Electronic Supplementary Material (ESI) for ChemComm.

Supplemental Information

Liposome Triggered Content Release Through Molecular Recognition of Inositol Trisphosphate

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1. Experimental Procedures

1.1 General Experimental

Reagents and solvents were generally purchased from Sigma-Aldrich or Fisher Scientific and used without further purification. PC (L-α-Phosphatidylcholine, mixed isomers from chicken egg), 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 18:1 Lissamine Rhodamine PE (Rd-PE, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (ammonium salt)) and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) were purchased from Avanti Polar Lipids, Inc (Alabaster, AL). D-*myo*-Inositol-1,4,5-triphosphate (sodium salt, IP_3, item # 10008205) was purchased from Cayman Chemical. Dry solvents were obtained from a Pure Solv MD-7 solvent purification system purchased from Innovative Technology, Inc (Newburyport, MA). Column chromatography was performed using 230-400 mesh silica gel purchased from Sorbent Technologies. NMR spectra were obtained using Varian 300 MHz or 500 MHz NMR spectrometers. Mass spectra were obtained with JEOL DART-AccuTOF and Waters Synapt G2-Si mass spectrometers (Milford, MA). Liposome extruder and polycarbonate membranes were obtained from Avestin (Ottawa, Canada) or Avanti Polar Lipids, Inc (Alabaster, AL). Ultrapure water was purified via a Millipore water system (≥ 18 MW·cm triple water purification system). Small quantities (< 5 mg) were weighed on a Mettler Toledo XS105 dual range analytical balance. Fluorescence studies were performed using a Cary Eclipse Fluorescence Spectrophotometer from Agilent Technologies. DLS analyses were carried out with a Malvern Zetasizer Nano ZS instrument equipped with a 4.0 mW laser operating at l = 633 nm. Plots were generated using Origin Pro 2021b. All error bars in plots indicate the standard errors of at least three experimental replicates.

1.2 Synthetic Procedures and Characterization Data

2,2',2''-((Benzene-1,3,5-triyltris(methylene))tris(oxy))tris(4-dodecylbenzaldehyde) (3)

Compound **2**[1] (155.5 mg, 0.535 mmol), potassium carbonate (665 mg, 4.82 mmol) and 1,3,5-tris(bromomethyl)benzene (63.5 mg, 0.178 mmol) were dissolved in 10 mL dry *N*,*N*-dimethylformamide in a 100 mL RBF under Argon and the mixture was heated at 110 ºC overnight. After completion, the reaction was cooled to rt and the solvent was removed using a rotary evaporator. The residue was re-dissolved in 25 mL methylene chloride and washed with 50 mL water. The aqueous layer was extracted with 2 x 25 mL methylene chloride. The combined organic layer was further washed with 1 x 50 mL water and 1 x 50 mL brine, respectively, before being dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude was purified via silica gel column chromatography using gradient elution from hexanes to 70% methylene chloride in hexanes. The product was obtained as a brown solid (0.128 g, 0.130 mmol, 73% yield). R_f = 0.25 (75% methylene chloride-hexanes).

^{1}H NMR (500 MHz, $CDCl_3$) δ 10.52 (s, 3H), 7.67 (d, *J* = 2.3 Hz, 3H), 7.51 (d, *J* = 3.4 Hz, 3H), 7.37 – 7.33 (m, 3H), 6.96 (d, *J* = 8.5 Hz, 3H), 5.21 (s, 6H), 2.63 – 2.54 (m, 6H), 1.58 (d, *J* = 7.5 Hz, 6H), 1.27 (m, 54H), 0.88 (t, *J* = 7.0 Hz, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 189.84, 159.11, 137.73, 136.13, 136.03, 128.23, 125.83, 125.01, 113.06, 70.25, 34.94, 32.03, 31.49, 29.78, 29.77, 29.76, 29.70, 29.59, 29.47, 29.32, 22.81, 14.24. ESI-MS: $[M+H]^+$ calcd for $C_{66}H_{96}O_6$: 985.7285; Found: 985.7243.

(((Benzene-1,3,5-triyltris(methylene))tris(oxy))tris(4-dodecylbenzene-2,1-diyl))trimethanol (4)

Compound **3** (0.105 g, 0.106 mmol) was dissolved in 12 mL methylene chloride/isopropanol (3/1, v/v) under argon in a 50 mL RBF at 0 ºC. Sodium borohydride (20.1 mg, 0.531 mmol) was then added. Next, the

reaction mixture was warmed up to rt and further stirred for 6h. After completion, the reaction mixture was brought back to 0 ºC with an ice bath and 10% citric acid was carefully added dropwise until no hydrogen gas was released, after which the reaction was further stirred for 20 min. The organic layer was collected, and the aqueous layer was further extracted with 2 x 25 mL methylene chloride. The combined organic layer was washed with 1 x 50 mL water and 1 x 50 mL brine. After being dried over sodium sulfate, filtered, and concentrated under reduced pressure, the product was obtained as a white solid in a quantitative yield (0.1057 g, 0.106 mmol). R_f = 0.45 (25% ethyl acetate-hexanes).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 (s, 3H), 7.15 (d, *J* = 2.1 Hz, 3H), 7.05 (dd, *J* = 8.3, 2.2 Hz, 3H), 6.83 (d, *J* = 8.3 Hz, 3H), 5.12 (s, 6H), 4.71 (s, 6H), 2.58 – 2.51 (m, 6H), 1.62 – 1.55 (m, 6H), 1.29 (m, 54H), 0.89 (t, *J* = 7.0 Hz, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.46, 138.27, 135.83, 129.24, 129.20, 128.58, 125.25, 111.68, 69.80, 61.96, 35.25, 32.06, 31.85, 29.82, 29.78, 29.76, 29.68, 29.49, 22.83, 14.26. ESI-MS: $[M+Na]^+$ calcd for $C_{66}H_{102}O_6Na$:1013.7574; Found: 1013.7584.

1,3,5-tris((2-(Bromomethyl)-5-dodecylphenoxy)methyl)benzene (5)

Tris-alcohol **4** (90.4 mg, 0.091 mmol) was weighed into a 100 mL RBF under argon and dissolved with 20 mL dry methylene chloride at 0 ºC in an ice bath. Phosphorus tribromide (0.386 mL, 4.10 mmol) was next carefully added into the RBF dropwise. The reaction was warmed to rt and further stirred for six hours. After being cooled back to 0 ºC, methanol was added dropwise to quench the unreacted PBr_3. The mixture was poured into 100 mL water in a separatory funnel and the methylene chloride layer was collected. The aqueous layer was further extracted with 2 x 20 mL methylene chloride. The combined organic layer was washed with 1 x 50 mL water and 1 x 50 mL brine before being dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude was purified with a short column packed with silica gel using elution of 10% ethyl acetate in hexanes as a brown oil (0.0882 g, 0.0747 mmol, 82% yield). R_f = 0.36 (5% EtOAc-hexanes).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.60 (s, 3H), 7.17 (d, *J* = 2.2 Hz, 3H), 7.06 (dd, *J* = 8.4, 2.2 Hz, 3H), 6.84 (d, *J* = 8.4 Hz, 3H), 5.18 (s, 6H), 4.61 (s, 6H), 2.57 – 2.51 (m, 6H), 1.61 – 1.55 (m, 6H), 1.29 (m, 54H), 0.89 (t, *J* = 7.0 Hz, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.65, 138.07, 135.69, 131.02, 130.07, 126.29, 125.55, 112.45, 70.11, 35.10, 32.07, 31.71, 30.11, 29.83, 29.82, 29.80, 29.76, 29.74, 29.66, 29.51, 29.47, 22.84, 14.27. ESI-MS: $[M+K]^+$ calcd for $C_{66}H_{99}Br_3O_3K$: 1215.4781; Found: 1215.6144.

1,1',1''-(((Benzene-1,3,5-triyltris(methylene))tris(oxy))tris(4-dodecylbenzene-2,1-diyl))tris(*N*,*N*-bis(pyridin-2-ylmethyl)methanamine) (6)

Compound **5** (43.4 mg, 0.0368 mmol) was dissolved with 10 mL dry tetrahydrofuran in a 50 mL RBF under Ar. *N,N*-Diisopropylethylamine (DIEA, 21.1 µL, 0.121 mmol) and dipicolylamine (DPA, 21.8 µL, 0.121 mmol) were next added, respectively. The reaction was refluxed at 70 ºC overnight, accompanied by a color change from pale yellow to brown. After evaporating the solvent under reduced pressure, the crude was subjected to neutral alumina column chromatography through gravity elution using 100% chloroform. Product **6** was obtained as a brown oil (25.9 mg, 0.0168 mmol, 46% yield). R_f = 0.5 (10% methanol in chloroform).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.44 (d, *J* = 4.1 Hz, 6H), 7.56 (d, *J* = 7.8 Hz, 6H), 7.48 (td, *J* = 7.7, 1.7 Hz, 6H), 7.39 – 7.32 (m, 6H), 7.05 – 6.99 (m, 6H), 6.96 (dd, *J* = 8.3, 2.1 Hz, 3H), 6.77 (d, *J* = 8.4 Hz, 3H), 5.00 (s, 6H), 3.84 (s, 12H), 3.78 (s, 6H), 2.60 – 2.49 (m, 6H), 1.55 (q, *J* = 7.1 Hz, 6H), 1.26 (m, 54H), 0.87 (t, *J* = 7.0 Hz, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.28, 155.22, 148.99, 138.33, 136.36, 135.24, 130.63, 127.87,

127.06, 125.76, 122.67, 121.84, 111.99, 70.20, 60.46, 52.87, 35.32, 32.07, 31.84, 29.87, 29.85, 29.81, 29.74, 29.51, 22.84, 14.27. ESI-MS: $[M+2H]^{2+}$ calcd for $C_{102}H_{137}N_9O_3$: 768.0422; Found: 768.0386. $[M+3H]^{3+}$ calcd for $C_{102}H_{138}N_9O_3$: 512.3641; Found: 512.3618.

1,1',1''-(((Benzene-1,3,5-triyltris(methylene))tris(oxy))tris(4-dodecylbenzene-2,1-diyl))triszinc(*N*,*N*-bis(pyridin-2-ylmethyl)methanamine) chloride (1)

Compound **6** (16.1 mg, 0.01 mmol) was dissolved with 2.2 mL chloroform/methanol (10/1, v/v) in a 4 dr vial. Anhydrous $ZnCl_2$ (4.29 mg, 0.031 mmol) was then added, and the reaction was stirred overnight. Next, the solvent was moved under nitrogen flow and the product was obtained as a brown solid in a quantitative yield.

^{1}H NMR (500 MHz, 10% CD_3OD-$CDCl_3$) δ 8.86 (d, J = 5.0 Hz, 6H), 7.58 – 7.53 (m, 6H), 7.24 – 7.19 (m, 6H), 7.07 (d, J = 7.8 Hz, 6H), 6.98 – 6.92 (m, 6H), 6.76 (d, J = 8.5 Hz, 3H), 6.70 (d, J = 1.7 Hz, 3H), 4.90 (s, 6H), 4.17 (s, 6H), 3.76 (s, 12H), 2.45 – 2.32 (m, 6H), 1.44 (s, 6H), 1.19 (m, 54H), 0.77 (t, J = 6.9 Hz, 9H).

1.3 Liposome Preparation and Assays

Preparation of liposomes for Nile red release assay

Stock solutions of 5 mM lipid switch **1** were prepared in chloroform/methanol solution (1/1, v/v). Stock solutions of 32.46 mM PC and 5 mM Nile red were prepared in chloroform. All the stock solutions were kept in a -20 ºC freezer after preparation. Proper volumes of each stock solution were pipetted into a 1 dr vial to reach a total lipid concentration of 2 mM with desired percentages of each lipid composition. Nile red was added as an extra 5% of the total lipid content. The organic solvents were next evaporated under a nitrogen stream, and the resulting lipid films were kept under vacuum for at least one hour. After that, the films were hydrated with proper volumes of 1×TBS buffer (pH 7.4, containing 25 mM Tris/Tris HCl, 0.13 M NaCl, 0.0027 M KCl) in a 60 ºC water bath for 1 hr. The vials were taken out and vortexed every 20 min. Next, ten freeze-thaw cycles were performed with a dry ice-acetone bath and 60 ºC water bath. Finally, the liposome solutions were extruded through a 200 nm polycarbonate membrane for 19 passes with an extruder purchased from either Avestin or Avanti. The resulting liposomes were stored at 4 ºC and were studied immediately after preparation.

