Supplementary Information

Traceable gene delivery using minimalistic far-red fluorescent pseudodendrimers

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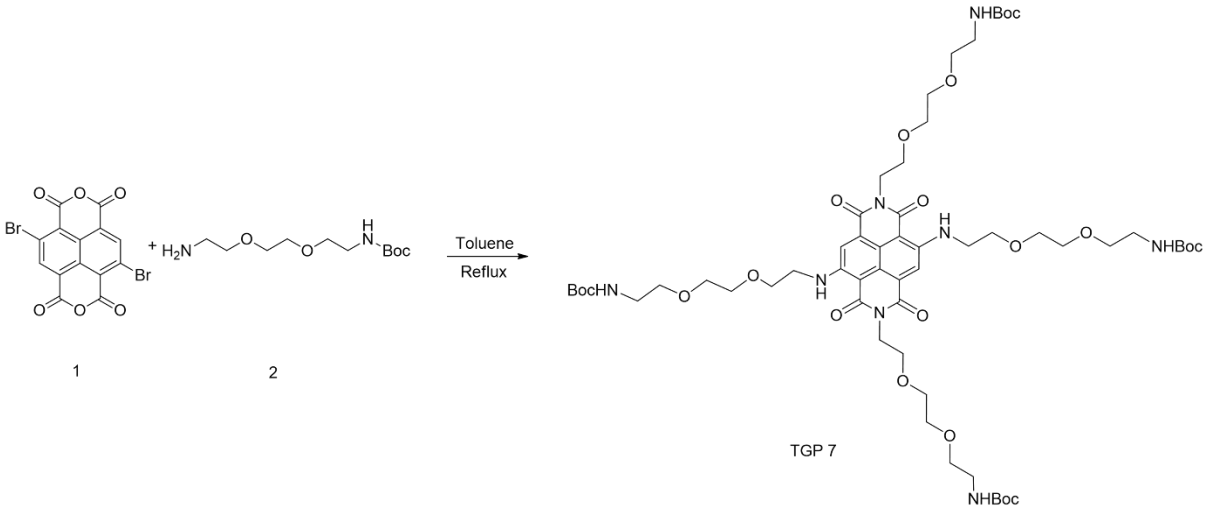
1. Methods and materials

1.1 General procedures

All reagents and solvents were procured from Spectrochem or Merck and used without further purification. Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (Cat #11320033), Roswell Park Memorial Institute (RPMI) 1640 (Cat #11835030), fetal bovine serum (FBS) (Cat #10270106), penicillin-streptomycin (PS) (Cat #15140122), and Anti-Anti (Antibiotic-Antimycotic) (Cat #15240062) were obtained from Gibco (ThermoFisher Scientific). HEK293T cells were obtained from the National Centre for Cell Science (NCCS) and murine microglial cell line N9 was kindly provided by Prof. Anirban Basu, National Brain Research Centre. Sterilized plastic wares for cell cultures were purchased from Nunc (ThermoFisher Scientific) and used directly. Mica discs for AFM studies were purchased from Ted Pella and used as per the standard protocol. Agilent Cary series UV-Vis-NIR absorption and Agilent Cary Eclipse fluorescence spectrophotometers, along with a SpectraMax i3x microplate reader, were utilized to monitor the absorbance and fluorescence properties of the pseudodendrimer. Either plasmid DNA (pDNA) or calf thymus DNA (ctDNA) was used, depending on the concentration required for the experiments. Purity was analysed performed using ^{1}H and ^{13}C NMR spectra recorded using Bruker AV-400 and JEOL 600 MHz spectrometers, with tetramethylsilane (TMS) as an internal standard. High-resolution mass spectra were recorded with an Agilent 6538 UHD HRMS/Q-TOF high-resolution spectrometer. Confocal imaging was performed using an Olympus Fluoview 3000 confocal laser scanning microscope, with image processing conducted *via* inbuilt software. Fluorescence imaging was carried out with a Leica DMi8 microscope equipped with a live cell imaging setup, and images were processed using Huygens software. Bruker atomic force microscopy was performed to study nanoscale

resolution. All data were quantified using ImageJ software and plotted using Origin 8.5 and Graphpad Prism 8.0.

1.2 Synthetic procedure and characterization data



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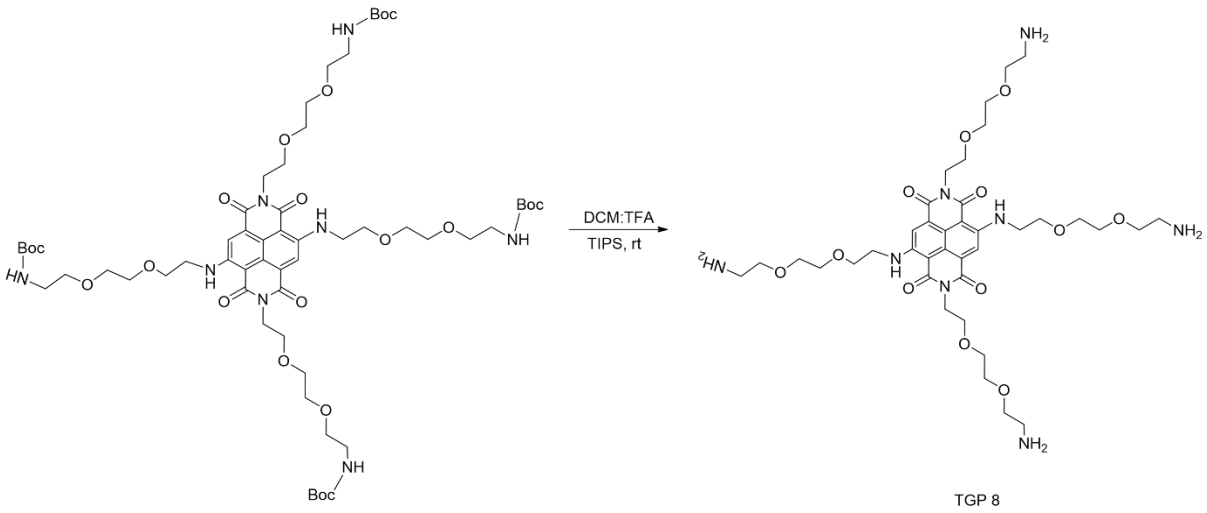
1.2.1 Synthesis of TGP 7. Into a 100 mL single-neck round-bottom flask containing a well-stirred solution of the dibromo NDI derivative 1 (10.42 g, 24.46 mmol, 1.0 equiv.) in toluene (20 mL) was added mono-Boc-2,2-(ethylenedioxy)bis-ethylamine 2 (26.72 g, 107.63 mmol, 4.4 equiv.). The reaction mixture was refluxed at 130 ºC under an N_2 atmosphere overnight in an oil bath. The progress of the reaction was monitored by TLC, LCMS and after completion of the reaction, the solvent was removed under reduced pressure. The crude compound was purified by column chromatography (silica gel 230-400 mesh) using 0-20% methanol in dichloromethane, while the desired product was eluted at 5% of methanol in dichloromethane. The pure fraction was concentrated under reduced pressure to obtain the desired product (**3**; **TGP 7**; 5.1 g, 4.17 mmol, Yield. 17%) as a blue coloured sticky solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.51 (s, 2H), 8.12 (s, 2H), 5.09 (d, J = 5.2 Hz, 2H), 4.42 (t, J = 6.0 Hz, 4H), 3.84 (t,

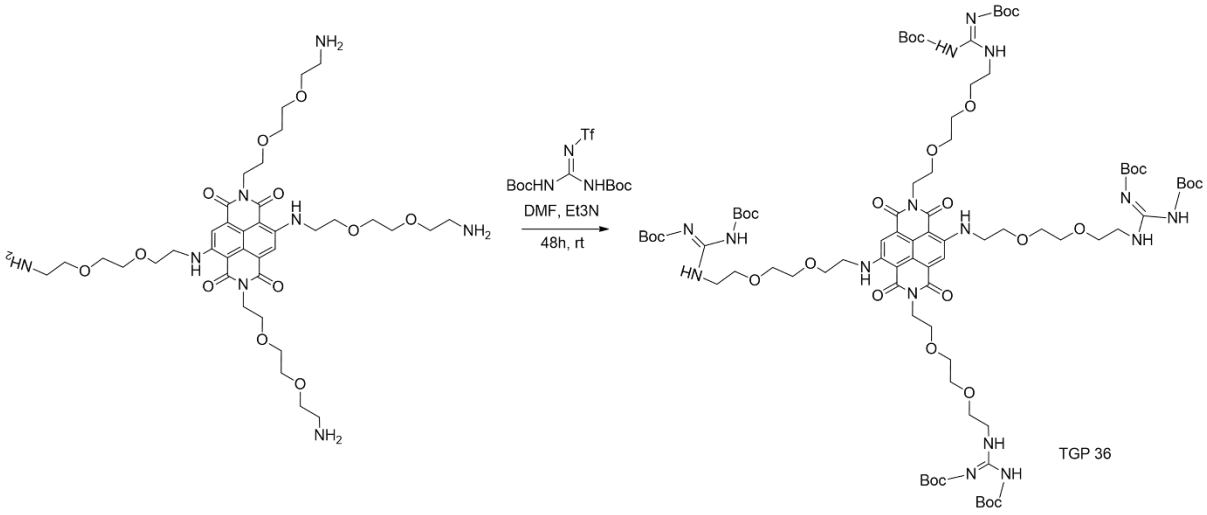
J = 5.1 Hz, 4H), 3.79 (t, J = 6.1 Hz, 4H), 3.72 –3.70 (m, 8H), 3.68 (s, 8H), 3.60 – 3.56 (m, 8H), 3.49 (t, J = 5.0 Hz, 4H), 3.33 (s, 4H), 3.28 – 3.23 (m, 4H), 1.41 (d, J = 6.5 Hz, 36H). ^{13}C NMR (151 MHz)

δ 166.0, 163.1, 156.0, 149.2, 125.8, 121.3, 118.5, 102.1, 82.9, 79.2, 77.3, 77.1, 76.8, 70.6, 70.4, 70.3, 70.3, 70.1, 69.6, 68.0, 42.9, 40.5, 39.1, 28.4. HRMS (ESI-TOF, $[M+H]^+$ calculated for $[C_{58}H_{93}N_8O_{20}]$ 1221.6501, found 1221.6453.



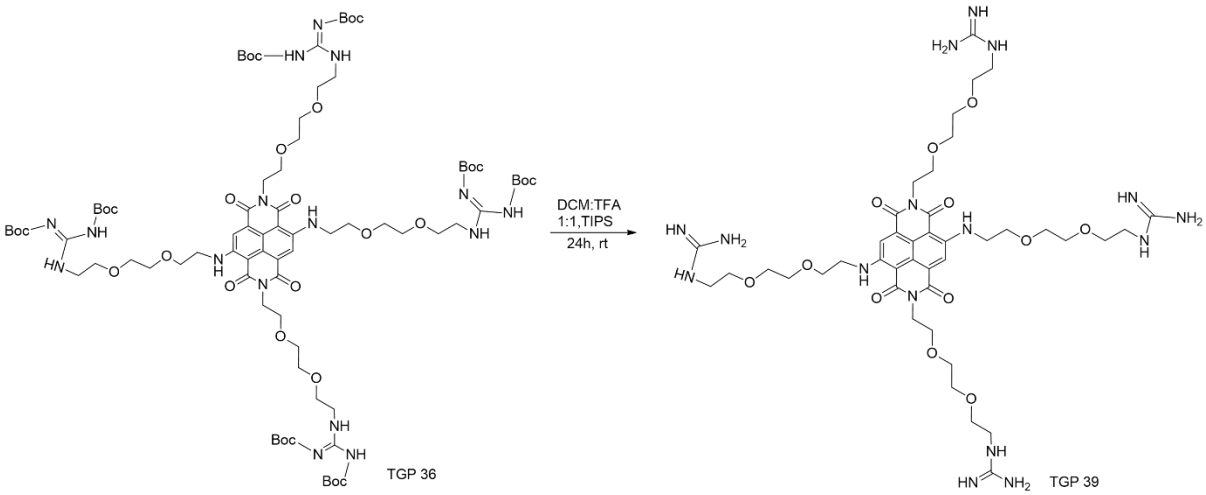
1.2.2 Synthesis of TGP 8. Into a 100 mL single-neck round-bottom flask containing a well-stirred solution of TGP-7 (5.1 g, 4.17 mmol, 1.0 equiv.) in dichloromethane (20 mL) were added trifluoroacetic acid (4 mL) and triisopropylsilane (TIPS, 0.4 mL). The reaction mixture was stirred at room temperature for 24 h. The progress of the reaction was monitored by LCMS after 24 h excess solvent was evaporated under reduced pressure yielded a gummy solid which was washed with diethyl ether (3x30 mL) and decanted to obtain a blue-coloured sticky solid. The crude compound was purified by reverse phase HPLC using acetonitrile: water (0.1% formic acid) system, the pure fractions were pooled together and frozen and lipolyzed to yield the desired compound **TGP 8** (2.2 g, 2.92 mmol, Yield 70%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.28 (d, J = 4.4 Hz, 2H), 8.39 (s, 2H), 7.78 (s, 1H), 4.18 (d, J = 6.2 Hz, 8H), 3.78 (t, J = 4.8 Hz, 4H), 3.71 – 3.66 (m, 4H), 3.66 – 3.58 (m, 24H), 3.53 (m, 4H), 3.48 (t, J = 5.4 Hz, 4H), 2.86 (t, J = 5.4 Hz, 4H), 2.79 (t, J = 5.4 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 165.0,

161.8, 158.6, 158.3, 148.2, 124.6, 120.0, 118.6, 117.4, 115.7, 112.7, 100.6, 69.7, 69.6, 69.5, 68.9, 66.9, 66.8, 42.1, 38.6, 38.5. HRMS (ESI-TOF, $[M + H]^+$) calculated for ($C_{38}H_{61}N_8O_{12}$) 821.4403 found 821.4380.



