Supplementary Information

Stimuli-responsive Non-ionic Gemini Amphiphiles for Drug Delivery Applications

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1. Experimental Section

1.1 Material

All the chemicals and solvents were purchased from Spectrochem Pvt. Ltd., India and Sigma-Aldrich Chemicals, USA. Immobilized *Candida antarctica* lipase (Novozym 435) was obtained from Novo Nordisk A/S Denmark. The dyes/drugs with maximum purity used for encapsulation studies were purchased from Fluka Chemie GmbH (Buchs, Switzerland) and Sigma-Aldrich Chemicals, USA. All the solvents were dried and distilled prior to use. To monitor the progress of the reaction, pre-coated TLC plate (Merck silica gel 60F254) was used with visualization of the spots on TLC using cerric solution. For column chromatography, silica gel (100-200 mesh) was used. Millipore water was used for preparation of samples for their physico-chemical characterization and transport studies.

1.2. Instrumentation and Methods

1.2.1 NMR, IR Spectroscopy, and GPC Analysis

The ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on JEOL 400 MHz, Bruker DRX 400, and Bruker AMX 500 MHz spectrometers with the residual solvent peak used as a reference. Infrared spectra (IR) of the samples were recorded using a Perkin-Elmer FT-IR model 9 spectrometer. The chemical shift values are on a δ scale and the coupling constant values (*J*) are in Hertz. High-resolution mass spectrometry (HRMS) data were recorded on Q-TOF LCMS-Agilent Technologies-6530 and HPLC/MS-Agilent 6210 (Agilent Technologies).To obtain the molecular weight of amphiphiles,Waters GPC system equipped with a Waters 515 HPLC pump, refractive index detector, and styragel ® HR columns, was used using tetrahydrofuran (THF) as an eluent at a flow rate of 1.2 mL min^{-1} and molecular weight calibration carried using polystyrene standards.

1.2.2 Critical aggregation concentration (CAC) measurements

The critical aggregation concentration of the synthesized amphiphiles was studied by fluorescence technique using 'Nile red' as a model dye. A stock solution of the dye of concentration 1 mg mL^{-1} (3.14×10^{-3} M) was prepared in THF. 10 µL from the stock solution was added in each empty vial followed by complete evaporation of THF to form a thin layer. For preparation of stock solutions, amphiphiles were taken 5 mg/mL in Milli-Q water and allowed to stir for 1 h. Different concentrations of the amphiphiles obtained by two-fold serial dilution of the stock solutions were then transferred to the vials having thin film of the dye followed by overnight stirring.The non-encapsulated dye in all the solutions was removed by filtration through 0.45 µm polytetrafluoroethylene (PTFE) filter with subsequent fluorescence measurements using Cary Eclipse fluorescence spectrophotometer. The plot of fluorescence intensity maxima values against log[amphiphile concentration] for different samples afforded the CAC value.

1.2.3 Dynamic Light Scattering (DLS)

Malvern Zetasizer Nano ZS analyzer, which was integrated with 4 mW He-Ne laser, λ= 633 nm, using backscattering detection (scattering angle θ = 173°) with an avalanche photodiode

detector, was used for determining the size of nanostructures (micelles/aggregates) formed by the supramolecular organization of amphiphiles in the aqueous solution (Milli-Q water) at a concentration of 5 mg/mL^{-1}. The samples were then further allowed to mix at 25 ^{o}C for 20 h with vigorous stirring. The obtained solutions were then filtered through 0.22 μm PTFE filters and equilibrated for 1 h at room temperature, then transferred to disposable micro BRAND ultraviolet (UV) cuvettes, and used for DLS measurements.

1.2.4 Cryogenic transmission electron microscopy (cryo-TEM)

Perforated carbon film-covered microscopical 200 mesh grids (R1/4 batch of Quantifoil, MicroTools GmbH, Jena, Germany) were cleaned with chloroform and hydrophilized by 60 s glow discharging at 8 W in a BAL-TEC MED 020 device (Leica Microsystems, Wetzlar, Germany) before 5 μl aliquots of the sample solution (5 mg ml^{-1}) were applied to the grids. The samples were automatically blotted and vitrified with a FEI Vitrobot Mark IV and (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) using liquid ethane as cryogen. *Cryo*-TEM measurements were carried out on a Tecnai F20 TEM (FEI Company, Oregon) equipped with a field emission gun (FEG) at an acceleration voltage of 160 kV using a Gatan cryo holder at 94 K sample temperature. By using the microscope's low-dose protocol, the micrographs were recorded with a FEI Eagle 4k × 4k CCD camera in a two-fold binding mode.

1.2.5 Encapsulation of Nile red and curcumin

Encapsulation of Nile red was studied by following the film method and for curcumin solid dispersion method was used. UV-Vis and fluorescence spectra measurements were used to quantify the encapsulated dye/drugs. The solubilization experiment was carried out at a concentration of 5 mg/mL^{-1} for all the amphiphiles using 0.12 mg of Nile red and for curcumin encapsulation, 1 mg of it and 5 mg of amphiphile were dissolved in methanol, stirred for 30 min and then the solvent evaporated, followed by the addition of 1 mL of 1X PBS buffer (pH 7.4). After stirring for 20 h at room temperature, the non-encapsulated portion of the dye/drug was removed by filtering it (twice), slowly through 0.45 μm PTFE filter. For the quantification of encapsulated dye/drug, the encapsulated samples were lyophilized and re-dissolved in a known quantity of anhydrous methanol. The absorbance (220-800 nm) spectra were recorded

on a Cary-300 series UV-Vis spectrophotometer (Agilent Technologies) using standard disposable PMMA UV/Vis cuvettes with a path length of 1 cm from PLASTIBRAND. Fluorescence measurements (450-800 nm) were performed on a Cary Eclipse fluorescence spectrophotometer (Agilent Technologies) using a variable slit system from 575-800 nm for Nile red and 450-700 nm for curcumin. The fluorescence emission spectra were recorded by carrying out the excitation at 550 nm for Nile red, and at 420 nm for curcumin. Microsoft Excel® and Origin 8 software were used for data analysis.

1.2.6 *Encapsulation of doxorubicin*

A solution of 1 mg of DOX.HCl in 400 µL THF was added to 1.1 eq of triethylamine and stirred for 3 h to get neutralized DOX. The obtained solution was added to 5 mg of amphiphile, 0.8 mL of milli Q water was added over 30 min with constant stirring, and then mixture was further dialyzed using 500D MWCO size membrane for 48 h using milli-Q water as a solvent. After the dialysis was completed, the solution inside the tube was collected and the total volume was adjusted to 1 mL. The final amphiphilic concentration was approximately 5 mg/mL. Furthermore, the UV/Vis and emission spectra were recorded, which confirmed the encapsulation of DOX inside the hydrophobic cavity of synthesized amphiphiles. For calculating the drug-loading content, lyophilized DOX-loaded samples were dissolved in DMSO and analyzed with UV/Vis spectroscopy: the absorption values were fitted to the calibration curve obtained from UV/Vis spectra of aqueous DOX solution in absence of any amphiphiles. DLE (DOX-loading efficiency) and DLC (DOX-loading content were determined according to the following formula:

DLC (%) = (weight of loaded drug)/ (weight of amphiphile) x 100%

DLE (%) = (weight of loaded drug)/ (weight of drug in feed) x100%.

1.2.7 Cell viability assay CCK8

Cytotoxicity of the compounds was analyzed by the Cell Counting Kit 8 (CCK-8) from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany) according to the manufactures' instructions. In short, A549 cells (DSMZ no.: ACC 107) that were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum (Biowest, Nuaillé,

France), 100 U/mL penicillin, and 100 µg/mL streptomycin were seeded in a 96-well plate (4,000 cells/well) and incubated overnight at 37 °C and 5% CO_2. Then compounds were added in serial dilutions. SDS and non-treated cells served as a control. For background subtraction, also wells containing no cells but only samples were used. Cells were incubated with the pure compounds for one day or the doxorubicin-containing compounds for two days, respectively, at 37 °C before the CCK-8 solution was added. After 3 hours, absorbance was measured at a measurement wavelength of 450 nm and a reference wavelength of 650 nm with a Tecan plate reader (Infinite pro200, TECAN-reader Tecan Group Ltd., Männedorf, Switzerland). Measurements were done in triplicates and repeated three times. The cell viability was calculated by setting the non-treated control to 100% and the non-cell control to 0% after subtracting the background using the GraphPad Prism software.

1.2.8 Cellular uptake study by a confocal laser-scanning microscopy (cLSM) and flow cytometry

Cellular uptake of the doxorubicin-loaded nanocarriers in A549 cells (routinely propagated as stated above) was monitored by a confocal laser-scanning microscopy (cLSM) and flow cytometry.

For cLSM, A549 cells were seeded in 8-well ibidi µ-slides (27.000 cells/well) in cell culture medium. After 1 day, the doxorubicin-loaded nanocarriers were added and the cells were grown for 4 hours or 24 hours, respectively. Cell nuclei were stained with 1 µg/ml Hoechst 33342 (Life Technologies GmbH, Darmstadt, Germany). Non-treated cells served as control. Confocal images were taken with an inverted confocal laser scanning microscope Leica DMI6000CSB SP8 (Leica, Wetzlar, Germany) with a 63x/1.4 HC PL APO CS2 oil immersion objective using the manufacture given LAS X software.

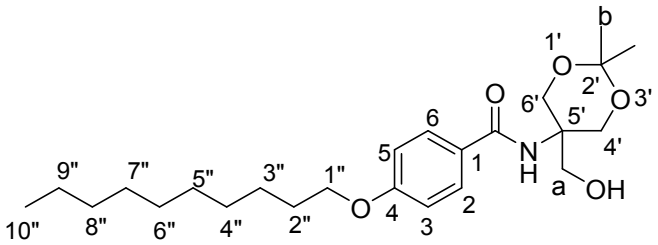
For flow cytometry, A549 cells were seeded in 24-well plates (100,000 cells per well) in cell culture medium. After 1 day, the doxorubicin-loaded nanocarriers were added and the cells were grown for 4 hours or 24 hours. Non-treated cells served as control. Cells were detached by trypsin, transferred to an Eppendorf tube, centrifuged at 140xg and 4 °C for 4 min, and resuspended in PBS. Fluorescence of the cells was measured in a BD Accuri C6 (Becton Dickinson, Heidelberg, Germany) and analysis was done by the BD Accuri C6 Software.

1.2.9 Redox- and enzyme-triggered release study

For the time-dependent redox-responsive and enzymatic release study, Nile red was used as a model dye and encapsulated in the amphiphilic solution following the same protocol as used for quantification. After removing the non-encapsulated dye through 0.45 μm PTFE filter, for redox responsive release, solid GSH was added to make different concentrations of the solution, i.e, 5 mM, 20 mM, and 30 mM. For enzymatic release study, a few drops of *n*-butanol and 200 wt % of the immobilized enzyme (Novozyme 435) were added. The final solutions were incubated at 37 ^{o}C and stirred at 200 rpm, and time-dependent fluorescence measurements were made after every 1 h. The time-dependent release was studied using fluorescence spectroscopy (Cary Eclipse fluorescence spectrophotometer, Agilent Technologies) by periodically measuring the emission maxima.

1.3 Experimental

*1.3.1 Synthesis of compound **11***



To an ice-cold stirring solution of 4-(decyloxy)benzoic acid (1 g, 3.99 mmol) in DMF, EDC (0.82 g, 4.2 mmol), HOBt (0.27 g, 2 mmol) and DIPEA (5.17 g, 4 mmol) were added. The reaction mixture was then stirred at 35 ^{o}C for 30 min, followed by the addition of amine (**7**). The resultant solution was stirred at 50 ^{o}C for 18 h. On completing reaction, which was monitored by TLC (petroleum ether:ethyl acetate :: 7:3), the solvent was evaporated under reduced pressure and the mixture extracted with ethyl acetate (4 x 150 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed by rotary evaporation. The crude product was purified by column chromatography to give pure product **11** in 70% yield.

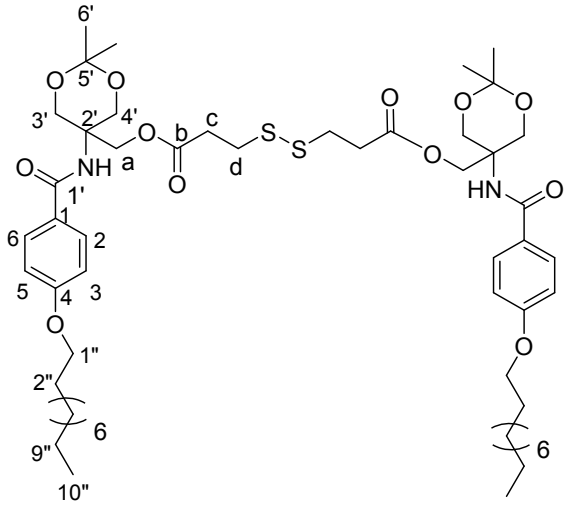
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (d, *J =8 Hz*, 2H, H-2 & H-6), 6.93 (d, *J =8Hz*, 2H, H-3 & H-5), 5.30 (t, *J =4 Hz*, 1H, OH), 3.99 (t, *J =6.4 Hz,* 2H, H-1"), 3.97 (d, *J =8 Hz*, 2H, H-4'a, H-6'a), 3.91 (d, *J =8 Hz*, 2H, H-4'b, H-6'b), 3.76 (d, *J =4 Hz*, 2H, H-a), 1.83-1.77 (m, 2H, H-2"), 1.49 & 1.47 (2s, 3H each, H-b), 1.34-1.27 (m, 14H, H-3"-H-9"), 0.88 (t, *J =8 Hz*, 3H, CH_3,(H-10")).

^{13}C NMR (100.5 MHz, $CDCl_3$): δ168.36 (-COO), 162.27 (C-4), 129.01 (C-2, C-6), 126.23 (C-1), 114.45 (C-3, C-5), 99.08 (C-2'), 68.33 (C-1"), 64.68 (C-4', C-6', C-a), 55.28 (C-5'), 32.03, 29.63, 29.59, 29.39, 28.46, 26.05, 22.82, 18.97 (C-2"-C-9"), 14.20 (C-10").

HRMS (positive, MeOH): *m/z* $[M + H]^+$ calculated for $C_{24}H_{39}NO_5$: 421.28; found: 422.24.

IR (Neat) ν_{max}: 3350, 2989, 1632, 1550 cm^{-1}.

*1.4.2 Synthesis of compound **14***



To an ice-cold stirring solution of compound **11** (2.2 g, 11.5 mmol) in DCM, EDC.HCl (1 g, 11.5 mmol) and DMAP (0.43 g, 5.35 mmol) were added. The reaction mixture was then

stirred at 30 ^{o}C for 30 min, followed by the addition of 3,3'dithiopropanoic acid (0.5 g, 2.38 mmol). The resultant solution was stirred at 35 ^{o}C for 12 h. On completion of the reaction, monitored by TLC, the solvent was evaporated under reduced pressure and the reaction mixture extracted with ethyl acetate (4 x 150 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed by rotary evaporation. The crude product was purified by column chromatography to give pure product in 78% yield.

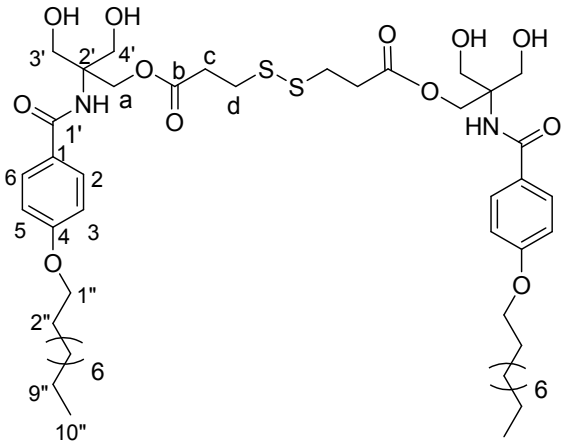
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69 (d, *J =8 Hz*, 4H, H-2 & H-6), 6.89 (d, *J =8Hz*, 4H, H-3 & H-5), 4.63 (s, 4H, H-a), 4.48 (d, *J =12 Hz*, 4H, H-3'a, H-4'a), 3.97 (t, *J =6.4 Hz,* 4H, H-1"), 3.80 (d, *J =12 Hz*, 4H, H-3'b, H-4'b), 2.87 (t, *J =6.4 Hz,* 4H, H-d) 2.75 (t, *J =8 Hz,* 4H, H-c), 1.79-1.75 (m, 4H, H-2"), 1.54 & 1.42 (2s, 6H each, 4 x CH_3 (H-6')), 1.31-1.23 (m, 28H, H-3"-H-9"), 0.87 (t, *J =8 Hz,* 6H, 2xCH_3 (H-10")).

^{13}C NMR (100.5 MHz, $CDCl_3$): δ172.58 (-COO), 167.32 (-CONH), 162.09 (C-4), 128.86 (C-2, C-6), 126.38 (C-1), 114.34 (C-3, C-5), 109.51 (C-5'), 68.30 (C-2"), 64.48 and 62.07 (C-3', C-4', C-a), 53.55 and 52.01 (C-2'), 33.84, 33.23, 33.09, 32.09, 31.88, 29.72, 29.01, 25.98, 24.53, 23.11, 22.69, 21.96 (C-c, C-d, C-2"-C-9"), 14.18 (C-10").

HRMS (positive, MeOH): *m/z* $[M + H]^+$ calculated for $C_{54}H_{84}N_2O_{12}S_2$: 1016.55; found: 1017.45.

IR (Neat) ν_{max} : 3198, 2978, 1631, 1607, 1548 cm^{-1}.

*1.4.3 Synthesis of compound **17***



The synthesized acetal-protected **12** in methanol was then subjected to deprotection using acidic ion exchange resin Dowex 50W along with 5-6 drops of water at 30 ^{o}C for 6 h. The resin was filtered off on completion of the reaction as monitored by TLC (MeOH: $CHCl_3$::1:9), which was followed by removal of solvent on a rotary evaporator to obtain the deprotected compound **17** as white solid in 80% yield.

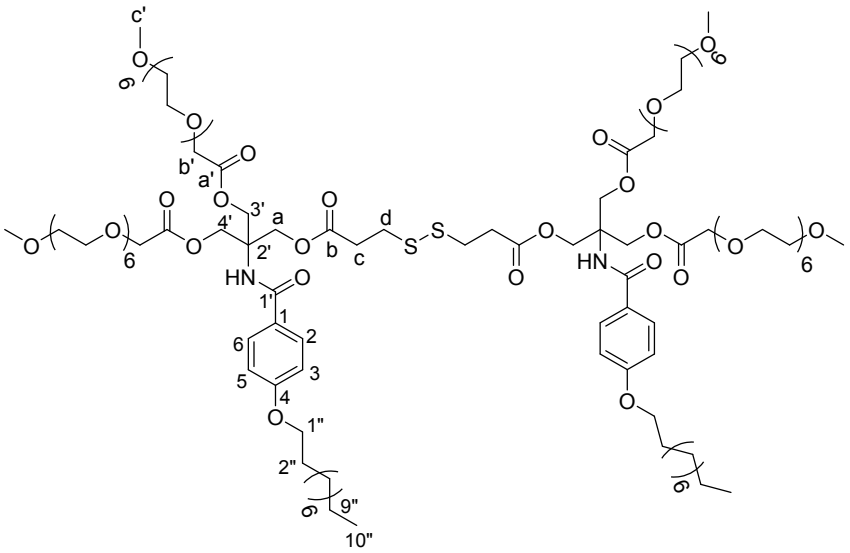
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72 (d*, J=8 Hz*, 4H, H-2 & H-6), 7.00 (bs, NH), (6.92 (d, *J=8Hz*, 4H,H-3 & H-5), 4.55 (bs, OH), 4.49 (s, 4H, H-a), 3.99 (t,*J =6.4 Hz,* 4H,H-1"), 3.79 (d, *J=12 Hz*, 4H, H-3a', H-4a'), 3.64 (d, *J=12 Hz*, 4H, H-3b', H-4b'), 2.95 (t*, J=6.4 Hz*, 4H, H-d), 2.81 (t*, J=6.4 Hz*, 4H, H-c), 1.82-1.79 (m, 4H, H-2"), 1.34-1.27 (m, 28H, H-3"-H-9"), 0.88 (t, *J =8 Hz,* 6H, 2xCH_3(H-10")).

^{13}C NMR (100.5 MHz, $CDCl_3$): δ172.73 (-COO), 168.41 (-CONH), 162.44 (C-4), 129.07 (C-2, C-6), 125.54 (C-1), 114.47 (C-3, C-5), 68.37, 63.00, 62.43, 61.52 (C-1", C-2', C-3', C-4', C-a), 34.08, 33.25, 31.97, 29.64, 29.45, 29.40, 29.18, 26.05, 22.76 (C-c, C-d, C-2"-C-9"), 14.21, 14.20 (C-10").

HRMS (positive, MeOH): *m/z* $[M + H]^+$ calculated for $C_{48}H_{76}N_2O_{12}S_2$: 937.25; found: 938.55.

IR (Neat) ν_{max}: 3450, 2980, 1751, 1607, 1548 cm^{-1}.

*1.4.4 Synthesis of compound **1***



To an ice-cold stirring solution of compound **17** (0.2 g, 0.213 mmol) in DCM, EDC.HCl (0.183 g, 4.3 mmol) and DMAP (0.065 g, 1.33 mmol) were added. The reaction mixture was then stirred at 35 ^oC for 30 min, followed by the addition of mPEG-350 acid (0.44 g, 7.5 mmol) The resultant solution was stirred at 35 ^oC for 24 h. On completion of the reaction monitored by TLC, the solvent was evaporated under reduced pressure and the reaction mixture extracted with chloroform (4 x 40 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and the solvent evaporated under reduced pressure. The crude product was purified by column chromatography to yield pure amphiphile in 75% yield.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69 (d, *J=8Hz*, 4H, H-2 & H-6), 6.87 (d, *J=8Hz*, 4H, H-3 & H-5), 6.75 (bs, NH), 4.61 (s, 8H, H-b'), 4.55 (s, 4H, H-a), 4.15(s, 8H, H-3', H-4'), 3.95 (t, *J=6.4 Hz,* 4H, H-1"), 3.72-3.52(m, PEG region), 3.35-3.32 (4s, 12H, 4xOCH_3), 2.86-2.83 (m, 4H, H-d) 2.74-2.71 (m, 4H, H-c), 1.78-1.74 (m, 4H, H-2"), 1.44-1.22 (m, 28H, H-3"-H-9"), 0.85 (t, 6H, 2xCH_3(H-10")).

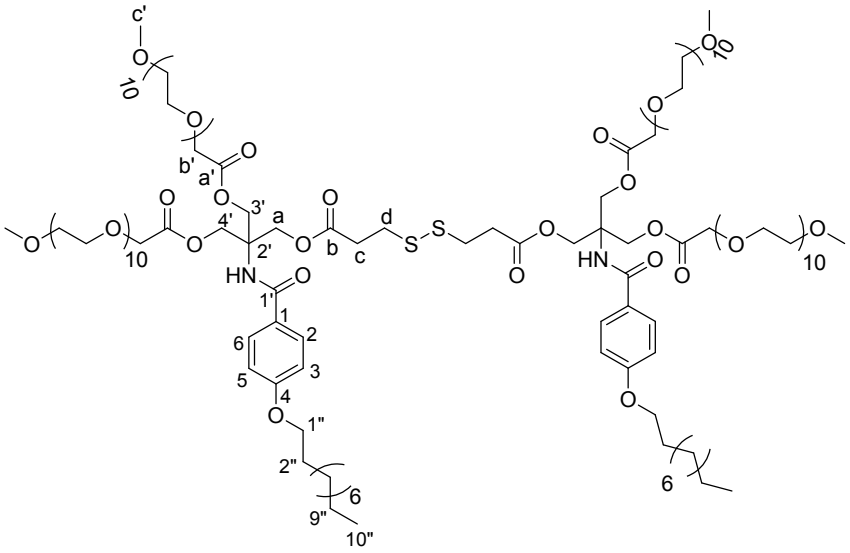
^{13}C NMR (100.5 MHz, $CDCl_3$): δ171.52, 170.39 (-COO), 167.15 (-CONH), 162.20 (C-4), 129.03 (C-2, C-6), 125.87 (C-1), 114.30 (C-3, C-5), 72.01, 71.02, 70.65, 70.54, 70.25, 70.03,

68.87, 68.53 (PEG), 68.21, 62.88, 59.07, 58.64 (C-1'', C-2', C-3', C-4', C-a), 51.84 (OCH_3), 33.84, 32.71, 31.94, 29.68, 29.52, 29.26, 29.19, 26.03, 22.73 (C-2”-C-9”), 14.22 (C-10'').

GPC (THF, 1.2 mL min^{-1}, polystyrene std.): Mw = 1975 g mol^{-1}, M_n = 1940 g mol^{-1}, PDI = 1.069.

IR (Neat) ν_{max}: 3272, 2912, 1745, 1645, 1609 cm^{-1}.

*1.4.5 Synthesis of compound **2***



To an ice-cold stirring solution of compound **17** (0.2 g, 0.213 mmol) in DCM, EDC.HCl (0.183 g, 4.3 mmol), DMAP (0.065g, 1.33 mmol) were added. The reaction mixture was then stirred at 35 ^oC for 30 min, followed by the addition of mPEG-550 acid (0.70 g, 7.6 mmol) The resultant solution was stirred at 35 ^oC for 24 h. On completion of the reaction monitored by TLC, the solvent was evaporated under reduced pressure and the reaction mixture extracted with chloroform (4 x 50 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed by rotary evaporation. The crude product was purified by column chromatography to give pure product in 78% yield.

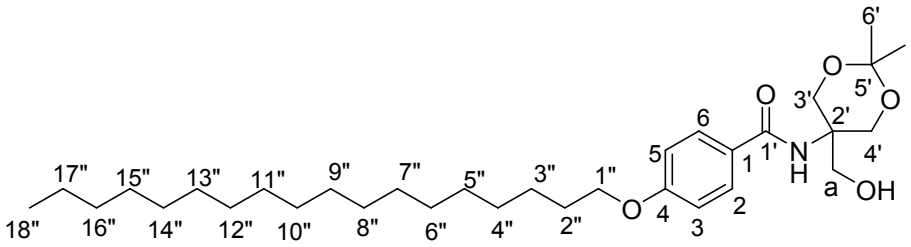
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (d, *J =8 Hz*, 4H, H2 & H-6), 6.88 (d, *J =8Hz*, 4H, H-3& H-5), 6.75 (bs, NH), 4.62-4.55 (m, 12H, H-a&H-b'), 4.15 (s, 8H, H-3', H-4'), 3.96 (t, *J=6.4 Hz*4H, H-1"), 3.73-3.52(m, PEG region), 3.36 (4s, 12H, H-c'), 2.85 (m, 4H, H-d), 2.74 (m, 4H, H-c), 1.77-1.73 (m, 4H, H-2"),1.42-1.22 (m, 28H, H-3"-H-9"), 0.86 (t, *J=8Hz* 6H, H-10").

