Electronic Supplementary Material (ESI) for Organic Chemistry Frontiers.

Supporting Information

Facile solid-phase synthesis of PNA-peptide conjugates using pNZ-protected PNA monomers

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1. General Information

a. Materials

All reagents and solvents were purchased from Sinopharm Chemical Reagent Co., Ltd., Alfa Aesar China Co., Ltd., J&K Chemical Co., Ltd. THF and Et_2O were distilled from sodium/diphenyl ketone immediately prior to use. DMF was distilled under reduced pressure from sodium sulfate and stored over 4 Å molecular sieves. CH_2Cl_2, pyridine and Et_3N were distilled from calcium hydride immediately prior to use. All other commercially available reagents and solvents were used as received without further purification unless otherwise indicated. All organic extracts were dried over sodium sulfate or magnesium sulfate. TLC was carried out on plates pre-coated with silica gel 60 F254 (250 layer thickness). Visualization was accomplished using UV light, iodine vapors, ninhydrin solution, permanganate solution and/or phosphomolybdic acid (PMA) solution. Flash column chromatographic purification of products was accomplished using forced-flow chromatography on Silica Gel (300-400 mesh on large-scale or 200-300 mesh on small-scale). Fmoc-protected amino acids were purchased from GL Biochem (Shanghai) Co., Ltd. Rink amide AM resins (100-200 mesh) were purchased from Tianjian Nankai HECHENG S&T Co., Ltd.

b. HPLC

Analytical HPLC was run on a SHIMADZU (Prominence LC-20AT) instrument using an analytical column (Grace Vydac "Peptide C4", 150 × 4.6 mm, 5 μm particle size, flow rate 1.2 mL/min, rt). Analytical injections were monitored at 214 nm and 254 nm. Semi preparative HPLC was run on a SHIMADZU (Prominence LC-20AT) instrument using a semi preparative column (Grace Vydac "Peptide C4", 250 × 10 mm, 10 μm particle size, flow rate 4.0 mL/min). Solvent A was 0.1% TFA in acetonitrile, and solvent B was 0.1% TFA in water. Both solvents were filtered through 0.22 μm filter paper and sonicated for 20 min before use.

c. Fmoc and pNZ SPPS

Screw-cap glass peptide synthesis reaction vessels were attained from commercial sources. Fmoc-protected Rink amide AM resins were initially swelled in DCM/DMF (1/1) for 10 min before use.

Capping reagent was acetic anhydride/2,6-lutidine/DMF (1:1:8) (1 × 3 min). The resin was washed with 5× DMF, 5× DCM and 5× DMF.

Fmoc deprotection reagent was 20% piperidine (0.1 M Oxyma)/DMF (5 min + 10 min). The resin was washed with 5× DMF, 5× DCM and 5× DMF.

pNZ deprotection reagent was 3 M $SnCl_2$ (20 mM HCl)/DMF (15 min at 60°C). The resin was washed with 5× DMF, 5× THF and 5× DMF.

The amino acid coupling was carried out at 25°C by pouring a preactivated solution of 4 eq protected amino acid (0.1 M in NMP) with 4 eq PyBOP, 8 eq DIEA to the resin. For the single coupling, after 40 min, the resin was washed with 5× DMF, 5× DCM and 5× DMF. For the double-coupling, the reaction time was changed to 20 min × 2.

The PNA monomer coupling was carried out at 25°C by pouring a preactivated solution of 2.5 eq protected PNA monomer (0.1 M in NMP) with 2.5 eq PyAOP, 5 eq NMM to the resin. For the single coupling, after 30 min, the resin was washed with 5× DMF, 5× DCM and 5× DMF. For the double-coupling, the reaction time was changed to 15 min × 2.

The coupling efficiency was checked with qualitative chloranil test. Prepare 4% chloranil (w/v) DMF solution stored at 4°C. Transfer a few resin beads to a 1.5 mL EP tube and add two drops of the chloranil reagent. Mix well and leave at room temperature for 3 min. Dark red or brown beads indicate positive results while colorless or light yellow beads indicate negative results.

Cleavage reagent was chosen as Reagent B. Typically a TFA cocktail of TFA/phenol/water/TIPS (88/5/5/2) was added to the dry resin prewashed with DCM. After 2-3 h, the resin was washed with an equal volume of TFA

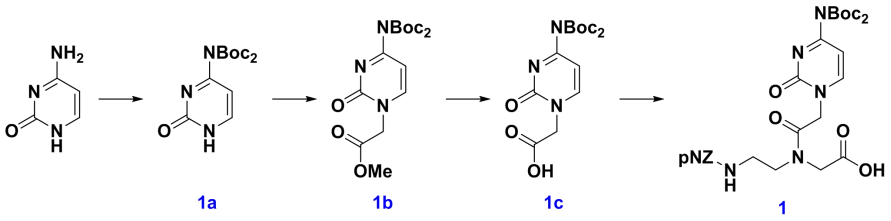
once. Combined elutents were concentrated by nitrogen blow. The crude peptides were obtained through precipitation with cold ether and centrifugation at 5000 rpm for 2 min. The peptide pellet was dissolved in 0.1% TFA containing CH_3CN/H_2O (1/1), characterized by analytical HPLC and ESI-MS, and purified, if necessary, by semi preparative HPLC and lyophilized immediately.

d. Mass spectrometry

MALDI-TOF mass spectra were measured on an Applied Biosystems 4700 Proteomics Analyzer 283. A solution of 10 mg/ml matrix α-cyano-4-hydroxy cinnamic acid containing 1:1 v/v (0.1% TFA in acetonitrile / 0.1% TFA in water) was used for generating the probe-matrix mixture. High-resolution ESI mass spectra were measured on Agilent 6210 Time of Flight Mass Spectrometer. Normal ESI mass spectra were measured on a Bruker Daltonics DataAnalysis 3.0 workstation.

2. Experimental Section

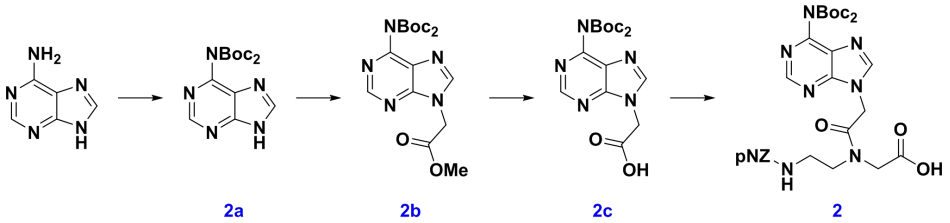
2a. Synthesis of pNZ/bis-Boc protected PNA monomers



Cytosine (1.11 g, 10 mmol, 1 eq) was mixed with THF (40 mL). DMAP (122 mg, 1 mmol, 0.1 eq) and Boc_2O (7.64 g, 35 mmol, 3.5 eq) were added stepwise. The slurry was stirred at RT for 5 h until the starting solid was entirely solubilized. THF was evaporated and the resulting residue was redissolved in MeOH (40 mL). Saturated $NaHCO_3$ solution (20 mL) was poured in and the mixture was kept at 50°C for 1 h. The reaction solution was concentrated and diluted with water. The aqeuous phase was extracted with CH_2Cl_2 (30 mL) three times, dried over Na_2SO_4, and filterd through celite. The crude **1a** was concentrated and redissolved in dry THF (40 mL). NaH (60% dispersed in mineral oil) (600 mg, 15 mmol, 1.5 eq) and $BrCH_2COOMe$ (3.7 mL, 40 mmol, 4 eq) were slowly added stepwise. The reaction was performed at 0°C -> RT for 12 h. H_2O was added to quench excess NaH (until the solution turned clear) and THF was evaporated in vacuo. The reaction was diluted with water and extracted with CH_2Cl_2 (20 mL) four times. The organic phase was dried over Na_2SO_4, concentrated and purified by column chromatography (CH_2Cl_2 : EtOAc = 10 : 1 -> CH_2Cl_2 : EtOAc = 5 : 1) to afford **1b** (3.33 g, 8.7 mmol, 87%). **1b** (3.33 g, 8.7 mmol, 1 eq) was dissolved in THF : H_2O = 1 : 1 (40 mL) and $LiOH{\cdot}H_2O$ (1.83 g, 43.5 mmol, 5 eq) was added in one portion. The mixture was stirred at 0°C for 10 min. The solution was adjusted to pH 4 with 1 M HCl, concentrated, extracted with EtOAc (40 mL) three times, washed with brine, and dried over Na_2SO_4. Concentration in vacuo afforded **1c** (2.98 g, 7.8 mmol, 90%, 4 steps 78%) which can be used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ(ppm) 8.14 (d, J = 7.2 Hz, 1H), 6.83 (d, J = 7.2 Hz, 1H), 4.57 (s, 2H), 1.50 (s, 18H). ^{13}C NMR (100 MHz, DMSO-d_6): δ(ppm) 169.1, 162.1, 154.1, 151.1, 149.2, 95.5, 84.6, 50.8, 27.2.