Phosphorylated metabolite selectivity screen for liposome Nile red release

Stock solutions (50 mM) of each phosphorylated metabolite were prepared by dissolving the appropriate salts (sodium phosphate dibasic heptahydrate (Pi, $Na_2HPO_4{\cdot}7H_2O$); sodium pyrophosphate dibasic (PPi, $Na_2H_2P_2O_7$); adenosine 5'-diphosphate sodium salt (ADP); adenosine 5'-triphosphate disodium salt, hydrate (ATP); D-fructose-6-phosphate disodium salt (FP); D-fructose-1,6-bisphosphate trisodium salt (FBP); sodium tripolyphosphate (TPi); cytidine-5'-triphosphate disodium salt (CTP); guanosine-5'-triphosphate trisodium salt (GTP); uridine 5′-triphosphate trisodium salt hydrate (UTP); and D-*myo*-inositol-1,4,5-triphosphate sodium salt (IP_3)) with MilliQ water. Adenosine 5′-monophosphate monohydrate (AMP) was prepared as 25 mM stock solutions in MilliQ water due to solubility issue. For each study, 50 µL of liposome solution encapsulating Nile red was first added into a sub-micro quartz cuvette. After an initial scan, 1 µL of a 50 mM stock solution of the phosphorylated metabolite under study was added to the cuvette (final concentration = 1 mM, 2 µL for AMP), and the fluorescence intensities were recorded after incubation for 5 min (excitation wavelength = 552 nm, excitation slit = 5 nm, emission slit = 5 nm). Control experiments were run by adding 1 or 2 µL MilliQ water. When processing the data, the fluorescence intensities at 630 nm were

selected and converted to percentage of initial fluorescence before phosphorylated metabolite addition. Experiments were run at least three times with different batches of liposomes. Averaged data were reported with error bars denoting standard error.

Expanded IP_3 titration experiment using Nile red release assay

IP_3 stock solutions (10 mM) were prepared by diluting the previously prepared 50 mM IP_3 stock solution 5× with MilliQ water. A 100 μL aliquot of 2 mM liposomes encapsulating Nile red prepared through the procedure described above was added into a sub-micro quartz cuvette. After an initial scan, 0.5 μL of 10 mM IP_3 stock solution was added directly into the cuvette for each measurement in the titration, and the fluorescence intensities were measured using the same detection method as described above. When processing the data, the fluorescence intensities at 630 nm were selected and converted to percentage of initial fluorescence before IP_3 addition. Experiments were run at least three times with different batches of liposomes. Averaged data were reported with error bars denoting standard errors. Kinetic experiments were run on a Cary Eclipse Fluorescence Spectrophotometer using kinetic mode. The excitation wavelength was set to 552 nm, and the fluorescence intensities at 630 nm were constantly collected over time.

Liposome stability test

The same PC liposome samples containing 0-20% lipid **1** for NR release studies were prepared as previously described. The stability and dye retention properties of these liposomes were tracked by measuring Nile red fluorescence. When not taking measurements, liposomes were stored in a 4 °C fridge. Fluorescence intensity was measured every 24 hours (excitation wavelength=552 nm, excitation slit=5 nm, emission slit=5 nm). When processing the data, fluorescence intensities at 630 nm were selected. Experiments were run with three different batches of liposomes, and averaged data are reported with error bars denoting standard error.

Preparation of liposomes for SRB release assay

The same stock solutions of **1** and PC prepared for Nile red release assay were used. Stock solutions of 33.6 mM DOPE and 34.1 mM DPPC were prepared in chloroform. SRB stock solutions (20 mM) were prepared by dissolving SRB with 1 × TBS (pH 7.4, containing 25 mM Tris/Tris HCl, 0.13 M NaCl, 0.0027 M KCl). Proper volumes of each lipid stock solution were pipetted into a clean 1 dr vial to reach a total lipid content of 5 mM scale with desired percentages of each lipid composition. The organic solvents were next removed with a nitrogen stream and the resulting lipid films were further dried under vacuum for at least 1 hr. The films were next hydrated with 20 mM SRB solution at 60 ºC in a water bath for four sets of 15 min with vortexing after each set. Next, the solutions were subjected to ten freeze-thaw cycles with a dry ice-acetone bath and a 60 ºC water bath, followed by extrusion through a 200 nm polycarbonate membrane for 21 passes with an extruder purchased from either Avestin or Avanti. Finally, the unencapsulated dye was removed by size-exclusive chromatography. To do so, a micro-column was first packed with Sephadex G-50 (pre-saturated with isotonic TBS buffer). The fractions were next collected every ~1 mL from the column and the second fraction showing significant turbidity was collected. The presence of liposomes was further verified by treating liposomes with Triton X-100 detergent, where an increase in fluorescence intensity was observed.

SRB release assay upon IP_3 treatment

An aliquot of 50 μL liposomes encapsulating SRB prepared through the procedure described above was added into a sub-micro quartz cuvette. After an initial scan, 1 μL of a 50 mM IP_3 stock solution in MilliQ water was added into the cuvette each time, and the fluorescence intensities were recorded after each addition (λ_{ex} = 550 nm, Ex/Em slit = 10/2.5 nm). After completion, the assay was calibrated by adding 1 μL of 10% Triton X-100 detergent to lyse the liposomes and induce 100% release. When processing the data, fluorescence intensities at 585 nm were selected and reported as a percentage of the fluorescence after Triton X-100 treatment for each sample. Experiments were run at least three times with different batches of liposomes. Averaged data were reported with error bars denoting standard errors.

Dynamic light scattering experiment

Samples were prepared by diluting the liposomes before and after triggered release 10-fold with 1×TBS buffer. All measurements were taken at a scattering angle of 173º at 20 ºC. Bar graphs were generated by the average of at least three experiments with error bars denoting standard error.

Zeta potential measurement of 1-liposomes before and after adding IP_3

Zeta potential values were also measured with a Malvern Zetasizer Nano ZS instrument equipped with a 4.0 mW laser operating at l = 633 nm. A 1 mM solution of PC-based liposomes containing 0%, 10% and 20% **1** without dye encapsulation were prepared in 1 mM HEPES (pH 7.4, containing 30 mM NaCl). 2.5 μL of 50 mM IP_3 was added into a 100 μL aliquot of liposomes before being diluted with 900 μL buffer (conc of liposomes = 0.1 mM, conc of IP_3 = 0.125 mM). The solutions were next transferred into an DTS1070 folded capillary cell ready for measurement. All measurements were taken at 20 ºC. Data were generated with at least three replicates.

Fluorescence microscopy studies for liposome aggregation after ATP treatment

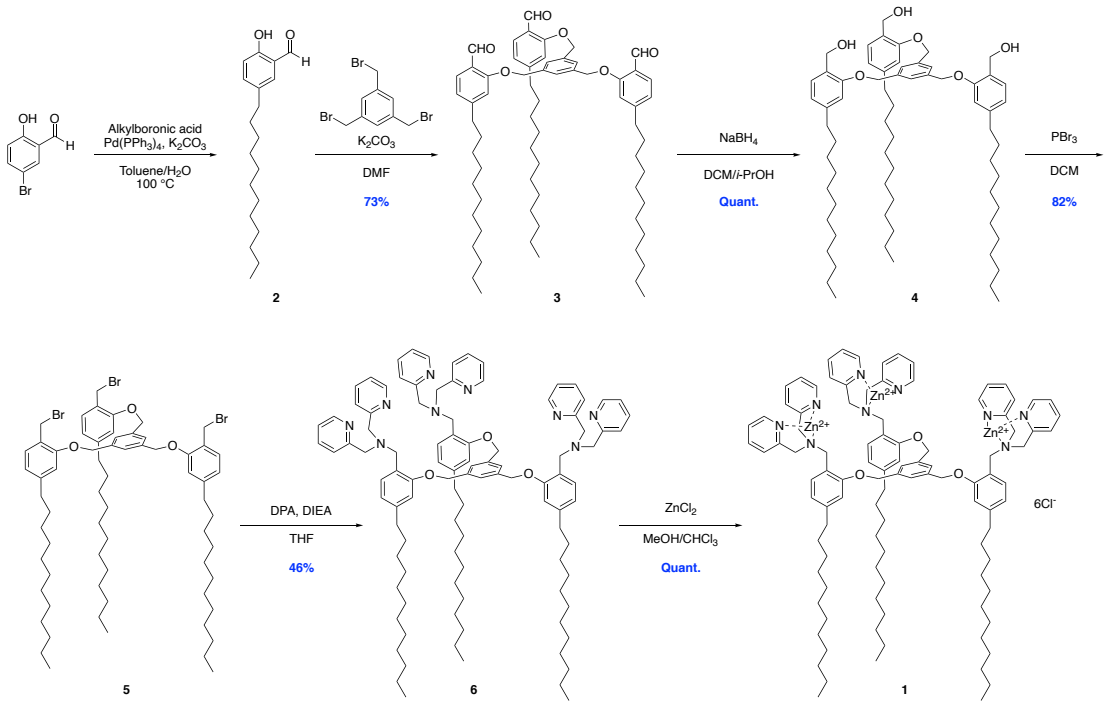
Solutions of 0% and 20% **1** liposomes in PC (1 mM) labeled with 0.08% rhodamine L-α-phosphatidylethanolamine (Rd-PE) in 1×TBS buffer (pH=7.4, containing 25 mM Tris/Tris HCl, 0.13 M NaCl, 0.0027 M KCl) were prepared using the previously described thin-film hydration procedures.

An aliquot of the liposome solution (100 μL) was first added into a FluoroDish Cell Culture Dish (WPI Inc.) and allowed to settle for 15 min before imaging using a 63x 1.4NA oil objective on a Leica SP8 White Light Laser Confocal Microscope (Wetzlar, Germany). 4.2 μL of a 25 mM IP_3 stock solution was added into the liposomes (final concentration = 1 mM), and the images were taken over time. Rd-PE was excited by using 561 nm laser line and the emission was collected between 566-620 nm.

Reference:

1. Lou, J.; Schuster, J. A.; Barrera, F. N.; Best, M. D., ATP-Responsive Liposomes via Screening of Lipid Switches Designed to Undergo Conformational Changes upon Binding Phosphorylated Metabolites. *J. Am. Chem. Soc.* **2022**, 144, 3746-3756.

3. Supplemental Figures



Scheme S1. Synthetic route to lipid switch **1**. 5-Bromo-2-hydroxybenzaldehyde was coupled with *N*-dodecylboronic acid to produce **2**, followed by trimerization using 1,3,5-tris(bromomethyl)benzene to **3**, aldehyde reduction to **4**, bromination to **5**, introduction of the DPA units of **6**, and finally zinc chelation to generate **1**.

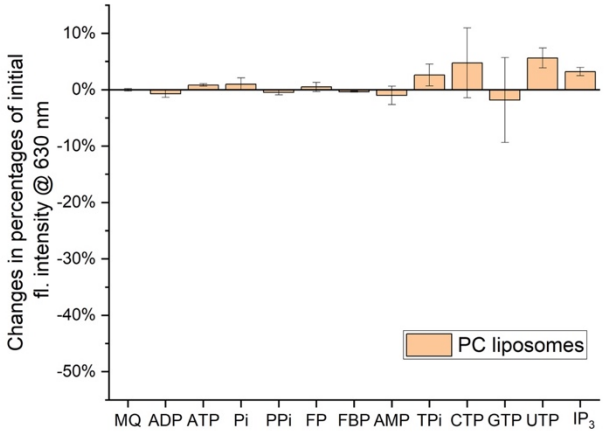


Figure S1. Selectivity screens for 100% PC liposomes toward different phosphorylated small molecules or simply milli-Q water (MQ). None of these induced content release, indicating there was no background leakage from PC. Error bars denotes standard errors from at least three replicates.

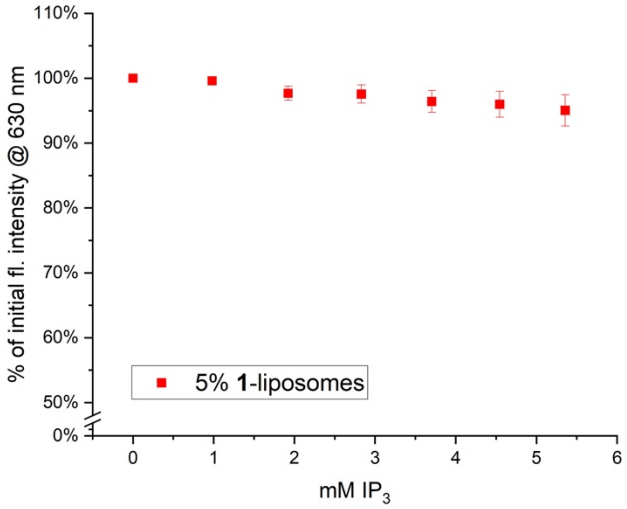


Figure S2. NR release titration curve for 5% **1**-liposomes by titrating with IP_3 to an excess amount (> 5 mM). Less than 5% background leakage was observed, indicating that higher amounts of IP_3 did not further affect release. Error bars denote standard errors from three replicates.

