Electronic Supplementary Material (ESI) for Chemical Science.

Biologically Targeted Probes for Zn^{2+}: A Diversity Oriented Modular "Click-S_NAr-Click" Approach

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1) General Experimental Information

All commercially available reagents, unless otherwise stated, and solvents for column chromatography were used as supplied without further purification. EDTA refers to ethylenediaminetetraacetic acid. EtOH was stored over 4 Å-molecular sieves. Et_3N was stored over KOH under a N_2 atmosphere. Reaction solvents were supplied dry from an MBRAUN MB SPS-800 solvent purification system. Petrol refers to the fraction of petroleum ether boiling in the range 40-60 °C. All glassware and needles were oven dried and cooled under an inert atmosphere prior to experimental use.

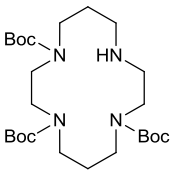
Infrared spectra were recorded in the range 4000-600 cm^{-1}, obtained directly from the compound as a solid or neat liquid on a Bruker Tensor 37 FTIR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV400 or AVIII 400 NMR spectrometer. Chemical shifts were reported in δ (ppm) and referenced to residual solvent. Multiplicities of signals are reported using standard abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, br = broad, and coupling constants measured in Hertz (Hz) and reported to 1 d.p.. Electrospray ionisation mass spectrometry was carried out by the EPSRC National Mass Spectrometry Service, University of Wales, Swansea on a Thermofisher LTQ Orbitrap XL. Melting points were measured on a Stuart SMP3 melting point apparatus and are uncorrected.

Flash column chromatography, unless stated otherwise was performed using VWR silica gel 60 (220-240 mesh), and TLC was carried out using pre-coated aluminium backed plates with Merck Kieselgel 60 F254. The plates were visualised under a UV lamp (254 nm), or by staining with basified aqueous $KMnO_4$ solution followed by gentle heating.

Fluorescence spectroscopic/spectrophotometric studies for characterisation were performed using a Jobin Yvon Horiba Fluorormax®-3 machine, in a 1 cm path length cell without an incident ray filter. Solutions of deprotected sensor were prepared from a stock solution in DMSO such that each 3 mL testing sample contained 1% DMSO. All aqueous testing was carried out using HEPES buffered water (0.1 mM, pH 7.4) at ambient temperature. Testing in MeCN was carried out using HPLC grade MeCN (VWR) which was used without any further purification.

Dynamic light scattering (DLS) measurements to determine average aggregate size of solutions of sensors **8** and **9b** were obtained on a Malvern Zetasizer Nano ZS instrument equipped with a 633 nm laser. The measurement angle was 173 degrees. Samples were prepared as for the fluorescence measurements.

Tri-Boc protected cyclam (**S1**),[1] tri-Boc protected cyclam acetylene (**S2**),[2] propargyl-dipicolylamine (**S3**),[3] diethyl iminodiacetate (**S4**),[4] (4-azidobutyl)triphenylphosphonium bromide (**S5**),[5] 3-azido-1-aminopropane (**S6**),[6] 2-chloro-*N*-(2-diethylamino-ethyl)acetamide hydrochloride (**S7**),[7] and 1-propargyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (**S8**),[8] were all prepared following previously reported literature procedures. All spectroscopic data obtained matched those previously reported.



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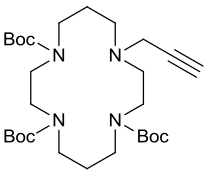

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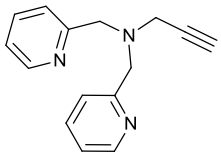

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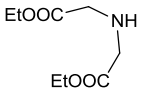

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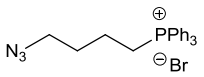

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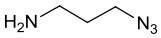

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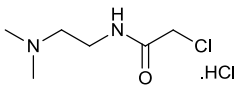

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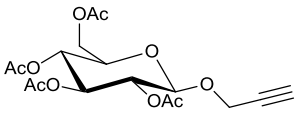
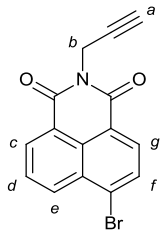


S8

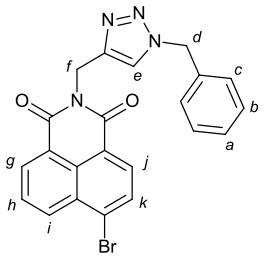
2) Experimental Procedures

6-Bromo-2-(prop-2-yn-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (2):



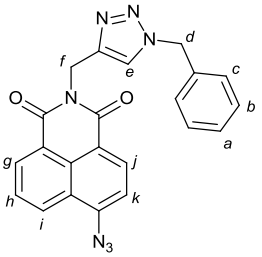
Following a modified literature procedure,[b] 4-bromo-1,8-naphthalic anhydride (1.0 g, 4.1 mmol) was dissolved in 1,4-dioxane (30 mL). One portion of propargyl amine (0.26 mL, 4.1 mmol) was added to the solution. This was stirred at r.t. for 1 h, and then heated at 70 °C for 2 h. After cooling the reaction mixture to r.t., a further portion of propargyl amine (0.05 mL, 0.8 mmol) was added, and the reaction left to stir at 70 °C overnight. After cooling to r.t, the reaction mixture was slowly poured into iced water (120 mL). The resulting precipitate was collected by suction filtration and dried *in vacuo* to give bromide **2** as a brown solid (1.1 g, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.70 (d, J = 7.3, 1H, H_c), 8.60 (d, J = 8.5, 1H, H_e), 8.46 (d, J = 7.9 , 1H, H_g), 8.06 (d, J = 7.9 , 1H, H_f), 7.87 (dd, J = 8.4, 7.4 , 1H, H_d), 4.95 (d, J = 2.4 , 2H, H_b), 2.20 (t, J = 2.4 , 1H, H_a); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 162.9, 162.9, 133.8, 132.5, 131.7, 131.2, 130.9, 130.8, 129.1, 128.2, 122.8, 121.9, 78.3, 70.7, 29.6; IR (ν_{max}/cm^{-1}) 3391, 3257, 2923, 2106, 1700, 1659, 778; M.p. (°C) 177-179; HRMS (EI) calcd for $C_{15}H_8{}^{79}BrNO_2$ $[M + H]^+$ 313.9811, found: 313.9809. UV: $\lambda_{max}(CH_2Cl_2)$/nm (ε / mol^{-1}cm^{-1}dm^3) 357 (8379), 343 (10173), 328 (6896).

2-((1-Benzyl-1H-1,2,3-triazol-4-yl)methyl)-6-bromo-1H-benzo[de]isoquinoline-1,3(2H)-dione (S9):



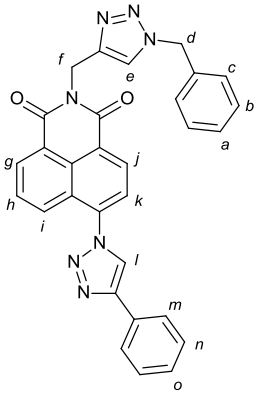
Acetylene **3** (1.0 g, 3.2 mmol), benzyl azide (0.43 g, 3.2 mmol), and CuI (60 mg, 10 mol%) were combined in a sealed CEM microwave vial which was purged with N_2. Anhydrous NMP (16 mL) was added, and the reaction stirred at r.t. for 1 h. The reaction mixture was diluted with a saturated solution of EDTA in 17.5% aqueous NH_3 (30 mL) and stirred for 5 min. The resulting precipitate was collected by suction filtration and purified by flash column chromatography (4:1 to 1:1 CH_2Cl_2/EtOAc) to yield triazole **S9** as a pale yellow powder (1.35 g, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.64 (d, *J* = 7.3, 1H, H_g), 8.55 (d, *J* = 8.5, 1H, H_i), 8.40 (d, *J* = 7.8, 1H, H_j), 8.02 (d, *J* = 7.8, 1H, H_k), 7.82 (dd, *J* = 8.5, 7.3, 1H, H_h), 7.56 (s, 1H, H_e), 7.37-7.29 (m, 4H, H_b and H_c), 7.26-7.22 (m, 1H, H_a), 5.46 (s, 4H, H_d and H_f); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 207.1, 163.5, 163.4, 134.7, 133.6, 132.5, 131.6, 131.2, 130.8, 130.8, 129.2, 128.8, 128.3, 128.2, 123.3, 123.1, 122.2, 54.3, 35.5, 31.1; IR: (ν_{max}/cm^{-1}) 3133, 1700, 1653, 1586, 1337, 1049, 959, 779. M.p. (°C) 128-131. HRMS (EI) calcd for $C_{22}H_{16}{}^{79}BrN_4O_2$ $[M + H]^+$ 447.0451, found: 447.0450. UV: $\lambda_{max}(CH_2Cl_2)$/nm (ε / mol^{-1}cm^{-1}dm^3) 358 (3459), 343 (4066), 328 (2753).

2-((1-Benzyl-1H-1,2,3-triazol-4-yl)methyl)-6-azido-1H-benzo[de]isoquinoline-1,3(2H)-dione (S10):



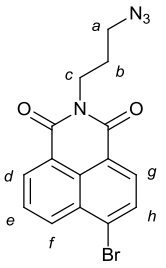
Bromo-triazole **S9** (1.4 g, 3.2 mmol), and NaN_3 (0.25 g, 3.80 mmol) were combined in a sealed CEM microwave vial which was purged with N_2 and wrapped in foil. Anhydrous NMP (16 mL) was added, and the reaction stirred at r.t. for 24 h. The reaction mixture was diluted with water (30 mL) and stirred for 5 min. The resulting precipitate was collected by suction filtration and purified by flash column chromatography (1:1 Petrol/EtOAc) to yield azide **S10** as a yellow powder (0.72 g, 55%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.64 (d, *J* = 7.2, 1H, H_g), 8.59 (d, *J* = 8.1, 1H, H_j), 8.44 (d, *J* = 8.4, 1H, H_i), 7.73 (dd, *J* = 7.4, 8.4, 1H, H_h), 7.55 (s, 1H, H_e), 7.46 (d, *J* = 8.0, 1H, H_k), 7.35-7.31 (m, 3H, H_a and H_b), 7.26-7.22 (m, 2H, H_c). 5.47 (s, 4H, H_d and H_f). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 174.6, 163.8, 163.3, 143.8, 134.7, 132.6, 132.2, 129.3, 129.2, 129.1, 128.8, 128.3, 126.9, 124.5, 123.3, 122.5, 118.8, 114.8, 54.2, 35.4. IR: (ν_{max}/cm^{-1}) 3142, 2923, 2120, 1655, 1579, 1325, 1233, 1046, 780. M.p. (°C) 160-162. HRMS (EI) calcd for $C_{22}H_{16}N_7O_2$ $[M + H]^+$ 410.1360, found: 410.1358. UV: $\lambda_{max}(CH_2Cl_2)$/nm (ε / mol^{-1}cm^{-1}dm^3) 384 (9103), 367 (11295), 344 (7235).

2-((1-Benzyl-1H-1,2,3-triazol-4-yl)methyl)-6-(4-phenyl-1H-1,2,3-triazol-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (4):



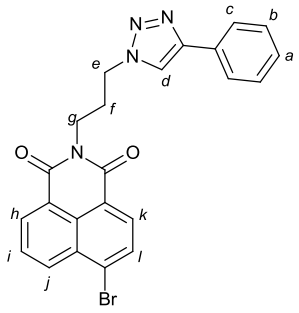
Triazole-azide **S10** (0.050 g, 0.12 mmol), phenylacetylene (0.014 mL, 0.12 mmol), CuI (2.3 mg, 10 mol%) and NaOAc (1.1 mg, 10 mol%) were combined in a sealed CEM microwave vial which was purged with N_2. Anhydrous NMP (0.61 mL) was added, and the reaction stirred at r.t. for 16 h. The reaction mixture was diluted with a saturated solution of EDTA in 17.5% aqueous NH_3 (5 mL) and stirred for 5 min. The resulting precipitate was collected by suction filtration and purified by flash column chromatography (100% CH_2Cl_2 to 1:1 CH_2Cl_2/EtOAc) to yield bis-triazole **4** as a pale yellow powder (0.051 g, 81%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.75-8.68 (m, 2H, H_g and H_j), 8.33 (d, J = 8.5, 1H, H_i), 8.25 (s, 1H, H_l), 7.96 (d, J = 7.4, 2H, H_m), 7.88 (d, J = 7.8, 1H, H_k), 7.86-7.82 (m, 1H, H_h), 7.59 (s, 1H, H_e), 7.52-7.48 (m, 2H, H_n), 7.43-7.40 (m, 1H, H_o), 7.36-7.31 (m, 3H, H_a and H_b), 7.27-7.23 (m, 2H, H_c), 5.51 (s, 2H, H_f), 5.49 (s, 2H, H_d). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 163.5, 162.9, 148.7, 143.7, 138.5, 134.7, 132.7, 131.1, 129.9, 129.7, 129.4, 129.3, 129.2, 129.0, 128.8, 128.7, 128.3, 126.7, 126.1, 123.9, 123.6, 123.3, 123.0, 121.9, 54.3, 35.6. IR: (ν_{max}/cm^{-1}) 3128, 3066, 1705, 1658, 1584, 1401, 1121, 1023, 953, 850, 781, 761, 718. M.p. (°C) >230. HRMS (EI) calcd for $C_{30}H_{22}N_7O_2$ $[M + H]^+$ 512.1829, found: 512.1829. UV: $\lambda_{max}(CH_2Cl_2)$/nm (ε / mol^{-1}cm^{-1}dm^3) 344 (9099).

2-(3-Azidopropyl)-6-bromo-1H-benzo[de]isoquinoline-1,3(2H)-dione (3):



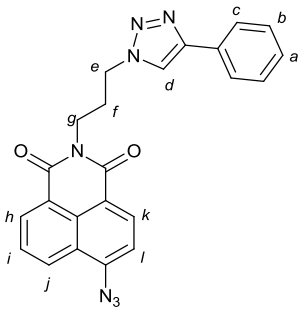
4-Bromo-1,8-naphthalic anhydride (0.25 g, 0.91 mmol) was dissolved in EtOH (30 mL). 3-azido-1-aminopropane **S6** (0.46 g, 4.6 mmol) was added to the solution and the reaction left to stir at reflux overnight. After slow cooling to r.t, the resulting precipitate was collected by suction filtration and purified by flash column chromatography (4:1 CH_2Cl_2/EtOAc) to give bromide **3** as a brown solid (0.26 g, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.67 (d, *J* = 7.2, 1H, H_d), 8.59 (d, *J* = 8.5, 1H, H_f), 8.42 (d, *J* = 7.8, 1H, H_g), 8.05 (d, *J* = 7.8, 1H, H_h), 7.86 (m, 1H, H_e), 4.28 (t, *J* = 7.0, 2H, H_c), 3.44 (t, *J* = 6.8, 2H, H_a), 2.04 (app. quint, *J* = 6.8, 2H, H_b). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 163.8, 163.8, 133.6, 132.4, 131.5, 131.3, 130.8, 130.6, 129.2, 128.3, 123.1, 122.2, 49.6, 38.2, 27.7. M.p. (°C) 92-93. HRMS (EI) calcd for $C_{15}H_{11}{}^{79}BrN_4O_2$ $[M + H]^+$ 359.0138, found 359.0141. UV: $\lambda_{max}(CH_2Cl_2)$/nm (ε / $mol^{-1}cm^{-1}dm^3$) 357 (11646), 343 (13675), 327 (9214).

6-Bromo-2-(3-(4-phenyl-1H-1,2,3-triazol-1-yl)propyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (S11):



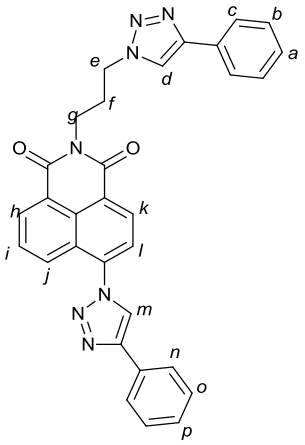
Azide **3** (0.20 g, 0.56 mmol), phenylacetylene (0.062 mL, 0.56 mmol), and CuI (10 mg, 10 mol%) were combined in a sealed CEM microwave vial which was purged with N_2. Anhydrous NMP (2.8 mL) was added, and the reaction stirred at r.t. for 1 h. The reaction mixture was diluted with a saturated solution of EDTA in 17.5% aqueous NH_3 and stirred for 5 min. The resulting precipitate was collected by suction filtration and purified by flash column chromatography (4:1 to 1:1 CH_2Cl_2/EtOAc) to yield triazole **S11** as a pale yellow powder (0.17 g, 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.65 (d, *J* = 7.3, 1H, H_h), 8.56 (d, *J* = 8.5, 1H, H_j), 8.40 (d, *J* = 7.9, 1H, H_k), 8.02 (d, *J* = 7.9, 1H, H_l), 7.94 (s, 1H, H_d), 7.83 (dd, *J* = 8.3, 7.4, 1H, H_i), 7.75 (m, 2H, H_c), 7.42-7.36 (m, 2H, H_b), 7.34-7.28 (m, 1H, H_a), 4.54 (t, *J* = 6.9, 2H, H_g), 4.33 (t, *J* = 6.6, 2H, H_e), 2.48 (app. quint, *J* = 6.8, 2H, H_f). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 163.9, 163.9, 147.8, 133.7, 132.4, 131.6, 131.3, 130.8, 130.7, 129.1, 128.9, 128.3, 128.2, 125.7, 122.9, 122.0, 120.0, 48.6, 37.9, 28.8; IR: (ν_{max}/cm^{-1}) 3142, 3061, 1698. 1659, 1586, 1568, 1460, 1345, 1225, 1055, 970, 918, 810, 767, 696. M.p. (°C) 200-202. HRMS (EI) calcd for $C_{23}H_{17}{}^{79}BrN_4O_2$ $[M + H]^+$ 461.0608, found 461.0604. UV: $\lambda_{max}(CH_2Cl_2)$/nm (ε / mol^{-1}cm^{-1}dm^3) 358 (20486), 343 (23859), 327 (15782).

6-Azido-2-(3-(4-phenyl-1H-1,2,3-triazol-1-yl)propyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (S12):



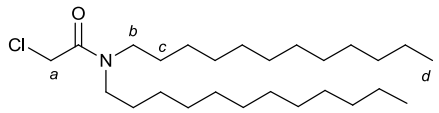
Bromo-triazole **S11** (50 mg, 0.11 mmol), and NaN_3 (9.0 mg, 1.2 equiv., 0.13 mmol) were combined in a sealed CEM microwave vial which was purged with N_2 and wrapped in foil. Anhydrous NMP (0.54 mL) was added, and the reaction stirred at r.t. for 24 h. The reaction mixture was diluted with H_2O (5 mL) and stirred for 5 min. The resulting precipitate was collected by suction filtration and purified by flash column chromatography (1:1 Petrol/EtOAc) to yield azide **S12** as a yellow powder (35 mg, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.63 (d, J = 7.3, 1H, H_h), 8.56 (d, J = 8.0, 1H, H_k), 8.44 (d, J = 8.4, 1H, H_j), 7.96 (s, 1H, H_d), 7.77-7.71 (m, 2H, H_i and H_l), 7.45-7.35 (m, 4H, H_b and H_c), 7.34-7.28 (m, 1H, H_a), 4.54 (t, J = 7.0, 2H, H_g), 4.33 (t, J = 6.6, 2H, H_e), 2.48 (app. quint, J = 6.8, 2H, H_f). Due to the instability of **S12** we were unable to obtain further characterisation data and it as used in subsequent reactions immediately.

6-(4-Phenyl-1H-1,2,3-triazol-1-yl)-2-(3-(4-phenyl-1H-1,2,3-triazol-1-yl)propyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (5):



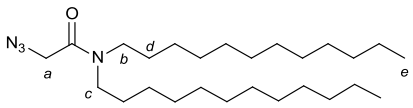
Triazole-azide **S12** (64 mg, 0.15 mmol), phenylacetylene (0.02 mL, 0.15 mmol), CuI (2.8 mg, 10 mol%) and NaOAc (1.2 mg, 10 mol%) were combined in a sealed CEM microwave vial which was purged with N_2. Anhydrous NMP (0.76 mL) was added, and the reaction stirred at r.t. for 16 h. The reaction mixture was diluted with a saturated solution of EDTA in 17.5% aqueous NH_3 (10 mL) and stirred for 5 min. The resulting precipitate was collected by suction filtration and purified by flash column chromatography (100% CH_2Cl_2 to 1:1 CH_2Cl_2/EtOAc) to yield bis-triazole **5** as a pale yellow powder (64 mg, 81%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.72 (d, J = 7.8, 1H, H_k), 8.71 (d, J = 7.2, 1H, H_h), 8.32 (d, J = 8.5, 1H, H_j), 8.21 (s, 1H, H_m), 7.97 (d, J = 7.3, 2H, H_n), 7.94 (s, 1H, H_d), 7.89-7.81 (m, 2H, H_i and H_l), 7.73 (d, J = 7.3, 2H, H_b), 7.55-7.49 (m, 2H, H_o), 7.47-7.35 (m, 3H, H_c and H_p), 7.34-7.28 (m, 1H, H_a), 4.57 (t, J = 6.8, 2H, H_g), 4.38 (t, J = 6.6, 2H, H_e), 2.53 (app. quint, J = 6.6, 2H, H_f). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 158.9, 158.9, 132.7, 131.1, 130.0, 129.7, 129.3, 129.1, 128.9, 128.8, 128.2, 126.2, 125.7, 123.6, 121.9, 120.0, 48.6, 38.2, 28.7. IR: (ν_{max}/cm^{-1}) 3126, 3064, 2923, 2112, 1698, 1654, 1583, 1517, 1465, 1432, 1400, 1348, 1232, 1188, 1152, 1057, 976, 898, 783. M.p. (°C) 185-188. HRMS (EI) calcd for $C_{31}H_{23}N_7O_2$ $[M + H]^+$ 526.1986, found 526.1979. UV: $\lambda_{max}(CH_2Cl_2)$/nm (ε / mol^{-1}cm^{-1}dm^3) 345 (30104).

2-Chloro-N,N-didodecylacetamide (S13):



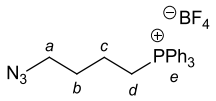
A solution of didodecylamine (2.00 g, 5.65 mmol) and NEt_3 (1.97 mL, 14.1 mmol) in dry CH_2Cl_2 (100 mL) was added in a slow dropwise manner to a solution of chloroacetyl chloride (0.54 mL, 6.79 mmol) in anhydrous CH_2Cl_2 (50 mL) at 0 °C under N_2. When the addition was complete, the red solution was warmed to r.t. and stirred for 24 h. The solution was filtered and the solid washed with chloroform (30 mL). The filtrate was further diluted with chloroform and washed with H_2O (2 x 200 mL), Na_2CO_3 solution (1 M, 2 x 200 mL), HCl solution (1 M, 2 x 200 mL) and brine (2 x 200 mL). The organic phase was dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude oil was purified by flash column chromatography (4:1 CH_2Cl_2/EtOAc) to yield chloride **S13** as a clear oil (1.86 g, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 4.05 (s, 2H, H_a), 3.36-3.22 (m, 4H, H_b), 1.65-1.47 (m, 4H, H_c), 1.36-1.17 (m, 36H, alkyl-H), 0.92-0.84 (m, 6H, H_d). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 166.2, 48.4, 46.3, 41.5, 32.0, 29.8, 29.7, 29.7, 29.6, 29.5, 29.5, 29.4, 29.4, 29.3, 27.5, 27.1, 27.0, 22.8, 14.3. IR: (ν_{max}/cm^{-1}) 2921, 2852, 1653, 1458, 1376, 1302, 1122, 925, 790. HRMS (EI) calcd for $C_{26}H_{52}ClNO$ $[M + H]^+$ 430.3810, found 430.3810.

2-Azido-N,N-didodecylacetamide (S14):



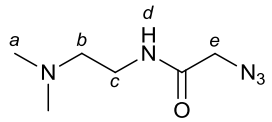
Chloride **S13** (1.86 g, 4.31 mmol) and NaN_3 (1.68 g, 25 mmol) were combined in a flask purged with N_2, dissolved in dry DMF (60 mL) and stirred for 24 h at 65 °C. After cooling to r.t, the reaction mixture was slowly poured into icy H_2O (200 mL). The resulting precipitate was extracted with EtOAc (3 x 100 mL), the organic extracts combined and washed with saturated $NaHCO_3$ solution (2 x 200 mL), brine (2 x 200 mL) and H_2O (2 x 100 mL). The organic phase was dried over $MgSO_4$ and the solvent was removed *in vacuo* to give azide **S14** as a viscous yellow oil (1.90 g, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 3.87 (s, 2H, H_a), 3.31 (t, *J* = 7.6, 2H, H_b), 3.11 (t, *J* = 7.6, 2H, H_c), 1.58-1.48 (m, 4H, H_d), 1.35-1.20 (m, 36H, alkyl-H), 0.91-0.84 (m, 6H, H_e). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 166.9, 50.4, 47.6, 46.6, 32.0, 29.7, 29.7, 29.7, 29.7, 29.6, 29.6, 29.5, 29.5, 29.4, 29.4, 29.2, 27.8, 27.1, 26.9, 22.8, 14.2. IR: (ν_{max}/cm^{-1}) 2921, 2852, 2103, 1654, 1458, 1425, 1376, 1273, 720. HRMS (EI) calcd for $C_{26}H_{53}N_4O$ $[M + H]^+$ 437.4214, found 437.4215.