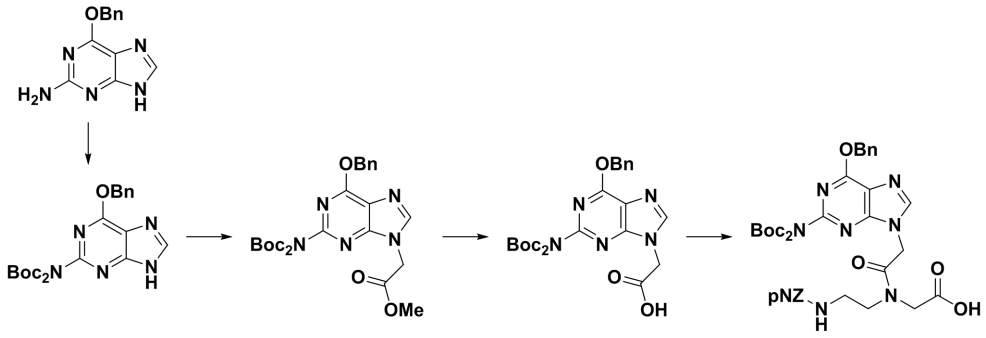
1c (738 mg, 2 mmol, 1 eq) and HOAt (324 mg, 2.4 mmol, 1.2 eq) were dissolved in DMF (10 mL). DIC (377 μL, 2.4 mmol, 1.2 eq) was added to prepare the HOAt ester of **1c**. **5** (650 mg, 2 mmol, 1 eq) dissolved in DMF (5 mL) was added in one portion. The reaction was kept at RT overnight. The resulting solution was diluted with sat. NH_4Cl and extracted with EtOAc (30 mL) twice. The organic phase was washed with sat. NH_4Cl, brine, dried over Na_2SO_4 and loaded onto flash column chromatography (CH_2Cl_2 : EtOAc = 1:1 -> CH_2Cl_2 : EtOAc = 2:3). The purified product was dissolved in THF : H_2O = 1 : 1 (8 mL) and $LiOH{\cdot}H_2O$ (420 mg, 10 mmol, 5 eq) was added in one portion. The mixture was stirred at 0°C for 20 min. The solution was adjusted to pH 4 with 1 M HCl, concentrated, extracted with EtOAc (20 mL) three times, washed with brine, dried over Na_2SO_4, concentrated in

vacuo to afford **1** (823 mg, 1.27 mmol, 2 steps 64%). ^{1}H NMR (400 MHz, $CDCl_3$): δ(ppm) 11.16 (d, 2H), 8.04 (d, *J* = 7.0 Hz, 2H, *rotamers*), 7.40 (d, *J* = 6.9 Hz, 2H, *rotamers*), 5.13-5.07 (s, 2H, *rotamers*), 4.68-4.61 (s, 2H, *rotamers*), 4.14-3.94 (s, 2H, *rotamers*), 3.46-3.23 (m, 4H, *rotamers*), 1.45 (s, 18H). ^{13}C NMR (100 MHz, $CDCl_3$): δ(ppm) 171.5, 168.2, 167.4, 163.1, 157.0, 149.9, 149.2, 147.6, 144.4, 144.0 (*rotamer*), 128.4, 128.0 (*rotamer*), 123.7, 97.8, 85.9, 85.8 (*rotamer*), 65.5, 65.2 (*rotamer*), 51.2, 48.5, 39.1, 27.8, 27.7 (*rotamer*), 24.2. ESI-MS (positive): 549.2, 649.2, 671.3 (observed, M-Boc+H, M+H, M+Na); 648.2 (calculated, M).



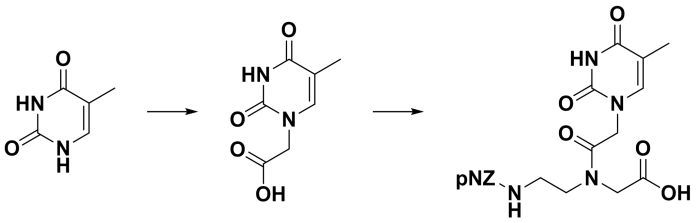
Adenine (1.35 g, 10 mmol, 1 eq) was mixed with THF (40 mL). DMAP (122 mg, 1 mmol, 0.1 eq) and Boc_2O (7.64 g, 35 mmol, 4 eq) were added stepwise. The slurry was stirred at RT for 5 h until the starting solid was entirely solubilized. THF was evaporated and the resulting residue was redissolved in MeOH (40 mL). Saturated $NaHCO_3$ solution (20 mL) was poured in and the mixture was kept at 50°C for 1 h. The reaction solution was concentrated and diluted with water. The aqeuous phase was extracted with CH_2Cl_2 (30 mL) three times, dried over Na_2SO_4, and filterd through celite. The crude **2a** was concentrated and redissolved in dry THF (40 mL). NaH (60% dispersed in mineral oil) (600 mg, 15 mmol, 1.5 eq) and $BrCH_2COOMe$ (3.7 mL, 40 mmol, 4 eq) were slowly added stepwise. The reaction was performed at 0°C -> RT for 12 h. H_2O was added to quench excess NaH (until the solution turned clear) and THF was evaporated in vacuo. The reaction was diluted with water and extracted with CH_2Cl_2 (20 mL) four times. The organic phase was dried over Na_2SO_4, concentrated and purified by column chromatography (PE : EtOAc = 2:1 -> PE : EtOAc = 3:2) to afford **2b** (2.50 g, 6.1 mmol, 61%). **2b** (2.50 g, 6.1 mmol, 1 eq) was dissolved in THF : H_2O = 1:1 (40 mL) and $LiOH{\cdot}H_2O$ (1.3 g, 30.5 mmol, 5 eq). The mixture was stirred at 0°C for 10 min. The solution was adjusted to pH 4 with 1 M HCl, concentrated, extracted with EtOAc (30 mL) three times, washed with brine, and dried over Na_2SO_4. Concentration in vacuo afforded **2c** (2.14 g, 5.5 mmol, 90%, 4 steps 55%) which can be used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ(ppm) 8.87 (s, 1H), 8.51 (s, 1H), 5.11 (s, 2H), 1.39 (s, 18H). ^{13}C NMR (100 MHz, $CDCl_3$): δ(ppm) 168.6, 153.3, 152.5, 150.1, 149.9, 146.6, 127.1, 84.5, 44.6, 27.9.

2c (2.63 g, 6.7 mmol, 1 eq) and HOAt (1.08 g, 8.0 mmol, 1.2 eq) were dissolved in DMF (30 mL). DIC (1.23 mL, 8.0 mmol, 1.2 eq) was added to prepare the HOAt ester of **2c**. **5** (2.12 g, 6.7 mmol, 1 eq) dissolved in DMF (15 mL) was added in one portion. The reaction was kept at RT overnight. The resulting solution was diluted with sat. NH_4Cl and extracted with EtOAc (60 mL) twice. The organic phase was washed with sat. NH_4Cl, brine, dried over Na_2SO_4 and loaded onto flash column chromatography (PE : EtOAc = 2:1 -> PE : EtOAc = 2:3). The purified product was dissolved in THF : H_2O = 1 : 1 (30 mL) and $LiOH{\cdot}H_2O$ (1.41 g, 33.5 mmol, 5 eq) was added in one portion. The mixture was stirred at 0°C for 20 min. The solution was adjusted to pH 4 with 1 M HCl, concentrated, extracted with EtOAc (30 mL) three times, washed with brine, dried over Na_2SO_4, concentrated in vacuo to afford **2** (853 mg, 1.27 mmol, 2 steps 57%). ^{1}H NMR (400 MHz, $CDCl_3$): δ(ppm) 11.28 (b, 2H), 8.79-8.74 (s, 1H, *rotamers*), 8.64-8.55 (s, 1H, *rotamers*), 8.10 (d, *J* = 8.4 Hz, 2H), 7.45-7.38 (d, *J* = 8.4 Hz, 2H, *rotamers*), 5.28-5.23 (s, 2H, *rotamers*), 5.17-5.09 (s, 2H, *rotamers*), 4.22-4.03 (s, 2H, *rotamers*), 3.55-3.31 (m, 4H, *rotamers*), 1.40 (s, 18H). ^{13}C NMR (100 MHz, $CDCl_3$): δ(ppm) 171.3, 167.2, 166.4 (*rotamer*), 160.6, 160.2 (*rotamer*), 156.8, 156.5 (*rotamer*), 153.2, 152.5 (*rotamer*), 150.2, 150.1 (*rotamer*), 149.5, 147.7, 147.6 (*rotamer*), 147.0, 144.2, 143.9, 128.2, 128.0 (*rotamer*), 125.7, 123.8, 123.7 (*rotamer*), 84.9, 65.6, 65.3 (*rotamer*), 48.9, 45.0, 44.5 (*rotamer*), 39.3, 27.8. ESI-MS (positive): 573.2, 673.3 (observed, M-Boc+H, M+H); 672.3 (calculated, M).



O6-Benzylguanine (1.70 g, 10 mmol, 1 eq) was mixed with THF (40 mL). DMAP (122 mg, 1 mmol, 0.1 eq) and Boc_2O (7.64 g, 35 mmol, 4 eq) were added stepwise. The slurry was stirred at RT for 5 h until the starting solid was entirely solubilized. THF was evaporated and the resulting residue was redissolved in MeOH (40 mL). Saturated $NaHCO_3$ solution (20 mL) was poured in and the mixture was kept at 50°C for 1 h. The reaction solution was concentrated and diluted with water. The aqeuous phase was extracted with CH_2Cl_2 (30 mL) three times, dried over Na_2SO_4, and filterd through celite. The crude **3a** was concentrated and redissolved in dry THF (40 mL). NaH (60% dispersed in mineral oil) (600 mg, 15 mmol, 1.5 eq) and $BrCH_2COOMe$ (3.7 mL, 40 mmol, 4 eq) were slowly added stepwise. The reaction was performed at 0°C -> RT for 12 h. H_2O was added to quench excess NaH (until the solution turned clear) and THF was evaporated in vacuo. The reaction was diluted with water and extracted with CH_2Cl_2 (20 mL) four times. The organic phase was dried over Na_2SO_4, concentrated and purified by column chromatography (PE : EtOAc = 2 : 1 -> PE : EtOAc = 1 : 2) to afford **3b** (3.00 g, 5.7 mmol, 57%). **3b** (3.00 g, 5.7 mmol, 1 eq) was dissolved in THF : H_2O = 1:1 (40 mL) and $LiOH{\cdot}H_2O$ (1.2 g, 28.5 mmol, 5 eq). The mixture was stirred at 0°C for 10 min. The solution was adjusted to pH 4 with 1 M HCl, concentrated, extracted with EtOAc (30 mL) three times, washed with brine, and dried over Na_2SO_4. Concentration in vacuo afforded **3c** (2.50 g, 5.0 mmol, 88%, 4 steps 50%) which can be used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ(ppm) 8.31 (s, 1H), 7.50 (d, J = 6.8 Hz, 2H), 7.41-7.34 (m, 3H), 5.60 (s, 2H), 4.57 (s, 2H), 1.37 (s, 18H). ^{13}C NMR (100 MHz, DMSO-d_6): δ(ppm) 168.8, 159.7, 153.2, 150.6, 150.5, 146.2, 136.1, 128.5, 128.3, 128.2, 118.4, 82.7, 67.9, 46.6, 27.4.

