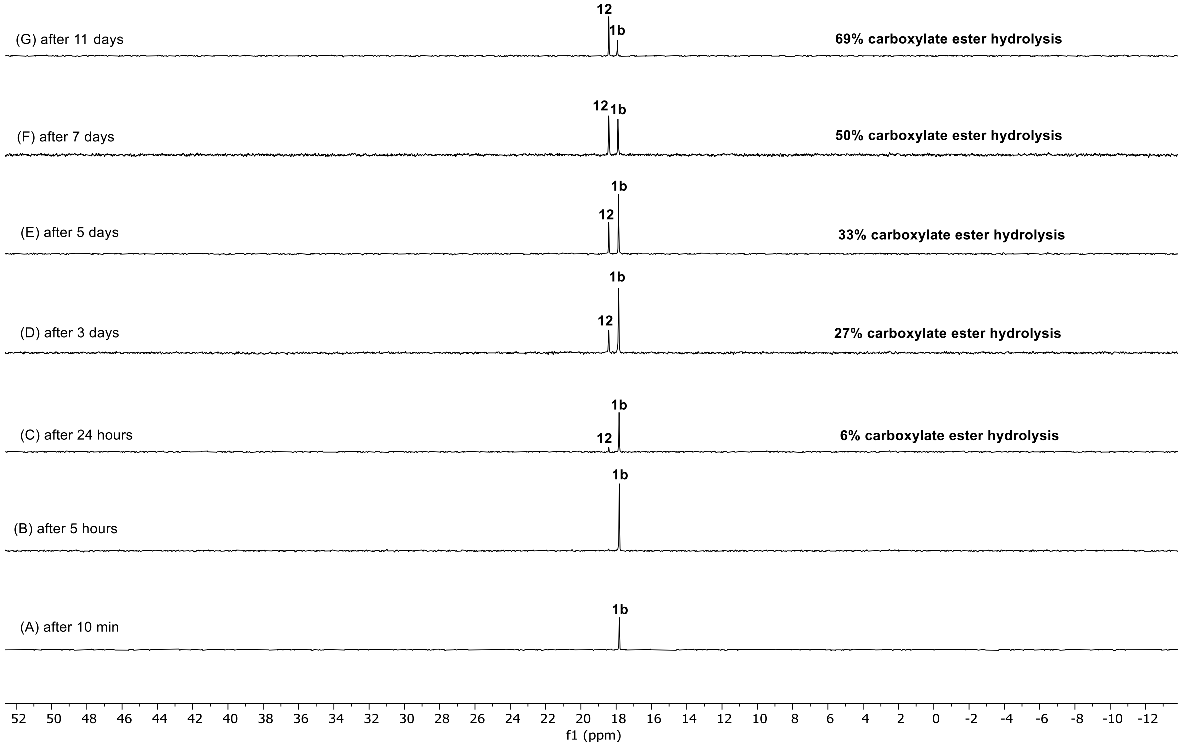


Figure S122. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 20 mM of cPA 10:0 **1b**, in 0.2 M bicarbonate buffer, pH 9.

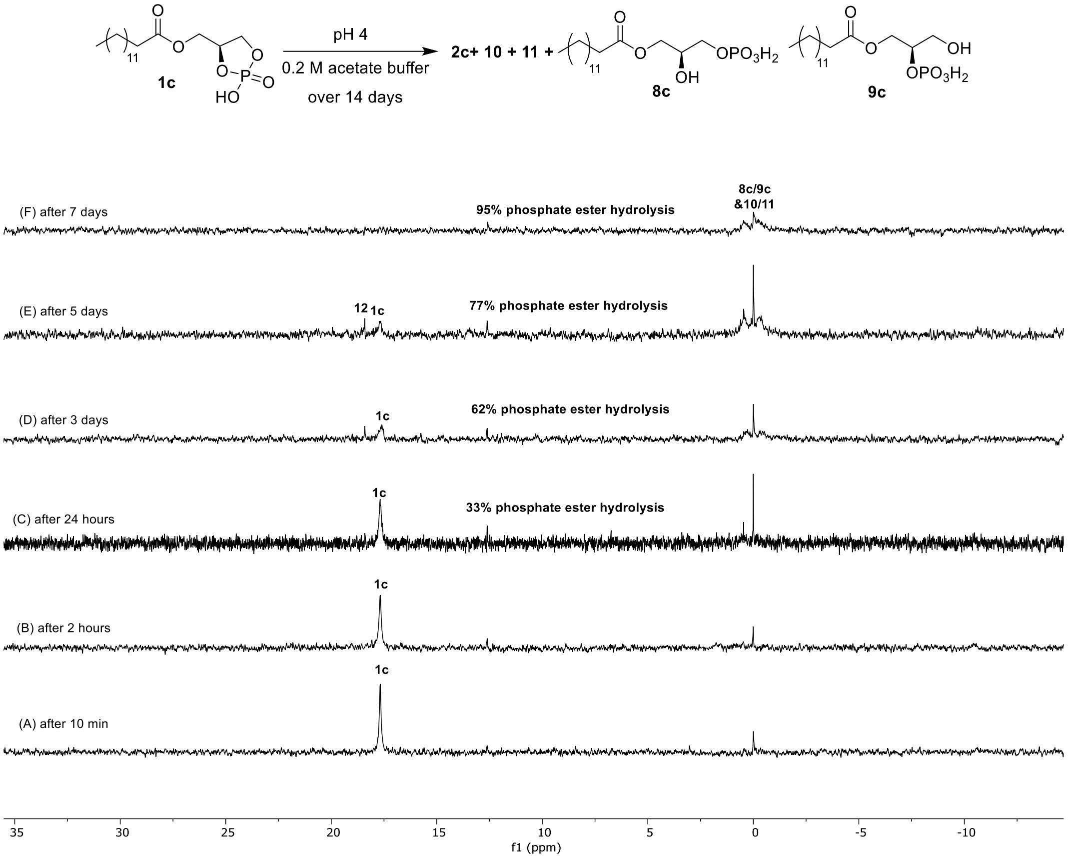


Figure S123. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 20 mM of cPA 14:0 **1c**, in 0.2 M acetate buffer at pH 4.

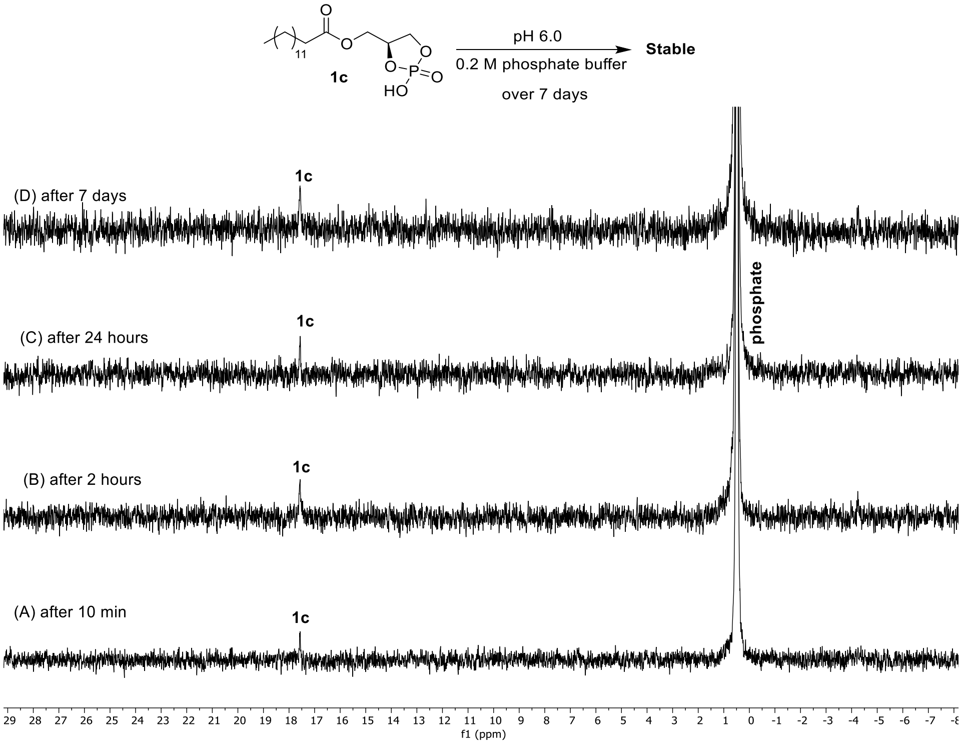


Figure S124. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 20 mM of cPA 14:0 **1c**, in 0.2 M phosphate buffer at pH 6.

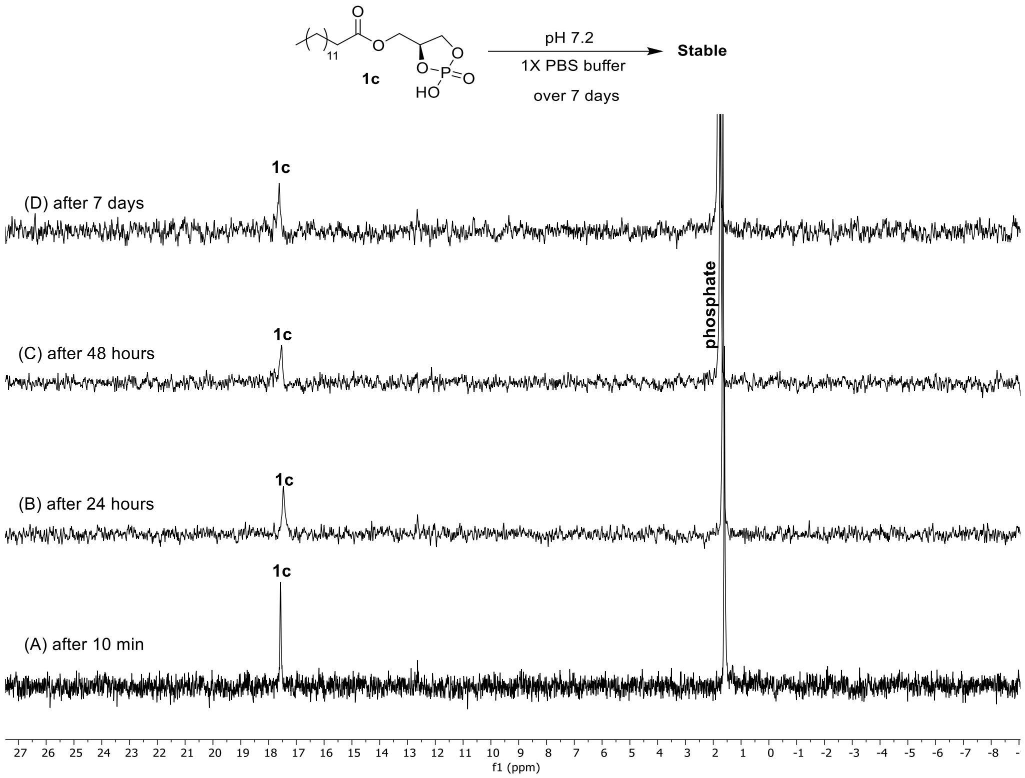


Figure S125. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 20 mM of cPA 14:0 **1c**, in 1X PBS buffer at pH 7.2.

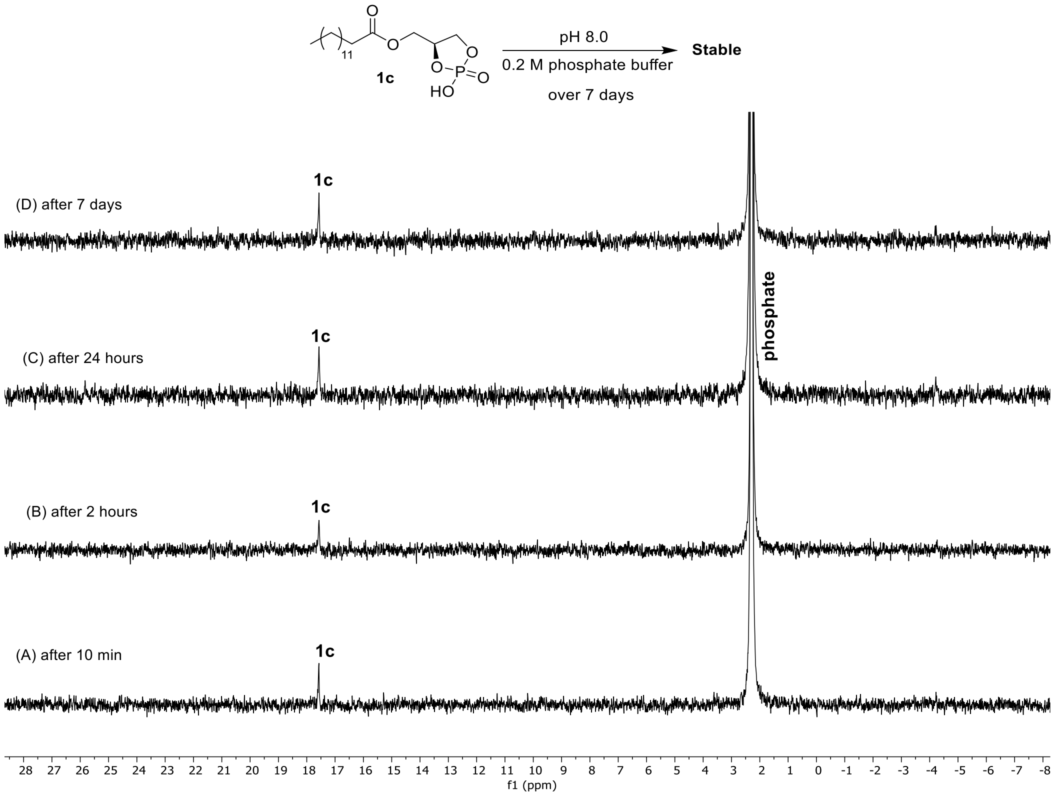
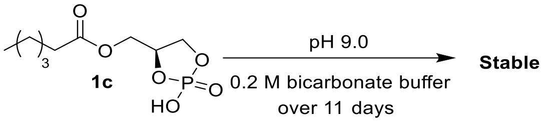


Figure S126. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 20 mM of cPA 14:0 **1c**, in 0.2 M phosphate buffer at pH 8.



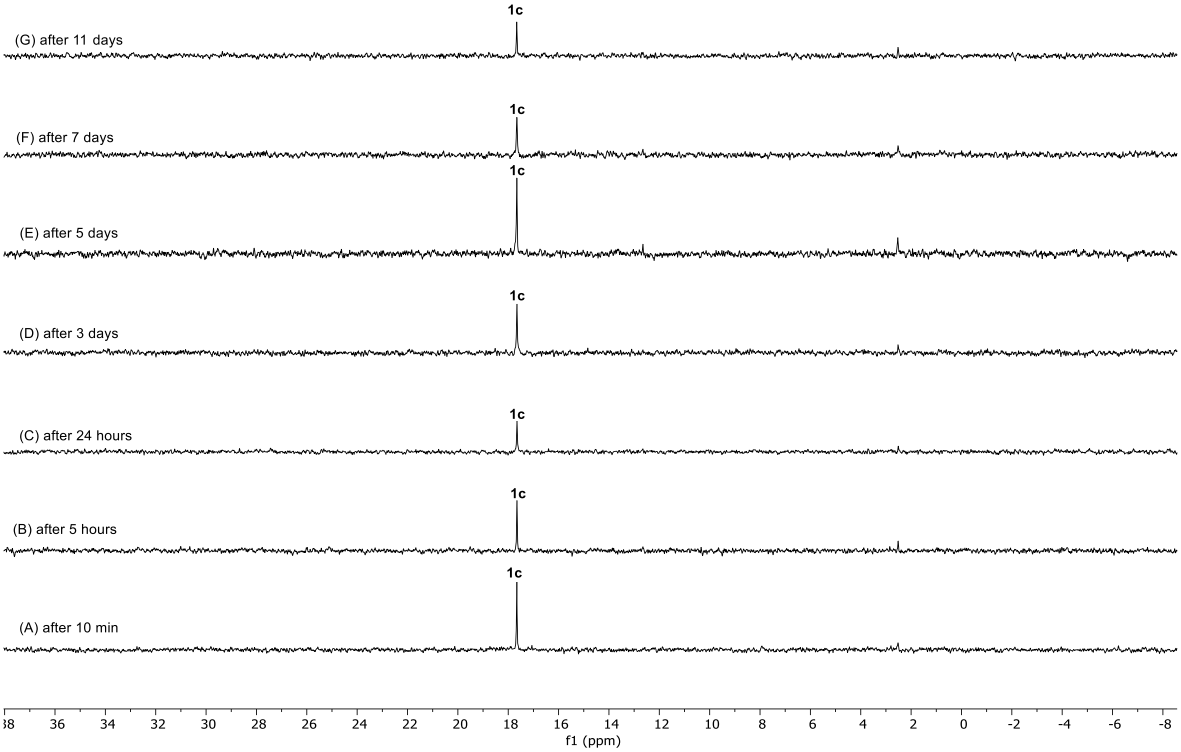


Figure S127. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 20 mM of cPA 14:0 **1c**, in 0.2 M bicarbonate buffer at pH 9.

