Supplementary Material

Cyanuric chloride as the basis for compositionally diverse lipids

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Materials and instrumentation

Beta-alanine-tert-butyl ester, cyanuric chloride, didodecylamine, diisopropylethylamine (DIPEA), 2-mercaptoethylamine HCl, morpholine, ninhydrin, N,N-dimethyl diaminopropane and trityl chloride were purchased from TCI America (Portland, OR). Dioctadecylamine was purchased from Sigma-Aldrich (Milwaukee, WI). *N*-Boc-1,3-diaminopropane was purchased from Matrix Scientific (Columbia, SC). 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dimyristoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (DMPG), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-di-O-octadecenyl-3-trimethylammonium propane (DOTMA) and cholesterol were purchased from Avanti Polar Lipids,

Inc. (Alabaster, AL). Solvents for reactions were purchased from various suppliers through VWR (Radnor, PA). Thin layer chromatography (TLC; Milipore Sigma, Silica gel 60 F_{254}) was visualized under UV light or with 2% ninhydrin in DMSO. Final compound purity was assessed via a Waters 2707 Autosampler, Waters 2545 Quaternary Gradient Module pump and Waters 2998 Photodiode Array Detector following injection into a Waters XBridge C18 3.5 μm column (part no. 186003034) using a water, acetonitrile and methanol mixture as described in the figures below and detected at 205 and 254 nm. ^{1}H and ^{13}C NMR spectra were recorded in deuterated chloroform using a Varian 400 MHz or Varian 500 MHz spectrometer equipped with a 5 mm OneProbe (Cambridge Isotope Laboratories, Inc.; Tewksbury, MA). HR-MS was performed on an Agilent 6230B TOF LC/MS instrument in positive ion by direct injection of the compounds. Lipopeptide purification was performed using the Waters system described above.

Synthesis

Two approaches were taken for the synthesis of the TZ lipids: a convergent and a divergent route. In the convergent approach, two small molecule nucleophiles with protected, ionizable moieties were reacted with cyanuric chloride through nucleophilic aromatic substitution (NAS). The resulting monochlorotriazine was then reacted with a long-chained secondary amine lipid tail (dioctadecylamine or didodecylamine) to yield the final protected lipid. In the divergent approach, the lipid tail was reacted first to form a dichlorotriazine, followed by headgroup diversification through addition of various nucleophilic small molecule moieties as headgroups. In both approaches, the first NAS was initiated on ice and allowed to stir at room temperature in chloroform for at least 4 hours. The second substituent was added at room temperature in chloroform and heated to 50 °C for at least 24 hours. The final NAS reaction was performed in xylenes or dioxane and heated from room temperature to 80 °C for at least 72 hours. In each reaction, excess nucleophile or DIPEA served as base. The reactions were monitored at each

step via thin layer chromatography and characterized by nuclear magnetic resonance and mass spectrometry. Small molecule nucleophiles with reactant pendant moieties were protected with acid labile protecting groups and deprotected as the final step in the lipid synthesis with trifluoroacetic acid in dichloromethane.

Intermediate A was prepared by adding 1 equiv. of cyanuric chloride to a stirring solution of chloroform with 2.4 equiv. of beta-alanine-tert-butyl ester and 10 equiv. of DIPEA on ice. The mixture was allowed to come to room temperature, then heated overnight at 50 °C. Remaining beta-alanine-tert-butyl ester was removed by washing the dried product three times with brine. The monochlorotriazine was purified using a 0-30% ethyl acetate/ CH_2Cl_2 mixture on silica gel and the final product was eluted from the column using ethyl acetate, which was evaporated to yield intermediate A (73.8%) (30% ethyl acetate:chloroform, R_f = 0.88). ^{1}H-NMR (500 MHz, $CHCl_3$) δ 5.65-5.86 (m, 2NH), 3.56-3.68 (m, 4H), 2.47-2.52 (m, 4H), 1.42-1.48 (m, 18H); ^{13}C-NMR (125 MHz, $CHCl_3$) δ 171.18, 165.57, 156.38, 81.13, 36.58, 34.94, 28.07; HRMS MW calculated for $C_{17}H_{28}ClN_5O_4$ $(M + H)^+$: = 402.1903; found: 402.1939.

Intermediate B was prepared by adding 1 equiv. of cyanuric chloride to a stirring solution of chloroform with 2.4 equiv. of *N*-Boc-1,3-diaminopropane and 10 equiv. of DIPEA on ice. The mixture was allowed to come to room temperature, then heated overnight at 50 °C. Remaining *N*-Boc-1,3-diaminopropane was removed by washing the dried product three times with brine. The monochlorotriazine was purified using a 0-30% ethyl acetate/ CH_2Cl_2 mixture on silica gel and the final product was eluted from the column using ethyl acetate, which was evaporated to yield intermediate B (86%) (50% ethyl acetate:chloroform, R_f = 0.51). ^{1}H-NMR (500 MHz, $CHCl_3$) δ 4.96-6.51 (m, 4NH), 3.38-3.49 (m, 4H), 3.19 (m, 4H), 1.74 (m, 4H), 1.44 (m, 18H); ^{13}C-NMR (125

MHz, $CHCl_3$) δ 168.02, 165.77, 156.17, 79.23, 37.97, 37.56, 30.04, 28.39; HRMS MW calculated for $C_{19}H_{34}ClN_7O_4$ $(M + H)^+$: = 460.2434; found: 460.2505.

2-[(Triphenylmethyl)thio]ethanamine (CAS number: 1095-85-8) was prepared by an adaptation of the procedure described by Watrelot et al.[1] To a stirred solution of 2-mercaptoethylamine HCl (1.1 equiv.) in dichloromethane at 0 °C under nitrogen was added dropwise trifluoracetic acid (TFA, 3 mL) followed by dropwise addition of trityl chloride (1 equiv.). The reaction was stirred for 2.5 hours at 0 °C then concentrated and diluted in $CHCl_3$ (10 mL) and washed 3 times with 1 M NaOH and once with brine. The organic layer was then dried over magnesium sulfate and filtered and evaporated to dryness to afford the desired compound (92%) without further purification. ^{1}H-NMR (500 MHz,$CDCl_3$) δ 7.43 (m, 6H), 7.28 (m, 6H), 7.21 (m, 3H), 2.6 (t, *J* = 6.5 Hz, 2H), 2.32 (t, *J* = 6.5 Hz, 2H), 1.21 (bs, 2H, NH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 144.87, 129.56, 127.82, 126.61, 66.50, 41.08, 36.35.

Intermediate C was prepared by adding 1 equiv. of cyanuric chloride to a stirring solution of chloroform with 1.2 equiv. of beta-alanine-tert-butyl ester and 10 equiv. of DIPEA on ice. The mixture was allowed to come to room temperature and reacted for 4 hours until the disappearance of cyanuric chloride was confirmed on TLC (chloroform, R_f = 0.58). To this mixture 1.1-1.5 equiv. of 2-[(triphenylmethyl)thio]ethanamine was added and stirred at room temperature for 24 hours. The final compound was dried and dissolved in ethyl acetate and then purified by washing with 0.5 M HCl three times then twice with brine. The organic phase was dried over magnesium sulfate and evaporated to yield intermediate C (97.7-99.3%). Of note, the formation of this product starting with 2-[(triphenylmethyl)thio]ethanamine yields an insoluble white solid following the addition of 2-[(triphenylmethyl)thio]ethanamine, which is extremely difficult to purify and dissolve for further reactions. ^{1}H-NMR (500 MHz, $CHCl_3$) δ 7.39 (m, 6H), 7.16-7.28 (m, 9H), 5.67-6.14 (m,

2NH), 3.51-3.66 (m, 2H), 3.14-3.30 (m, 2H), 2.39-2.50 (m, H4), 1.42-1.47 (m, 9H); ^{13}C-NMR (125 MHz, $CHCl_3$) δ 171.20, 168.31, 165.39, 146.84, 144.67, 129.45, 127.85, 127.17, 126.69, 81.09, 66.73, 39.60, 36.43, 34.86, 31.40, 28.07; HRMS MW calculated for $C_{31}H_{34}ClN_5O_4S$ $(M + H)^+$: = 576.2195; found: 576.2198.

Intermediate D was prepared by adding 1.1-1.5 equiv. of cyanuric chloride to a solution of chloroform with 1 equiv. of dioctadecylamine and 10 equiv. of DIPEA. The solution was started at -78 °C and allowed to come to 4 °C overnight. In the morning the reaction was assessed for the disappearance of the secondary amine using 2% ninhydrin in DMSO on TLC (3:2 CH_2Cl_2:hexanes, R_f = 0.95). The completed reaction was dried by rotary evaporation, then precipitated from chloroform with MeOH and filtered. This process was repeated twice, and the resulting white powder was resuspended in $CHCl_3$, dried over magnesium sulfate, filtered and evaporated to dryness to afford intermediate D (92-95%). ^{1}H-NMR (500 MHz, $CHCl_3$) δ 3.51 (t, J = 10, 4H), 1.55-1.61 (m, 4H), 1.20-1.32 (m, 60H), 0.86 (t, J = 10, 6H); ^{13}C-NMR (125 MHz, $CHCl_3$) δ 169.66, 164.16, 47.75, 31.84, 29.61, 29.58, 29.57, 29.55, 29.49, 29.40, 29.28, 29.15, 27.06, 26.59, 22.60, 14.03; HRMS MW calculated for $C_{39}H_{74}Cl_2N_4$ $(M + H)^+$: = 669.5363; found: 669.5361.

Intermediate E was prepared by adding 1.2 equiv. of beta-alanine-tert-butyl ester to a solution of chloroform with 1 equiv. of intermediate D and 10 equiv. of DIPEA. The mixture was stirred at room temperature for 2 hours then heated to 50 °C and allowed to react overnight. Remaining beta-alanine-tert-butyl ester was removed by washing the reaction mixture three times with brine. The compound was further purified on a silica gel column using a 10% ethyl acetate/chloroform mixture to yield intermediate E (51.2%) ($CHCl_3$, R_f = 0.50). ^{1}H-NMR (500 MHz, $CHCl_3$) δ 5.52-5.6 (m, NH), 3.57-3.66 (m, 2H), 3.35-3.51 (m, 4H), 2.49 (t, J = 7.5, 2H), 1.52-1.62 (m, 4H), 1.44 (s,

9H), 1.20-1.32 (m, 60H), 0.88 (t, *J* = 7.5, 6H); ^{13}C-NMR (125 MHz, $CHCl_3$) δ 171.13, 168.61, 165.12, 164.44, 80.90, 47.33, 47.09, 35.00, 31.83, 29.61, 29.57, 29.54, 29.49, 29.37, 29.30, 29.27, 28.02, 27.73, 26.97, 26.70, 22.60, 14.03; HRMS MW calculated for $C_{46}H_{88}ClN_5O_2$ $(M + H)^+$: = 778.6699; found: 778.6692.

Intermediate F was prepared in the same manner as intermediate D using didodecylamine with a similar product yield (93-95%). ^{1}H-NMR (500 MHz, $CHCl_3$) δ 3.51 (t, *J* = 9.5, 4H), 1.54-1.62 (m, 4H), 1.22-1.32 (m, 36H), 0.86 (t, *J* = 8.5, 6H); ^{13}C-NMR (125 MHz, $CHCl_3$) δ 169.60, 164.08, 47.71, 31.86, 29.55, 29.26, 29.14, 27.02, 26.56, 22.63, 14.07; HRMS MW calculated for $C_{27}H_{50}Cl_2N_4$ $(M + H)^+$: = 501.3485; found: 501.3489.

Intermediate G was prepared in the same manner as described for intermediate E using intermediate F with a similar product yield (50.4%). ^{1}H-NMR (500 MHz, $CHCl_3$) δ 5.52-5.6 (t, *J* = 6.1, NH), 3.58-3.62 (m, 2H), 3.35-3.49 (m, 4H), 2.49 (t, *J* = 6.5, 2H), 1.50-1.61 (m, 4H), 1.42 (s, 9H), 1.20-1.29 (m, 36H), 0.86 (t, *J* = 7, 6H); ^{13}C-NMR (125 MHz, $CHCl_3$) δ 171.03, 168.45, 165.11, 164.38, 80.67, 47.28, 47.01, 35.02, 31.78, 29.52, 29.49, 29.48, 29.43, 29.33, 29.24, 29.21, 27.95, 27.69, 26.92, 26.64, 22.54, 13.97; HRMS MW calculated for $C_{34}H_{64}ClN_5O_2$ $(M + H)^+$: = 610.4821; found: 610.4842.

Lipid 1 was prepared by adding 1 equiv. of didodecylamine to a stirring solution of dioxane containing 2 equiv. of intermediate A and 10 equiv. of DIPEA. The solution was heated to 80 °C. After at least 48 hours (shorter reaction periods led to reduction in product yield) the reaction was evaporated using a rotary evaporator and re-dissolved in chloroform then washed three times with brine. The organic phase was then dried over magnesium sulfate, filtered, and dried in a rotary evaporator. The resulting solid was purified on a silica gel column using a chloroform to

ethyl acetate mobile phase gradient (1:9 ethyl acetate:chloroform R_f = 0.5) and confirmed on NMR before being deprotected using a mixture of 1:1 TFA and dichloromethane and evaporated to dryness to yield lipid 1 (39.7-52.7%, final product).^{1}H-NMR (500 MHz, $CHCl_3$) δ 8.30 (s, 2OH), 3.60-3.70 (m, 4H), 3.44-3.54 (t, J = 7.5, 4H), 2.63 (t, J = 5, 4H), 1.56-1.64 (m, 4H), 1.23-1.32 (m, 36H), 0.87 (t, J = 5, 6H) ; ^{13}C-NMR (125 MHz, $CHCl_3$) δ 196.46, 175.78, 161.64, 154.61, 107.24, 48.06, 36.65, 33.71, 31.82, 29.57, 29.55, 29.53, 29.29, 29.26, 27.77, 26.95, 22.59, 14.02; HRMS MW calculated for $C_{33}H_{62}N_6O_4$ $(M + H)^+$: = 607.4905; found: 697.4904.

Lipid 2 was prepared in the same manner as compound 1 using dioctadecylamine and yielded compound 2 (21.7-27.6 %, final product). ^{1}H-NMR (400 MHz, $CHCl_3$) δ 8.18 (s, 2COOH), 3.39-3.74 (m, 8H), 2.53-2.79 (m, 4H), 1.52-1.64 (m, 4H), 1.18-1.33 (m, 60H), 0.86 (t, J = 6, 6H); ^{13}C-NMR (100 MHz, $CHCl_3$) δ 175.64, 161.67, 154.74, 48.24, 36.62, 33.44, 31.89, 29.68, 29.63, 29.60, 29.58, 29.37, 29.33, 27.84, 27.01, 22.66, 14.08; HRMS MW calculated for $C_{45}H_{86}N_6O_4$ $(M + H)^+$: = 775.6783; found: 775.6790.

Lipid 3 was prepared by adding 1 equiv. of didodecylamine to a stirring solution of dioxane containing 2 equiv. of intermediate B and 10 equiv. of DIPEA. The solution was heated to 80 °C. After at least 48 hours (shorter reaction periods led to reduction in product yield) the reaction was evaporated and dissolved in chloroform then washed three times with brine. The organic phase was then dried over magnesium sulfate, filtered, and dried using a rotary evaporator. The resulting solid was purified on a silica gel column using a chloroform to ethyl acetate mobile phase gradient (ethyl acetate R_f = 0.46) and confirmed on NMR before being deprotected using a mixture of 1:1 TFA in dichloromethane and evaporated to dryness to yield lipid 3 (32-46.0%, final product).^{1}H-NMR (500 MHz, $CHCl_3$) δ 3.28-3.48 (m, 8H), 2.77 (t, J = 7.5, 4H), 1.68 (t, J = 7.5, 4H), 1.48-1.58 (m, 4H), 1.16-1.32 (m, 36H), 0.86 (t, J = 7.5, 6H); ^{13}C-NMR (125 MHz, $CHCl_3$) δ 164.88, 46.71,

31.90, 29.67, 29.65, 29.63, 29.53, 29.34, 28.04, 27.11, 22.66, 14.10; HRMS MW calculated for $C_{33}H_{68}N_8$ $(M + H)^+$: = 557.6540; found: 577.5639.

Lipid 4 was prepared in the same manner as compound 3 using dioctadecylamine and yielded (55.8-56%, final product). ^{1}H-NMR (500 MHz, $CHCl_3$) δ 3.31-3.49 (m, 8H), 2.82-3.04 (m, 4H), 1.72-1.92 (m, 4H), 1.48-1.58 (m, 4H), 1.17-1.34 (m, 60H), 0.86 (t, *J* = 7.5, 6H); ^{13}C-NMR (125 MHz, $CHCl_3$) δ 164.54, 46.78, 31.83, 29.62, 29.56, 29.45, 29.26, 27.93, 27.05, 22.59, 14.02; HRMS MW calculated for $C_{45}H_{92}N_8$ $(M + H)^+$: = 745.7518; found: 745.7526. Of note, the peak resolution of this compound was poor and while several attempts were made to improve the quality of the spectra using various solvents alone and in combination, as well as various additives, the definition could not be improved beyond that presented here.

Lipid 5 was prepared by adding 1 equiv. of didodecylamine to a stirring solution of dioxane containing 2 equiv. of intermediate C and 10 equiv. DIPEA. The solution was heated to 80 °C. After at least 48 hours (shorter reaction periods led to reduction in product yield) the reaction was concentrated by rotary evaporation and re-dissolved in chloroform then washed three times with brine. The organic phase was then dried over magnesium sulfate, filtered, and dried using a rotary evaporator. The resulting solid was deprotected using a mixture of 1:1 TFA in dichloromethane and evaporated to dryness. The resulting solid was purified by silica gel chromatography by first eluting impurities with chloroform and ethyl acetate, then eluting the final product with methanol. The methanol fraction was dried and re-dissolved in chloroform before being filtered over magnesium sulfate to yield lipid 5 (90.6%, final product).^{1}H-NMR (500 MHz, $CHCl_3$) δ 8.40 (OH), 7.67 (s, NH), 3.47-3.70 (m, 8H), 2.62-2.75 (m, 4H), 1.55-1.66 (m, 4H), 1.42 (t, *J* = 8.6, SH), 1.23-1.33 (m, 36H), 0.87 (t, J = 7, 6H); ^{13}C-NMR (125 MHz, $CHCl_3$) δ 176.27, 162.51, 161.61, 154.95, 154.41, 117.19, 114.89, 93.02, 48.19 43.93, 36.35, 33.06, 31.81, 30.91, 29.53, 29.52, 29.35,

29.29, 29.24, 27.83, 27.77, 27.61, 26.99, 26.93, 23.31, 22.58, 14.00; HRMS MW calculated for $C_{32}H_{62}N_6O_2S$ $(M + H)^+$: = 595.4728; found: 595.4735.

Lipid 6 was prepared in the same manner as compound 5 using dioctadecylamine and yielded lipid 6 (72.6%, final product). ^{1}H-NMR (500 MHz, $CHCl_3$) δ 9.01 (s, OH), 7.78 (s, NH), 3.44-3.73 (m, 8H), 2.65-2.74 (m, 4H), 1.53-1.65 (m, 4H), 1.42 (t, *J* = 8.6, SH), 1.22-1.31 (m, 6oH), 0.86 (t, *J* = 7, 6H); ^{13}C-NMR (125 MHz, $CHCl_3$) δ 175.46, 162.97, 161.61, 155.09, 154.54, 117.59, 114.71, 48.32, 44.02, 36.83, 33.24, 30.89, 31.01, 29.68, 29.63, 29.59, 29.43, 29.37, 29.33, 27.91, 27.07, 27.02, 23.41, 22.66, 14.08, 13.08; HRMS MW calculated for $C_{44}H_{86}N_6O_2S$ $(M + H)^+$: = 763.6606; found: 763.6604.

Lipid 7 was prepared by adding 8 equiv. of morpholine to 1 equiv. of intermediate D dissolved in chloroform and refluxed overnight. After 48 hours, the reaction was first washed with 0.5 M NaOH, then brine and the organic phase was evaporated to yield lipid 7 (99.3%, final product) (ethyl acetate, R_f = 0.75). ^{1}H-NMR (500 MHz, $CHCl_3$) δ 3.67-3.75 (m, 16H), 3.44 (t, *J* = 7.5, 4H), 1.50-1.57 (m, 4H), 1.22-1.32 (m, 60H), 0.88 (t, *J* = 7.5, 6H); ^{13}C-NMR (125 MHz, $CHCl_3$) δ 165.34, 164.96, 66.84, 46.74, 43.55, 31.84, 29.62, 29.60, 29.58, 29.57, 29.56, 29.42, 29.27, 27.84, 27.01, 22.60, 14.03; HRMS MW calculated for $C_{47}H_{90}N_6O_2$ $(M + H)^+$: = 771.7198; found: 771.7197.

Lipid 8 was prepared by adding 1 equiv. of intermediate E to 8 equiv. of morpholine in xylenes or dioxane and heating to 80 °C for 48 hours. The solvent was removed using a rotary evaporator at 80-90°C and the resulting solid was dissolved in chloroform and washed three times with 0.5 M HCl then twice with brine. The organic phase contained a number of impurities and was purified by silica gel chromatography using at 0-10% ethyl acetate:chloroform mobile phase gradient. The pure product was then confirmed on NMR before being deprotected using a mixture of 1:1 TFA

in dichloromethane and evaporated to dryness to yield lipid 8 (86.6%, final product). ^{1}H-NMR (500 MHz, $CHCl_3$) δ 8.23 (m, OH), 3.66-3.88 (m, 10H), 3.32-3.52 (m, 4H), 2.57-2.75 (m, 2H), 1.50-1.62 (m, 4H), 1.22-1.32 (m, 60H), 0.86 (t, *J* = 8, 6H); ^{13}C-NMR (125 MHz, $CHCl_3$) δ 171.88, 166.05, 165.33, 165.00, 80.95, 66.91, 46.80, 43.56, 36.48, 35.77, 31.90, 29.68, 29.65, 29.64, 29.64, 27.52, 29.34, 28.13, 27.09, 22.67, 14.09; HRMS MW calculated for $C_{46}H_{88}N_6O_3$ $(M + H)^+$: = 773.6991; found: 773.6991.