^{13}C NMR (100.5 MHz, $CDCl_3$): δ171.54, 170.40 (-COO), 167.14 (-CONH), 162.21 (C-4), 129.14 (C-2, C-6), 125.86 (C-1), 114.21 (C-3, C-5), 72.07, 71.16, 70.30, 69.57, 68.93, 68.08 (PEG), 67.83 (C-1"), 63.74, 62.86, 61.76, 59.10 (C-3', C-4', C-a), 52.14, 51.79 (OCH_3), 31.86, 29.76, 29.33, 29.00, 26.06, 22.74 (C-2"-C-9"), 14.19 (C-10").

GPC (THF, 1.2 mL min^{-1}, polystyrene): Mw = 3528 g mol^{-1}, M_n= 3322 g mol^{-1}, PDI = 1.067

IR (Neat) ν_{max}: 3224, 2920, 1745, 1640, 1607 cm^{-1}.

*1.4.6 Synthesis of compound **12***



To an ice-cold stirring solution of 4-(octadecyloxy)benzoic acid (1 g, 2.82 mmol) in DMF, EDC (0.58 g, 3.07 mmol), HOBt (0.41 g, 3.07 mmol), and DIPEA (0.99 g, 7.6 mmol) were added. The reaction mixture was then stirred at 35 ^oC for 30 min, followed by the addition of amine (**7**) (0.49 g, 3.0 mmol). The resultant solution was stirred at 50 ^oC for 18 h. On completion of the reaction, monitored by TLC (petroleum ether:ethyl acetate :: 7:3), the solvent was evaporated under reduced pressure and the reaction mixture was extracted with

ethyl acetate (4 x 150 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure using rotary evaporation. The crude product was purified by column chromatography to give pure product **12** as a white solid in 70% yield.

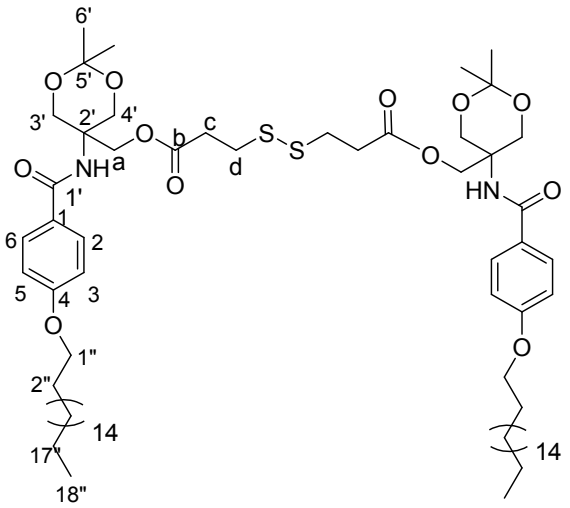
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (d, *J=8 Hz*, 2H, H-2 & H-6), 6.93 (d, *J=8Hz*, 2H, H-3 & H-5), 4.00 (t, *J=6.4 Hz* 2H, H-1''), 3.97 (d, *J=8 Hz*, 4H, H-3a', H-4a') and 3.91(d, *J=8 Hz*, 4H, H-3b', H-4b'), 3.77 (s, 2H, H-a), 1.83-1.76 (m, 2H, H-2''), 1.49 & 1.46 (2s, 3H each, 2 x CH_3 (H-6′)), 1.35-1.20 (m, 30H, H-3''-H-17''), 0.87 (t, *J=8 Hz,* 3H, H-18'').

^{13}C NMR (100.5 MHz, $CDCl_3$): δ 168.37(-CONH), 162.28 (C-4), 129.01 (C-2, C-6), 126.21 (C-1), 114.45 (C-3, C-5), 99.10 (C-5'), 68.34 (C-1''), 64.74, 64.66, 55.35 (C-3',C-4',C-a, C-2') 36.58, 32.01, 31.52, 29.78, 29.74, 29.68, 29.65, 29.45, 29.19, 28.60, 26.06, 22.76, 18.85 (C-2''-C-17''), 14.22 (CH_3).

HRMS (positive, MeOH): *m/z* $[M + H]^+$ calculated for $C_{32}H_{55}NO_5$: 533.78; found: 534.80.

IR (Neat) ν_{max} : 3410, 2990, 1632, 1550 cm^{-1}.

*1.4.7 Synthesis of compound **15***



To an ice-cold stirring solution of compound **12** (1.67 g, 3.1 mmol) in DCM, EDC.HCl (0.6 g, 3.1 mmol), DMAP (0.26 g, 2.1 mmol) were added. The reaction mixture was then stirred at 35 ^{o}C for 30 min, followed by the addition of 3,3'-dithiopropanoic acid (0.3 g, 1.4 mmol). The resultant solution was stirred at 35 ^{o}C for 12 h. On completion of the reaction, monitored by TLC, the solvent was evaporated under reduced pressure and mixture extracted with ethyl acetate (4 x 150 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed by rotary evaporation. The crude product was purified by column chromatography to give pure product in a 80% yield.

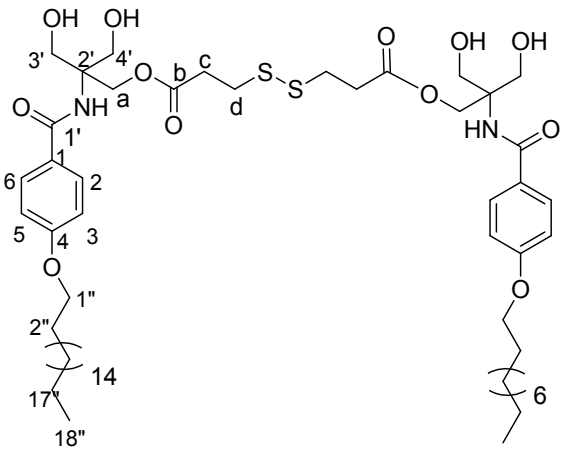
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69 (d, *J=8 Hz*, 4H, H-2 & H-6), 6.89 (d, *J=8Hz*, 4H, H-3 & H-5), 6.58 (bs, NH), 4.63 (s, 4H, H-a), 4.50 (d, *J=12 Hz,* 4H, H-3'), 3.97 (t, *J=6.4 Hz* 4H, H-1"), 3.81 (d, *J=12 Hz,* 4H, H-4'), 2.87 (t, *J=6.4 Hz,* 4H, H-d) 2.75 (t, *J=6.4 Hz*, 4H, H-c), 1.81-1.74 (m, 4H, H-2"), 1.54 & 143 (2s, 6H each, H-6′), 1.32-1.20 (m, 60H, H-3"-H-17"), 0.87 (t, *J=8 Hz,* 6H, H-18").

^{13}C NMR (100.5 MHz, $CDCl_3$): δ172.53 (-COO), 167.34 (-CONH), 162.29 (C-4), 128.85 (C-2, C-6), 126.56 (C-1), 114.00 (C-3, C-5), 98.86 (C-5'), 68.31 (C-1"), 64.70, 64.48, 62.25(C-3', C-4', C-a), 53.51 (C-2'), 33.98, 33.00, 32.01, 30.01, 29.89, 29.74, 29.62, 29.49, 29.21, 26.08, 24.37, 23.16, 22.75 (C-c, C-d , C-2" - C-17"), 14.22 (CH_3).

HRMS (positive, MeOH): *m/z* $[M + H]^+$ calculated for $C_{70}H_{116}N_2O_{12}S_2$: 1241.81; found: 1242.78

IR (Neat) ν_{max} : 3241, 2950, 1645, 1607, 1544cm^{-1}.

*1.4.8 Synthesis of compound **18***



The synthesized acetal-protected compound **15** in methanol was then subjected to deprotection using acidic ion exchange resin Dowex 50W along with 5-6 drops of water at 30 ^{o}C for 6 h. The resin was filtered off on completion of reaction, which was monitored by TLC (MeOH: $CHCl_3$:: 1:9), followed by the removal of solvent on a rotary evaporator to obtain the deprotected compound **18** in 80% yield.

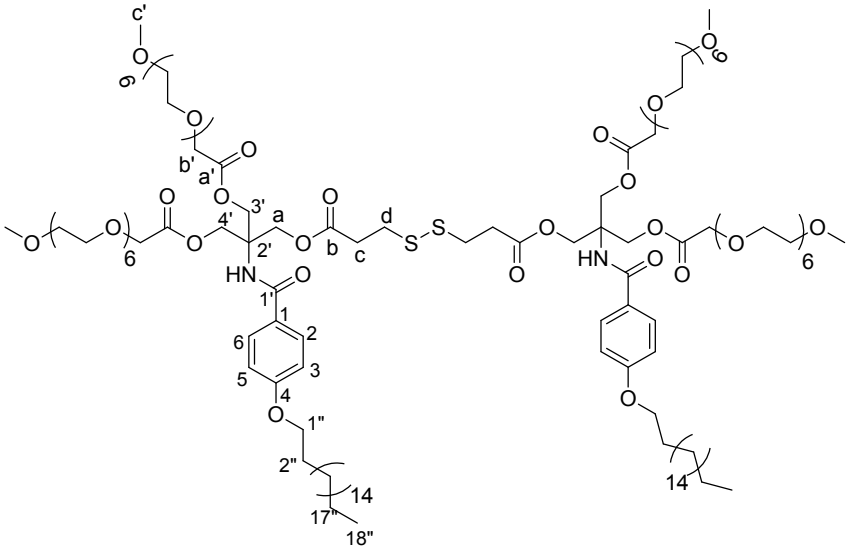
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72 (d, *J=8 Hz*, 4H, H-2 & H-6), 7.01 (bs, NH), 6.91 (d, *J= 8Hz*, 4H, H-3 & H-5), 4.59 (broad peak, OH), 4.46 (s, 4H, H-a), 3.97 (t, *J=6.4 Hz,* 4H, H-1"), 3.80 (d, *J=12 Hz*, 4H, H-3a', H-4a') and 3.62 (d, *J=12 Hz*, 4H, H-3a', H-4a'), 2.93 (t, *J=6.4 Hz,* 4H, H-d), 2.80 (t, *J=6.4 Hz,* 4H, H-c), 1.79-1.75 (m, 4H, H-2"), 1.45-1.25 (m, 60H, H-3" - H-17"), 0.87 (t, *J=8 Hz,* 6H, H-18").

^{13}C NMR (100.5 MHz, $CDCl_3$): δ 172.68 (-COO), 168.29 (-CONH), 162.51 (C-4), 129.07 (C-2, C-6), 125.55 (C-1), 114.57 (C-3, C-5), 68.43 (C-1"), 62.98, 62.00, 61.50 (C-2', C-3', C-4', C-a), 34.08, 33.26, 31.96, 30.25, 30.08, 29.54, 29.09, 26.07, 22.82 (C-c, C-d, C-2"-C-17"), 14.14 (C-18").

HRMS (positive, MeOH):*m/z* $[M + H]^+$ calculated for $C_{64}H_{108}N_2O_{12}S_2$: 1160.73; found: 1162.02.

IR (Neat) ν_{max} : 3340, 2981, 1650, 1607, 1545cm^{-1}.

*1.4.9 Synthesis of compound **3***



To an ice-cold stirring solution of compound **18** (0.2 g, 0.175 mmol) in DCM, EDC.HCl (0.148 g, 0.7 mmol), DMAP (0.052 g, 0.4 mmol) were added. The reaction mixture was then stirred at 35 ^{o}C for 30 min, followed by the addition of mPEG-350 acid (0.36 g, 1.03 mmol) The resultant solution was stirred at 35 ^{o}C for 24 h. On completion of reaction, monitored by TLC, the solvent was evaporated under reduced pressure and the mixture extracted with chlorform (4 x 50 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed by rotary evaporation. The crude product was purified by column chromatography to give pure product in a 70 % yield.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72(d, *J=8 Hz*, 4H, H-2 & H-6), 6.88 (d, *J=8Hz*, 4H, H-3 & H-5), 6.87 (bs, NH),4.63-4.57 (m, 12H, H-a, H-b'), 4.16 (s, 8H, H-3', H-4'), 3.97 (t, *J=6.4 Hz,* 4H, H-1") 3.67-3.62(m, PEG region), 3.37-3.36 (m, 12H, H-c'), 2.88-2.85 (m, 4H, H-d) 2.77-2.73 (m, 4H, H-c), 1.80-1.74 (m, 4H, H-2"), 1.31-1.22 (m, 60H, H-3"-H-17"), 0.87 (t, *J=8 Hz*, 6H, H- 18").

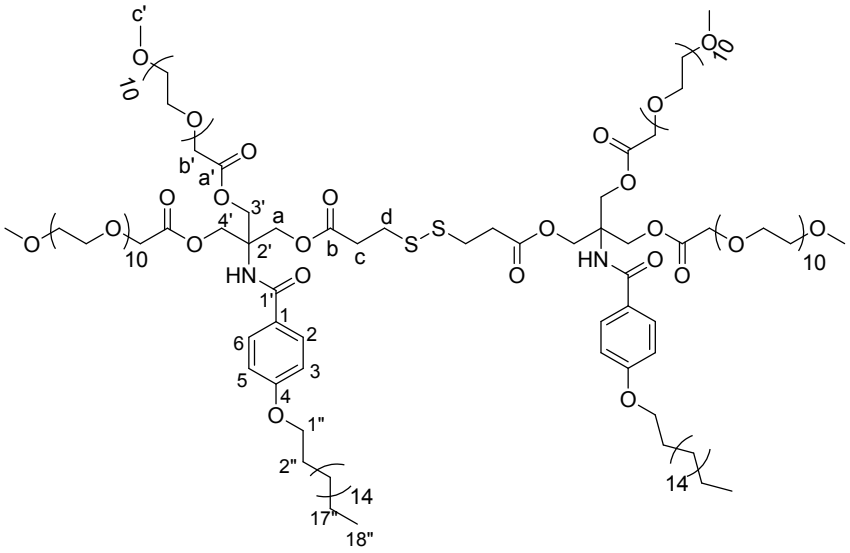
^{13}C NMR (100.5 MHz, $CDCl_3$): δ171.55, 170.47 (-COO), 167.13 (-CONH), 162.19 (C-4), 129.06 (C-2, C-6), 125.90 (C-1), 114.36 (C-3, C-5), 71.99, 71.00, 70.63, 70.60, 70.56, 70.26, 68.44, 68.33 (PEG), 63.85 (C-1"), 63.49, 62.90, 59.14, 59.06, 58.55 (C-3', C-4', C-a, C-2'

OCH_3), 33.75, 32.06, 29.80, 29.67, 29.32, 29.11, 26.09, 22.77 (C-c, C-d, C-2"-C-17"), 14.21(C-18").

GPC (THF, 1.2mL min^{-1}, polystyrene): Mw = 3254 g mol^{-1}, M_n= 3167 g mol^{-1}, PDI = 1.02.

IR (Neat) ν_{max} : 3290, 2981, 1750, 1645, 1607 cm^{-1}.

*1.4.10 Synthesis of compound **4***



To an ice-cold stirring solution of compound **18** (0.2 g, 0.175 mmol) in DCM, EDC.HCl (0.148 g, 0.7 mmol), DMAP (0.052 g, 0.4 mmol) were added. The reaction mixture was then stirred at 35 ^oC for 30 min, followed by the addition of mPEG-550 acid (0.57 g, 1.03 mmol) The resultant solution was stirred at 35 ^oC for 24 h. On completion of reaction monitored by TLC, the solvent was evaporated under reduced pressure and mixture extracted with chloroform(4 x 50 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed by rotary evaporation. The crude product was purified by column chromatography to give pure product in a 72 % yield.

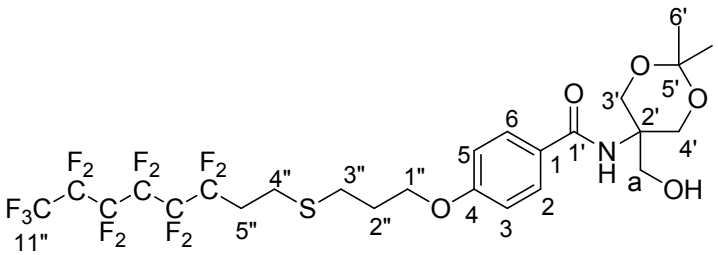
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (d, *J=8 Hz*, 4H, H-2 & H-6), 6.87 (d, *J=8Hz*, 4H, H-3 & H-5), 6.8 (bs, NH), 4.61-4.55 (m, 12H, H-a, H-b'),4.15(s, 8H, H-3', H-4'), 3.96 (t, *J=6.4 Hz,* 4H, H-1"), 3.62-3.53(m, PEG region), 3.35 (4s, 12H, H-c'), 2.85-2.84 (m, 4H, H-d), 2.75-2.73 (m, 4H, H-c), 1.78-1.73 (m, 4H, H-2"), 1.46-1.21 (m, 60 H, H-3"-H-17"), 0.87 (t, *J=8 Hz*, 6H, H- 18").

^{13}C NMR (100.5 MHz, $CDCl_3$): δ 171.54, 170.41 (-COO), 167.20 (-CONH), 162.22 (C-4), 129.06 (C-2, C-6), 125.86 (C-1), 114.30 (C-3,C-5), 72.71, 71.98, 71.01, 70.62, 70.31, 69.03, 68.58, 68.44, 68.33 (PEG), 64.24, 63.85, 62.89, 61.74, 59.12, 58.58 (C-3', C-4', C-a, C-2' OCH_3), 33.85, 32.00, 29.78, 29.51, 29.23, 26.09, 22.77 (C-c, C-d, C-2"-C-17"), 14.21(C-18").

GPC (THF, 1.2mL min^{-1}, polystyrene): M_w = 3878 g mol^{-1}, M_n= 3727 g mol^{-1}, PDI = 1.04.

IR (Neat) ν_{max} : 3290, 2981, 1750, 1645, 1607 cm^{-1}.

*1.4.11 Synthesis of compound **13***



To an ice-cold stirring solution of compound **10** (1 g, 2.82 mmol) in DMF, EDC (0.41 g, 2.1 mmol), HOBt (0.29 g, 2.1 mmol) and DIPEA (0.69 g, 5.3 mmol) were added. The reaction mixture was then stirred at 35 ^oC for 30 min, followed by the addition of amine (**7**)(0.34 g, 2.1mmol). The resultant solution was stirred at 35 ^oC for 18 h. On completion of reaction monitored by TLC (petroleum ether:ethylacetate :: 7:3), the solvent was evaporated under reduced pressure and the mixture extracted with ethyl acetate (4 x 150 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed by rotary evaporation. The crude product was purified by column chromatography to give pure compound **13** in a 70% yield.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (d, *J= 8 Hz*, 2H, H-2 & H-6), 6.93 (d, *J=8 Hz*, 2H, H-3 & H-5), 5.26 (bs, OH), 4.11 (t, *J=6.4 Hz,*2H, H-1"), 3.97-3.89 (m, 4H, H-3', H-4'), 3.76 (s, 2H, H-a), 2.77-2.74 (m, 4H, H-3", H-4"), 2.40-2.36 (m, 2H, H-2"), 2.13-2.06 (m, 2H, H-5"), 1.48 &1.46 (s, 3H, H-6').

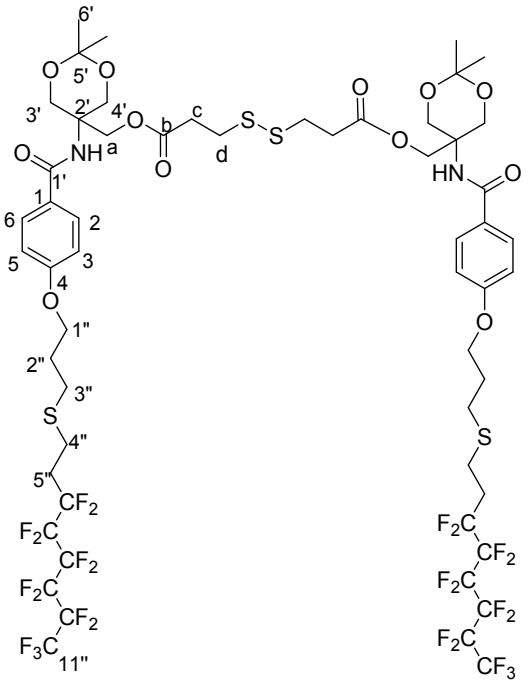
^{13}C NMR (100.5 MHz, $CDCl_3$): δ 168.29 (-CONH), 161.81(C-4), 129.09(C-2, C-6), 126.70 (C-1), 114.39 (C-3, C-5), 99.14 (C-5'), 66.19 (C-1"), 64.73, 64.62 (C-3', C-4', C-a), 55.39 (C-2'), 32.02, 28.91, 28.77, 28.59, 22.73, 18.81 (C-2"-C-5", C-6').

^{19}F NMR (376 MHz, $CDCl_3$): δ -80.83, -114.30, -121.67, -121.90, -122.71, -123.34, -126.12.

HRMS (positive, MeOH):*m/z* $[M + H]^+$ calculated for $C_{25}H_{28}F_{13}NO_5S$: 701.55; found: 702.67.

IR (Neat) ν_{max} : 33340, 2989, 1650, 1545cm^{-1}.

*1.4.12 Synthesis of compound **16***



To an ice-cold stirring solution of compound **13** (0.73 g, 1.04 mmol) in DCM, EDC.HCl (0.2 g, 1.04 mmol), DMAP (0.12 g, 1.04 mmol) were added. The reaction mixture was then stirred at 35 ^{o}C for 30 min, followed by the addition of 3,3'dithiopropanoic acid (0.1 g, 0.47 mmol) The resultant solution was stirred at 35 ^{o}C for 12 h. On completion of reaction, which was monitored by TLC, the solvent was evaporated under reduced pressure and the mixture extracted with chloroform (4 x 50 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed by rotary evaporation. The crude product was purified by column chromatography to give pure product in 65% yield.

^{1}H NMR (400 MHz): δ 7.71 (d, *J=8 Hz*, 4H, H-2 & H-6), 6.90 (d, *J=8Hz*, 4H, H-3 & H-5), 6.59 (bs, NH), 4.63 (s, 4H, H-a), 4.59 (d, *J=12 Hz*,4H, H-3'a, H-4'a), 4.10 (t, *J=6.4Hz*, H-1"), 3.81 (d, *J=12 Hz,*4H, H-3'b, H-4'b), 2.90-2.86 (m, 4H, H-d), 2.77-2.74 (m, 12H, H-c, H-3", H-4"), 2.41-2.36 (m, 4H, H-2"), 2.11- 2.07 (m, 4H, H-5"), 1.54 &1.44 (s, 6H each, H-6').

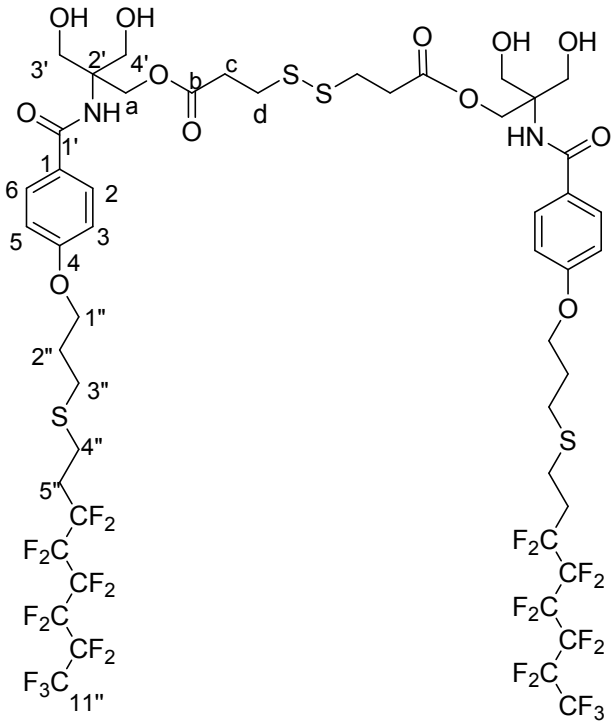
^{13}C NMR (100.5 MHz, $CDCl_3$): δ 172.41(-COO), 167.20 (-CONH), 161.64 (C-4), 128.95 (C-2, C-5), 124.71 (C-1), 114.31 (C-3, C-5), 98.82 (C-5'), 66.27, 64.43, 62.21, 53.49 (C-1",C-3', C-4', C-a), 33.96, 33.00, 28.91, 28.77, 24.21, 23.26 (C-2"-C-5", C-6').

^{19}F NMR (376 MHz, $CDCl_3$): δ -80.67, -114.27, -121.58, -121.85, -122.61, -123.22, -126.01.

HRMS (positive, MeOH): *m/z* $[M + H]^+$ calculated for $C_{56}H_{62}F_{26}N_2O_{12}S_4$: 1577.28; found: 1578.35.

IR (Neat) ν_{max} : 3130, 2980, 1650, 1545cm^{-1}.

*1.4.13 Synthesis of compound **19***



The synthesized acetal-protected **16** in methanol was then subjected to deprotection using acidic ion exchange resin Dowex 50W along with 5-6 drops of water at 30 ^oC for 6 h. The resin was filtered off on completion of reaction, as monitored by TLC (MeOH: $CHCl_3$:: 1:9), followed by removal of solvent on a rotary evaporator to obtained the deprotected compound **19** in a 80% yield.

^{1}H NMR (**400 MHz**): δ 7.73 (d, *J =8 Hz*, 4H, H-2 &H-6), 7.01 (bs, NH), 6.92 (d, *J =8Hz*, 4H, H-3 & H-5), 4.46 (s, 4H, H-a), 4.11 (t, *J=6.4 Hz* ,4H, J= H-1"), 3.81(d, *J=12 Hz,*4H, H-3'), 3.64 (d, *J=12 Hz,*4H, H-4'), 2.96-2.93 (m, 4H, H-d), 2.81-2.76 (m, 12H, H-c, H-3", H-4"), 2.41-2.36 (m, 4H, H-2"), 2.13- 2.08 (m, 4H, H-5").