(4-Azidobutyl)triphenylphosphonium tetrafluoroborate (S15):



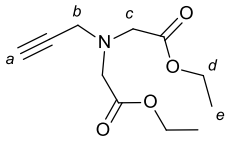
(4-Azidobutyl)triphenylphosphonium bromide **S5** (2.27 g, 5.15 mmol) was dissolved in CH_2Cl_2 (40 mL). This solution was thoroughly washed with a saturated aqueous solution of ammonium tetrafluoroborate (50 mL). The organic phase was dried over $MgSO_4$ and the solvent was removed *in vacuo* to yield tetrafluoroborate **S15** as a white solid (2.18 g, 95%)**.** ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.86-7.65 (m, 15H, H_e), 3.46-3.28 (m, 4H, H_a and H_d), 1.96-1.85 (m, 2H, H_b), 1.78-1.63 (m, 2H, H_c). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 135.0 ($J_{C\text{-}P}$ = 2.9), 133.2 ($J_{C\text{-}P}$ = 9.9), 130.4 ($J_{C\text{-}P}$ = 12.7), 117.9 ($J_{C\text{-}P}$ = 86.4), 50.1, 28.8 ($J_{C\text{-}P}$ = 16.9), 21.2 ($J_{C\text{-}P}$ = 52.0), 19.5 ($J_{C\text{-}P}$ = 3.9). IR: (ν_{max}/cm^{-1}) 2943, 2923, 2877, 2098, 1588, 1486, 1438, 1342, 1276, 1239, 1025, 749, 722, 689. M.p. (°C) 115-118. HRMS (EI) calcd for $C_{22}H_{23}N_3P_1$ $[M - BF_4]^+$ 360.1624, found 360.1627.

2-Azido-*N*-(2-diethylamino-ethyl)acetamide (S16):



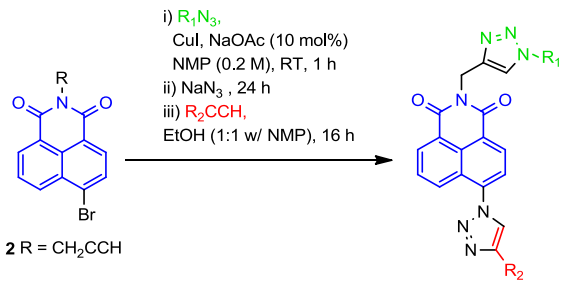
2-Chloro-*N*-(2-diethylamino-ethyl)acetamide hydrochloride **S7** (0.83 g, 2.50 mmol) was dissolved in H_2O (12.5 mL), NaN_3 (1.61 g, 15.0 mmol) was added to the flask, and the reaction mixture stirred for 48 h at 65 °C. After cooling, the solution was adjusted to pH 10 using aqueous $NaHCO_3$ (1.0 M), and extracted with EtOAc (3 x 20 mL). The remaining aqueous layer was adjusted to pH 14 with aqueous NaOH (1.0 M), and extracted with EtOAc (3 x 20 mL). All organic extracts were combined, dried over $MgSO_4$ and the solvent was removed *in vacuo* (400 mbar – volatile product) to give azide **S16** as a pale yellow oil (0.31 g, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 6.82 (br s, 1H, H_d), 3.96 (s, 2H, H_e), 3.34 (q, *J* = 5.9, 2H, H_c), 2.42 (t, *J* = 6.0, 2H, H_b), 2.23 (s, 6H, H_a). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 166.8, 57.8, 52.8, 45.3, 36.9. IR: (ν_{max}/cm^{-1}) 3292 (b), 3079, 2978, 2948, 2872, 2783, 2101, 1658, 1541, 1461, 1252, 1189, 1166, 1040, 906, 849, 776, 645. HRMS (EI) calcd for $C_6H_{13}N_5O$ $[M + H]^+$ 172.1193, found 172.1191.

Diethyl 2,2'-(prop-2-yn-1-ylazanediyl)diacetate (S17):



Diethyl iminodiacetate **S4** (1.00 g, 5.28 mmol) was dissolved in MeCN (30 mL). K_2CO_3 (1.46 g, 10.5 mmol) was added, and propargyl bromide (0.94 mL, 6.34 mmol) was added slowly to the stirring reaction mixture and refluxed for 16 h. After cooling, the suspension was filtered, and the solvent removed *in vacuo*. The crude oil was purified by flash column chromatography (2:8 EtOAc/Petrol) to yield alkyne **S17** as a colourless oil (0.71 g, 58%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 4.17 (q, *J* = 7.1, 4H, H_d), 3.66 (d, *J* = 2.3, 2H, H_b), 3.54 (s, 4H, H_c), 2.25 (t, *J* = 2.4, 1H, H_a), 1.27 (t, *J* = 7.1, 6H, H_e). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 170.5, 78.4, 73.8, 60.8, 54.4, 43.4, 14.3.IR: (ν_{max}/cm^{-1}) 3278, 2978, 2870, 2105, 1733, 1444, 1372, 1190, 1027, 990. HRMS (EI) calcd for $C_{11}H_{18}N_1O_4$ $[M + H]^+$ 228.1230, found 228.1224.

General procedure A: a one-pot synthesis of a sensor from building block 2.



Acetylene **2** (314 mg, 1.0 mmol), an azide (1.0 mmol), CuI (19.0 mg, 10 mol%) and NaOAc (8.2 mg, 10 mol%) were combined in a sealed CEM microwave vial which was wrapped in foil and purged with N_2. Anhydrous NMP was added, and the reaction was stirred at r.t. for 1 h. Upon complete consumption of **2** (determined by ^{1}H NMR spectroscopy), NaN_3 (78 mg, 1.2 mmol) was added in a single portion to the reaction vial, which was resealed and the reaction stirred for a further 24 h. Upon completion of azide formation (determined by ^{1}H NMR spectroscopy), an acetylene (1 mmol) and EtOH (1:1 w/ NMP) were added to the reaction vial, and the final mixture was stirred for a further 16 h, at which point the reaction was complete (determined by ^{1}H NMR spectroscopy).

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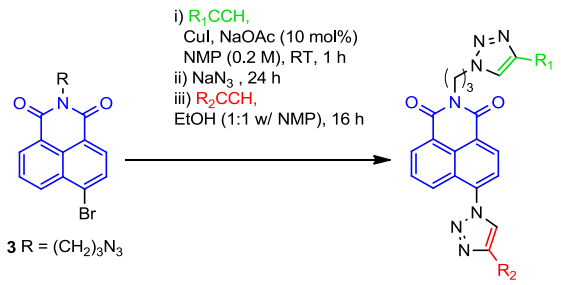
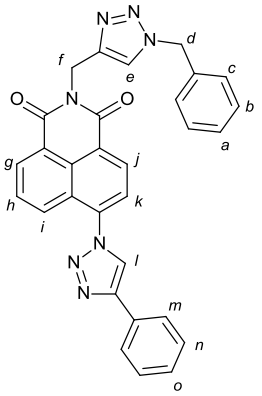
General procedure B: a one-pot synthesis of a sensor from building block 3.



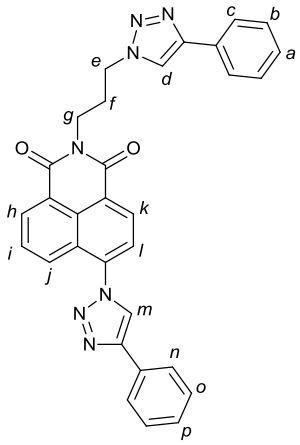
Azide **3** (359 mg, 1.0 mmol), an acetylene (1.0 mmol), CuI (19.0 mg, 10 mol%) and NaOAc (8.2 mg, 10 mol%) were combined in a sealed CEM microwave vial which was wrapped in foil and purged with N_2. Anhydrous NMP was added, and the reaction was stirred at r.t. for 1 h. Upon complete consumption of **3** (determined by ^{1}H NMR spectroscopy), NaN_3 (78 mg, 1.2 mmol) was added in a single portion to the reaction vial, which was resealed and the reaction stirred for a further 24 h. Upon completion of azide formation (determined by ^{1}H NMR spectroscopy), an acetylene (1 mmol) and EtOH (1:1 w/ NMP) were added to the reaction vial, and the final mixture was stirred for a further 16 h, at which point the reaction was complete (determined by ^{1}H NMR spectroscopy).

2-((1-Benzyl-1H-1,2,3-triazol-4-yl)methyl)-6-(4-phenyl-1H-1,2,3-triazol-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (4):



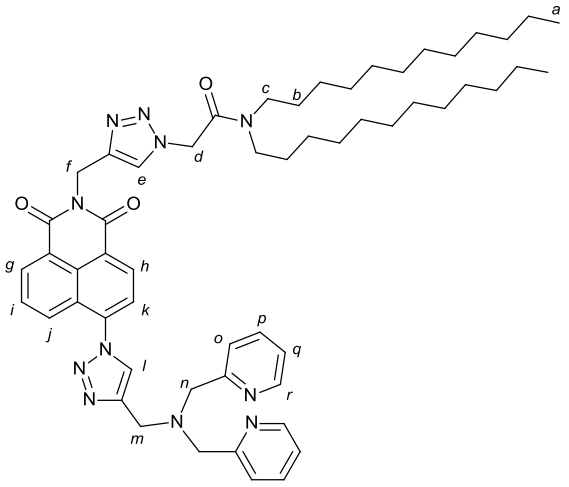
General procedure A was employed with **2** (0.31 g, 1.0 mmol), benzyl azide (0.13 g, 1.0 mmol) in NMP (3.33 mL), followed by phenylacetylene (0.11 mL, 1.0 mmol) and EtOH (3.33 mL). The crude product was obtained obtained *via* precipitation with a saturated solution of EDTA in 17.5% aqueous NH_3 (20 mL) and purified by flash column chromatography (100% CH_2Cl_2 to CH_2Cl_2/EtOAc 1:1) to yield bis-triazole **4** as a pale yellow powder (0.43 g, 85%). All spectroscopic data in accordance with double triazole **4** *vide supra*.

6-(4-Phenyl-1H-1,2,3-triazol-1-yl)-2-(3-(4-phenyl-1H-1,2,3-triazol-1-yl)propyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (5):



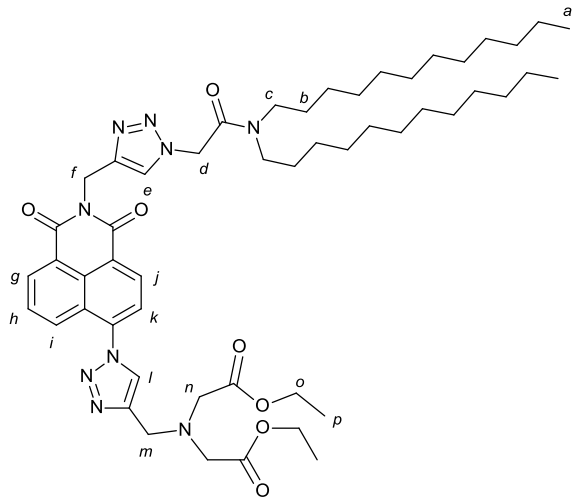
General procedure B was employed with **3** (0.36 g, 1.0 mmol)**,** phenylacetylene (0.11 mL, 1.0 mmol) in NMP (3.33 mL), followed by a second portion of phenylacetylene (0.11 mL, 1.0 mmol) and EtOH (3.33 mL). The crude product was obtained obtained *via* precipitation with a saturated solution of EDTA in 17.5% aqueous NH_3 (20 mL) and purified by flash column chromatography (100% CH_2Cl_2 to CH_2Cl_2/EtOAc 1:1) to yield bis-triazole **5** a pale yellow powder (0.37 g, 72%). All spectroscopic data in accordance with double triazole **5** *vide supra*.

2-(4-((6-(4-((bis(Pyridin-2-ylmethyl)amino)methyl)-1H-1,2,3-triazol-1-yl)-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)methyl)-1H-1,2,3-triazol-1-yl)-N,N-didodecylacetamide (6):



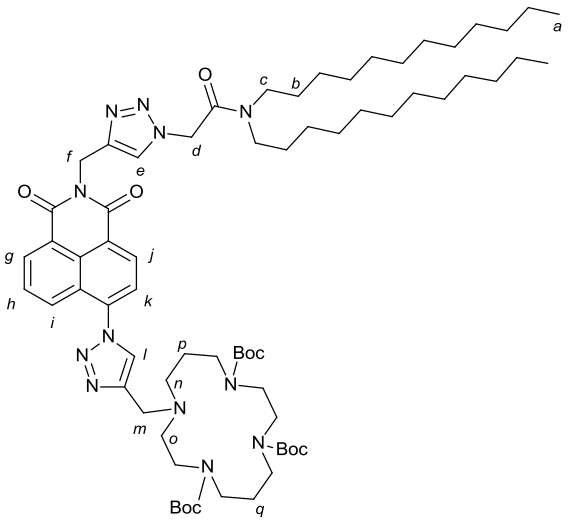
General procedure A was employed with **2** (314 mg, 1.00 mmol)**,** azide **S14** (0.48 g, 1.0 mmol) in NMP (3.33 mL), followed by propargyl dipicolylamine **S3** (0.29 g, 1.1 mmol) and EtOH (3.33 mL). Crude product obtained after dilution of the reaction mixture with CH_2Cl_2 (10 mL), and washing the organic mixture with a saturated solution of EDTA in 17.5% aqueous NH_3 (3 x 15 mL). The organic phase was dried over $MgSO_4$, concentrated *in vacuo* and purified by flash column chromatography on alumina (99:1 CH_2Cl_2/MeOH) to yield sensor **6** as a brown oil (0.78 g, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.68 (d, *J* = 7.8, 1H, H_h), 8.67 (d, *J* = 7.1, 1H, H_g), 8.55-8.51 (m, 2H, H_r), 8.25 (d, *J* = 8.5, 1H, H_j), 8.15 (s, 1H, H_l), 7.91 (s, 1H, H_e), 7.82-7.75 (m, 2H, H_i and H_k), 7.66 (app. td, *J* = 7.7, 1.6, 2H, H_p), 7.62-7.57 (m, 2H, H_o), 7.18-7.12 (m, 2H, H_q), 5.52 (s, 2H, H_f), 5.14 (s, 2H, H_d), 4.06 (s, 2H, H_m), 3.94 (s, 4H, H_n), 3.31-3.23 (m, 4H, H_c), 1.64-1.43 (m, 4H, H_b), 1.34-1.12 (m, 36H, alkyl-H), 0.85 (app. t, 6H, H_a). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 164.3, 163.4, 162.9, 159.1, 149.3, 145.2, 143.4, 138.5, 136.6, 132.5, 131.1, 129.9, 129.3, 128.5, 126.5, 125.7, 125.3, 123.7, 123.5, 122.9, 122.3, 59.9, 50.8, 48.6, 47.9, 46.8, 35.5, 31.9, 29.7, 29.7, 29.6, 29.6, 29.5, 29.4, 29.3, 29.2, 27.6, 27.1, 26.9, 22.8, 14.2. IR: (ν_{max}/cm^{-1}) 2922, 2852, 1703, 1661, 1587, 1467, 1430, 1369, 1233, 1040, 995, 783. HRMS (EI) calcd for $C_{56}H_{76}N_{11}O_3$ $[M + H]^+$ 950.6127, found 950.6117. UV: $\lambda_{max}(CH_2Cl_2)$/nm (ε/ mol^{-1}cm^{-1}dm^3) 344 (20365)

Diethyl 2,2'-(((1-(2-((1-(2-(didodecylamino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl)-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-6-yl)-1H-1,2,3-triazol-4-yl)methyl)azanediyl)diacetate (7):



General procedure A was employed with **2** (314 mg, 1.00 mmol), azide **S14** (0.48 g, 1.0 mmol) in NMP (3.33 mL), followed by acetylene **S17** (0.59 g, 1.1 mmol) and EtOH (3.33 mL). Crude product obtained after dilution of the reaction mixture with CH_2Cl_2 (10 mL), and washing the organic mixture with a saturated solution of EDTA in 17.5% aqueous NH_3 (3 x 15 mL). The organic phase was dried over $MgSO_4$, concentrated *in vacuo* and purified by flash column chromatography (4:1 CH_2Cl_2/EtOAc to EtOAc to 4:1 EtOAc/MeOH) to yield **7** as an orange foam (0.72 g, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.70 (d, *J* = 7.8, 1H, H_j), 8.69 (d, *J* = 7.2, 1H, H_g), 8.28 (d, *J* = 8.6, 1H, H_i), 8.13 (s, 1H, H_l), 7.91 (s, 1H, H_e), 7.92-7.78 (m, 2H, H_h and H_k), 5.54 (s, 2H, H_f), 5.15 (s, 2H, H_d), 4.23 (s, 2H, H_m), 4.18 (q, *J* = 7.1, H_o), 3.69 (s, 4H, H_n), 3.33-3.22 (m, 4H, H_c), 1.65-1.41 (m, 4H, H_b), 1.35-1.14 (m, 42H, H_p (6H) and alkyl-H (36H)), 0.90-0.80 (m, 6H, H_a). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 171.2, 164.3, 163.4, 162.9, 146.4, 143.5, 138.5, 132.6, 131.1, 129.9, 129.3, 128.6, 126.5, 125.7, 125.4, 123.9, 123.6, 122.9, 65.9, 60.8, 55.1, 50.9, 49.3, 48.0, 46.9, 35.6, 32.0, 29.8, 29.7, 29.7, 29.6, 29.4, 29.4, 29.3, 27.6, 27.1, 26.9, 22.8, 15.4, 14.4, 14.2. IR: (ν_{max}/cm^{-1}) 3145, 2921, 2852, 1739, 1704, 1659, 1585, 1465, 1369, 1232, 1184, 1026, 949, 783. M.p. (°C) 48-51. HRMS (EI) calcd for $C_{52}H_{78}N_9O_7$ $[M + H]^+$ 940.6019, found 940.6010. UV: $\lambda_{max}(CH_2Cl_2)$/nm (ε/ mol^{-1}cm^{-1}dm^3) 344 (18804) .

Tri-*tert*-butyl 11-((1-(2-((1-(2-(didodecylamino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl)-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-6-yl)-1H-1,2,3-triazol-4-yl)methyl)-1,4,8,11-tetraazacyclotetradecane-1,4,8-tricarboxylate (8a):



General procedure A was employed with **2** (314 mg, 1.00 mmol)**,** azide **S14** in NMP (3.33 mL), followed by cyclam acetylene **S2** (0.59 g, 1.1 mmol) and EtOH (3.33 mL). Crude product was obtained by dilution of the reaction mixture with CH_2Cl_2 (10 mL), and washing the organic mixture with a saturated solution of EDTA in 17.5% aqueous NH_3 (3 x 15 mL). The organic phase was dried over $MgSO_4$, concentrated *in vacuo* and purified by flash column chromatography (4:1 CH_2Cl_2/EtOAC to EtOAc to 4:1 EtOAc/MeOH) to yield **8a** as a brown oil (0.93 g, 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.67-8.59 (m, 2H, H_g and H_j), 8.20 (app. br s, 1H, H_i), 7.98-7.72 (m, 4H, H_e and H_h, H_l and H_k), 5.47 (s, 2H, H_f), 5.09 (s, 2H, H_d), 3.89 (br s, 2H, H_m), 3.50-3.08 (m, 16H, H_c and Cyclam-H (12H)), 2.70-2.62 (m, 2H, H_o), 2.54-2.48 (m, 2H, H_n), 1.92-1.78 (m, 2H, H_q), 1.76-1.66 (m, 2H, H_p), 1.60-0.93 (m, 67H, H_b (4H), ^{t}Bu (27H) and alkyl-H (36H)), 0.86-0.72 (m, 6H, H_a). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 164.2, 163.4, 162.8, 155.9, 155.6, 143.4, 138.4, 132.5, 131.1, 129.8, 129.2, 128.7, 126.5, 125.3, 123.7, 123.5, 122.9, 79.7, 50.8, 47.9, 46.8, 35.5, 31.9, 29.7, 29.6, 29.6, 29.5, 29.4, 29.3, 29.2, 28.6, 28.5, 27.6, 27.0, 26.9, 22.7, 14.2. IR: (ν_{max}/cm^{-1}) 2927, 2855, 1691, 1588, 1466, 1414, 1365, 1236, 1166, 782. HRMS (EI) calcd for $C_{29}H_{48}N_3O_2$ $[M + H]^+$ 1251.8149, found 1251.8577. UV: $\lambda_{max}(CH_2Cl_2)$/nm (ε / mol^{-1}cm^{-1}dm^3) 344 (25178).

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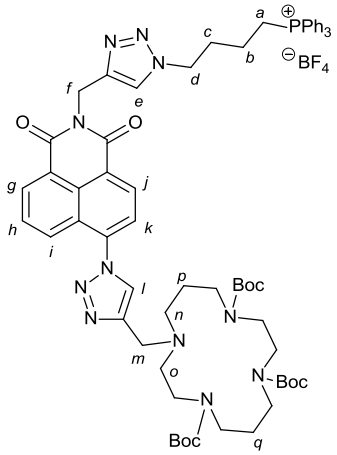
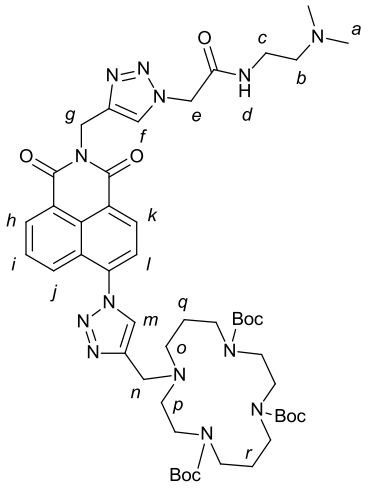
(4-(4-((1,3-Dioxo-6-(4-((4,8,11-tris(tert-butoxycarbonyl)-1,4,8,11-tetraazacyclotetradecan-1-yl)methyl)-1H-1,2,3-triazol-1-yl)-1H-benzo[de]isoquinolin-2(3H)-yl)methyl)-1H-1,2,3-triazol-1-yl)butyl)triphenylphosphonium (9a):



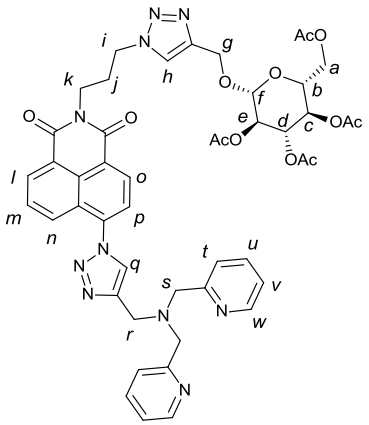
General procedure A was employed with **2** (314 mg, 1.00 mmol)**,** azide **S15** (0.45 g, 1.0 mmol) in NMP (5 mL), followed by cyclam acetylene **S2** (0.59 g, 1.1 mmol) and EtOH (5 mL). Crude product obtained by dilution of the reaction mixture with CH_2Cl_2 (10 mL), and washing the organic mixture with a saturated solution of EDTA in 17.5% aqueous NH_3 (3 x 15 mL). The organic phase was dried over $MgSO_4$, concentrated *in vacuo* and triturated with EtOAc (3 x 30 mL). The resulting suspension was filtered, the filtrate concentrated *in vacuo* and purified by flash column chromatography (CH_2Cl_2/MeOH 99:1 to 9:1) to yield **9a** as a yellow foam (0.84 g, 66%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.65-8.61 (m, 2H, H_g and H_j), 8.26-8.21 (m, 1H, H_i), 8.02 (br s, 1H, H_l), 7.88-7.83 (m, 1H, H_k), 7.80 (dd, *J* = 7.4, 8.4, 1H, H_h), 7.75 (s, 1H, H_e), 7.75-7.59 (m, 15H, 3 x Ph), 5.41 (s, 2H, H_f), 4.38 (t, *J* = 6.5, 2H, H_d), 3.93 (br s, 2H, H_m), 3.45-3.25 (m, 14H, cyclam-H (12H) and H_c), 2.74-2.68 (m, 2H, H_o), 2.55 (t, *J* = 5.5, 2H, H_n), 2.16 (quint, *J* = 6.7, 2H, H_b), 1.93-1.86 (m, 2H, H_q), 1.80-1.72 (m, 2H, H_p), 1.63-1.51 (m, 2H, H_a), 1.48-1.30 (m, 27H, 3 x Boc). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 163.5, 163.0, 155.7, 143.6, 138.6, 135.3, 135.3, 133.6, 133.5, 132.6, 131.2, 130.7, 130.6, 129.3, 128.7, 123.7, 123.7, 122.9, 118.4, 117.5, 79.8, 48.8, 35.7, 30.0, 29.8, 28.7, 28.6, 21.5, 21.0, 19.4, 19.3 (overlapping signals). IR: (ν_{max}/cm^{-1}) 2981, 2930, 1684, 1664, 1588, 1478, 1441, 1411, 1364, 1237, 1157, 1111, 1050, 1049, 882. HRMS (EI) calcd for $C_{29}H_{48}N_3O_2$ $[M - BF_4]^+$ 1174.6002, found 1174.5983. UV: $\lambda_{max}(CH_2Cl_2)$/nm (ε/ mol^{-1}cm^{-1}dm^3) 344 (3139)

tri-*tert*-Butyl 11-((1-(2-((1-(2-((2-(dimethylamino)ethyl)amino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl)-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-6-yl)-1H-1,2,3-triazol-4-yl)methyl)-1,4,8,11-tetraazacyclotetradecane-1,4,8-tricarboxylate (10a):



