Supplementary Information

Understanding the assembly of amphiphilic additives in bulk and dispersed non-lamellar lipid-based matrices: phosphorylation, H-bonding and ionisation.

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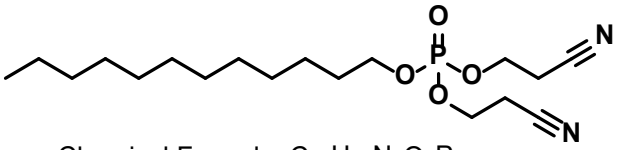
Synthesis

General Information Related to Synthesis and pH-Measurements

All chemicals were used as received, unless otherwise stated. All solvents were of technical grade and distilled prior to their use. Evaporation of the solvents *in vacuo* was performed with the rotary evaporator at the standard bath temperature and pressure.[1] Column chromatography was performed using silica gel *Merck* 60 (particle size 40-63 µm) or Q-Sepharose® Fast Flow (preswollen, 45-165 µm) (*Sigma–Aldrich*) with the indicated solvent system. Analytical thin-layer chromatography (TLC) was performed using *Merck* pre-coated silica gel plates 60 F_{254}, visualisation was performed by UV light (254 nm and 366 nm) and stains if indicated. Medium Pressure Liquid Chromatography (MPLC) was performed by CombiFlash® Rf+ Lumen^{TM} (*TELEDYNE ISCO*) with indicated column size, solvents, time and flow rate. Freeze-drying of aqueous samples was performed at a *CHRIST* alpha 1-4 LD plus after freezing the samples in liquid nitrogen. ^{1}H-NMR, ^{13}C-NMR, ^{31}P-NMR and ^{31}P[^{1}H]-NMR spectra were recorded on the following machines: *Bruker* AV-300 (300 MHz), *Bruker* AV-400 (400 MHz) or *Bruker* AV-500 (500 MHz); Chemical shifts are given in parts per million (ppm) relative to the internal standard. Coupling constants J are expressed in Hz and multiplicities are abbreviated as follows: s (singlet), br (broad), d (doublet), t (triplet), q (quadruplet), quint (quintet), m (multiplet) If signals fall together they are reported as X seen as Y. Peaks in ^{13}C-NMR that were not completely separated are named 2p for two peaks and mp for more. Mass spectra were recorded by the Mass Spectroscopy Service of UZH on *Finnigan* MAT95 MS, *Bruker* LC MS and *Finnigan* TSQ700 MS machines.

bis(2-cyanoethyl) dodecyl phosphate (2):

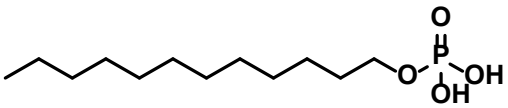


Chemical Formula: $C_{18}H_{33}N_2O_4P$
Molecular Weight: 372.45

Bis(2-cyanoethyl)diisopropylphosphoramidite **1** (0.85 g, 3.13 mmol, 1.2 eq.), synthesised after a published procedure was dissolved in MeCN (2 mL).[2] After the addition of dodecanol (0.49 g, 2.62 mmol, 1 eq.) the solvent was evaporated under high vacuum. This drying by coevaporation was repeated twice with MeCN (2 mL). Subsequently the mixture was dissolved in a tetrazole solution (11.8 mL, 4 wt% in MeCN, 1.5 eq.) and stirred for four hours at room temperature. After completion the reaction mixture was cooled to 0 °C and *t*-butyl hydroperoxide solution (0.707 mL, 5.5 M in decane, 1.5 eq.) was added. After 20 min of stirring, the mixture was warmed to room temperature and half of the solvents were evaporated at high vacuum. After dilution with EtOAc (30 mL) the organic phase was washed with brine (3 x 30 mL), then dried over $MgSO_4$ and subsequently applied to vacuum. Then the compound was purified by column chromatography (Cyclohexane/EtOAc 0 to 80 %) to yield **2** as a yellowish oil (0.87 g, 2.34 mmol, 88 %).

***R**f*:(SiO_2: EtOAc:Cyclohexane 8:2) = 0.31 **^{1}H-NMR** (400 MHz, Chloroform-d, with aceton 2.01) δ [ppm]: 4.33 – 4.23 (m, 4H), 4.12 (q, J = 6.8 Hz, 2H), 2.78 (t, J = 6.1 Hz, 4H), 1.76 – 1.65 (m, 2H), 1.44 – 1.20 (m, 18H), 0.87 (t, J = 6.8 Hz, 3H). **^{31}P-NMR** (162 MHz, Chloroform-d) δ [ppm]: -2.26. **^{31}P[^{1}H]-NMR** (162 MHz, Chloroform-d) δ [ppm]: -2.26 (dt seen as sept). **^{13}C-NMR** (101 MHz, Chloroform-d, with EtOAc 180.79, 60.09, 20.74, 13.94) δ [ppm] 116.43, 69.09 (2p), 62.15, 62.10, 31.88, 30.17, 30.11, 29.59, 29.52, 29.45, 29.31, 29.07, 25.32, 22.66, 19.73, 19.66, 14.09. **(+)-HR-ESI-MS**: calculated 372.2178, m/z found 395.2070 [M + Na^+].

dodecyl dihydrogen phosphate (3):



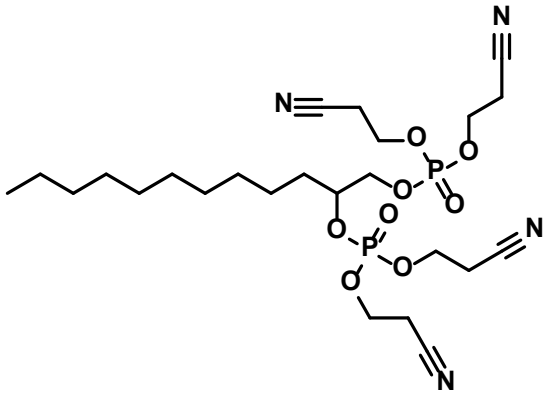
Chemical Formula: $C_{12}H_{27}O_4P$
Molecular Weight: 266.32

2 (0.87 g, 2.34 mmol, 1 eq.) was dissolved in a 1:1 mixture of methanol and THF (9 mL), subsequently aq. NH_4OH (25%, 8.8 mL) was added and the reaction was stirred for 1 hour at room temperature and 550 mbar. Subsequently the organic solvents were evaporated and more aq. NH_4OH (25 %, 8.8 mL) was added and the reaction stirred overnight (60 °C and 550 mbar). After completion of the deprotection the product was lyophilised twice to afford pure **3** (0.61 g, 2.29 mmol, 98 %) as a white powder.

^{1}H-NMR (400 MHz, D_2O) δ [ppm]: 3.85 (q, J = 6.7 Hz, 1H), 1.65 – 1.58 (m, 2H), 1.28 (s, 18H), 0.85 (t, J = 6.3 Hz, 3H). **^{31}P-NMR** (162 MHz, D_2O) δ [ppm]: 0.60. **^{31}P[^{1}H]-NMR** (162 MHz, D_2O-d) δ [ppm]: -0.60 (t, *J*= 6.6 Hz). **^{13}C-**

NMR (101 MHz, D_2O) δ [ppm]: 67.85, 53.40, 31.93, 29.76 – 29.52 (mp), 29.37 (2p), 29.21, 25.47, 22.69, 14.10. **(-)-HR-ESI-MS**: calculated 266.1647, m/z found 265.1566 [M - H^+].

tetrakis(2-cyanoethyl) dodecane-1,2-diyl bis(phosphate) (5):

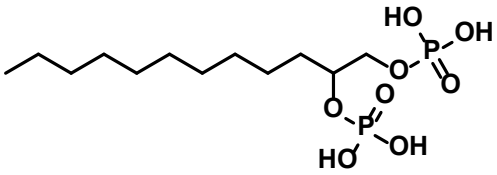


Chemical Formula: $C_{24}H_{40}N_4O_8P_2$
Molecular Weight: 574.55

1,2-dodecanediol (500 mg, 2.10 mmol, 1 eq.) and bis(2-cyanoethyl)diisopropylphosphoramidite **1** (2.62 g, 5.02 mmol 2.4 eq.) were dried by coevaporation with MeCN (3 x 2 mL) and subsequently dissolved in dry DMF (18 mL) at 0 °C and under N_2. After the addition of 5-phenyl-1*H*-tetrazole (0.919 g, 6.29 mmol, 3 eq.) the reaction was stirred for 6 hours, then cooled to 0 °C before a *tert*-butyl hydroperoxide solution (1.10 mL, 5.5 M in decane, 3 eq.) was added. After further stirring for 20 minutes half of the solvents were removed by reduced pressure before mixture was diluted with EtOAc (30 mL). The organic phase was washed with brine (3 x 20 mL), dried over $MgSO_4$ and concentrated *in vacuo*. By column chromatography (EtOAc/MeOH 24:1) the product was isolated in 37 % yield (526 mg, 0.915 mmol).

***R**f*:(SiO_2: EtOAc:MeOH 24:1) = 0.21; **^{1}H-NMR** (400 MHz, Chloroform-d, with EtOAc 4.27, 2.03, 1.23) δ [ppm]: 4.71 – 4.58 (m, 1H), 4.40 – 4.25 (m, 8H), 4.20 – 4.06 (m, 2H), 2.87 – 2.74 (m, 8H), 1.79 – 1.59 (m, 2H), 1.49 – 1.19 (m, 16H), 0.87 (t, J = 6.9 Hz, 3H). **^{31}P-NMR** (162 MHz, Chloroform-d) δ [ppm]: -2.48, -2.97. **^{31}P[^{1}H]-NMR** (162 MHz, Chloroform-d) δ [ppm]: -2.48 (dt seen as hept), -2.97 (dd seen as hex). **^{13}C-NMR** (101 MHz, Chloroform-d, with EtOAc 171.13, 60.37, 21.02, 14.19) δ [ppm]: 116.73, 116.59, 116.54, 116.43, 78.92 – 78.42 (mp), 69.39, 62.62, 62.57, 62.54, 62.48, 62.38, 62.33, 31.87, 31.52, 31.48, 29.54, 29.51, 29.37, 29.29, 24.82, 22.65, 19.76, 19.69, 14.09. **(+)-HR-ESI-MS**: calculated 574.2321, m/z found 575.2403 [M + H^+], 597.2218 [M + Na^+].

dodecane-1,2-diyl bis(dihydrogen phosphate) (6):

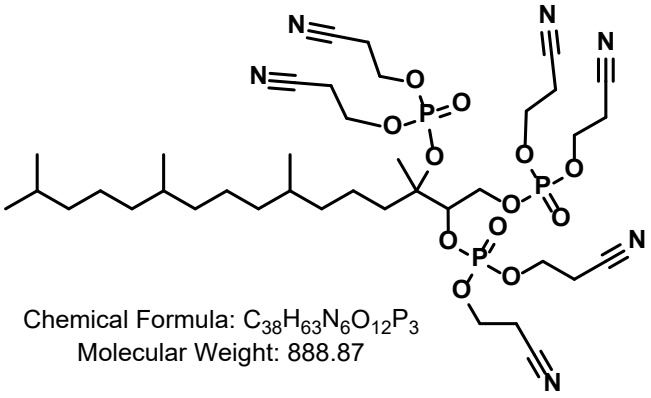


Chemical Formula: $C_{12}H_{28}O_8P_2$
Molecular Weight: 362.30

5 (0.29 g, 0.52 mmol, 1 eq.) was dissolved in a 1:1 mixture of methanol and THF (3.4 mL). After the addition of aq. NH_4OH (25 %, 3.7 mL) the reaction was stirred for 1 hour at room temperature. Subsequently the organic solvents were evaporated and more aq. NH_4OH (25 %, 7.6 mL) was added and the reaction was stirred for 3 more hours (60 °C and 550 mbar). After ^{31}P-NMR showed completion of the deprotection the product was lyophilised twice. The crude mixture was purified by Q-sepharose® (25 mM to 1 M NH_4HCO_3 in H_2O) to afford a **6** as a white powder (0.176 g, 0.486 mmol, 96 %).

^{1}H-NMR (400 MHz, D_2O) δ [ppm]: 4.20 – 4.09 (m, 1H), 3.85 – 3.69 (m, 2H), 1.61 – 1.46 (m, 2H), 1.21 (s, 16H), 0.79 (t, J = 6.9 Hz, 3H). **^{31}P-NMR** (162 MHz, D_2O) δ [ppm]: 2.95, 1.13. **^{31}P[^{1}H]-NMR** (162 MHz, Chloroform-d) δ [ppm]: 3.10 (t, J = 5.3 Hz), 1.32 (d, J = 8.3 Hz). **^{13}C-NMR** (101 MHz, D_2O) δ [ppm]: 75.25, 66.78, 31.96 (2p), 31.18, 29.01, 28.82, 28.77, 28.75, 28.48, 24.59, 22.02, 13.41. **(-)-HR-ESI-MS**: calculated 362.1259, m/z found 361.1183 [M - H^+].

hexakis(2-cyanoethyl) (3,7,11,15-tetramethylhexadecane-1,2,3-triyl) tris(phosphate) (8)
(as diastereoisomeric mixture):



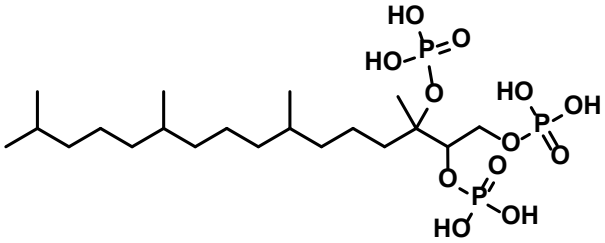
Chemical Formula: $C_{38}H_{63}N_6O_{12}P_3$
Molecular Weight: 888.87

bis(2-cyanoethyl)diisopropylphosphoramidite **1** (1.30 g, 2.45 mmol 3.6 eq.) was dried by coevaporation with THF (2 x 0.5 mL) and subsequently dissolved in dry THF (6 mL) at 0 °C and under N_2. After the addition of 5-phenyl-1*H*-tetrazole (451 mg, 6.29 mmol, 3 eq.) and the dropwise addition of phytantriol (diastereoisomeric mixture, 225 mg, 0.68 mmol, 1 eq.) in dry THF (3 mL) the reaction was stirred for 6 hours, then cooled to 0 °C before a *tert*-butyl hydroperoxide solution (0.535 mL, 5.5 M in decane, 4.5 eq.) was added. After further stirring for 20 minutes the solvents were removed by reduced pressure before the crude product was dissolved in EtOAc (20 mL). The organic phase was washed with

brine (3 x 20 mL), dried over $MgSO_4$ and concentrated *in vacuo*. By column chromatography (EtOAc/MeOH 19:1 to 9:1) the product was isolated in 78 % yield (470 mg, 0.529 mmol).

***R*$_f$**(SiO_2: EtOAc:MeOH 19:1) = 0.17 **^{1}H-NMR** (300 MHz, Chloroform-d, with EtOAc 4.08, 2.01, 1.22) δ [ppm]: 4.83 – 4.67 (m, 1H), 4.52 – 4.42 (m, 1H), 4.42 – 4.22 (m, 12H), 2.88 – 2.71 (m, 12H), 1.73 – 1.56 (m, 3H), 1.51 – 0.97 (m, 21H), 0.82 (t, J = 6.5 Hz, 12H). **^{31}P-NMR** (121 MHz, Chloroform-d) δ [ppm]: -2.59 (2p), -2.86 (2p) -7.26 (2p). **^{31}P[^{1}H]-NMR** (121 MHz, Chloroform-d) δ [ppm]: -2.22 – 3.23 (m), -6.94 – 7.65 (m). **^{13}C-NMR** (101 MHz, Chloroform-d, with EtOAc 171.21, 60.43, 14.23) δ [ppm]: 117.86 – 116.34 (mp), 87.34, 77.16, 66.84, 63.43 – 62.01 (mp), 39.39, 38.18, 37.61 – 37.05 (mp), 33.10 – 32.41 (mp), 28.01, 24.85, 24.55, 22.77, 22.67, 21.54, 21.09, 20.04 – 19.65 (mp), 19.61 – 19.26 (mp). **(+)-HR-ESI-MS**: calculated 888.3717, m/z found 911.367 [M + Na^+].

3,7,11,15-tetramethylhexadecane-1,2,3-triyl tris(dihydrogen phosphate) (9):

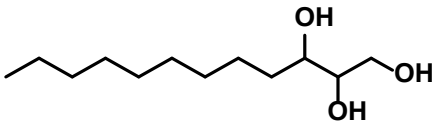


Chemical Formula: $C_{20}H_{45}O_{12}P_3$
Molecular Weight: 570.49

8 (470 mg, 0.529 mmol, 1 eq.) was dissolved in a 1:1 mixture of methanol and THF (2.4 mL). After addition of aq. NH_4OH (25 %, 2.5 mL) the reaction was stirred for 1 hour at room temperature. Subsequently the organic solvents were evaporated and more aq. NH_4OH (25 %, 4 mL) was added and the reaction was stirred o.n. at 60 °C. After ^{31}P-NMR showed complete deprotection, the product was lyophilised twice. The crude mixture was purified by Q-sepharose® (25 mM to 1 M NH_4HCO_3 in H_2O) to afford a **9** as a white powder (0.270 g, 0.473 mmol, 90 %).

^{1}H-NMR (400 MHz, D_2O) δ [ppm]: 4.57 – 4.48 (m, 1H), 4.48 – 4.38 (m, 1H), 4.32 – 4.15 (m, 1H), 4.11 – 3.94 (m, 1H), 1.99 – 1.07 (m, 25H), 1.04 – 0.83 (m, 12H). **^{31}P-NMR** (162 MHz, D_2O) δ [ppm]: 1.55, 0.00, -3.86. **^{31}P[^{1}H]-NMR** (203 MHz, D_2O) δ [ppm]: 2.48 – 0.77 (m), 0.51 – -0.63 (m), -2.64 – -5.07 (m). **^{13}C-NMR** (101 MHz, D_2O) δ [ppm]: 75.25, 66.78, 31.96 (2p), 31.18, 29.01, 28.82, 28.77, 28.75, 28.48, 24.59, 22.02, 13.41.^{13}C NMR (126 MHz, D_2O) δ [ppm]: 83.25, 80.30, 66.01, 39.35, 38.40, 37.79, 37.56, 37.30, 37.10, 32.54, 27.85, 24.62, 24.34, 22.60 (2p), 21.73, 21.39 (2p), 19.74 (2p), 19.55. **(-)-HR-ESI-MS**: calculated 570.2124, m/z found 569.2054 [M - H^+].

dodecane-1,2,3-triol (11):

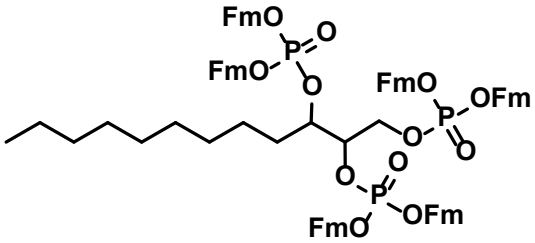


Chemical Formula: $C_{12}H_{26}O_3$
Molecular Weight: 218.34

To the stirred solution of AD-mix-α (7.97 g, 10.2 mM, 1.88 eq.) in a 1:1 mixture of *t*BuOH and H_2O (42 mL) at 0 °C (*E*)-dodec-2-en-1-ol **10** (1.18 mL, 5.43 mmol, 1 eq.) was added and stirred overnight at room temperature. Subsequently the reaction was quenched with a tip of the spatula Na_2SO_3 and stirred for 1 more hour. Then the organic phase was extracted with EtOAc (3 x 20 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The product was purified by column chromatography (Cyclohexane/EtOAc 1 to 100 %) to afford the desired molecule in 42 % yield (0.501 g, 2.29 mmol).

***R**f*:(SiO_2: Cyclohexane:EtOAc 2:8) = 0.17 **^{1}H-NMR** (400 MHz, Chloroform-d) δ [ppm]: 5.70 – 5.47 (m, 2H), 3.97 (d, J = 5.5 Hz, 2H), 3.09 (d, J = 16.1 Hz, 1H), 1.97 (dd, J = 13.9, 6.7 Hz, 2H), 1.39 – 1.14 (m, 14H), 0.82 (t, J = 6.8 Hz, 3H). **^{13}C-NMR** (101 MHz, Chloroform-d) δ [ppm]: 132.87, 128.91, 63.24, 32.21, 31.89, 29.56, 29.51, 29.32, 29.21, 29.17, 22.63, 14.00.

hexakis((9*H*-fluoren-9-yl)methyl) dodecane-1,2,3-triyl tris(phosphate) (12):

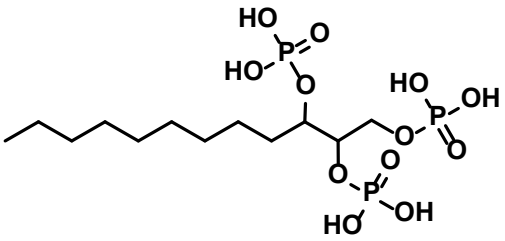


Chemical Formula: $C_{96}H_{89}O_{12}P_3$
Molecular Weight: 1527.68

bis(9-Fluorenylmethyl)diisopropylphosphoramidite (5.49 g, 10.05 mmol, 3.6 eq.) and triol **11** (0.64 g, 2.93 mmol, 1 eq.) were coevaporated with dry CH_2Cl_2/MeCN (3 x 5 mL). Under nitrogen atmosphere the two starting materials were subsequently dissolved in a 1:1 MeCN and CH_2Cl_2 mixture (40 mL). Then 5-phenyl-1*H*-tetrazole solution (1.71 g, 11.7 mmol, 4 eq.) was added at room temperature before the reaction was stirred for 3.5 hours. After ^{31}P-NMR showed the product, the reaction mixture was cooled to 0 °C and *t*-butyl hydroperoxide solution (4.04 mL, 5.5 M in decane, 1.5 eq.) was added. After 20 minutes of stirring the mixture was warmed to room temperature and half of the solvents were evaporated at high vacuum. After dilution with EtOAc (50 mL) the organic phase was washed with brine (3 x 50 mL), dried over $MgSO_4$ and subsequently applied to vacuum. Then the compound was purified by column chromatography (Cyclohexane/EtOAc 0 to 60 %) to yield **12** (0.71 g, 0.468 mmol, 30 %).

***R*$_f$**:(SiO_2: Cyclohexane:EtOAc 4:6) = 0.54 **^{1}H-NMR** (400 MHz, Chloroform-d, with EtOAc 4.13, 2.08, 1.29 and H_2O 1.47) δ [ppm]: 7.74 – 7.60 (m, 12H), 7.44 (dt, J = 6.4, 5.2 Hz, 12H), 7.35 – 7.24 (m, 12H), 7.23 – 7.09 (m, 12H), 4.63 – 4.39 (m, 2H), 4.31 – 4.15 (m, 14H), 4.10 – 3.94 (m, 6H), 1.66 – 1.48 (m, 2H), 1.29 – 1.10 (m, 14H), 0.91 (t, J = 7.0 Hz, 3H). **^{1}P-NMR** (162 MHz, Chloroform-d) δ [ppm]: -1.56, -1.92. **^{31}P[^{1}H]-NMR** (162 MHz, Chloroform-d) δ [ppm]: -1.56 (dt seen as hept.), -1.92 (dd seen as hex.). **^{13}C-NMR** (101 MHz, Chloroform-d, with EtOAc 171.18, 65.98, 14.16) δ [ppm]: 142.94 (mp), 141.85 – 140.93 (mp), 127.84, 127.09, 125.36 – 124.89 (mp), 119.93 (2p), 69.70 – 69.02 (mp), 65.98, 60.42, 47.98 – 47.67 (mp), 31.91, 29.64, 29.56 (2p), 29.35, 26.94, 24.75, 22.72, 21.07, 14.23.

dodecane-1,2,3-triyl tris(dihydrogen phosphate) (13):

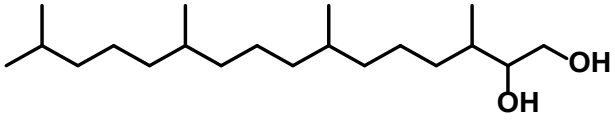


Chemical Formula: $C_{12}H_{29}O_{12}P_3$
Molecular Weight: 458.27

Triphosphate **12** (0.71 g, 0.462 mmol, 1 eq.) was dissolved in a 10 % piperidine solution in CH_2Cl_2 and stirred for 2.5 hours at room temperature. Subsequently the solvents were removed under reduced pressure, then the remainings were dissolved in Et_2O (20 mL) and 0.5 M NaOH solution (20 mL). The water soluble molecule was extracted with water (3 x 20 mL), concentrated by freeze-drying and purified by Q-Sepharose® (25 mM to 1 M NH_4HCO_3 in H_2O). After three rounds of dissolving in water and freeze-drying the final product was obtained as a white powder (189 mg, 0.412 mmol, 90 %).

^{1}H-NMR (400 MHz, D_2O) δ [ppm]: 4.24 – 4.13 (m, 1H), 3.92 – 3.77 (m, 1H), 1.71 – 1.43 (m, 1H), 1.38 – 1.10 (m, 14H), 0.77 (t, J = 7.0 Hz, 3H). **^{31}P-NMR** (162 MHz, D_2O) δ [ppm]: 0.59, -0.26, -0.43. **^{31}P[^{1}H]-NMR** (162 MHz, D_2O) δ [ppm]: 0.59 (t, J = 4.3 Hz), -0.31 (d, J = 8.9 Hz), -0.54 (d, J = 8.6 Hz). **^{13}C-NMR** (126 MHz, D_2O) δ [ppm]: 75.54, 75.36, 64.39, 31.18, 30.70, 30.20, 28.84, 28.76 (2p), 28.50, 24.64, 22.04, 13.40. **(-)-HR-ESI-MS**: calculated 458.0872, m/z found 457.0795 [M - H^+].

3,7,11,15-tetramethylhexadecane-1,2-diol (15):



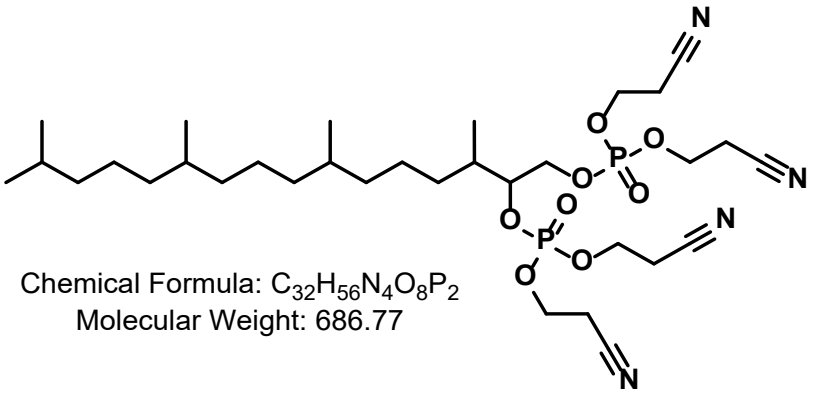
Chemical Formula: $C_{20}H_{42}O_2$
Molecular Weight: 314.55

To phytol (0.17 mL, 0.50 mmol, 1 eq.), dissolved in dry THF (2 mL), $Me_2S{\cdot}BH_3$ (2 M in THF, 0.25 mL) was dropwise added at 0 °C and under nitrogen atmosphere. After the addition, the reaction was stirred at room temperature for 4 hours before it was cooled to 0 °C again. As soon as this temperature was reached aq. NaOH (3 M, 0.5 mL) and then aq.

H_2O_2 (30 %, 0.7 mL) were added slowly. After another stirring for 30 minutes at room temperature the reaction mixture was diluted with EtOAc (10 mL) and the organic phase was washed with brine (3 x 10 mL). Subsequently this layer was dried over $MgSO_4$ and the solvents were removed *in vacuo*. Then the compound was purified by chromatography (CH_2Cl_2/EtOAc 0 to 70 %) affording a yellowish oil (0.130 g, 0.41 mmol, 72 %).

R_f:(SiO_2: CH_2Cl_2:EtOAc 7:3) = 0.29; **^{1}H-NMR** (400 MHz, Chloroform-d) δ [ppm]: 3.69 – 3.60 (m, 1H), 3.55 (s, 2H), 3.51 – 3.43 (m, 2H), 1.59 – 0.99 (m, 22H), 0.90 – 0.79 (m, 15H); **^{13}C-NMR** (101 MHz, Chloroform-d) δ [ppm]: 76.30 (2p), 64.57, 39.37, 37.57, 37.52 – 37.24 (mp), 36.21 (2p), 32.94 – 32.62 (mp), 27.96, 24.79, 24.65 – 24.34 (mp), 22.71, 22.62, 19.76, 19.74, 19.63, 15.21 (2p). **(+)-HR-ESI-MS**: calculated 314.3185, m/z found 337.3075 [M + Na^+].

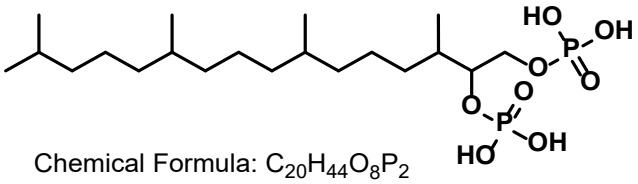
tetrakis(2-cyanoethyl) (3,7,11,15-tetramethylhexadecane-1,2-diyl) bis(phosphate) (16):



Bis(2-cyanoethyl)diisopropylphosphoramidite **1** (269 mg, 0.99 mmol, 2.4 eq.) and **15** (130 mg, 0.41 mmol, 1 eq.) were dissolved in MeCN (0.33 mL), then the solvent was evaporated under high vacuum. This was repeated twice before the mixture was dissolved in DMF (3.3 mL). Then 5-phenyl-1*H*-tetrazole (181 mg, 1.24 mmol, 3 eq.) was added and the reaction mixture was stirred for 4 hours at room temperature. As soon as ^{31}P-NMR showed full conversion, the mixture was cooled to 0 °C and *t*-butyl hydroperoxide solution (0.49 mL, 5.5 M in decane, 3 eq.) was added. After 20 minutes of stirring the mixture was warmed to room temperature and half of the solvents were evaporated at high vacuum. Subsequently the remaining mixture was diluted with EtOAc (10 mL) and the organic phase was washed with brine (3 x 10 mL), dried over $MgSO_4$ and applied to vacuum. The compound was purified by column chromatography (EtOAc/MeOH 0 to 20 %) to yield **16** as a colourless oil (101 mg, 0.15 mmol, 36 %).

R_f:(SiO_2: EtOAc:MeOH 99:1) = 0.16 **^{1}H-NMR** (400 MHz, Chloroform-d) δ [ppm]: 4.58 – 4.49 (m, 1H), 4.42 – 4.14 (m, 10H), 2.91 – 2.74 (m, 8H), 1.58 – 1.46 (m, 1H), 1.45 – 0.97 (m, 21H), 0.89 – 0.81 (m, 12H). **^{31}P-NMR** (162 MHz, Chloroform-d) δ [ppm]: -2.34, -2.84. **^{31}P[^{1}H]-NMR** (162 MHz, Chloroform-d) δ [ppm]: -2.34 (dt, J = 14.5, 7.3 Hz), -2.84 (dd, J = 15.2, 7.5 Hz). **^{13}C-NMR** (101 MHz, Chloroform-d) δ [ppm]: 117.40, 117.22, 117.19, 117.04, 83.23 (2p), 68.55, 63.24 – 63.08 (mp), 62.94, 62.90, 39.96, 38.08 (2p), 37.91 (2p), 37.74 (2p), 36.04 (2p), 33.40, 32.92 (2p), 28.57, 25.40, 25.09 (2p), 23.28 (2p), 20.54 – 20.20 (mp), 20.18, 15.27 (2p).

3,7,11,15-tetramethylhexadecane-1,2-diyl bis(dihydrogen phosphate) (17):



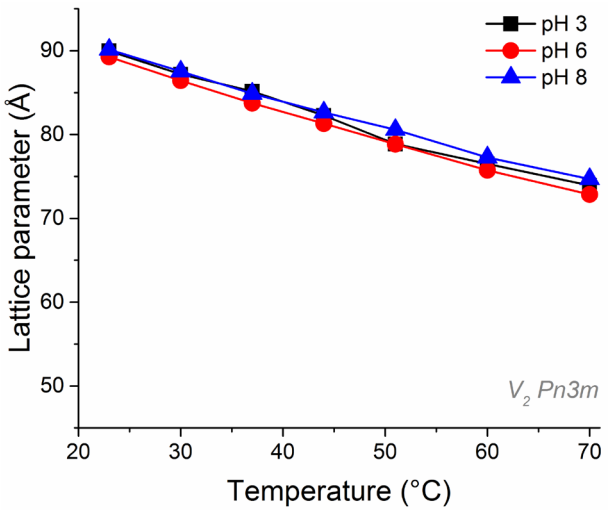
Chemical Formula: $C_{20}H_{44}O_8P_2$
Molecular Weight: 474.51

Diphospholipid **16** (330 mg, 0.481 mmol, 1 eq.) was dissolved in a 1:1 mixture of MeOH and THF (3.7 mL) and after the addition of aq. NH_4OH (25%, 3.9 mL), stirred for 1 hour at room temperature. Subsequently the organic solvents were evaporated and more aq. NH_4OH (25%, 16 mL) was added and the reaction was stirred for 4 hours. at 60 °C. After ^{31}P-NMR showed complete deprotection, the product was freeze-dried. The crude mixture was purified by Q-sepharose® (25 mM to 1 M NH_4HCO_3 in H_2O) to afford a white powder (220 g, 0.464 mmol, 96 %).

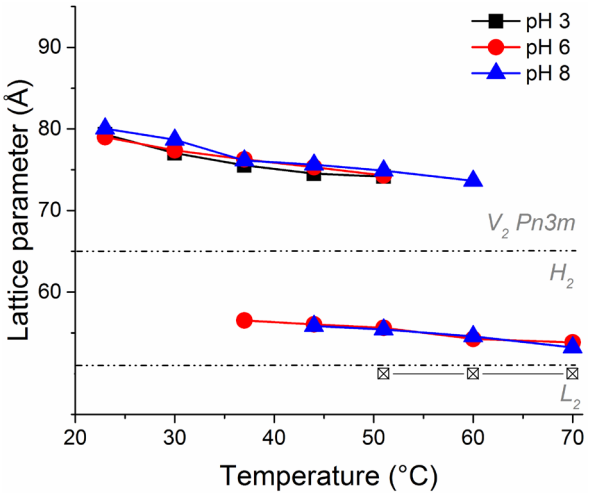
^{1}H-NMR (400 MHz, D_2O) δ [ppm]: 4.08 (s, 1H), 3.96 – 3.81 (m, 2H), 1.77 (s, 1H), 1.53 – 0.97 (m, 1H), 0.93 – 0.73 (m, 15H). **^{31}P-NMR** (162 MHz, D_2O) δ [ppm]: 0.80, -0.21. **^{31}P[^{1}H]-NMR** (162 MHz, D_2O) δ [ppm]: 1.00 – 0.60 (m), -0.22 (d, J = 7.8 Hz). **^{13}C-NMR** (101 MHz, D_2O/methanol-d4) δ [ppm]: 79.52, 67.13, 39.15, 37.57 – 37.13 (mp), 37.05, 32.51, 30.33, 27.67, 24.57, 24.29, 22.41, 22.32, 19.44, 19.32, 19.22, 14.23. **(-)-HR-ESI-MS**: calculated 474.2511, m/z found 473.2430 [M - H^+], 495.2252 [M - 2 H^+ + Na^+].

Bulk phase behaviour

A. MO

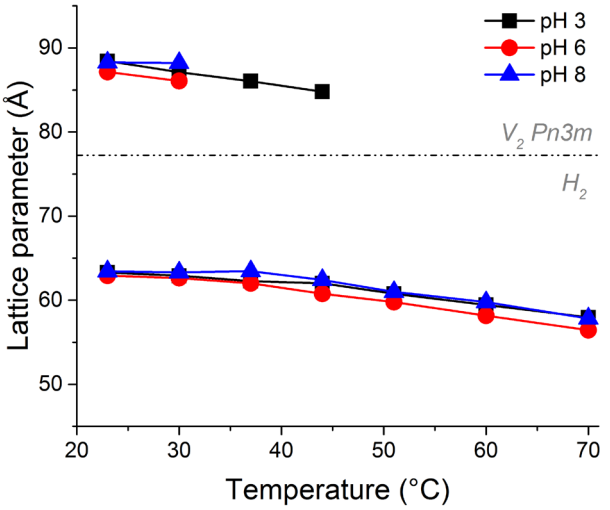


B. MO + C12P1 0.5 mol%



☒ indicates the formation of inverse micelles (L2) which do not exhibit long range order.

C. MO + C12P1 1 mol%



D. MO + C12P1 2 mol%

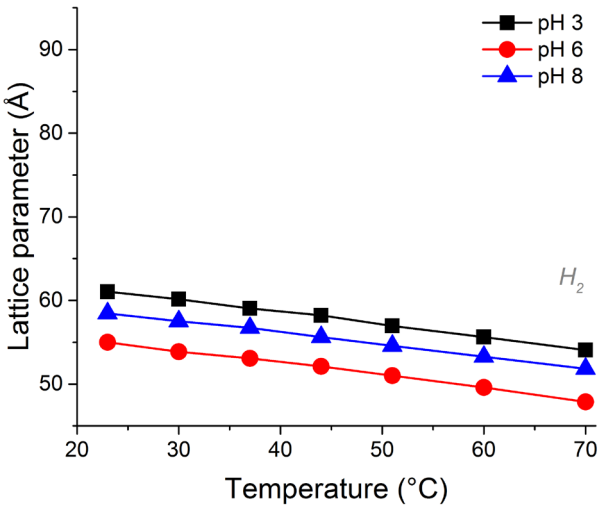


Fig. SI1. Composition-, temperature- and pH-dependent phase behaviour of the singly phosphorylated C12 guest lipid (C12P1) in host monoolein bulk phase formulations in excess water as determined by SAXS. Increasing amounts of C12P1 additives result in the formation of inverse hexagonal phase (H_2) at various pH values.

