Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

# Cyclohexane Rings Reduce Membrane Permeability to Small Ions in Archaea-Inspired Tetraether Lipids

*Takaoki Koyanagi, Geoffray Leriche, David Onofrei, Gregory P. Holland, Michael Mayer, and Jerry Yang**

ange_201510445_sm_miscellaneous_information.pdf

## Table of Contents

## 1. General Information

All reagents were purchased from commercial sources and used without further purification. EggPC was purchased from Avanti Polar Lipids. The EggPC lipids were stored under Argon at -20°C and used within 3 months of purchase. Glassware was dried at 115°C overnight. Air and moisture-sensitive reagents were transferred using a syringe or stainless steel cannula. Intermediates were purified over silica (60Å, particle size 40-63 μm) purchased from Dynamic Adsorbents, Inc. Reactions were monitored by thin-layer chromatography (TLC) using 0.25 mm silica gel plates (60F-254) from Dynamic Adsorbents, Inc. Deuterated solvents were purchased from Cambridge Isotope Laboratories, Inc. $^{1}H$, $^{13}C$, $^{31}P$ NMR spectra were obtained on either JEOL ECA 500 spectrometer or Varian 400 MHz/500MHz spectrometer. Chemical shifts are reported in ppm relative to residual solvent. The FID file was analyzed using NMRnotebook version 2.70 build 0.10 by NMRTEC.

Dynamic Light Scattering (DLS) measurements were performed on a Wyatt DynaPro NanoStar (Wyatt Technology, Santa Barbara, CA) instrument using a disposable cuvette (Eppendorf UVette 220 nm – 1,600 nm) and data processed using Wyatt DYNAMICS V7 software. Each analysis involved an average of 10 measurements. The data was exported for final plotting using GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA).

Low resolution MS analysis was performed on a Micromass Quattro Ultima triple quadrupole mass spectrometer with an electrospray ionization (ESI) source. High resolution MS analysis was performed using Agilent 6230 Accurate-Mass TOFMS with an electrospray ionization (ESI) source by Molecular Mass Spectrometry Facility (MMSF) in the department of chemistry and biochemistry at University of California, San Diego.

Stopped-Flow fluorescence measurements were taken using Applied Photophysics SX-17MV stopped-flow. The slit was set at 4 nm and the PMU set to 600. The experiments were run using asymmetrical mixing; one solution comprised of the lipid solution in buffer A (0.1 mg/mL) and the other solution with either buffer A/B. The kinetic was measured when 25 μL of the liposome solution was mixed with 225 μl of buffer A/B (0.01 mg/mL final liposome concentration) for 1000 seconds. The excitation was set at 485 nm and the fluorescence data was collected using a high pass cut-off filter at 505 nm. The data was exported for final plotting using GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA).

Longer fluorescence decay measurements were taken on a Perkin Elmer Enspire© multimode plate reader (excitation 485 nm, emission 516 nm and 75 flashes; initially, each measurement was taken every second for 500 seconds, followed by every 60 seconds for 6 hours). Costar EIA/RIA plates were used (96 well half area, no lid, flat bottom, non-treated black polystyrene). The data were

exported for final plotting using GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA).

## 2. General Procedure for Lipid Synthesis

### 2.1 General Synthetic Procedures

**Alcohol oxidation using Albright–Onodera conditions:**

To a cold solution of the starting alcohol (1 eq) in a mixture of dry dichloromethane (DCM)/dimethyl sulfoxide (DMSO) (1:1) (0.15 M), phosphorus pentoxide (3 eq) was slowly added and reaction was stirred at room temperature for 30 minutes. Then, the reaction mixture was cooled using an ice-water bath and triethylamine ($Et_3N$) (10 eq) was slowly added. After 30 min of stirring at room temperature, the reaction mixture was cooled down again and 10% aqueous HCl was added. The resulting mixture was extracted with DCM, washed with water, dried over $Na_2SO_4$ and purified by column chromatography on silica gel.

Note: When a diol was used, the number of equivalents for $P_2O_5$ and $Et_3N$ were 6 and 20 respectively.

**Wittig olefination:**

To a suspension of phosphonium salt (1 eq) in dry THF (0.07 M), butyllithium (1.2 eq) was added dropwise at 0°C. The reaction mixture was stirred for 15 min at 0°C, and the reactive aldehyde (1.1 eq) dissolved in dry tetrahydrofuran (THF) (0.09 M) was added dropwise. After 30 minutes of stirring at room temperature, the reaction was quenched with water and solvent was removed under vacuum. The aqueous residue was extracted with DCM, washed successively with water and brine, dried over $Na_2SO_4$ and purified by column chromatography on silica gel.

Note: For double Wittig reactions, the amount of butyllithium was increased to 2.2 equivalents.

**Bromination of diol using hydrobromic acid solution:**

To a suspension of diol (0.02 M, 1 eq) in a solution of hydrobromic acid (48 wt. % in $H_2O$), tetrabutylammonium bromide (0.5 eq) was added and the reaction mixture was stirred at reflux for 24 hours. The solution was cooled down and extracted with DCM. The combined organic layers were washed with water until the aqueous layer remained at neutral pH, and dried over $Na_2SO_4$. Unless noted otherwise, the desired product was obtained after purification by column chromatography on silica gel using hexane/Ethyl Acetate (EtOAc) (99:1) as the eluent.

**Formation of tetraether lipid scaffold by $S_N2$ reaction:**

To a cold solution of **3** (0.21 M, 2.5 eq) in dry THF, sodium hydride (2.7 eq) was added portionwise. The solution was stirred for 1 hour at room temperature and cooled down again. A solution of dibromoalkane (0.09 M, 1 eq) in dry THF was added and the reaction was stirred at reflux for 16 hours. The reaction was quenched with water and the solvent was removed under vacuum. The aqueous residue was extracted with DCM, washed successively with water and brine, then dried over $Na_2SO_4$ and purified by column chromatography on silica gel.

**Debenzylation of lipid scaffold by hydrogenation:**

Benzylated lipid (1 eq) was dissolved in a degassed mixture of ethanol (EtOH)/THF (1:1) (0.01 M) and 20% $Pd(OH)_2$ (10% w/w) was added. The reaction was stirred under 1 atm $H_2$ at room temperature for 16 hours. The catalyst was removed by filtration through a pad of celite, and the resulting residue was purified by column chromatography on silica gel.

**Formation of phosphocholine lipid:**

First, bromoethyldichlorophosphate was prepared following a reported protocol.[1] To a solution of bromoethyldichlorophosphate (8 eq) in dry THF (0.33 M), a solution of the diol (1 eq) and $Et_3N$ (11 eq) in dry THF (0.04 M) was added dropwise. After stirring the mixture for 3 days in the dark at room temperature, toluene was added to precipitate triethylammonium chloride. Then, the solution was filtered through a small pad of celite and the filtrate concentrated. The resulting residue was dissolved in a mixture of THF/$NaHCO_3$ (sat) (2.8 mM) and the reaction was stirred for 16 hours at room temperature. THF was evaporated under vacuum and the resulting aqueous solution was acidified to pH 1 using a dilution solution of hydrochloric acid (1M) and extracted using several portions of DCM/Methanol (MeOH) (8:2). The organic layers were combined, dried over $Na_2SO_4$ and concentrated under reduced pressure.

To a solution of the previous crude in a mixture of THF/chloroform ($CHCl_3$) (2:1) (0.03 M), $Me_3N$ (33% in EtOH) (180 eq) was added and the reaction was stirred in a sealed tube at room temperature for 5 days. The reaction mixture was concentrated to dryness, purified on sephadex LH-20 using DCM/MeOH (1:1) as eluent and purified by column chromatography on silica gel.

## 2.2 Synthesis of Glycerol Scaffold

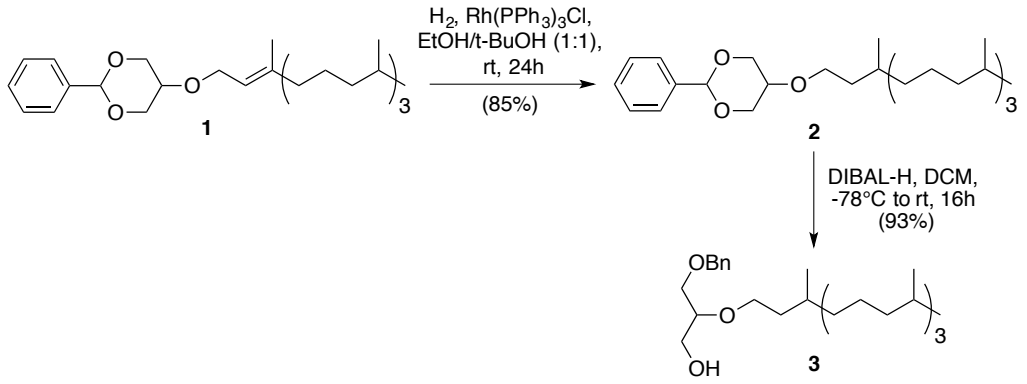
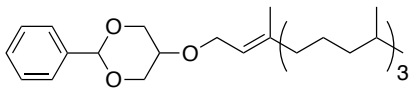


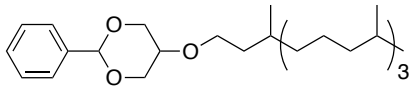

**Figure S1. Synthesis of glycerol scaffold 3.**

*(E)-2-phenyl-5-((3,7,11,15-tetramethylhexadec-2-en-1-yl)oxy)-1,3-dioxane* (**1**)



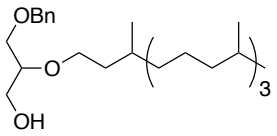
Compound **1** was synthesized following a reported protocol.[2]

*2-phenyl-5-((3,7,11,15-tetramethylhexadecyl)oxy)-1,3-dioxane* (**2**)



**1** (0.50 g, 1.10 mmol) was dissolved in a degassed solution of *tert*-butanol (tBuOH)/THF (1:1) (10 mL) and Wilkinson's catalyst ($Rh(PPh_3)_3Cl$) (0.02 g, 4% w/w) was added. The mixture was stirred under 1 atm $H_2$, at room temperature for 24 hours, and filtered through a small pad of alumina. The solvent was removed under vacuum and the crude product was purified by column chromatography on silica gel using hexane/EtOAc (95:5) as the eluent. Compound **2** was obtained as a yellow oil (0.43 g, 85%) and $^1H$ NMR spectrum matched previously reported data.[2]

*3-(benzyloxy)-2-((3,7,11,15-tetramethylhexadecyl)oxy)propan-1-ol* (**3**)



Compound **3** was synthesized following a reported protocol.[2]

## 2.3 Synthetic Procedure for GMGTPC

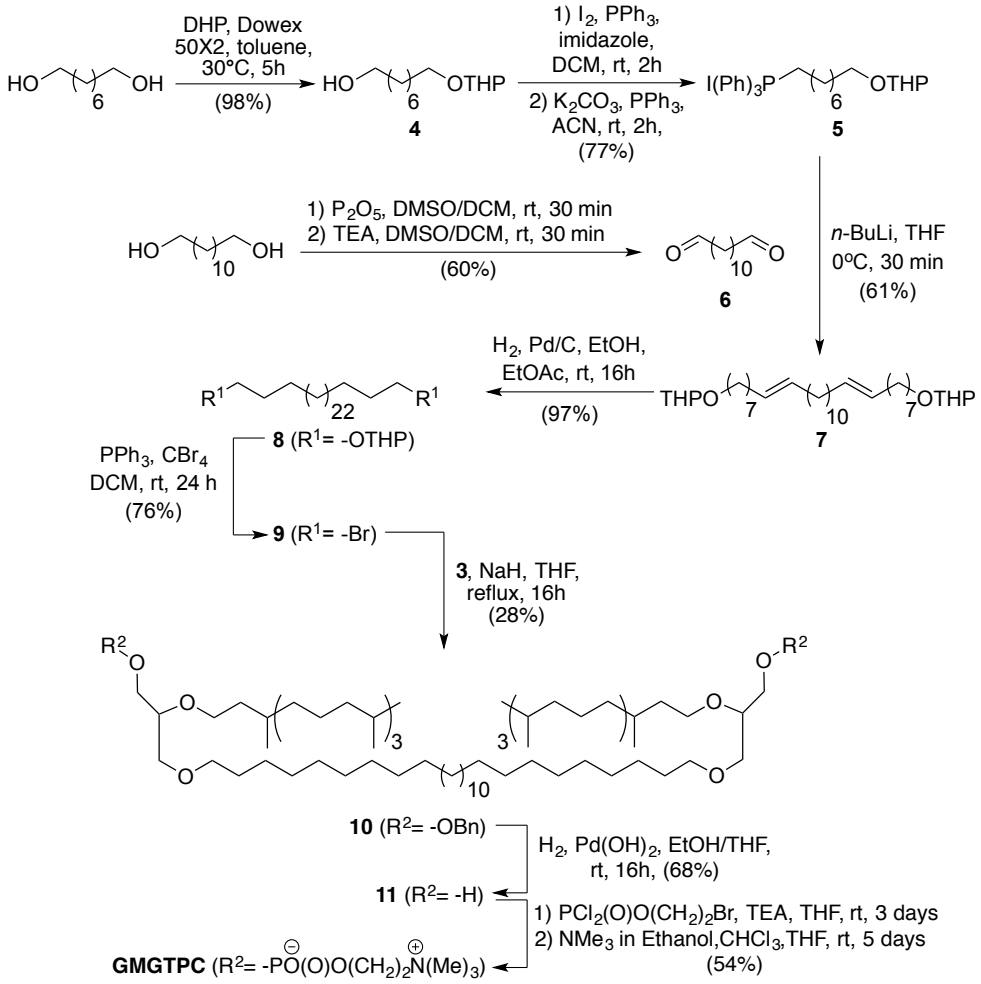



**Figure S2. Synthesis of GMGTPC.**

*8-((tetrahydro-2H-pyran-2-yl)oxy)octan-1-ol* (**4**)

Compound **4** was synthesized following a reported protocol.[3]

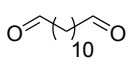
*triphenyl(8-((tetrahydro-2H-pyran-2-yl)oxy)octyl)phosphonium iodide* (**5**)

To a cold solution of triphenylphosphine (6.1 g, 23.5 mmol) and imidazole (1.6 g, 23.5 mmol) in DCM (45 mL), iodine (6.0 g, 23.5 mmol) was added. After stirring of the mixture at room temperature in the dark for 10 min, a solution of compound **4** (4.5 g, 19.6 mmol) in DCM (12 mL) was added. The reaction mixture was stirred for 2 hours at room temperature in the dark. The reaction mixture was washed with 10% $Na_2S_2O_3$ aqueous solution and extracted with DCM. The combined organic layers were washed with water, dried over $Na_2SO_4$, and purified by silica gel chromatography using hexane/EtOAc (8:2) as eluent. The resulting oil was carried out to the next step

without further purification and characterization. To a solution of the purified crude in acetonitrile (CAN) (100 mL), potassium carbonate (3.2 g, 23.5 mmol) and triphenylphosphine (6.0 g, 23.5 mmol) were added. After stirring at reflux for two days, the reaction mixture was cooled down, evaporated and purified by column chromatography on silica gel using DCM/MeOH (100:0 to 95:5) as the eluent. Phosphonium salt **5** (9.1 g, 77%) was obtained as a white fluffy solid.

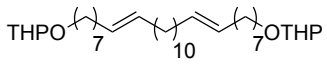
Rf: 0.78 (DCM/MeOH 95:5); $^{1}$H NMR (500 MHz, $CDCl_3$-$d_1$) δ 7.76-7.63 (m, 15H), 4.45 (dd, *J* = 3.2, 4.4 Hz, 1H), 3.76 (ddd, *J* = 3.2, 7.4, 11.3 Hz, 1H), 3.60 (td, *J* = 6.9, 9.6 Hz, 1H), 3.54-3.48 (m, 2H), 3.42-3.38 (m, 1H), 3.26 (td, *J* = 6.9, 9.6 Hz, 1H), 1.74-1.40 (m, 12H), 1.23-1.15 (m, 6H); $^{13}$C NMR (126 MHz, $CDCl_3$-$d_1$) δ 135.2 (d, *J*C-P= 3.0 Hz), 133.7 (d, *J*C-P = 10.0 Hz), 130.7 (d, *J*C-P = 12.5 Hz), 118.1 (d, *J*C-P= 86.0 Hz), 99.0, 67.6, 62.6, 30.8, 30.5, 30.3, 29.6, 29.1, 29.0, 26.1, 25.5, 23.3, 22.9, 22.6, 22.5, 19.8; ESI-MS: 475.3 $[M+Na]^+$; HRMS 475.2760 calcd for $[C_{31}H_{40}O_2P]^+$, found 475.2757.

dodecanedial (**6**)



Dialdehyde **6** was synthesized from 1,12-dodecandiol (3.50 g, 17.3 mmol) according to the general procedure for Alcohol oxidation (Albright–Onodera conditions) (see section 2.1). **6** (2.06 g, 60%) was obtained as a white solid after purification by column chromatography on silica gel using hexane/EtOAc (95:5 to 90:10) as the eluent. $^{1}$H NMR data matched previously reported data.[4]

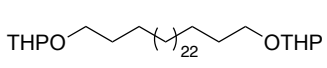
(8E,20E)-1,28-bis((tetrahydro-2H-pyran-2-yl)oxy)octacosa-8,20-diene (**7**)



Compound **7** was synthesized by reaction of **5** (4.44 g, 7.37 mmol) and **6** (0.66 g, 3.4 mmol) according to the general procedure for Wittig olefination (see section 2.1). Olefin **7** (1.27 g, 61%) was obtained as a colorless oil after purification by column chromatography on silica gel using hexane/EtOAc (97:3 to 95:5) as the eluent.

Rf: 0.29 (hexane/EtOAc 97:3); $^{1}$H NMR (500 MHz, $CDCl_3$-$d_1$) δ 5.34-5.26 (m, 4H), 4.53 (dd, *J* = 2.8, 4.4 Hz, 2H), 3.84-3.80 (m, 2H), 3.71-3.66 (m, 2H), 3.47-3.43 (m, 2H), 3.35-3.31 (m, 2H), 1.98-1.45 (m, 25H), 1.27-1.22 (m, 33H); $^{13}$C NMR (126 MHz, $CDCl_3$-$d_1$) δ 130.5, 130.4, 130.1, 129.9, 99.0, 67.8, 62.4, 32.8, 32.7, 29.9, 29.9, 29.9, 29.8, 29.7, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 27.2, 27.3, 26.4, 25.7, 19.8; ESI-MS: 613.6 $[M+Na]^+$; HRMS 613.5166 calcd for $[C_{38}H_{70}O_4Na]^+$, found 613.5167.

*1,28-bis((tetrahydro-2H-pyran-2-yl)oxy)octacosane* (**8**)

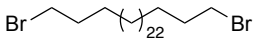


**7** (1.27 g, 2.15 mmol) was dissolved in a degassed mixture of EtOH/EtOAc/pyridine (9:1:0.02) (55 mL) and 10% Pd/C (0.65 g, 50% w/w) was added. The reaction was stirred under 1 atm

$H_2$ at room temperature for 16 hours. The catalyst was removed by filtration on celite after the solvent was heated to 40°C, and evaporation of the filtrate gave **8** (1.24 g, 97%) as a white solid without further purification.

$^1$H NMR (400 MHz, $CDCl_3$-$d_1$) δ 4.55 (dd, *J* = 2.8, 4.4 Hz, 2H), 3.87-3.83 (m, 2H), 3.70 (td, *J* = 6.9, 9.6 Hz, 2H), 3.49-3.44 (m, 2H), 3.35 (td, *J* = 6.9, 9.6 Hz, 2H), 1.84-1.76 (m, 2H), 1.72-1.66 (m, 2H), 1.58-1.46 (12H), 1.33-1.16 (m, 48H); $^{13}$C NMR (100 MHz, $CDCl_3$-$d_1$) δ 99.0, 67.9, 62.5, 31.0, 29.9, 29.8, 29.7, 26.4, 25.7, 19.9; ESI-MS: 617.5 $[M+Na]^+$; HRMS 617.5479 calcd for $[C_{38}H_{74}O_4Na]^+$, found 617.5480.

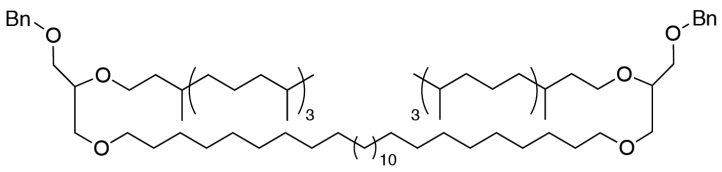
*1,28-dibromooctacosane* (**9**)




1,28-dibromooctacosane was synthesized following a reported protocol for conversion of tetrahydropyranylated alcohols to their corresponding bromides.[5] Carbon tetrabromide (1.80 g, 5.44 mmol) was added to a solution of **8** (1.08 g, 1.81 mmol) in dry DCM (30 mL). After stirring for 10 min, the solution was cooled down and triphenylphosphine (2.85 g, 10.88 mmol) was added. The mixture was stirred 24 hours at room temperature and purified by column chromatography on silica gel using DCM/hexane (1:1) as the eluent. Compound **9** (0.76 g, 76%) was obtained as a white solid.

Rf: 0.90 (DCM/hexane 1:1); $^1$H NMR (500 MHz, $CDCl_3$-$d_1$) δ 3.39 (t, *J* = 6.9 Hz, 4H), 1.86-1.80 (m, 4H), 1.42-1.37 (m, 4H), 1.29-1.23 (m, 44H); $^{13}$C NMR (126 MHz, $CDCl_3$-$d_1$) δ 34.3, 33.0, 29.9, 29.8, 29.8, 29.7, 29.0, 28.4.

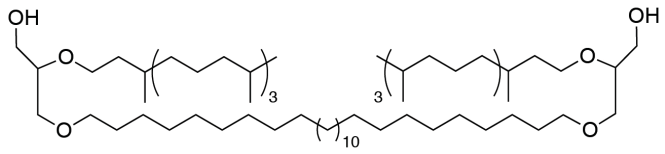
*18,51-bis((benzyloxy)methyl)-2,6,10,14,55,59,63,67-octamethyl-17,20,49,52-tetraoxaoctahexacontane* (**10**)




Compound **10** was synthesized by reaction of **3** (1.21 g, 2.62 mmol) and **9** (0.58 g, 1.05 mmol) according to the general procedure for formation of tetraether lipid scaffold by $S_N2$ reaction (see section 2.1). Compound **10** (0.39 g, 28%) was obtained as a colorless oil after purification by column chromatography on silica gel using hexane/EtOAc (98:2 to 95:5) as the eluent.

Rf: 0.26 (hexane/EtOAc 95:5); $^1$H NMR (500 MHz, $CDCl_3$-$d_1$) δ 7.32-7.25 (m, 10H), 4.54 (s, 4H), 3.64-3.45 (m, 14H), 3.41 (t, *J* = 6.9 Hz, 4H), 1.67-1.48 (m, 10H), 1.36-1.02 (m, 90H), 0.86-0.82 (m, 30H); $^{13}$C NMR (126 MHz, $CDCl_3$-$d_1$) δ 138.6, 128.5, 127.8, 127.7, 78.1, 73.5, 71.9, 70.9, 70.5, 69.1, 39.6, 37.7, 37.7, 37.6, 37.6, 37.5, 37.4, 37.3, 33.0, 30.0, 29.9, 29.9, 29.7, 28.2, 26.3, 25.0, 24.7, 24.6, 22.9, 22.8, 20.0, 19.9, 19.8.

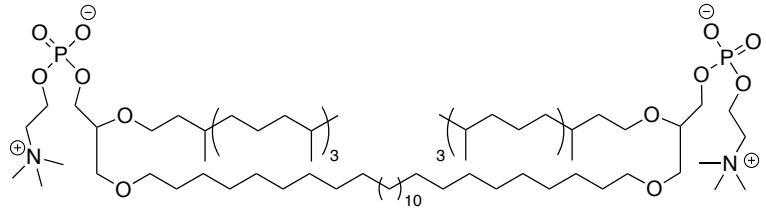
*3-((28-(3-hydroxy-2-((3,7,11,15-tetramethylhexadecyl)oxy)propoxy)octacosyl) oxy)-2-((3,7,11,15-tetramethylhexadecyl) oxy)propan-1-ol* (**11**)



Compound **11** was synthesized by hydrogenation of **10** (0.39 g, 0.30 mmol) according to the general procedure for debenzylation of the lipid scaffold by hydrogenation (see section 2.1). Diol **11** (0.23 g, 68%) was obtained as a white solid after purification by column chromatography on silica gel using hexane/EtOAc (9:1 to 8:2) as the eluent.

Rf: 0.52 (hexane/EtOAc 8:2); $^{1}$H NMR (500 MHz, $CDCl_3$-$d_1$) δ 3.71-3.39 (m, 18H), 2.16 (brs, 2H), 1.62-1.45 (m, 10H), 1.39-1.02 (m, 90H), 0.85-0.81 (m, 30H); $^{13}$C NMR (126 MHz, $CDCl_3$-$d_1$) δ 78.5, 72.1, 72.1, 68.9, 63.3, 39.6, 37.7, 37.7, 37.6, 37.5, 37.5, 37.3, 37.2, 33.0, 30.0, 29.9, 29.8, 29.7, 28.2, 26.3, 25.0, 24.7, 24.6, 22.9, 22.8, 20.0, 19.9,19.8; ESI-MS: 1135.8 $[M+H]^+$.

*(octacosane-1,28-diylbis(oxy))bis(2-((3,7,11,15-tetramethylhexadecyl)oxy) propane-3, 1-diyl) bis(2-(trimethylammonio)ethyl) bis(phosphate)* (**GMGTPC**)



Lipid **GMGTPC** was synthesized from diol **11** (0.23 g, 0.21 mmol) following the general procedure for formation of phosphocholine lipid (see section 2.1). Lipid **GMGTPC** (0.16 g, 54%) was obtained as a white gum after purification by column chromatography on silica gel using DCM/MeOH/$H_2O$ (70:30:5) as the eluent.

Rf: 0.33 (DCM/MeOH/$H_2O$ 70:30:5); $^{1}$H NMR (500 MHz, MeOD-$d_4$/$CDCl_3$-$d_1$ 1:1) δ 4.00-3.96 (m, 4H), 3.63 (t, *J* = 5.5 Hz, 4H), 3.40-3.31 (m, 12H), 3.23-3.17 (m, 6H), 2.95 (s, 18H), 1.39-1.22 (m, 10H), 1.14-0.79 (m, 90H), 0.62-0.58 (30H); $^{13}$C NMR (126 MHz, MeOD-$d_4$/$CDCl_3$-$d_1$ 1:1) δ 77.8, 77.7, 77.7, 77.6, 71.3, 70.2, 68.6, 68.3, 66.0, 64.7, 64.6, 58.6, 58.5, 53.5, 39.0, 37.3, 37.1, 37.0, 36.8, 36.8, 36.7, 32.4, 29.5, 29.3, 29.1, 27.5, 25.6, 24.3, 24.0, 22.0, 21.9, 19.1, 19.0, 19.0, 18.9, 18.9; $^{31}$P NMR (202 MHz, MeOD-$d_4$/$CDCl_3$-$d_1$ 1:1) δ 0.16; ESI-MS: 1466.0 $[M+H]^+$; HRMS calcd 1466.2615 for $[C_{84}H_{175}N_2O_{12}P_2]^+$, found 1466.2597.

## 2.4 Synthetic Procedure for GMGTPC-CP1

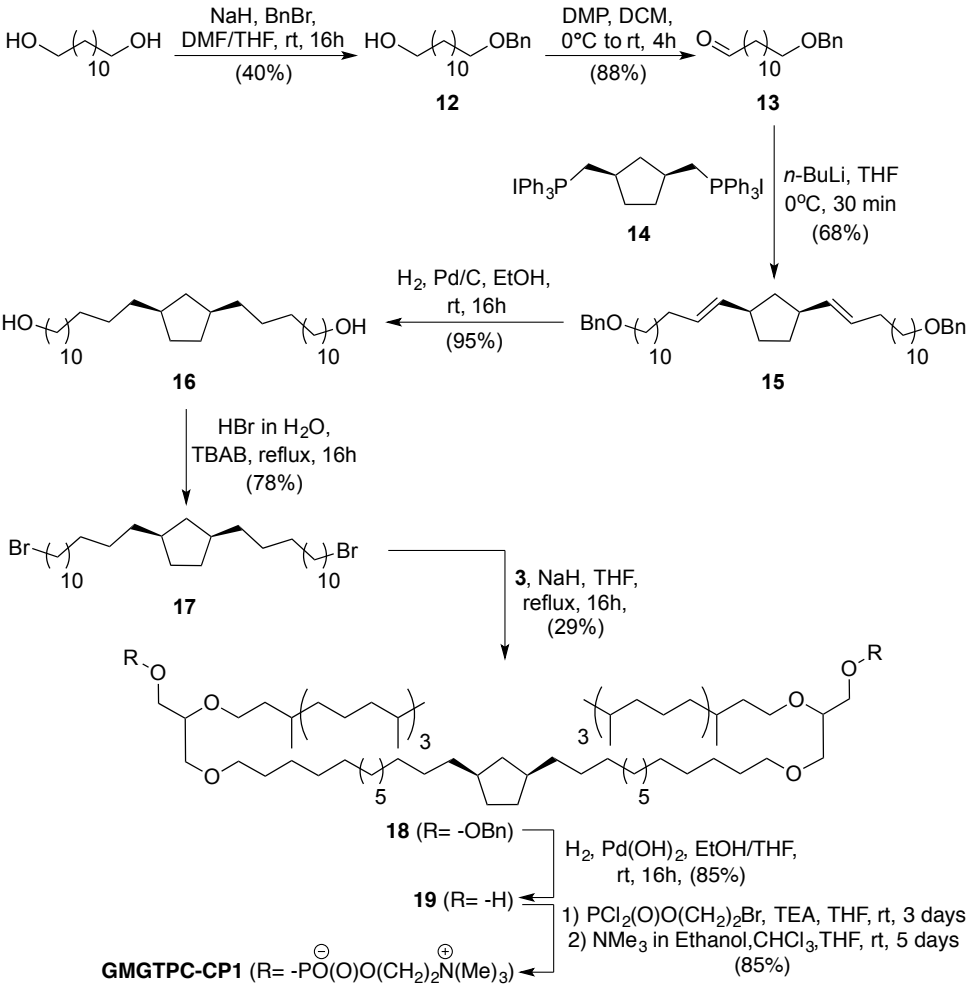
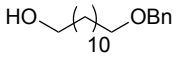


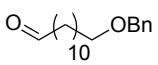

**Figure S3. Synthesis of GMGTPC-CP1.**

12-(benzyloxy)dodecan-1-ol (**12**)



Compound **12** was synthesized following a reported protocol.[6]

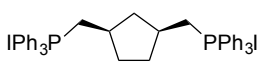
12-(benzyloxy)dodecanal (**13**)



To a cold solution of **12** (1.44 g, 4.90 mmol) in dry DCM (45 mL), Dess-Martin Periodinane (DMP) (2.50 g, 5.89 mmol) was added portionwise. The resulting mixture was allowed to reach room temperature over 1 hour. After 3 more hours of stirring at room temperature, the mixture was diluted with DCM, washed successively with 1M NaOH solution, water and brine. The organic layer was then dried over $Na_2SO_4$, concentrated under reduce pressure and purified by column chromatography on silica gel using hexane/EtOAc (95:5) as the eluent. Aldehyde **13** (1.25 g, 88%) was obtained as a colorless oil.

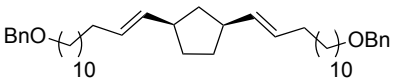
Rf: 0.47 (hexane/EtOAc 95:5); $^1$H NMR (400 MHz, $CDCl_3$-$d_1$) δ 9.74 (t, *J* = 1.7 Hz, 1H), 7.33-7.25 (m, 5H), 4.48 (s, 2H), 3.44 (t, *J* = 6.7 Hz, 2H), 2.40 (tt, *J* = 1.7, 6.7 Hz, 2H), 1.62-1.55 (m, 4H), 1.35-1.25 (m, 14H); ESI-MS: 291.2 $[M+H]^+$; HRMS 291.2319 calcd for $[C_{19}H_{31}O_2]^+$, found 291.2322.

(((1R,3S)-cyclopentane-1,3-diyl)bis(methylene))bis(triphenylphosphonium) iodide (**14**)




Compound **14** was synthesized following a reported protocol.[7]

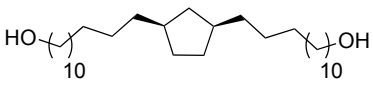
*(1R,3S)-1,3-bis((E)-13-(benzyloxy)tridec-1-en-1-yl)cyclopentane* (**15**)




Compound **15** was synthesized by reaction of **13** (3.84 g, 13.24 mmol) and **14** (4.87 g, 5.57 mmol) according to the general procedure for Wittig olefination (see section 2.1). Olefin **15** (2.44 g, 68%) was obtained as a colorless oil after purification by column chromatography on silica gel using hexane/EtOAc (99:1) as the eluent.

Rf: 0.45 (hexane/EtOAc 99:1); $^1$H NMR (500 MHz, $CDCl_3$-$d_1$) δ 7.35-7.26 (m, 10H), 5.37-5.27 (m, 4H), 4.50 (s, 4H), 3.46 (dd, *J* = 6.5, 6.8 Hz, 4H), 2.82-2.76 (m, 1.5H), 2.49-2.43 (m, 0.5H), 2.04-1.77 (m, 7H), 1.64-1.58 (m, 4H), 1.36-1.26 (m, 34H), 1.06-0.96 (m, 1H); $^{13}$C NMR (126 MHz, $CDCl_3$-$d_1$) δ 138.9, 135.3, 135.2, 135.0, 134.9, 128.8, 128.8, 128.7, 128.5, 127.8, 127.6, 73.0, 70.7, 43.7, 42.3, 41.9, 38.4, 38.2, 33.0, 32.8, 32.7, 32.5, 32.4, 30.1, 30.0, 29.8, 29.7, 29.7, 29.5, 29.4, 27.7, 26.4; ESI-MS: 660.6 $[M+NH_4]^+$; HRMS calcd 660.5714 for $[C_{45}H_{70}O_2NH_4]^+$, found 660.5715.

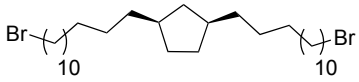
*13,13'-((1R,3S)-cyclopentane-1,3-diyl)bis(tridecan-1-ol)* (**16**)




To a degassed solution of **15** (2.38 g, 3.71 mmol) in EtOH (220 mL), 10% Pd/C (0.60 g, 25% w/w) was added. The reaction was stirred under 1 atm $H_2$ at room temperature for 24 hours. The solution was then filtered through celite, and the solvent was evaporated. Diol **16** (1.64 g, 95%) was obtained as a white solid and used without further purification.

Rf: 0.26 (hexane/EtOAc 95:5); $^1$H NMR (500 MHz, MeOD-$d_4$/$CDCl_3$-$d_1$ 1:1) δ 3.58 (t, *J* = 7.0 Hz, 4H), 1.92-1.68 (m, 5H), 1.58-1.51 (m, 4H), 1.39-1.21 (m, 44H), 1.18-1.11 (m, 2H), 0.67-0.59 (m, 1H); $^{13}$C NMR (126 MHz, MeOD-$d_4$/$CDCl_3$-$d_1$ 1:1) δ 62.4, 40.7, 40.1, 36.7, 32.5, 31.6, 29.9, 29.7, 29.6, 29.5, 28.7, 28.6, 25.7; ESI-MS: 467.6 $[M+H]^+$.

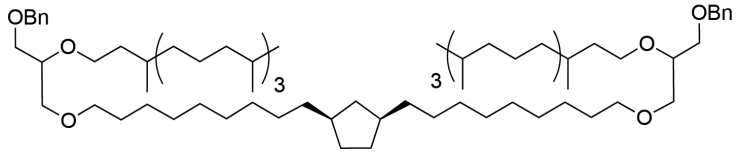
(*1R,3S)-1,3-bis(13-bromotridecyl)cyclopentane* (**17**)




Dibromo alkane **17** (1.57 g, 78%) was obtained as a white solid from diol **16** (1.58 g, 3.39 mmol) following the general procedure for bromination of diol using hydrobromic acid solution (see section 2.1).

Rf: 0.81 (hexane/EtOAc 99:1); (500 MHz, $CDCl_3$-$d_1$) δ 3.41 (t, *J* = 7.0 Hz, 4H), 1.89-1.69 (m, 9H), 1.44-1.14 (m, 44H), 0.91-0.85 (m, 2H), 0.66-0.50 (m, 1H); $^{13}C$ NMR (126 MHz, $CDCl_3$-$d_1$) δ 40.9, 40.4, 39.0, 37.0, 37.0, 34.2, 33.2, 33.0, 31.9, 30.2, 29.9, 29.9, 29.8, 29.7, 29.0, 29.0, 28.9, 28.4.

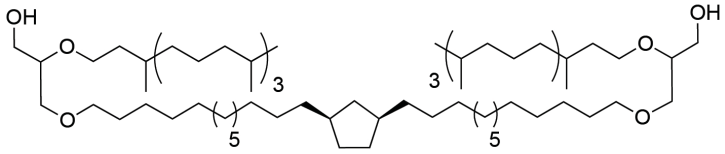
*(1R,3S)-1,3-bis(13-(3-(benzyloxy)-2-((3,7,11,15-tetramethylhexadecyl)oxy) propoxy)tridecyl)cyclopentane* (**18**)




Compound **18** was synthesized by reaction of **3** (0.39 g, 0.84 mmol) and **17** (0.20 g, 0.34 mmol) according to the general procedure for formation of tetraether lipid scaffold by $S_N2$ reaction (see section 2.1). Compound **18** (0.13 g, 29%) was obtained as a colorless oil after purification by column chromatography on silica gel using hexane/EtOAc (99:1 to 95:5) as the eluent.

Rf: 0.27 (hexane/EtOAc 95:5); $^1H$ NMR (500 MHz, $CDCl_3$-$d_1$) δ 7.25-7.26 (m, 10H), 4.57 (s, 4H), 3.68-3.43 (m, 18H), 1.94-1.89 (m, 1H), 1.81-1.71 (m, 4H), 1.69-1.50 (m, 10H), 1.42-1.07 (m, 88H), 0.89-0.60 (m, 31H); $^{13}C$ NMR (126 MHz, $CDCl_3$-$d_1$) δ 138.6, 128.6, 128.5, 127.8, 127.8, 128.7, 100.1, 78.1, 73.5, 71.8, 70.9, 70.5, 69.0, 40.9, 40.3, 39.6, 39.0, 37.7, 37.7, 37.6, 37.6, 37.5, 37.5, 36.9, 33.0, 31.8, 30.2, 30.0, 29.9, 29.8, 29.7, 29.0, 28.1, 26.3, 25.0, 24.7, 24.6, 22.9, 22.8, 20.0, 19.9, 19.8.

3,3'-((((1R,3S)-cyclopentane-1,3-diyl)bis(tridecane-13,1-diyl))bis(oxy))bis(2-((3,7,11,15-tetramethylhexadecyl)oxy)propan-1-ol) (**19**)

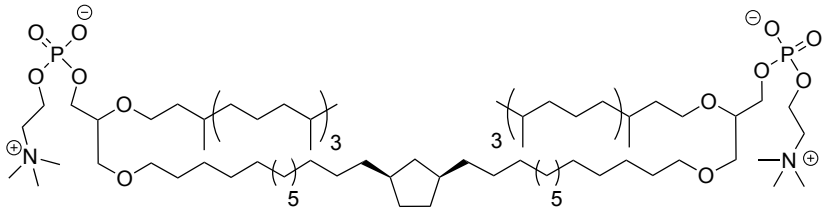



Compound **19** was synthesized by hydrogenation of **18** (0.28 g, 0.28 mmol) according to the general procedure for debenzylation of lipid scaffold by hydrogenation (see section 2.1). Diol **19** (0.28 g, 85%) was obtained as a colorless oil after purification by column chromatography on silica gel using hexane/EtOAc (9:1 to 8:2) as the eluent.

Rf: 0.43 (hexane/EtOAc 8:2); $^1H$ NMR (500 MHz, $CDCl_3$-$d_1$) δ 3.75-3.37 (m, 18H), 2.22 (t, *J* = 7.0 Hz, 2H), 1.91-1.48 (m, 17H), 1.43-1.01 (m, 86H), 0.88-0.84

(m, 30H), 0.75-0.59 (m, 1H); $^{13}$C NMR (126 MHz, $CDCl_3$-$d_1$) δ 78.4, 72.1, 71.1, 68.9, 63.3, 40.9, 40.3, 39.6, 39.0, 37.7, 37.7, 37.6, 37.6, 37.5, 37.3, 37.3, 37.0, 33.2, 33.0, 31.8, 30.2, 30.0, 29.9, 29.8, 29.7, 29.0, 28.9, 28.2, 26.3, 25.0, 24.7, 24.6, 22.9, 22.8, 20.0, 19.9, 19.8; ESI-MS: 1175.9 $[M+H]^+$; HRMS calcd 1176.1818 for $[C_{77}H_{155}O_6]^+$, found 1176.1817.

((((1R,3S)-cyclopentane-1,3-diyl)bis(tridecane-13,1-diyl))bis(oxy))bis(2-((3,7,11,15-tetramethylhexadecyl)oxy)propane-3,1-diyl) bis(2-(trimethylammonio) ethyl) bis(phosphate) (**GMGTPC-CP1**)



Lipid **GMGTPC-CP1** was synthesized from diol **19** (0.38 g, 0.32 mmol) following the general procedure for formation of phosphocholine lipid (see section 2.1). Lipid **GMGTPC-CP1** (0.42 g, 85%) was obtained as a white gum after purification by column chromatography on silica gel using DCM/MeOH/$H_2O$ (70:30:5) as the eluent.