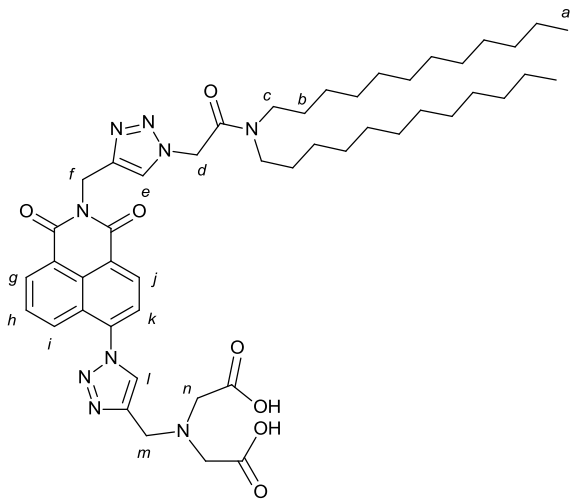
General procedure A was employed with **2** (314 mg, 1.0 mmol)**,** azide **S16** (0.17 g, 1.0 mmol) in NMP (5 mL), followed by cyclam acetylene **S2** (0.59 g, 1.1 mmol) and EtOH (5 mL). The crude product was obtained by dilution of the reaction mixture with CH_2Cl_2 (10 mL), and washing the organic mixture with a saturated solution of EDTA in 17.5% aqueous NH_3 (3 x 15 mL). The organic phase was dried over $MgSO_4$, concentrated *in vacuo* and purified by flash column chromatography on alumina (EtOAC to 9:1 EtOAc/MeOH) to yield **10a** as a brown oil (0.67 g, 68%). ^{1}H NMR (400 MHz, $CDCl_3$) 8.75-8.69 (m, 2H, H_h and H_k), 8.34-8,26 (m, 1H,H_j), 7.98-7.82 (m, 4H, H_f, H_i, H_l and H_m), 6.49 (br s, 1H, H_d), 5.56 (s, 2H, H_g), 4.99 (s, 2H, H_e), 3.96 (br s, 2H, H_n), 3.49-3.25 (m, 12H, cyclam-H), 3.29 (q, *J* = 5.8, 2H, H_c), 2.70 (t, *J* = 5.2, 2H, H_p), 2.58 (t, *J* = 5.8, 2H, H_o), 2.35 (t, *J* = 6.0, 2H, H_b), 2.15 (s, 6H, H_a (2 x CH_3), 1.95-1.70 (m, 4H, H_p + H_q), 1.49-1.35 (m, 27H, 3 x ^{t}Bu). δ_H ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 164.9, 163.5, 163.0, 143.9, 132.7, 131.2, 130.2, 130.1, 130.0, 129.4, 128.8, 126.6, 124.9, 123.7, 123.7, 122.9, 79.8, 57.4, 53.2, 49.6, 45.1, 37.2, 35.5, 30.8, 28.7, 28.6, 17.8 (overlapping signals). IR: (ν_{max}/cm^{-1}) 3409, 2966, 2919, 2850, 1691, 1688, 1593, 1476, 1420, 1369, 1260, 1165, 1096, 1017, 864, 801. HRMS (EI) calcd for $C_{49}H_{71}N_{13}O_9$ [M + H]$^+$ 986.5570, found 986.5562. UV: λ_{max}(CH_2Cl_2)/nm (ε/ mol^{-1}cm^{-1}dm^3) 344 (43812).

(2*R*,3*R*,4*S*,5*R*,6*R*)-2-(Acetoxymethyl)-6-((1-(3-(6-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1H-1,2,3-triazol-1-yl)-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)propyl)-1H-1,2,3-triazol-4-yl)methoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (11a):



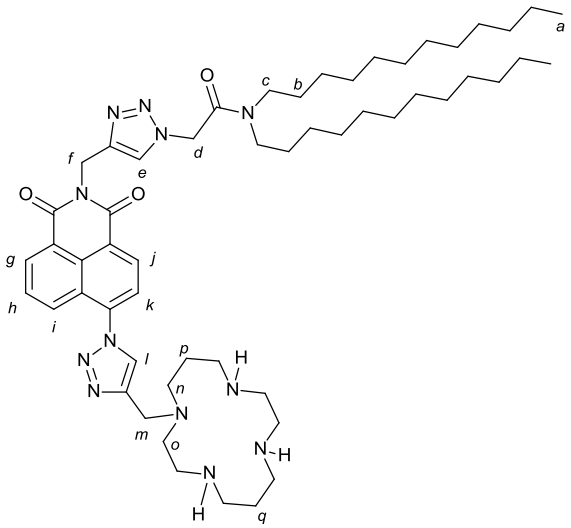
General procedure B was employed with **3** (359 mg, 1.00 mmol)**,** propargyl-glucose **S8** (0.36 g, 1.0 mmol) in NMP (3.33 mL), followed by propargyl dipicolylamine **S3** (0.26 g, 1.1 mmol) and EtOH (3.33 mL). Crude product obtained by dilution of the reaction mixture with CH_2Cl_2 (10 mL), and washing the organic mixture with a saturated solution of EDTA in 17.5% aqueous NH_3 (3 x 15 mL). The organic phase was dried over $MgSO_4$, concentrated *in vacuo* and purified by flash column chromatography on alumina (gradient from 99:1 to 90:10 CH_2Cl_2/MeOH) to yield **11a** as a yellow foam (0.58 g, 61%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.75-8.70 (m, 2H, H_l + H_o), 8.58-8.54 (m, 2H, H_w), 8.32 (dd, *J* = 8.6, 1.0, 1H, H_n), 8.19 (s, 1H, H_q), 7.88-7.82 (m, 2H, H_m + H_p), 7.76 (s, 1H, H_h), 7.69 (td, *J* = 7.7, 1.8, 2H, H_u), 7.63-7.59 (m, 2H, H_t), 7.20-7.16 (m, 2H, H_v), 5.22 (t, *J* = 9.5, 1H, H_d), 5.10 (t, *J* = 9.5, 1H, H_c), 5.03 (dd, *J* = 9.5, 8.0, 1H, H_e), 4.94 (d, *J* = 12.7, 1H, one of H_g), 4.84 (d, *J* = 12.7, 1H, one of H_g), (4.69 (d, *J* = 7.9, 1H, H_f), 4.56-4.48 (m, 2H, H_k), 4.33-4.24 (m, 3H, H_i and one of H_a), 4.17 (dd, *J* = 12.2, 2.2, 1H, one of H_a), 4.08 (s, 2H, H_r), 3.96 (s, 4H, H_s), 3.80-3.74 (m, 1H, H_b), 2.43 (app. quint, *J* = 6.5, 2H, H_j), 2.09 (s, 3H, Ac), 2.02 (s, 3H, Ac), 2.00 (s, 3H, Ac), 1.99 (s, 3H, Ac). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 170.8, 170.3, 169.5, 163.9, 163.4, 159.1, 149.3, 145.1, 144.1, 138.8, 136.7, 132.6, 131.2, 130.3, 129.3, 128.7, 126.6, 125.8, 123.6, 123.6, 123.5, 123.3, 122.7, 122.4, 99.6, 72.9, 72.0, 71.4, 68.6, 62.9, 62.0, 59.9, 53,5, 48.6, 48.4, 37.9, 29.0, 20.9, 20.8, 20.7 (overlapping signals). IR: (ν_{max}/cm^{-1}) 3454 (weak), 3138, 3006, 2958, 1753, 1704, 1660, 1588, 1435, 1361, 1218, 1038, 995, 905, 785, 755. HRMS (EI) calcd for $C_{47}H_{48}N_{10}O_{12}$ $[M + H]^+$ 945.3526**,** found 945.3517. UV: $\lambda_{max}(CH_2Cl_2)$/nm (ε/ mol^{-1}cm^{-1}dm^3) 344 (16280)

2,2'-(((1-(2-((1-(2-(Didodecylamino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl)-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-6-yl)-1H-1,2,3-triazol-4-yl)methyl)azanediyl)diacetic acid (7b):



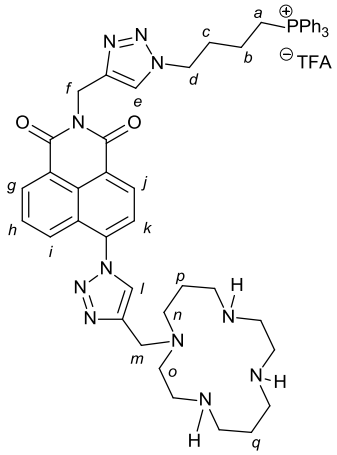
To a stirred solution of **7a** (28 mg, 0.030 mmol) in THF (0.15 mL) was added an aqueous solution of LiOH (2 equiv., 0.15 mL). The reaction mixture was stirred for 18 h at r.t., and the solvent removed *in vacuo*. The residue was re-suspended in $CHCl_3$ (5 mL), washed with aqueous citric acid solution (5% w/v, 5 mL), the organic phase dried over $MgSO_4$ and the solvent removed *in vacuo* to yield deprotected sensor **7b** as an orange residue (14.0 mg, 53%). ^{1}H NMR (400 MHz, MeOD) δ_H 8.72-8.66 (m, 2H, H_g and H_j), 8.50 (s, 1H, H_l), 8.24 (d, J = 5.6, 1H, H_i), 7.98 (s, 1H, H_e), 7.96 (d, J = 5.2, 1H, H_k), 7.91-7.86 (m, 1H, H_h), 5.49 (s, 2H, H_f), 5.34 (s, 2H, H_d), 4.35 (s, 2H, H_m), 3.73 (s, 4H, H_n), 3.39-3.11 (m, 2H, H_c – obscured by solvent peak), 1.68-1.46 (m, 4H, H_b), 1.37-1.13 (m, 36H, alkyl-H), 0.89-0.84 (m, 6H, H_a). ^{13}C NMR (101 MHz, MeOD) δ_C 166.5, 164.5, 164.0, 156.7, 144.3, 140.3, 133.1, 132.2, 131.8, 131.4, 131.0, 130.6, 130.6, 130.1, 129.6, 128.4, 128.3, 127.6, 127.1, 125.0, 124.9, 123.8, 121.2, 51.8, 49.6, 47.7, 35.9, 32.8, 30.5, 30.4, 30.4, 30.2, 30.1, 29.6, 28.3, 27.7, 27.6, 23.5, 14.4. IR: (ν_{max}/cm^{-1}) 3357 (broad), 2923, 2854, 1705, 1661, 1588, 1465, 1423, 1377, 1235, 1120, 1052, 952, 785. HRMS (EI) calcd for $C_{48}H_{69}N_9O_7$ $[M + H]^+$ 884.5393, found 884.5391. UV: $\lambda_{max}(CH_2Cl_2)$/nm (ε / mol^{-1}cm^{-1}dm^3) 344 (9173)

2-(4-((6-(4-((1,4,8,11-Tetraazacyclotetradecan-1-yl)methyl)-1H-1,2,3-triazol-1-yl)-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)methyl)-1H-1,2,3-triazol-1-yl)-N,N-didodecylacetamide (8b):



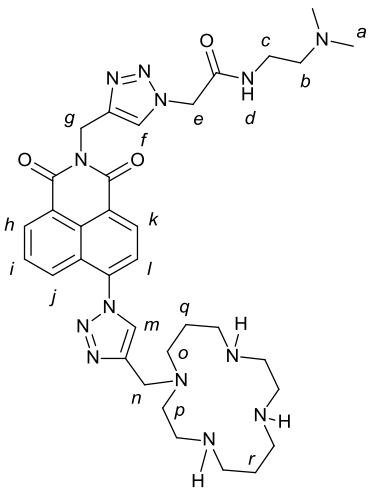
Compound **8a** (50 mg, 0.040 mmol) was dissolved in a solution of TFA (20%) in CH_2Cl_2 (1 mL), and for 6 h at r.t. The reaction mixture was concentrated *in vacuo*, re-suspended in $CHCl_3$ (5 mL) and washed with aqueous NaOH (1 M, 5 mL). The organic phase was dried over $MgSO_4$ and the solvent was removed *in vacuo* to yield deprotected sensor **8b** as a yellow residue (34.0 mg, 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.72-8.64 (m, 2H, H_g and H_j), 8.30 (d, *J* = 8.5, 1H, H_i), 8.15 (s, 1H, H_l), 7.90 (s, 1H, H_e), 7.85 (d, J = 7.8, 1H, H_k), 7.83-7.78 (m, 1H, H_h), 5.52 (s, 2H, H_f), 5.14 (s, 2H, H_d), 3.98 (s, 2H, H_m), 3.33-3.20 (m, 4H, H_c), 2.84-2.62 (m, 12H, cyclam-H), 2.00-1.87 (m, 2H, H_o), 1.70-1.42 (m, 6H, H_n, H_q, H_p), 1.36-1.12 (m, 40H, H_b (4H), and alkyl-H (36H)), 0.90-0.82 (m, 6H, H_a). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 164.3, 163.4, 162.9, 145.8, 143.5, 138.6, 132.5, 131.1, 129.9, 129.3, 128.6, 126.5, 125.3, 125.2, 123.8, 123.6, 123.0, 55.2, 53.8, 51.0, 50.9, 50.0, 49.4, 48.8, 48.2, 48.0, 47.8, 47.4, 46.9, 35.6, 32.0, 29.7, 29.7, 29.7, 29.6, 29.4, 29.3, 29.2, 28.8, 27.6, 27.1, 26.9, 26.3, 22.8, 14.2. IR: (ν_{max}/cm^{-1}) 2927, 2850, 1695, 1578, 1460, 1403, 1368, 1244, 1156, 807, 782. HRMS (EI) calcd for $C_{54}H_{86}N_{12}O_3$ $[M + H]^+$ 951.7019, found 951.7022. UV: $\lambda_{max}(CH_2Cl_2)$/nm (ε / mol^{-1}cm^{-1}dm^3) 345 (8389)

(4-(4-((6-(4-((1,4,8,11-Tetraazacyclotetradecan-1-yl)methyl)-1H-1,2,3-triazol-1-yl)-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)methyl)-1H-1,2,3-triazol-1-yl)butyl)triphenylphosphonium trifluoroacetate (9b):



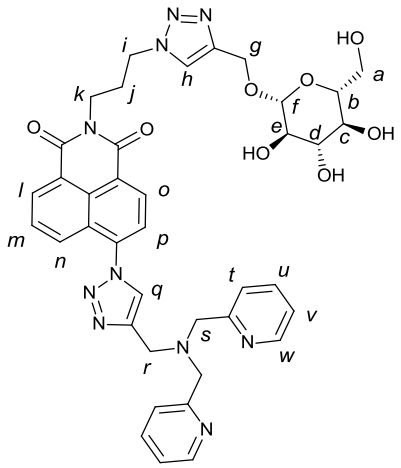
Compound **9a** (20 mg, 0.016 mmol) was dissolved in a solution of TFA (20%) in CH_2Cl_2 (1 mL), and for 6 h at r.t. The reaction mixture was concentrated *in vacuo*, re-suspended in $CHCl_3$ (5 mL) and washed with aqueous NaOH (1 M, 5 mL). The organic phase was dried over $MgSO_4$ and the solvent was removed *in vacuo* to yield deprotected sensor **9b** as a yellow residue (12.0 mg, 74%) ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.71-8.64 (m, 2H, H_g and H_j), 8.30 (dd, *J* = 8.6, 0.9, 1H, H_i), 8.16 (s, 1H, H_l), 7.87 (d, *J* = 7.8, 1H, H_k), 7.83-7.58 (m, 16H, H_h and 3 x Ph), 7.80 (s, 1H, H_e), 5.46 (s, 2H, H_f), 4.47 (t, *J* = 6.3, 2H, H_d), 3.98 (s, 2H, H_m), 3.64-3.55 (m, 2H, H_c), 2.87-2.64 (m, 16H, cyclam-H (12H) and H_n + H_o), 2.22 (app. quint, *J* = 6.6, 2H, H_b), 1.99-1.90 (m, 2H, H_q), 1.74-1.65 (m, 2H, H_p), 1.63-1.52 (m, 2H, H_a). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 163.5, 163.1, 145.6, 143.6, 138.7, 138.2, 135.2, 135.2, 133.7, 133.7, 132.6, 131.2, 130.7, 130.6, 129.4, 128.7, 127.2, 126.7, 125.4, 123.8, 123.7, 123.0, 118.5, 117.9, 53.9, 50.9, 49.4, 48.8, 48.7, 47.7, 44.0, 35.8, 29.8, 29.8, 21.2, 19.4. ^{19}F NMR (376MHz, $CDCl_3$) δ_F -75.0. ^{31}P NMR (162 MHz, $CDCl_3$) δ_P 24.3. IR: (ν_{max}/cm^{-1}) 3509 (b), 2966, 2943, 1663, 1585, 1482, 1443, 1419, 1363, 1325, 1232, 1167, 1108, 1030, 863, 802. HRMS (EI) submitted calcd for [M – anion]$^+$ 874.4429, found 874.4425. UV: $\lambda_{max}(CH_2Cl_2)$/nm (ε/ mol^{-1}cm^{-1}dm^3) 345 (10549)

2-(4-((6-(4-((1,4,8,11-Tetraazacyclotetradecan-1-yl)methyl)-1H-1,2,3-triazol-1-yl)-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)methyl)-1H-1,2,3-triazol-1-yl)-N-(2-(dimethylamino)ethyl)acetamide (10b):



Compound **10a** (20.0 mg, 0.016 mmol) was dissolved in a solution of TFA (20%) in CH_2Cl_2 (1 mL), and for 6 h at r.t.. The reaction mixture was concentrated *in vacuo*, re-suspended in $CHCl_3$ (5 mL) and washed with aqueous NaOH (1 M, 5 mL). The organic phase was dried over $MgSO_4$ and the solvent was removed *in vacuo* to yield deprotected sensor **10b** as a yellow residue (8.9 mg, 63%) ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.72-8.66 (m, 2H, H_h + H_k), 8.31 (d, J = 6.5, 1H, H_j), 8.15 (s, 1H, H_m), 7.87 (d, J = 7.8, 1H, H_l), 7.84 (s, 1H, H_m), 7.81 (dd, J = 7.4, 8.5, 1H, H_i and H_l), 6.57 (br s, 1H, H_d), 5.53 (s, 2H, H_g), 4.99 (s, 2H, H_e), 3.98 (s, 2H, H_n), 3.28 (q, J = 5.7, 2H, H_c), 2.85-2.60 (m, 16H, H_o and H_p and cyclam-H), 2.33 (t, J = 6.0, 2H, H_b), 2.13 (s, 6H, H_a (2 x CH_3)), 1.96-1.89 (m, 2H, H_r), 1.70-1.62 (m, 2H, H_q). ^{13}C NMR (101 MHz, CD_3CN) δ_C 168.4, 164.4, 163.8, 160.9, 160.7, 160.5, 144.1, 138.8, 132.9, 131.6, 130.4, 129.6, 129.6, 127.1, 127.0, 126.6, 124.9, 118.3, 116.0, 58.5, 55.5, 52.9, 50.7, 49.0, 47.9, 47.7, 45.9, 45.5, 44.1, 36.1, 35.7, 31.0, 24.7, 23.4, 18.2. IR: (ν_{max}/cm^{-1}). UV: λ_{max}(CH_2Cl_2)/nm (ε / $mol^{-1}cm^{-1}dm^3$) 345 (11800)

6-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1H-1,2,3-triazol-1-yl)-2-(3-(4-((((2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)propyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (11b):



Following a modified literature procedure[h], sensor **11a** (25 mg, 0.026 mmol) was dissolved in MeOH (0.5 mL) and NaOMe (0.3 eq) was added to the solution. The reaction mixture was stirred for 4 h, and evaporated to dryness *in vacuo* to yield deprotected sensor **11b** as a yellow residue (16.0 mg, 79%). ^{1}H NMR (400 MHz, MeOD) δ_H 8.84-8.77 (m, 2H, H_l + H_o), 8.60 (s, 1H, H_q), 8.58-8.55 (m, 2H, H_w), 8.33 (d, *J* = 8.6, 1H, H_n), 8.17 (s, 1H, H_h), 8.07 (d, *J* = 7.8, 1H, H_p), 8.04-7.98 (m, 1H, H_m), 7.90 (td, *J* = 7.7, 1.6, 2H, H_u), 7.86-7.81 (m, 2H, H_t), 7.41-7.35 (m, 2H, H_m), 5.01 (d, *J* = 12.5, 1H, one of H_g), 4.86-4.82 (m, 1H, one of H_g – obscured by solvent peak, identified by cross peak on COSY), 4.66 (t, *J* = 6.8, 2H, H_k), 4.48 (d, *J* = 7.7, 1H, H_f), 4.39 (t, *J* = 7.0, 2H, H_i), 4.16 (s, 2H, H_r), 4.06 (s, 4H, H_s), 4.00 (dd, *J* = 12.1, 1.6, 1H, one of H_a), 3.80 (dd, *J* = 11.8, 5.3, 1H, one of H_a), 3.50-3.40 (m, 3H, H_c and H_d and H_e – obscured by solvent peak, identified by cross peak on COSY), 3.34 (dd, *J* = 9.0, 7.8, 1H, H_b), 2.54 (quint, *J* = 7.0, 2H, H_j). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 159.8, 149.3, 145.3, 139.4, 138.5, 133.2, 131.8, 130.1, 127.5, 125.2, 124.8, 123.7, 120.7, 103.1, 95.6, 77.7, 77.6, 74.7, 71.4, 62.7, 62.6, 60.5, 49.8, 49.6, 38.7. IR: (ν_{max}/cm^{-1}) 3378 (broad), 2929, 2831, 1699, 1659, 1623, 1592, 1476, 1434, 1394, 1352, 1268, 1233, 1103, 1082, 1046, 996, 842. HRMS (EI) calcd for $C_{39}H_{40}N_{10}O_8$ $[M + H]^+$ 777.3103, found 777.3104. UV: $\lambda_{max}(CH_2Cl_2)$/nm (ε/ mol^{-1}cm^{-1}dm^3) 345 (13126).

3) UV and Fluorescence Spectroscopic Characterisation

a) Sensor **6** - for full aggregation phenomena studies see Section **4**.

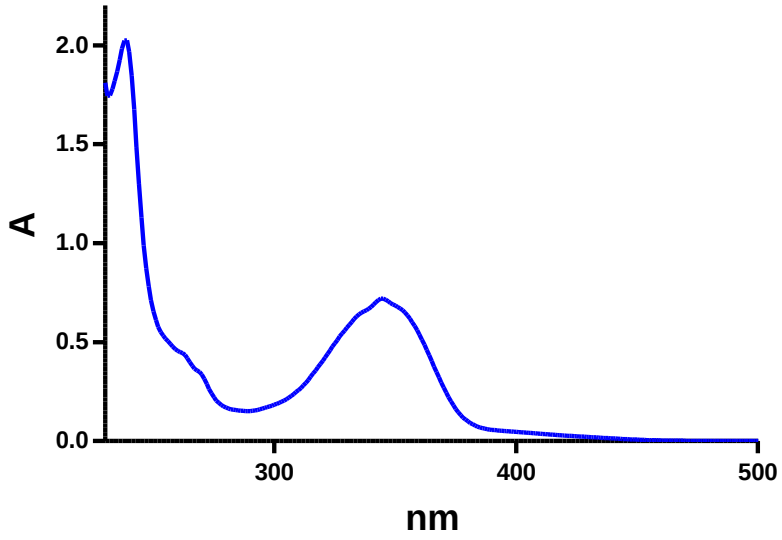


Fig. S1 UV-vis absorption spectrum of sensor **6** (MeCN)

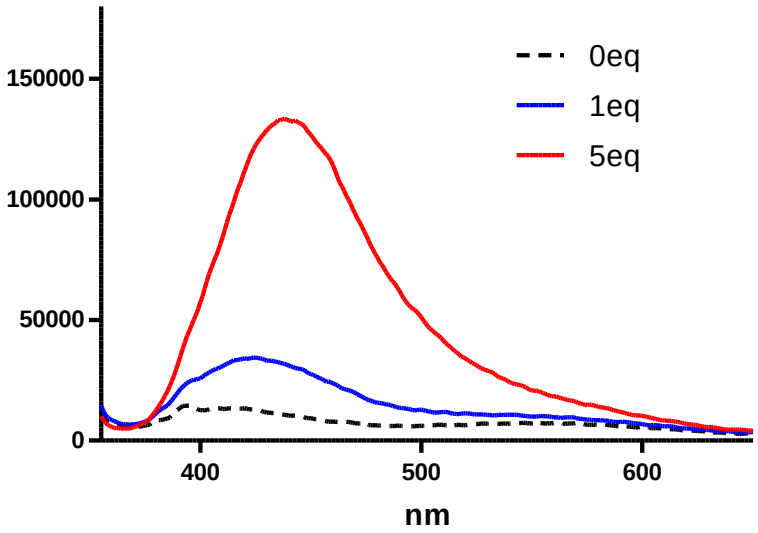


Fig. S2 Fluorescence response of sensor **6** (50 μM) with increasing amounts of Zn^{2+} in aqueous buffer (0.1 mM HEPES, pH 7.4, ambient temperature, λ_{ex} = 347 nm).