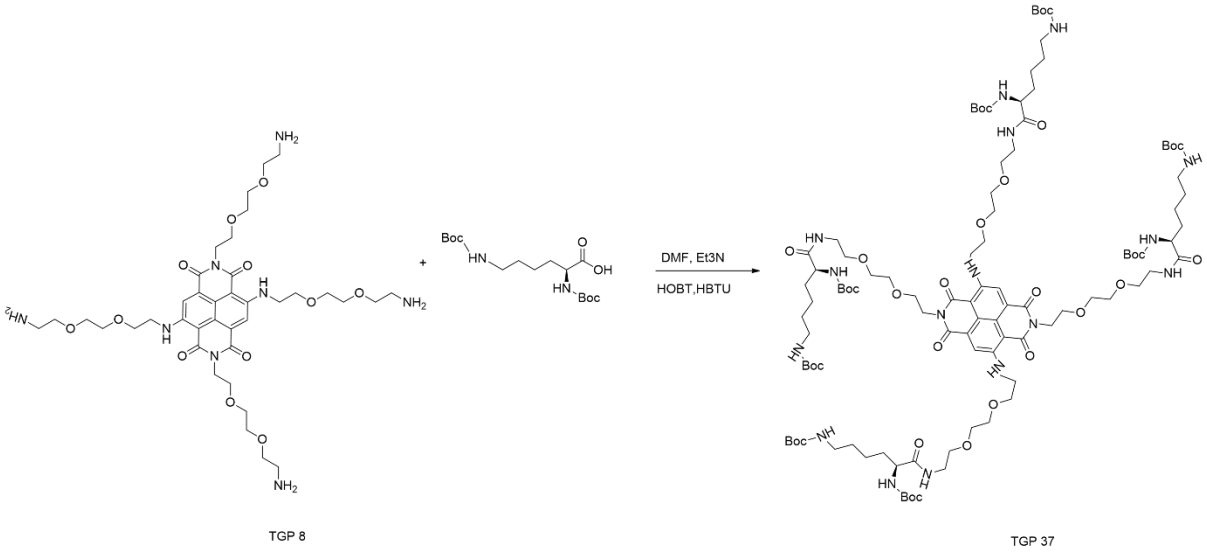
1.2.3 Synthesis of TGP 36. Into a 25 mL single-neck round-bottom flask containing a well-stirred solution of TGP-8 (0.40 g, 0.48 mmol, 1.0 equiv.) in N,N-dimethylformamide (2 mL) were added triethylamine (0.5 mL), followed by N,N′-di-Boc-N-triflylguanidine (1.1 g, 2.92 mmol, 6.0 equiv.). The reaction mixture was stirred at room temperature for 48 h. After 48 h excess solvent was evaporated under reduced pressure, the resultant sticky product was treated with water (10 mL) and extracted with 5% methanol in dichloromethane (3 x 30 mL). The organics were further washed with brine, the organic phases were dried under anhydrous sodium sulphate, filtered and the solvent was evaporated under reduced pressure. The crude compound was purified by column chromatography (silica gel 230-400 mesh) using 0-10% methanol in dichloromethane, while the desired product was eluted at 1-2% of methanol in dichloromethane. The pure fraction was concentrated under reduced pressure to obtain the desired product a blue-coloured sticky solid. **TGP 36** (0.25 g, 0.14 mmol, 29% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ *ppm*: 11.45 (s, 4H), 9.52 (t, 5.2Hz 2H), 8.61 (d, 13.9Hz, 4H), 8.14 (s, 2H), 4.43 (t, 6.1Hz, 4H), 3.85 (t, 5.2Hz, 4H), 3.79(t, 6.2Hz 4H), 3.73 (m, 4H), 3.69 (dd,

4.7Hz, 12H), 3.63 (m, 8H), 3.60 (m, 4H), (3.58, bs, 8H), 1.48 (s, 36H), 1.46 (s, 36H). ^{13}C NMR (100 MHz, $CDCl_3$) *δ ppm* 166.0, 163.5, 163.0, 156.2, 153.0, 152.9, 149.1, 125.7, 121.3, 118.4, 102.1, 82.9, 82.9, 79.2, 79.1, 70.6, 70.5, 70.4, 70.1, 69.6, 69.4, 69.3, 67.9, 42.9, 40.6, 40.6, 39.1, 29.7. HRMS (ESI-TOF, $[M+H]^+$) calculated for $[C_{82}H_{133}N_{16}O_{28}]^+$ 1789.9431, found 1789.9366



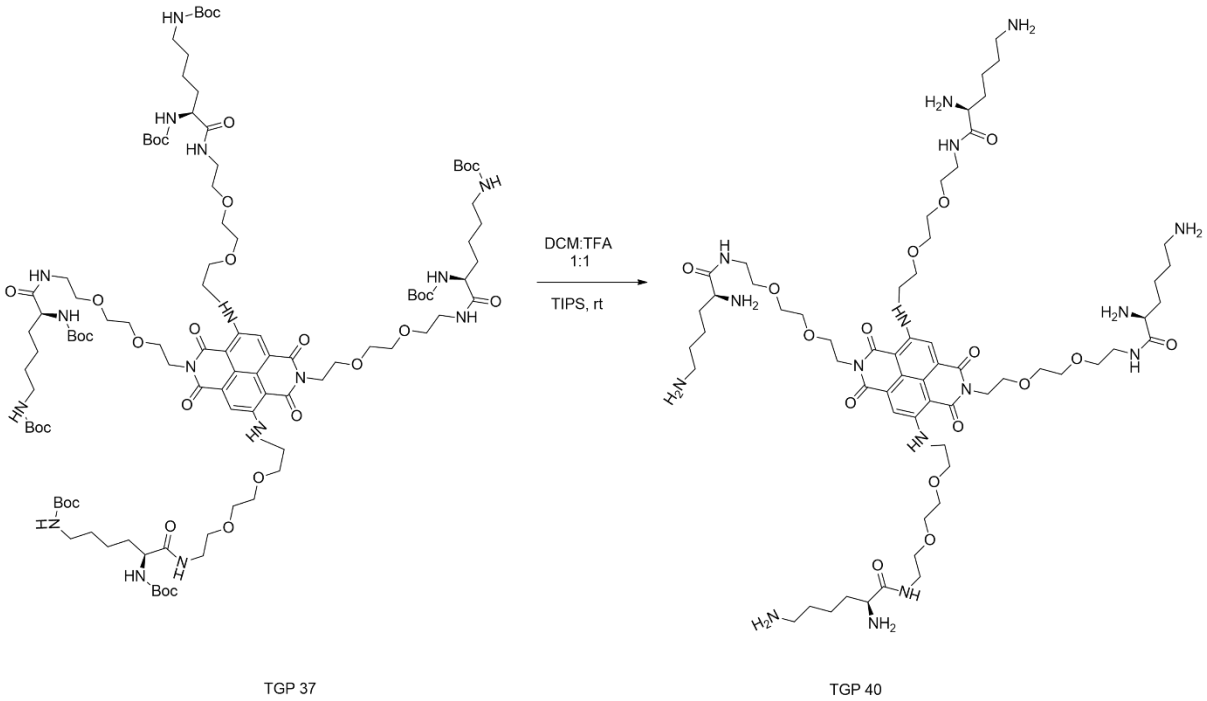
1.2.4 Synthesis of TGP 39. Into a 25 mL single-neck round-bottom flask containing a well-stirred solution of TGP-36 (0.25 g, 0.14 mmol, 1.0 equiv.) in dichloromethane (2 mL) were added trifluoroacetic acid (2 mL) and triisopropylsilane (TIPS, 0.2 mL). The reaction mixture was stirred at room temperature for 24 h, and the progress of the reaction was monitored by LC-MS. After 24 h, the excess solvent was removed under reduced pressure, and the resulting solid was washed with diethyl ether (3 × 10 mL) and decanted to afford a blue-colored sticky solid. The crude product was purified by reverse-phase HPLC using an acetonitrile/1% formic acid in water system as the mobile phase. The pure fractions were pooled, frozen, and lyophilized to yield the desired compound TGP-39 (50 mg, 0.05 mmol, Yield 35.7%). ^{1}H NMR (400 MHz, DMSO-d_6) δ *ppm*: 9.42 (d, *J* = 4.8 Hz, 2H), 8.10 (s, 2H), 7.58 (s, 2H), 7.54 (s, 2H), 7.36 (bs, 2H), 4.26 (t, *J* = 4.7 Hz, 4H), 3.76(t, *J* = 4.7 Hz,4H), 3.69 (d, *J* = 4.7 Hz, 4H), 3.64 (dd, *J* = 8.0 Hz, 8H), 3.58 (m, 10H), 3.52 (m, 10H) 3.46(m, 6H), 3.30 (m, 6H), 3.25 (m, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) *δ ppm* 166.0, 162.8, 157.0, 157.5, 149.1, 125.5, 121.1, 118.1

101.3, 69.8, 69.7, 69.6, 69.0, 68.6, 68.5, 66.7, 42.2, 40.9, 40.8. HRMS (ESI-TOF, $[M+H]^+$) calculated for $[C_{42}H_{69}N_{16}O_{12}]^+$ 989.5236 found 989.5212.



1.2.5 Synthesis of TGP 37. Into a 25 mL single-neck round-bottom flask containing a well-stirred solution of Boc-Lys(Boc)-OH (6) (0.5 g, 1.4 mmol, 6.0 equiv.) in N,N-dimethylformamide (1 mL) were added HBTU (0.92 g, 2.4 mmol, 10.0 equiv.) and N,N-diisopropylethylamine (DIPEA, 0.85 mL), and the mixture was stirred at room temperature for 15 min. HOBt (0.16 g, 1.2 mmol, 5.0 equiv.) was then added, and the reaction was stirred for an additional 15 min. TGP-8 (0.20 g, 0.24 mmol, 1.0 equiv.) was dissolved in N,N-dimethylformamide (1 mL) with N,N-diisopropylethylamine (0.5 mL) and added to the activated Boc-Lys(Boc)-OH solution. The reaction mixture was stirred at room temperature for 24 h. After completion, the solvent was removed under reduced pressure, and the resulting gum was treated with water (10 mL) and extracted with 3% methanol in dichloromethane (3 × 30 mL). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (230–400 mesh) using 0–10% methanol in dichloromethane as the eluent. The desired product eluted at 1–2% methanol in

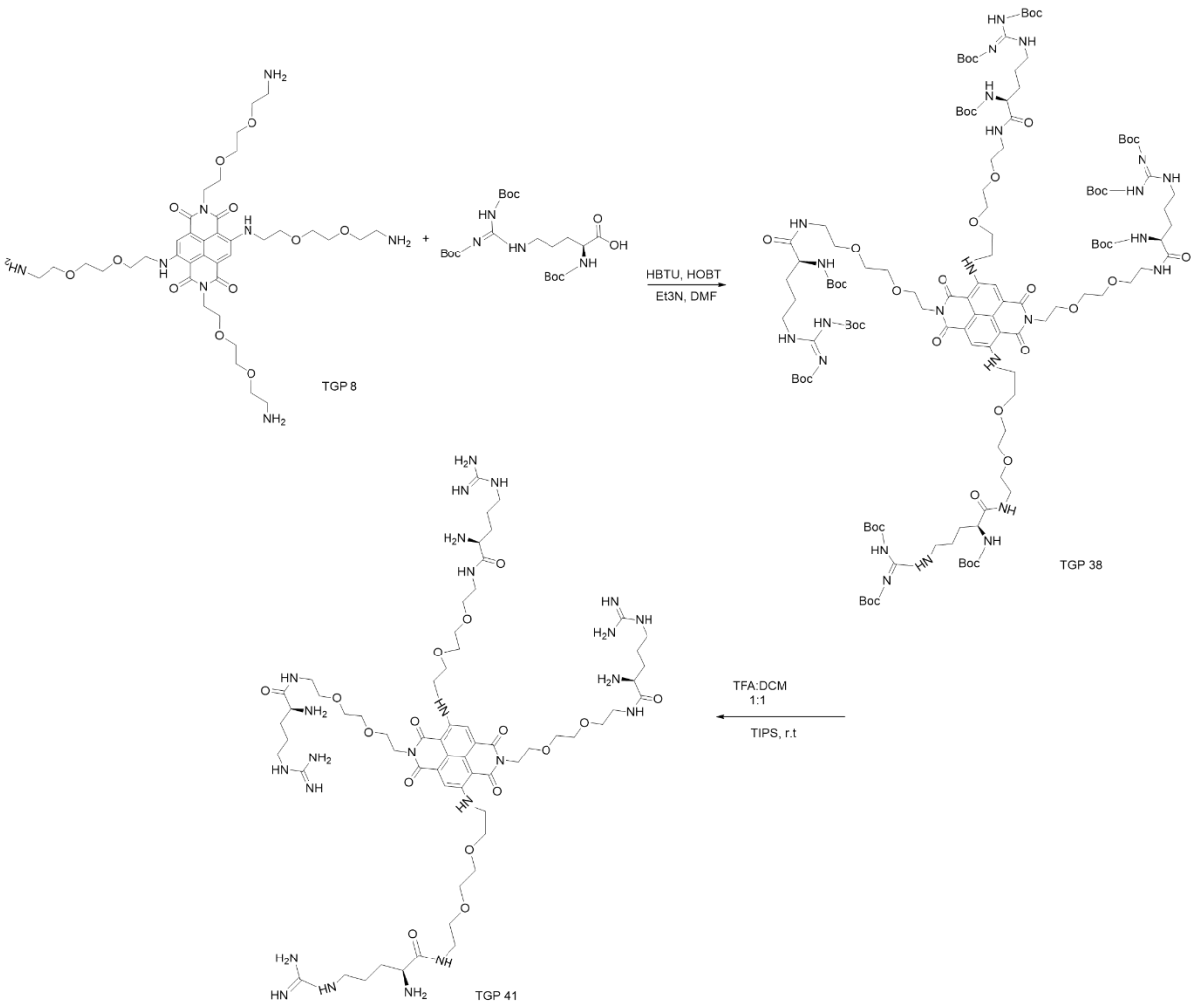
dichloromethane. Concentration of the pure fractions under reduced pressure afforded TGP-37 as a blue-colored sticky solid (0.18 g, 0.084 mmol, Yield 35.1%). ^{1}H NMR (400 MHz, $CDCl_3$) δ *ppm* δ 9.48 (s, 2H), 8.09 (s, 2H), 7.08 – 6.90 (m, 4H), 5.41 – 5.3 (m, 4H), 4.78 (s, 4H), 4.40 (dd, *J* = 10.9, 5.9 Hz, 4H), 4.04 (t, *J* = 30.4 Hz, 4H), 3.82 (t, *J* = 5.1 Hz, 4H), 3.69 (d, *J* = 5.0 Hz, 4H), 3.65 (dd, *J* = 5.5, 2.5 Hz, 18H), 3.56 (dt, *J* = 9.1, 4.4 Hz, 10H), 3.51 – 3.44 (m, 10H), 3.41 – 3.33 (m, 6H), 3.06-3.0 (s, 8H), 2.33-2.28 (m, 4H), 1.77-1.60 (m, 3H), 1.69 – 1.53 (m, 3H), 1.52 – 1.43 (m, 8H), 1.44 – 1.30 (m, 72H), 0.86 – 0.80 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) *δ ppm* δ 172.3, 166.0, 163.0, 156.1, 155.6, 149.1, 125.6, 121.2, 118.4, 102.04, 79.6, 79.0, 70.5, 70.2, 70.1, 69.7, 69.7, 69.5, 67.9, 54.3, 49.4, 42.8, 40.1, 39.3, 39.2, 32.6, 32.5, 30.6, 30.3, 29.6, 22.6, 17.6 HRMS (ESI-TOF, $[M+H]^+$) calculated for $(C_{102}H_{172}N_{16}O_{32})^+$ 2134.2396 found 2034.1747(1-Boc cleaved).