Lipid 9 was prepared by adding 20 equiv. of *N,N*-dimethyl-1,3-diaminopropane to a stirring solution of intermediate F and 10 equiv. of DIPEA in dioxane. The reaction was allowed to stir at room temperature for 24 hours then heated at 80 °C for another 48 hours. The reaction was then concentrated using a rotary evaporator and the product was dissolved in ethyl acetate and washed three times with brine. The organic phase was collected, dried over magnesium sulfate and concentrated to yield lipid 9 (92.3 %, final product). ^{1}H-NMR (500 MHz, $CHCl_3$) δ 5.15 (s, 2NH), 3.36-3.49 (m, 4H), 3.28-3.36 (m, 4H), 2.27 (t, J = 9.6, 4H), 2.16 (s, 12H), 1.59-1.76 (m, 4H), 1.45-1.57 (m, 4H), 1.17-1.28 (m, 36H), 0.83 (t, *J* = 8.6, 6H); ^{13}C-NMR (125 MHz, $CHCl_3$) δ 165.90, 164.89, 57.63, 46.71, 45.44, 39.17, 31.83, 29.62, 29.61, 29.58, 29.56, 29.46, 29.27, 27.99, 27.72, 27.05, 22.59, 14.02; HRMS MW calculated for $C_{37}H_{76}N_8$ $(M + H)^+$: = 633.6266; found: 633.6270.

Lipid 10 was prepared in the same manner as compound 9 using intermediate D and yielded lipid 10 (93.4 %, final product). ^{1}H-NMR (500 MHz, $CHCl_3$) δ 5.20 (s, 2NH), 3.30-3.50 (m, 8H), 2.35 (t, *J* = 8.4, 4H), 2.22 (s, 12H), 1.64-1.78 (m, 4H), 1.46-1.59 (m, 4H), 1.18-1.32 (m, 60H), 0.83 (t, *J* = 8.6, 6H); ^{13}C-NMR (125 MHz, $CHCl_3$) δ 165.42, 164.73, 57.63, 46.79, 45.37, 39.23, 31.89, 29.68, 29.53, 29.33, 28.03, 27.55, 27.11, 22.66, 14.09; HRMS MW calculated for $C_{49}H_{101}N_8$ $(M + H)^+$: = 801.8144; found: 801.8126.

Lipid 11 was prepared by adding 4-8 equiv. of *N*-Boc-1,3-diaminopropane to a stirring solution of dioxane containing 1 equiv. of intermediate G and 10 equiv. of DIPEA. The solution was stirred at 80 °C for 72 hours after which the solvent was removed using a rotary evaporator. The resulting solid was then dissolved in chloroform and washed three times with 0.5 M HCl then twice with brine. The organic phase was dried then purified by silica gel chromatography using a chloroform to ethyl acetate gradient and the product was confirmed on NMR before being deprotected using a mixture of 1:1 TFA in dichloromethane and evaporated to dryness to yield pure lipid 11 (90.3%, final product). ^{1}H-NMR (500 MHz, $CHCl_3$) δ 7.98 (s, 3NH), 7.66 (s, OH), 3.38-3.69 (m, 8H), 2.95-3.13 (m, 2H), 2.54-2.69 (m, 2H), 1.92-2.09 (m, 2H), 1.51-1.64 (m, 4H), 1.22-1.32 (m, 36H), 0.87 (t, J = 5, 6H); ^{13}C-NMR (125 MHz, $CHCl_3$) δ 175.48, 154.54, 48.23, 31.82, 29.53, 29.26, 27.73, 27.60, 26.94, 22.59, 14.00; HRMS MW calculated for $C_{33}H_{65}N_7O_2$ $(M + H)^+$: = 592.5273; found: 592.5277.

Lipid 12 was prepared in the same manner as compound 11 using intermediate E and yielded lipid 12 (44.4%, final product). ^{1}H-NMR (500 MHz, $CHCl_3$) δ 7.92 (s, 3NH), 7.64 (s, OH), 3.30-3.72 (m, 8H), 2.92-3.20 (m, 2H), 2.51-2.72 (m, 2H), 1.89-2.15 (m, 2H), 1.53-1.63 (m, 4H), 1.20-1.34 (m, 60H), 0.87 (t, J = 7.5, 6H); ^{13}C-NMR (125 MHz, $CHCl_3$) δ 175.51, 154.49, 48.18, 31.84, 29.63, 29.28, 27.73, 26.95, 24.78, 22.60, 14.02; HRMS MW calculated for $C_{45}H_{89}N_7O_2$ $(M + H)^+$: = 760.7151; found: 760.7159.

Lipopeptide synthesis

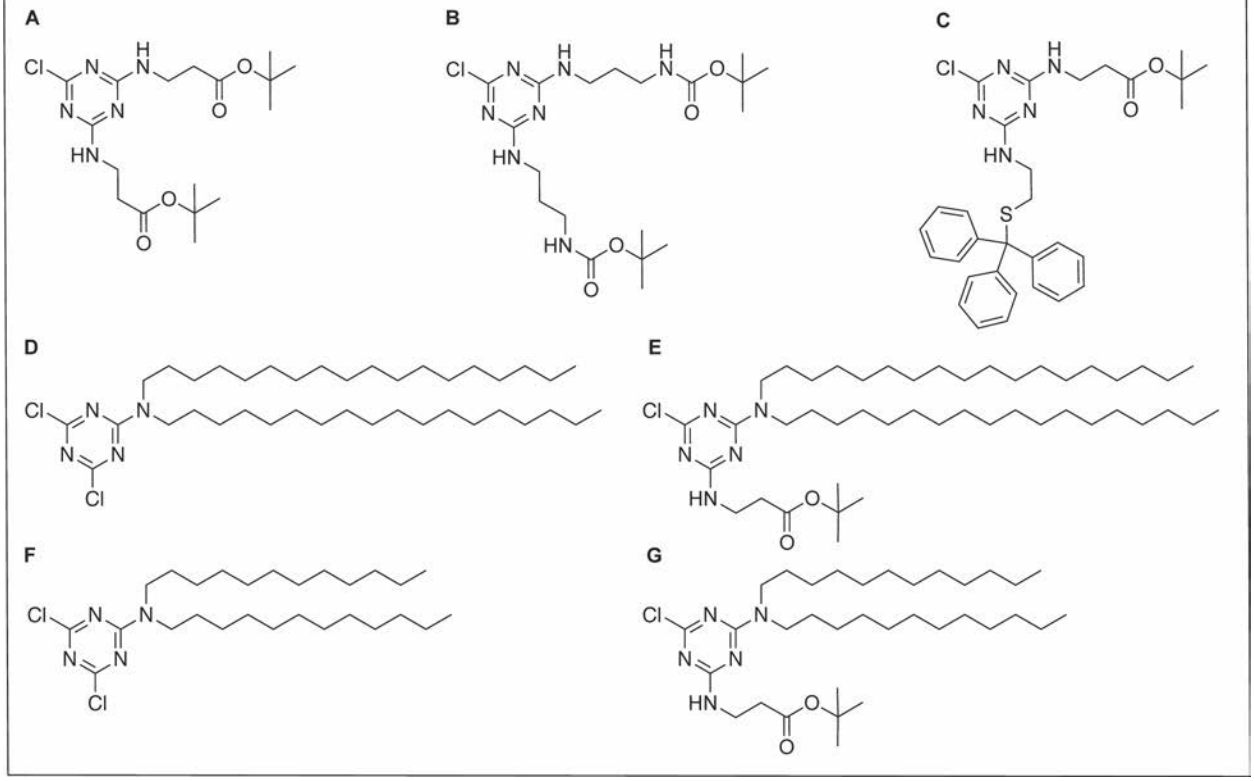
Lipidation of an ApoA-I peptide spanning the residues 141-184 of the mouse sequence (ApoA-$I_{141\text{-}184}$) was completed using intermediate D (C18 TZ linker). Resin was added to a vial, based on 22-40 mg of resin-cleaved and deprotected peptide (sequence *β*AGGLSPVAEEFRDRMRTHVDSLRTQLAPHSEQMRESLAQRLAELKSN) (Elim Biopharm, Inc.)

containing 200 mg of intermediate D and 10 equiv. of DIPEA and stirred slowly at 35°C for 72 hours (10.3-23 mg yield). After the reaction was completed, both compounds were washed extensively with chloroform to remove excess reactants and the peptide was cleaved from resin and deprotected in 4.7 mL of trifluoracetic acid, 125 µL ethanedithiol, 125 µL water and 50 µL triisopropylsilane. After 30 minutes this solution was pipetted through a glass wool filter into a conical vial containing cold diethyl ether (-20 ˚C) and left overnight at -20 ˚C. The following morning, the conical vial was centrifuged at 4000 rpm for 5 minutes and the peptide pellet was resuspended in cold ether and allowed to sit for one more day at -20 ˚C. Centrifugation was repeated and the resulting pellet was dried, weighed, and resuspended in a 1:1 mixture of water and tetrahydrofuran at 2 mg/mL. Concentration was confirmed by absorbance at 205 nm. The resulting products were further purified via HPLC using a gradient of 50 to 95% acetonitrile in water with 0.1% TFA and detected at 215 nm using the ChromeScope software provided by Waters. The purified lipopeptide isolated by HPLC yielded 10.3-23.0 mg (46.8-57.5% yield).

References:

1. Watrelot, A.A., et al., *Immobilization of flavan-3-ols onto sensor chips to study their interactions with proteins and pectins by SPR.* Applied Surface Science, 2016. **371**: p. 512-518.