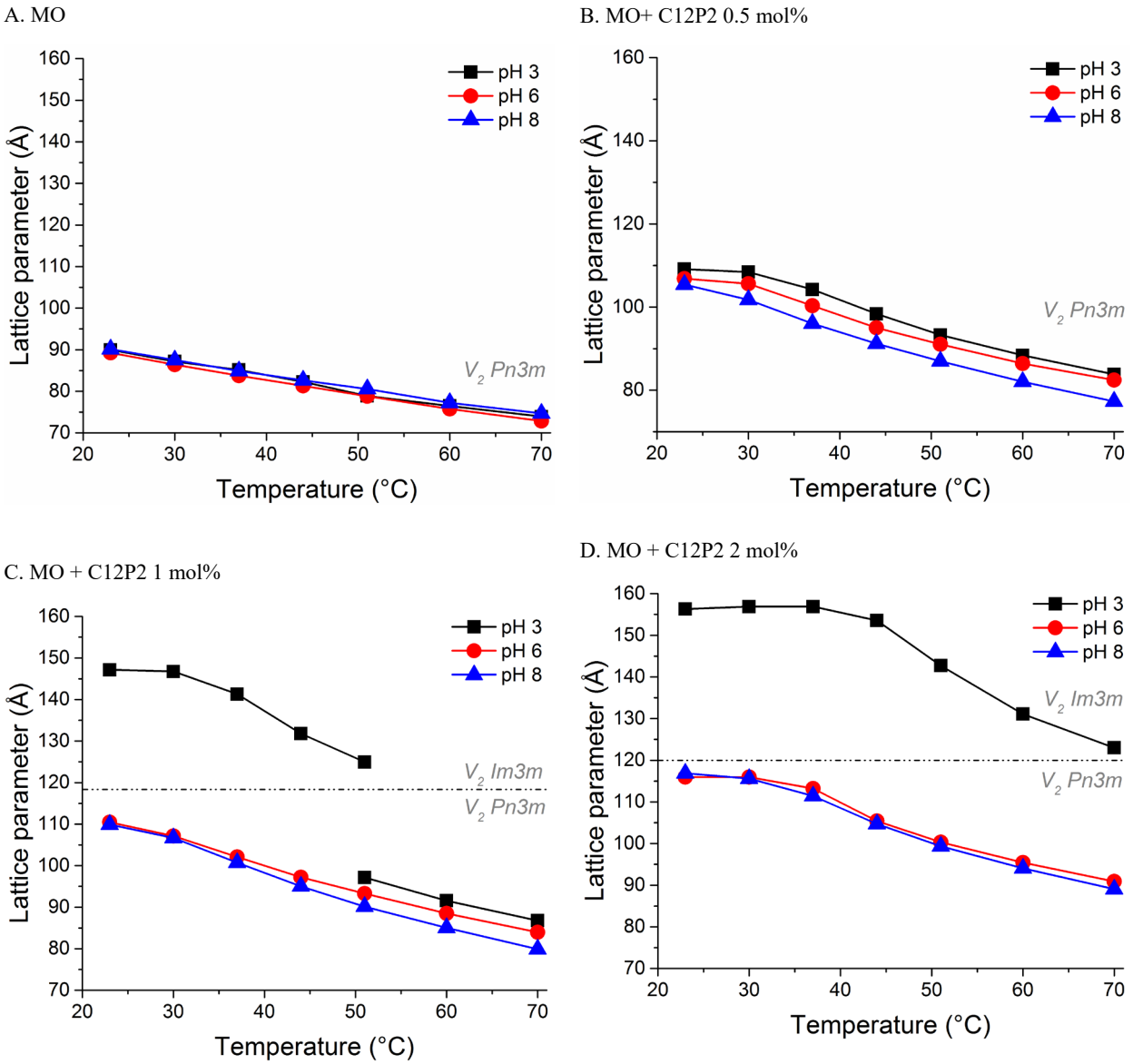


Fig. SI2. Composition-, temperature- and pH-dependent phase behaviour of the doubly phosphorylated C12 guest lipid (C12P2) in host monoolein bulk phase formulations in excess water as determined by SAXS. Increasing amounts of C12P2 additives result in swelling of the unit cells, as demonstrated by the increase in lattice parameter, followed by the formation of $V_{2\ Im3m}$ at <1 mol% (at pH 3 only).

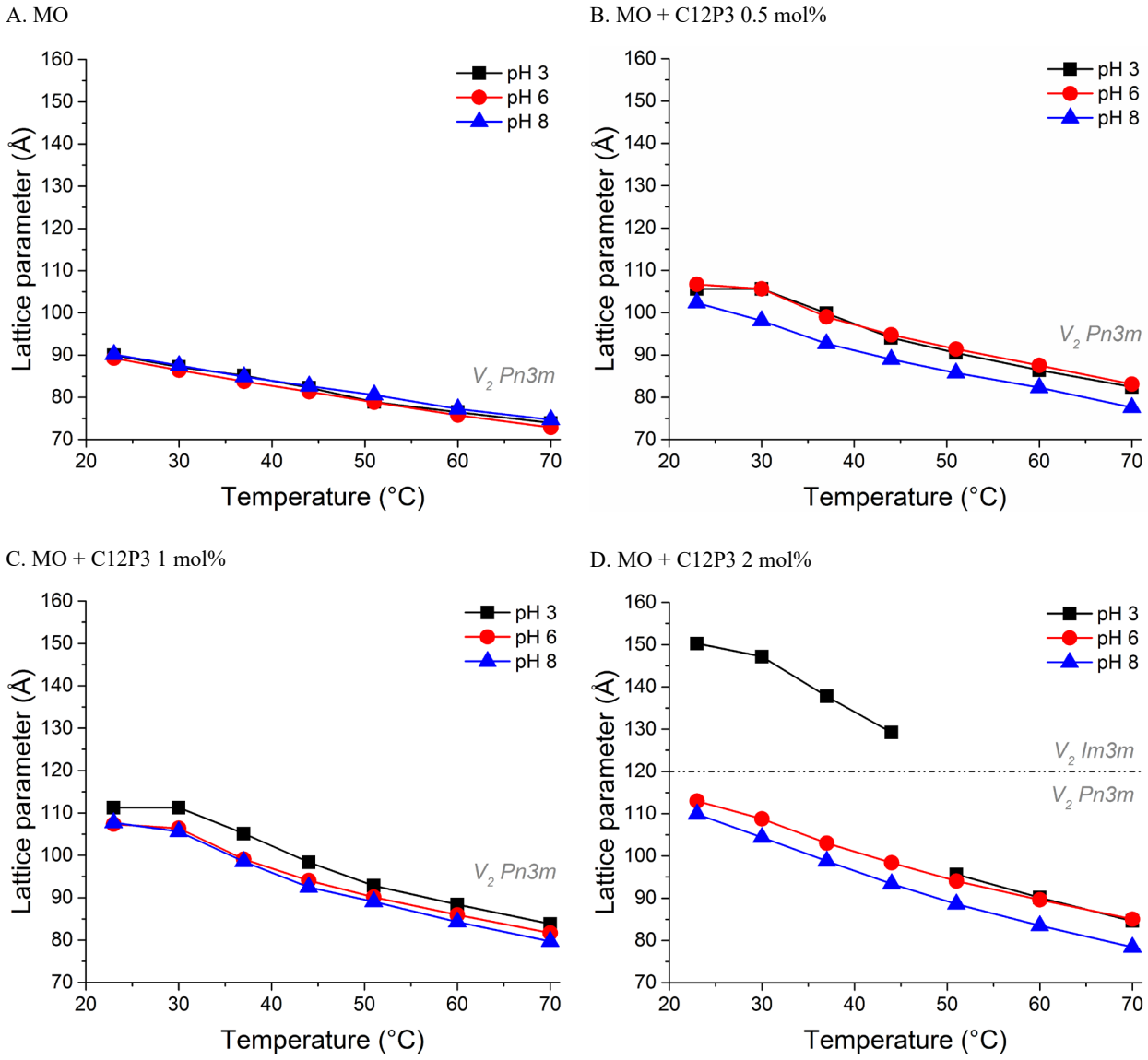


Fig. SI3. Composition-, temperature- and pH-dependent phase behaviour of the triply phosphorylated C12 guest lipid (C12P3) in host monoolein bulk phase formulations in excess water as determined by SAXS. Increasing amounts of C12P3 additives result in swelling of the unit cells, as demonstrated by the increase in lattice parameter, followed by the formation of V2 Im3m at 2 mol% (at pH 3 only).

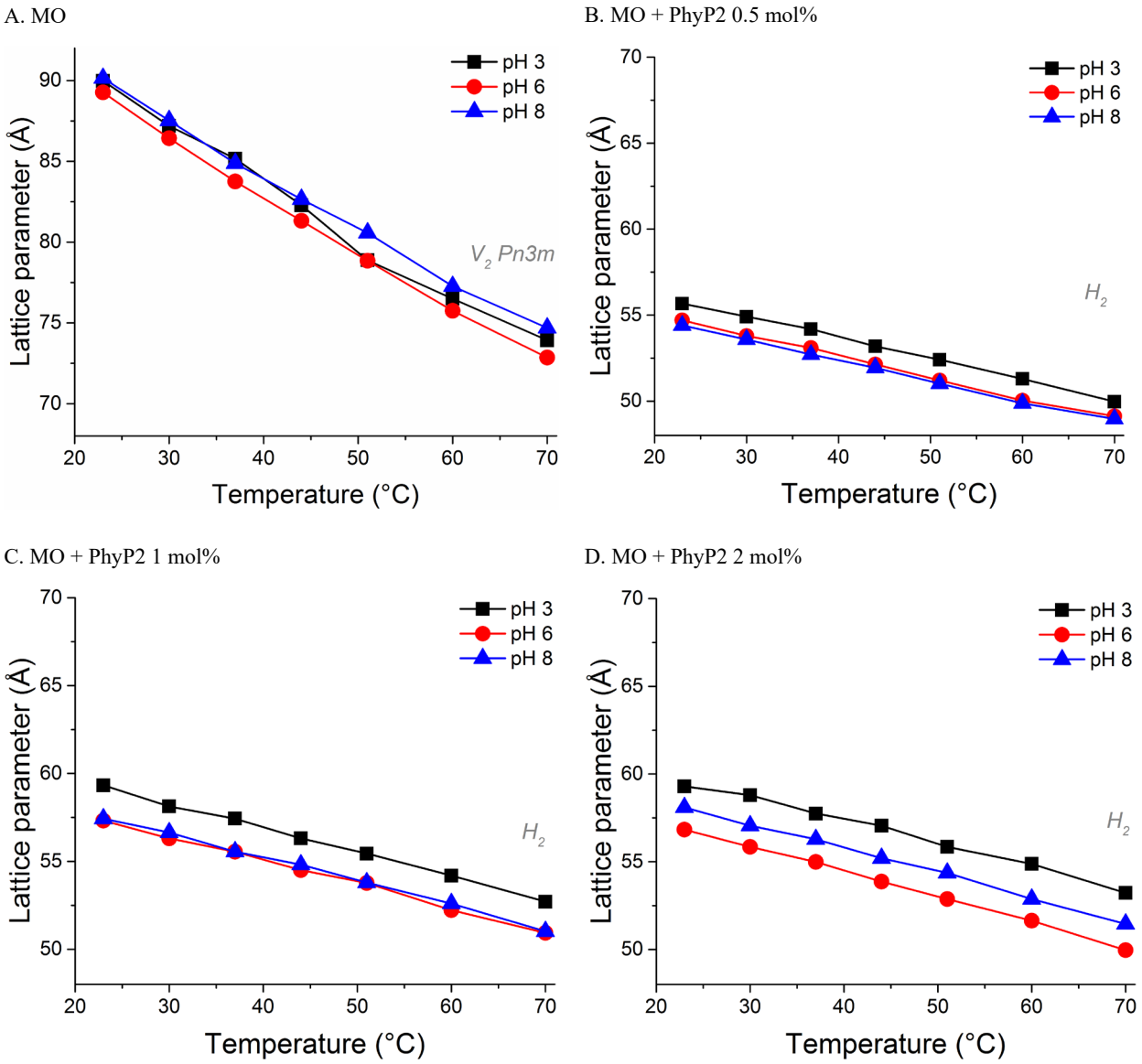
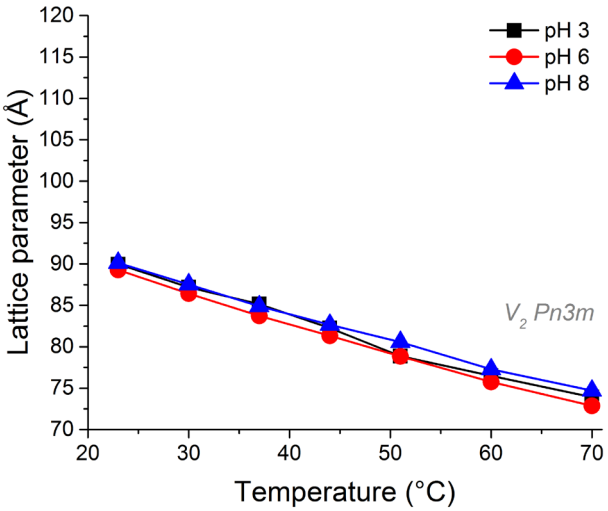
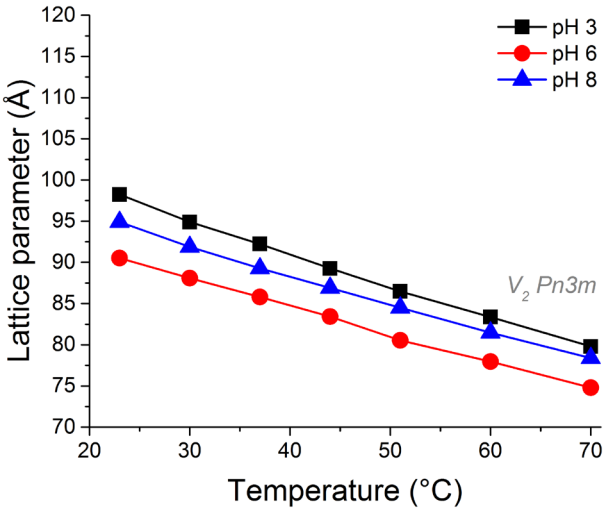


Fig. SI4. Composition-, temperature- and pH-dependent phase behaviour of the doubly phosphorylated phytol guest lipid (PhyP2) in host monoolein bulk phase formulations in excess water as determined by SAXS. Increasing amounts of the PhyP2 additive result in formation of inverse hexagonal phase (H_2) at various pH values.

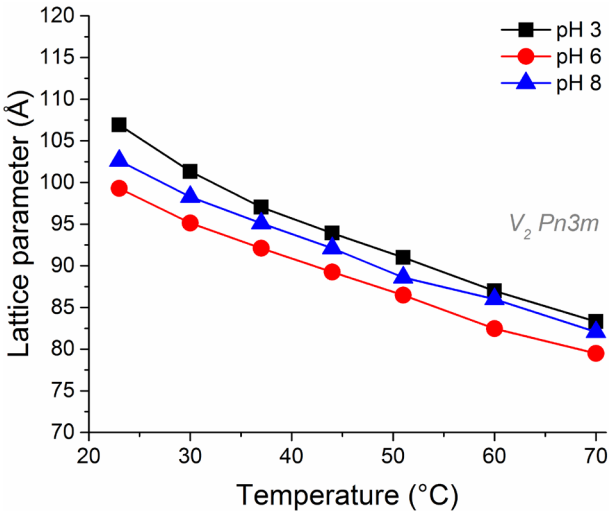
A. MO



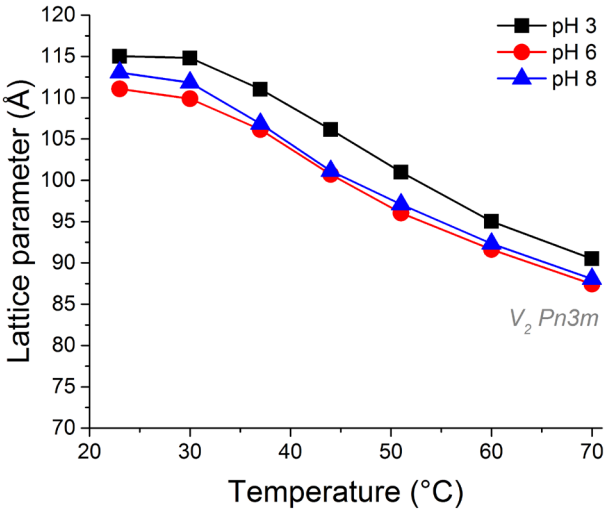
B. MO + PhyP3 0.5 mol%



C. MO + PhyP3 1 mol%



D. MO + PhyP3 2 mol%



E. MO + PhyP3 2.5 mol%

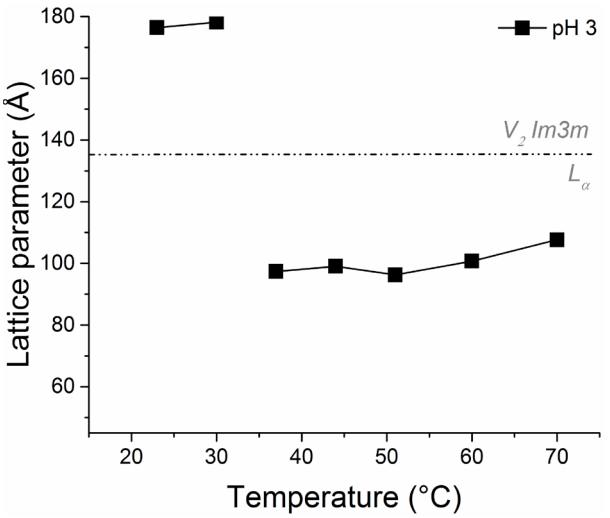


Fig. SI5. Composition-, temperature- and pH-dependent phase behaviour of the triply phosphorylated phytol guest lipid (PhyP3) in host monoolein bulk phase formulations in excess water as determined by SAXS. Increasing amounts of the PhyP3 additive result in swelling of the unit cells, as demonstrated by the increase in lattice parameter. No phase transitions were observed until 2.5 mol%. E: The lattice parameters of the vesicles formed at pH 6 and pH 8 could not be determined as they did not exhibit Bragg peaks characteristic of multilamellar systems.

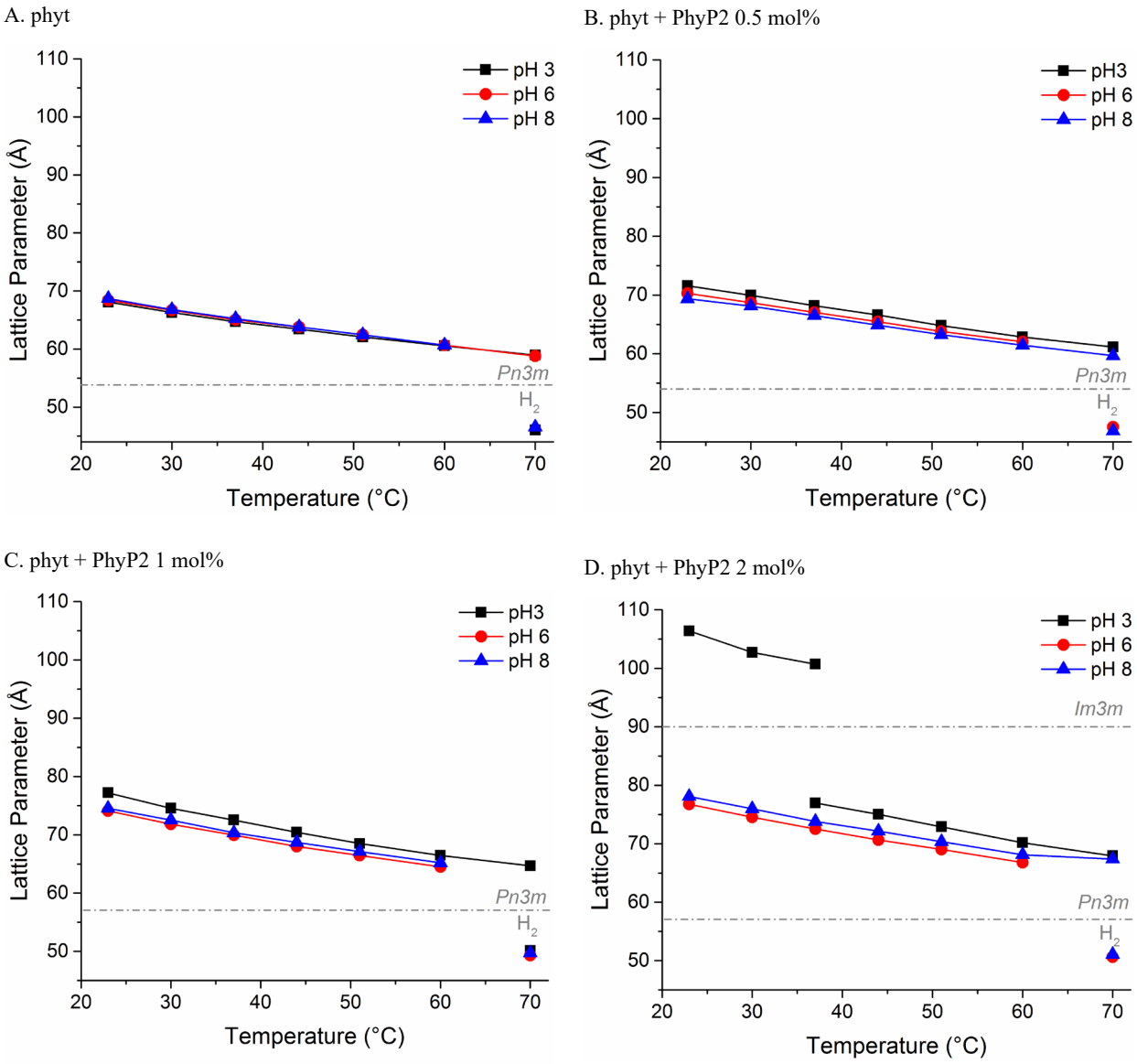


Fig. SI6. Composition-, temperature- and pH-dependent phase behaviour of the doubly phosphorylated phytol guest lipid (PhyP2) in host phytantriol bulk phase formulations in excess water. Increasing amounts of the PhyP2 additive result in swelling of the unit cells, as demonstrated by the increase in lattice parameter, followed by the formation of $V_{2\ Im3m}$ at 2 mol% (at pH 3 only).

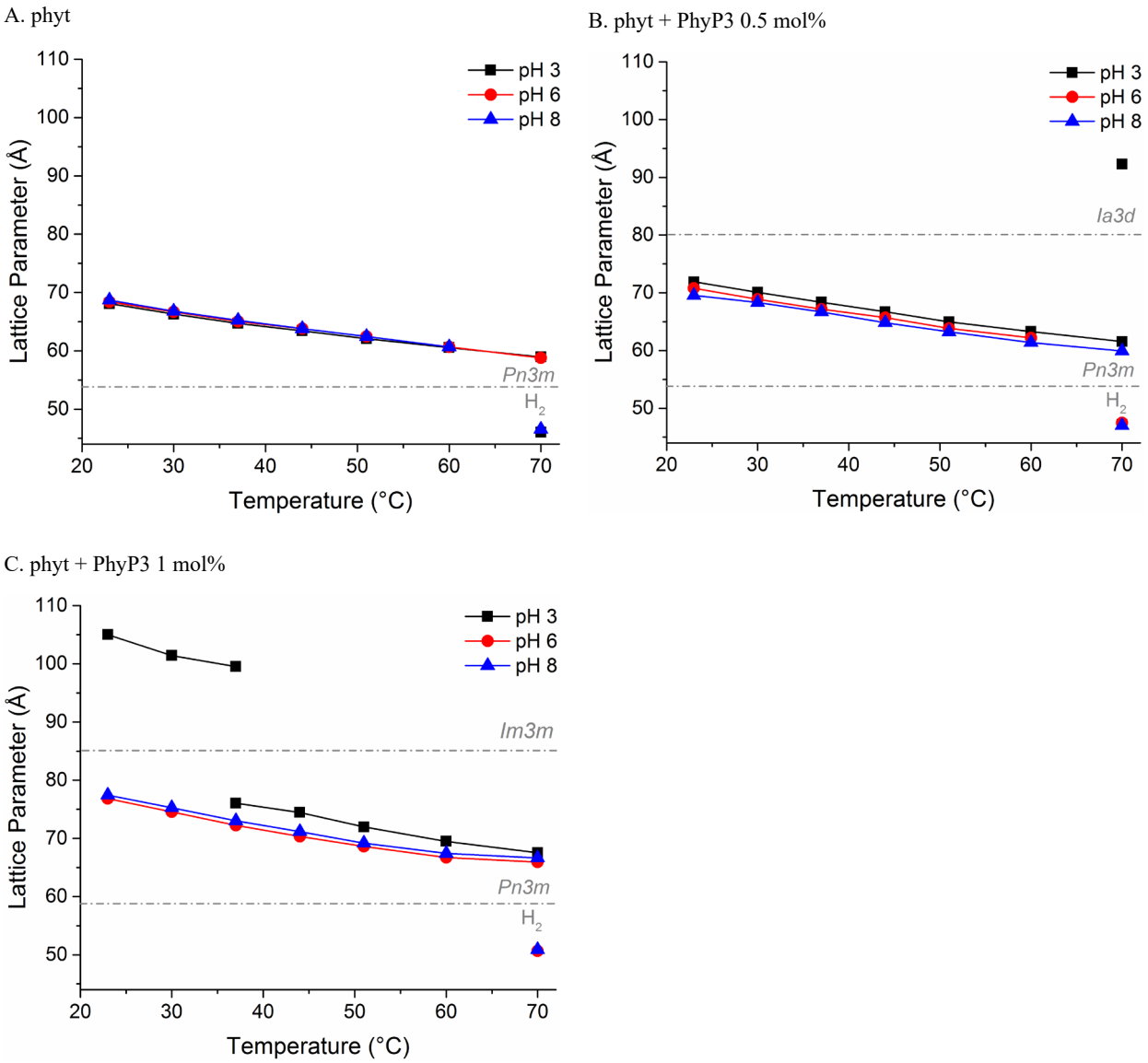
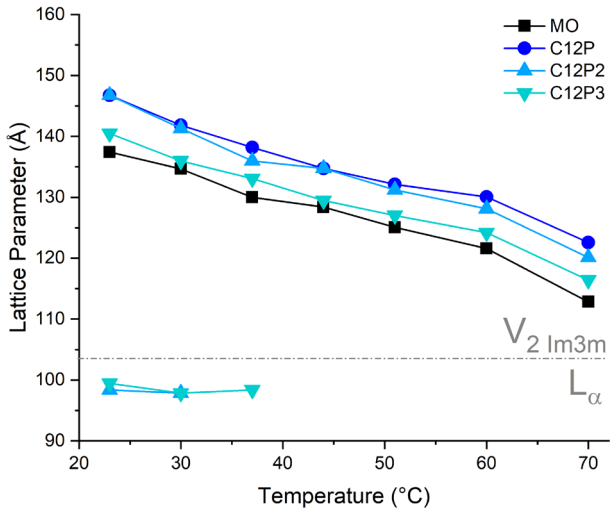


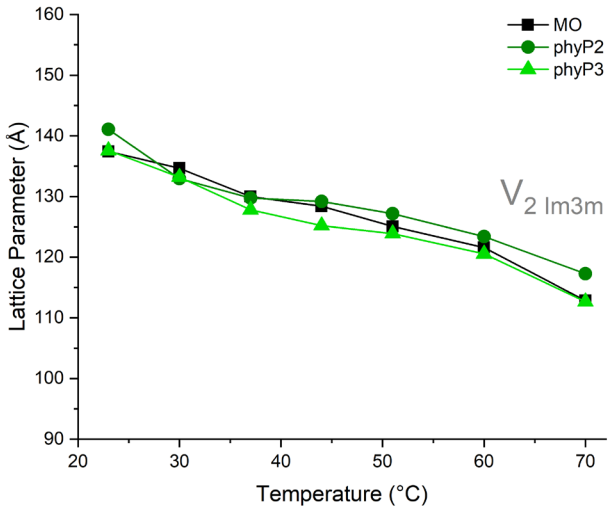
Fig. SI7. Composition-, temperature- and pH-dependent phase behaviour of the triply phosphorylated phytol guest lipid (PhyP3) in host phytantriol bulk phase formulations in excess water. Increasing amounts of the PhyP3 additive result in swelling of the unit cells, as demonstrated by the increase in lattice parameter, followed by the formation of $V_{2\ Im3m}$ upon at 1 mol% (at pH 3 only). Unusually, $V_{2\ Ia3d}$ was observed at high temperatures in system containing 0.5 mol%. Note that PhyP3 could not be incorporated into phytantriol at 2 mol% due to its limited solubility in the lipid.

Temperature dependent phase behaviour of phosphorylated additives in cubosomes.

A. MLO + 0.5 mol% C12P1, C12P2 or C12P3 – temperature



B. MLO + 0.5 mol% PhyP2 or PhyP3 – temperature



C. phyt + 0.5 mol% PhyP2 or PhyP3 – temperature

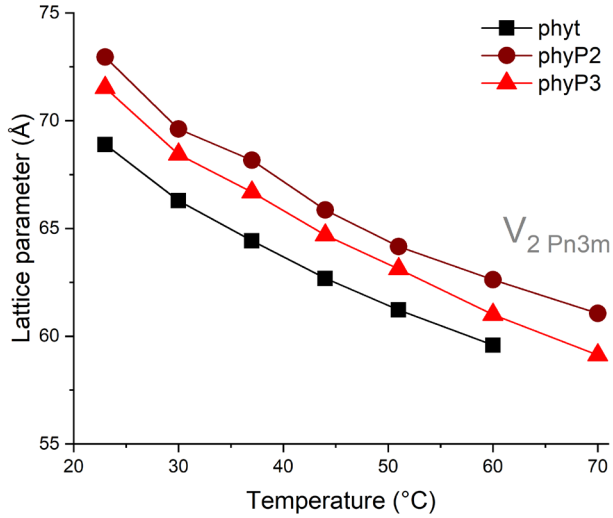


Fig. SI8. Temperature-dependent phase behaviour of cubosomes at pH ~6 as determined by SAXS. Phase boundaries are indicated by dotted lines and annotated accordingly. All formulations contained 0.5% of phosphorylated lipid as additive. Note that in Panel C, phytantriol at 70 °C forms inverse micellar phase; from which a lattice parameter cannot be determined as the mesophase lacks long range order.

Raw SAXS data used to create Fig. 2 & 3, and Fig. SI1-SI8.

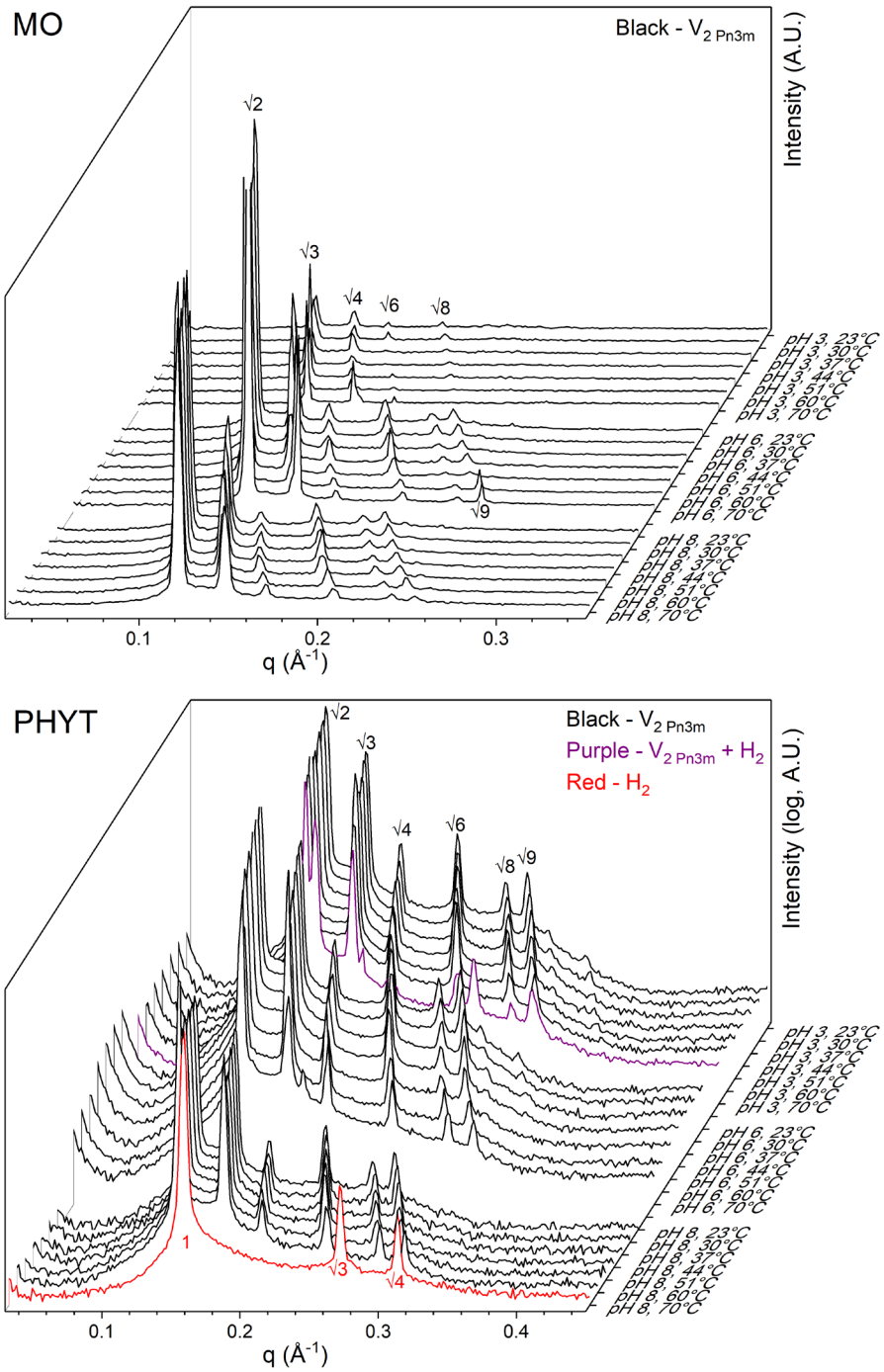


Fig. SI9a. SAXS patterns used to determine the phase behaviour of each sample in Fig. 2

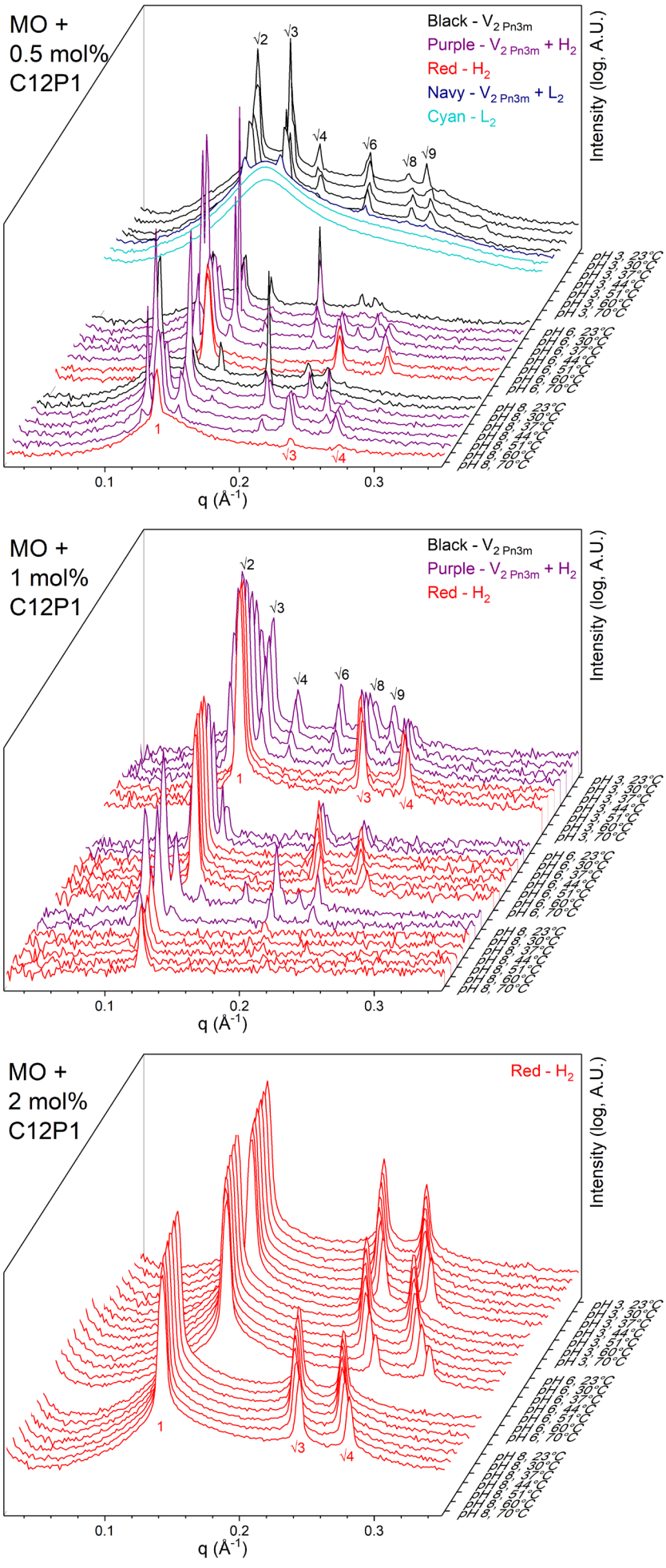


Fig. SI9b. SAXS patterns used to determine the phase behaviour of each sample in Fig. 2

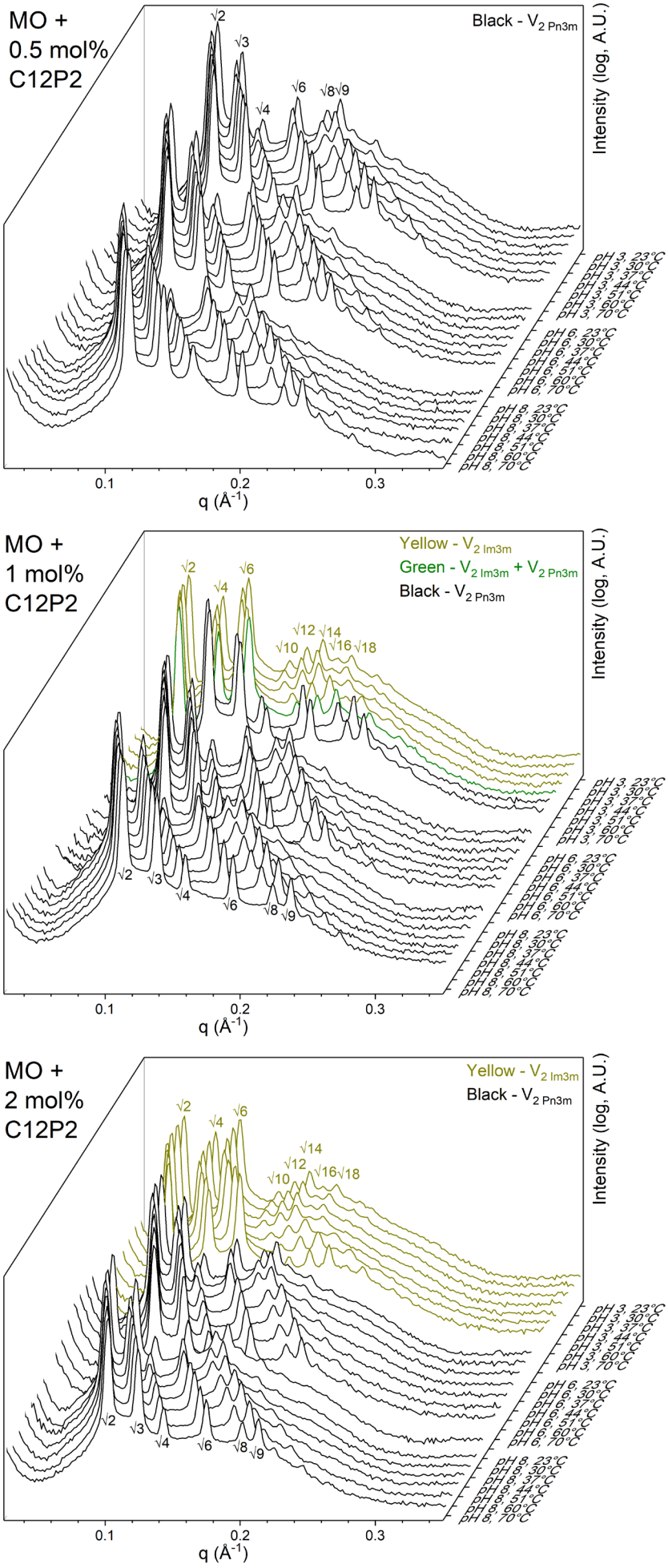


Fig. SI9c. SAXS patterns used to determine the phase behaviour of each sample in Fig. 2