TGP 37 TGP 40

1.2.6 Synthesis of TGP 40. Into a 25 mL single-neck round-bottom flask containing a well-stirred solution of TGP-Lys-Boc (TGP-37) (0.18 g, 0.08 mmol, 1.0 equiv.) in dichloromethane (2 mL) were added trifluoroacetic acid (2 mL) and triisopropylsilane (TIPS, 0.2 mL). The reaction mixture was stirred at room temperature for 24 h, and the progress of the reaction was

monitored by LC-MS. After 24 h, the excess solvent was removed under reduced pressure, and the resulting solid was washed with diethyl ether (3 × 10 mL) and decanted to obtain a crude blue-colored sticky solid. The crude residue was purified by reverse-phase HPLC using an acetonitrile/1% formic acid in water system as the mobile phase. The pure fractions were pooled, frozen, and lyophilized to yield the desired compound TGP-40 (42 mg, 0.03 mmol, Yield 38.7%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.35 (s, 2H), 8.38 (s, 4H), 8.13 (d, J = 5.1 Hz, 4H), 7.89 (s, 2H), 4.23 (s, 6H), 3.79 (s, 4H), 3.65 (s, 12H), 3.60-3.58 (m, 8H), 3.51-3.47 (m, 10H), 3.39 (s, 4H), 3.24- 3.19 (m, 14H), 2.73 (s, 8H), 1.52 (bs, 15H), 1.45 – 1.25 (m, 15H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.5, 165.6, 165.1, 161.9, 158.3, 148.3, 124.8, 120.2, 118.7, 115.7, 100.8, 69.8, 69.6, 69.5, 69.0, 68.9, 66.9, 53.3, 42.2, 38.4, 32.7, 26.7, 21.7. HRMS (ESI-TOF, $[M+H]^+$) calculated for $(C_{62}H_{111}N_{16}O_{16})^+$ 1335.8348 found 1335.7824. MALDI (TOF) calculated for $C_{62}H_{108}N_{16}O_{16}$ $[M+Na+H]^+$ 1356.809, observed 1356.043



1.2.7 Synthesis of TGP 41. Into a 25 mL single-neck round-bottom flask containing a well-stirred solution of Boc-Arg(Boc)$_2$-OH (0.68 g, 1.44 mmol, 6.0 equiv.) in N,N-dimethylformamide (1 mL) were added HBTU (0.92 g, 2.4 mmol, 10.0 equiv.) and N,N-diisopropylethylamine (DIPEA, 0.85 mL), and the mixture was stirred at room temperature for 15 min. HOBt (0.16 g, 1.2 mmol, 5.0 equiv.) was then added, and the reaction was stirred for an additional 15 min. TGP-8 (0.20 g, 0.24 mmol, 1.0 equiv.) was dissolved in N,N-dimethylformamide (1 mL) with N,N-diisopropylethylamine (0.5 mL) and added to the activated Boc-Arg(Boc)$_2$-OH solution. The reaction mixture was stirred at room temperature for 24 h. After 24 h, the solvent was removed under reduced pressure, and the resulting gum was treated with water (10 mL) and extracted with 5% methanol in dichloromethane (3 × 30 mL). The combined organic layers were washed with brine, dried over anhydrous sodium

sulfate, filtered, and concentrated under reduced pressure to give a crude residue. The crude compound was used directly in the next reaction without further purification. The Boc-protected compound was deprotected by dissolving it in dichloromethane (2 mL), followed by the addition of trifluoroacetic acid (2 mL) and triisopropylsilane (TIPS, 0.2 mL). The reaction mixture was stirred at room temperature for 24 h, and the progress of the reaction was monitored by LC-MS. After 24 h, the excess solvent was removed under reduced pressure, and the resulting solid was washed with diethyl ether (3 × 10 mL) and decanted to obtain a crude blue-colored sticky solid. The crude residue was purified by reverse-phase HPLC using an acetonitrile/1% formic acid in water system as the mobile phase. The pure fractions were pooled, frozen, and lyophilized to yield the desired compound TGP-41 (50 mg, 0.029 mmol, Yield 12.1%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.43 (s, 2H), 8.38 (s, 6H), 8.07 (s, 2H), 8.04 – 8.00 (m, 4H), 4.26 (s, 4H), 3.76 (d, J = 4.8 Hz, 4H), 3.69 (d, J = 4.9 Hz, 4H), 3.65 (t, J = 6.5 Hz, 6H), 3.64 – 3.62 (m, 6H), 3.57 (dd, J = 8.1, 4.9 Hz, 10H), 3.50 – 3.48 (m, 8H), 3.23 (d, J = 5.4 Hz, 14H), 3.19 – 3.15 (m, 7H), 3.15 – 3.11 (m, 7H), 3.03 (t, J = 6.7 Hz, 10H), 1.57 (s, 6H), 1.48 (dd, J = 22.2, 15.3, 7.2 Hz, 11H), 1.35 (dd, J = 12.2, 9.4 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm δ 13C NMR (151 MHz) δ 173.3, 166.8, 165.3, 162.1, 157.2, 148.4, 125.0, 120.3, 69.9, 69.7, 69.6, 69.1, 69.1, 69.0, 67.0, 53.5, 42.3, 40.4, 40.0, 39.9, 39.7, 39.6, 39.5, 39.3, 39.2, 39.1, 38.9, 38.7, 38.6, 38.5, 30.9, 24.7, 23.8. HRMS (ESI-TOF, $[M+3H]^{+}$) calculated for $(C_{62}H_{109}N_{24}O_{16})^{+}$ 1445.8448 found 1445.8072.

1.3 UV-vis absorption spectroscopy

All measurements were conducted in Tris.HCl buffer (10 mM, pH 7.4) at room temperature. Stock solutions of TGP-8 and TGP-39-41 (10 mM) were prepared in water and used for subsequent experiments. The absorption and fluorescence spectra of the TGPs were recorded in Tris.HCl buffer (10 mM, pH 7.4).

1.4 Fluorescence dye displacement assay

1.4.1 EtBr displacement assay Fluorescence intensity of EtBr bound to pDNA emitted at 605 nm was monitored using Agilent Cary Eclipse fluorescence spectrophotometer with a slit width of excitation and emission set at 10 nm. The apparent binding constants (K_{app}) of the TGPs ligand were calculated using the following equation:

$K_{app}[C_{50}$ of ligand$] = K_{EtBr}$[EtBr]

Where [EtBr] is the concentration of EtBr used (5μM) and [C_{50} of ligand] is the concentration of ligand that caused a 50% reduction in the EtBr fluorescence. $K_{EtBr} = 9.5\times10^{6}$ M^{-1}.

1.4.2 Hoechst displacement assay Fluorescence intensity of Hoechst bound pDNA emit at 450 nm upon excitation with 350 nm as recorded by Agilent Cary Eclipse fluorescence spectrophotometer with a slit width of 10 nm for excitation and emission. The K_{app} of the ligand was calculated using the following equation:

$K_{app}[C_{50}$ of ligand$] = K_{Hoechst}$[Hoechst]

Where [Hoechst] is the Hoechst concentration used (5 μM) and [C_{50} of ligand] is the concentration of the TGPs that caused a 50% reduction in Hoechst fluorescence intensity. $K_{Hoechst} = 4.75 \times 10^{7}$ M^{-1}

1.5 AFM studies

Samples were dropcasted on freshly cleaved mica discs (Agar scientific), and incubated for 15 min under room temperature, washed with DI water thrice for 5 min and dried for 1 h at 37 °C. For imaging with AFM, we used a SCANASYST-AIR probe with a tip radius of 5 nm and spring constant of 0.4N/m sample at 1 Hz with 256 samples/line. Peak force Quantitative Nanomechanical Mapping in the air (PGQNM) was utilized for sample scanning and Nanoscope Analysis 1.8 software was utilized for processing.

1.6 Nanocondensate formation

pDNA (100-150 ng) was dissolved in Tris.HCl buffer (10 mM, pH 7.4) and subsequently treated concentrations of TGP 8, 39-41 (10-40 μM) and incubated for 30 min. Size distribution and zeta potential measurement were performed using Malvern Zeta Nanosizer.

1.7 CD measurement

The characteristic CD spectra were recorded using Jasco J-815 spectropolarimeter, with a path length of 10 mm. ctDNA (100 μM) independently and upon addition of varying concentrations of TGP 39-41 (25-150 μM) were used in Tris.HCl buffer (10 mM, pH 7.4) for the measurement of CD spectra.

1.8 DNA melting Studies

DNA melting experiments were performed by monitoring the UV absorbance at 260 nm as a function of temperature using a temperature-controlled spectrophotometer. ctDNA (100 μM) and PD-NC were prepared at identical DNA concentrations, with PD added at increasing molar ratios (1:1, 1:3, and 1:5). Samples were heated from 25 to 90 °C at a controlled ramp rate, allowing thermal equilibration at each temperature point. The melting temperature (T_m) was determined from the inflection point of the normalized absorbance (ΔA_{260}) versus temperature plots. All measurements were performed under identical buffer conditions to ensure comparability.

1.9 Agarose gel electrophoresis

Electrophoretic mobility shift assay (EMSA) was performed to understand the interaction between DNA and dendrimers. pDNA (150 ng) was incubated with varying concentrations of TGP 8, 39-41 (1-40 μM) in Tris.HCl buffer (10 mM, pH 7.4). Prior to loading, the mixtures were incubated at room temperature for 30 min to form complexes. These mixtures were treated with 2.5 μL of loading dye (20% glycerol, 25 mM EDTA, 0.05% bromophenol blue and xylene cyanol) and loaded into the gel with a run time of 45 minutes and 90 V.

1.10 Cellular studies

1.10.1 Cell culture

HEK293-T and N9 cells were cultured using DMEM and RPMI with 10% fetal bovine serum (FBS) and 1% pen-strep (PS) or 1% Anti-Anti under the ambient cell growing conditions of 37 °C and 5% CO_2 atmosphere.

1.10.2 Cellular toxicity study

The HEK-293T cells were seeded in 96 well plates with a density of 25,000 cells per well and were incubated for 24 h. After that the media was changed, and the cells were treated with TGP 39-41 and further incubated for 24 h in the ambient cell growing conditions. Post 24 h, 10 µL of MTT (5 mg/mL) was added to the cells and incubated for an additional 3 h followed by media replacement with 100 µL MeOH: DMSO (1:1) to dissolve the formazan crystals. The absorbance was measured at 570 nm with the background corrections at 640 nm.

1.10.3 Cellular transfection experiment

Cellular transfection experiments were performed to assess the ability of the nanocondensates to deliver GFP plasmid. DNA: TGP 39-41 complexes were prepared by adding varying concentrations of TGP 39-41 (25 to 150 µM) to EGFP DNA (150 ng) in OptiMEM media (50 µL), vortexed gently to mix the components and allowed to equilibrate for 30 min. After 30 min Ca^{2+} (2 mM) was added to the dendriplex and incubated further for 10 min. Prior to the treatment of the nanocondensate, the media was replaced with that containing condensate Ca^{2+} composite and incubated for 16 h, post which DMEM with 10% FBS and 1% PS were added. The cells were incubated for an additional 48 h and imaged for the expression of EGFP at different regions.

1.10.4 Flow cytometry

HEK-293T cells were seeded in a 24-well culture plate (150,000 cells/well) followed by incubation for 30 h at 37 °C under optimum cell culture conditions. Post 30 h, the media was

replaced with 2.5% FBS DMEMF-12, with or without the inhibitors such as sucrose (0.45 M), amiloride (50 µM), chloroquine (100 µM), methyl-β-cyclodextrin (5 mM), or colchicine (10 µM) for 30 min. Following this, TGP41 NC and TGP41NC+ Ca^{2+} formulation was added to each well, and the cells were incubated for an additional 6 h. Post incubation, the cells were gently washed with PBS thrice to remove free TGP41 NC. The cells were then trypsinized and centrifuged at 1000 rpm for 5 min at 4 °C. The resulting cell pellets were resuspended in 300 µL PBS and used for flow cytometry analysis.

2. Results

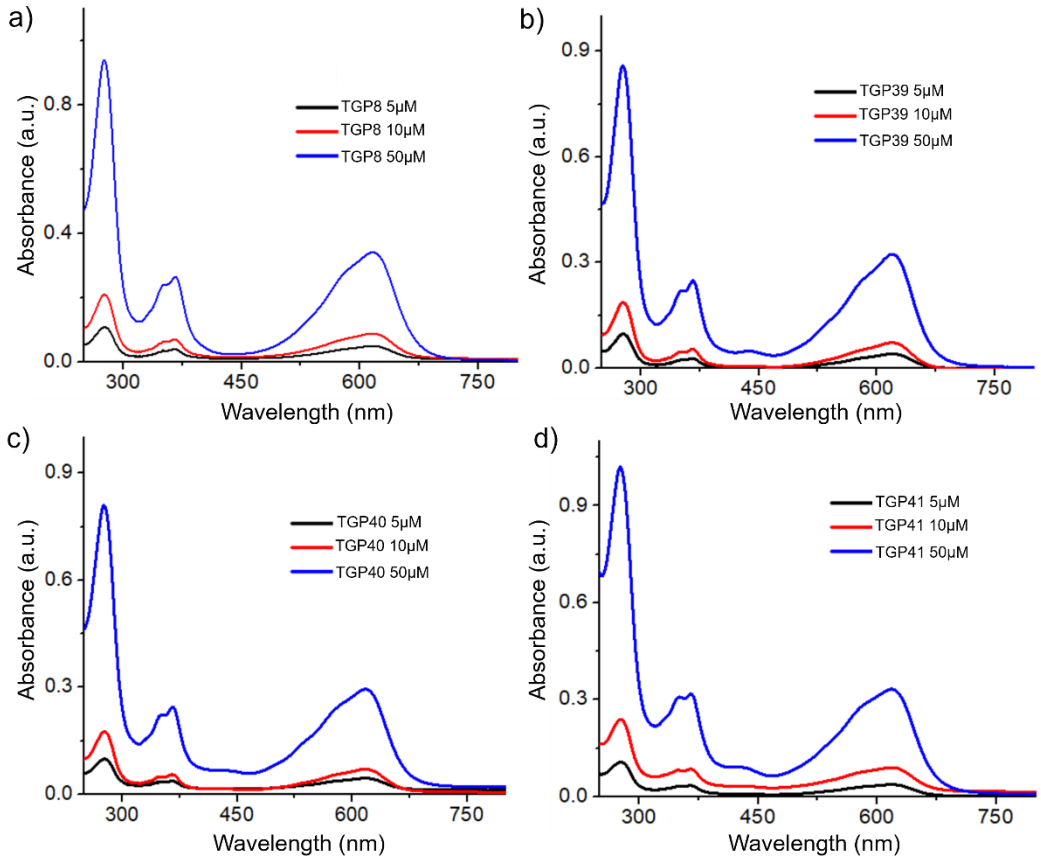


Fig. S1 Absorbance of a) TGP 8, b) TGP 39, c) TGP 40 and d) TGP 41 in PBS (10 mM, pH 7.4).

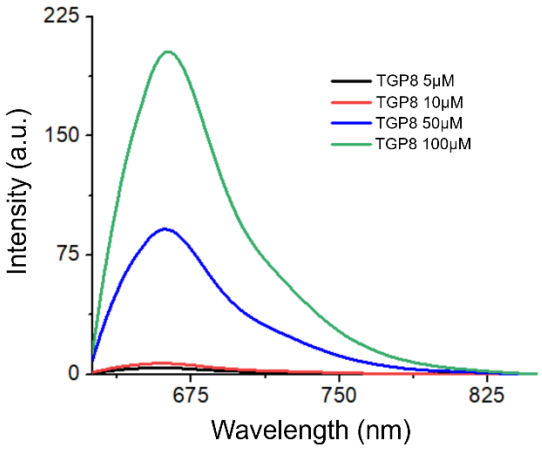


Fig. S2 Fluorescence of TGP 8 in PBS (10 mM, pH 7.4).

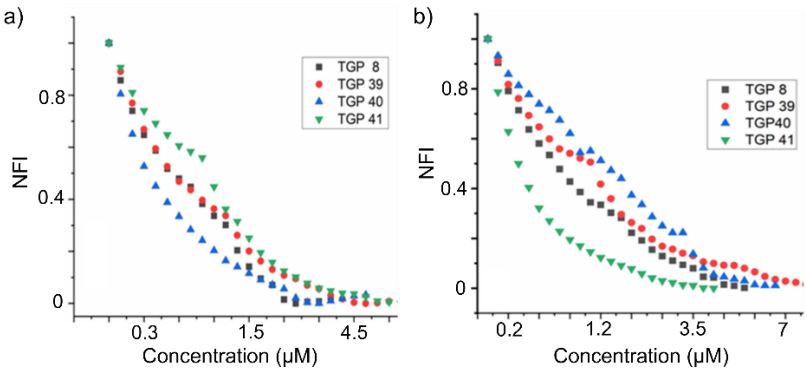


Fig. S3 DNA binding assay for TGP 8, 39-41 monitored by A) EtBr and B) Hoechst displacement.

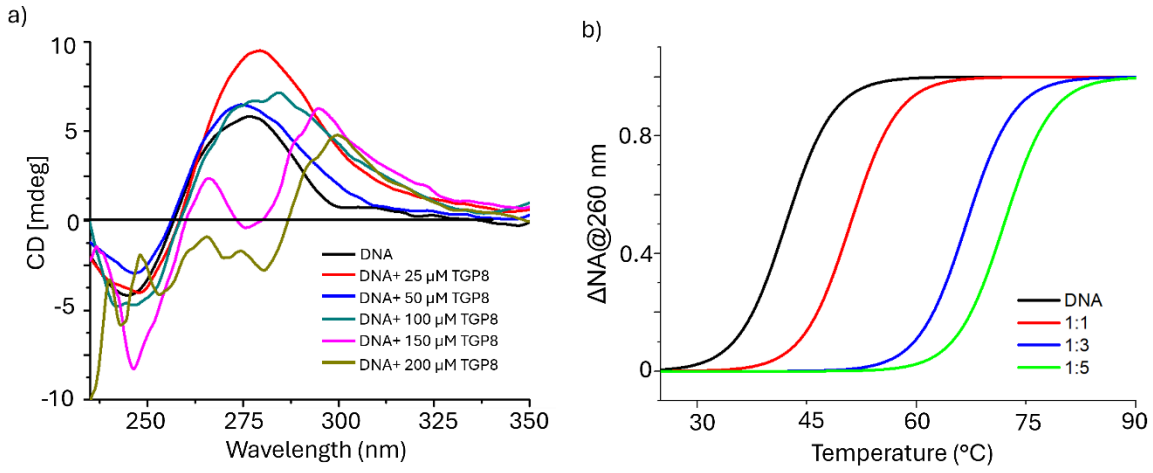


Fig S4. TGP8 NC formation as assessed with a) CD analysis and b) thermal melt curve.