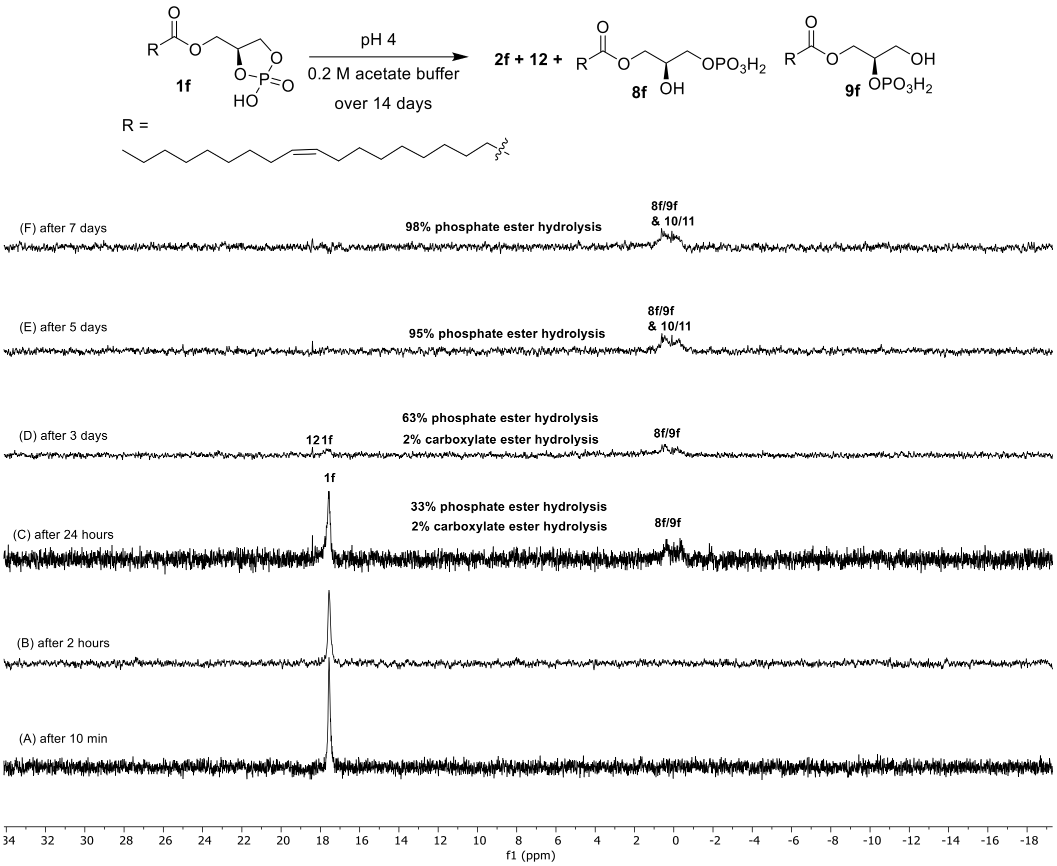


Figure S128. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 20 mM of cPA 18:1 **1f**, in 0.2 M acetate buffer at pH 4.

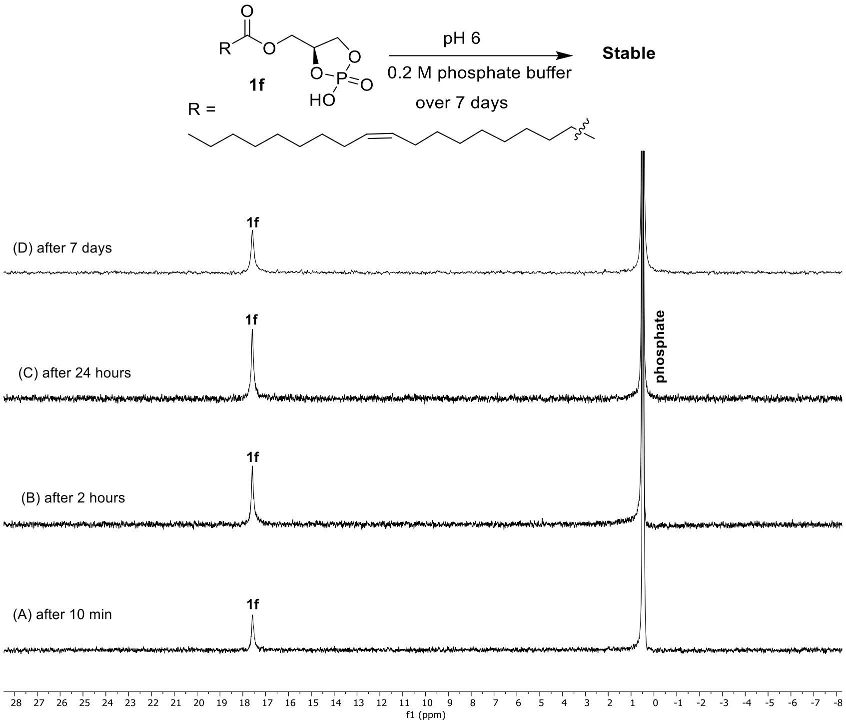


Figure S129. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 20 mM of cPA 18:1 **1f**, in 0.2 M phosphate buffer at pH 6.

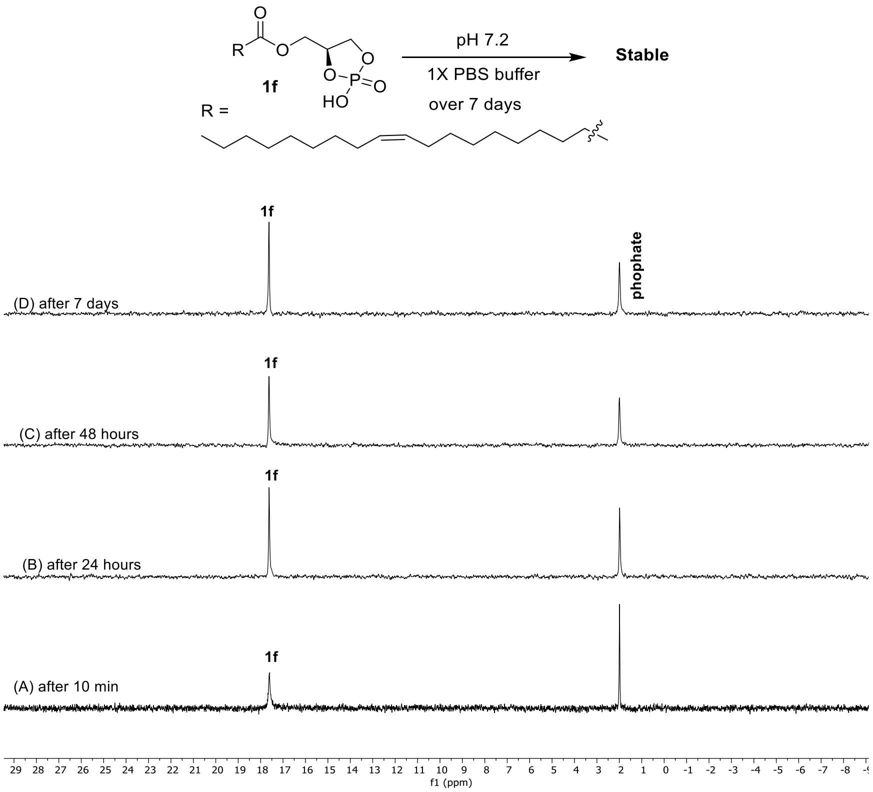


Figure S130. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 20 mM of cPA 18:1 **1f**, in 1X PBS buffer at pH 7.2.

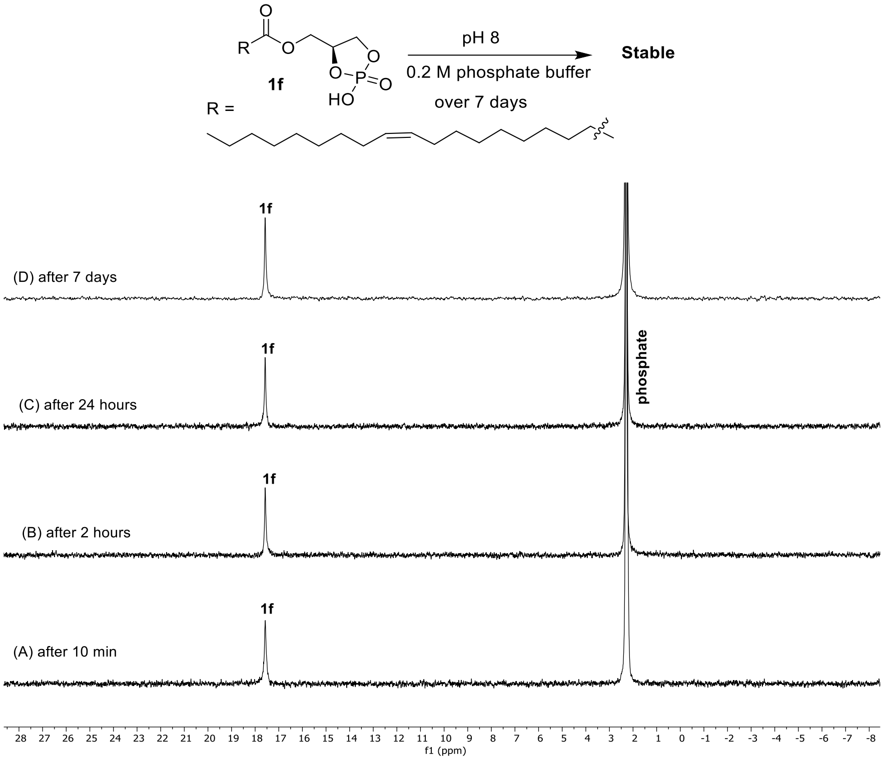


Figure S131. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 20 mM of cPA 18:1 **1f**, in 0.2 M phosphate buffer at pH 8.

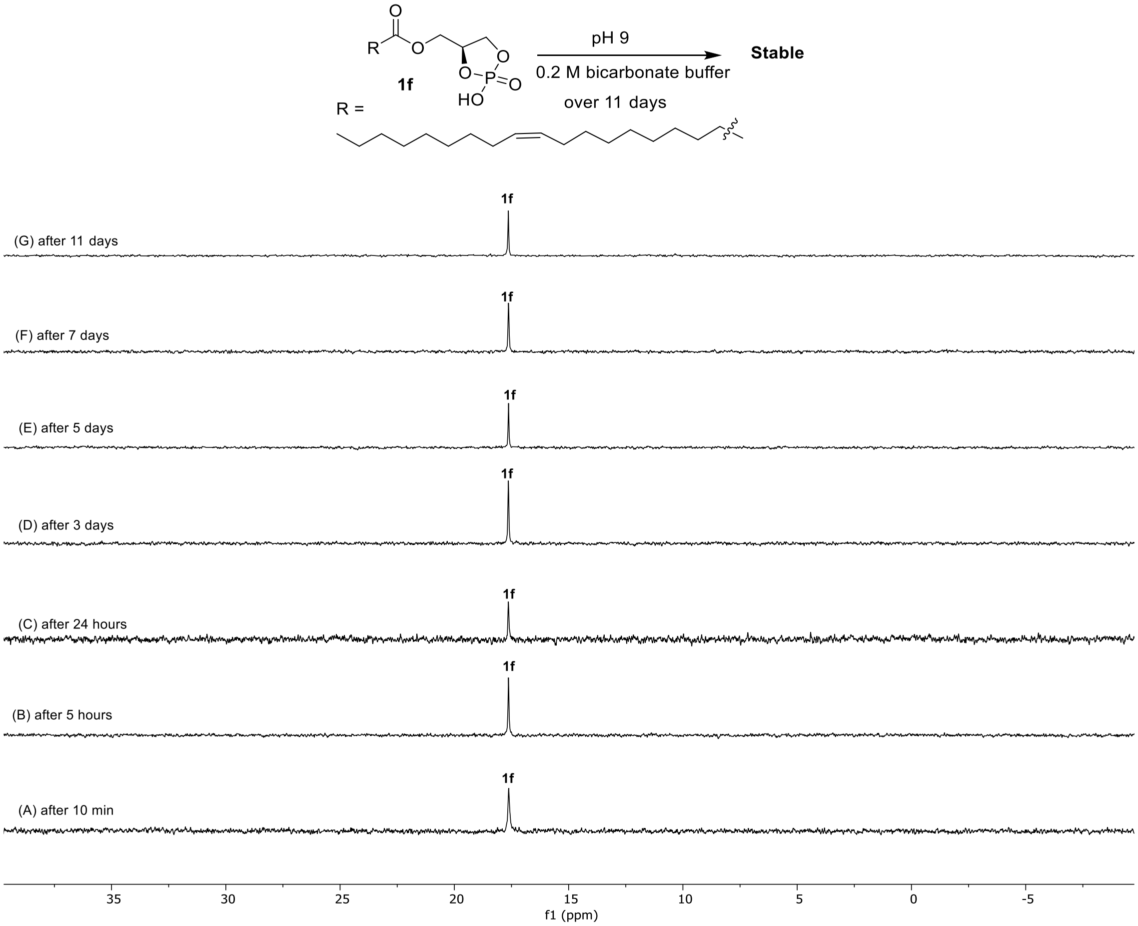


Figure S132. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 20 mM of cPA 18:1 **1f**, in 0.2 M bicarbonate buffer at pH 9.

Summary of Stability studies of 20 mM of cPAs at various pHs (4-9) in 0.2M buffer

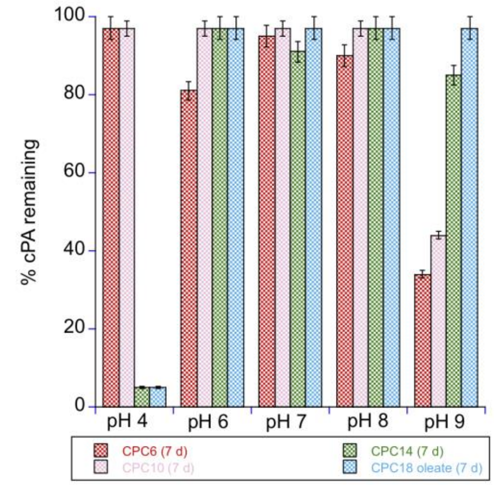


Figure S133. Summary of hydrolytic stability of 20 mM of cPAs at various pHs (4-9) in 0.2M buffer based on data from the ^{31}P-NMR monitoring from Figs. 113-132.

Stability studies of 40 mM cyclic phosphatidic acids (cPAs) over range of pH 4-9 in 0.2M buffer (for buffers see Table S2)

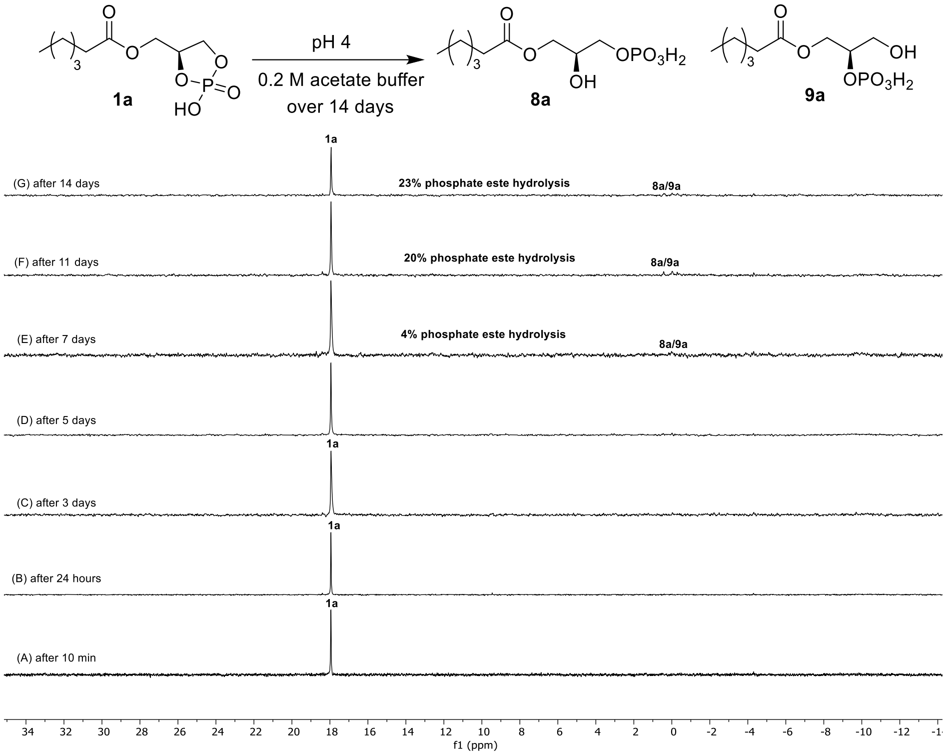


Figure S134. ^{31}P NMR (^{1}H-coupled for A, ^{1}H-decoupled for B and C) of 40 mM of cPA 6:0 **1a**, in 0.2 M acetate buffer at pH 4.0.

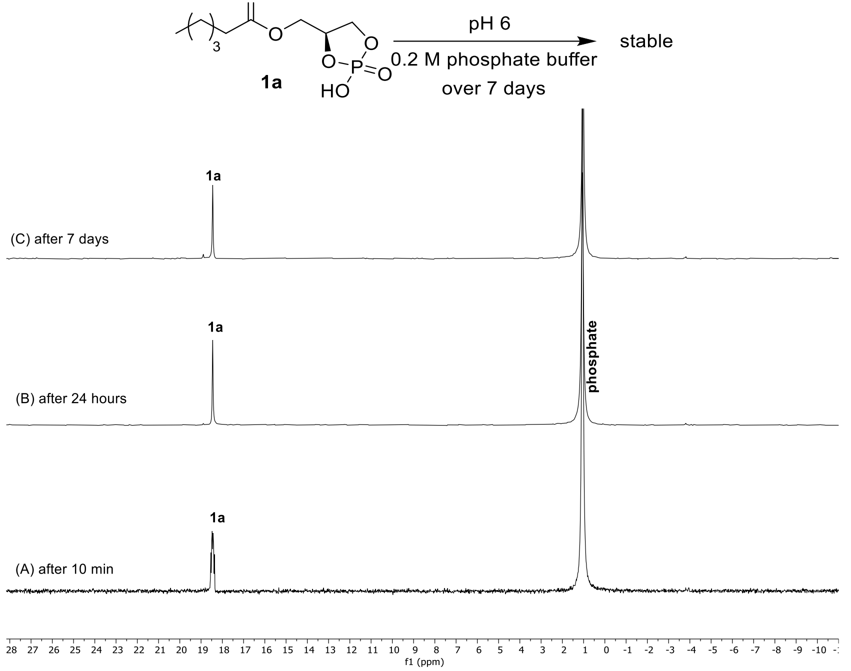


Figure S135. ^{31}P NMR (^{1}H-coupled for A, ^{1}H-decoupled for B and C) of 40 mM of cPAs 6:0 **1a**, in 0.2 M phosphate buffer at pH 6.0.