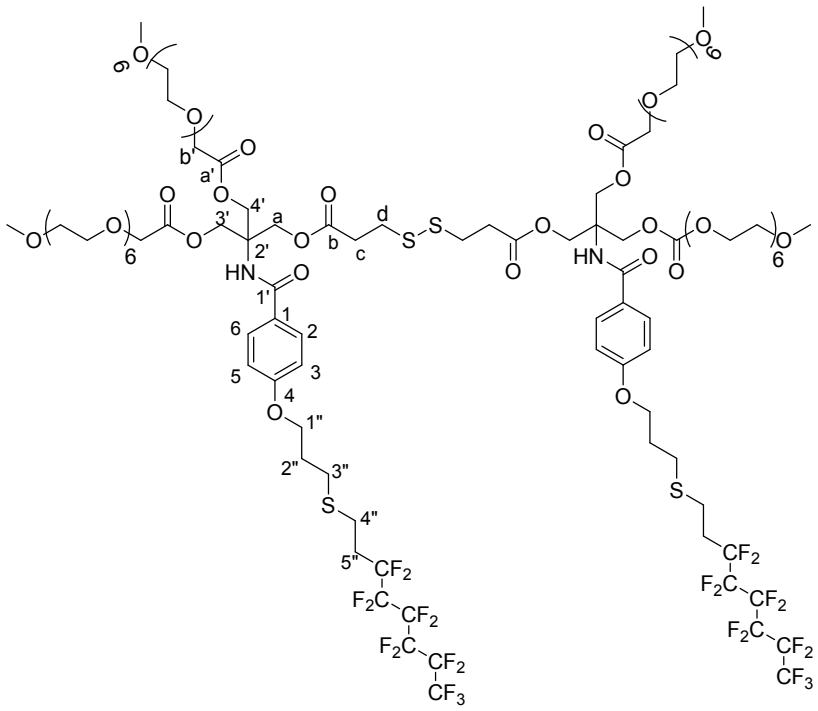
^{13}C NMR (**100.5 MHz, $CDCl_3$**): δ 172.72 (-COO), 168.33 (-CONH), 161.98 (C-4), 129.15 (C-2, C-6), 126.01 (C-1), 114.45 (C-3, C-5), 66.23, 62.96, 62.48, 61.56 (C-1",C-3', C-4', C-a) 34.07, 33.26, 29.77, 28.88, 28.72, 22.76 (C-2"-C-5").

^{19}F NMR (**376 MHz, $CDCl_3$**): δ -80.67, -114.22, -114.32, -121.80, -122.61, -123.27, -126.06.

HRMS (positive, MeOH): *m/z* $[M + H]^+$ calculated for $C_{50}H_{54}F_{26}N_2O_{12}S_4$: 1497.19; found: 1498.50.

IR (Neat) ν_{max} : 3450, 2980, 1645, 1550cm^{-1}.

*1.4.14 Synthesis of compound **5***



To an ice-cold stirring solution of compound **19** (0.2 g, 0.175 mmol) in DCM, EDC.HCl (0.11 g, 0.6 mmol), DMAP (0.05 g, 0.4 mmol) were added. The reaction mixture was then stirred at 35 ^{o}C for 30 min, followed by the addition of mPEG-350 acid (0.28 g, 0.8 mmol). The resultant solution was stirred at at 35 ^{o}C for 24 h. On completion of reaction monitored by TLC, the solvent was evaporated under reduced pressure and mixture extracted with chloroform (4 x 50 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed by rotary evaporation. The crude product was purified by column chromatography to give pure product in a 74% yield.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71 (d, *J=8 Hz*, 4H, H-2 &H-6), 6.88 (d, *J=8Hz*, 2H, H-3 & H-5), 4.61-4.44 (m, 12H, H-a , H-b'), 4.14 (s, 8H, H-3', H-4'), 4.11-4.09 (m, 4H, H-1''), 3.82-3.51 (mPEG region), 3.35-3.30 (m, 12H, H-c'), 2.86-2.85 (m, 4H, H-c), 2.75-2.71 (m, 12H, H-d, H-3'', H-4''), 2.41-2.32(m, 4H, H-2''), 2.10- 2.05(m, 4H, H-5'').

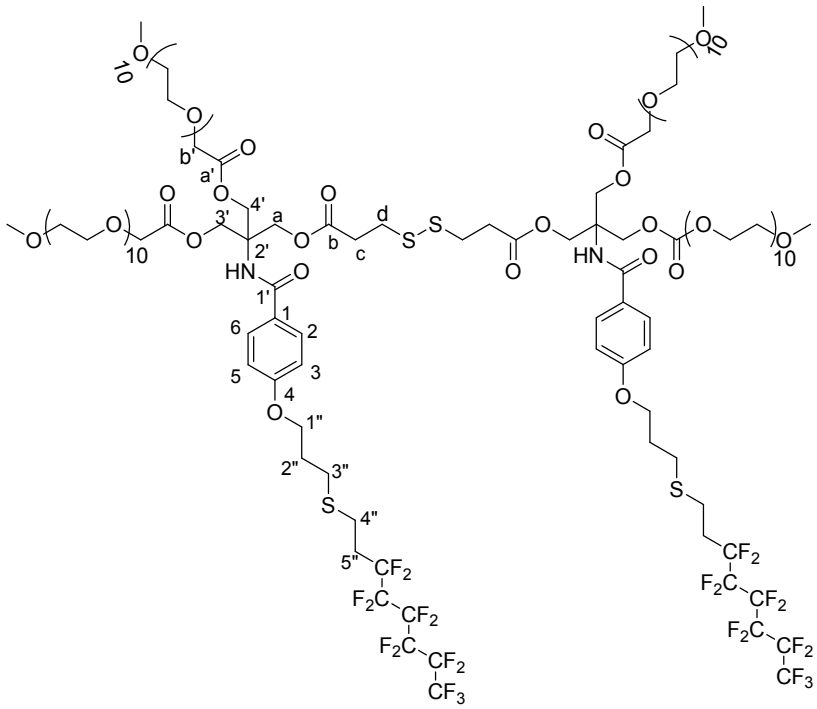
^{13}C NMR (100.5 MHz, $CDCl_3$): δ 171.56, 170.41 (-COO) 167.14 (-CONH), 161.76 (C-4), 129.15 (C-2,C-6), 126.30 (C-1), 114.28 (C-3,C-5), 71.97, 70.98, 70.61, 69.03, 68.68, 68.44 (PEG), 66.20, 63.85, 62.89, 59.10, 58.60 (C-1'',C-3', C-4', C-a, OCH_3), 33.86, 32.13, 31.34, 29.77, 28.52, 22.71 (C-2''-C-5'').

^{19}F NMR (376 MHz, $CDCl_3$): δ -80.67, -114.11, -121.58, -121.85, -122.61, -123.22, -126.01.

GPC (THF, 1.2mL min^{-1}, polystyrene): M_w = 3589 g mol^{-1}, M_n= 3490 g mol^{-1}, PDI = 1.02.

IR (Neat) ν_{max} : 3310, 2990, 1750, 1645, 1607 cm^{-1}.

1.4.15 Synthesis of compound ***6***



To an ice-cold stirring solution of compound **19** (0.2 g, 0.175 mmol) in DCM, EDC.HCl (0.11 g, 0.6 mmol), DMAP (0.05 g, 0.4 mmol) were added. The reaction mixture was then stirred at 35 ^oC for 30 min, followed by the addition of mPEG-550 acid (0.44 g, 0.8 mmol) The resultant solution was stirred at 35 ^oC for 24 h. On completion of reaction monitored by TLC, the solvent was evaporated under reduced pressure and the mixture extracted with chloroform (4 x 50 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and

the solvent was removed by rotary evaporation. The crude product was purified by column chromatography to give pure product in a 73% yield.

^{1}H NMR (**400 MHz, $CDCl_3$**): δ 7.71 (d, *J=8 Hz*, 4H, H-2 & H-6), 6.88 (d, *J=8Hz*, 4H, H-3 & H-5), 4.61-4.41 (m, 12H, H-a, H-b'), 4.14 (s, 8H, H-3', H-4'), 4.11-4.09 (m, 4H, H-1") 3.66-3.53 (mPEG region), 3.35-3.31 (m, 12H, H-c'), 2.87-2.83 (m, 4H, H-c), 2.76-2.70 (m, 12H, H-d, H-3", H-4"), 2.42-2.32 (m, 4H, H-2"), 2.09- 2.06 (m, 4H, H-5").

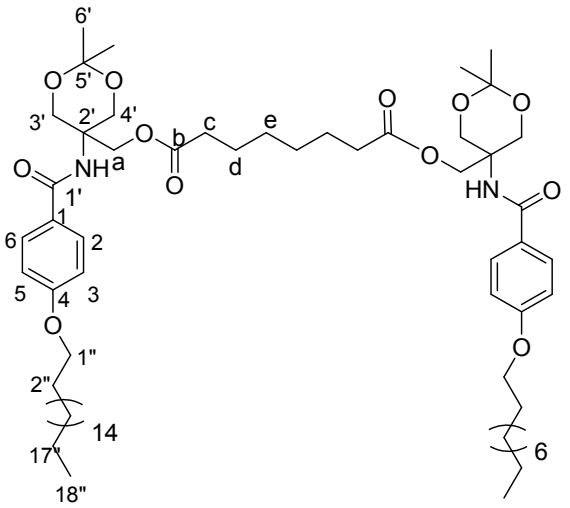
^{13}C NMR (**100.5 MHz, $CDCl_3$**): δ170.39 (-COO), 168.71 (-CONH), 161.73 (C-4), 129.07 (C-2, C-6), 124.56 (C-1), 114.27 (C-3,C-5), 72.11, 71.97, 70.98, 70.61, 70.18, 68.94 (PEG), 64.04, 63.21, 62.89, 60.51, 58.76 (C-1",C-3', C-4', C-a, OCH_3), 33.94, 31.70, 29.39, 28.33, 23.07 (C-2"-C-5").

^{19}F NMR (376 MHz,$CDCl_3$): δ -80.67, -114.20, -121.58, -121.82, -122.62, -123.25, -126.02.

GPC (THF, 1.2 mL min^{-1}, polystyrene): M_w = 4005 g mol^{-1}, M_n= 3805 g mol^{-1}, PDI = 1.05.

IR (Neat) ν_{max} : 3285, 2979, 1745, 1640, 1607 cm^{-1}.

*1.3.16 Synthesis of compound **20***



To an ice-cold stirring solution of compound **12** (2.2 g, 11.5 mmol) in DCM, EDC.HCl (1 g, 11.5 mmol), DMAP (0.43 g, 5.35 mmol) were added. The reaction mixture was then stirred at 30 ^{o}C for 30 min, followed by the addition of suberic acid (0.5 g, 2.38 mmol). The resultant solution was stirred at 30^{o}C for 24 h. On completion of reaction, monitored by TLC, the solvent was evaporated under reduced pressure and the mixture extracted with ethyl acetate (4 x 150 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed by rotary evaporation. The crude product was purified by column chromatography to give pure product in a 67% yield.

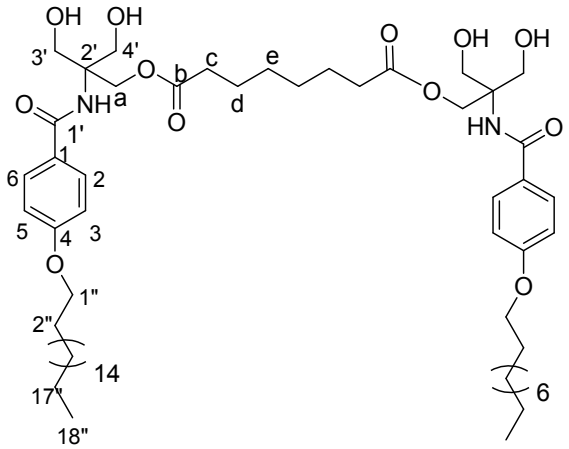
^{1}H NMR (**400 MHz, $CDCl_3$**): δ 7.68 (d, *=8 Hz*, 4H, H-2 & H-6), 6.88 (d, *J=8 Hz*, 4H, H-3 & H-5), 6.70 (bs, NH), 4.58-4.55 (m, 6H, H-a, H-3a', H-4a'),3.95 (t, *J=6.4 Hz*,4H, H-1"), 3.76-3.73 (d, *J=12 Hz,*4H, H-3b', H-4b'), 2.31 (m, *J=6.4 Hz,*4H, H-c), 1.80-1.76 (m, 4H, H-2"), 1-62-1.58 (m, 4H, H-d), 1.56 &1.43 (2s, 6H each, H-6'), 1.34-1.23 (m, 60H, H-3"-H-17"), 0.86 (t, *J=8 Hz,* 6H, H-18").

^{13}C NMR (**100.5 MHz, $CDCl_3$**): 174.84 (-COO), 167.19 (-CONH), 162.09 (C-4), 128.80 (C-2, C-6), 126.34 (C-1), 114.34 (C-3, C-5), 98.80 (C-5'), 68.31, 64.17, 61.88, 53.55 (C-1", C-3', C-4', C-a), 32.01, 29.79, 29.75, 29.70, 29.66, 29.48, 29.45, 29.21, 28.70, 26.07, 24.76, 22.83, 22.78, 22.83, 22.78, 14.22 (C-2"-C-18").

HRMS (positive, MeOH): *m/z* [M + H]$^+$ calculated for $C_{72}H_{120}N_2O_{12}$: 1205.73; found: 1206.78.

IR (Neat) ν_{max} : 3291, 2985, 1650, 1607cm^{-1}.

*1.3.17 Synthesis of compound **21***



The synthesized acetal-protected **20** in methanol was then subjected to deprotection using acidic ion exchange resin Dowex 50W along with 5-6 drops of water at 30 ^oC for 6 h. The resin was filtered off on completion of reaction as monitored by TLC (MeOH: $CHCl_3$:: 1:9), followed by removal of solvent on a rotary evaporator to obtain the deprotected compound **21** in a 80% yield.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (d, *J=8 Hz*, 4H, H-2 & H-6), 7.01 (bs, NH), 6.90 (d, *J =8Hz*, 4H, H-3 & H-5), 4.40 (s, 4H, H-a), 3.97 (t, *J=4H*, H-1"), 3.79-3.76 (d, *J=12 Hz* 4H,H-3'), 3.62(d, *J=12 Hz,* 4H, H-4'), 2.37-2.33 (m, 4H, H-c), 1.81-1.74 (m, 4H, H-2"), 1.62-1.59 (m, 4H, H-d), 1.47-1.40 (m, 4H, H-e), 1.30-1.24 (m, 60H, H-3"-H-17"), 0.86 (t „*J=8 Hz*, 6H, H-18").

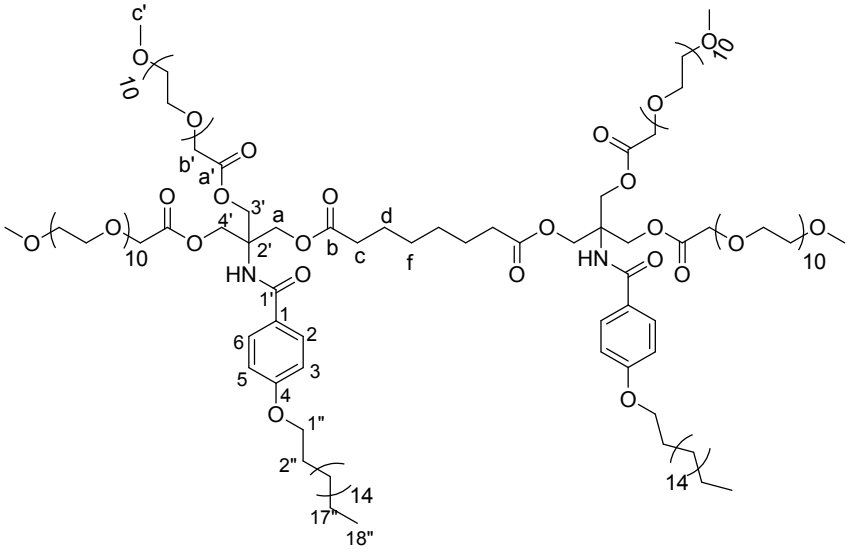
^{13}C NMR (100.5 MHz, $CDCl_3$): 175.00 (-COO), 168.39 (-CONH), 162.41 (C-4), 129.03 (C-2, C-6), 125.49 (C-1), 114.46 (C-3, C-5), 68.43, 63.01, 61.50, 61.74 (C-1", C-3', C-4'. C-

a),34.11, 33.02, 29.80, 29.76, 29.71, 29.67, 29.49, 29.47, 29.19, 28.52, 26.07, 24.65, 22.80, 14.25(C-2"-C-18").

HRMS (positive, MeOH):*m/z* $[M + H]^+$ calculated for $C_{66}H_{112}N_2O_{12}$: 1125.60; found: 1126.85.

IR (Neat) ν_{max} : 3450, 2990, 1650, 1607cm^{-1}.

*1.3.18 Synthesis of compound **22***



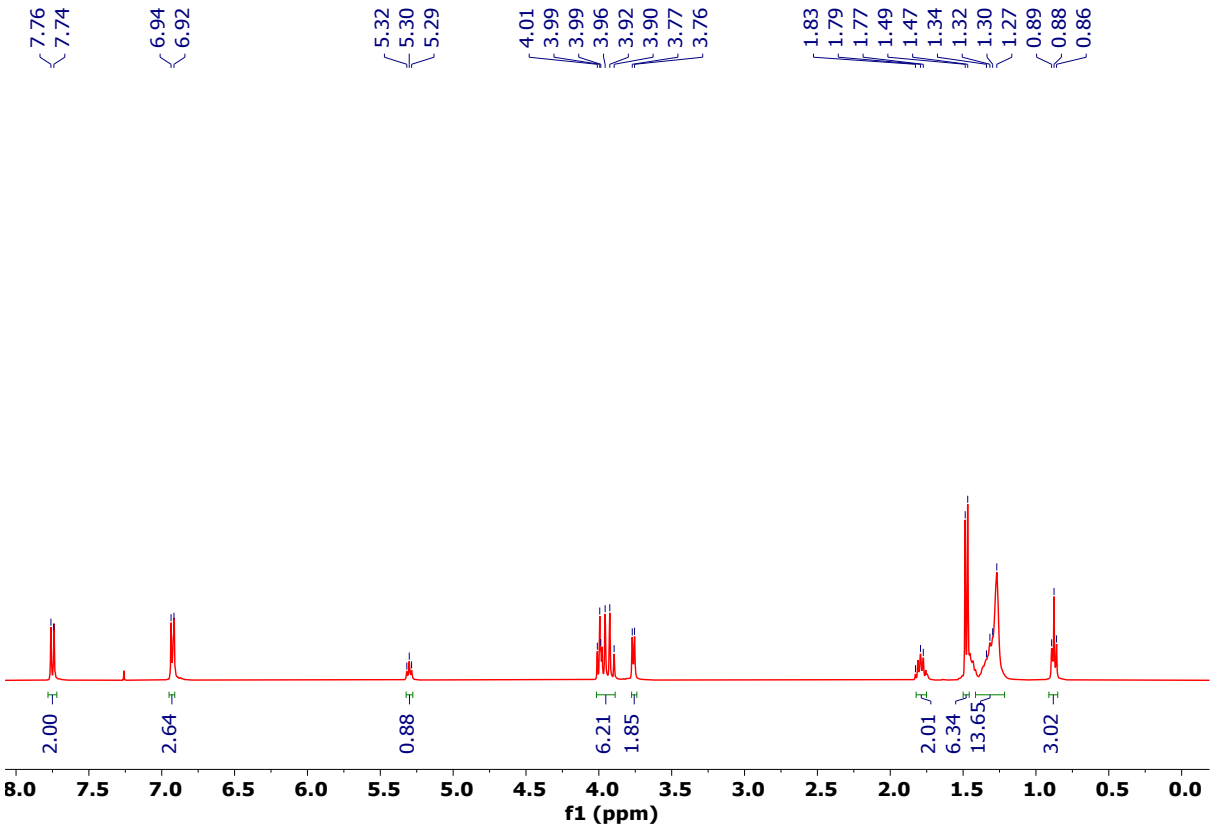
To an ice-cold stirring solution of compound **21** (0.2 g, 0.175 mmol) in DCM, EDC.HCl (0.153 g, 3.6 mmol), DMAP (0.053 g, 1.09 mmol) were added. The reaction mixture was then stirred at 30°C for 30 min, followed by the addition of mPEG-550 acid (0.58 g, 6.3 mmol) The resultant solution was stirred at 30°C for 24 h. On completion of reaction monitored by TLC, the solvent was evaporated under reduced pressure and mixture extracted with chloroform (4 x 50 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed by rotary evaporation. The crude product was purified by column chromatography to give pure product in a 72% yield.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.66 (d, *J=8Hz*, 4H, H-2 & H-6), 6.84 (d, *J=4Hz*, 4H, H-3 &H-5), 4.58 (s, 8H, H-b'), 4.47 (s, 4H, H-a), 4.11 (s, 8H, H-3', H-4'), 3.93 (t, *J=6.4 Hz,*4H, H-1''), 3.70-3.48 (m, PEG region), 3.32-3.30 (m, 12H, H-c'), 2.28-2.24 (m, 4H, H-c), 1.73-1.71 (m, 4H, H-d), 1.53-1.50 (m, 4H, H-2''), 1.41-1.35 (m, 4H, H-e) 1.23-1.20 (m, 60 H, H-3''-H-17''), 0.82 (t, *J=8 Hz,* 6H, H-18'').

^{13}C NMR (100.5 MHz, $CDCl_3$): δ 173.50, 170.35 (-COO), 167.17 (-CONH), 162.17 (C-4), 129.00 (C-2, C-6), 125.93 (C-1), 114.28 (C-3, C-5), 71.94, 70.98, 70.94, 70.91, 70.59, 70.56, 70.50, 68.99, 68.64, 68.53, 68.41 (PEG), 68.30, 63.83, 59.05, 58.63, 51.84 (C-1'', C-3', C-4', C-a, OCH_3) 33.92, 31.96, 29.73, 29.69, 29.67, 29.63, 29.46, 29.40, 29.19, 28.68, 26.05, 24.60, 22.73, 14.17 (C-2''-C-18'').

GPC (THF, 1.2mL min^{-1}, polystyrene): M_w = 2541 g mol^{-1}, M_n= 2495 g mol^{-1}, PDI = 1.02,

IR (Neat) ν_{max} : 3290, 2965, 1750, 1645, 1607 cm^{-1}.


7.76
7.74
6.94
6.92
5.32
5.30
5.29
4.01
3.99
3.99
3.96
3.92
3.90
3.77
3.76
1.83
1.79
1.77
1.49
1.47
1.34
1.32
1.30
1.27
0.89
0.88
0.86
2.00
2.64
0.88
6.21
1.85
2.01
6.34
13.65
3.02
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

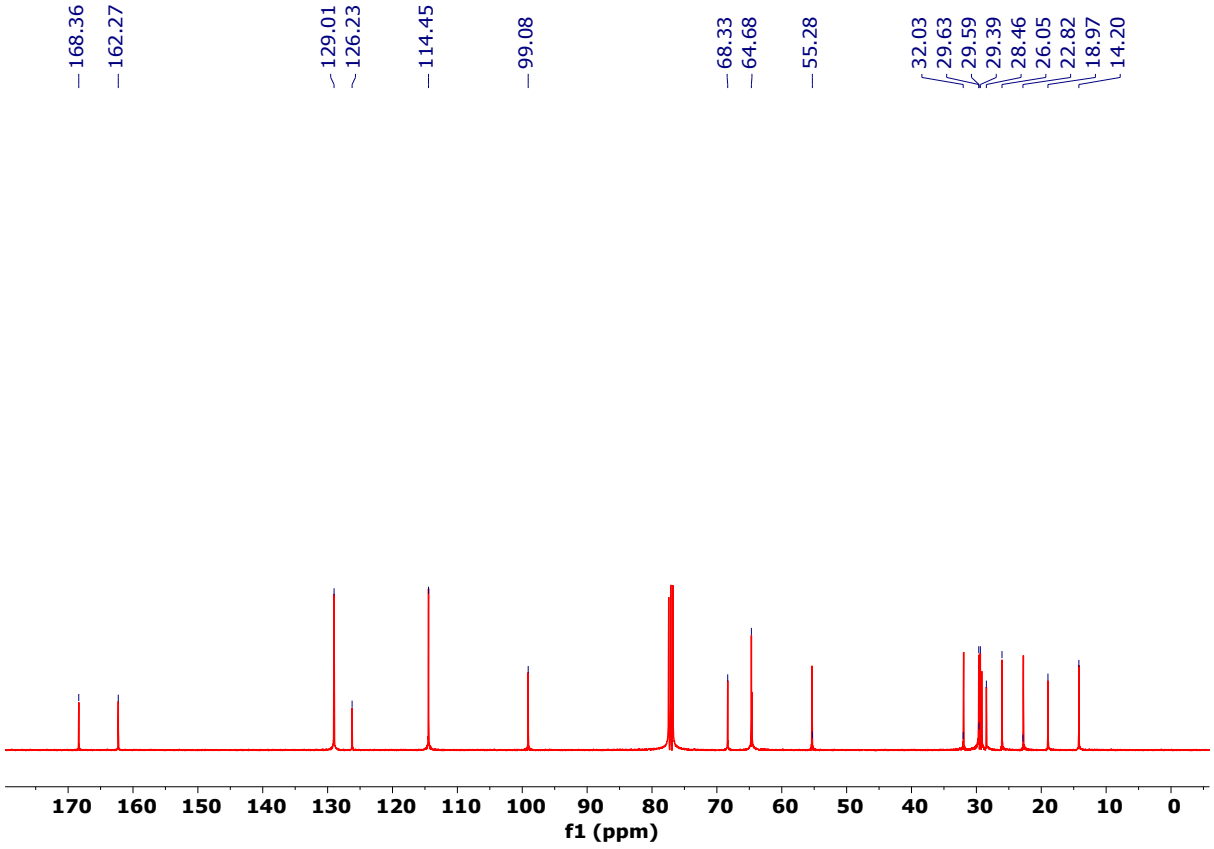

168.36
162.27
129.01
126.23
114.45
99.08
68.33
64.68
55.28
32.03
29.63
29.59
29.39
28.46
26.05
22.82
18.97
14.20
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

Figure S1. ^{1}H and ^{13}C NMR spectra of Compound **11**.