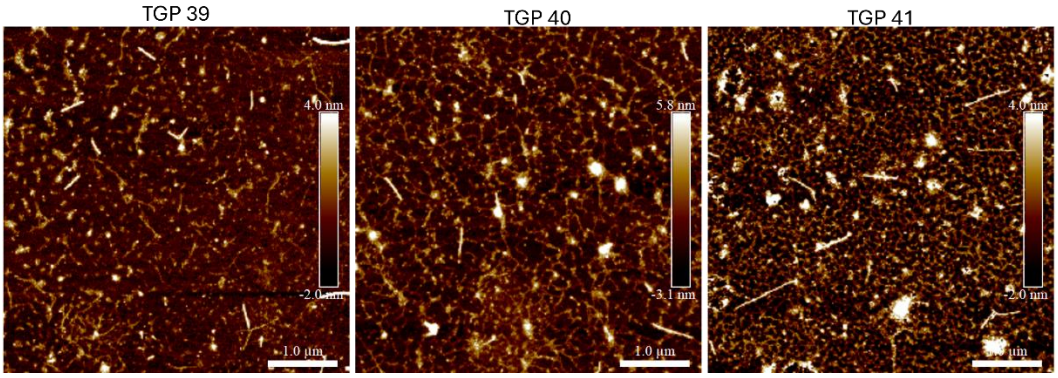


Fig S5. Atomic force microscopy analysis of PDs.

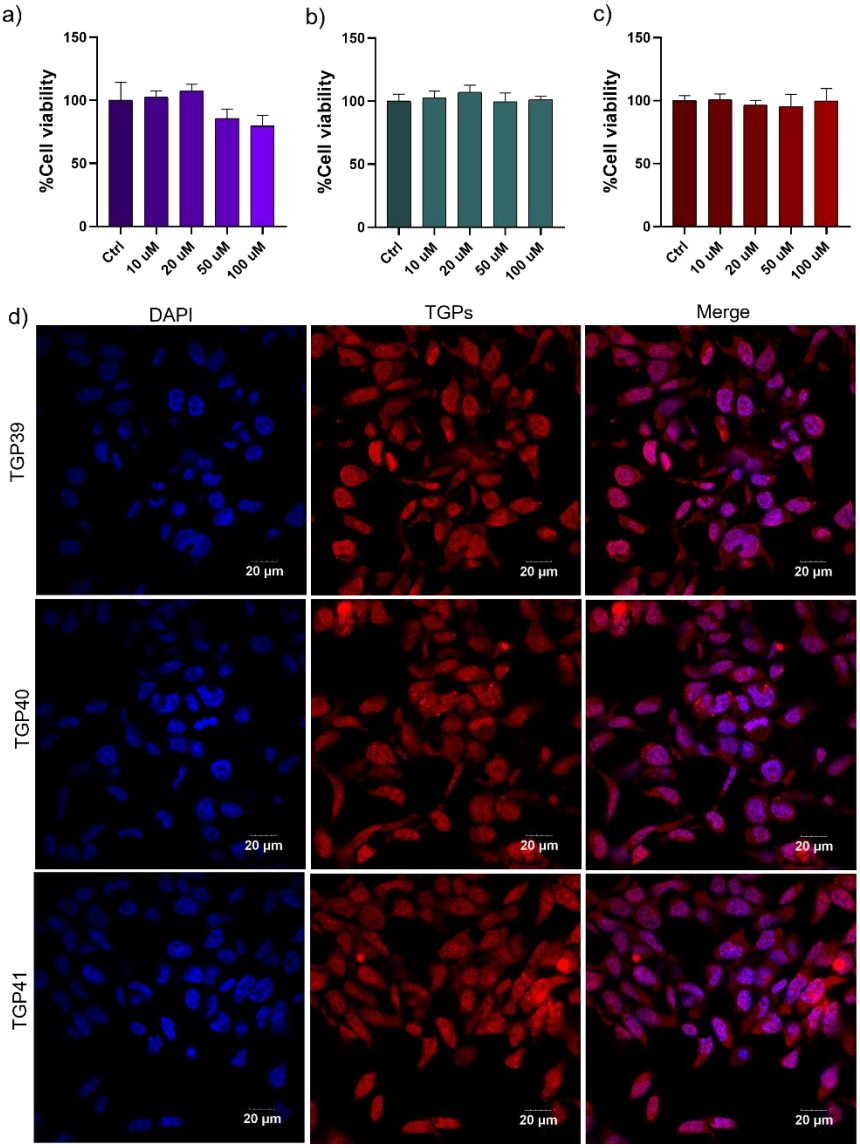


Fig. S6 Cell viability assessment using MTT assay for a) TGP 39, b) TGP 40 and c) TGP 41 in HEK-293T cells and their d) cellular uptake study.

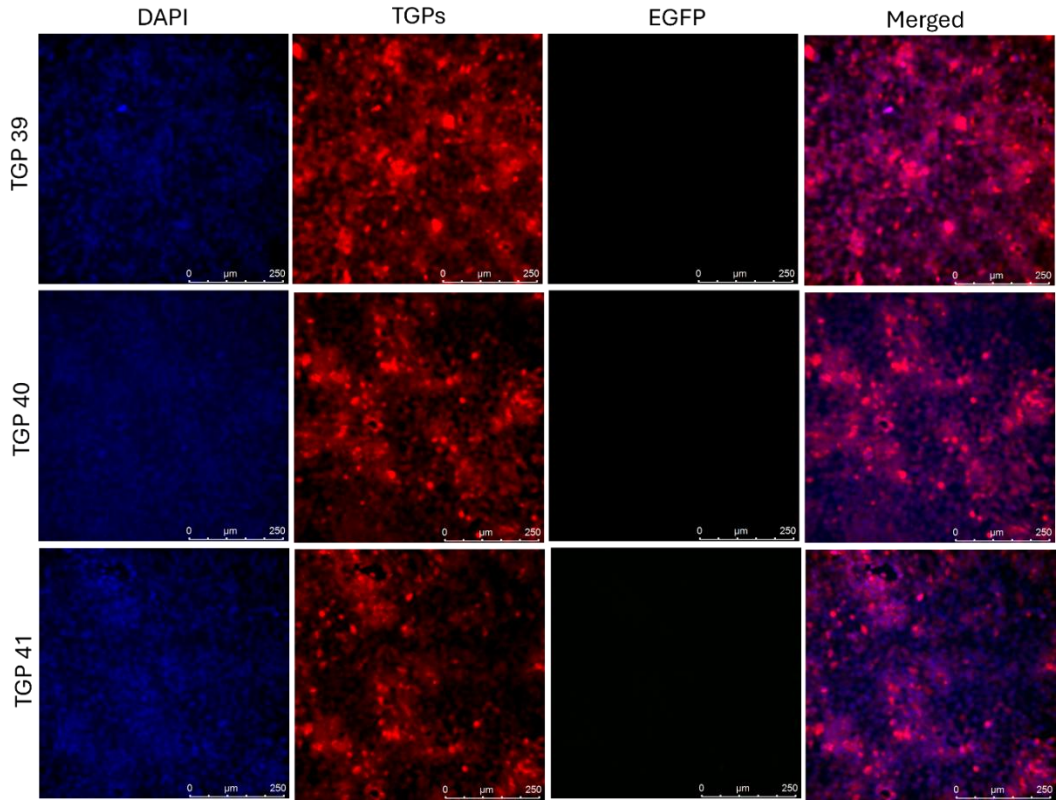


Fig. S7 Transfection experiment TGP39-41 with EGFP plasmid DNA.

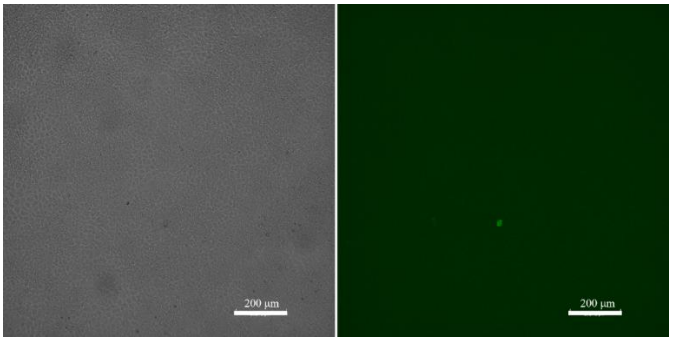


Fig. S8 EGFP plasmid transfection experiments carried out in only in the presence of Ca^{2+} (2 mM).

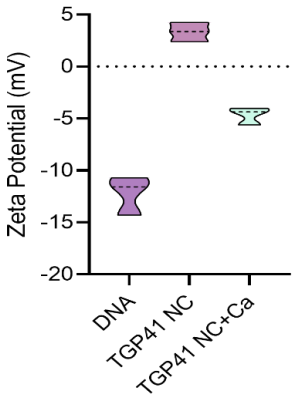


Fig. S9 Zeta potential of DNA and NC alongside its Ca^{2+} formulation.

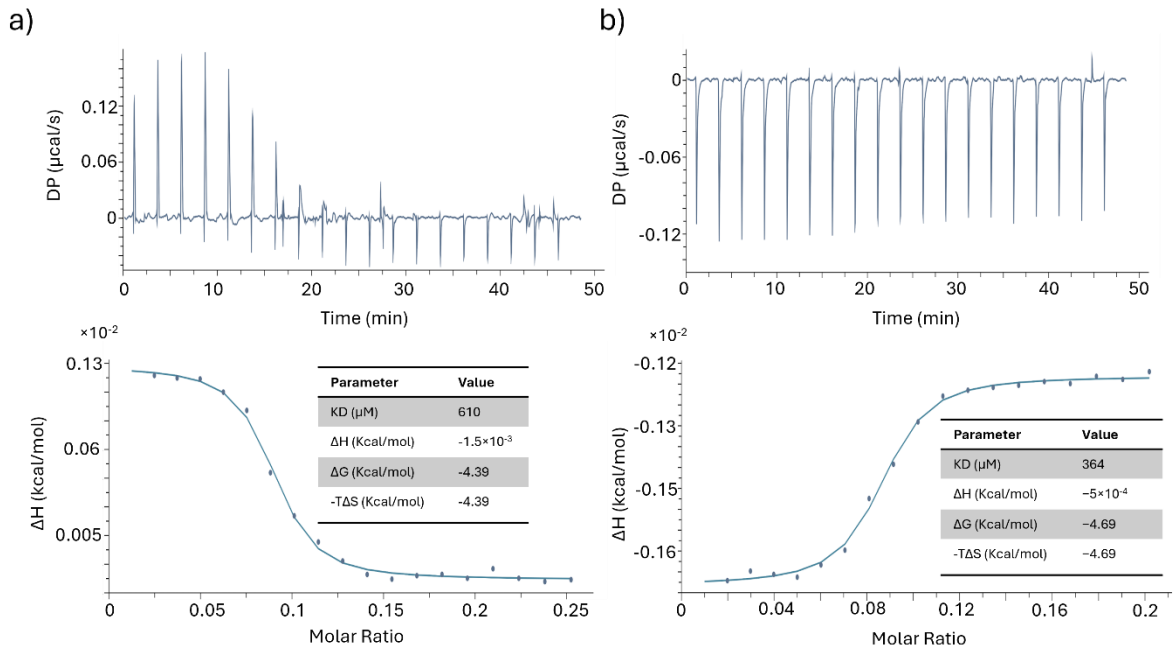


Fig. S10. ITC analysis of a) TGP 39 and b)TGP 40 in NC formation.

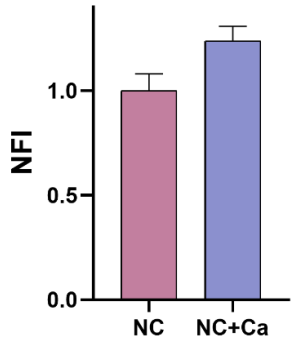
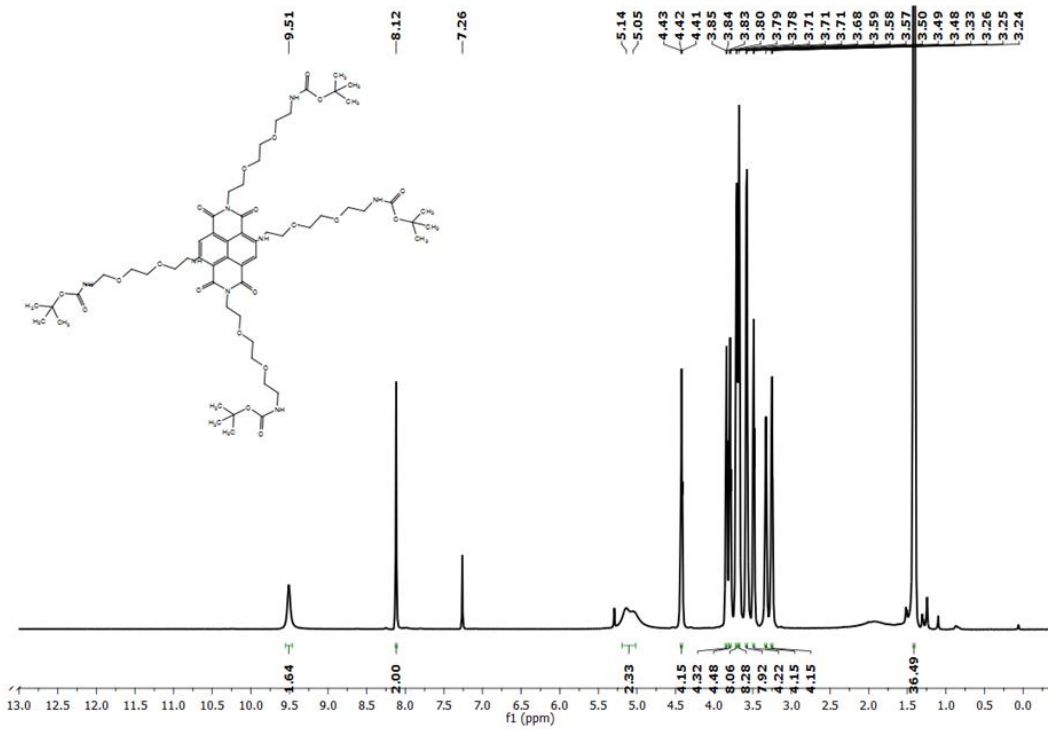


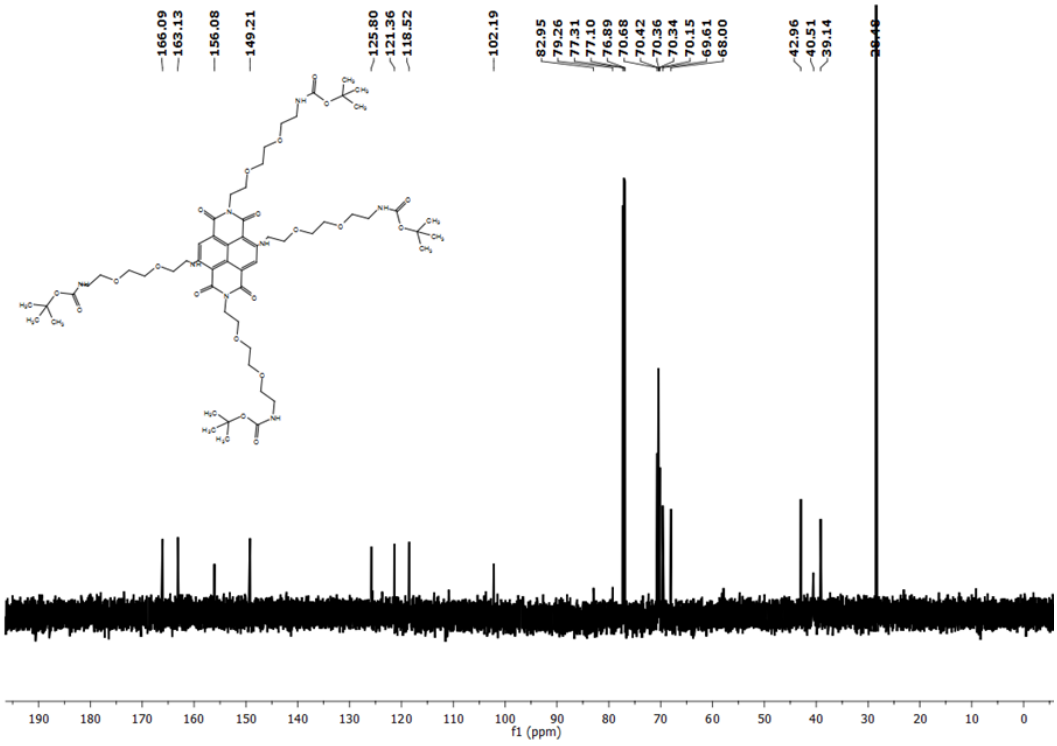
Fig. S11 Cellular uptake of NC and NC+ Ca indicating higher uptake of NC+Ca^{2+} compared to pristine NC.