Figure S1. Intermediate compounds for synthesis of trizine lipids



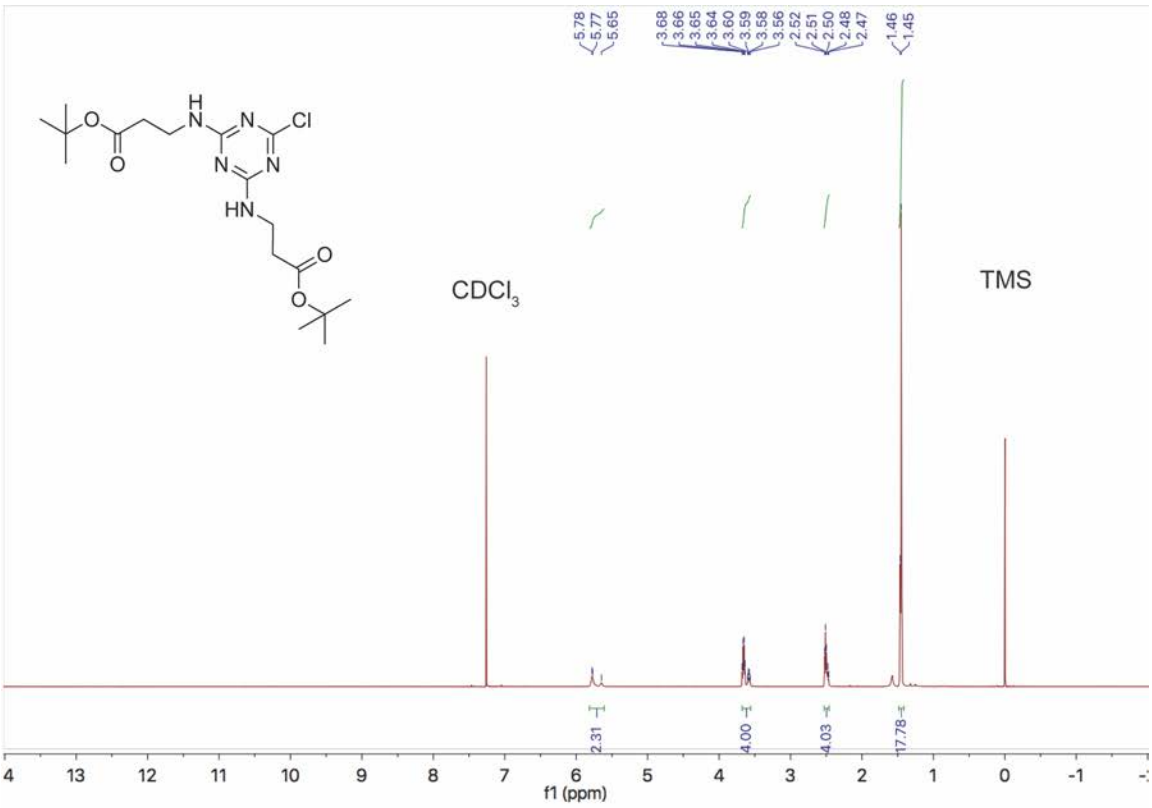


Figure S2. ^{1}H NMR of intermediate A

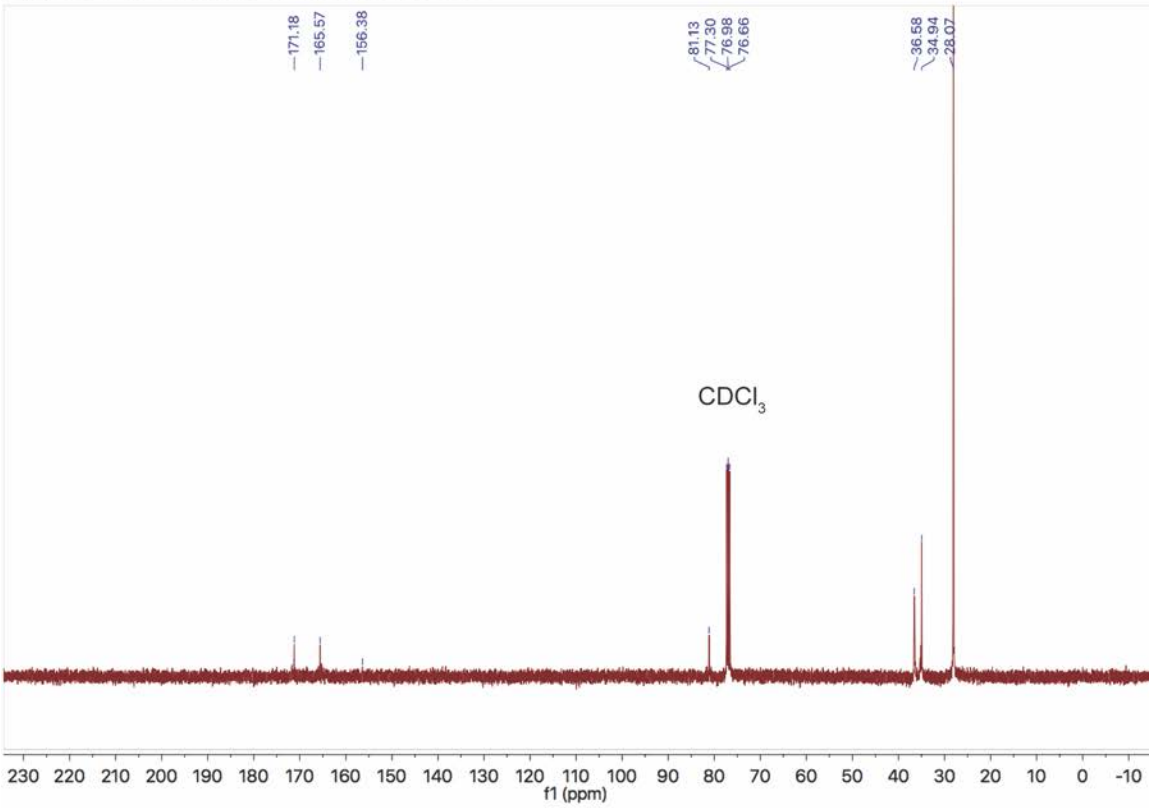


Figure S3. ^{13}C NMR of intermediate A

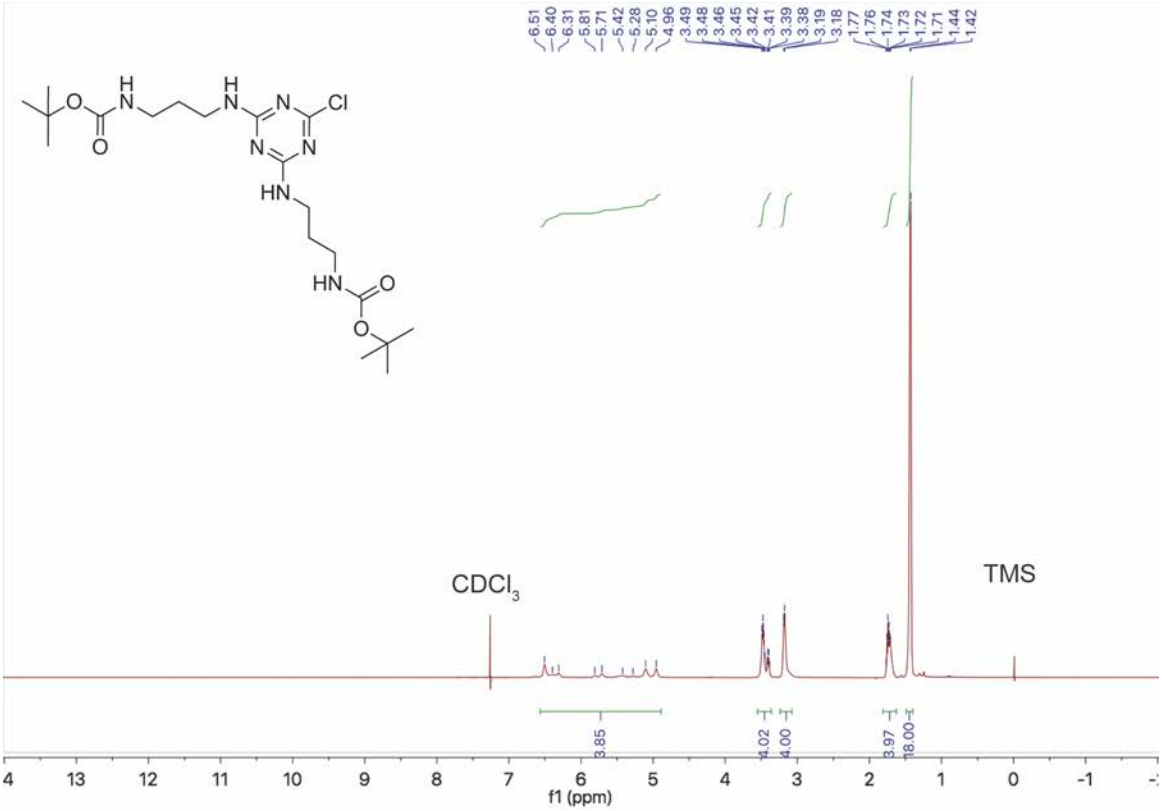


Figure S4. ^{1}H NMR of intermediate B

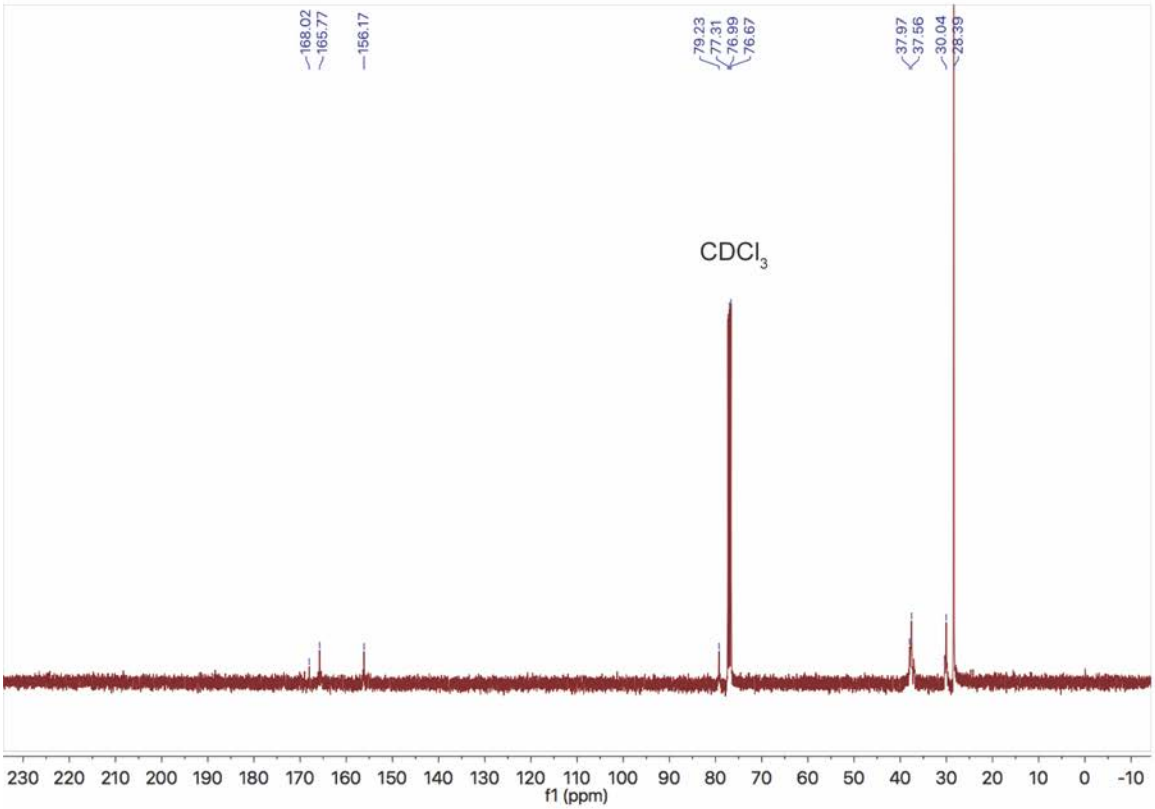


Figure S5. ^{13}C NMR of intermediate B

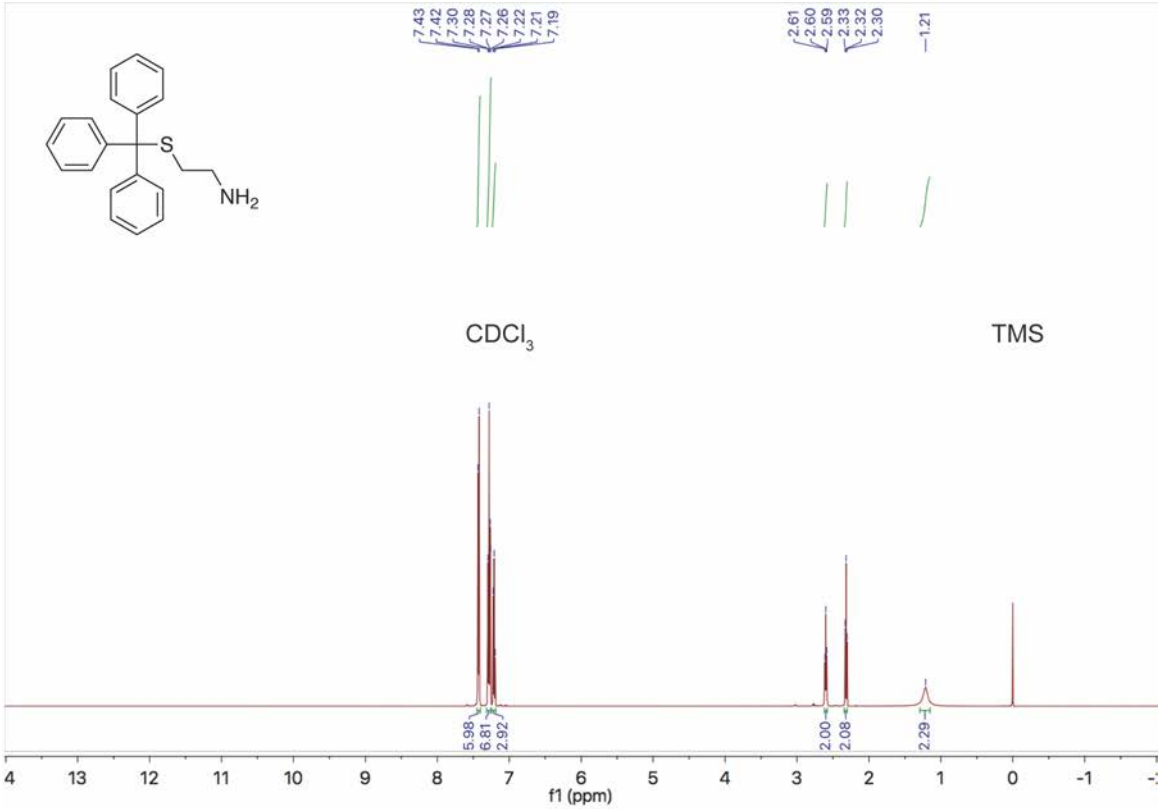


Figure S6. ^{1}H NMR of 2-[(Triphenylmethyl)thio]ethanamine

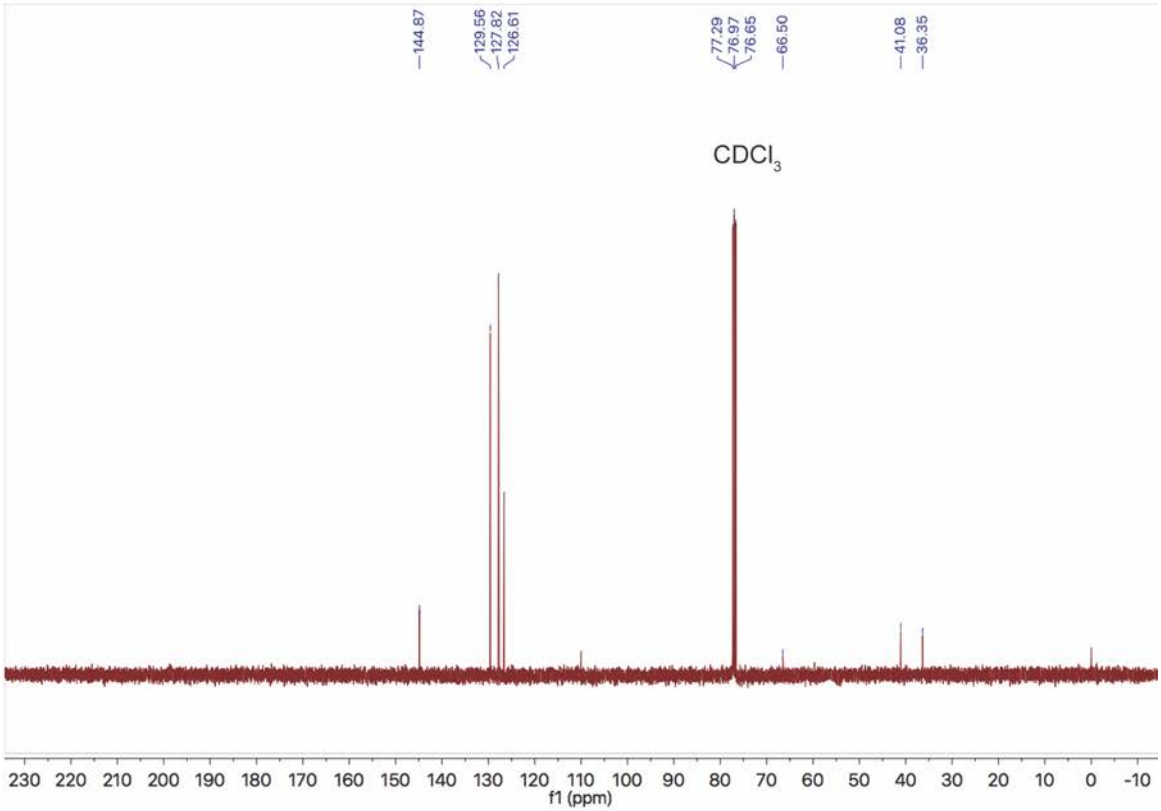


Figure S7. ^{13}C NMR of 2-[(Triphenylmethyl)thio]ethanamine

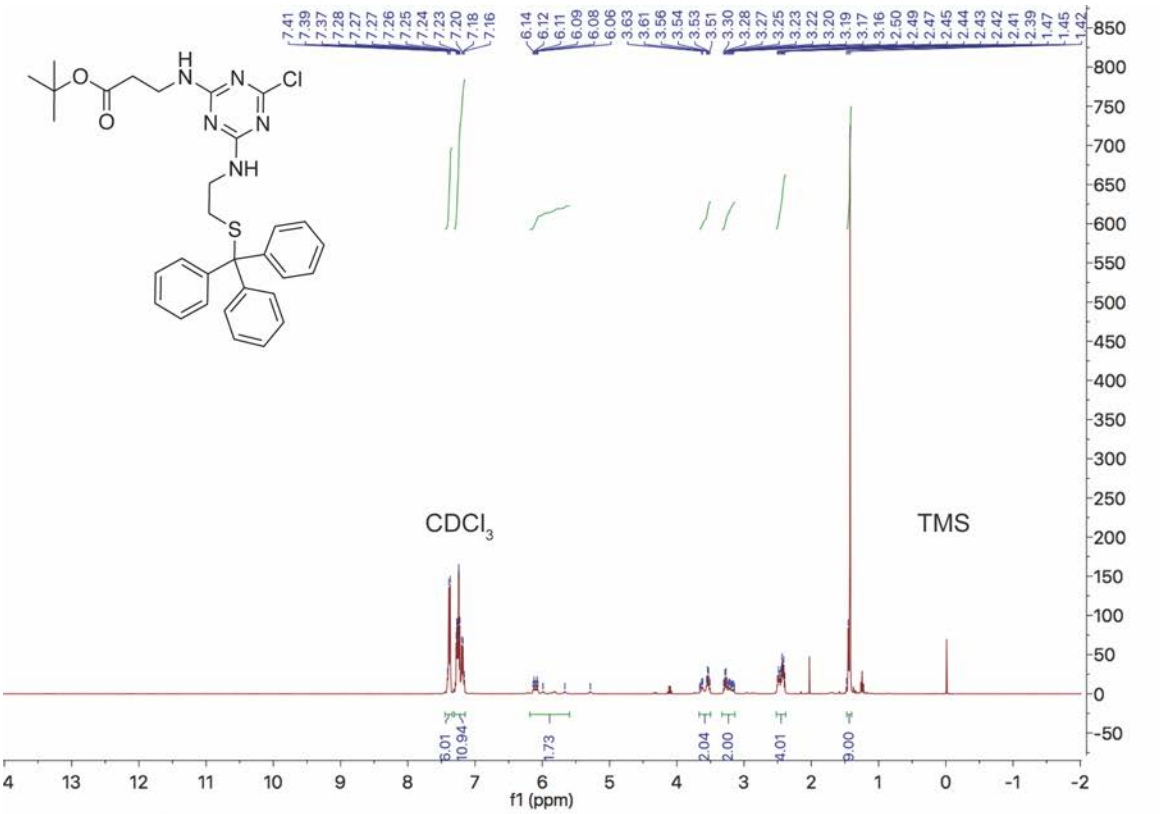


Figure S8. ^{1}H NMR of intermediate C

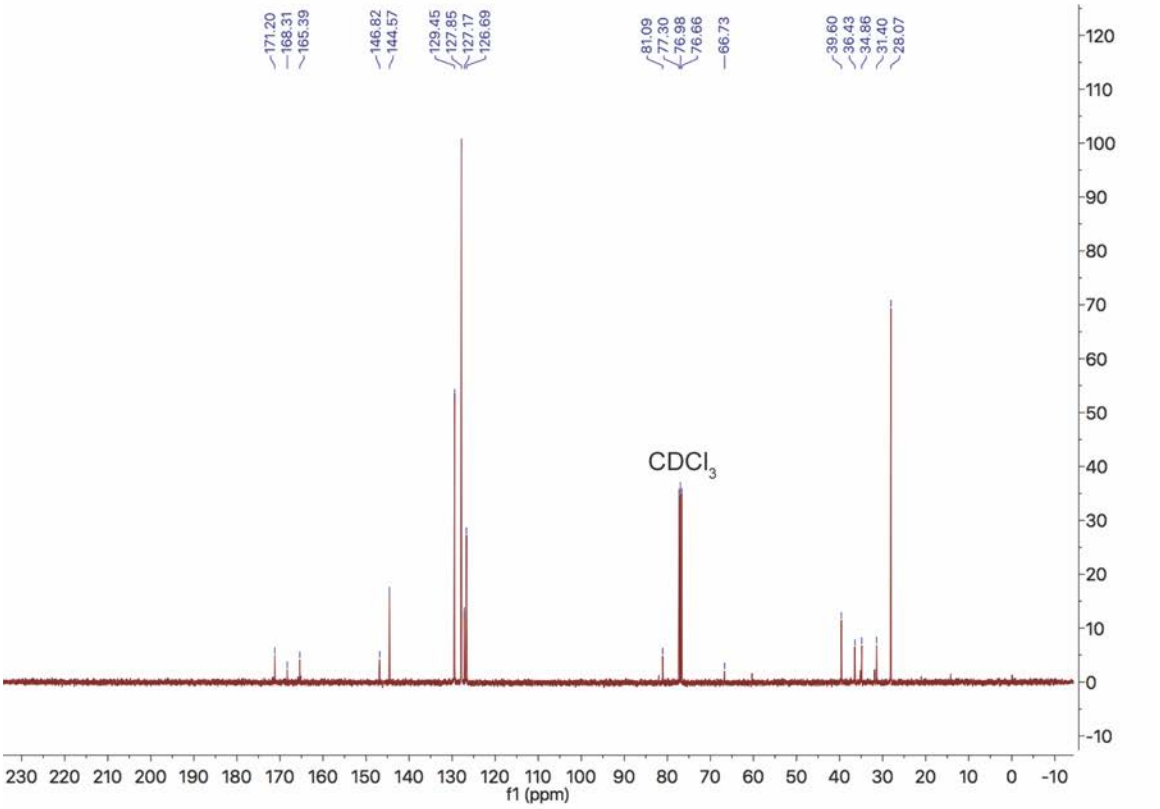


Figure S9. ^{13}C NMR of intermediate C

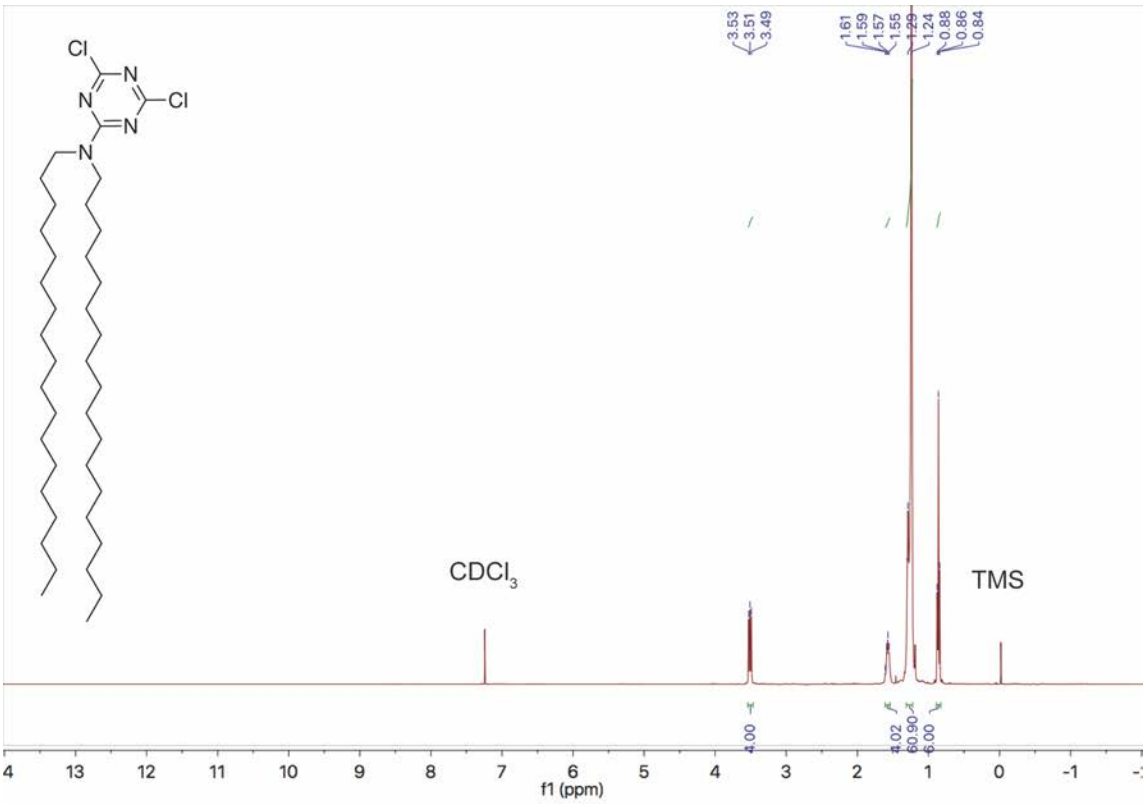


Figure S10. ^{1}H NMR of intermediate D

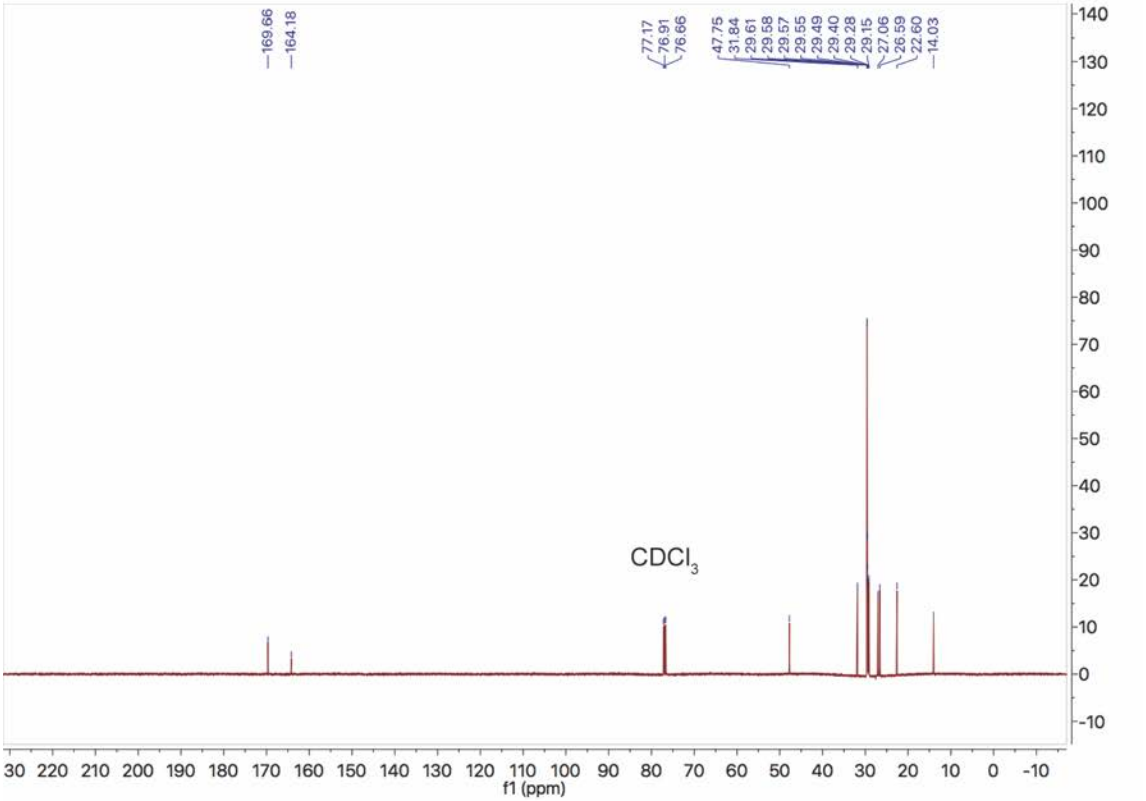


Figure S11. ^{13}C NMR of intermediate D

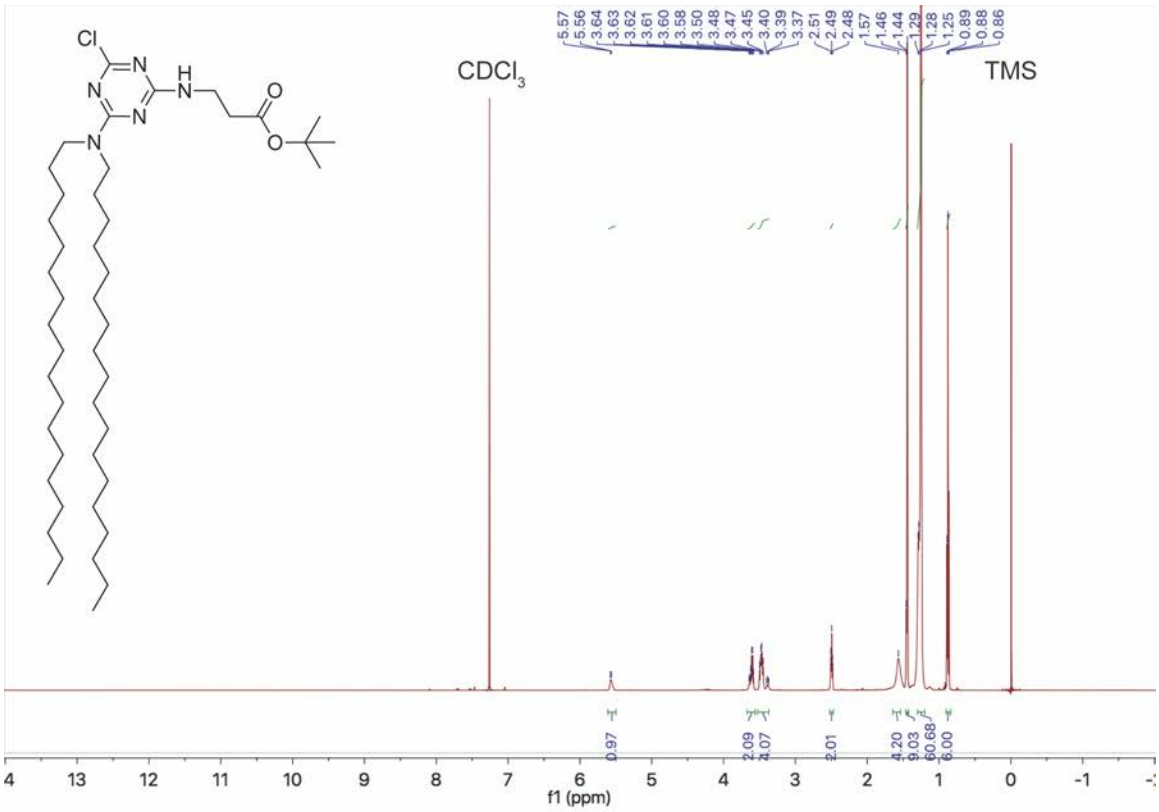