Rf: 0.32 (DCM/MeOH/$H_2O$ 70:30:5); $^1$H NMR (500 MHz, MeOD-$d_4$/$CDCl_3$-$d_1$ 1:1) δ 3.91 (m, 4H), 3.56 (t, *J* = 5.5 Hz, 4H), 3.33-3.10 (m, 18H), 2.89 (s, 18H), 1.75-1.15 (m, 14H), 1.05-0.72 (m, 89H), 0.55-0.51 (m, 31H), 0.33-0.26 (m, 1H); $^{13}$C NMR (126 MHz, MeOD-$d_4$/$CDCl_3$-$d_1$ 1:1) δ 77.8, 77.8, 77.7, 71.4, 70.2, 68.7, 68.5, 66.1, 64.7, 64.7, 58.6, 58.6, 53.6, 40.4, 39.8, 39.0, 37.3, 37.1, 37.0, 36.9, 36.8, 36.7, 36.4, 32.4, 31.2, 29.6, 29.4, 29.3, 29.3, 29.2, 28.4, 27.6, 25.8, 24.4, 24.1, 24.0, 22.1, 22.0, 19.2, 19.1, 19.1, 19.0; $^{31}$P NMR (202 MHz, MeOD-$d_4$/$CDCl_3$-$d_1$ 1:1) δ 0.22; ESI-MS: 1505.9 $[M+H]^+$; HRMS calcd 1506.2928 for $[C_{87}H_{179}N_2O_{12}P_2]^+$, found 1506.2930.

## 2.5 Synthetic Procedure for GMGTPC-CP2

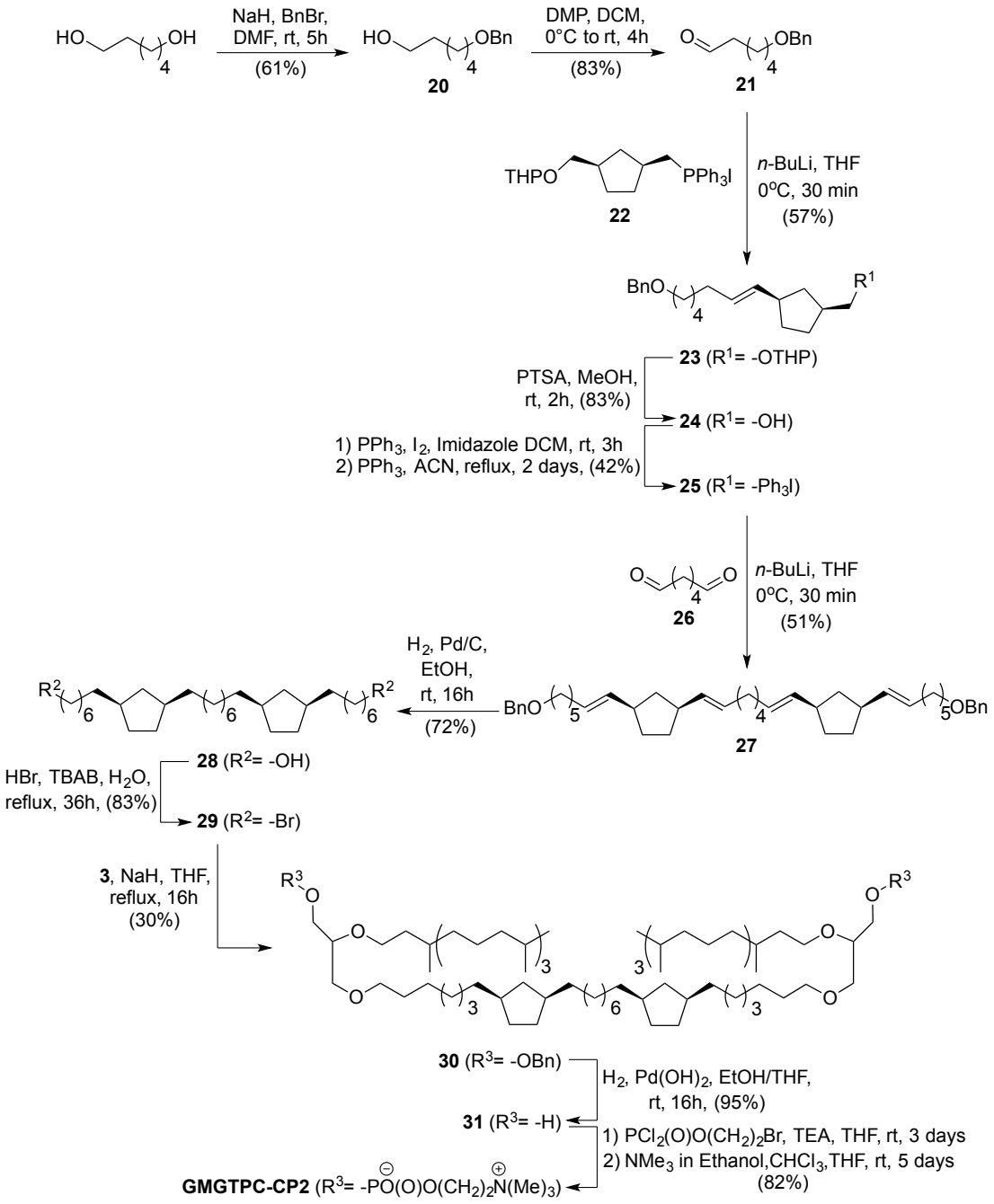
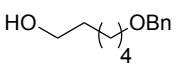


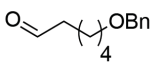

**Figure S4. Synthesis of GMGTPC-CP2.**

*6-(benzyloxy)hexan-1-ol* (**20**)



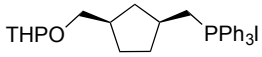
Compound **20** was synthesized following a reported protocol[6] and $^1$H NMR data match previously reported data.[8]

*6-(benzyloxy)hexanal* (**21**)



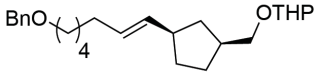
To a cold solution of **20** (2.15 g, 10.34 mmol) in dry DCM (100 mL), DMP (5.26 g, 12.40 mmol) was added portionwise. The resulting mixture was allowed to reach room temperature over 1 hour. After 3 more hours of stirring at room temperature, the mixture was diluted with DCM, washed successively with 1M NaOH solution, water and brine. The organic solution was dried over $Na_2SO_4$, concentrated under reduce pressure and purified by column chromatography on silica gel using hexane/EtOAc (95:5 to 90:10) as the eluent. Aldehyde **21** (1.77 g, 83%) was obtained as a colorless oil and $^1$H NMR data match previously reported data.[9]

*triphenyl(((1S,3R)-3-(((tetrahydro-2H-pyran-2-yl)oxy)methyl)cyclopentyl)methyl) phosphonium iodide* (**22**)



Compound **22** was synthesized following a reported protocol.[10]

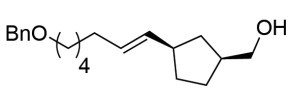
*2-(((1S,3R)-3-((E)-7-(benzyloxy)hept-1-en-1-yl)cyclopentyl)methoxy)tetrahydro-2H-pyran* (**23**)



Compound **23** was synthesized by reaction of **21** (0.85 g, 4.14 mmol) and **22** (2.02 g, 3.45 mmol) according to the general procedure for Wittig olefination (see section 2.1). Olefin **23** (0.76 g, 57%) was obtained as a colorless oil after purification by column chromatography on silica gel using hexane/EtOAc (99:1 to 95:5) as the eluent.

Rf: 0.39 (hexane/EtOAc 95:5); $^1$H NMR (500 MHz, $CDCl_3$-$d_1$) δ 7.34-7.25 (m, 5H), 5.39-5.24 (m, 2H), 4.58 (ddd, *J* = 1.9, 2.9, 4.2 Hz, 1H), 4.50 (s, 2H), 3.89-3.84 (m, 1H), 3.65-3.60 (m, 1H), 3.51-3.45 (m, 3H), 3.31-3.26 (m, 1H), 2.79-2.71 (m, 0.8H), 2.45-2.42 (m, 0.2H), 2.28-2.20 (m, 1H), 2.08-2.89 (m, 3H), 1.86-1.67 (m, 4H), 1.65-1.23 (m, 12H), 1.03-0.90 (m, 1H); $^{13}$C NMR (126 MHz, $CDCl_3$-$d_1$) δ 138.8, 135.2, 134.9, 128.8, 128.7, 128.5, 127.8, 127.6, 99.0, 73.0, 72.5, 72.4, 70.6, 62.4, 62.3, 43.7, 43.6, 39.7, 39.5, 38.5, 38.4, 38.4, 38.1, 37.9, 37.7, 32.9, 32.9, 32.6, 32.5, 30.9, 30.0, 29.9, 29.8, 29.6, 29.1, 29.0, 28.8, 27.6, 36.0, 25.9, 25.7, 19.8, 19.7; ESI-MS: 409.3 $[M+Na]^+$; HRMS calcd 409.2713 for $[C_{25}H_{38}O_3Na]^+$, found 409.2714.

((1S,3R)-3-((E)-7-(benzyloxy)hept-1-en-1-yl)cyclopentyl)methanol (**24**)

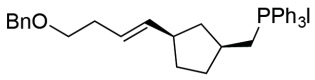


To a solution of **23** (740 mg, 1.92 mmol) in MeOH (32 mL), p-toluenesulfonic acid (18 mg, 0.3 mmol) was added. The solution was stirred 2 hours at room temperature,

concentrated and purified by column chromatography on silica gel using hexane/EtOAc (9:1 to 8:2) as the eluent. Alcohol **24** (477 mg, 83%) was obtained as a colorless oil.

Rf: 0.41 (hexane/EtOAc 8:2); $^{1}$H NMR (500 MHz, $CDCl_3$-$d_1$) δ 7.34-7.25 (m, 5H), 5.31-5.24 (m, 2H), 4.49 (s, 2H), 3,51-3.44 (m, 4H), 2.79-2.71 (m, 0.8H), 2.47-2.39 (m, 0.2H), 2.19-2.13 (m, 1H), 2.06-1.95 (m, 2H), 1.94-1.87 (m, 1H), 1.79-1.59 (m, 5H), 1.44-1.22 (m, 6H), 0.98-0.88 (m, 1H); $^{13}$C NMR (126 MHz, $CDCl_3$-$d_1$) δ 138.8, 134.9, 134.7, 129.0, 128.8, 128.5, 127.8, 127.6, 73.0, 70.6, 67.7, 67.6, 43.6, 42.2, 42.0, 38.5, 37.6, 37.1, 32.9, 32.6, 32.4, 29.9, 29.8, 29.8, 29.5, 28.4, 28.2, 27.6, 26.0, 25.8; ESI-MS: 303.2 $[M+H]^+$; HRMS calcd 325.2138 for $[C_{20}H_{30}O_2Na]^+$, found 325.2137.

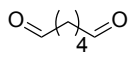
*(((1S,3R)-3-((E)-7-(benzyloxy)hept-1-en-1-yl)cyclopentyl)methyl)triphenyl-phosphonium iodide* (**25**)




To a cold solution of triphenylphosphine (476 mg, 1.82 mmol) and imidazole (218 mg, 3.20 mmol) in DCM (11 mL), iodine (539 mg, 2.13 mmol) was added. After stirring of the mixture at room temperature in the dark for 10 min, a solution of compound **24** (460 mg, 1.52 mmol) in DCM (11 mL) was added. The reaction mixture was stirred for 2 hours at room temperature in the dark. The reaction mixture was washed with 10% $Na_2S_2O_3$ aqueous solution and extracted with DCM. The combined organic layers were washed with water, dried over $Na_2SO_4$, and purified by silica gel chromatography using DCM as the eluent. The resulting oil was carried out to the next step without further purification and characterization. To a solution of the purified crude in ACN (70 mL), triphenylphosphine (476 mg, 1.82 mmol) was added. After stirring at reflux for two days, the reaction mixture was cooled down, evaporated and purified by column chromatography on silica gel using DCM/MeOH (100:0 to 98:2) as the eluent. Phosphonium salt **25** (427 mg, 42%) was obtained as a yellow pale solid.

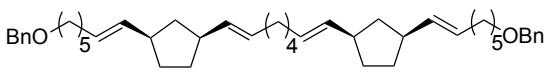
Rf: 0.46 (DCM/MeOH 95:5); $^{1}$H NMR (500 MHz, $CDCl_3$-$d_1$) δ 7.79-7.62 (m, 15H), 7.25-7.17 (m, 5H), 5.20-5.13 (m, 2H), 4.40 (s, 2H), 3.77-3.64 (m, 2H), 3.38-3.35 (m, 2H), 2.57-2.49 (m, 0.8H), 2.24-2.13 (m, 1.2H), 1.91-1.80 (m, 2H), 1.74-1.66 (m, 1H), 1.62-1.13 (m, 11H); $^{13}$C NMR (126 MHz, $CDCl_3$-$d_1$) δ 138.6, 138.5, 135.1, 133.8, 133.7, 133.6, 132.0, 132.0, 130.6, 130.5, 129.2, 129.1, 128.5 (*J*C-P = 12.5 Hz), 128.3, 127.6, 127.4, 118.4 (*J*C-P= 86.0 Hz), 72.8, 70.3, 42.8, 42.5, 42.4, 42.2, 42.1, 37.5, 34.5, 24.5, 34.3, 33.2, 33.1, 32.3, 32.0, 31.5, 29.6, 29.2, 29.1, 28.7, 27.3, 25.6, 25.6; ESI-MS: 547.4 $[M+H]^+$.

*adipaldehyde* (**26**)




Dialdehyde **26** was synthesized following a reported protocol.[11]

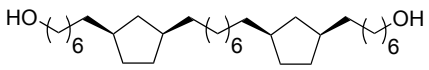
(1E,7E)-1-((1R,3S)-3-((E)-7-(benzyloxy)hept-1-en-1-yl)cyclopentyl)-8-((1S,3R)-3-((E)-7-(benzyloxy)hept-1-en-1-yl)cyclopentyl)octa-1,7-diene (**27**)



Compound **27** was synthesized by reaction of **25** (2.71 g, 4.02 mmol) and **26** (0.21 g, 1.83 mmol) according to the general procedure for Wittig olefination (see section 2.1). Olefin **23** (0.61 g, 51%) was obtained as a colorless oil after purification by column chromatography on silica gel using hexane/EtOAc (98:2) as the eluent. This product represents a possible mixture of isotactic and syndiotactic isomers. For simplicity, only the isotactic isomer is shown in Figure 1 and throughout the supporting information.

Rf: 0.28 (hexane/EtOAc 98:2); $^{1}$H NMR (500 MHz, $CDCl_3$-$d_1$) δ 7.34-7.25 (m, 10H), 5.36-5.26 (m, 8H), 4.48 (s, 4H), 3.44 (t, *J* = 6.9 Hz, 4H), 2.82-2.73 (m, 3H), 2.47-2.41 (m, 0.8H), 2.30-2.27 (m, 0.2H), 2.01-1.92 (m, 7H), 1.88-1.74 (m, 6H), 1.65-1.55 (m, 5H), 1.41-1.30 (m, 16H), 1.05-0.91 (m, 2H); $^{13}$C NMR (126 MHz, $CDCl_3$-$d_1$) δ 138.8, 135.5, 135.4, 135.4, 135.3, 135.1, 135.0, 134.0, 133.8, 128.9, 128.7, 128.6, 128.6, 128.5, 127.8, 127.6, 73.0, 70.6, 43.7, 43.4, 42.3, 41.9, 38.4, 38.1, 33.0, 32.8, 32.6, 32.6, 32.5, 32.3, 29.9, 29.8, 29.8, 29.7, 29.6, 29.3, 27.6, 27.6, 26.0, 25.9.

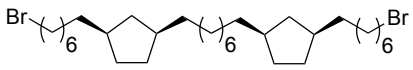
1-((1R,3S)-3-(heptyl-7-ol)cyclopentyl)-8-((1S,3R)-3-(heptyl-7-ol)cyclopentyl) octane (**28**)



To a degassed solution of olefin **27** (0.85 g, 1.31 mmol) in EtOH (76 mL), 10% Pd/C (0.21 g, 25% w/w) was added. The reaction was stirred under 1 atm $H_2$ at room temperature for 24 hours. The solution was then filtered through celite, and the solvent was evaporated. Diol **28** (0.45 g, 72%) was obtained as a white solid.

$^{1}$H NMR (500 MHz, MeOD-$d_4$/$CDCl_3$-$d_1$ 9:1) δ 3.34 (t, *J* = 6.9 Hz, 4H), 1.71-1.66 (m, 2H), 1.56-1.47 (m, 8H), 1.35-1.29 (m, 4H), 1.14-0,98 (m, 40H), 0.96-0.80 (m, 6H), 0.43-0.37 (m, 2H); $^{13}$C NMR (126 MHz, MeOD-$d_4$/$CDCl_3$-$d_1$ 9:1) δ 62.2, 40.6, 40.0, 38.6, 38.6, 32.9, 32.4, 31.5, 29.8, 29.6, 29.4, 28.6, 28.5, 25.6.

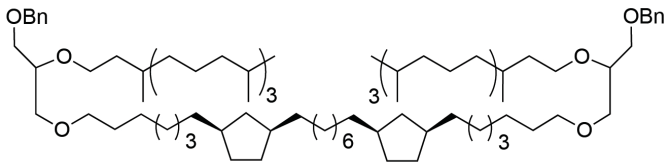
*1-((1R,3S)-3-(7-bromoheptyl)cyclopentyl)-8-((1S,3R)-3-(7-bromoheptyl) cyclopentyl)octane* (**29**)



Dibromo alkane **29** (0.47 g, 83%) was obtained as a white solid from diol **28** (0.45 g, 0.94 mmol) following the general procedure for bromination of diol using hydrobromic acid solution (see section 2.1).

Rf: 0.75 (hexane); $^{1}$H NMR (500 MHz, $CDCl_3$-$d_1$) δ 3.38 (t, *J* = 6.9 Hz, 4H), 1.90-1.66 (m, 13H), 1.42-1.00 (m, 40H), 0.87-0.83 (m, 1H), 0.63-0.56 (m, 2H); $^{13}$C NMR (126 MHz, $CDCl_3$-$d_1$) δ 40.9, 40.3, 40.3, 39.0, 38.9, 36.9, 36.8, 34.3, 33.2, 33.0, 31.8, 30.2, 29.9, 29.0, 29.0, 28.9, 28.8, 28.7, 28.4.

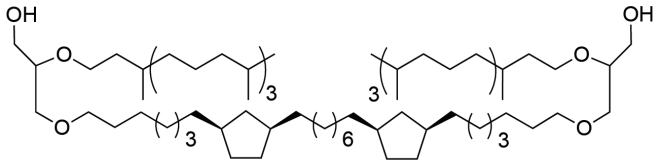
*1-((1R,3S)-3-(7-(3-(benzyloxy)-2-((3,7,11,15-tetramethylhexadecyl)oxy)propoxy) heptyl)cyclopentyl)-8-((1S,3R)-3-(7-(3-(benzyloxy)-2-((3,7,11,15-tetramethyl-hexadecyl)oxy)propoxy)heptyl)cyclopentyl)octane* (**30**)




Compound **30** was synthesized by reaction of **3** (1.10 g, 2.35 mmol) and **29** (0.47 g, 0.78 mmol) according to the general procedure for formation of tetraether lipid scaffold by $S_N2$ reaction (see section 2.1). Product **30** (0.32 g, 30%) was obtained as a colorless oil after purification by column chromatography on silica gel using hexane/EtOAc (99:1 to 95:5) as the eluent.

Rf: 0.50 (hexane/EtOAc 95:5); $^1$H NMR (500 MHz, $CDCl_3$-$d_1$) δ 7.32-7.25 (m, 10H), 4.53 (s, 4H), 3.63-3.92 (m, 18H), 1.88-1.85 (m, 2H), 1.76-1.66 (m, 6H), 1.62-1.47 (m, 10H), 1.37-1.01 (m, 84H), 0.85-0.81 (m, 30H), 0.73-0.56 (m, 2H); $^{13}$C NMR (126 MHz, $CDCl_3$-$d_1$) δ 138.6, 128.5, 127.8, 127.7, 78.1, 73.6, 71.9, 70.9, 70.5, 69.1, 40.9, 40.3, 39.6, 39.0, 37.7, 37.7, 37.6, 37.6, 37.5, 37.4, 37.3, 37.0, 36.9, 33.3, 33.0, 31.8, 30.2, 30.1, 30.0, 30.0, 29.9, 29.8, 29.0, 28.9, 28.2, 26.4, 25.0, 24.7, 24.6, 23.0, 22.9, 20.0, 19.9, 19.8.

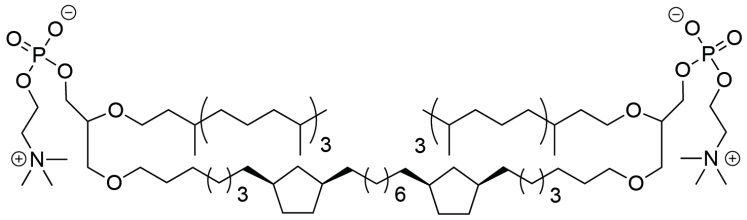
*3-((7-((1R,3S)-3-(8-((1R,3S)-3-(7-(3-hydroxy-2-((3,7,11,15-tetramethylhexadecyl) oxy)propoxy)heptyl)cyclopentyl)octyl)cyclopentyl)heptyl)oxy)-2-((3,7,11,15-tetramethylhexadecyl)oxy)propan-1-ol* (**31**)




Compound **31** was synthesized by hydrogenation of **30** (0.22 g, 0.16 mmol) according to the general procedure for debenzylation of lipid scaffold by hydrogenation (see section 2.1). Diol **31** (0.18 g, 95%) was obtained as a colorless oil after purification by column chromatography on silica gel using hexane/EtOAc (95:5 to 85:15) as the eluent.

Rf: 0.41 (hexane/EtOAc 80:20); $^1$H NMR (500 MHz, $CDCl_3$-$d_1$) δ 3.71-3.40 (m, 18H), 1.90-1.85 (m, 2H), 1.74-1.47 (m, 20H), 1.38-1.01 (m, 82H), 0.86-0.81 (m, 30H), 0.63-0.56 (m, 1H); $^{13}$C NMR (126 MHz, $CDCl_3$-$d_1$) δ 78.5, 72.1, 71.1, 68.9, 40.9, 40.3, 39.6, 39.0, 37.7, 37.7, 37.6, 37.5, 37.4, 37.3, 37.0, 36.9, 33.2, 33.0, 31.9, 30.2, 30.1, 30.0, 30.0, 29.9, 29.7, 29.0, 28.9, 28.8, 28.2, 26.3, 25.0, 24.7, 24.6, 23.0, 22.9, 20.0, 19.9, 19.8; ESI-MS: 1187.8 $[M+H]^+$.

3-((7-((1S,3R)-3-(8-((1S,3R)-3-(7-(3-((oxido(2-(trimethylammonio)ethoxy) phosphoryl)oxy)-2-((3,7,11,15-tetramethylhexadecyl)oxy)propoxy)heptyl) cyclopentyl)octyl)cyclopentyl)heptyl)oxy)-2-((3,7,11,15-tetramethylhexadecyl)oxy) propyl-(2-(trimethylammonio)ethyl) phosphate (**GMGTPC-CP2**)



Lipid **GMGTPC-CP2** was synthesized from diol **31** (0.17 g, 0.14 mmol) following the general procedure for formation of phosphocholine lipid (see section 2.1). Lipid **GMGTPC-CP2** (0.15 g, 82%) was obtained as a white gum after purification by column chromatography on silica gel using DCM/MeOH/$H_2O$ (70:30:5) as the eluent.

Rf: 0.50 (DCM/MeOH/$H_2O$ 70:30:5); $^1$H NMR (500 MHz, MeOD-$d_4$/$CDCl_3$-$d_1$ 1:1) δ 3.90-3.87 (m, 4H), 3.53 (t, *J* = 5.5 Hz, 4H), 3.32-3.06 (m, 18H), 2.86 (s, 18H), 1.58-1.34 (m, 10H), 1.28-1.13 (m, 10H), 1.03-0.70 (m, 82H), 0.53-0.48 (m 30H), 0.30-0.24 (m, 2H); $^{13}$C NMR (126 MHz, MeOD-$d_4$/$CDCl_3$-$d_1$ 1:1) δ 77.8, 77.7, 77.6, 77.6, 71.3, 70.2, 68.6, 68.4, 66.0, 64.7, 64.6, 58.6, 58.5, 53.5, 40.3, 39.8, 39.0, 38.4, 38.3, 37.3, 37.1, 37.0, 37.0, 36.9, 36.8, 36.7, 36.4, 36.3, 32.6, 32.4, 31.2, 29.5, 29.4, 29.3, 29.2, 29.1, 28.3, 27.5, 25.7, 24.4, 24.0, 24.0, 22.1, 22.0, 19.1, 19.1, 19.0, 19.0, 18.9; $^{31}$P NMR (202 MHz, MeOD-$d_4$/$CDCl_3$-$d_1$ 1:1) δ 0.20; ESI-MS: 1518.2 $[M+H]^+$; HRMS calcd 1518.2928 for $[C_{88}H_{179}N_2O_{12}P_2]^+$, found 1518.2923.

## 2.6 Synthetic Procedure for GMGTPC-CP3

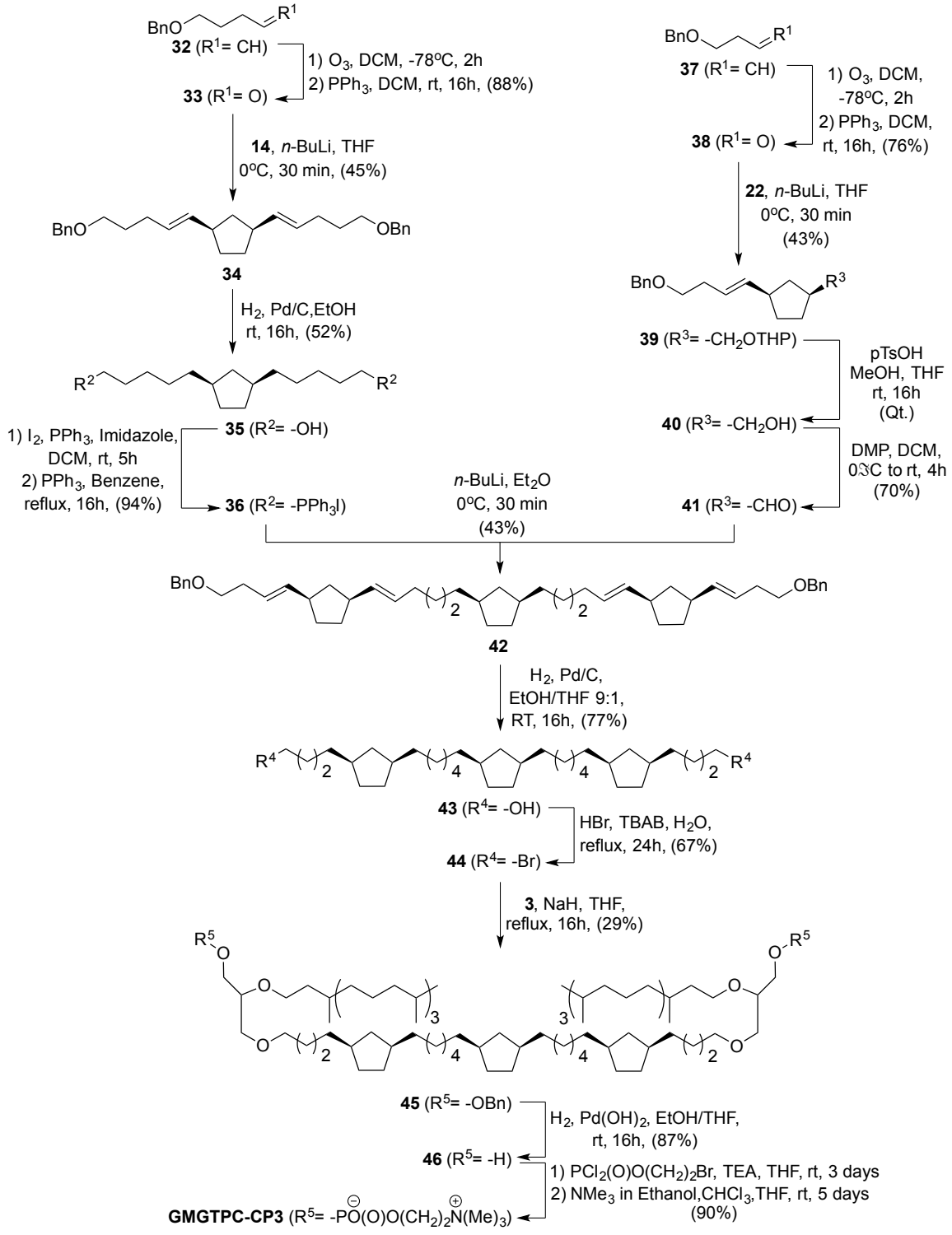
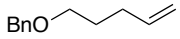


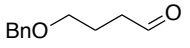

**Figure S5. Synthesis of GMGTPC-CP3.**

*((pent-4-en-1-yloxy)methyl)benzene* (**32**)



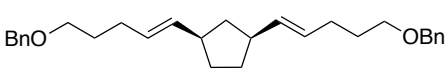

Compound **32** was synthesized following a reported protocol.[12]

*4-(benzyloxy)butanal* (**33**)




To a solution of **32** (6.63 g, 37.6 mmol) in DCM (300 mL) at -78 °C ozone was bubbled until the solution turned blue. Then, oxygen was bubbled for 10 min and triphenylphosphine (10.8 g, 41.4 mmol) was added portionwise at -78 °C. The reaction mixture was allowed to warm up slowly to room temperature and stirred overnight. The solvent was removed under reduced pressure and the resulting solid was purified by column chromatography on silica gel using hexane/EtOAc (90:10 to 80:20) as the eluent. Aldehyde **33** (5.85 g, 88%) was obtained as a colorless oil and $^{1}$H NMR data match previously reported data.[13]

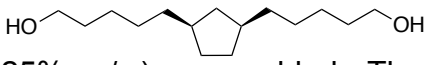
*(1R,3S)-1,3-bis((E)-5-(benzyloxy)pent-1-en-1-yl)cyclopentane* (**34**)




Compound **34** was synthesized by reaction of **14** (13.1 g, 14.9 mmol) and **33** (5.9 g, 32.9 mmol) according to the general procedure for Wittig olefination (see section 2.1). Olefin **34** (2.8 g, 45%) was obtained as a colorless oil after purification by column chromatography on silica gel using hexane/EtOAc (98:2) as the eluent.

Rf: 0.45 (hexane/EtOAc 95:5); $^{1}$H NMR (500 MHz, $CDCl_3$-$d_1$) δ 7.36-7.28 (m, 10H); 5.41-5.29 (m, 4H); 4.52 (s, 4H); 3.51-3.48 (m, 4H), 2.86-2.77 (m, 1.7H), 2.50-2.45 (m, 0.3H); 2.19-2.08 (m, 4H), 1.93-1.78 (m, 3H), 1.73-1.67 (m, 4H), 1.41-1.34 (m, 2H), 1.08-0.99 (m, 1H); $^{13}$C NMR (126 MHz, $CDCl_3$-$d_1$) δ 138.8, 136.0, 135.9, 135.7, 135.5, 128.5, 127.9, 127.8, 127.6, 73.1, 73.0, 70.0, 69.9, 43.6, 43.4, 42.2, 41.8, 38.4, 38.1, 32.9, 32.8, 32.4, 32.3, 30.1, 29.8, 29.2, 24.3.

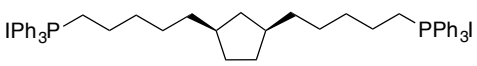
*5,5'-((1R,3S)-cyclopentane-1,3-diyl)bis(pentan-1-ol)* (**35**)




To a degassed solution of **34** (2.75 g, 6.58 mmol) in EtOH/THF (9:1) (75 mL), 10% Pd/C (0.69 g, 25% w/w) was added. The reaction was stirred under 1 atm $H_2$ at room temperature for 16 hours. The solution was then filtered through celite, and the solvent was evaporated. Diol **35** (0.83 g, 52%) was obtained as a white solid after purification by column chromatography on silica gel using hexane/EtOAc (75:25 to 50:50) as the eluent.

Rf: 0.42 (hexane/EtOAc 1:1); $^{1}$H NMR (500 MHz, $CDCl_3$-$d_1$) δ 3.49 (t, *J* = 6.9 Hz, 4H), 3.23 (brs, 2H), 1.84-1.79 (m, 1H), 1.73-1.61 (m, 4H), 1.48-1.42 (m, 4H), 1.25-1.04 (m, 14.2H), 0.57-0.51 (m, 0.8H); $^{13}$C NMR (126 MHz, $CDCl_3$-$d_1$) δ 62.6, 40.1, 38.8, 36.8, 36.7, 33.0, 32.7, 31.6, 28.6, 28.5, 26.1; ESI-MS: 265.1 $[M+Na]^+$; HRMS calcd 265.2138 for $[C_{15}H_{30}O_2Na]^+$, found 265.2141.

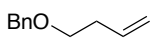
*(((1R,3S)-cyclopentane-1,3-diyl)bis(pentane-5,1-diyl))bis(triphenylphosphonium) iodide* (**36**)




To a cold solution of triphenylphosphine (1.98 g, 7.55 mmol) and imidazole (0.52 g, 7.55 mmol) in DCM (18 mL), iodine (1.92 g, 7.55 mmol) was added. After stirring of the mixture at room temperature in the dark for 10 min, a solution of compound **35** (0.83 g, 3.43 mmol) in DCM (18 mL) was added. The reaction mixture was stirred for 2 hours at room temperature in the dark. The reaction mixture was washed with 10% $Na_2S_2O_3$ aqueous solution and extracted with DCM. The combined organic layers were washed with water, dried over $Na_2SO_4$, and purified by silica gel chromatography using a mixture of hexane/EtOAc (8:2) as eluent. The resulting oil was carried out to the next step without further purification and characterization. To a solution of the purified crude in benzene (15 mL), triphenylphosphine (2.70 g, 10.29 mmol) was added. After stirring at reflux for 16 hours, the reaction mixture was cooled down, evaporated and purified by column chromatography on silica gel using DCM/MeOH (1:0 to 9:1) as the eluent. diphosphonium salt **36** (3.19 g, 94%) was obtained as a white solid.

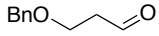
$^1$H NMR (500 MHz, $CDCl_3$-$d_1$) δ 7.74-.61 (m, 30H), 3.48-3.42 (m, 4H), 1.76-1.71 (m, 1H), 1.57-1.50 (m, 12H), 1.16-0.85 (10.2H), 0.43-0.37 (m, 0.8H); $^{13}$C NMR (126 MHz, $CDCl_3$-$d_1$) δ 135.2, 133.6 (d, *JC-P* = 10.0 Hz), 130.6 (d, *JC-P* = 12.5 Hz), 117.9 (d, *JC-P* = 85.7 Hz), 53.6, 40.2, 39.7, 38.3, 38.2, 36.0, 32.8, 31.5, 30.6, 30.5, 28.0, 23.2, 22.8, 22.5, 22.5; ESI-MS: 366.3 $[M\text{-}2I]^{2+}$; HRMS calcd 366.2001 for $[C_{51}H_{58}P_2]^{2+}$, found 366.2003.

*((but-3-en-1-yloxy)methyl)benzene* (**37**)



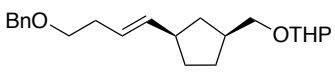

Compound **37** was synthesized following a reported protocol.[14]

*3-(benzyloxy)propanal* (**38**)




Compound **38** was synthesized following a reported protocol.[15]

*2-(((1S,3R)-3-((E)-4-(benzyloxy)but-1-en-1-yl)cyclopentyl)methoxy)tetrahydro-2H-pyran* (**39**)

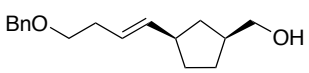



Compound **39** was synthesized by reaction of **22** (4.41 g, 7.52 mmol) and **38** (1.45 g, 9.02 mmol) according to the general procedure for Wittig olefination (see section 2.1). Olefin **39** (1.10 g, 43%) was obtained as a colorless oil after purification by column chromatography on silica gel using hexane/EtOAc (98:2 to 95:5) as the eluent.

Rf: 0.33 (hexane/EtOAc 95:5); $^1$H NMR (500 MHz, $CDCl_3$-$d_1$) δ 7.32-7.24 (m, 5H), 5.49-5.30 (m, 2H), 4.57 (dd, *J* = 2.9, 4.2 Hz, 1H), 4.50 (s, 2H), 3.87-3.83 (m, 1H), 3.63-3.59 (m, 1H), 3.49-3.44 (m, 3H), 3.29-3.24 (m, 1H), 2.79-2.71 (m,

0.8H), 2.44-2.22 (m, 3.3H); 1.98-1.90 (m, 1H), 1.84-1.66 (m, 4H), 1.59-1.40 (m, 5H), 1.34-1.24 (m, 1H), 1.00-0.89 (m, 1H); $^{13}$C NMR (126 MHz, $CDCl_3$-$d_1$) δ 138.7, 138.6, 137.1, 136.9, 128.4, 127.7, 127.6, 124.7, 124.4, 98.9, 72.9, 72.9, 72.4, 72.3, 72.2, 70.4, 70.3, 62.3, 62.2, 43.7, 43.6, 41.7, 39.7, 39.4, 38.6, 38.5, 38.2, 38.0, 37.7, 37.5, 33.1, 32.8, 32.8, 32.3, 30.8, 29.0, 28.9, 28.7, 28.4, 25.6, 19.7, 19.6.

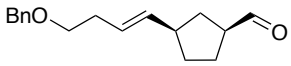
*((1S,3R)-3-((E)-4-(benzyloxy)but-1-en-1-yl)cyclopentyl)methanol* (**40**)




To a solution of **39** (1.10 g, 3.20 mmol) in a mixture of MeOH (150 mL) and THF (30 mL), p-toluenesulfonic acid (30 mg, 0.16 mmol) was added and the solution was stirred for 16 hours at room temperature. Then, the solvent was evaporated and the resulting residue was dissolved in EtOAc. The organic solution was washed successively with 1M NaOH solution, water, brine and dried over $Na_2SO_4$ to yield the alcohol **40** (0.84 g, Qt.) as a colorless oil.

$^1$H NMR (500 MHz, $CDCl_3$-$d_1$) δ 7.33-7.24 (m, 5H), 5.49-5.30 (m, 2H), 4.50 (s, 2H), 3.48-3.44 (m, 4H), 2.80-2.71 (m, 0.8H), 2.40-2.28 (M, 3.3H), 2.17-2.10 (m, 1H), 1.93-1.88 (m, 1H), 1.78-1.69 (m, 2H), 1.44-1.23 (m, 2H), 0.96-0.88 (m, 1H); $^{13}$C NMR (126 MHz, $CDCl_3$-$d_1$) δ 138.5, 138.4, 136.9, 136.7, 128.4, 127.7, 127.6, 124.7, 124.5, 72.9, 72.8, 70.3, 70.2, 67.3, 67.2, 43.5, 42.1, 41.9, 38.5, 37.5, 36.9, 33.0, 32.7, 32.2, 28.4, 28.3, 28.2; ESI-MS: 283.1 $[M+Na]^+$; HRMS calcd 283.1669 for $[C_{17}H_{24}O_2Na]^+$, found 283.1668.

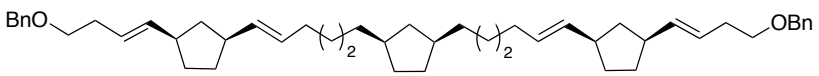
*(1S,3R)-3-((E)-4-(benzyloxy)but-1-en-1-yl)cyclopentane-1-carbaldehyde* (**41**)




To a cold solution of **40** (0.84 g, 3.23 mmol) in dry DCM (30 mL), DMP (1.64 g, 3.88 mmol) was added portionwise. The resulting mixture was allowed to reach room temperature over 1 hour. After 3 more hours of stirring at room temperature, the mixture was diluted with DCM, washed successively with 1M NaOH solution, water and brine. The organic solution was dried over $Na_2SO_4$, concentrated under reduce pressure and purified by column chromatography on silica gel using hexane/EtOAc (90:10 to 75:25) as the eluent. Aldehyde **41** (0.59 g, 70%) was obtained as a colorless oil.

Rf: 0.63 (hexane/EtOAc 75:25); $^1$H NMR (500 MHz, $CDCl_3$-$d_1$) δ 9.64 (d, $J$ = 2.5 Hz, 1H), 7.34-7.30 (m, 5H), 5.52-5.38 (m, 2H), 4.55 (s, 2H), 3.54-3.50 (m, 2H), 2.92-2.80 (m, 1.8H), 2.60-2.33 (m, 2.2H), 2.07-1.98 (m, 2H), 1.89-1.90 (m, 2H), 1.63-1.51 (m, 1H), 1.38-1.27 (m, 1H); $^{13}$C NMR (126 MHz, $CDCl_3$-$d_1$) δ 203.6, 203.4, 138.6, 138.5, 135.3, 135.1, 128.4, 127.7, 127.6, 126.0, 125.7, 73.0, 72.9, 70.1, 70.0, 51.5, 51.3, 43.7, 38.7, 33.9, 33.8, 33.4, 33.1, 33.0, 32.7, 28.4, 25.9, 25.7; ESI-MS: 281.1 $[M+Na]^+$; HRMS calcd 281.1512 for $[C_{18}H_{22}O_2Na]^+$, found 281.1514.