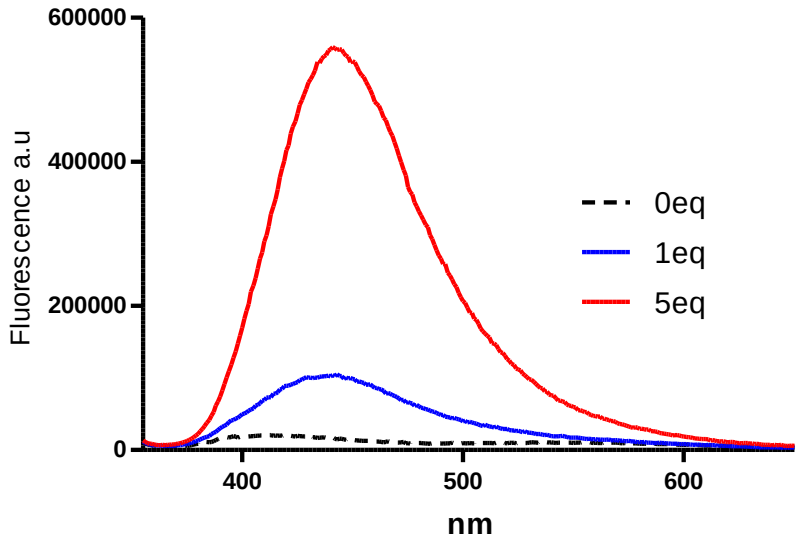


Fig. S3 Fluorescence response of sensor **6** (100 μM) with increasing amounts of Zn^{2+} in aqueous buffer (0.1 mM HEPES, pH 7.4, ambient temperature, λ_{ex} = 347 nm.

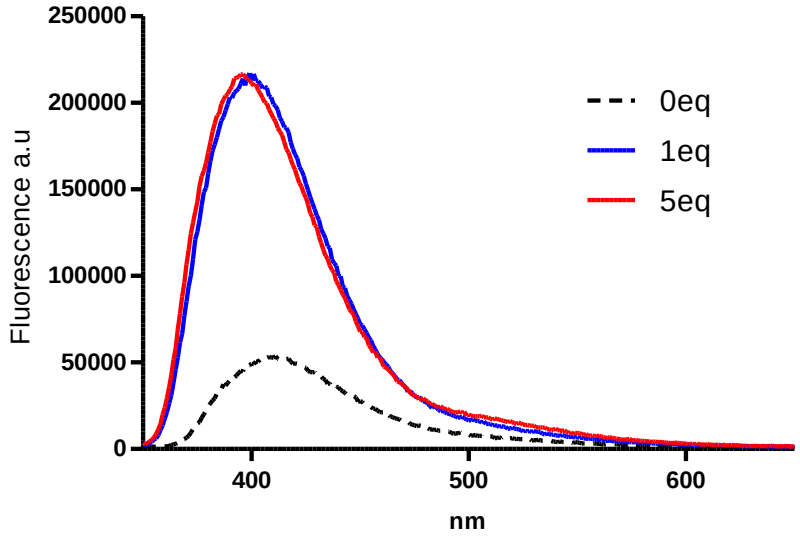


Fig. S4 Fluorescence response of sensor **6** (100 μM) with increasing amounts of Zn^{2+} in MeCN (ambient temperature, λ_{ex} = 347 nm).

b) Sensor **7b**

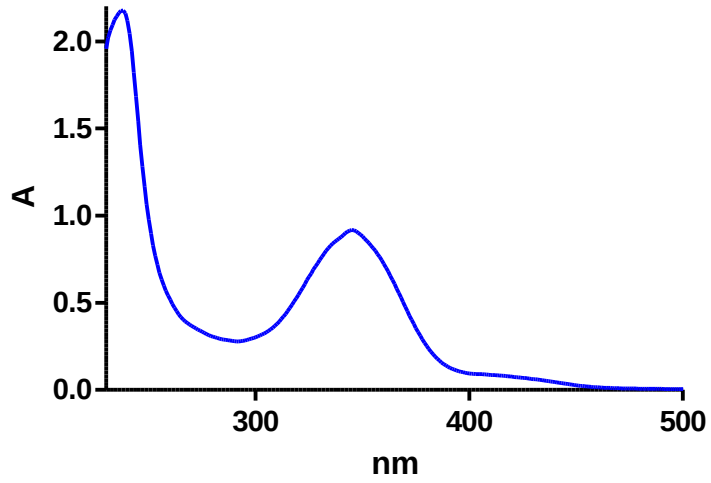


Fig. S5 UV-vis absorption spectrum of sensor **7b** (MeCN)

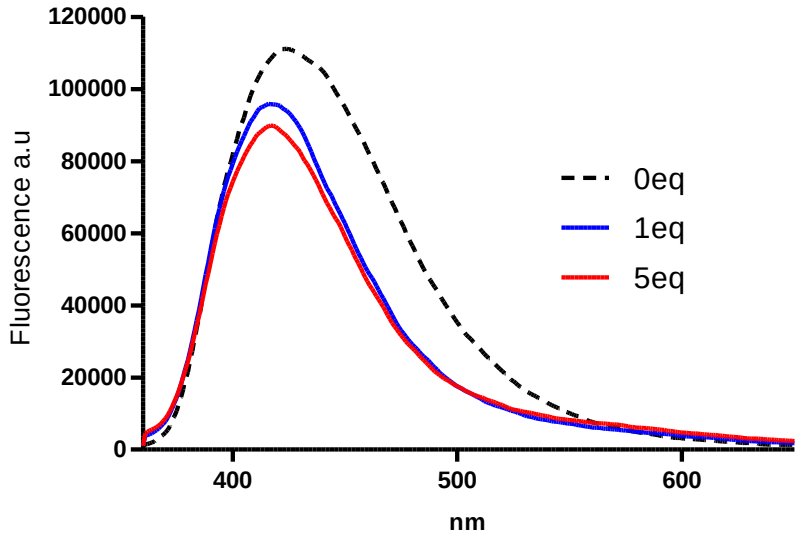


Fig. S6 Fluorescence response of sensor **7b** (100 μM) with increasing amounts of Zn^{2+} in aqueous buffer (0.1 mM HEPES, pH 7.4, ambient temperature, λ_{ex} = 347 nm).

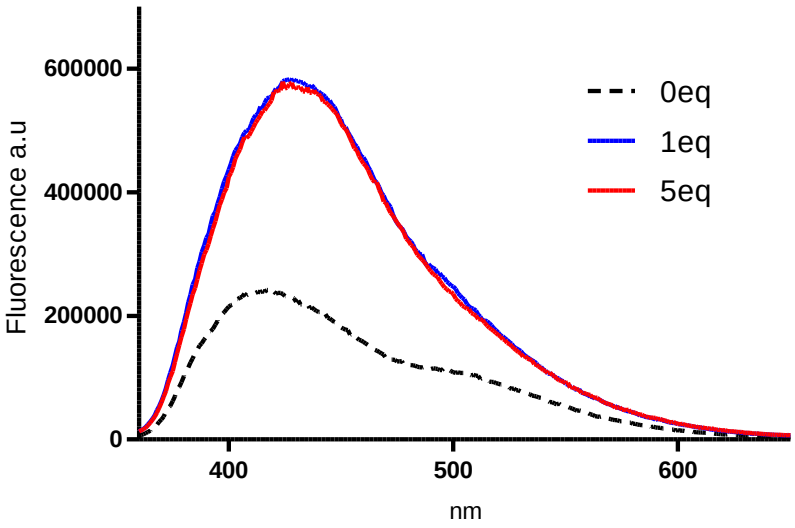


Fig. S7 Fluorescence response of sensor **7b** (100 μM) with increasing amounts of Zn^{2+} in MeCN (ambient temperature, λ_{ex} = 347 nm).

c) Sensor **8b**

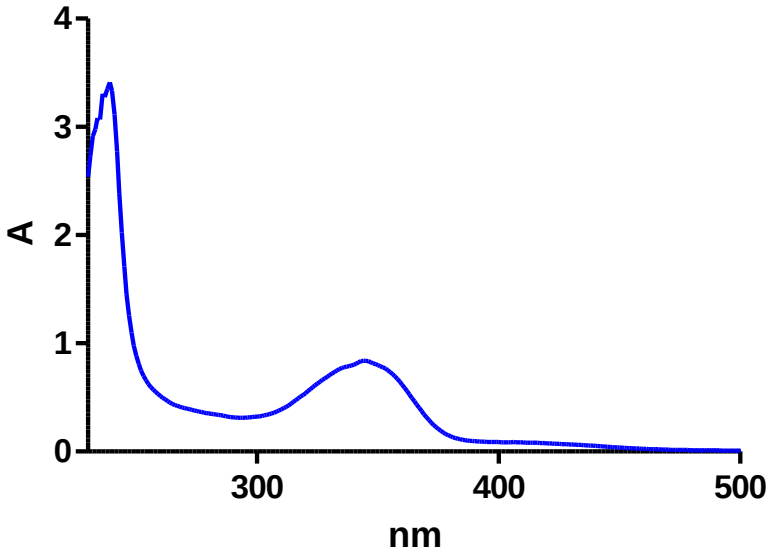


Fig. S8 UV-vis absorption spectrum of sensor **8b** (MeCN)

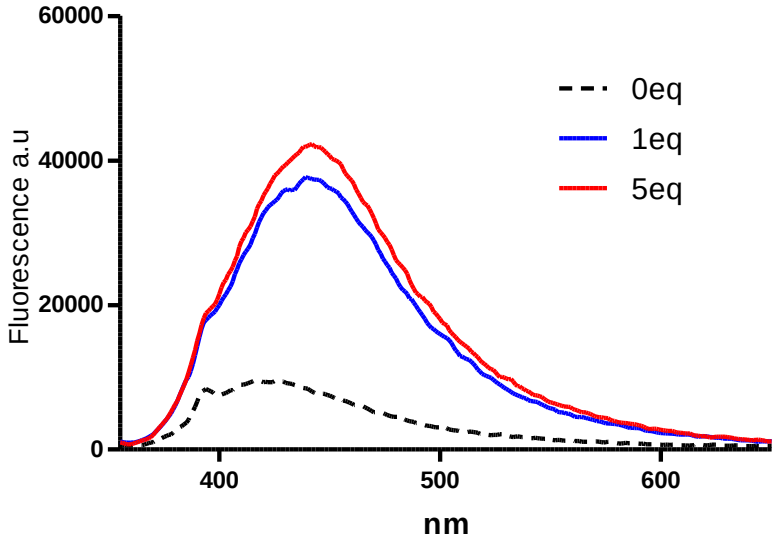


Fig. S9 Fluorescence response of sensor **8b** (50 μM) with increasing amounts of Zn^{2+} in aqueous buffer (0.1 mM HEPES, pH 7.4, ambient temperature, λ_{ex} = 347 nm).

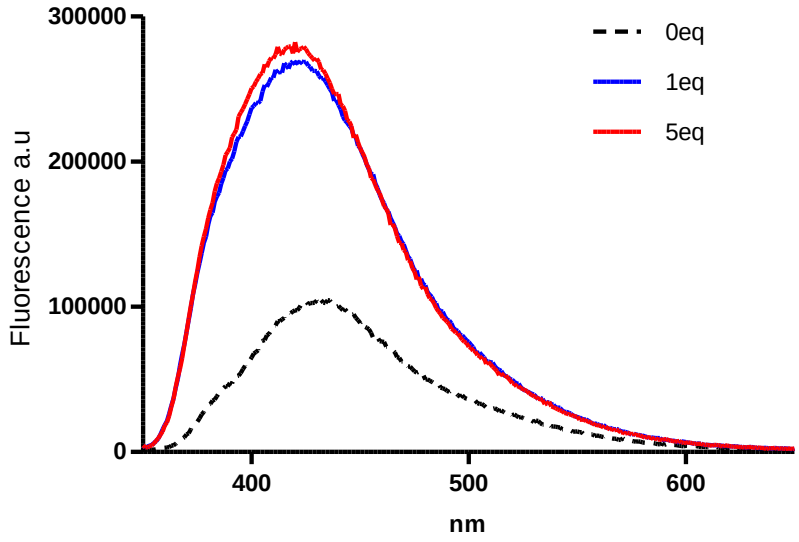


Fig. S10 Fluorescence response of sensor **8b** (100 μM) with increasing amounts of Zn^{2+} in MeCN (ambient temperature, λ_{ex} = 347 nm.

d) Sensor **9b**

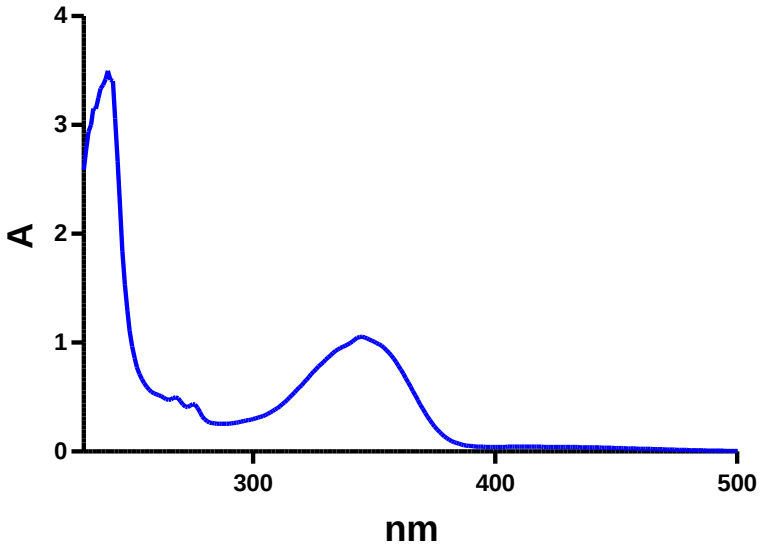


Fig. S11 UV-vis absorption spectrum of sensor **9b** (MeCN)

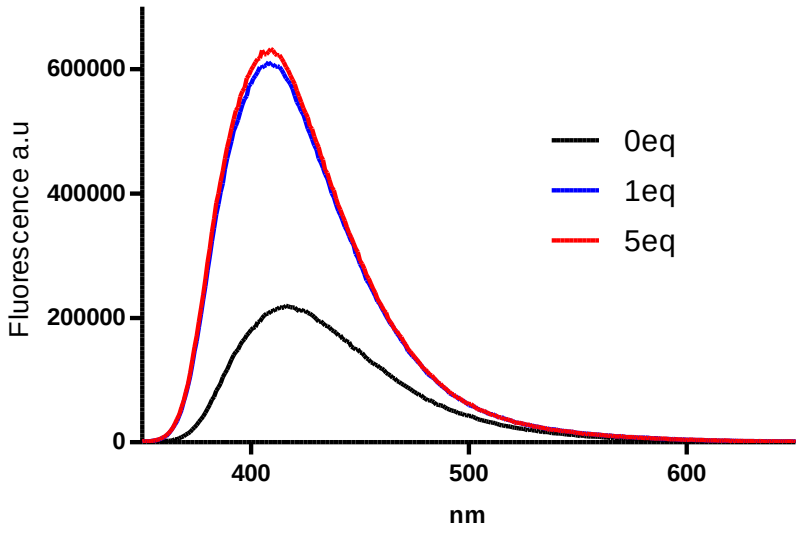


Fig. S12 Fluorescence response of sensor **9b** (50 μM) with increasing amounts of Zn^{2+} in aqueous buffer (0.1 mM HEPES, pH 7.4, ambient temperature, λ_{ex} = 347 nm

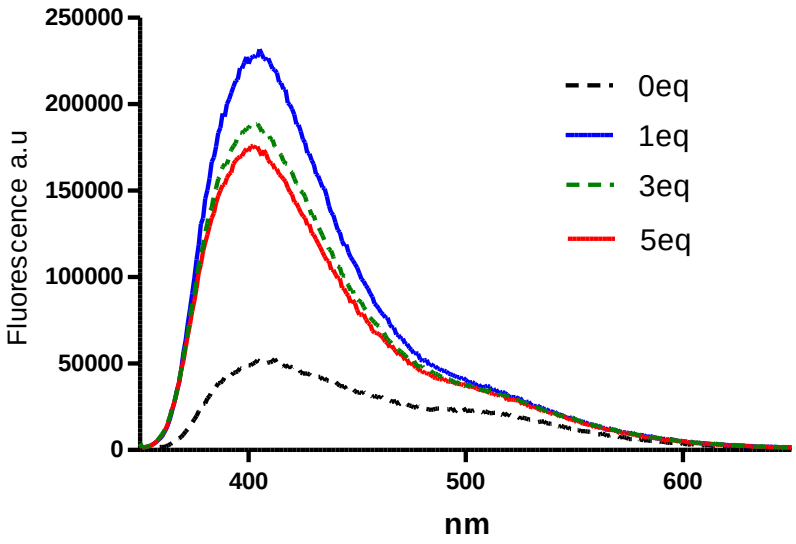


Fig. S13 Fluorescence response of sensor **9b** (100 μM) with increasing amounts of Zn^{2+} in MeCN (ambient temperature, λ_{ex} = 347 nm).

d) Sensor **10b**

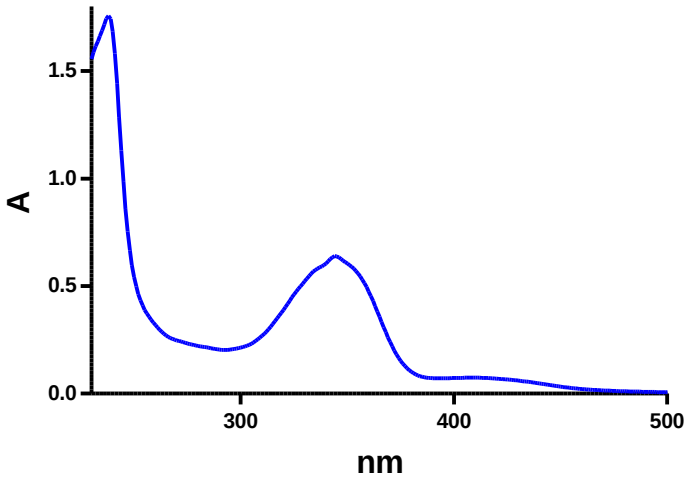


Fig. S14 UV-vis absorption spectrum of sensor **10b** (MeCN)

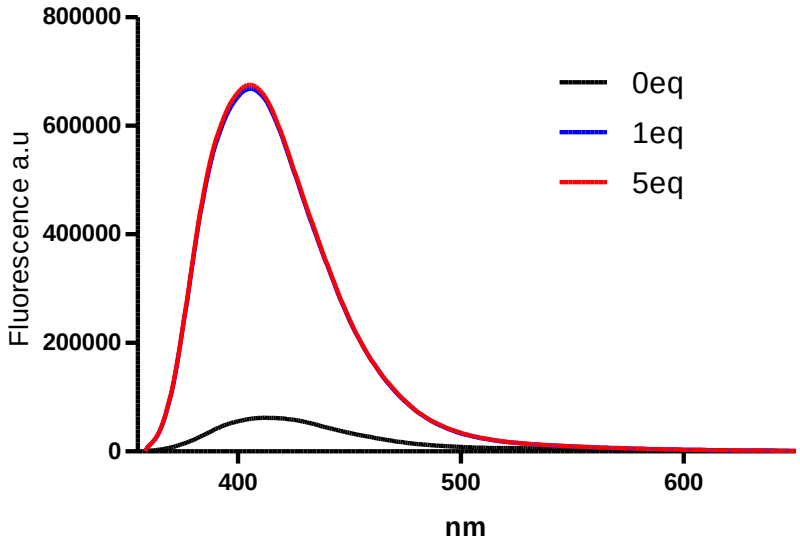


Fig. S15 Fluorescence response of sensor **10b** (100 μM) with increasing amounts of Zn^{2+} in aqueous buffer (10 mM HEPES, pH 7.4, ambient temperature, λ_{ex} = 347 nm.

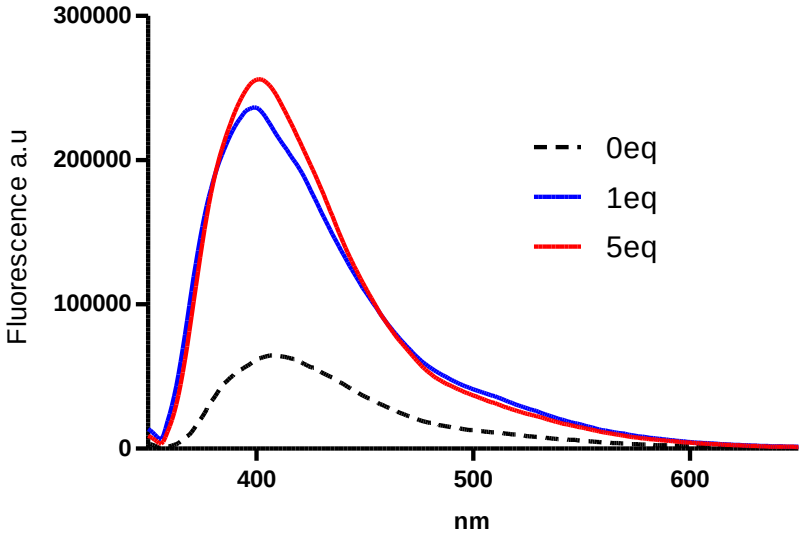


Fig. S16 Fluorescence response of sensor **10b** (100 μM) with increasing amounts of Zn^{2+} in MeCN (ambient temperature, λ_{ex} = 347 nm).

f) Sensor **11b**

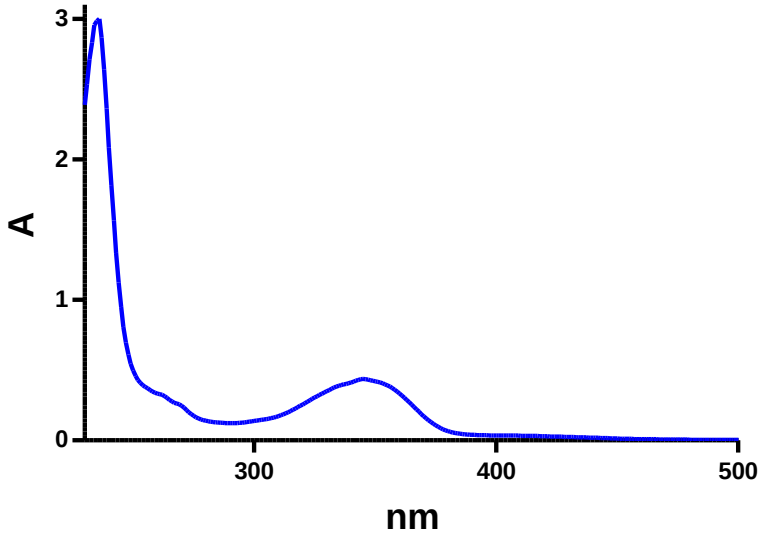


Fig. S17 UV-vis absorption spectrum of sensor **11b** (MeCN)

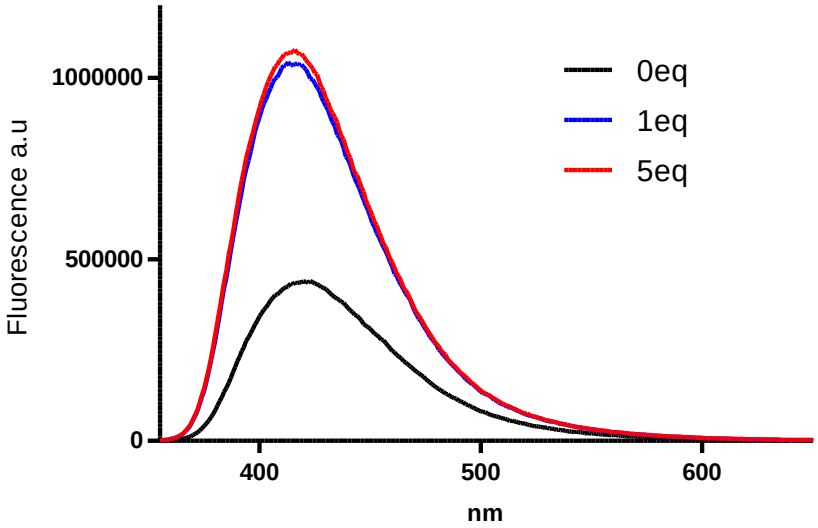


Fig. S18 Fluorescence response of sensor **11b** (50 μM) with increasing amounts of Zn^{2+} in aqueous buffer (0.1 mM HEPES, pH 7.4, ambient temperature, λ_{ex} = 347 nm).