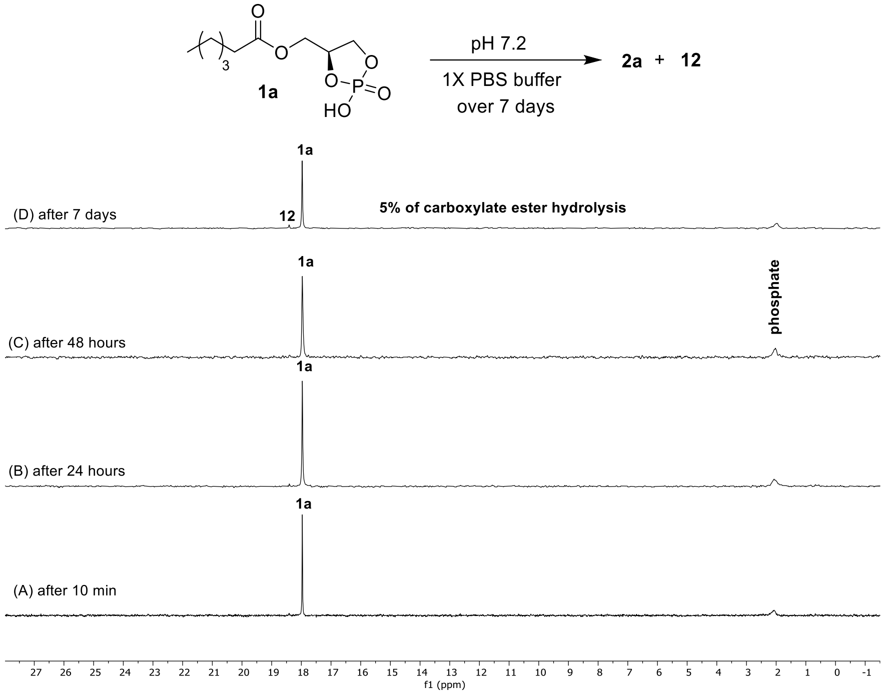


Figure S136. ^{31}P NMR (^{1}H-decoupled) of 40 mM of cPA 6:0 **1a**, in 1X PBS buffer at pH 7.2.

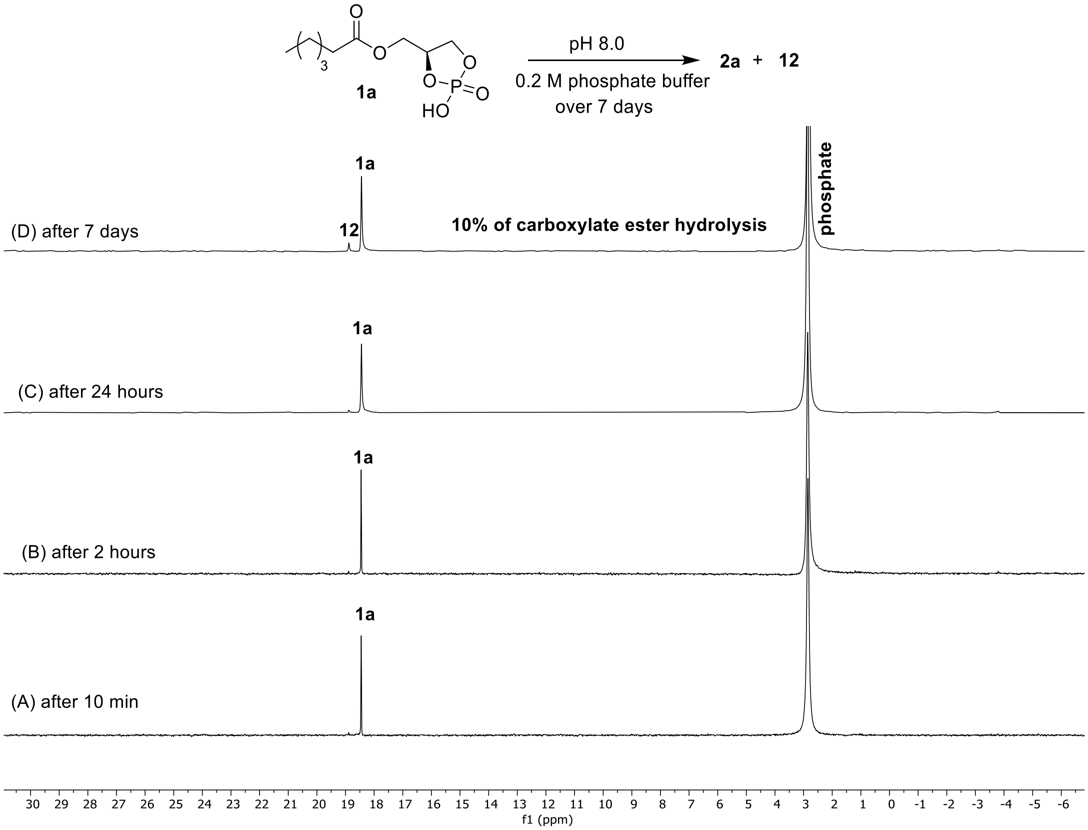


Figure S137. ^{31}P NMR (^{1}H-coupled for A, ^{1}H-decoupled for B and C) of 40 mM of cPA 6:0 **1a**, in 0.2 M phosphate buffer at pH 8.0. % Carboxylate ester hydrolysis is based on the relative ^{31}P (^{1}H-decoupled) NMR integration of **12** versus **1a**.

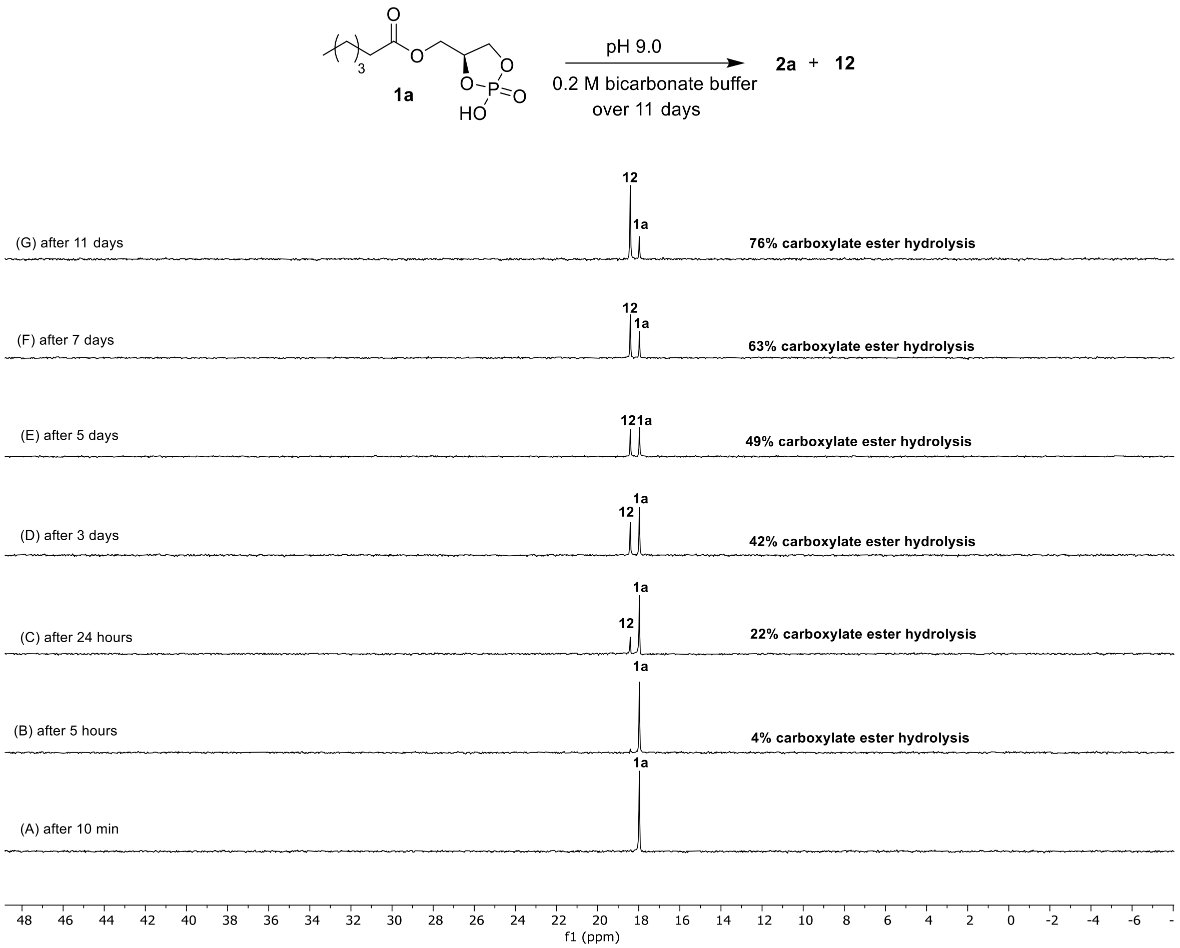


Figure S138. ^{31}P NMR (^{1}H-decoupled) of 40 mM of cPA 6:0 **1a**, in 0.2 M bicarbonate buffer at pH 9.0. (A) after 10 min.

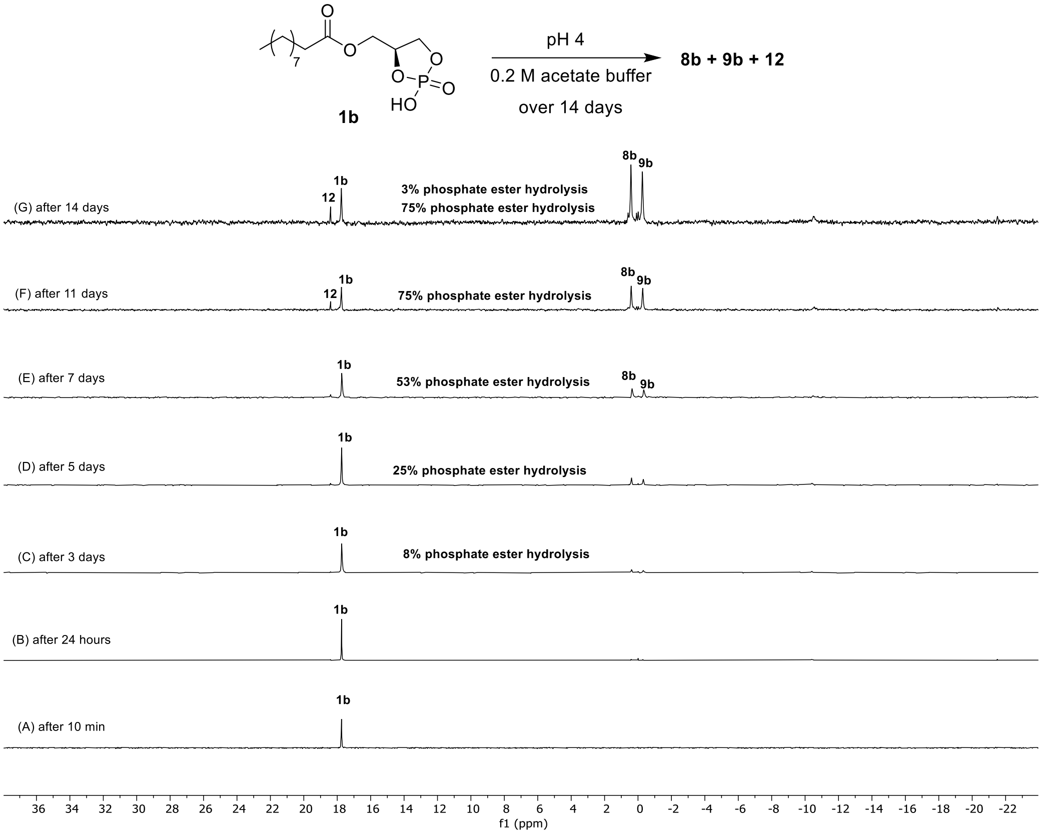


Figure S139. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 40 mM of cPA 10:0 **1b**, in 0.2 M acetate buffer at pH 4.0.

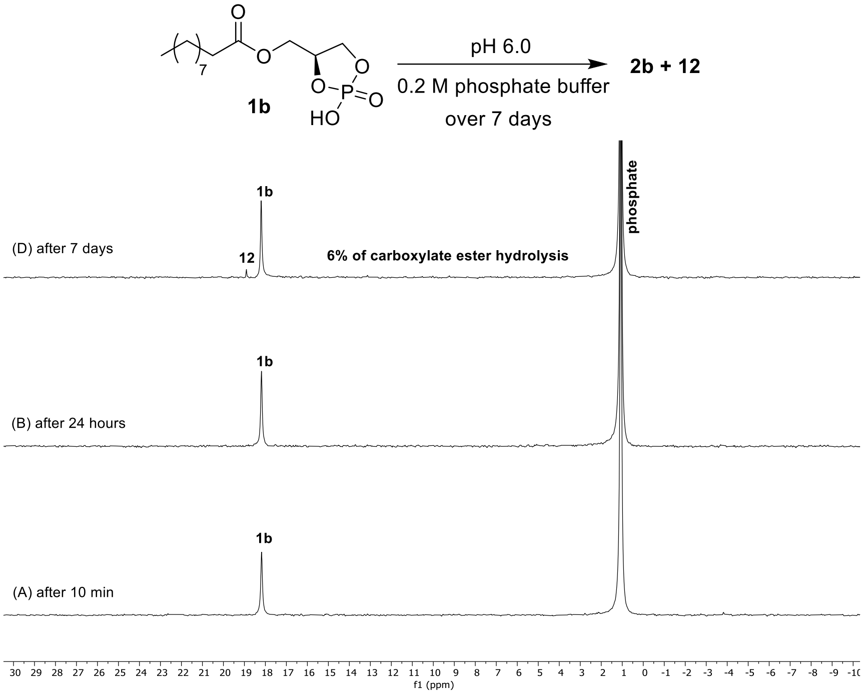


Figure S140. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 40 mM of cPA 10:0 **1b**, in 0.2 M phosphate buffer at pH 6.0.

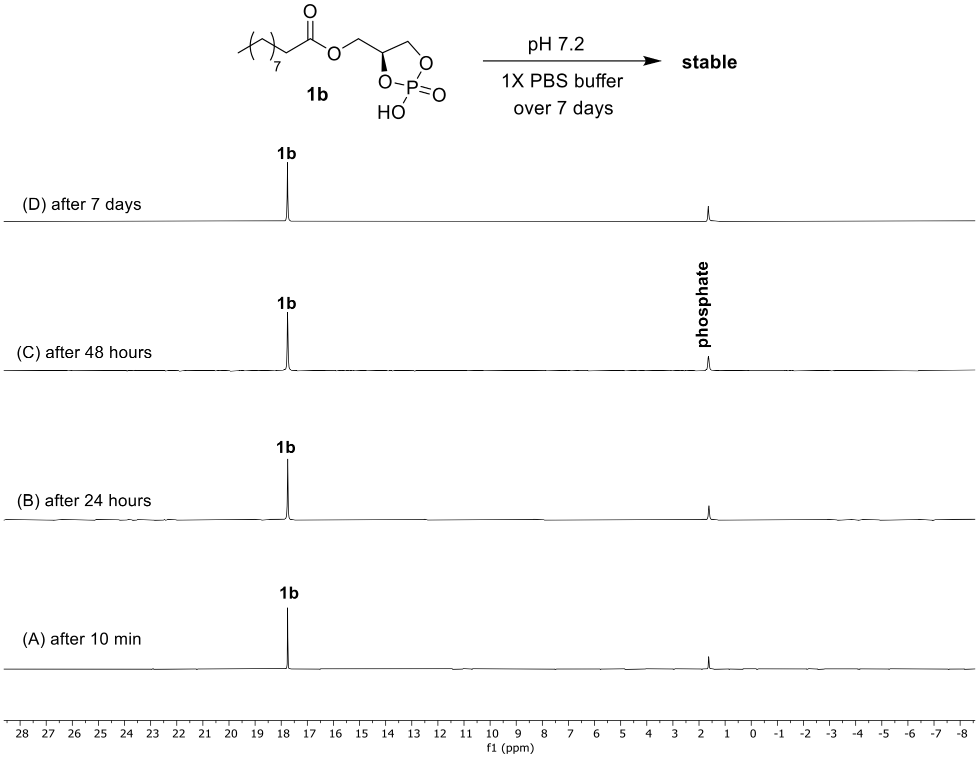


Figure S141. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 40 mM of cPA 10:0 **1b**, in 1X PBS buffer at pH 7.2.

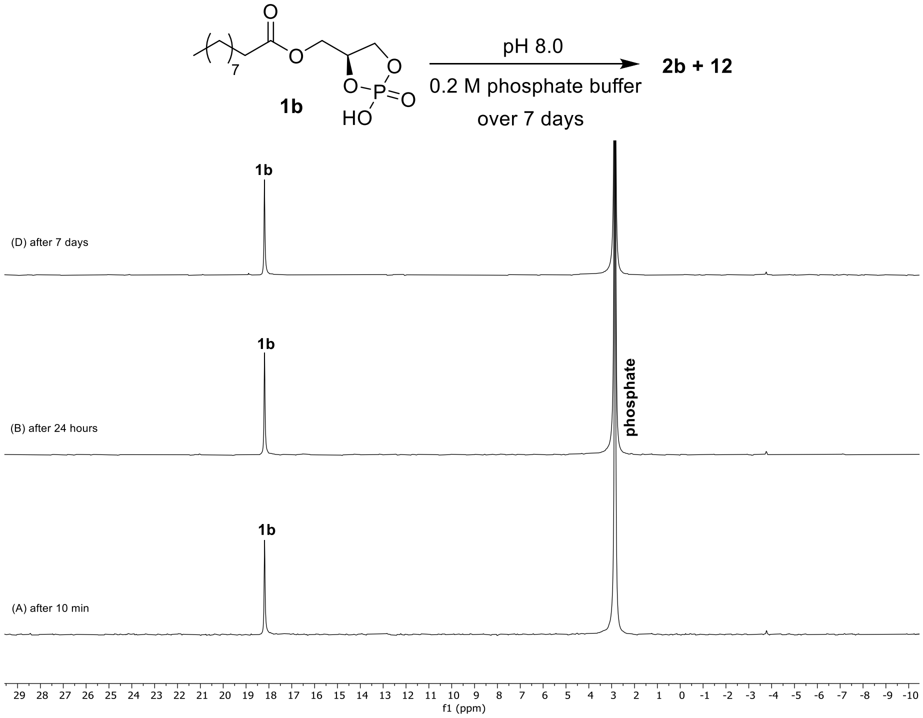


Figure S142. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 40 mM of cPA 10:0 **1b**, in 0.2 M phosphate buffer at pH 8.0.