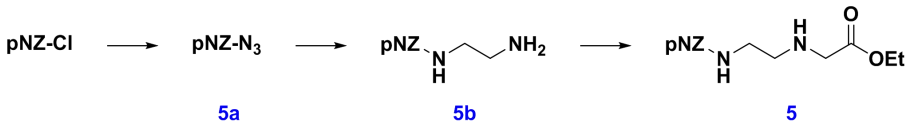
3c (1.30 g, 2.6 mmol, 1 eq) and HOAt (421 mg, 3.12 mmol, 1.2 eq) were dissolved in DMF (20 mL). DIC (490 µL, 3.12 mmol, 1.2 eq) was added to prepare the HOAt ester of **3c**. **5** (845 mg, 2.6 mmol, 1 eq) dissolved in DMF (10 mL) was added in one portion. The reaction was kept at RT overnight. The resulting solution was diluted with sat. NH_4Cl and extracted with EtOAc (40 mL) twice. The organic phase was washed with sat. NH_4Cl, brine, dried over Na_2SO_4 and loaded onto flash column chromatography (PE : EtOAc = 2:1 -> PE : EtOAc = 1:2). The purified product was dissolved in THF : H_2O = 1 : 1 (25 mL) and $LiOH{\cdot}H_2O$ (546 mg, 13 mmol, 5 eq) was added in one portion. The mixture was stirred at 0°C for 20 min. The solution was adjusted to pH 4 with 1 M HCl, concentrated, extracted with EtOAc (20 mL) three times, washed with brine, dried over Na_2SO_4, concentrated in vacuo to afford **3** (1.12 g, 1.44 mmol, 2 steps 55%). ^{1}H NMR (400 MHz, $CDCl_3$): δ(ppm) 11.7 (b, 3H), 8.6 (s, 1H), 8.07-8.03 (m, 2H), 7.44-7.39 (m, 2H), 7.36-7.28 (m, 5H), 5.57-5.55 (s, 2H, *rotamers*), 5.20-5.06 (s, 2H, s, 2H, *rotamers*), 4.20-4.01 (s, 2H, *rotamers*), 3.55-3.28 (m, 4H, *rotamers*), 1.43-1.41 (s, 18H, *rotamers*). ^{13}C NMR (100 MHz, $CDCl_3$): δ(ppm) 167.0, 166.4, 160.2, 156.8, 153.0, 152.2, 150.9, 150.8 (*rotamer*), 147.6, 144.1, 143.9 (*rotamer*), 135.2, 128.7, 128.6 (*rotamer*), 128.3, 128.1 (*rotamer*), 128.0, 123.7, 115.4, 84.2, 69.5, 65.4, 48.6, 44.6, 39.2, 27.9, 27.7 (*rotamer*) . ESI-MS (positive): 679.3, 779.3, 801.3 (observed, M-Boc+H, M+H, M+Na); 778.3 (calculated, M).



4a **4**

Thymine (2.52 g, 20 mmol, 1 eq) and KOH (4.48 g, 80 mmol, 4 eq) were dissolved in H_2O (25 mL). $BrCH_2CO_2H$ (2.09 g, 30 mmol, 1.5 eq) was then added. The reaction mixture was stirred at 50°C for 1 h. During the workup, the solution was adjusted to pH 2 with conc. HCl and kept at 4°C for 1 h, and filtered through a Büchner funnel. The filter cake was washed with H_2O, collected, dissolved in EtOH and concentrated in vacuo to afford **4a** (2.19 g, 11.9 mmol, 60%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ(ppm) 13.11 (b, 1H), 11.34 (s, 1H), 7.49 (s, 1H), 4.36 (s, 2H), 3.34 (b, 1H), 1.75 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ(ppm) 169.7, 164.4, 151.0, 141.8, 108.3, 48.4, 11.9.

4a (350 mg, 1.9 mmol, 1 eq) and HOAt (308 mg, 2.28 mmol, 1.2 eq) were dissolved in DMF (20 mL). DIC (358 μL, 2.28 mmol, 1.2 eq) was added to prepare the HOAt ester of **4a**. **5** (556 mg, 1.71 mmol, 0.9 eq) dissolved in DMF (10 mL) was added in one portion. The reaction was kept at RT overnight. The resulting solution was diluted with sat. NH_4Cl and extracted with EtOAc (30 mL) twice. The organic phase was washed with sat. NH_4Cl, brine, dried over Na_2SO_4 and loaded onto flash column chromatography (CH_2Cl_2 : MeOH = 40:1 -> CH_2Cl_2 : MeOH = 25:1). The purified product was dissolved in THF : H_2O = 1 : 1 (25 mL) and $LiOH{\cdot}H_2O$ (399 mg, 9.5 mmol, 5 eq) was added in one portion. The mixture was stirred at 0°C for 20 min. The solution was adjusted to pH 4 with 1 M HCl, concentrated, extracted with EtOAc (20 mL) three times, washed with brine, dried over Na_2SO_4, concentrated in vacuo to afford **4** (703 mg, 1.52 mmol, 2 steps 89%). ^{1}H NMR (400 MHz, DMSO-d_6): δ(ppm) 12.92 (b, 1H), 11.28-11.26 (s, 1H, *rotamers*), 8.20 (d, *J* = 7.6 Hz, 2H), 7.63-7.58 (d, *J* = 7.6 Hz, 2H, *rotamers*), 7.51-7.33 (m, 1H, *rotamers*), 7.27 (d, *J* = 6.0 Hz, 1H), 5.18-5.16 (s, 2H, *rotamers*), 4.64-4.48 (s, 2H, *rotamers*), 4.21-3.98 (s, 2H, *rotamers*), 3.45-3.14 (m, 4H, *rotamers*), 1.72 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ(ppm) 170.8, 170.4 (*rotamer*), 167.7, 167.3 (*rotamer*), 164.4, 156.1, 155.9 (*rotamer*), 151.0, 146.9, 145.2, 145.0 (*rotamer*), 142.1, 128.3, 128.1 (*rotamer*), 123.5, 108.2, 108.1 (*rotamer*), 64.3, 64.1 (*rotamer*), 49.0, 47.8, 47.7 (*rotamer*), 47.5, 46.7, 38.6, 11.9. ESI-MS (positive): 464.2 (observed, M+H); 463.1 (calculated, M).