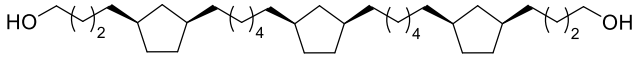
*(1S,3R)-1-((E)-6-((1R,3S)-3-((E)-4-(benzyloxy)but-1-en-1-yl)cyclopentyl)hex-5-en-1-yl)-3-((E)-6-((1S,3R)-3-((E)-4-(benzyloxy)but-1-en-1-yl)cyclopentyl)hex-5-en-1-yl)cyclopentane* (**42**)




Compound **42** was synthesized by reaction of **36** (2.41 g, 2.44 mmol) and **41** (1.40 g, 5.43 mmol) according to the general procedure for Wittig olefination (see section 2.1). Olefin **42** (0.80 g, 43%) was obtained as a colorless oil after purification by column chromatography on silica gel using hexane/EtOAc (98:2) as the eluent. This product represents a possible mixture of isotactic and syndiotactic isomers. For simplicity, only the isotactic isomer is shown in Figure 1 and throughout the supporting information.

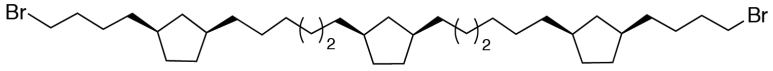
Rf: 0.32 (hexane/EtOAc 95:5); $^{1}$H NMR (500 MHz, $CDCl_3$-$d_1$) δ 7.33-7.25 (m, 10H), 5.49-5.23 (m, 8H), 4.51 (s, 4H), 3.48-3.44 (m, 4H), 2.82-2.75 (m, 3H), 2.50-2.28 (m, 5H), 2.02-1.70 (m, 15H), 1.38-1.24 (m, 16H), 1.14-0.96 (m, 4.2H), 0.66-0.58 (m, 0.8H); $^{13}$C NMR (126 MHz, $CDCl_3$-$d_1$) δ 138.7, 137.6, 137.4, 137.4, 137.2, 135.3, 135.1, 134.8, 129.0, 128.9, 128.9, 128.6, 127.8, 127.7, 124.6, 124.3, 124.2, 73.1, 73.0, 70.5, 70.4, 43.7, 43.7, 42.3, 41.9, 41.8, 40.9, 40.3, 40.3, 39.0, 38.6, 38.5, 38.4, 38.3, 38.2, 36.9, 36.8, 36.7, 33.2, 33.0, 32.9, 32.8, 32.8, 32.5, 32.4, 31.8, 30.4, 30.1, 28.6, 28.5, 28.4, 27.7.

*4-((1R,3S)-3-(6-((1R,3S)-3-(6-((1R,3S)-3-(4-hydroxybutyl)cyclopentyl)hexyl) cyclopentyl)hexyl)cyclopentyl)butan-1-ol* (**43**)




To a degassed solution of olefin **42** (0.77 g, 1.16 mmol) in EtOH/THF (8:2) (45 mL), 10% Pd/C (0.28 g, 25% w/w) was added. The reaction was stirred under 1 atm $H_2$ at room temperature for 24 hours. The solution was then filtered through celite, and the solvent was evaporated to yield a white solid which was used in the next step without further purification and characterization.

*((1S,3R)-1-(6-((1R,3S)-3-(4-bromobutyl)cyclopentyl)hexyl)-3-(6-((1S,3R)-3-(4-bromobutyl)cyclopentyl)hexyl)cyclopentane* (**44**)

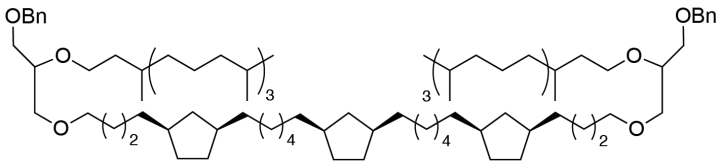



Dibromo alkane **44** (0.38 g, 67%) was obtained as a white solid from diol **28** (0.46 g, 0.89 mmol) following the general procedure for bromination of diol using hydrobromic acid solution (see section 2.1). The purification was accomplished by column chromatography on silica gel using hexane/DCM (1:1) as the eluent.

Rf: 0.86 (hexane/DCM); $^{1}$H NMR (500 MHz, $CDCl_3$-$d_1$) δ 3.38 (dd, *J* = 6.6, 6.9 Hz, 4H), 1.90-1.67 (m, 19H), 1.43-1.02 (m, 40H), 0.87-0.59 (m, 3H); $^{13}$C NMR

(126 MHz, $CDCl_3$-$d_1$) δ 40.9, 40.8, 40.3, 40.1, 39.0, 38.9, 38.7, 37.0, 37.0, 36.9, 36.0, 35.9, 34.2, 33.3, 33.2, 31.8, 31.8, 31.7, 30.2, 28.9, 28.9, 27.5, 27.4.

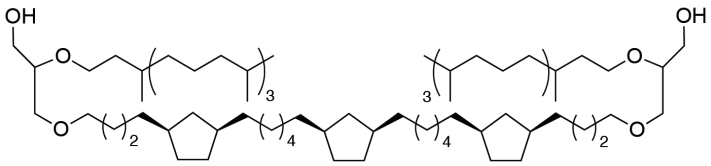
(1*S*,3*R*)-1-(6-((1*R*,3*S*)-3-(4-(3-(benzyloxy)-2-((3,7,11,15-tetramethylhexadecyl) oxy)propoxy)butyl)cyclopentyl)hexyl)-3-(6-((1*S*,3*R*)-3-(4-(3-(benzyloxy)-2-((3,7,11,15-tetramethylhexadecyl)oxy)propoxy)butyl)cyclopentyl)hexyl) cyclopentane (**45**)




Compound **45** was synthesized by reaction of **3** (0.85 g, 1.84 mmol) and **44** (0.38 g, 0.60 mmol) according to the general procedure for formation of tetraether lipid scaffold by $S_N2$ reaction (see section 2.1). Compound **45** (0.24 g, 29%) was obtained as a colorless oil after purification by column chromatography on silica gel using hexane/EtOAc (98:2 to 95:5) as eluent.

Rf: 0.29 (hexane/EtOAc 95:5); $^1$H NMR (500 MHz, $CDCl_3$-$d_1$) δ 7.32-7.25 (m, 10H), 4.55 (s, 4H), 3.63-3.40 (m, 18H), 1.91-1.68 (m, 15H), 1.65-1.49 (m, 10H), 1.39-1.03 (m, 80H), 0.87-0.83 (m, 30H), 0.75-0.59 (m, 3H); $^{13}$C NMR (126 MHz, $CDCl_3$-$d_1$) δ 138.6, 128.5, 127.8, 127.7, 78.1, 73.5, 71.9, 71.0, 70.5, 69.1, 40.9, 40.9, 40.3, 40.3, 39.6, 39.0, 38.9, 37.7, 37.7, 37.6, 37.6, 37.5, 37.4, 37.3, 37.0, 36.9, 36.8, 36.7, 33.2, 32.0, 31.8, 31.8, 30.2, 30.1, 30.0, 29.0, 28.9, 28.2, 25.4, 25.4, 25.0, 24.7, 24.6, 22.9, 22.8, 20.0, 19.9, 19.8; ESI-MS: 1408.9 $[M-H]^-$.

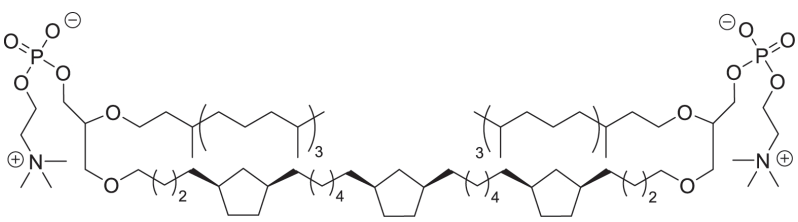
*3-(4-((1R,3S)-3-(6-((1R,3S)-3-(6-((1R,3S)-3-(4-(3-hydroxy-2-((3,7,11,15-tetra methylhexadecyl)oxy)propoxy)butyl)cyclopentyl)hexyl)cyclopentyl)hexyl)cyclopen tyl )butoxy)-2-((3,7,11,15-tetramethylhexadecyl)oxy)propan-1-ol* (**46**)




Compound **46** was synthesized by hydrogenation of **45** (0.24 g, 0.17 mmol) according to the general procedure for debenzylation of lipid scaffold by hydrogenation (see section 2.1). Diol **46** (0.18 g, 87%) was obtained as a colorless oil after purification by column chromatography on silica gel using hexane/EtOAc (9:1 to 8:2) as the eluent.

Rf: 0.38 (hexane/EtOAc); $^1$H NMR (500 MHz, $CDCl_3$-$d_1$) δ 3.70-3.39 (m, 18H), 2.24 (brs, 2H), 1.90-1.47 (m, 25H), 1.37-1.00 (m, 80H), 0.85-0.81 (m, 30H), 0.72-0.58 (m, 3H); $^{13}$C NMR (126 MHz, $CDCl_3$-$d_1$) δ 78.5, 72.0, 71.1, 68.8, 40.9, 40.8, 40.3, 40.2, 39.6, 39.0, 38.9, 37.7, 37.6, 37.6, 37.5, 37.5, 37.3, 37.3, 37.0, 36.9, 36.9, 36.7, 36.6, 33.2, 33.2, 33.0, 31.8, 31.8, 30.2, 30.1, 30.0, 28.9, 28.9, 28.2, 25.4, 25.3, 25.0, 24.7, 24.6, 22.9, 22.8, 20.0, 19.9, 19.8; ESI-MS: 1226.6 $[M-H]^-$.

*3-(4-((1S,3R)-3-(6-((1S,3R)-3-(6-((1S,3R)-3-(4-(3-((oxido(2-(trimethylammonio) ethoxy)phosphoryl)oxy)-2-((3,7,11,15-tetramethylhexadecyl)oxy)propoxy)butyl) cyclopentyl)hexyl)cyclopentyl)hexyl)cyclopentyl)butoxy)-2-((3,7,11,15-tetramethylhexadecyl)oxy)propyl (2-(trimethylammonio)ethyl) phosphate* (**GMGTPC-CP3**)



Lipid **GMGTPC-CP3** was synthesized from diol **46** (0.18 g, 0.14 mmol) following the general procedure for formation of phosphocholine lipid (see section 2.1). Lipid **GMGTPC-CP3** (0.20 g, 90%) was obtained as a white gum after purification by column chromatography on silica gel using DCM/MeOH/$H_2O$ (70:30:5) as the eluent.

Rf: 0.59 (DCM/MeOH/$H_2O$ 70:30:5); $^1$H NMR (500 MHz, MeOD-$d_4$/$CDCl_3$-$d_1$ 1:1) δ 3.91-3.86 (m, 4H), 3.53 (t, *J* = 5.4 Hz, 4H), 3.29-3.07 (m, 18H), 2.85 (m, 18H), 1.56-1.31 (m, 15H), 1.28-1.12 (m, 10H), 1.02-0.69 (m, 80H), 0.52-0.45 (m, 30H), 0.38-0.24 (m, 3H); $^{13}$C NMR (126 MHz, MeOD-$d_4$/$CDCl_3$-$d_1$ 1:1) δ 77.8, 77.7, 77.6, 77.5, 71.3, 70.2, 68.6, 68.4, 66.0, 66.0, 64.7, 58.6, 58.6, 53.5, 48.8, 39.8, 39.7, 39.0, 38.4, 38.3, 37.3, 37.1, 37.0, 37.0, 36.9, 36.8, 36.7, 36.4, 36.3, 36.2, 36.1, 32.6, 32.4, 31.2, 31.1, 29.5, 29.5, 29.4, 28.3, 28.2, 27.5, 24.8, 24.7, 24.4, 24.0, 24.0, 22.0, 21.9, 19.1, 19.0, 19.0, 18.9, 18.9; $^{31}$P NMR (202 MHz, MeOD-$d_4$/$CDCl_3$-$d_1$ 1:1) δ 0.16; ESI-MS: 1558.2 $[M-H]^-$; HRMS calcd 1558.3241 for $[C_{91}H_{183}N_2O_{12}P_2]^+$, found 1558.3241.

## 2.7 Synthetic Procedure for GMGTPC-CH1

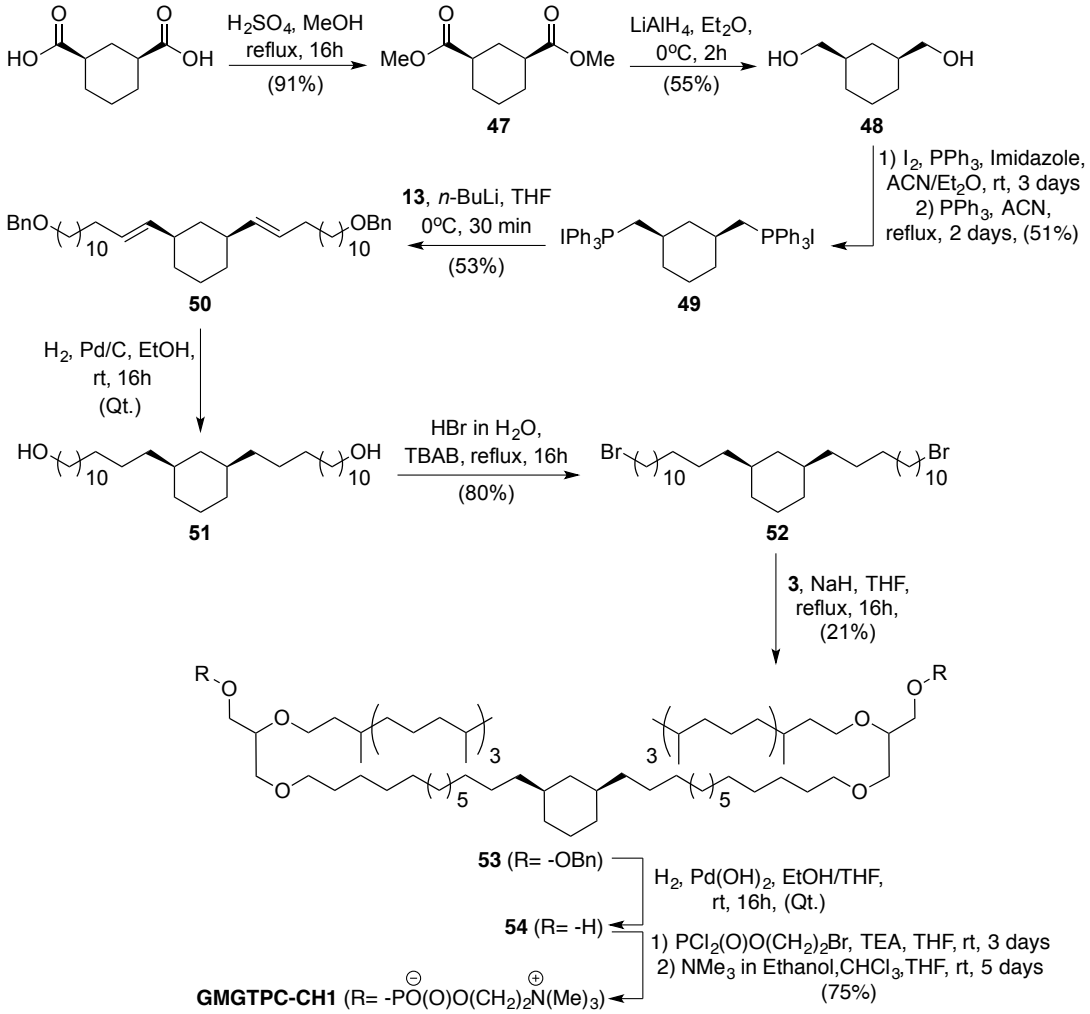
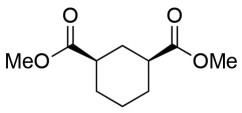


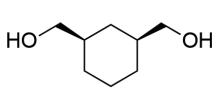

**Figure S6. Synthesis of GMGTPC-CH1.**

*Dimethyl (1R,3S)-cyclohexane-1,3-dicarboxylate* (**47**)



To a solution of *cis*-1,3-cyclohexanedicarboxylic acid (10.3 g, 60.1 mmol) in MeOH (82 mL), concentrated sulfuric acid (2 mL) was added. The solution was stirred for 16 hours at reflux and the solvent was removed under vaccum. Ice was added to the residue and the aqueous solution was extracted with EtOAc. The combined organic layer was washed with sat. $NaHCO_3$ solution, water and dried over $Na_2SO_4$. Diester **47** (10.9 g, 91%) was obtained as a colorless oil, which was used in the next step without further purification.

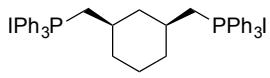
*((1R,3S)-cyclohexane-1,3-diyl)dimethanol* (**48**)



To a cold suspension of $LiAlH_4$ (10.4 g, 272.0 mmol) in dry $Et_2O$ (300 mL), a solution of **47** (10.9 g, 54.4 mmol) in dry $Et_2O$ (230 mL) was added dropwise. After stirring at 0°C for 1.5 hours, 1M HCl solution was added slowly to quench the reaction. The reaction mixture was extracted with EtOAc and the combined organic layer was washed with water, brine and dried over $Na_2SO_4$. Diol **48** (4.4 g, 55%) was obtained as a white solid after solvent evaporation.

$^1$H NMR (500 MHz, $CDCl_3$-$d_1$) δ 3.38-3.40 (m, 4H), 2.04-2.00 (m, 3H), 1.88-1.73 (m, 4H), 1.55-1.47 (m, 2H), 1.32-1.22 (m, 1H), 0.89-0.81 (m, 2H), 0.65-0.58 (m, 1H).

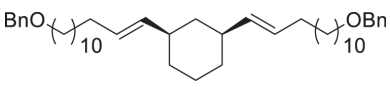
*(((1R,3S)-cyclohexane-1,3-diyl)bis(methylene))bis(triphenylphosphonium) iodide* (**49**)



To a cold solution of triphenylphosphine (17.4 g, 66.5 mmol) and imidazole (4.5 g, 66.5 mmol) in mixture of dry ACN/$Et_2O$ (1:3) (150 mL), iodine (16.8 g, 66.5 mmol) was added. After stirring of the mixture at room temperature in the dark for 10 min, a solution of compound **48** (4.4 g, 30.2 mmol) in dry $Et_2O$ (30 mL) was added. The reaction mixture was stirred for 2 hours at room temperature in the dark. Reaction mixture was washed with 10% $Na_2S_2O_3$ aqueous solution and extracted with $Et_2O$. The combined organic layers were washed with water, dried over $Na_2SO_4$, and purified by silica gel chromatography using a mixture of hexane/EtOAc (8:2) as the eluent. The resulting oil was carried out to the next step without further purification and characterization. To a solution of the purified crude in ACN (80 mL), triphenylphosphine (23.7 g, 90.6 mmol) was added and the reaction mixture was stirred for 16 hours at reflux. After the reaction mixture was allowed to cool down to room temperature, the product was precipitated by adding toluene. Pure compound **49** (13.7 g, 51%) was obtained as a white solid after recrystallization from MeOH and $Et_2O$.

$^1$H NMR (500 MHz, MeOD-$d_4$) δ 7.89-7.72 (m, 30H), 3.52-3.36 (m, 4H), 1.88-1.70 (m, 3H), 1.64-1.57 (m, 1H), 1.45-1.42 (m, 1H), 1.33-1.30 (m, 2H), 1.20-1.12 (m, 2H), 1.03-0.94 (m, 1H); $^{13}$C NMR (126 MHz, MeOD-$d_4$) δ 136.4, 135.0 (d, *JC-P* = 10.1 Hz), 131.7 (d, *J* C-P= 12.6 Hz), 120.5 (d, *J* C-P= 86.0 Hz), 34.7, 43.6, 43.6, 34.6, 34.5, 34.1, 29.4, 29.0, 29.2; $^{31}$P NMR (202 MHz, MeOD-$d_4$) δ 22.78; ESI-MS: 317.5 $[M-2I]^{2+}$; HRMS calcd 317.1454 for $[C_{44}H_{44}P_2]^{2+}$, found 317.1452.

*(1R,3S)-1,3-bis((E)-13-(benzyloxy)tridec-1-en-1-yl)cyclohexane* **(50)**

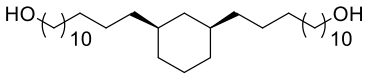


Compound **50** was synthesized by reaction of **43** (2.57 g, 2.89 mmol) and **13** (2.01 g, 6.93 mmol) according to the general procedure for Wittig olefination (see section 2.1). Olefin **50** (1.00 g, 53%) was obtained as a colorless

oil after purification by column chromatography on silica gel using hexane/EtOAc (99:1) as the eluent.

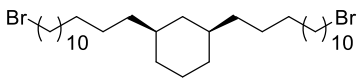
Rf: 0.40 (Hexane/EtOAc 99:1); $^{1}$H NMR (500 MHz, $CDCl_3$-$d_1$) δ 7.34-7.25 (m, 10H), 5.40-5.14 (m, 4H), 4.50 (s, 4H), 3.46 (t, *J* = 6.6 Hz, 4H), 2.35 (m, 1H), 2.06-1.92 (m, 5H), 1.78-1.52 (m, 8H), 1.38-1.26 (m, 33H), 1.02-0.81 (m, 3H); $^{13}$C NMR (126 MHz, $CDCl_3$-$d_1$) δ 138.9, 136.3, 136.2, 135.9, 135.8, 134.0, 133.8, 128.5, 128.5, 128.1, 127.8, 127.6, 73.0, 70.7, 40.8, 40.5, 40.1, 40.0, 36.3, 36.2, 33.1, 32.9, 32.9, 32.8, 30.2, 30.0, 29.9, 29.8, 29.7, 29.5, 29.3, 27.7, 27.6, 26.4, 26.0, 25.9; ESI-MS: 657.5 $[M+H]^+$; HRMS calcd 679.5425 for $[C_{46}H_{72}O_2Na]^+$, found 679.5424.

*13,13'-((1R,3S)-cyclohexane-1,3-diyl)bis(tridecan-1-ol)* (**51**)




To a degassed solution of olefin **50** (0.95 g, 1.45 mmol) in EtOH (88 mL), 10% Pd/C (0.24 g, 25% w/w) was added. The reaction was stirred under 1 atm $H_2$ at room temperature for 24 hours. The solution was then filtered through celite, and the solvent was evaporated to yield a white solid which was used in the next step without further purification and characterization.

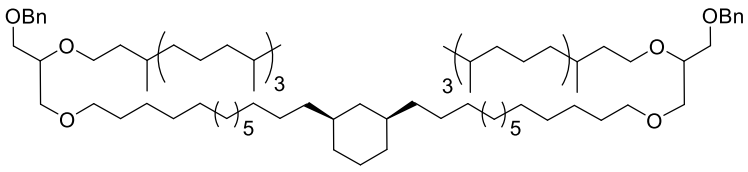
*(1R,3S)-1,3-bis(13-bromotridecyl)cyclohexane* (**52**)




Dibromo alkane **52** (0.70 g, 80%) was obtained as a white solid from diol **51** (0.70 g, 1.45 mmol) following the general procedure for bromination of diol using hydrobromic acid solution (see section 2.1).

Rf: 0.80 (hexane/EtOAc 99:1); $^{1}$H NMR (500 MHz, $CDCl_3$-$d_1$) δ 3.39 (t, *J* = 6.9 Hz, 4H), 1.86-1.80 (m, 4H), 1.69-1.66 (m, 3H), 1.43-1.37 (m, 4H), 1.29-1.05 (m, 43H), 0.87-0.69 (m, 3H), 0.49-0.42 (m, 1H); $^{13}$C NMR (126 MHz, $CDCl_3$-$d_1$) δ 40.6, 37.9, 37.8, 34.2, 33.5, 32.9, 30.1, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 28.9, 28.3, 27.0, 26.5.

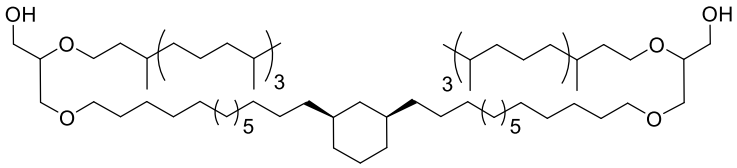
*(1R,3S)-1,3-bis(13-(3-(benzyloxy)-2-((3,7,11,15-tetramethylhexadecyl)oxy) propoxy)tridecyl)cyclohexane* (**53**)




Compound **53** was synthesized by reaction of **3** (1.53 g, 3.32 mmol) and **44** (0.67 g, 1.11 mmol) according to the general procedure for formation of tetraether lipid scaffold by $S_N2$ reaction (see section 2.1). Compound **53** (0.32 g, 21%) was obtained as a colorless oil after purification by column chromatography on silica gel using hexane/EtOAc (98:2 to 95:5) as the eluent.

Rf: 0.35 (hexane/EtOAc 95:5); $^{1}$H NMR (500 MHz, $CDCl_3$-$d_1$) δ 7.33-7.24 (m, 10H), 4.55 (s, 4H), 3.65-3.41 (m, 18H), 1.72-1.50 (m, 14H), 1.39-1.04 (m, 89H), 0.88-0.72 (m, 32H), 0.52-0.45 (m, 1H); $^{13}$C NMR (126 MHz, $CDCl_3$-$d_1$) δ 138.6, 128.4, 127.7, 127.6, 78.1, 73.5, 71.8, 70.9, 70.4, 69.0, 40.7, 39.5, 38.0, 37.9, 37.8, 37.7, 37.6, 37.5, 37.4, 37.3, 37.2, 33.6, 33.0, 30.3, 29.9, 29.8, 29.7, 28.1, 27.1, 26.6, 26.3, 25.0, 24.7, 24.5, 22.9, 22.8, 20.0, 19.9, 19.8.

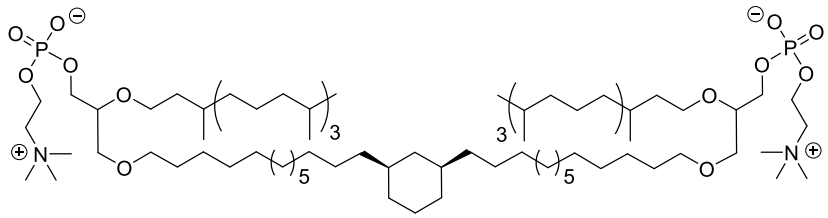
*3,3'-((((1R,3S)-cyclohexane-1,3-diyl)bis(tridecane-13,1-diyl))bis(oxy))bis(2-((3,7,11,15-tetramethylhexadecyl)oxy)propan-1-ol)* (**54**)



Compound **54** was synthesized by hydrogenation of **53** (0.32 g, 0.23 mmol) according to the general procedure for debenzylation of lipid scaffold by hydrogenation (see section 2.1). Diol **54** (0.28 g, Qt.) was obtained as a colorless oil after purification by column chromatography on silica gel using hexane/EtOAc (8:2) as the eluent.

Rf: 0.48 (hexane/EtOAc 8:2); $^{1}$H NMR (500 MHz, $CDCl_3$-$d_1$) δ 3.71-3.39 (m, 18H), 2.11 (brs, 2H), 1.69-1.46 (m, 14H), 1.39-1.00 (m, 89H), 0.86-0.68 (m, 32H), 0.49-0.42 (m, 1H); $^{13}$C NMR (126 MHz, $CDCl_3$-$d_1$) δ 78.4, 72.0, 71.1, 71.0, 68.8, 63.3, 63.2, 40.7, 39.6, 38.0, 37.9, 37.8, 37.7, 37.6, 37.5, 37.4, 37.3, 37.2, 33.6, 33.0, 30.3, 30.1, 30.0, 29.9, 29.8, 29.7, 28.2, 27.1, 26.6, 26.3, 25.0, 24.7, 24.5, 22.9, 22.8, 20.0, 19.9, 19.8; ESI-MS: 1190.2 $[M+H]^+$; HRMS calcd 1190.1975 for $[C_{78}H_{157}O_6]^+$, found 1190.1979.

*((((1R,3S)-cyclohexane-1,3-diyl)bis(tridecane-13,1-diyl))bis(oxy))bis(2-((3,7,11,15-tetramethylhexadecyl)oxy)propane-3,1-diyl)bis(2-(trimethylammonio)ethyl) bis(phosphate)* (**GMGTPC-CH1**)



Lipid **GMGTPC-CH1** was synthesized from diol **54** (0.26 g, 0.22 mmol) following the general procedure for formation of phosphocholine lipid (section 2.1). Lipid **GMGTPC-CH1** (0.25 g, 75%) was obtained as a white gum after purification by column chromatography on silica gel using DCM/MeOH/$H_2O$ (70:30:5) as the eluent.

Rf: 0.55 (DCM/MeOH/$H_2O$ 70:30:5); $^{1}$H NMR (500 MHz, MeOD-$d_4$/$CDCl_3$-$d_1$ 1:1) δ 3.90-3.86 (m, 4H), 3.53 (t, *J* = 5.6 Hz, 4H), 3.32-3.20 (m, 12H), 3.13-3.06 (m, 6H), 2.04 (s, 18H), 1.37-1.32 (m, 4H), 1.25-1.12 (m, 10H), 1.02-0.69 (m, 89H), 0.52-0.48 (m, 30H), 0.43-0.35 (m, 2H), 0.15-0.08 (m, 1H); $^{13}$C NMR (126 MHz,

MeOD-$d_4$/$CDCl_3$-$d_1$ 1:1) δ 77.8, 77.7, 77.6, 71.3, 70.2, 68.6, 68.4, 66.0, 64.7, 64.6, 58.6, 58.5, 53.5, 40.2, 39.0, 37.5, 37.4, 37.3, 37.2, 37.1, 37.0, 36.9, 36.8, 36.7, 33.1, 32.4, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 27.5, 26.5, 26.0, 25.7, 24.4, 24.0, 23.9, 22.1, 22.0, 19.2, 19.1, 19.0, 18.9, 18.8; $^{31}P$ NMR (202 MHz, MeOD-$d_4$/$CDCl_3$-$d_1$ 1:1) δ 0.15; ESI-MS: 1520.4 $[M+H]^+$; HRMS calcd 1520.3084 for $[C_{88}H_{181}N_2O_{12}P_2]^+$, found 1520.3093.

## 3. General Procedure for Differential Scanning Calorimetry (DSC) Measurement of Lipids in Liposome Form

Suspensions of liposomes were prepared by sonication of lipids for 30 min in DI water. All liposome samples contained a final concentration of ~5% lipid by weight. DSC experiments were performed in duplicate using a Thermal Analysis Q2000 DSC. Each experiment involved a 5 °C/min ramp from 5 °C to 70 °C under high purity $N_2$ at 50 mL/min. TA Universal Analysis was used to extract the $T_m$ for these samples.
All of the synthetic lipids did not exhibit a phase transition from 5 - 70 °C shown in Figure S7. DMPC was used as a positive control which showed a phase transition at 24 °C, which was consistent with the literature value for the $T_m$ for this lipid.[16]

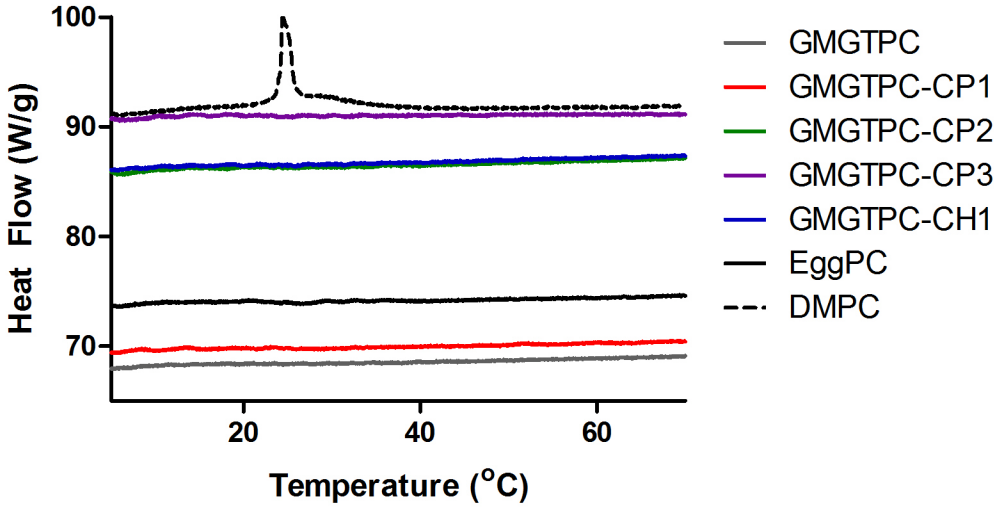



**Figure S7. DSC measurements of Lipids in Liposome Form.**

## 4. General Buffer Preparation Procedure

Preparation of Buffer A- 4.18 g of Bis Tris (10 mM) and 11.68 g of NaCl (100 mM) was dissolved in 2 L of Milli-Q filtered Deionized water. The pH was then adjusted to 7.2 by minimal addition of 2 M HCl.

Preparation of Buffer B - 4.18 g of Bis Tris (10 mM) and 11.68 g of NaCl (100 mM) was dissolved in 2 L of Milli-Q filtered Deionized water. The pH was then adjusted to 5.8 by minimal addition of 2 M HCl.

**5. General Procedure for Liposome Extrusion**

10 mg/mL liposome solution was prepared by first dissolving 5 mg of lipid of interest into a 5 mL round bottom flask in a DCM/MeOH (7/3) solution. A thin lipid film was achieved by evaporating the solvent using a rotary evaporator (BUCHI RE111) then dried further over a hi-vacuum pump (Welch 1402) for 4 hrs. The thin lipid film was then hydrated, in a 4 mM Carboxyfluorescein (CF) solution prepared in buffer A, by vortexing the solution for 30 seconds followed by sonication in a water bath sonicator (Branson 2510) for 30 mins. After sonication, the lipid mixture underwent 5 freeze thaw cycles that consisted of 2 mins at -78°C followed by 2 mins at 50°C. The lipid solution was then extruded (Avanti mini-extruder) through 200 nm polycarbonate membrane 25 times followed by another extrusion with a 100 nm polycarbonate membrane 51 times. The lipid solution was then stored at 4°C in Protein Lo-Bind Eppendorf tube. Liposome radius is shown in Figure S8.

*Note: Depending on the lipids, EggPC or GMGTPC derivatives, the time window between DLS measurements were different. For EggPC, the rate of small ion leakage was very fast, requiring us to use a stop flow experiment for 0.28 h. Since GMGTPC derivatives leak slower, the acquisition times are longer (up to 6h). Figure S8 demonstrates that the different liposomes were stables over the time of the membrane leakage experiments.

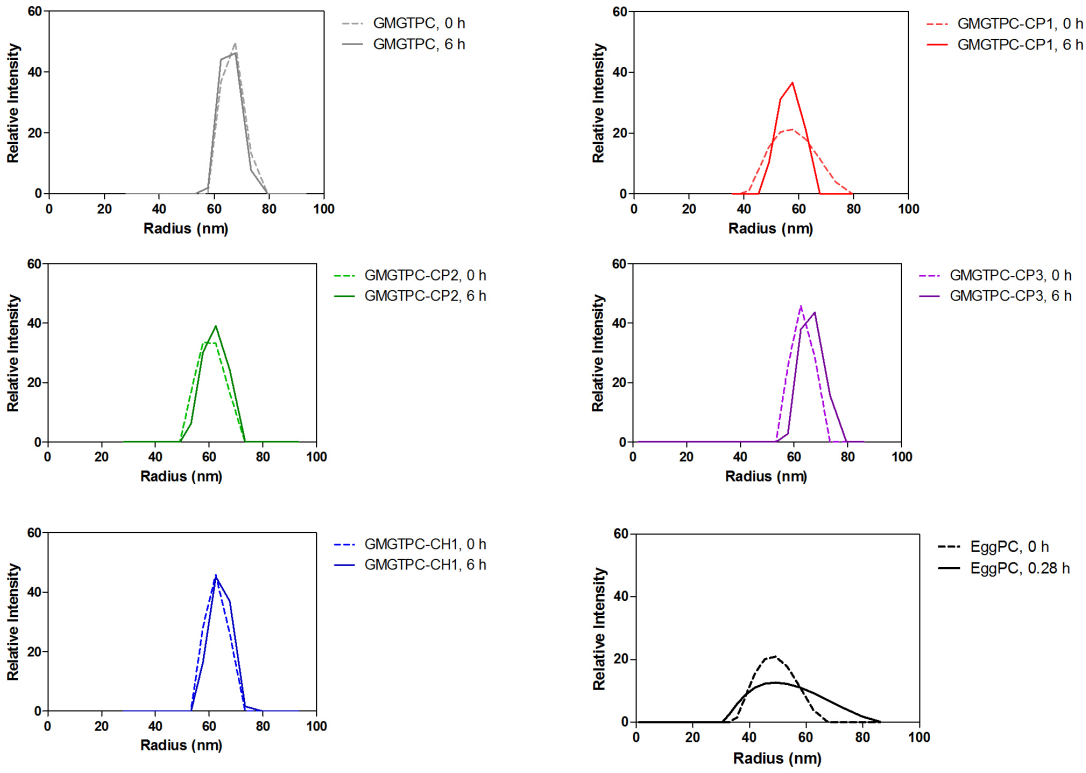



**Figure S8. DLS hydrodynamic radius of GMGTPC liposomes.**

## 6. General Procedure of Assessing Liposomal Fusion

Fusion of **GMGTPC** liposomes was assessed using a Förster Resonance Energy Transfer based (FRET) lipid mixing assay[17] and 1,2-dipalmitoyl-*sn*-glycero-3-phospho-L-serine (DPPS) liposomes as a positive fusogenic system.[18–20]

Liposomes (10 mg/mL) containing 0.5% of 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-*N*-(7-nitro-2-1,3-benzoxadiazol-4-yl) (NBD-PE) and 0.5% of 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-l-(lissamine rhodamine B sulfonyl) (Lissamine-PE) (Avanti Polar Lipids, USA) were prepared using either EggPC/DPPS (7:3) or **GMGTPC**. Unlabeled liposomes (10 mg/mL) were also prepared using either EggPC/DPPS (7:3) or **GMGTPC**. ≈100 nm liposomes (100 nm diameter) were prepared in a 2 mM *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES) buffer solution (2 mM histidine, 100 mM NaCl, pH 7.4)[20] according to the procedure described above. Labeled and unlabeled solutions of liposomes were diluted 50- and 5-times respectively in a 2 mM TES buffer solution (2 mM histidine, 100 mM NaCl, pH 7.4).

For calcium-mediated fusion with EggPC/DPPS (7:3) liposomes, 100 µL of fluorescent and nonfluorescent liposome solutions were added to a microtube

containing 800 µL of 20 mM $CaCl_2$ in a buffer solution (2 mM TES, 2 mM histidine, 100 mM NaCl, pH 7.4). The ratio of labeled to unlabeled liposomes was 1:10. The sample was incubated at 37°C and fluorescence was measured at different times.

For **GMGTPC** liposomes, 100 µL of fluorescent and nonfluorescent liposome solutions were added to a microtube containing 800 µL of a 2 mM TES buffer solution (2 mM histidine, 100 mM NaCl, pH 7.4). The ratio of labeled to unlabeled liposomes was 1:10. The sample was incubated at room temperature and fluorescence was measured at different times shown in Figure S9.

Measurements were performed on a PTI spectrofluorometer (75W, Photon Technology). Lissamine fluorescence was used as a read-out for membrane fusion. The excitation wavelength was λ = 460 nm and the fluorescence emission of Lissamine-PE was recorded from 550 to 650 nm. To determine the extent of fusion as a percentage of the total fluorescence change, 10 µL of a detergent (10% (w/w) C12E8 solution in water) was added to the cuvette at the end of the experiment.

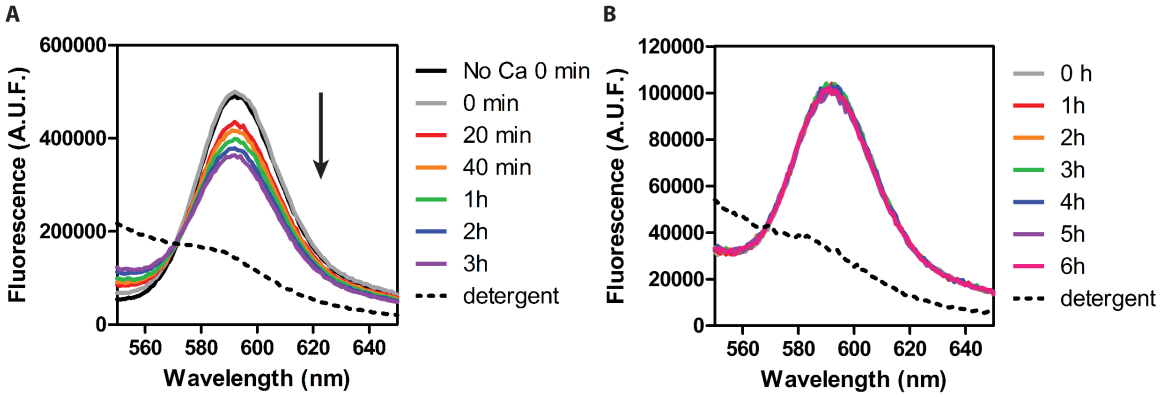



**Figure S9. FRET-based lipid mixing assay for liposome fusion experiments. A) Calcium-induced fusion with EggPC/DPPS (7:3) liposomes. A substantial decrease of rhodamine signal over time indicates fusion of labeled and unlabeled liposomes. B) Absence of observed fusion with GMGTPC liposomes.**

## 7. General Procedure to Measure pH Equilibrium of CF

First, to evaluate whether CF permeates through the liposomal membrane at room temperature, liposomes incorporating 100 mM of CF were prepared. At high intravesicular concentrations, CF has negligible fluorescence due to self-quenching properties.[21] When the dye is released from the liposome, CF becomes fluorescent due to liberation from the high concentration quenching. No appreciable fluorescence was measured, results shown in Figure S10.