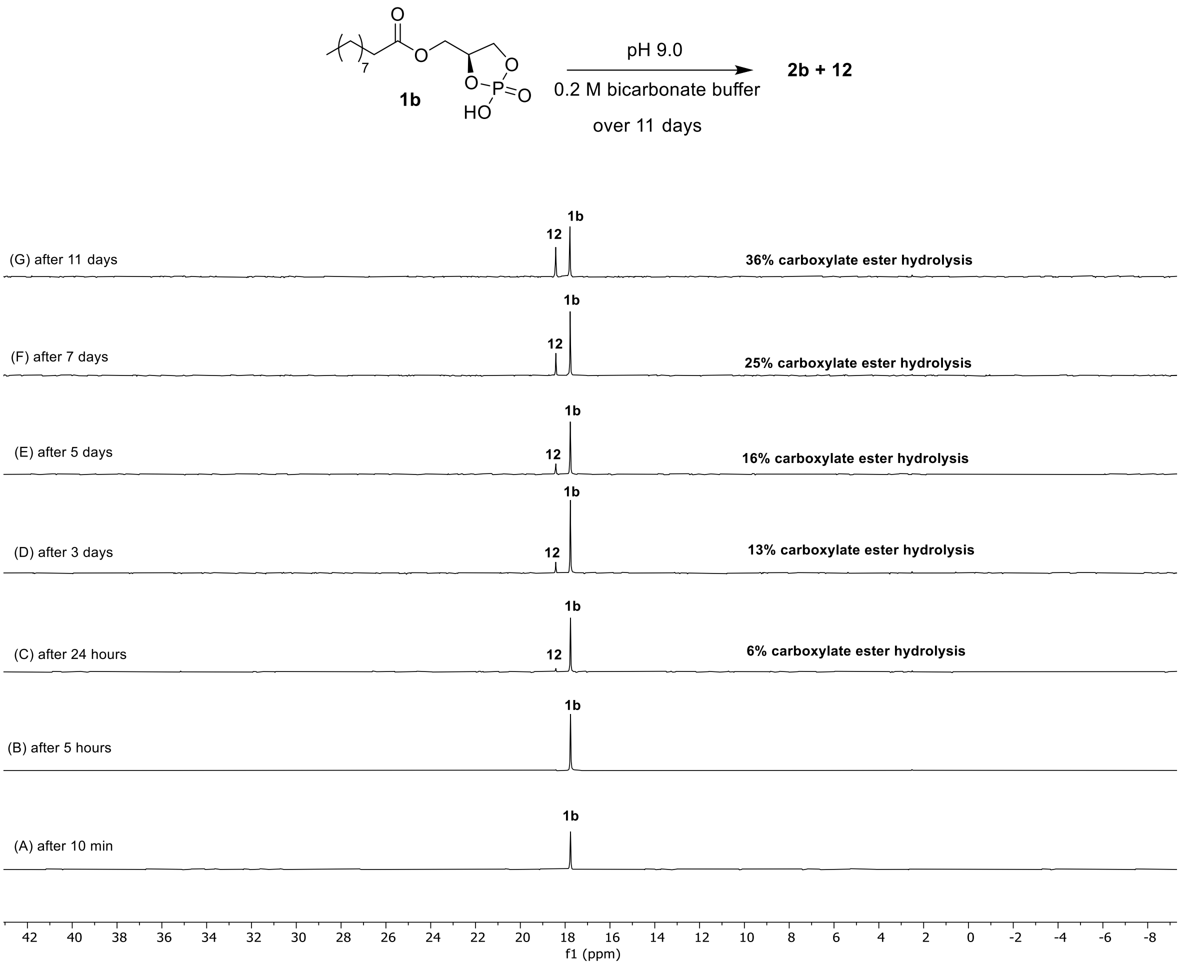


Figure S143. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 40 mM of cPA 10:0 **1b**, in 0.2 M bicarbonate buffer at pH 9.0.

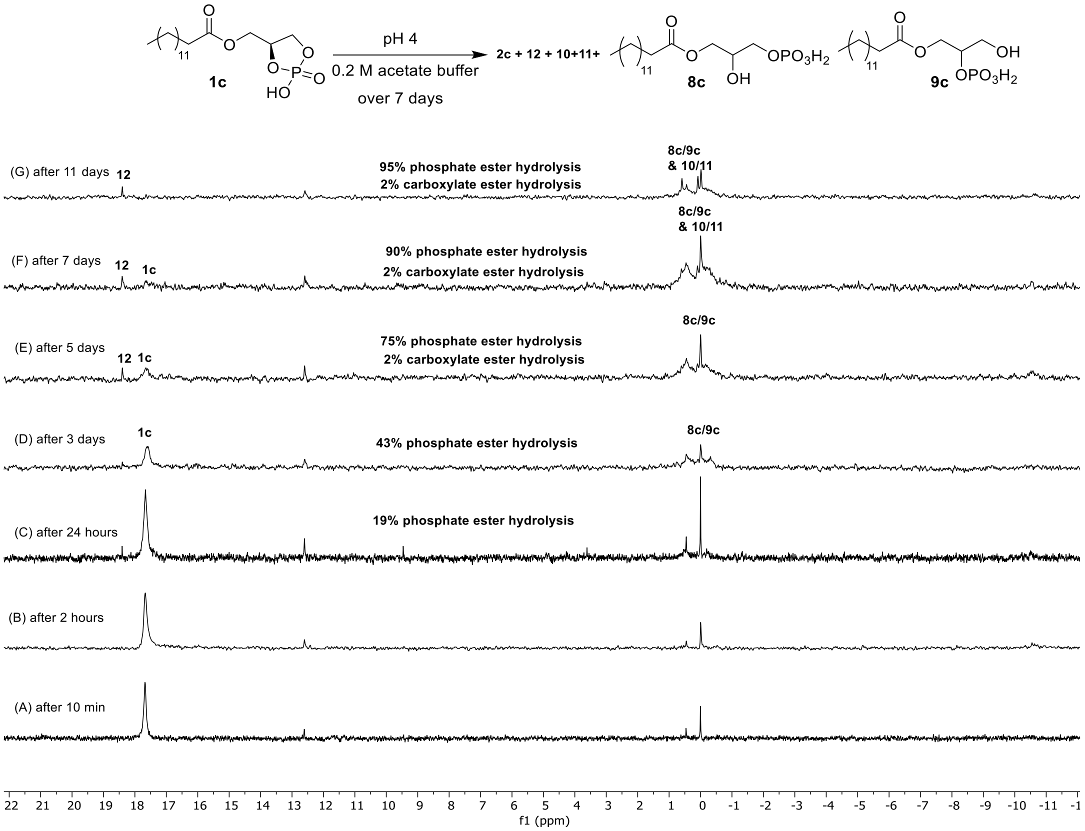


Figure S144. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 40 mM of cPA 14:0 **1c**, in 0.2 M acetate buffer at pH 4.0.

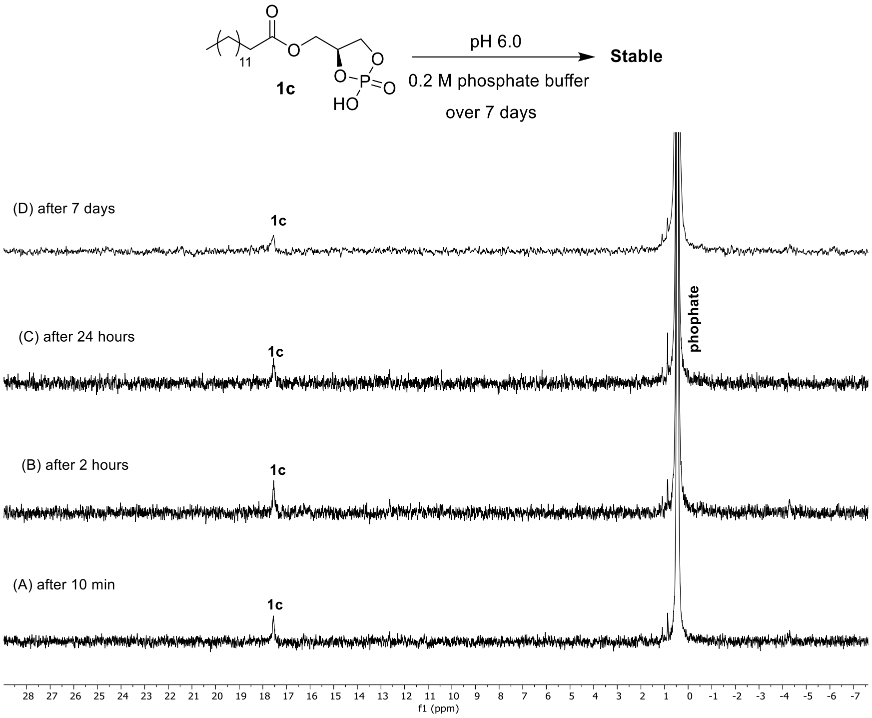


Figure S145. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 40 mM of cPA 14:0 **1c**, in 0.2 M phosphate buffer at pH 6.0.

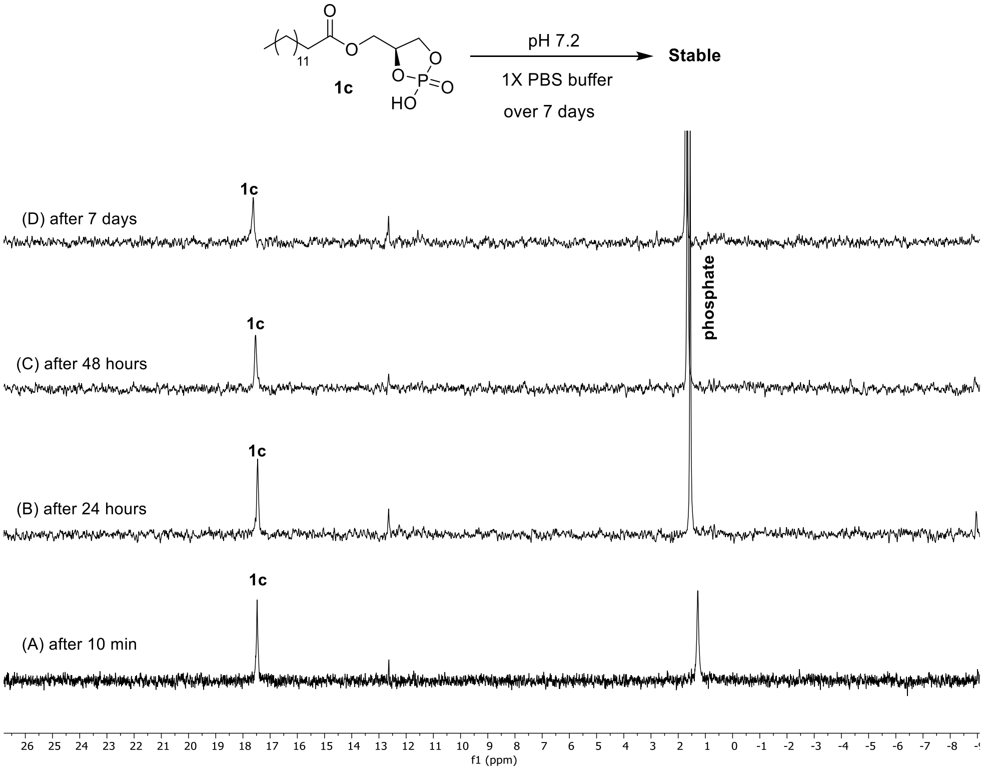


Figure S146. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 20 mM of cPA 14:0 **1c**, in 1X PBS buffer at pH 7.2.

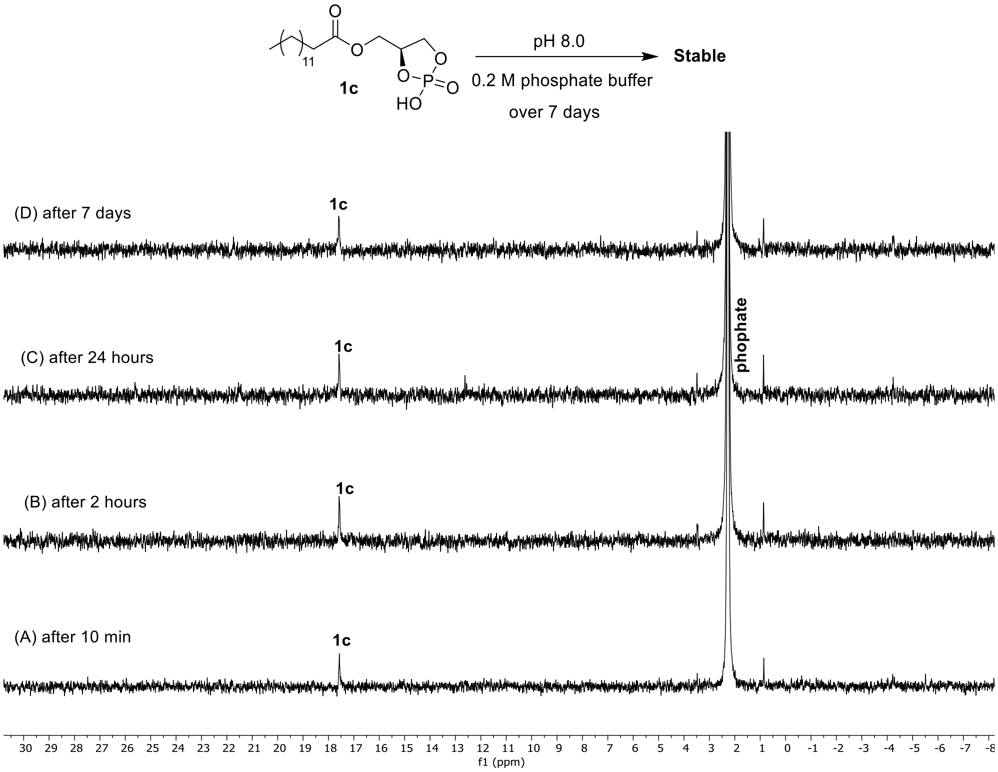


Figure S147. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 40 mM of cPA 14:0 **1c**, in 0.2 M phosphate buffer at pH 8.

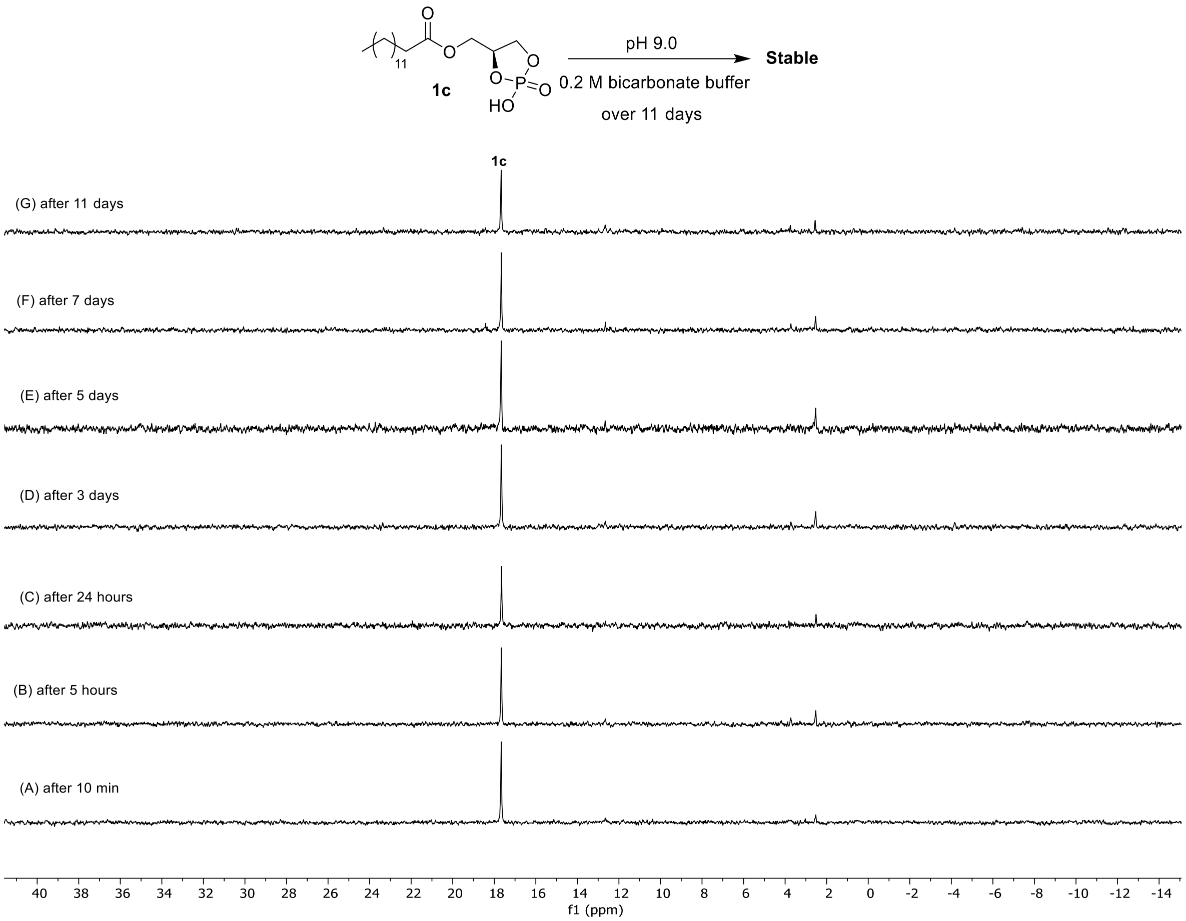


Figure S148. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 40 mM of cPA 14:0 **1c**, in 0.2 M bicarbonate buffer at pH 9.