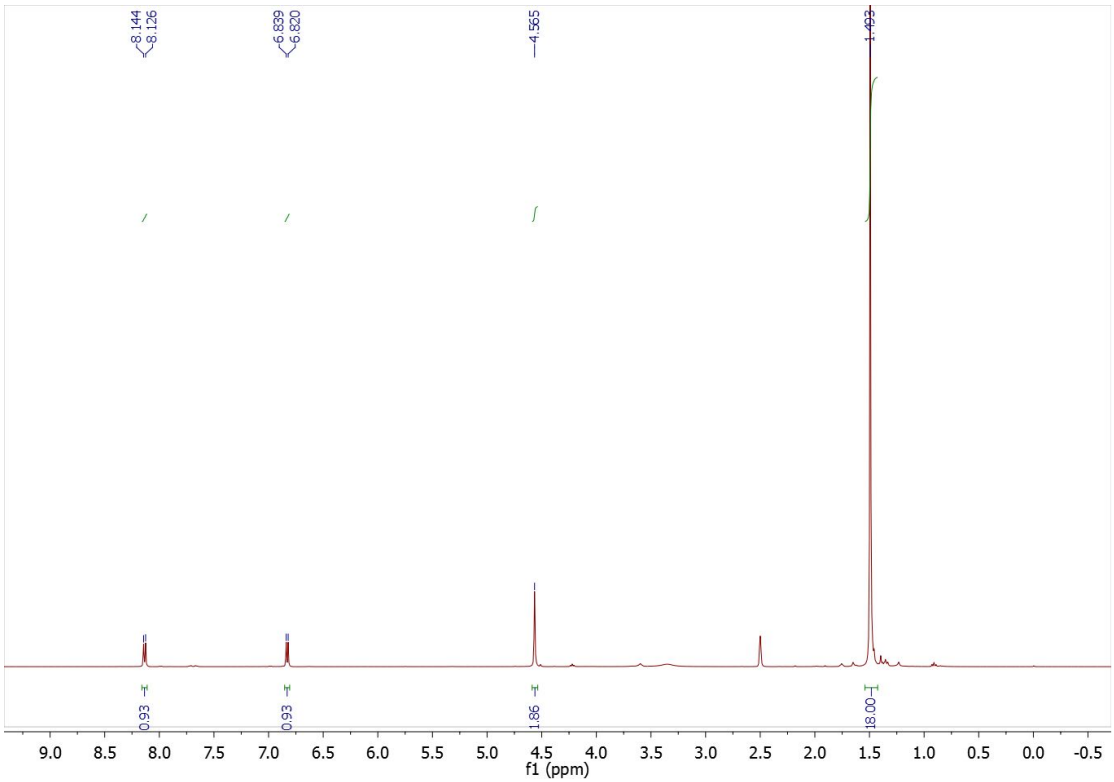
5a **5b** **5**

pNZ chloride (8.64 g, 40 mmol) dissolved in THF (40 mL) was added into NaN_3 (3.12 g, 48 mmol, 1.2 eq) dissolved in H_2O (20 mL). After 15 min, the mixture was extracted with EtOAc (50 mL). The organic phase was washed with water and brine once, dried over Na_2SO_4 and concentrated to afford **5a** as a white solid which can be used in the next step without further purification. Ethylenediamine (7.4 mL, 110 mmol, 10 eq) dissolved in $CHCl_3$ (40 mL) was kept at 0°C. To this solution was added dropwise **5a** (2.44 g, 11 mmol, 1 eq) dissolved in $CHCl_3$ (20 mL) within 20 min. The reaction mixture was stirred at RT for 1 h. The organic phase was washed with water three times and brine once, dried over Na_2SO_4 and concentrated to afford **5b** (2.05 g, 8.6 mmol) as a pale yellow solid. **5b** (2.05 g, 8.6 mmol, 1 eq) was dissolved in DMF (30 mL). Triethylamine (1.43 mL, 10.3 mmol, 1.2 eq) and $BrCH_2COOEt$ (1.14 mL, 10.3 mmol, 1.2 eq) were added. The reaction mixture was stirred for 2 h at RT. The reaction mixture was diluted with sat. NH_4Cl and extracted with EtOAc (40 mL) twice, washed with sat. NH_4Cl and brine, dried over Na_2SO_4 and concentrated. The crude product was purifed by column chromatography (CH_2Cl_2 : MeOH = 50 : 1) to afford **5** as a yellow oil (1.76 g, 5.41 mmol, 3 steps 63%). ^{1}H NMR (400 MHz, $CDCl_3$): δ(ppm) 8.12 (d, *J* = 8.4 Hz, 2H), 7.44 (d, *J* = 8.0 Hz, 2H), 5.74 (b, 1H), 5.12 (s, 2H), 4.12 (q, *J* = 7.2 Hz,

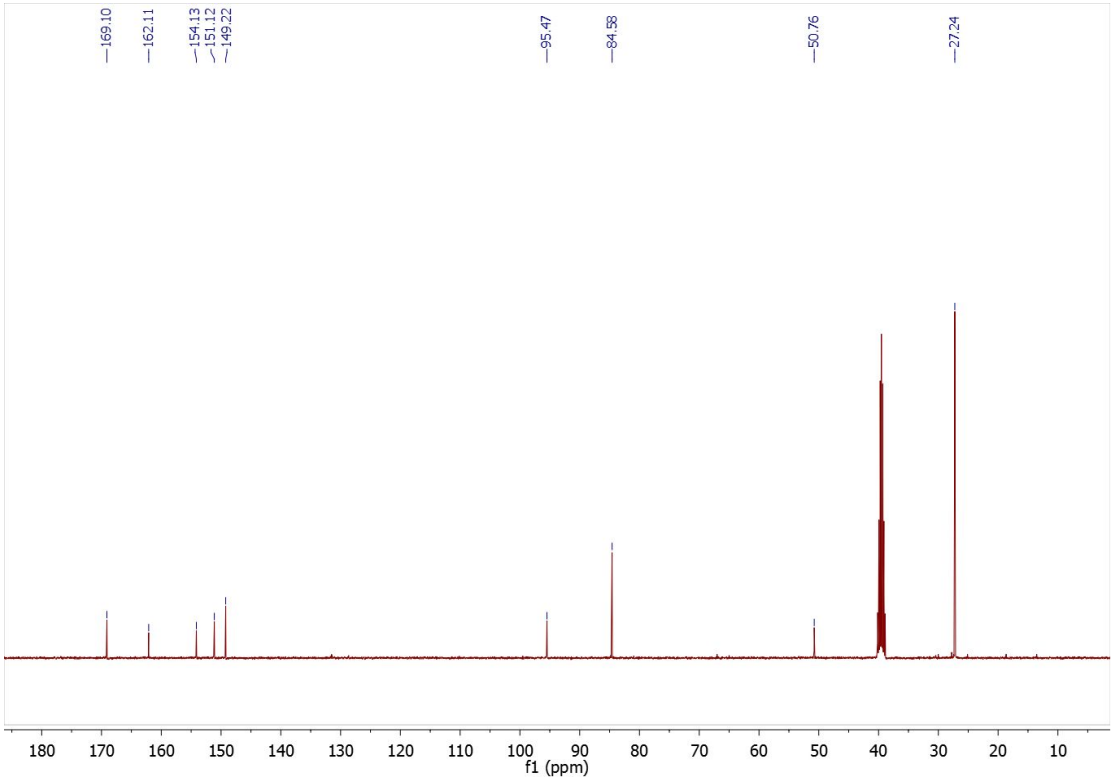
2H), 3.34 (s, 2H), 3.24 (q, J = 5.6 Hz, 2H), 2.72 (t, J = 5.6 Hz, 2H), 1.93 (s, 1H), 1.20 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ(ppm) 172.4, 156.0, 147.4, 144.3, 128.0, 123.6, 65.0, 60.9, 50.3, 48.5, 40.7, 14.2.

2b. NMR spectra

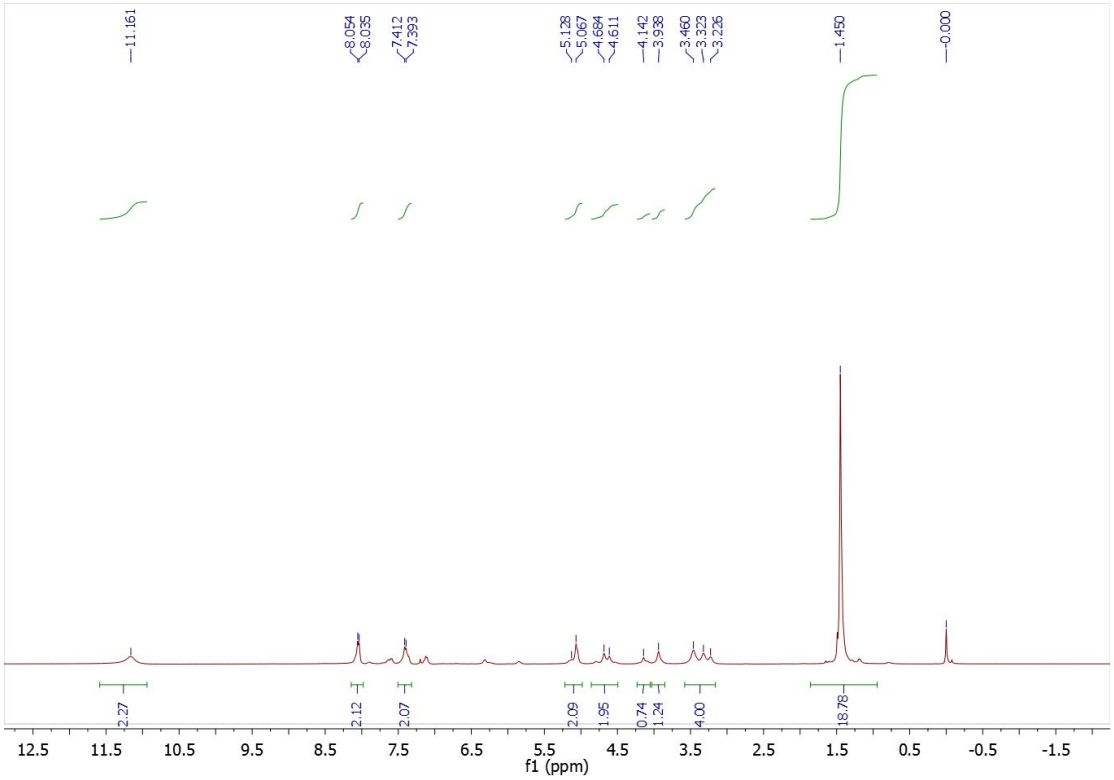
Compound **1c** (^{1}H NMR)