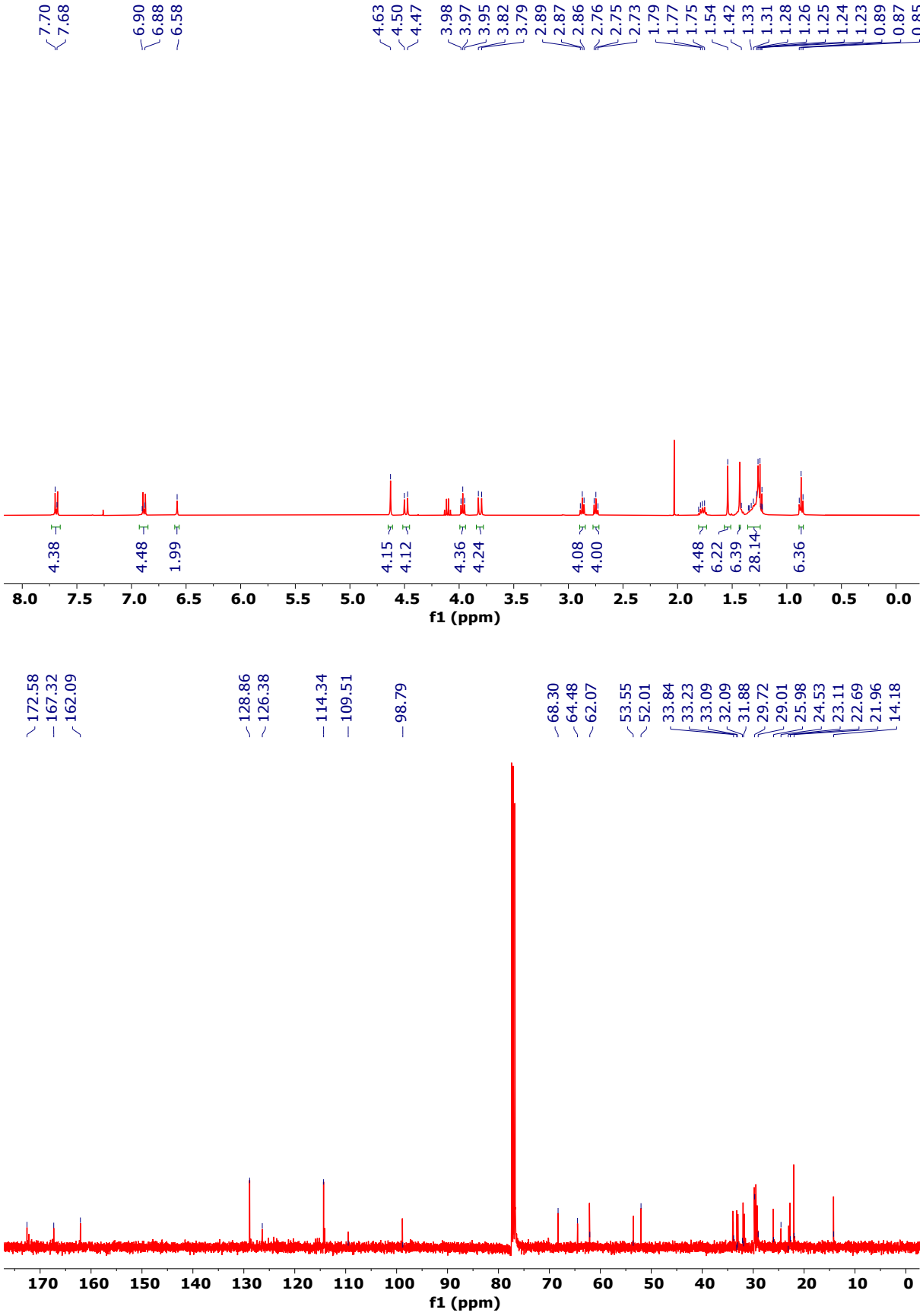


Figure S2. ^{1}H and ^{13}C NMR spectra of Compound **14**.

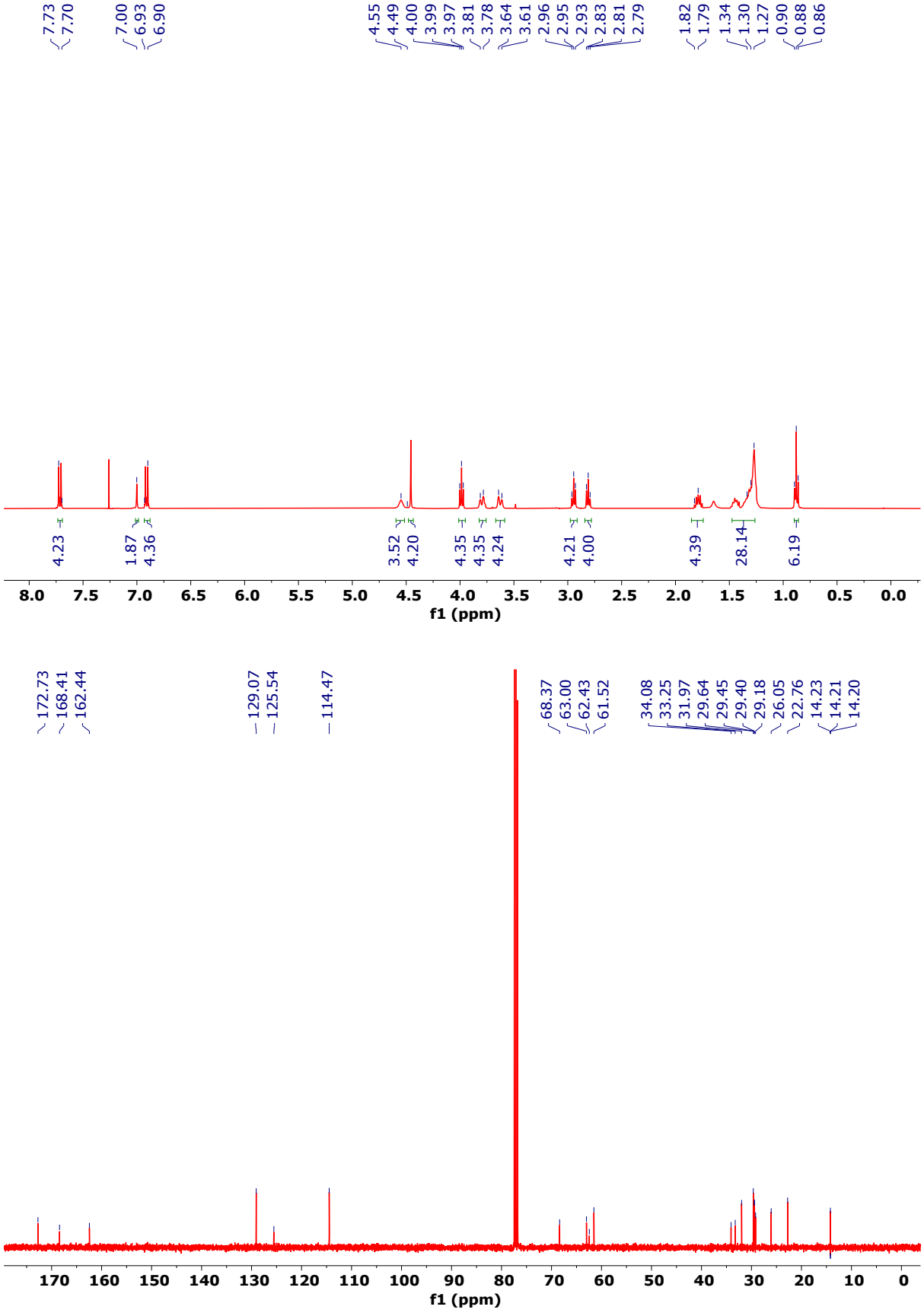


Figure S3. ^{1}H and ^{13}C NMR spectra of Compound **17**.

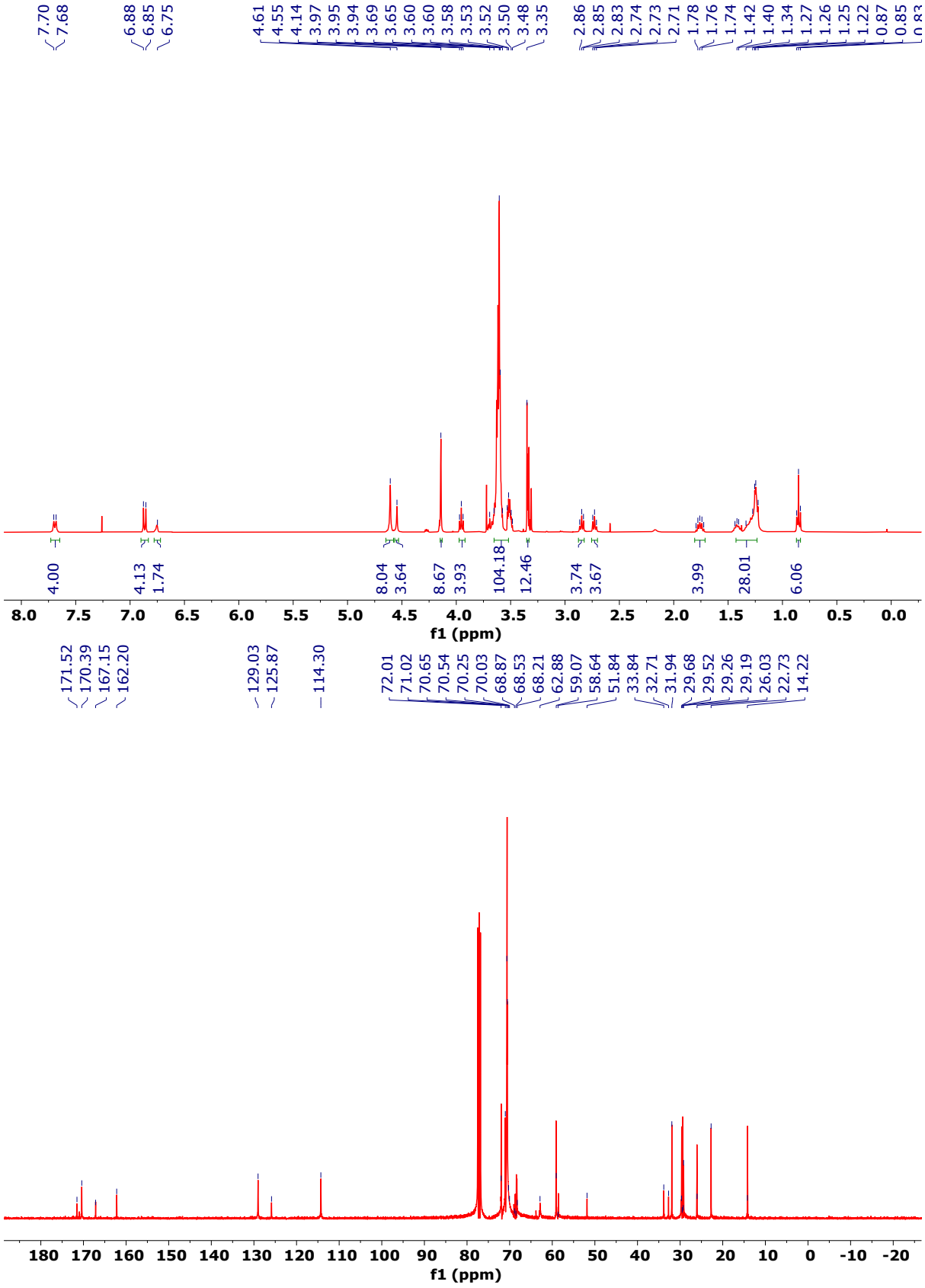


Figure S4. ^{1}H and ^{13}C NMR spectra of Compound **1**.

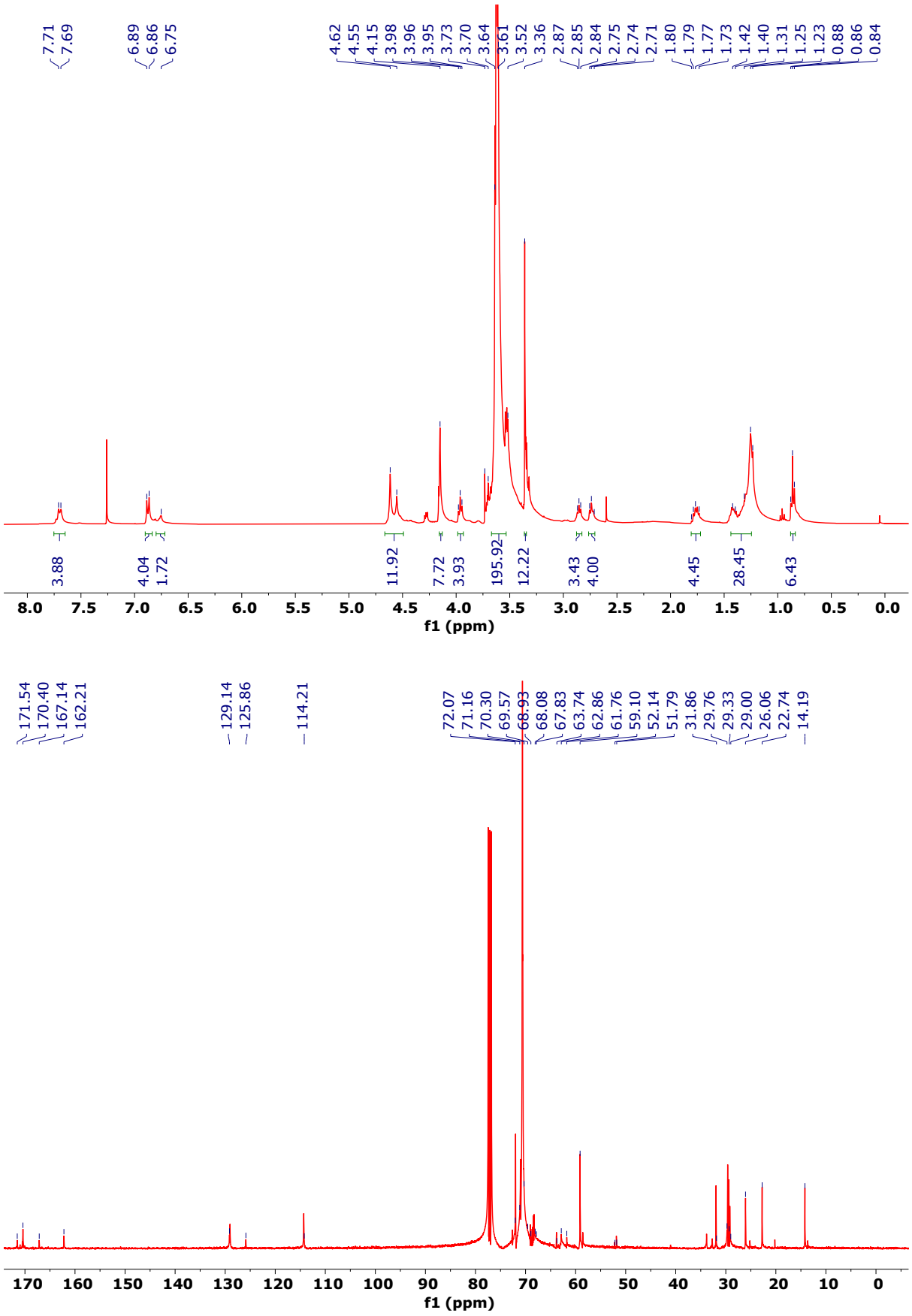
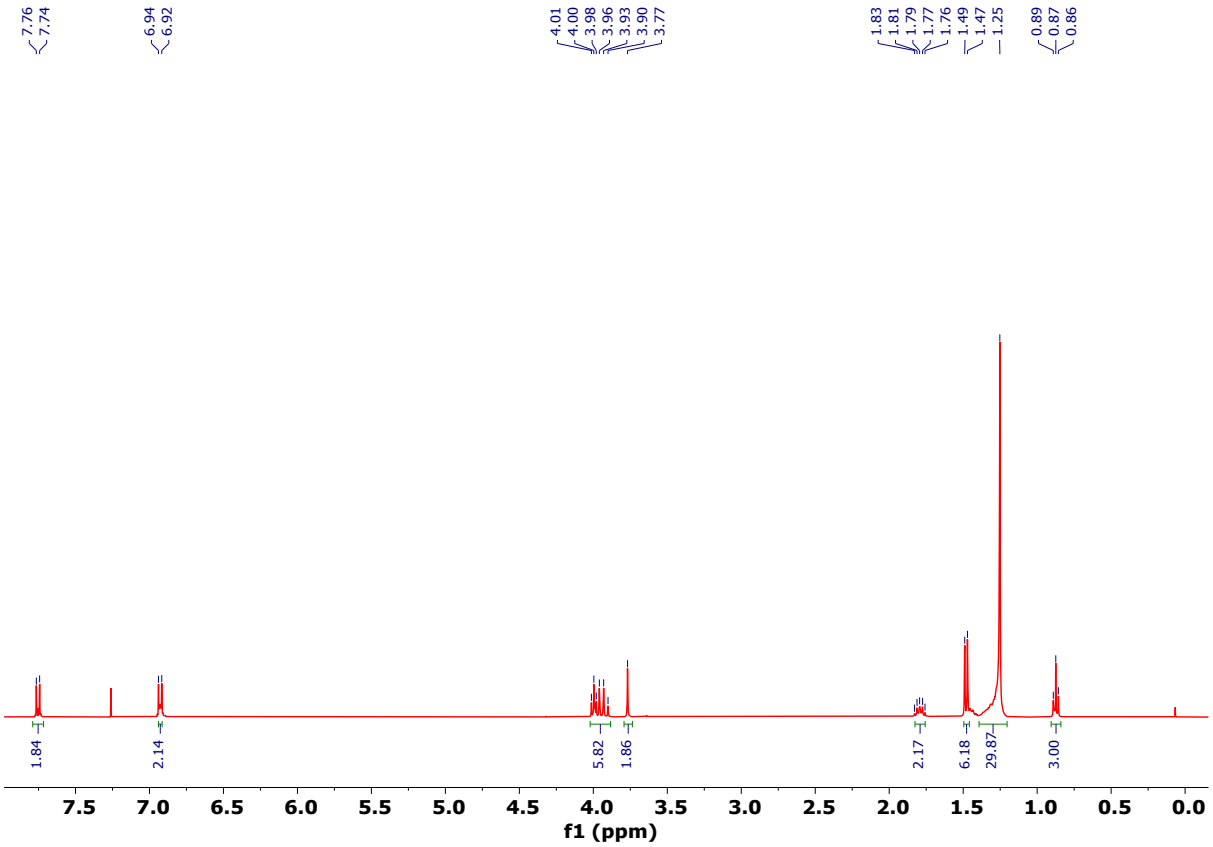


Figure S5. ^{1}H and ^{13}C NMR spectra of Compound **2**.


7.76
7.74
6.94
6.92
4.01
4.00
3.98
3.96
3.93
3.90
3.77
1.83
1.81
1.79
1.77
1.76
1.49
1.47
1.25
0.89
0.87
0.86
1.84
2.14
5.82
1.86
2.17
6.18
29.87
3.00
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

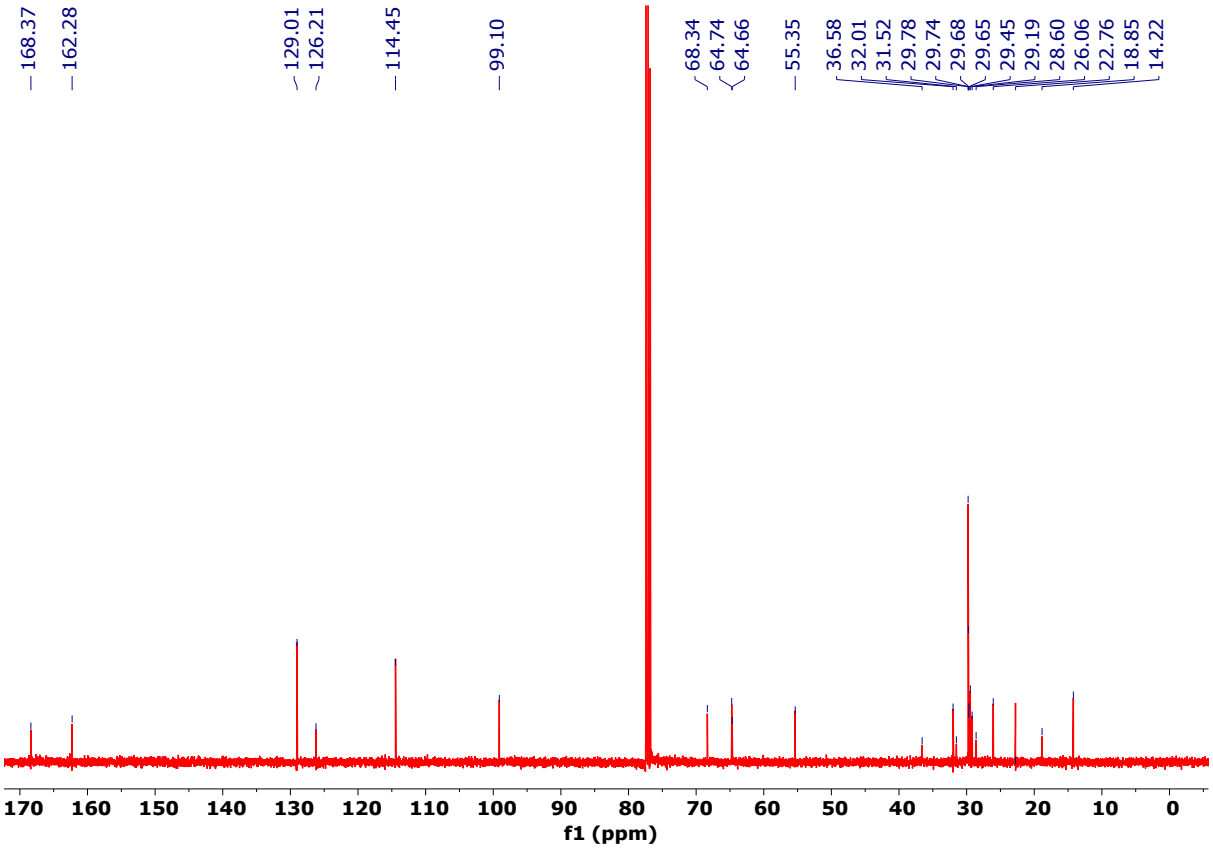
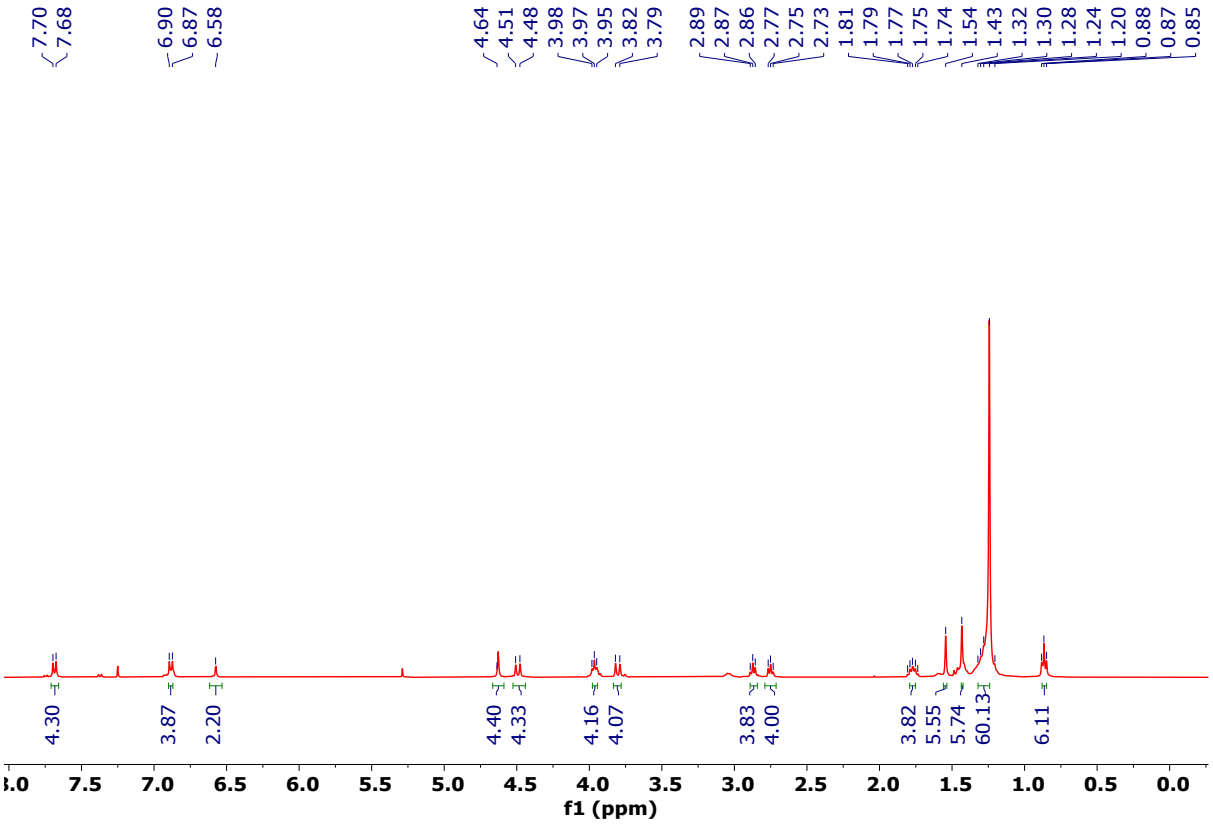

168.37
162.28
129.01
126.21
114.45
99.10
68.34
64.74
64.66
55.35
36.58
32.01
31.52
29.78
29.74
29.68
29.65
29.45
29.19
28.60
26.06
22.76
18.85
14.22
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

Figure S6. ^{1}H and ^{13}C NMR spectra of Compound **12**.