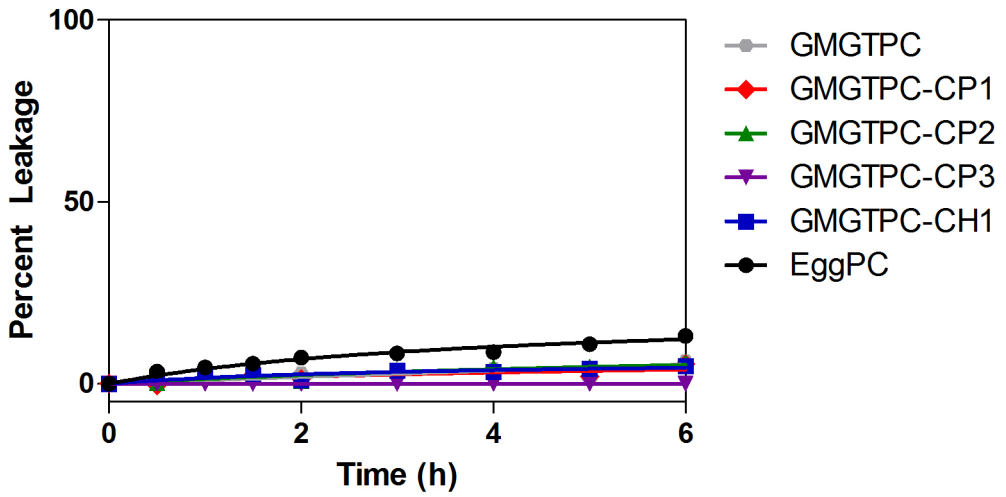



**Figure S10. CF Leakage Measurements of Lipids at Room Temperature.**

After determining that CF does not leak through the liposomal membrane, pH induced reduction of intravesicular CF fluorescence was monitored. In order to obtain the fast rate of pH induced decrease of CF for EggPC, a stopped-flow assay was necessary. Before each assay, 10 µL of the stock extruded lipid solution was diluted in 500 µL of buffer A. Free CF was removed using a PD miniTrap™ G-25 Sephadex™ column from GE Healthcare ending 100 times dilution from the stock extruded solution (0.1 mg/mL). The reaction cell was cleaned using buffer A (15 drives) until a steady signal was obtained before running any experiments. After a steady fluorescence signal was achieved and free CF was removed from the liposome solution, 25 µl of the lipid solution was mixed with 225 µL of buffer A to obtain the relative maximum fluorescence at pH 7.2 (5 acquisitions). The reaction cell was again cleaned until a steady signal was observed and repeated with buffer B (5 acquisitions). To confirm 100% change of fluorescence at pH 5.8 from the assay, the relative fluorescence at 1000 seconds was confirmed by comparison to the relative fluorescence of liposomes in Buffer B doped with 1 µL of a solution of Nigericin in Ethanol (100 µM) using the plate reader.

To obtain the pH equilibration observed rate of each lipid, the decrease in fluorescence of CF was followed using Perkin Elmer Enspire© multimode plate reader. Before each assay, 10 µL of the stock extruded lipid solution was diluted in 500 µL of buffer A. Free CF was removed using a PD miniTrap™ G-25 Sephadex™ column from GE Healthcare ending in 100 times dilution from the stock extruded solution (0.1 mg/mL). 45 µL of purified liposome solution was next added into three 0.5 mL Protein Lo-Bind tubes for each lipid solution. In one tube, 405 µL of Buffer A was added. In the second tube, 405 µL of Buffer B was added with 1 µL of 100 µM solution of Nigericin in Ethanol. In the third tube, right before starting the measurement, 405 µL of Buffer B was added. 125 µL was added to

each well of the plate three times for each tubes resulting in three measurements with three replicates with a total of 9 measurements per lipid solution. The 9 average measurements are plotted in Figure S11 below. No significant morphology change was observed after the assay as shown in Figure S8 above.

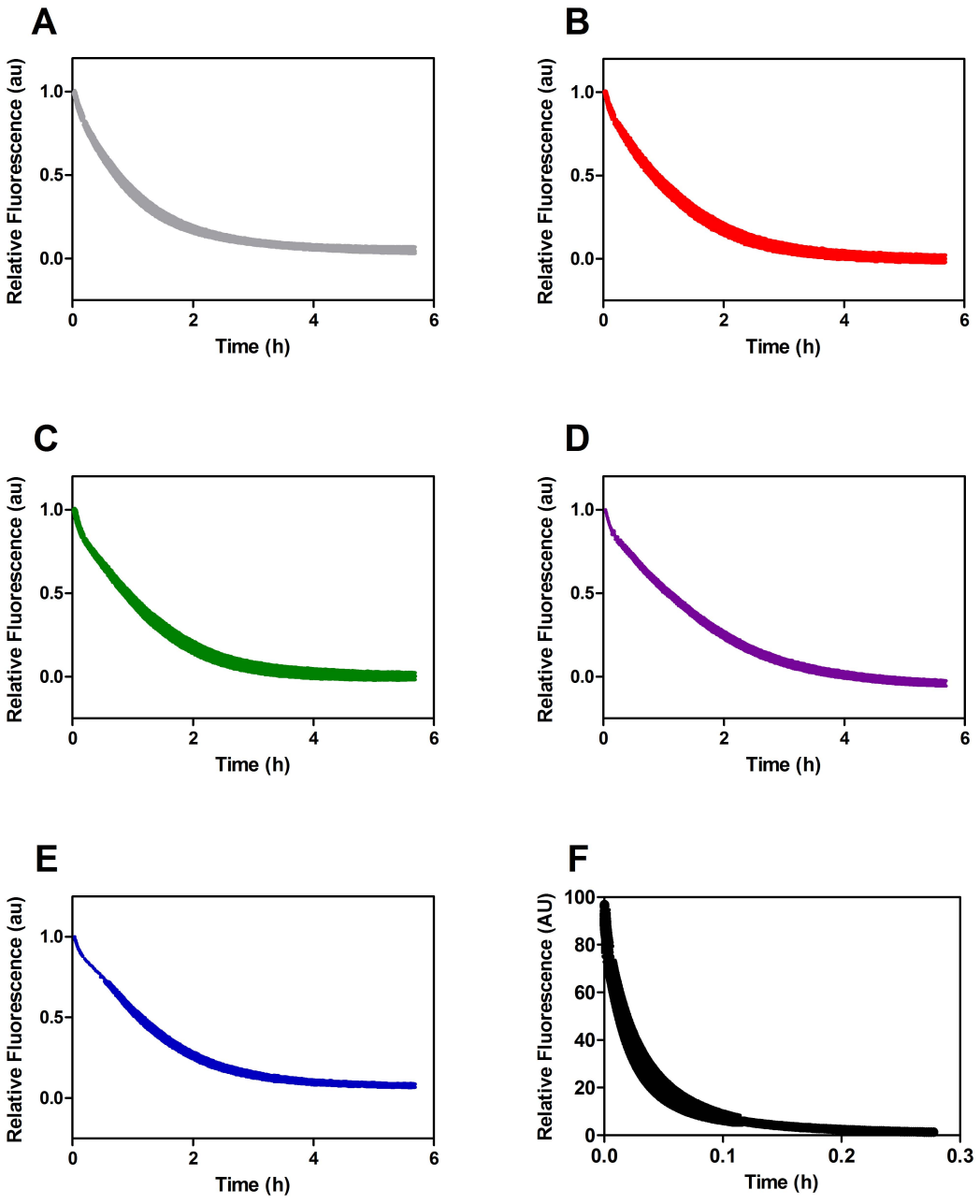



**Figure S11. pH Equilibration of CF vs Time (h). (A) GMGTPC; (B) GMGTPC-CP1; (C) GMGTPC-CP2; (D) GMGTPC-CP3; (E) GMGTPC-CH1; (F) Egg-PC.**

## 8. General Calculation Procedure for Kinetic Analysis

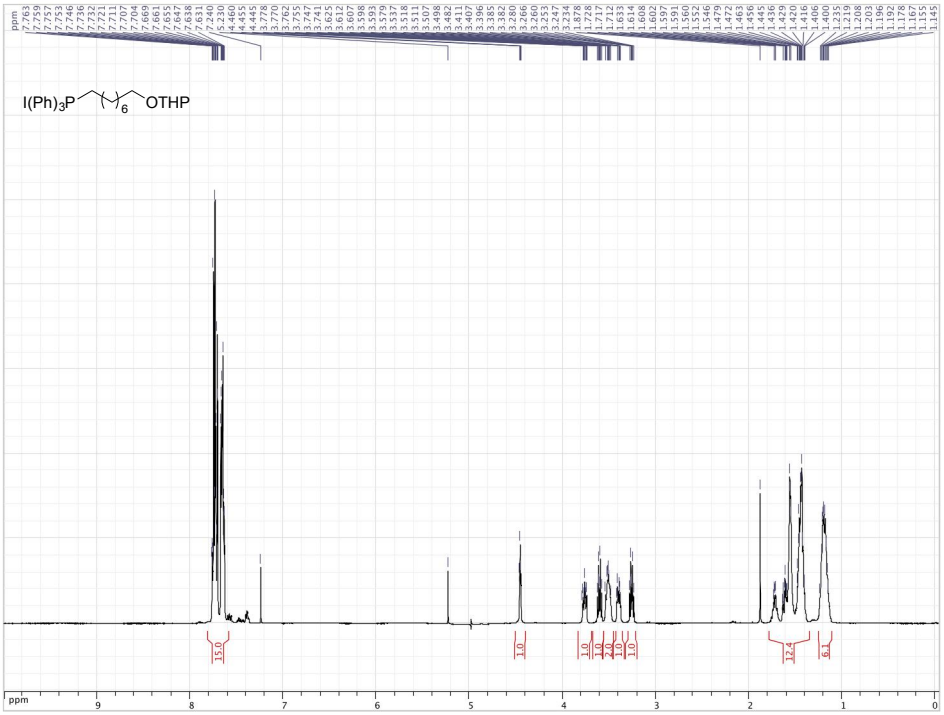
For each assay, the relative fluorescence ($F_{rel}$) was normalized using equation (1) below. $F_0$ represents fluorescence at $T_0$, $F_A$ represents fluorescence measurements at different times, and $F_{Nig}$ is the fluorescence measurement of the liposome solution including Nigericin at 600 second in buffer B. After the data was normalized, equation (2) was used to determine the initial rate (i.e., up to 15% decrease in CF fluorescence) of the decrease in CF fluorescence by combining individual measurements using GraphPad Prism 5 software.

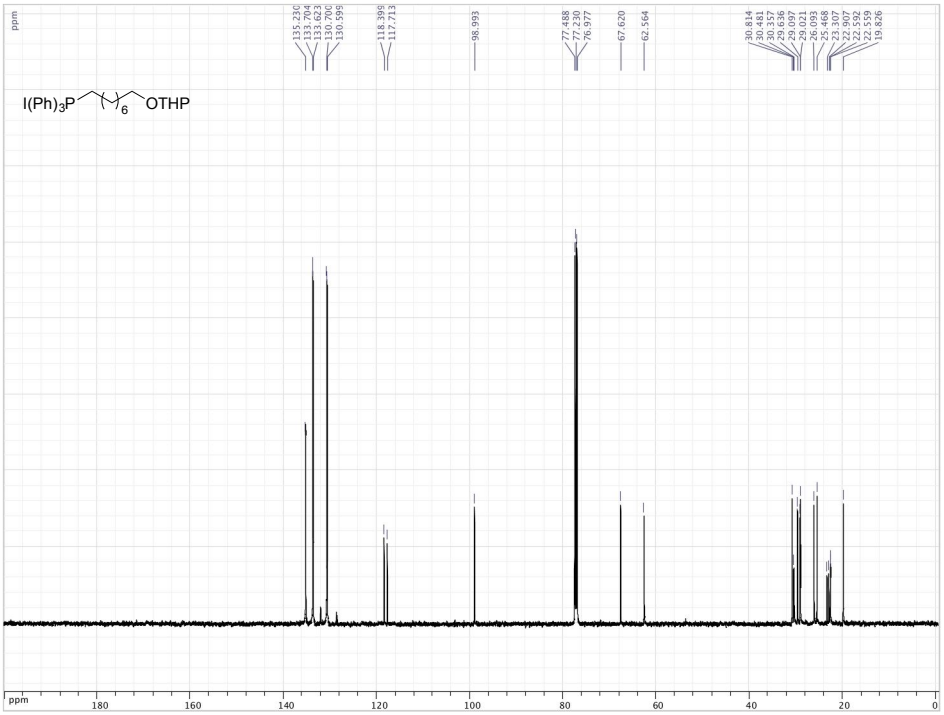
$$F_{rel} = \left(1 - \frac{(F_0 - F_A)}{(F_0 - F_{Nig})}\right) X\ 100 \quad (1)$$

$$ln\ (F_{rel})\ =\ -kt \quad (2)$$

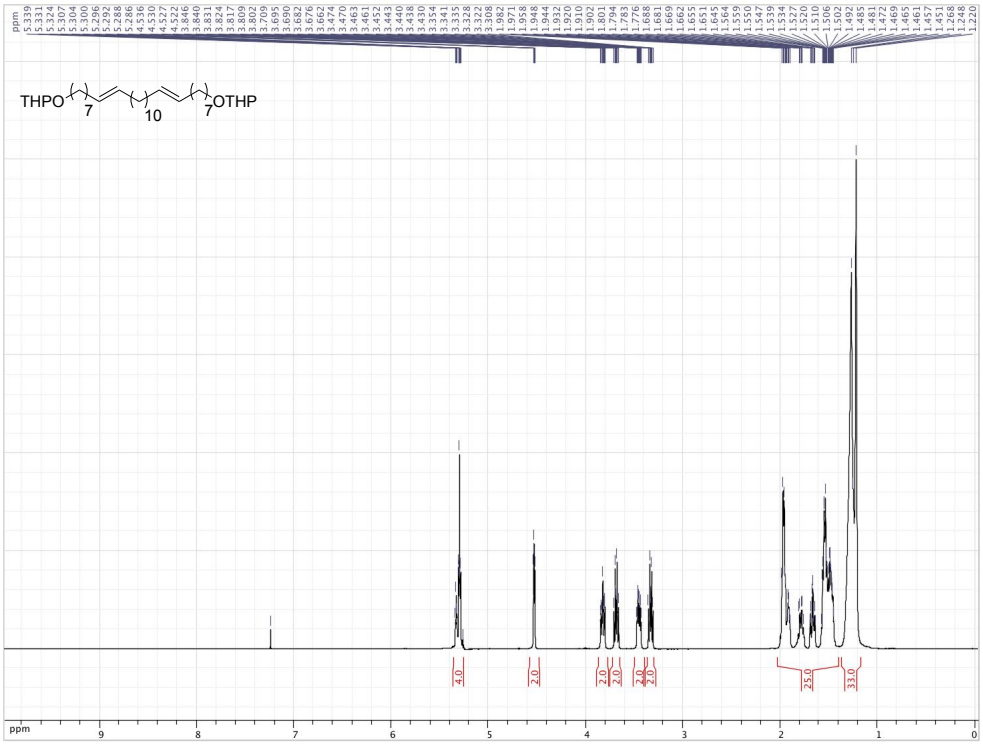
## 9. NMR Spectra

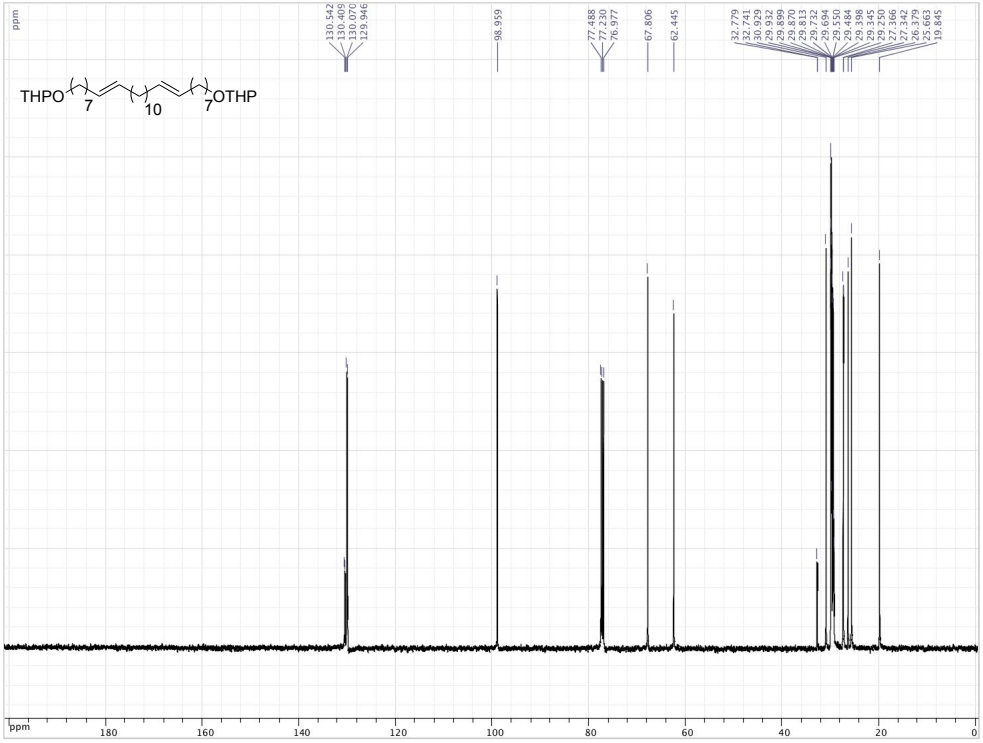
### Compound 5



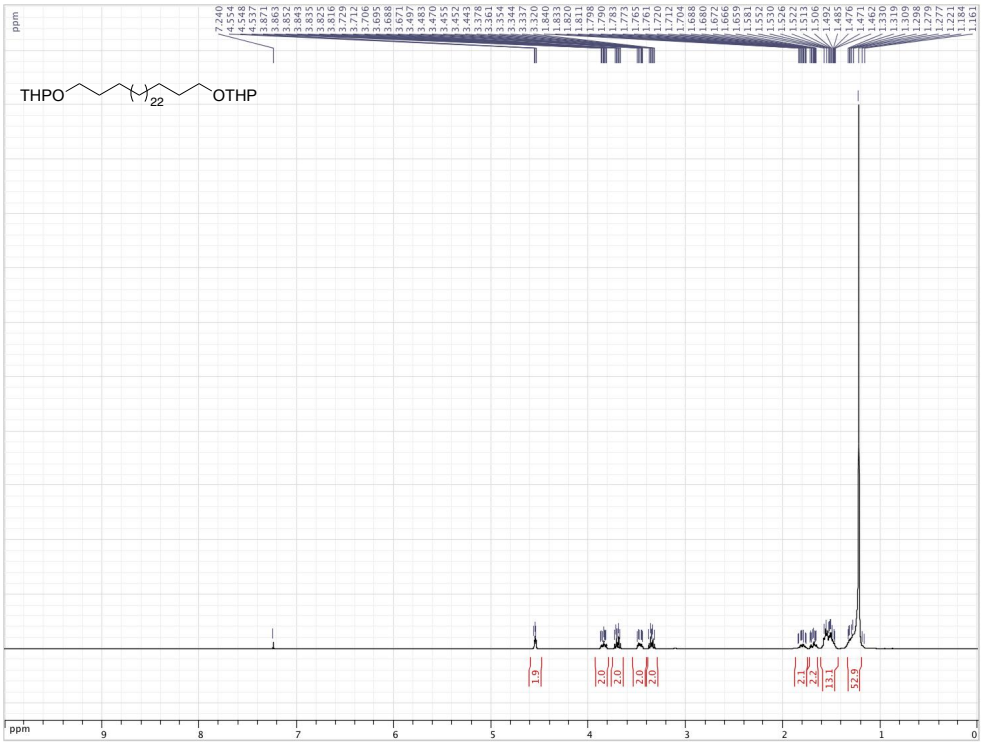



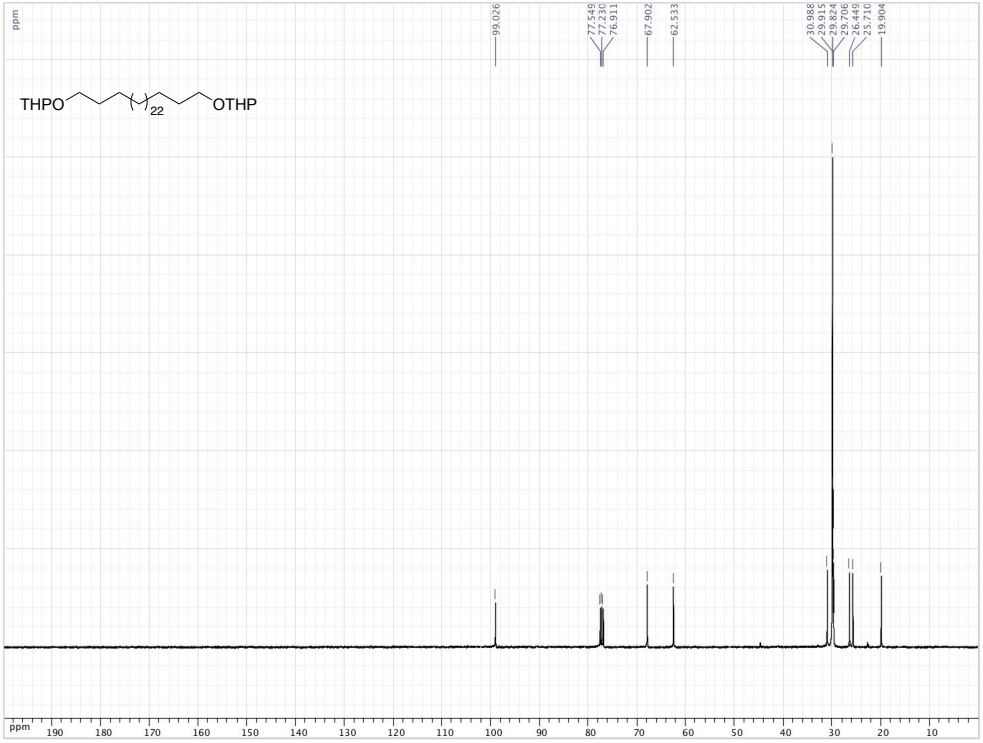
## Compound 7



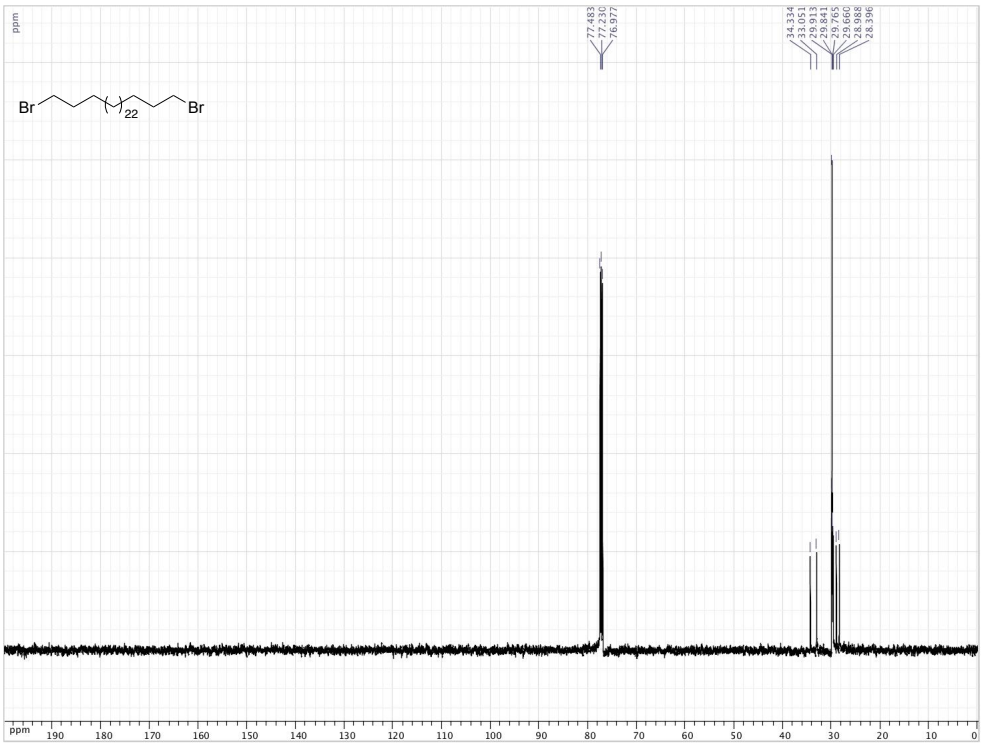
## Compound 8



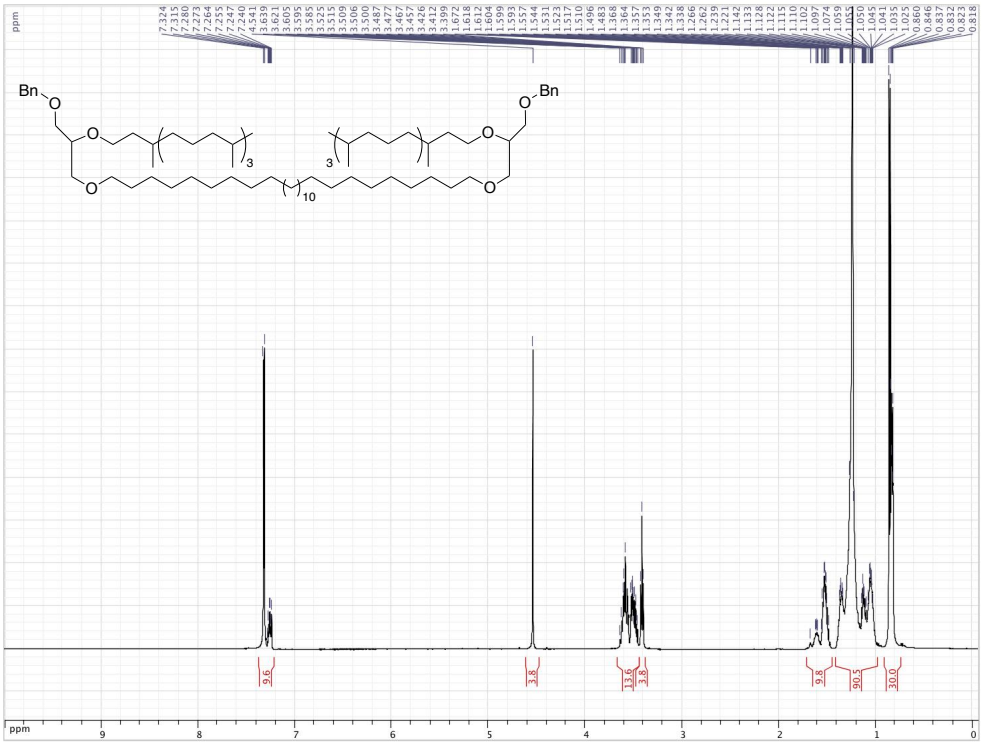



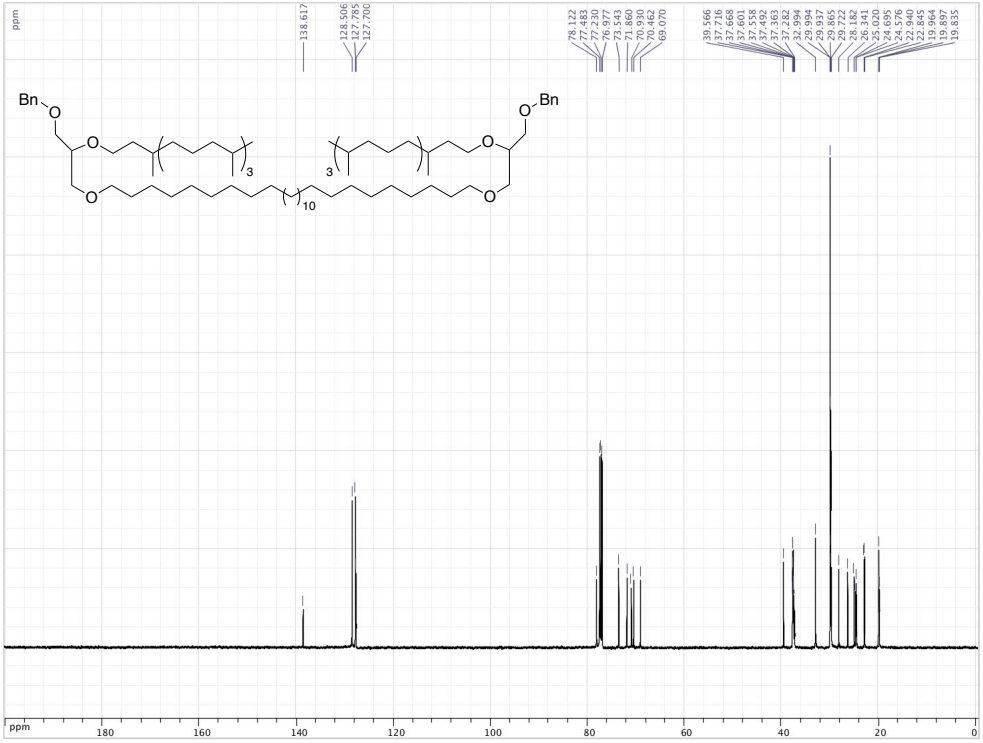
## Compound 9


ppm
7.240
3.400
3.386
3.373
1.258
1.229
Br
22
Br
4.0
4.0
4.2
44.3
ppm
9.5
9
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0

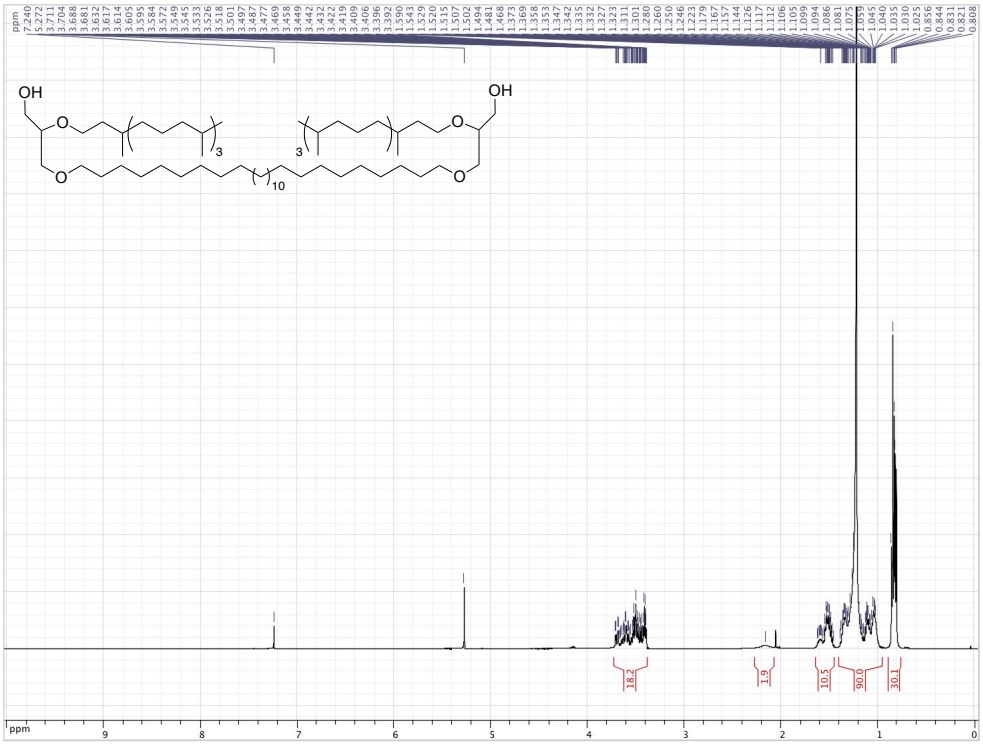

ppm
77.483
77.230
76.977
34.334
33.051
29.913
29.841
29.765
29.660
28.988
28.396
Br
22
Br
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

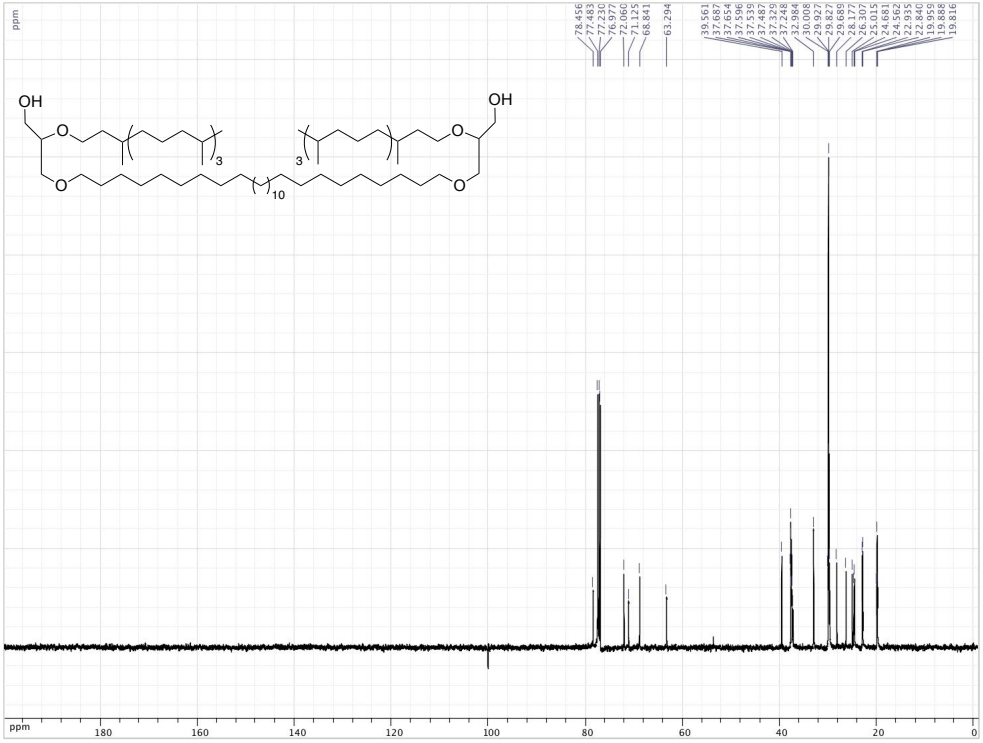
## Compound 10



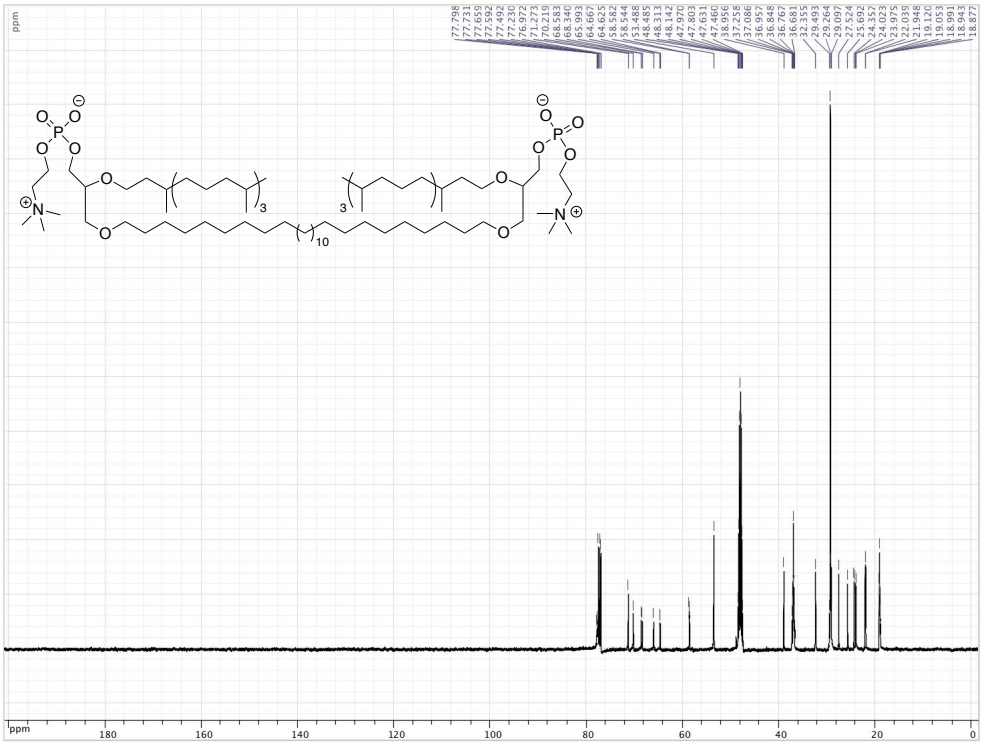



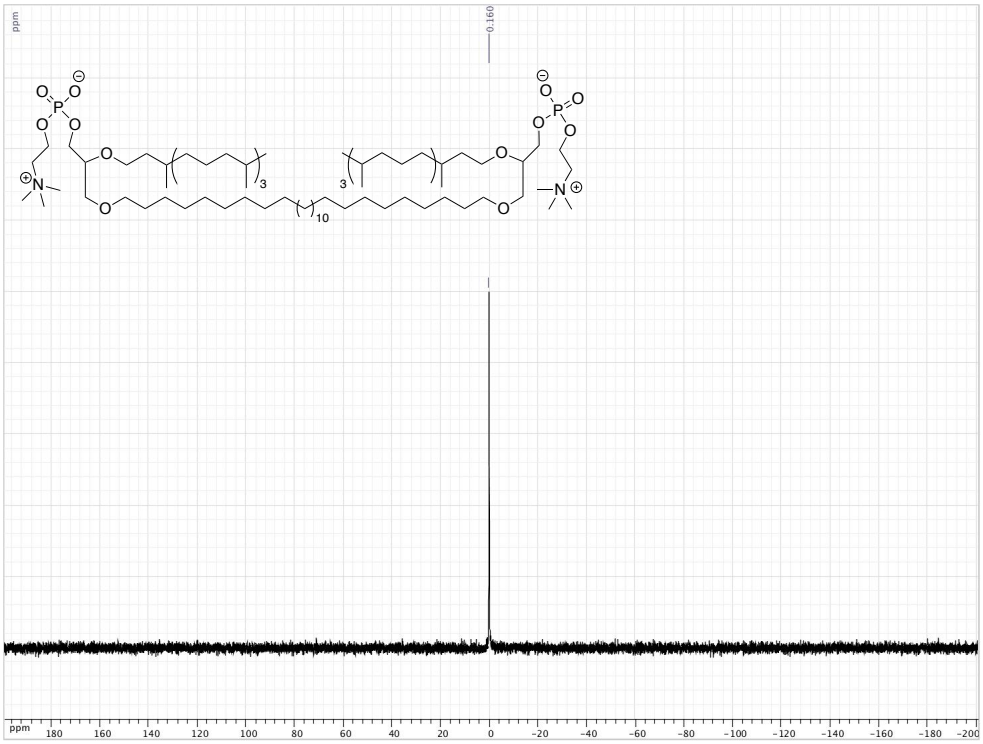
## Compound 11



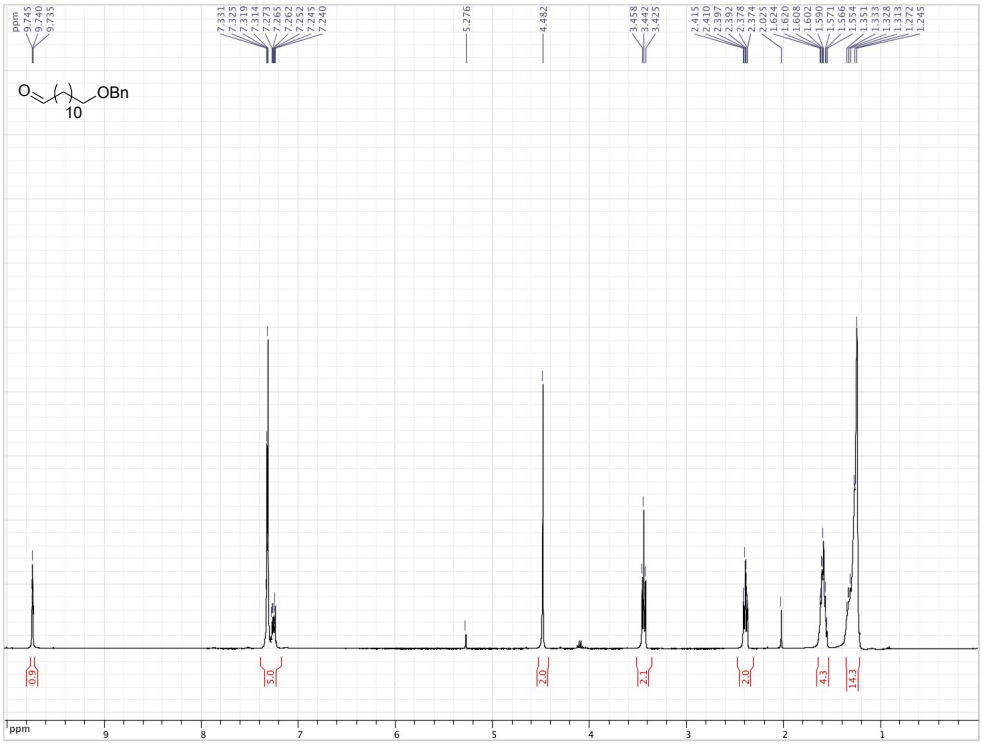



## Compound GMGTPC

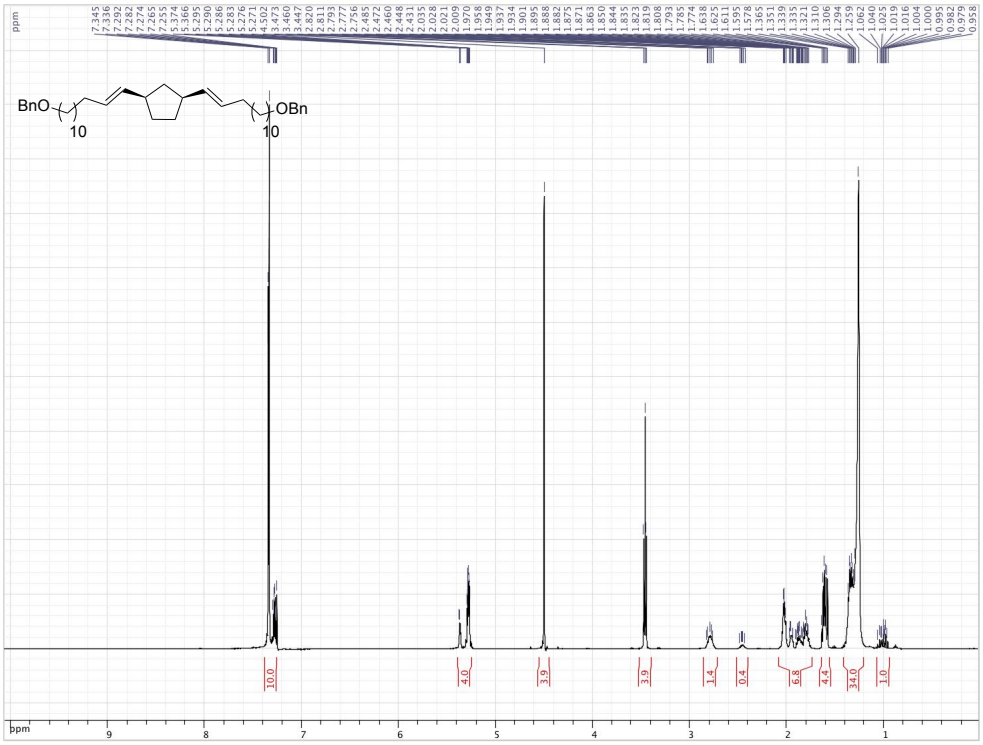


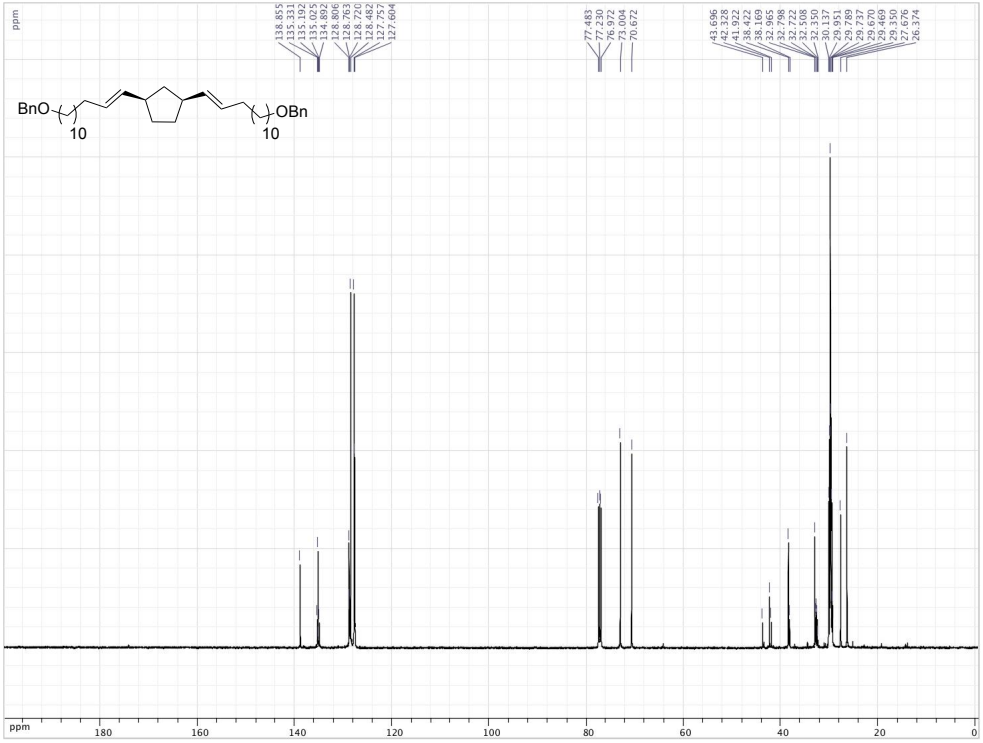

ppm
0.160
O
P
N
3
10
ppm
180
160
140
120
100
80
60
40
20
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200