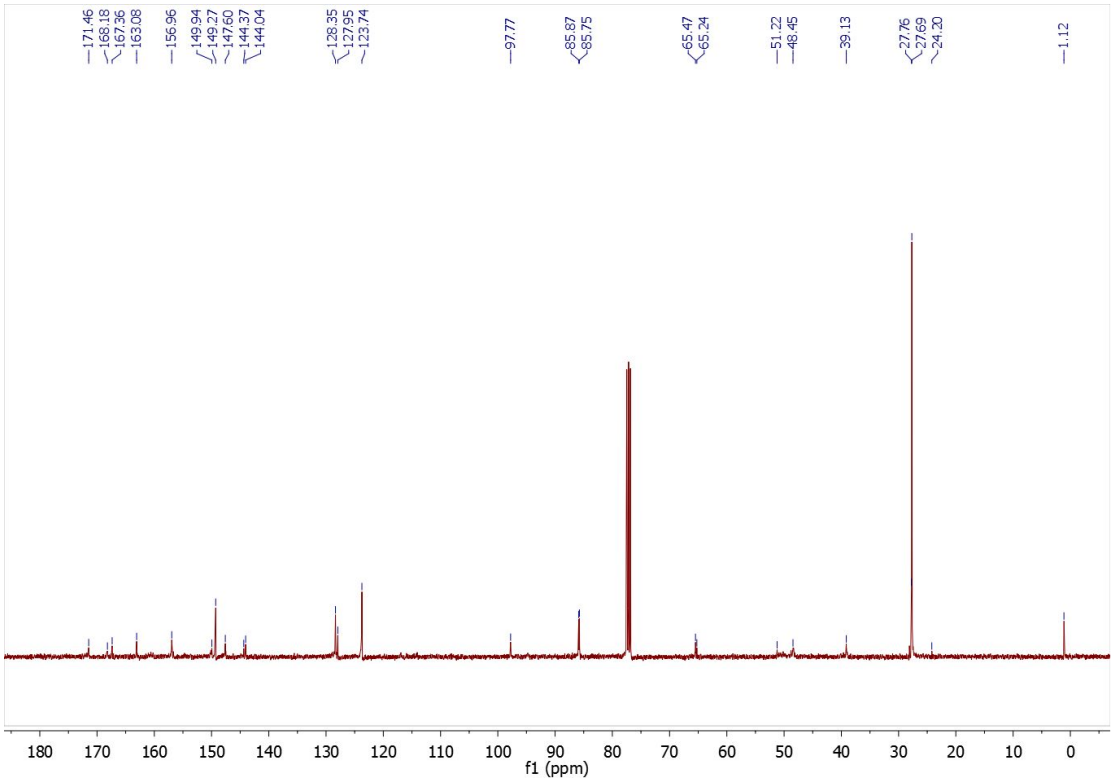
Compound **1c** (^{13}C NMR)



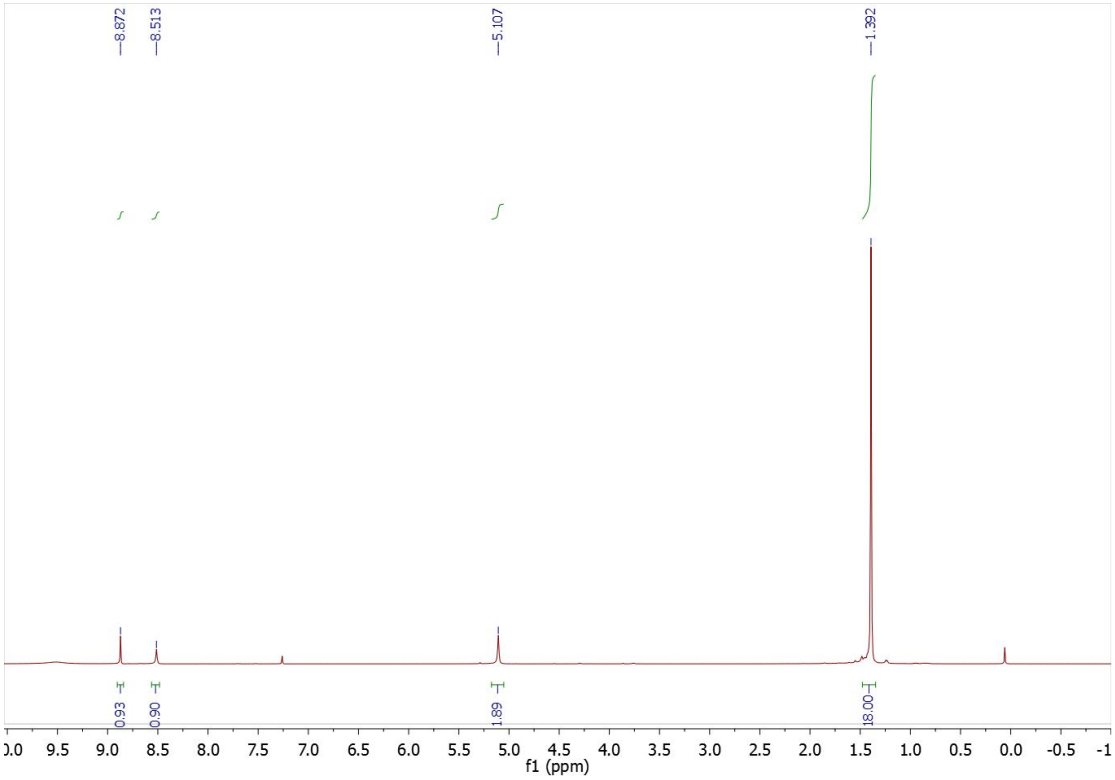
Compound **1** (^{1}H NMR)



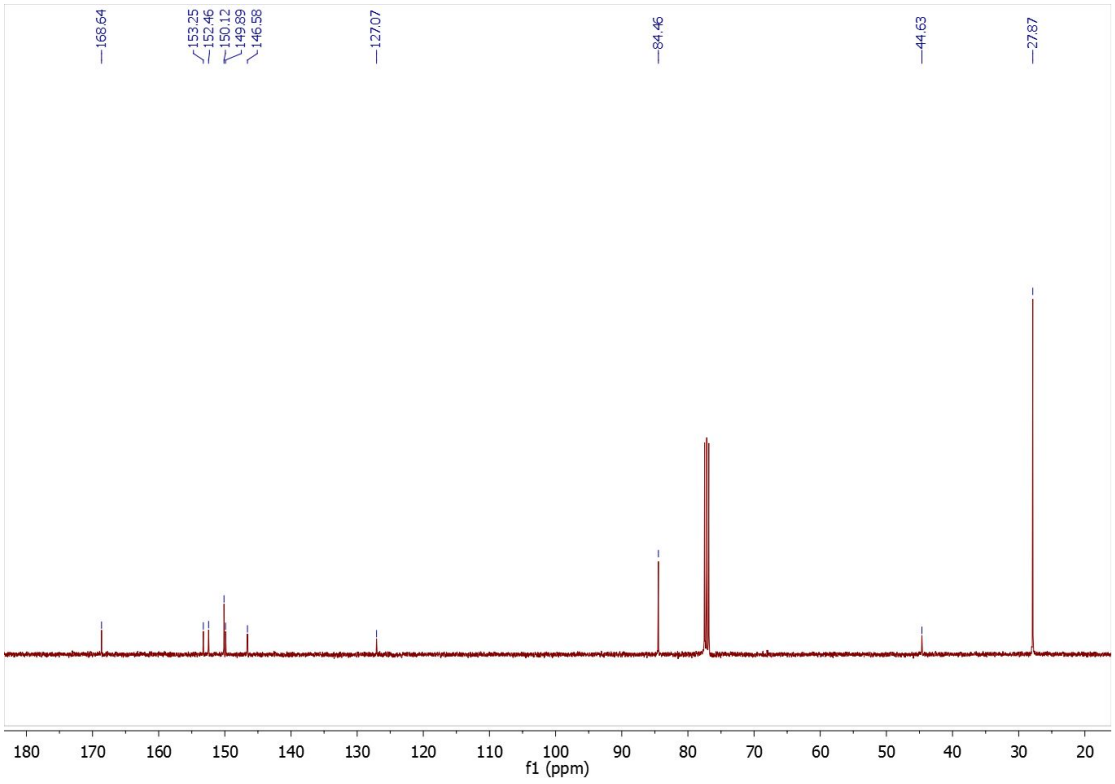
Compound **1** (^{13}C NMR)



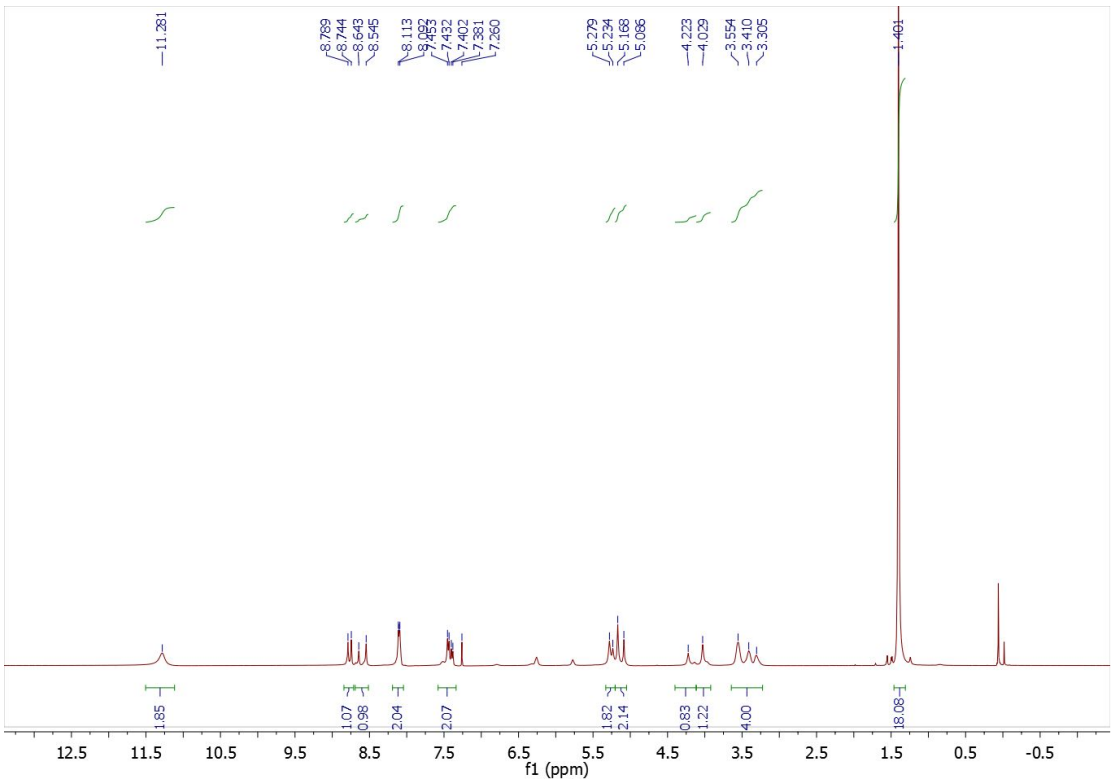
Compound **2c** (^{1}H NMR)