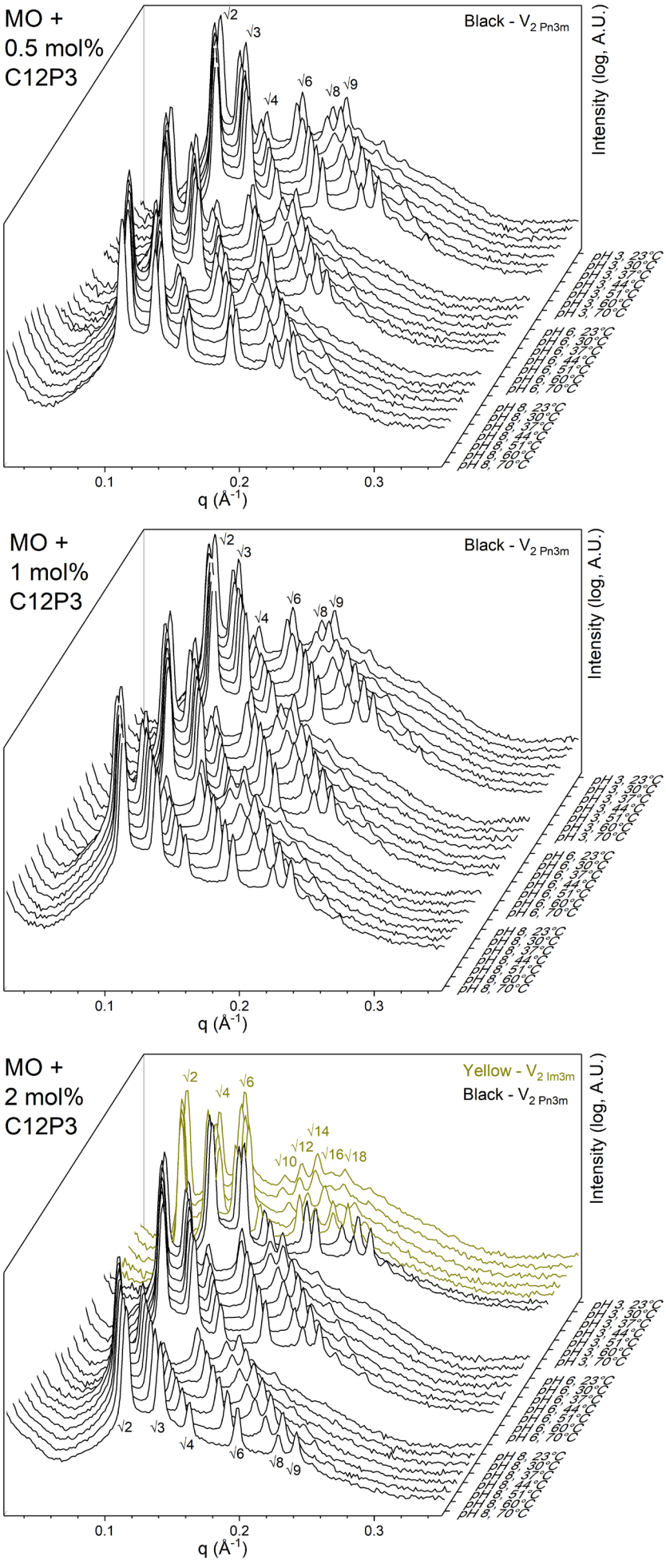


Fig. SI9d. SAXS patterns used to determine the phase behaviour of each sample in Fig. 2

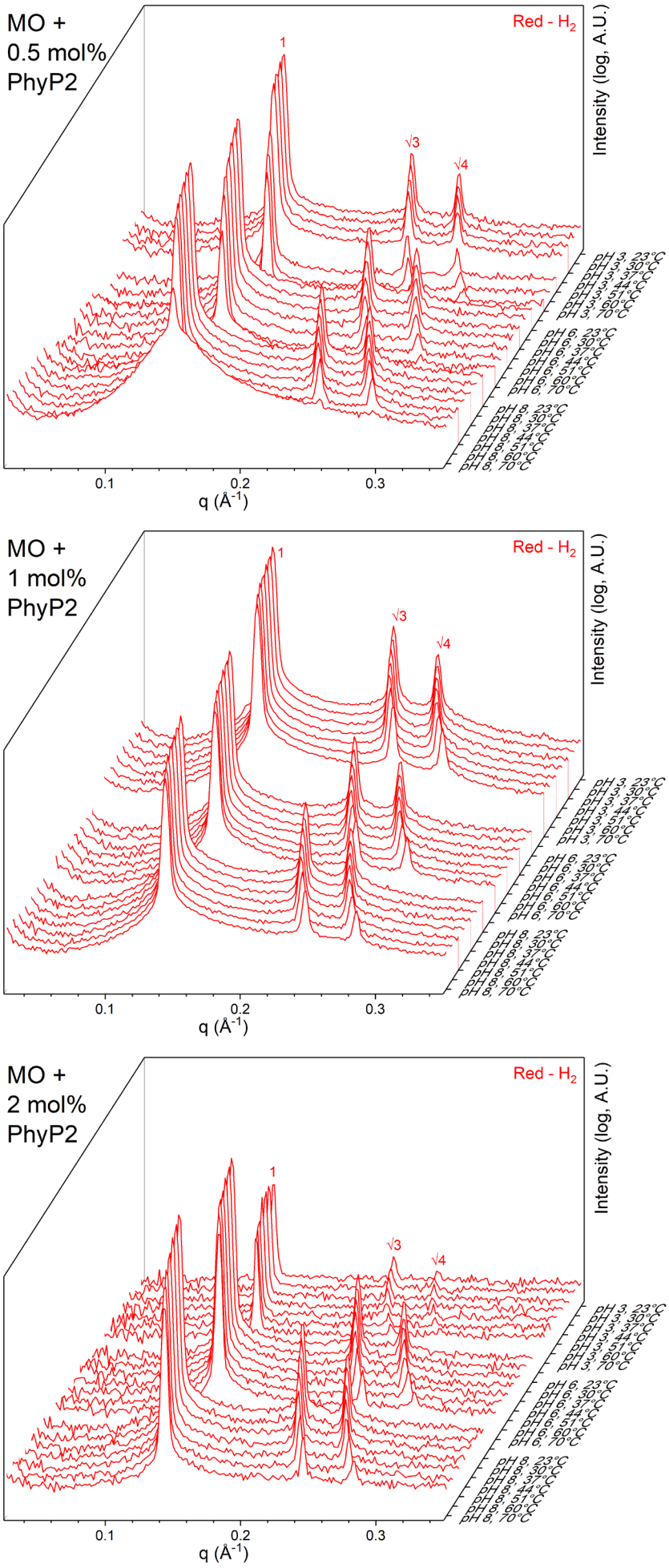


Fig. SI9e. SAXS patterns used to determine the phase behaviour of each sample in Fig. 2

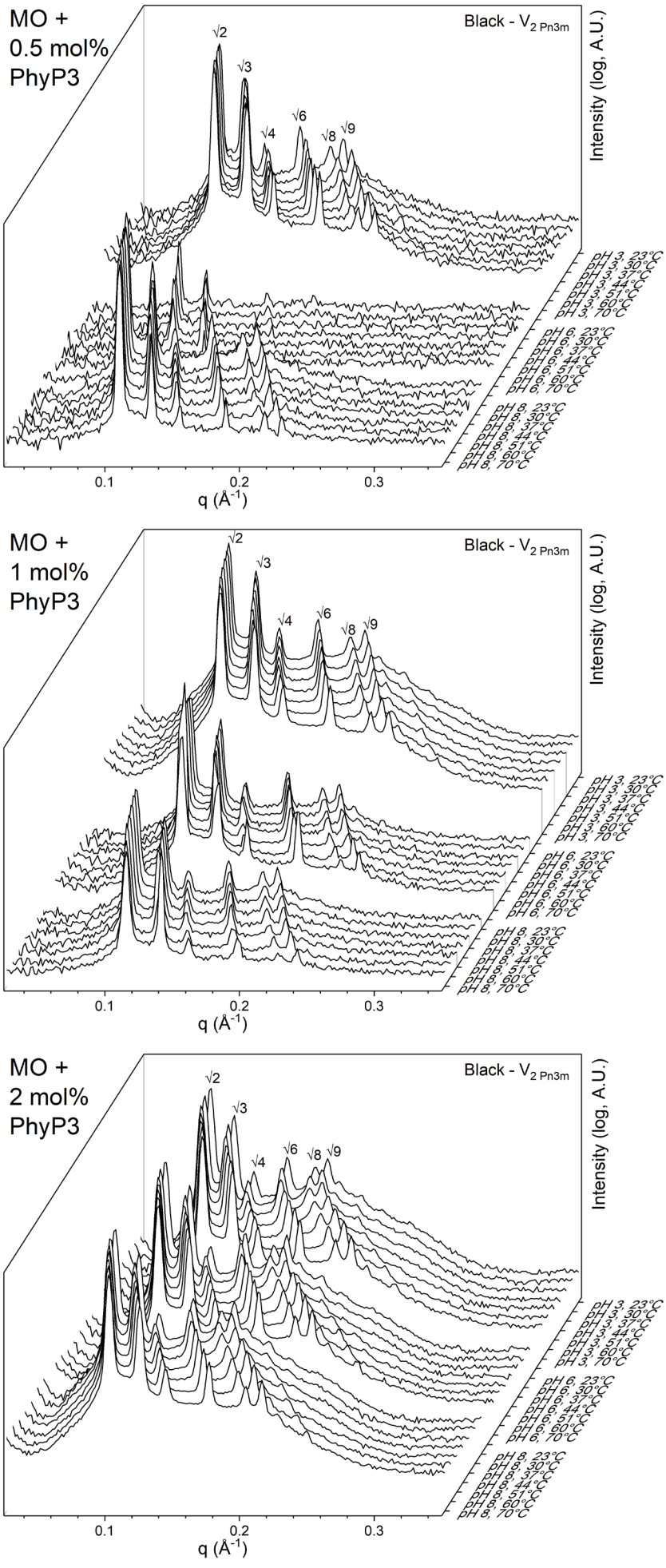


Fig. SI9f. SAXS patterns used to determine the phase behaviour of each sample in Fig. 2

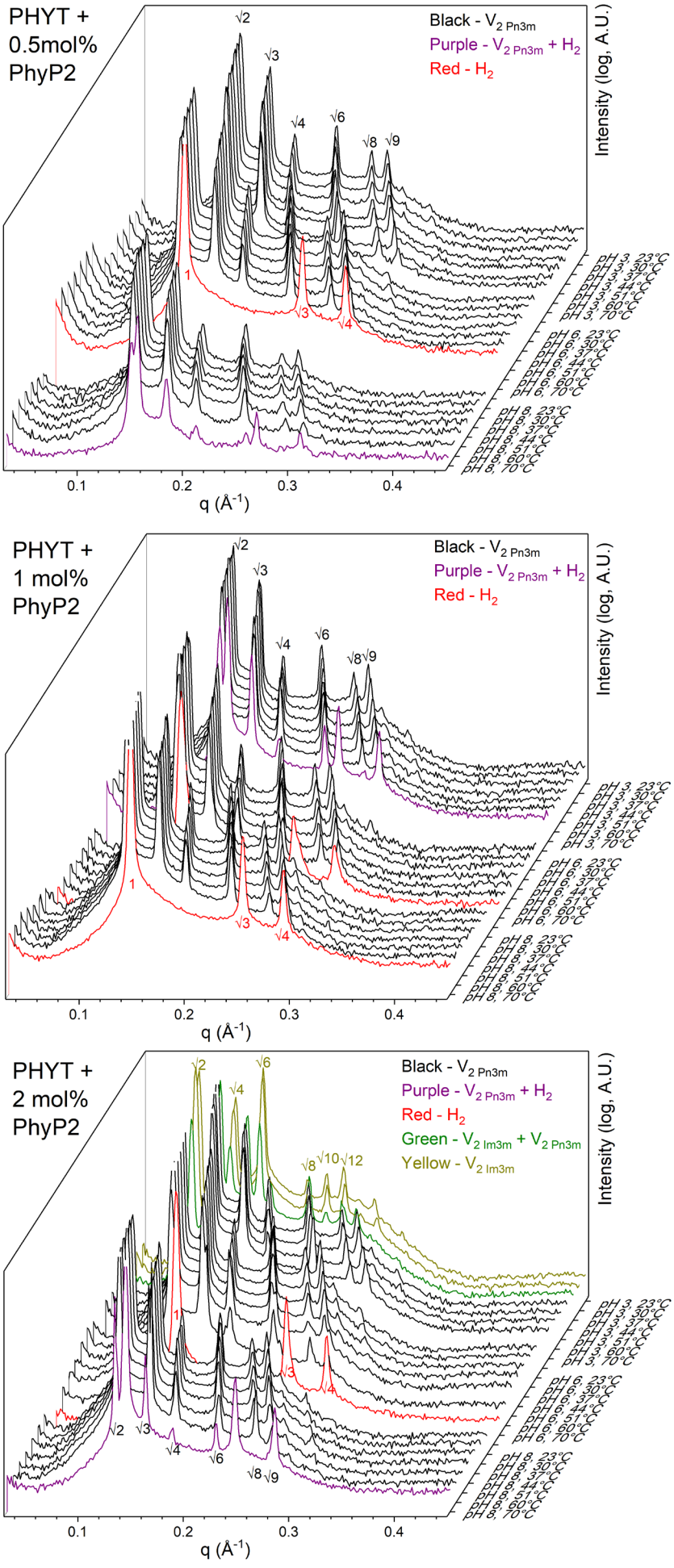


Fig. SI9g. SAXS patterns used to determine the phase behaviour of each sample in Fig. 2

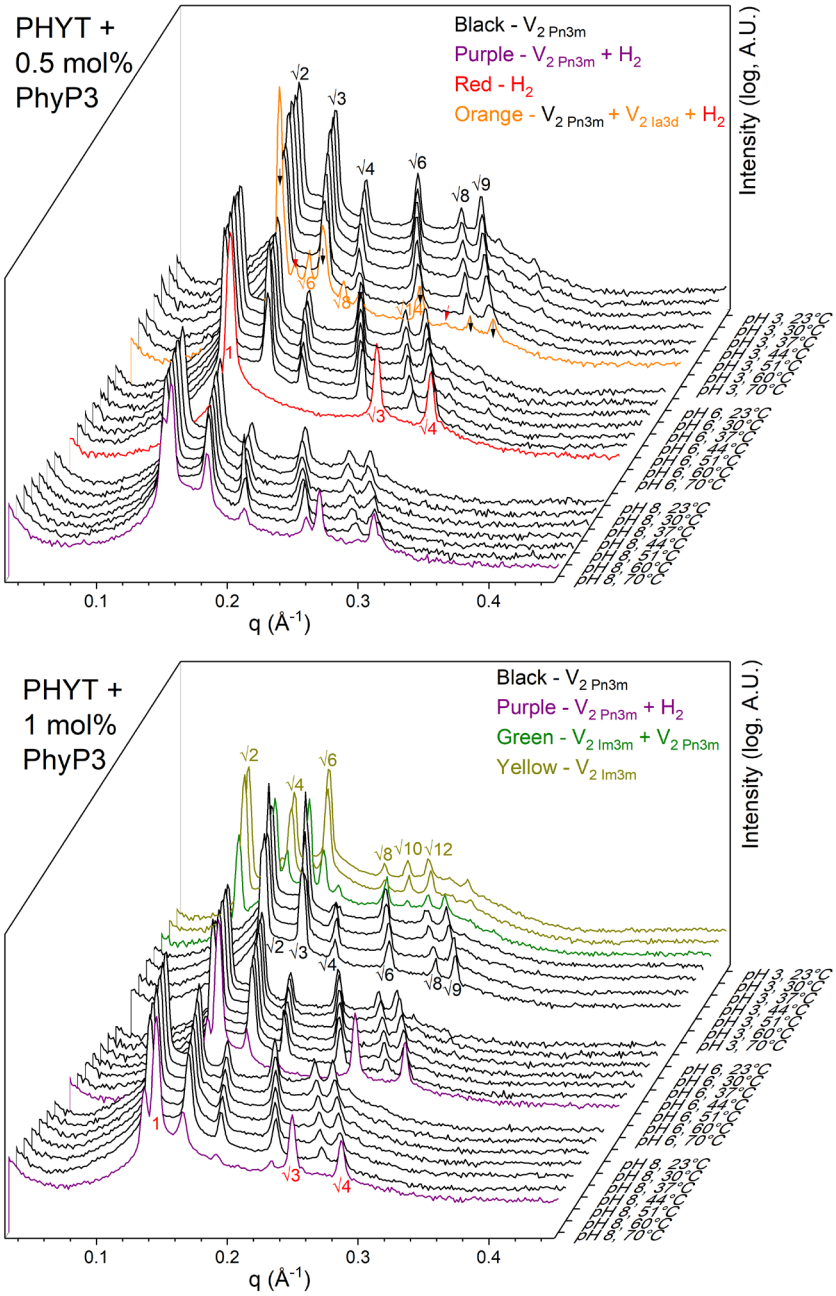


Fig. SI9h. SAXS patterns used to determine the phase behaviour of each sample in Fig. 2. In PHYT + 0.5 mol% PhyP3, at pH 3 and 70°C, black and red arrows are used to indicate the reflections of the $V_{2\ Pn3m}$ and H_2 phases respectively.

SAXS patterns of dispersions

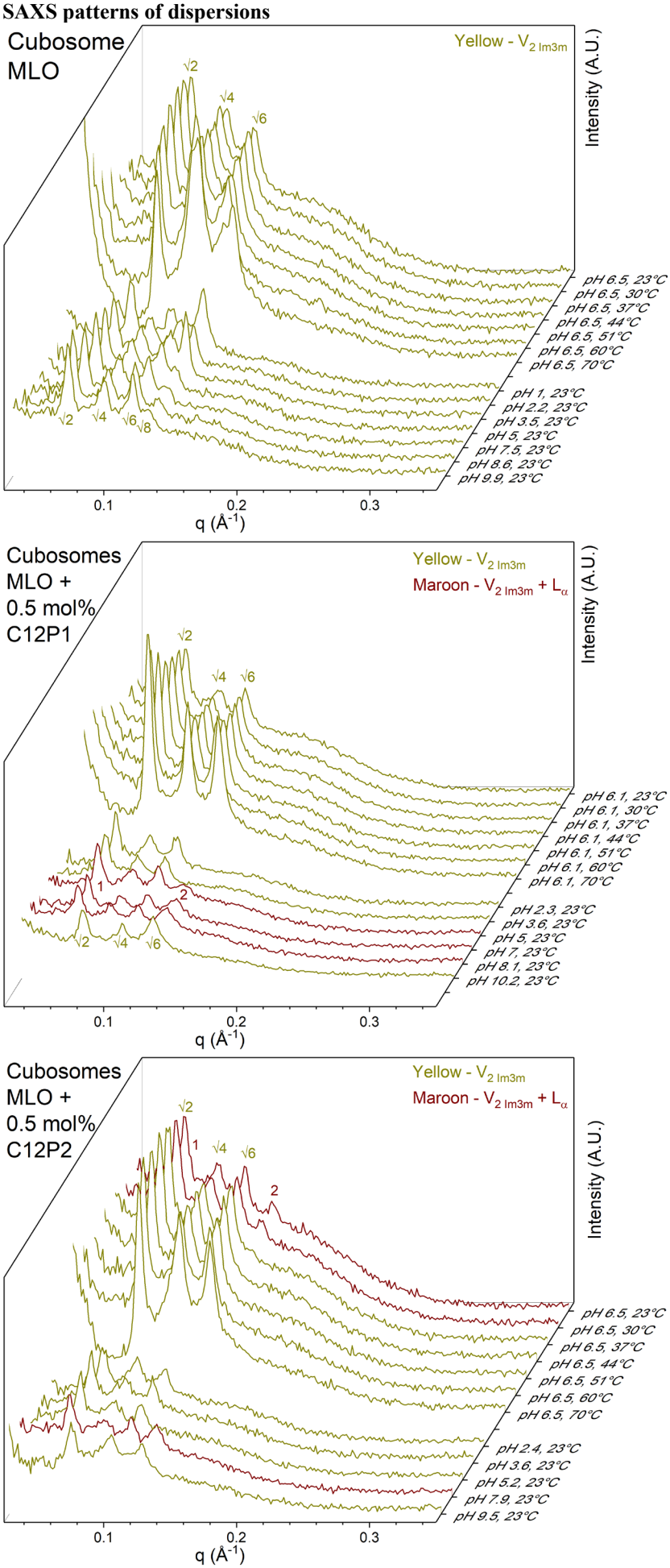
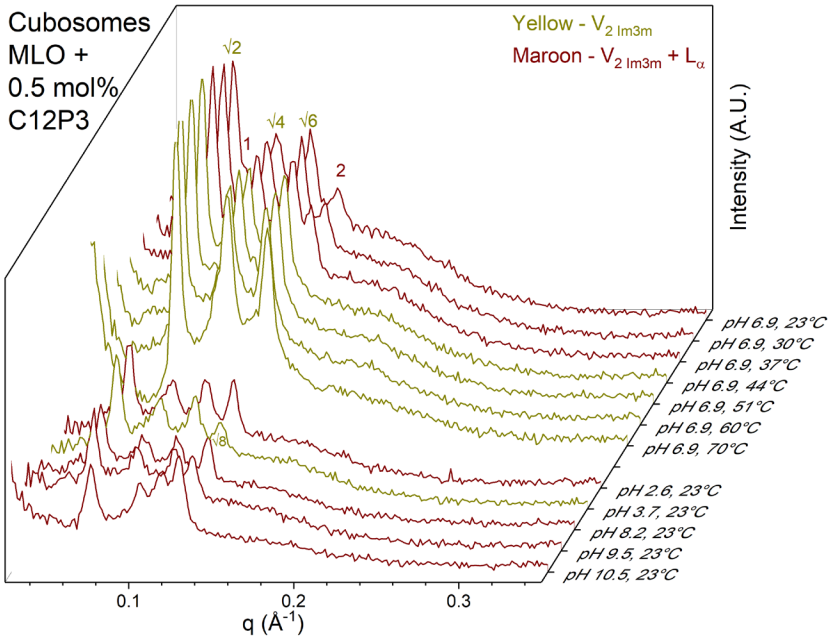
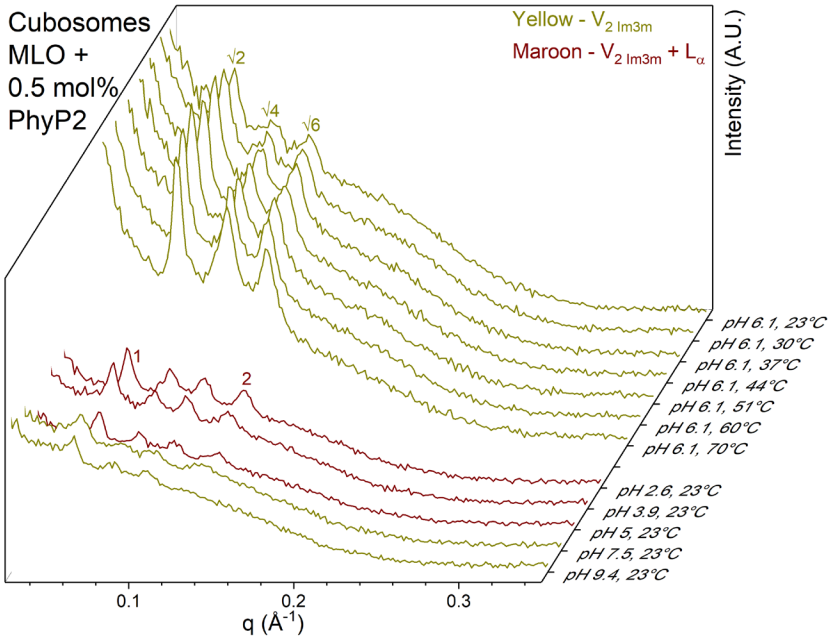


Fig. SI9i. SAXS patterns used to determine the phase behaviour of each sample in Fig. 3 and Fig. SI8.





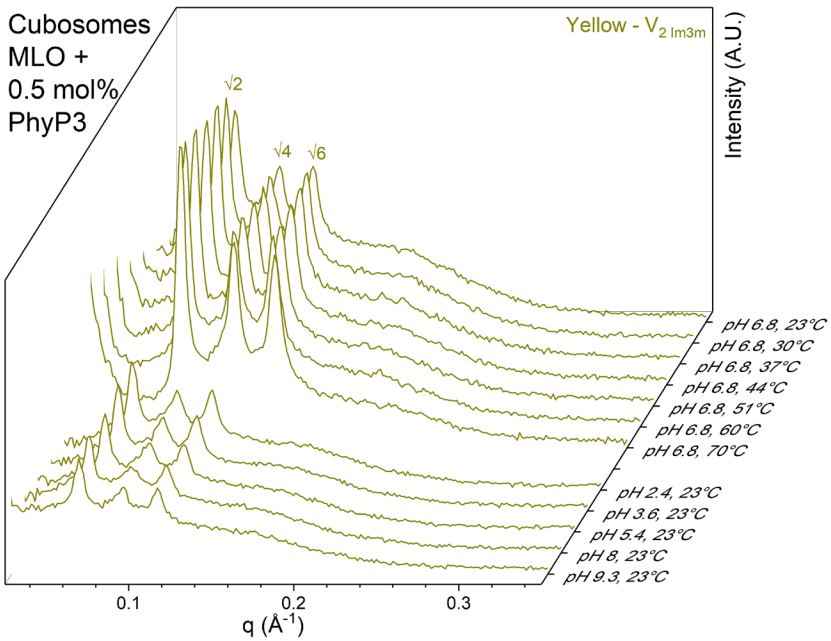
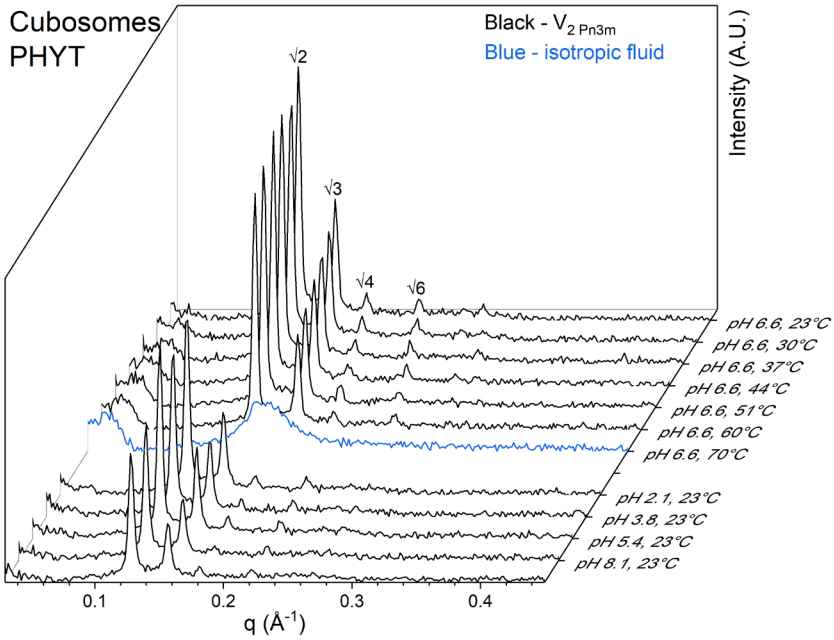
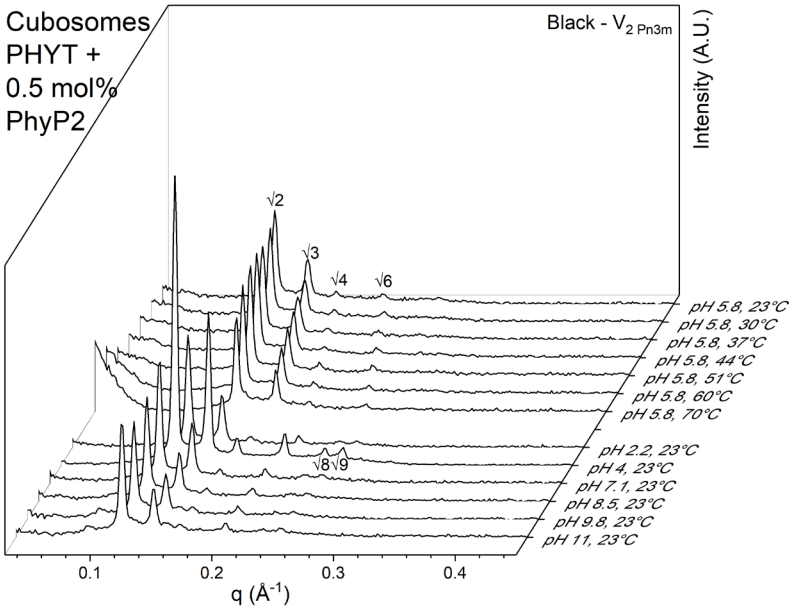


Fig. SI9j. SAXS patterns used to determine the phase behaviour of each sample in Fig. 3 and Fig. SI8.





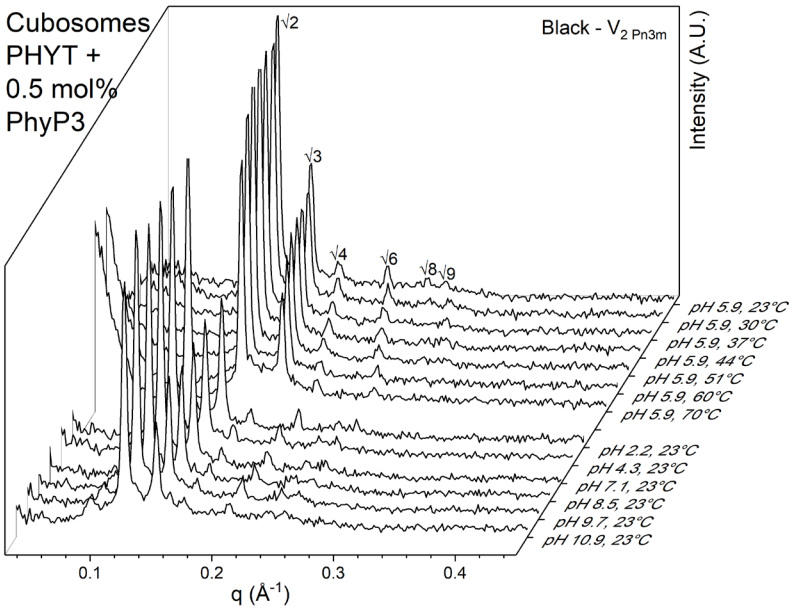


Fig. SI9k. SAXS patterns used to determine the phase behaviour of each sample in Fig. 3 and Fig. SI8.

pK_a measurements of micellar solutions of phosphorylated lipids

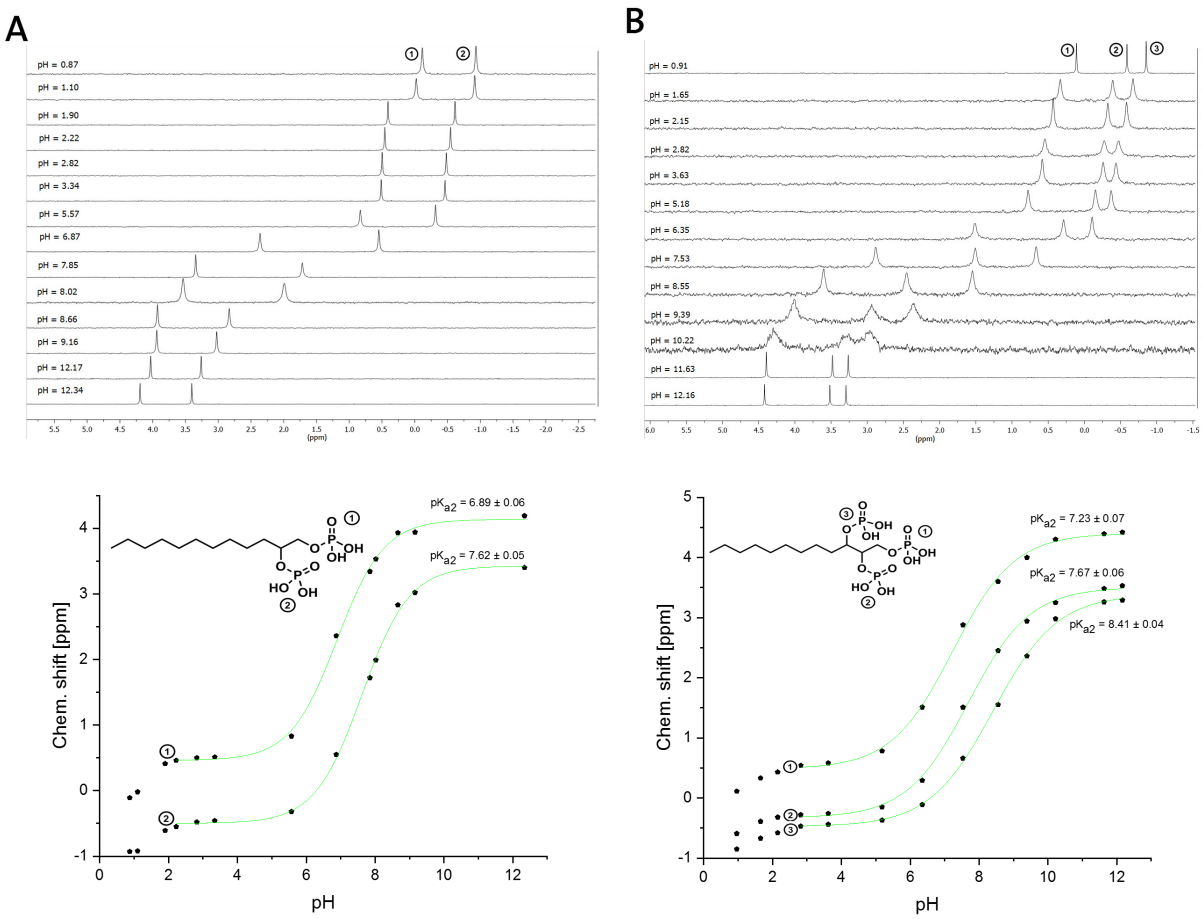


Fig SI10. Representative $^{31}P[^{1}H]$-NMR spectra (upper panel) and titration curves (lower panel) of the indicated phosphorylated lipids with a C_{12} chain as a function of pH. Titration curves were constructed by plotting the chemical shift derived from the NMR spectra against the pH value. The pK_a was subsequently determined from the fitting curve, using a published procedure.[3] A) Data of C12P2. B) Data of C12P3.

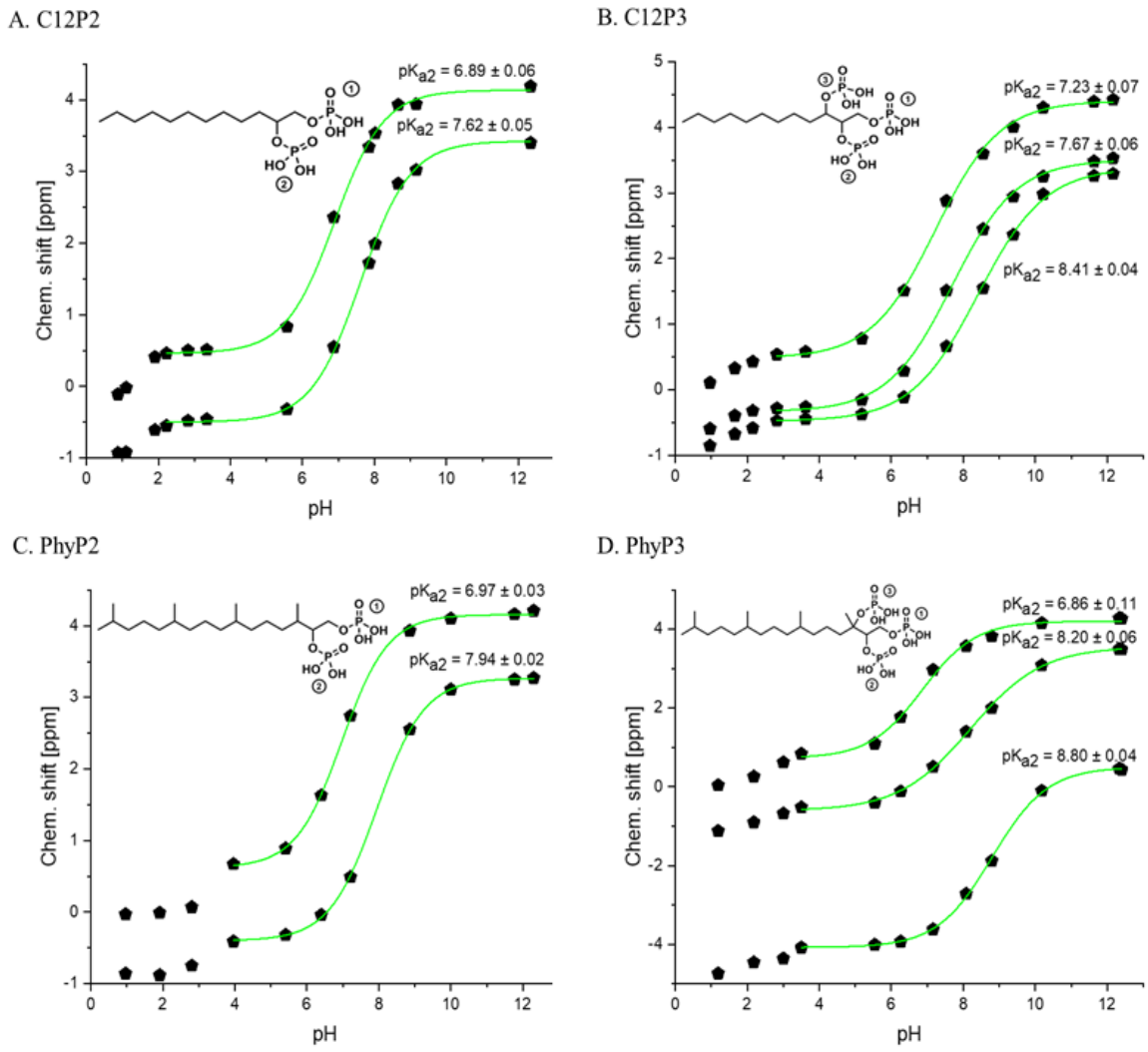


Fig. SI11. Titration curves of micellar solutions of the phosphorylated additive constructed by plotting the chemical shift against the pH value. The calculated pKa was derived from the fitting curve, using a published procedure.[3]

Fitted parameters from ^{31}PNMR titration data used to determine pKa

Table 1. Curve fitting of pH titration data for C12P2 and C12P3 micellar solutions.