## Compound 13

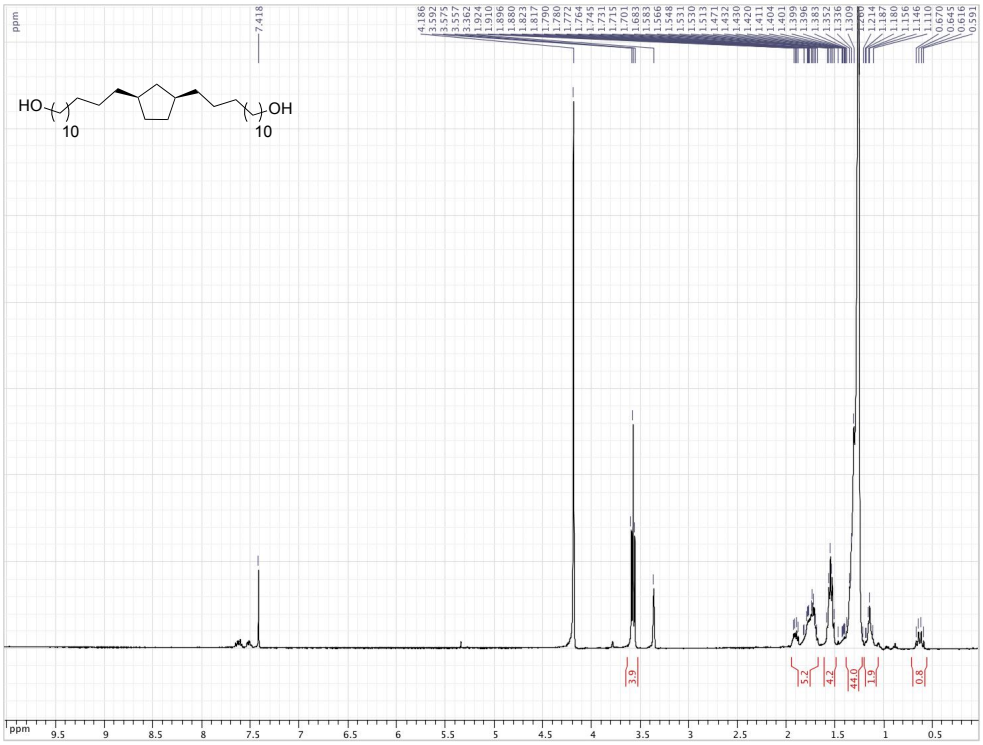

O
OBn
10
ppm
9.745
9.740
9.735
7.331
7.325
7.319
7.314
7.273
7.265
7.262
7.252
7.245
7.240
5.276
4.482
3.458
3.442
3.425
2.415
2.410
2.397
2.392
2.378
2.374
2.025
1.624
1.620
1.608
1.602
1.590
1.571
1.566
1.554
1.551
1.333
1.328
1.313
1.272
1.245
0.9
5.0
2.0
2.1
2.0
4.3
14.3
ppm
9
8
7
6
5
4
3
2
1

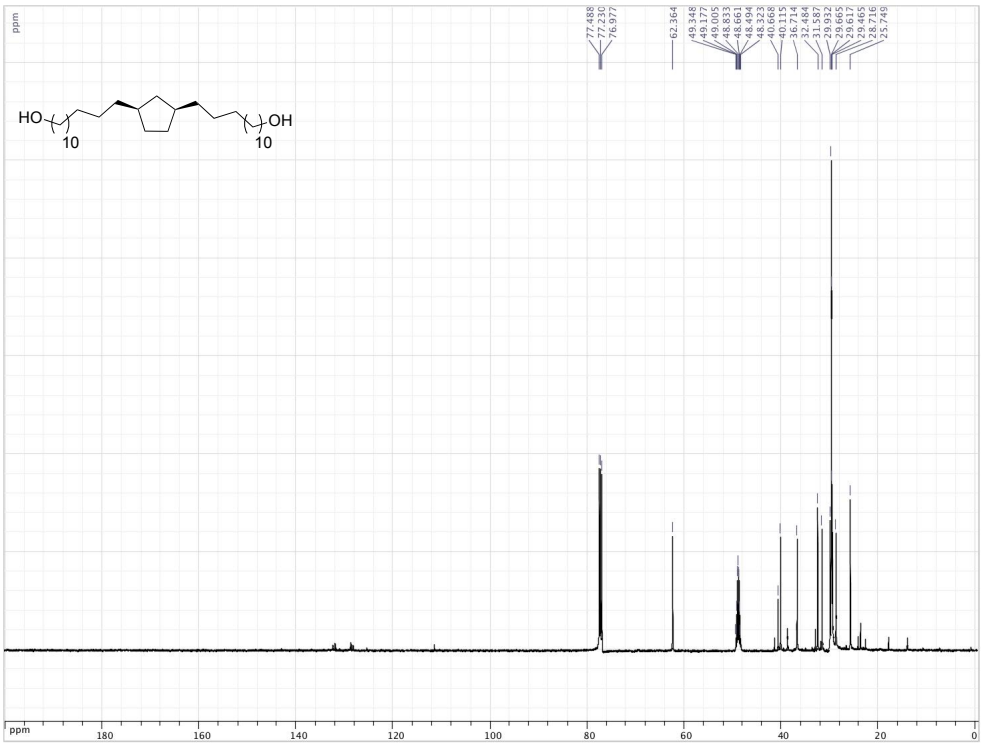
## Compound 15



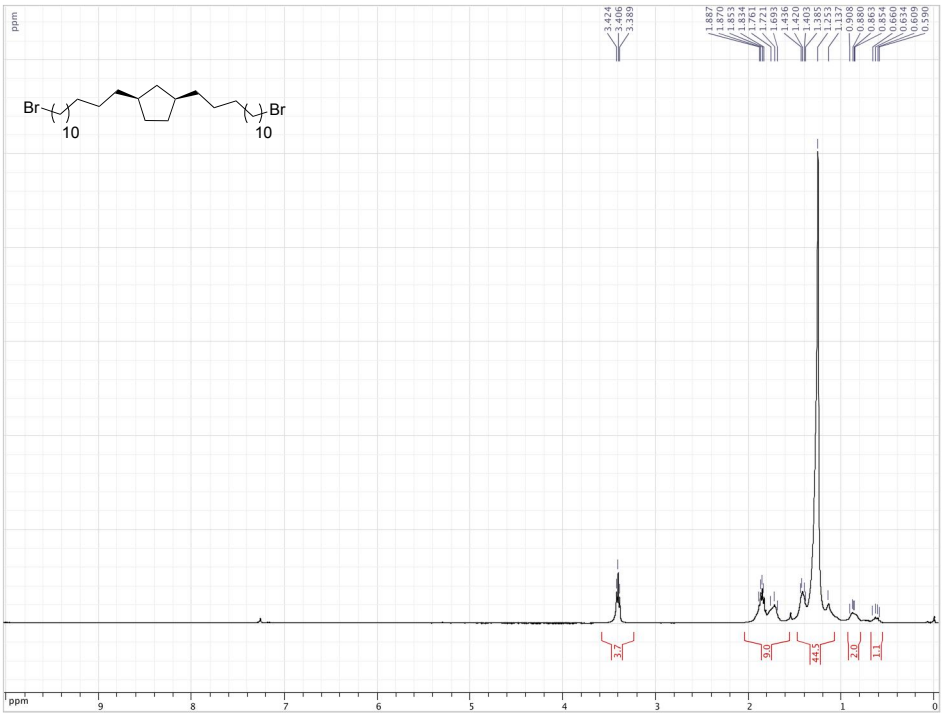



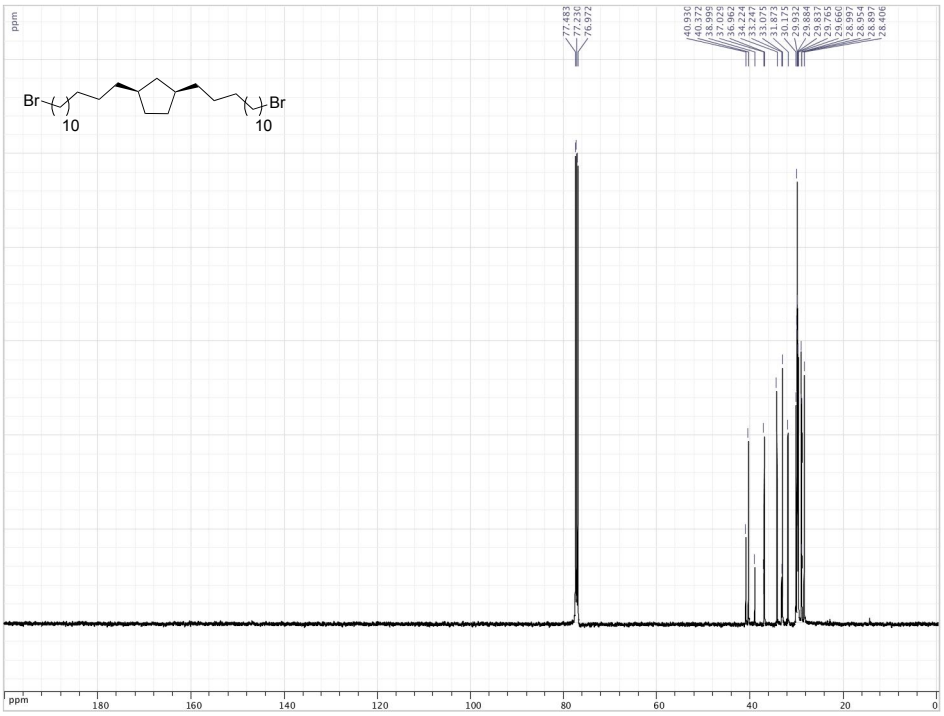
## Compound 16



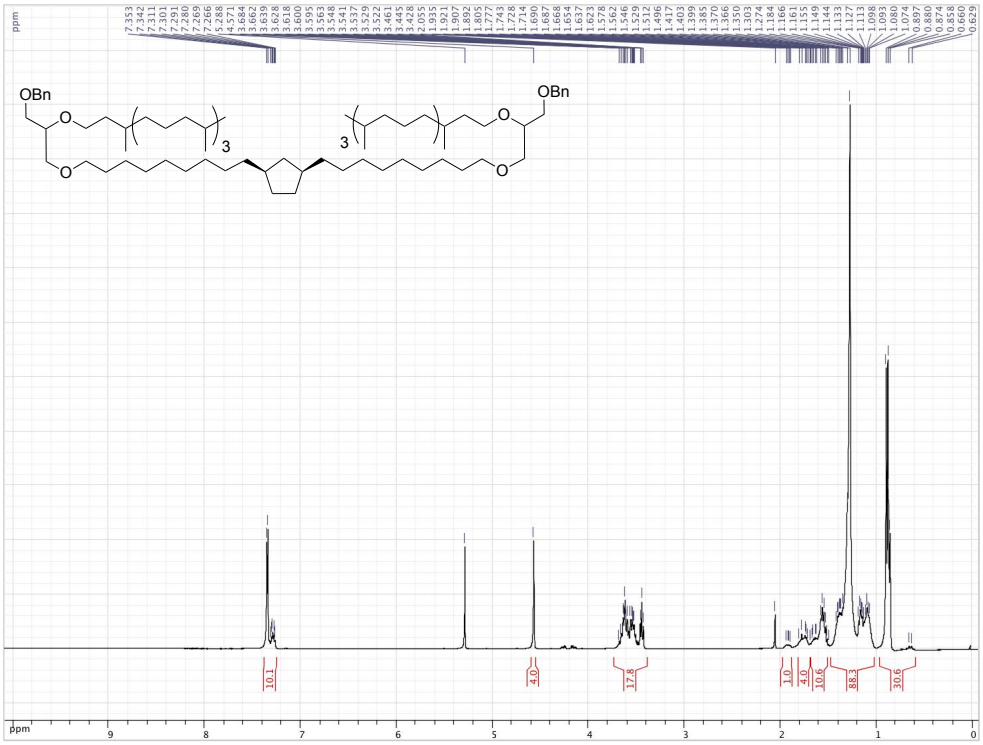



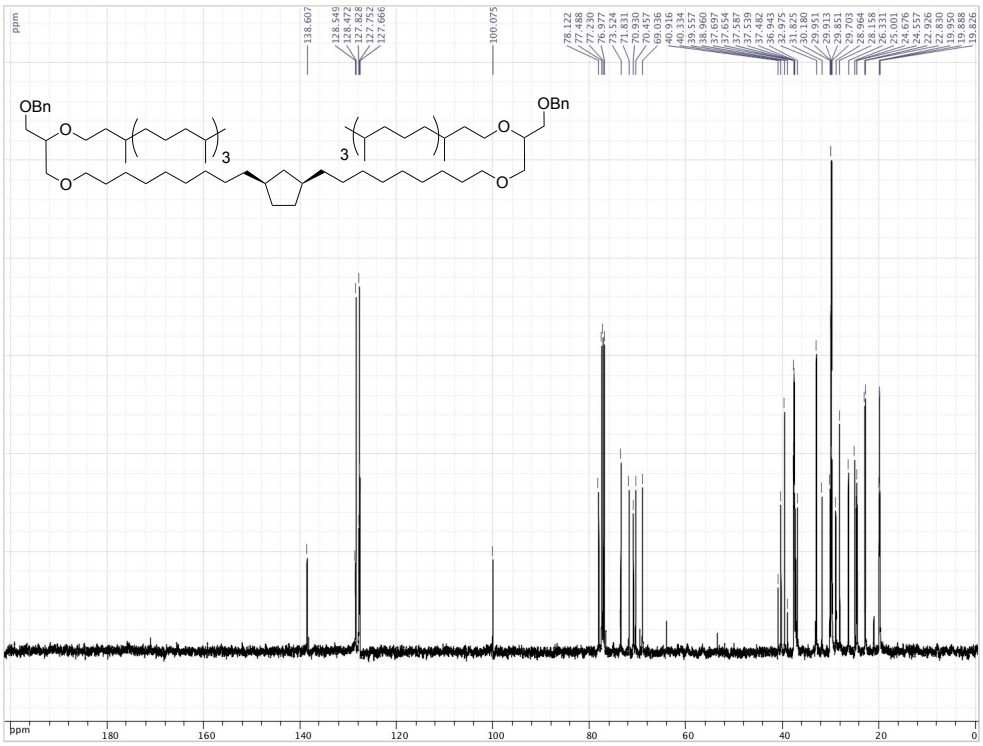
## Compound 17


Br
10
10
Br
3.424
3.406
3.389
1.887
1.870
1.853
1.834
1.761
1.721
1.436
1.420
1.403
1.355
1.253
1.137
0.908
0.889
0.869
0.854
0.660
0.634
0.609
0.590
3.7
9.0
44.5
2.0
1.1
ppm
9
8
7
6
5
4
3
2
1
0

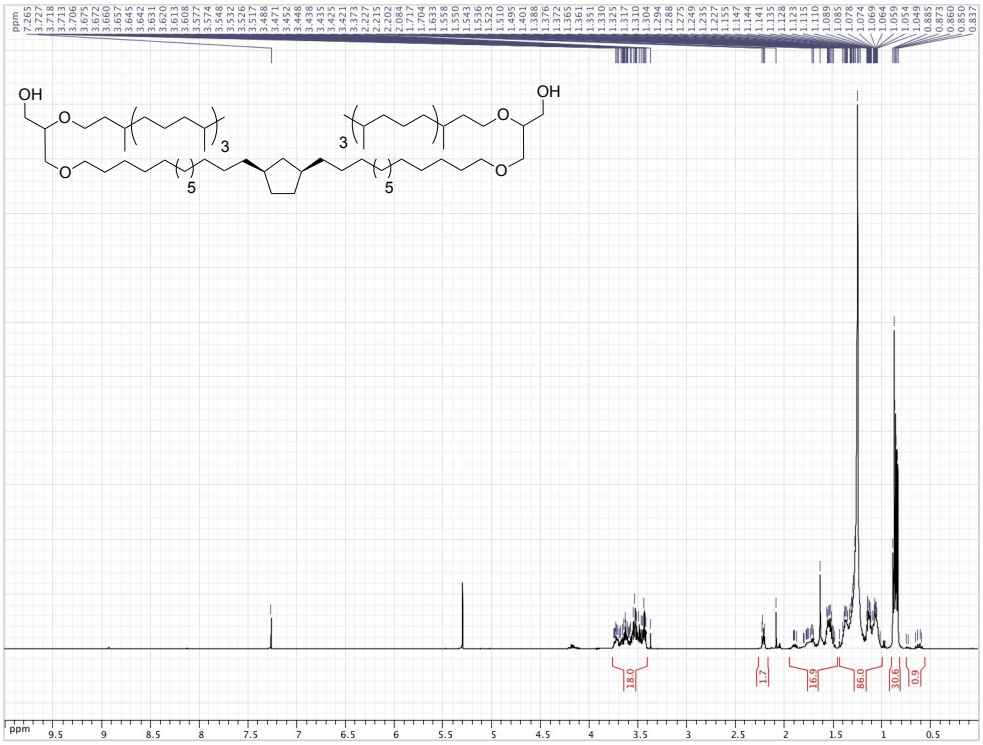

Br
10
10
Br
77.483
77.230
76.972
40.930
40.372
38.999
37.709
36.962
34.224
33.247
33.075
31.928
30.175
29.932
29.884
29.837
29.765
29.660
28.997
28.854
28.893
28.406
ppm
180
160
140
120
100
80
60
40
20
0

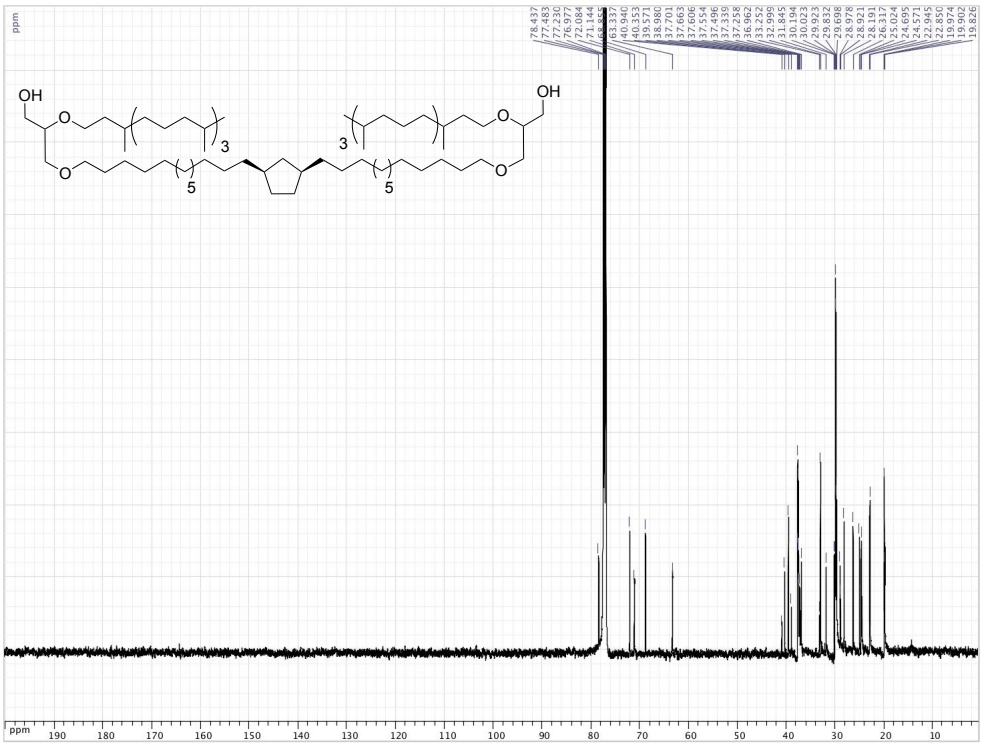
## Compound 18


OBn
OBn
O
O
O
O
3
3
10.1
4.0
17.8
1.0
4.0
10.6
88.3
30.6
ppm

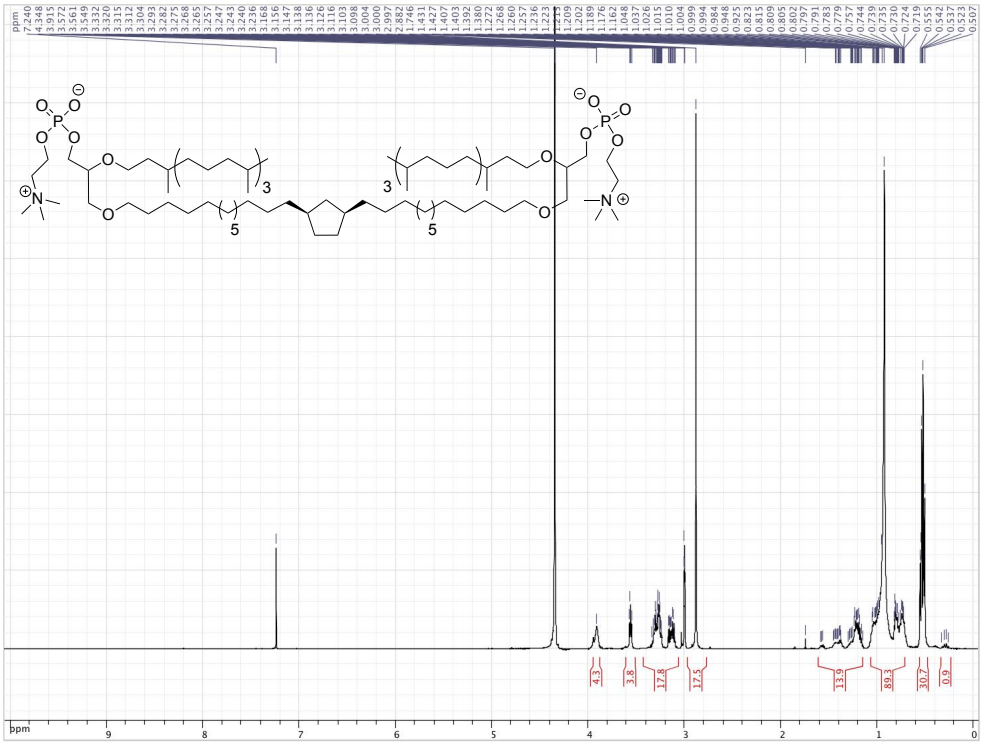

OBn
OBn
O
O
O
O
3
3
ppm

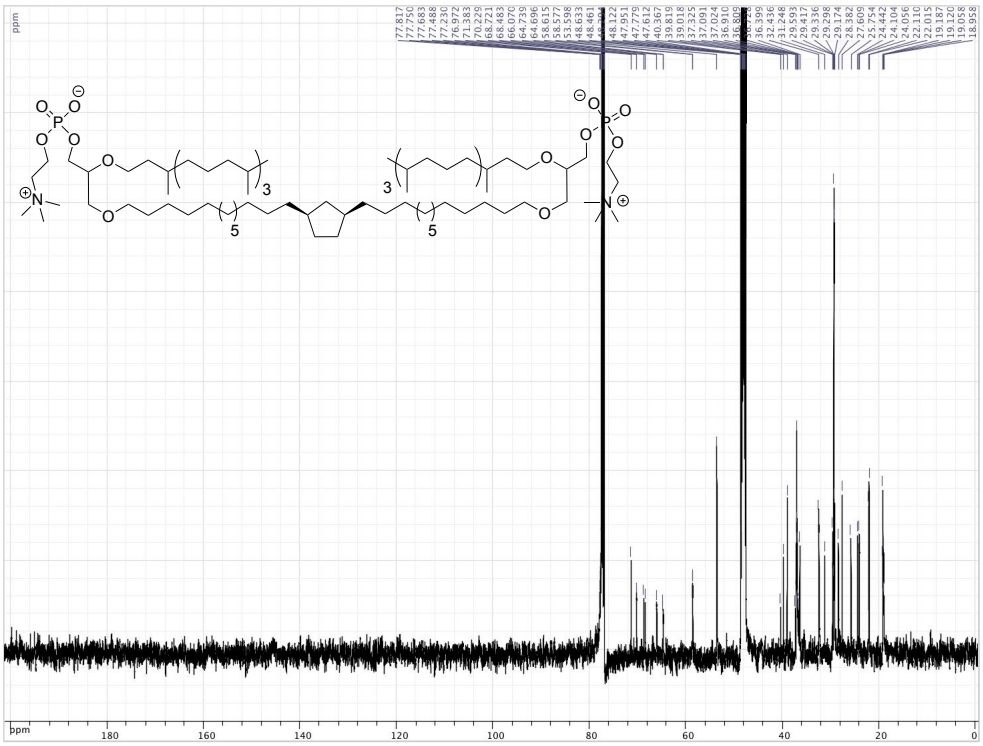
## Compound 19

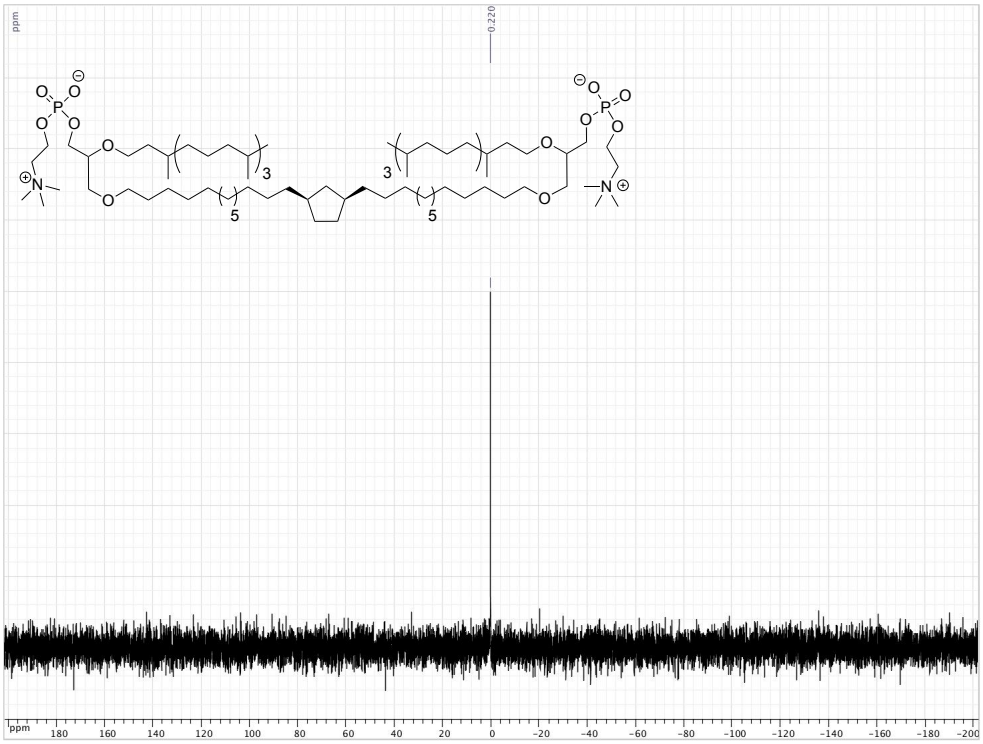


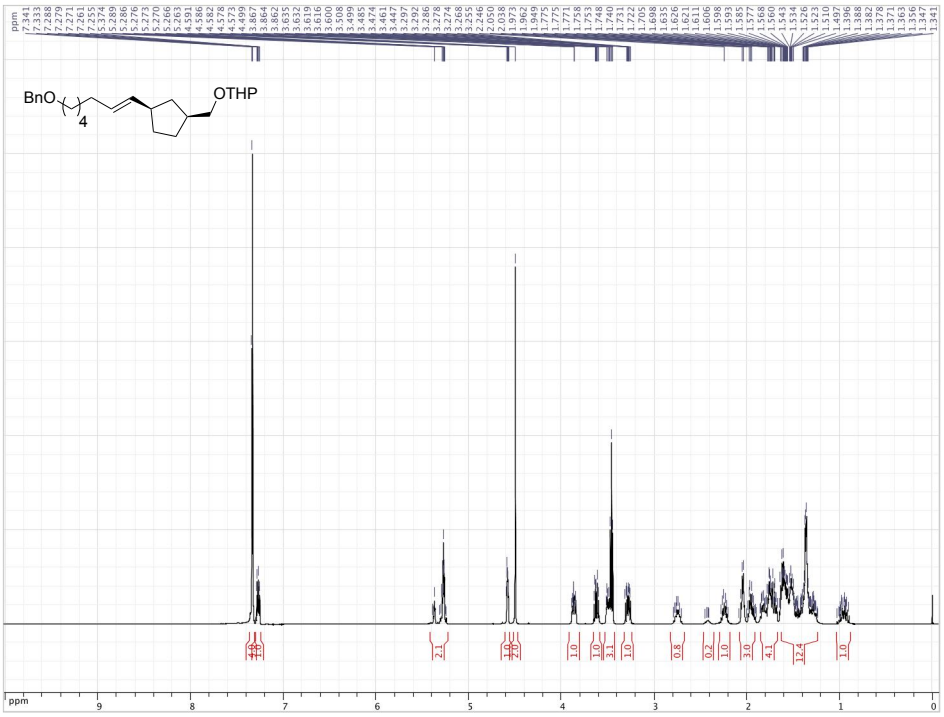
## Compound GMGTPC-CP1

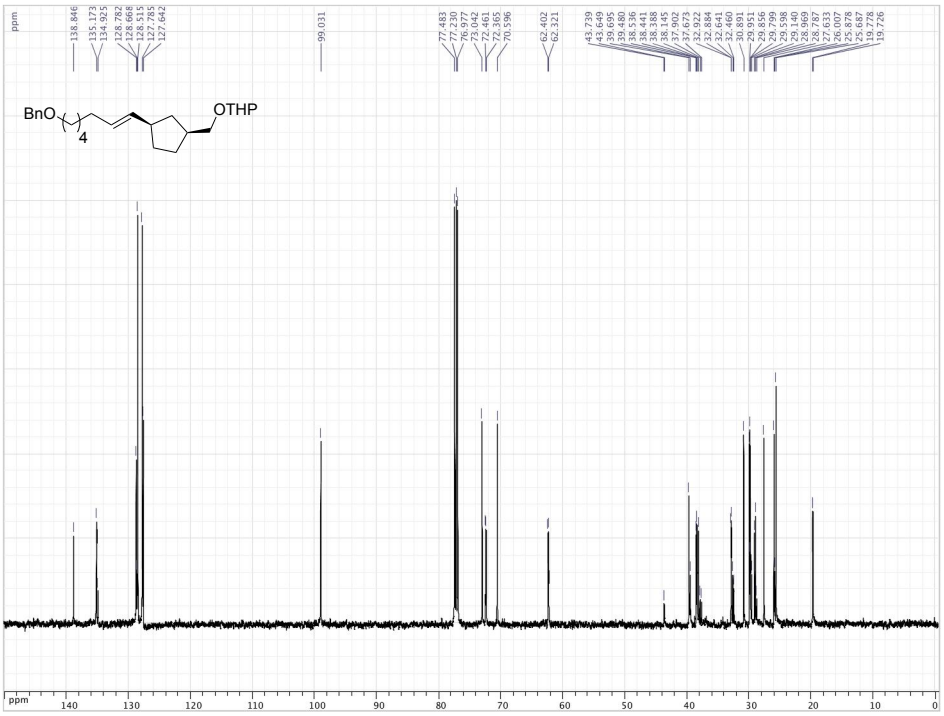




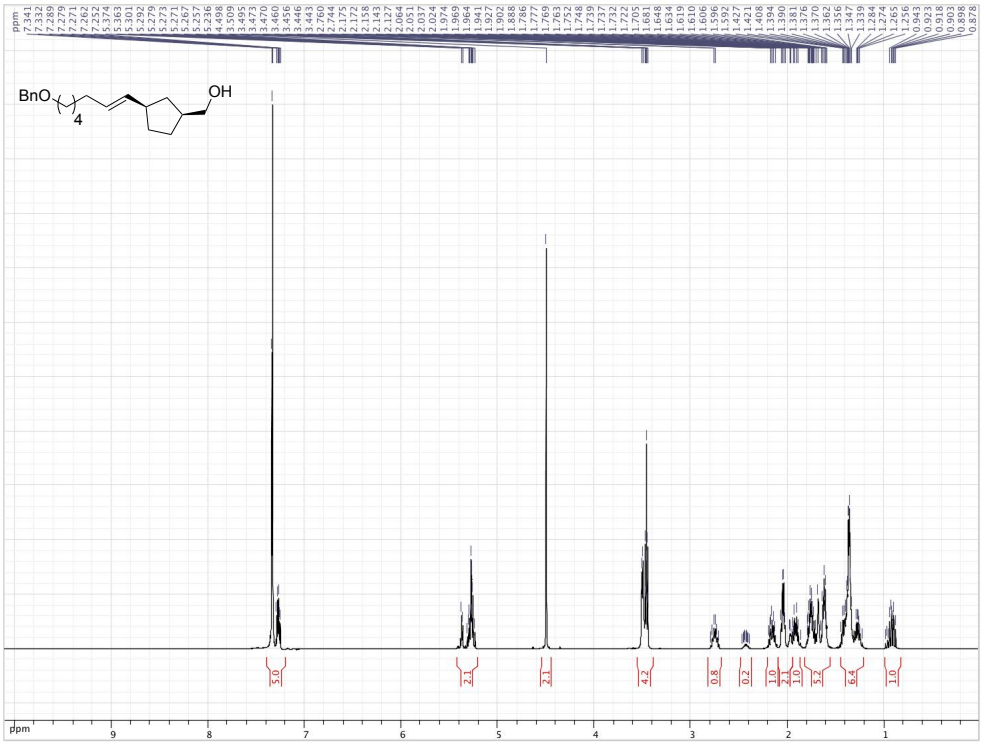

ppm
0.220
3
3
5
5
ppm
180
160
140
120
100
80
60
40
20
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200