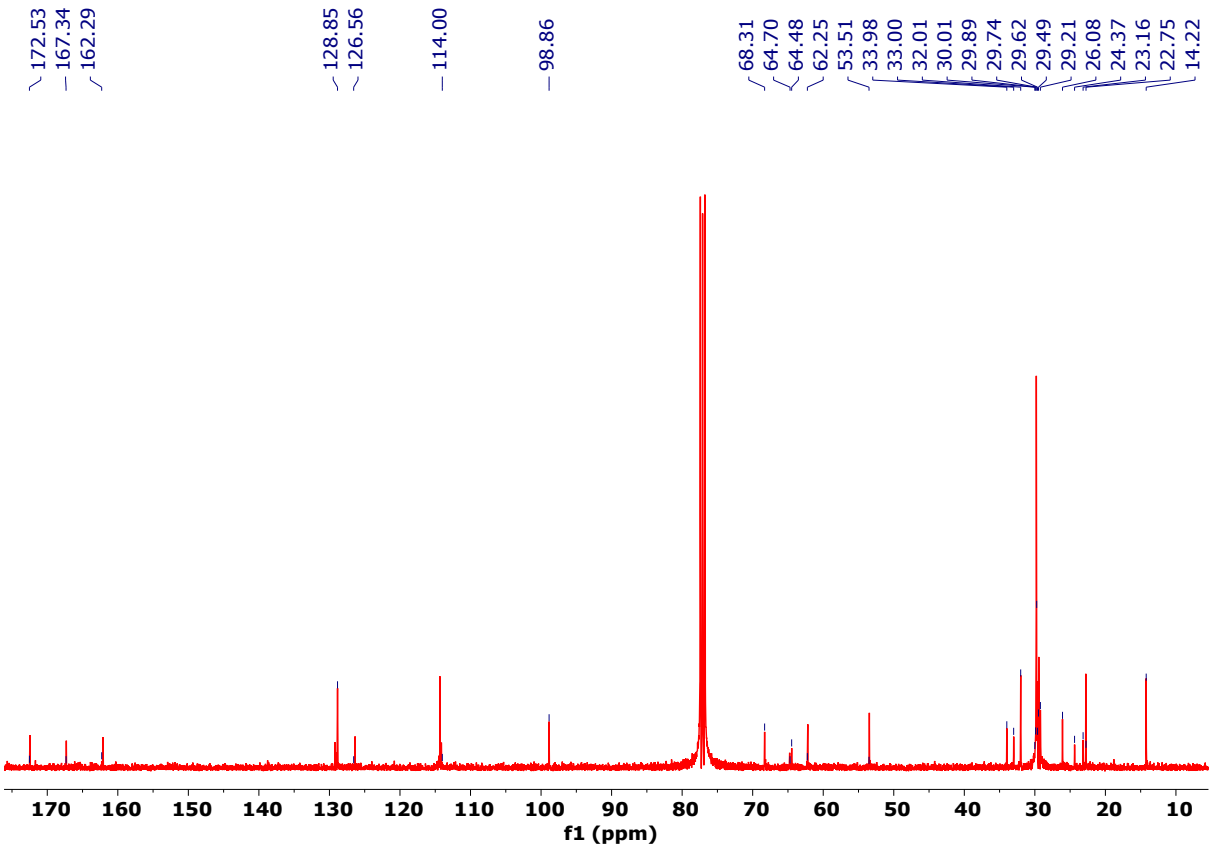
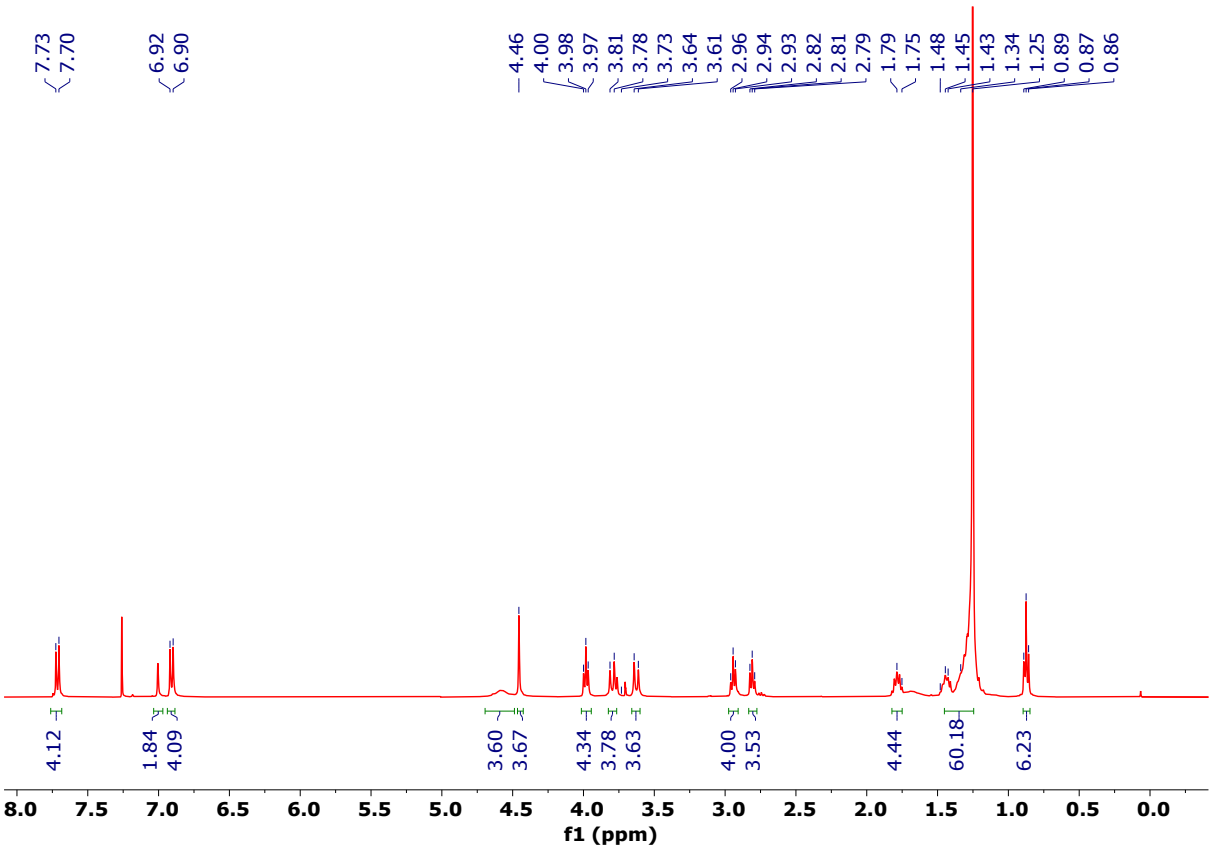


Figure S7. ^{1}H and ^{13}C NMR spectra of Compound **15**.



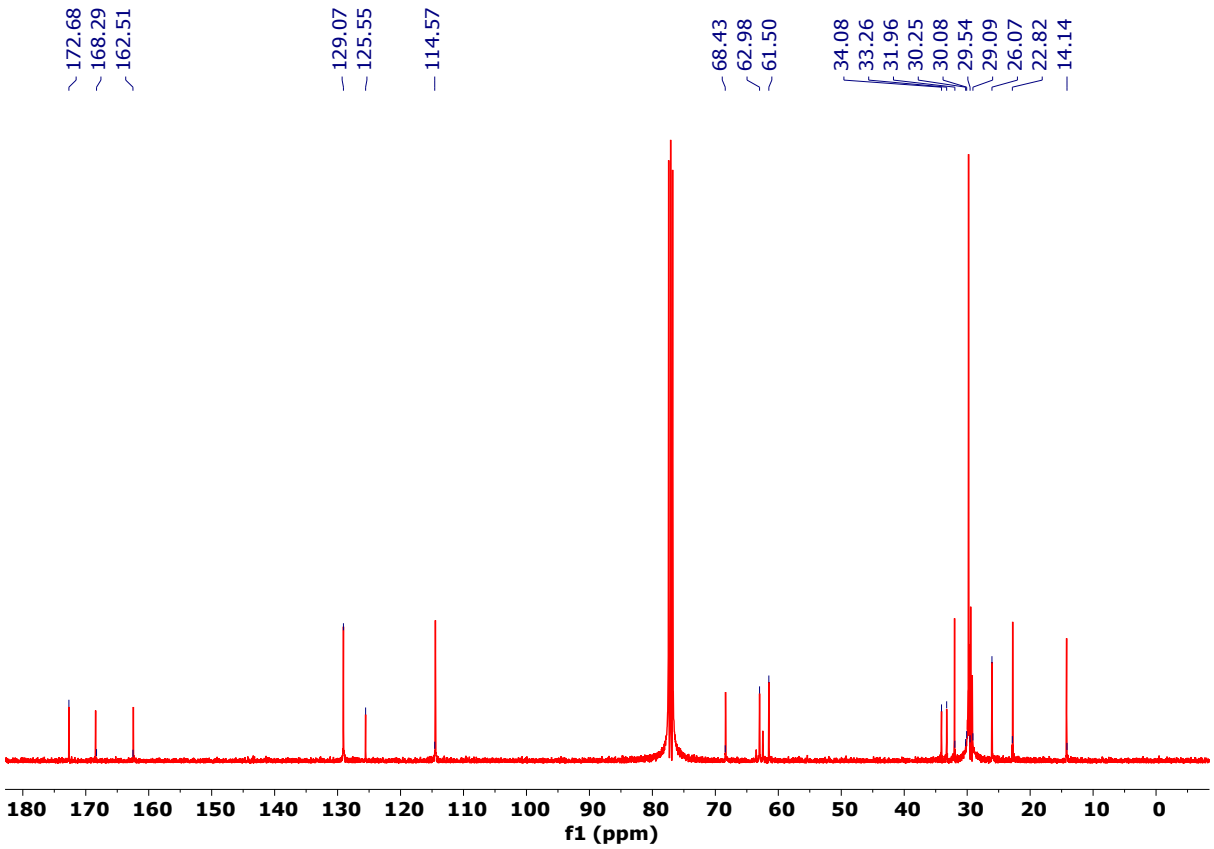


Figure S8. ^{1}H and ^{13}C NMR spectra of Compound **18**.

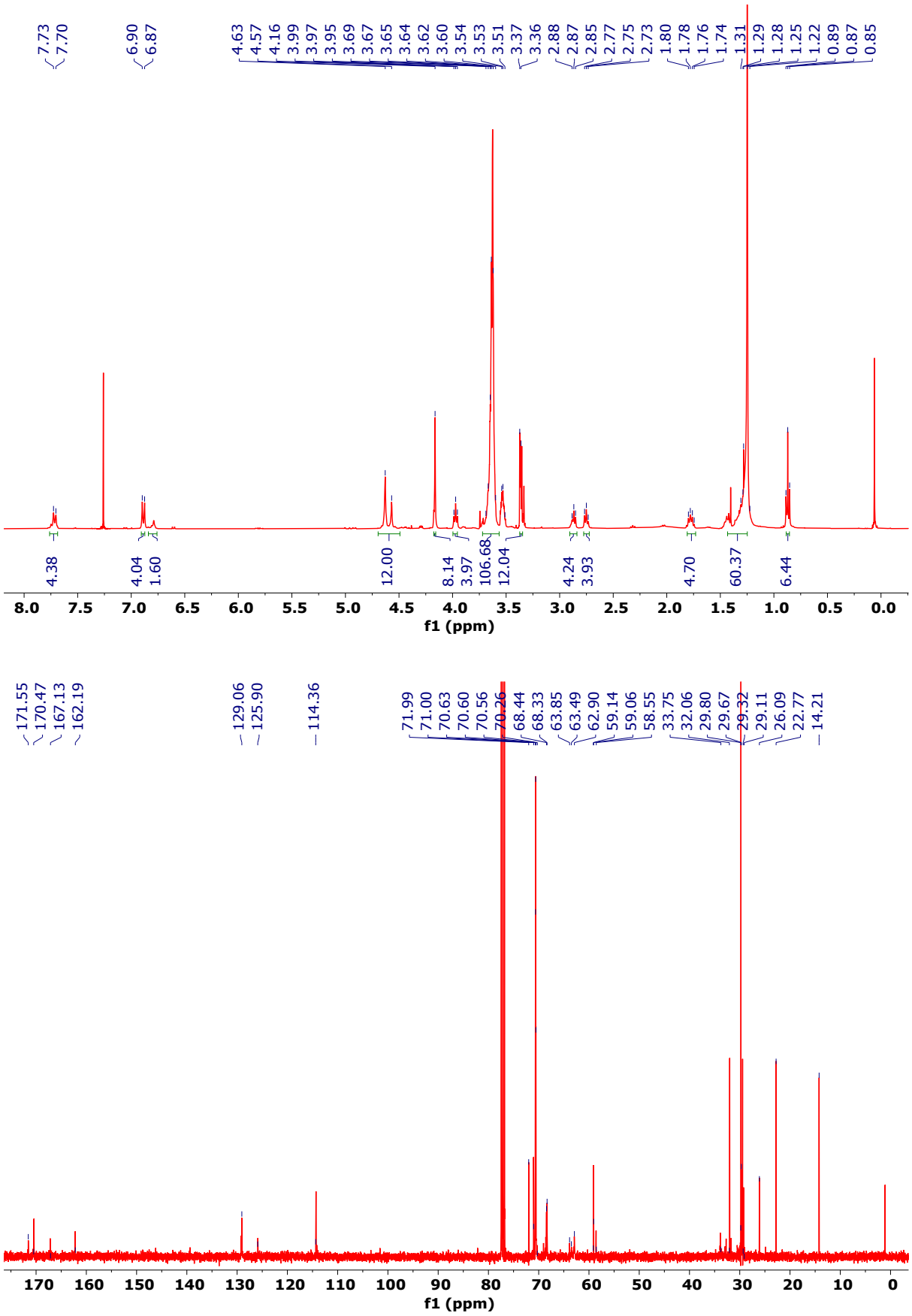
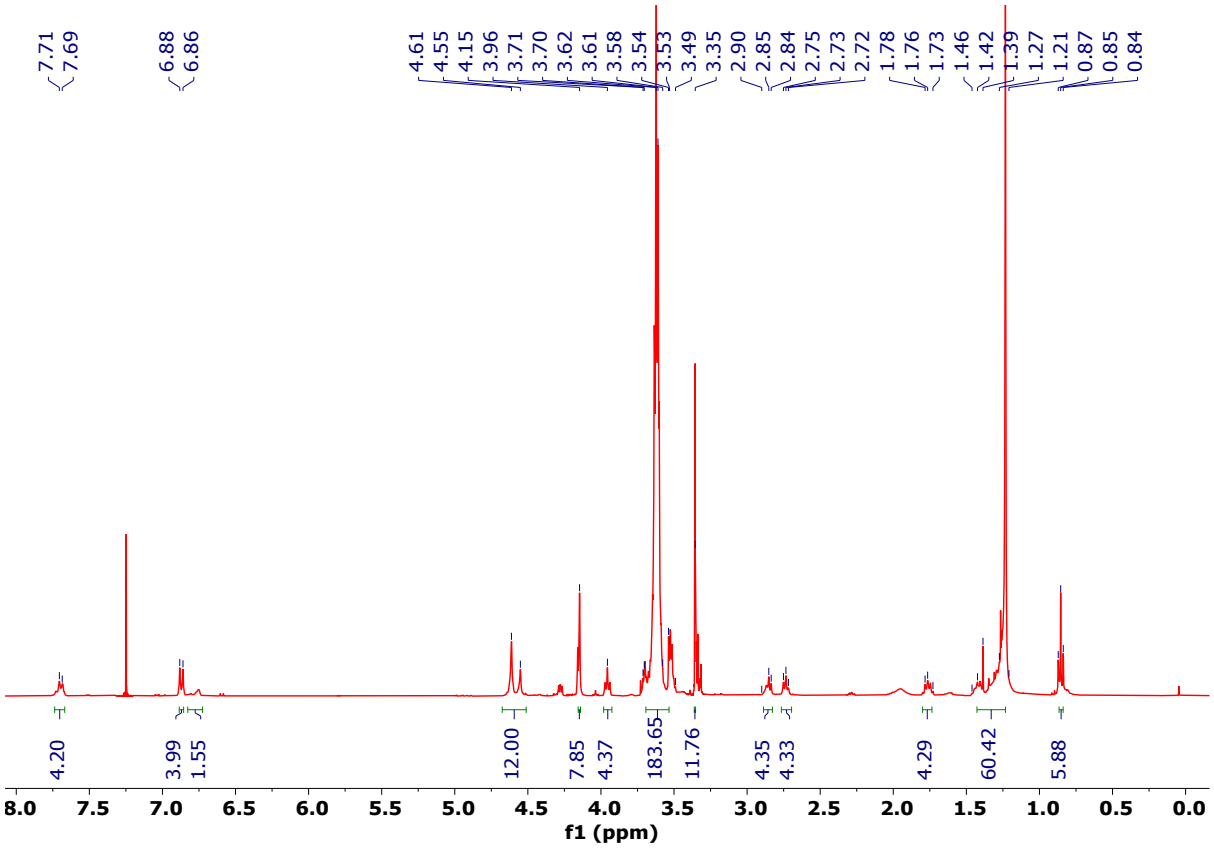


Figure S9. ^{1}H and ^{13}C NMR spectra of Compound **3**.


7.71
7.69
6.88
6.86
4.61
4.55
4.15
3.96
3.71
3.70
3.62
3.61
3.58
3.54
3.53
3.49
3.35
2.90
2.85
2.84
2.75
2.73
2.72
1.78
1.76
1.73
1.46
1.42
1.39
1.27
1.21
0.87
0.85
0.84
4.20
3.99
1.55
12.00
7.85
4.37
183.65
11.76
4.35
4.33
4.29
60.42
5.88
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

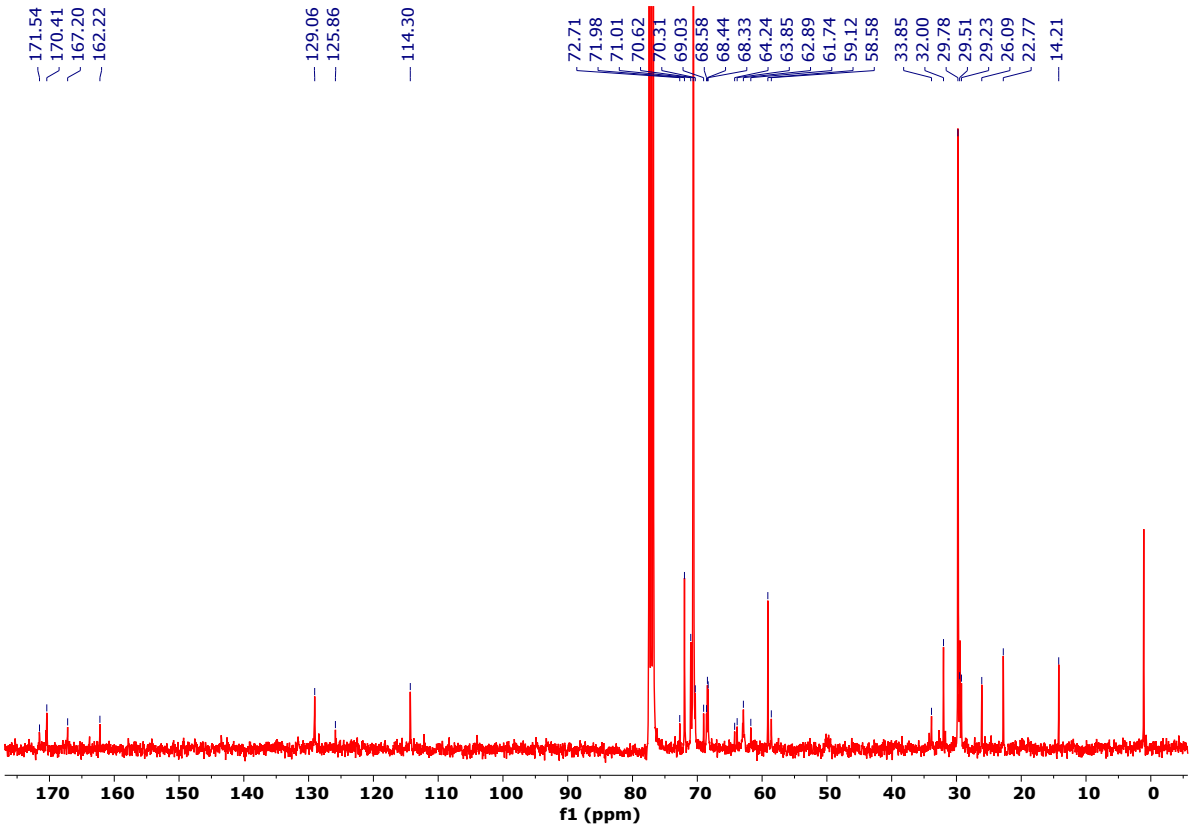
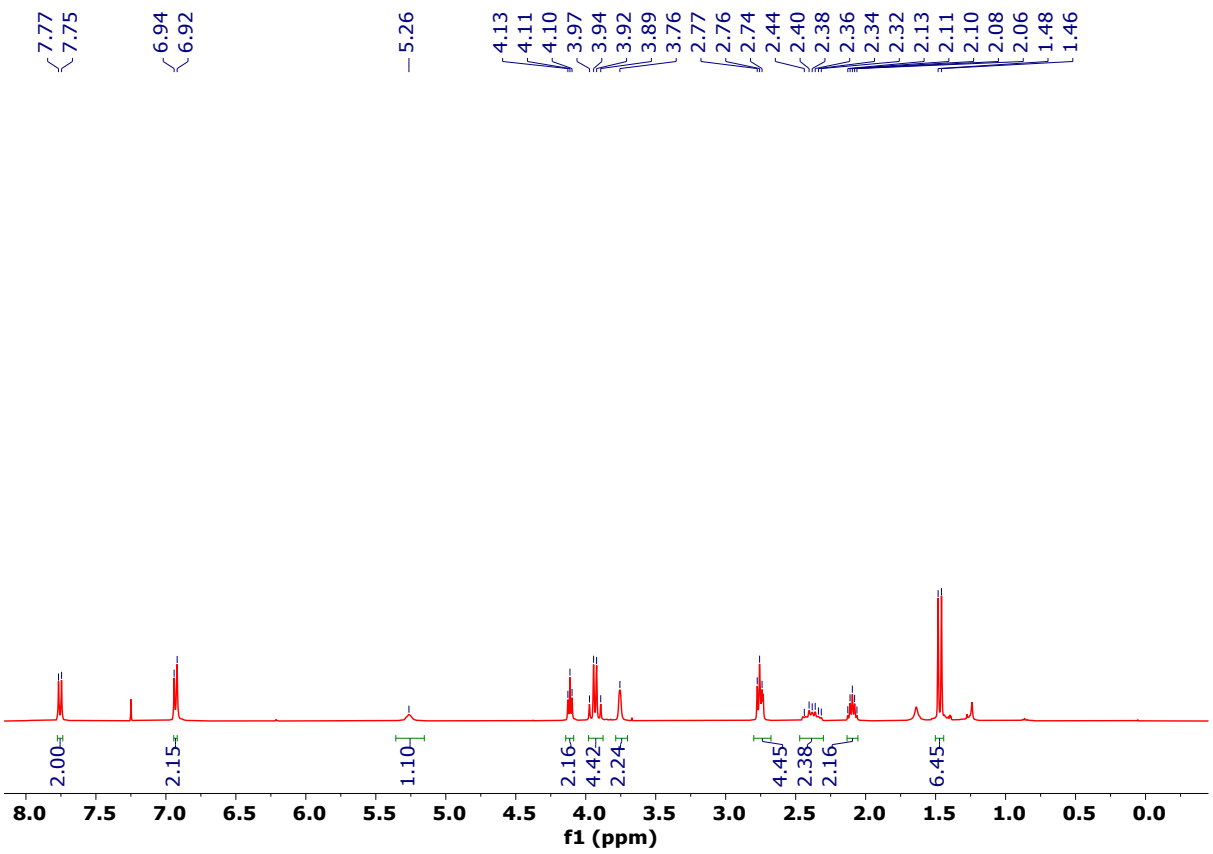

171.54
170.41
167.20
162.22
129.06
125.86
114.30
72.71
71.98
71.01
70.62
70.31
69.03
68.58
68.44
68.33
64.24
63.85
62.89
61.74
59.12
58.58
33.85
32.00
29.78
29.51
29.23
26.09
22.77
14.21
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

Figure S10. ^{1}H and ^{13}C NMR spectra of Compound **4**.