	C12P2		**C12P3**		
	Peak 1	Peak 2	Peak 1	Peak 2	Peak 3
$P_{A^{2-}}$	4.14 ± 0.06	3.43 ± 0.06	4.40 ± 0.05	3.50 ± 0.05	3.39 ± 0.04
$P_{AH^{-}}$	0.46 ± 0.04	-0.50 ± 0.04	0.48 ± 0.06	-0.33 ± 0.05	-0.47 ± 0.03
pK_{a2}	6.89 ± 0.06	7.62 ± 0.04	7.23 ± 0.07	7.69 ± 0.06	8.41 ± 0.04
n	0.64 ± 0.04	0.63 ± 0.04	0.49 ± 0.03	0.49 ± 0.03	0.47 ± 0.02

Table 2. Curve fitting of pH titration data for PhyP2 and PhyP3 micellar solutions.

	PhyP2		PhyP3		
	Peak 1	Peak 2	Peak 1	Peak 2	Peak 3
$P_{A^{2-}}$	4.16 ± 0.03	3.27 ± 0.02	4.21 ± 0.08	3.56 ± 0.05	0.50 ± 0.04
$P_{AH^{-}}$	0.62 ± 0.05	-0.40 ± 0.02	0.73 ± 0.01	-0.61 ± 0.06	-4.08 ± 0.04
pK_{a2}	6.97 ± 0.03	7.94 ± 0.02	6.86 ± 0.01	8.20 ± 0.06	8.80 ± 0.04
n	0.68 ± 0.04	0.66 ± 0.02	0.59 ± 0.07	0.44 ± 0.02	0.57 ± 0.03

pK_a measurements in cubosomes via ^{31}P-NMR

The cubosome dispersion (0.63 mL), as described in part 5.6.3, was mixed with D_2O (63 µL) and subsequently the pH was adjusted with solutions of NaOH and HCl in H_2O as described in the previous part. The samples were analysed with the previously described standard methodology.

The commercially available Pluronic® F-127, used as standard stabiliser for the cubosomes, was found to contain trace amounts of phosphates. This additional peak in the ^{31}P-NMR did not influence the measurement of the phosphorylated lipids and was utilised as internal standard to validate the measurements. Furthermore, the NMR assignments are a working hypothesis and if not indicated, assigned without any special techniques.

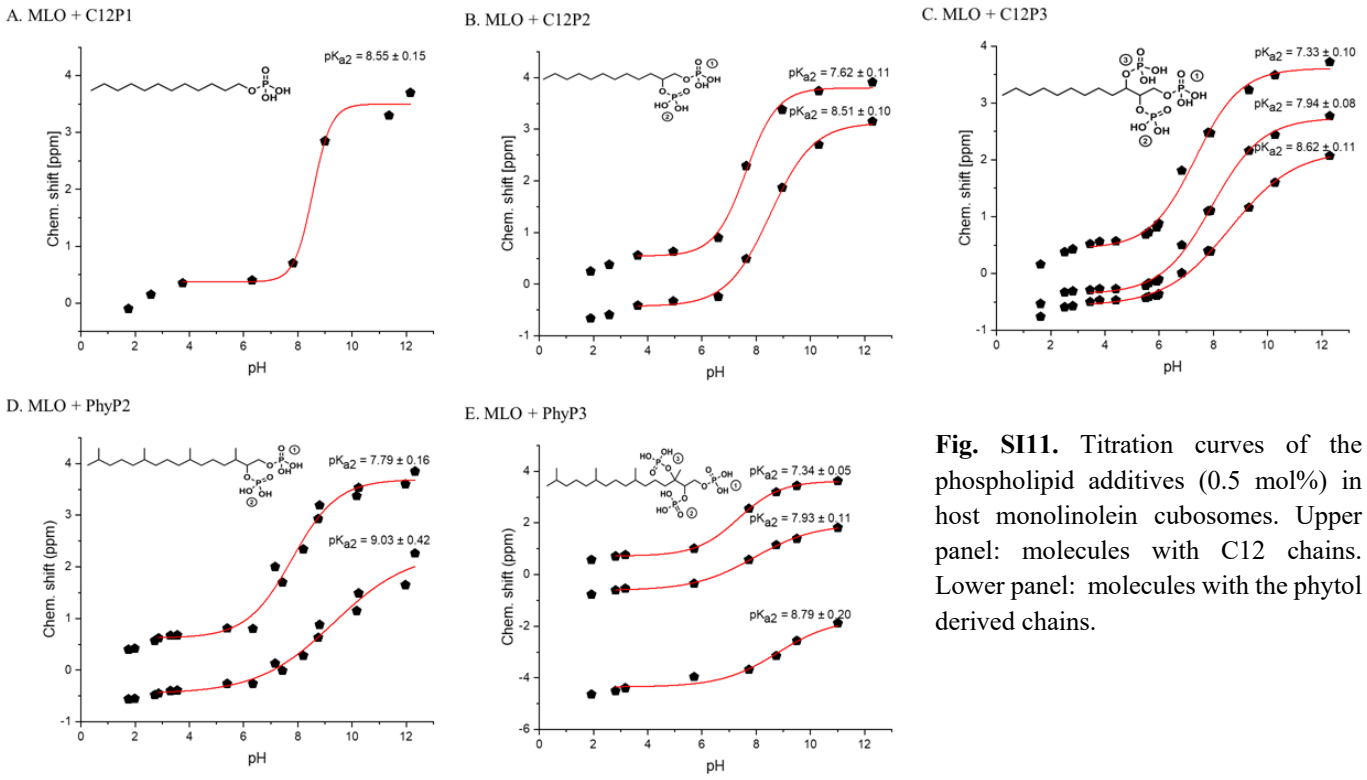


Fig. SI11. Titration curves of the phospholipid additives (0.5 mol%) in host monolinolein cubosomes. Upper panel: molecules with C12 chains. Lower panel: molecules with the phytol derived chains.

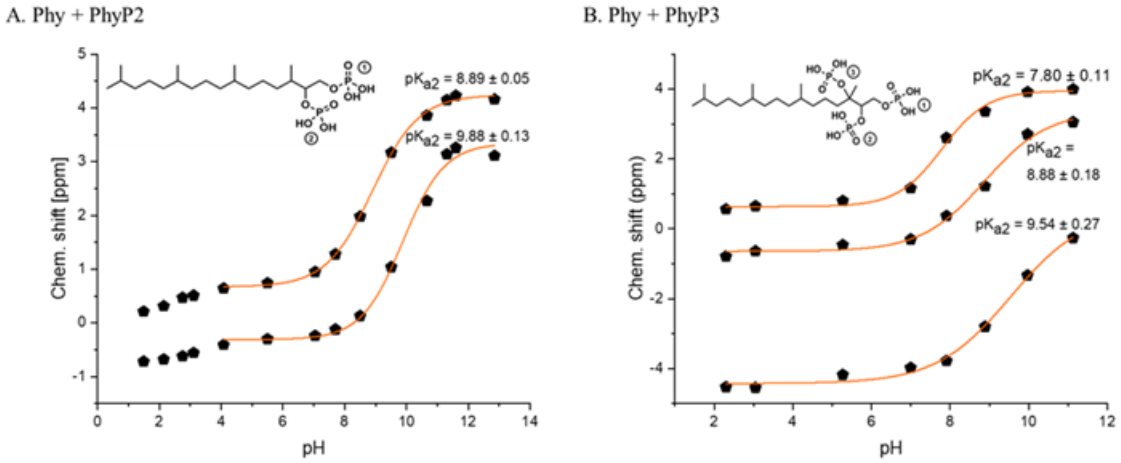


Fig. SI12. Titration curves of the phytol-derived phospholipid additives (0.5 mol%) in host phytantriol cubosomes.

Table 3. Curve fitting of pH titration data for the host MLO cubosomes with additives containing a dodecanol chain.

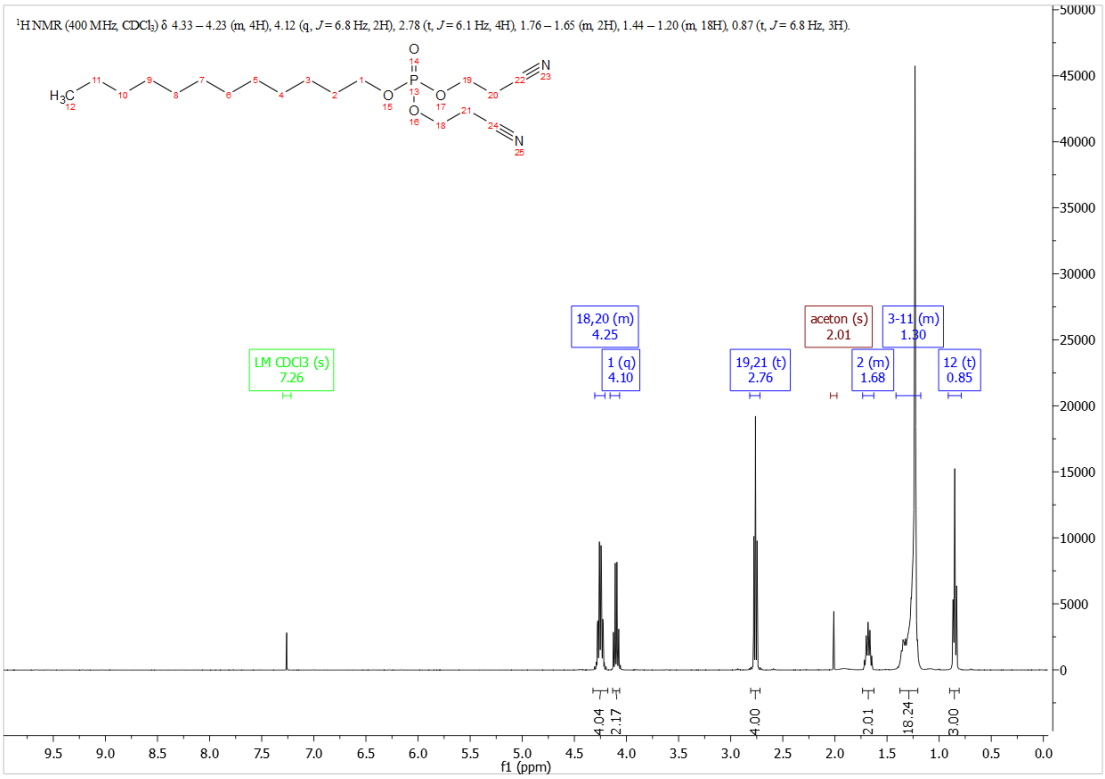
Additive	**C12P1**	**C12P2**		**C12P3**		
	Peak1	Peak 1	Peak 2	Peak 1	Peak 2	Peak 3
$P_{A^{2-}}$	3.50 ± 0.14	3.80 ± 0.10	3.13 ± 0.10	3.61 ± 0.09	2.74 ± 0.08	2.17 ± 0.08
$P_{AH^{-}}$	0.37 ± 0.14	0.54 ± 0.10	-0.43 ± 0.07	0.44 ± 0.09	-0.36 ± 0.05	-0.57 ± 0.04
pK_{a2}	8.55 ± 0.15	7.62 ± 0.11	8.51 ± 0.10	7.33 ± 0.11	7.94 ± 0.08	8.62 ± 0.11
n	1.28 ± 0.34	0.75 ± 0.14	0.54 ± 0.06	0.52 ± 0.06	0.47 ± 0.04	0.36 ± 0.03

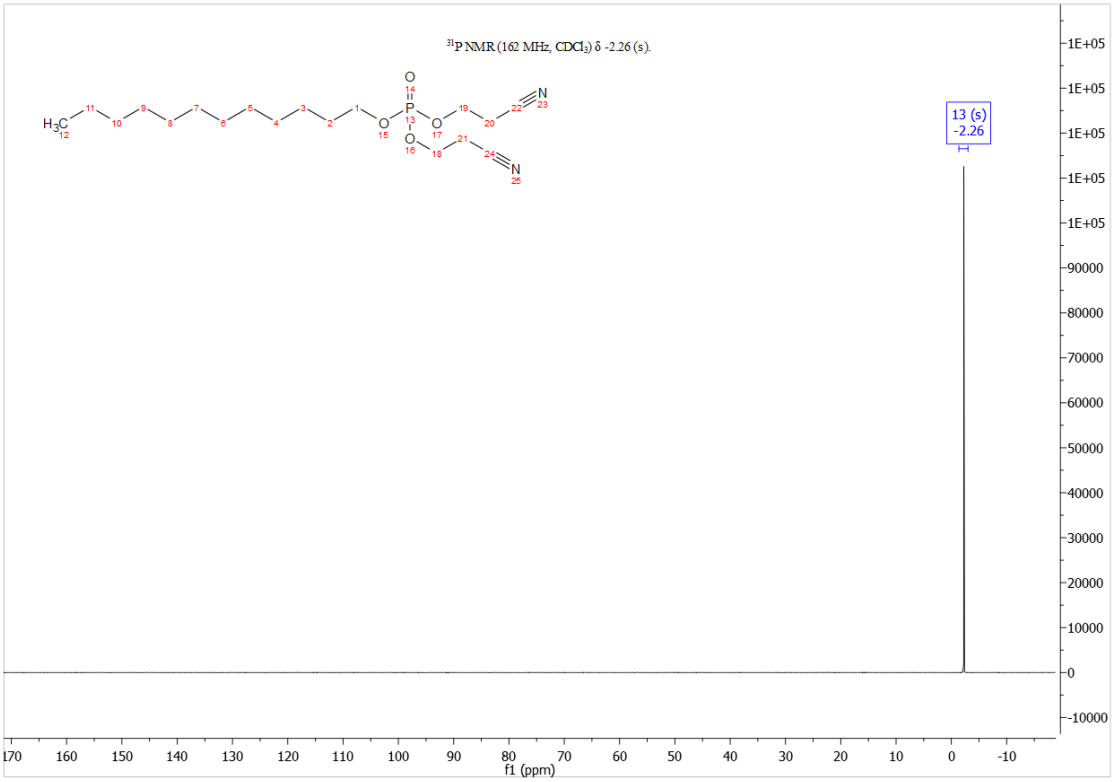
Table 4. Curve fitting of pH titration data for MLO cubosomes with additives containing a phytol chain.

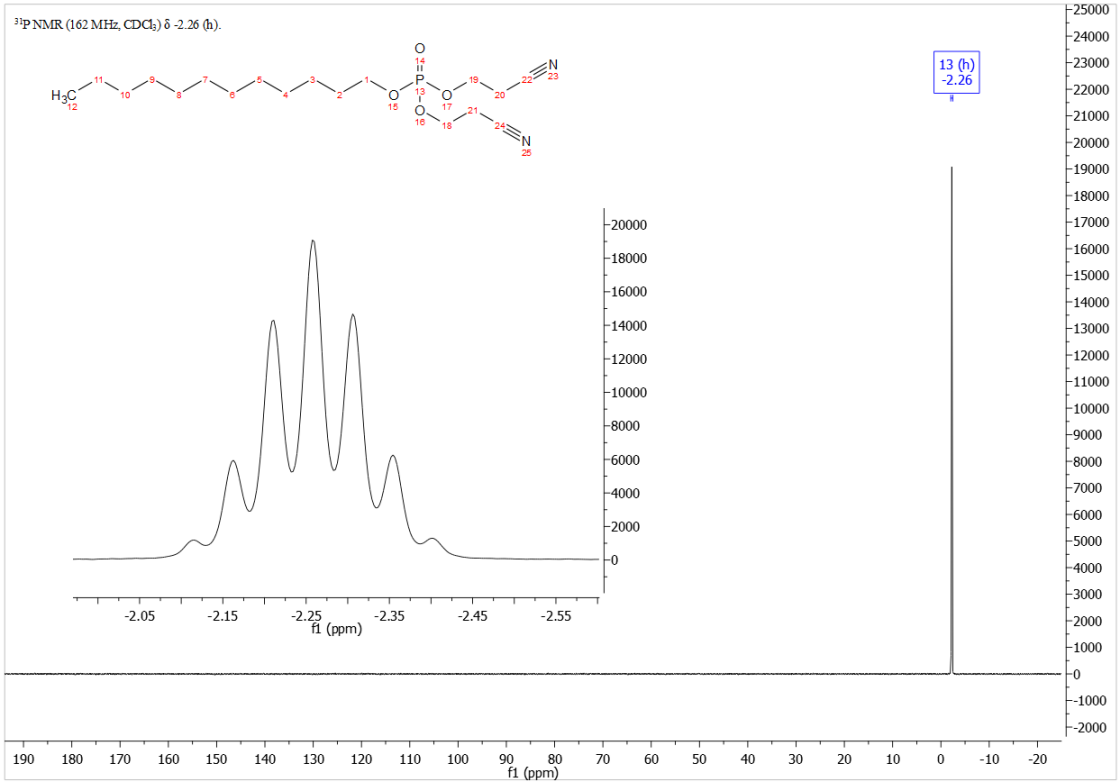
	PhyP2		**PhyP3**		
	Peak 1	Peak 2	Peak 1	Peak 2	Peak 3
$P_{A^{2-}}$	3.69 ± 0.13	2.92 ± 0.33	3.62 ± 0.03	1.94 ± 0.09	-1.7 (fixed)
$P_{AH^{-}}$	0.62 ± 0.11	-0.46 ± 0.12	0.72 ± 0.02	-0.61 ± 0.04	-4.35 ± 0.11
pK_{a2}	7.79 ± 0.16	9.32 ± 0.43	7.34 ± 0.01	7.93 ± 0.11	8.79 ± 0.20
n	0.52 ± 0.09	0.31 ± 0.08	0.39 ± 0.04	0.44 ± 0.02	0.42 ± 0.09

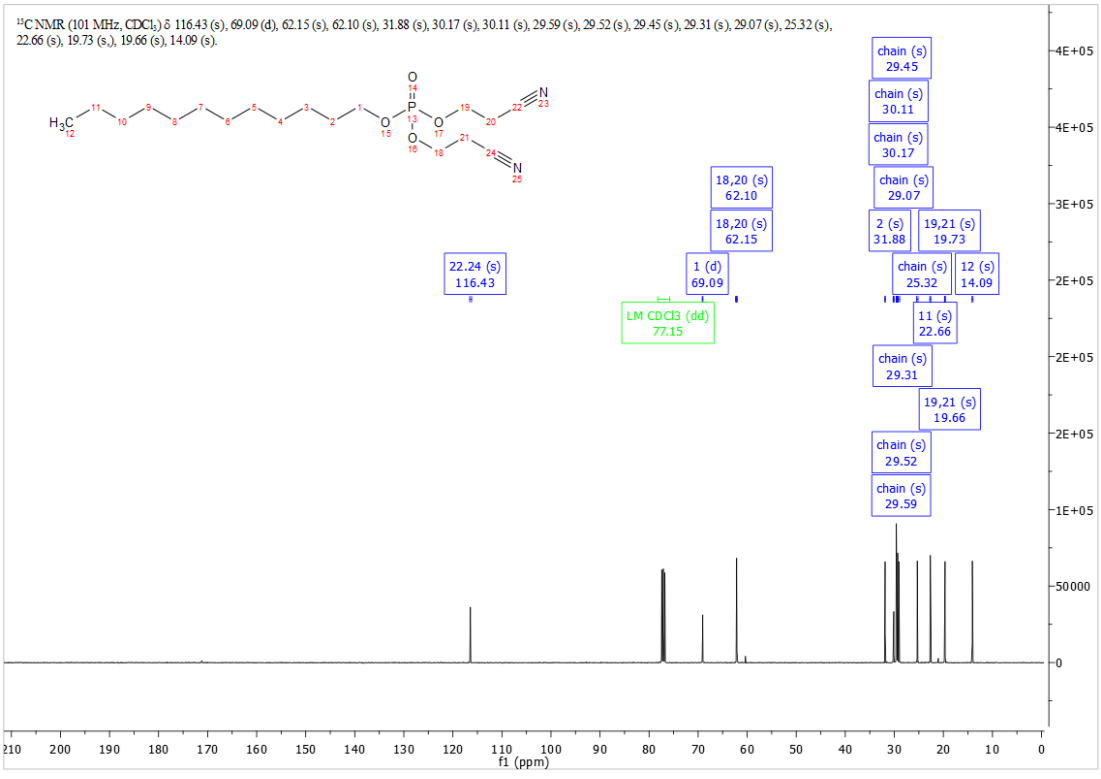
The NMR assignments are a working hypothesis and if not indicated, assigned without special NMR-techniques.

bis(2-cyanoethyl) dodecyl phosphate (2):

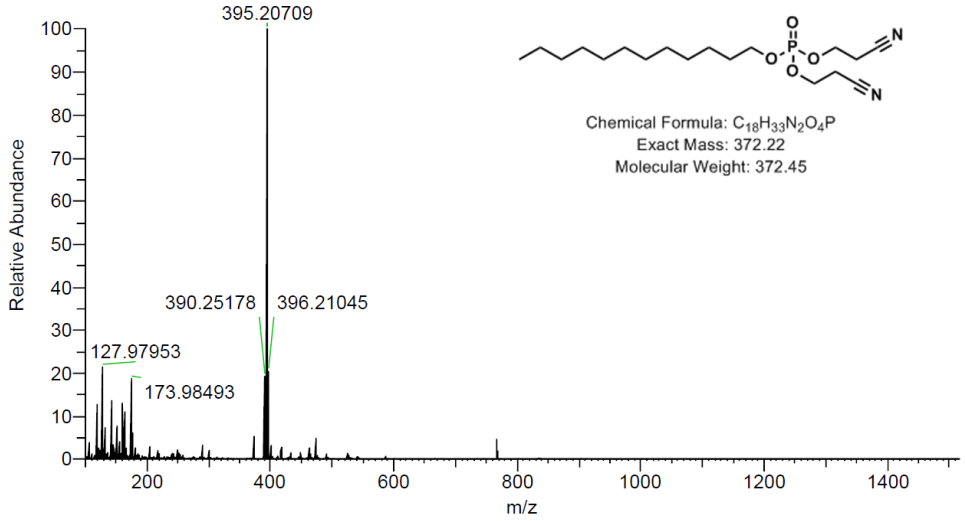





31P NMR (162 MHz, CDCl3) δ -2.26 (h).
13 (h)
-2.26
f1 (ppm)

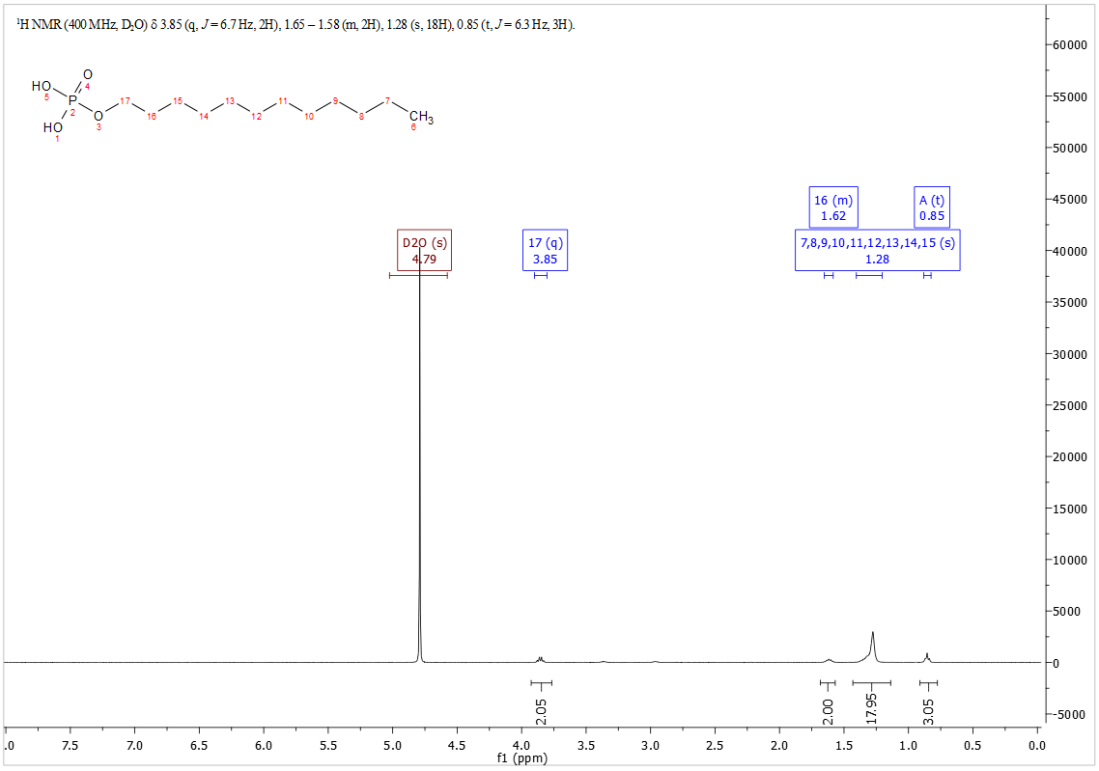

13C NMR (101 MHz, CDCl3) δ 116.43 (s), 69.09 (d), 62.15 (s), 62.10 (s), 31.88 (s), 30.17 (s), 30.11 (s), 29.59 (s), 29.52 (s), 29.45 (s), 29.31 (s), 29.07 (s), 25.32 (s), 22.66 (s), 19.73 (s), 19.66 (s), 14.09 (s).
chain (s) 29.45
chain (s) 30.11
chain (s) 30.17
chain (s) 29.07
18,20 (s) 62.10
18,20 (s) 62.15
2 (s) 31.88
19,21 (s) 19.73
22.24 (s) 116.43
1 (d) 69.09
chain (s) 25.32
12 (s) 14.09
LM CDCl3 (dd) 77.15
11 (s) 22.66
chain (s) 29.31
19,21 (s) 19.66
chain (s) 29.52
chain (s) 29.59
f1 (ppm)

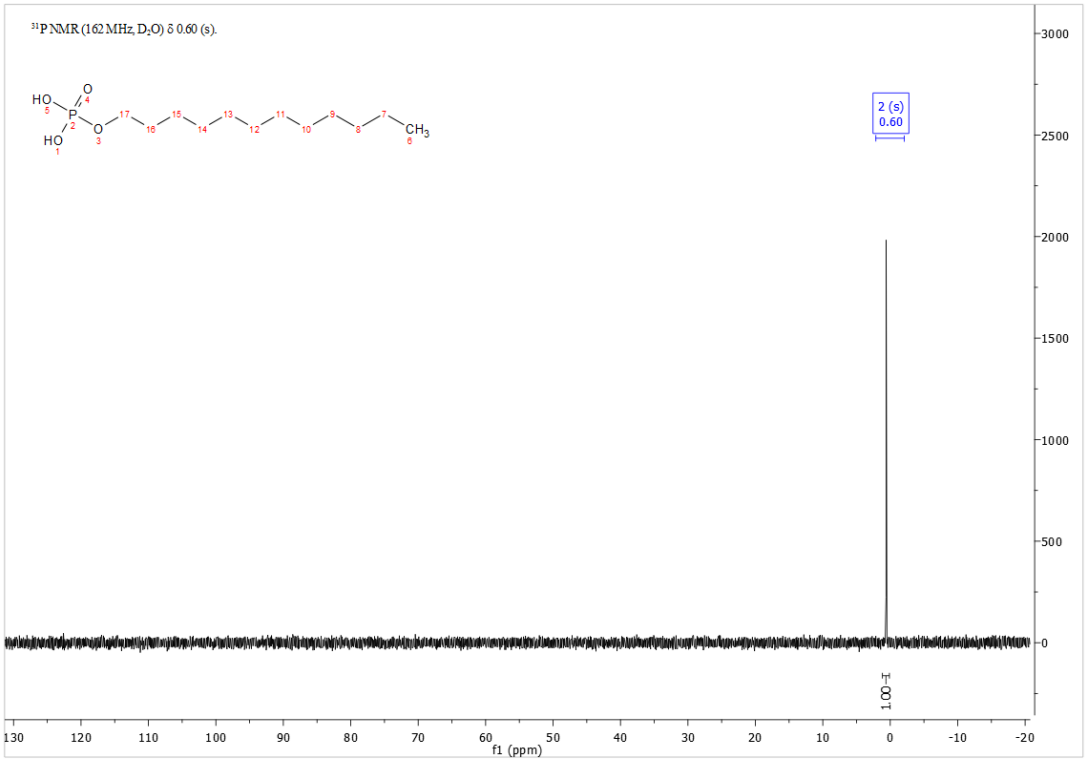
laQEx6261 #55-68 RT: 0.54-0.67 AV: 5 SB: 12 0.03-0.25 NL: 1.32E+008
T: FTMS + p ESI Full lock ms [100.0000-1500.0000]

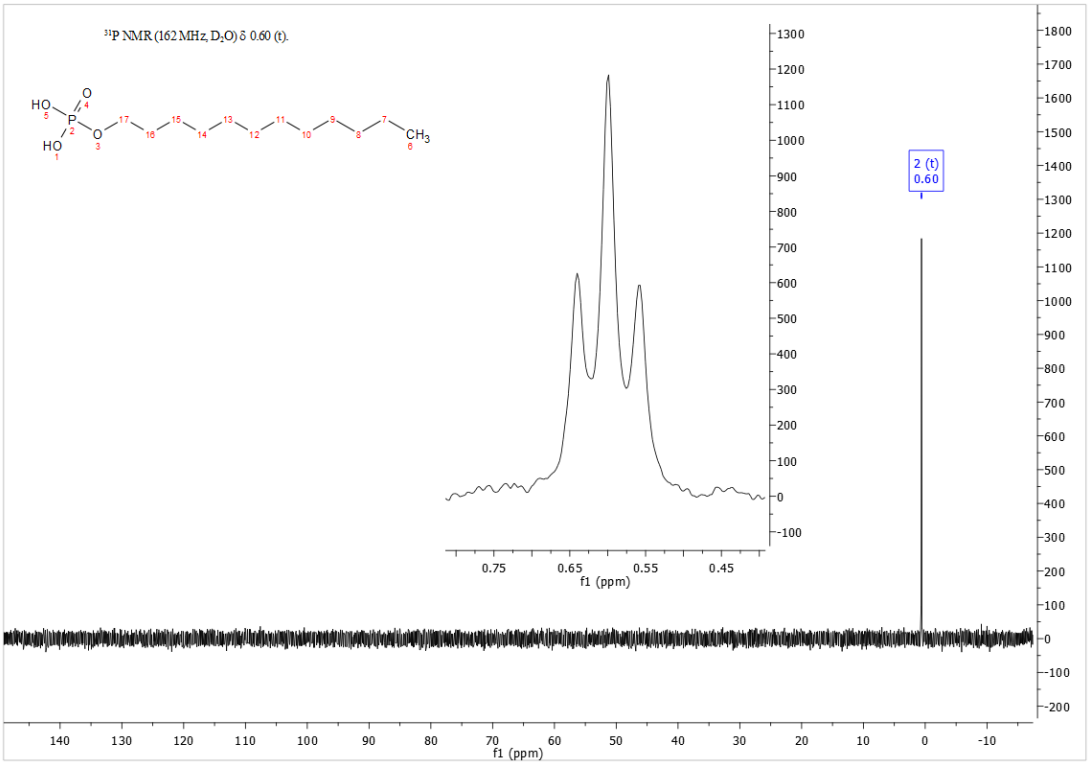


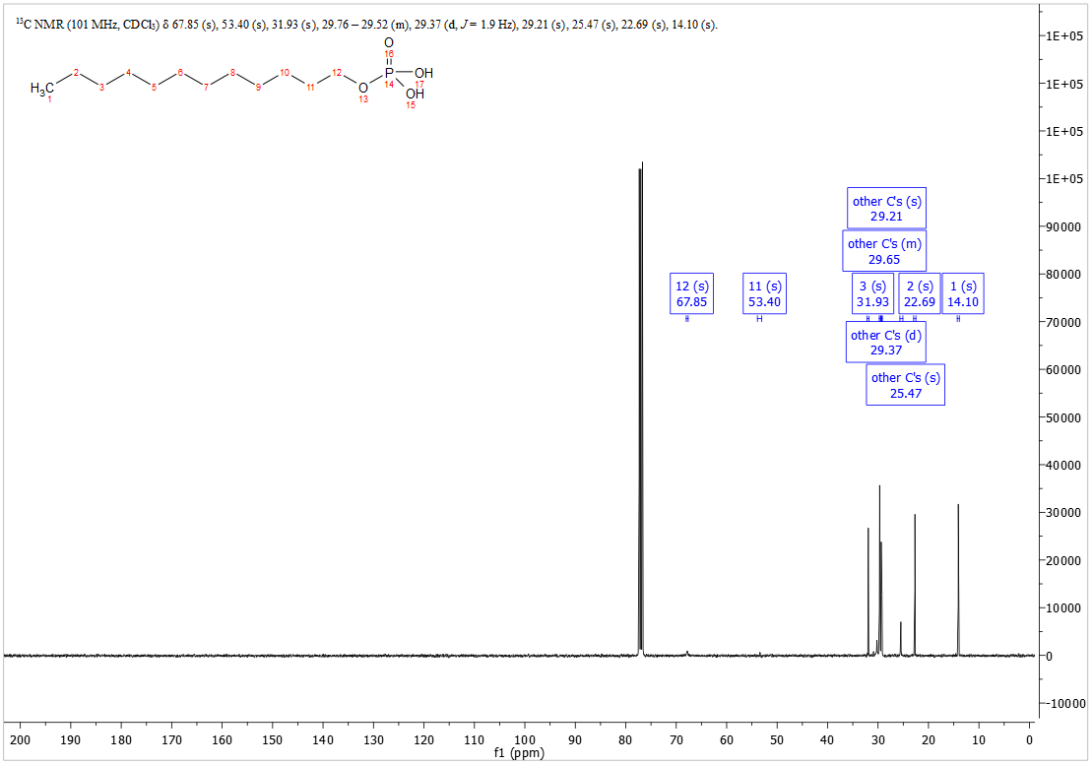
Rank	Peak Mass	Theo. mass	Display Formula	Combined Fit	Delta [ppm]	MSMS Matched Fr...
1	395.20709	395.20702	$C_{18}H_{33}O_4N_2{}^{23}NaP$	99.64781324867...	0.19	(Collection)

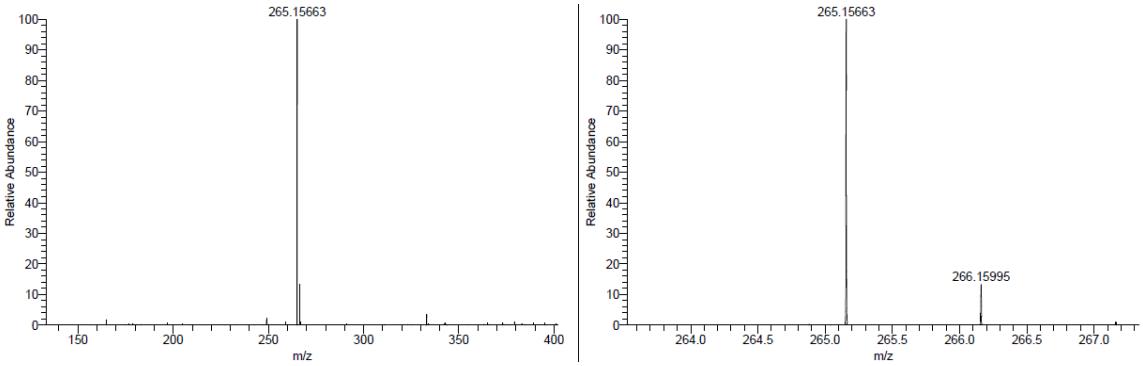
dodecyl dihydrogen phosphate (3):




31P NMR (162 MHz, D2O) δ 0.60 (s).
2 (s)
0.60
1.00
f1 (ppm)

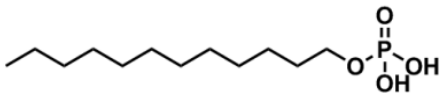

31P NMR (162 MHz, D2O) δ 0.60 (t).
2 (t)
0.60
f1 (ppm)





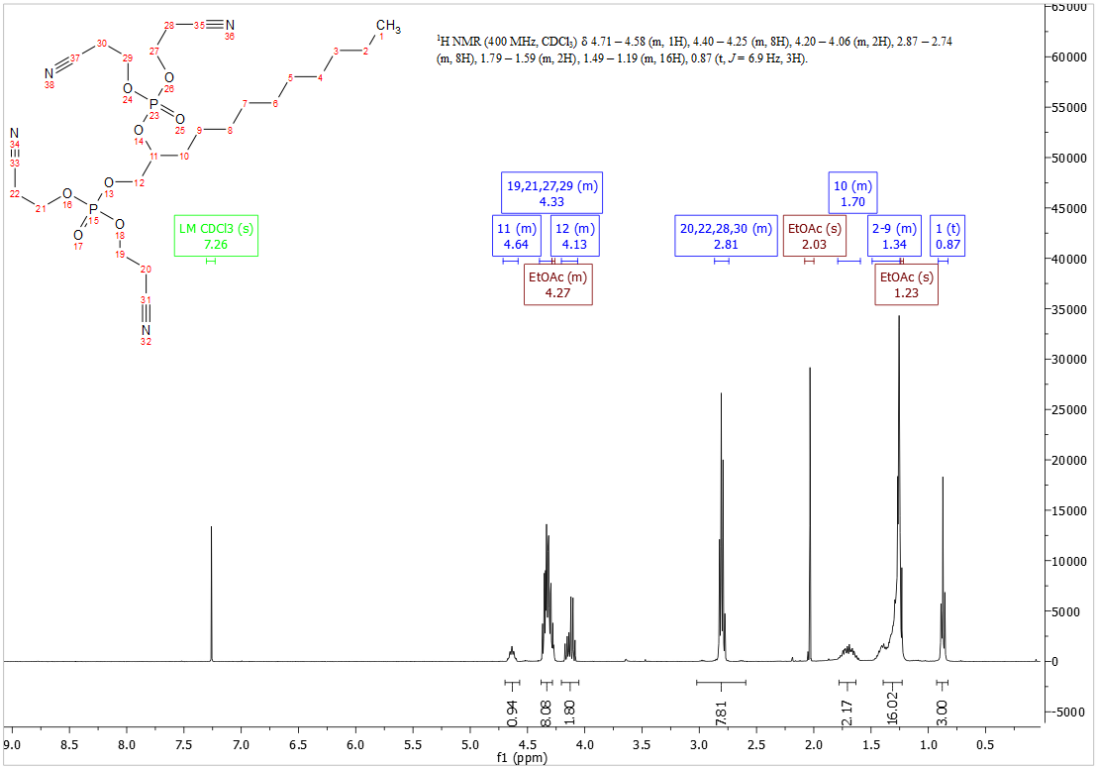
laQEx2415#42-64 RT: 0.41-0.63 AV: 12
SB: 26 0.07-0.28 , 0.71-0.99
T: FTMS - p ESI Full ms [100.00-1500.00]