Figure S12. ^{1}H NMR of intermediate E

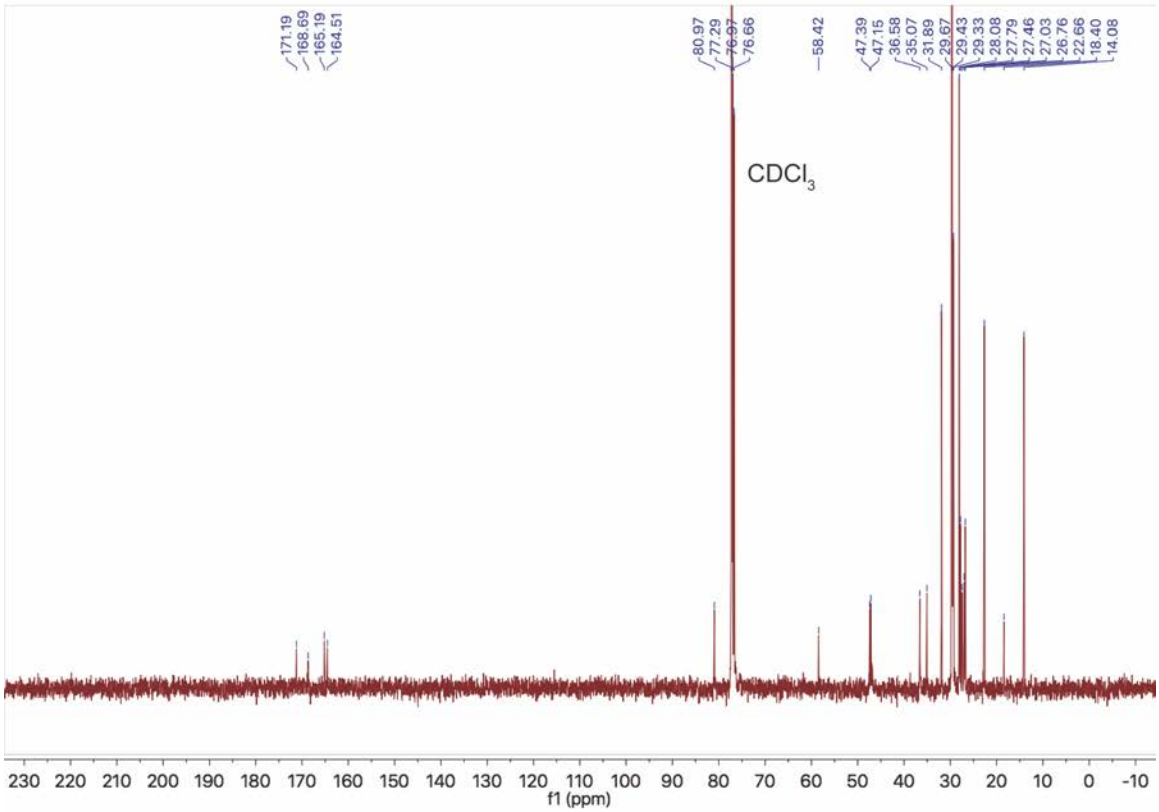


Figure S13. ^{13}C NMR of intermediate E

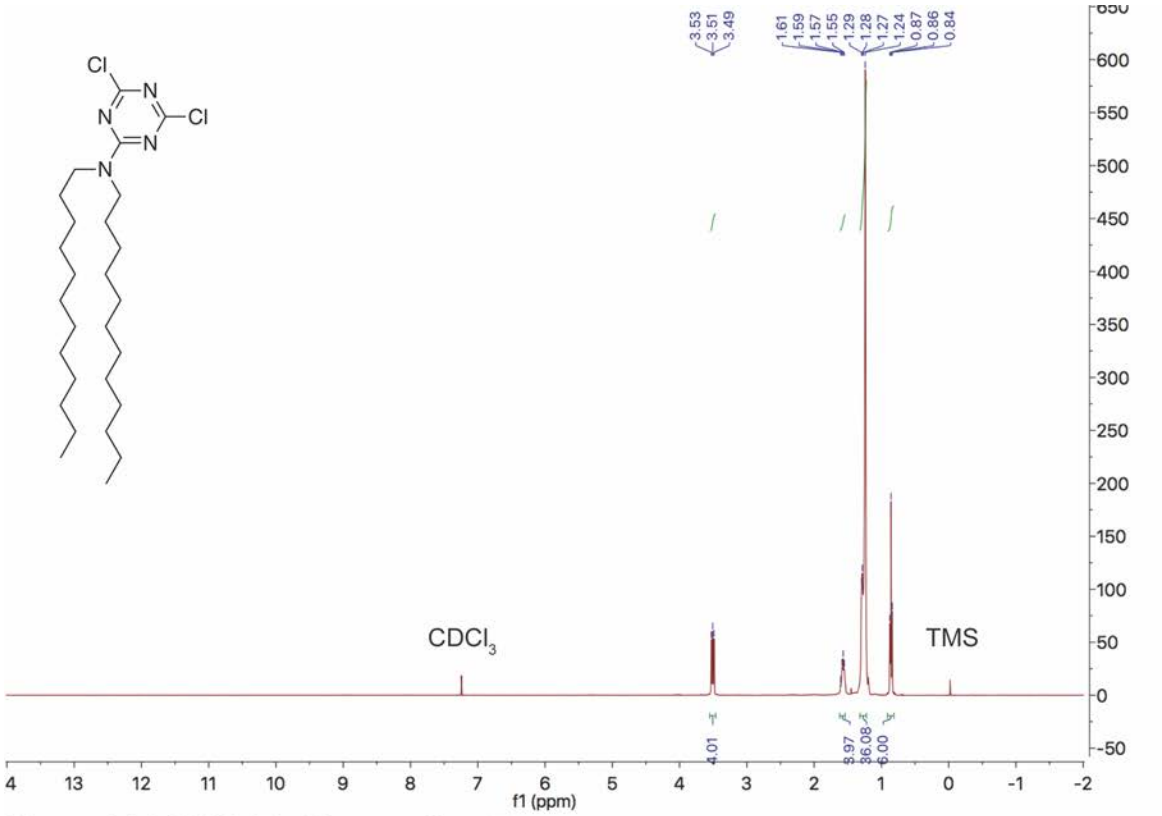


Figure S14. ^{1}H NMR of intermediate F

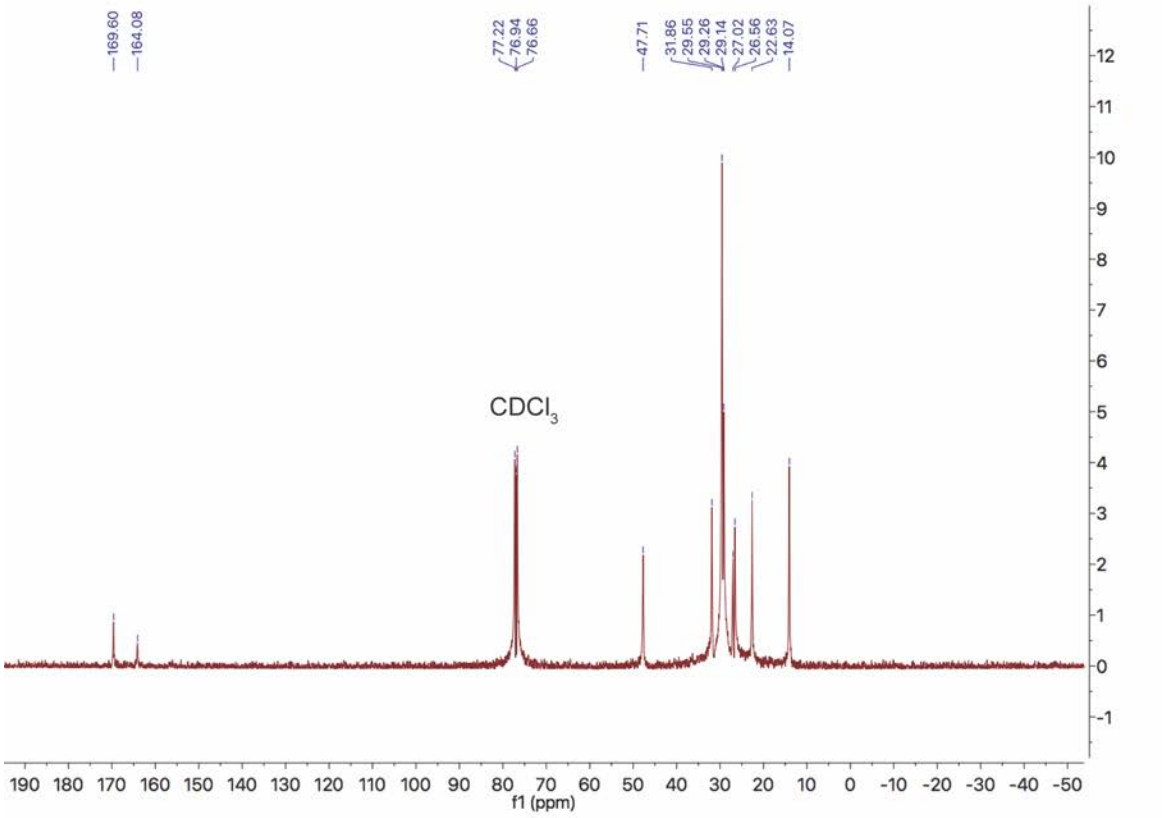


Figure S15. ^{13}C NMR of intermediate F

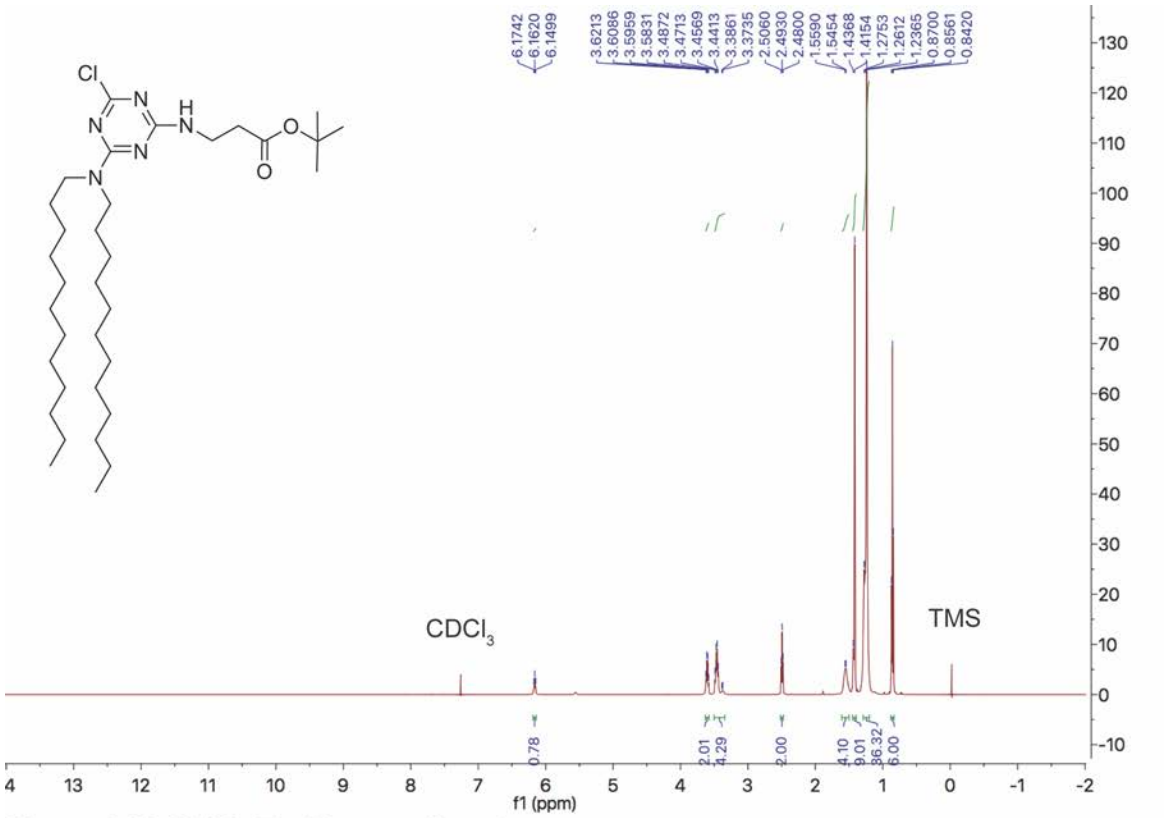


Figure S16. ^{1}H NMR of intermediate G

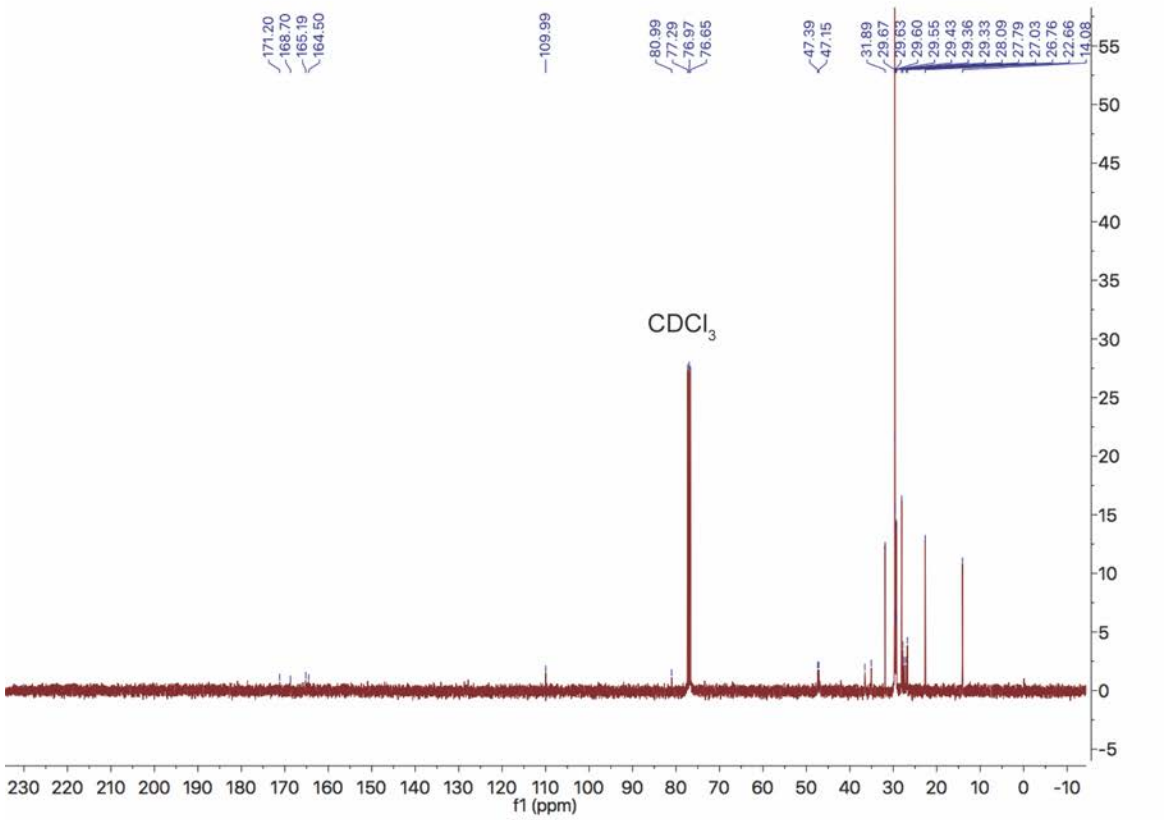


Figure S17. ^{13}C NMR of intermediate G

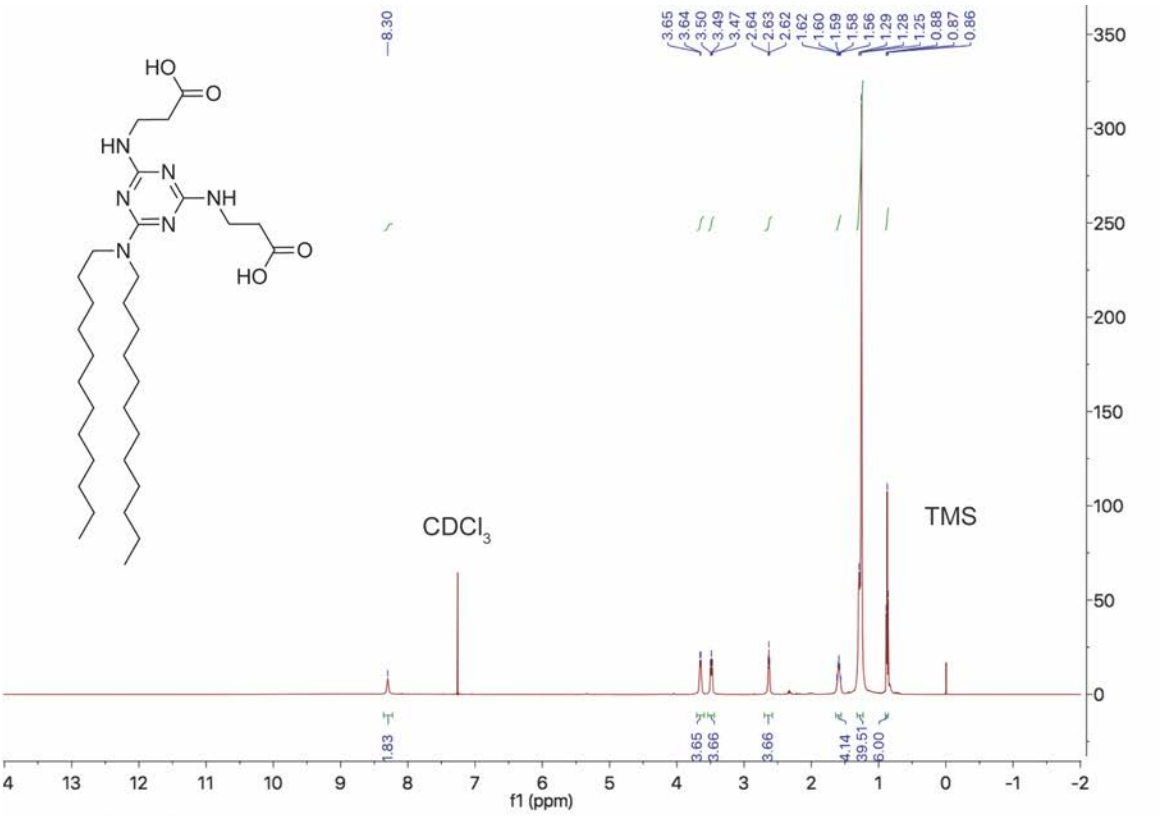


Figure S18. ^{1}H NMR of lipid 1

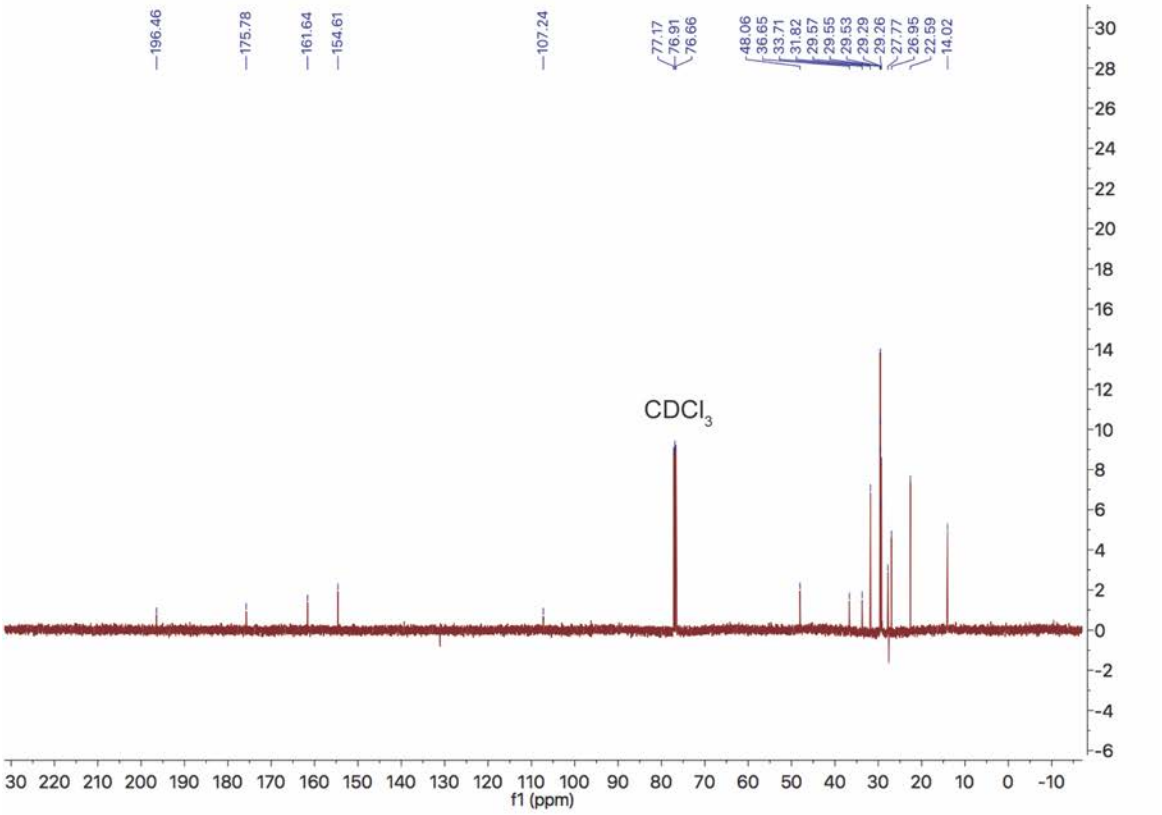


Figure S19. ^{13}C NMR of lipid 1

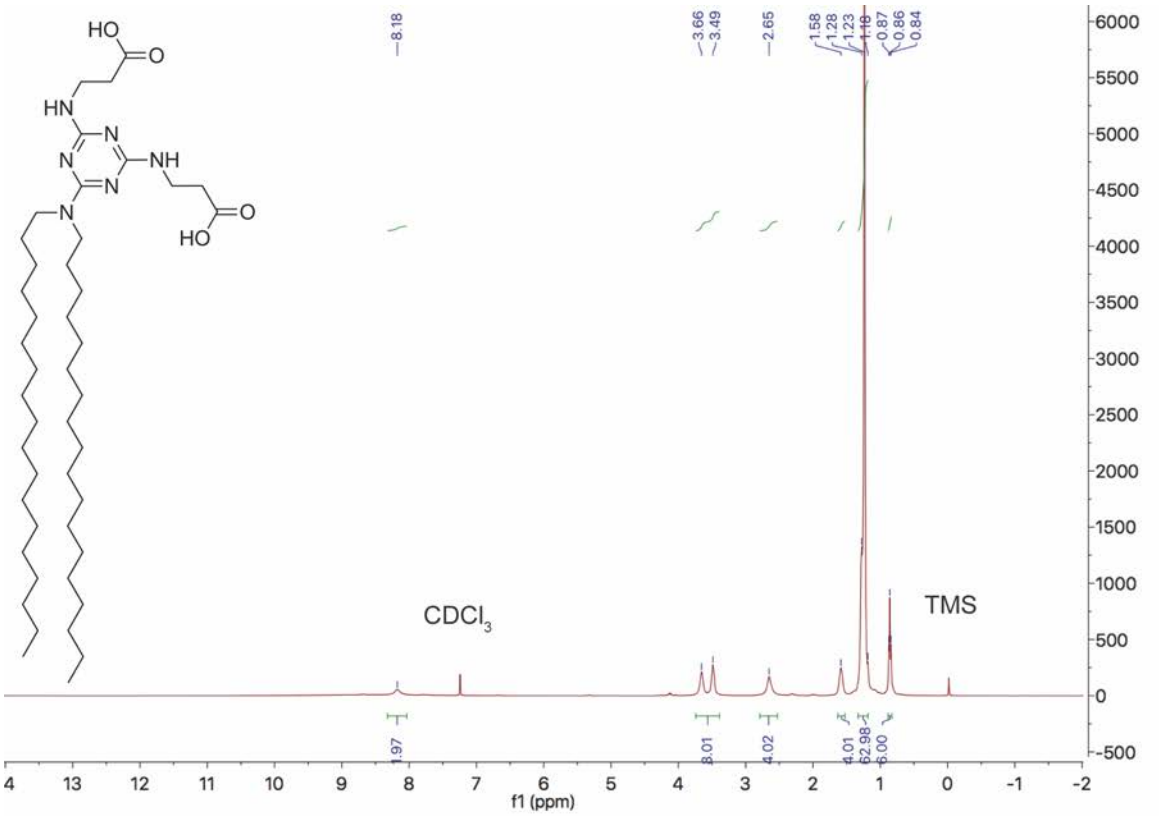


Figure S20. ^{1}H NMR of lipid 2

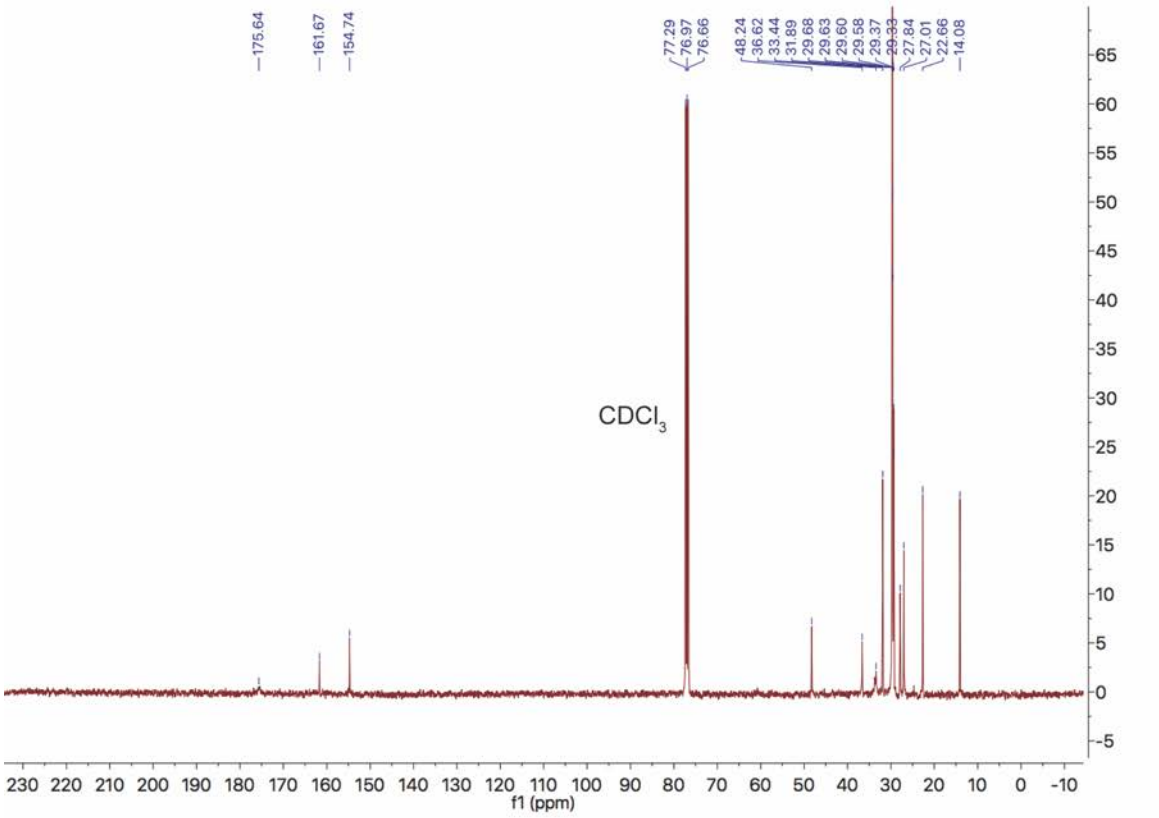


Figure S21. ^{13}C NMR of lipid 2

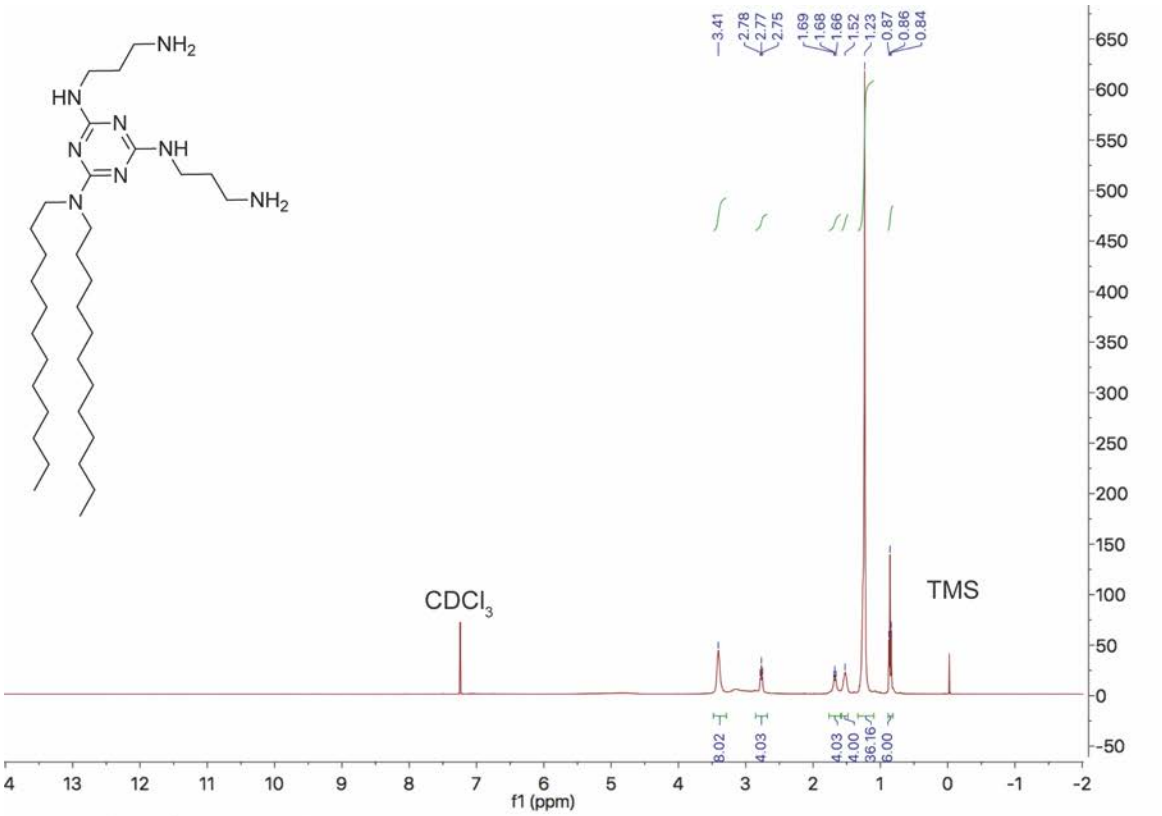


Figure S22. ^{1}H NMR of lipid 3

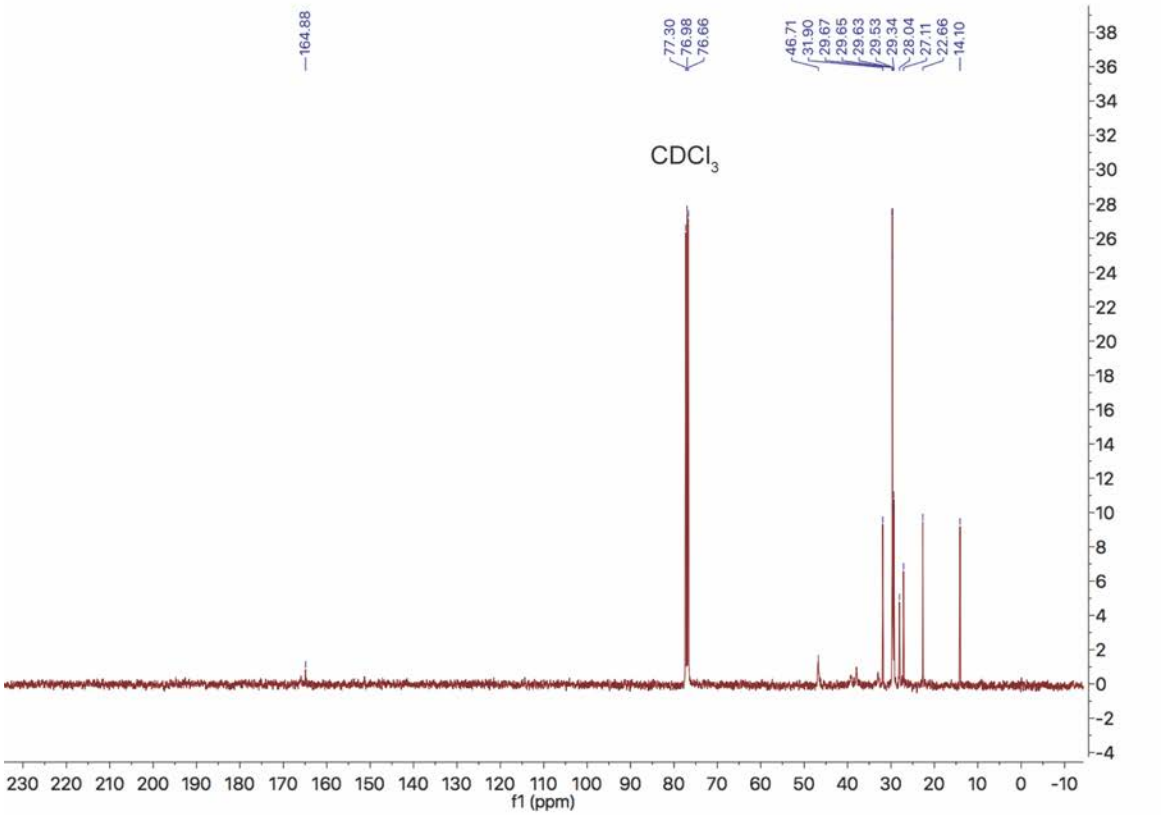


Figure S23. ^{13}C NMR of lipid 3

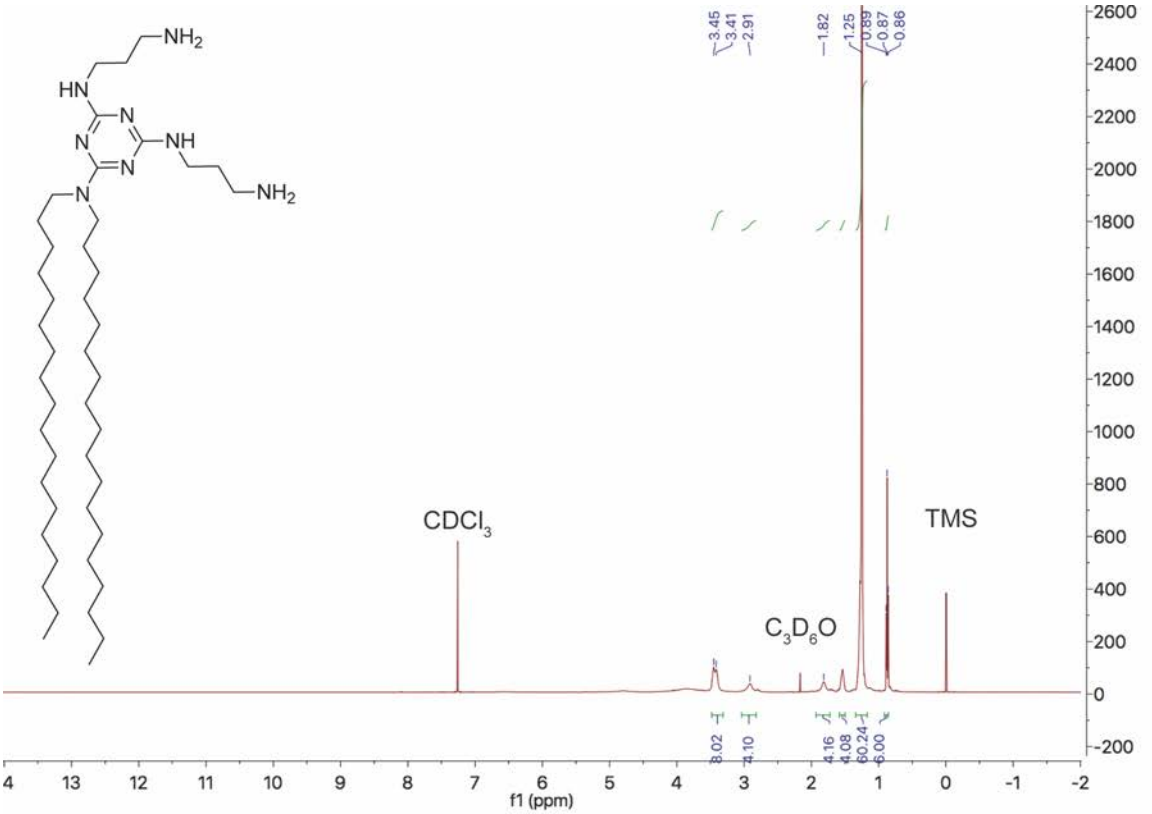