Characterization data

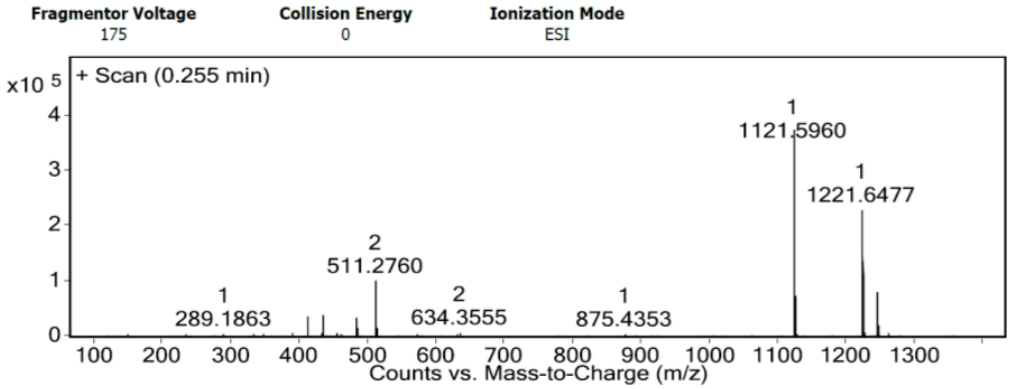
^{1}H NMR spectrum of TGP 7 in $CDCl_3$



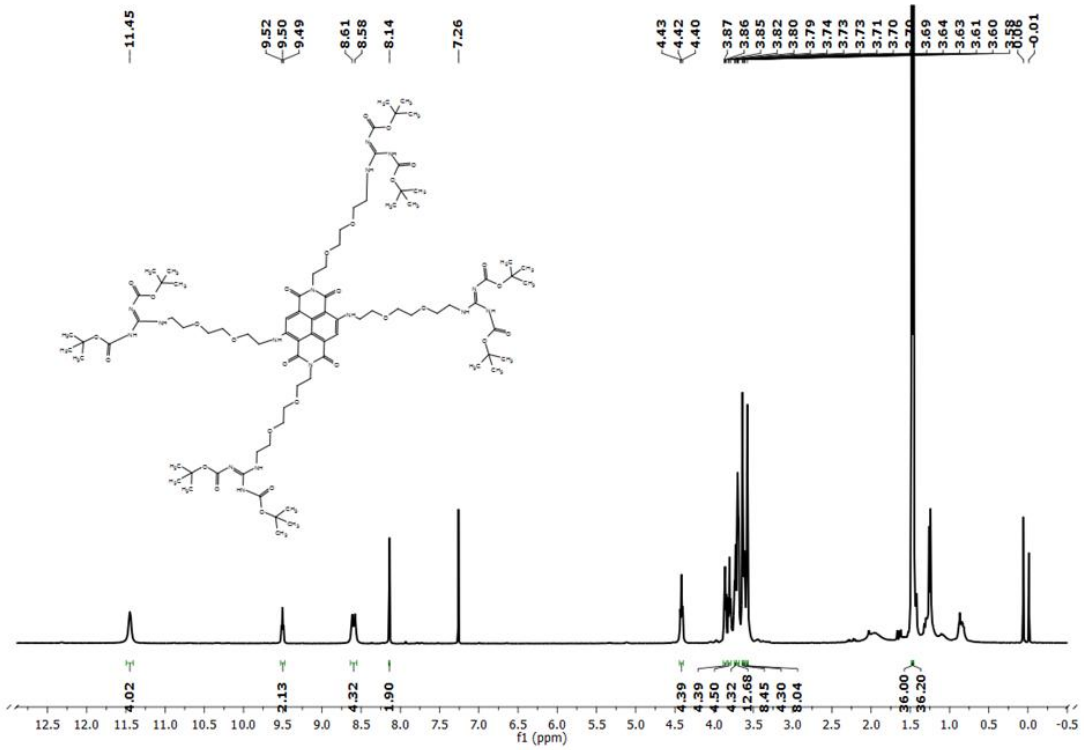
^{13}C NMR spectrum of TGP 7 in $CDCl_3$



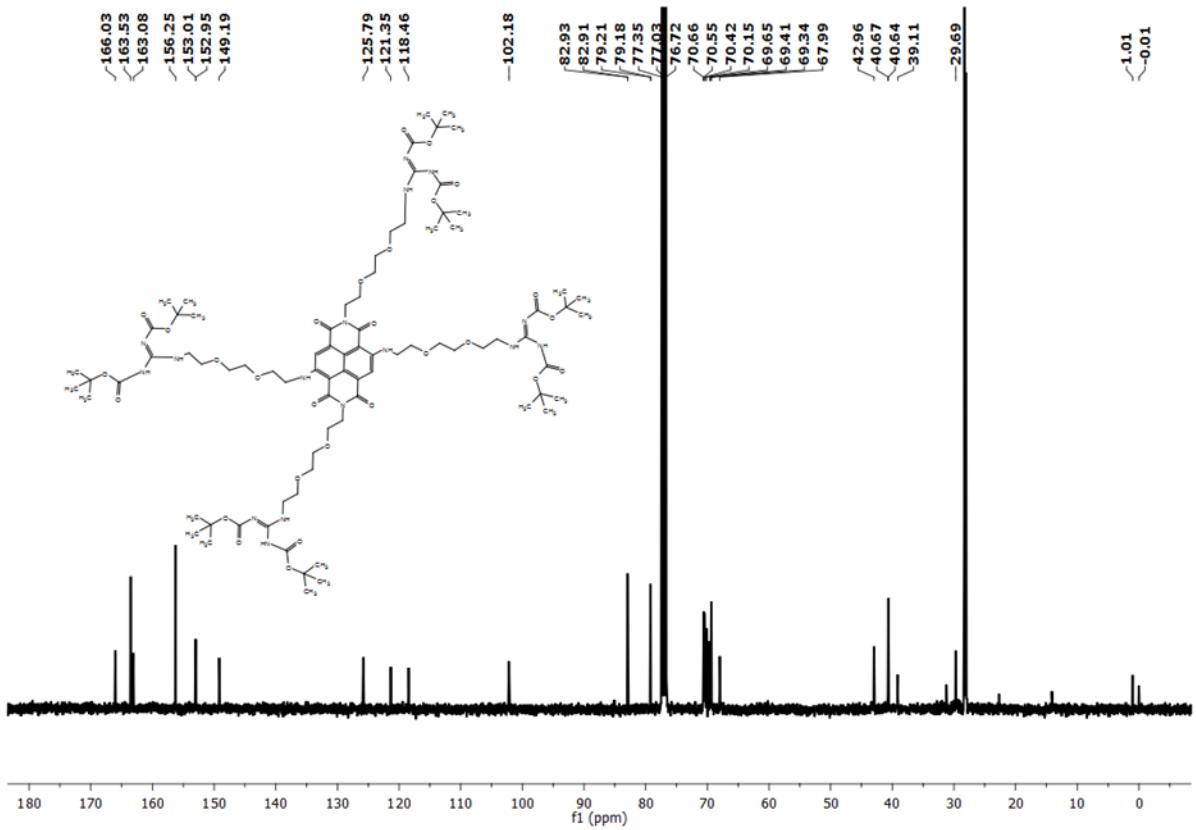
HRMS characterization of TGP 7



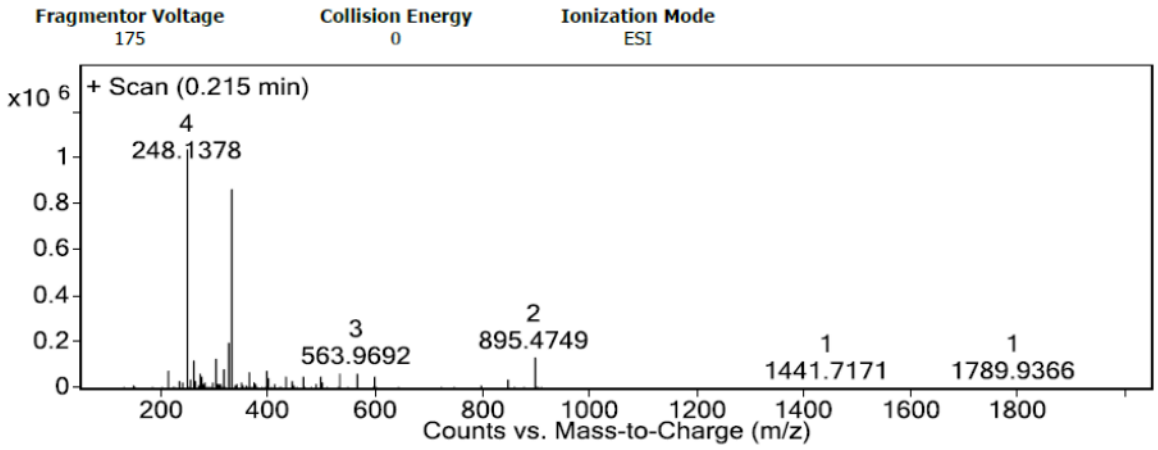
^{1}H NMR spectrum of TGP 36 in $CDCl_3$



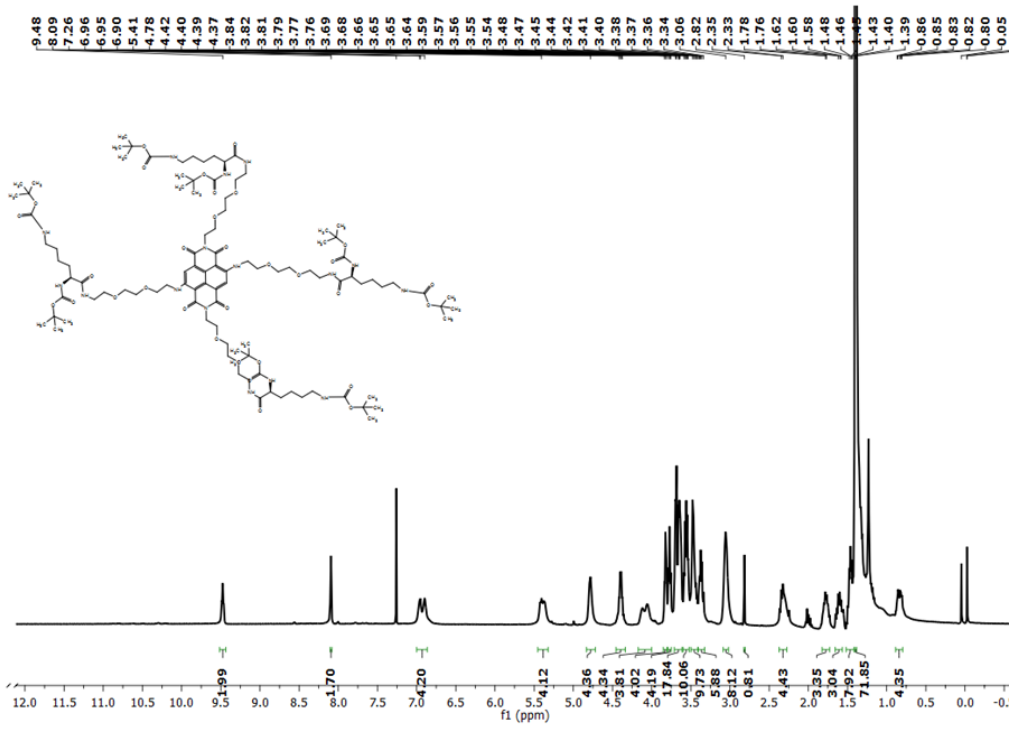
^{13}C NMR spectrum of TGP 36 in $CDCl_3$



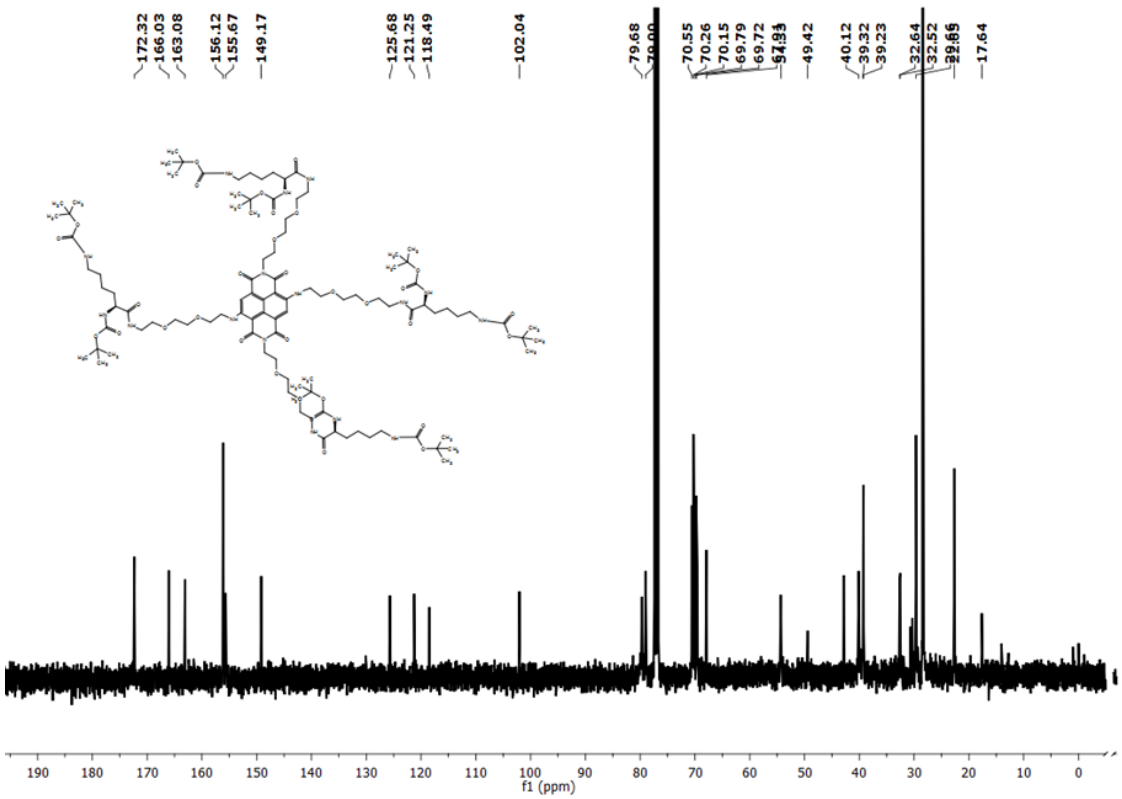
HRMS characterization of TGP 36



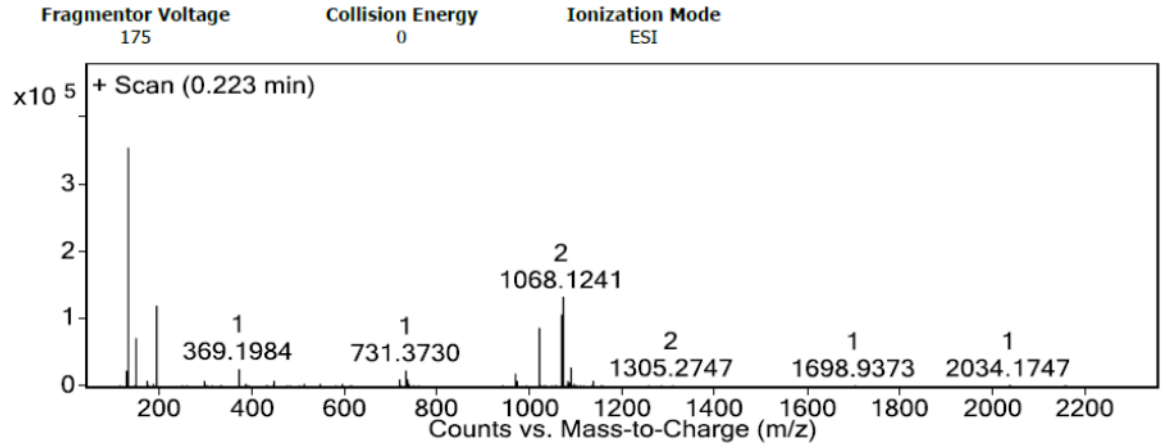
^{1}H NMR spectrum of TGP 37 in $CDCl_3$