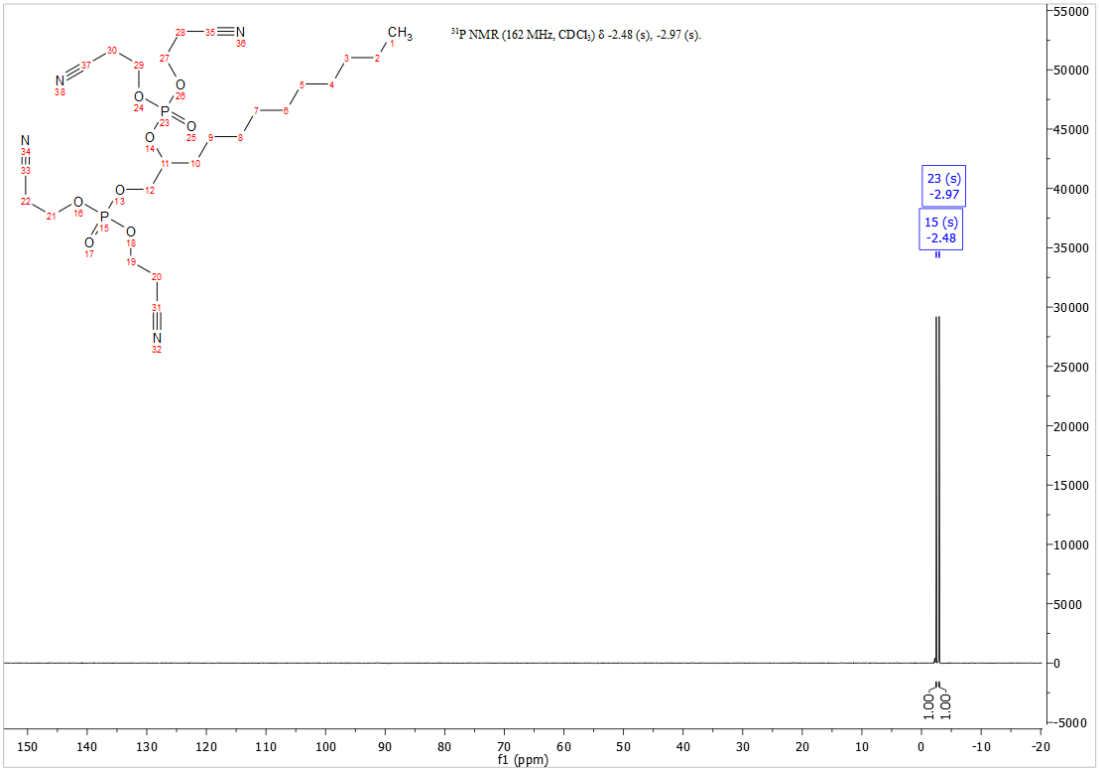
m/z	Intensity	Relative	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
265.15663	91739952.0	100.00	265.15738	-2.82	6.5	$C_{17}H_{22}ONa$
			265.15742	-2.96	0.5	$C_{12}H_{26}O_4P$

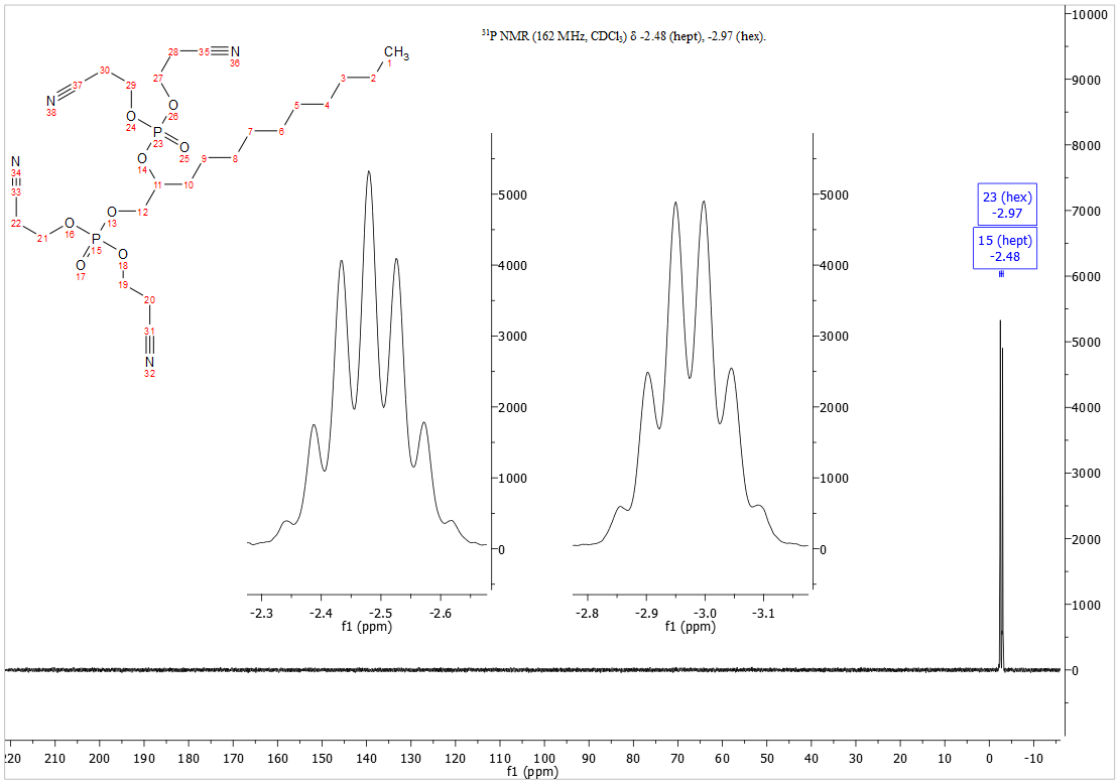


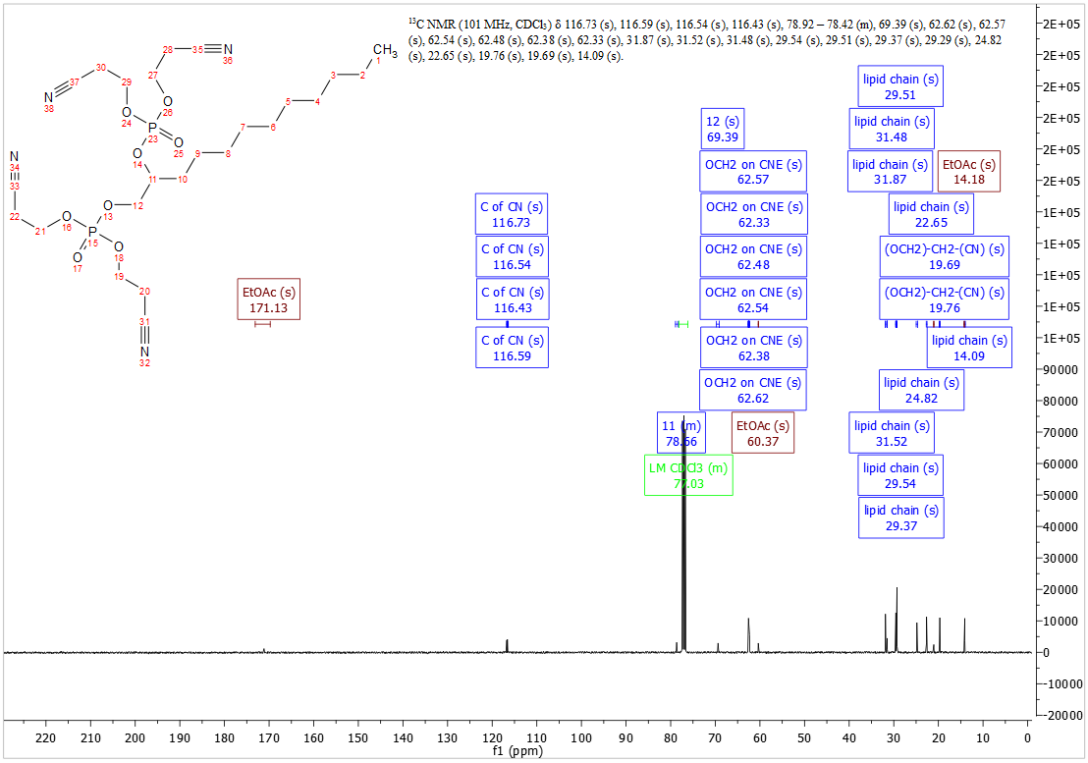
Chemical Formula: $C_{12}H_{27}O_4P$
Exact Mass: 266.16
Molecular Weight: 266.32

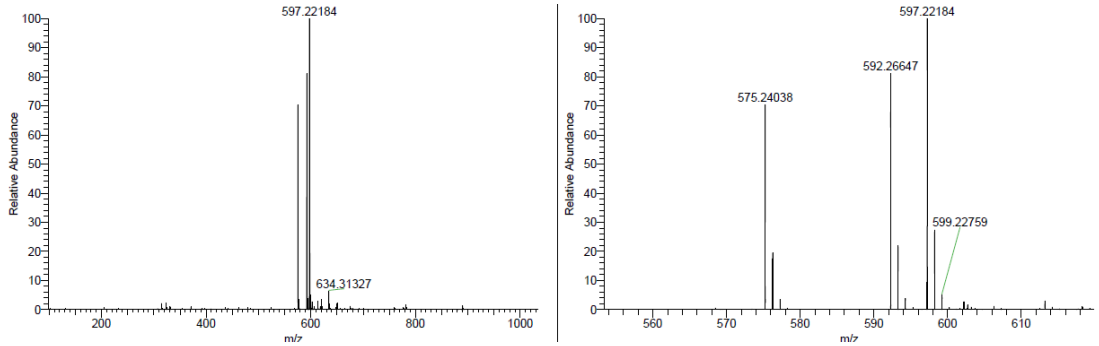
tetrakis(2-cyanoethyl) dodecane-1,2-diyl bis(phosphate) (5):





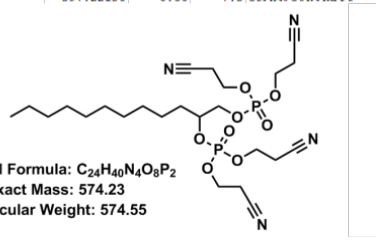

31P NMR (162 MHz, CDCl3) δ -2.48 (hept), -2.97 (hex).
23 (hex)
-2.97
15 (hept)
-2.48
f1 (ppm)


13C NMR (101 MHz, CDCl3) δ 116.73 (s), 116.59 (s), 116.54 (s), 116.43 (s), 78.92 – 78.42 (m), 69.39 (s), 62.62 (s), 62.57 (s), 62.54 (s), 62.48 (s), 62.38 (s), 62.33 (s), 31.87 (s), 31.52 (s), 31.48 (s), 29.54 (s), 29.51 (s), 29.37 (s), 29.29 (s), 24.82 (s), 22.65 (s), 19.76 (s), 19.69 (s), 14.09 (s).
EtOAc (s) 171.13
C of CN (s) 116.73
C of CN (s) 116.54
C of CN (s) 116.43
C of CN (s) 116.59
12 (s) 69.39
OCH2 on CNE (s) 62.57
OCH2 on CNE (s) 62.33
OCH2 on CNE (s) 62.48
OCH2 on CNE (s) 62.54
OCH2 on CNE (s) 62.38
OCH2 on CNE (s) 62.62
11 (m) 78.66
EtOAc (s) 60.37
LM CDCl3 (m) 77.03
lipid chain (s) 29.51
lipid chain (s) 31.48
lipid chain (s) 31.87
EtOAc (s) 14.18
lipid chain (s) 22.65
(OCH2)-CH2-(CN) (s) 19.69
(OCH2)-CH2-(CN) (s) 19.76
lipid chain (s) 14.09
lipid chain (s) 24.82
lipid chain (s) 31.52
lipid chain (s) 29.54
lipid chain (s) 29.37
f1 (ppm)



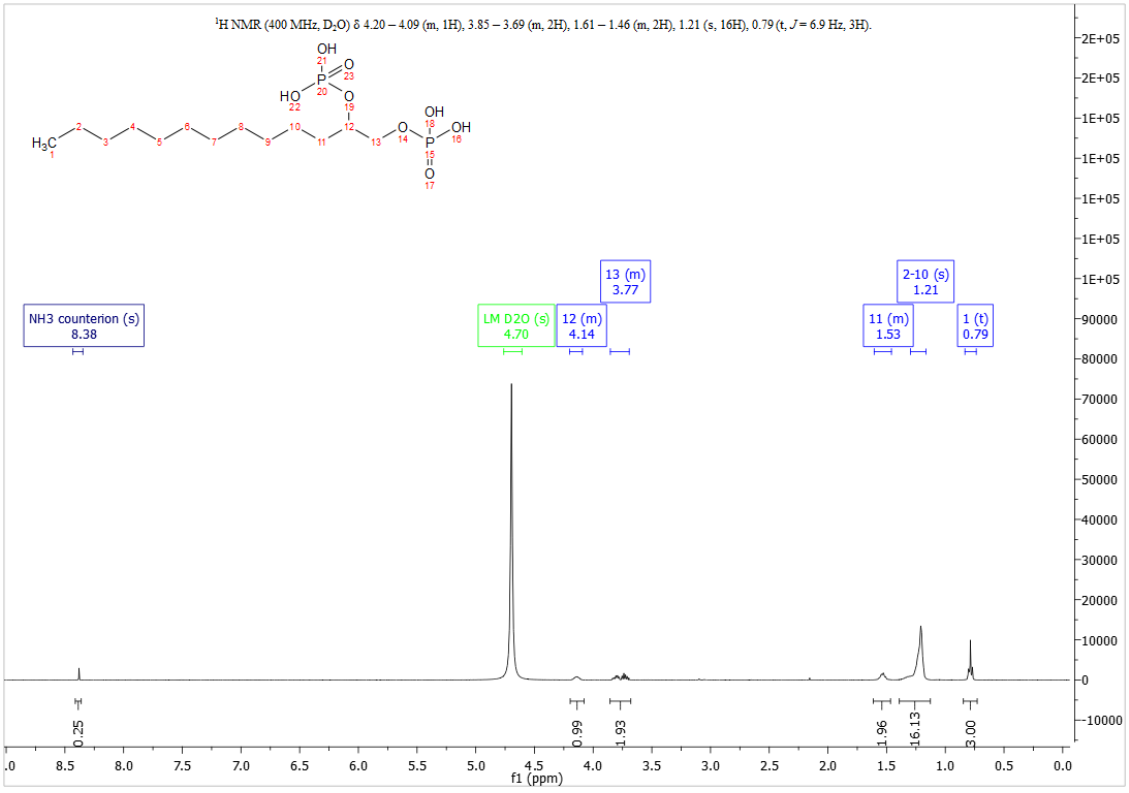
laQEx3504#35-51 RT: 0.35-0.51 AV: 5
SB: 25 0.07-0.29 , 0.71-0.99
T: FTMS + p ESI Full lock ms [100.00-1500.00]
m/z= 595.97044-597.82017

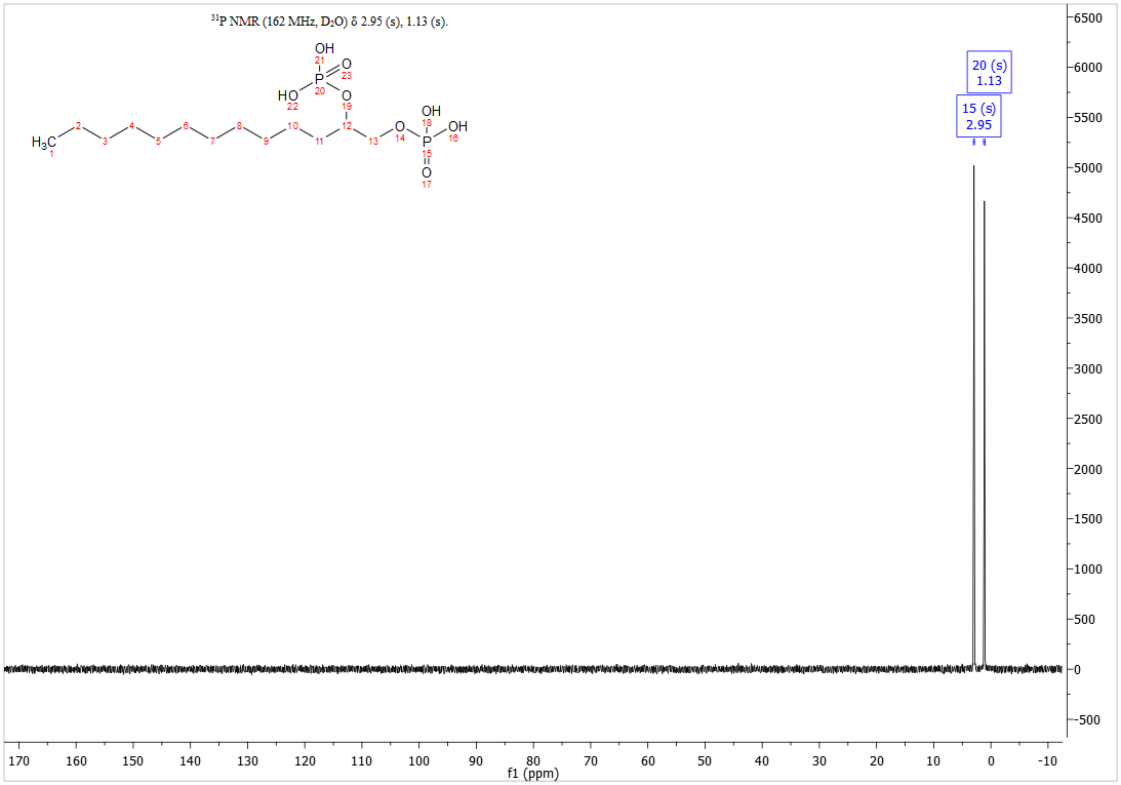
m/z	Intensity	Relative	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
597.22184	170475312.0	100.00	597.22180	0.06	20.0	$C_{32}H_{31}O_7N_5$
			597.22191	-0.13	23.5	$C_{38}H_{35}ON_2P_2$
			597.22136	0.80	7.5	$C_{24}H_{40}O_8N_4NaP_2$

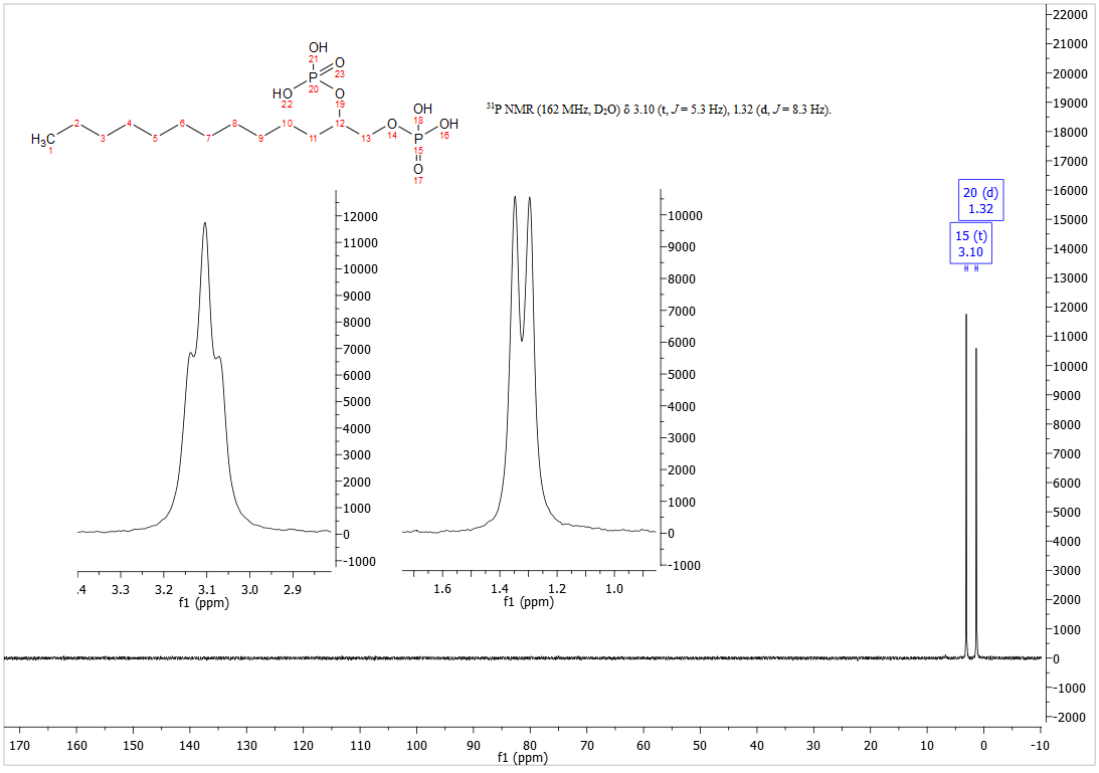


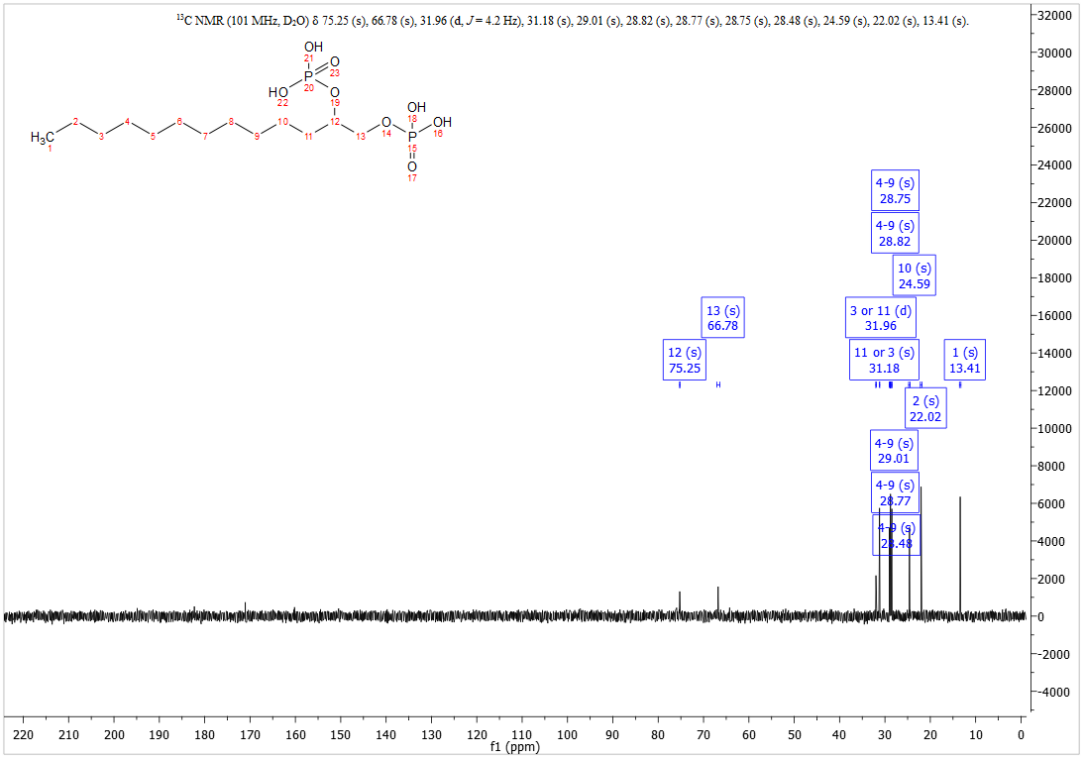
Chemical Formula: $C_{24}H_{40}N_4O_8P_2$
Exact Mass: 574.23
Molecular Weight: 574.55

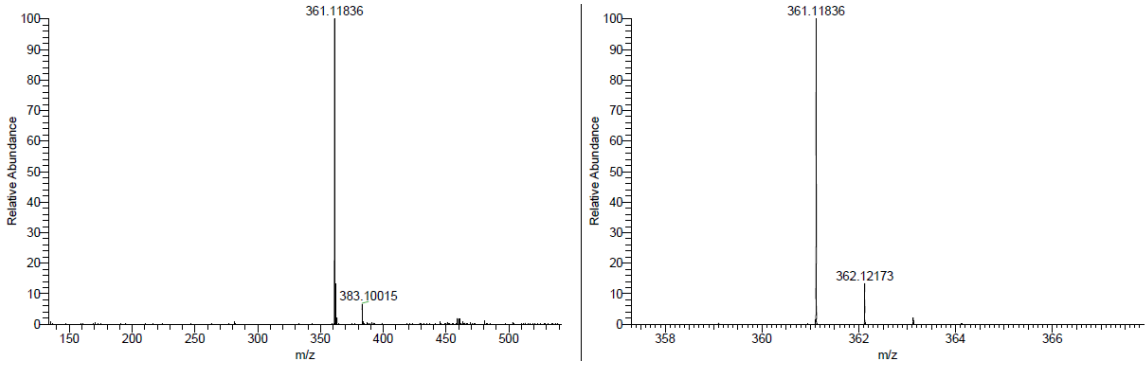
dodecane-1,2-diyl bis(dihydrogen phosphate) (6):




31P NMR (162 MHz, D2O) δ 2.95 (s), 1.13 (s).
20 (s)
1.13
15 (s)
2.95
f1 (ppm)

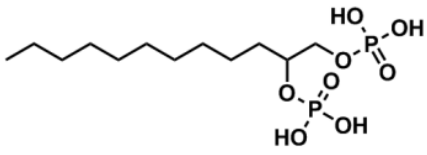

31P NMR (162 MHz, D2O) δ 3.10 (t, J = 5.3 Hz), 1.32 (d, J = 8.3 Hz).
20 (d)
1.32
15 (t)
3.10
f1 (ppm)





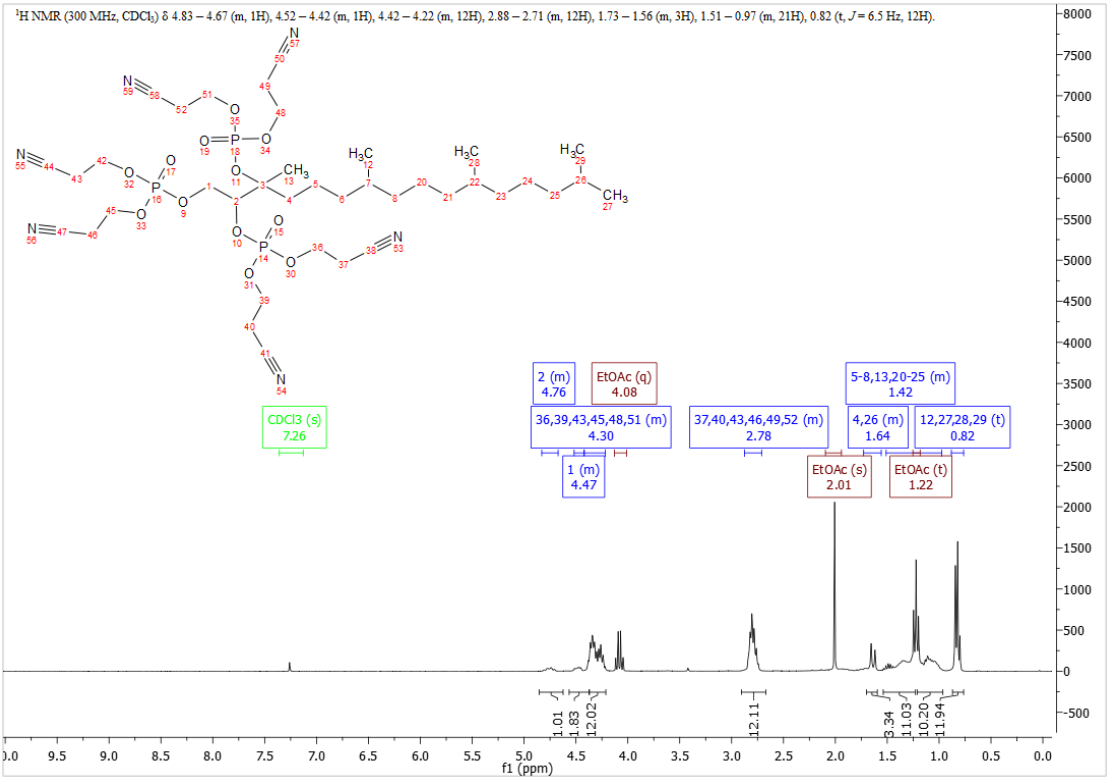
laQEx3541#36-68 RT: 0.35-0.66 AV: 17
SB: 26 0.07-0.29 , 0.71-0.98
T: FTMS - p ESI Full ms [100.00-1500.00]
m/z= 360.65838-361.61747

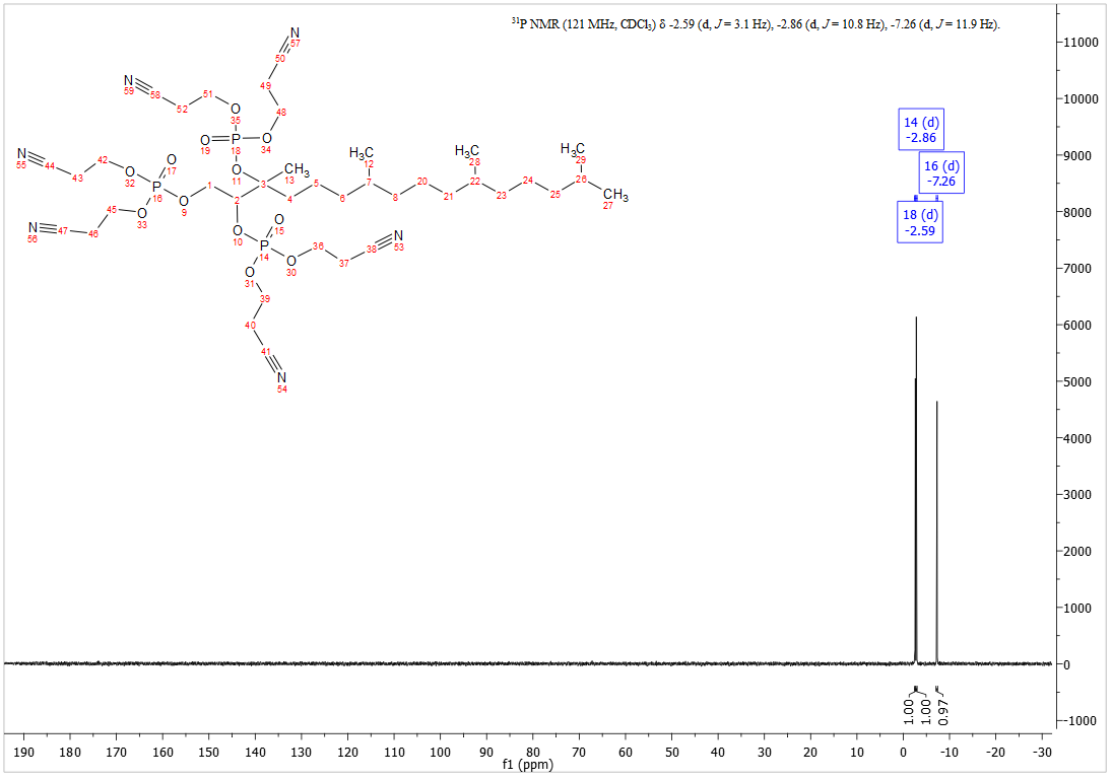
m/z	Intensity	Relative	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
361.11836	104060392.0	100.00	361.11835	0.03	10.5	$C_{15}H_{18}O_3N_6P$
			361.11855	-0.51	2.5	$C_5H_{17}O_9N_{10}$
			361.11866	-0.83	6.0	$C_{11}H_{21}O_3N_7P_2$
			361.11866	-0.84	0.5	$C_{12}H_{27}O_8P_2$

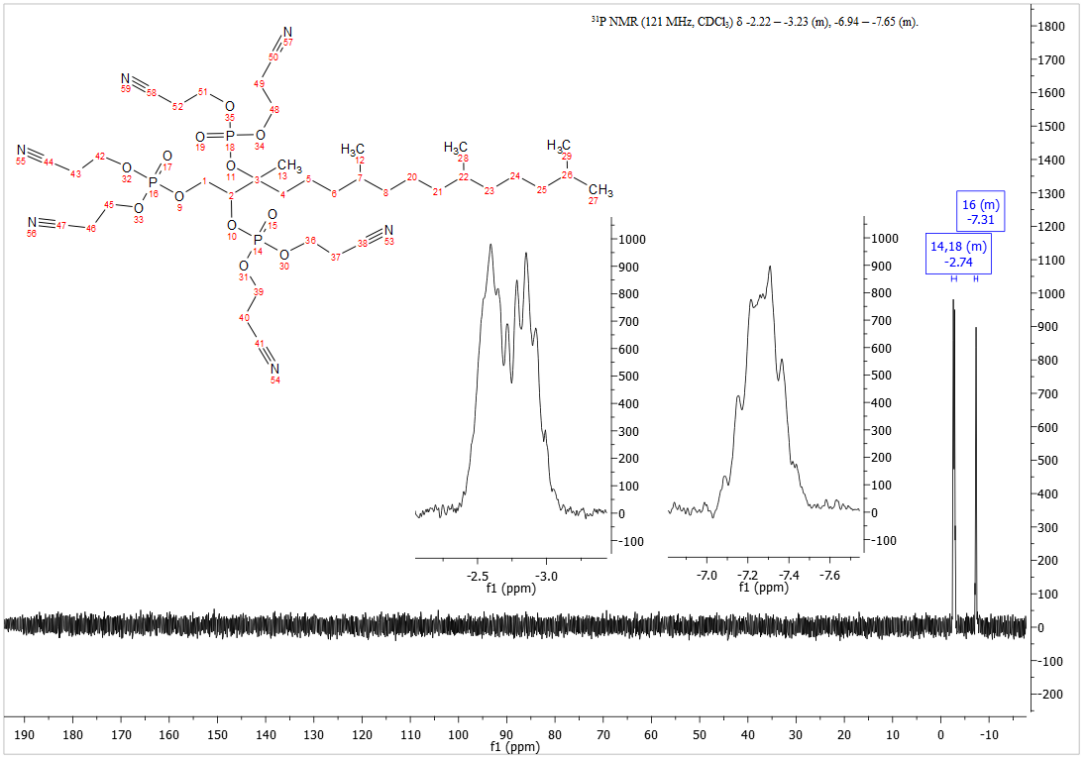


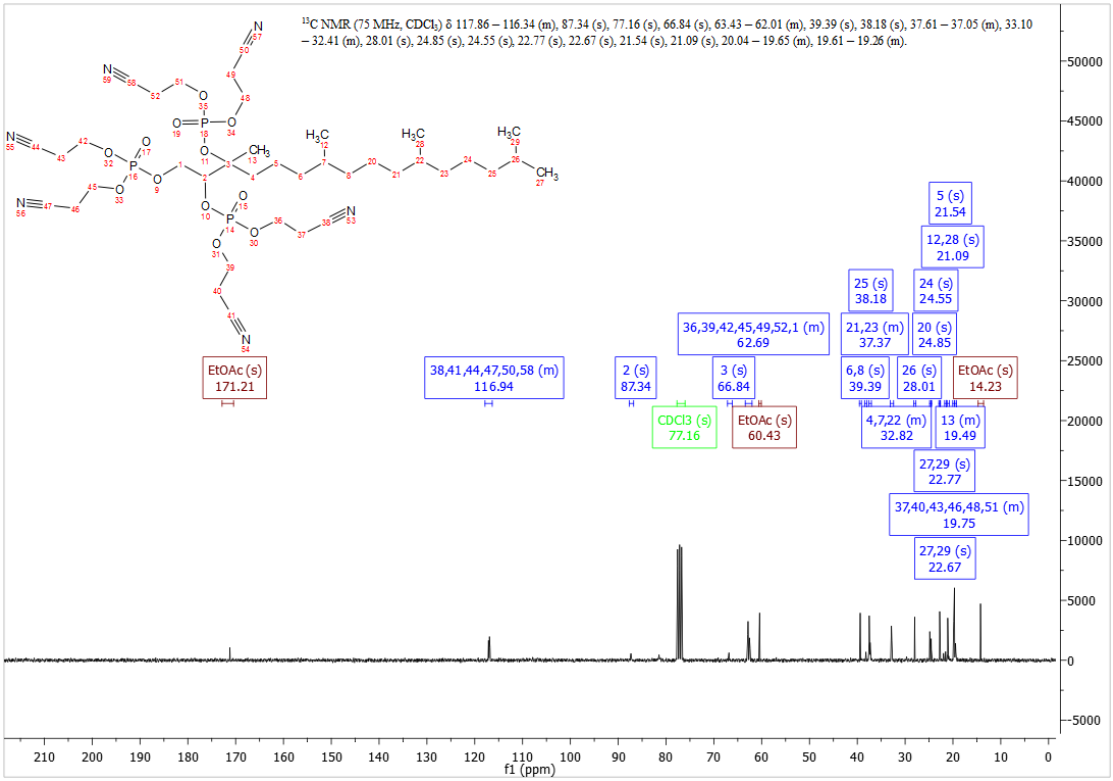
Chemical Formula: $C_{12}H_{28}O_8P_2$
Exact Mass: 362.13
Molecular Weight: 362.30