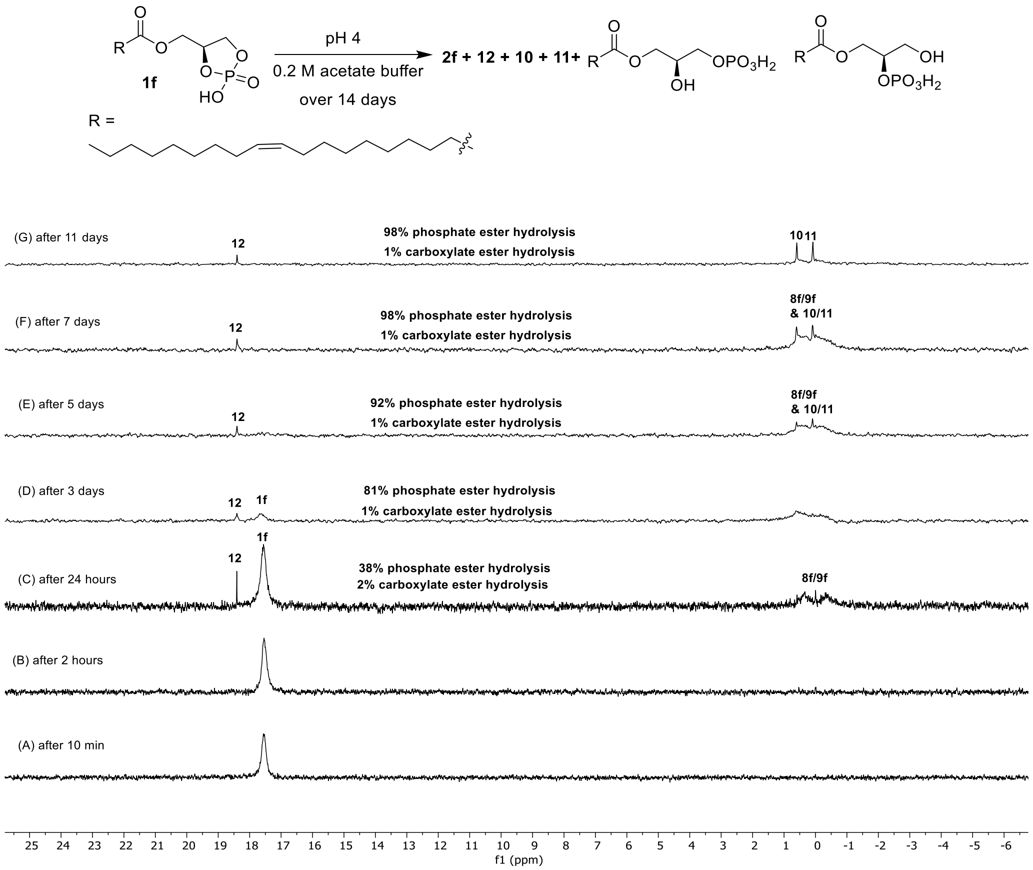


Figure S149. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 40 mM of cPA 18:1 **1f**, in 0.2M acetate buffer at pH 4.0.

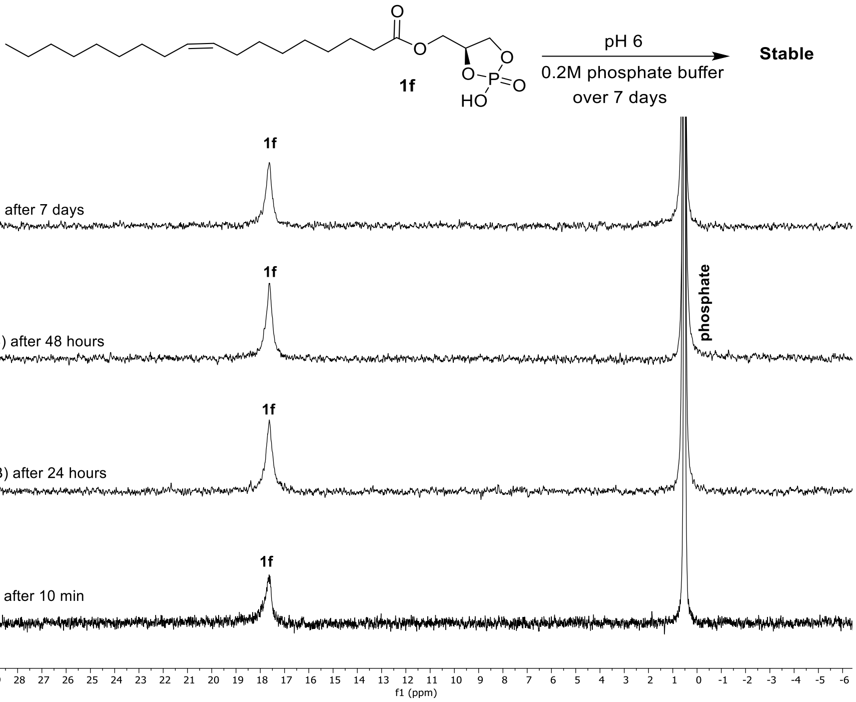


Figure S150. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 40 mM of cPA 18:1 **1f**, in 0.2M phosphate buffer at pH 6.0.

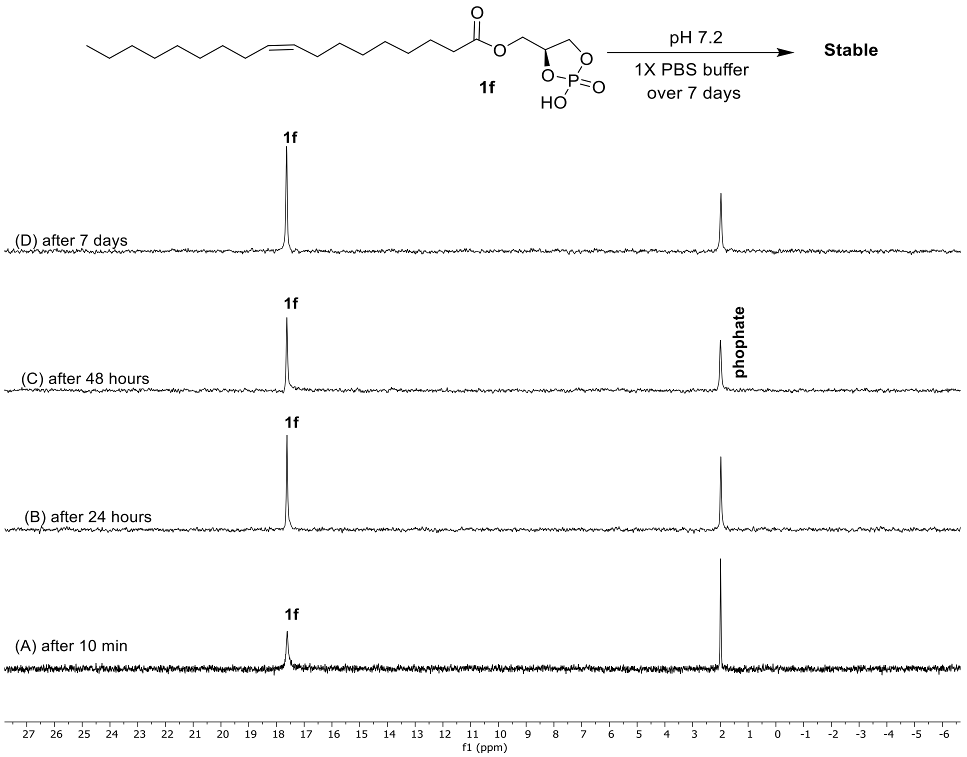


Figure S151. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 40 mM of cPA 18:1 **1f**, in 1X PBS buffer at pH 7.2.

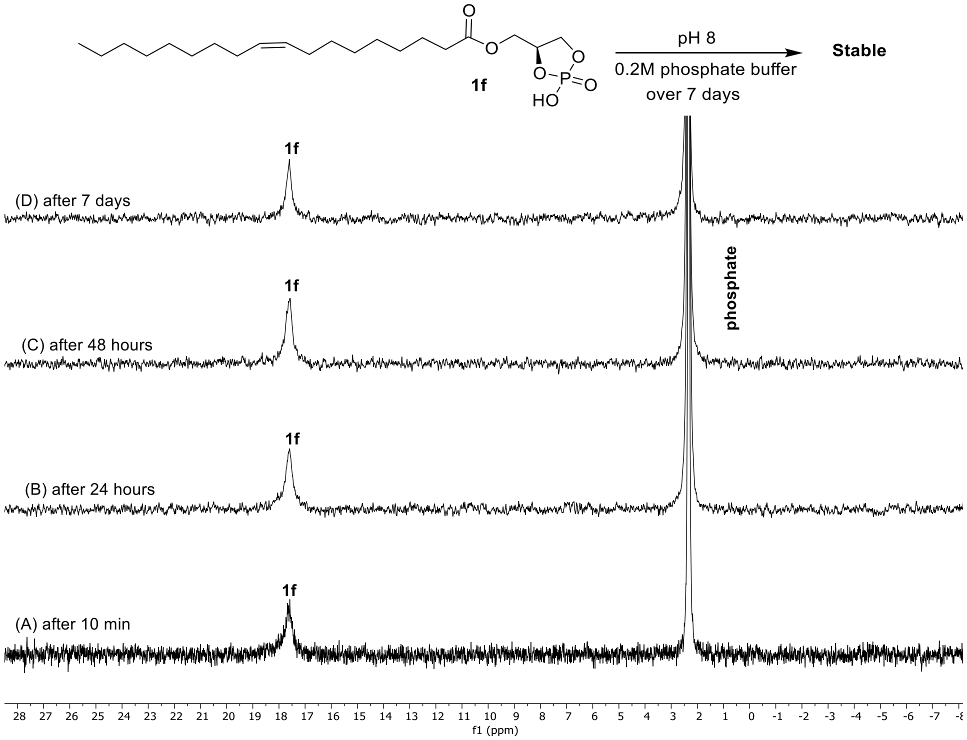
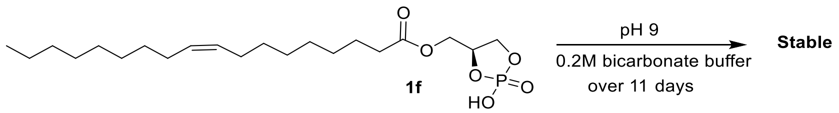


Figure S152. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 40 mM of cPA 18:1 **1f**, in 0.2M phosphate buffer at pH 8.0.



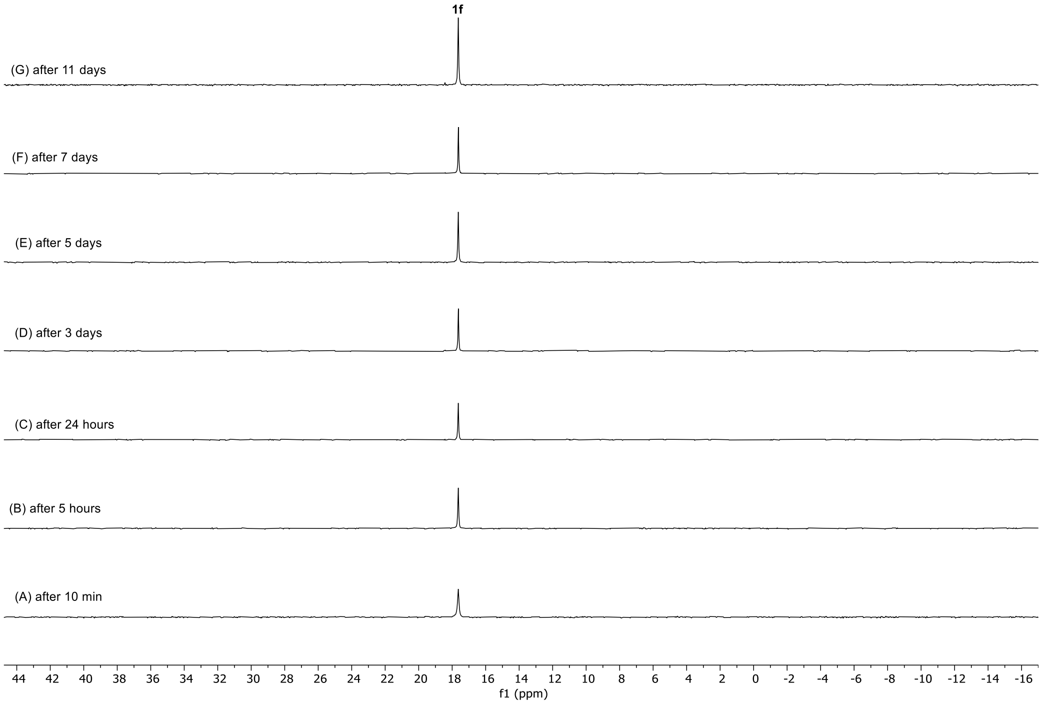


Figure S153. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 40 mM of cPA 18:1 **1f**, in 0.2M bicarbonate buffer at pH 9.0.

Summary of Stability studies of 40 mM of cPAs at various pHs (4-9) in 0.2M buffer

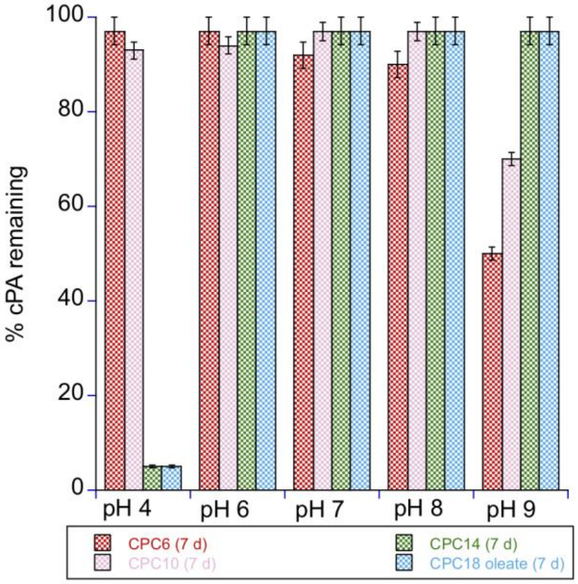


Figure S154. Summary of hydrolytic stability of 40 mM of cPAs at various pHs (4-9) in 0.2M buffer based on data from the ^{31}P-NMR monitoring from Figs. 34-153.

Kinetic Studies of the hydrolysis of cPAs:

The kinetics of hydrolysis of cPAs (**1a**, **1b**, **1c**, and **1f**) were obtained from the data at pH 4.0 in 0.2 M acetate buffer and at pH 9.0 in 0.2M bicarbonate buffer as these were the two pHs where hydrolysis (of either the phosphate group and/or the carboxylate ester group) was observed. The studies were carried out over 14 days monitored by ^{31}P NMR. However, only the data collected up to 7 days were used for kinetic studies (plotting conc. or Ln(conc) vs time) as reliable integration for the peaks of cPAs beyond 7 days could not be obtained for some of the cases.

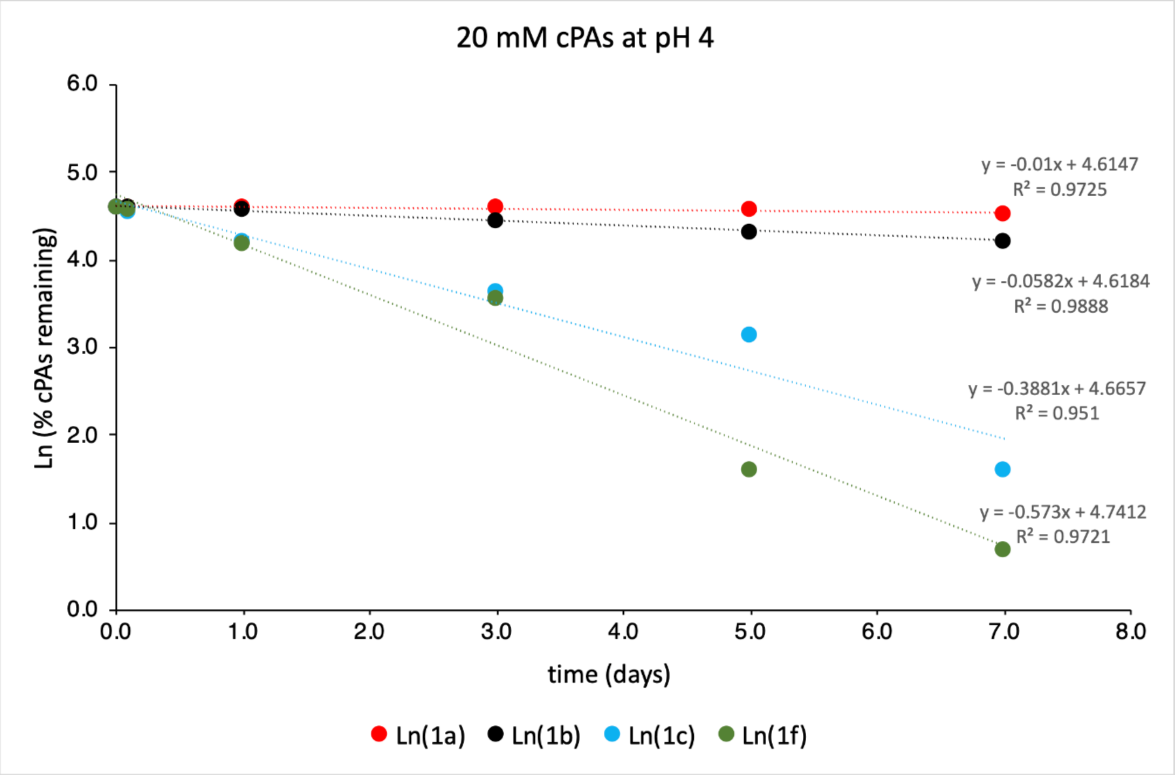


Figure S155. Kinetic profile of hydrolysis of 20 mM of cPAs at pH 4 in 0.2M acetate buffer over 7days. The hydrolysis behavior of cPAS **1c** and **1f** over time fit best with first-order rate (Ln(c) vs time) profile. The $t_{1/2}$ on the average is about 2 days for **1c** and 1.5 days for **1f**. The cPAs **1a** and **1b** remained relatively stable with little hydrolysis.