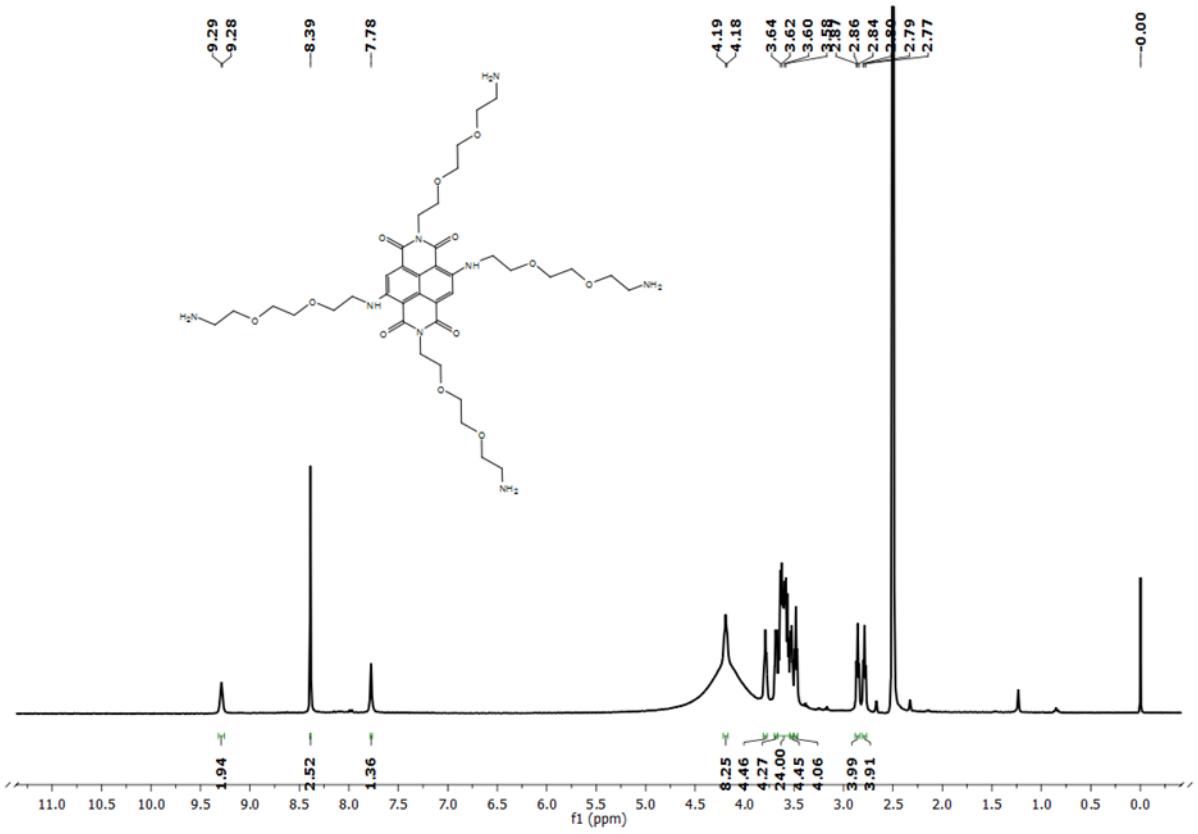
^{13}C NMR spectrum of TGP 37 in $CDCl_3$



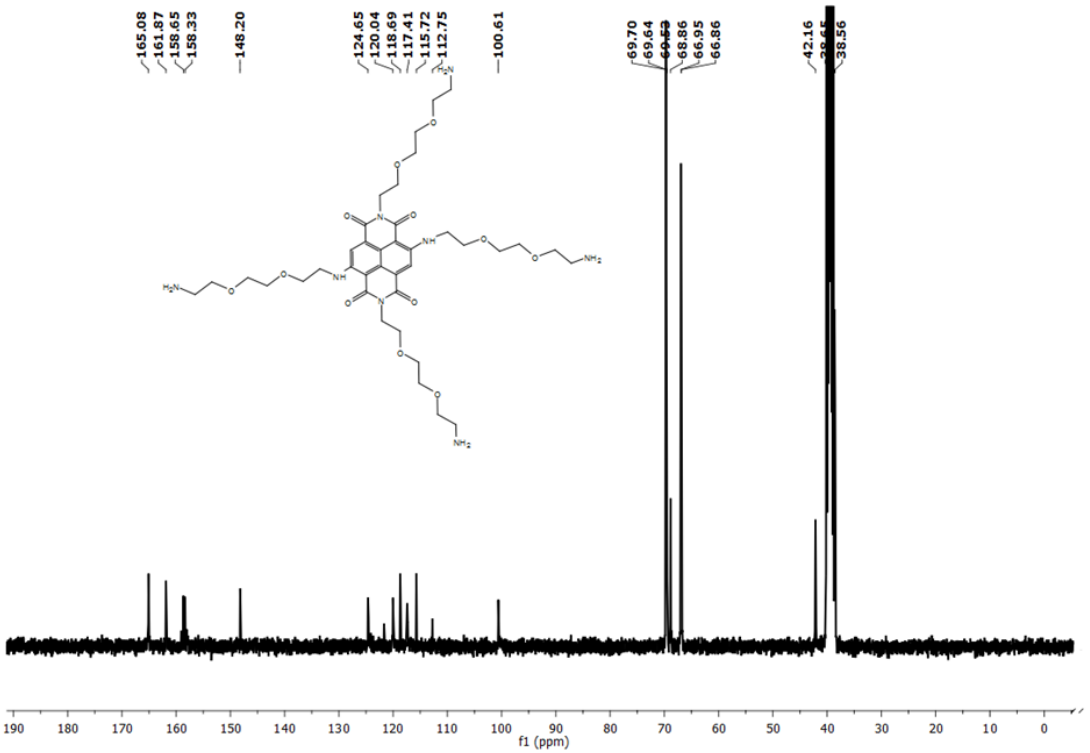
HRMS characterization of TGP 37



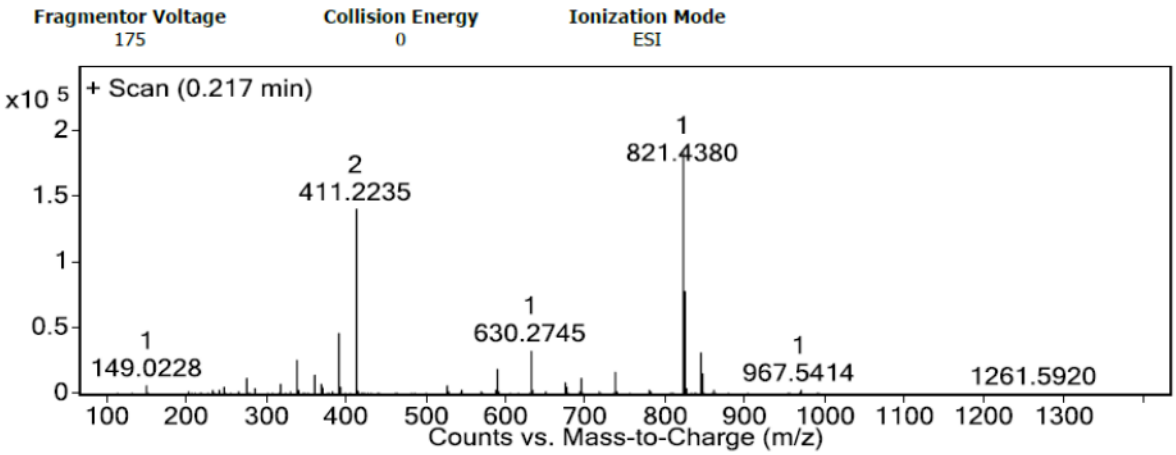
^{1}H NMR spectrum of TGP 8 in DMSO-d_6



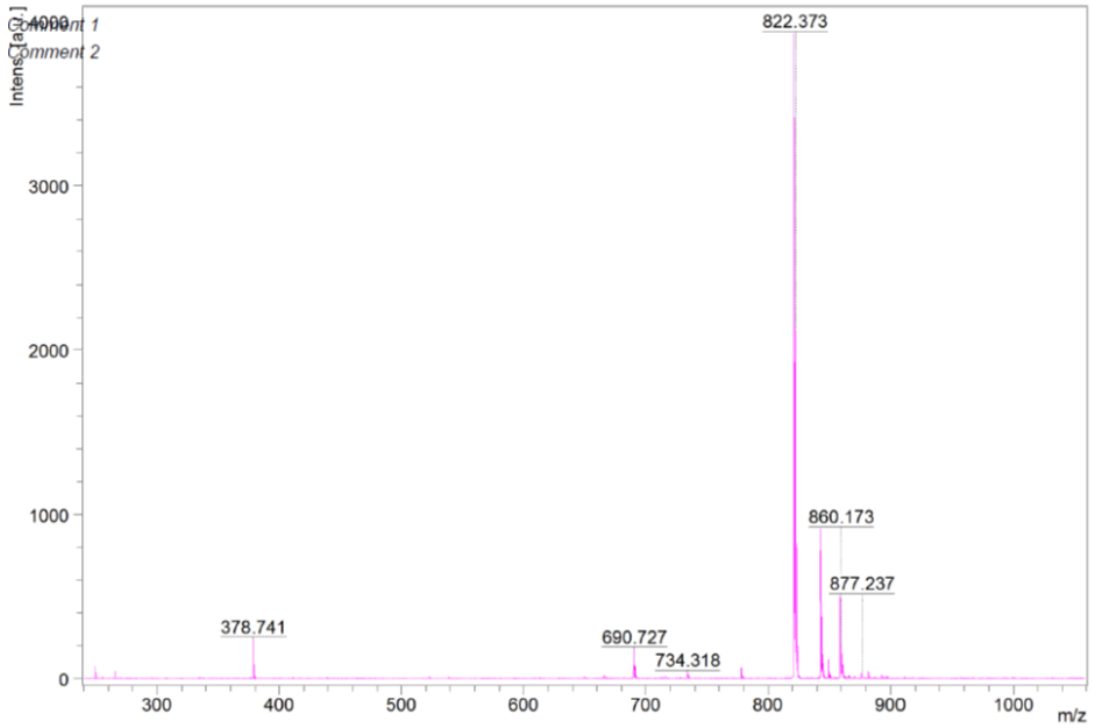
^{13}C NMR spectrum of TGP 8 in DMSO-d_6