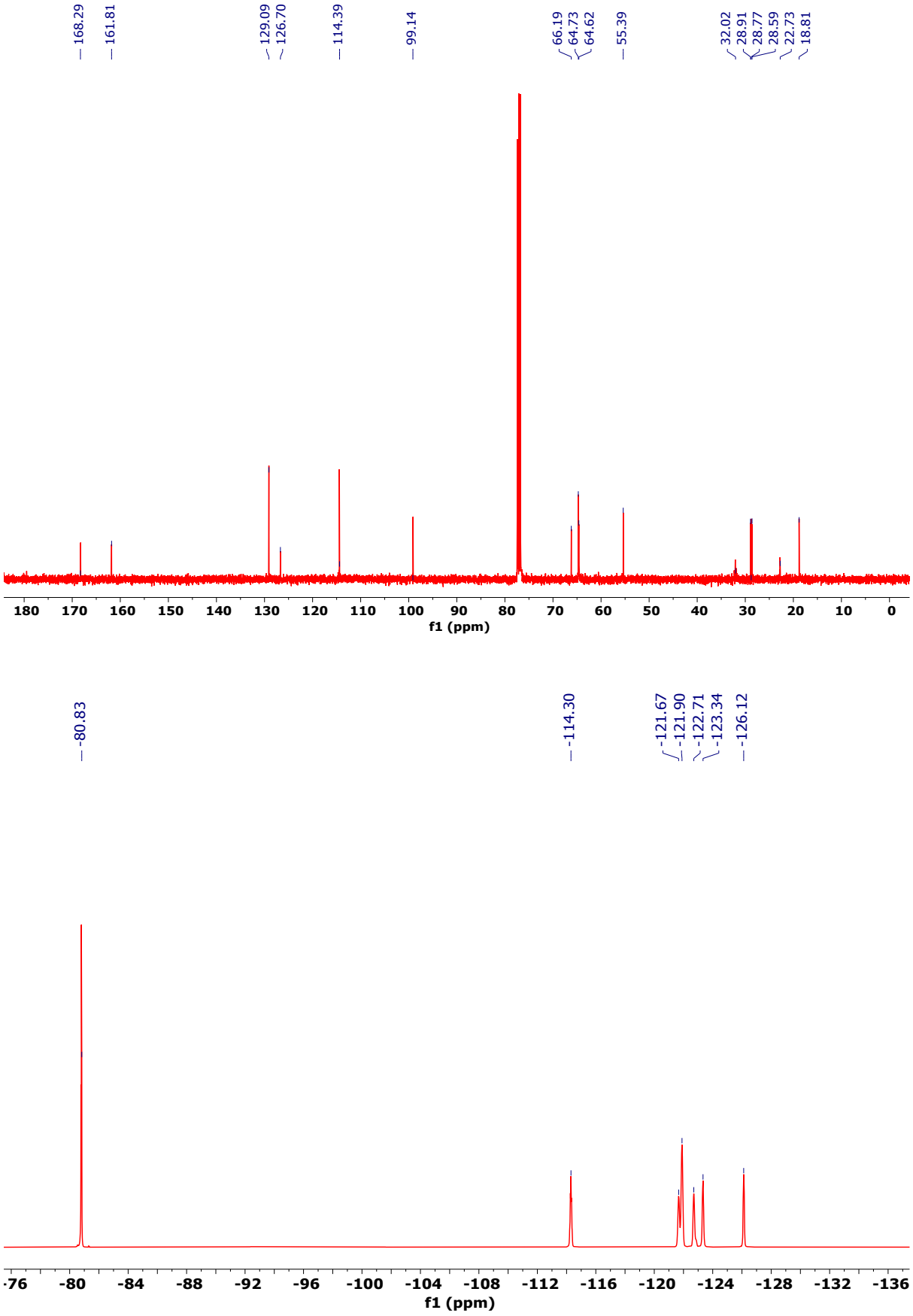
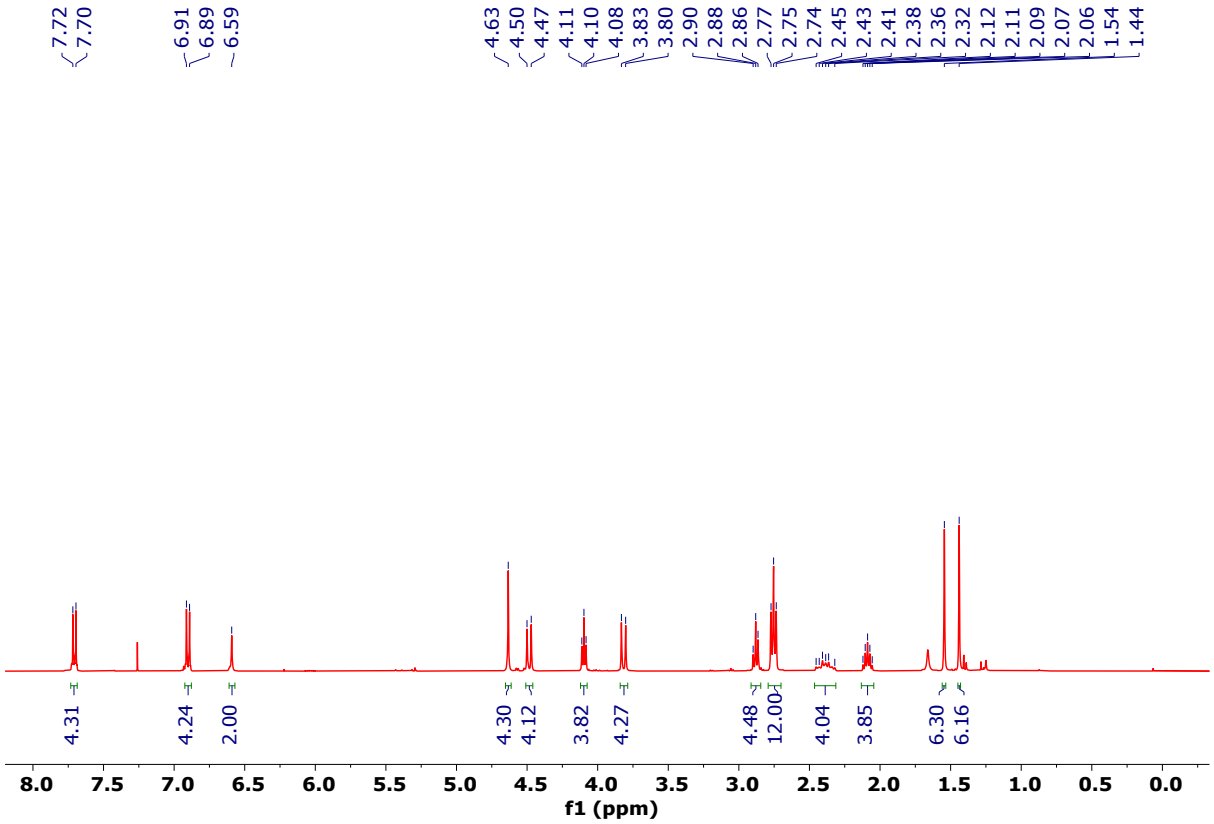
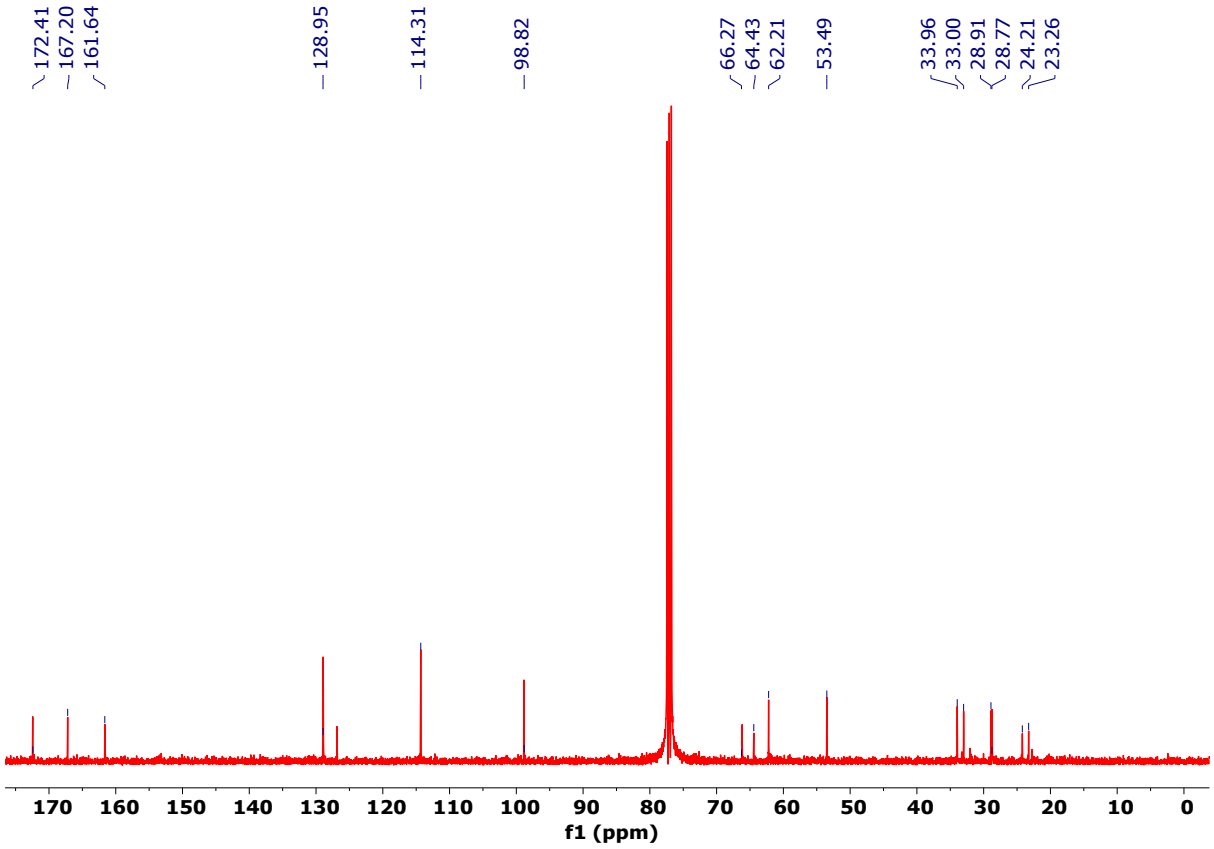


Figure S11. ^{1}H, ^{13}C and ^{19}F NMR spectra of Compound **13**.


7.72
7.70
6.91
6.89
6.59
4.63
4.50
4.47
4.11
4.10
4.08
3.83
3.80
2.90
2.88
2.86
2.77
2.75
2.74
2.45
2.43
2.41
2.38
2.36
2.32
2.12
2.11
2.09
2.07
2.06
1.54
1.44
4.31
4.24
2.00
4.30
4.12
3.82
4.27
4.48
12.00
4.04
3.85
6.30
6.16
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


172.41
167.20
161.64
128.95
114.31
98.82
66.27
64.43
62.21
53.49
33.96
33.00
28.91
28.77
24.21
23.26
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

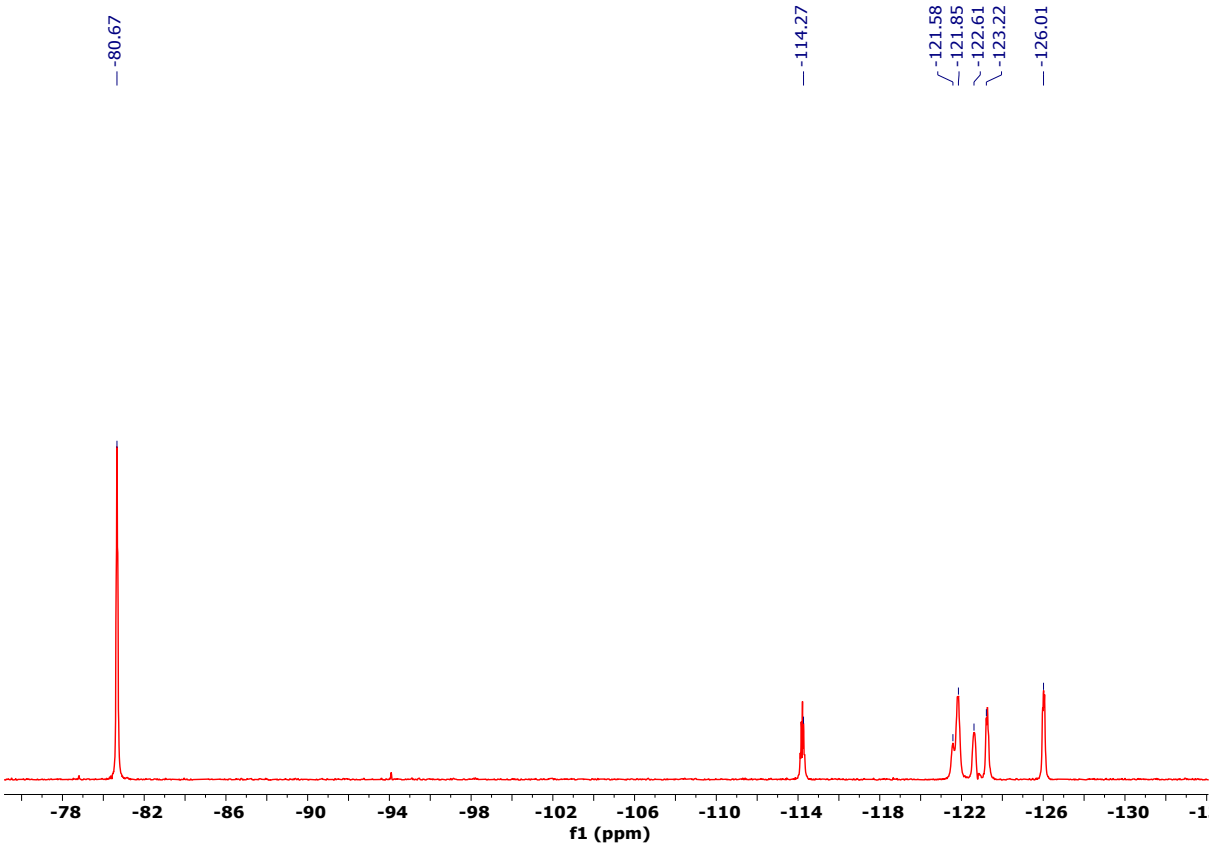
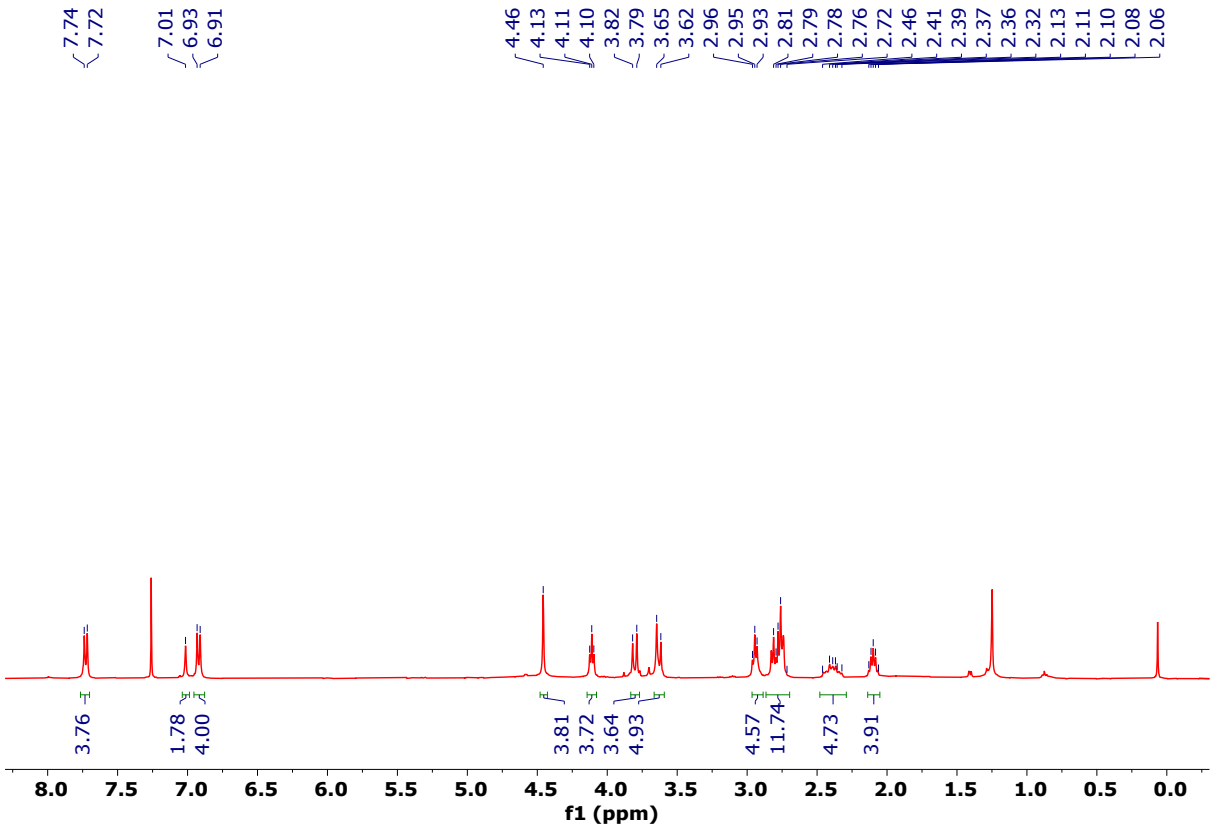
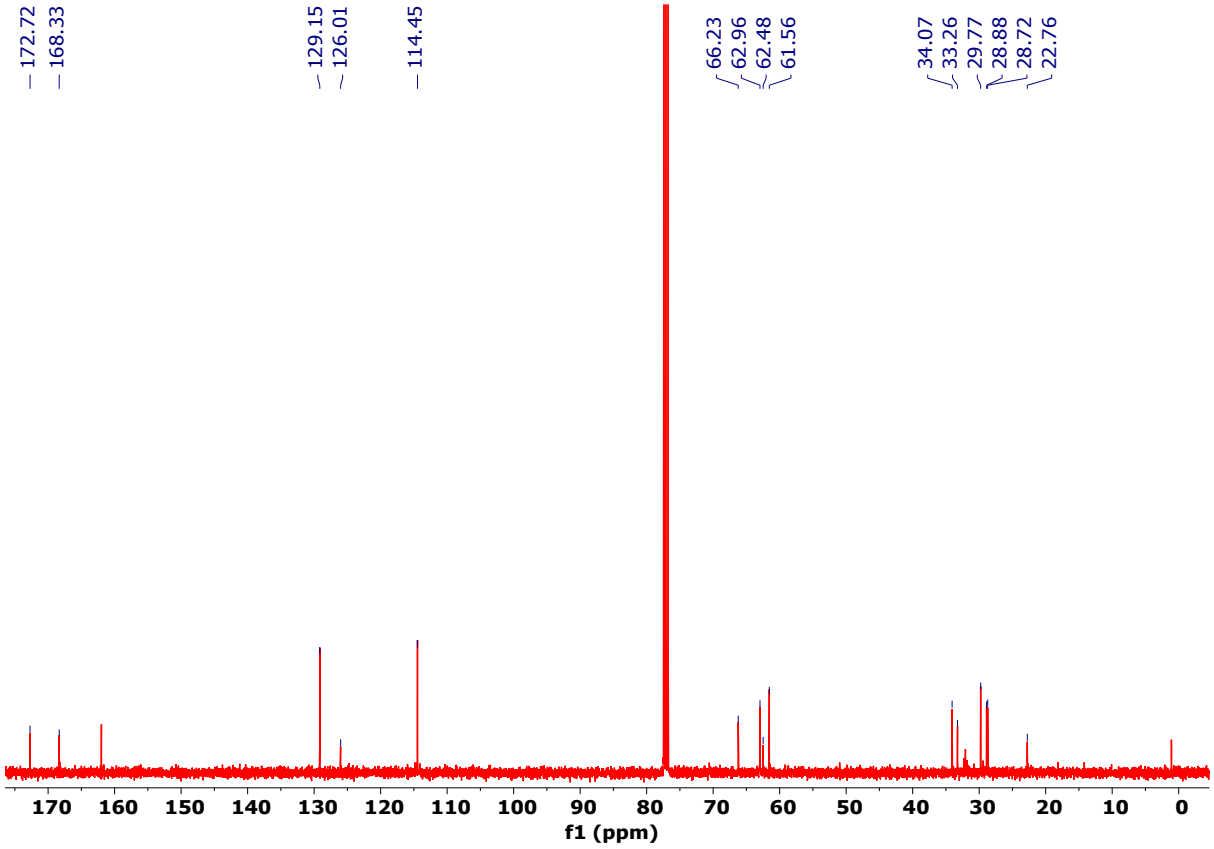


Figure S12. ^{1}H, ^{13}C and ^{19}F NMR spectra of Compound **16**.


7.74
7.72
7.01
6.93
6.91
4.46
4.13
4.11
4.10
3.82
3.79
3.65
3.62
2.96
2.95
2.93
2.81
2.79
2.78
2.76
2.72
2.46
2.41
2.39
2.37
2.36
2.32
2.13
2.11
2.10
2.08
2.06
3.76
1.78
4.00
3.81
3.72
3.64
4.93
4.57
11.74
4.73
3.91
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


172.72
168.33
129.15
126.01
114.45
66.23
62.96
62.48
61.56
34.07
33.26
29.77
28.88
28.72
22.76
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

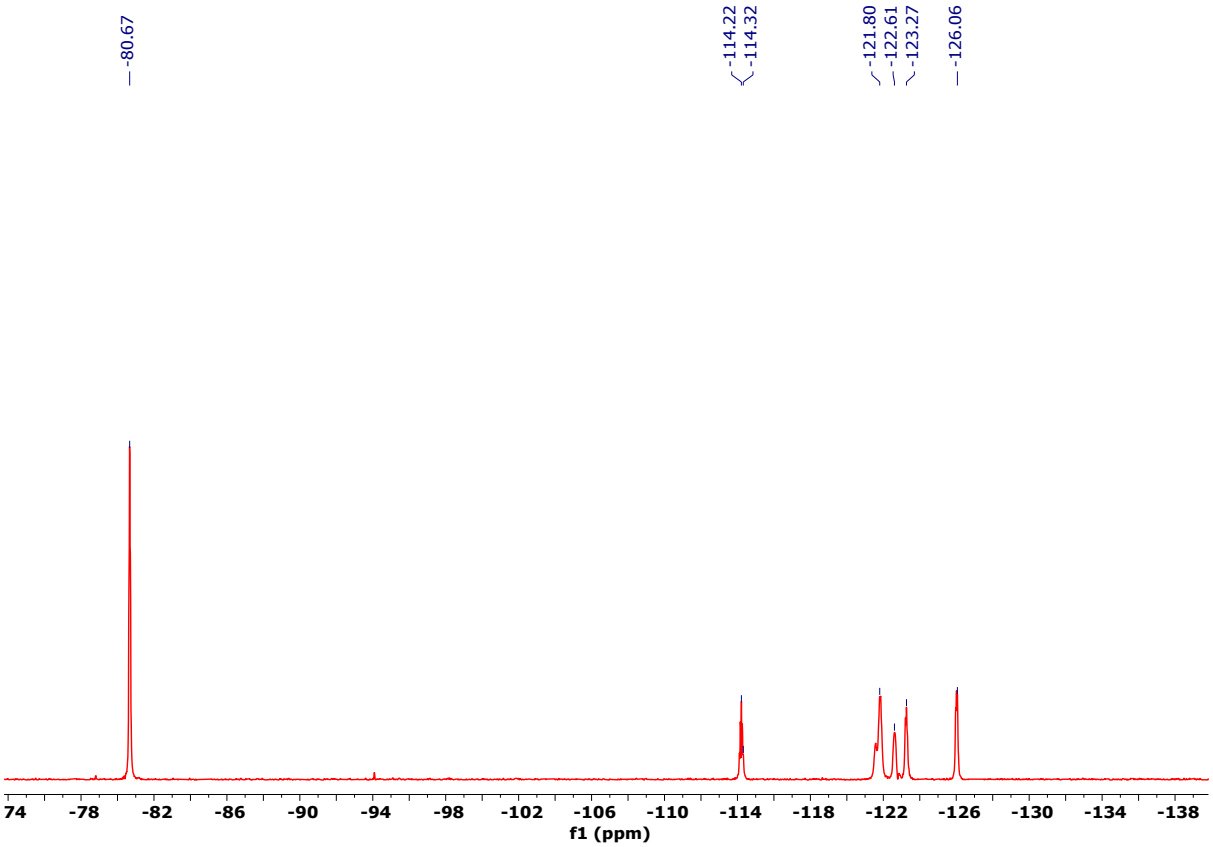
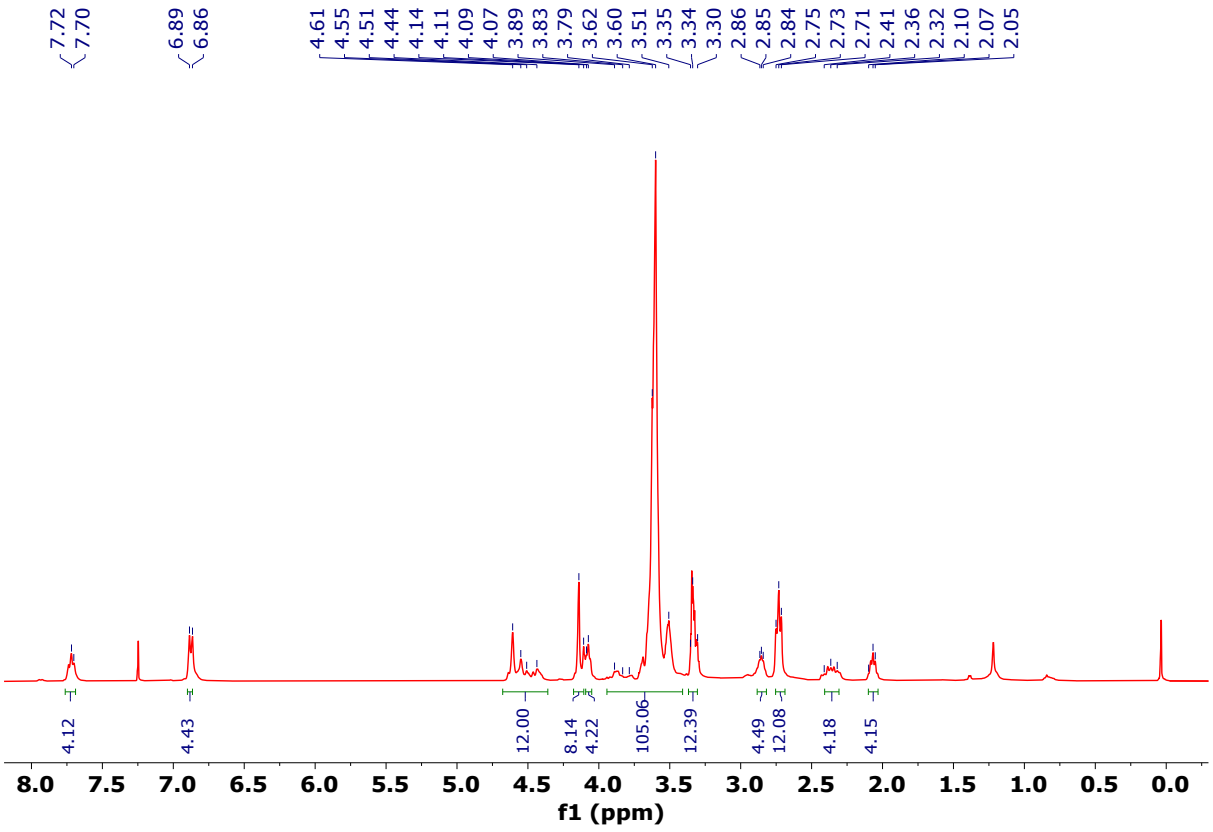
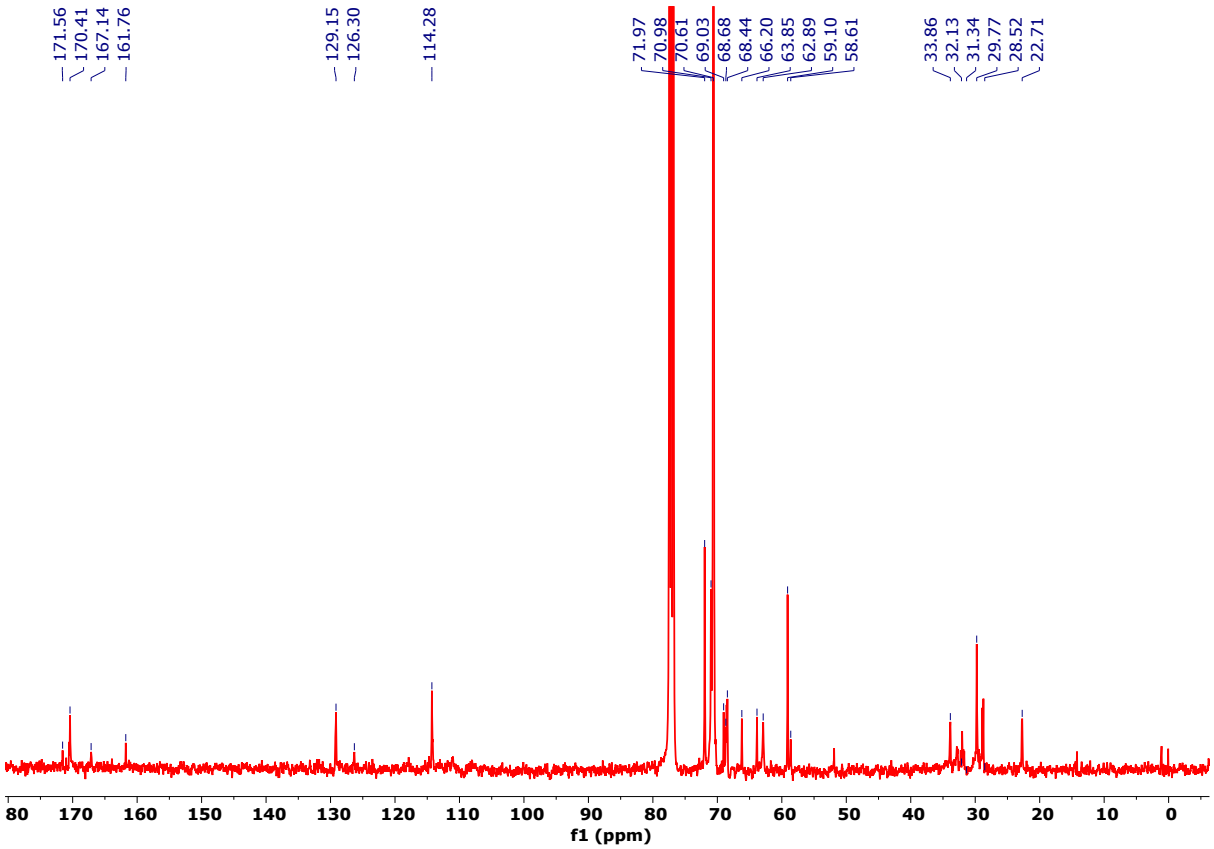


Figure S13. ^{1}H, ^{13}C and ^{19}F NMR spectra of Compound **19**.