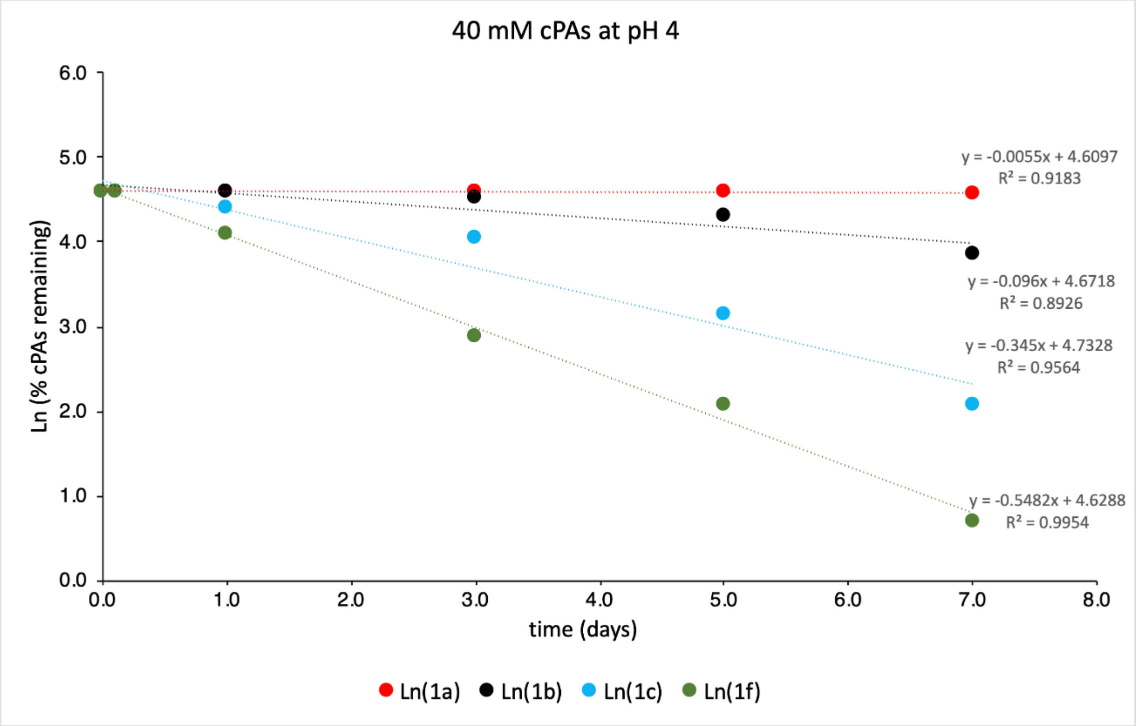


Figure S156. Kinetic profile of hydrolysis of 40 mM of cPAs at pH 4 in 0.2M acetate buffer over 7 days. The hydrolysis behavior remained relatively the same as observed at 20 mM (Figure S155) with slight change in t1/2 values (2.5 days for **1c** and 1.2 days for **1f**) .

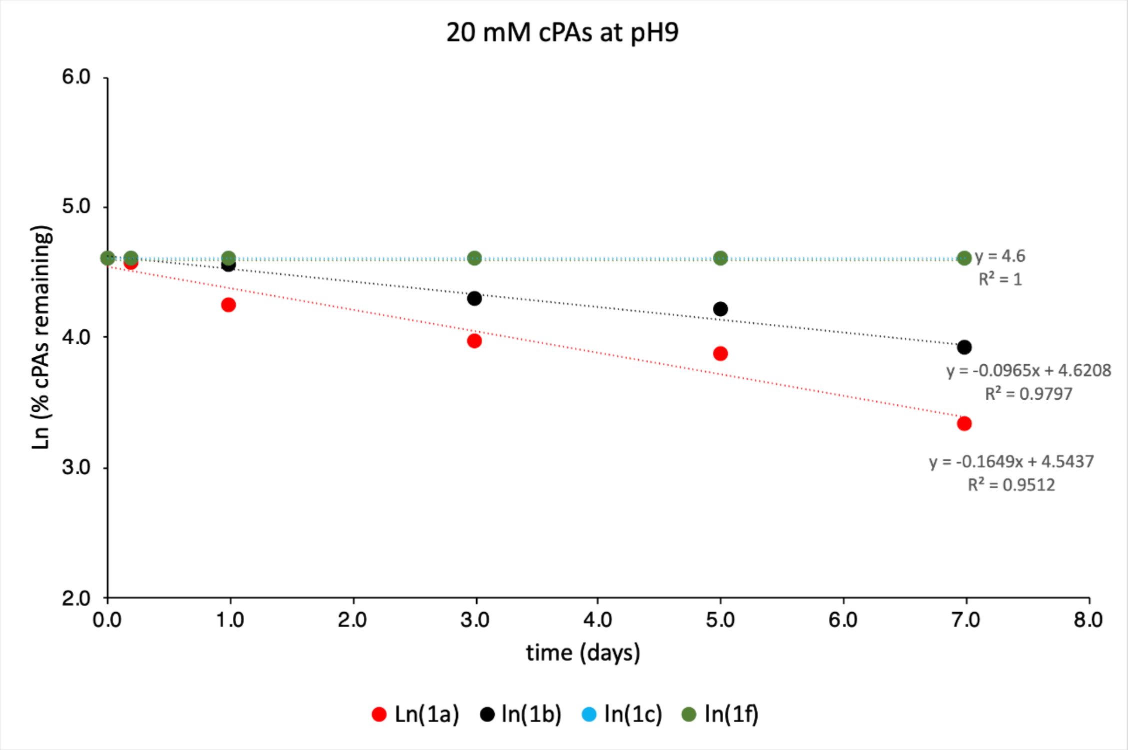


Figure S157. Kinetic profile of hydrolysis of 20 mM of cPAs at pH 4 in 0.2M bicarbonate buffer over 7 days. The hydrolysis behavior of cPAS **1a** and **1b** over time fit best with first-order rate (Ln(c) vs time) profile. The $t_{1/2}$ is about 4 days for **1a** and 7 days for **1b**. The cPAs **1c** and **1f** remained stable with no discernible hydrolysis. Note: data of **1c** and **1f** overlap with each other.

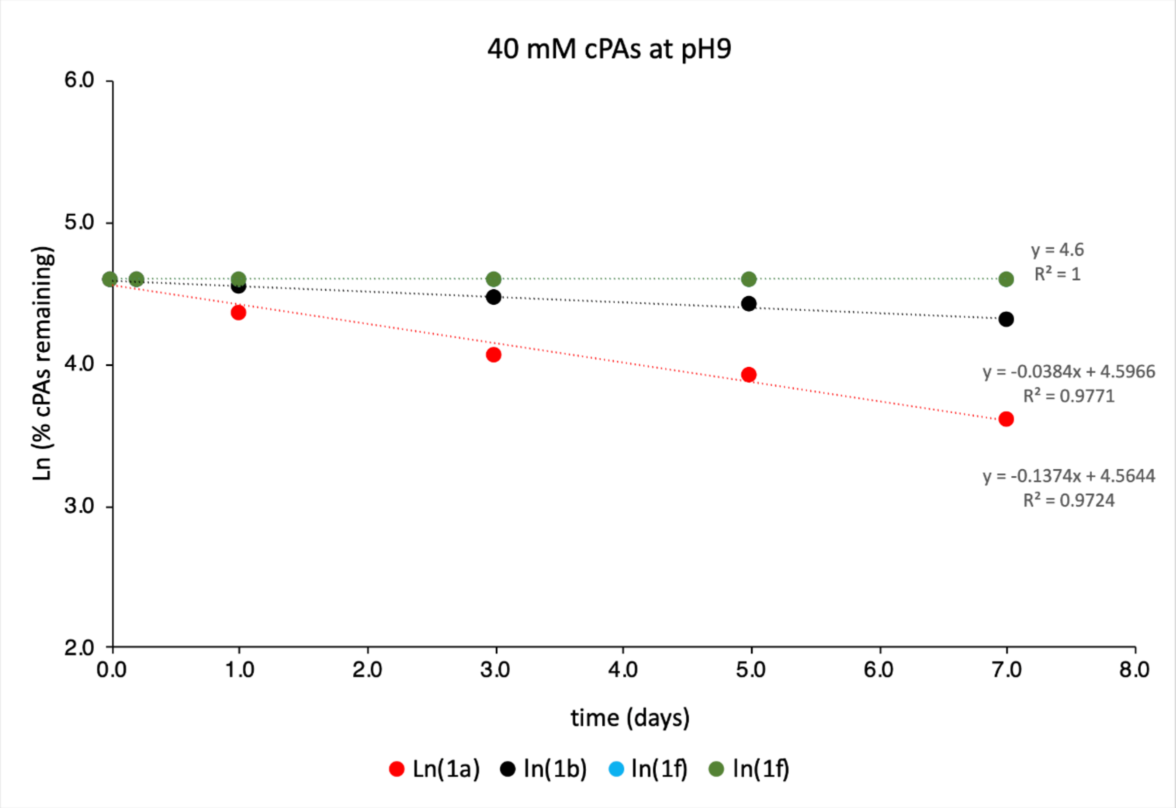


Figure S158. Kinetic profile of hydrolysis of 40 mM of cPAs at pH 9 in 0.2M bicarbonate buffer over 7 days. The hydrolysis behavior remained relatively the same as observed at 20 mM (Figure S157) with change in $t_{1/2}$ values (5 days for **1a** and 18 days for **1b**). Note: data of **1c** and **1f** overlap with each other.

Stability studies of 40 mM of glyceride 5a at various pHs (4-9) in 0.2M buffer

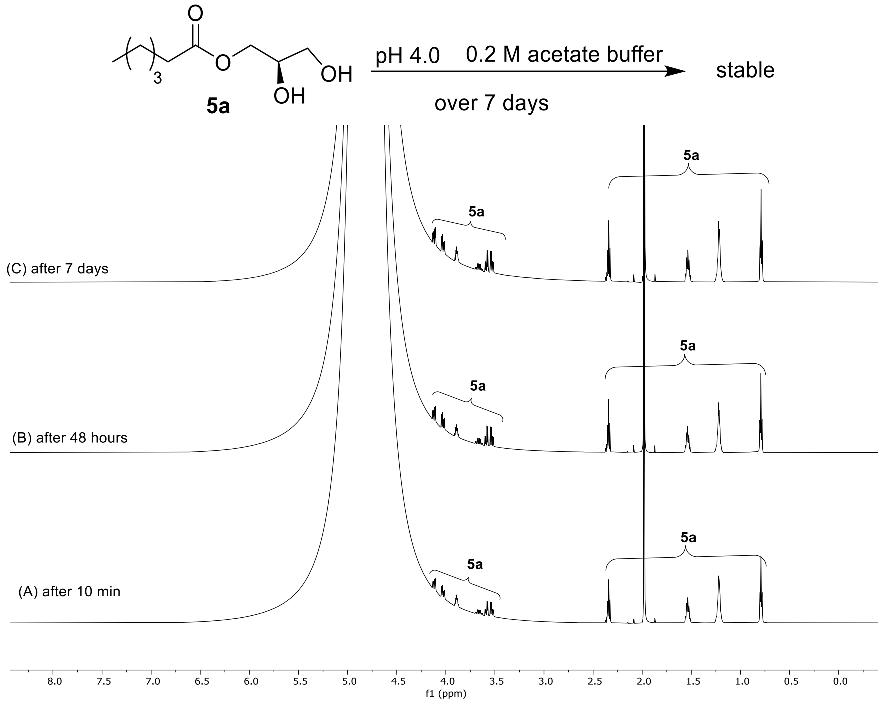


Figure S159. ^{1}H NMR (H_2O-D_2O) of 40 mM of C6 glyceride **5a**, in 0.2 M acetate buffer at pH 4.0.

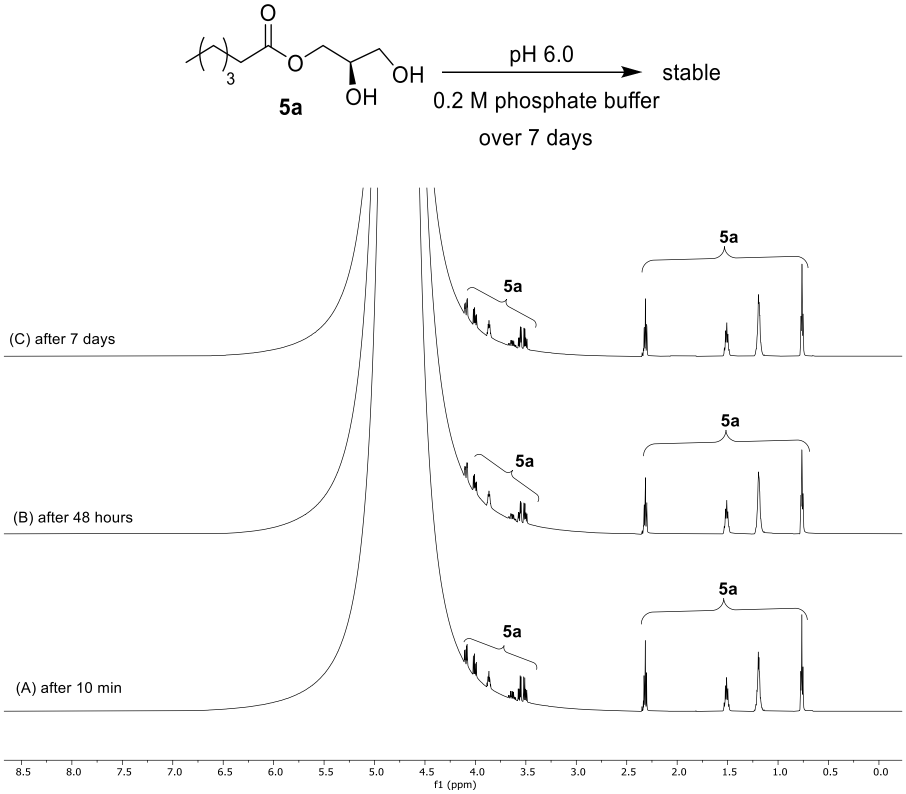


Figure S160. ^{1}H NMR (H_2O-D_2O) of 40 mM of C6 glyceride **5a**, in 0.2 M phosphate buffer at pH 6.0.

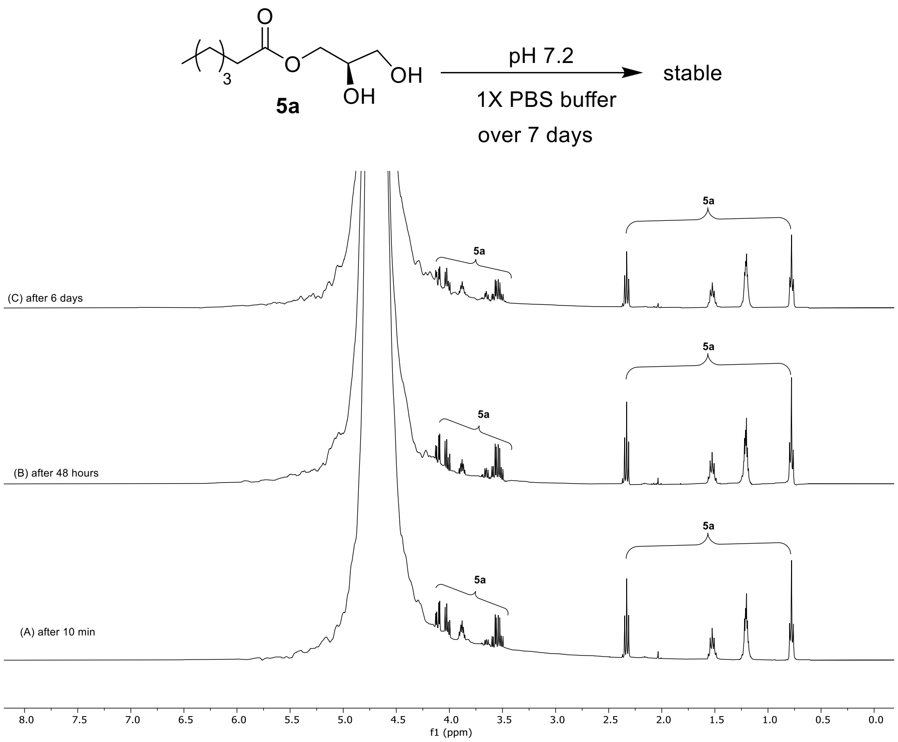


Figure S161. ^{1}H NMR (H_2O-D_2O) of 40 mM of C6 glyceride **5a**, in 1X PBS buffer at pH 7.2. % Carboxylate ester hydrolysis is based on the ^{1}H NMR integration of fatty acid, **2a** (~2.2 ppm) vs C6 glyceride, **5a** (~2.4 ppm).

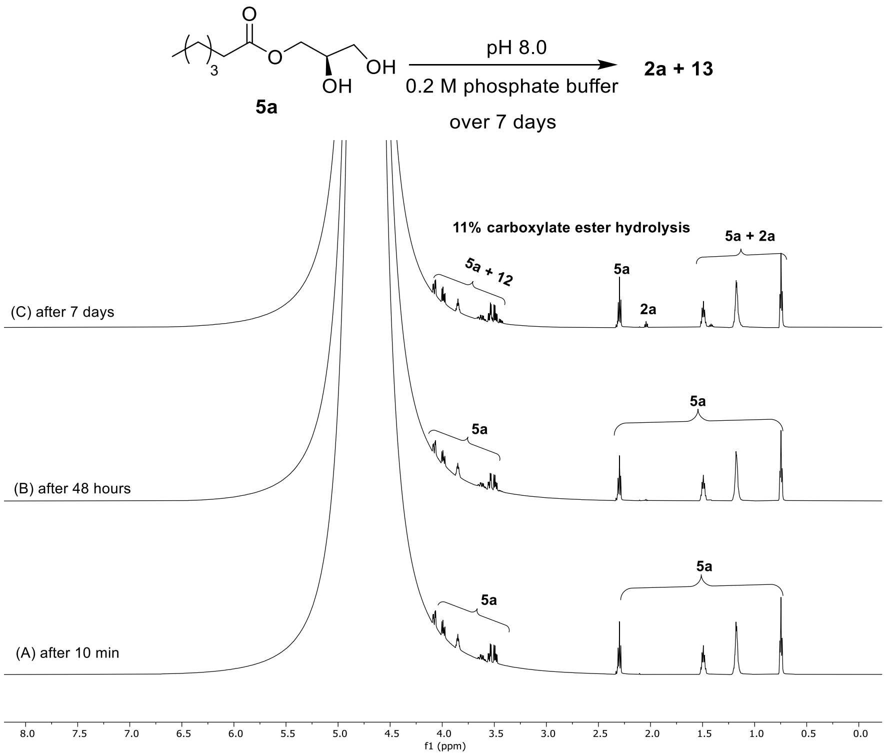


Figure S162. ^{1}H NMR (H_2O-D_2O) of 40 mM of C6 glyceride **5a**, in 0.2 M phosphate buffer at pH 8.0.