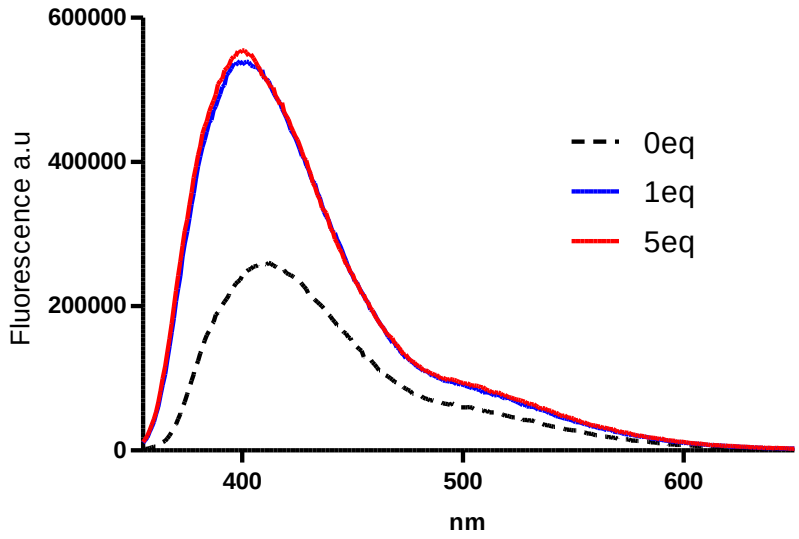


Fig. S19 Fluorescence response of sensor **11b** (100 μM) with increasing amounts of Zn^{2+} in MeCN (ambient temperature, λ_{ex} = 347 nm).

g) **Determination of Kd values for sensors 8b, 9b, 10b, and 11b**

Fluorescence titration was used to determine the dissociation constant (K_d) and F(max) of probes **8b**, **9b**, **10b**, and **11b** in HEPES buffer (1% DMSO). Each sensor was titrated at a constant sensor concentration of 50 μM with increasing equivalents of Zn^{2+} and the fluorescence output (F) measured. The relative increase in fluorescence (F/F(0)) was plotted against the ratio of probe to Zn^{2+} (CM/CL) and fit to the 1:1 binding isotherm (equation shown in Fig. S20) using the non-linear regression function of Graphpad Prism 5.[9] The values obtained are tabulated in Table S1. In the case of probes **9b** and **10b** that target the lysosome and mitochondria respectively titrations were also performed at the relevant pH of the organelle. The poor fitting of sensors **9b** and **11b** is currently under investigation.

$$\frac{F}{F(0)} = 1 + \left(\frac{F(max)}{2F(0)} - 0.5\right) \times \left(1 + \frac{CM}{CL} + \frac{Kd}{CL} - \left[\left(1 + \frac{CM}{CL} + \frac{Kd}{CL}\right)^2 - \frac{4CM}{CL}\right]^{0.5}\right)$$

Fig. S20 Equation used to determine K_d values for sensors **8b**, **9b**, **10b** and **11b** where CM and CL concentrations of Zn^{2+} and probe respectively, F is the observed fluorescence, F(0) is the observed fluorescence of the probe alone (ie CM = 0). Non-linear regression analysis was used to determine F(max)/F(0) and K_d.

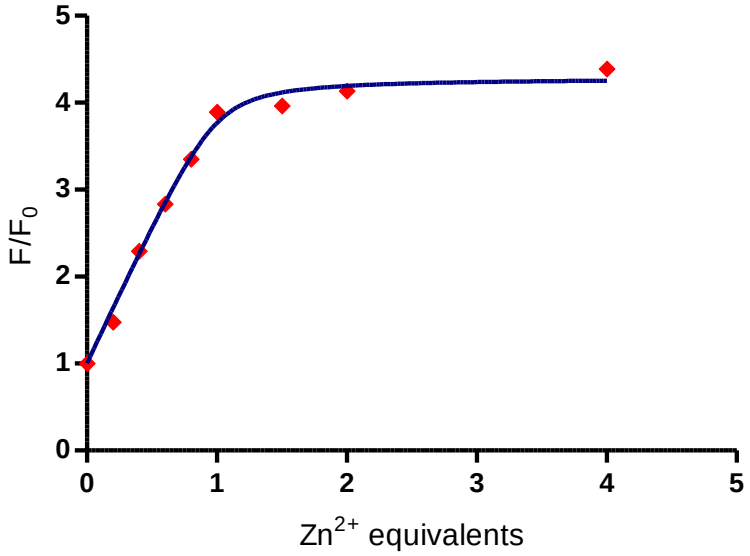


Fig. S21 Example titration plot and non-linear curve fitting of Sensor **8b**.

Sensor	K_d (calculated)	F(max)/F(0)	R^2	K_d Lit. value[2,10]
8b	2.96 x10^{-6} (pH7.4)	4.3	0.9926	4.3 x 10^{-8} M^{-1}
9b	2.13 x10^{-7} (pH 7.4)	3.1	0.7438	4.3 x 10^{-8} M^{-1}
	1.85 x10^{-7} (pH 7.8)	3.3	0.7150	
10b	9.80 x10^{-8} (pH 7.4)	11.5	0.9223	4.3 x 10^{-8} M^{-1}
	1.38 x10^{-7} (pH 5.8)	10.0	0.9019	
11b	2.12 x10^{-7} (pH 7.4)	2.6	0.6482	1.2 × 10^{-8} M^{-1}

Table S1 Values for K_d and F(max)/F(0) obtained by non-linear regression analysis.

4) Aggregation Studies of Sensor 6

The aggregation of probe **6** was studied by investigating the effect of concentration on its response to Zn^{2+}, the effect of solvent composition and dynamic light scattering.

a) Effect of Probe Concentration of the Fluorescence Response of Probe 6

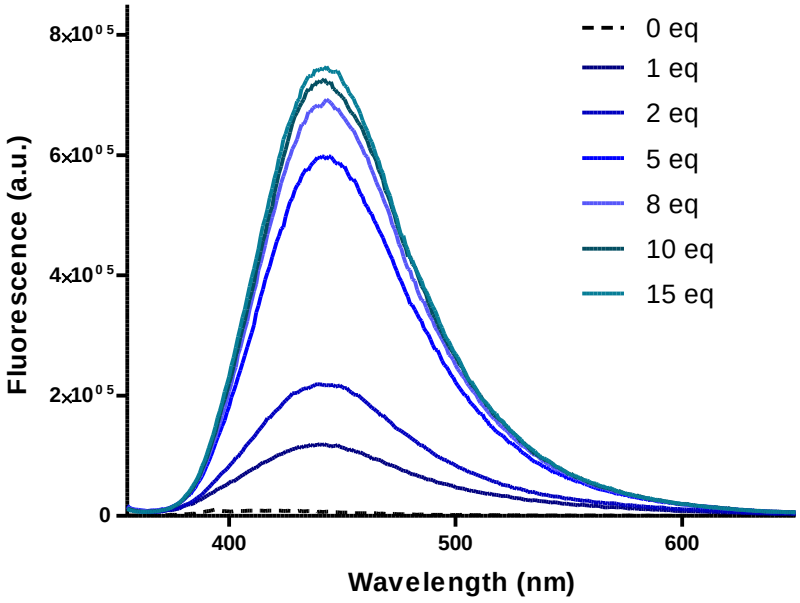


Fig. S22 Fluorescence response of the titration of sensor **6** (100 μM) with increasing amounts of Zn^{2+} in aqueous buffer (0.1 mM HEPES, pH 7.4, ambient temperature, λ_{ex} = 347 nm.

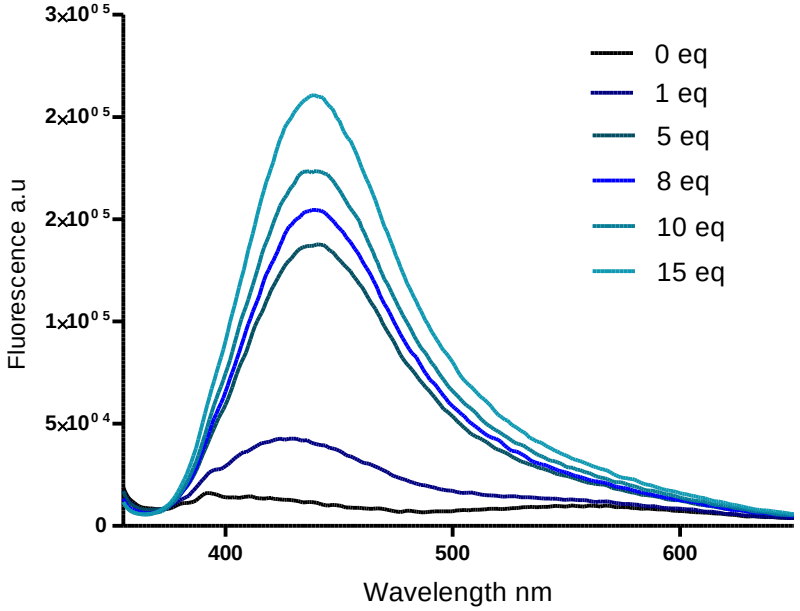


Fig. S23 Fluorescence response of the titration of sensor **6** (50 μM) with increasing amounts of Zn^{2+} in aqueous buffer (0.1 mM HEPES, pH 7.4, ambient temperature, λ_{ex} = 347 nm).

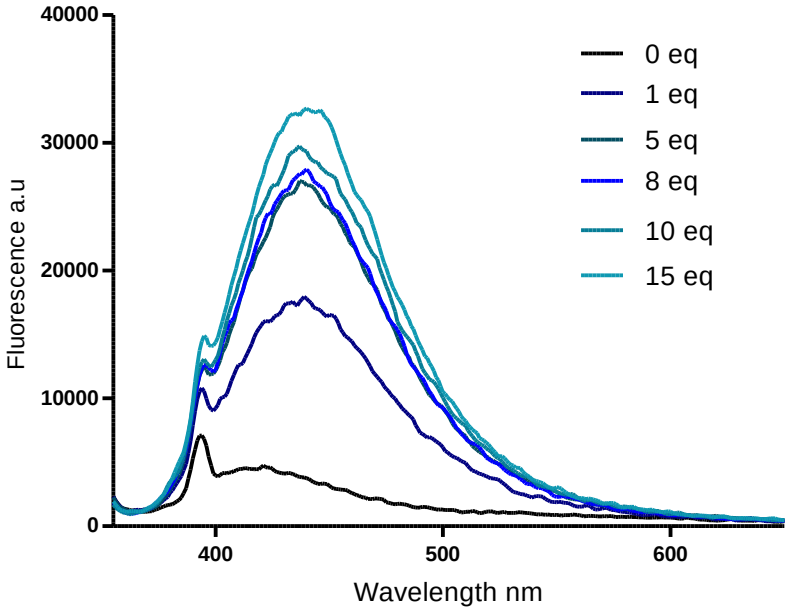


Fig. S24 Fluorescence response of the titration of sensor **6** (10 μM) with increasing amounts of Zn^{2+} in aqueous buffer (0.1 mM HEPES, pH 7.4, ambient temperature, λ_{ex} = 347 nm).

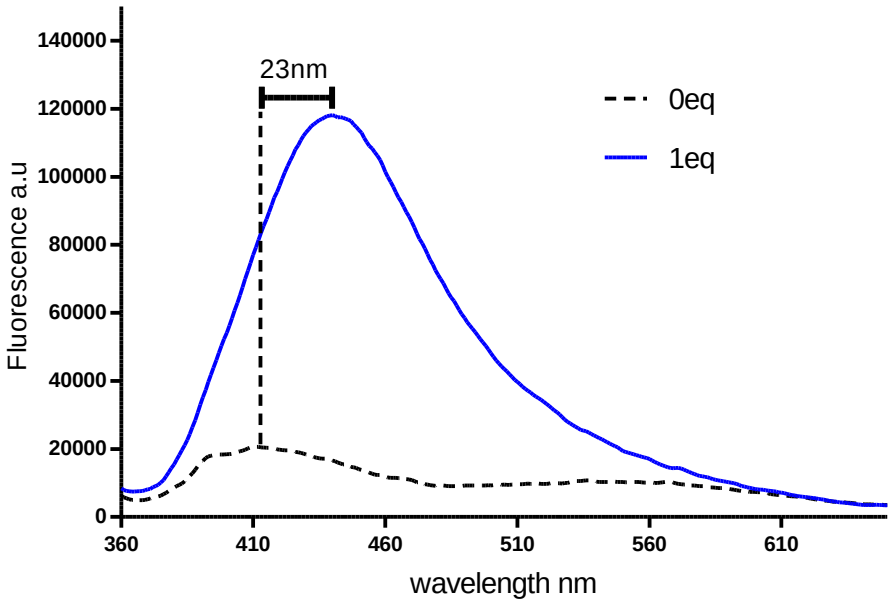


Fig. S25 Graphical representation of the red-shift in λ_{em} upon binding of 1 equiv. Zn^{2+} with sensor **6** (100 μM) in aqueous buffer (0.1 mM HEPES, pH 7.4, ambient temperature, λ_{ex} = 347 nm).

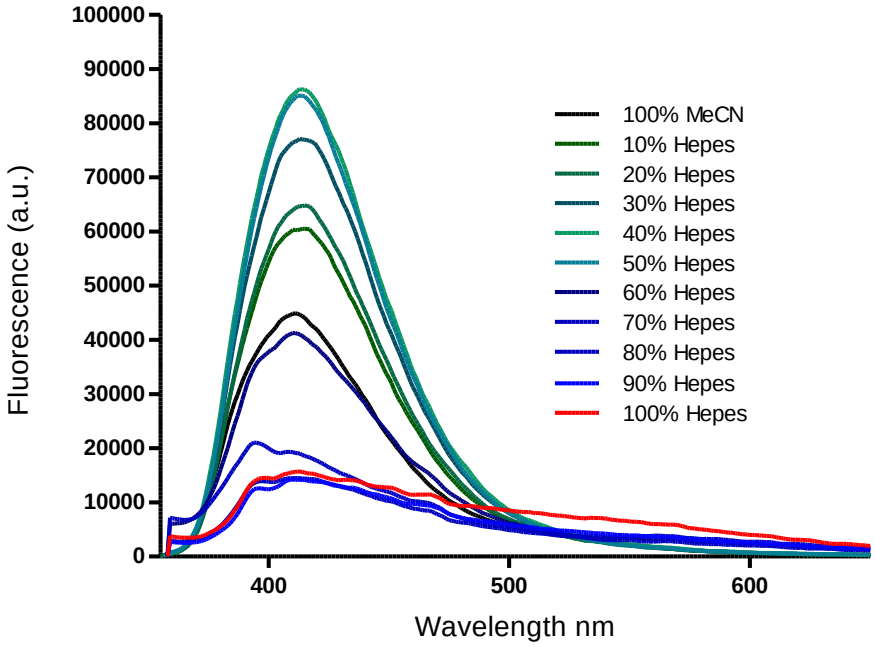


Fig. S26 HEPES/MeCN titration of sensor **6** (100 μM) (0.1 mM HEPES, pH 7.4, ambient temperature, λ_{ex} = 347 nm).

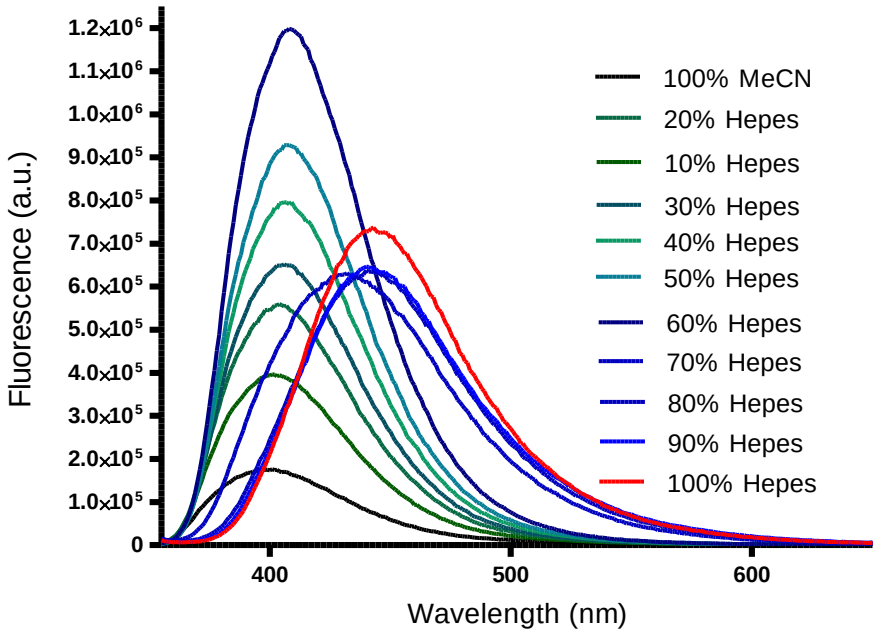


Fig. S27 HEPES/MeCN titration of sensor **6** (100 μM) with 5 equiv. of Zn^{2+} (0.1 mM HEPES, pH 7.4, ambient temperature, λ_{ex} = 347 nm).

b) Dynamic Light Scattering Study

Dynamic light scattering (DLS) experiments were performed at a sensor concentration of 30 μM in HEPES buffer (1% DMSO). Sensor **6** demonstrates aggregation phenomena in aqueous solution, as demonstrated by the large particle size observed in these samples. The aggregates are at their largest with no Zn^{2+} present; in the presence of Zn^{2+} the particle size decreases greatly, coinciding

with an increase in fluorescence emission upon addition of Zn^{2+} to the sensor. Samples containing **11b** on the other hand, structurally similar to **6** but with a different biological targeting unit, show no significant aggregation as demonstrated by the low degree of scattering and poor signal quality, demonstrating the difference small structural changes can have on the behaviour of these probes.

- Sensor **6**

Size Distribution Report by Intensity
v2.2

Malvern

Sample Details

Sample Name:	JP2107 Water 30uM 0 1		
SOP Name:	mansettings.nano		
General Notes:	Average result created from record number(s): 1 2 3		
File Name:	ZET0002884.dts	Dispersant Name:	Water
Record Number:	16	Dispersant RI:	1.330
Material RI:	1.59	Viscosity (cP):	0.8872
Material Absorbtion:	0.010	Measurement Date and Time:	04 October 2013 10:15:43

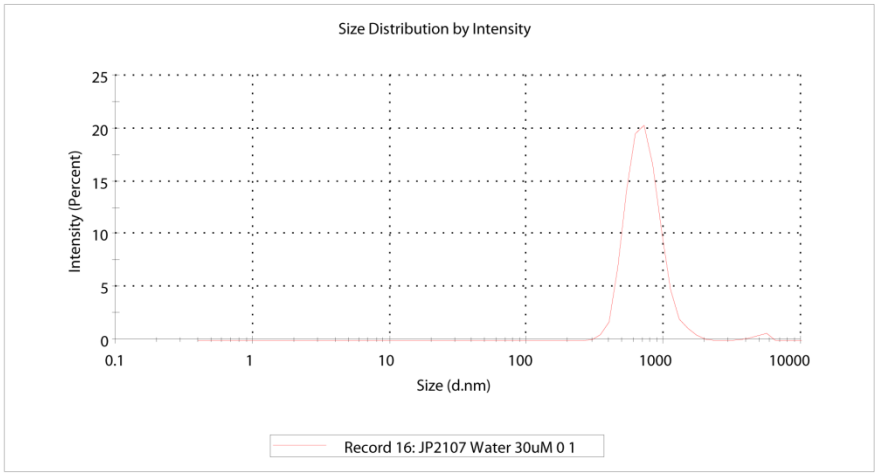
System

Temperature (℃):	25.0	Duration Used (s):	50
Count Rate (kcps):	155.5	Measurement Position (mm):	1.25
Cell Description:	Disposable sizing cuvette	Attenuator:	7

Results

			Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	772.6	Peak 1:	732.1	98.6	222.7
PdI:	0.250	Peak 2:	5011	1.4	603.9
Intercept:	0.972	Peak 3:	0.000	0.0	0.000

Result quality : Refer to quality report



- Sensor **6** + 1 equiv. Zn^{2+}:

Size Distribution Report by Intensity

v2.2

Malvern

Sample Details

Sample Name:	JP2-107 30uM 1 zinc 1
SOP Name:	mansettings.nano
General Notes:	

File Name:	ZET0002894.dts	Dispersant Name:	Water
Record Number:	2	Dispersant RI:	1.330
Material RI:	1.59	Viscosity (cP):	0.9781
Material Absorbtion:	0.010	Measurement Date and Time:	17 October 2013 14:38:36

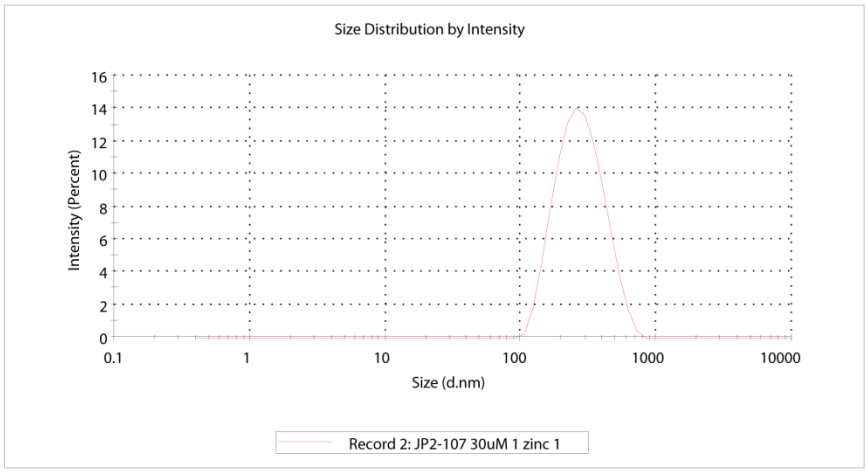
System

Temperature (℃):	21.0	Duration Used (s):	70
Count Rate (kcps):	191.6	Measurement Position (mm):	4.65
Cell Description:	Disposable sizing cuvette	Attenuator:	7

Results

			Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	260.6	Peak 1:	290.0	100.0	113.5
PdI:	0.165	Peak 2:	0.000	0.0	0.000
Intercept:	0.968	Peak 3:	0.000	0.0	0.000

Result quality : Good



- Sensor **6** + 5 equiv. Zn^{2+}:

Size Distribution Report by Intensity

v2.2

Malvern

Sample Details

Sample Name:	JP2-107 30uM 5 zinc 1
SOP Name:	mansettings.nano
General Notes:	

File Name:	ZET0002894.dts	Dispersant Name:	Water
Record Number:	3	Dispersant RI:	1.330
Material RI:	1.59	Viscosity (cP):	0.9781
Material Absorbtion:	0.010	Measurement Date and Time:	17 October 2013 14:45:15

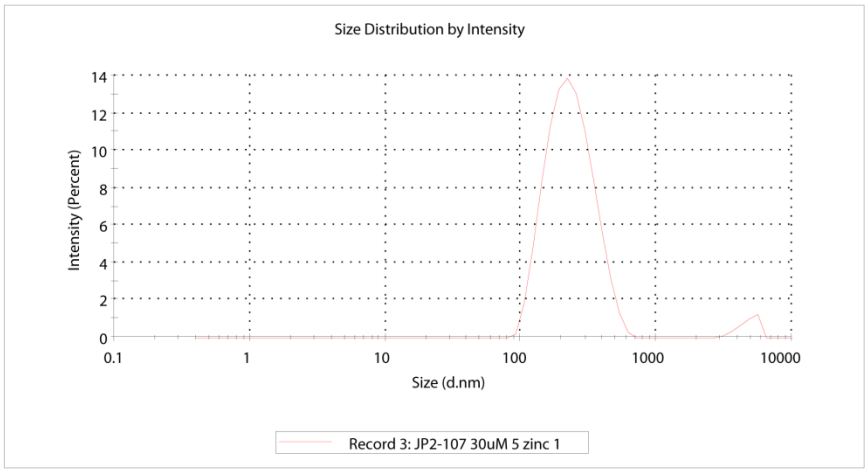
System

Temperature (℃):	21.0	Duration Used (s):	80
Count Rate (kcps):	150.6	Measurement Position (mm):	4.65
Cell Description:	Disposable sizing cuvette	Attenuator:	7