Figure S24. ^{1}H NMR of lipid 4

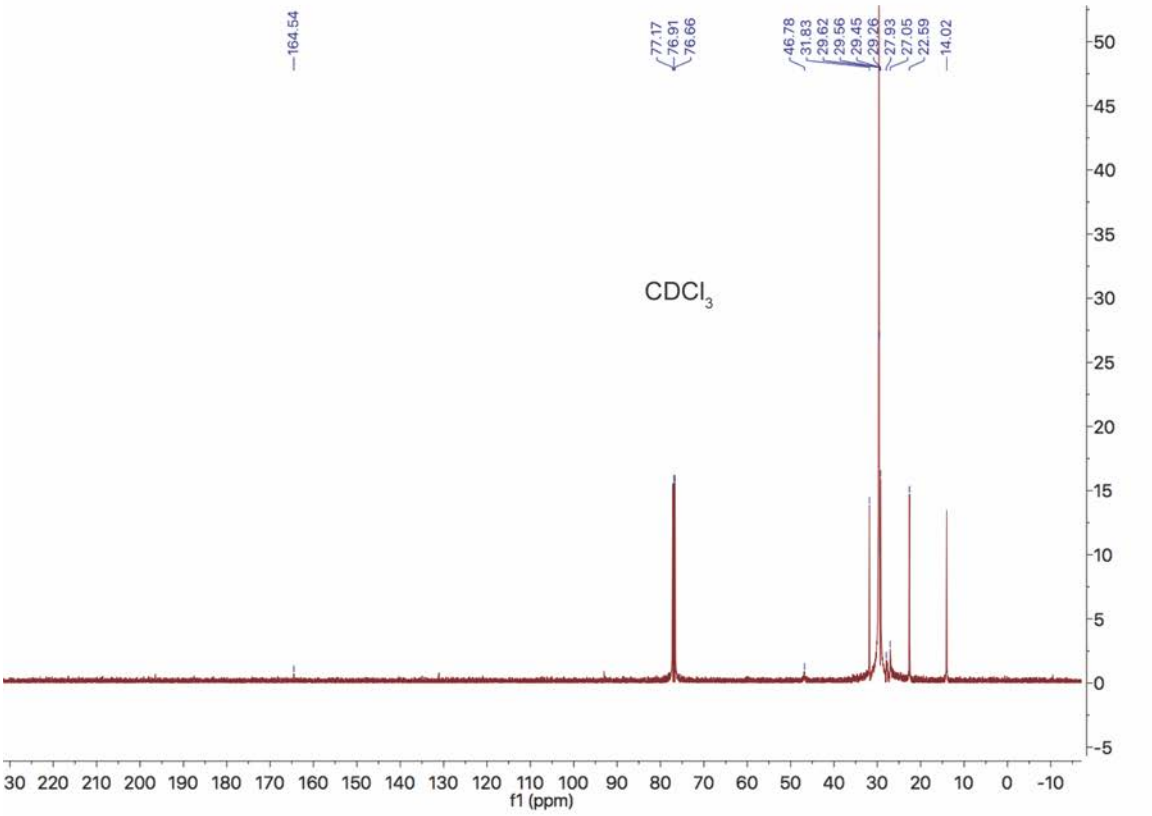


Figure S25. ^{13}C NMR of lipid 4

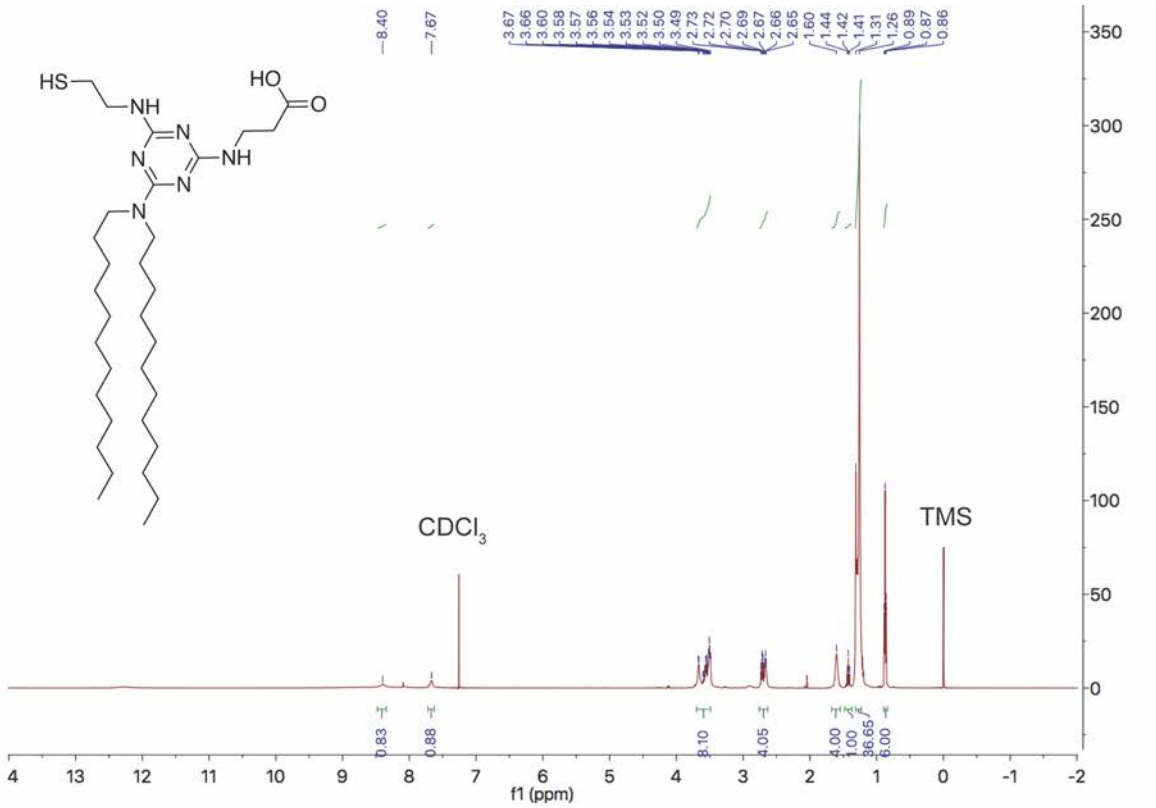


Figure S26. ^{1}H NMR of lipid 5

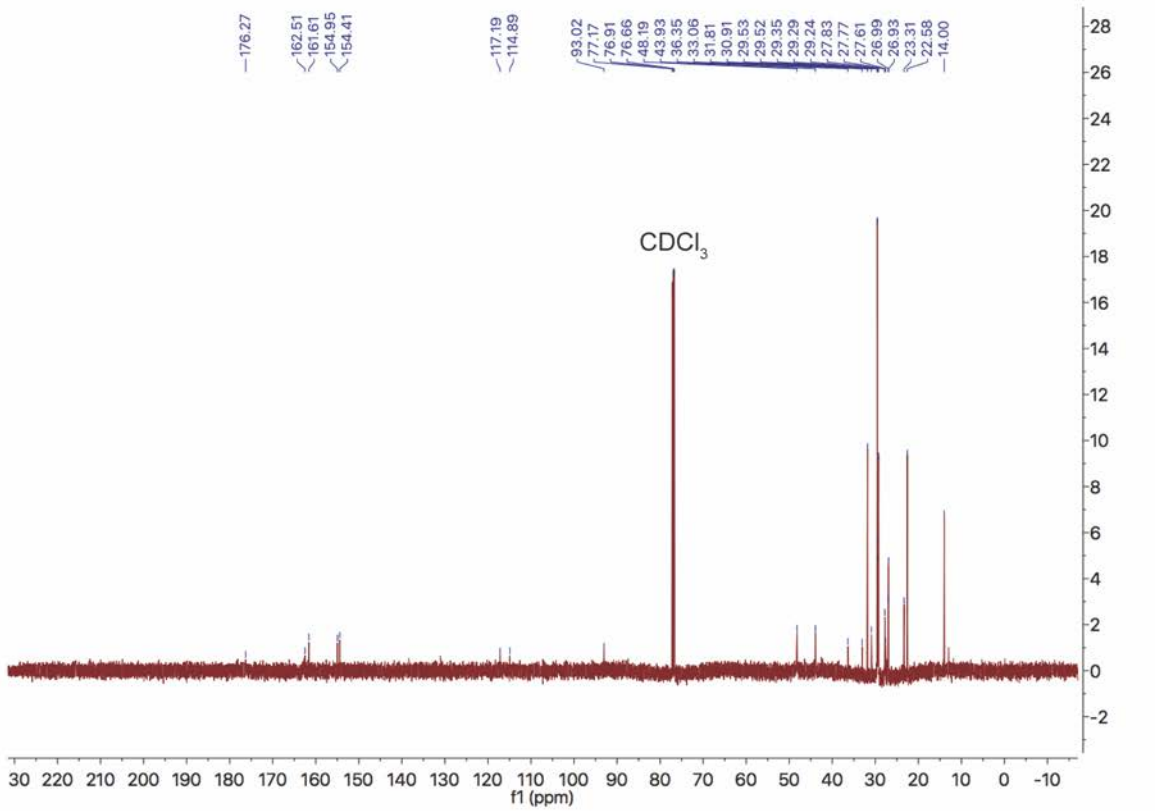


Figure S27. ^{13}C NMR of lipid 5

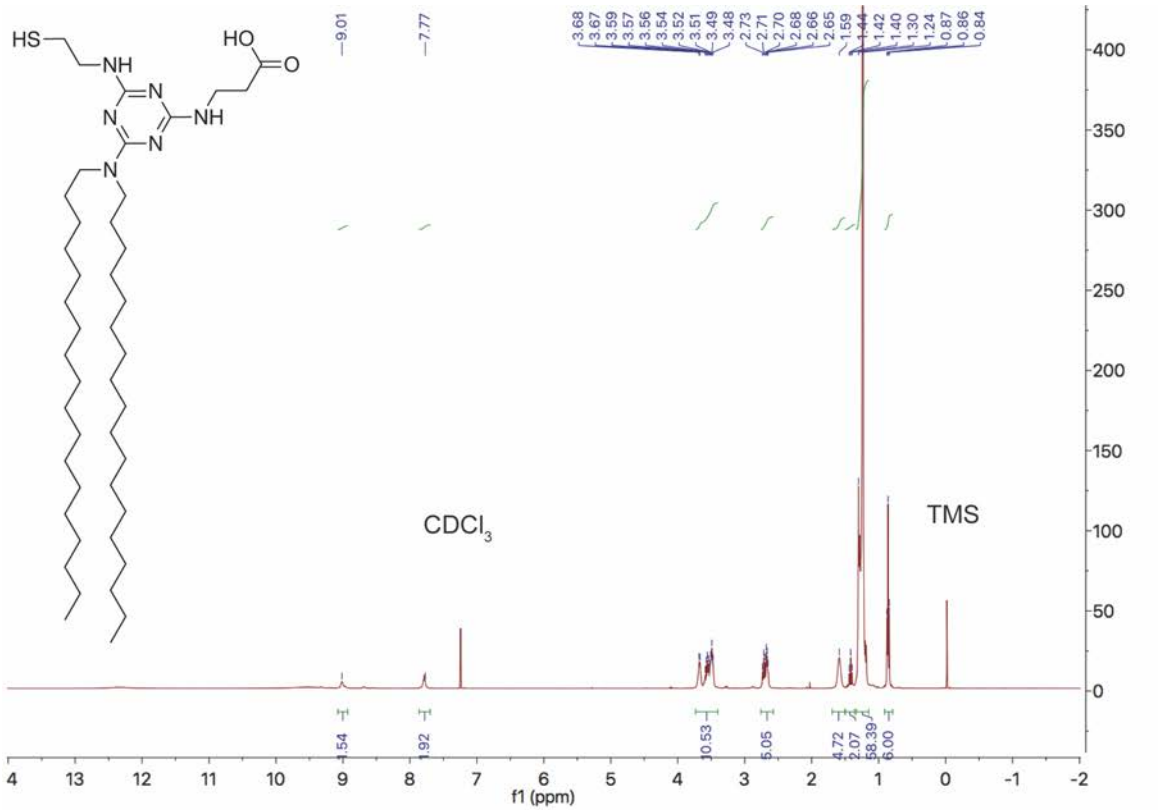


Figure S28. ^{1}H NMR of lipid 6

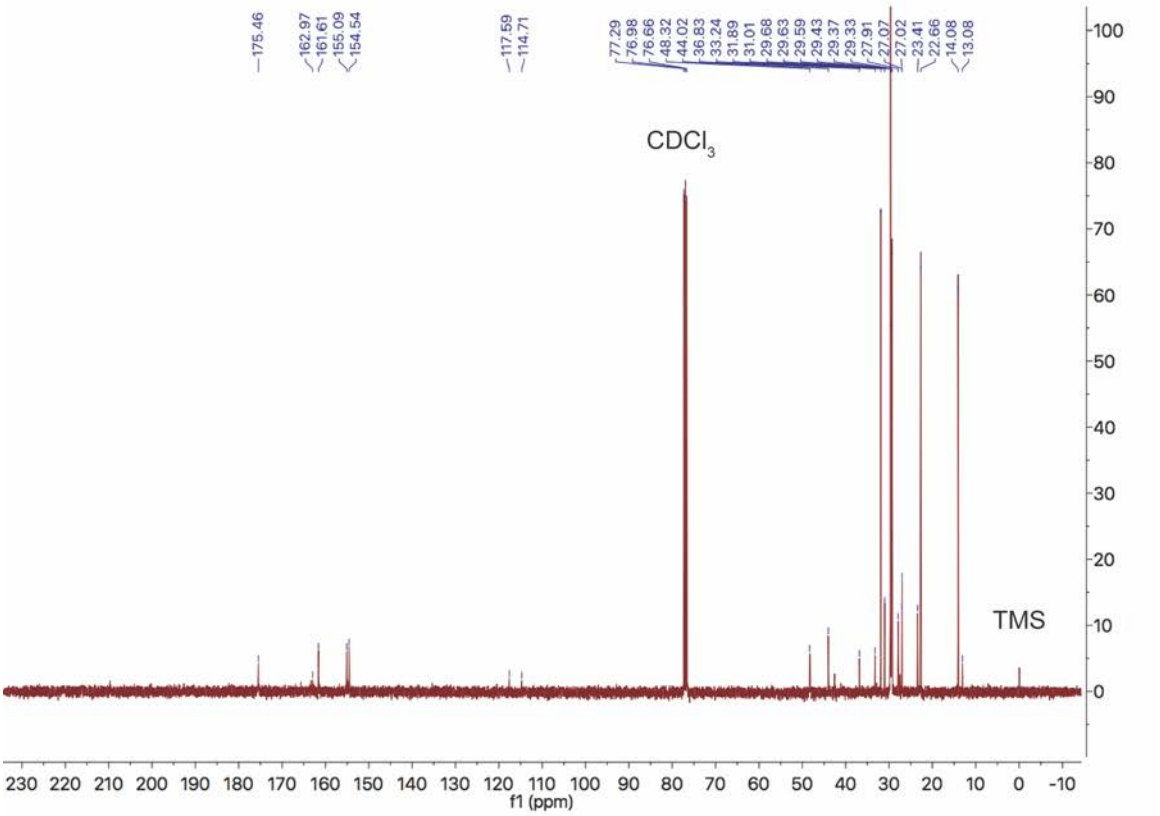


Figure S29. ^{13}C NMR of lipid 6

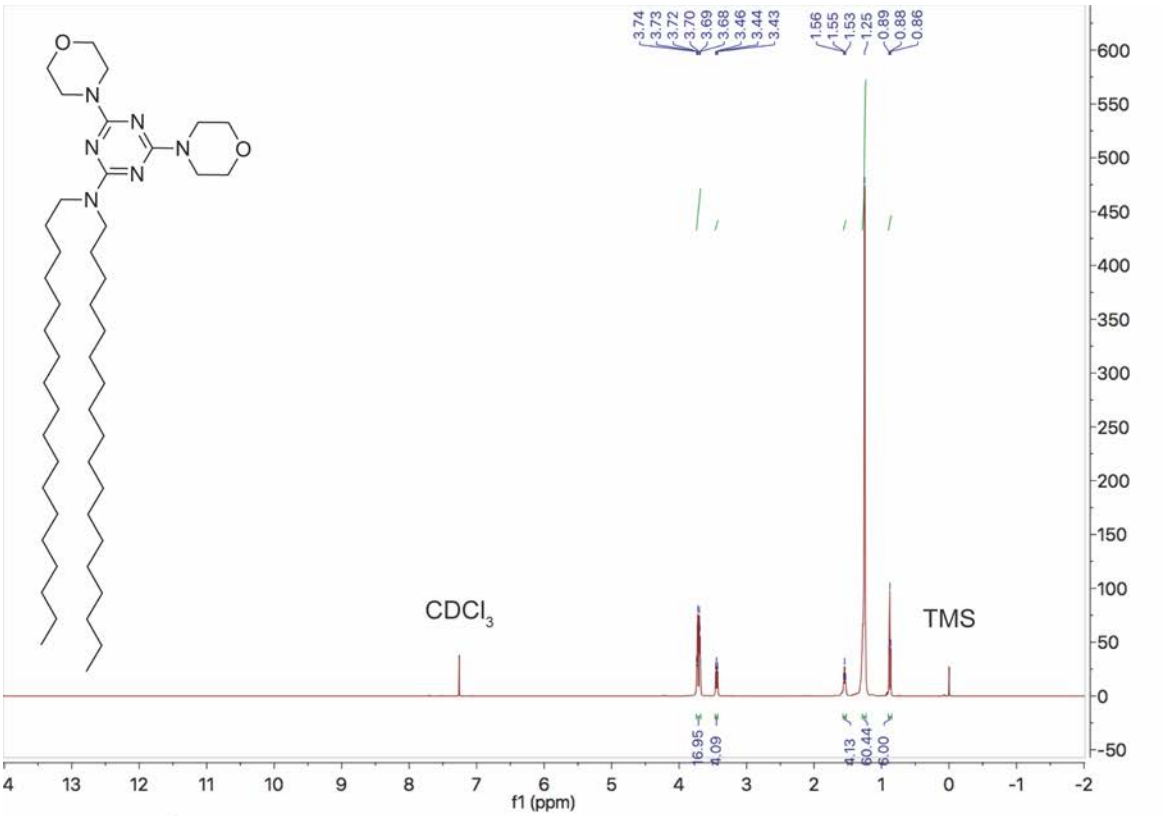


Figure S30. ^{1}H NMR of lipid 7

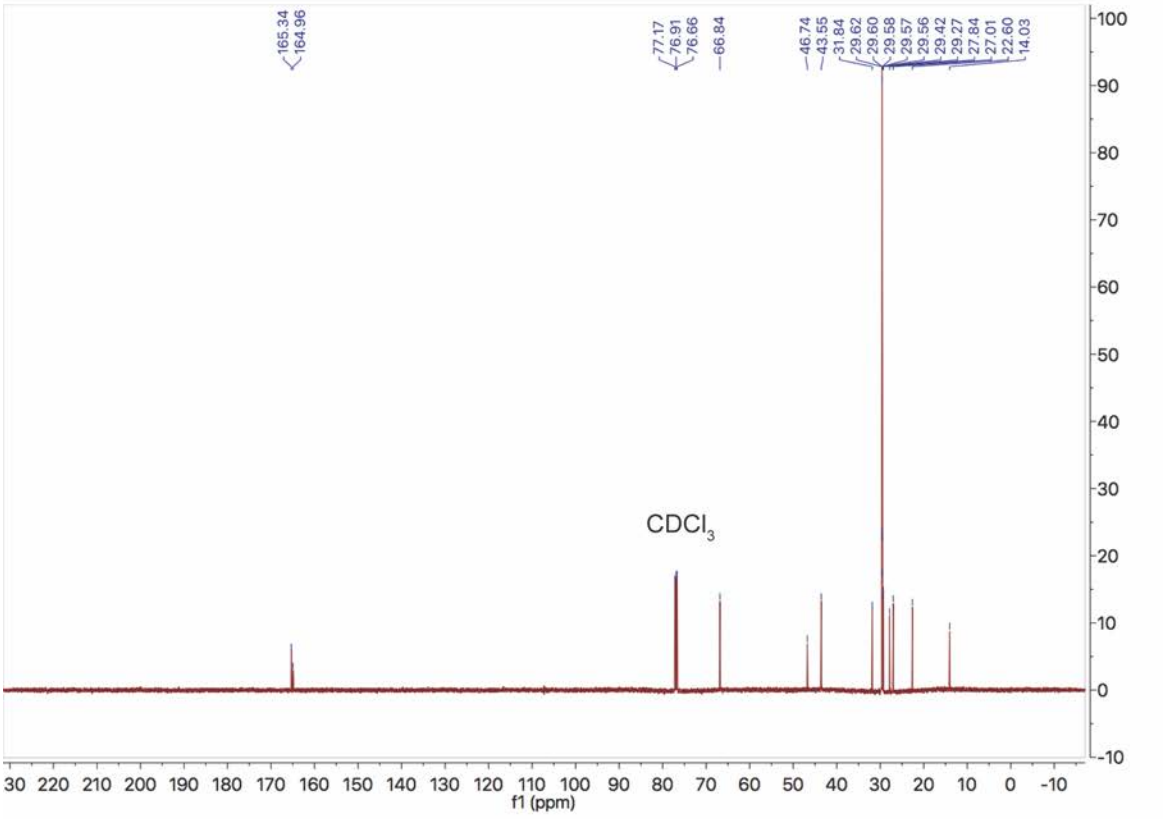


Figure S31. ^{13}C NMR of lipid 7

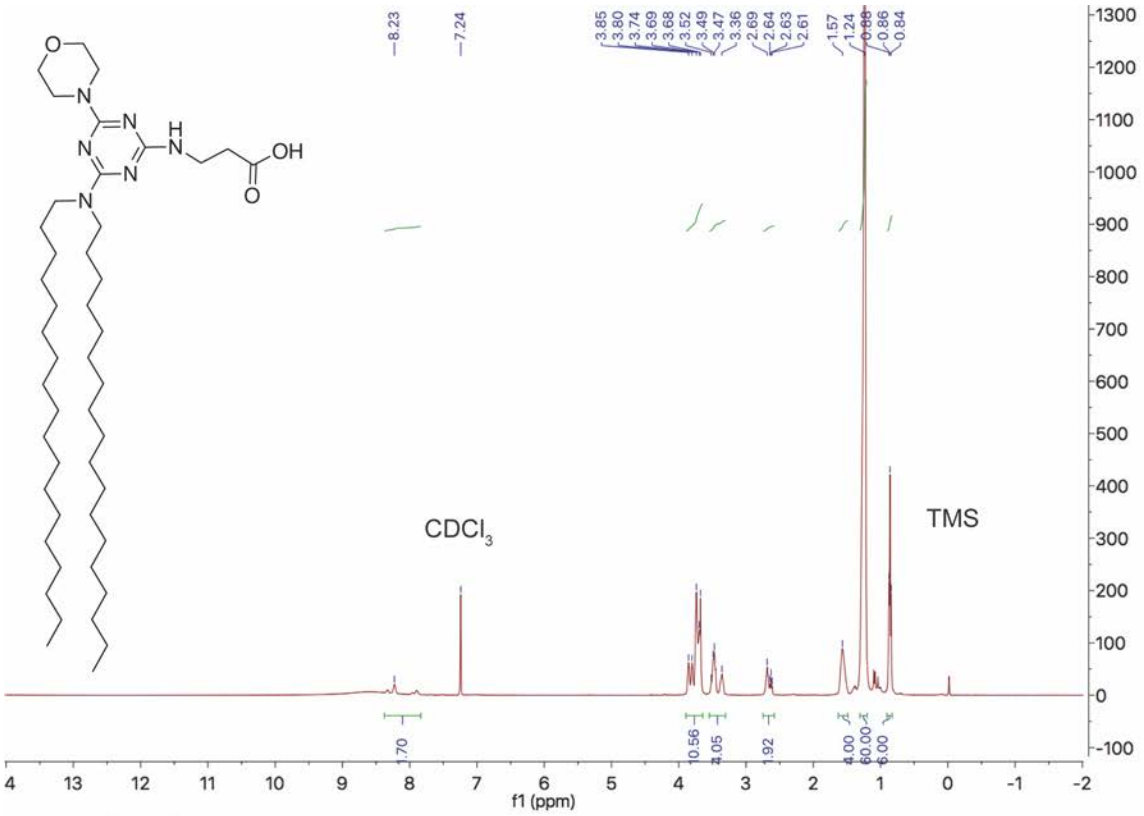


Figure S32. ^{1}H NMR of lipid 8

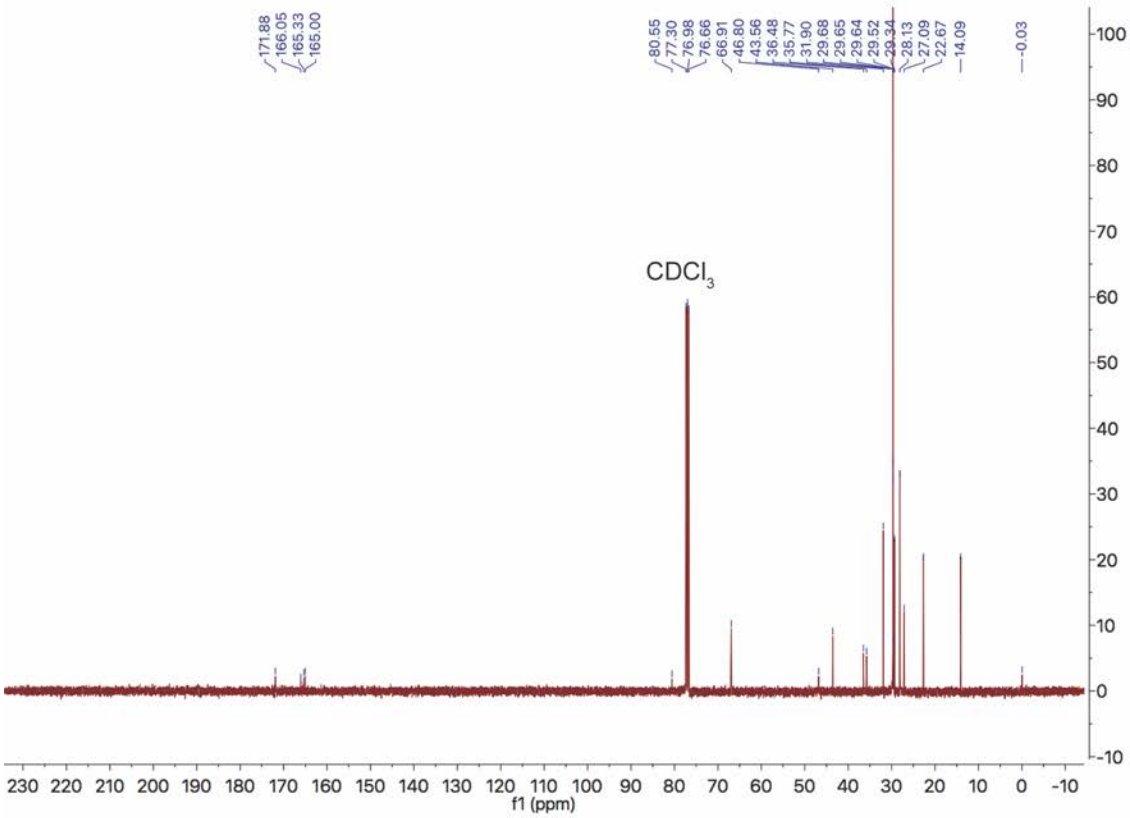


Figure S33. ^{13}C NMR of lipid 8

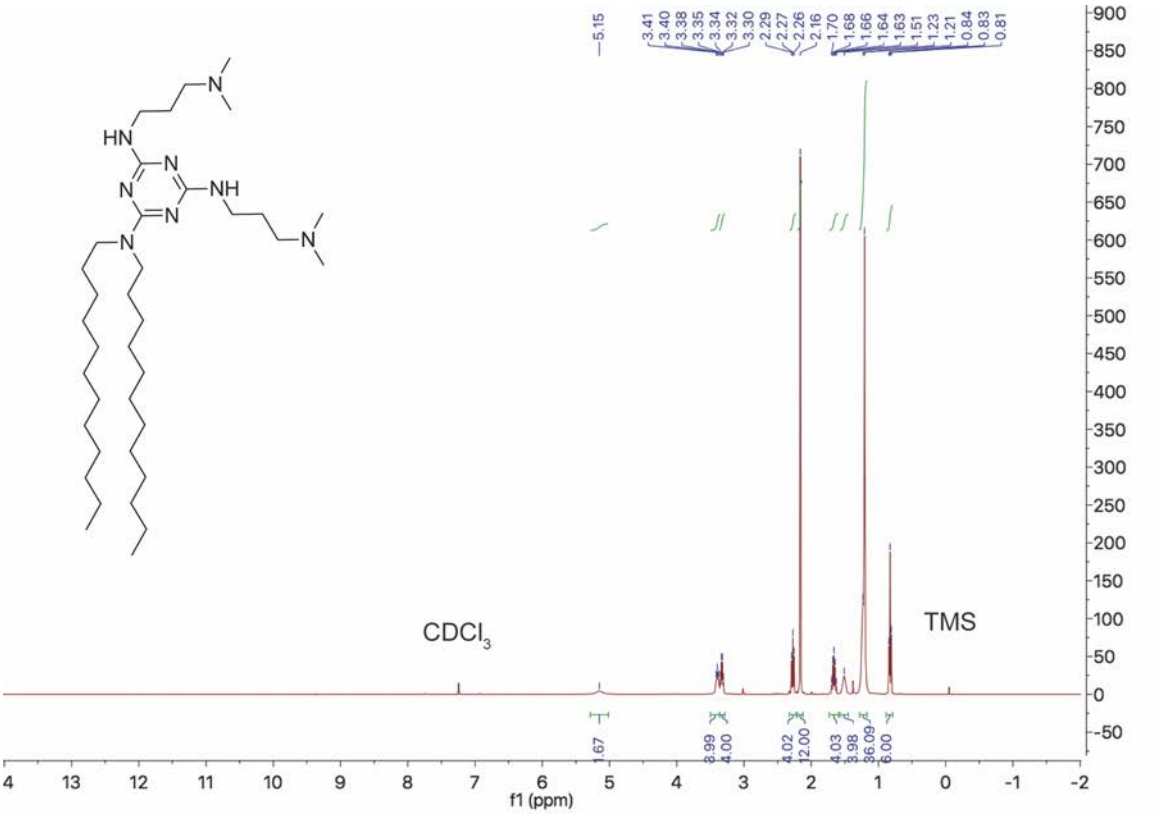


Figure S34. ^{1}H NMR of lipid 9

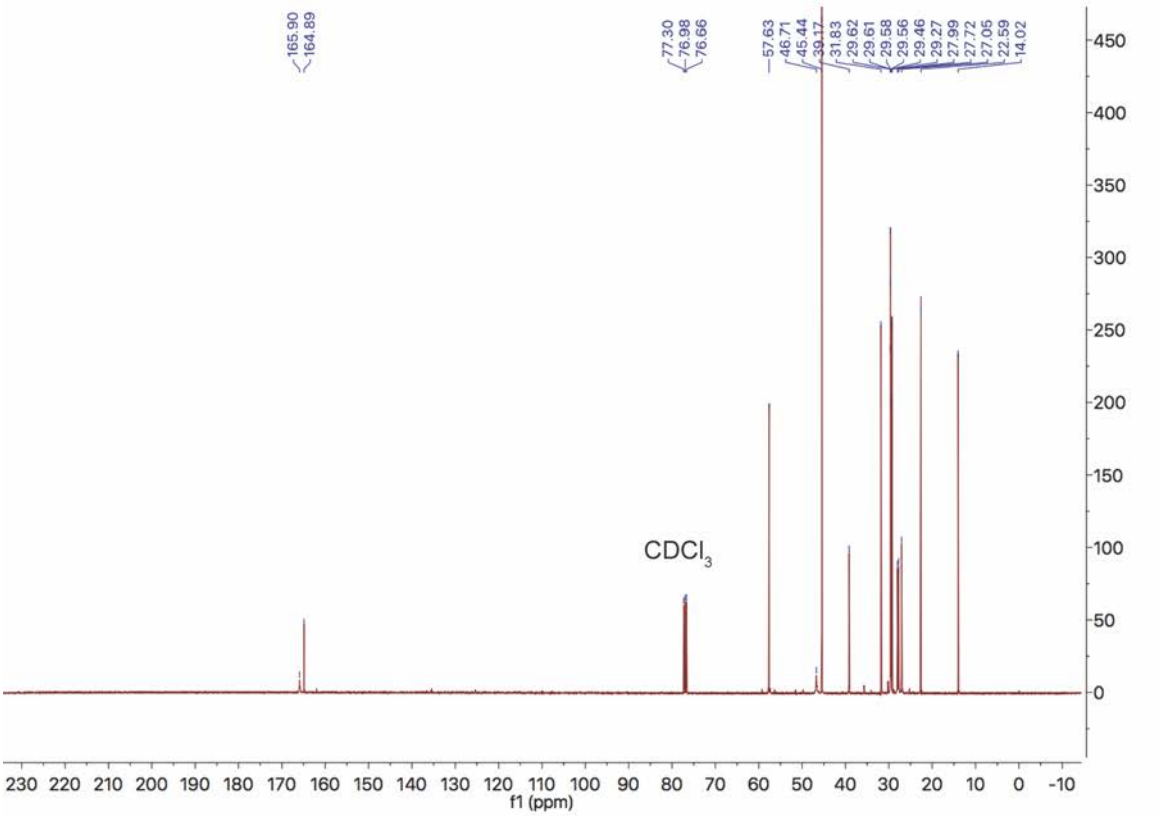


Figure S35. ^{13}C NMR of lipid 9

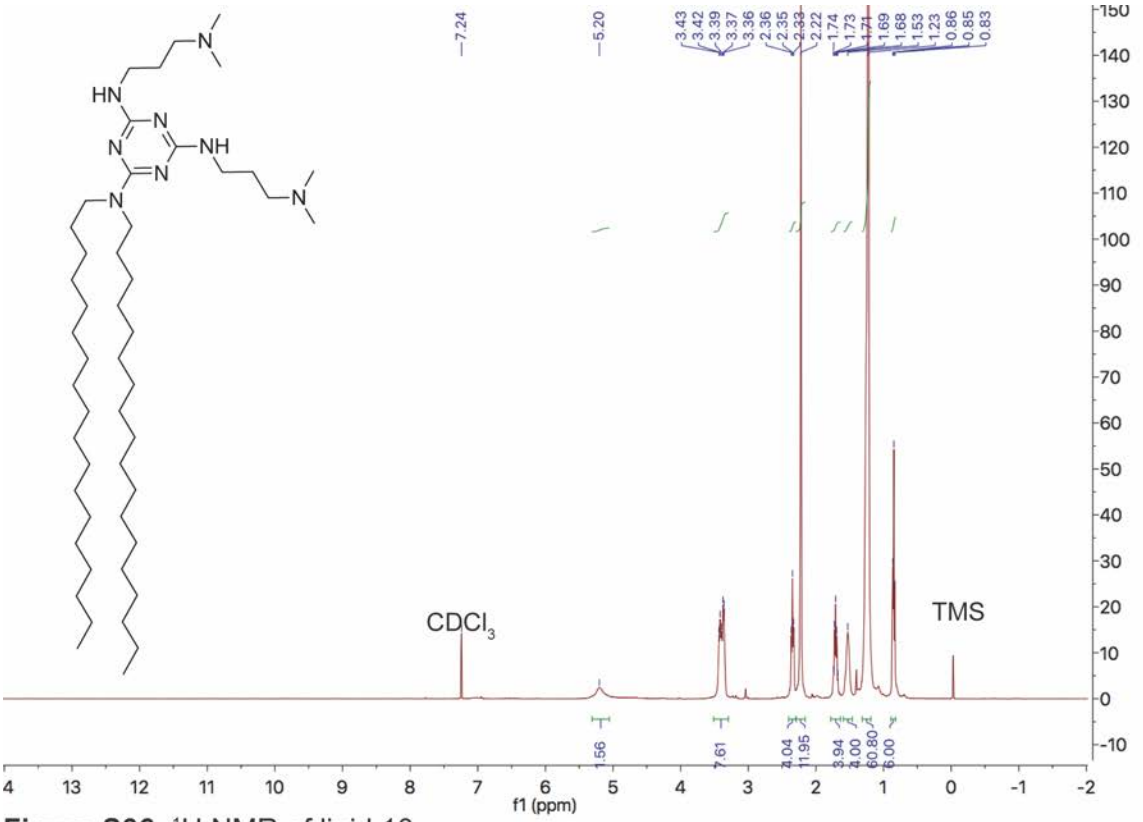


Figure S36. ^{1}H NMR of lipid 10