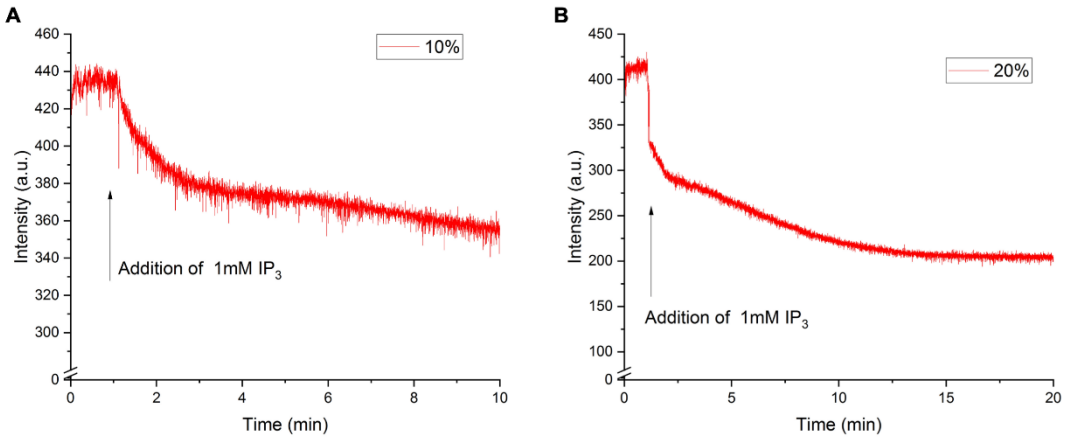


Figure S3. Representative kinetic curves for NR release from 10% and 20% **1**/PC liposomes (2 mM) after adding IP_3. In both cases, release reached a plateau within ten minutes after adding IP_3 to a concentration of 1 mM.

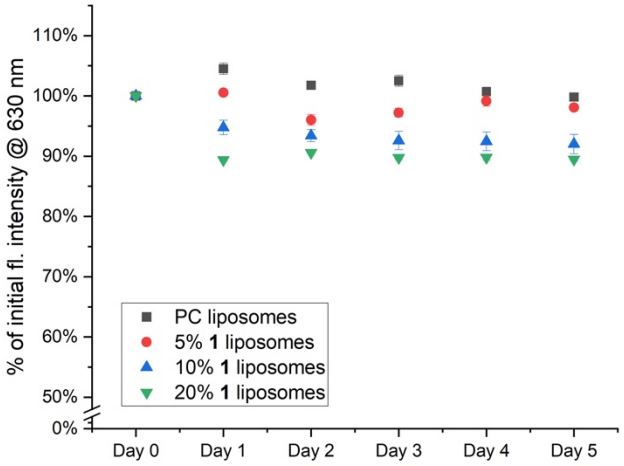


Figure S4. Liposome stability test for NR encapsulated PC-based liposomes containing 0-20% lipid **1**. All liposomes generally showed only minor fluctuations in fluorescence over time, although these were greater for 10% and 20% **1**-liposomes, which showed ~10% fluorescence decreases over five days. Error bars denote standard errors from at least three replicates.

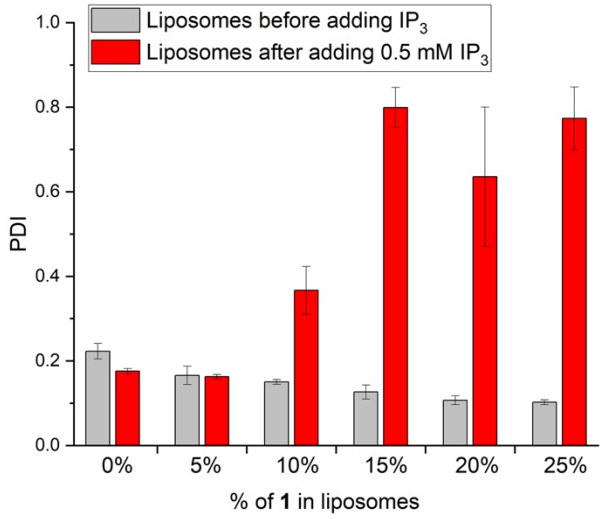


Figure S5. Polydispersity indeces (PDIs) for 0-25% **1**/PC liposomes before and after 0.5 mM IP_3 treatment. While 0% and 5% **1**-liposomes remained uniform in size upon IP_3 addition, 10-25% **1**-liposomes all showed dramatic changes in PDI, indicating alterations to particle sizes. Error bars denote standard errors from at least three replicates.

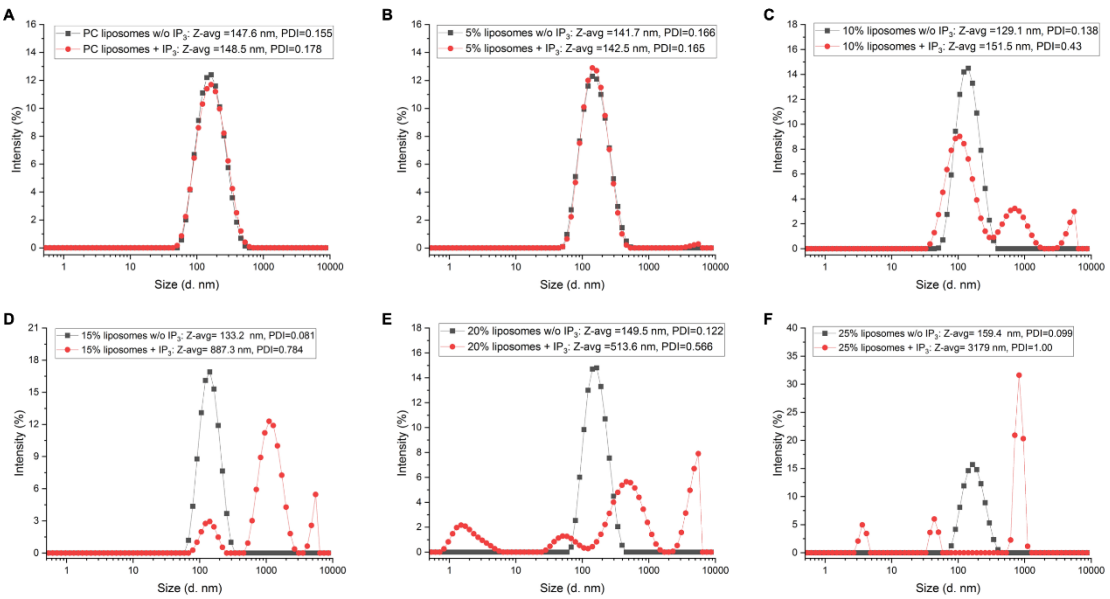


Figure S6. DLS raw distribution curves for PC and 5-25% **1**/PC liposomes before and after IP_3 addition. Liposomes of uniform size were formed in all cases before IP_3 treatment. After adding IP_3, PC and 5% liposomes did not show any change, while significant changes were observed for 10-25% liposomes. Note: only representative sets of distribution curves are shown for 10-25% liposomes after IP_3 treatment.

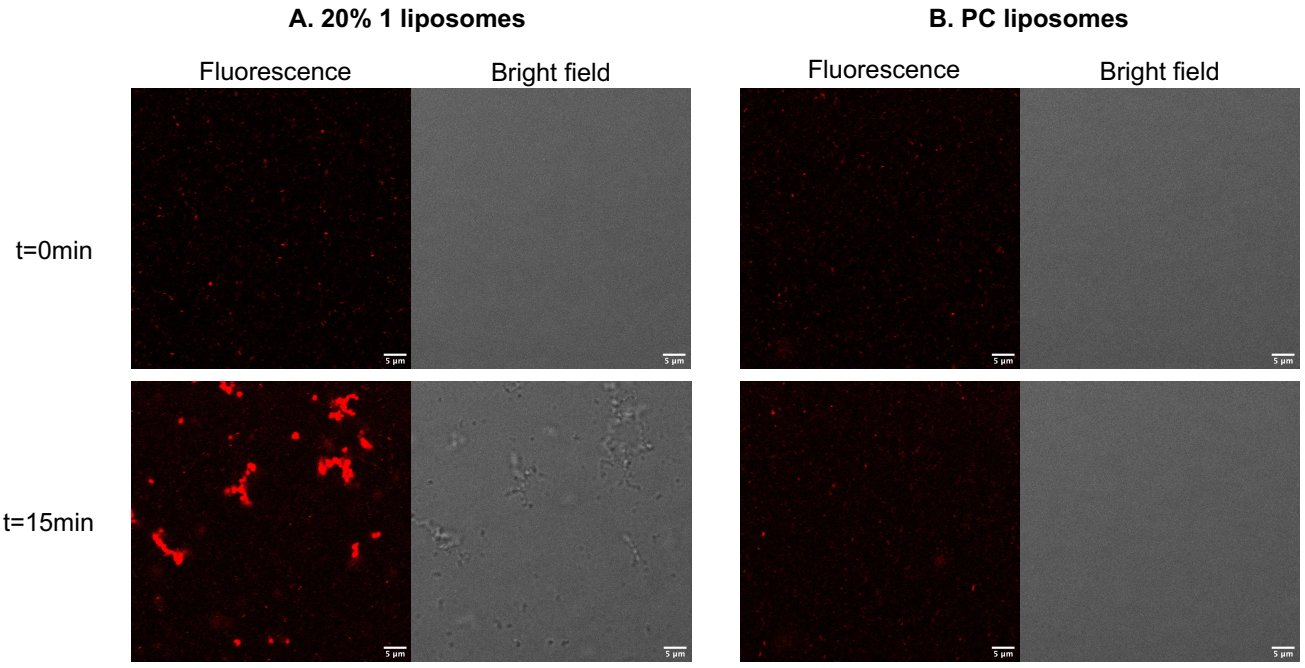


Figure S7. Fluorescence microscopy images for Rd-PE labeled 0% (**B**) and 20% (**A**) **1**-liposomes (1 mM) before and after 15 minute 1 mM IP_3 incubation. IP_3 treatment resulted in aggregation of **1** liposomes, indicated by the larger fluorescent particles under the microscope. No aggregation was observed for PC liposomes after IP_3 treatment over time. Scale bars denote 5 μm.

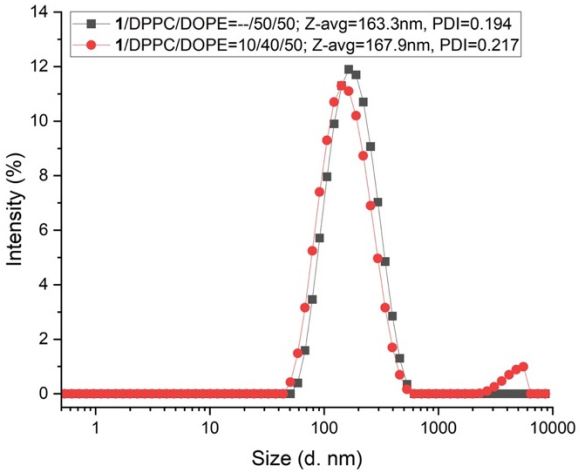


Figure S8. DLS raw distribution curves for SRB-encapsulated DPPC/DOPE liposomes containing or lacking lipid **1**. Stable and uniform liposomes were formed in all cases.