Results

			Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	226.1	Peak 1:	243.2	96.6	94.35
PdI:	0.210	Peak 2:	4769	3.4	737.7
Intercept:	0.969	Peak 3:	0.000	0.0	0.000

Result quality : Good



- Sensor **6** + 15 equiv. Zn^{2+}:

Size Distribution Report by Intensity

v2.2

Malvern

Sample Details

Sample Name:	JP2-107 30uM 15 zinc 1
SOP Name:	mansettings.nano
General Notes:	

File Name:	ZET0002894.dts	Dispersant Name:	Water
Record Number:	4	Dispersant RI:	1.330
Material RI:	1.59	Viscosity (cP):	0.9781
Material Absorbtion:	0.010	Measurement Date and Time:	17 October 2013 14:50:33

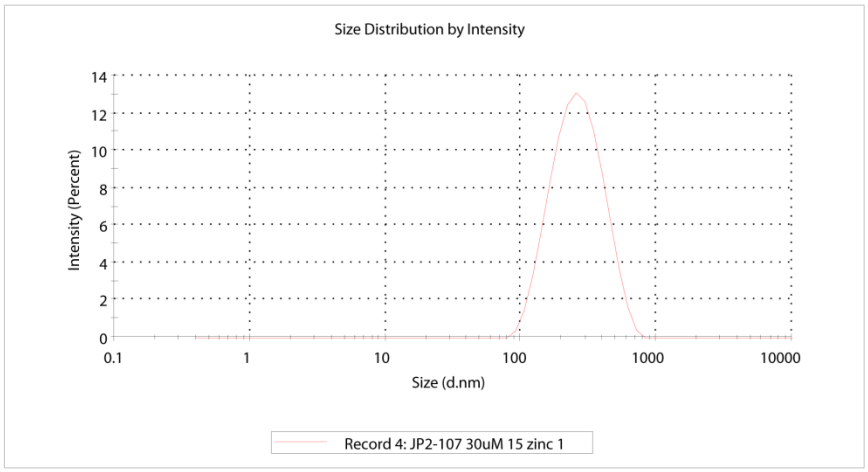
System

Temperature (℃):	21.0	Duration Used (s):	70
Count Rate (kcps):	185.7	Measurement Position (mm):	4.65
Cell Description:	Disposable sizing cuvette	Attenuator:	7

Results

			Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	247.2	Peak 1:	279.7	100.0	116.4
PdI:	0.187	Peak 2:	0.000	0.0	0.000
Intercept:	0.972	Peak 3:	0.000	0.0	0.000

Result quality : Good



- Sensor **11b** blank:

Size Distribution Report by Intensity

v2.2

Malvern

Sample Details

Sample Name:	JP2-156D 30uM 0 zinc 1
SOP Name:	mansettings.nano
General Notes:	

File Name:	ZET0002894.dts	Dispersant Name:	Water
Record Number:	5	Dispersant RI:	1.330
Material RI:	1.59	Viscosity (cP):	0.9781
Material Absorbtion:	0.010	Measurement Date and Time:	17 October 2013 14:56:37

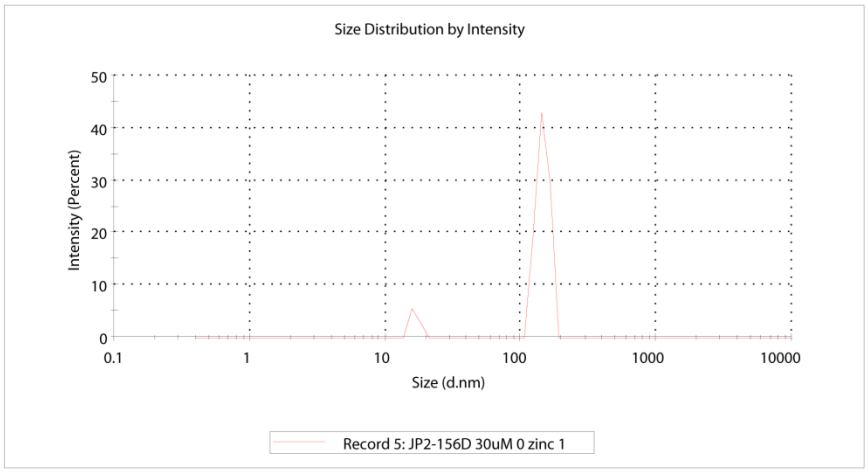
System

Temperature (℃):	21.0	Duration Used (s):	70
Count Rate (kcps):	116.7	Measurement Position (mm):	4.65
Cell Description:	Disposable sizing cuvette	Attenuator:	10

Results

			Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	552.7	Peak 1:	144.9	91.5	15.18
PdI:	0.638	Peak 2:	16.53	8.5	1.176
Intercept:	1.03	Peak 3:	0.000	0.0	0.000

Result quality : Refer to quality report



- Sensor **11b** + 1 equiv. Zn^{2+}:

Size Distribution Report by Intensity

v2.2

Malvern

Sample Details

Sample Name:	JP2-156D 30uM 1 zinc 1
SOP Name:	mansettings.nano
General Notes:	

File Name:	ZET0002894.dts	Dispersant Name:	Water
Record Number:	6	Dispersant RI:	1.330
Material RI:	1.59	Viscosity (cP):	0.9781
Material Absorbtion:	0.010	Measurement Date and Time:	17 October 2013 15:02:53

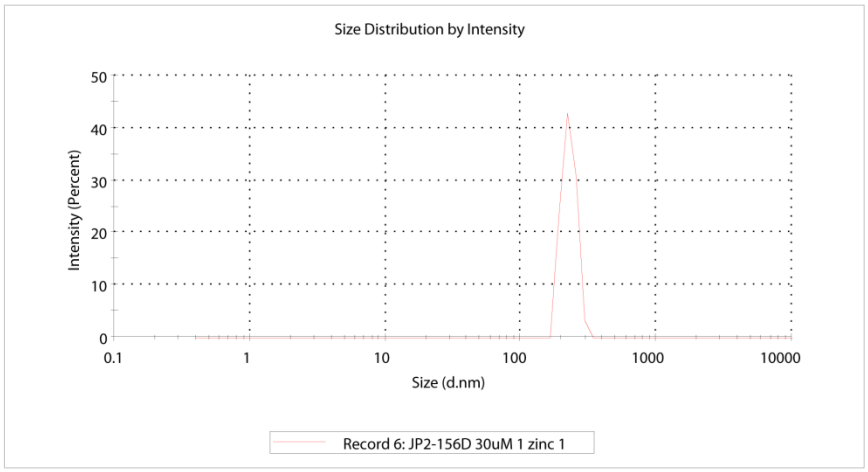
System

Temperature (℃):	21.0	Duration Used (s):	60
Count Rate (kcps):	337.7	Measurement Position (mm):	4.65
Cell Description:	Disposable sizing cuvette	Attenuator:	10

Results

			Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	452.2	Peak 1:	226.4	100.0	27.00
PdI:	0.468	Peak 2:	0.000	0.0	0.000
Intercept:	1.01	Peak 3:	0.000	0.0	0.000

Result quality : Refer to quality report



- Sensor **11b** + 5 equiv. Zn^{2+}:

Size Distribution Report by Intensity

v2.2

Malvern

Sample Details

Sample Name:	JP2-156D 30uM 5 zinc 1
SOP Name:	mansettings.nano
General Notes:	

File Name:	ZET0002894.dts	Dispersant Name:	Water
Record Number:	7	Dispersant RI:	1.330
Material RI:	1.59	Viscosity (cP):	0.9781
Material Absorbtion:	0.010	Measurement Date and Time:	17 October 2013 15:08:06

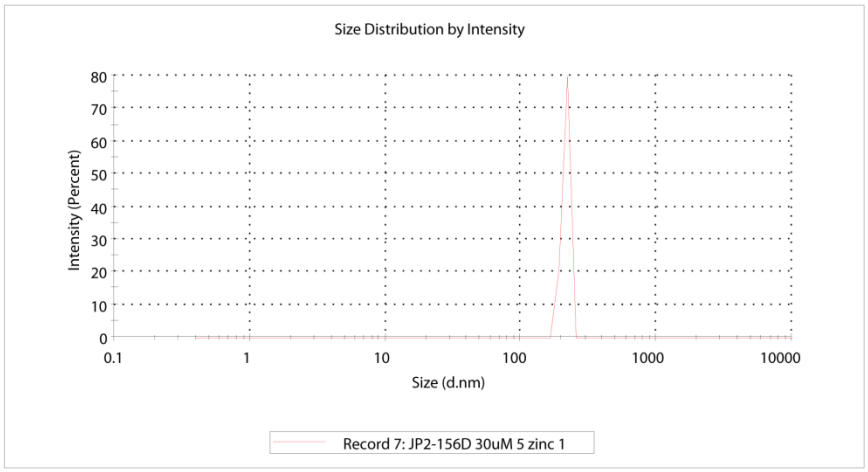
System

Temperature (℃):	21.0	Duration Used (s):	60
Count Rate (kcps):	302.3	Measurement Position (mm):	4.65
Cell Description:	Disposable sizing cuvette	Attenuator:	10

Results

			Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	1791	Peak 1:	214.1	100.0	12.08
PdI:	1.000	Peak 2:	0.000	0.0	0.000
Intercept:	1.25	Peak 3:	0.000	0.0	0.000

Result quality : Refer to quality report



- Sensor **11b** + 15 equiv. Zn^{2+}:

Size Distribution Report by Intensity

v2.2

Malvern

Sample Details

Sample Name:	JP2-156D 30uM 15 zinc 1
SOP Name:	mansettings.nano
General Notes:	

File Name:	ZET0002894.dts	Dispersant Name:	Water
Record Number:	8	Dispersant RI:	1.330
Material RI:	1.59	Viscosity (cP):	0.9781
Material Absorbtion:	0.010	Measurement Date and Time:	17 October 2013 15:14:23

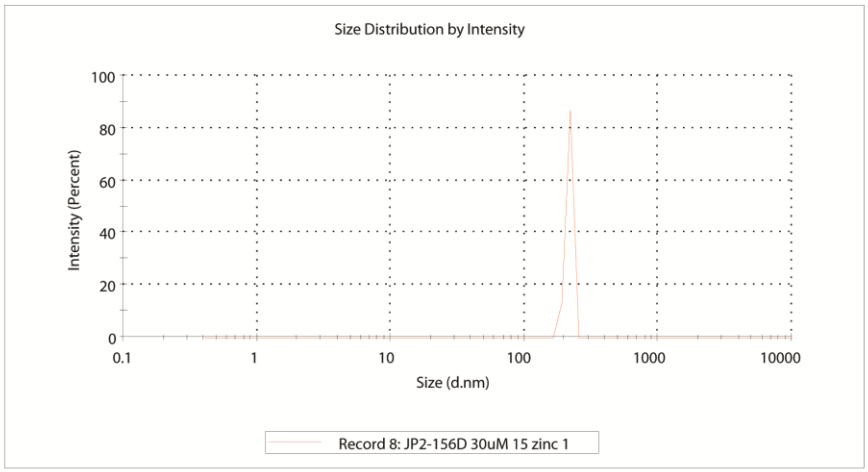
System

Temperature (℃):	21.0	Duration Used (s):	60
Count Rate (kcps):	279.3	Measurement Position (mm):	4.65
Cell Description:	Disposable sizing cuvette	Attenuator:	10

Results

			Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	1679	Peak 1:	216.2	100.0	10.22
PdI:	1.000	Peak 2:	0.000	0.0	0.000
Intercept:	1.29	Peak 3:	0.000	0.0	0.000

Result quality : Refer to quality report



5) Response of Probe 6 to Other Metal Ions

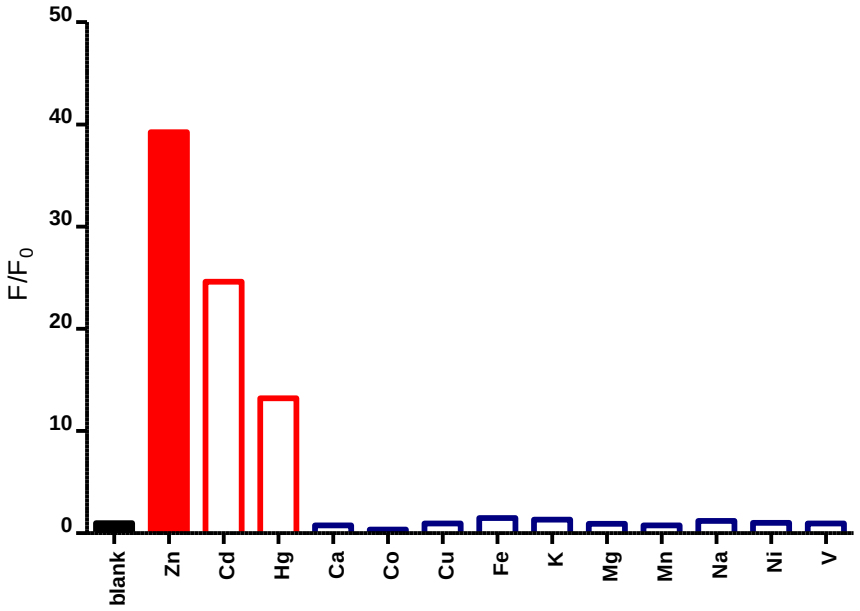


Fig. S29 Fluorescence response of sensor **6** (100 μM) in HEPES buffer (1% DMSO) to 5 equiv of metal ions (cations were used as the perchlorate salt).

6) Experimental Procedures for the Evaluation of Sensors in Pancreatic Islets

Female CD1 mice (8-12 weeks of age) were housed under specific pathogen free conditions with *ad libitum* access to food and water. Animals were euthanized by cervical dislocation before isolation of islets by collagenase digestion, as previously described (PMID: 20204627). Animal procedures were approved by the Home Office according to the Animals (Scientific Procedures) Act 1986 of the United Kingdom (PPL 70/7349). Following 24-48 h culture, islets were incubated for 60 min. with the sensor under study before imaging using a Zeiss Axiovert 200 inverted widefield stereomicroscope. Illumination was delivered through a 20× 0.4NA objective (LD Plan Neofluar) using a halogen light source and a DAPI filter set (λ_{ex} = 365/12, λ_{em} = 447/60). Emitted signals were detected using a highly-sensitive 1344 x 1024 CCD camera (Hamamatsu ORCA-ER). Throughout, islets were incubated at 36 ^{o}C and irrigated with HEPES-bicarbonate buffer (120 mM NaCl, 4.8 mM KCl, 24 mM $NaHCO_3$, 0.5 mM Na_2HPO_4, 5 mM HEPES, 2.5 mM $CaCl_2$ and 1.2 mM $MgCl_2$) saturated with 95% O_2/5% CO_2 and adjusted to pH 7.4. Offline signal analysis was performed using Volocity (Perkin Elmer) and Igor Pro (Wavemetrics) softwares. For two-photon imaging of probe distribution, islets were incubated with sensor as above before imaging using a Leica SP5 multiphoton microscope equipped with a 25× 0.95NA water-dipping objective adjusted for infrared wavelengths (HCX IRAPO). Two-photon excitation was achieved using a Spectraphysics Mai Tai femtosecond-pulsed laser (λ_{ex} = 850 nm) and emitted signals were collected using a PMT (λ_{em} = 400-550 nm). Image analysis was performed using Image J (NIH).

a) Cytotoxicity Assay

Islets were incubated with 3 μM calcein-AM (Life Technologies) and 2.5 μM propidium iodide (PI; Sigma-Aldrich) before detection of absorbance/emission at 491/525nm and 561/620nm, respectively. Calcein AM is a vital stain and requires cleavage by intracellular esterases for

fluorescence. Conversely, PI is a necrosis stain which only enters the nucleus in membrane-compromised cells. The islet area occupied by dead cells was expressed as a unitary ratio versus that occupied by live cells. Non-multifactorial pairwise comparisons were performed using Student's t-test. Interactions between multiple treatments were assessed using Kruskal-Wallis test followed by pairwise comparisons using Dunn's post-hoc test. In all cases, analysis was performed using Graphpad Prism (Graphpad Software) and results considered significant at $P<0.05$.

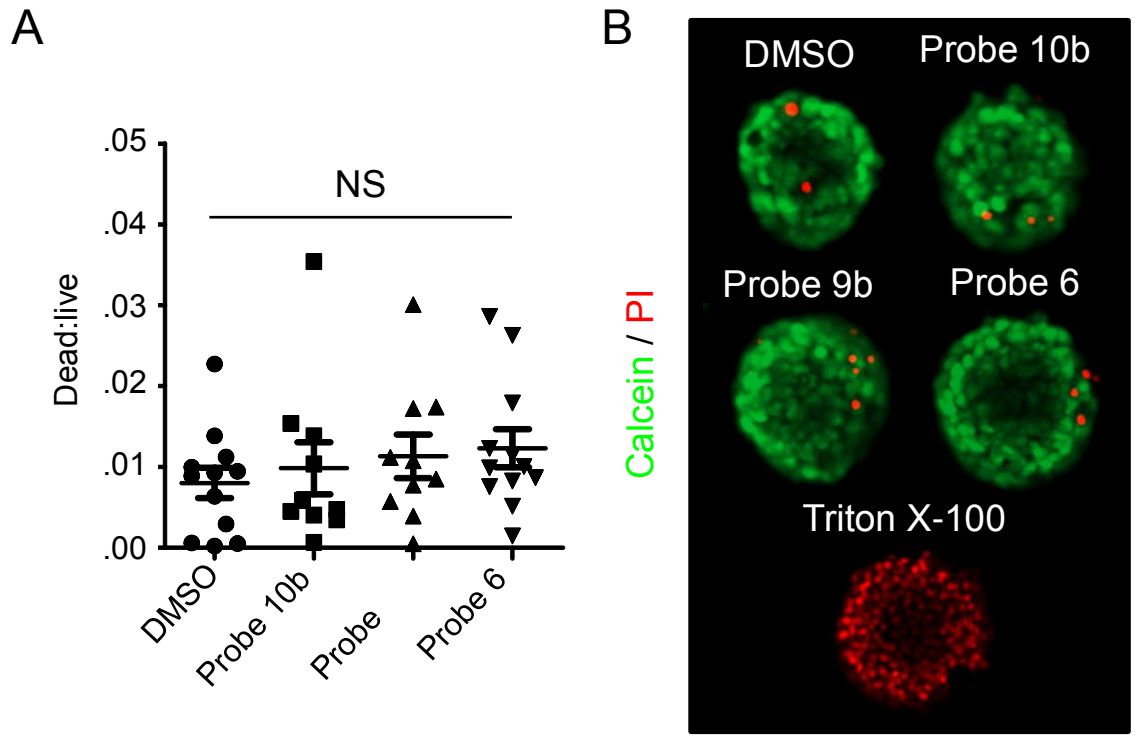


Fig. S30: Cytotoxicity assays for probes **6**, **9b** and **10b**. A. Incubation of mouse islets with probes **6**, **9b** and **10b** does not significantly induce cell death (necrosis) versus DMSO-alone (1:333) (NS, non-significant; $P<0.54$). B. Representative images showing calcein and PI staining in islets treated with DMSO and probes **6**, **9b** and **10b**. Below is a positive control (Triton X-100; to permeabilise the membrane).

a) Live imaging of dye co-localisation

MIN6 beta cells were incubated for 1-2 h with each Zn^{2+}-binding probe (30 µM for probes **6** and **9b**; 300 µM for probe **10b** before 30 min incubation with either 200 nM Mitotracker Red FM or 200 nM Lysotracker DND-99 (both Life Technologies). Cells were washed three times with PBS before live-imaging using a confocal microscope equipped with an acousto optical beam splitter (AOBS) and spectral detectors (Leica TCS SP5). The Zn^{2+}-binding probes were excited using a 405 nm diode laser and emissions collected at 460/70 nm (63 x oil-immersion objective; NA 1.4). The organelle-specific dyes were excited using a 543 HeNe laser and emitted light captured using PMTs centred on 650/50 nm and 595/50 nm for Mitotracker and Lysotracker, respectively. Analysis of co-localisation was performed after background fluorescence correction using the Pearson's correlation coefficient embedded within the Intensity Correlation Analysis (ICA) plugin for ImageJ (NIH). Uniform linear adjustments were applied to contrast/brightness to improve image quality for analysis/presentation purposes.

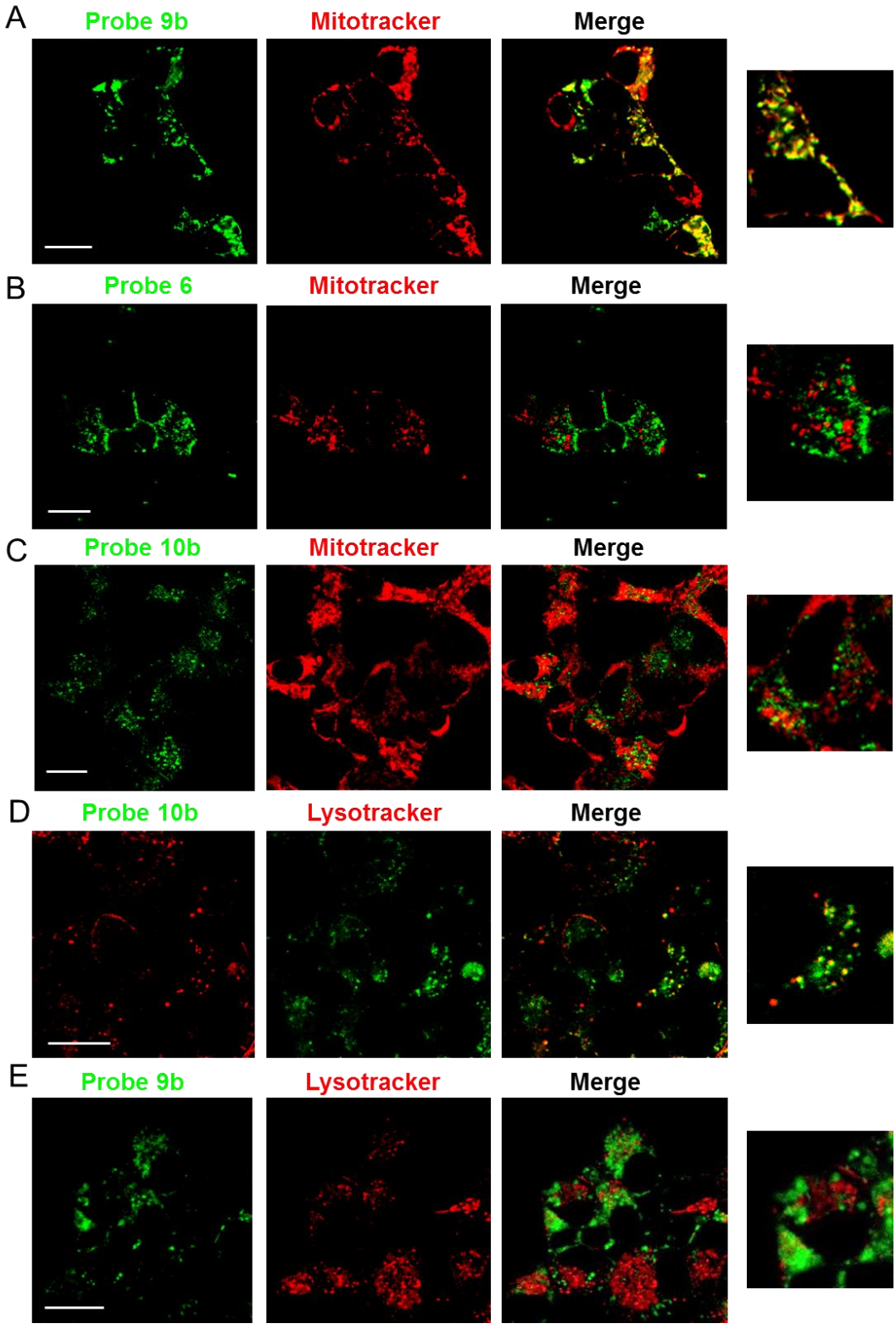


***Fig. S31*:** Cellular distribution of probes **6**, **9b** and **10b**. A. Probe **9b**, expected to sequester in mitochondria, is co-localised with Mitotracker. B. Probe **6**, expected to aggregate at the plasma membrane, does not co-localise with Mitotracker. C. Probe **10b**, expected to aggregate in lysosomes, does not co-localise with Mitotracker. D. Probe **10b** co-localises with Lysotracker, a marker of acidic organelles. E. Probe **9b** does not co-localise with Lysotracker, further confirming its mitochondrial specificity. Scale bar represents 10 µm.