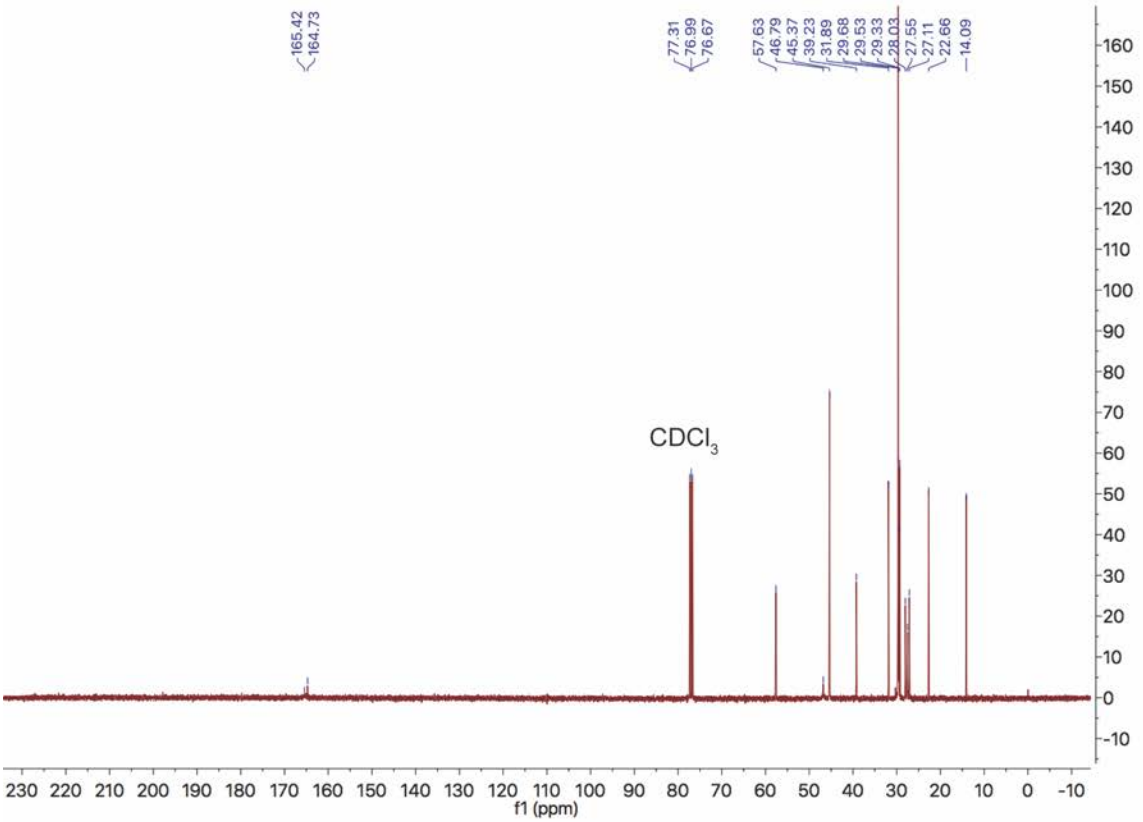


Figure S37. ^{13}C NMR of lipid 10

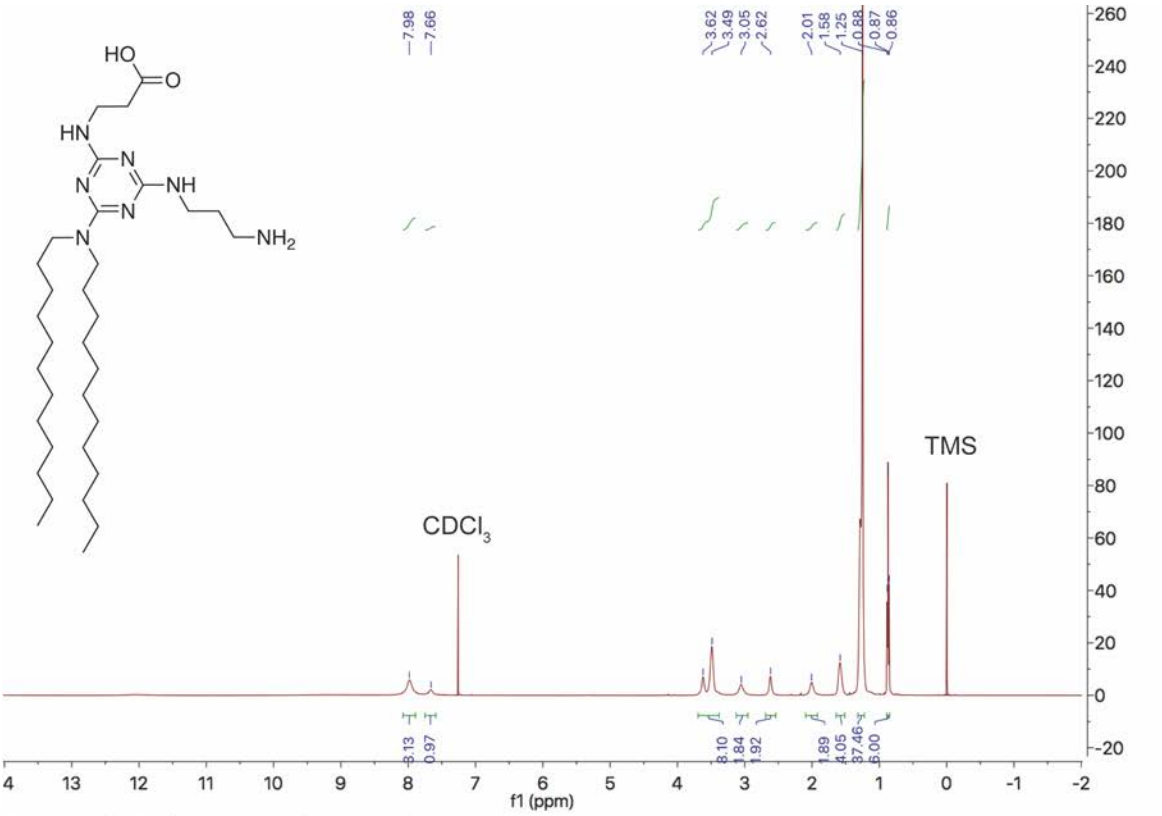


Figure S38. ^{1}H NMR of lipid 11

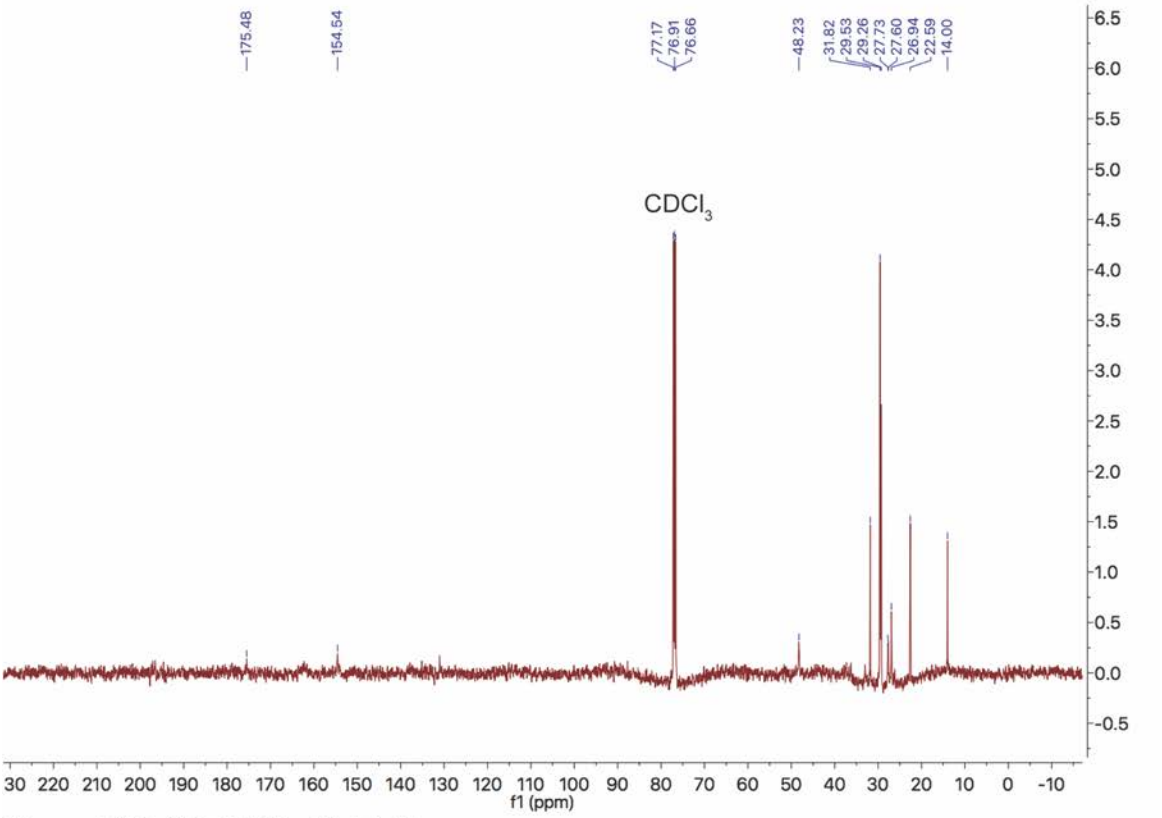


Figure S39. ^{13}C NMR of lipid 11

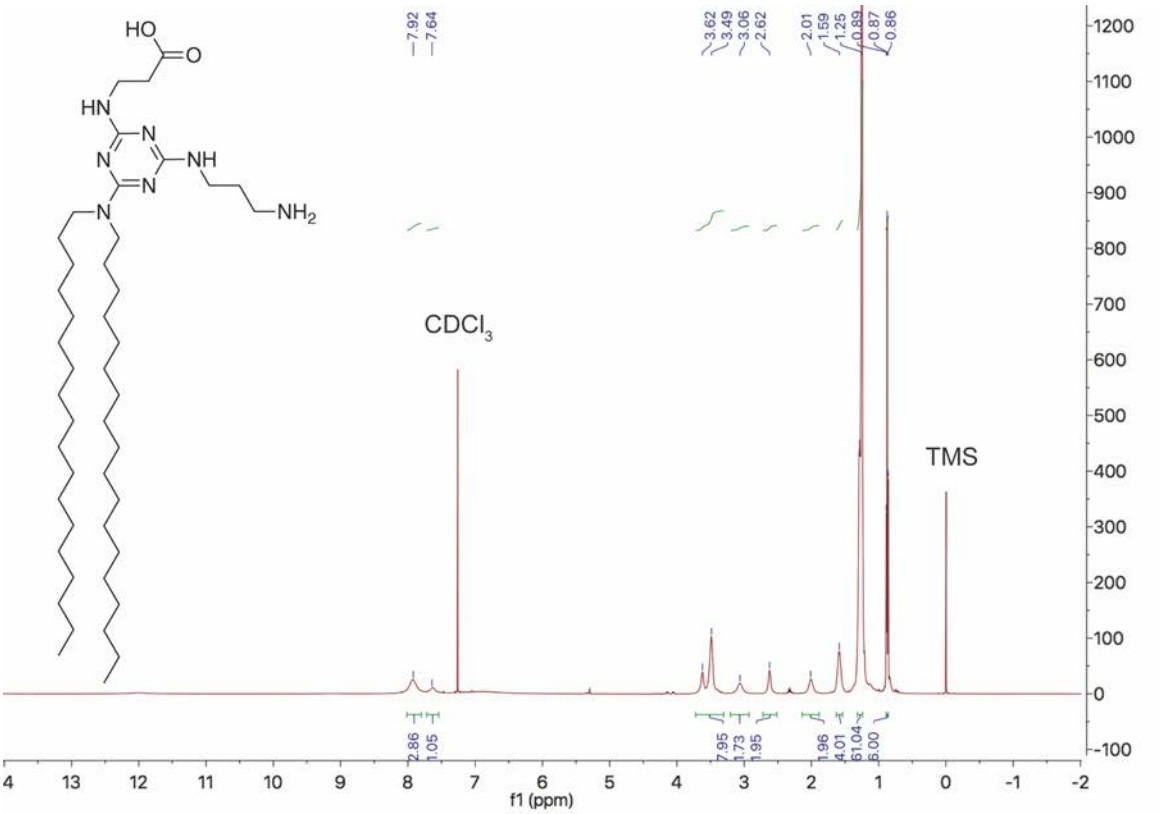


Figure S40. ^{1}H NMR of lipid 12

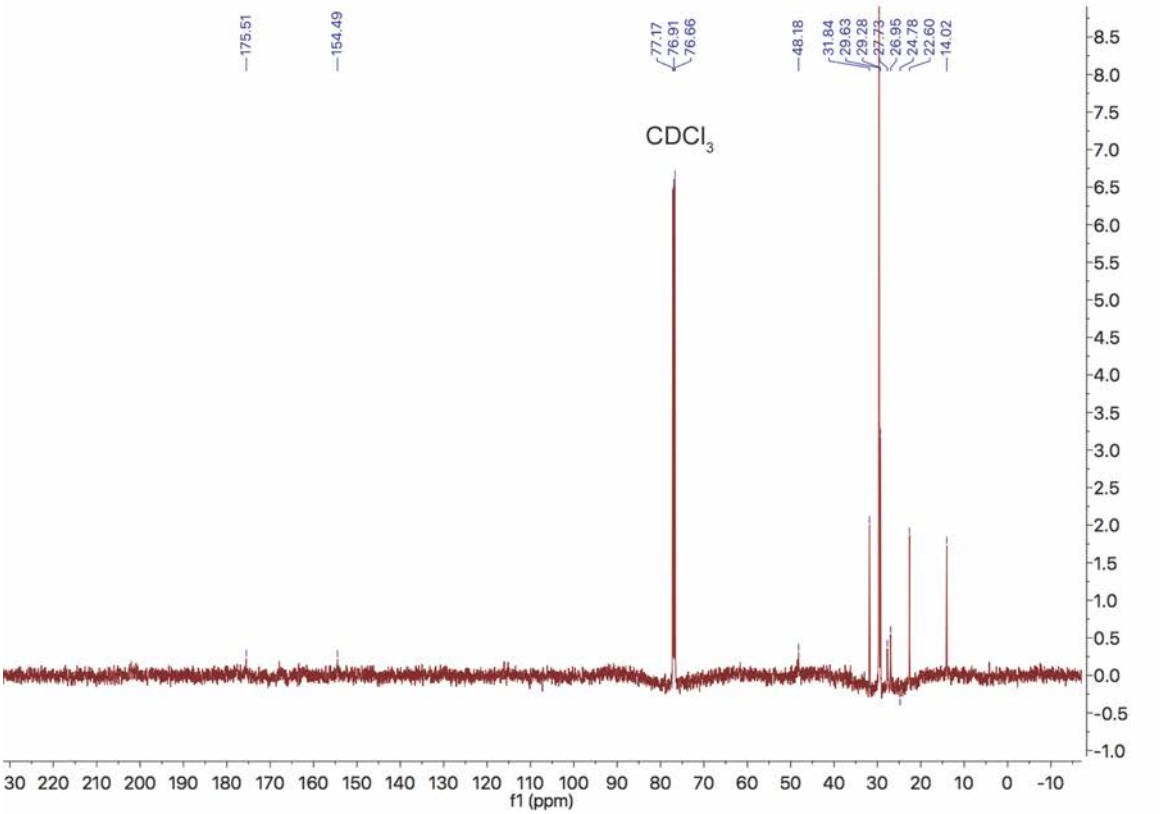


Figure S41. ^{13}C NMR of lipid 12

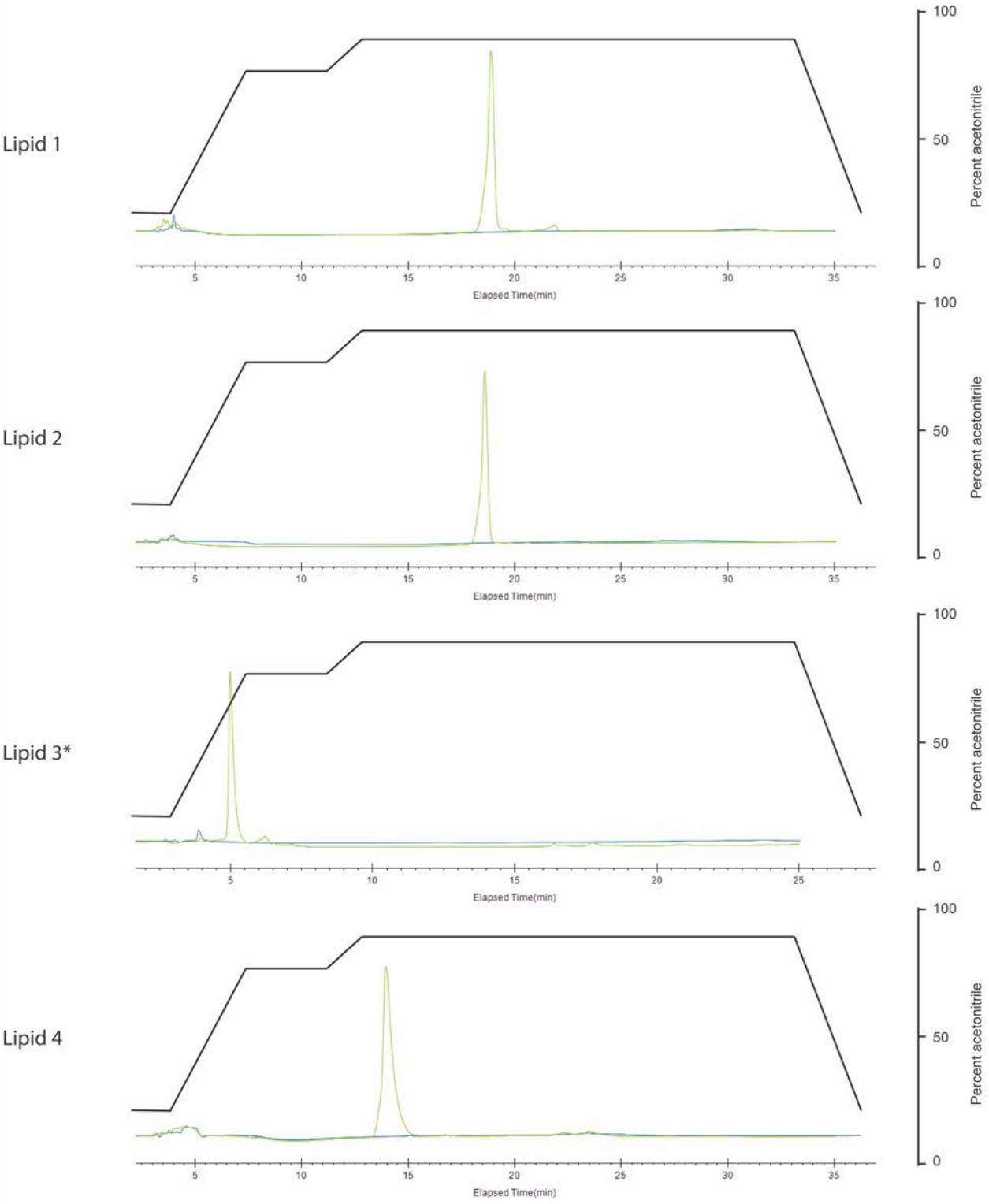


Figure S42. HPLC traces of lipids 1-4 and chloroform (used as solvent), detected at 205 and 254 (254 shown). The mobile phase was a gradient of water and acetonitrile with 0.1% trifluoroacetic acid, as indicated, and constant 5% methanol with 0.1% trifluoroacetic acid. *Shortened due to speed of compound elution.

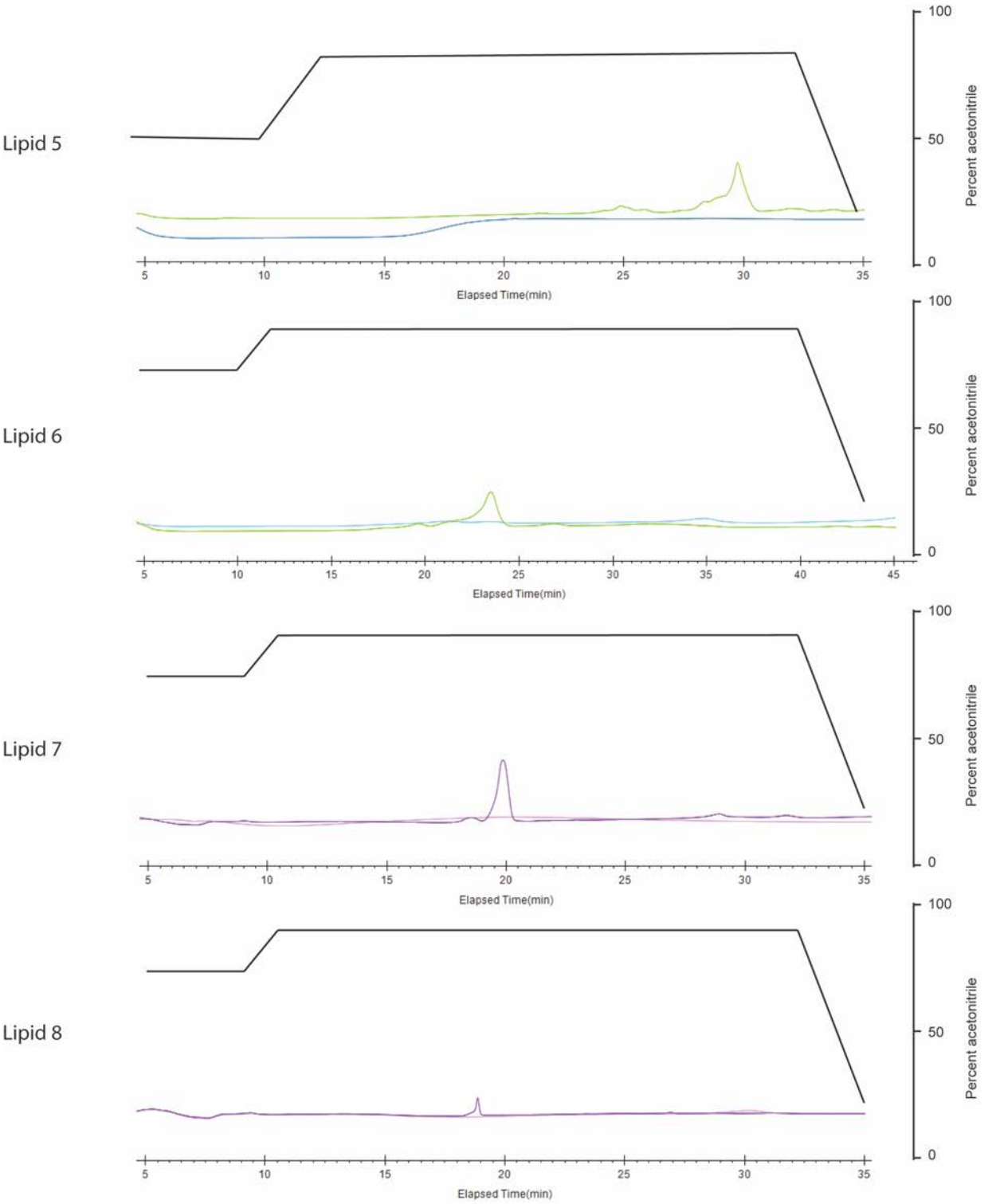


Figure S43. HPLC traces of lipids 5-8 and chloroform (used as solvent), detected at 205 and 254 (254 shown). The mobile phase was a gradient of water and acetonitrile with 0.1% trifluoroacetic acid, as