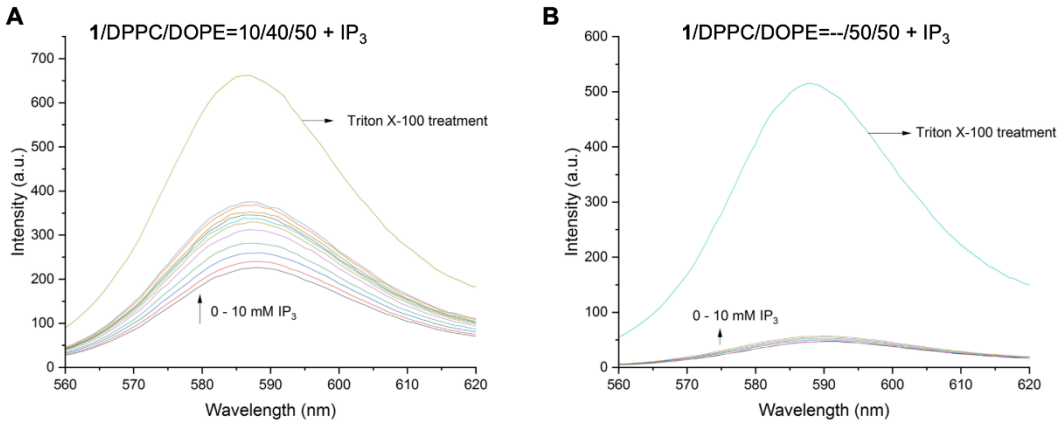
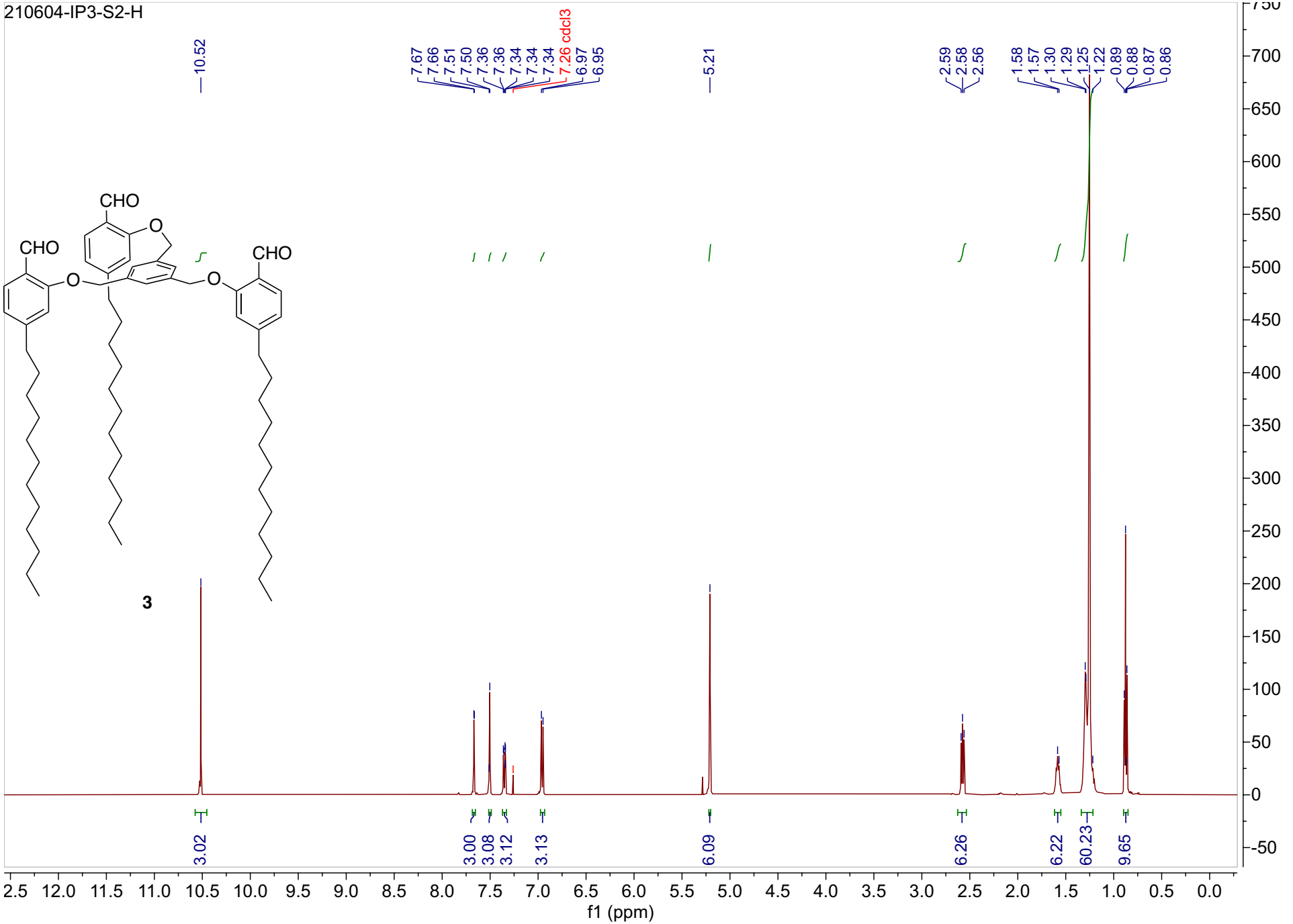


Figure S9. Sample raw fluorescence emission spectra for liposomes containing 0 or 10% lipid switch **1**, 50 or 40% DPPC and 50% DOPE used in SRB release assay after IP_3 addition. Fluorescence intensities were recorded after each addition. (λ_{ex} = 550 nm, Ex/Em slit = 10/2.5 nm).

Video S1. Time-course fluorescence microscopy video of 20% **1**/PC liposomes labeled with 0.08% Rd-PE shows the gradual formation of larger particles after IP_3 addition.

Video S2. Time-course fluorescence microscopy video of negative control PC liposomes labeled with 0.08% Rd-PE shows that larger particles are not observed after IP_3 treatment.

^{1}H NMR


210604-IP3-S2-H
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7.67
7.66
7.51
7.50
7.36
7.36
7.34
7.34
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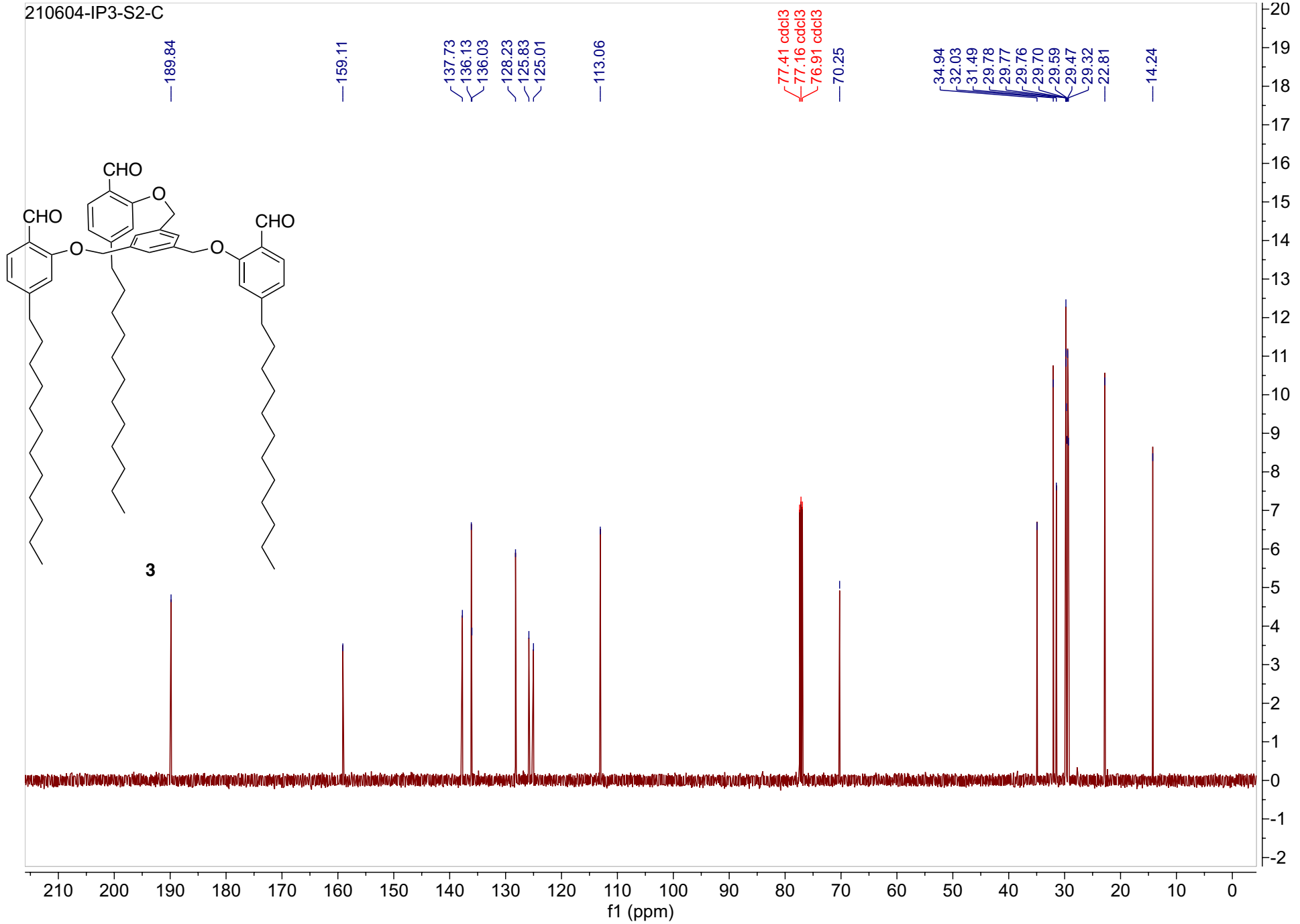
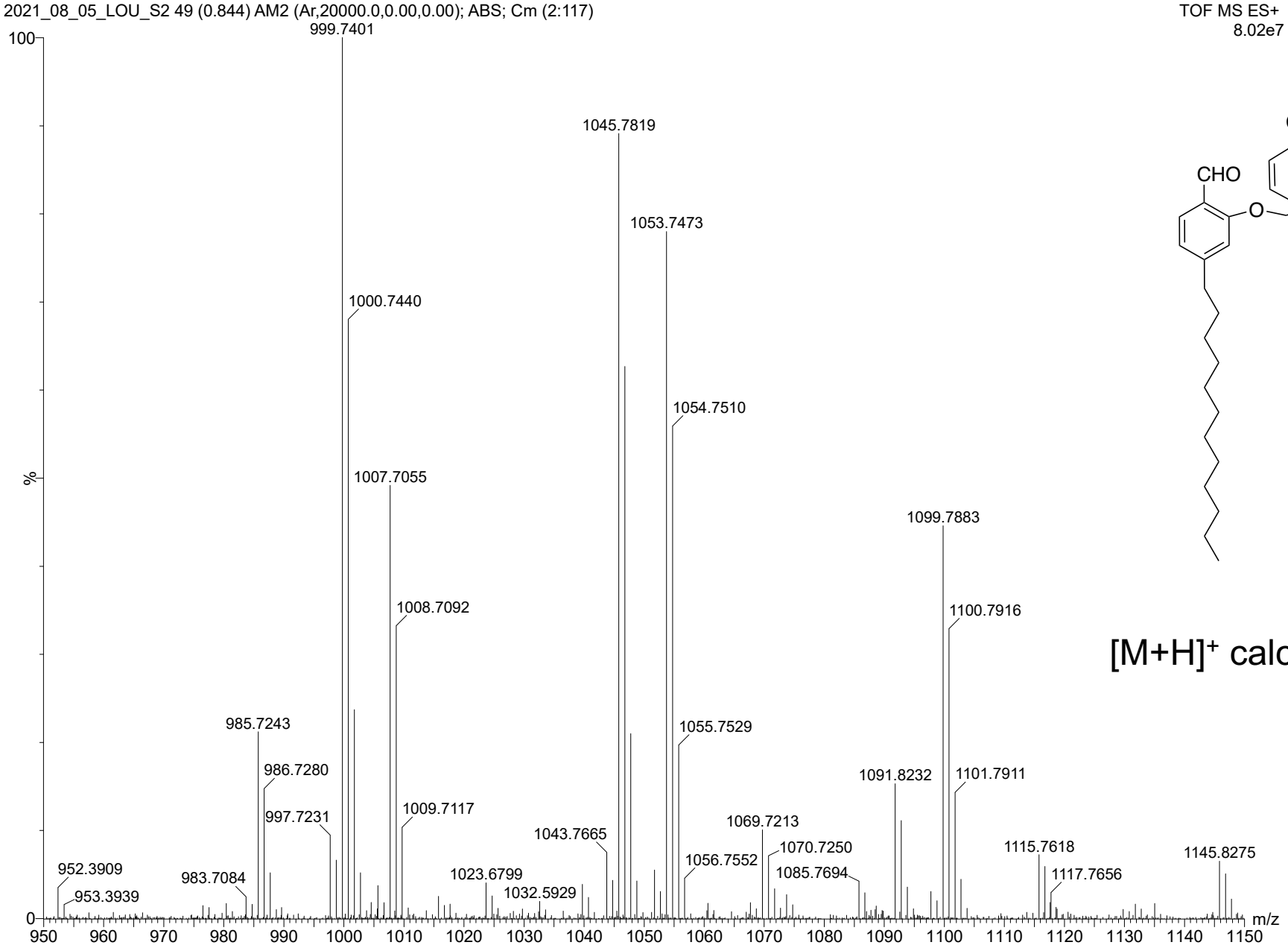
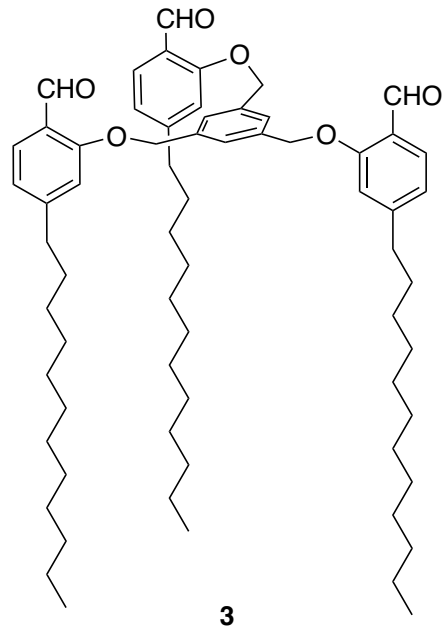
6.97
6.95
5.21
2.59
2.58
2.56
1.58
1.57
1.30
1.29
1.25
1.22
0.89
0.88
0.87
0.86
CHO
CHO
CHO
O
O
O
3
3.02
3.00
3.08
3.12
3.13
6.09
6.26
6.22
60.23
9.65
f1 (ppm)