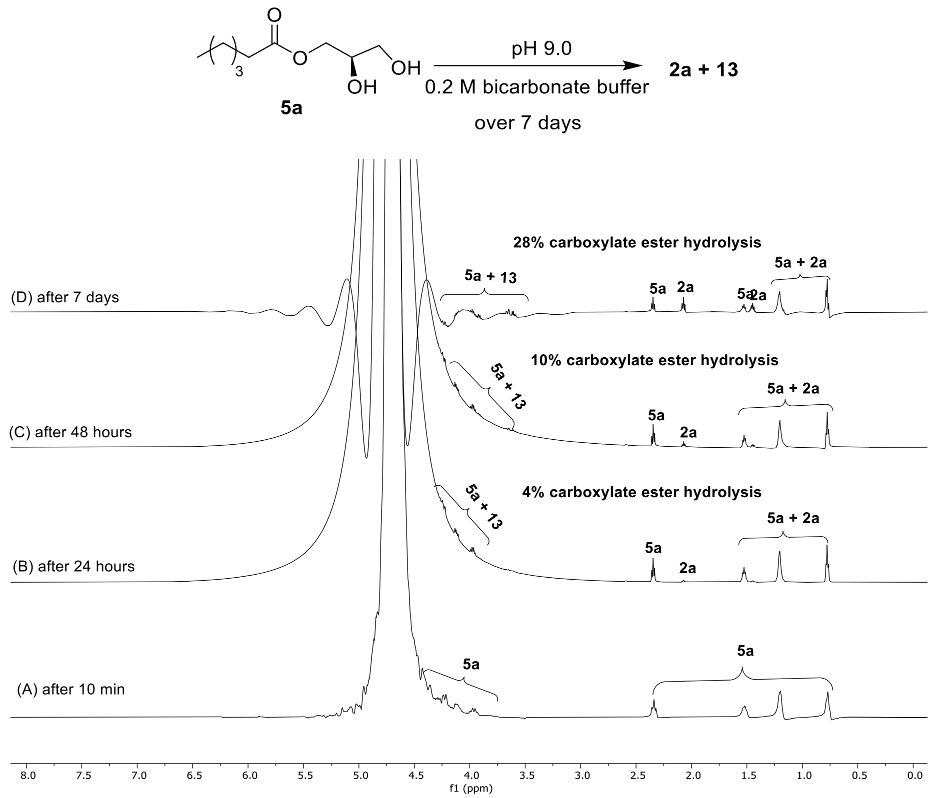


Figure S163. ^{1}H NMR (H_2O-D_2O) of 40 mM of C6 glyceride **5a**, in 0.2 M bicarbonate buffer at pH 9.0 by ^{1}H NMR.

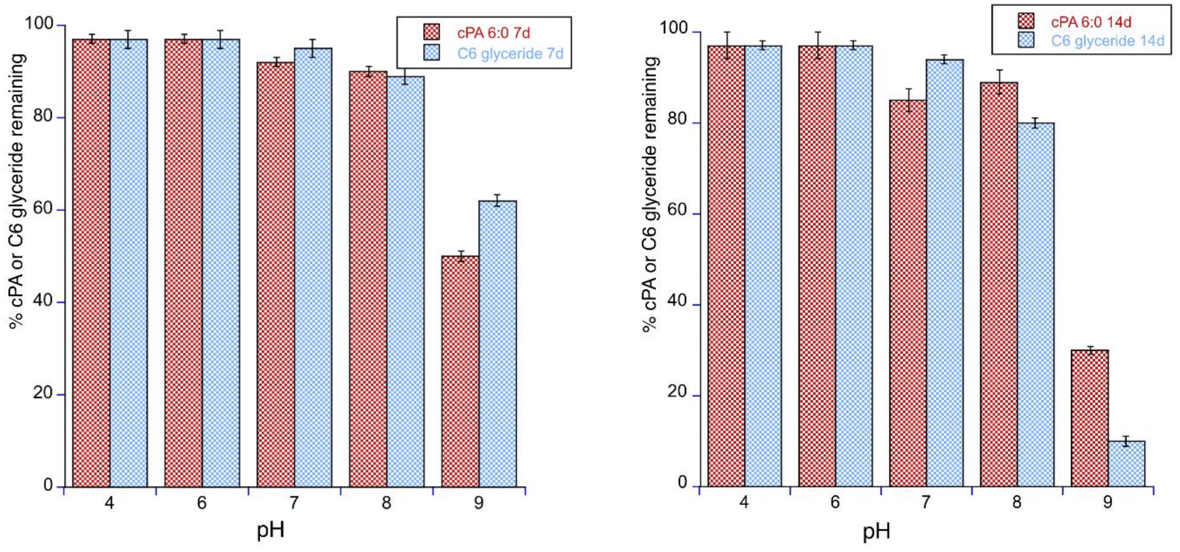


Figure S164. Comparison of hydrolytic stability of 40 mM of cPA **1a** 6:0 and of C6 glyceride **5a** 6:0 in 0.2 M acetate buffer (pH 4), 0.2 M phosphate buffer (pH 6, pH 8), 0.2M HEPES buffer (pH 7.2), 0.2 M bicarbonate buffer (pH 9) over 7 and 14 days. Data from figures 134-138 and 159-163.

Table S3: pKa determination of cPAs

cPA	Apparent pKa
Hexanoate **1a**	2.2
Decanoate **1b**	2.9
Myristic **1c**	4.1
Oleate **1e**	“>4.0”

Measurement of apparent pKa of cPA**s** **1a**, **1b**, **1c** and **1e** by titration following the method described in J. R. Kanicky and D. O. Shah, Langmuir 2003, 19, 2034-2038. For the oleate **cPA** 1e, the end points were not well defined, and the value determined is not exact and is left as “greater than 4” based on the behavior of myristate cpA **1c**.

Temperature dependent stability studies of CPAs (40 mM) in 0.2M 1X PBS buffer, pH 7.5

General procedure: cPA (**1a**-**1c, 1f**) was taken in 0.65 mL of 1X PBS buffer, pH 7.5 such that the concentration of cPA is 40 mM. The resulting solution was vortexed for ~1 min, followed by sonication for ~1 min for complete dissolution (cPA **1a, 1b** and **1f** were clear solutions, cPA, **1c** was partially soluble) and heated at three different temperatures (40 °C, 60 °C and 90 °C) on heating block without stirring. ^{31}P NMR of the solution was measured at different time intervals.

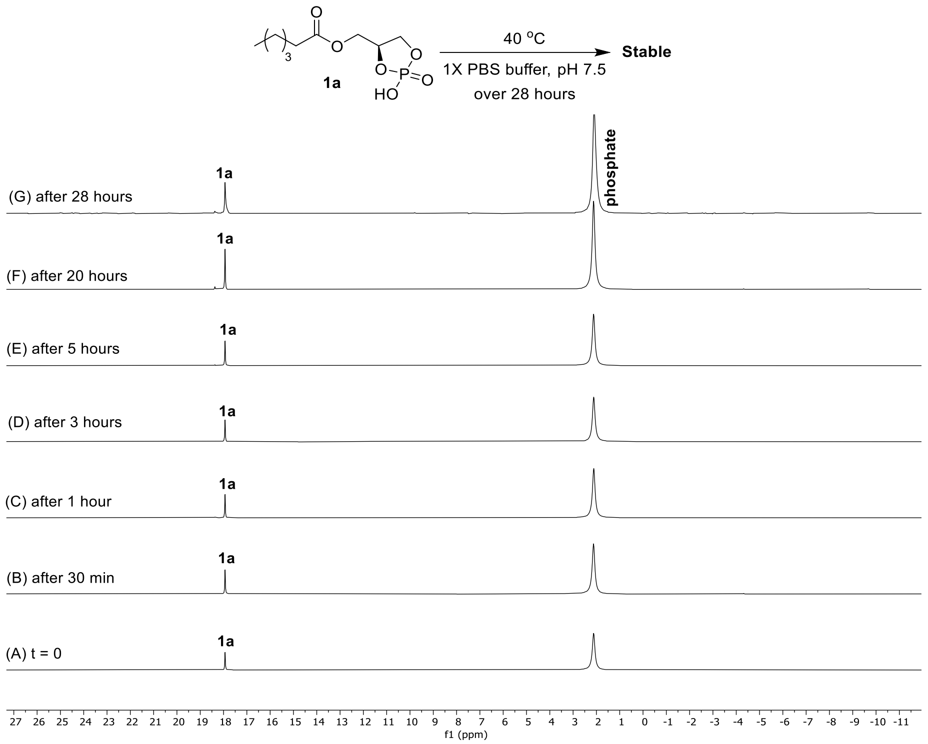


Figure S165. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 40 mM of cPA 6:0 **1a**, in 1X PBS buffer, pH 7.5 at 40 °C.

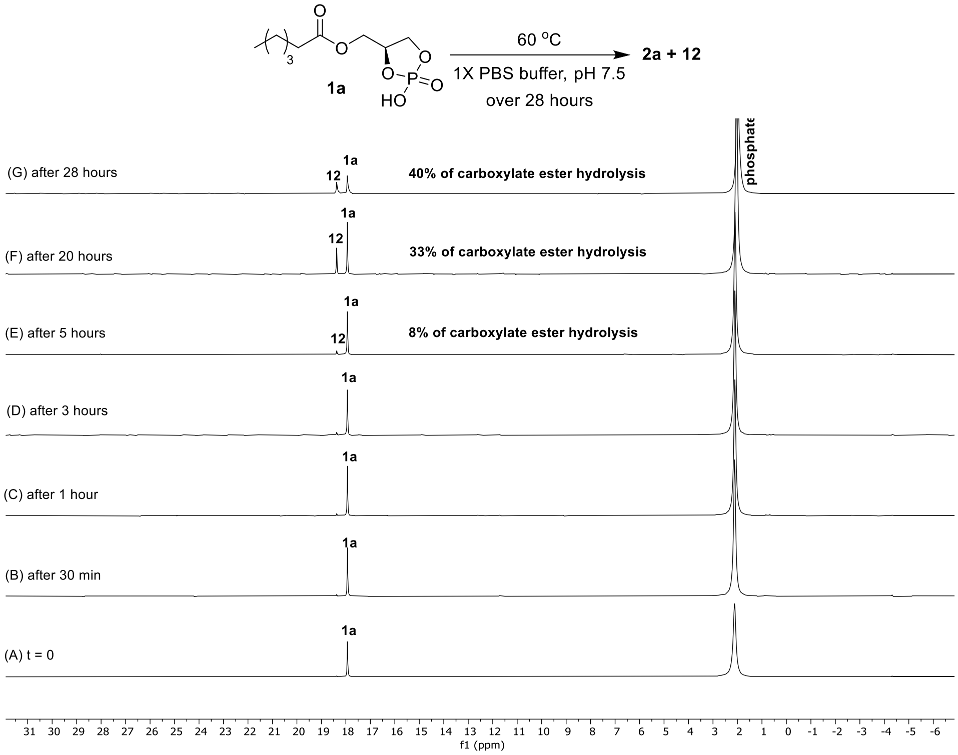


Figure S166. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 40 mM of CPA 6:0 **1a**, in 1X PBS buffer, pH 7.5 at 60 °C. % Carboxylate ester hydrolysis is based on the relative ^{31}P (^{1}H-decoupled) NMR integration of **12** versus **1a**.

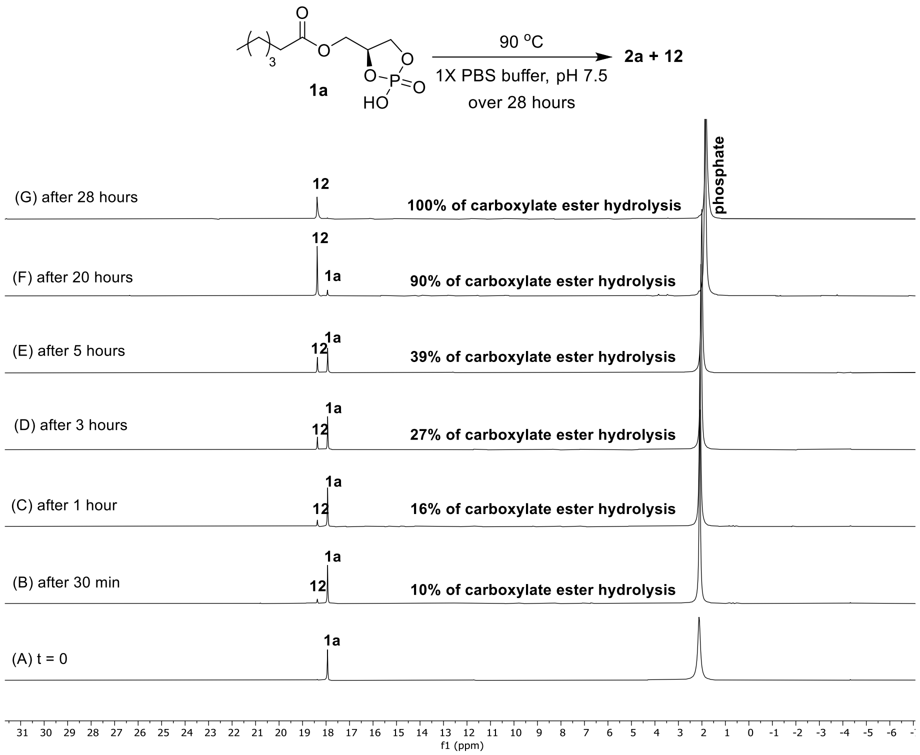


Figure S167. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 40 mM of cPA 6:0 **1a**, in 1X PBS buffer, pH 7.5 at 90 °C.

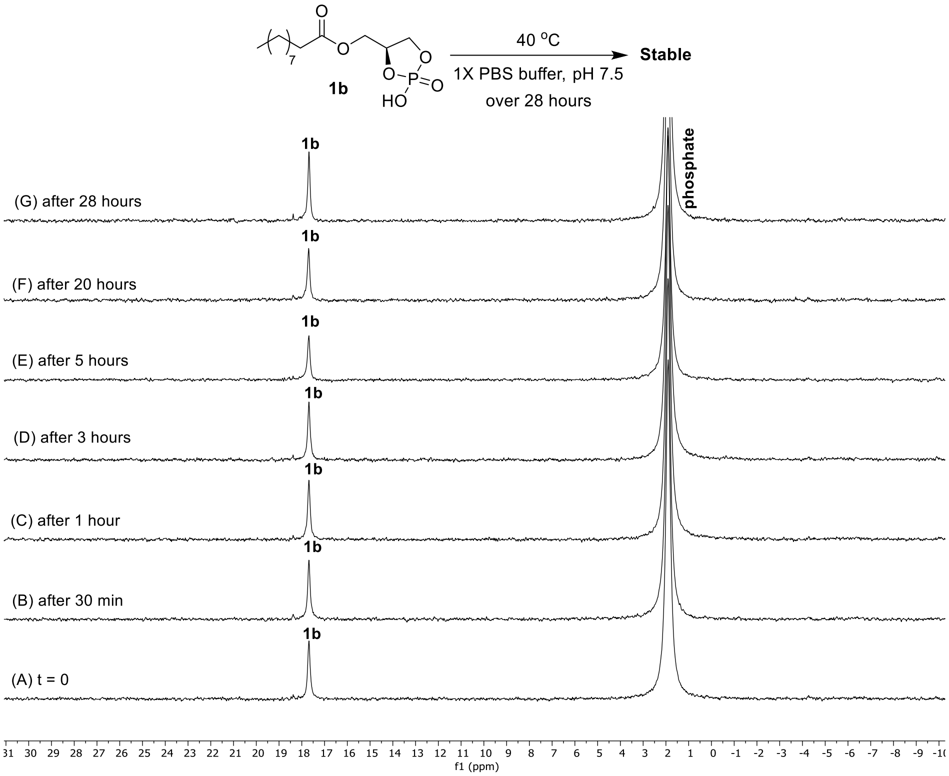


Figure S168. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 40 mM of cPA 10:0 **1b**, in 1X PBS buffer, pH 7.5 at 40 °C.

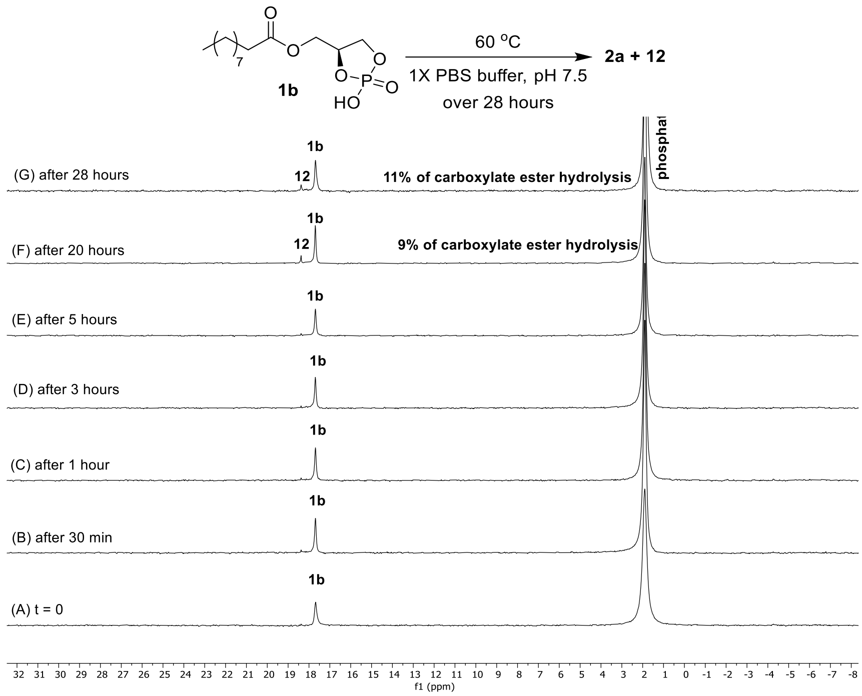


Figure S169. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 40 mM of cPA 10:0 **1b**, in 1X PBS buffer, pH 7.5 at 60 °C. % Carboxylate ester hydrolysis is based on the relative ^{31}P (^{1}H-decoupled) NMR integration of **12** versus **1b**.