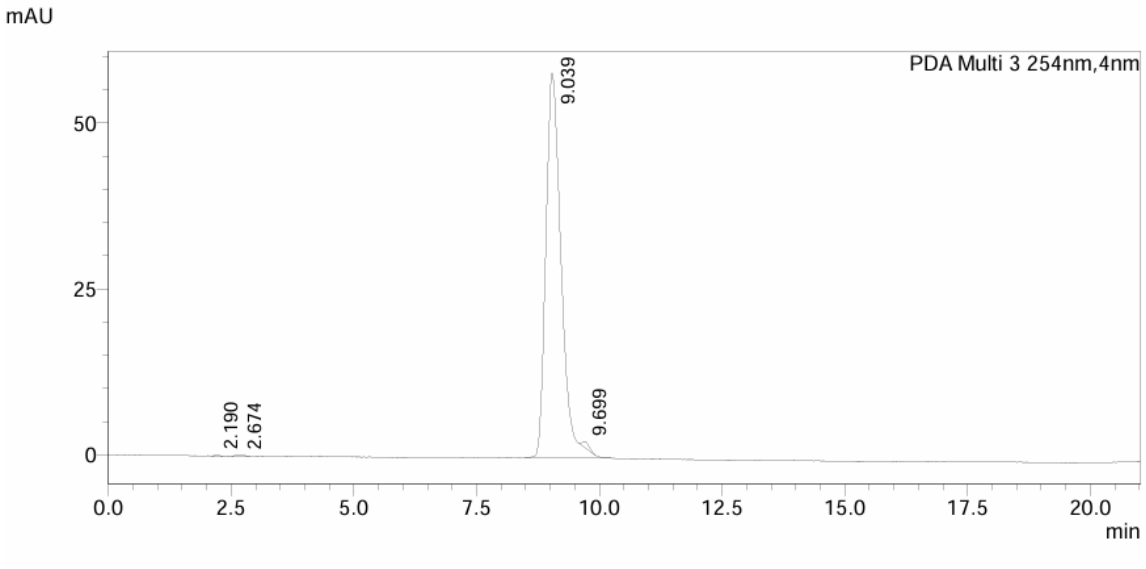
HRMS characterization of TGP 8



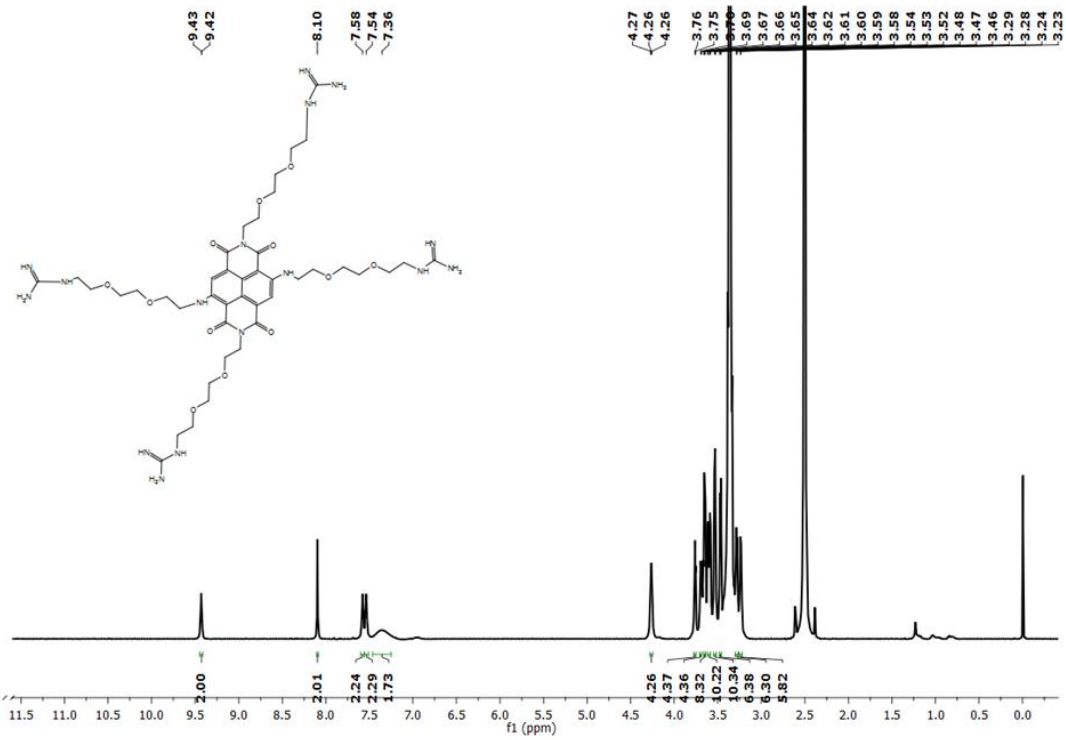
MALDI-TOF characterization of TGP 8



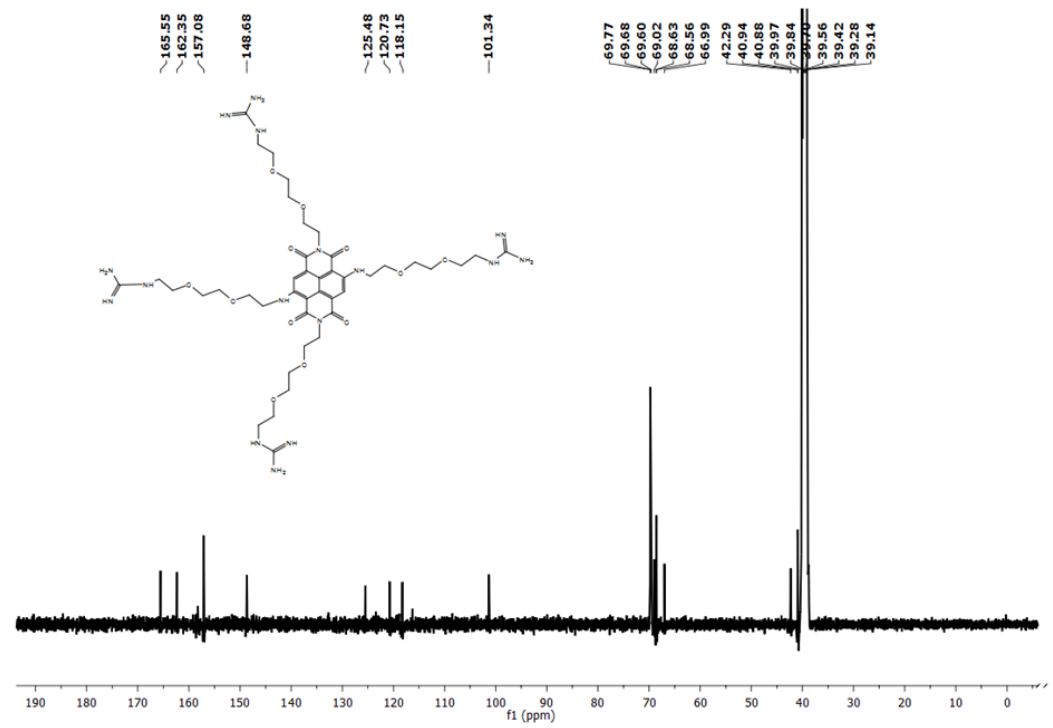
HPLC analysis of TGP 8



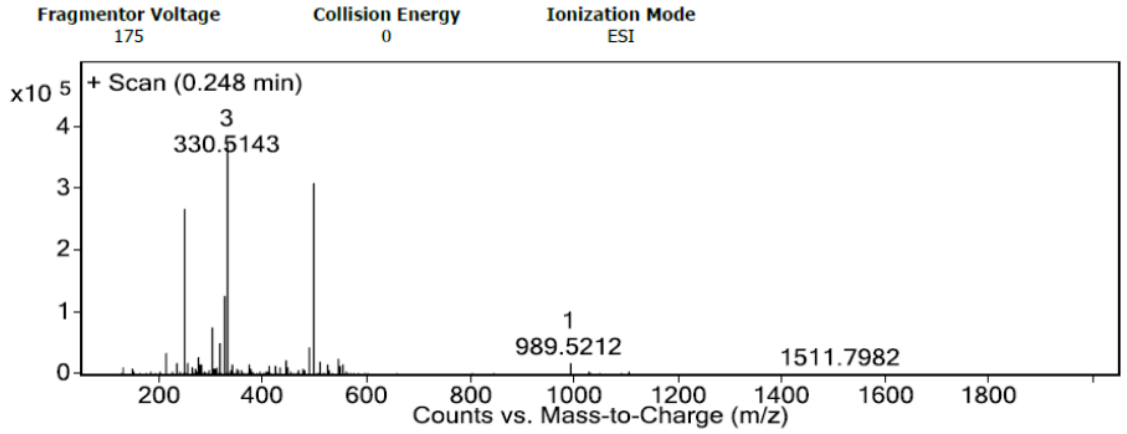
^{1}H NMR spectrum of TGP 39 in DMSO-d_6



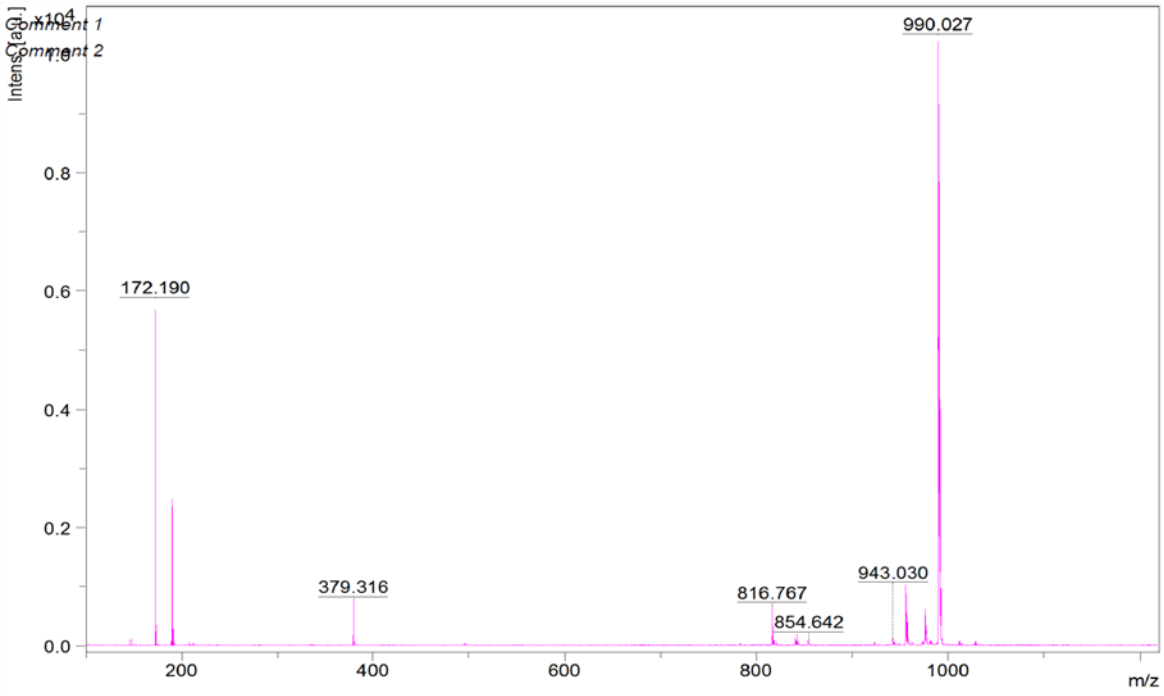
^{13}C NMR spectrum of TGP 39 in DMSO-d_6



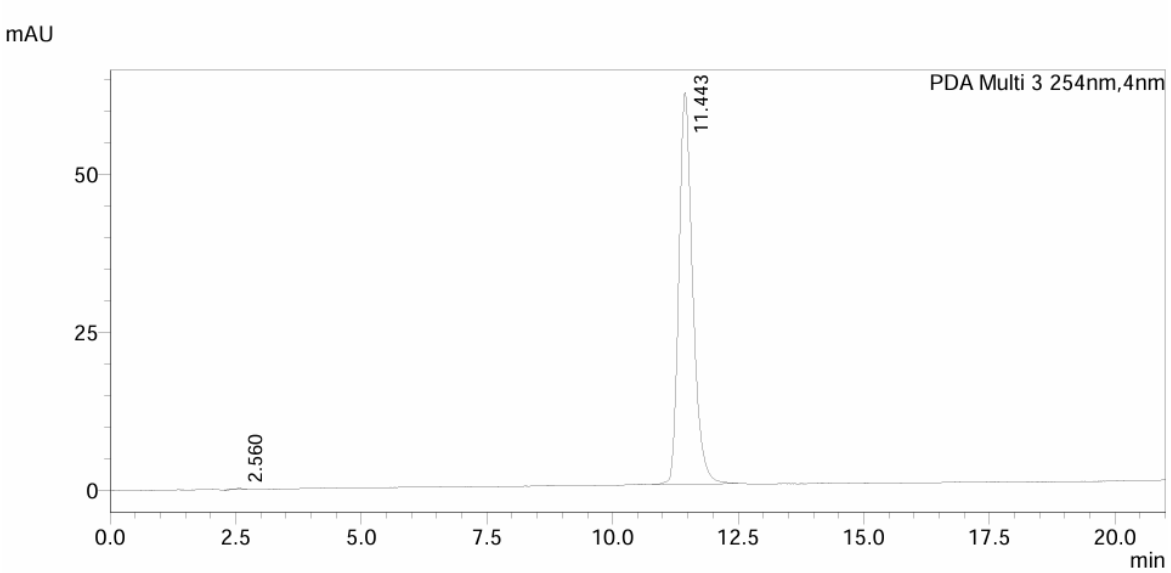
HRMS characterization of TGP 39



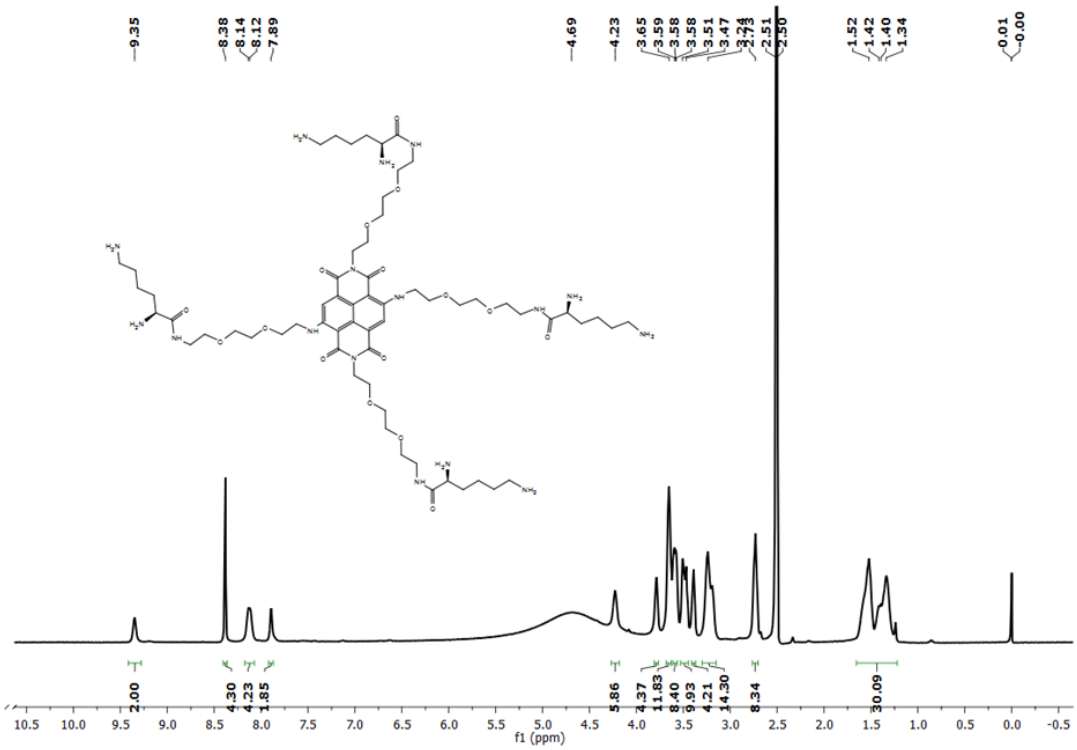
MALDI-TOF characterization of TGP 39



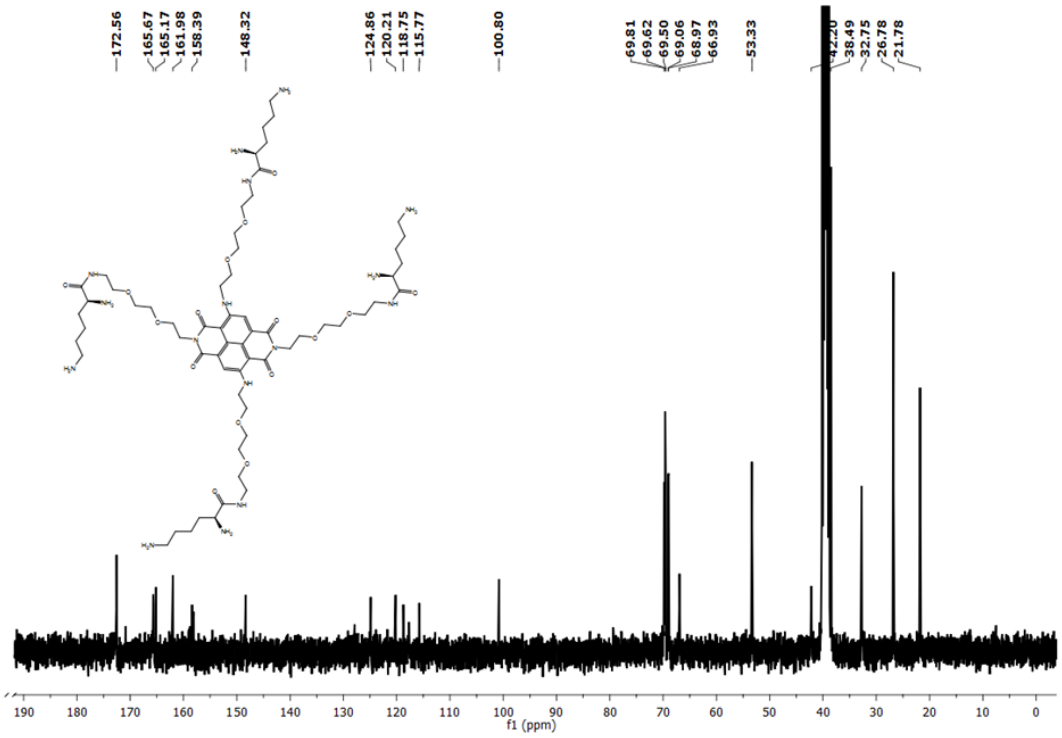
HPLC trace of TGP 39



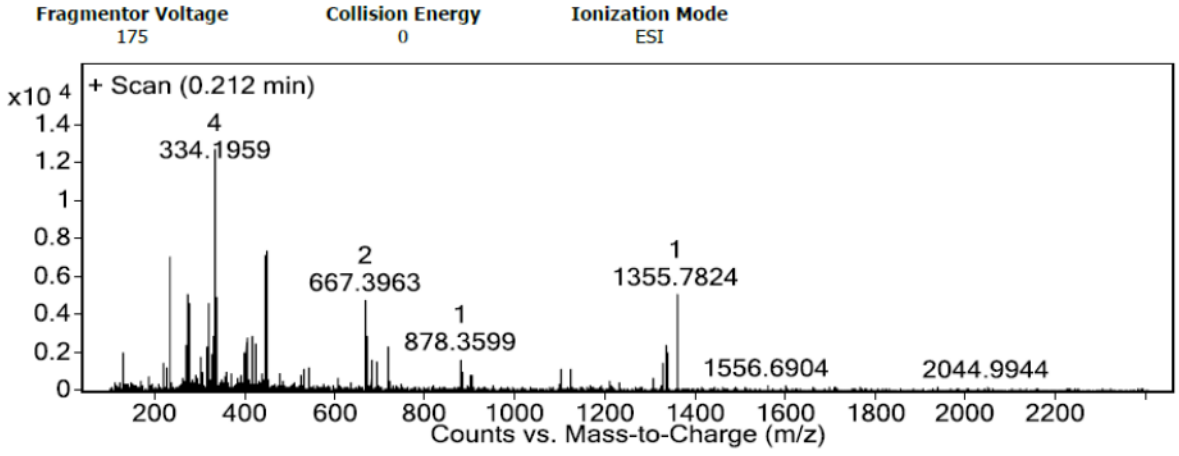
^{1}H NMR spectrum of TGP 40 in DMSO-d_6