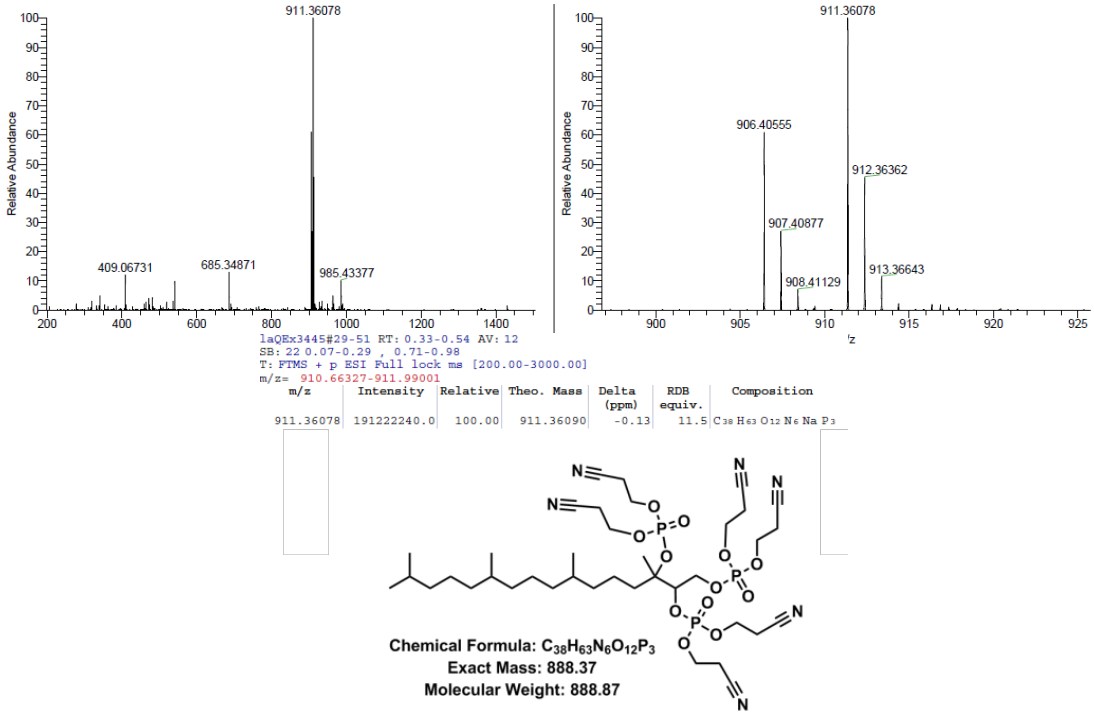
hexakis(2-cyanoethyl) (3,7,11,15-tetramethylhexadecane-1,2,3-triyl) tris(phosphate) (8):



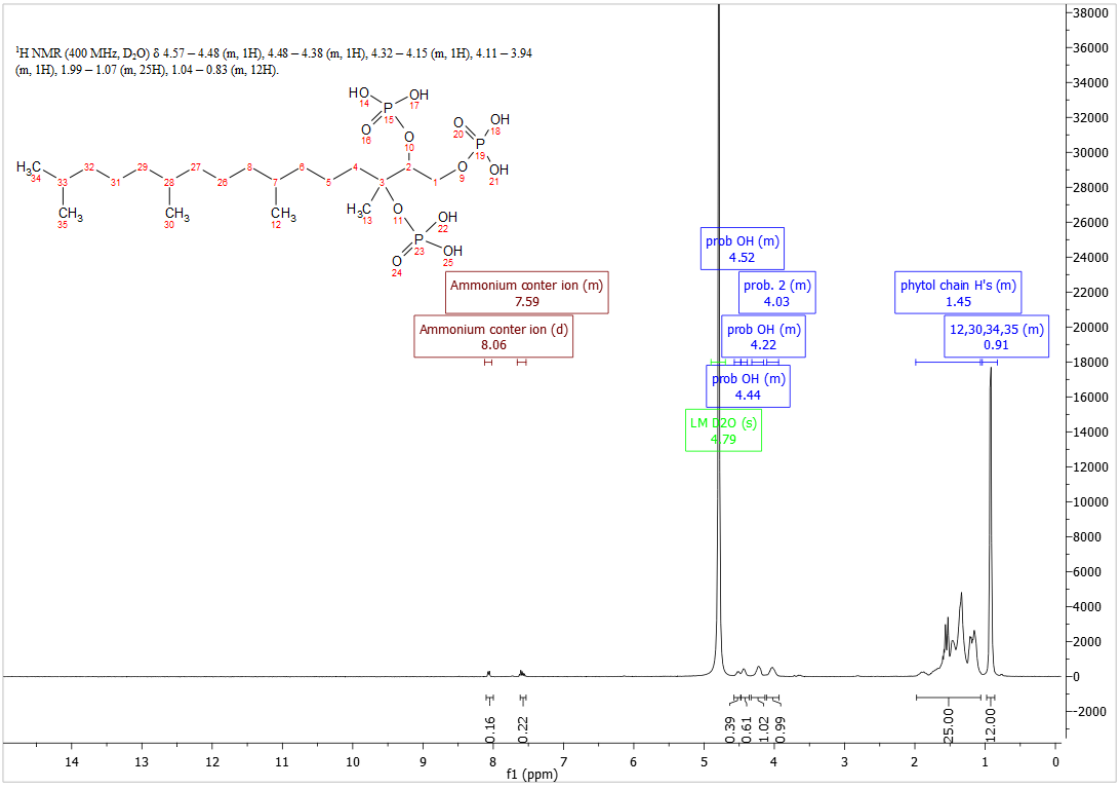


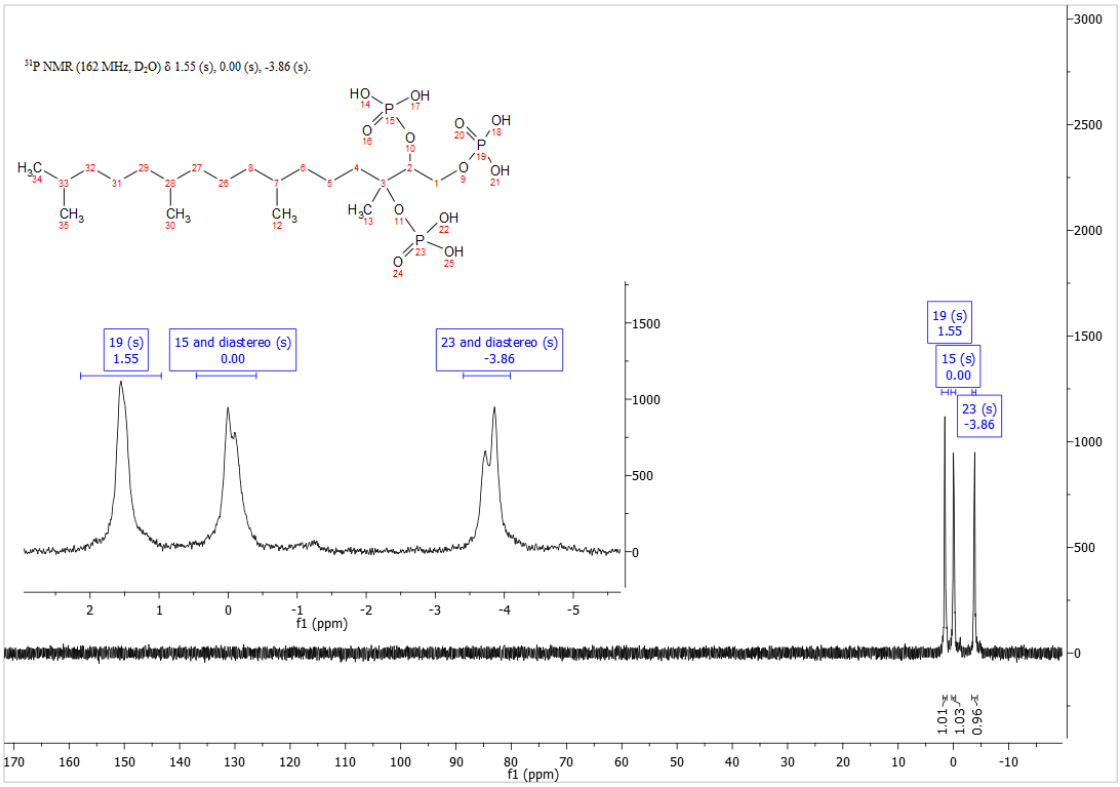

^{31}P NMR (121 MHz, CDCl$_3$) δ -2.22 – -3.23 (m), -6.94 – -7.65 (m).
16 (m)
-7.31
14,18 (m)
-2.74
f1 (ppm)

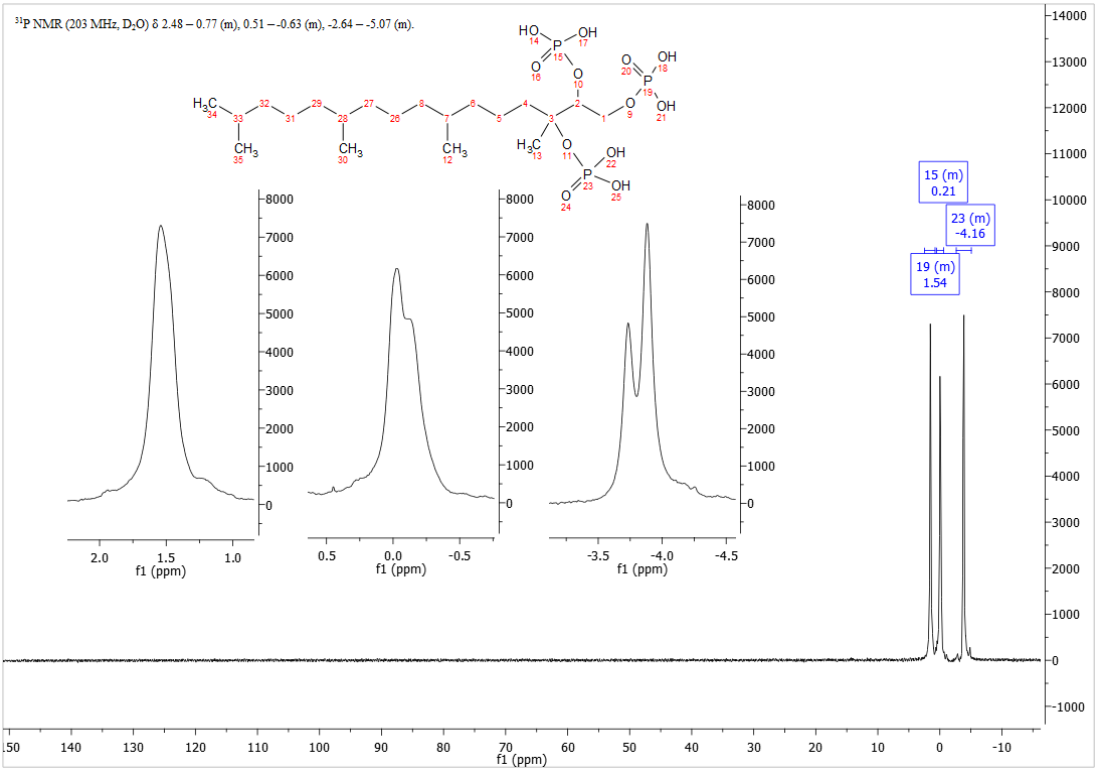

^{13}C NMR (75 MHz, CDCl$_3$) δ 117.86 – 116.34 (m), 87.34 (s), 77.16 (s), 66.84 (s), 63.43 – 62.01 (m), 39.39 (s), 38.18 (s), 37.61 – 37.05 (m), 33.10 – 32.41 (m), 28.01 (s), 24.85 (s), 24.55 (s), 22.77 (s), 22.67 (s), 21.54 (s), 21.09 (s), 20.04 – 19.65 (m), 19.61 – 19.26 (m).
EtOAc (s)
171.21
38,41,44,47,50,58 (m)
116.94
2 (s)
87.34
CDCl3 (s)
77.16
3 (s)
66.84
EtOAc (s)
60.43
36,39,42,45,49,52,1 (m)
62.69
25 (s)
38.18
21,23 (m)
37.37
20 (s)
24.85
24 (s)
24.55
12,28 (s)
21.09
5 (s)
21.54
6,8 (s)
39.39
26 (s)
28.01
EtOAc (s)
14.23
4,7,22 (m)
32.82
13 (m)
19.49
27,29 (s)
22.77
37,40,43,46,48,51 (m)
19.75
27,29 (s)
22.67
f1 (ppm)

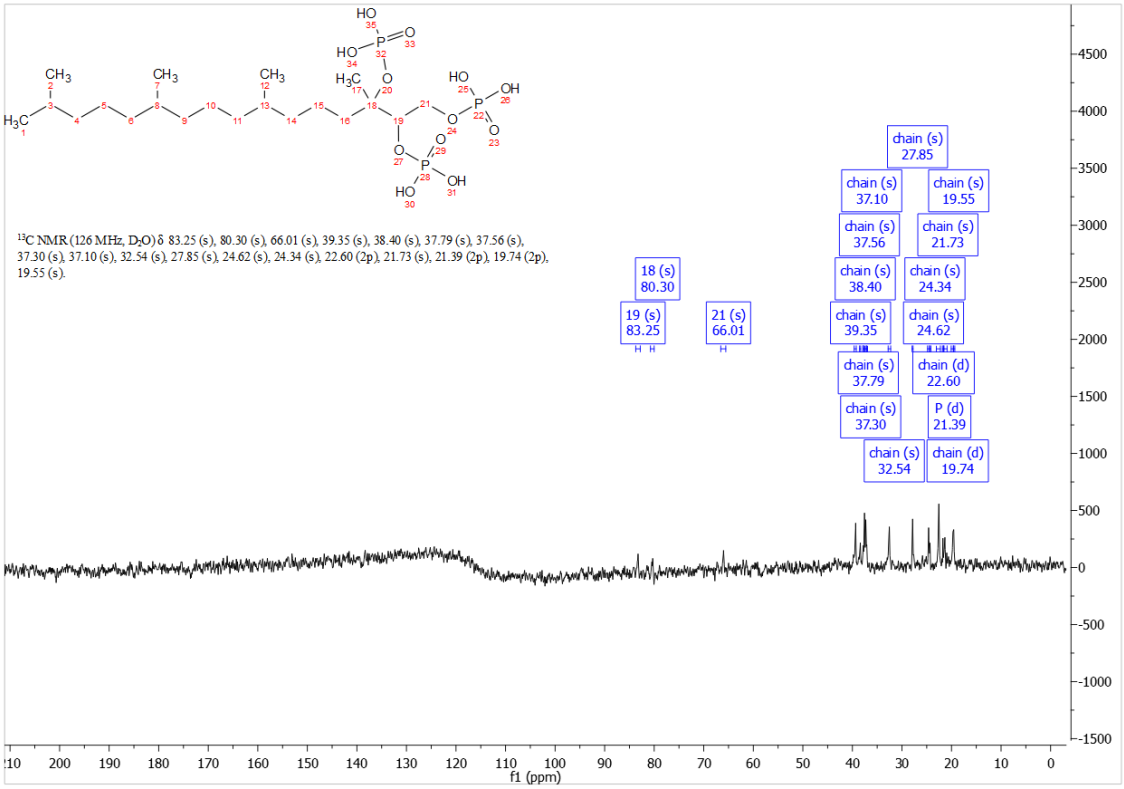


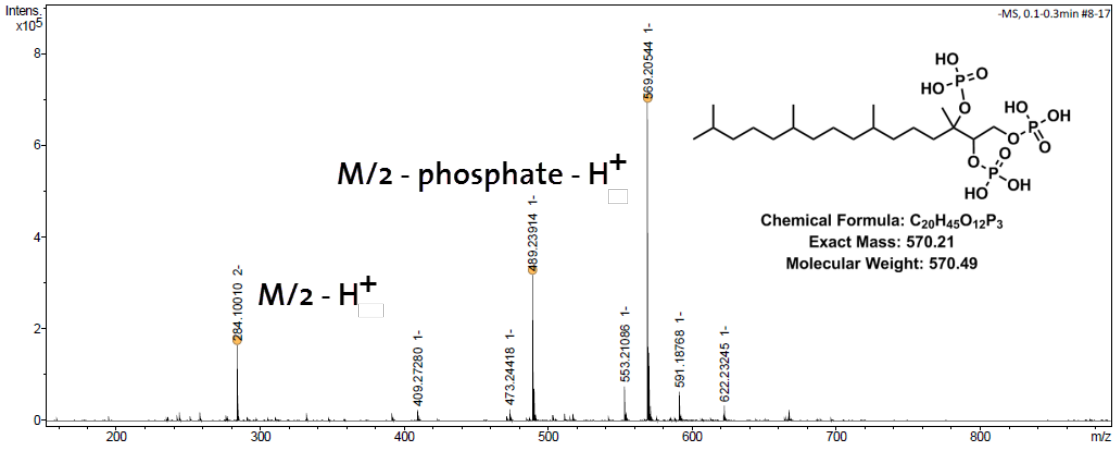
3,7,11,15-tetramethylhexadecane-1,2,3-triyl tris(dihydrogen phosphate) (9):




31P NMR (162 MHz, D2O) δ 1.55 (s), 0.00 (s), -3.86 (s).
19 (s)
1.55
15 and diastereo (s)
0.00
23 and diastereo (s)
-3.86
19 (s)
1.55
15 (s)
0.00
23 (s)
-3.86
1.01
1.03
0.96
f1 (ppm)

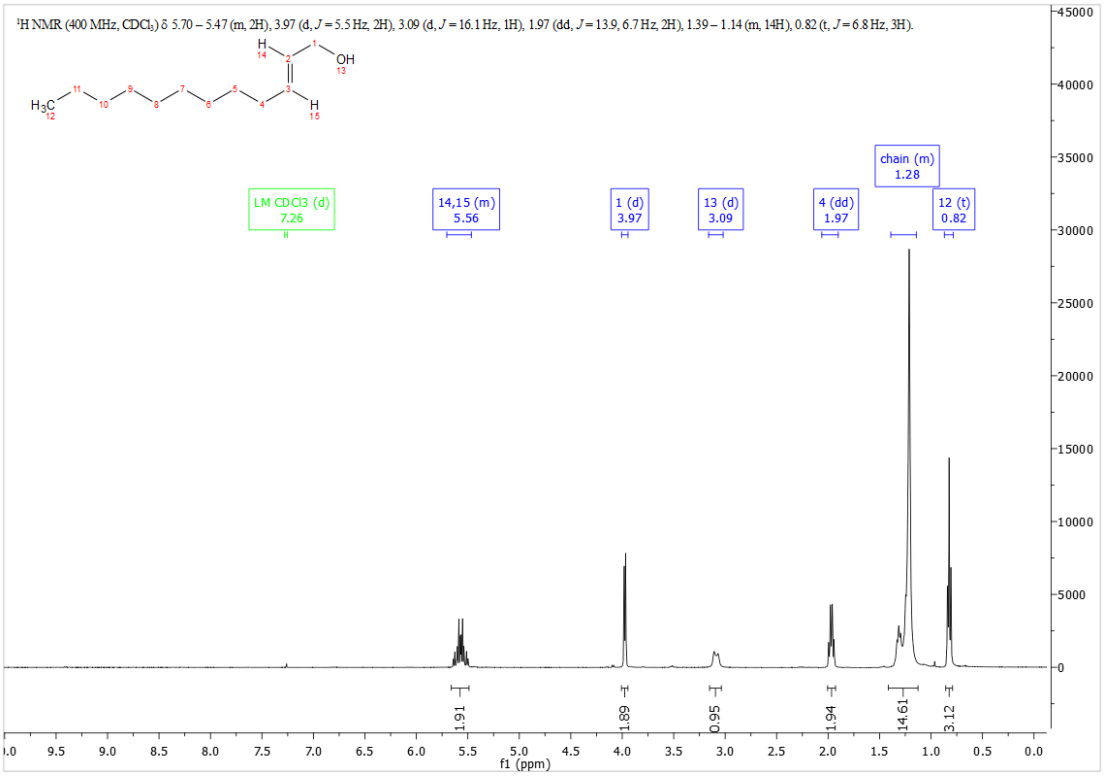

31P NMR (203 MHz, D2O) δ 2.48 – 0.77 (m), 0.51 – -0.63 (m), -2.64 – -5.07 (m).
15 (m)
0.21
23 (m)
-4.16
19 (m)
1.54
f1 (ppm)

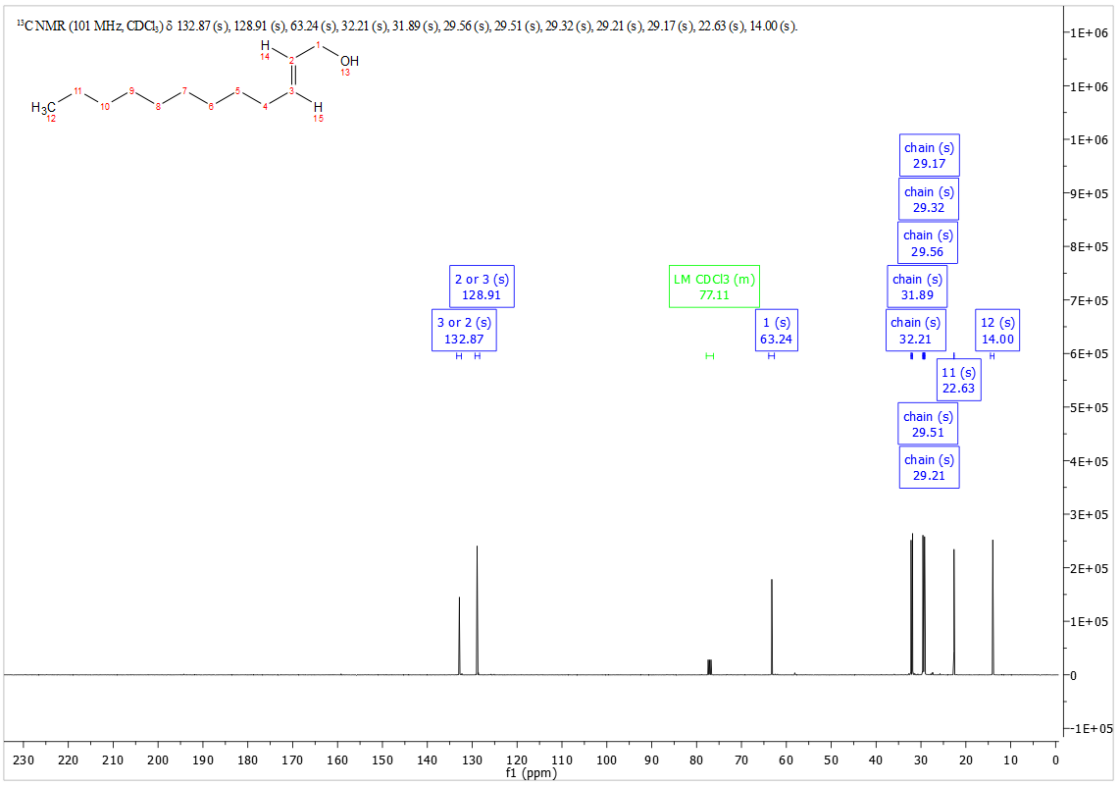




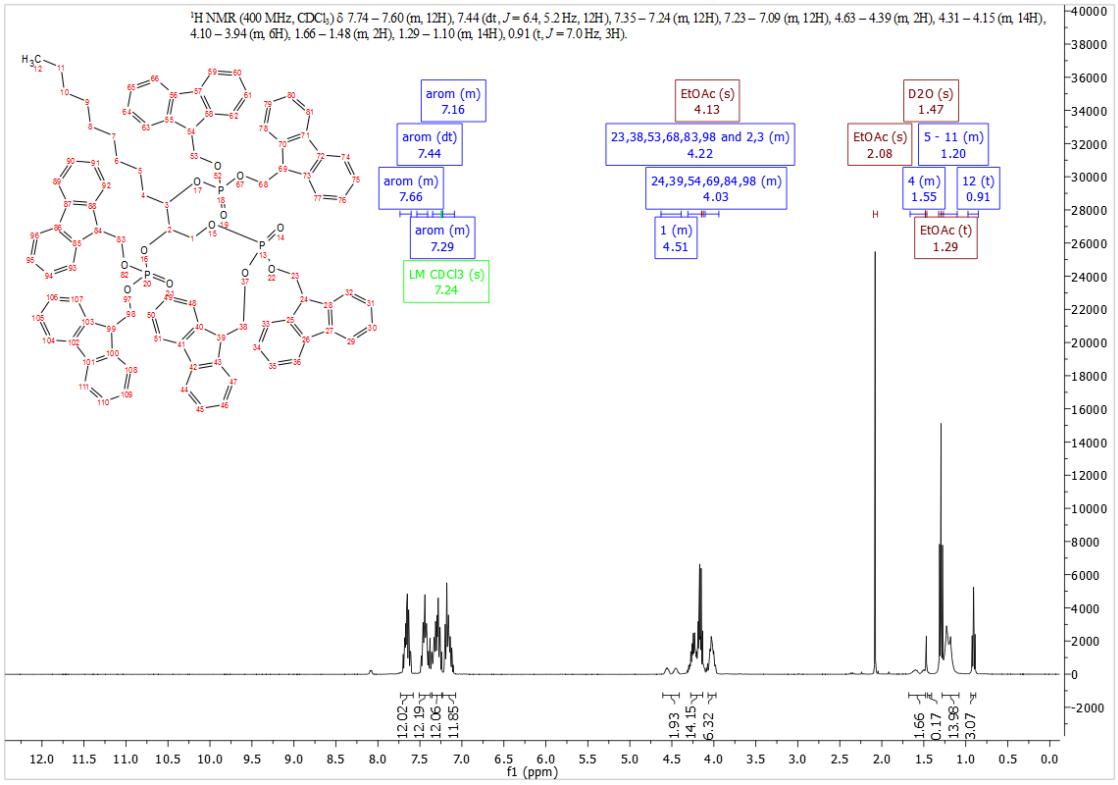
Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	Score	rdb	e¯ Conf	N-Rule
284.10010	1	C18H33N8O11P	284.10087	2.70	1.5	85.21	7.0	even	ok
	2	C20H43O12P3	284.09892	-4.17	1.6	67.21	1.0	even	ok
489.23914	1	C20H43O9P2	489.23878	-0.73	4.1	100.00	0.5	even	ok
	2	C17H35N10O3P2	489.23743	-3.48	6.7	43.86	6.5	even	ok
569.20544	1	C14H30N14O9P	569.20633	1.56	3.6	59.77	7.5	even	ok
	2	C20H44O12P3	569.20511	-0.58	6.6	100.00	0.5	even	ok

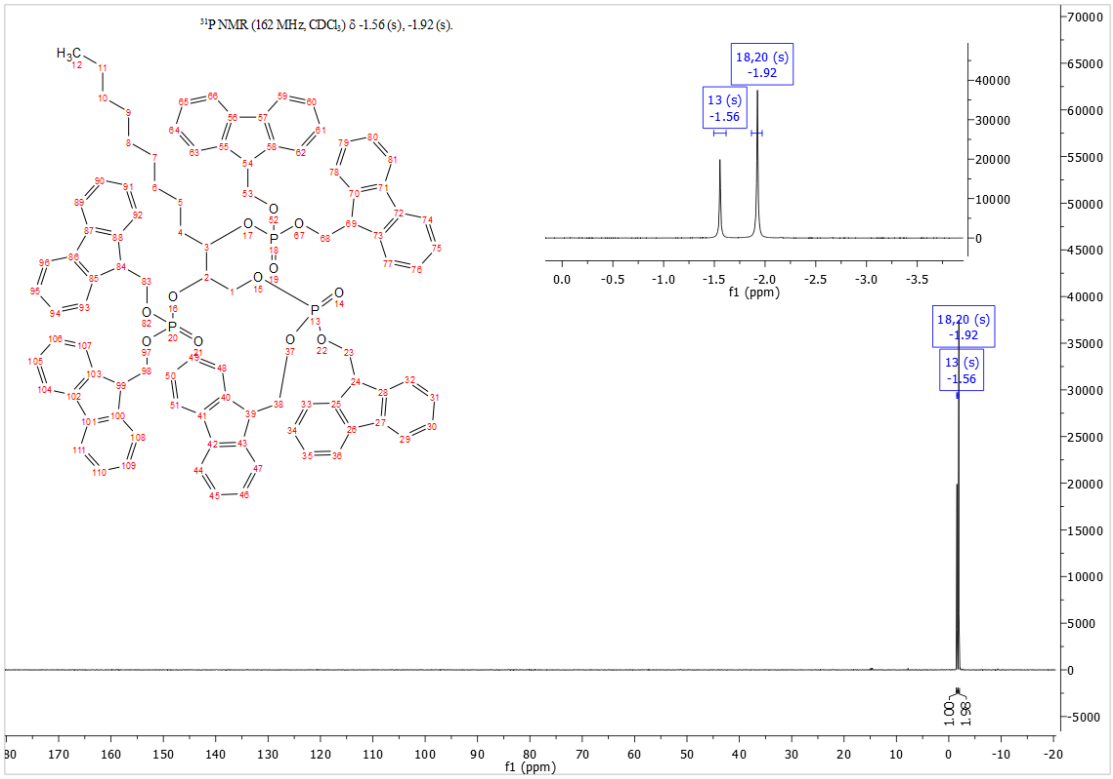
dodecane-1,2,3-triol (11):

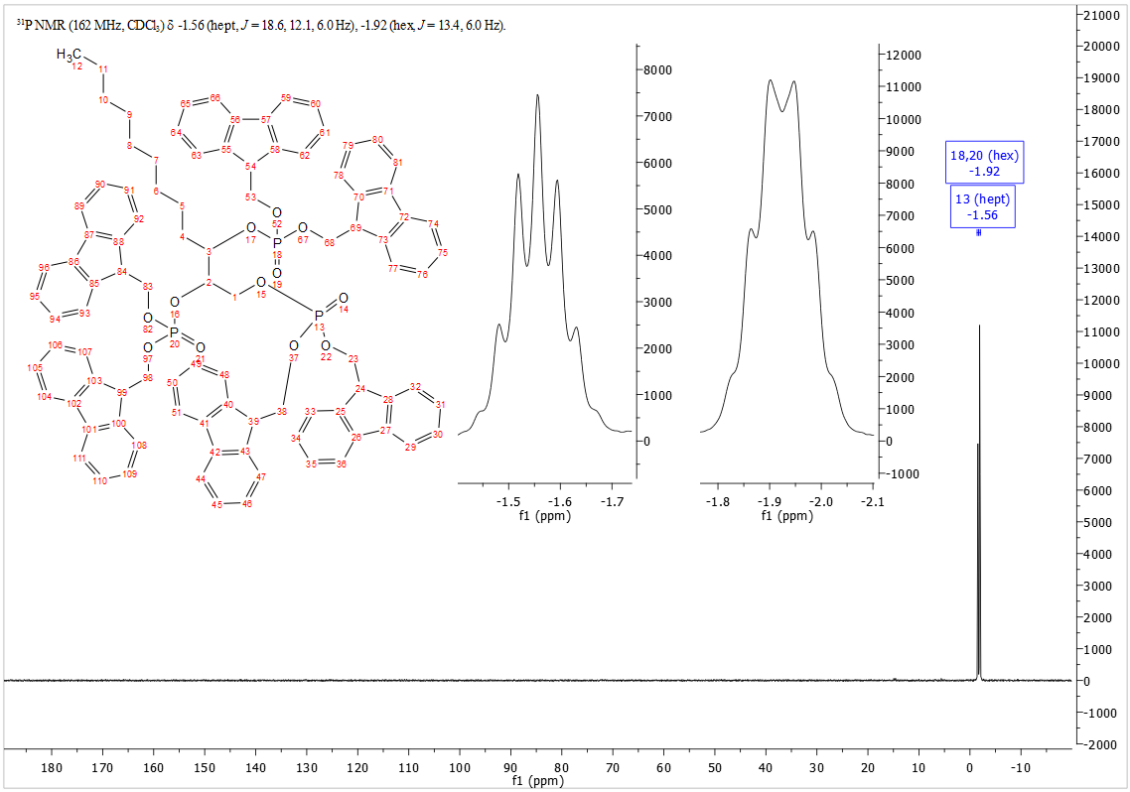


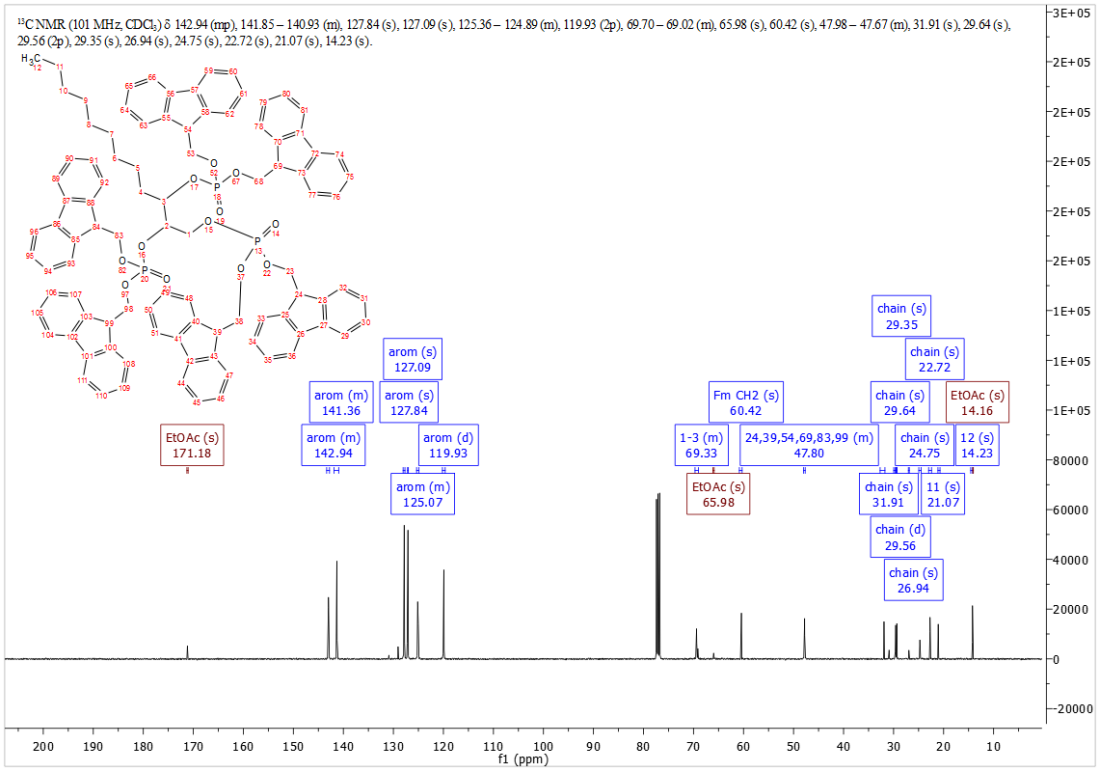


hexakis((9*H*-fluoren-9-yl)methyl) dodecane-1,2,3-triyl tris(phosphate) (12):

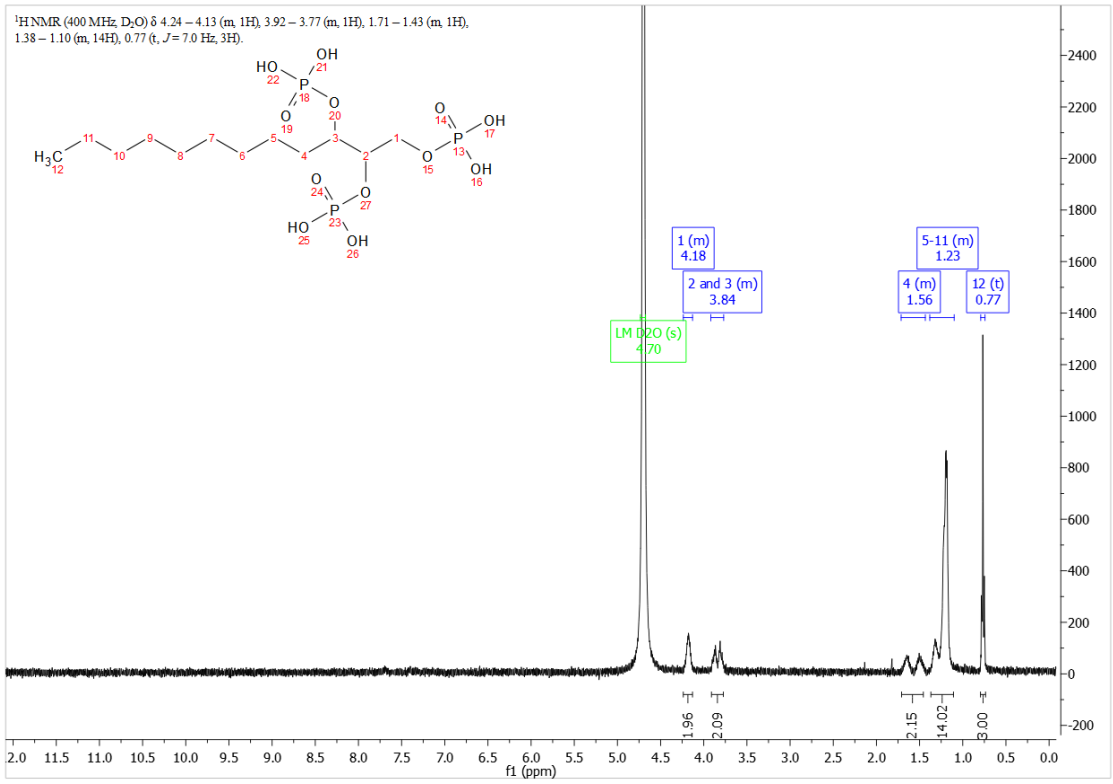


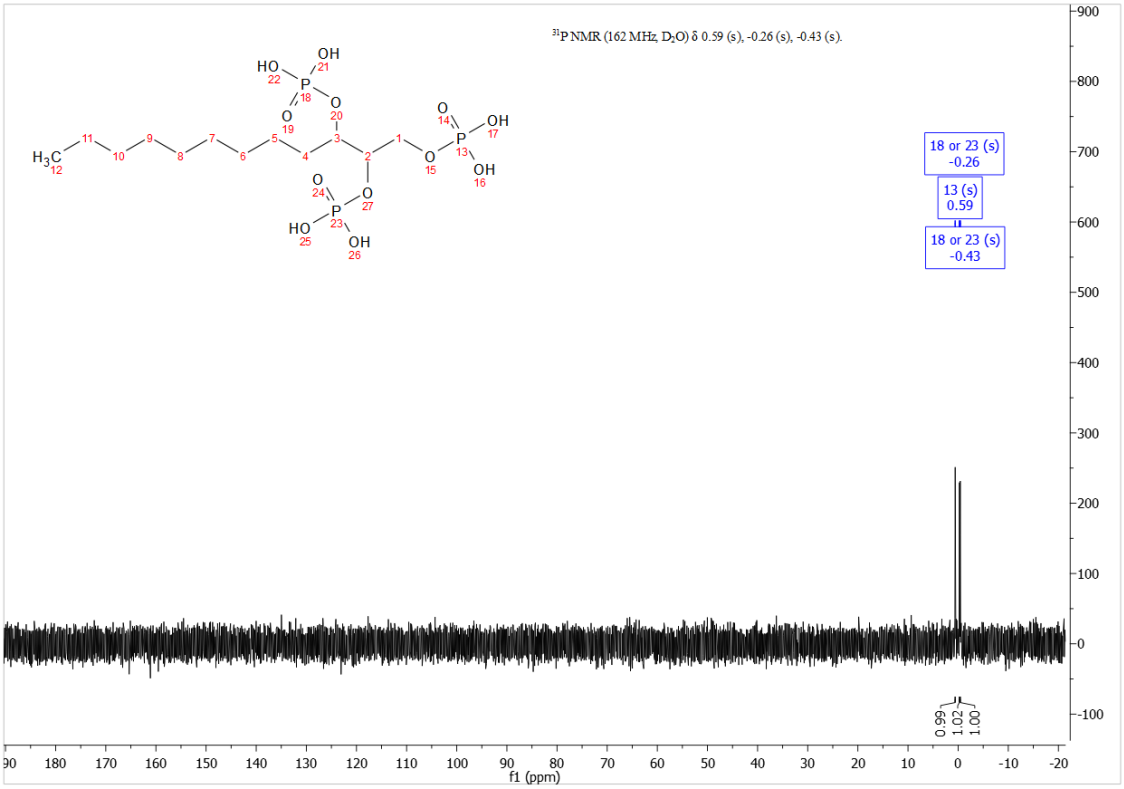


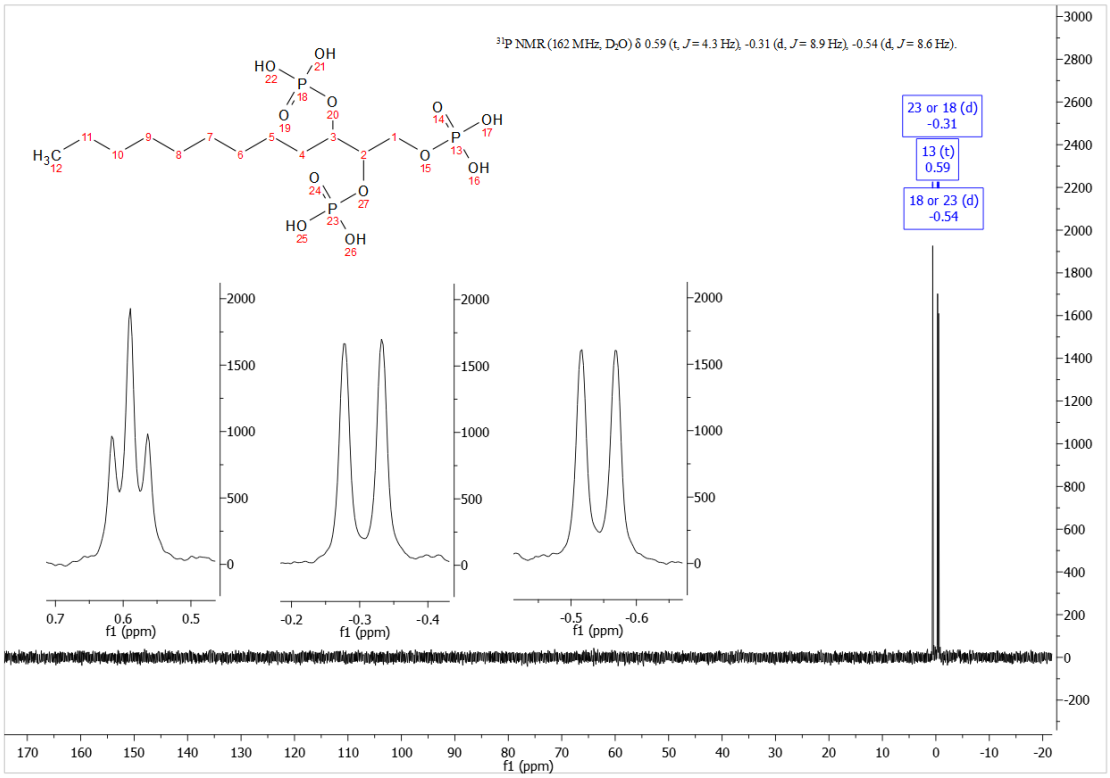

31P NMR (162 MHz, CDCl3) δ -1.56 (hept, J = 18.6, 12.1, 6.0 Hz), -1.92 (hex, J = 13.4, 6.0 Hz).
18,20 (hex)
-1.92
13 (hept)
-1.56
f1 (ppm)