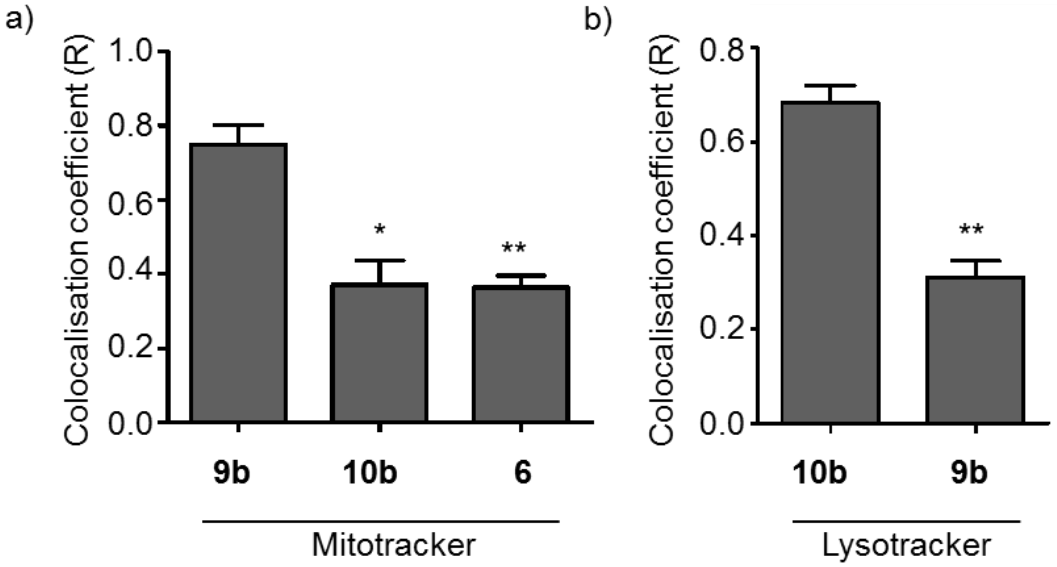


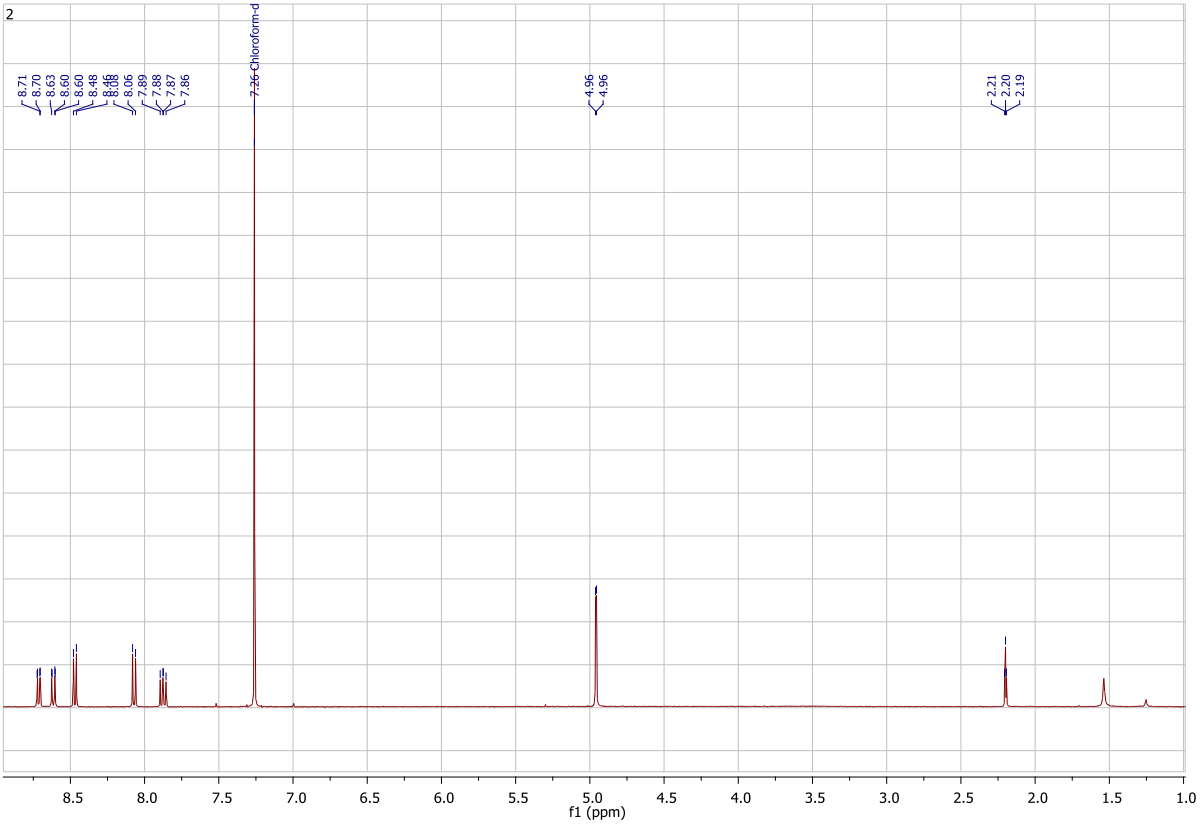
Fig. S32 Statistical analysis of probe localisation with a) mito- and b) lyso-tracker.

Image analysis demonstrates that the signals from probe **9b** and Mitotracker are strongly correlated. By contrast, fluorescence from both probe **10b** and probe **6** poorly correlates with that of the organelle-specific dye (*P<0.05 and **P<0.01 versus probe **9b**) (Kruskal-Wallis test). Note that some co-localisation is still apparent due to the inability to fully resolve sub-cellular structures close to the diffraction limit. We also observe a strong correlation between probe **10b** and Lysotracker DND-99, but not probe **9b** and the organelle-specific dye (**P<0.001 probe 11b versus probe **10b**) (Student's t-test).