^{13}C NMR


210604-IP3-S2-C
189.84
159.11
137.73
136.13
136.03
128.23
125.83
125.01
113.06
77.41 cdcl3
77.16 cdcl3
76.91 cdcl3
70.25
34.94
32.03
31.49
29.78
29.77
29.76
29.70
29.59
29.47
29.32
22.81
14.24
CHO
CHO
CHO
O
O
O
3
f1 (ppm)

ESI-MS

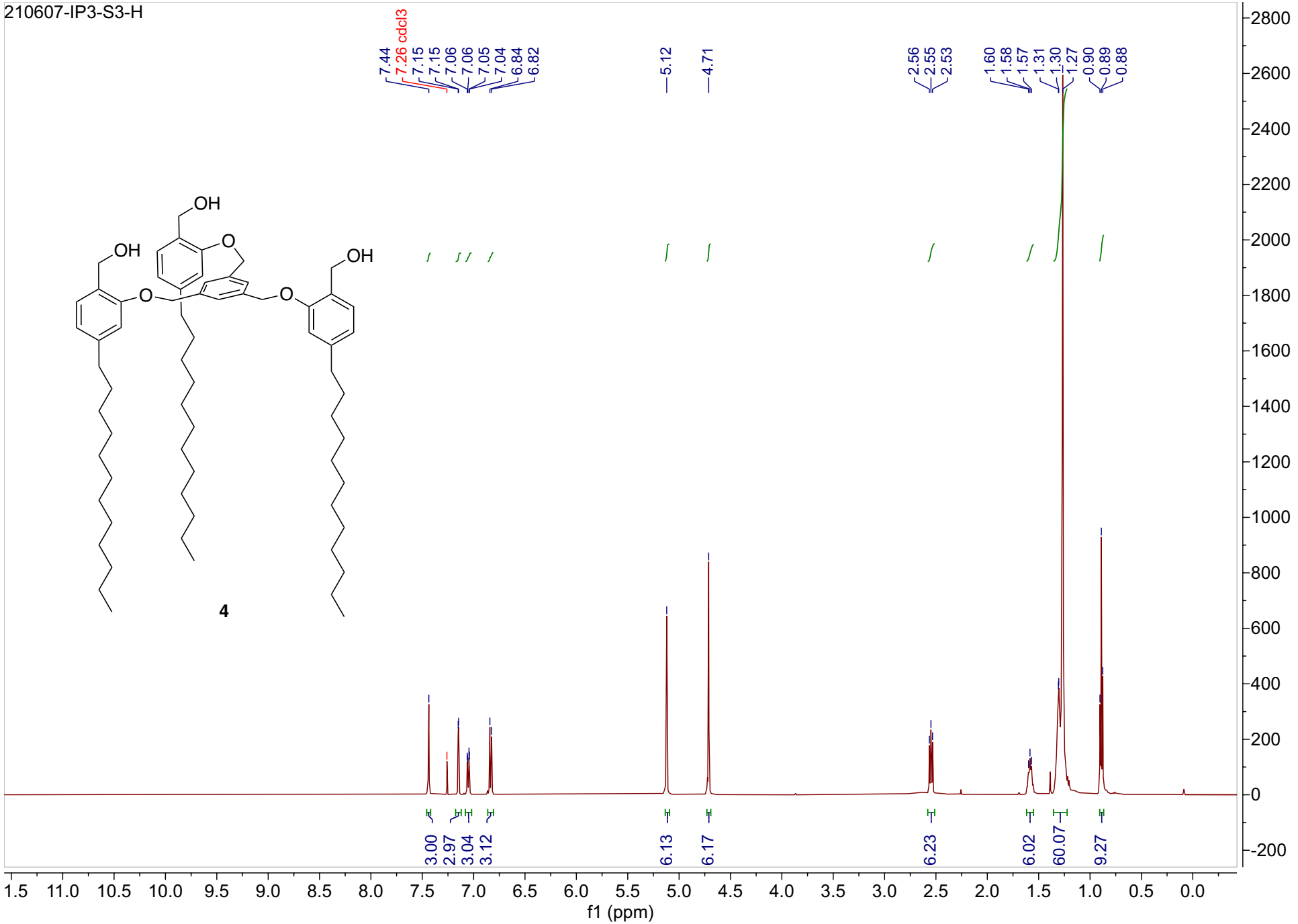




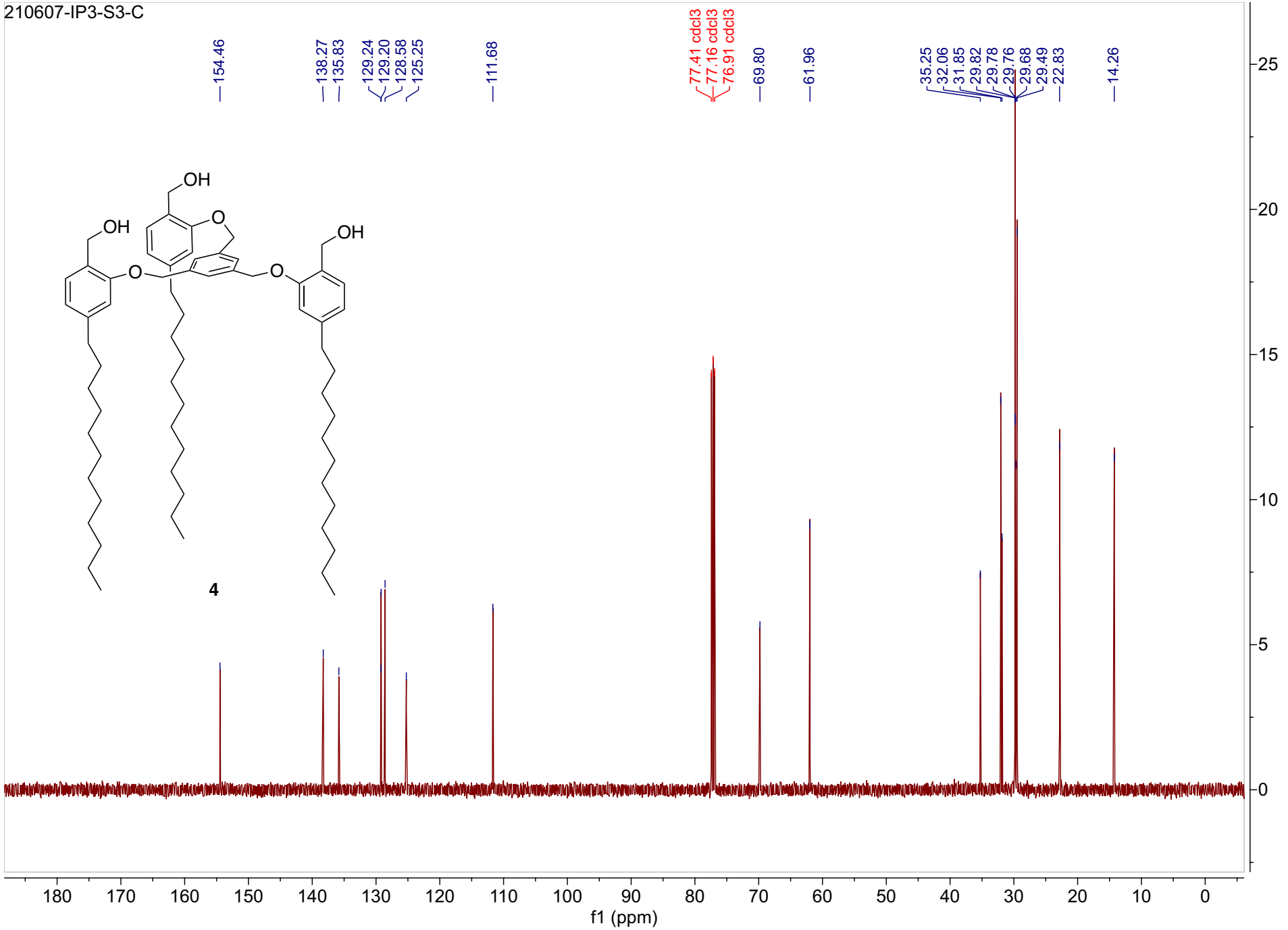
3

$[M+H]^+$ calcd for $C_{66}H_{96}O_6$: 985.7285
Found: 985.7243

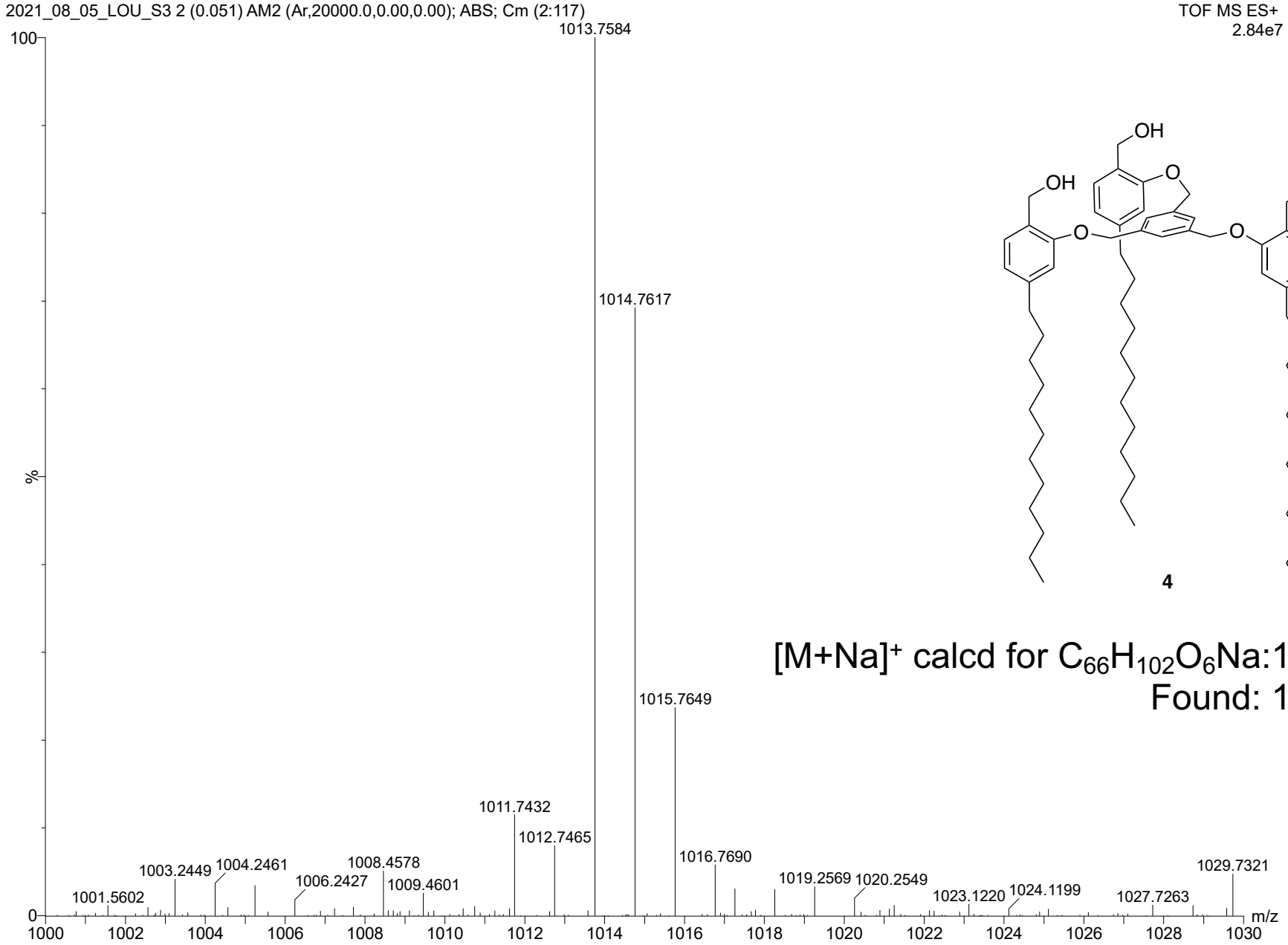
^{1}H NMR


210607-IP3-S3-H
7.44
7.26 cdcl3
7.15
7.15
7.06
7.06
7.05
7.04
6.84
6.82
5.12
4.71
2.56
2.55
2.53
1.60
1.58
1.57
1.31
1.30
1.27
0.90
0.89
0.88
OH
OH
OH
O
O
O
4
3.00
2.97
3.04
3.12
6.13
6.17
6.23
6.02
60.07
9.27
f1 (ppm)

^{13}C NMR


210607-IP3-S3-C
154.46
138.27
135.83
129.24
129.20
128.58
125.25
111.68
77.41 cdcl3
77.16 cdcl3
76.91 cdcl3
69.80
61.96
35.25
32.06
31.85
29.82
29.78
29.76
29.68
29.49
22.83
14.26
OH
OH
OH
O
O
O
4
f1 (ppm)