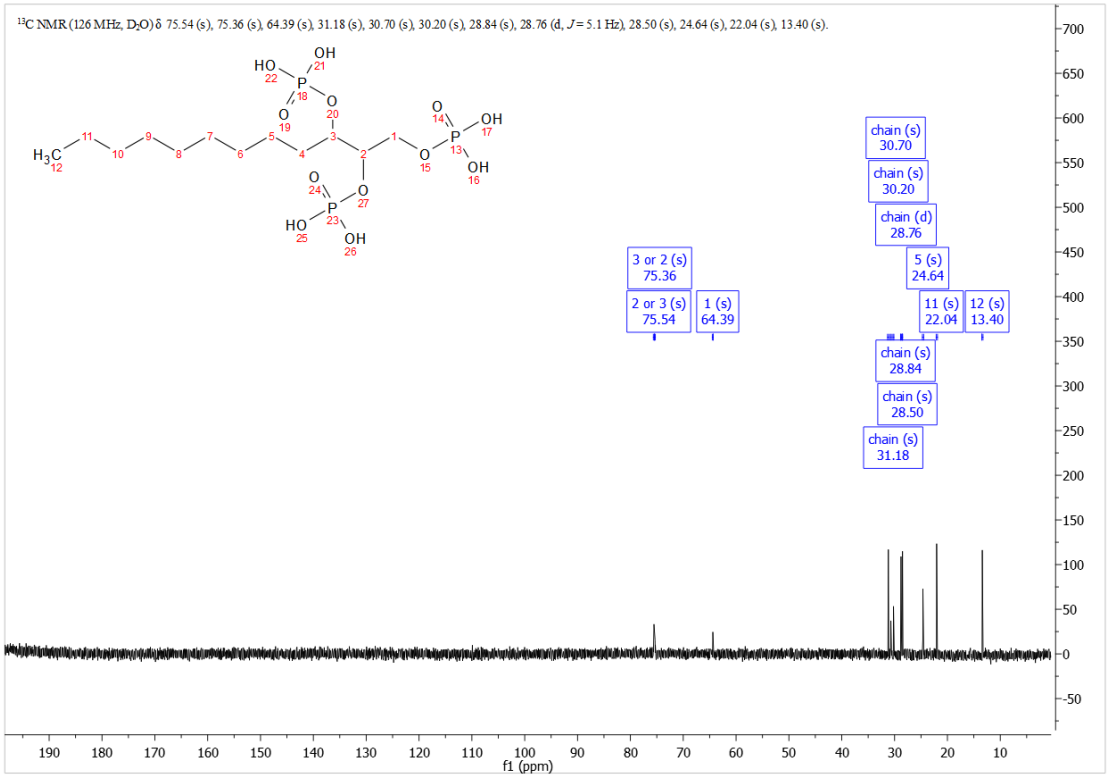

13C NMR (101 MHz, CDCl3) δ 142.94 (mp), 141.85 – 140.93 (m), 127.84 (s), 127.09 (s), 125.36 – 124.89 (m), 119.93 (2p), 69.70 – 69.02 (m), 65.98 (s), 60.42 (s), 47.98 – 47.67 (m), 31.91 (s), 29.64 (s), 29.56 (2p), 29.35 (s), 26.94 (s), 24.75 (s), 22.72 (s), 21.07 (s), 14.23 (s).
EtOAc (s) 171.18
arom (m) 141.36
arom (m) 142.94
arom (s) 127.09
arom (s) 127.84
arom (d) 119.93
arom (m) 125.07
Fm CH2 (s) 60.42
1-3 (m) 69.33
24,39,54,69,83,99 (m) 47.80
EtOAc (s) 65.98
chain (s) 29.35
chain (s) 22.72
chain (s) 29.64
EtOAc (s) 14.16
chain (s) 24.75
12 (s) 14.23
chain (s) 31.91
11 (s) 21.07
chain (d) 29.56
chain (s) 26.94
f1 (ppm)

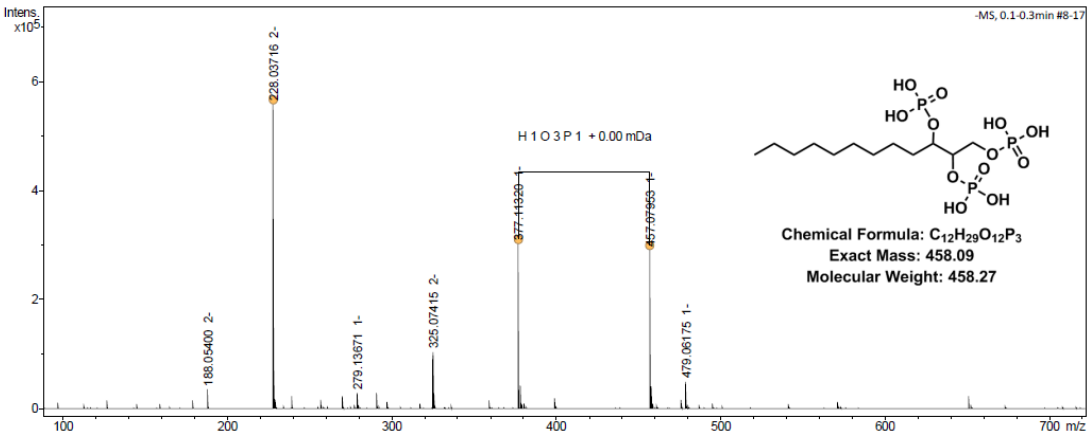
dodecane-1,2,3-triyl tris(dihydrogen phosphate) (13):





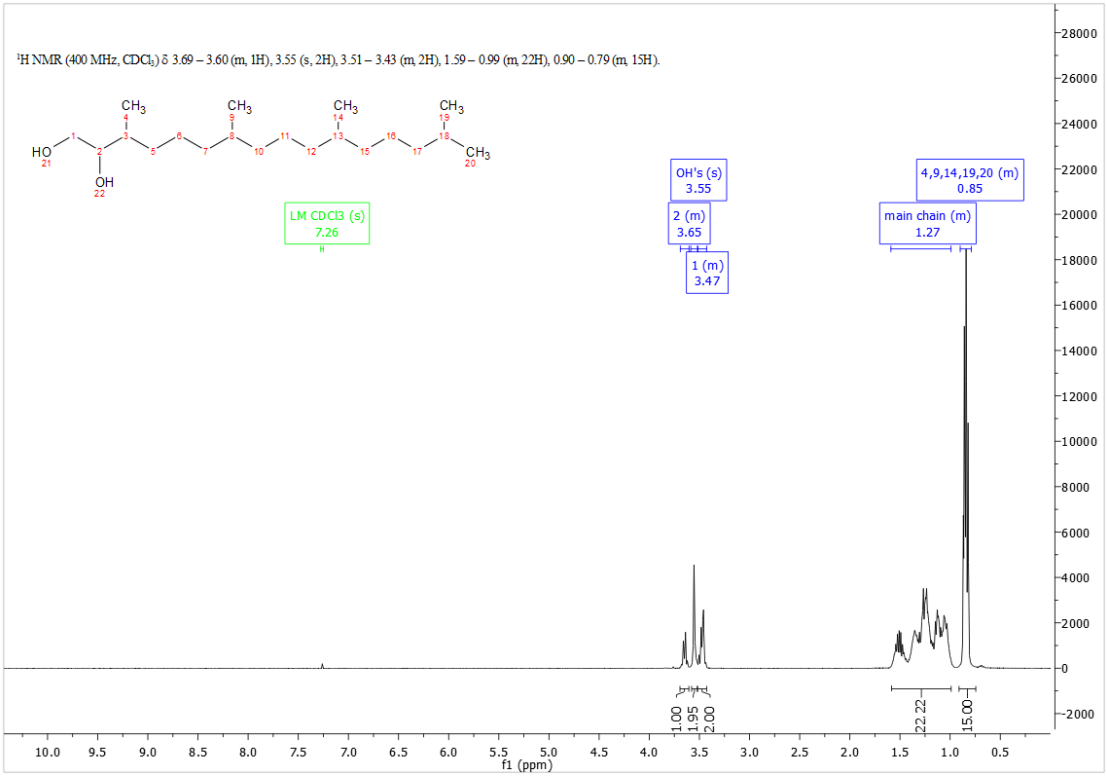

^{31}P NMR (162 MHz, D_2O) δ 0.59 (t, J = 4.3 Hz), -0.31 (d, J = 8.9 Hz), -0.54 (d, J = 8.6 Hz).
23 or 18 (d)
-0.31
13 (t)
0.59
18 or 23 (d)
-0.54
f1 (ppm)

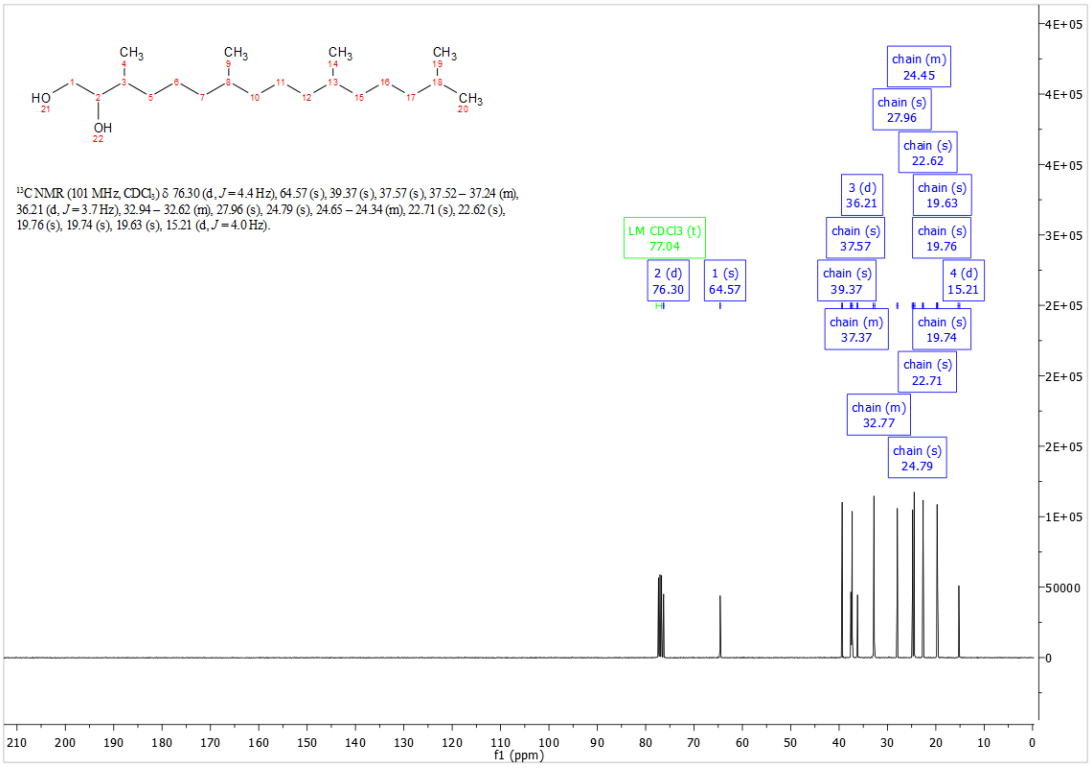

^{13}C NMR (126 MHz, D_2O) δ 75.54 (s), 75.36 (s), 64.39 (s), 31.18 (s), 30.70 (s), 30.20 (s), 28.84 (s), 28.76 (d, J = 5.1 Hz), 28.50 (s), 24.64 (s), 22.04 (s), 13.40 (s).
chain (s)
30.70
chain (s)
30.20
chain (d)
28.76
5 (s)
24.64
11 (s)
22.04
12 (s)
13.40
chain (s)
28.84
chain (s)
28.50
chain (s)
31.18
3 or 2 (s)
75.36
2 or 3 (s)
75.54
1 (s)
64.39
f1 (ppm)



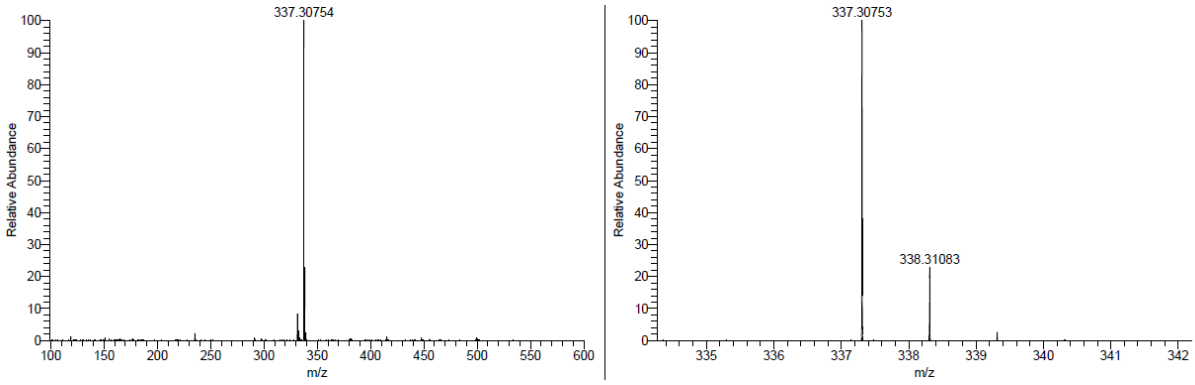
Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	Score	rdb	e¯ Conf	N-Rule
228.03716	1	C12H27O12P3	228.03632	-3.72	0.1	89.08	1.0	even	ok
377.11320	1	C12H27O9P2	377.11358	1.01	3.5	100.00	0.5	even	ok
457.07953	1	C12H28O12P3	457.07991	0.84	1.6	93.34	0.5	even	ok

3,7,11,15-tetramethylhexadecane-1,2-diol (15):



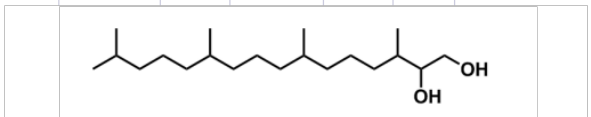


^{13}C NMR (101 MHz, $CDCl_3$) δ 76.30 (d, J = 4.4 Hz), 64.57 (s), 39.37 (s), 37.57 (s), 37.52 – 37.24 (m), 36.21 (d, J = 3.7 Hz), 32.94 – 32.62 (m), 27.96 (s), 24.79 (s), 24.65 – 24.34 (m), 22.71 (s), 22.62 (s), 19.76 (s), 19.74 (s), 19.63 (s), 15.21 (d, J = 4.0 Hz).



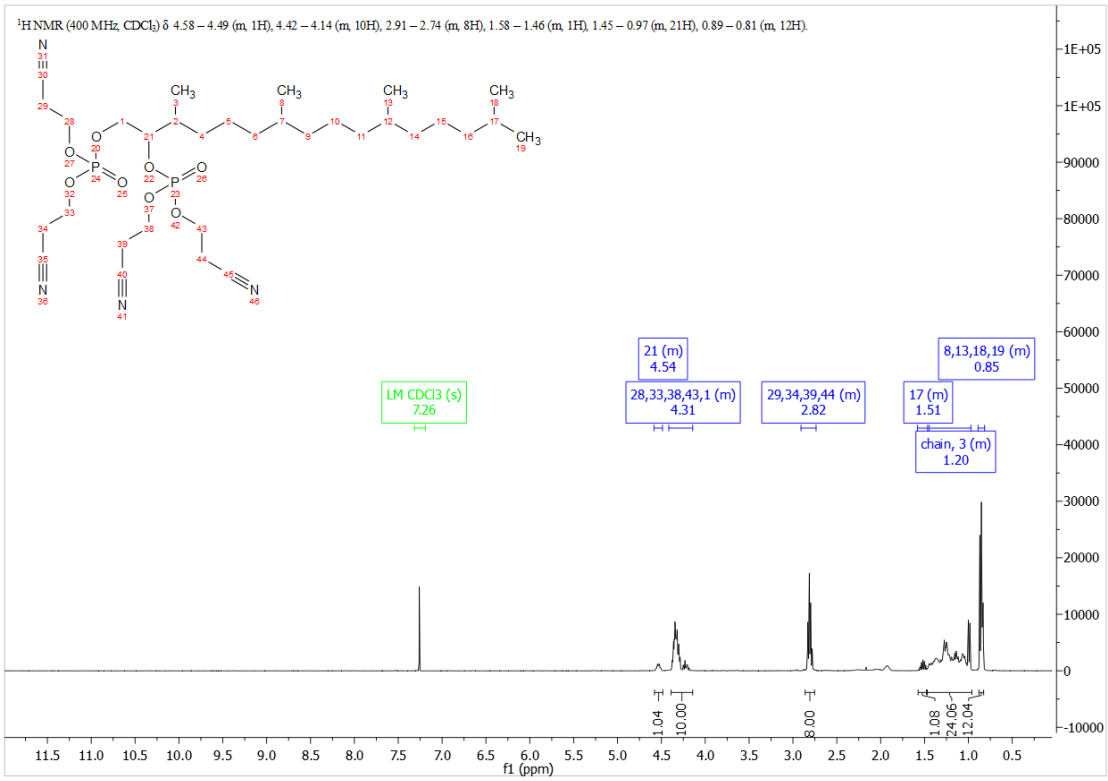
laQEx5844#39-51 RT: 0.39-0.50 AV: 7
SB: 24 0.08-0.30 , 0.74-0.99
T: FTMS + p ESI Full lock ms [100.00-1500.00]
m/z= 337.06647-337.51855

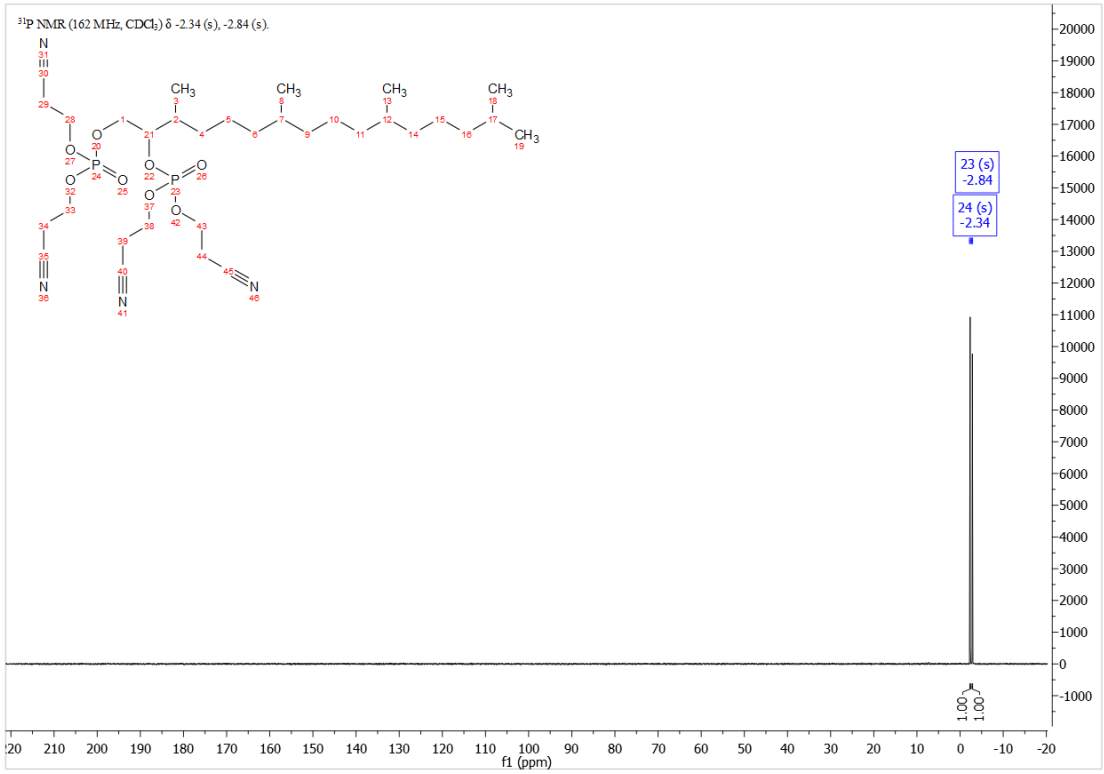
m/z	Intensity	Relative	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
337.30754	558704064.0	100.00	337.30742	0.12	3.5	$C_{18}H_{37}N_6$
			337.30770	-0.16	-0.5	$C_{20}H_{42}O_2Na$

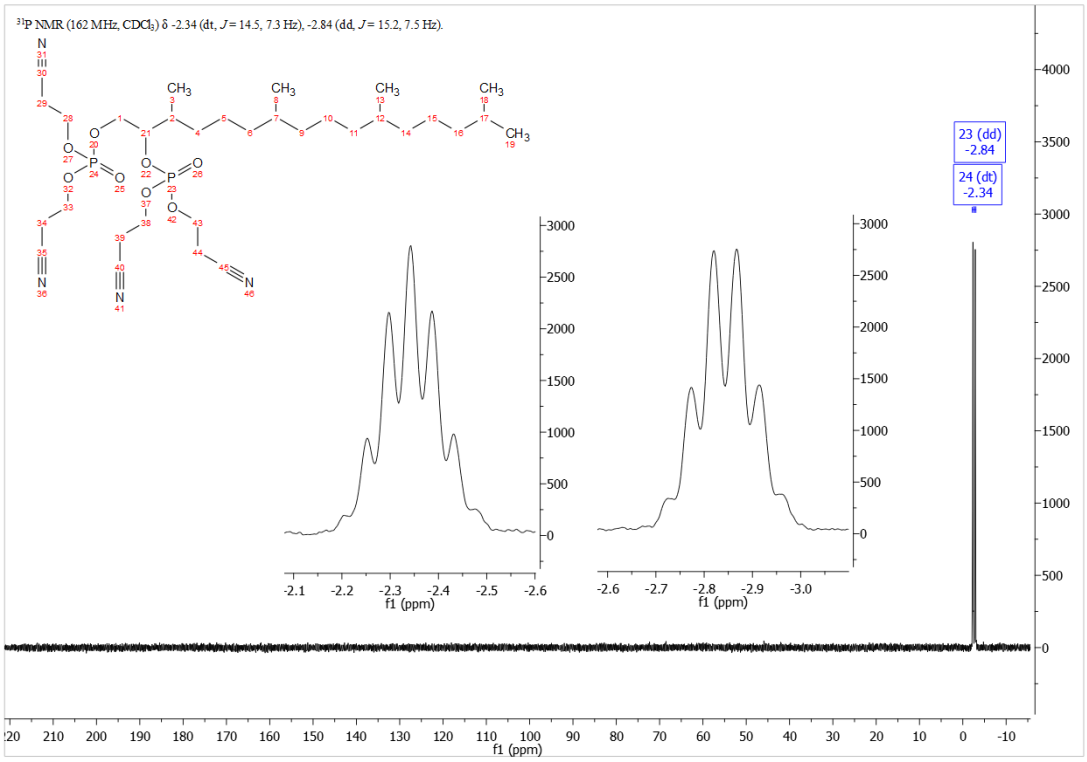


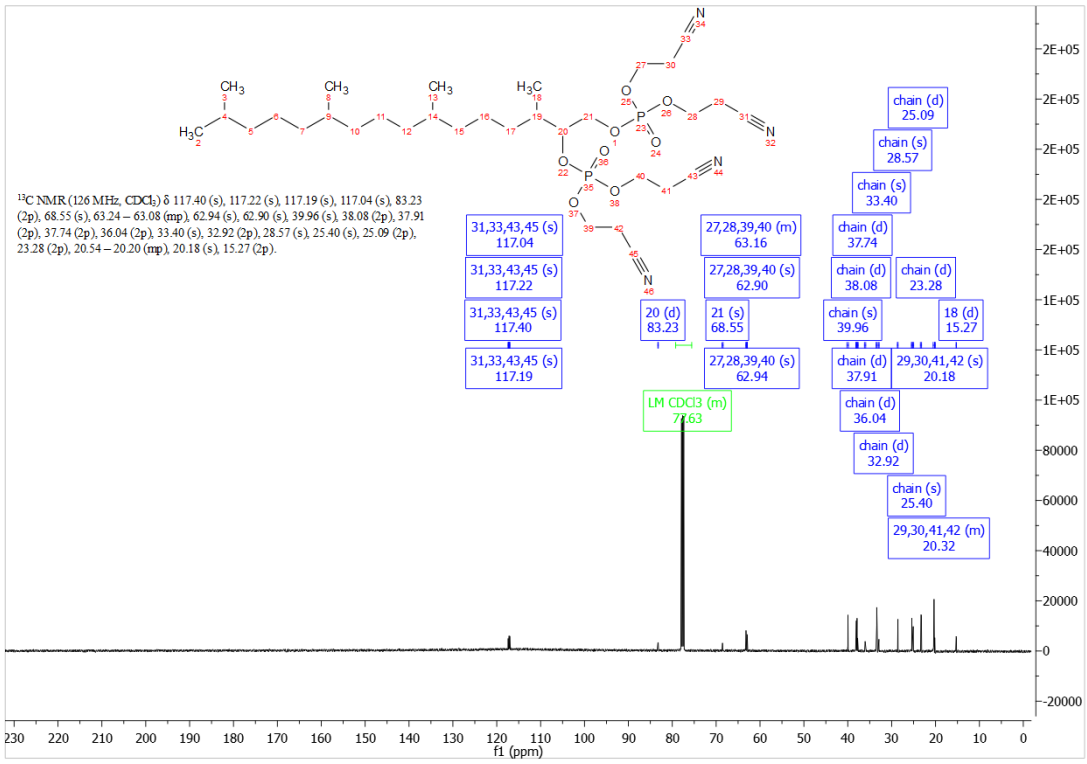
Chemical Formula: $C_{20}H_{42}O_2$
Exact Mass: 314.32
Molecular Weight: 314.55

tetrakis(2-cyanoethyl) (3,7,11,15-tetramethylhexadecane-1,2-diyl) bis(phosphate) (16):

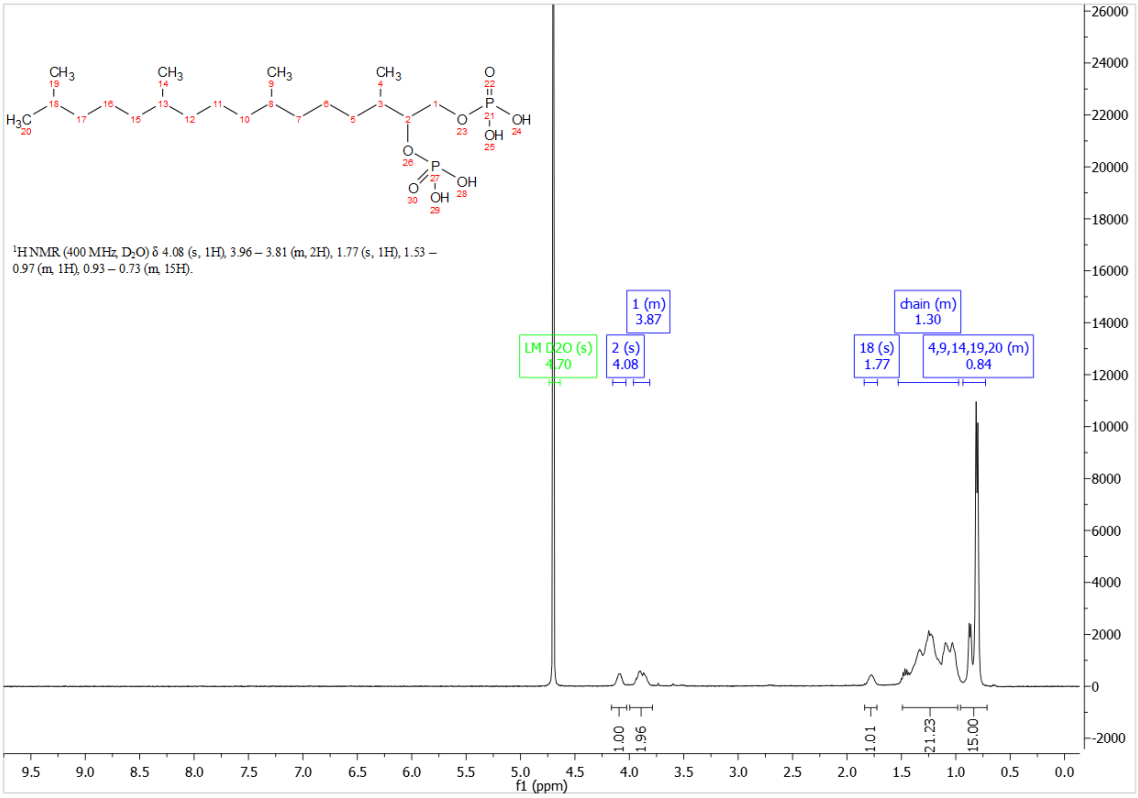


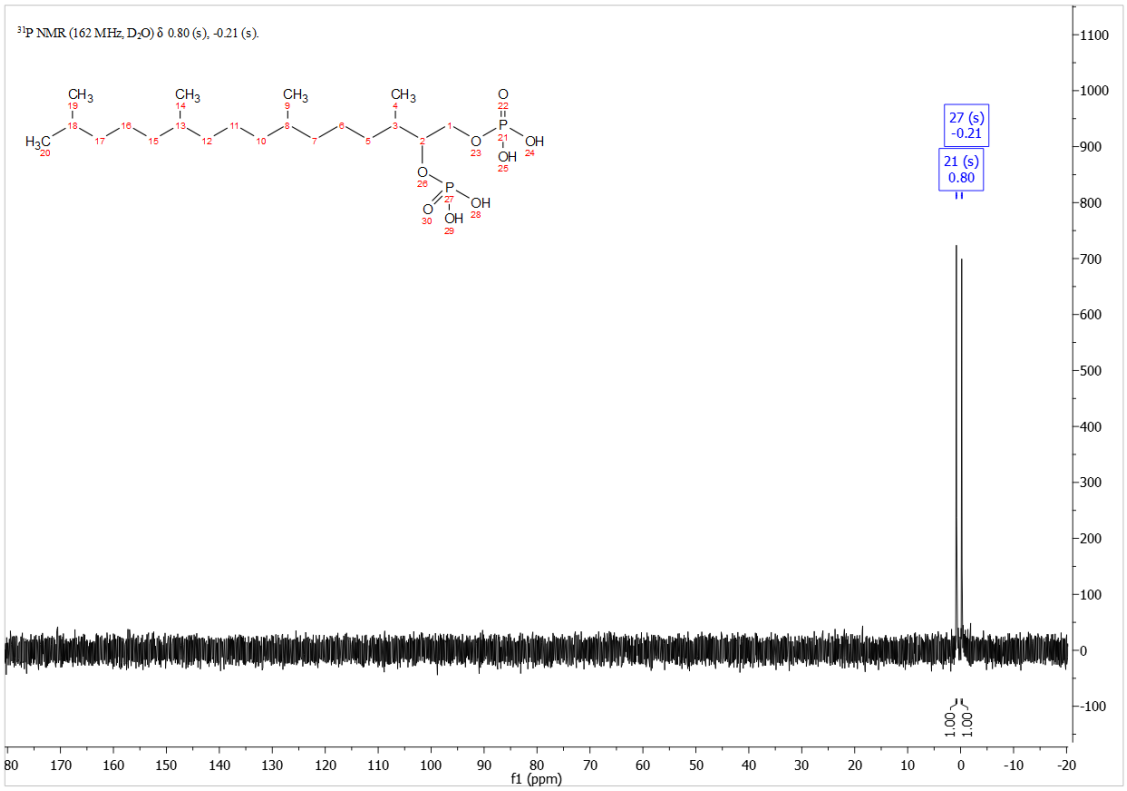


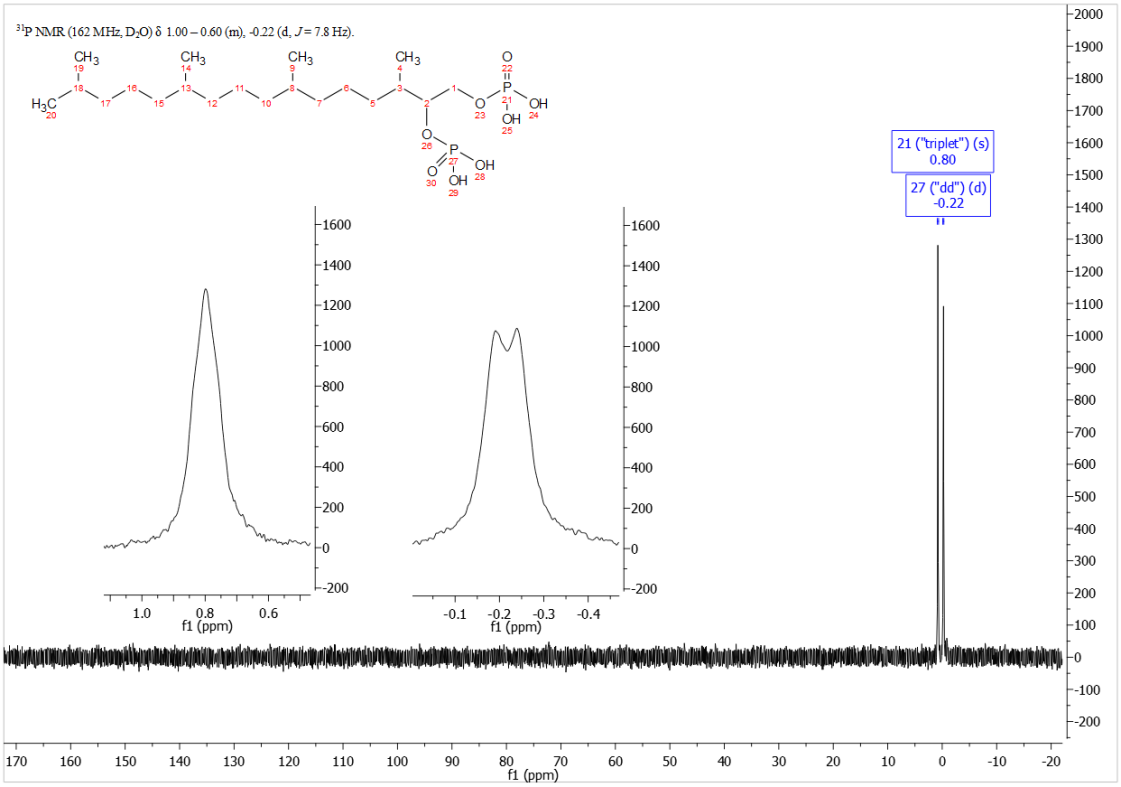

31P NMR (162 MHz, CDCl3) δ -2.34 (dt, J = 14.5, 7.3 Hz), -2.84 (dd, J = 15.2, 7.5 Hz).
23 (dd) -2.84
24 (dt) -2.34
f1 (ppm)