171.56
170.41
167.14
161.76
129.15
126.30
114.28
71.97
70.98
70.61
69.03
68.68
68.44
66.20
63.85
62.89
59.10
58.61
33.86
32.13
31.34
29.77
28.52
22.71
80 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

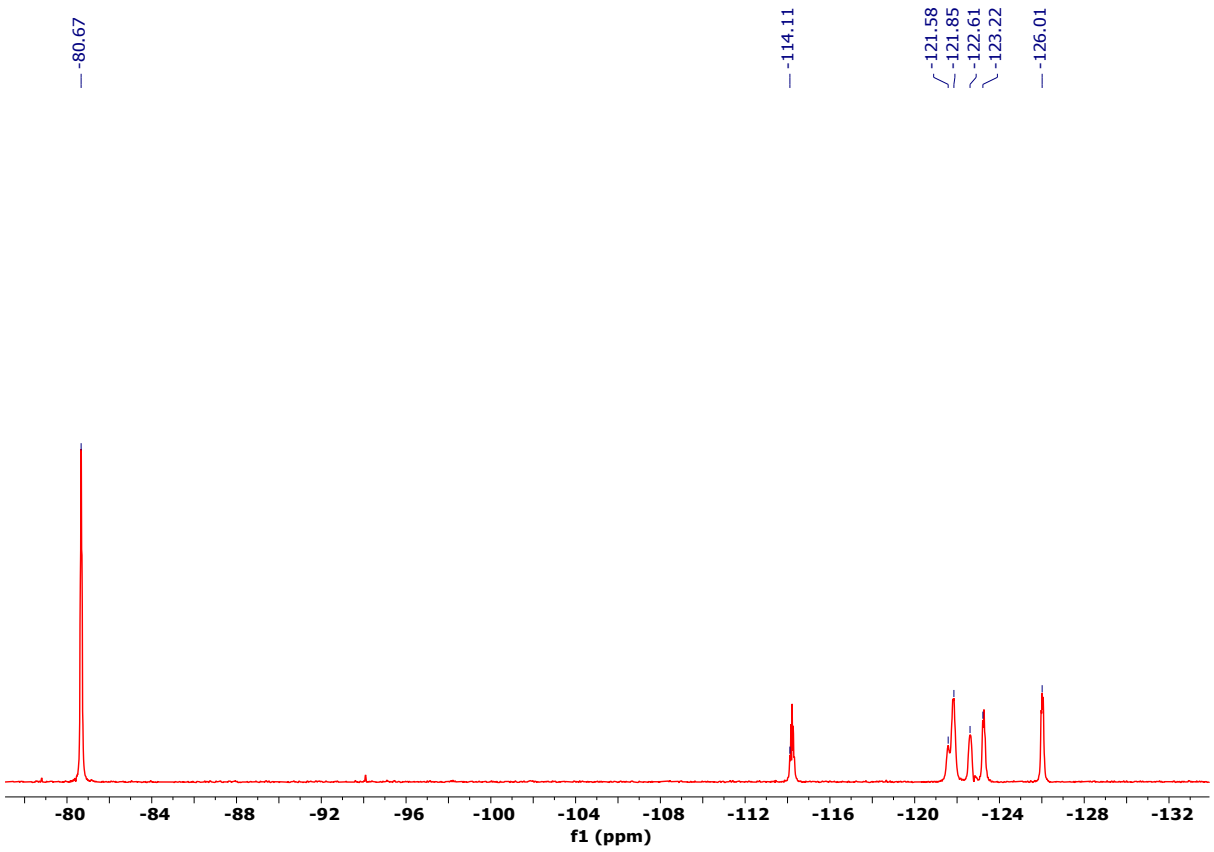
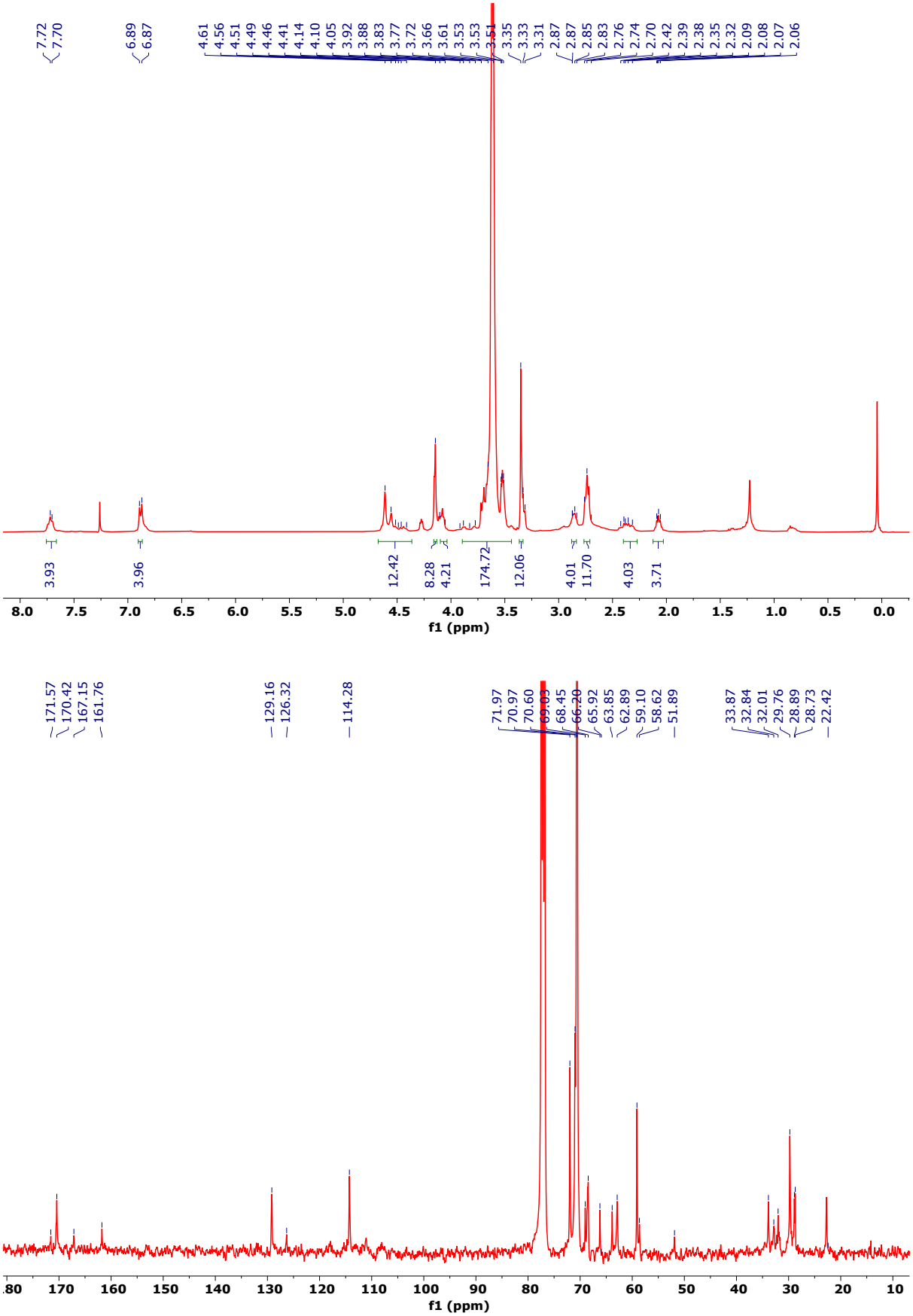

-80.67
-114.11
-121.58
-121.85
-122.61
-123.22
-126.01
-80 -84 -88 -92 -96 -100 -104 -108 -112 -116 -120 -124 -128 -132
f1 (ppm)

Figure S14. ^{1}H, ^{13}C and ^{19}F NMR spectra of Compound **5**.



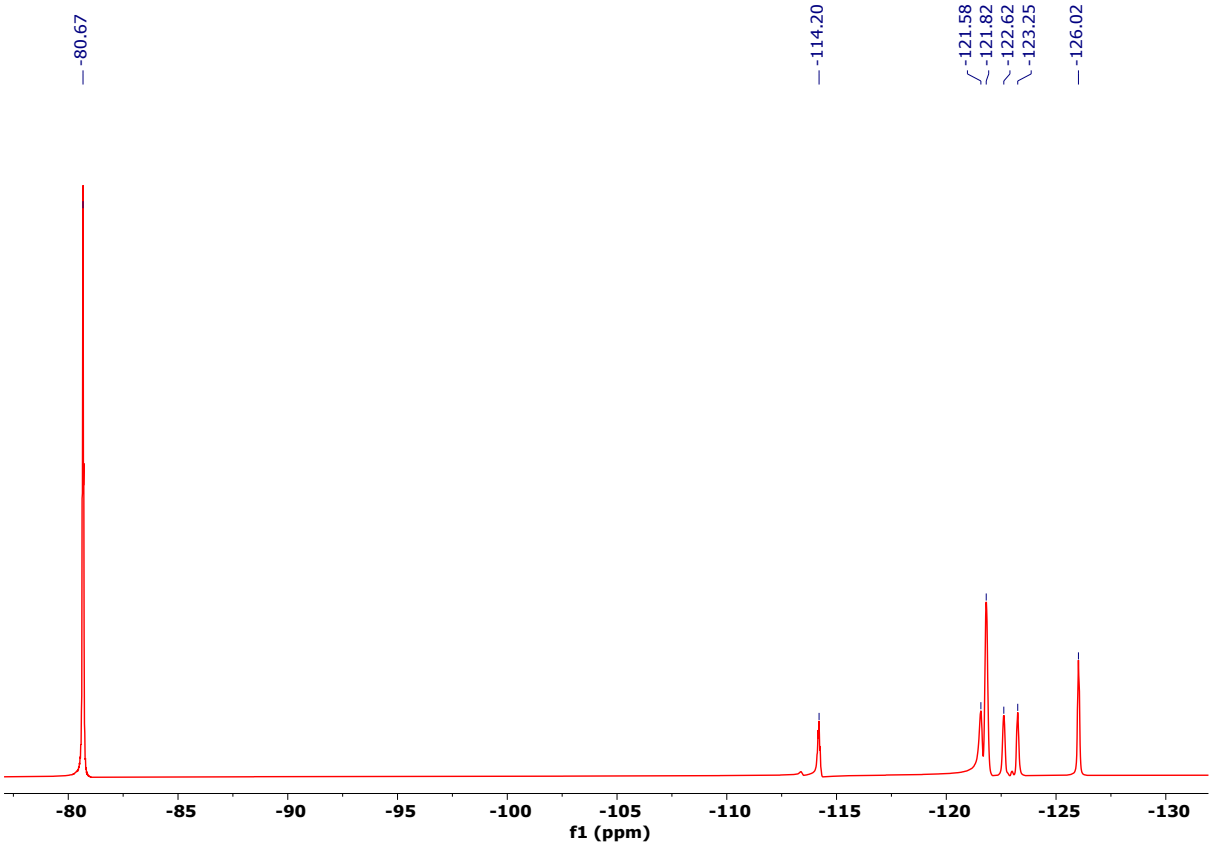
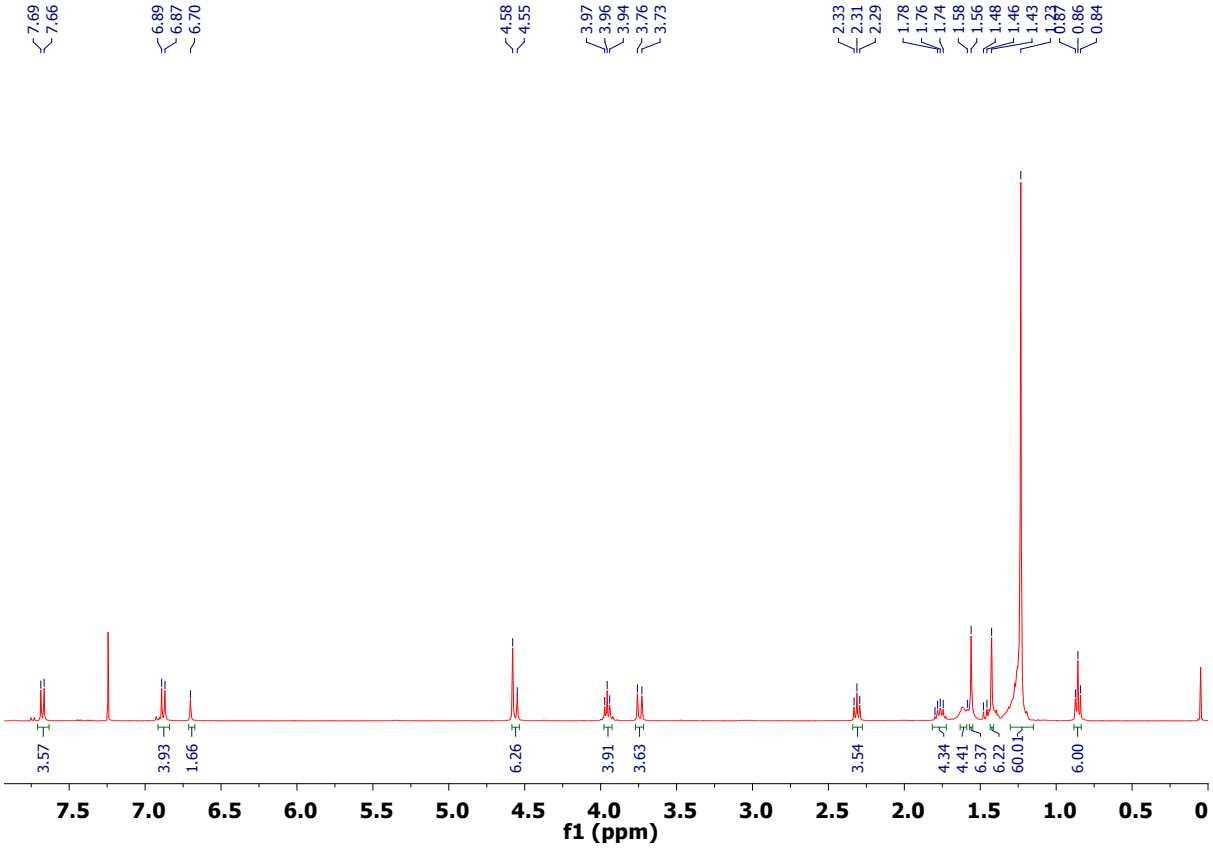


Figure S15. ^{1}H, ^{13}C and ^{19}F NMR spectra of Compound **6**.


7.69
7.66
6.89
6.87
6.70
4.58
4.55
3.97
3.96
3.94
3.76
3.73
2.33
2.31
2.29
1.78
1.76
1.74
1.58
1.56
1.48
1.46
1.43
0.87
0.86
0.84
3.57
3.93
1.66
6.26
3.91
3.63
3.54
4.34
4.41
6.37
6.22
60.01
6.00
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0
f1 (ppm)

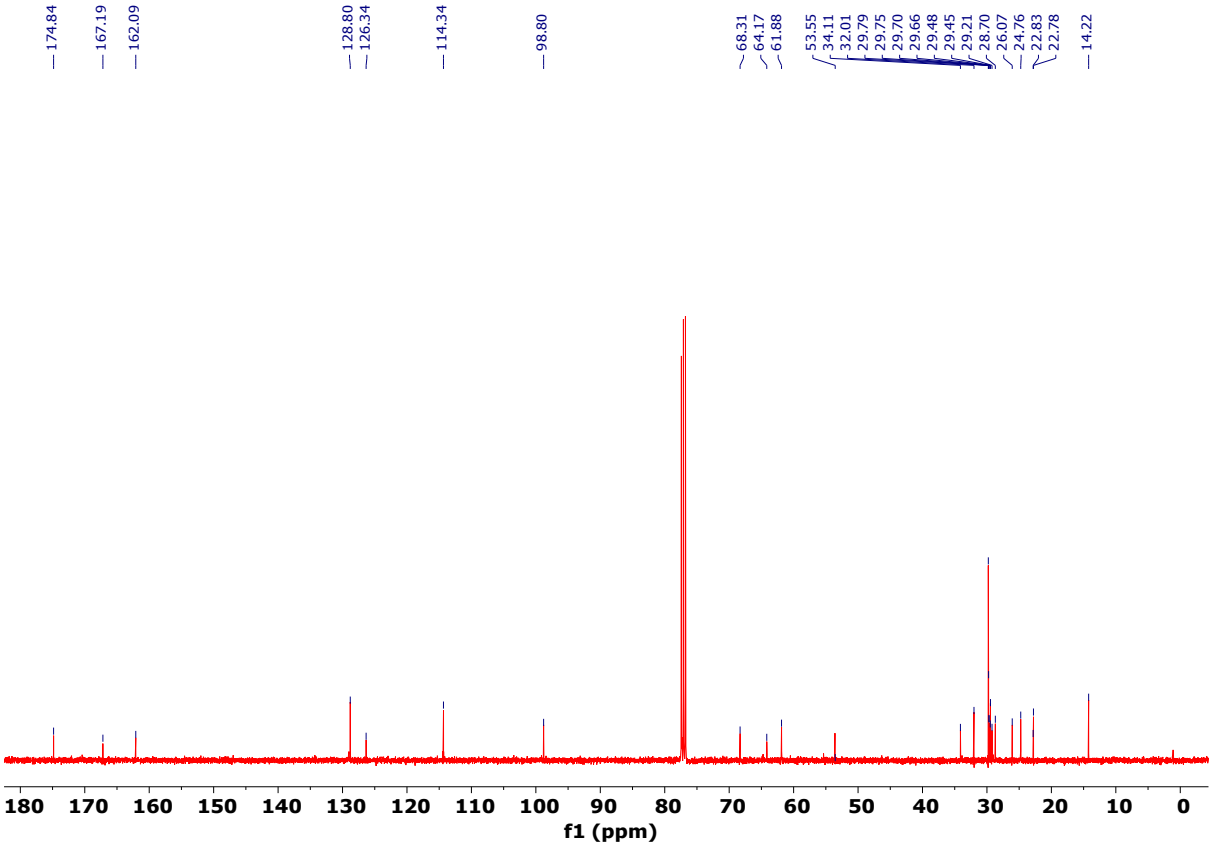
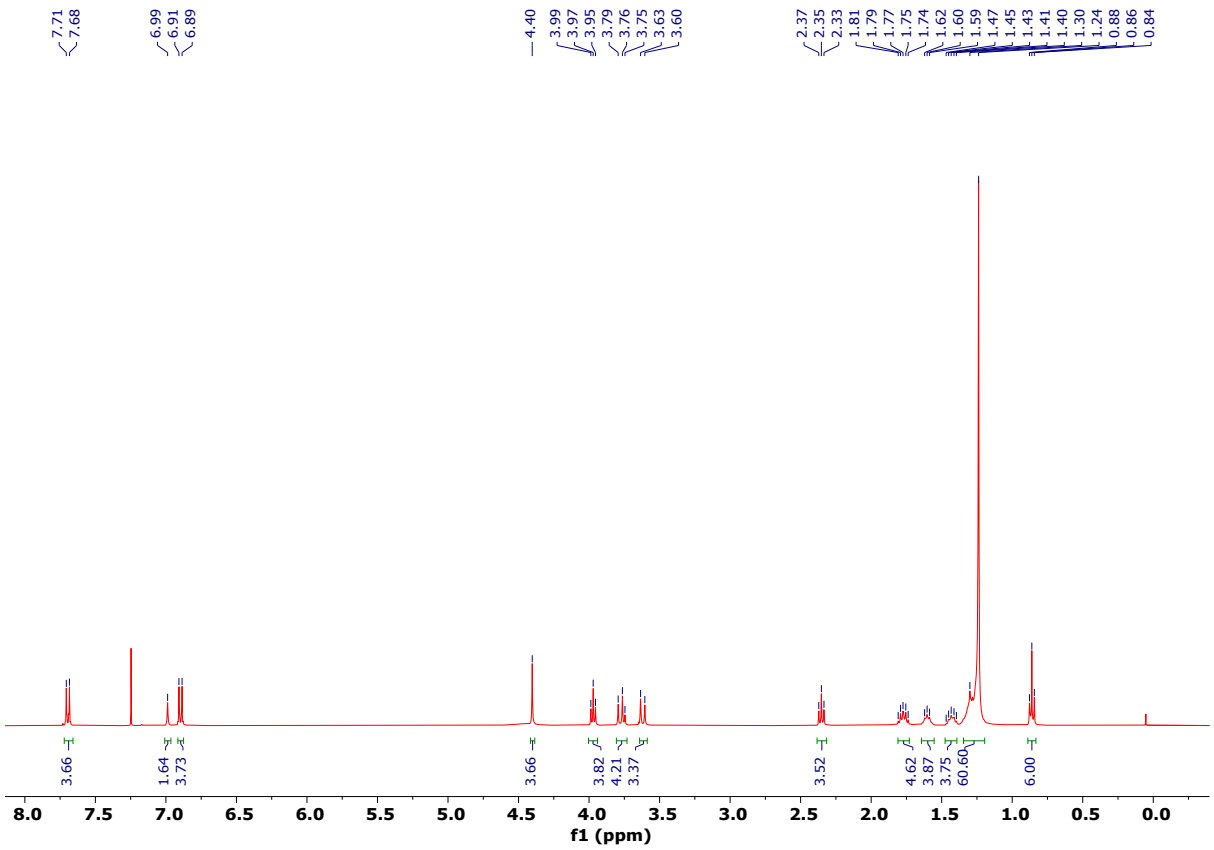

174.84
167.19
162.09
128.80
126.34
114.34
98.80
68.31
64.17
61.88
53.55
34.11
32.01
29.79
29.75
29.70
29.66
29.48
29.45
29.21
28.70
26.07
24.76
22.83
22.78
14.22
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

Figure S16. ^{1}H, and ^{13}C NMR spectra of Compound **20**.



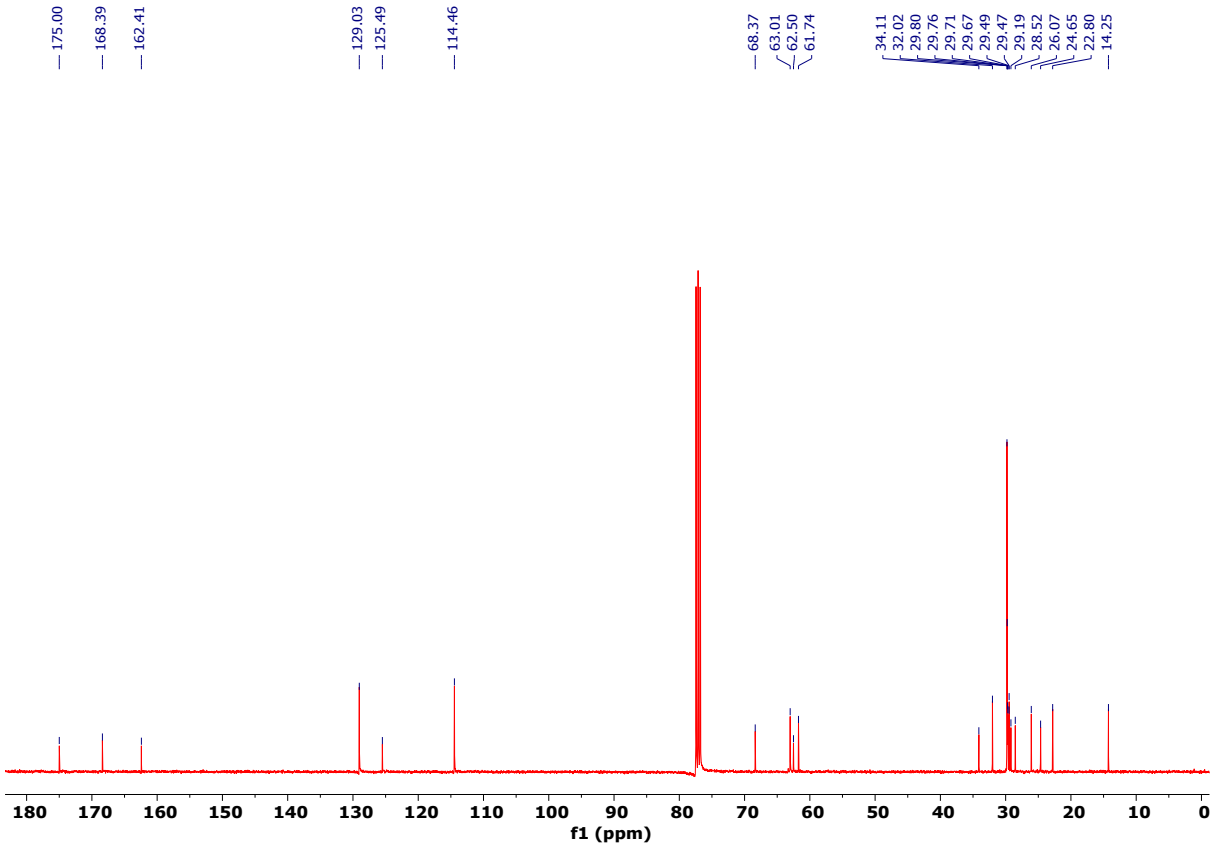


Figure S17. ^{1}H, and ^{13}C NMR spectra of Compound **21**.

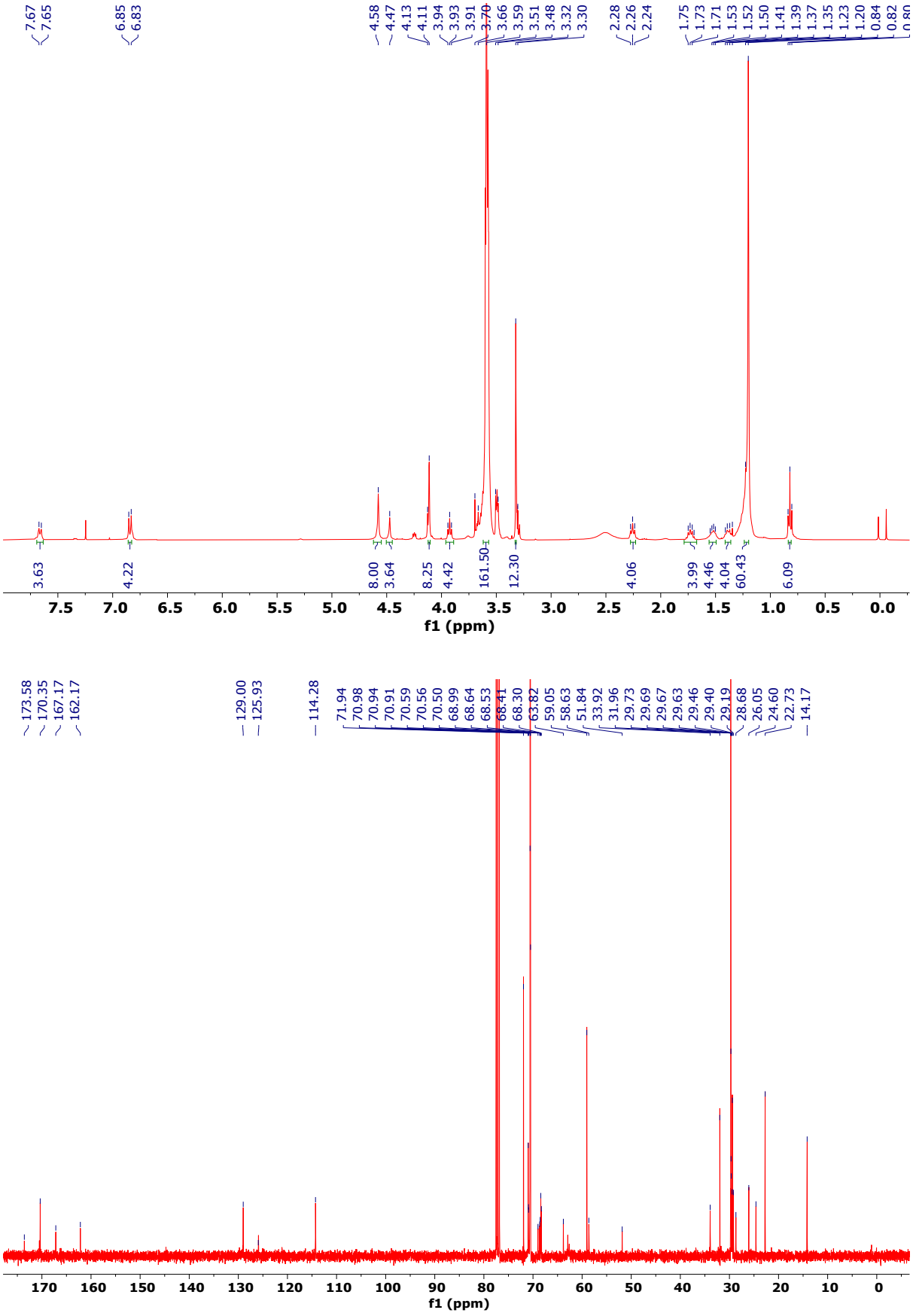


Figure S18. ^{1}H, and ^{13}C NMR spectra of Compound **22**.