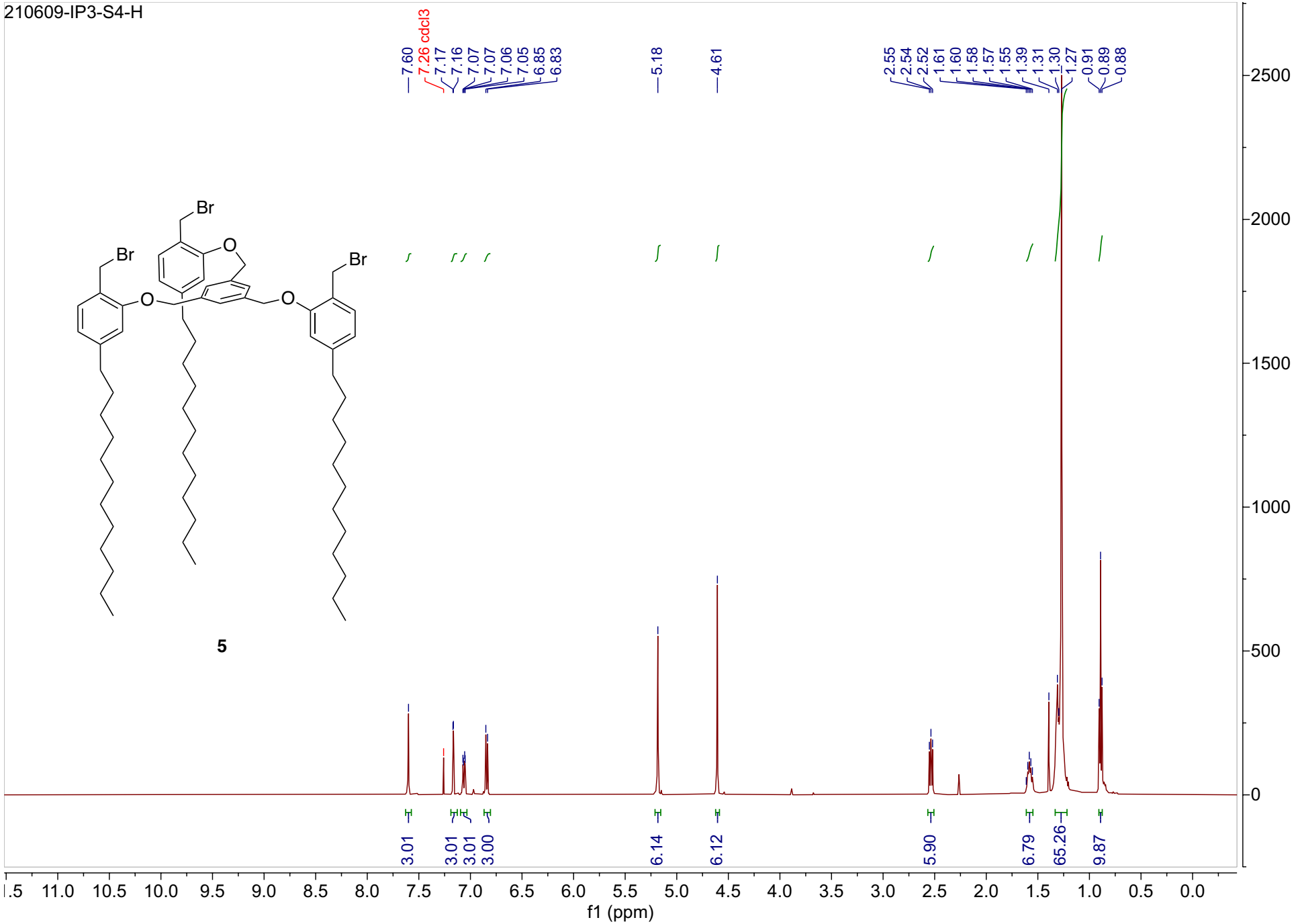
ESI-MS



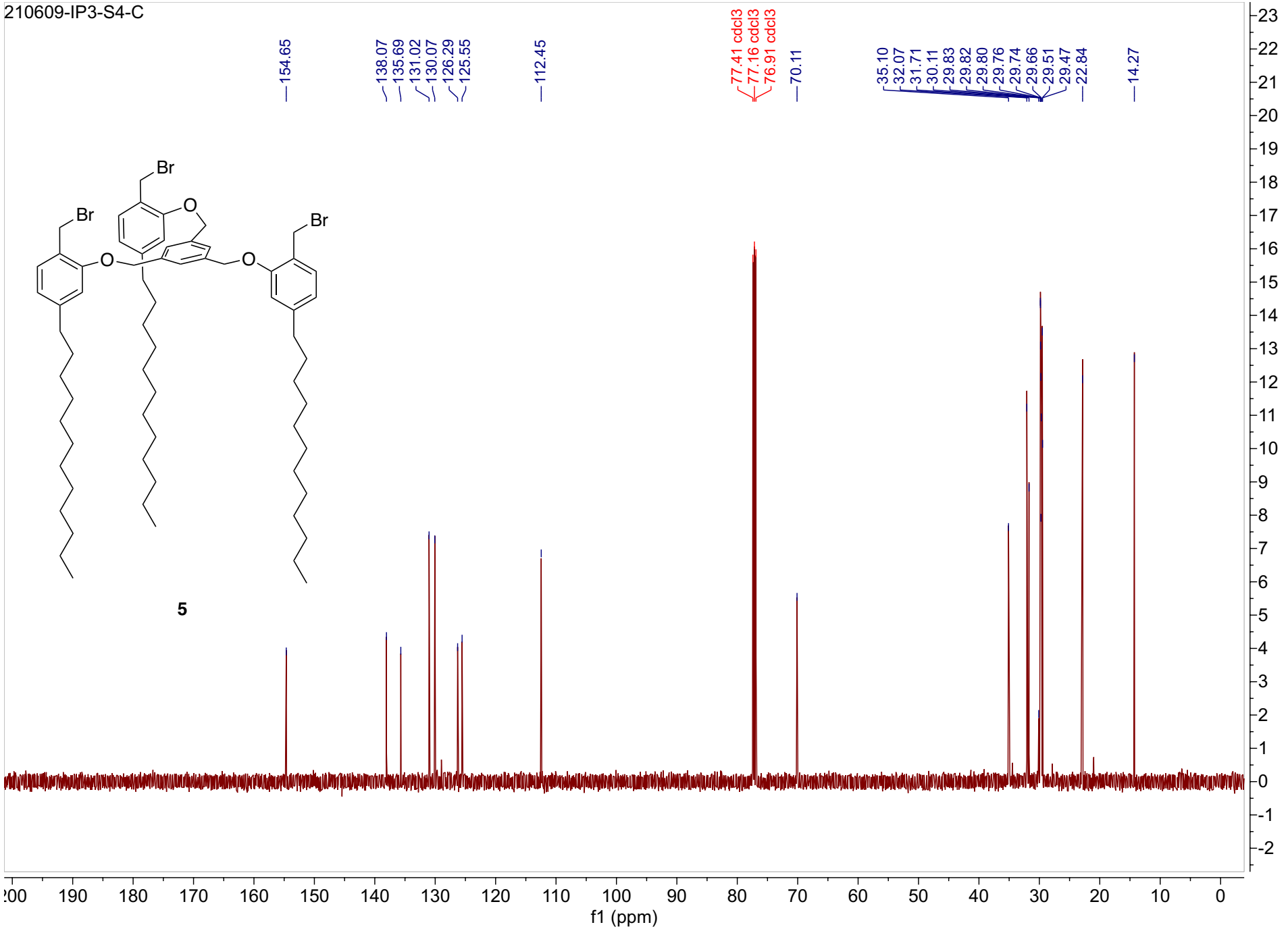
4

$[M+Na]^+$ calcd for $C_{66}H_{102}O_6Na$:1013.7574
Found: 1013.7584

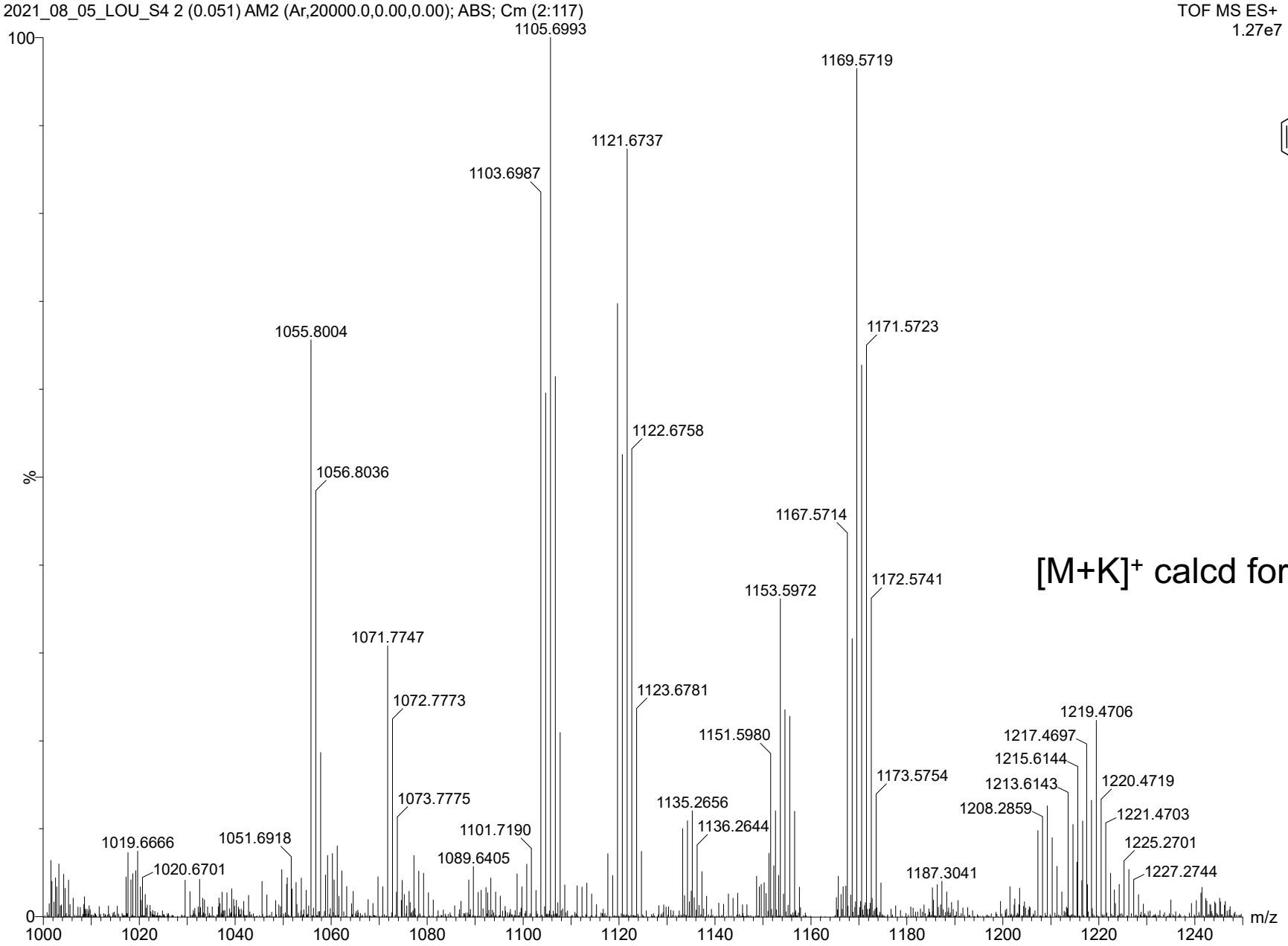
^{1}H NMR


210609-IP3-S4-H
7.60
7.26 cdcl3
7.17
7.16
7.07
7.07
7.06
7.05
6.85
6.83
5.18
4.61
2.55
2.54
2.52
1.61
1.60
1.58
1.57
1.55
1.39
1.31
1.30
1.27
0.91
0.89
0.88
Br
Br
Br
O
O
O
5
3.01
3.01
3.01
3.00
6.14
6.12
5.90
6.79
65.26
9.87
2500
2000
1500
1000
500
0
f1 (ppm)

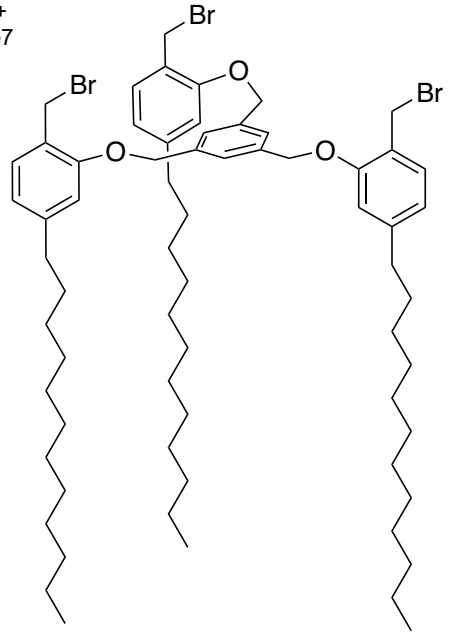
^{13}C NMR


210609-IP3-S4-C
154.65
138.07
135.69
131.02
130.07
126.29
125.55
112.45
77.41 cdcl3
77.16 cdcl3
76.91 cdcl3
70.11
35.10
32.07
31.71
30.11
29.83
29.82
29.80
29.76
29.74
29.66
29.51
29.47
22.84
14.27
Br
Br
Br
O
O
O
5
f1 (ppm)