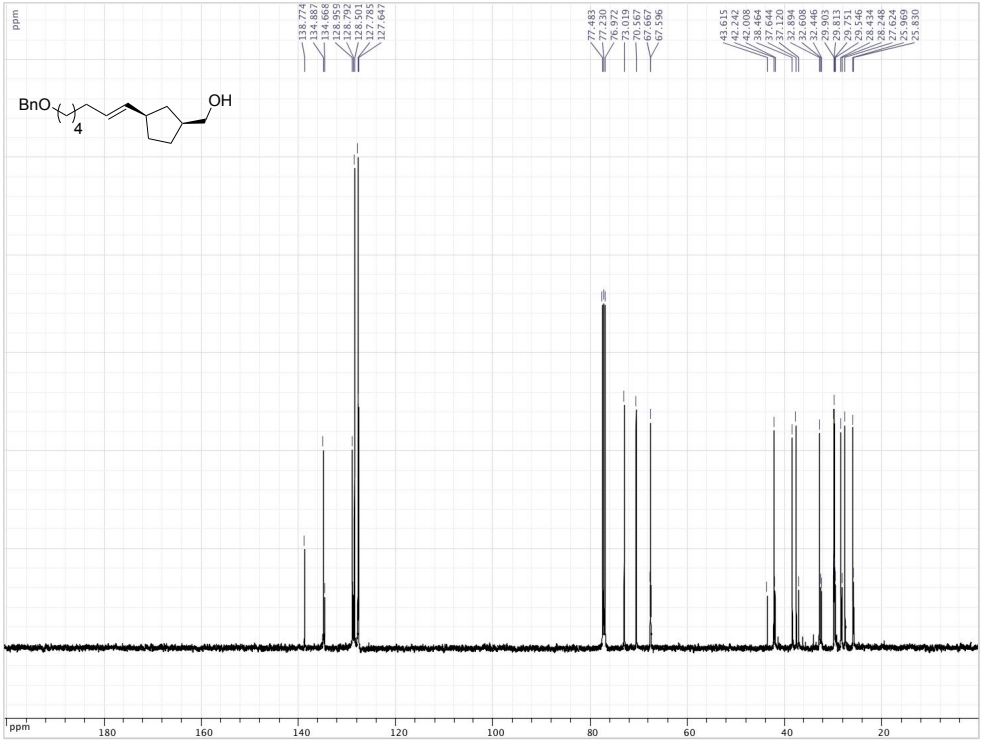
## Compound 23



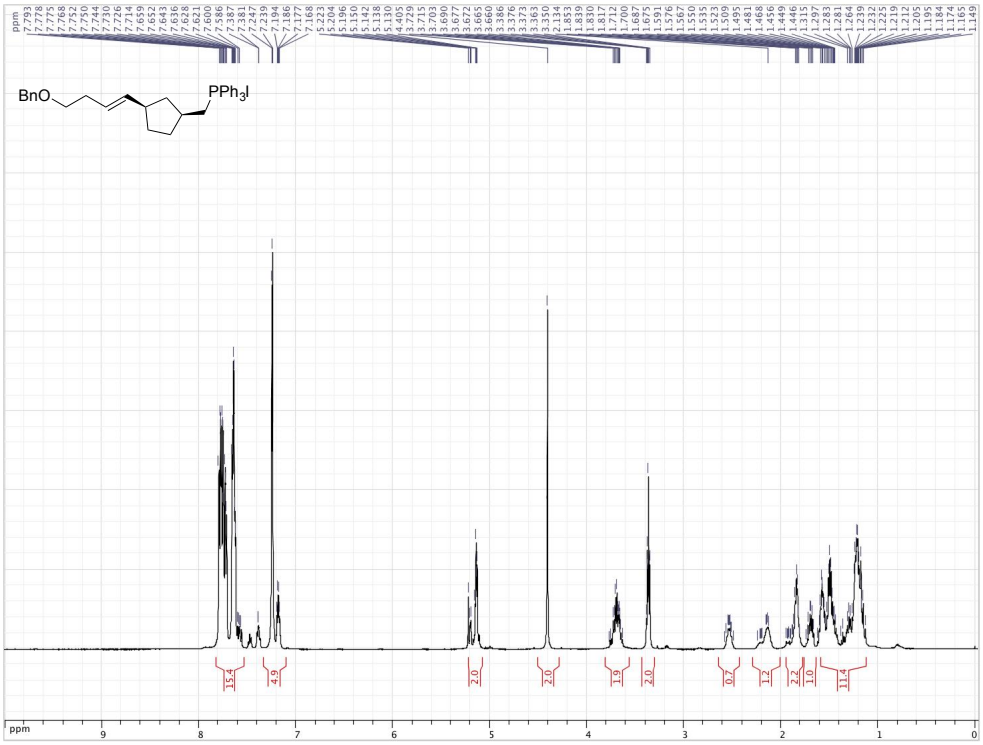



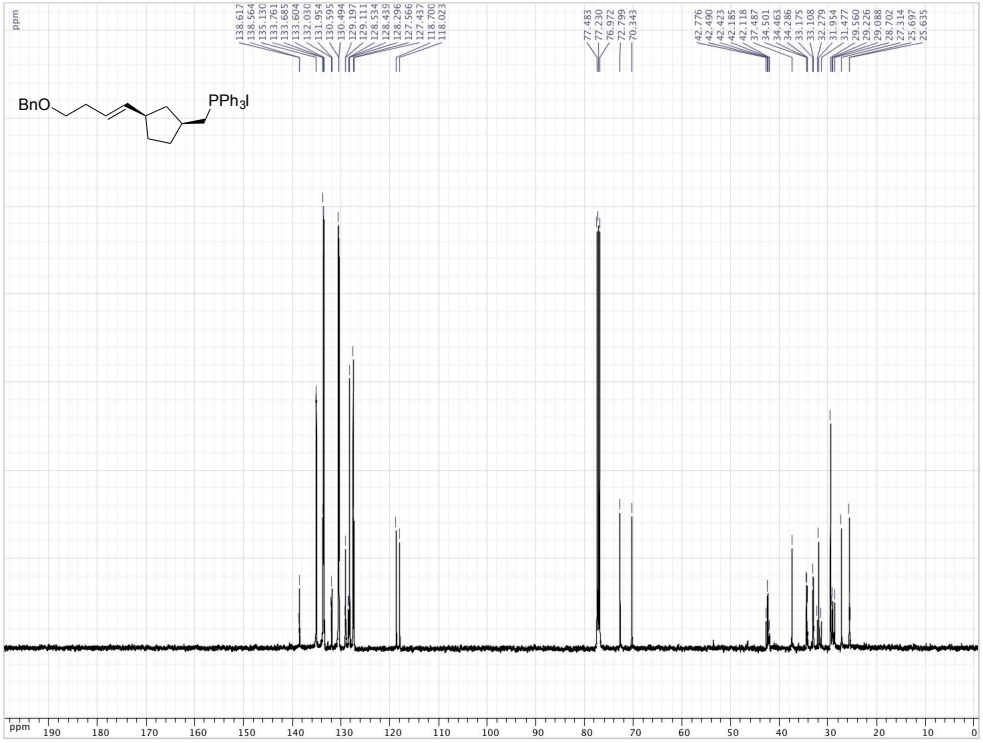
## Compound 24



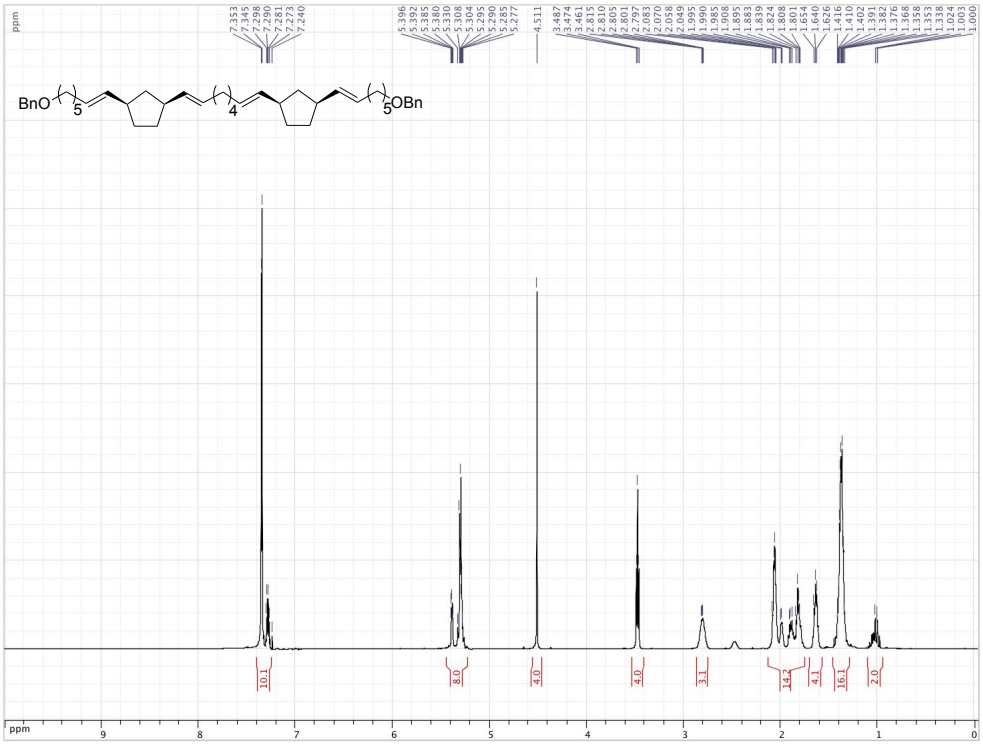



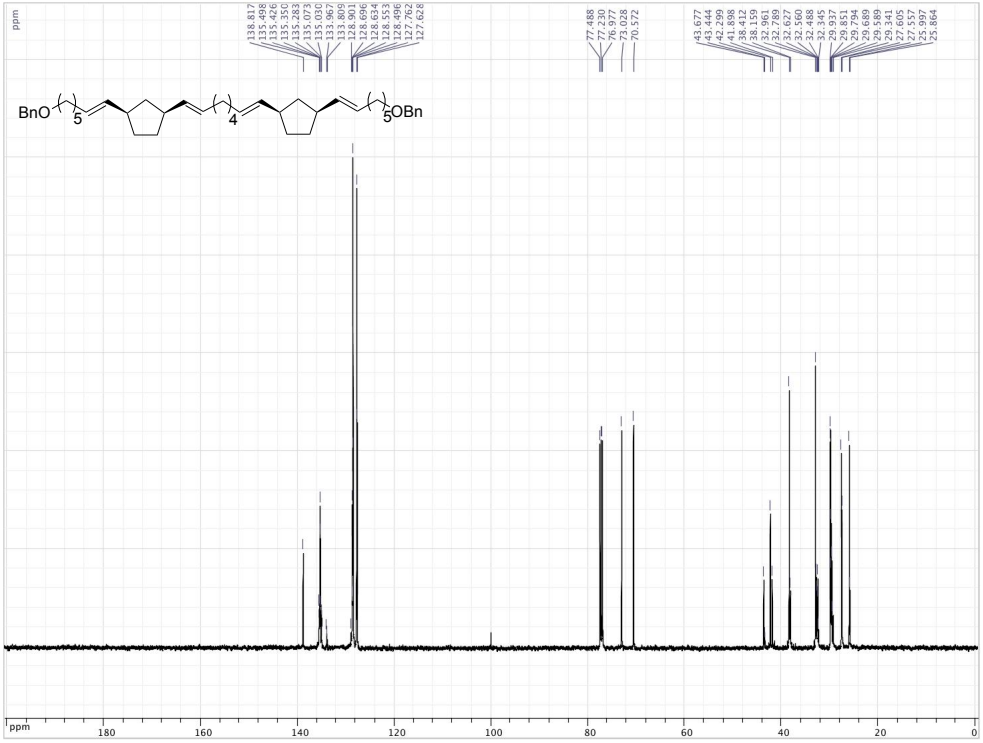
## Compound 25



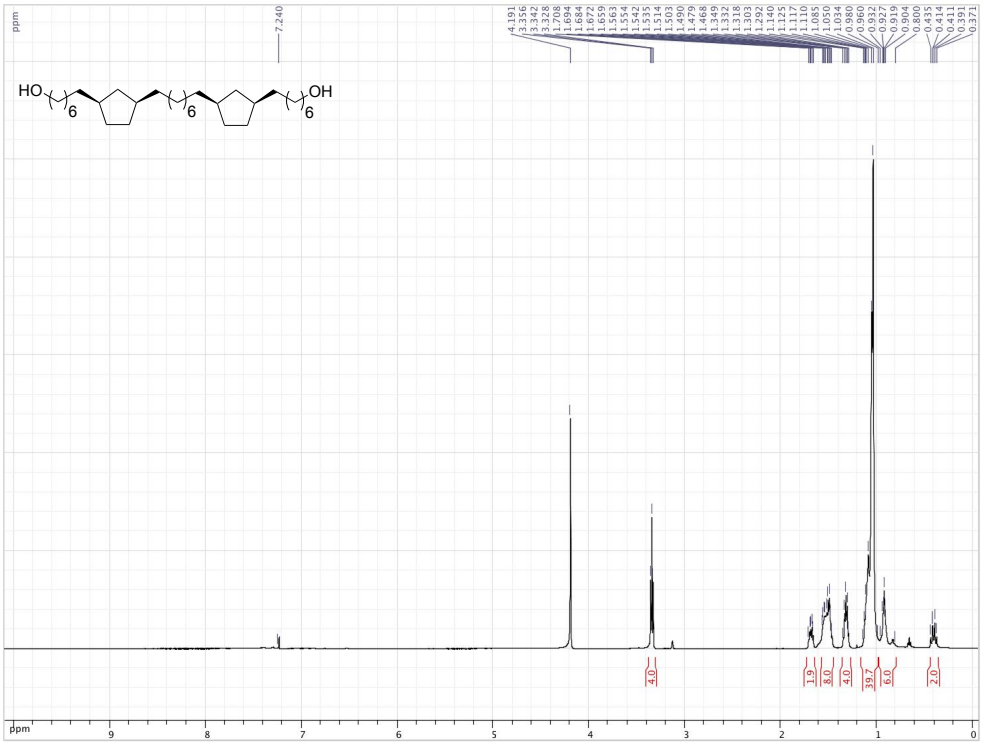



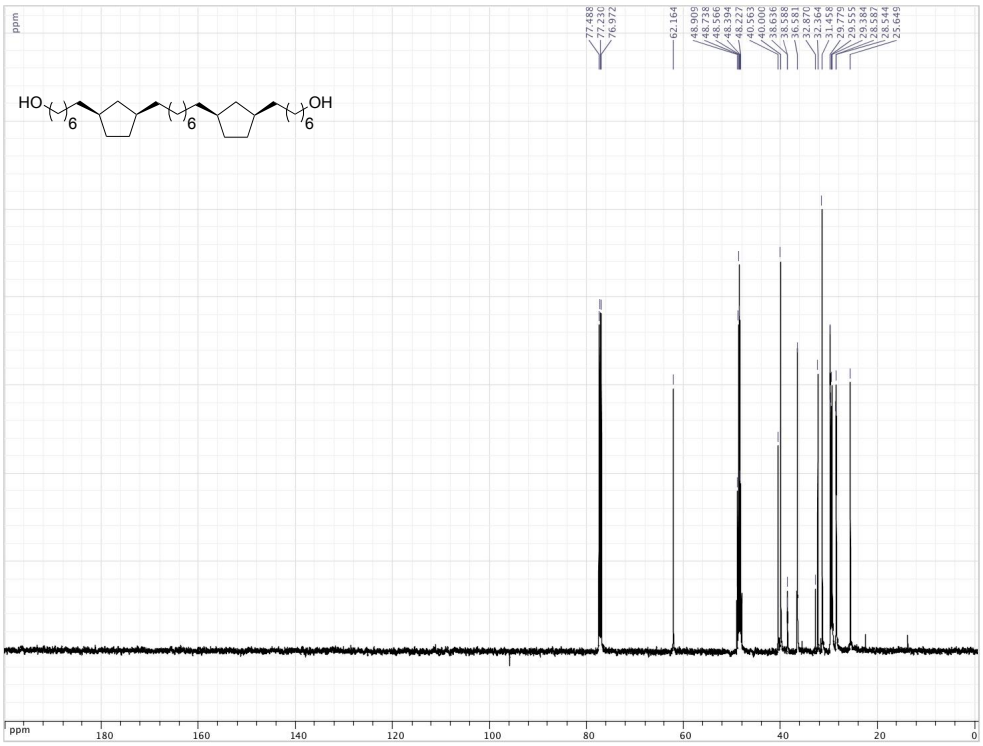
## Compound 27



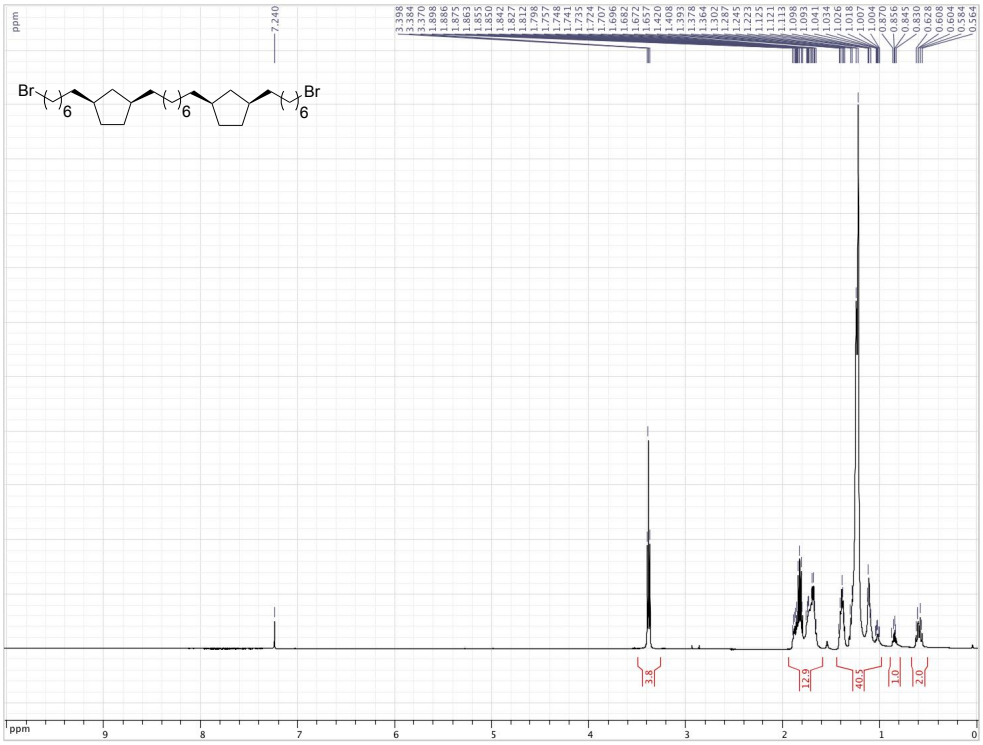



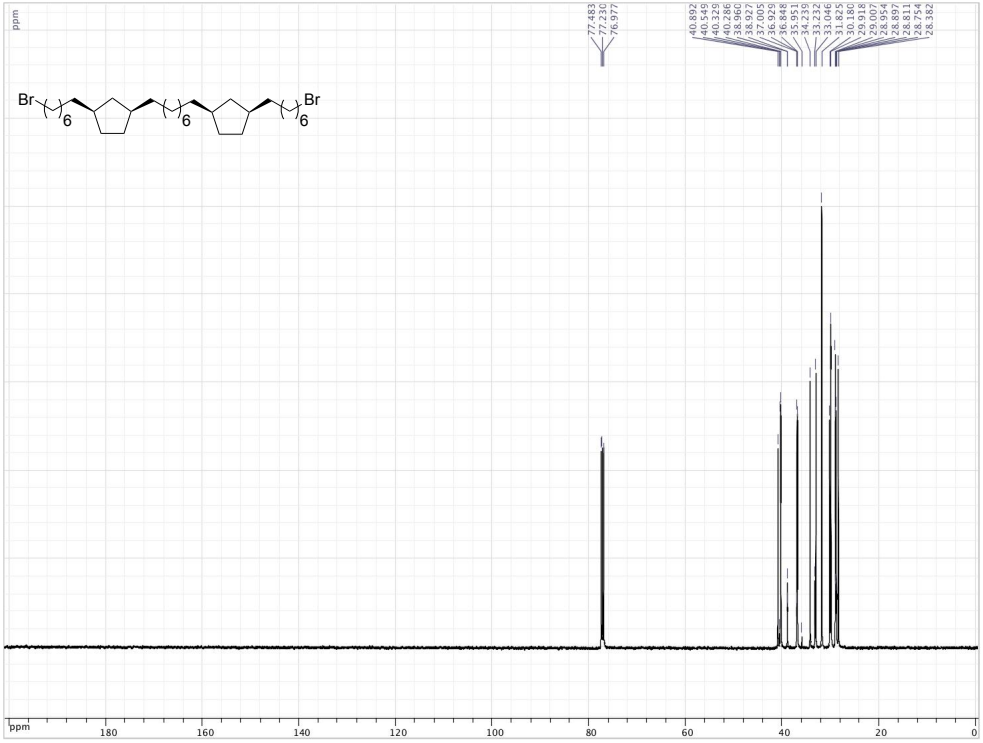
## Compound 28


HO
6
6
6
OH
ppm
7.240
4.191
3.356
3.342
3.328
3.286
1.694
1.684
1.672
1.659
1.555
1.554
1.542
1.535
1.514
1.503
1.490
1.479
1.468
1.349
1.332
1.318
1.303
1.292
1.140
1.125
1.117
1.110
1.085
1.030
1.004
0.980
0.960
0.932
0.927
0.919
0.904
0.800
0.435
0.414
0.411
0.391
0.371
4.0
1.9
8.0
4.0
39.7
6.0
2.0
9
8
7
6
5
4
3
2
1
0

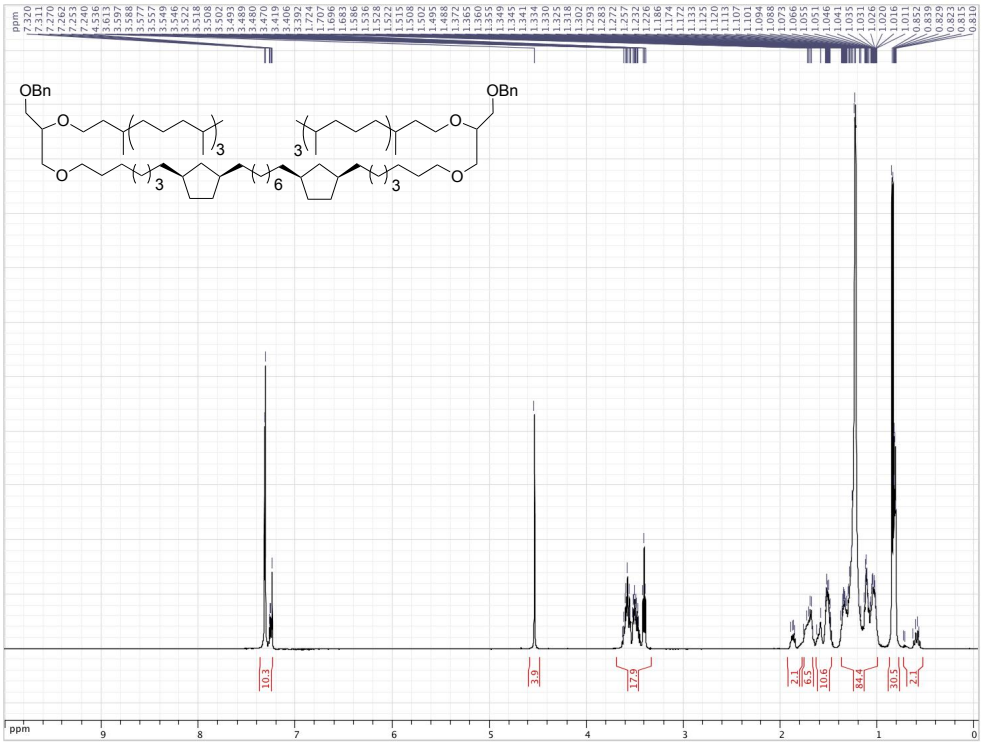

HO
6
6
6
OH
ppm
77.488
77.250
76.972
62.164
48.909
48.738
48.566
48.394
48.223
40.563
40.000
38.636
36.588
36.531
32.870
32.364
31.458
29.779
29.555
29.384
28.587
28.544
25.649
180
160
140
120
100
80
60
40
20
0

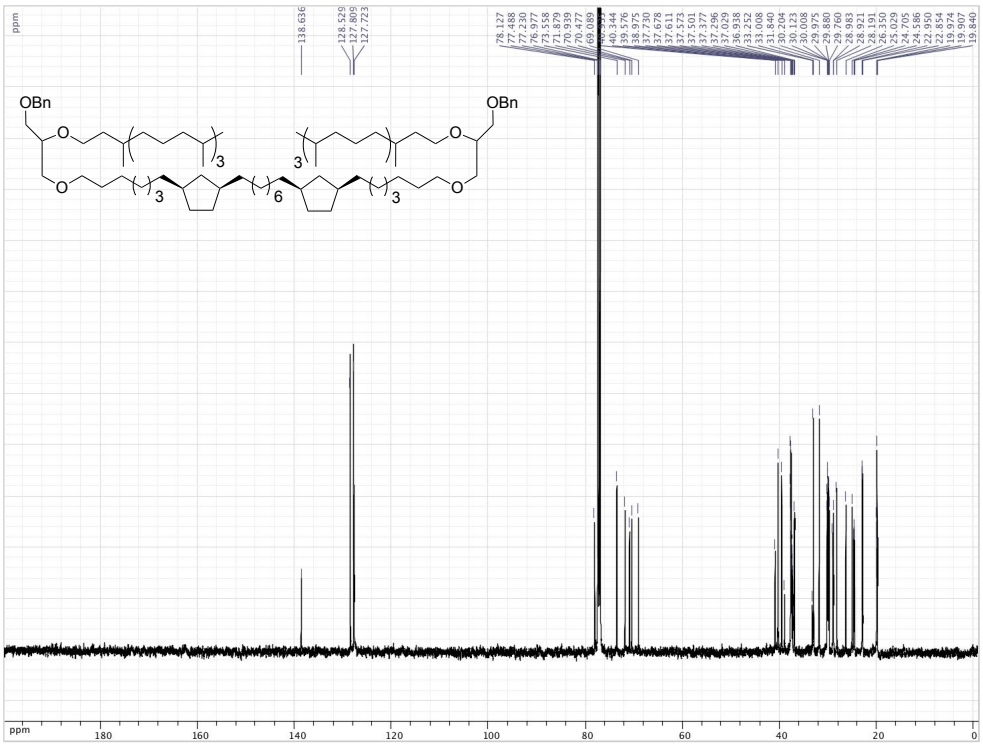
## Compound 29



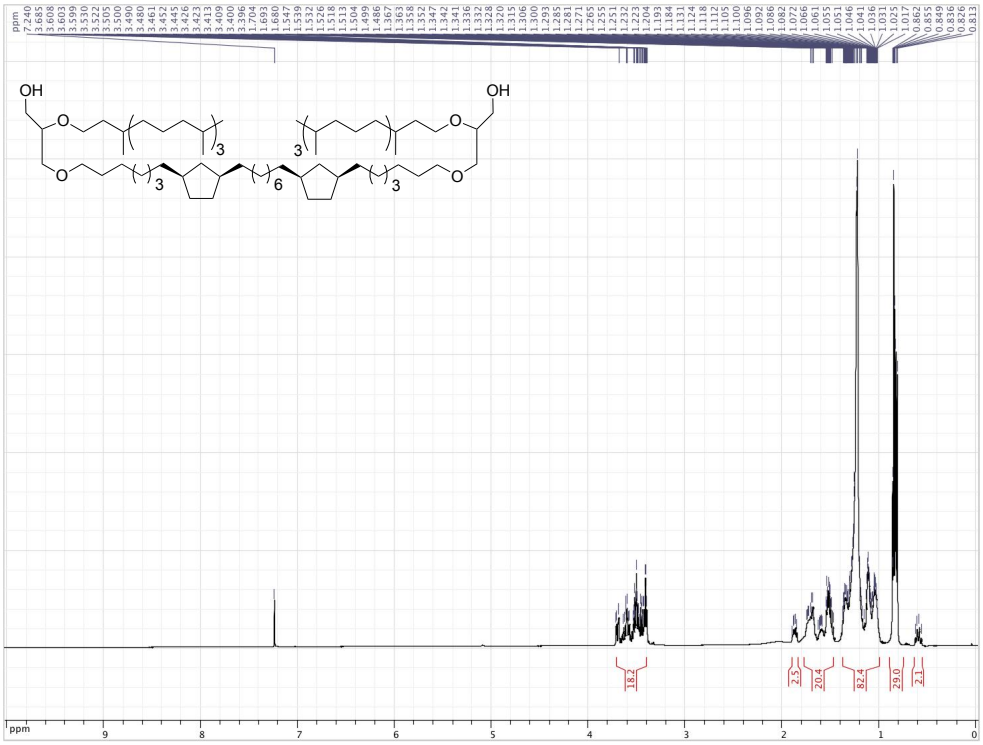



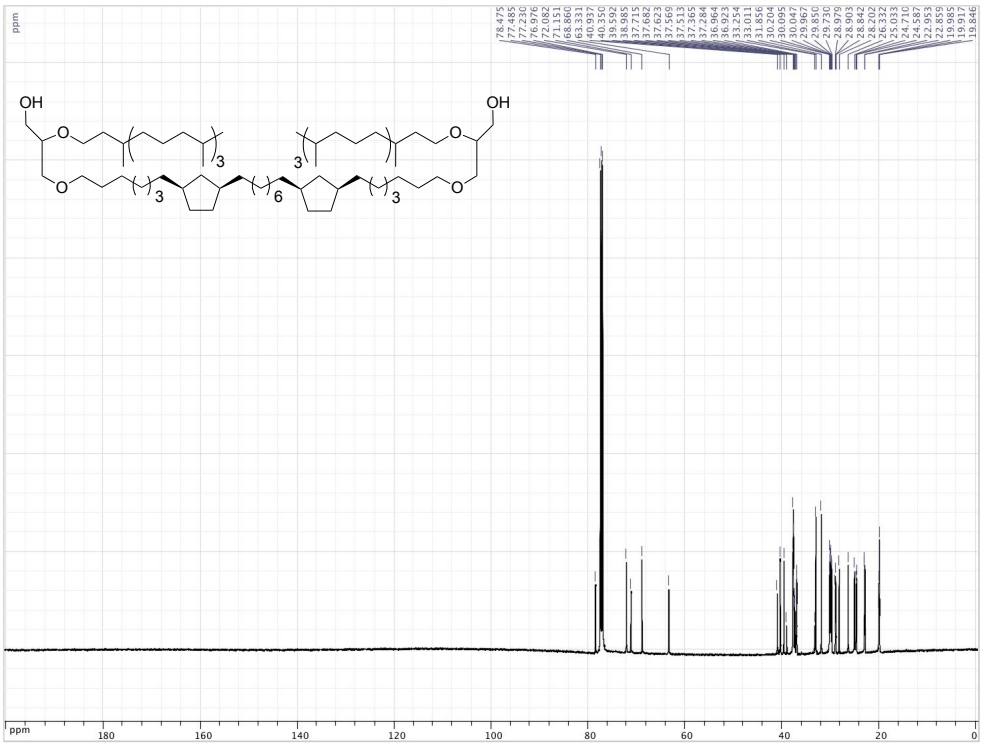
## Compound 30



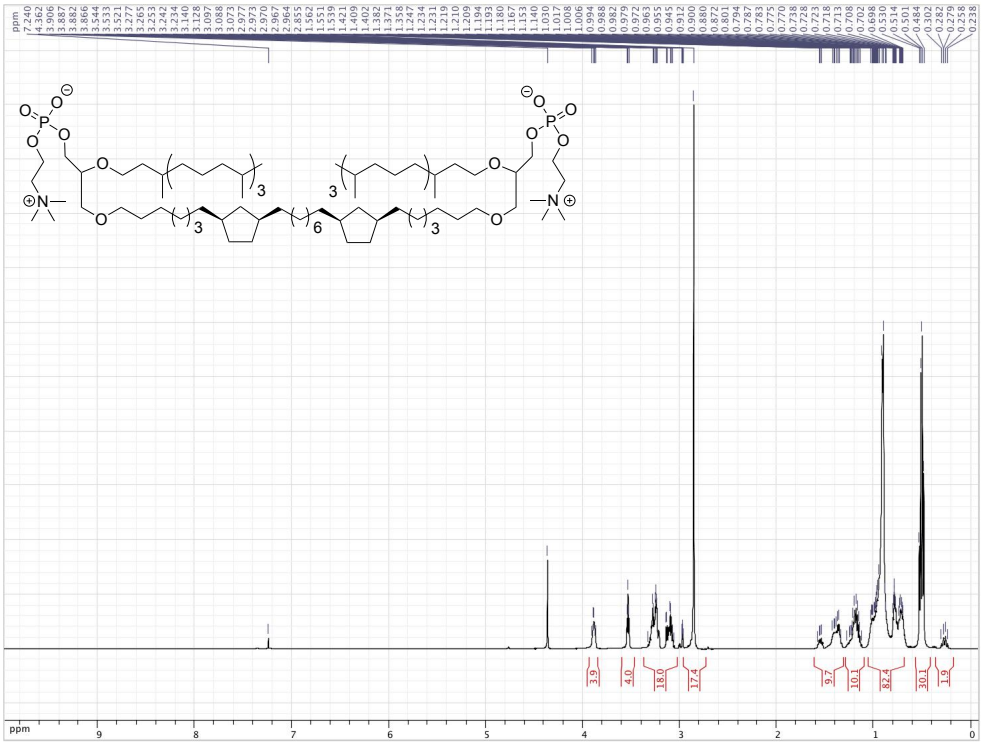



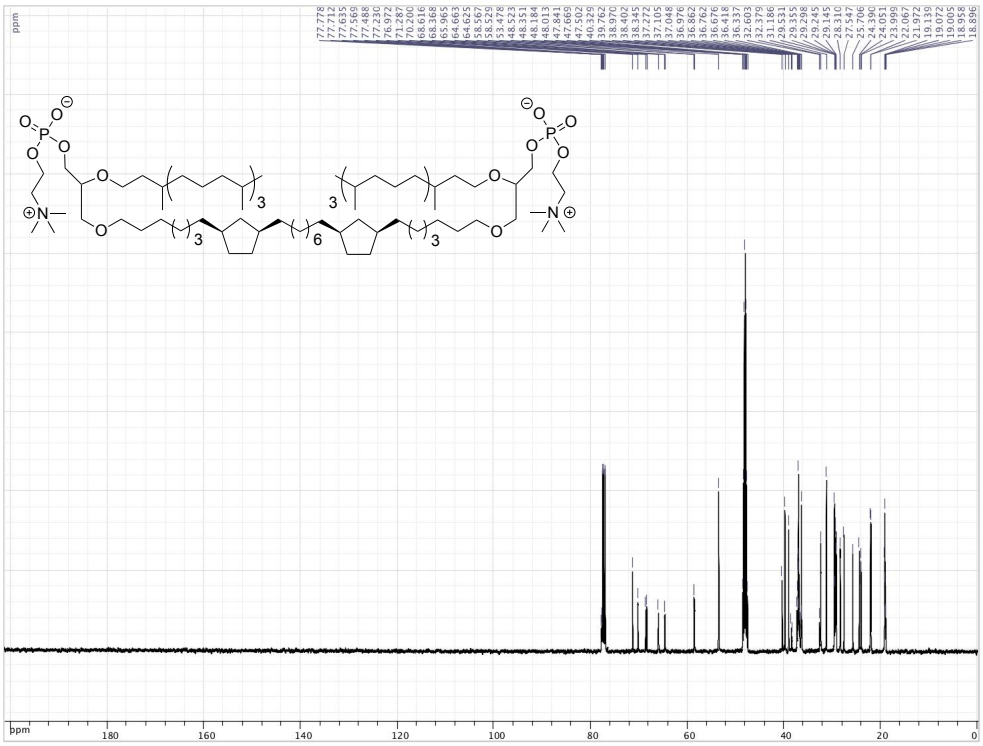
## Compound 31


OH
OH
O
O
O
O
3
3
3
6
3
18.2
2.5
20.4
82.4
29.0
2.1
ppm
9
8
7
6
5
4
3
2
1
0


OH
OH
O
O
O
O
3
3
3
6
3
ppm
180
160
140
120
100
80
60
40
20
0

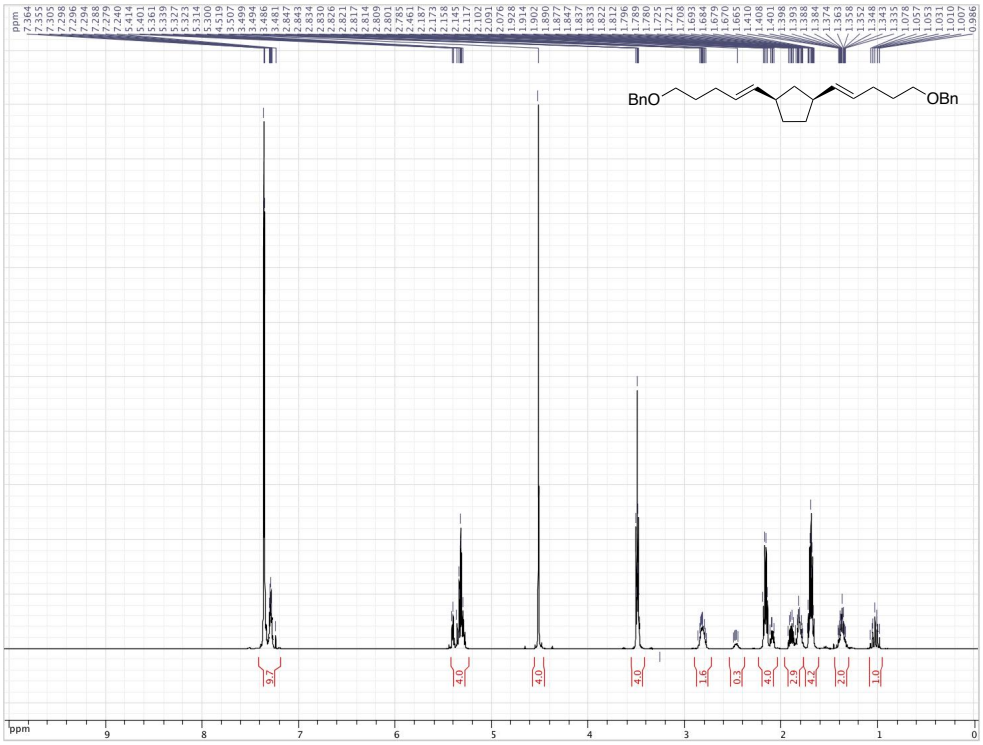
## Compound GMGTPC-CP2

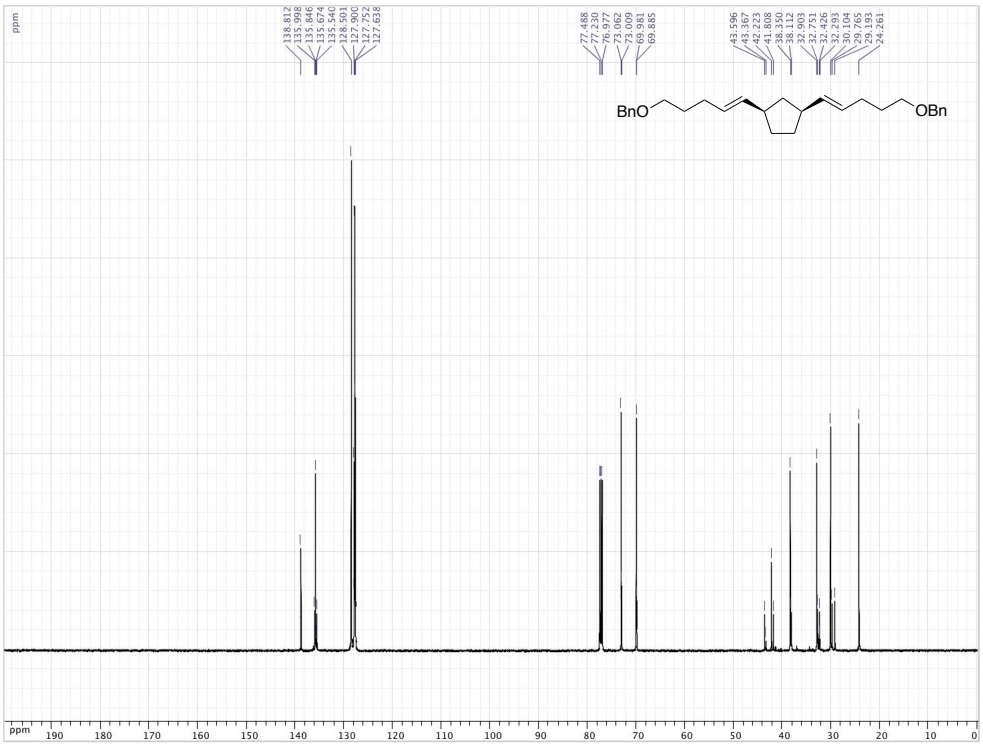




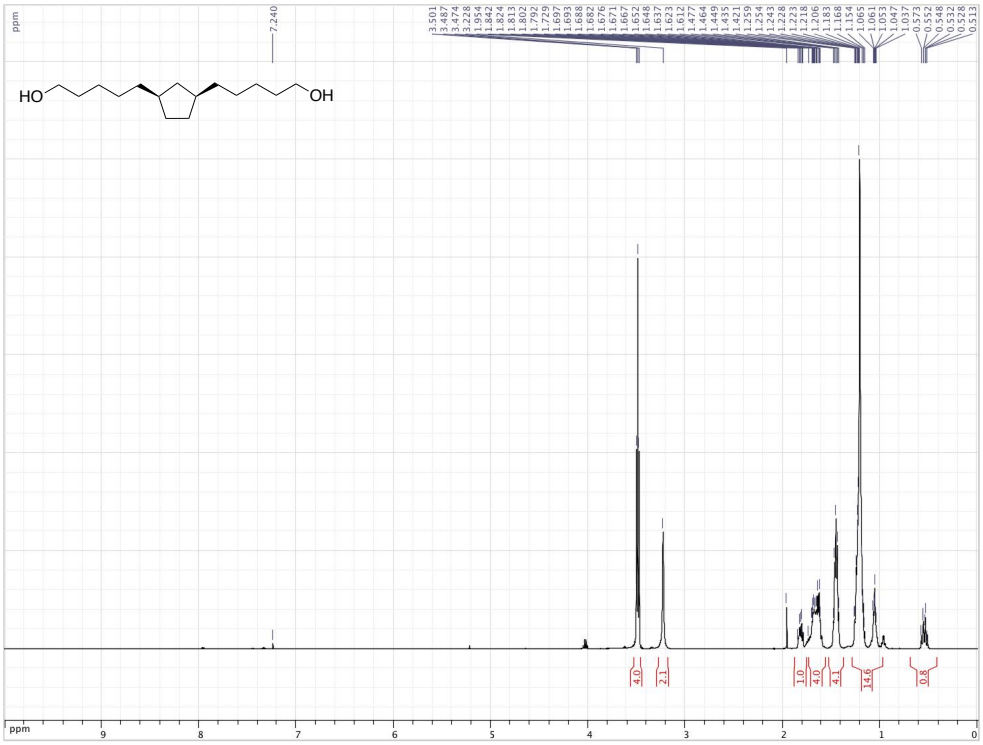

ppm
0.200
ppm
180
160
140
120
100
80
60
40
20
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200