^{13}C NMR spectrum of TGP 40 in DMSO-d_6



HRMS characterization of TGP 40



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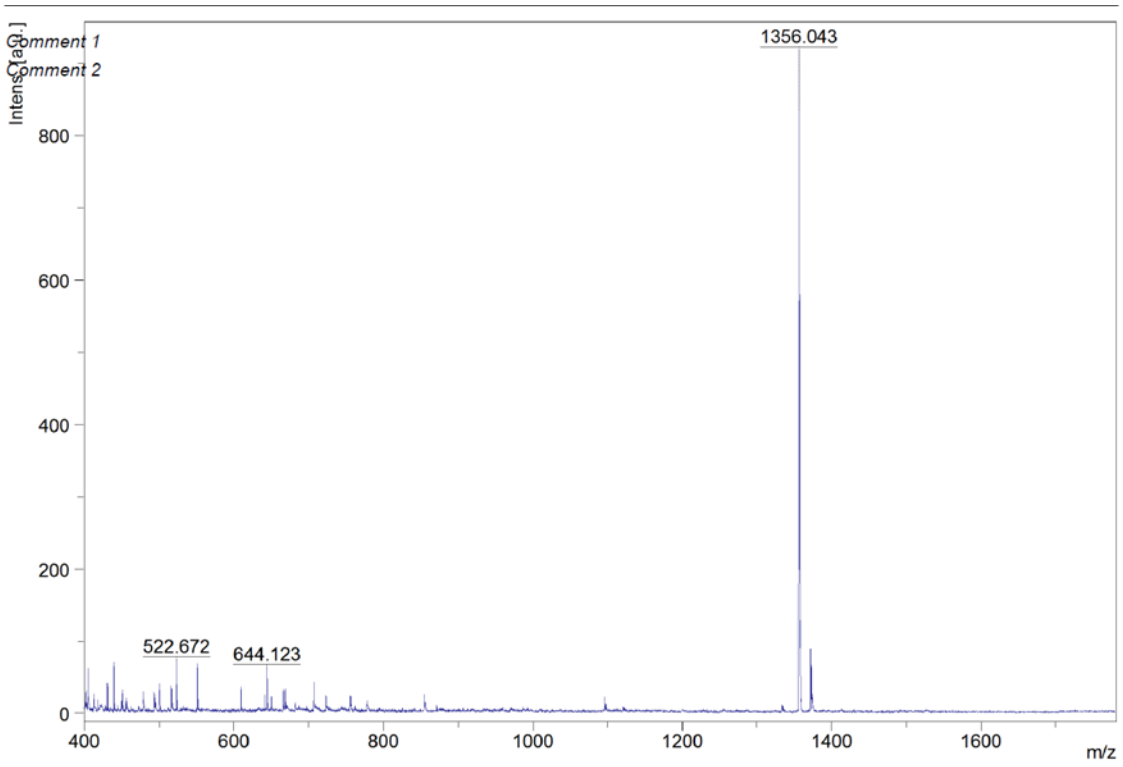
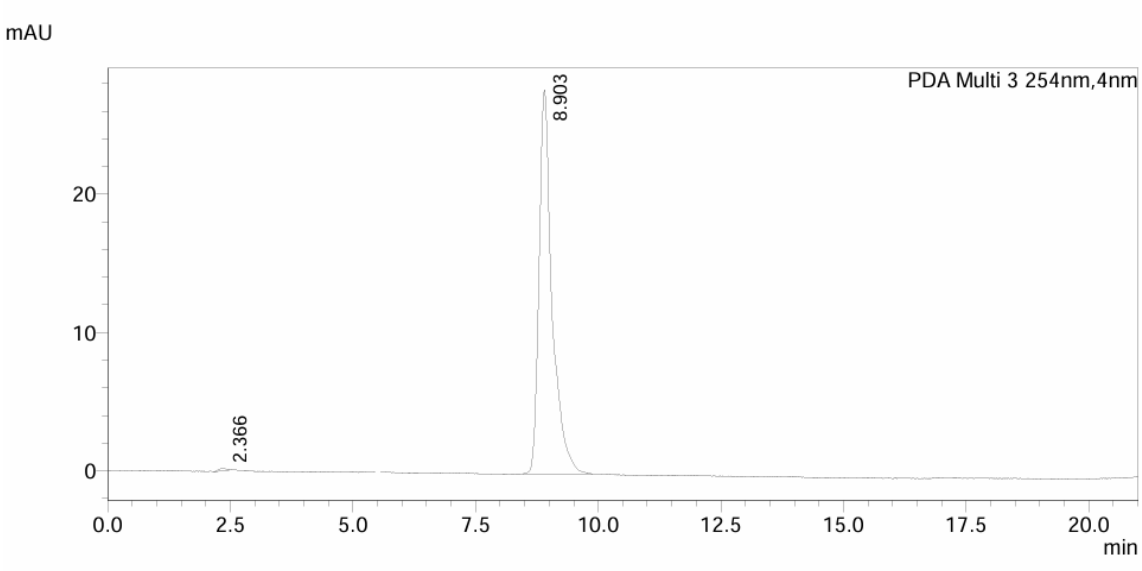
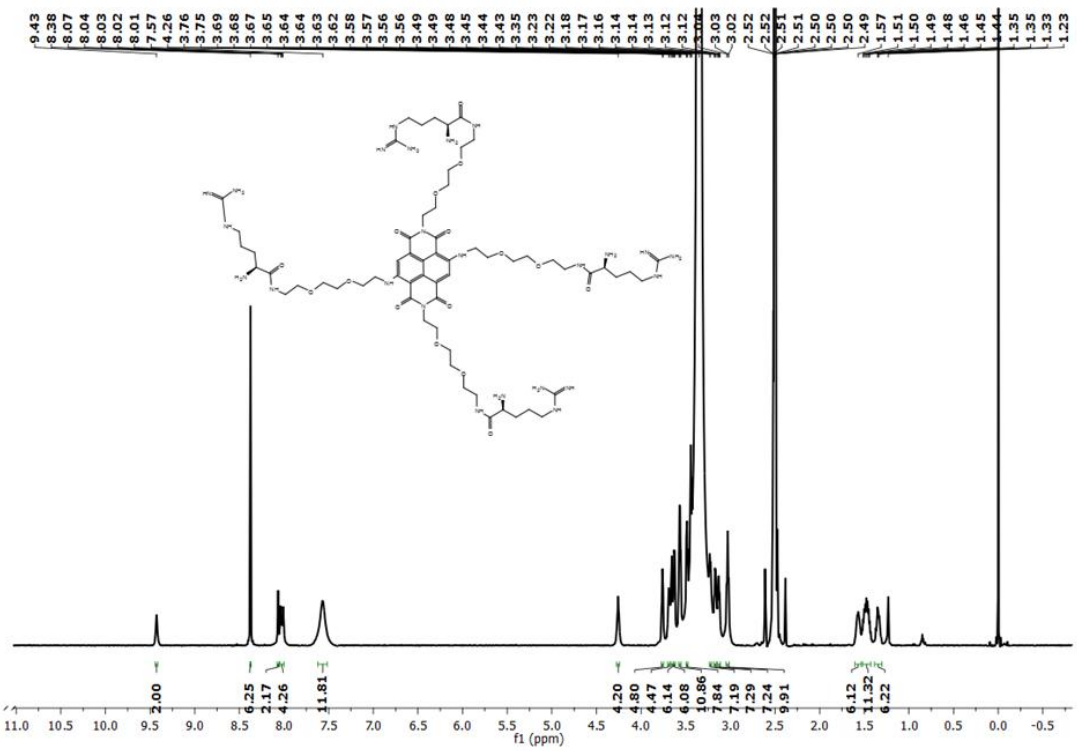


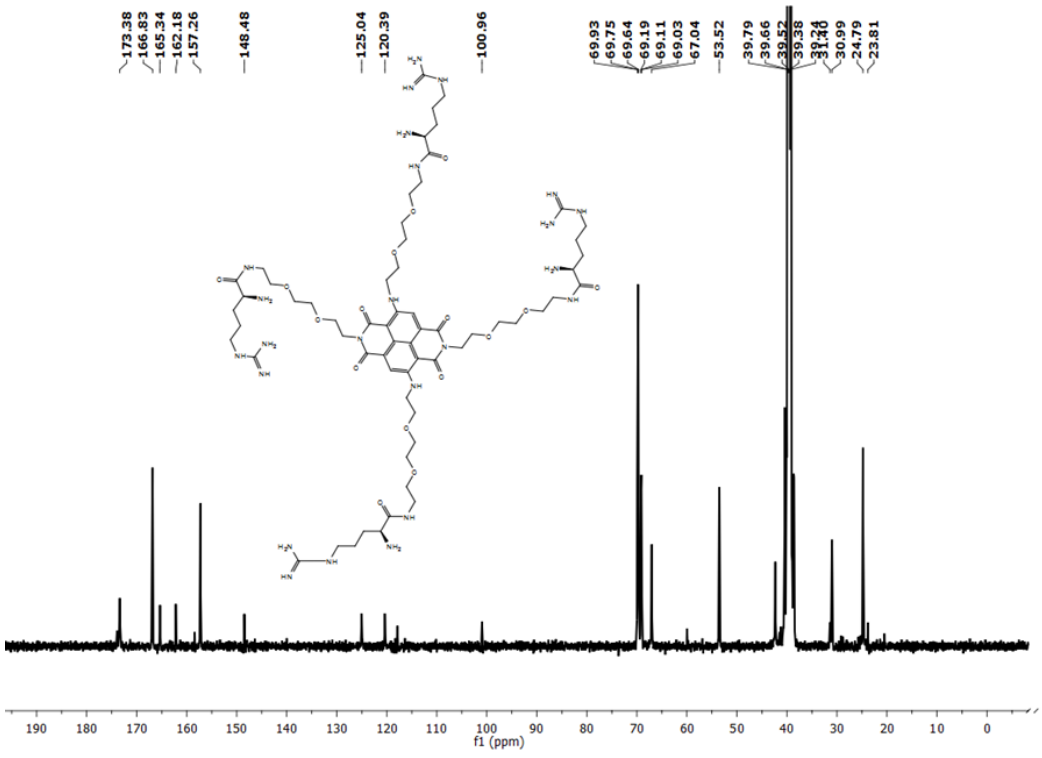
HPLC trace of TGP 40



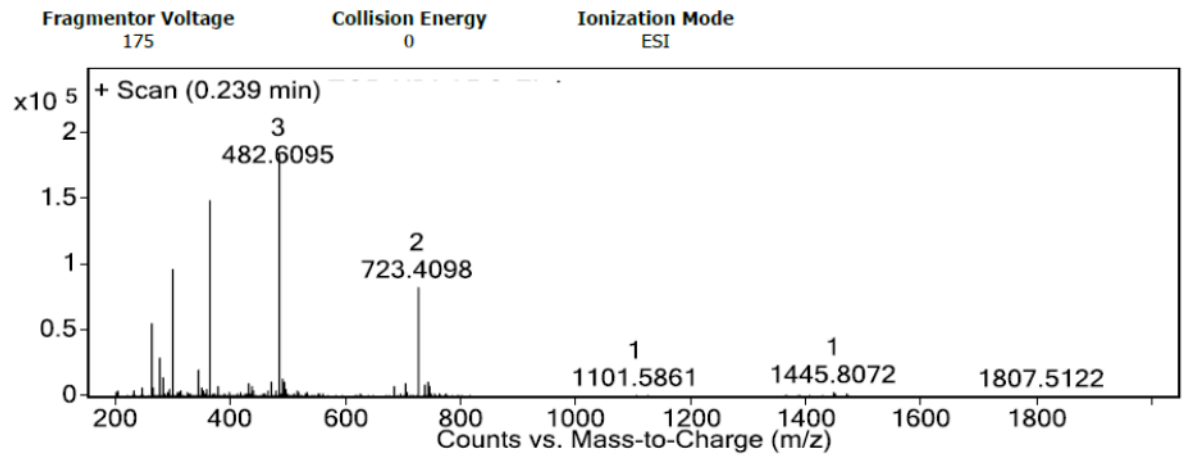
^{1}H NMR spectrum of TGP 41 in DMSO-d_6



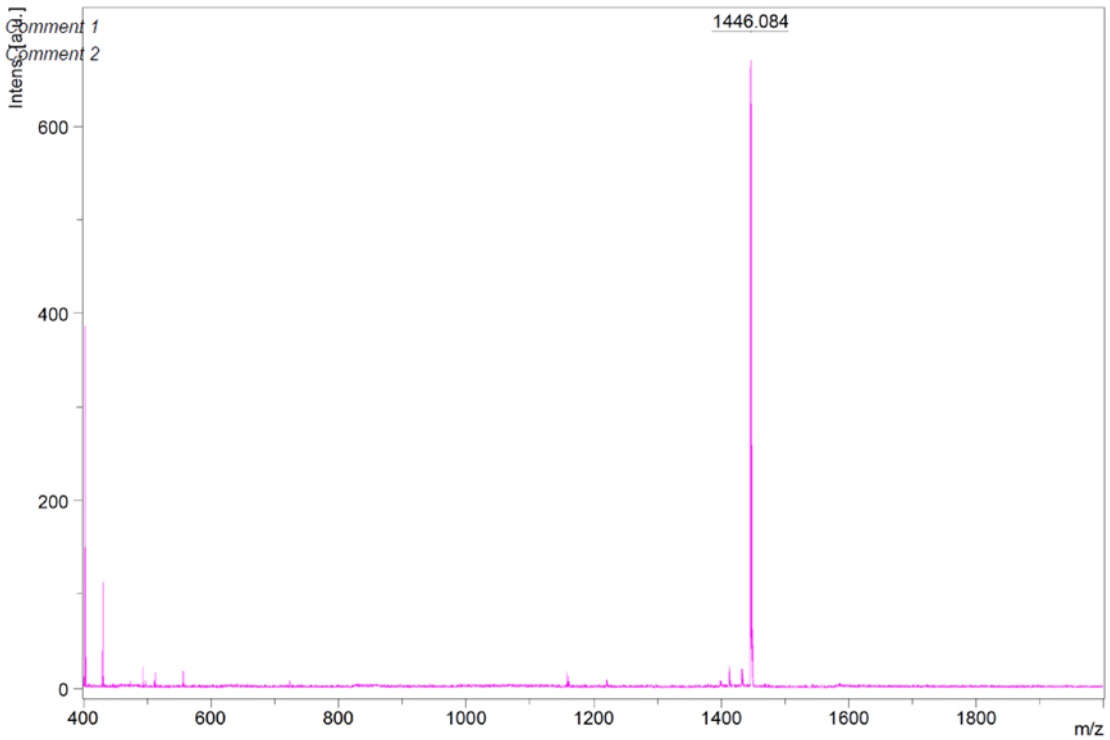
^{13}C NMR spectrum of TGP 41 in DMSO-d_6



HRMS characterization of TGP 41



MALDI characterization of TGP 41



HPLC trace of TGP 41