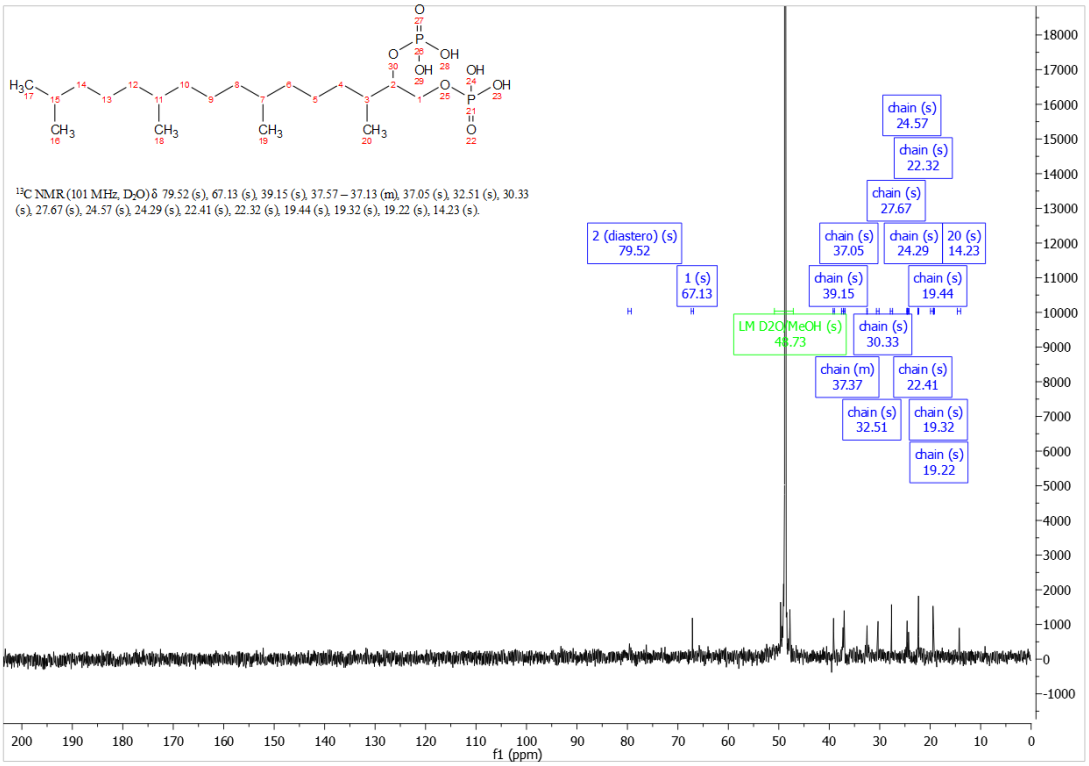

13C NMR (126 MHz, CDCl3) δ 117.40 (s), 117.22 (s), 117.19 (s), 117.04 (s), 83.23 (2p), 68.55 (s), 63.24 – 63.08 (mp), 62.94 (s), 62.90 (s), 39.96 (s), 38.08 (2p), 37.91 (2p), 37.74 (2p), 36.04 (2p), 33.40 (s), 32.92 (2p), 28.57 (s), 25.40 (s), 25.09 (2p), 23.28 (2p), 20.54 – 20.20 (mp), 20.18 (s), 15.27 (2p).
31,33,43,45 (s) 117.04
31,33,43,45 (s) 117.22
31,33,43,45 (s) 117.40
31,33,43,45 (s) 117.19
27,28,39,40 (m) 63.16
27,28,39,40 (s) 62.90
20 (d) 83.23
21 (s) 68.55
27,28,39,40 (s) 62.94
LM CDCl3 (m) 77.63
chain (d) 25.09
chain (s) 28.57
chain (s) 33.40
chain (d) 37.74
chain (d) 38.08
chain (d) 23.28
chain (s) 39.96
18 (d) 15.27
chain (d) 37.91
29,30,41,42 (s) 20.18
chain (d) 36.04
chain (d) 32.92
chain (s) 25.40
29,30,41,42 (m) 20.32
f1 (ppm)

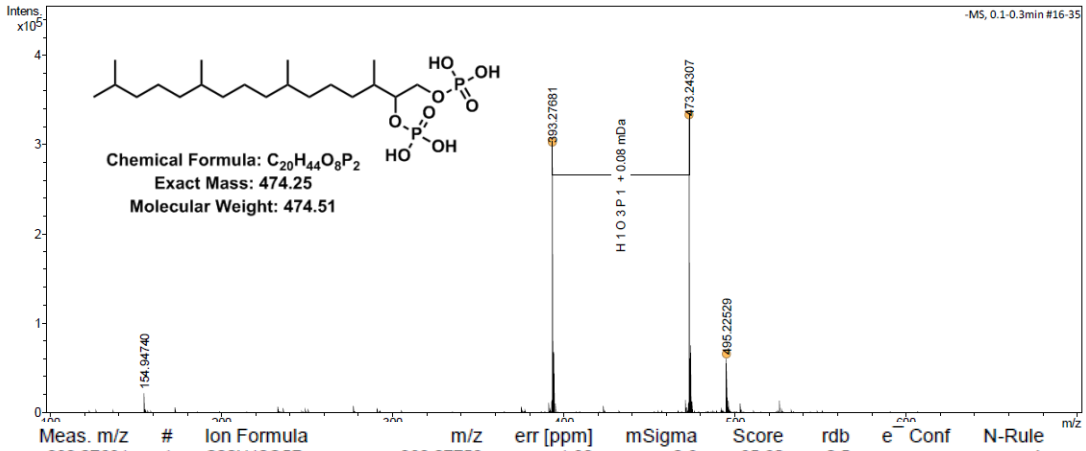
3,7,11,15-tetramethylhexadecane-1,2-diyl bis(dihydrogen phosphate) (17):






31P NMR (162 MHz, D2O) δ 1.00 – 0.60 (m), -0.22 (d, J = 7.8 Hz).
21 ("triplet") (s)
0.80
27 ("dd") (d)
-0.22
f1 (ppm)

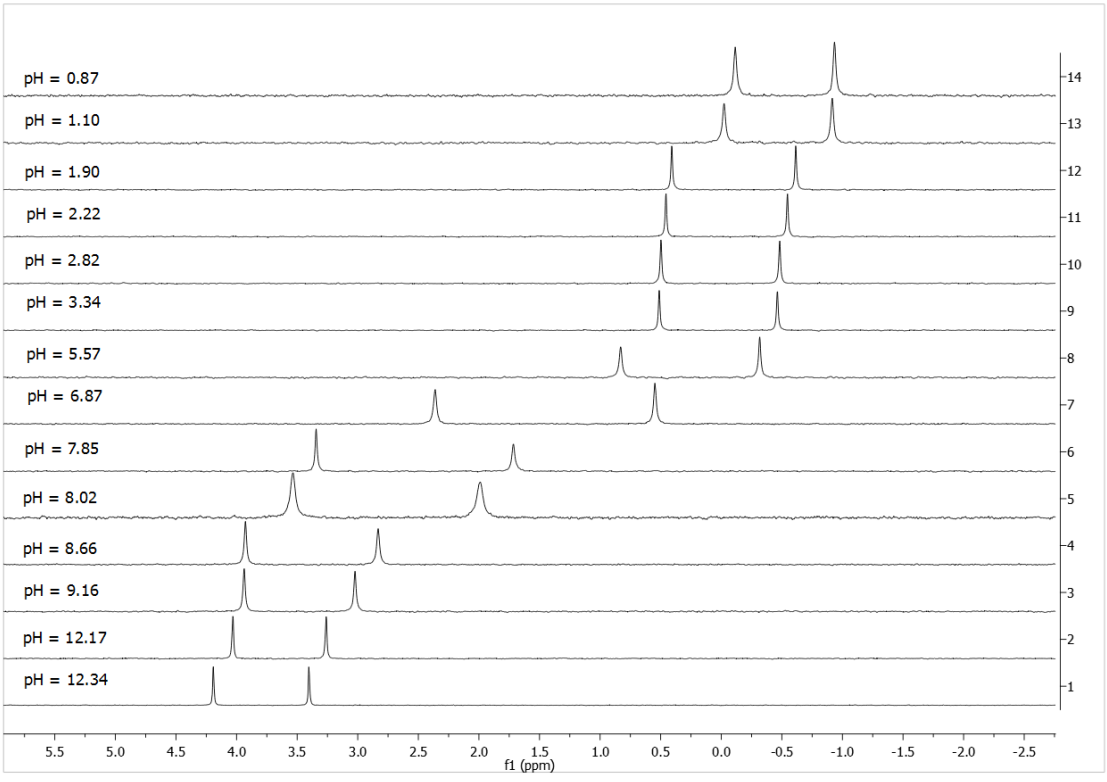

13C NMR (101 MHz, D2O) δ 79.52 (s), 67.13 (s), 39.15 (s), 37.57 – 37.13 (m), 37.05 (s), 32.51 (s), 30.33 (s), 27.67 (s), 24.57 (s), 24.29 (s), 22.41 (s), 22.32 (s), 19.44 (s), 19.32 (s), 19.22 (s), 14.23 (s).
2 (diastero) (s) 79.52
1 (s) 67.13
LM D2O/MeOH (s) 48.73
chain (s) 39.15
chain (s) 37.05
chain (m) 37.37
chain (s) 32.51
chain (s) 30.33
chain (s) 27.67
chain (s) 24.57
chain (s) 24.29
chain (s) 22.41
chain (s) 22.32
chain (s) 19.44
chain (s) 19.32
chain (s) 19.22
20 (s) 14.23
f1 (ppm)



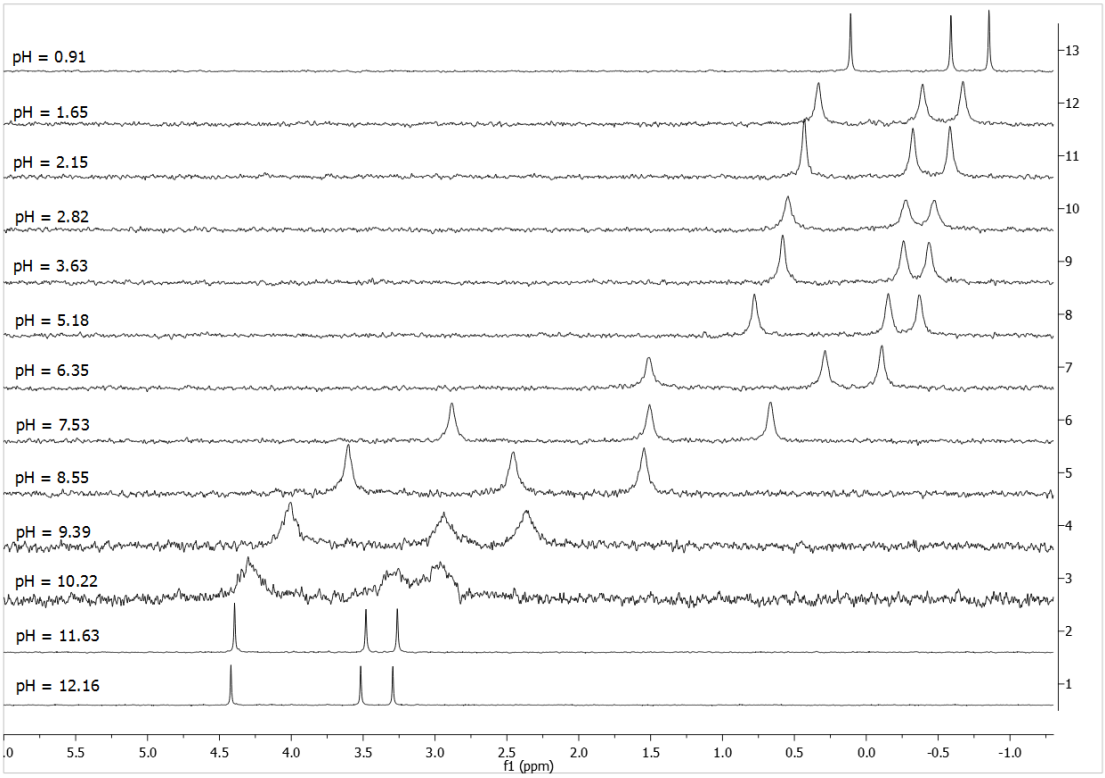
Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	Score	rdb	e⁻ Conf	N-Rule
393.27681	1	C20H42O5P	393.27753	1.83	2.9	85.60	0.5	even	ok
	2	C19H39N4NaOP	393.27647	-0.88	4.4	100.00	2.5	even	ok
	3	C23H33N6	393.27722	1.03	26.9	61.86	10.5	even	ok
	4	C25H38NaO2	393.27750	1.74	27.8	52.84	6.5	even	ok
473.24307	1	C20H43O8P2	473.24387	1.69	3.3	78.33	0.5	even	ok
495.22529	1	C20H42NaO8P2	495.22581	1.06	1.2	90.26	0.5	even	ok

pH-Measurements and plotted titration curves

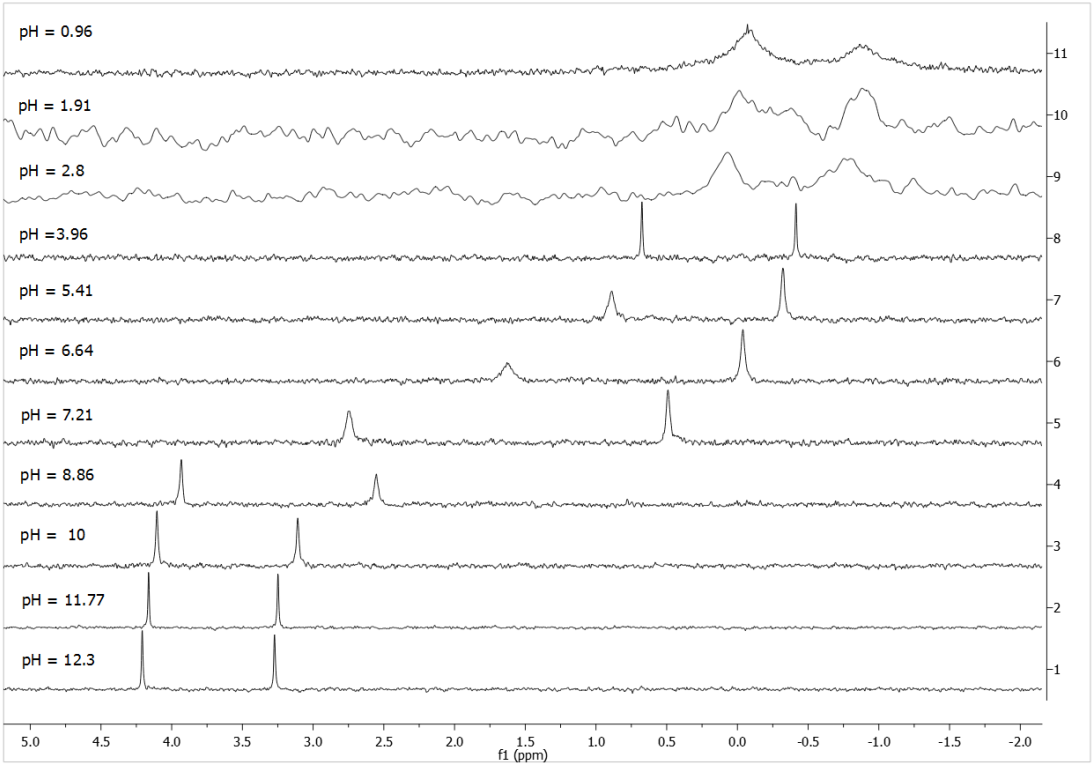
Titration curve of 6, C12P2



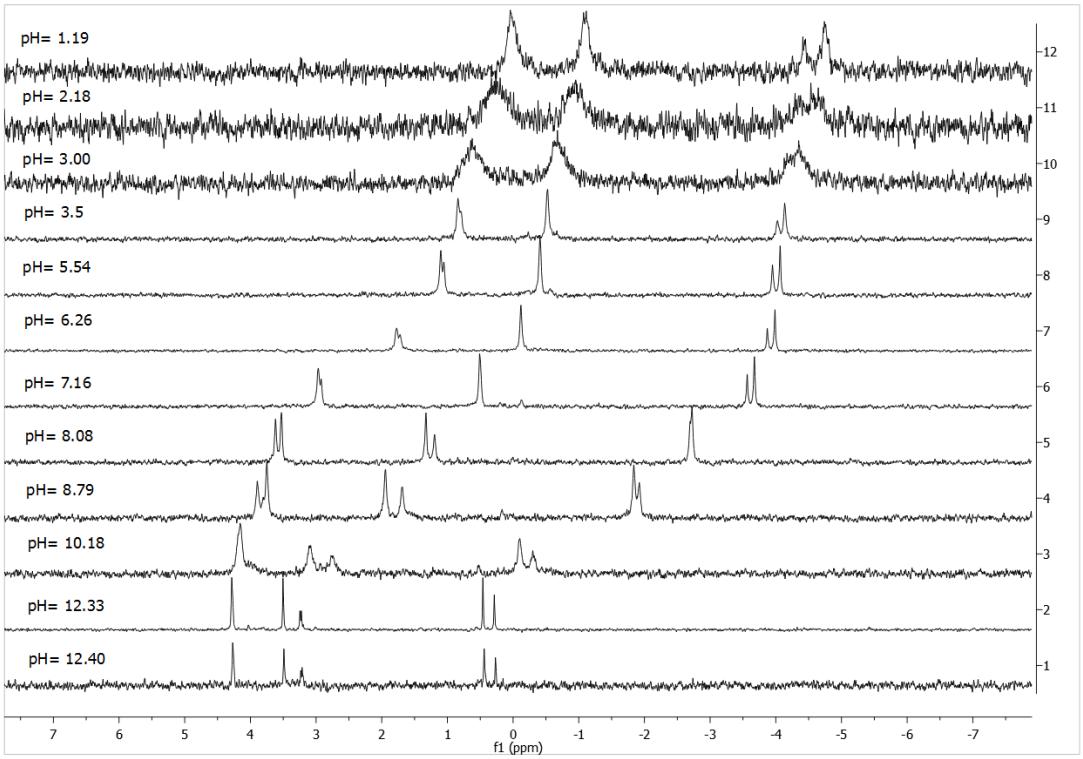
Titration curve of 13, C12P3



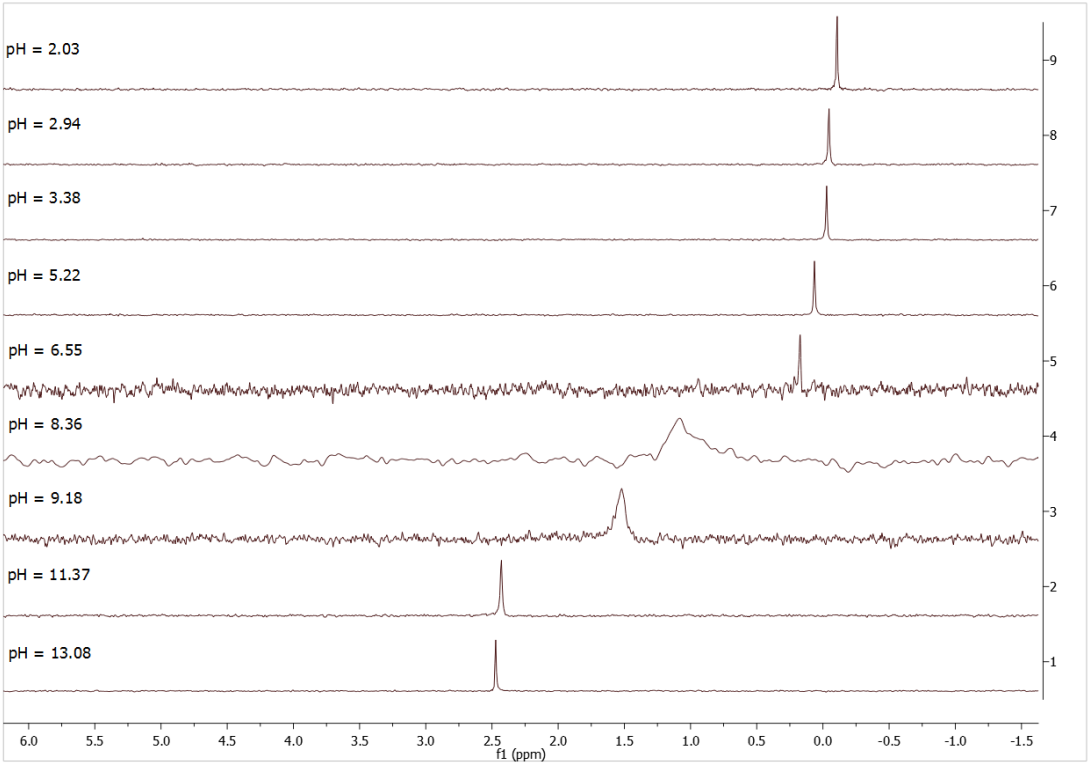
Titration curve of 17, PhyP2



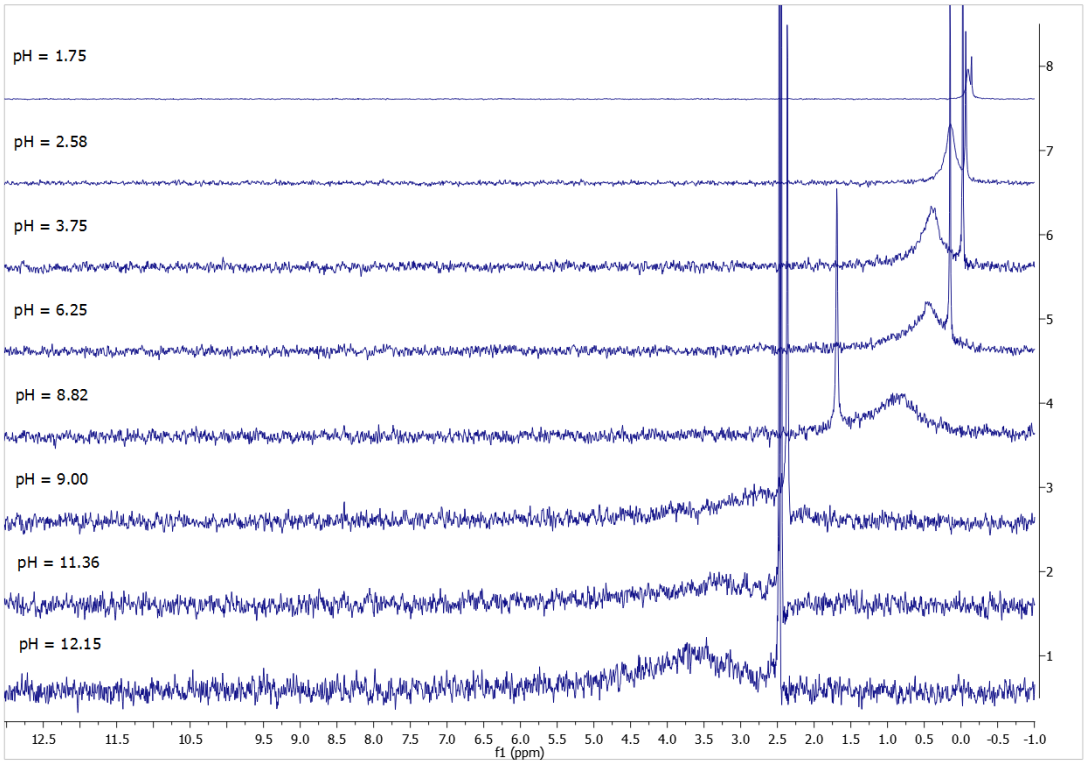
Titration curve of 9, PhyP3



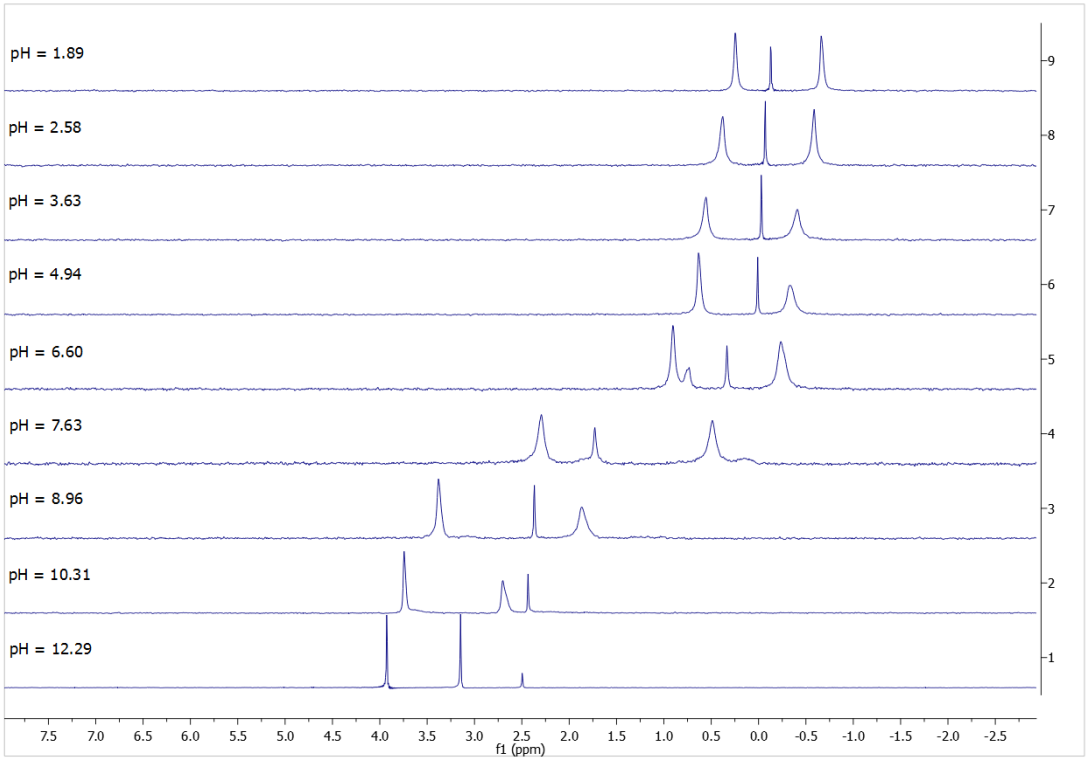
Cubosomes without additive (Signal of phosphate from Pluronic® F-127)



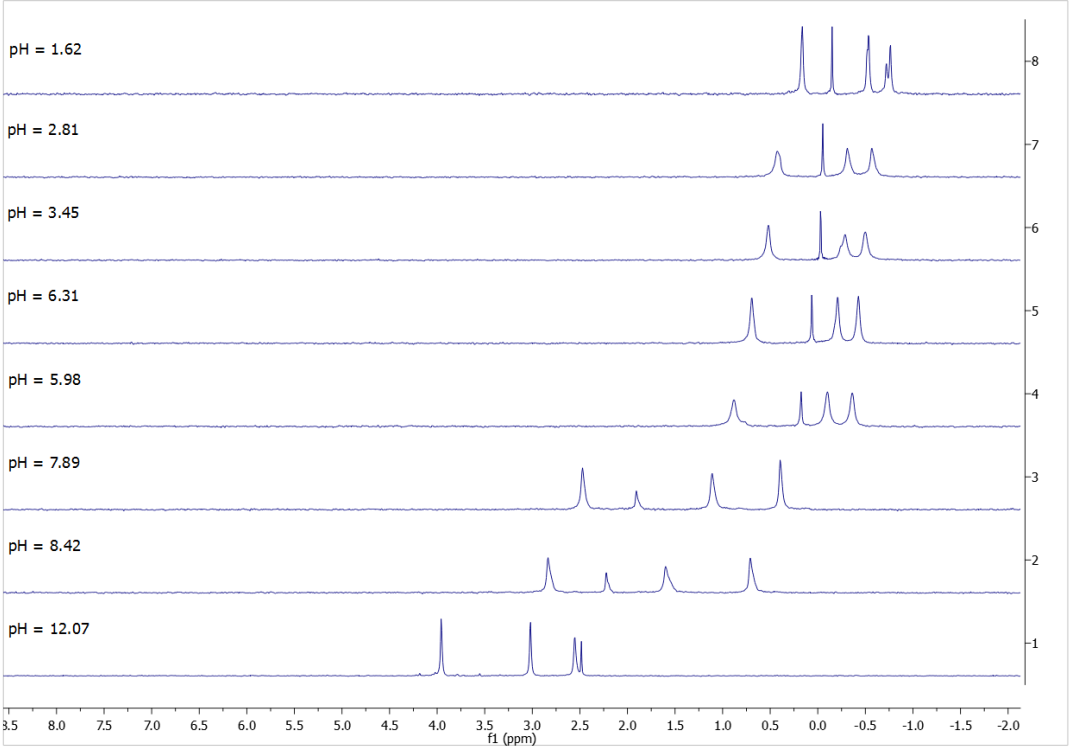
Monolinolein cubosomes with 3 as additive



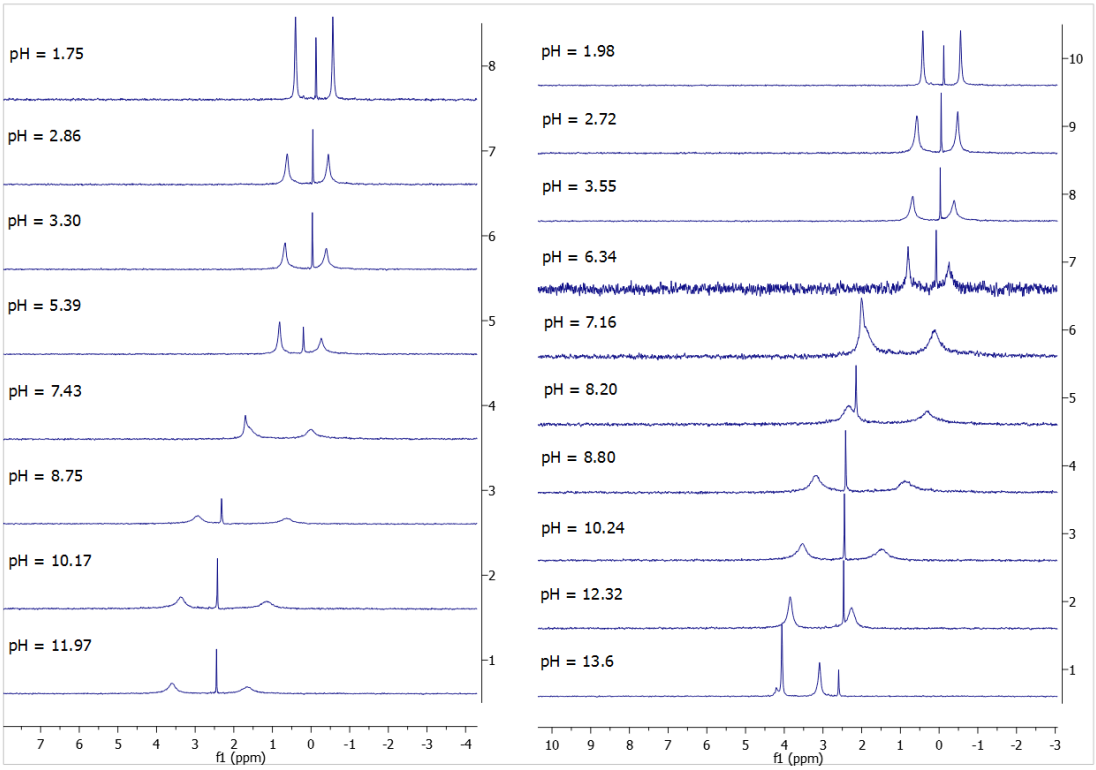
Monolinolein cubosomes with 6 as additive



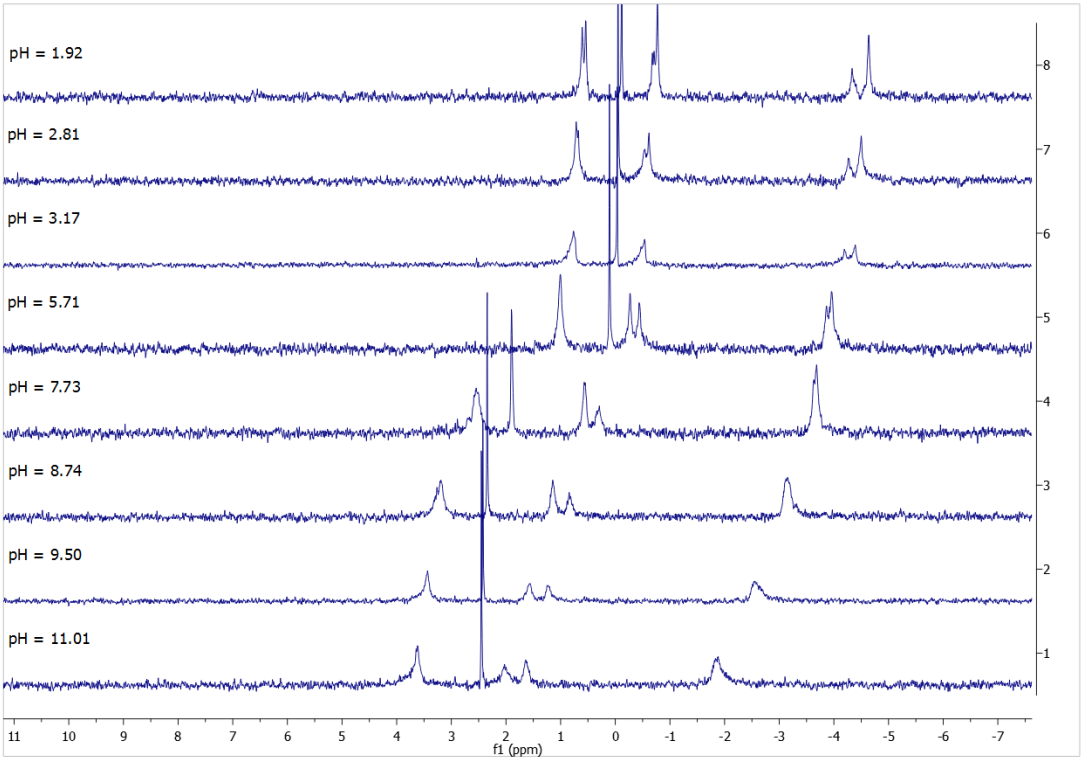
Monolinolein cubosomes with 13 as additive



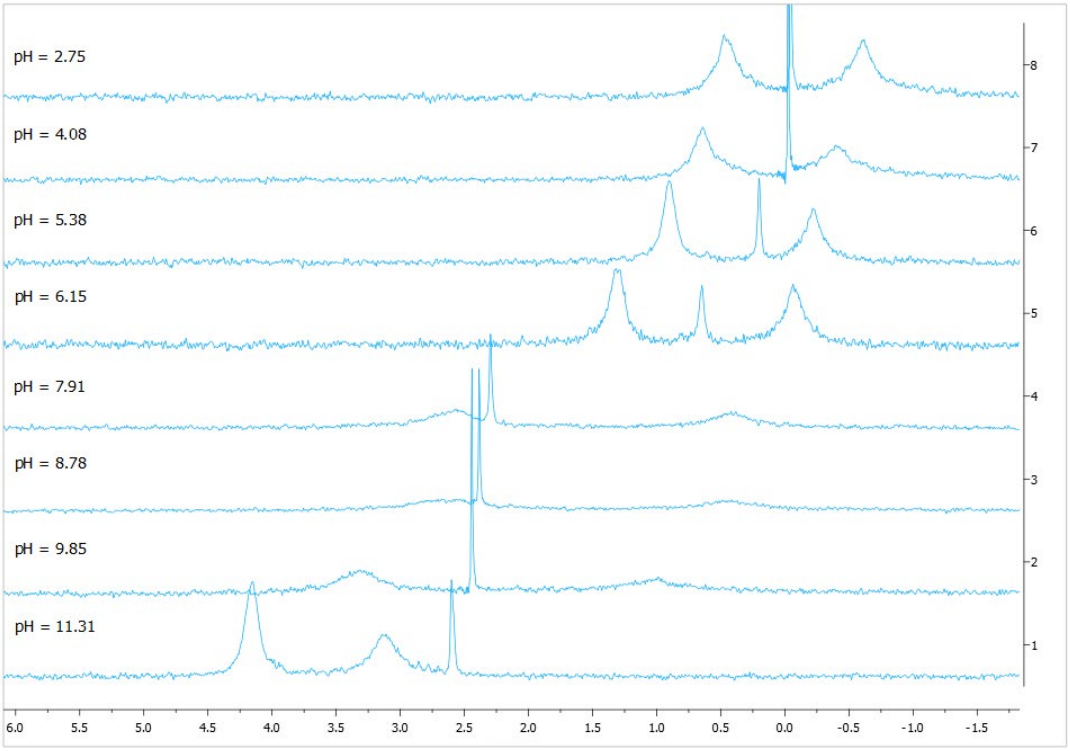
Monolinolein cubosomes with 17 as additive



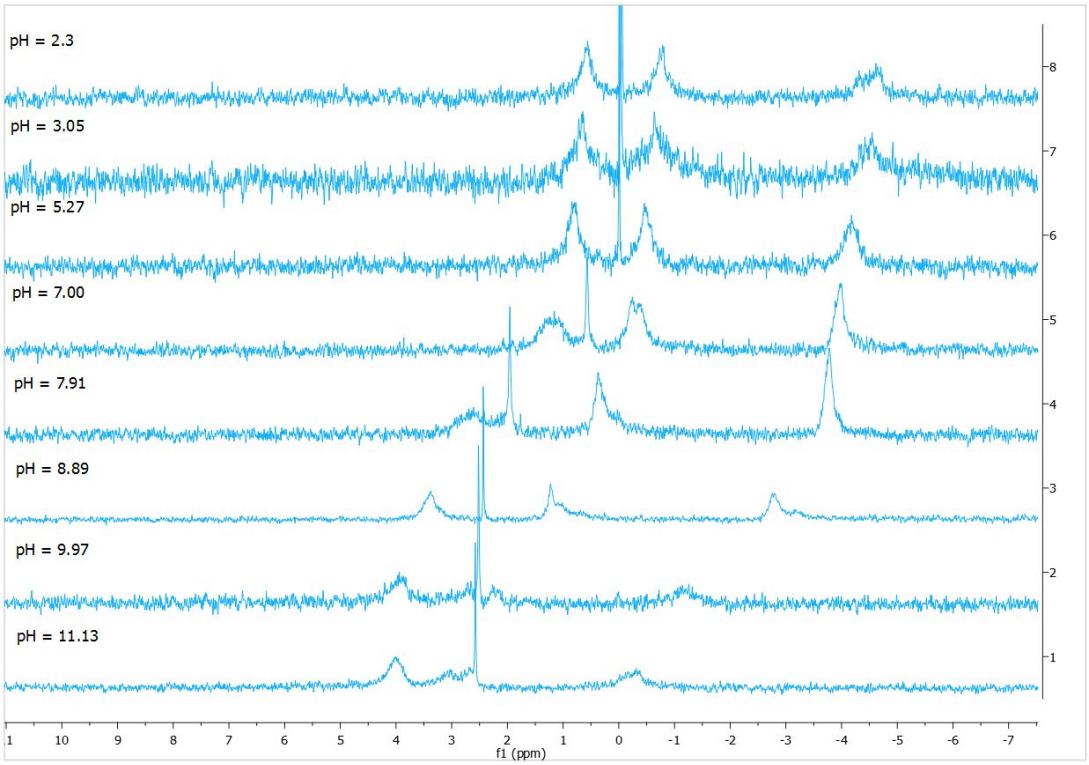
Monolinolein cubosomes with 9 as additive



Phytol cubosomes with 17 as additive



Phytol cubosomes with 9 as additive



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