indicated, and constant 5% methanol with 0.1% trifluoroacetic acid. These four compounds needed a mixture of isopropanol and chloroform for proper dissolution for HPLC.

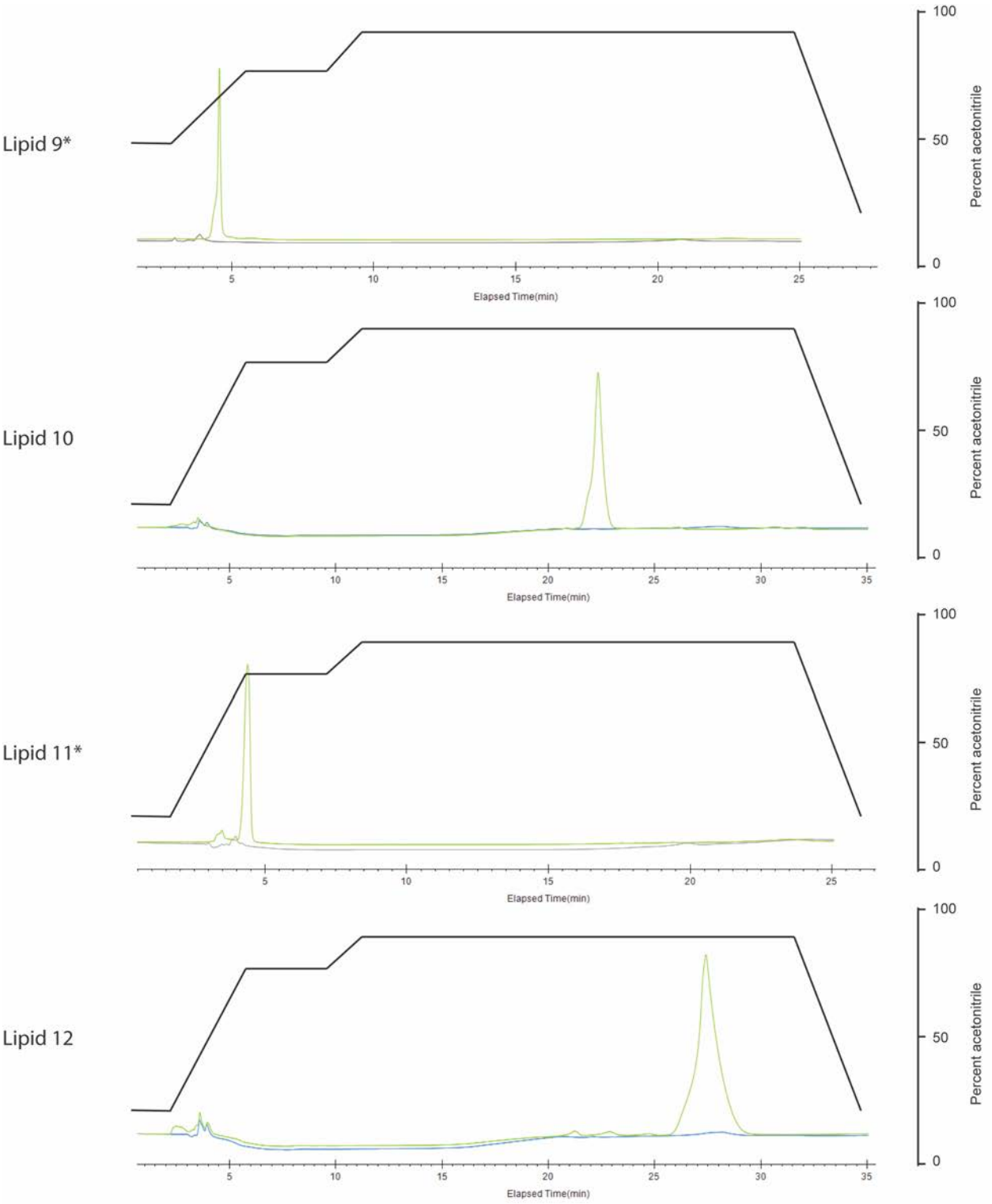


Figure S44. HPLC traces of lipids 9-12 and chloroform (used as solvent), detected at 205 and 254 (254 shown). The mobile phase was a gradient of water and acetonitrile with 0.1% trifluoroacetic acid, as indicated, and constant 5% methanol with 0.1% trifluoroacetic acid. *Shortened due to speed of compound elution.

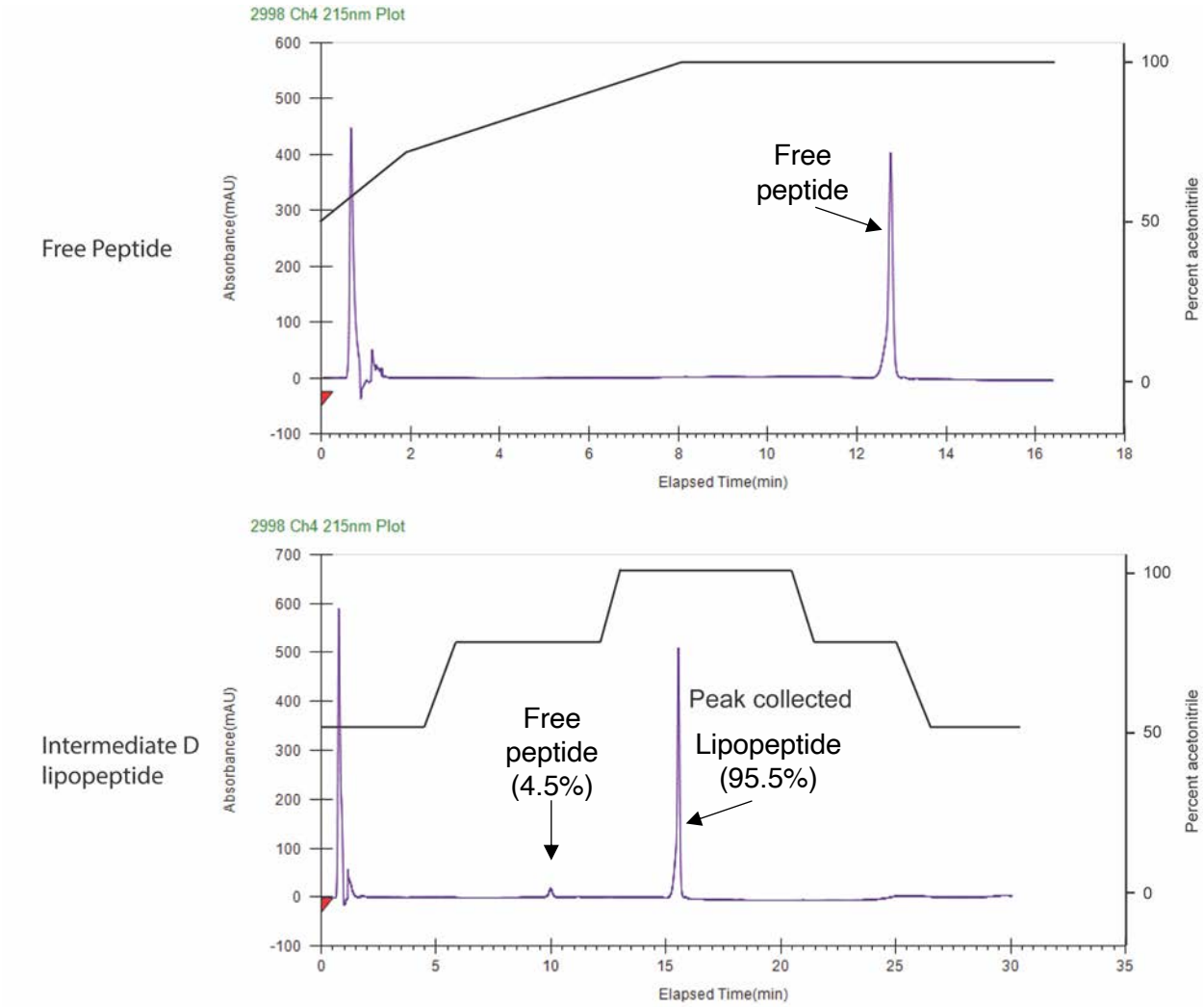


Figure S45. HPLC traces of free apolipoprotein A-I and apolipoprotein A-I lipopeptide

Table S1. Characteristics of liposomes made with DMPC, DOTMA, and various lipid combinations, as well as immunization liposomes.

Lipid	Size (nm)	PDI	Charge (mV)	LD_{50} (μM)
DMPC	277 ± 45	0.39 ± 0.01	-9 ± 0.3	969
DOTMA	105 ± 2	0.43 ± 0.06	70 ± 4	78
DOTMA:DOPE (1:1)	104 ± 5	0.28 ± 0.04	28 ± 1	ND
1:DOPE (1:1)	89 ± 1	0.45 ± 0.05	-61 ± 4	ND