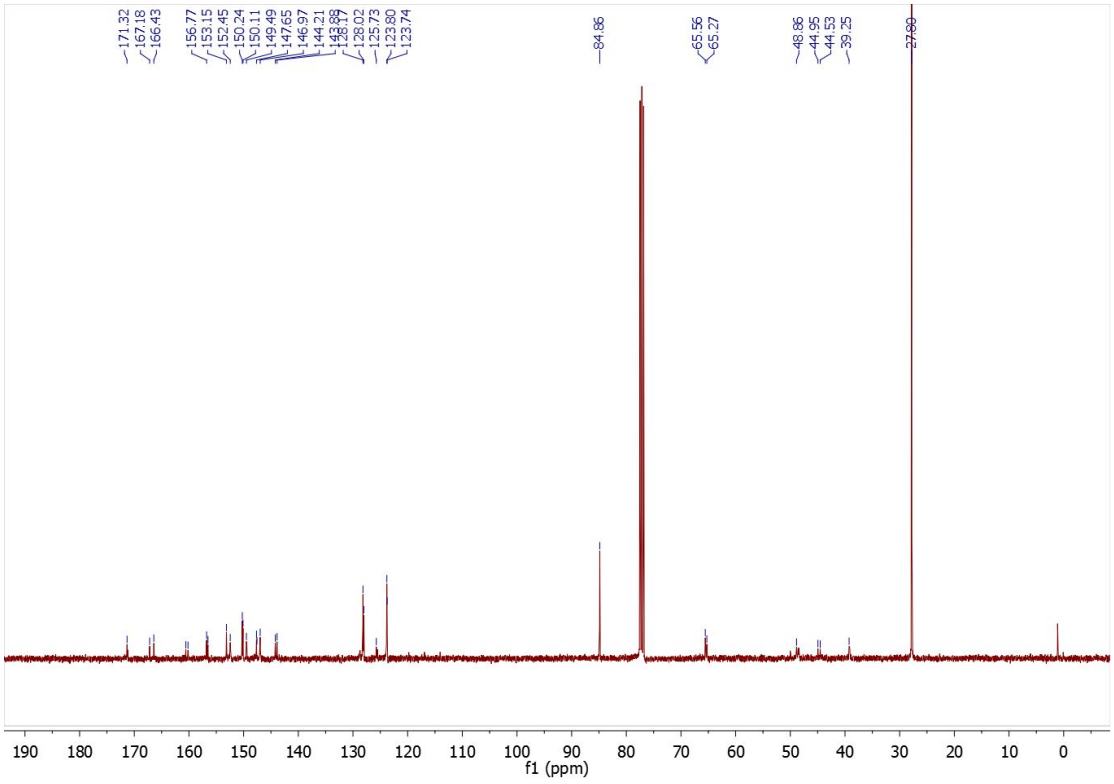
Compound **2c** (^{13}C NMR)



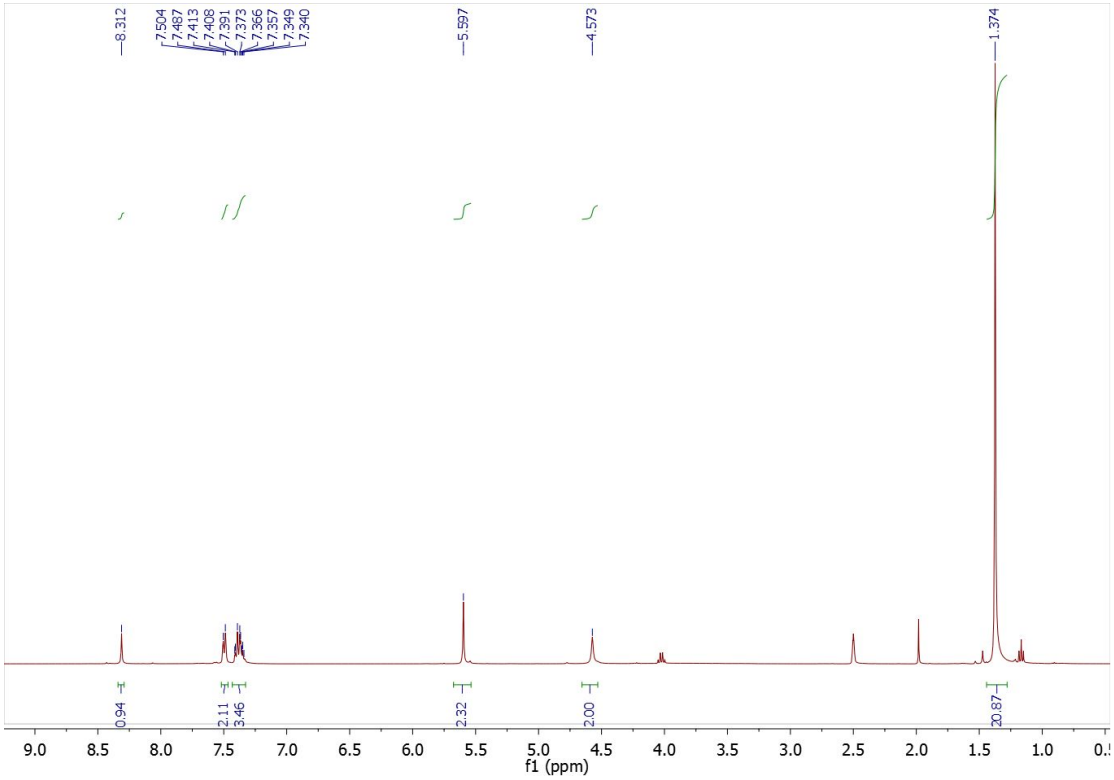
Compound **2** (^{1}H NMR)



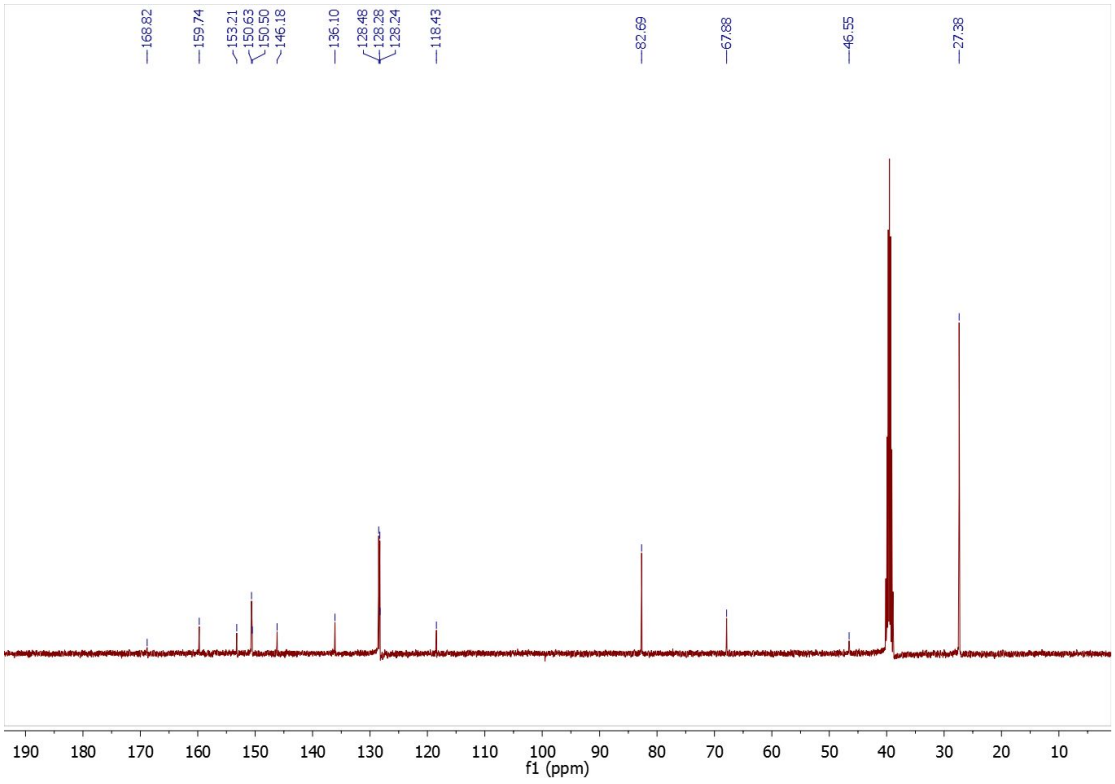
Compound **2** (^{13}C NMR)