ESI-MS



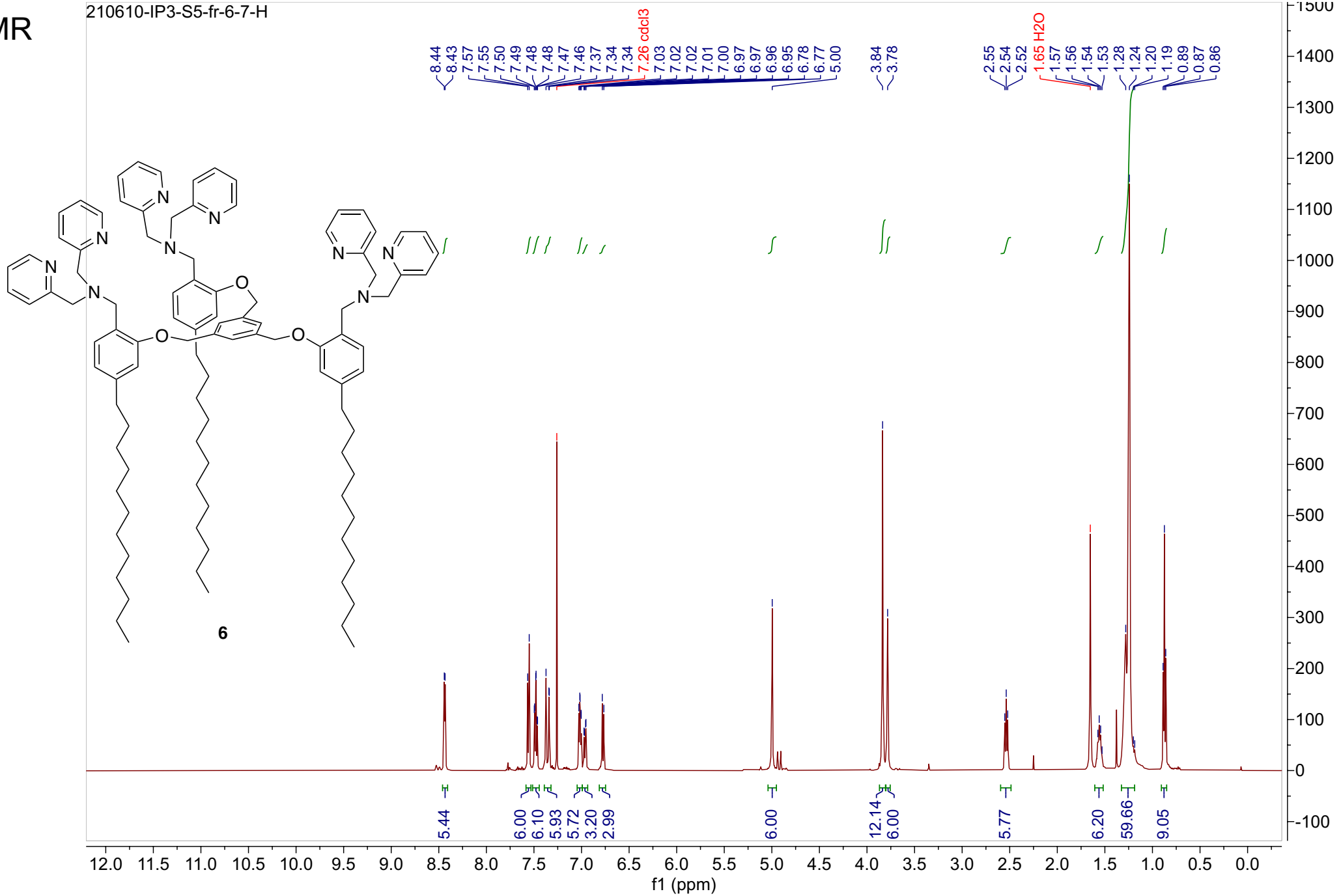
TOF MS ES+
1.27e7



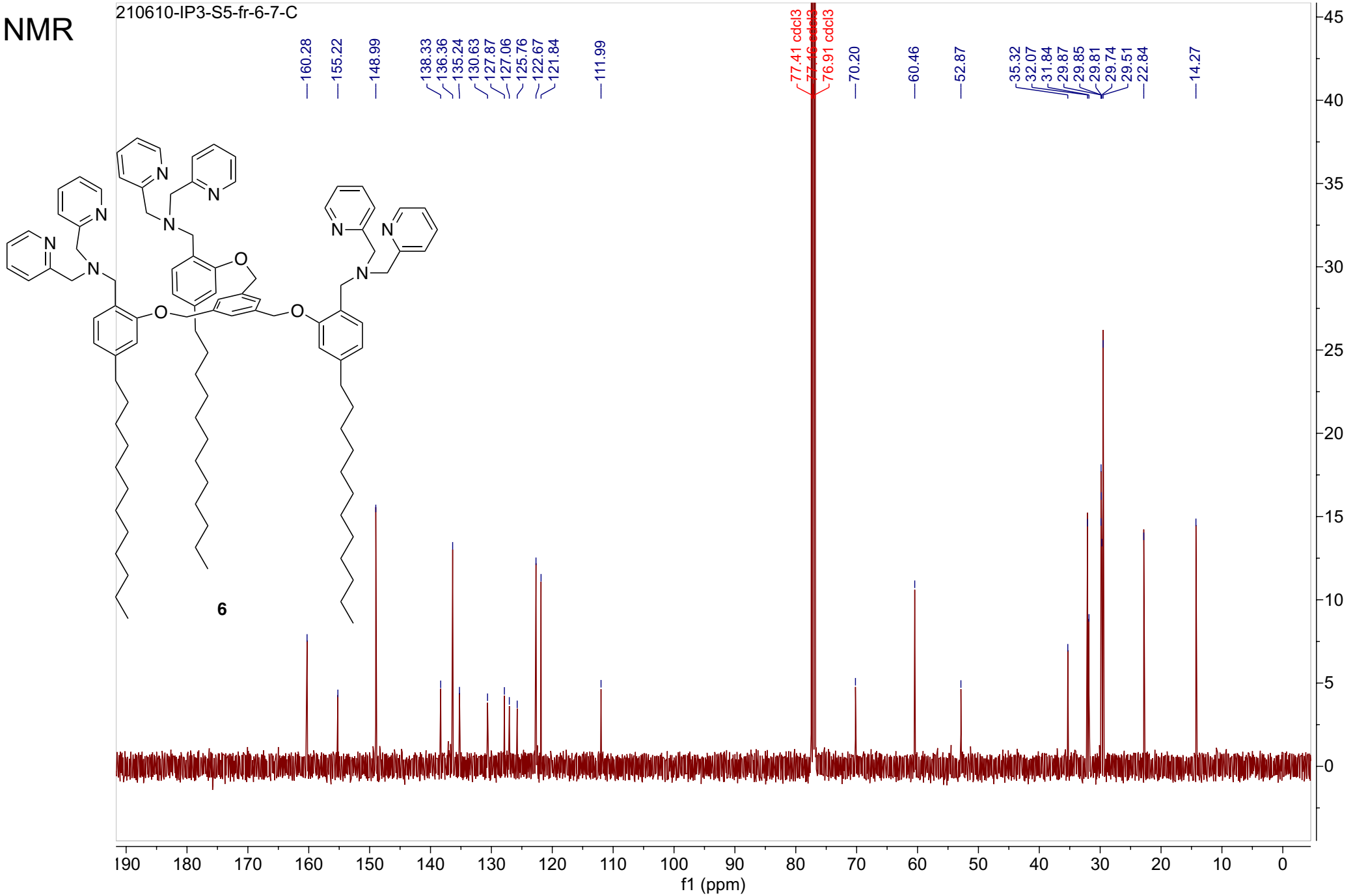
5

$[M+K]^+$ calcd for $C_{66}H_{99}Br_3O_3K$: 1215.4781
Found: 1215.6144

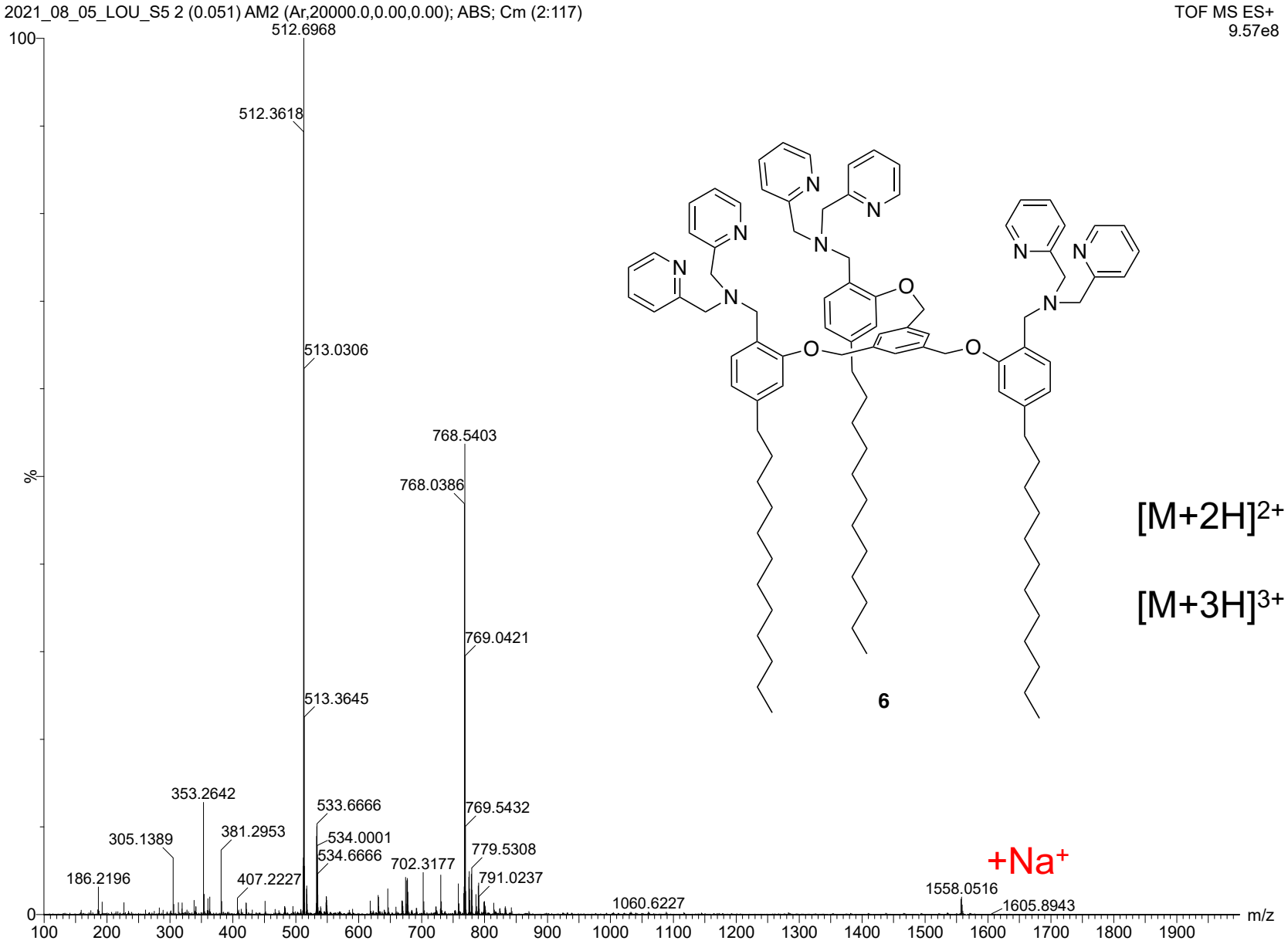
^{1}H NMR


210610-IP3-S5-fr-6-7-H
8.44
8.43
7.57
7.55
7.50
7.49
7.48
7.48
7.47
7.46
7.37
7.34
7.34
7.26 cdcl3
7.03
7.02
7.02
7.01
7.00
6.97
6.97
6.96
6.95
6.78
6.77
5.00
3.84
3.78
2.55
2.54
2.52
1.65 H2O
1.57
1.56
1.54
1.53
1.28
1.24
1.20
1.19
0.89
0.87
0.86
6
5.44
6.00
6.10
5.93
5.72
3.20
2.99
6.00
12.14
6.00
5.77
6.20
59.66
9.05
f1 (ppm)