2:DOPE (1:1)	85 ± 2	0.29 ± 0.04	-46 ± 2	ND
3:DOPE (1:1)	61 ± 1	0.38 ± 0.01	44 ± 2	ND
4:DOPE (1:1)	64 ± 0.2	0.28 ± 0.02	30 ± 7	ND
9:DOPE (1:1)	76 ± 4	0.57 ± 0.01	38 ± 2	ND
10:DOPE (1:1)	107 ± 2	0.23 ± 0.01	44 ± 2	ND
11:DOPE (1:1)	93 ± 5	0.24 ± 0.01	47 ± 1	ND
12:DOPE (1:1)	98 ± 1	0.34 ± 0.04	52 ± 3	ND
3 Lipoplex (N:P 1)	91 ± 3	0.45 ± 0.01	27 ± 6	ND
3 Lipoplex (N:P 5)	66 ± 1	0.25 ± 0.01	21 ± 1	ND
4 Lipoplex (N:P 1)	225 ± 7	0.39 ± 0.02	35 ± 6	ND
4 Lipoplex (N:P 5)	194 ± 5	0.22 ± 0.00	35 ± 3	ND
9 Lipoplex (N:P 1)	95 ± 4	0.46 ± 0.01	19 ± 1	ND
9 Lipoplex (N:P 5)	65 ± 2	0.35 ± 0.05	24 ± 3	ND
10 Lipoplex (N:P 1)	102 ± 4	0.55 ± 0.08	50 ± 8	ND
10 Lipoplex (N:P 5)	78 ± 1	0.44 ± 0.01	56 ± 5	ND
DOTMA Lipoplex (N:P 1)	97 ± 2	0.23 ± 0.01	34 ± 4	ND
DOTMA Lipoplex (N:P 5)	90 ± 1	0.21 ± 0.00	2 ± 0	ND
Peptide free liposome	113 ± 4	0.40 ± 0.02	ND	ND
CHEMS peptide liposome	194 ± 4	0.23 ± 0.02	ND	ND
Dioctadecylamine peptide liposome	177 ± 4	0.22 ± 0.01	ND	ND

ND = Not determined.