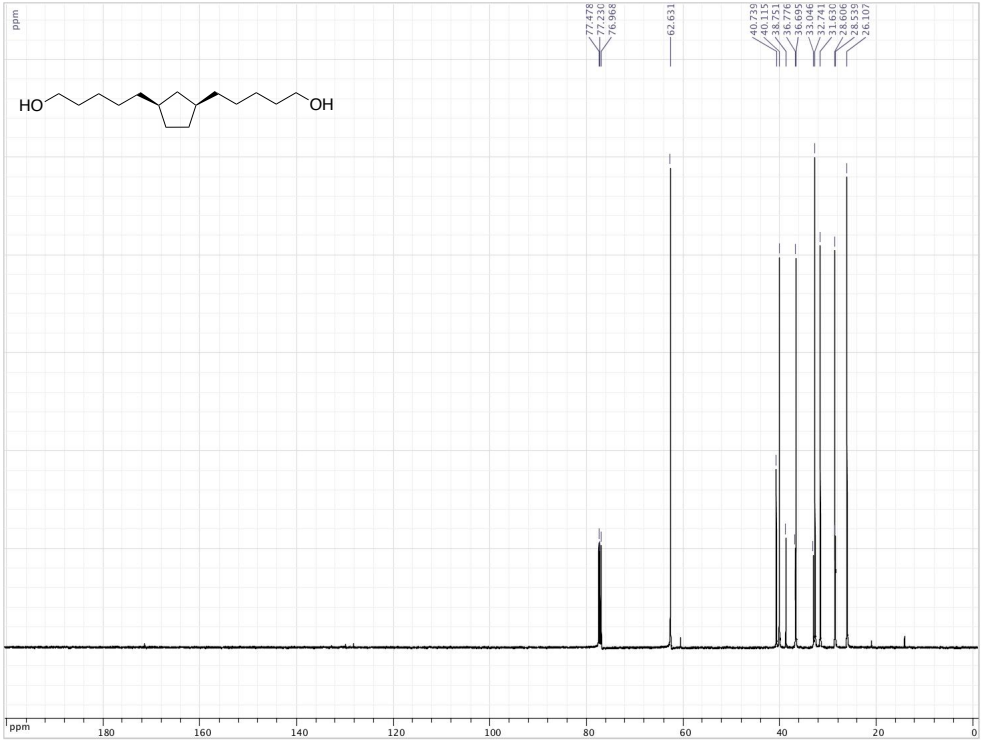
## Compound 34



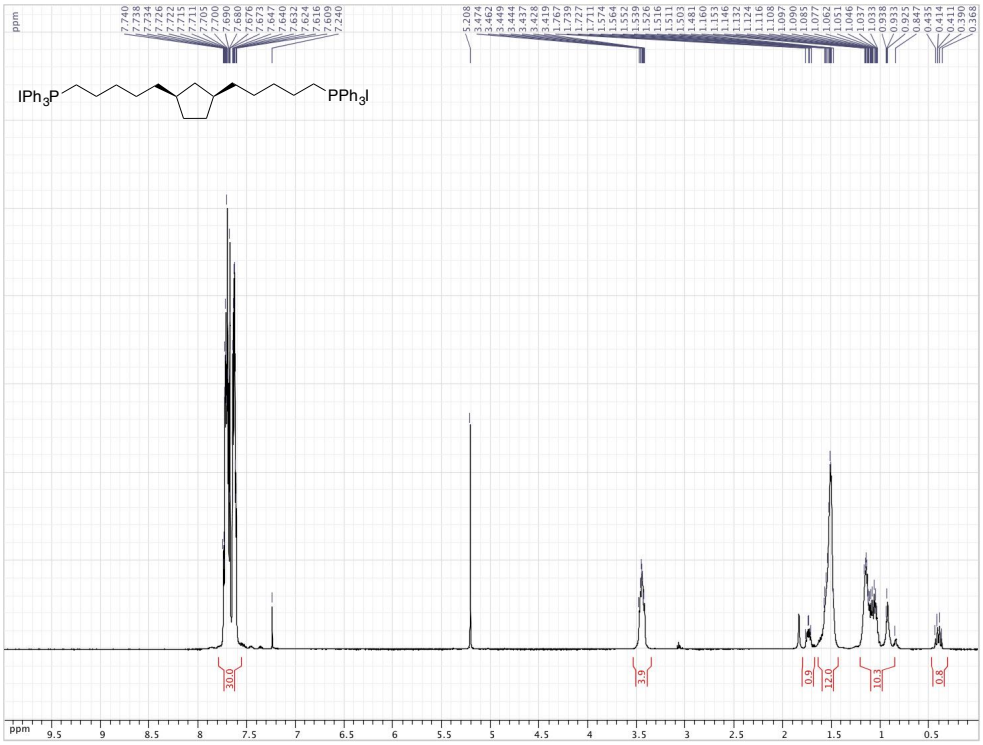



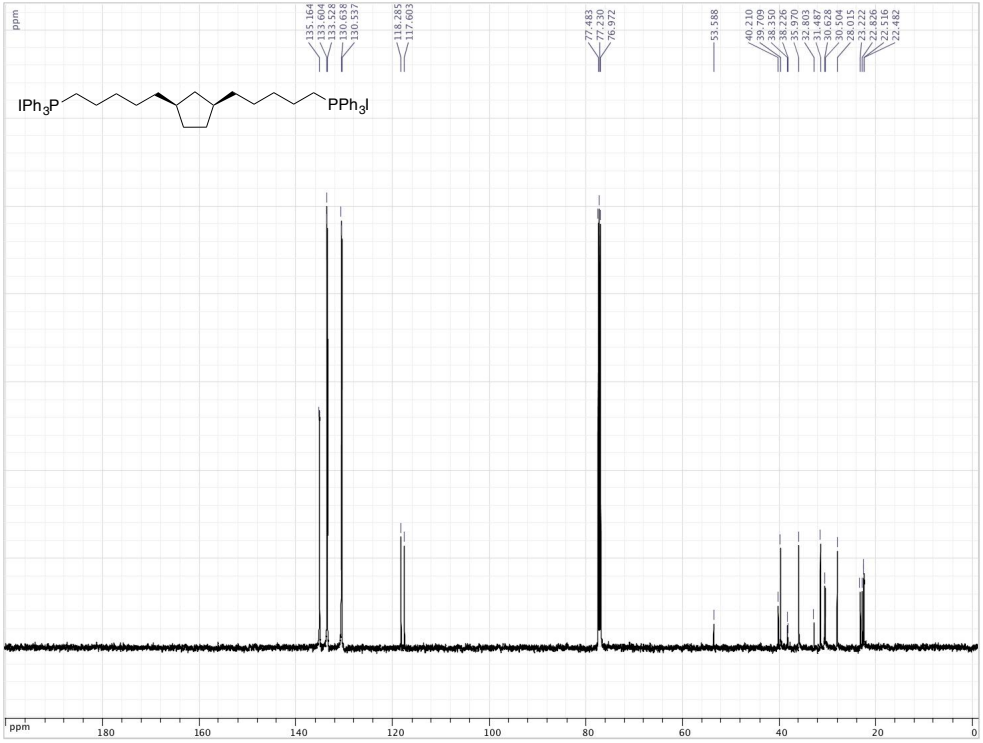
## Compound 35



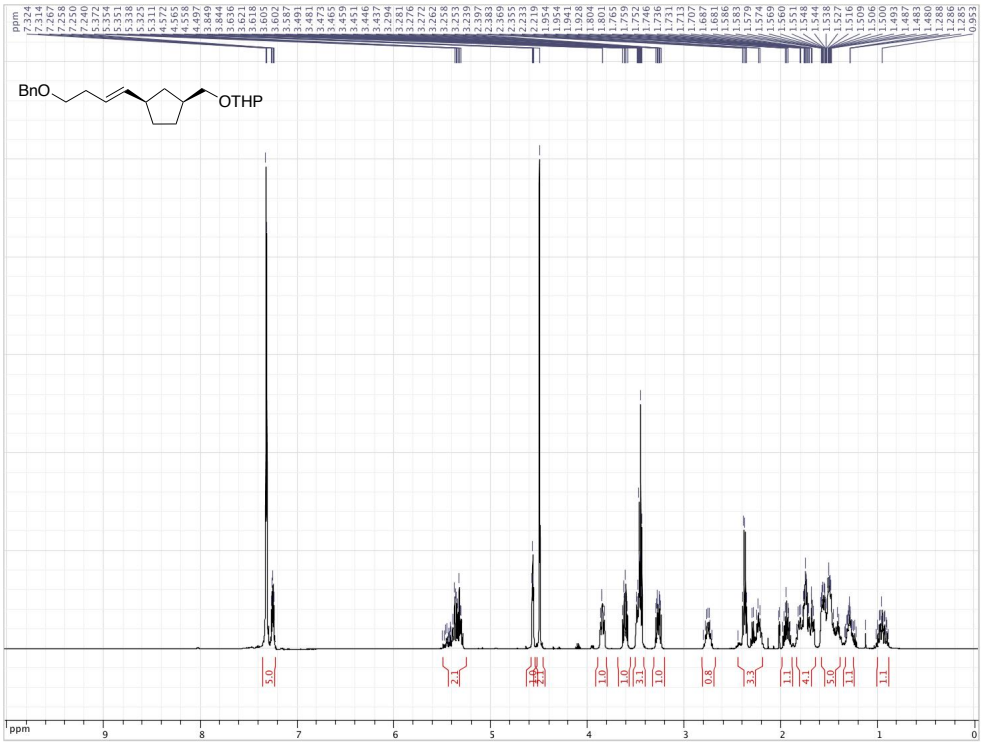



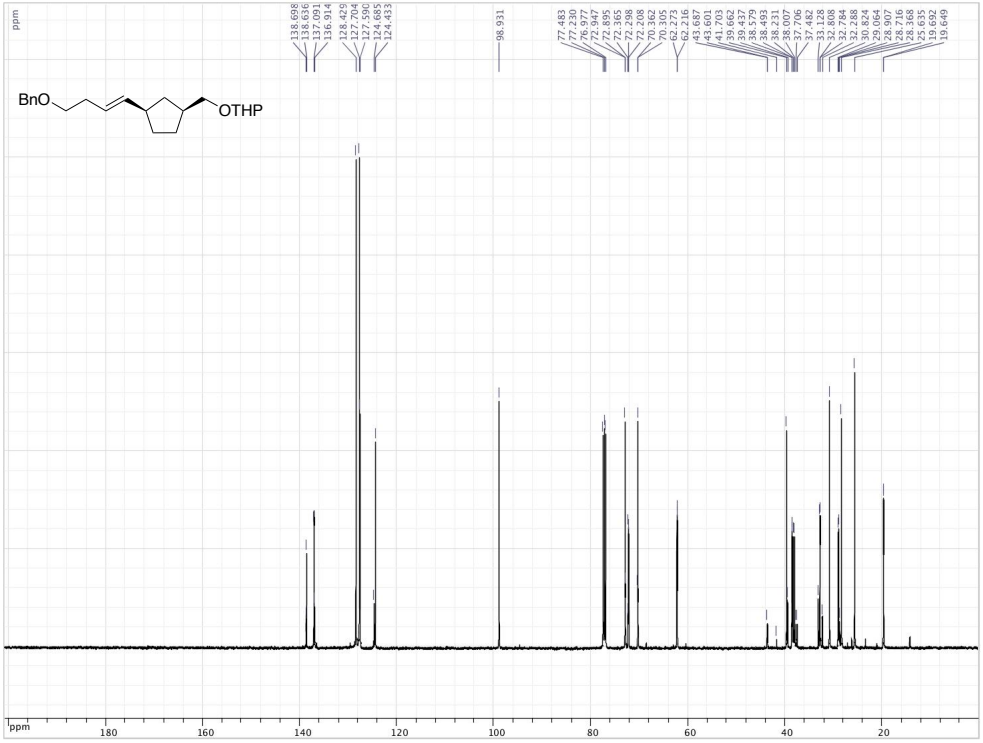
## Compound 36



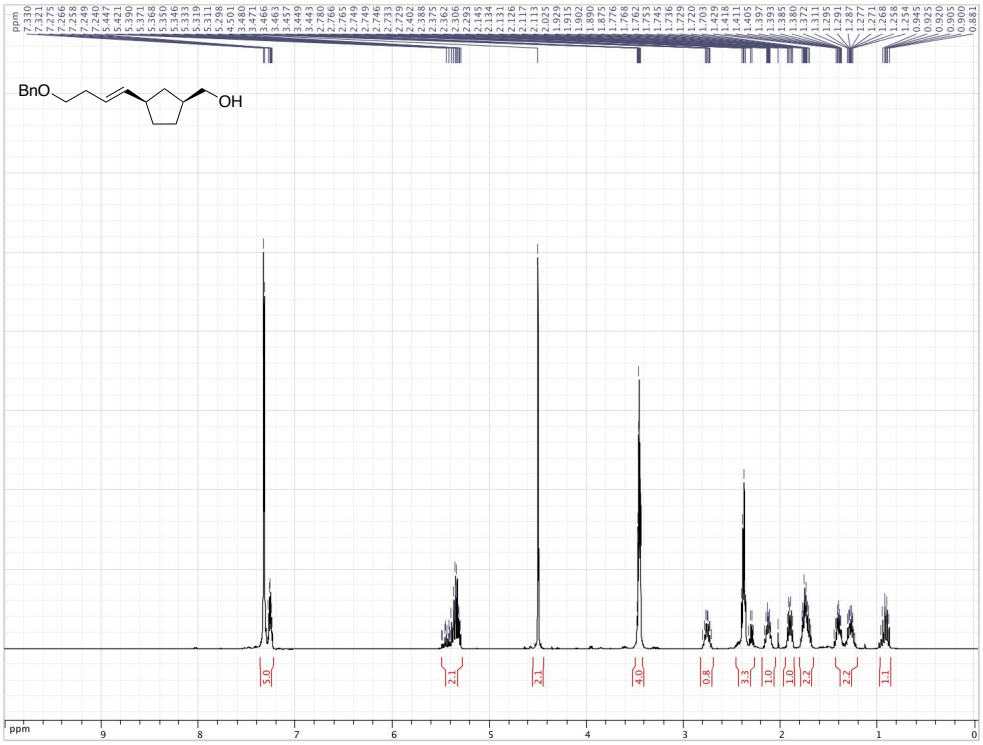



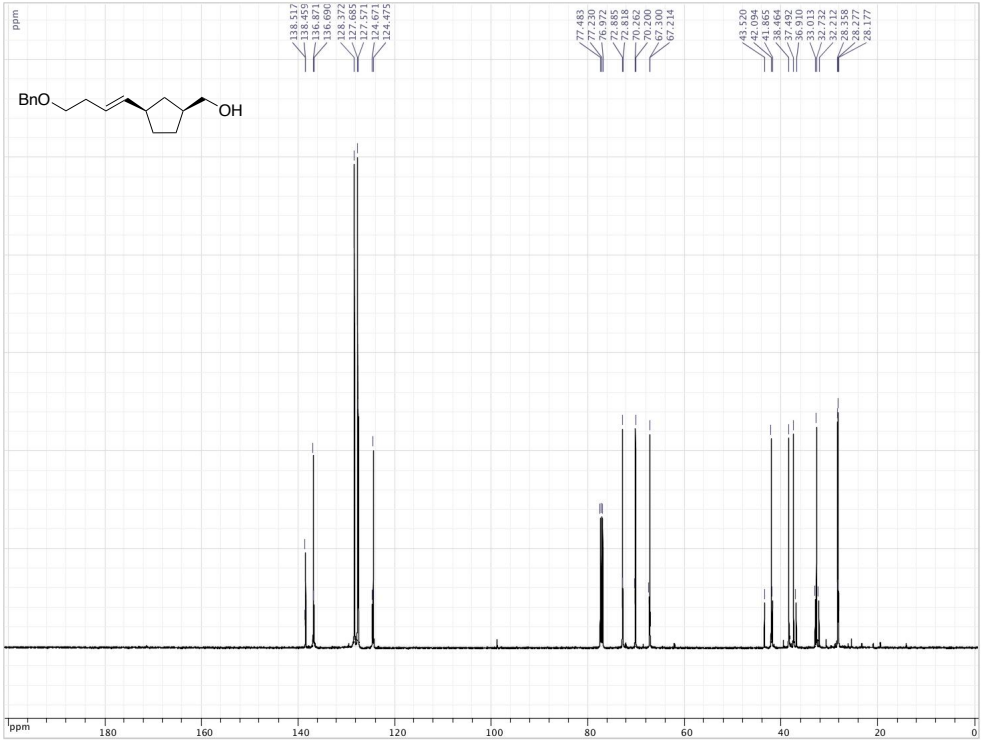
## Compound 39



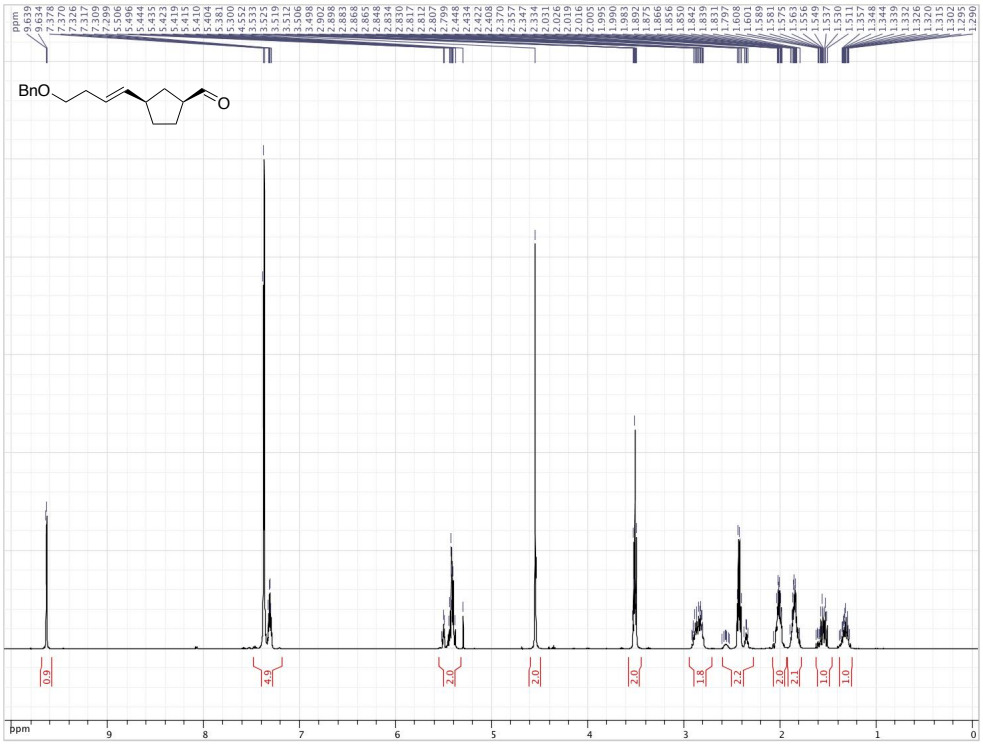



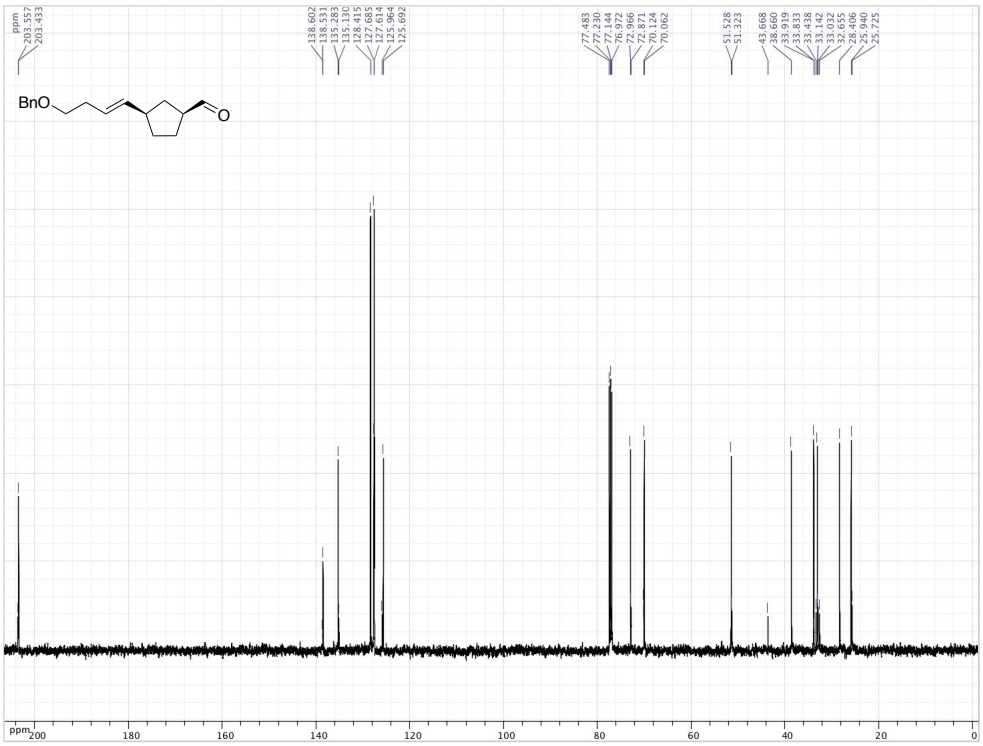
## Compound 40



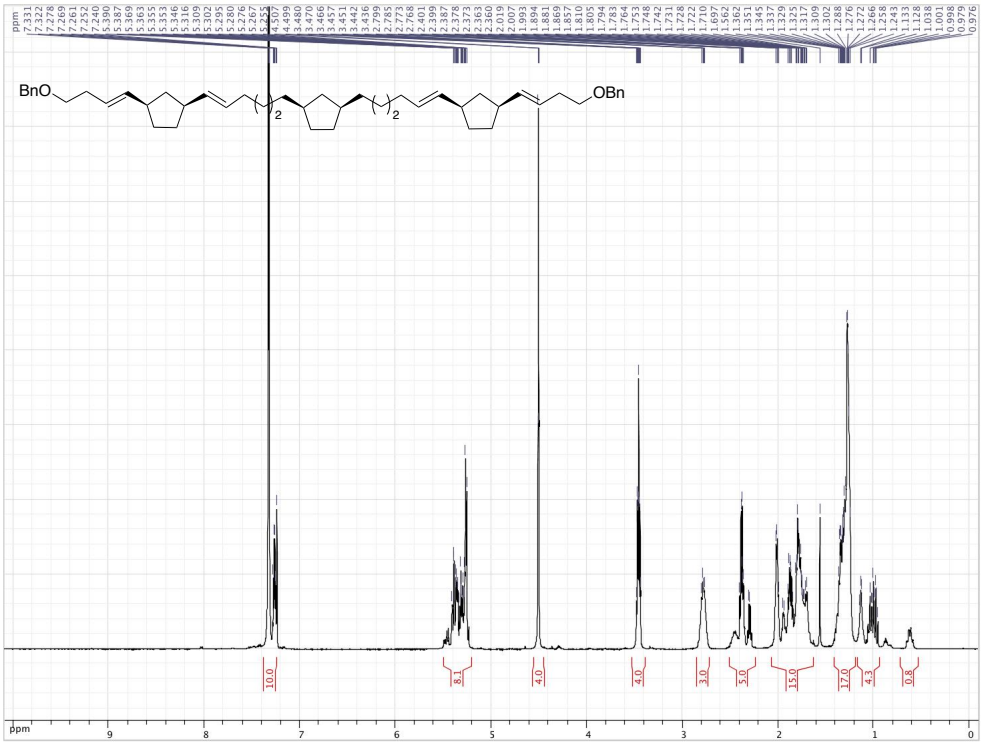



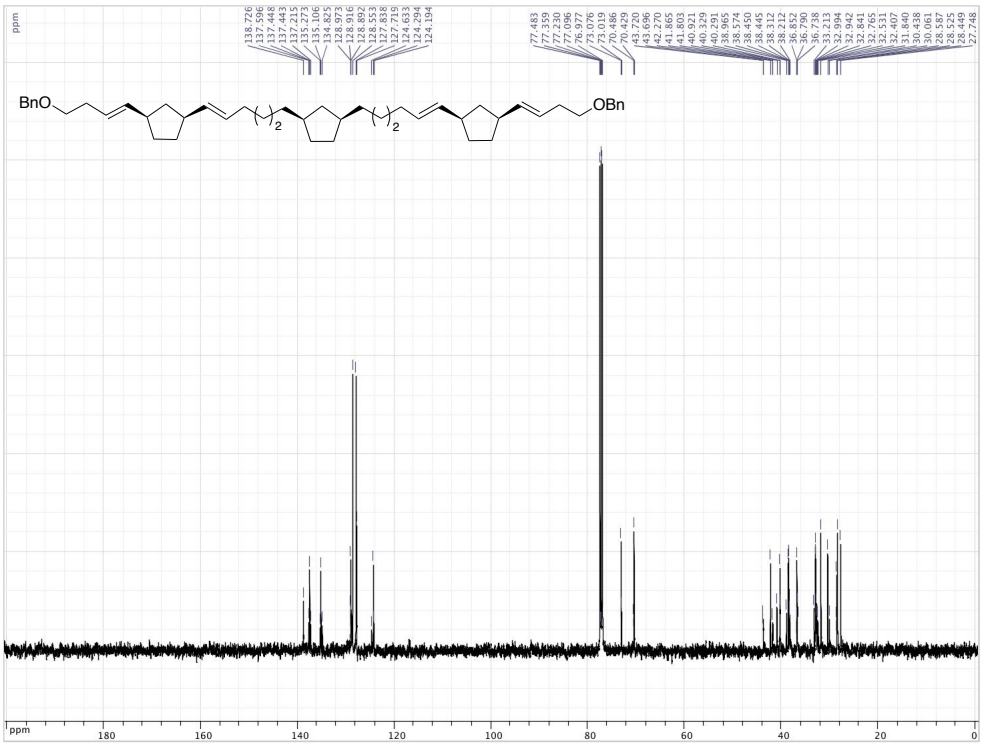
## Compound 41



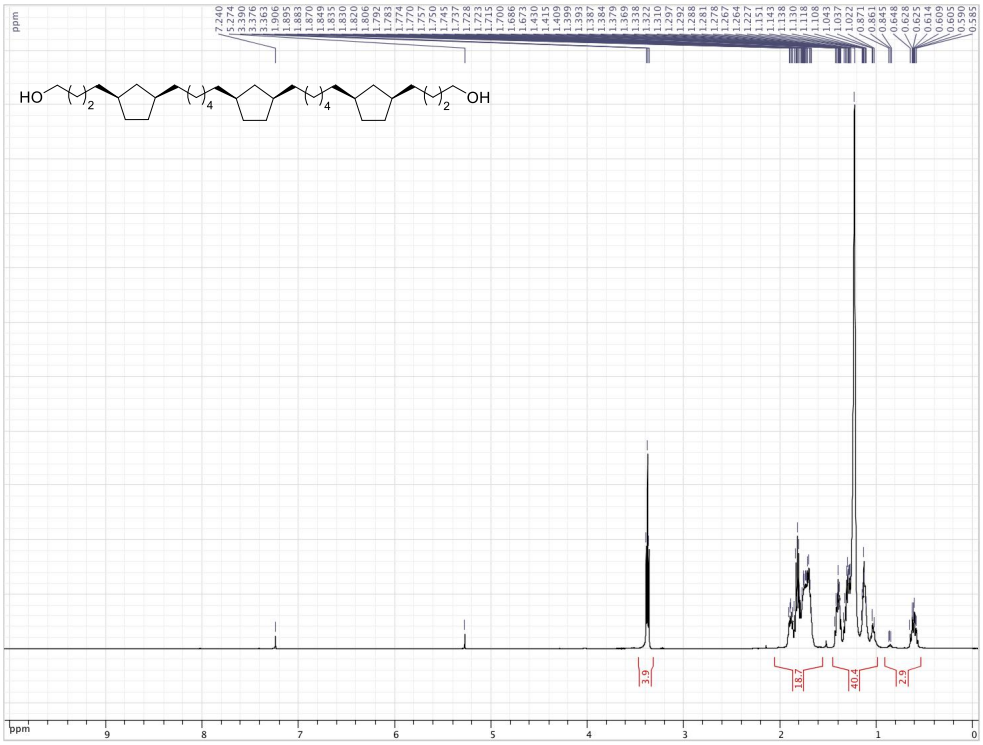



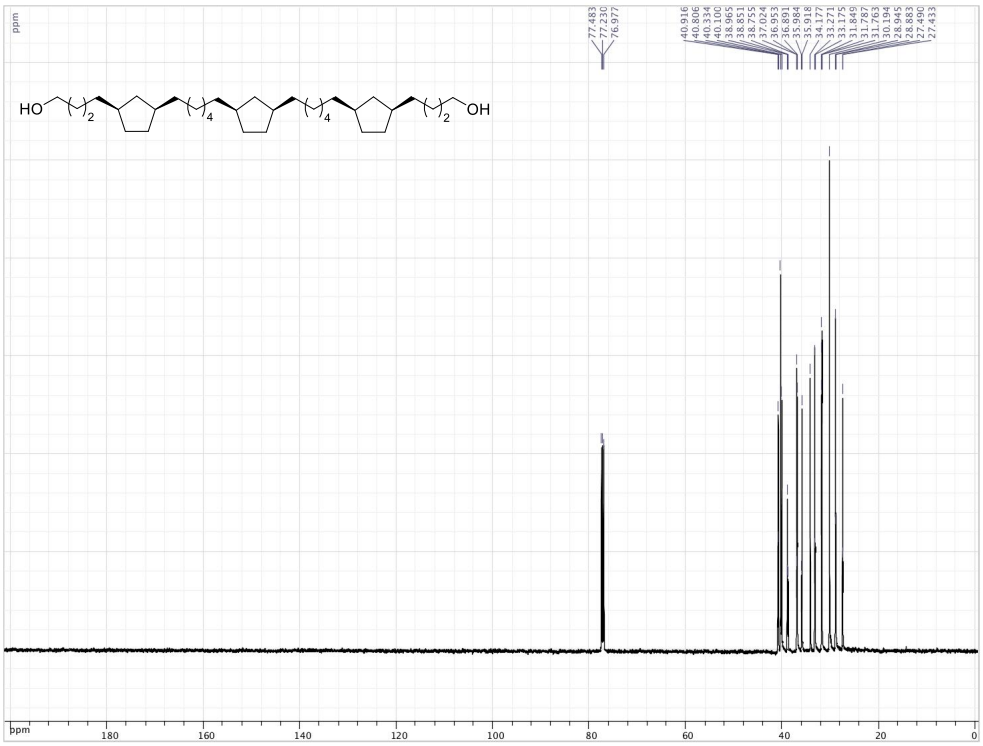
## Compound 42



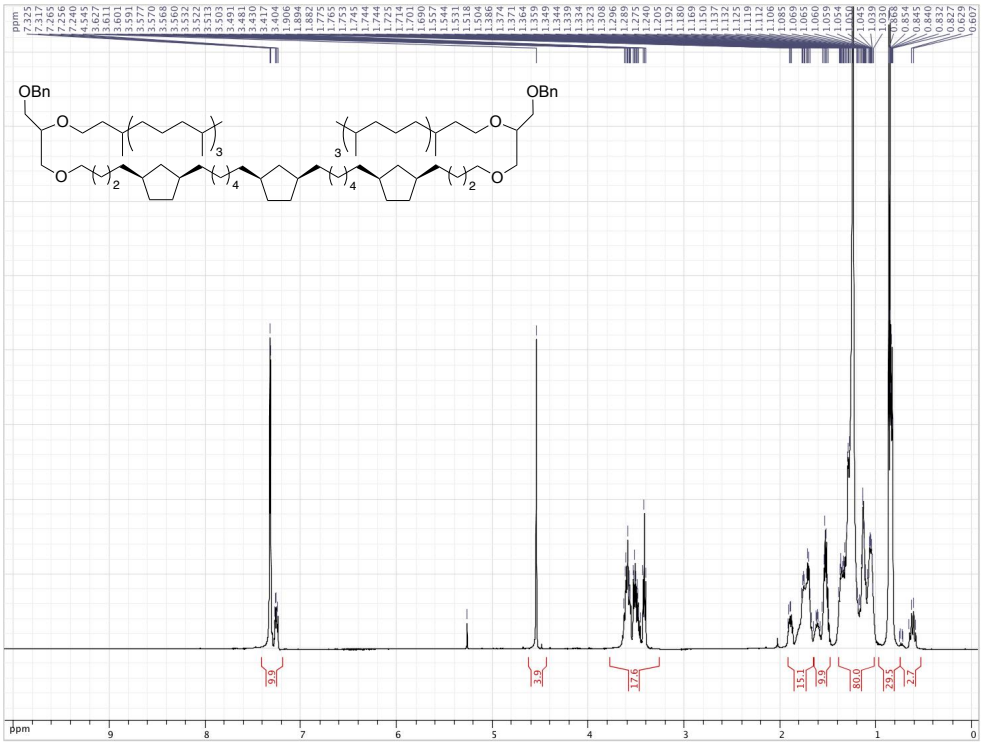



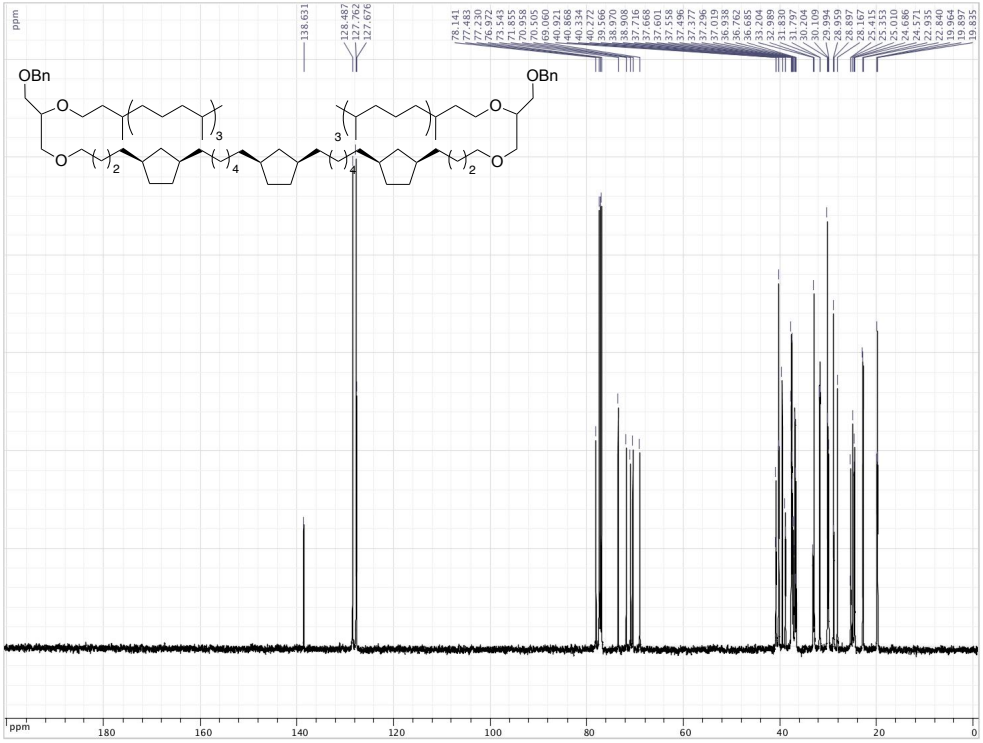
## Compound 44



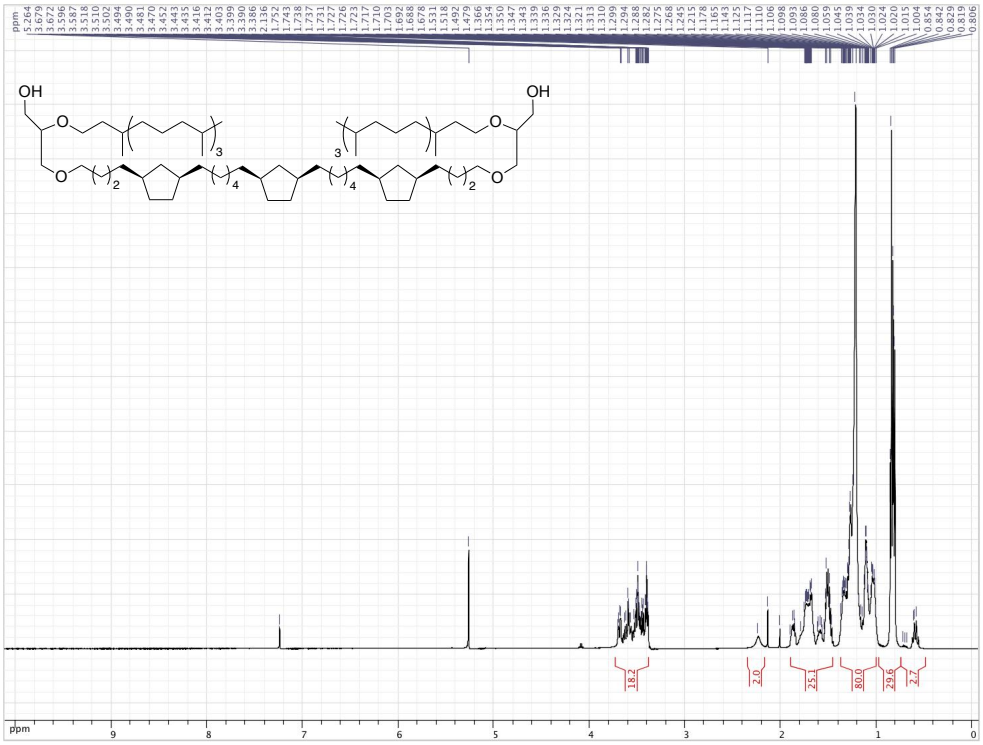



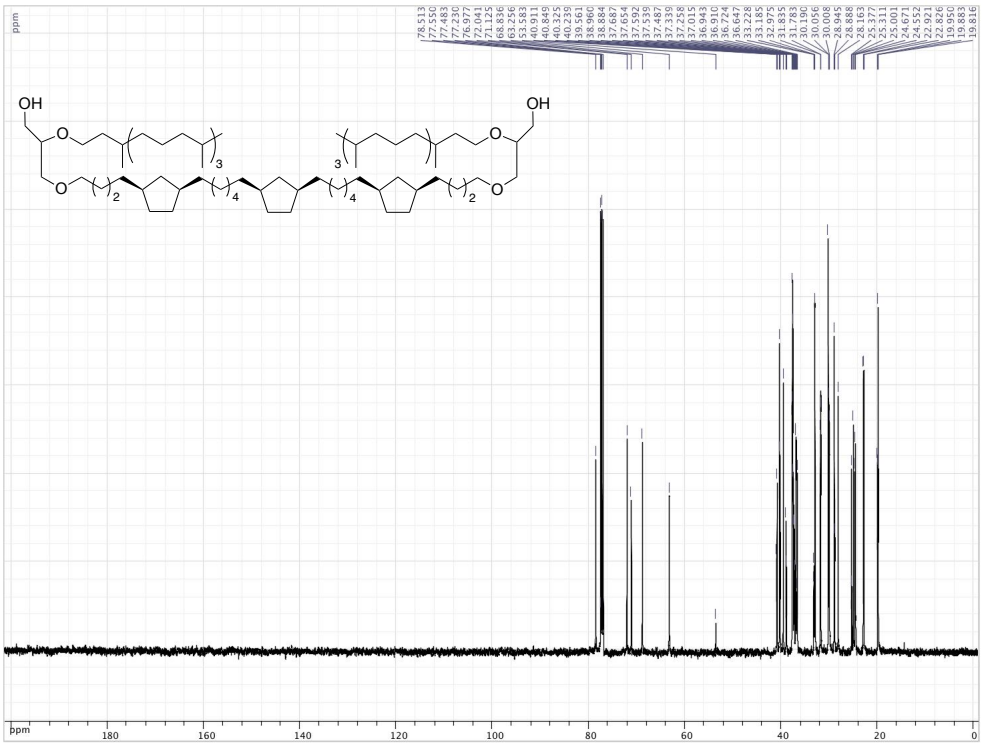
## Compound 45



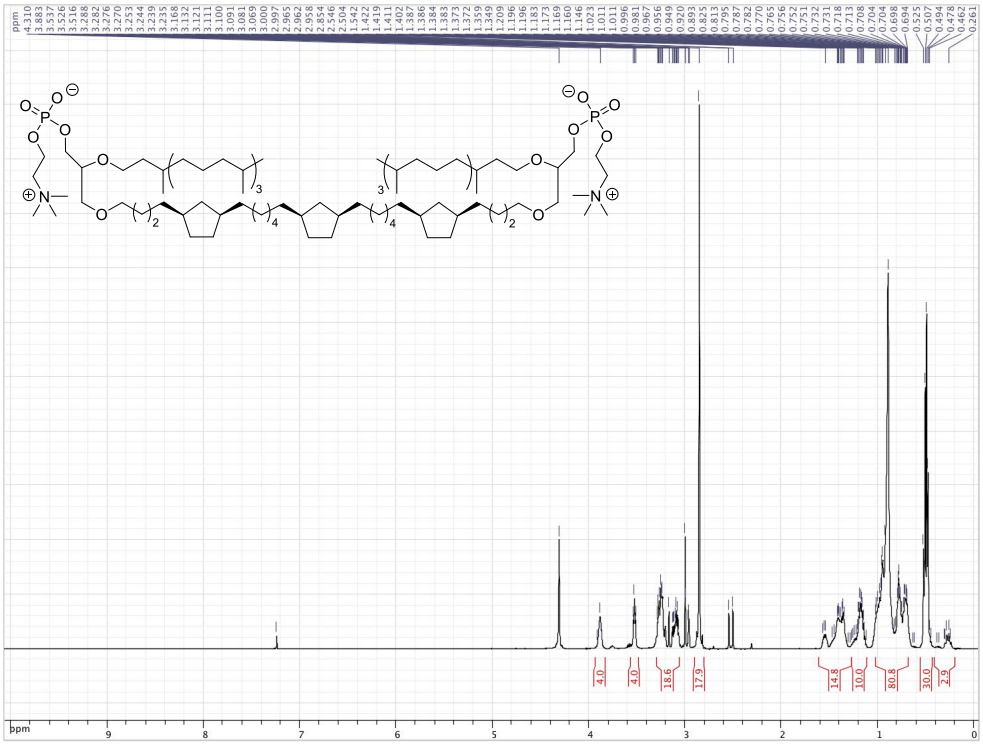



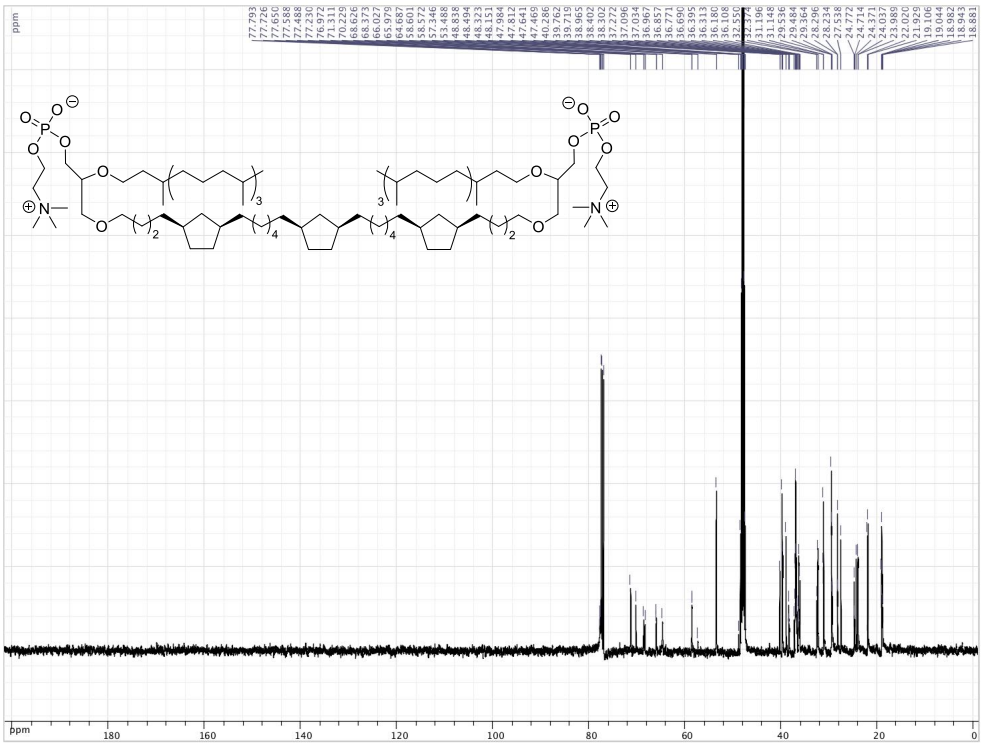
## Compound 46

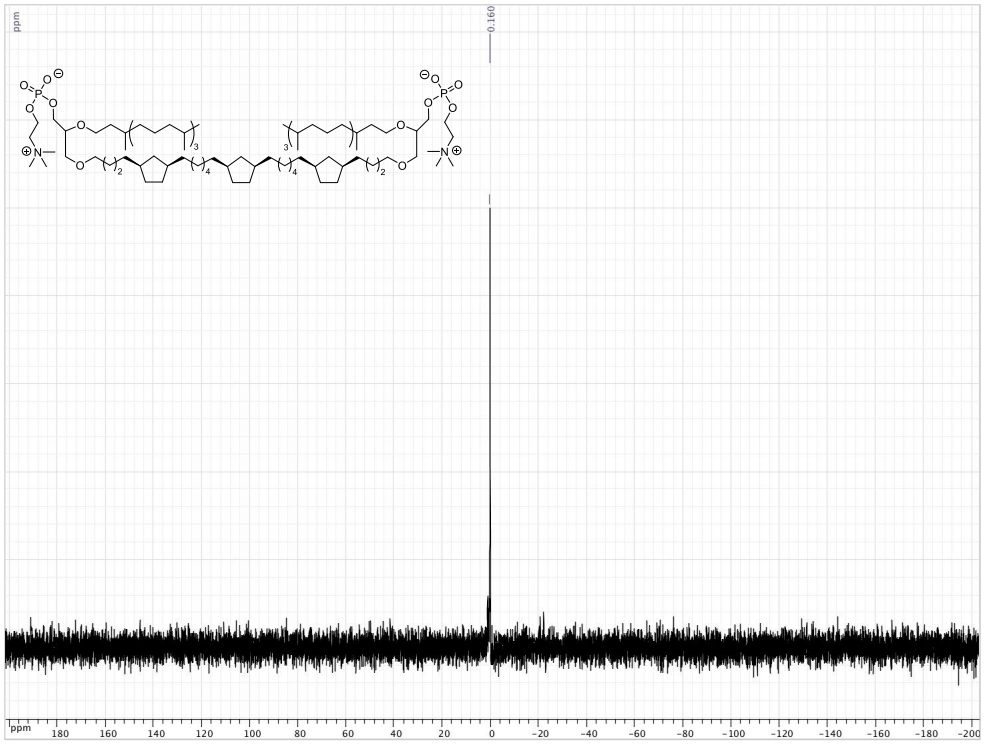


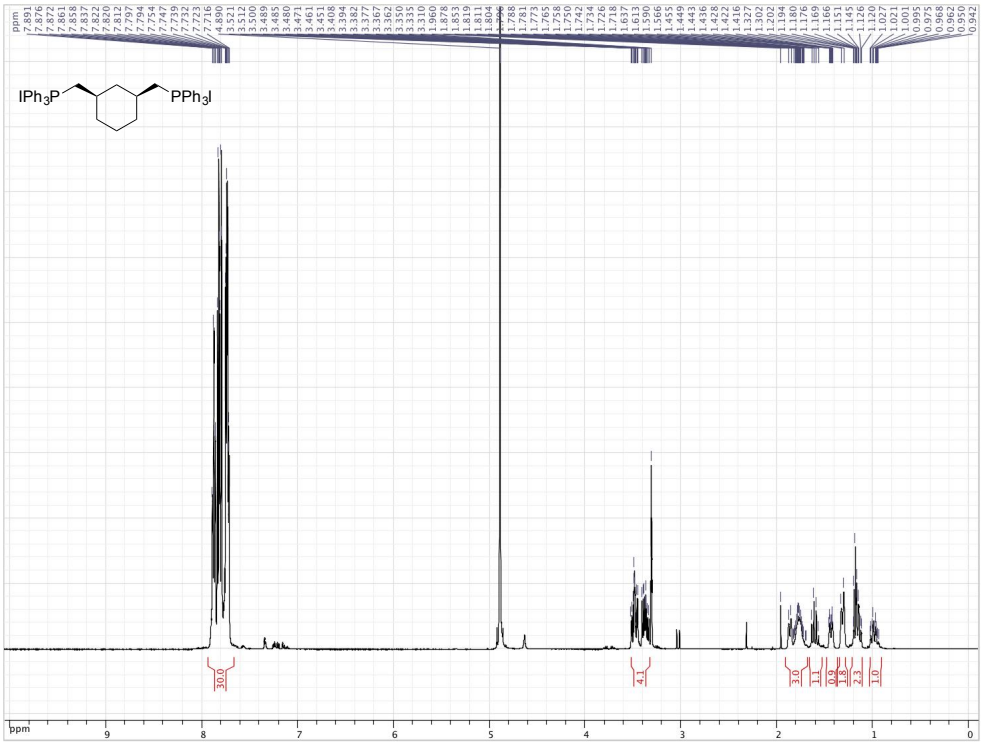
## Compound GMGTPC-CP3

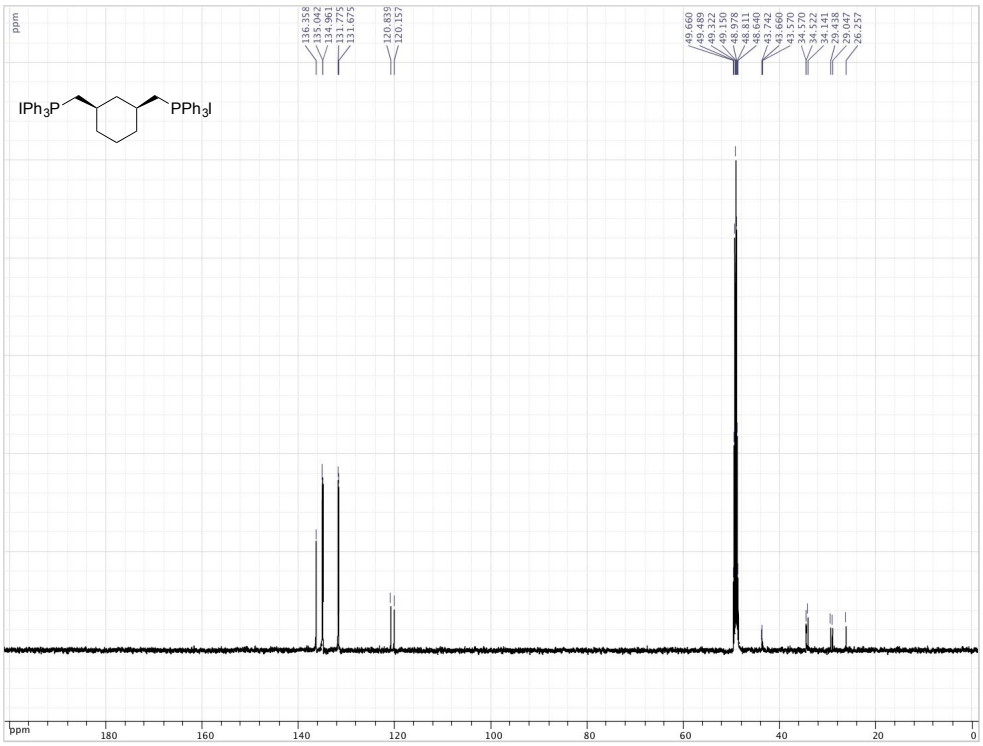


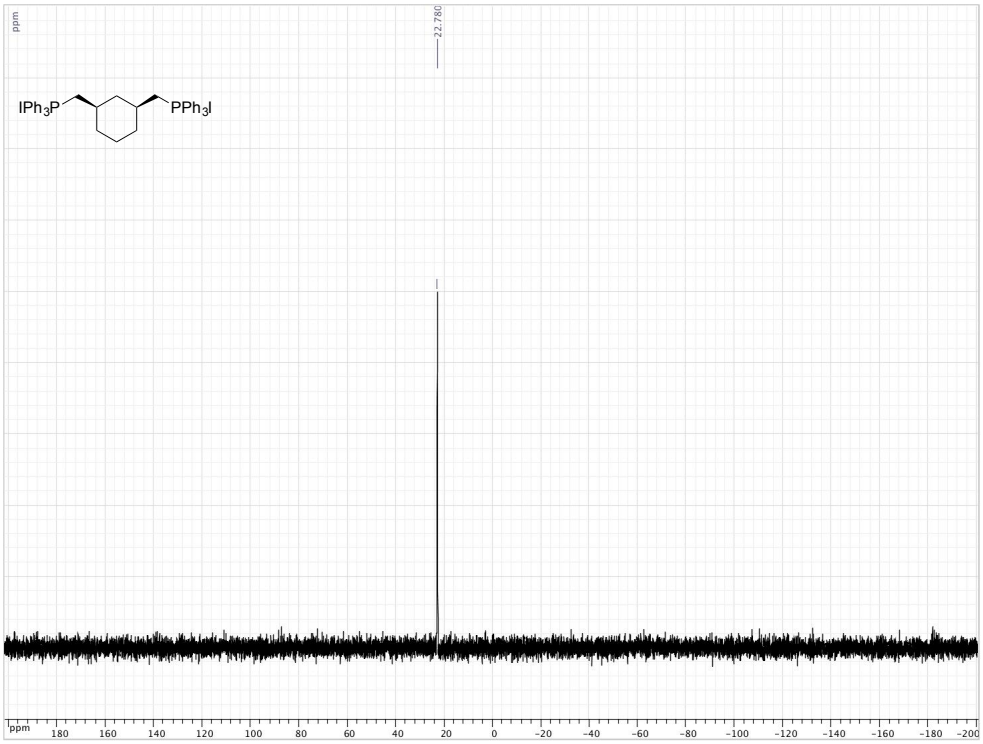



ppm
0.160
ppm
180
160
140
120
100
80
60
40
20
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200

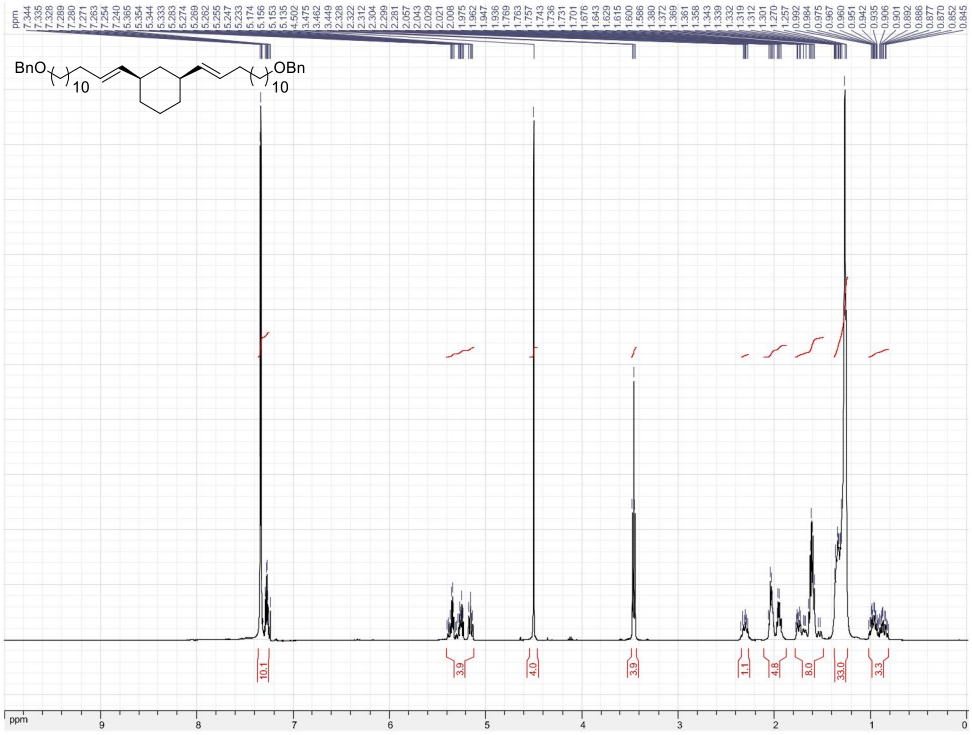
## Compound 49

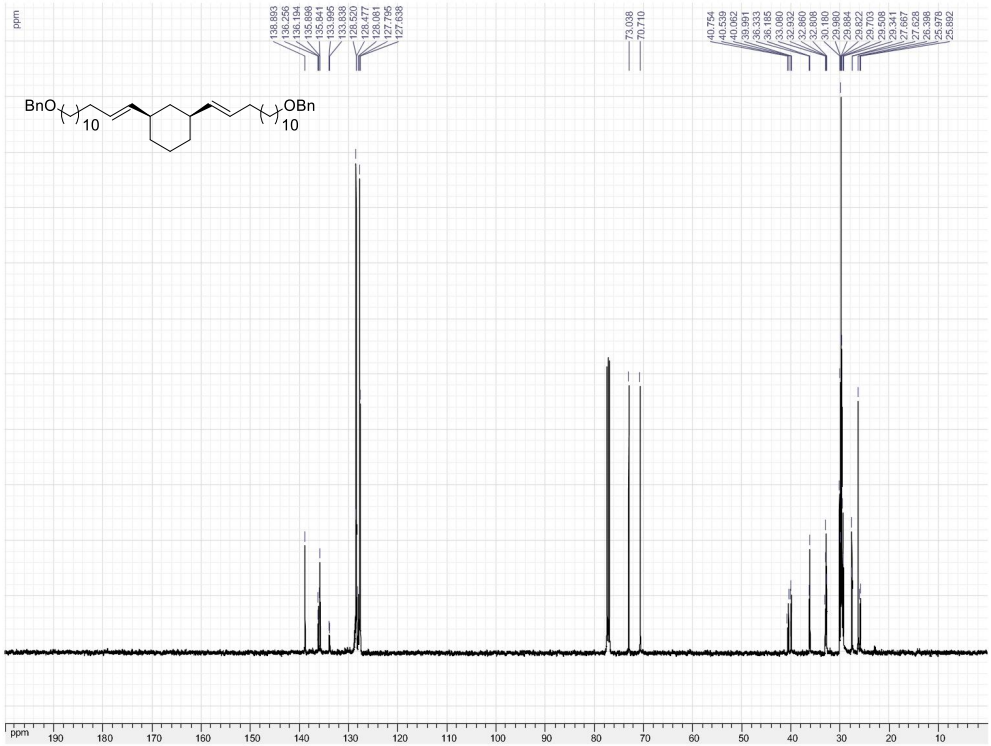

IPh3P
PPh3I
ppm
30.0
4.1
3.0
1.1
0.9
1.8
2.3
1.0


IPh3P
PPh3I
ppm
136.358
135.042
134.961
131.775
131.675
120.839
120.157
49.660
49.489
49.322
49.150
48.978
48.811
48.640
43.722
43.660
43.570
34.570
34.522
34.141
29.438
29.047
26.257

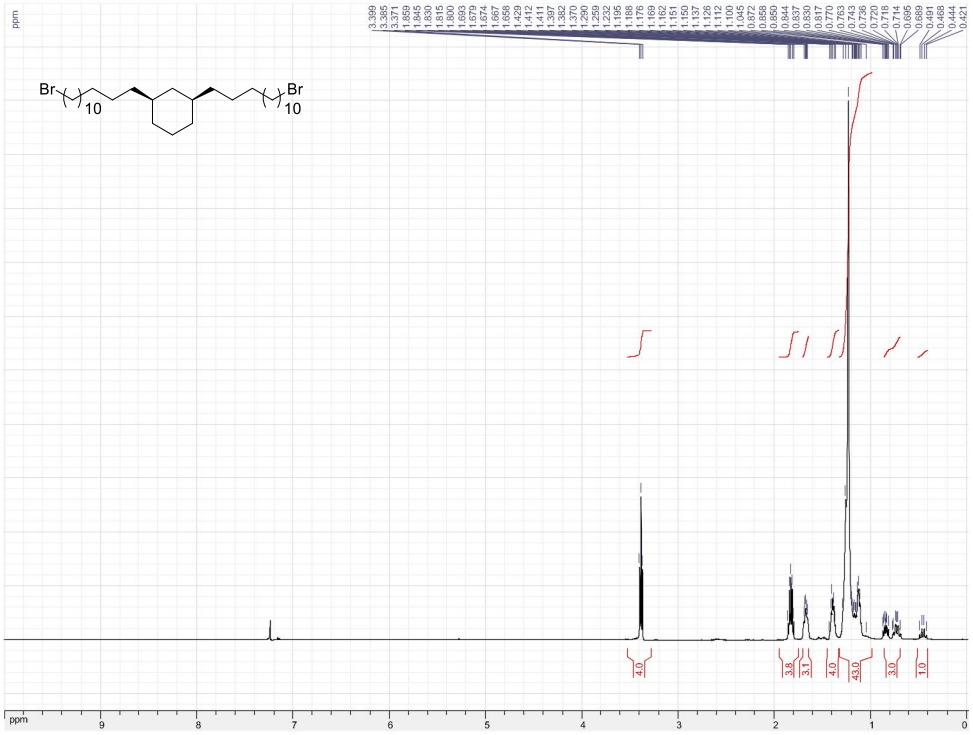

ppm
22.780
IPh3P
PPh3I
ppm
180
160
140
120
100
80
60
40
20
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200

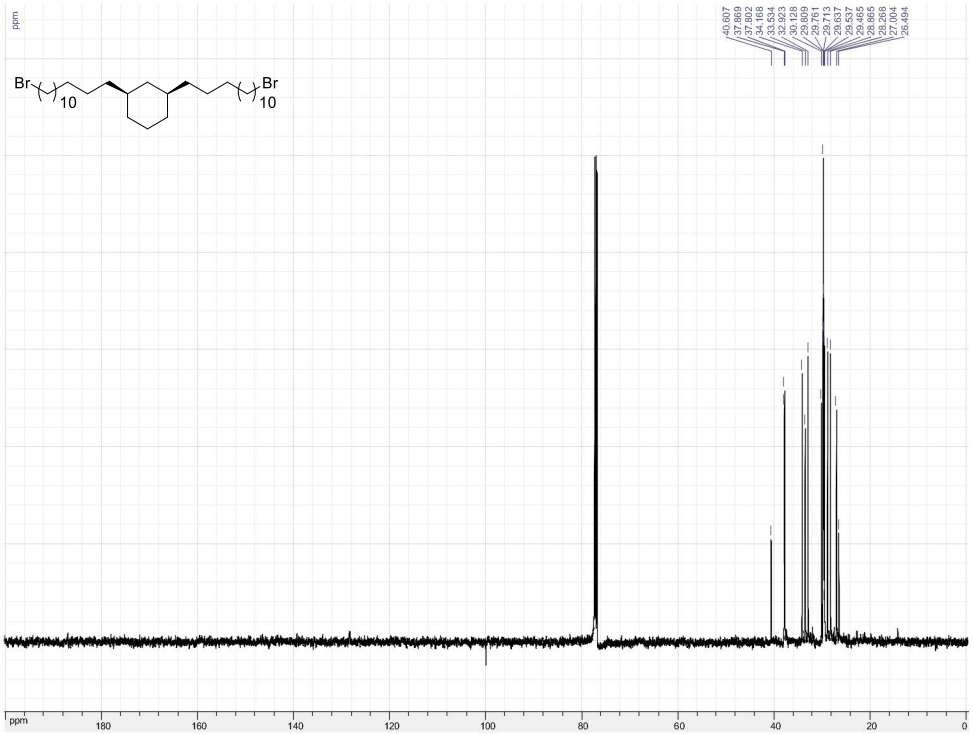
## Compound 50



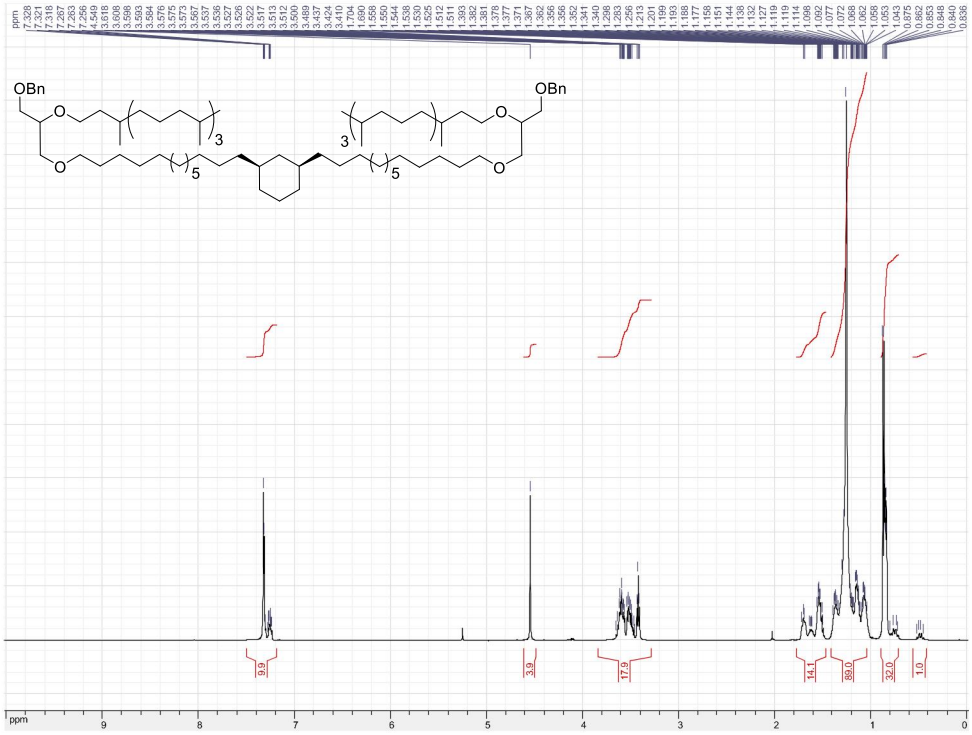



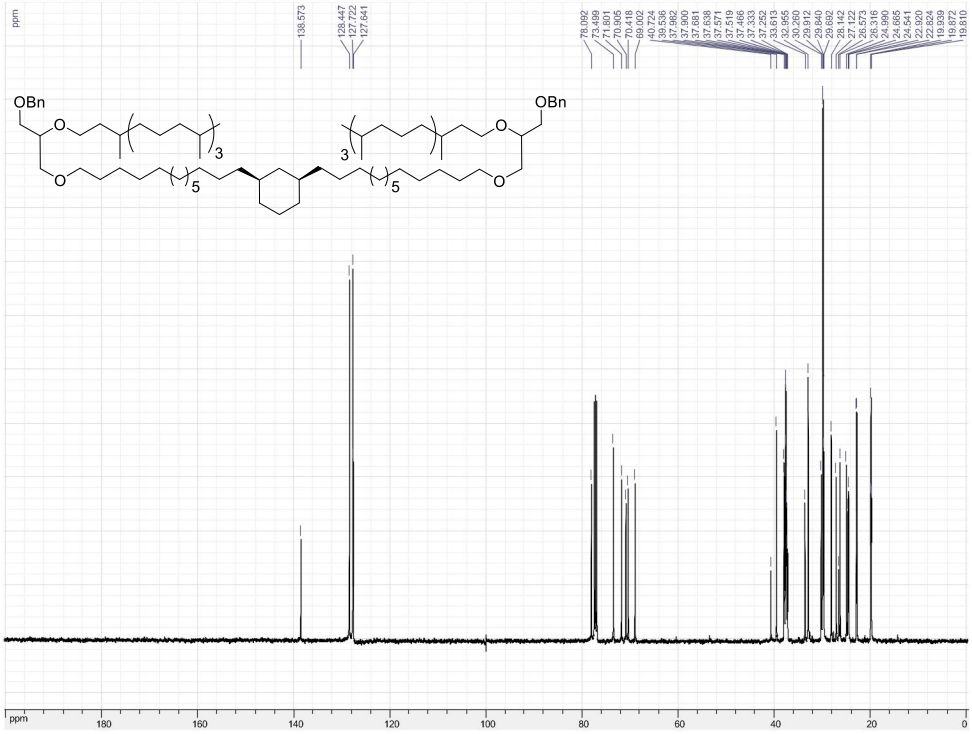
## Compound 52



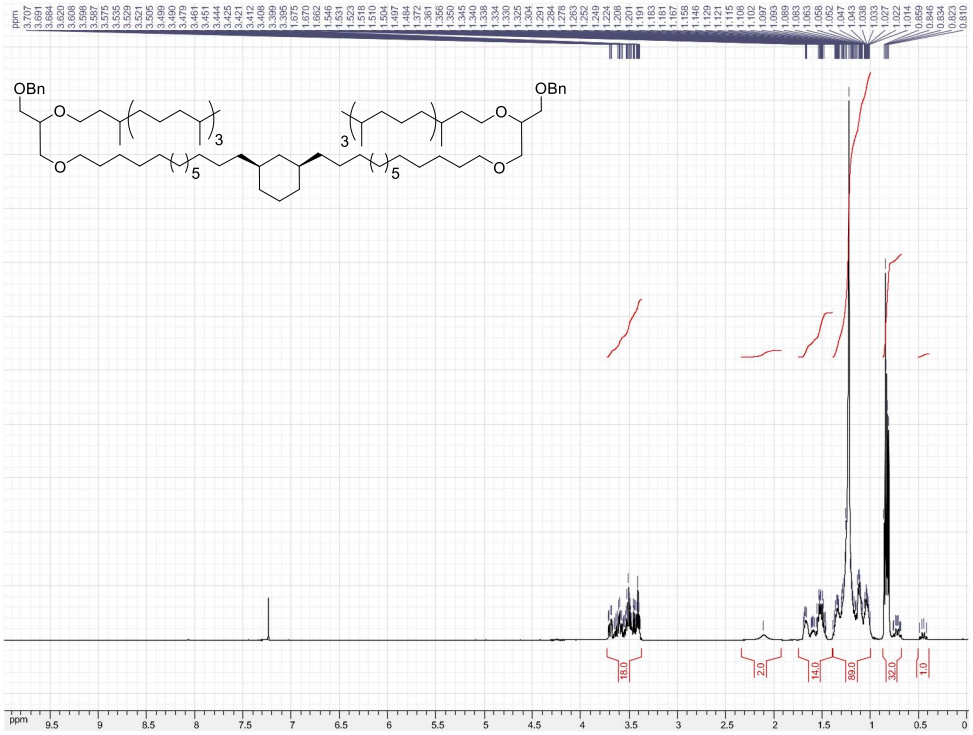



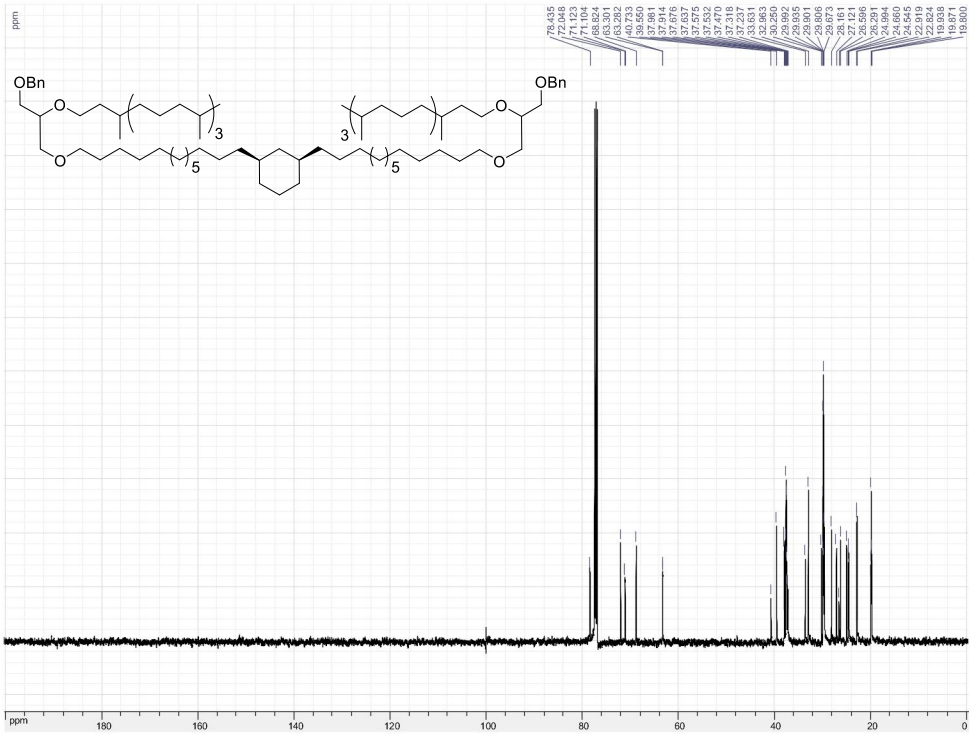
## Compound 53


OBn
OBn
O
O
O
O
3
3
5
5
ppm
9.9
3.9
17.9
14.1
89.0
32.0
1.0

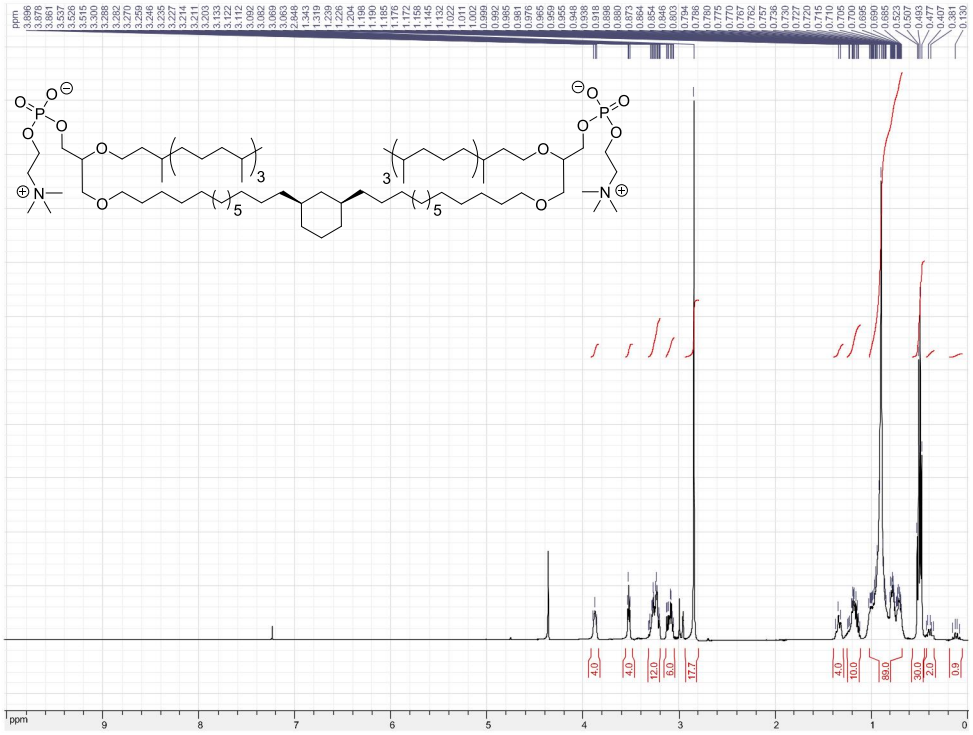

OBn
OBn
O
O
O
O
3
3
5
5
ppm
138.573
128.447
127.722
127.641

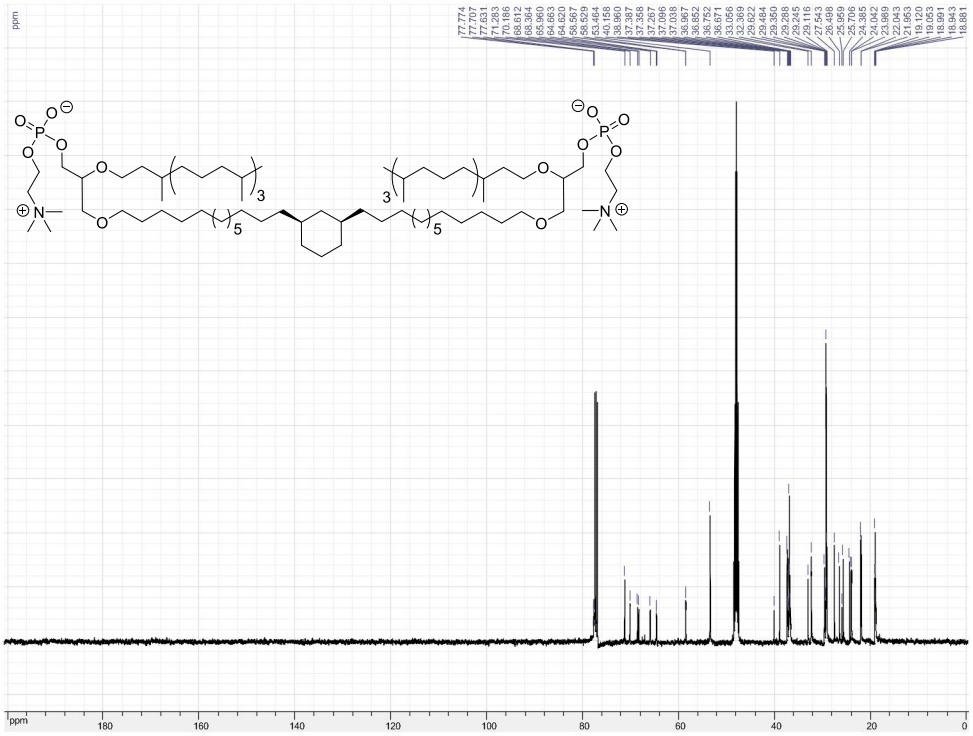
## Compound 54

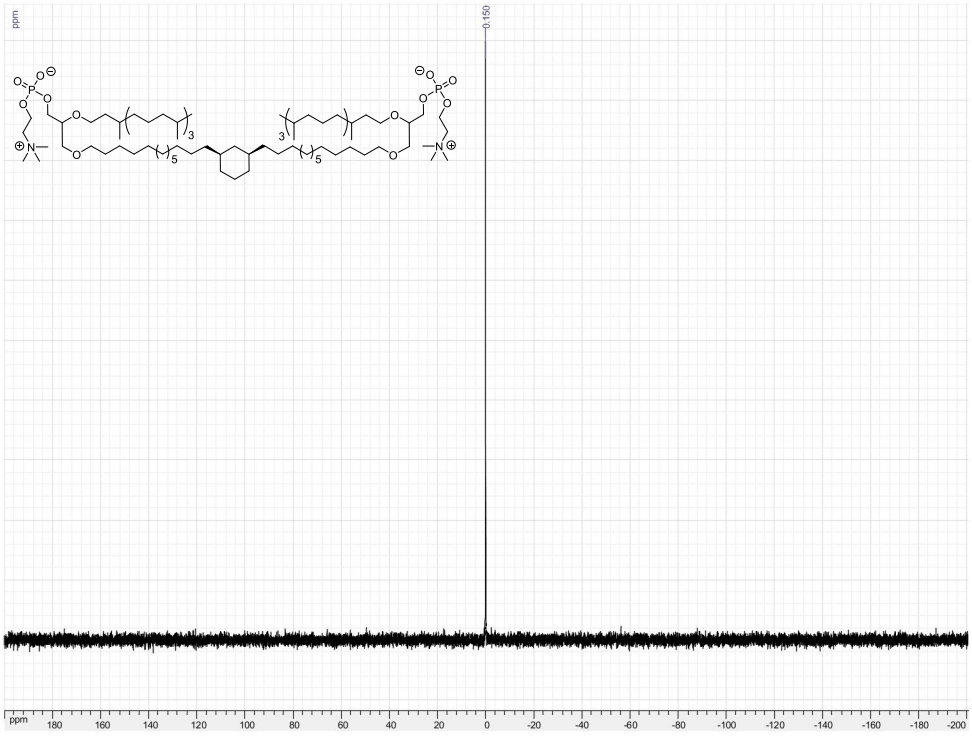

OBn
OBn
O
O
O
O
3
3
5
5
ppm
9.5
9
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
16.0
2.0
14.0
88.0
32.0
1.0


OBn