^{13}C NMR


210610-IP3-S5-fr-6-7-C
160.28
155.22
148.99
138.33
136.36
135.24
130.63
127.87
127.06
125.76
122.67
121.84
111.99
77.41 cdcl3
77.16 cdcl3
76.91 cdcl3
70.20
60.46
52.87
35.32
32.07
31.84
29.87
29.85
29.81
29.74
29.51
22.84
14.27
6
f1 (ppm)

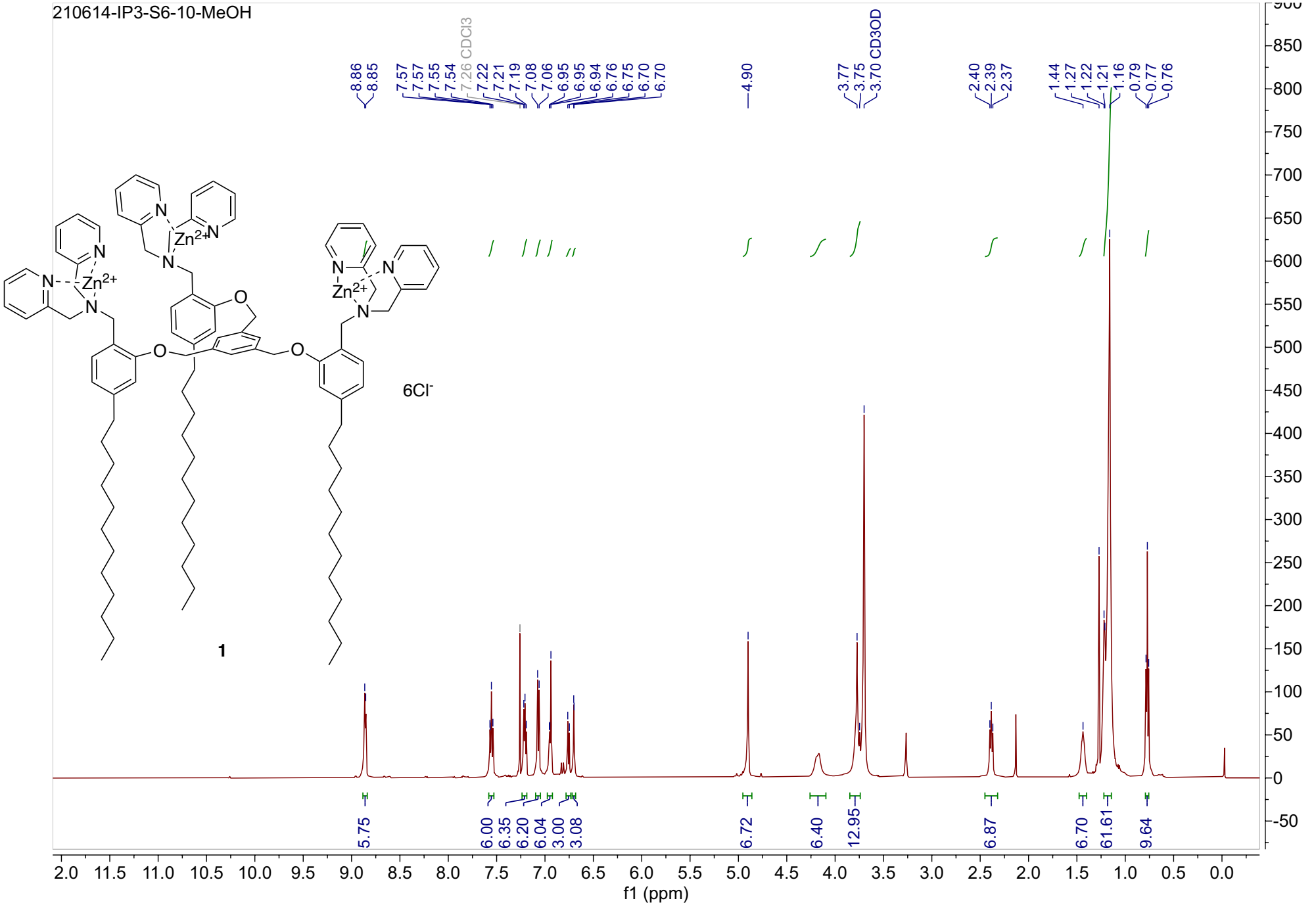
ESI-MS



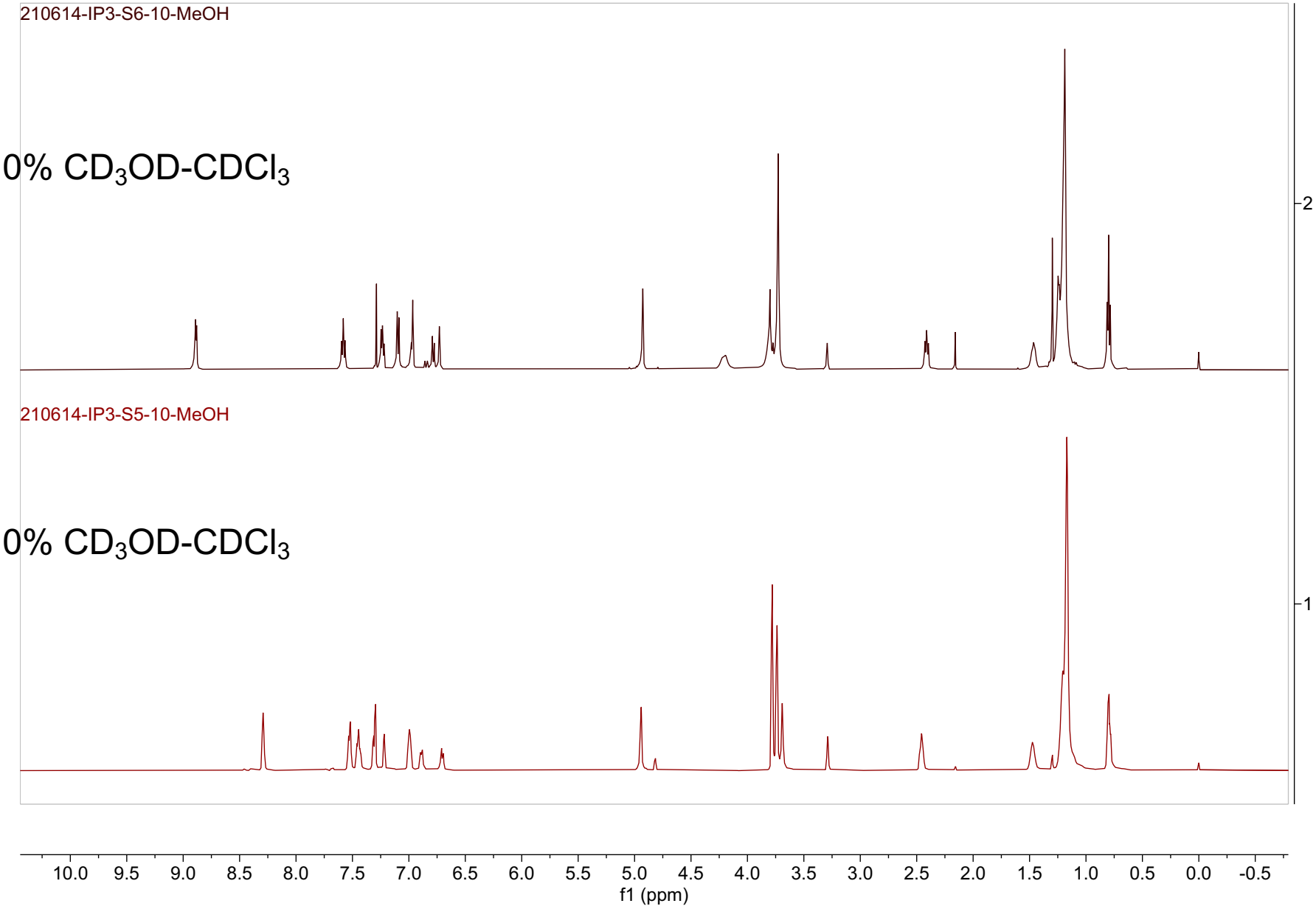
6

$[M+2H]^{2+}$ calcd for $C_{102}H_{137}N_9O_3$: 768.0422
Found: 768.0386
$[M+3H]^{3+}$ calcd for $C_{102}H_{138}N_9O_3$: 512.3641
Found: 512.3618

^{1}H NMR


210614-IP3-S6-10-MeOH
8.86
8.85
7.57
7.57
7.55
7.54
7.26 CDCl3
7.22
7.21
7.19
7.08
7.06
6.95
6.95
6.94
6.76
6.75
6.70
6.70
4.90
3.77
3.75
3.70 CD3OD
2.40
2.39
2.37
1.44
1.27
1.22
1.21
1.16
0.79
0.77
0.76
6Cl-
1
5.75
6.00
6.35
6.20
6.04
3.00
3.08
6.72
6.40
12.95
6.87
6.70
61.61
9.64
f1 (ppm)

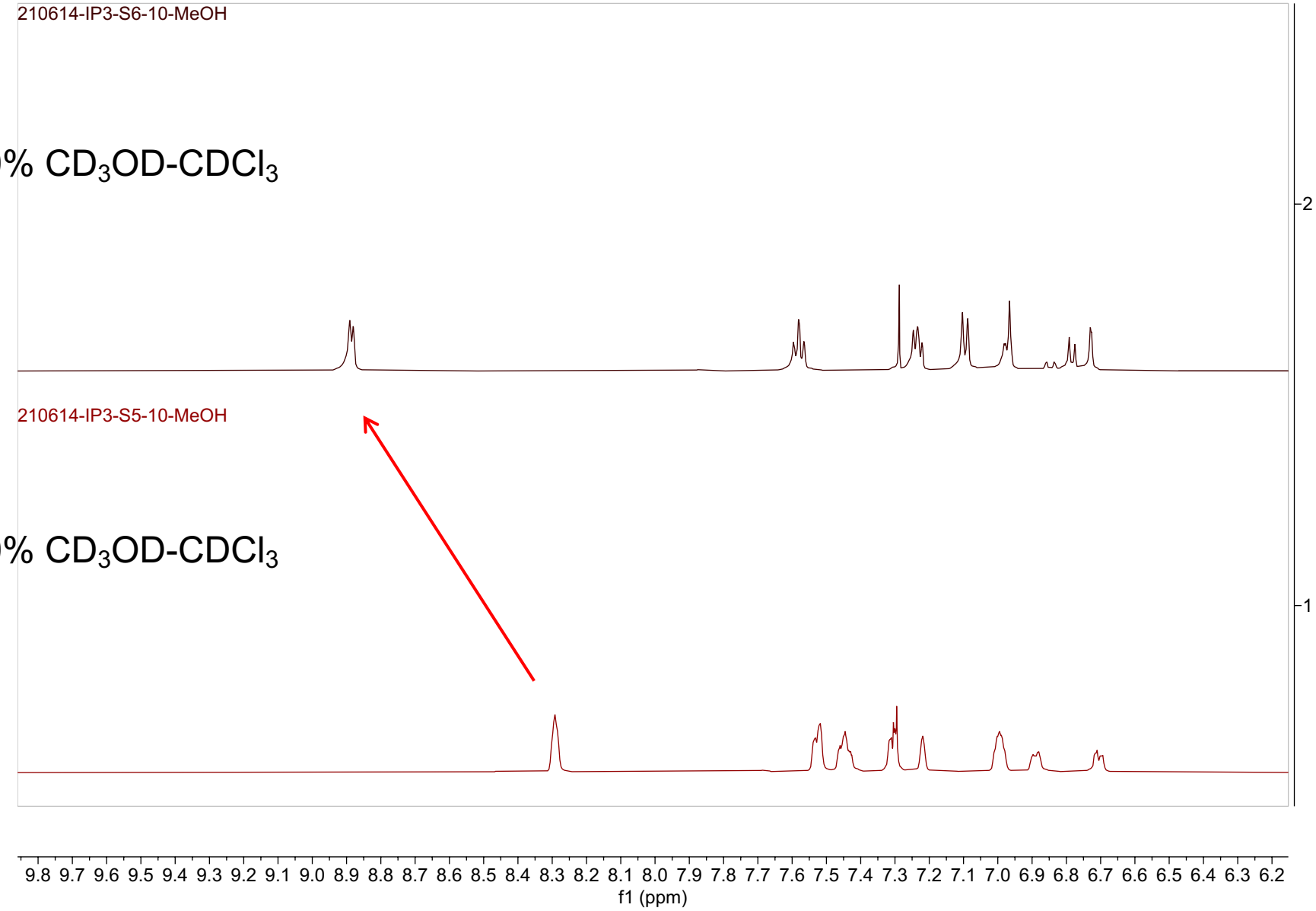
^{1}H NMR shift upon Zn chelation - Full spectra



1 in 10% CD_3OD-$CDCl_3$

6 in 10% CD_3OD-$CDCl_3$

^{1}H NMR shift upon Zn chelation - Full spectra



1 in 10% CD_3OD-$CDCl_3$

6 in 10% CD_3OD-$CDCl_3$