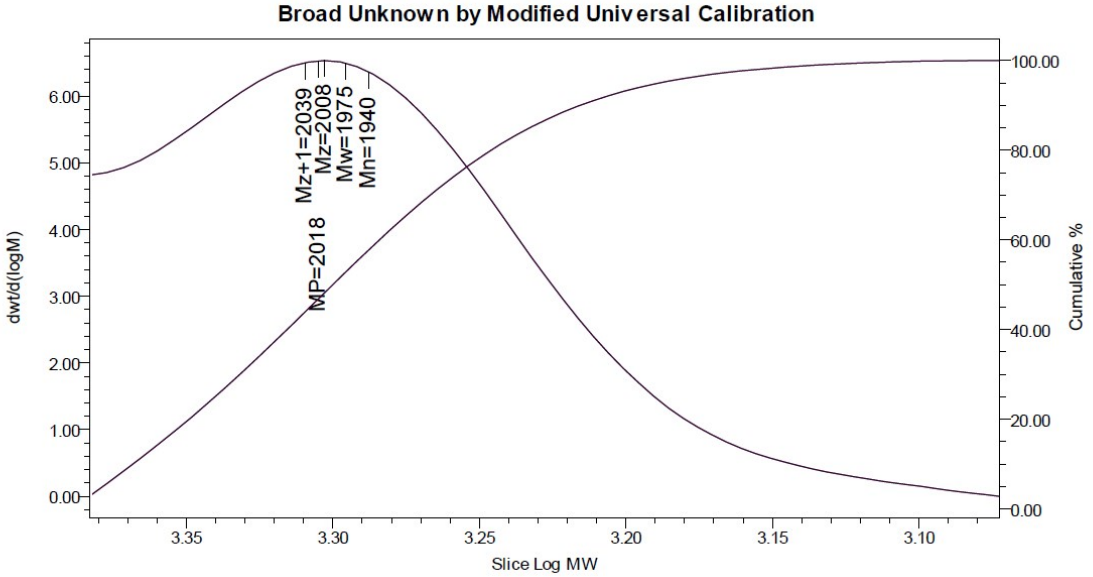


Figure S19. Gel permeation chromatography profile of **1**.

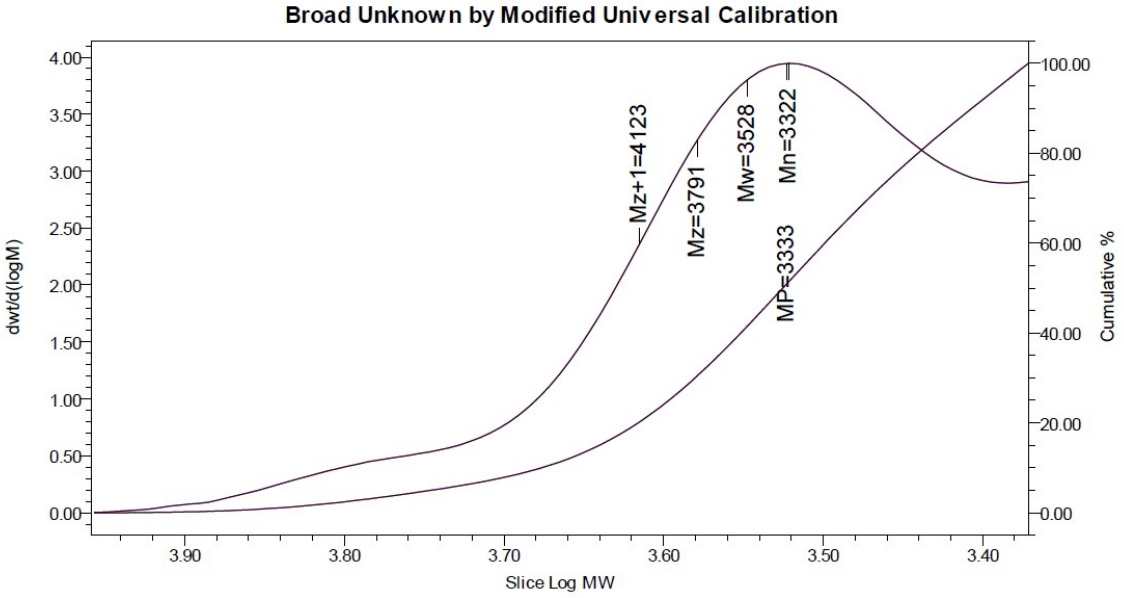


Figure S20. Gel permeation chromatography profile of **2**.

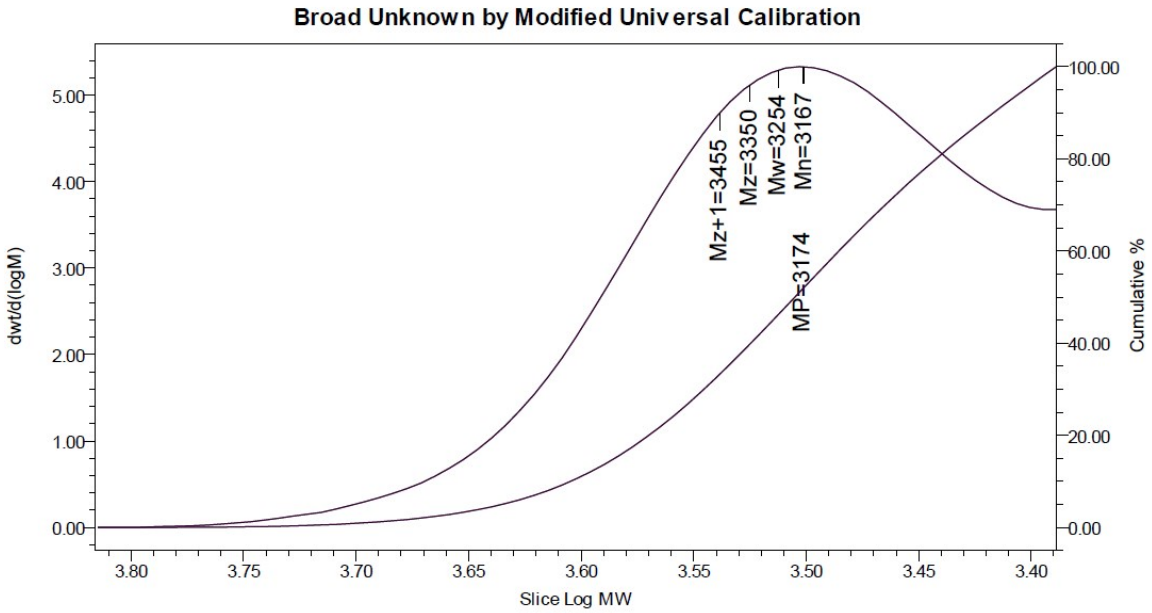


Figure S21. Gel permeation chromatography profile of **3**.

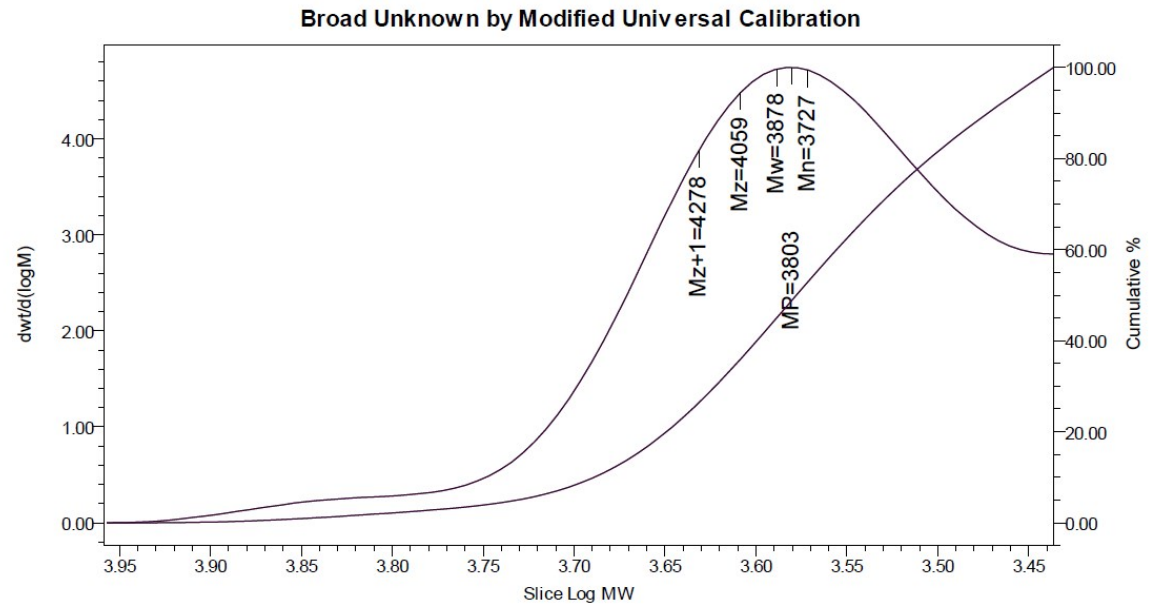


Figure S22. Gel permeation chromatography profile of **4**.

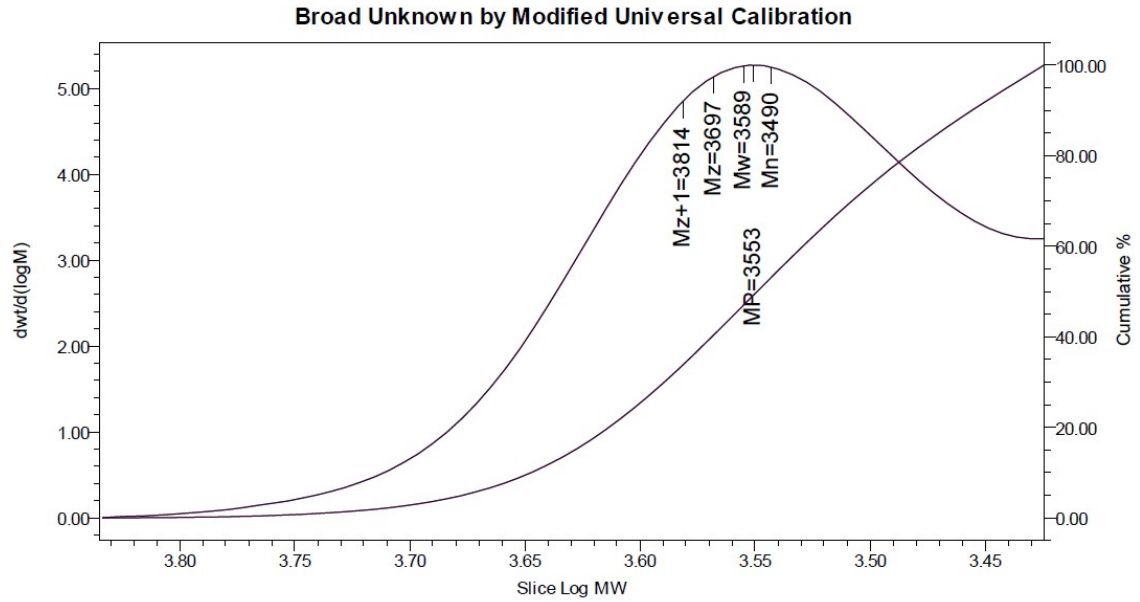


Figure S23. Gel permeation chromatography profile of **5**.

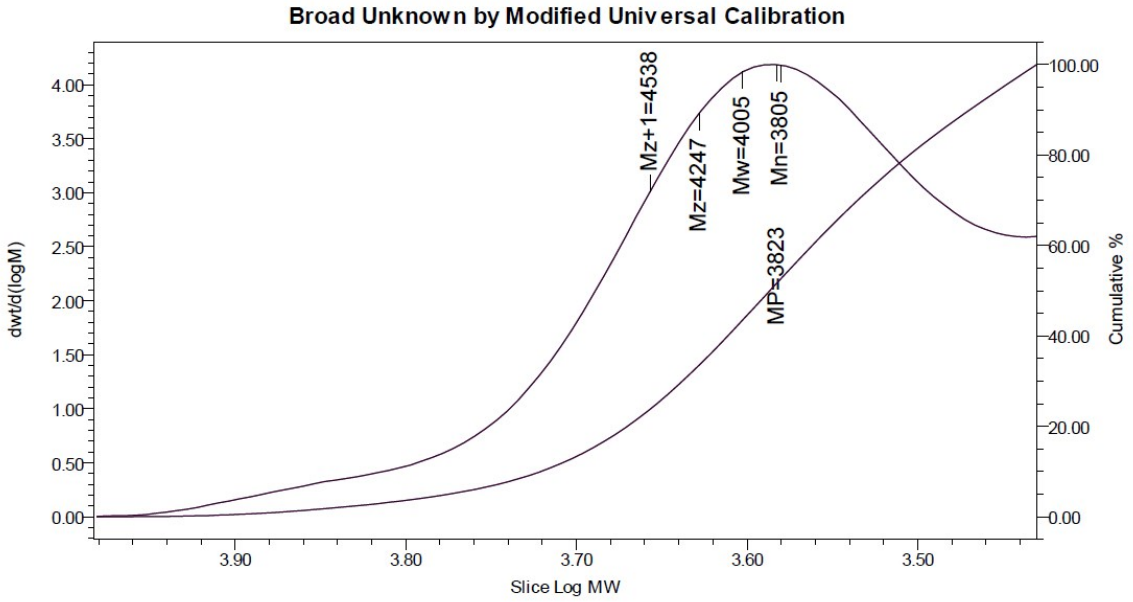


Figure S24. Gel permeation chromatography profile of **6**.

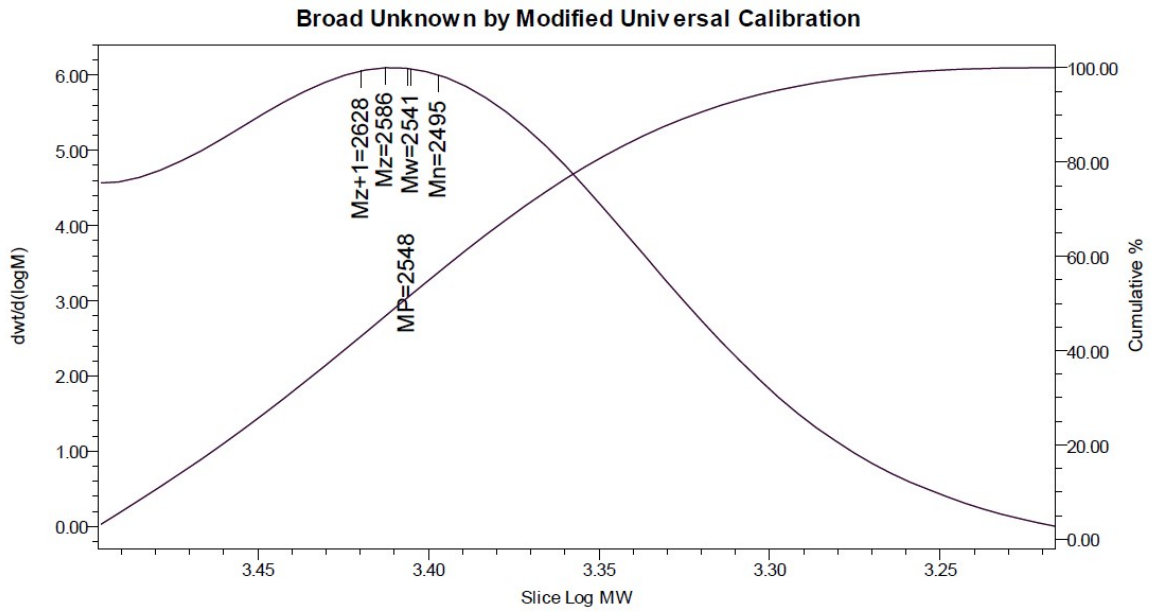


Figure S25. Gel permeation chromatography profile of **22**.

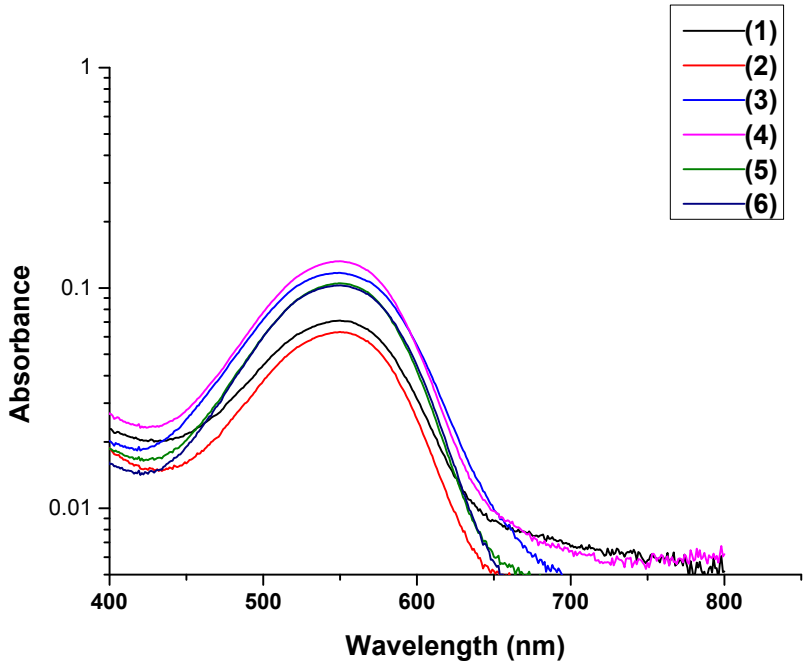


Figure S26. UV Absorbance spectra of Nile red in methanol.

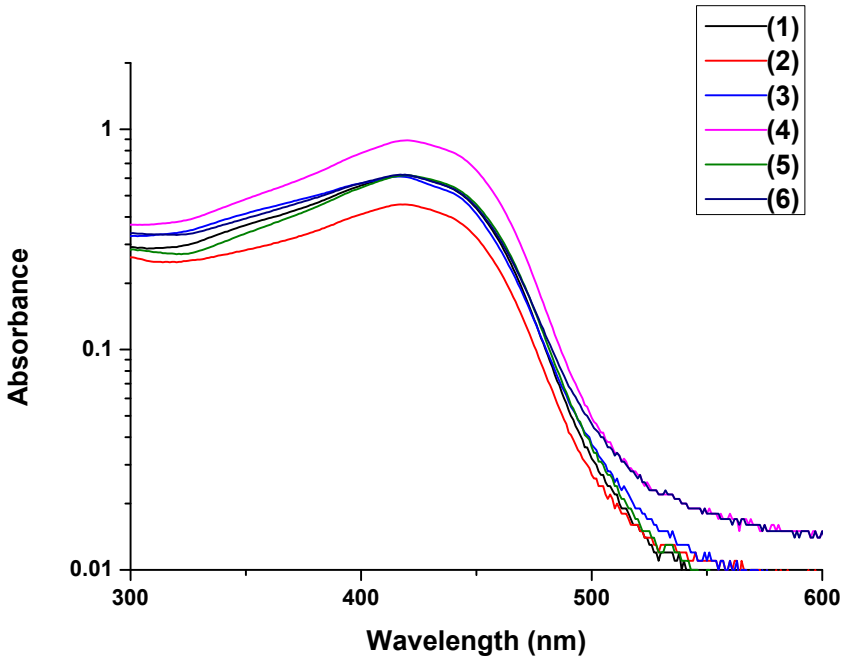


Figure S27. UV Absorbance spectra of Curcumin in methanol.

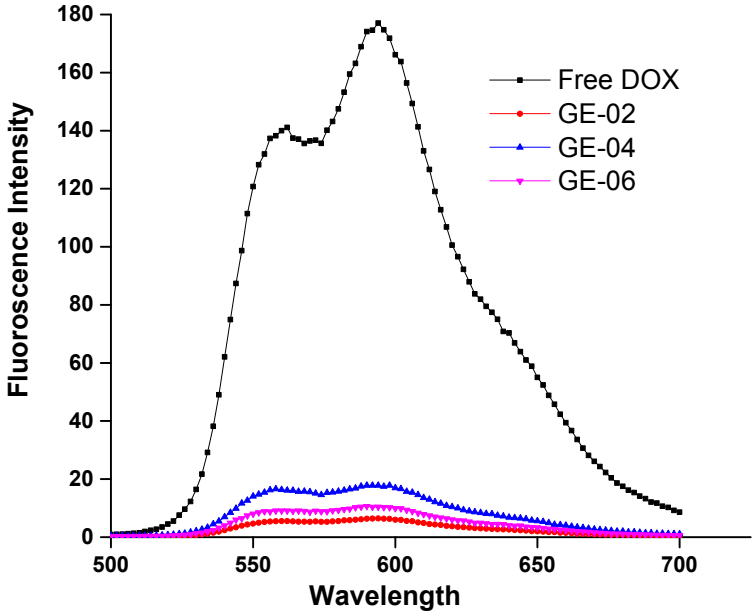


Figure S28. Fluroscence intensity spectra of Doxorubicin in water.

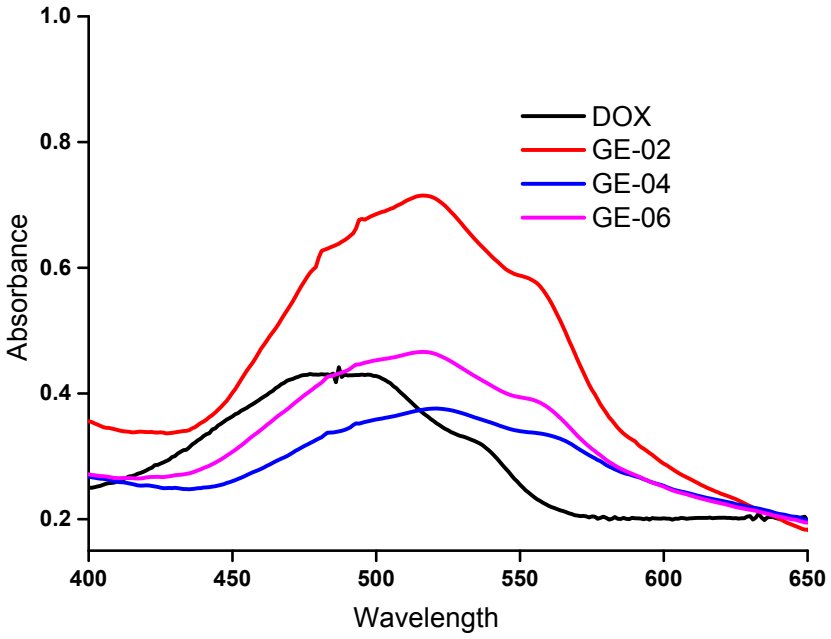


Figure S29. UV Absorbance spectra of Doxorubicin in DMSO.

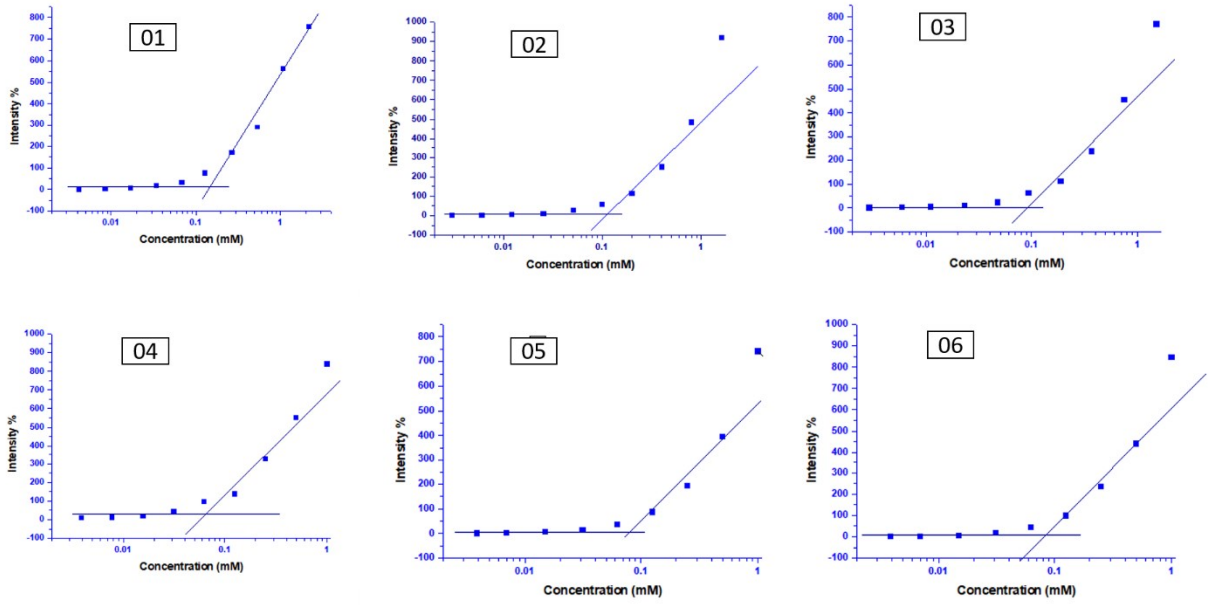


Figure S30. Plot of fluorescence intensity versus concentration for calculating critical aggregation concentration.