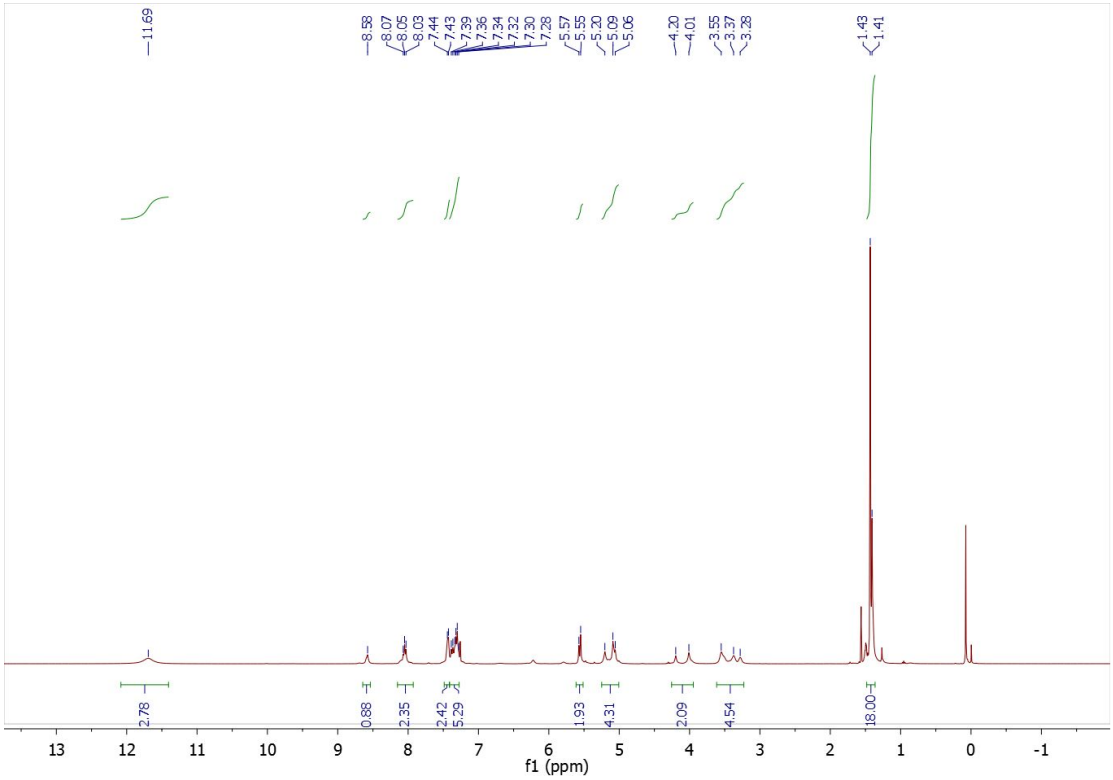
Compound **3c** (^{1}H NMR)



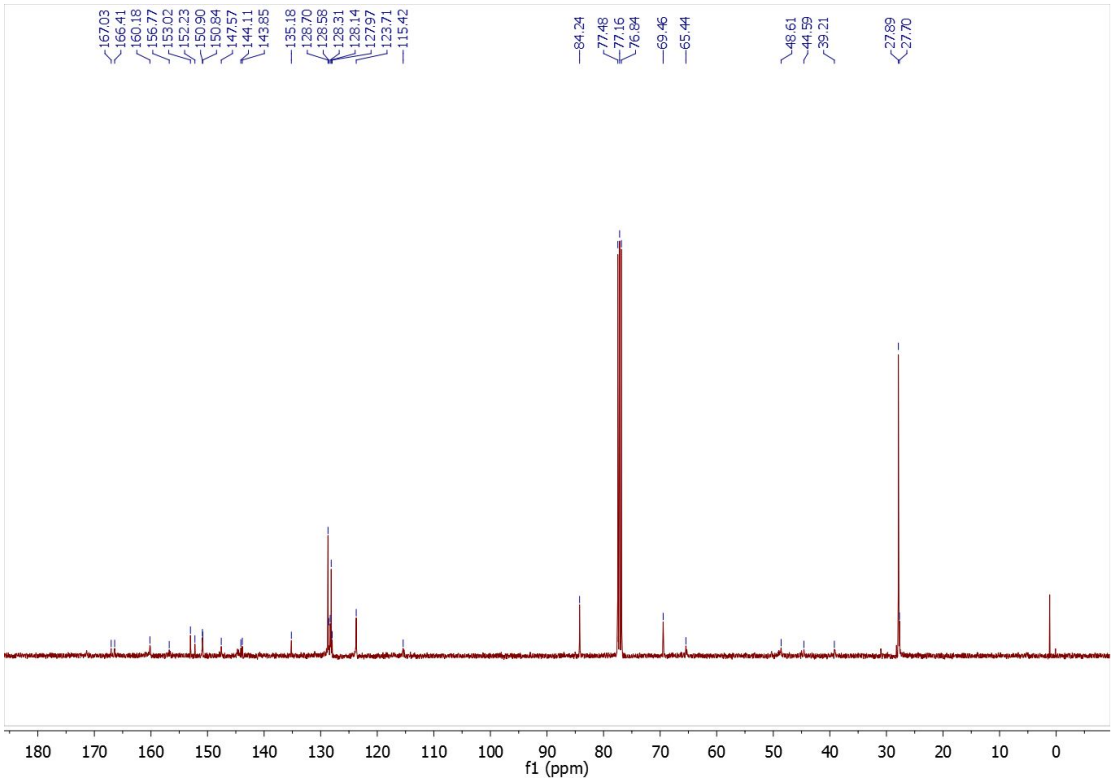
Compound **3c** (^{13}C NMR)



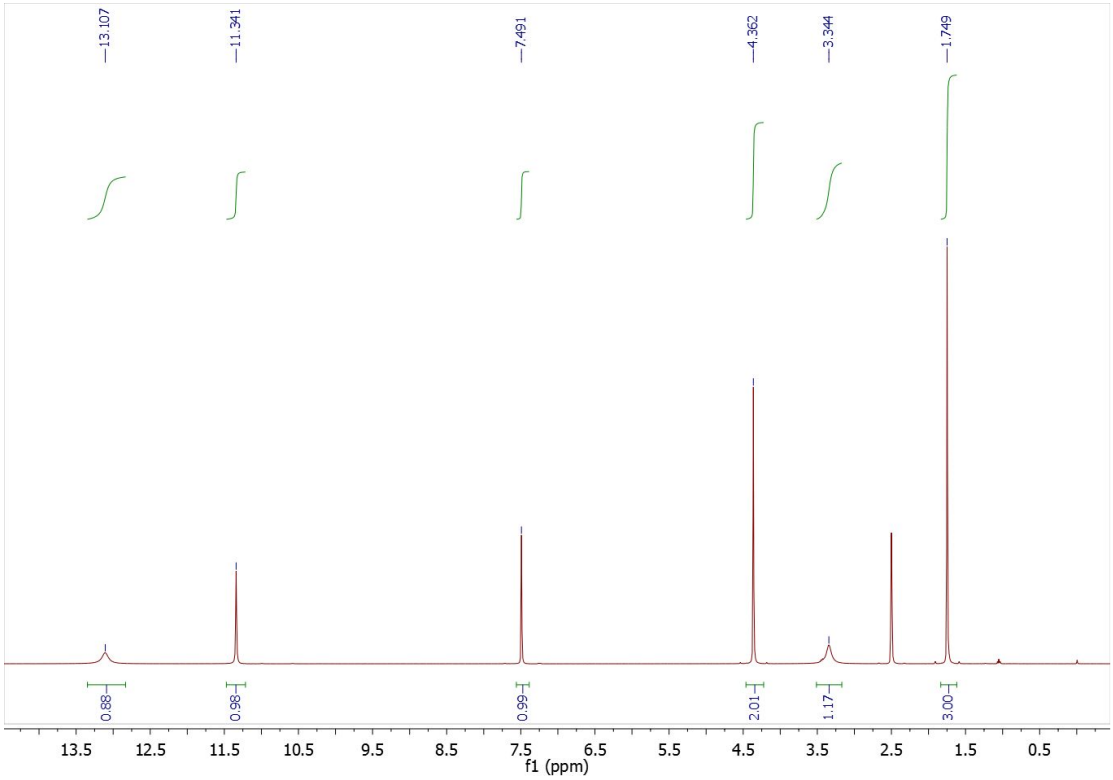
Compound **3** (^{1}H NMR)



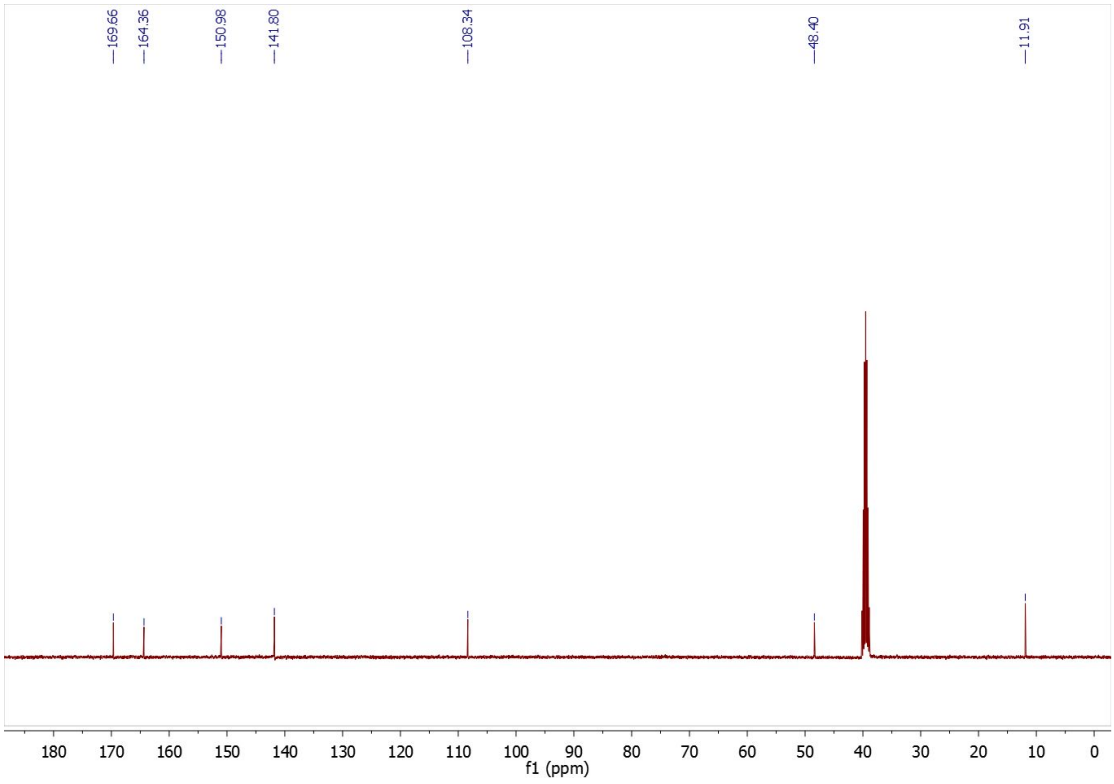
Compound **3** (^{13}C NMR)