OBn
O
O
O
O
3
3
5
5
ppm
180
160
140
120
100
80
60
40
20
0

## Compound GMGTPC-CH1






ppm
0.150
ppm
180
160
140
120
100
80
60
40
20
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200

## 10. References


(1) Chang Chung, Y.; Hong Chiu, Y.; Wei Wu, Y.; Tai Tao, Y. *Biomaterials* **2005**, *26* (15), 2313–2324.

(2) Febo-Ayala, W.; Morera-Félix, S. L.; Hrycyna, C. A.; Thompson, D. H. *Biochemistry* **2006**, *45* (49), 14683–14694.

(3) Babu, K. V.; Sharma, G. V. M. *Tetrahedron Asym.* **2008**, *19* (5), 577–583.

(4) Shirouzu, T.; Watari, K.; Ono, M.; Koizumi, K.; Saiki, I.; Tanaka, C.; van Soest, R. W. M.; Miyamoto, T. *J. Nat. Prod.* **2013**, *76* (7), 1337–1342.

(5) Wagner, A.; Heitz, M.-P.; Mioskowski, C. *Tetrahedron Lett.* **1989**, *30* (5), 557–558.

(6) *WO 2009/135977*.

(7) Brard, M.; Lainé, C.; Réthoré, G.; Laurent, I.; Neveu, C.; Lemiègre, L.; Benvegnu, T. *J. Org. Chem.* **2007**, *72* (22), 8267–8279.

(8) Shimojo, M.; Matsumoto, K.; Hatanaka, M. *Tetrahedron* **2000**, *56* (47), 9281–9288.

(9) Pan, X.; Lacôte, E.; Lalevée, J.; Curran, D. P. *J. Am. Chem. Soc.* **2012**, *134* (12), 5669–5674.

(10) Brard, M.; Richter, W.; Benvegnu, T.; Plusquellec, D. *J. Am. Chem. Soc.* **2004**, *126* (32), 10003–10012.

(11) Trigo, G. G.; Muñoz, E. M.; Llama-Hurtado, E. *J. Heterocycl. Chem.* **1984**, *21* (5), 1479–1483.

(12) Lowik, D. W. P. M.; Liskamp, R. M. J. *Eur. J. Org. Chem.* **2000**, *2000* (7), 1219–1228.

(13) Prabhakar, P.; Rajaram, S.; Reddy, D. K.; Shekar, V.; Venkateswarlu, Y. *Tetrahedron Asym.* **2010**, *21* (2), 216–221.

(14) Qin, L.; Ren, X.; Lu, Y.; Li, Y.; Zhou, J. *Angew. Chem., Int. Ed. Engl.* **2012**, *51* (24), 5915–5919.

(15) Flowers, C. L.; Vogel, P. *Chem. - A Eur. J.* **2010**, *16* (47), 14074–14082.

(16) Mabrey, S.; Sturtevant, J. M. *Proc. Natl. Acad. Sci. U. S. A.* **1976**, *73* (11), 3862–3866.

(17) Struck, D. K.; Hoekstra, D.; Pagano, R. E. *Biochemistry* **1981**, *20* (14), 4093–4099.

(18) Düzgüne, N.; Nir, S.; Wilschut, J.; Bentz, J.; Newton, C.; Portis, A.; Papahadjopoulos, D. *J. Membr. Biol.* **1981**, *59* (2), 115–125.

(19) Portis, A.; Newton, C.; Pangborn, W.; Papahadjopoulos, D. *Biochemistry* **1979**, *18* (5), 780–790.

(20) Wilschut, J.; Papahadjopoulos, D. *Nature*. 1979, pp 690–692.

(21) Weinstein, J.; Yoshikami, S.; Henkart, P.; Blumenthal, R.; Hagins, W. *Science (80-. ).* **1977**, *195* (4277), 489–492.