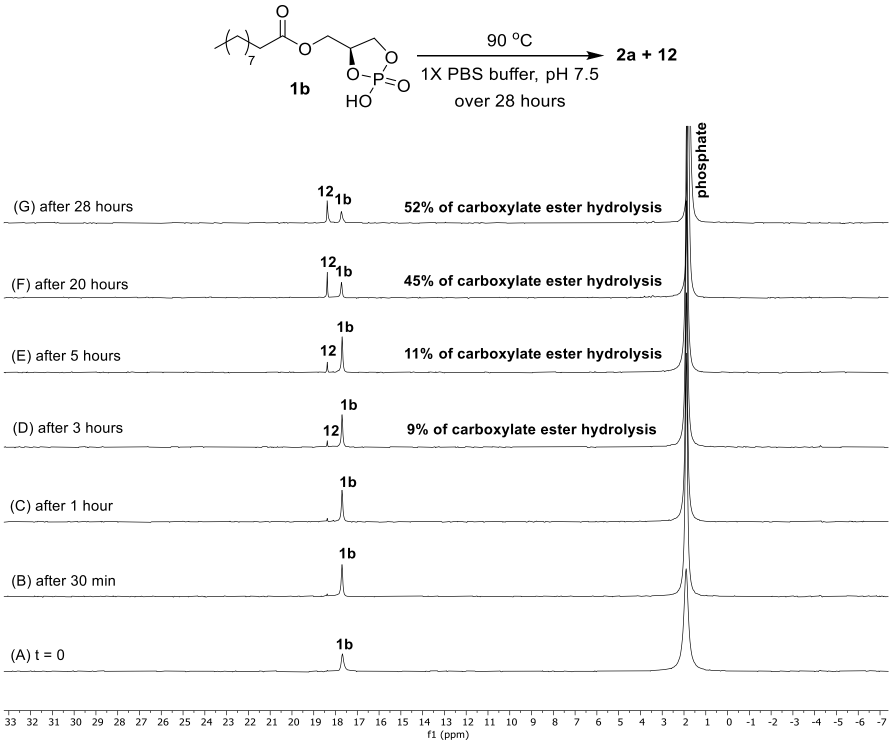


Figure S170. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 40 mM of cPA 10:0 **1b**, in 1X PBS buffer, pH 7.5 at 90 °C.

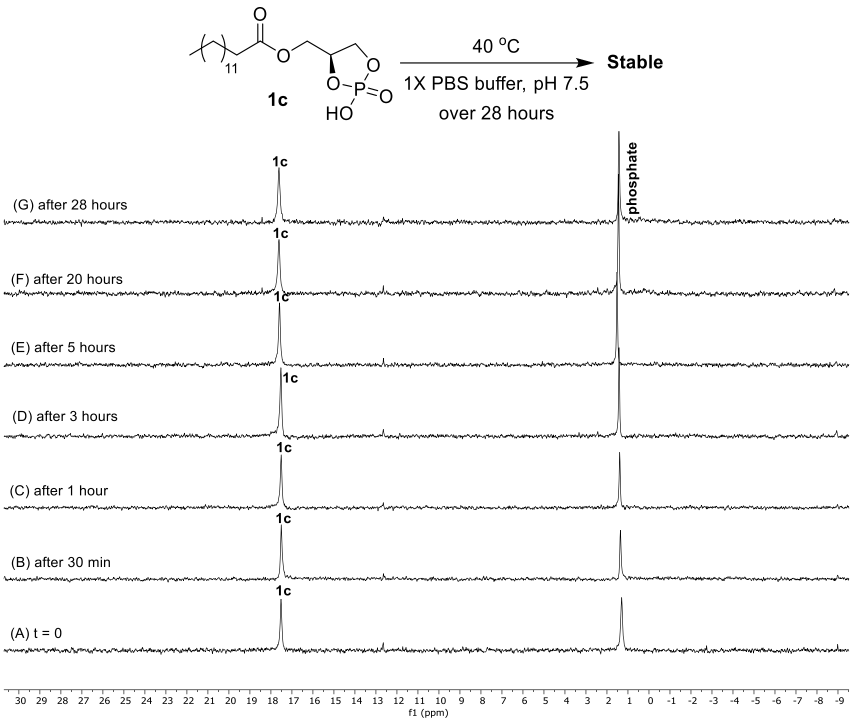


Figure S171. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 40 mM of cPA 14:0 **1c**, in 1X PBS buffer, pH 7.5 at 40 °C.

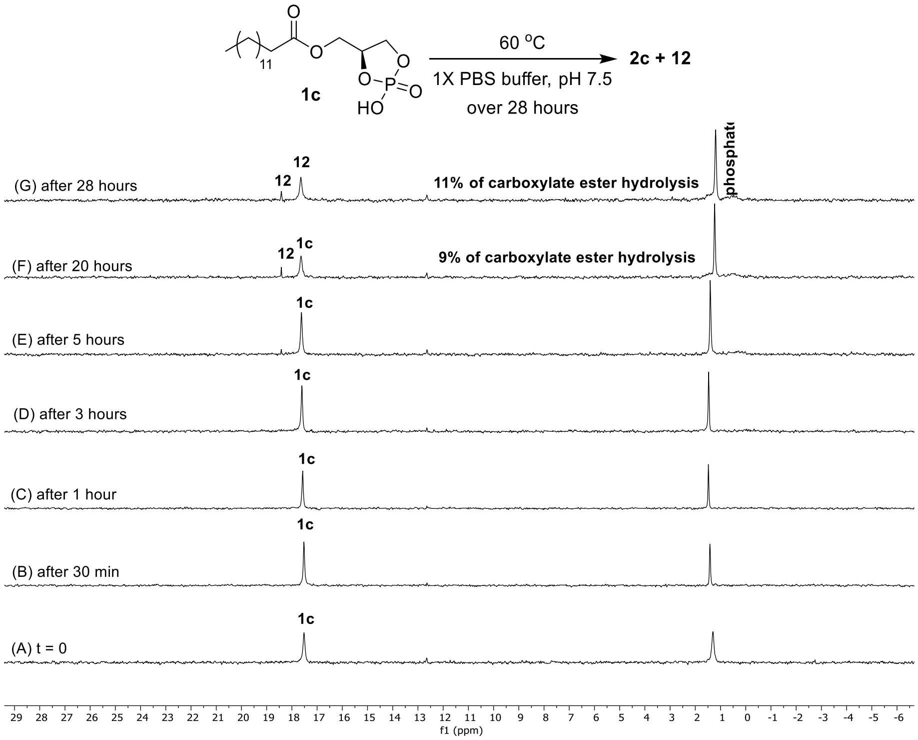


Figure S172. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 40 mM of cPA 14:0 **1c**, in 1X PBS buffer, pH 7.5 at 60 °C. % Carboxylate ester hydrolysis is based on the relative ^{31}P (^{1}H-decoupled) NMR integration of **12** versus **1c**.

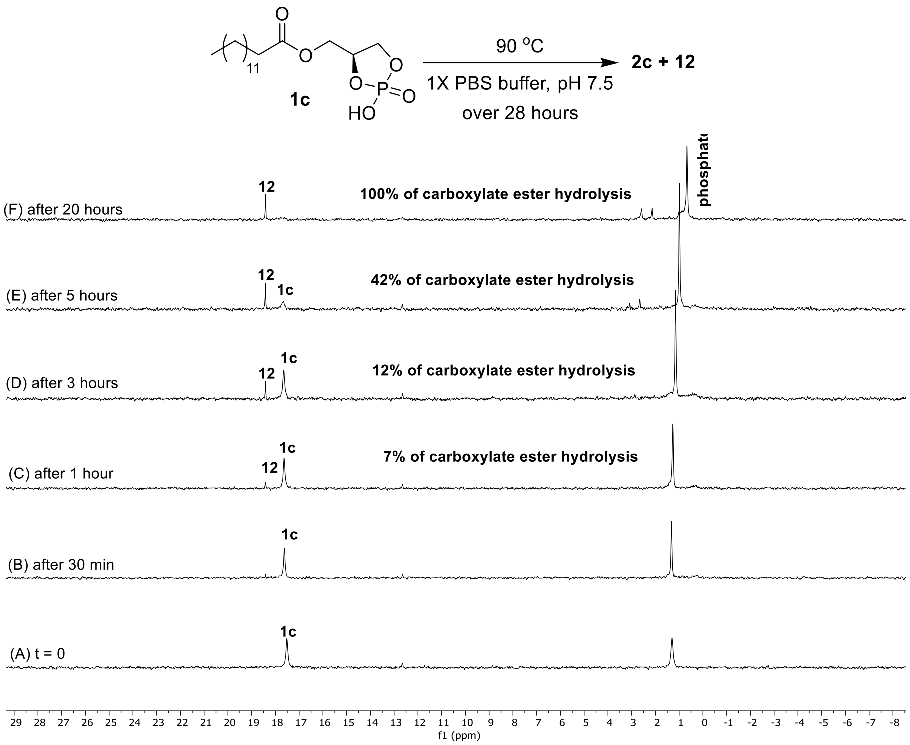


Figure S173. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 40 mM of cPA 14:0 **1c**, in 1X PBS buffer, pH 7.5 at 90 °C.

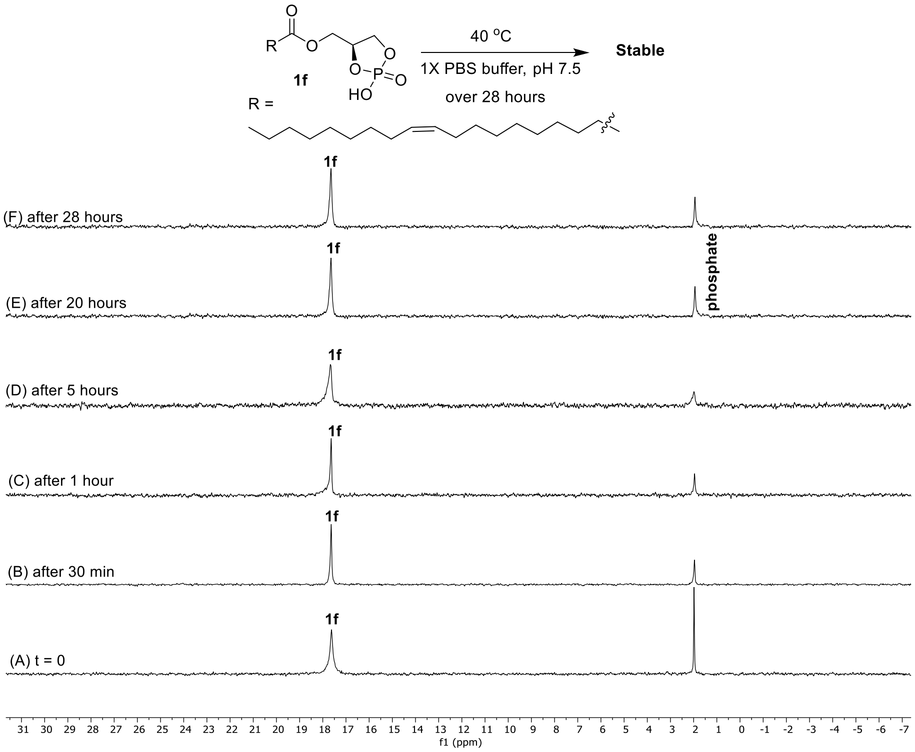


Figure S174. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 40 mM of cPA 18:1 **1f**, in 1X PBS buffer, pH 7.5 at 40 °C.

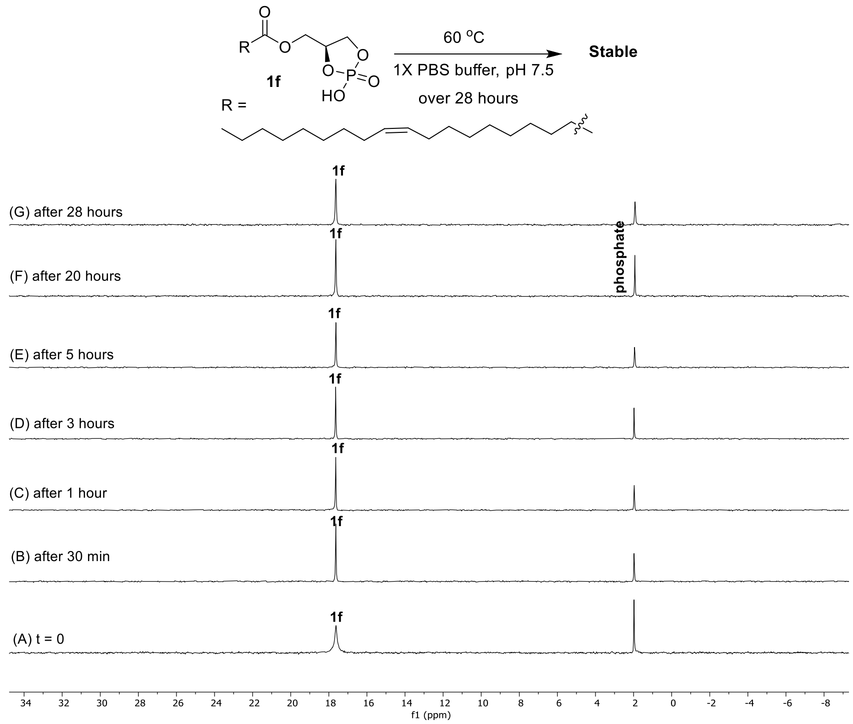


Figure S175. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 40 mM of cPA 18:1 **1f**, in 1X PBS buffer, pH 7.5 at 40 °C.

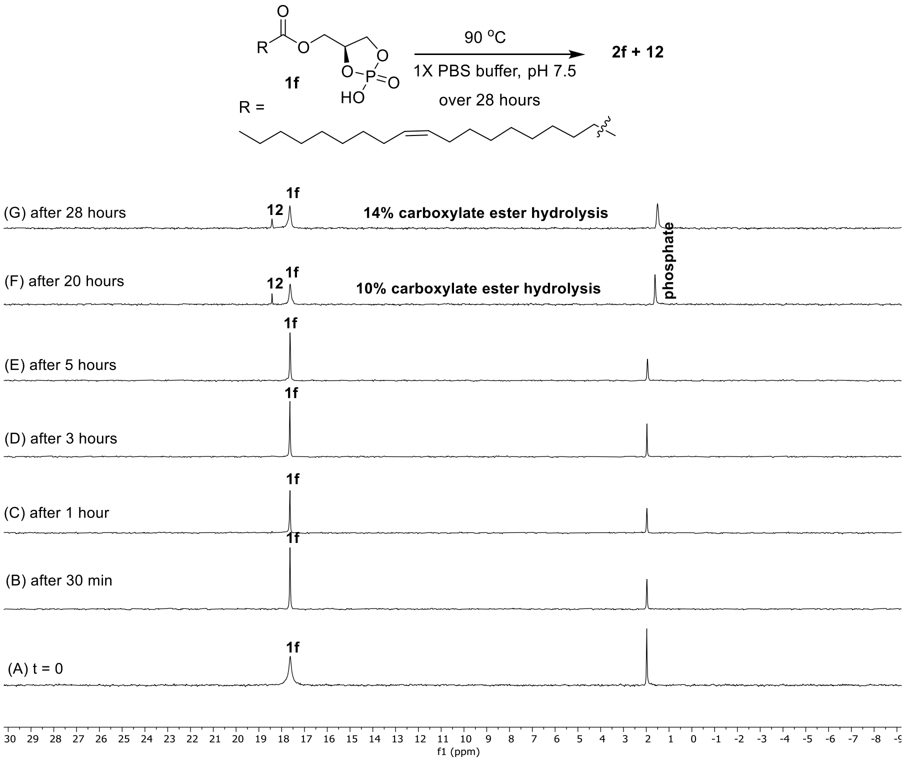


Figure S176. ^{31}P NMR (^{1}H-decoupled, H_2O-D_2O) of 40 mM of cPA 18:1 **1f**, in 1X PBS buffer, pH 7.5 at 90 °C. % Carboxylate ester hydrolysis is based on the relative ^{31}P (^{1}H-decoupled) NMR integration of **12** versus **1f**.

Distribution of products from the hydrolysis of cPAs

The data of product distribution was obtained from the 31P-NMR spectra of the hydrolysis of each of the cPAs (1a-1f). Relative % values calculated from the integration of the peaks corresponding to the various species observed in 31P-NMR.

Table S4: Distribution of products from the hydrolysis of 20mM of cPAs at pH 4.

	1a[#]			**1b[#]**			**1c[#]**			**1f[#]**		
time (days)	**12 (%)**	**8a (+10) (%)[a]**	**9a (+11)(%)[b]**	**12 (%)**	**8b (+10) (%)[a]**	**9b (+11) (%)[b]**	**12 (%)**	**8c (+10) (%)[a]**	**9c (+11) (%)[b]**	**12 (%)**	**8f (+10) (%)[a]**	**9f (+11) (%)[b]**
0	0	0	0	0	0	0	0	0	0	0	0	0
0.1	0	0	0	0	0	0	0	3	2	0	1	1
1	0	0	0	0	1	1	2	14	17	4	18	18
3	0	0	0	2	11	10	1	30	30	2	33	30
5	0	1	1	4	18	14	1	37	34	2	47	43
7	0	1	1	5	25	24	7	47	44	2	50	47

[a] Contains the product 10 after day 5

[b] Contains the product 11 after day 5

[#] ±2% error

Table S5: Distribution of products from the hydrolysis of 40mM of cPAs at pH 4

	1a[#]			**1b[#]**			**1c[#]**			**1f[#]**		
time (days)	**12 (%)**	**8a (+10) (%)[a]**	**9a (+11) (%)[b]**	**12 (%)**	**8b (+10) (%)[a]**	**9b (+11) (%)[b]**	**12 (%)**	**8c (+10) (%)[a]**	**9c (+11) (%)[b]**	**12 (%)**	**8f (+10) (%)[a]**	**9f (+11) (%)[b]**
0	0	0	0	0	0	0	0	0	0	0	0	0
0.1	0	0	0	0	0	0	0	1	1	0	1	1
1	0	0	0	0	2	2	0	10	9	2	20	18
3	0	0	0	0	5	3	0	23	20	1	41	40
5	0	1	1	0	13	12	2	39	35	1	47	45
7	0	2	2	3	28	25	2	45	45	1	48	44

[a] Contains the product 10 after day 5

[b] Contains the product 11 after day 5

[#] ±2% error

Table S6: Distribution of products from the hydrolysis of 20mM of cPAs at pH 9

	1a[#]	1b[#]	1c[#]	1f[#]
time (days)	12 (%)[*]	12 (%)[*]	12 (%)[*]	12 (%)[*]
0	0	0	0	0
0.2	4	0	0	0
1	30	6	0	0
3	47	27	0	0
5	52	33	0	0
7	72	50	0	0

[#] ±2% error

*Remaining %ge is starting cPA

Table S7: Distribution of products from the hydrolysis of 40mM of cPAs at pH 9

	1a[#]	1b[#]	1c[#]	1f[#]
time (days)	12 (%)[*]	12 (%)[*]	12 (%)[*]	12 (%)[*]
0	0	0	0	0
0.2	4	2	0	0
1	22	6	0	0
3	42	13	0	0
5	49	16	0	0
7	63	25	0	0

[#] ±2% error

*Remaining %ge is starting cPA

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