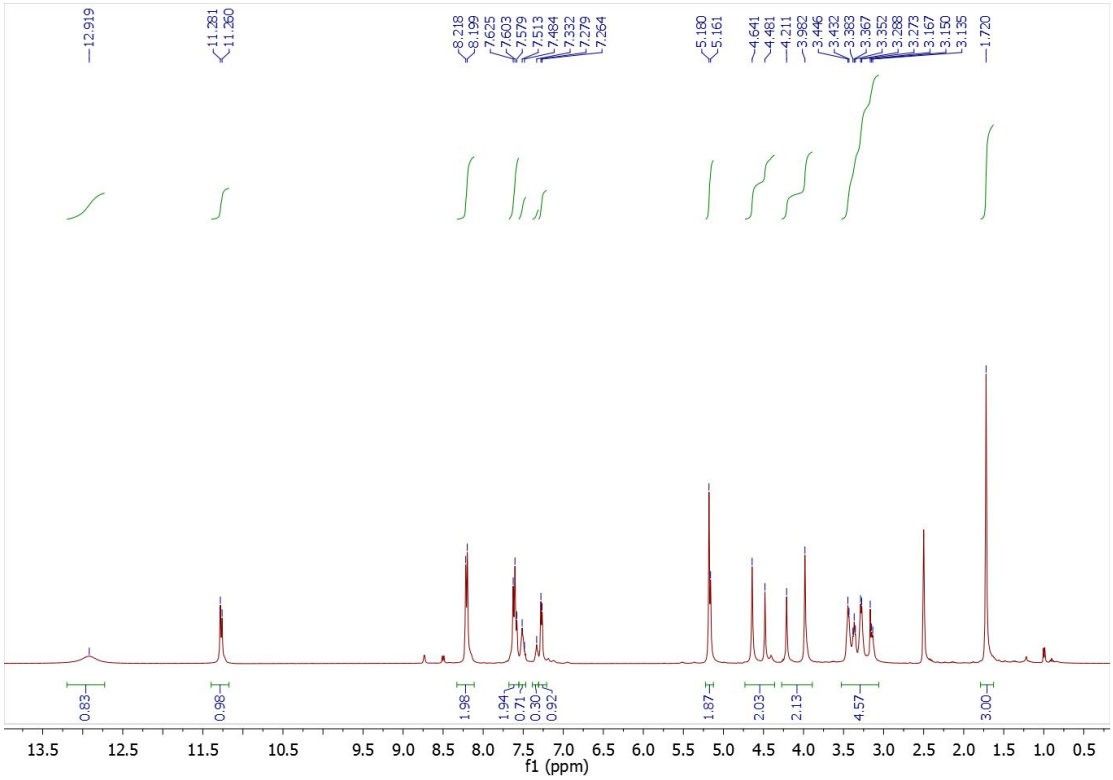
Compound **4a** (^{1}H NMR)



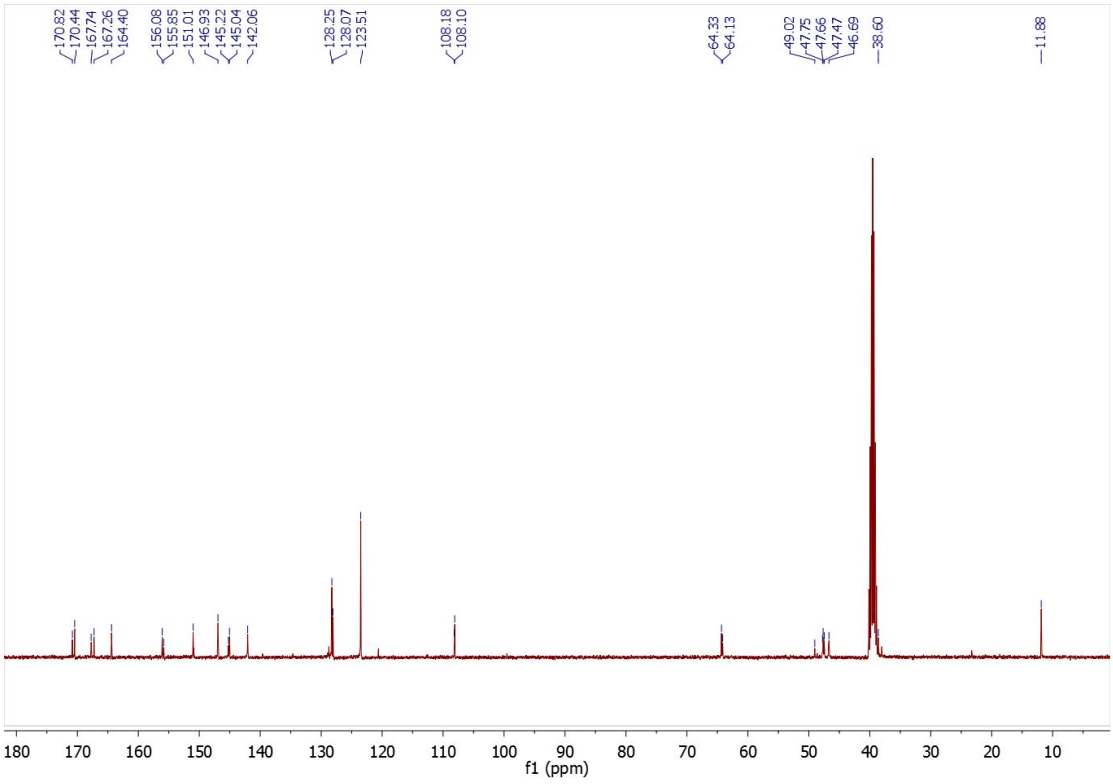
Compound **4a** (^{13}C NMR)



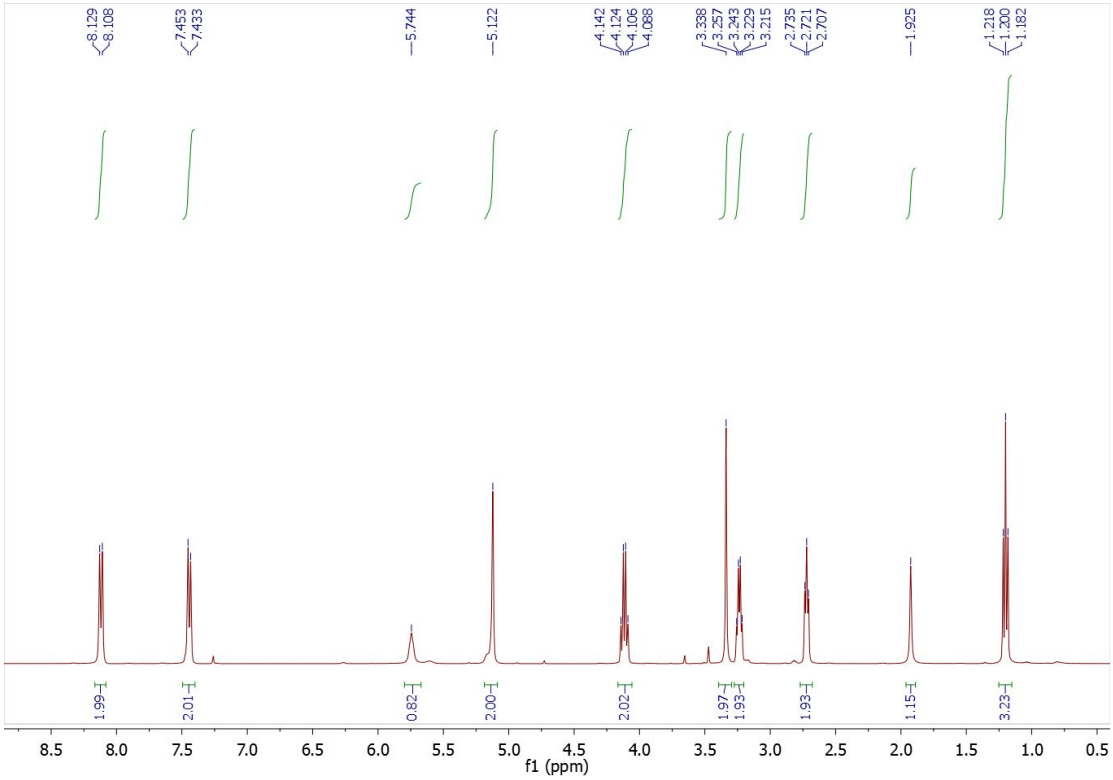
Compound **4** (^{1}H NMR)



Compound **4** (^{13}C NMR)



Compound **5** (^{1}H NMR)



Compound **5** (^{13}C NMR)