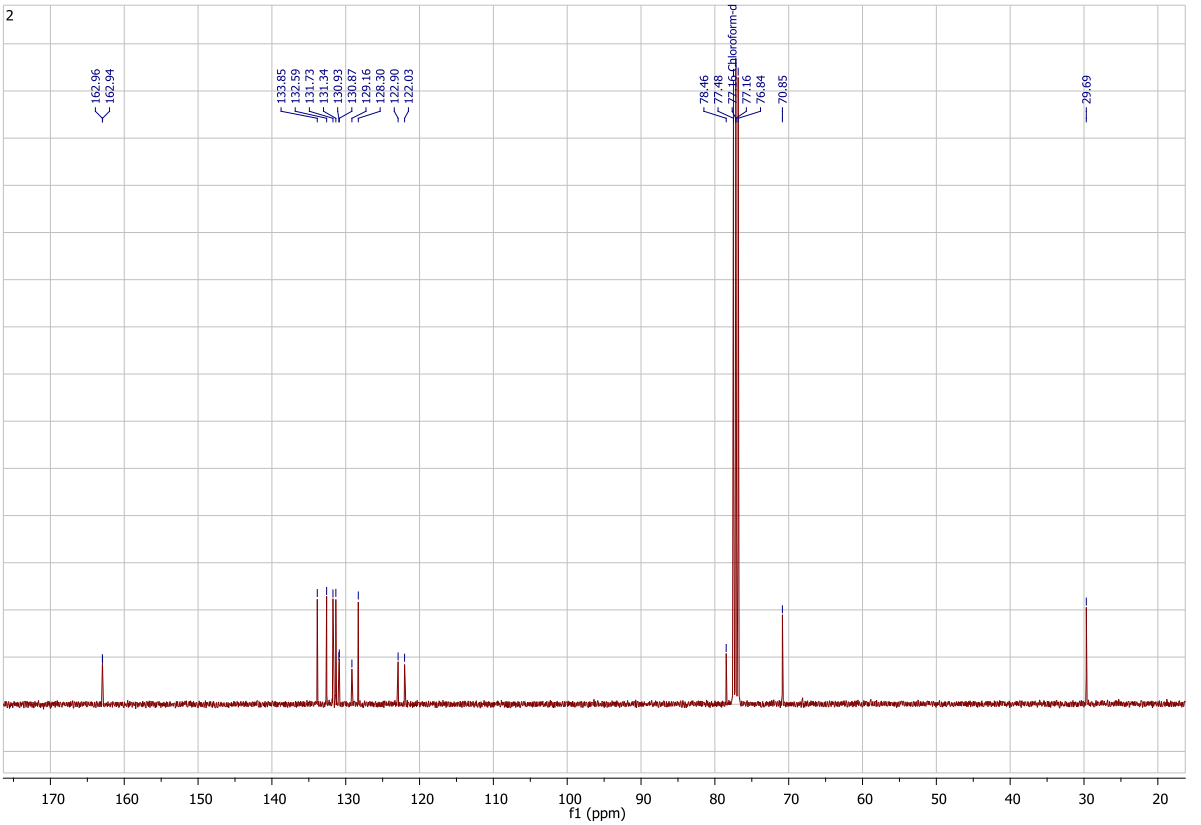
7) NMR Spectroscopic Characterisation

a) Compounds reported in the manuscript

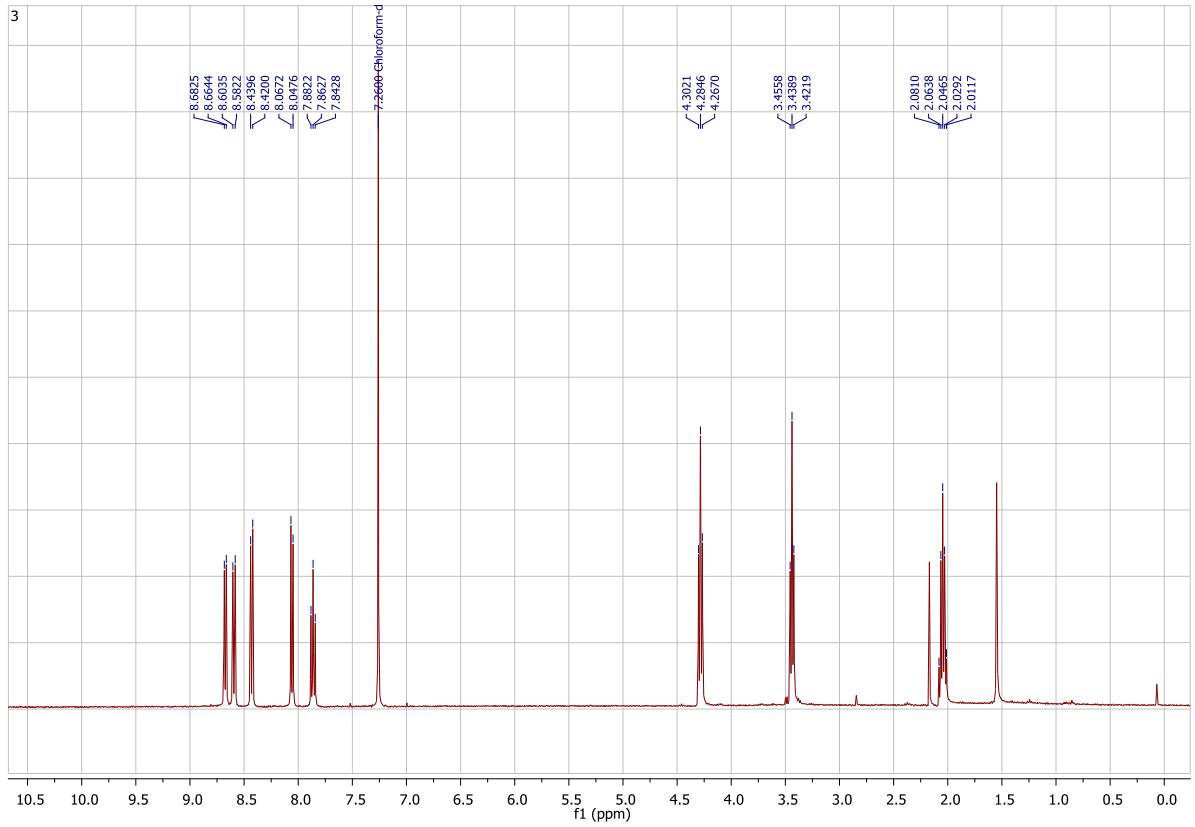
^{1}H NMR of **2**



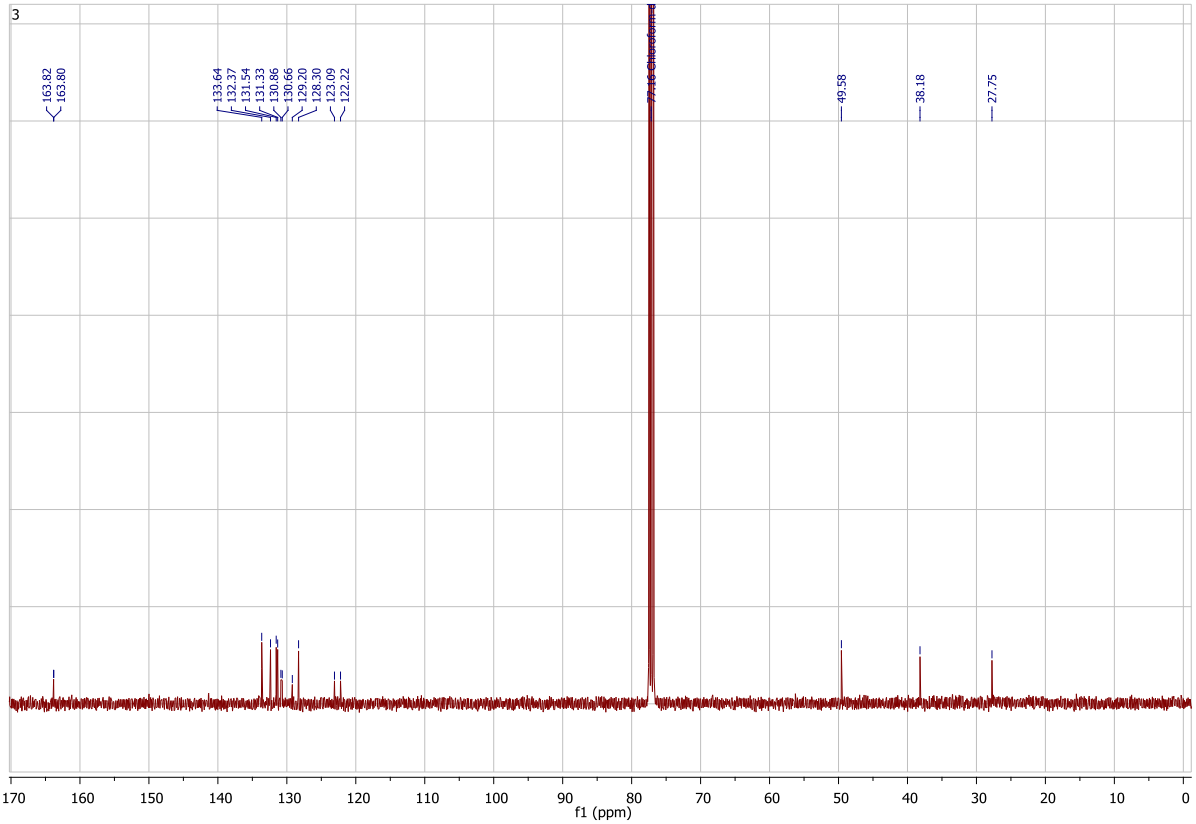
^{13}C NMR of **2**



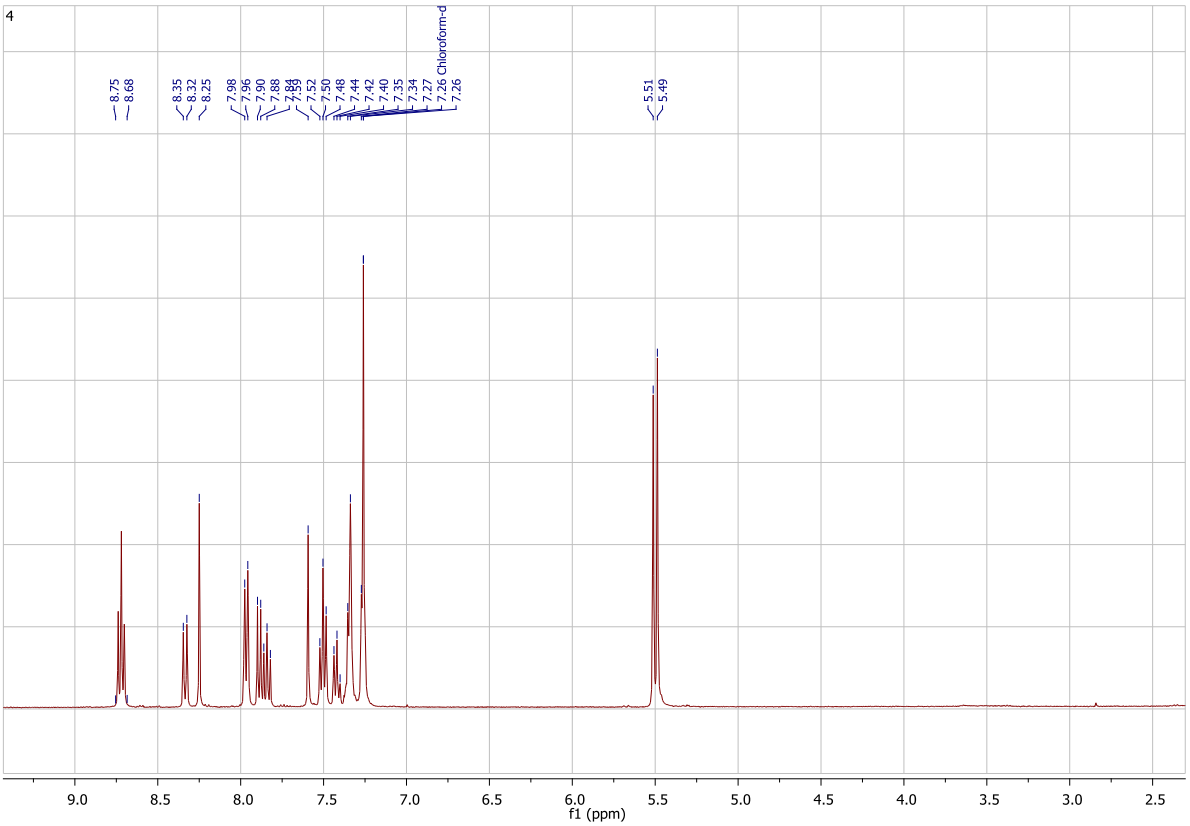
^{1}H NMR of **3**



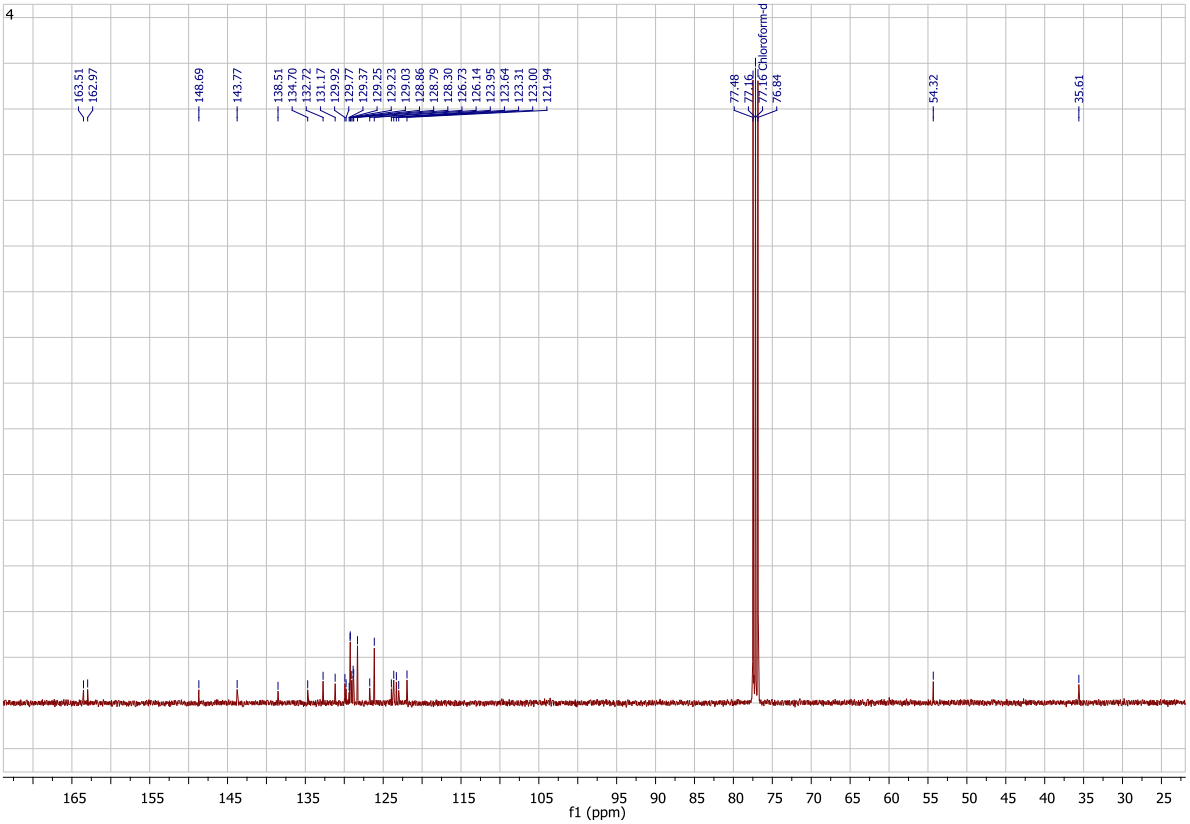
^{13}C NMR of **3**



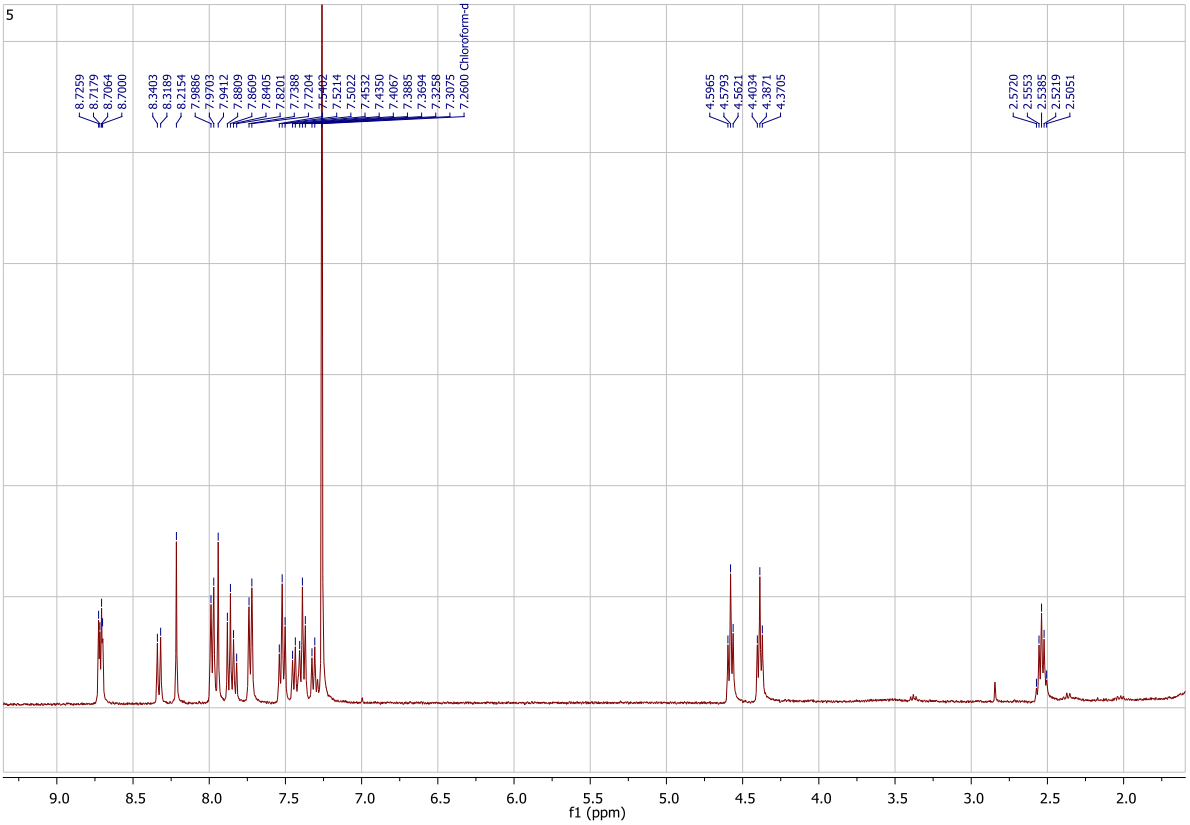
^{1}H NMR of **4**



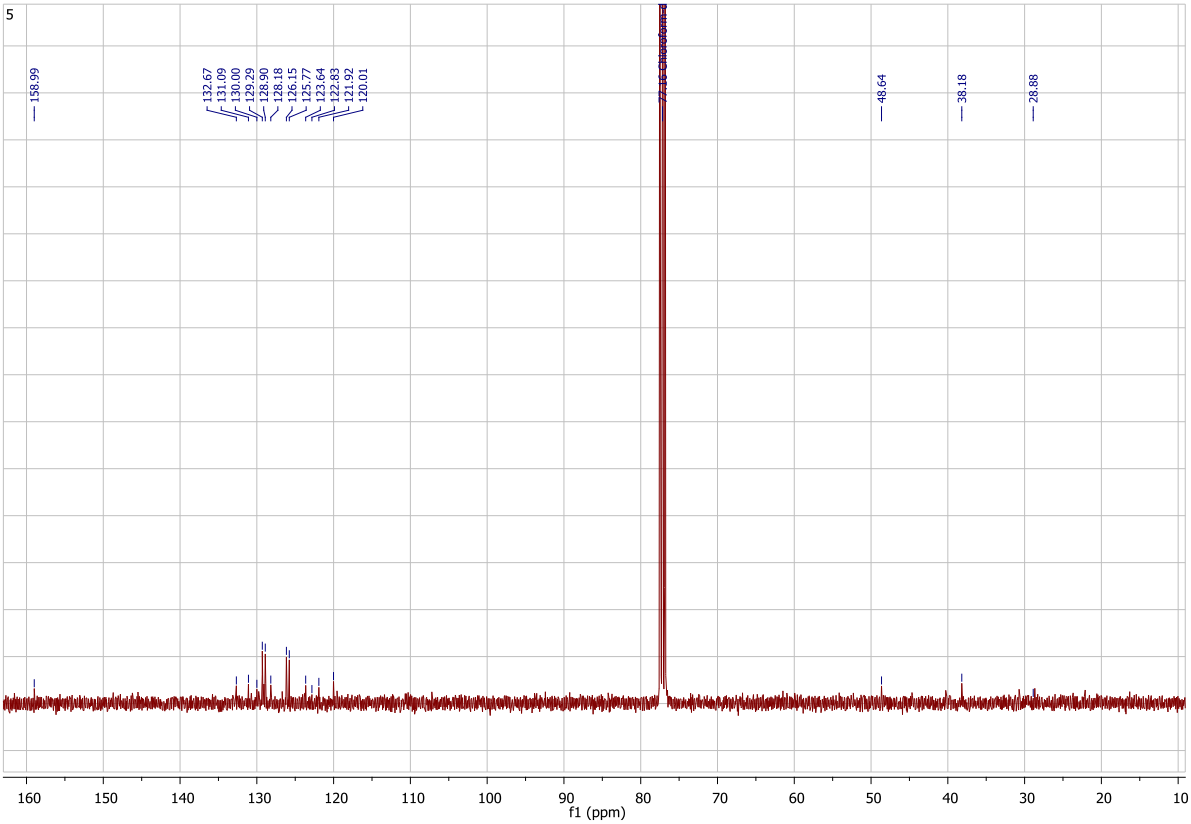
^{13}C NMR of **4**



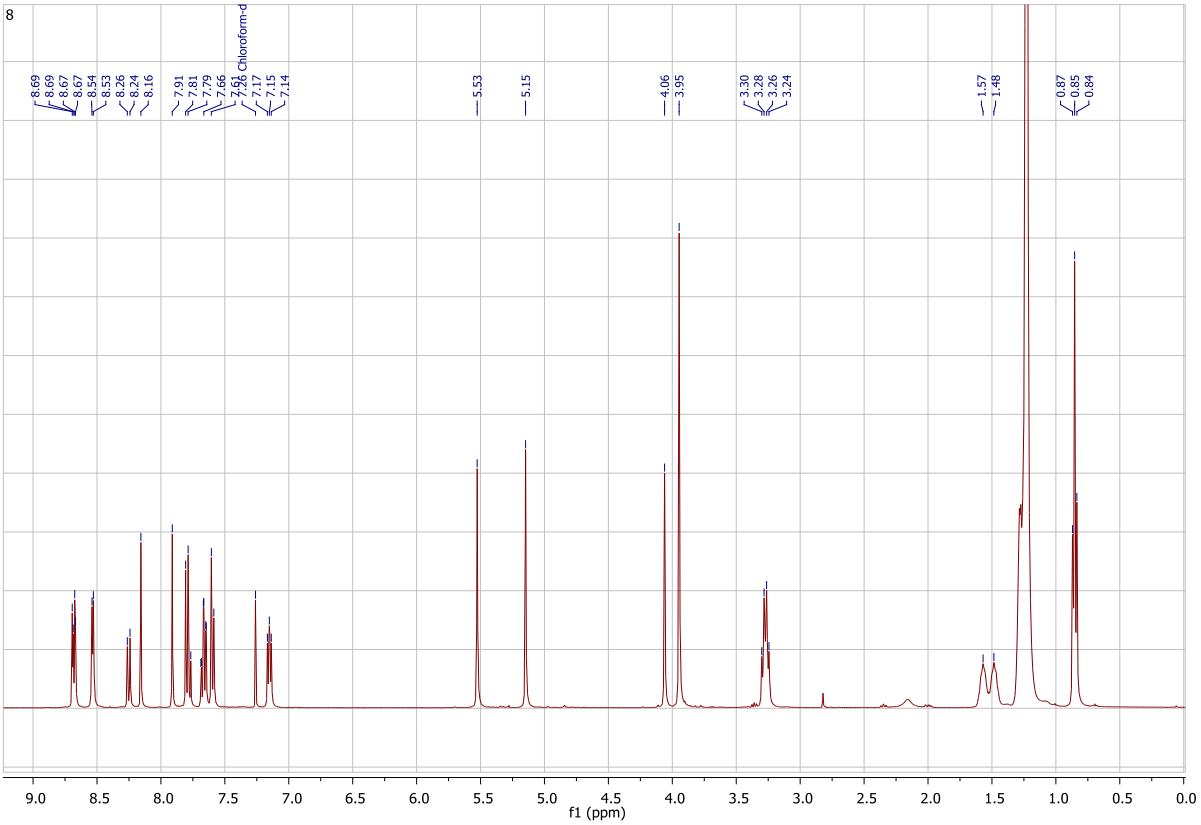
^{1}H NMR of **5**



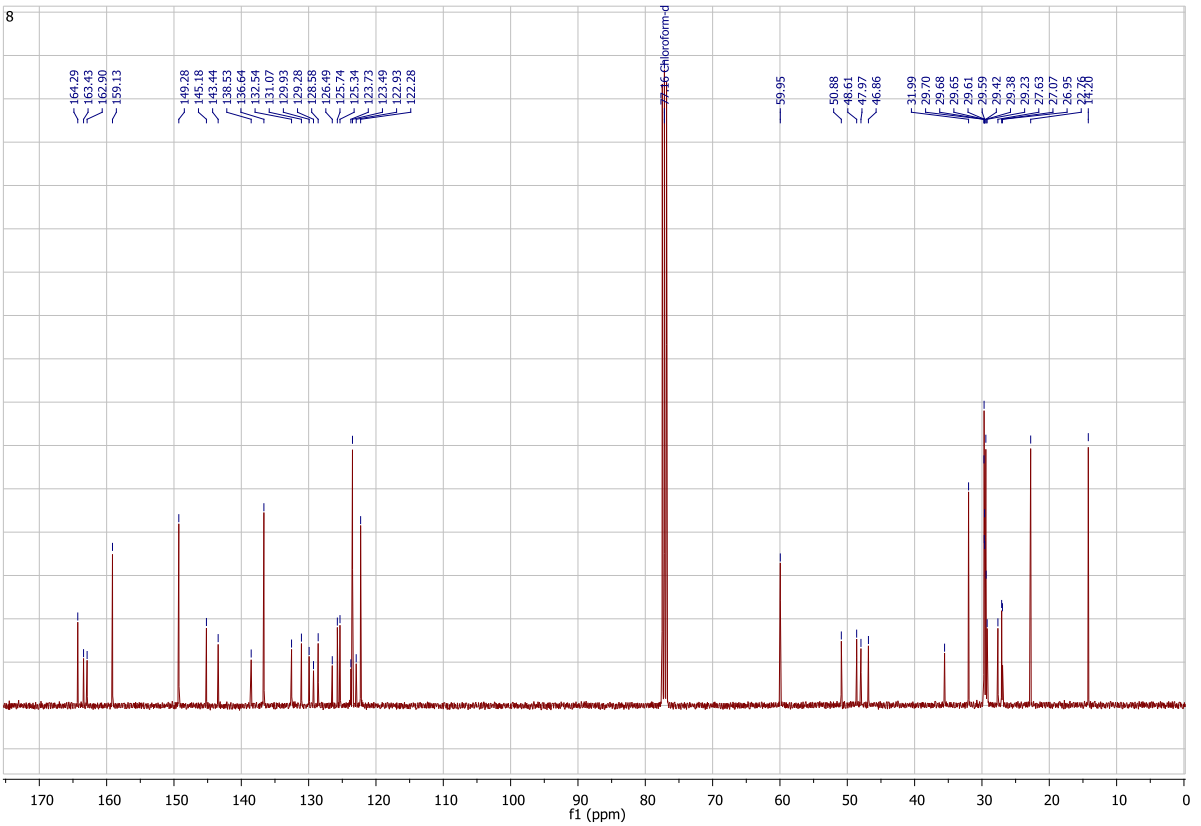
^{13}C NMR of **5**



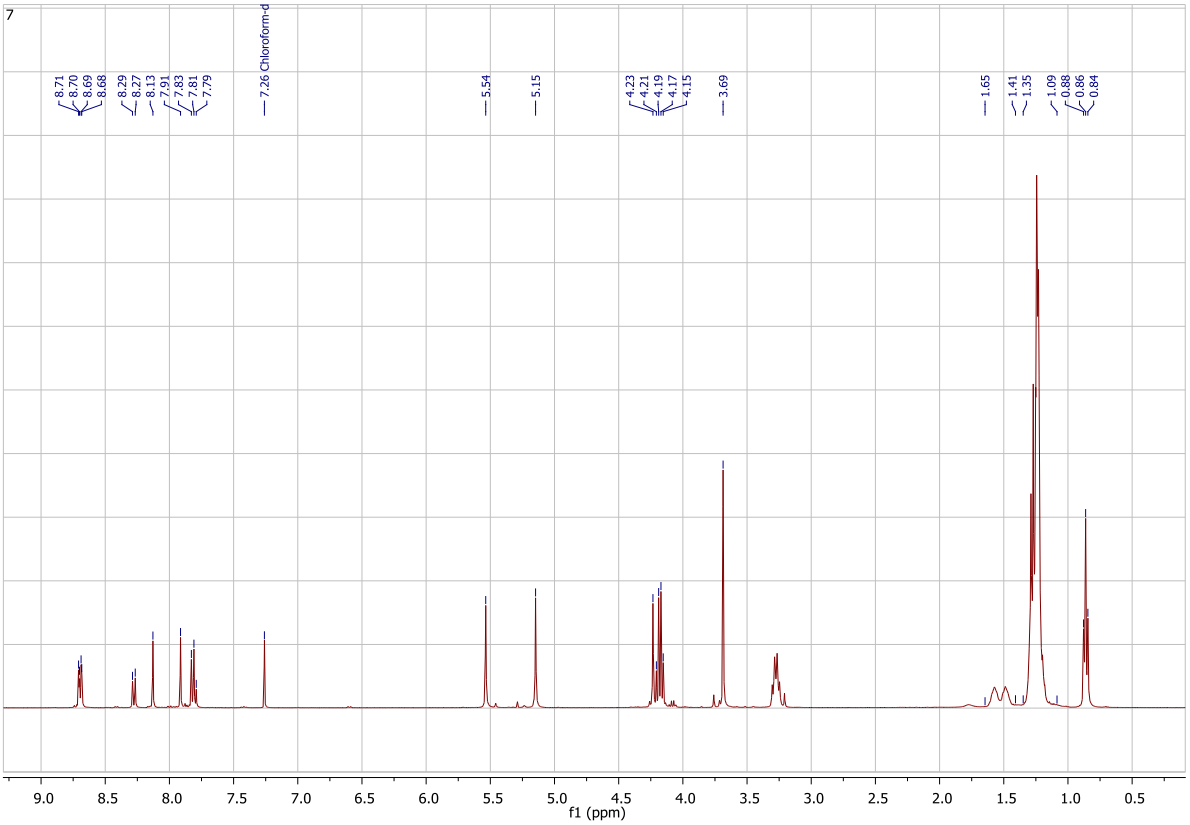
^{1}H NMR of **6**



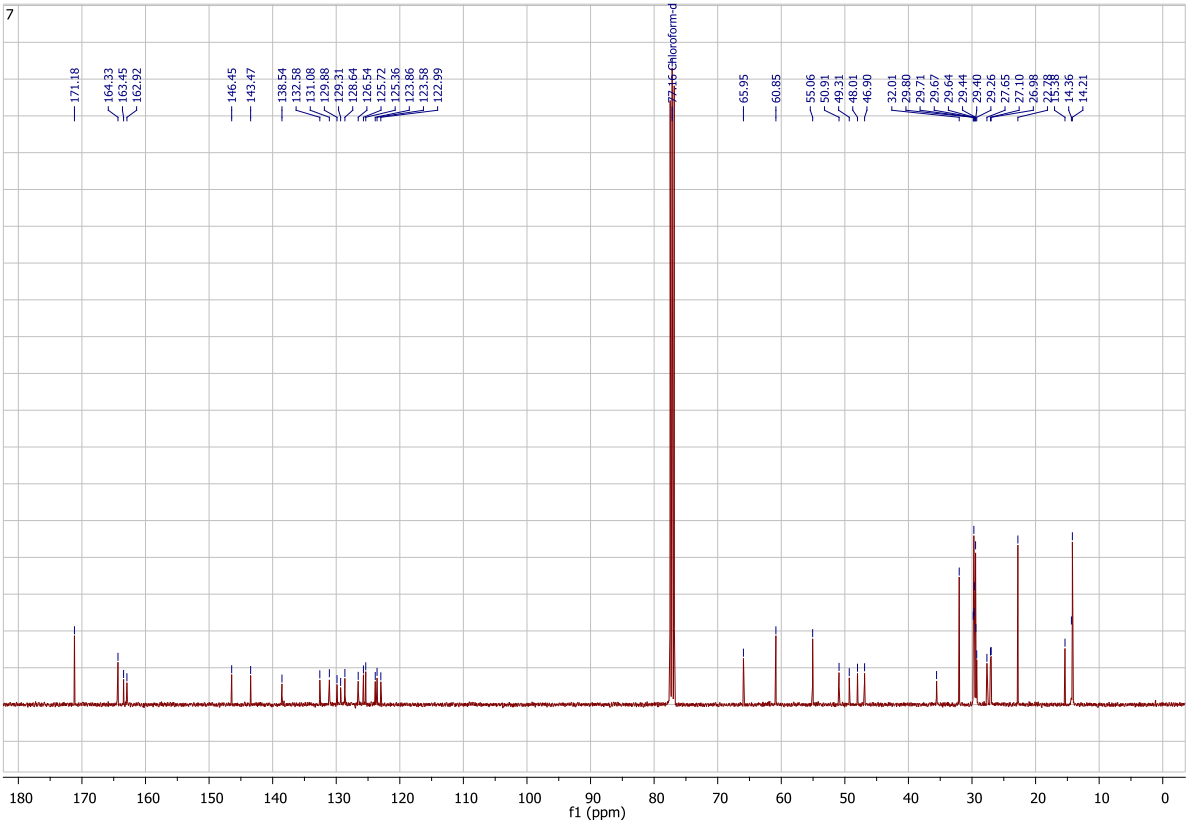
^{13}C NMR of **6**



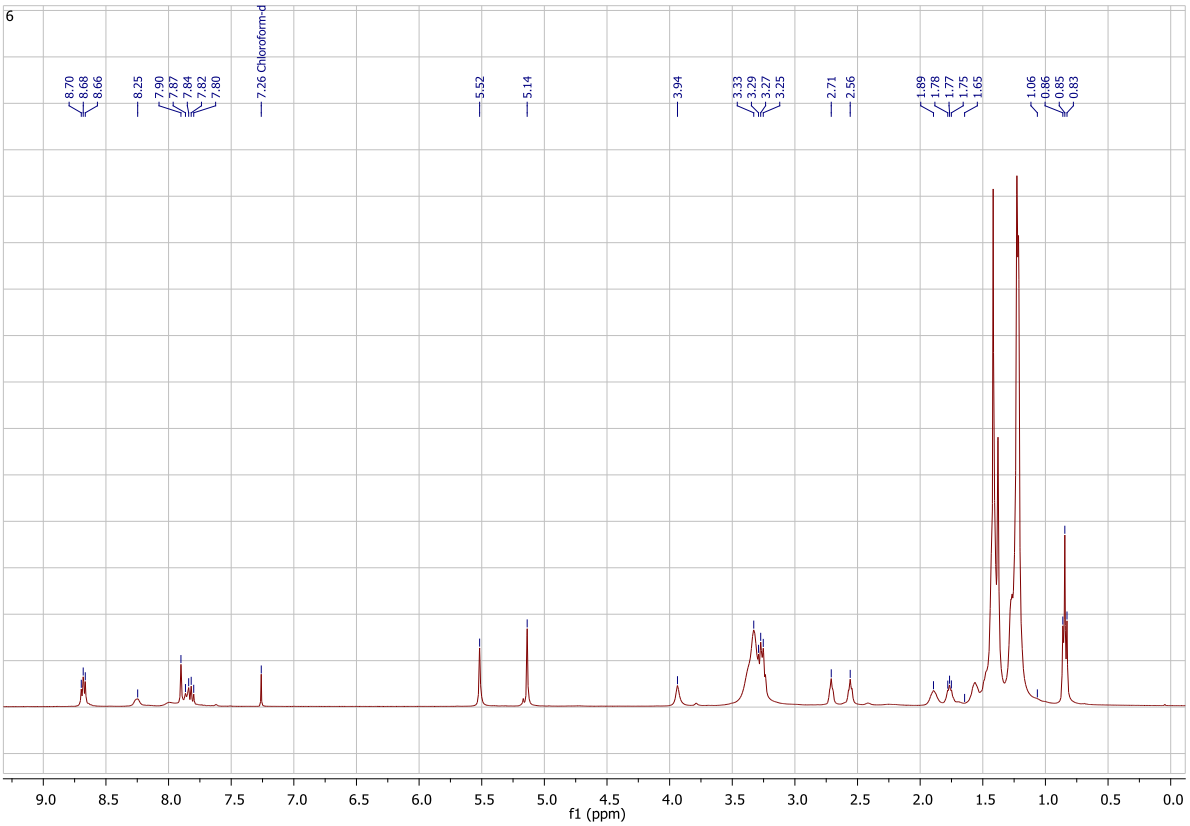
^{1}H NMR of **7a**



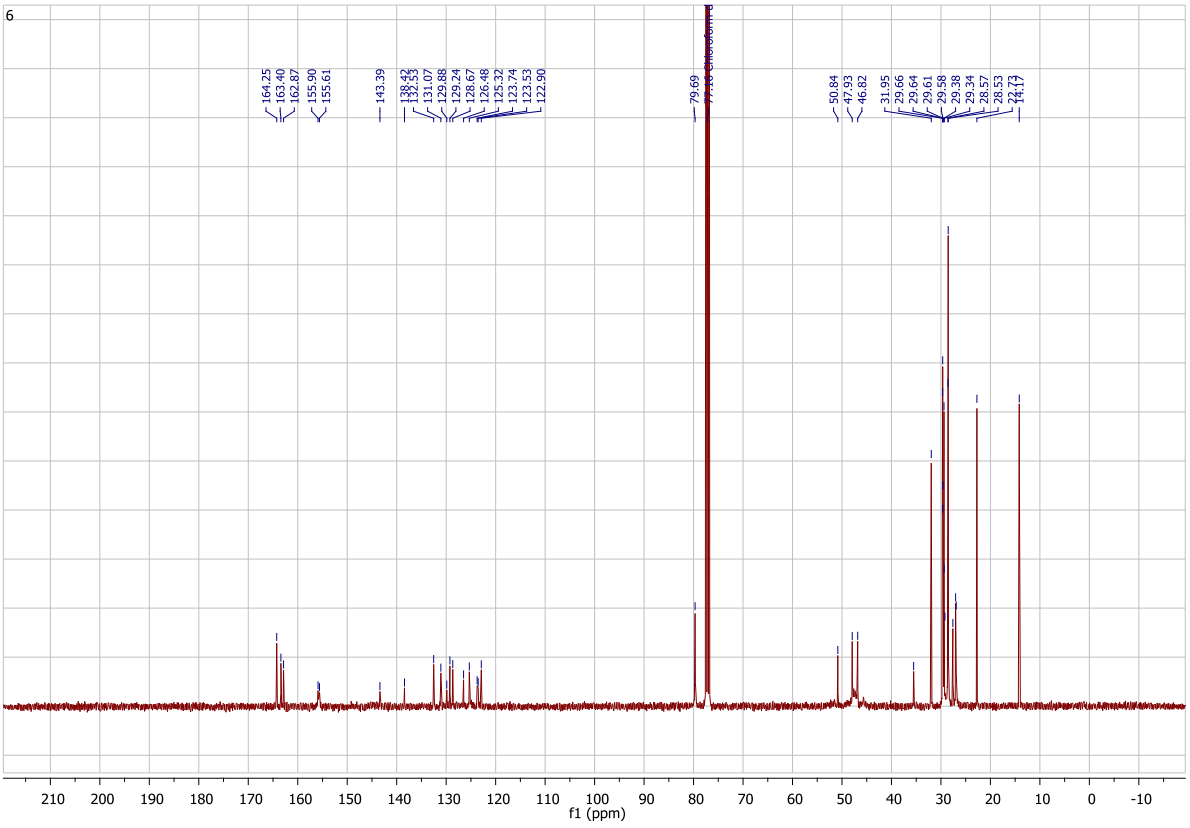
^{13}C NMR of **7a**



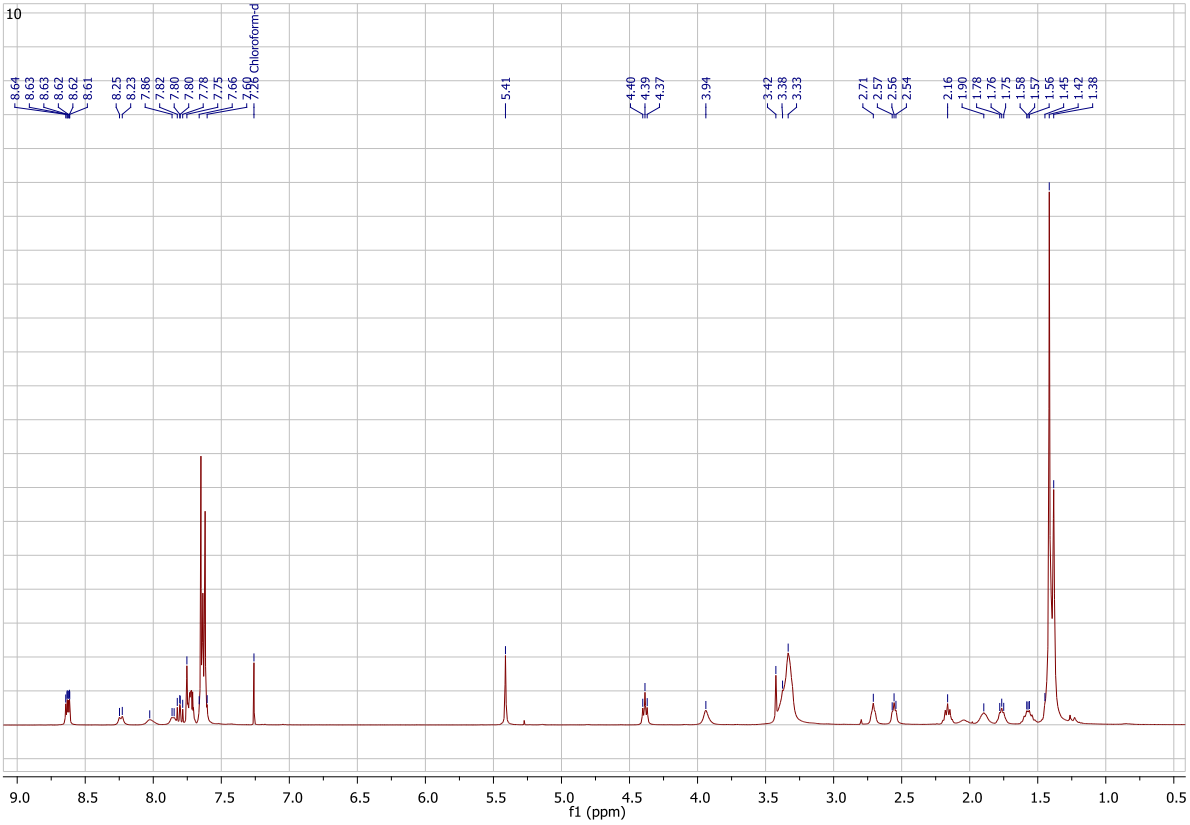
^{1}H NMR of **8a**



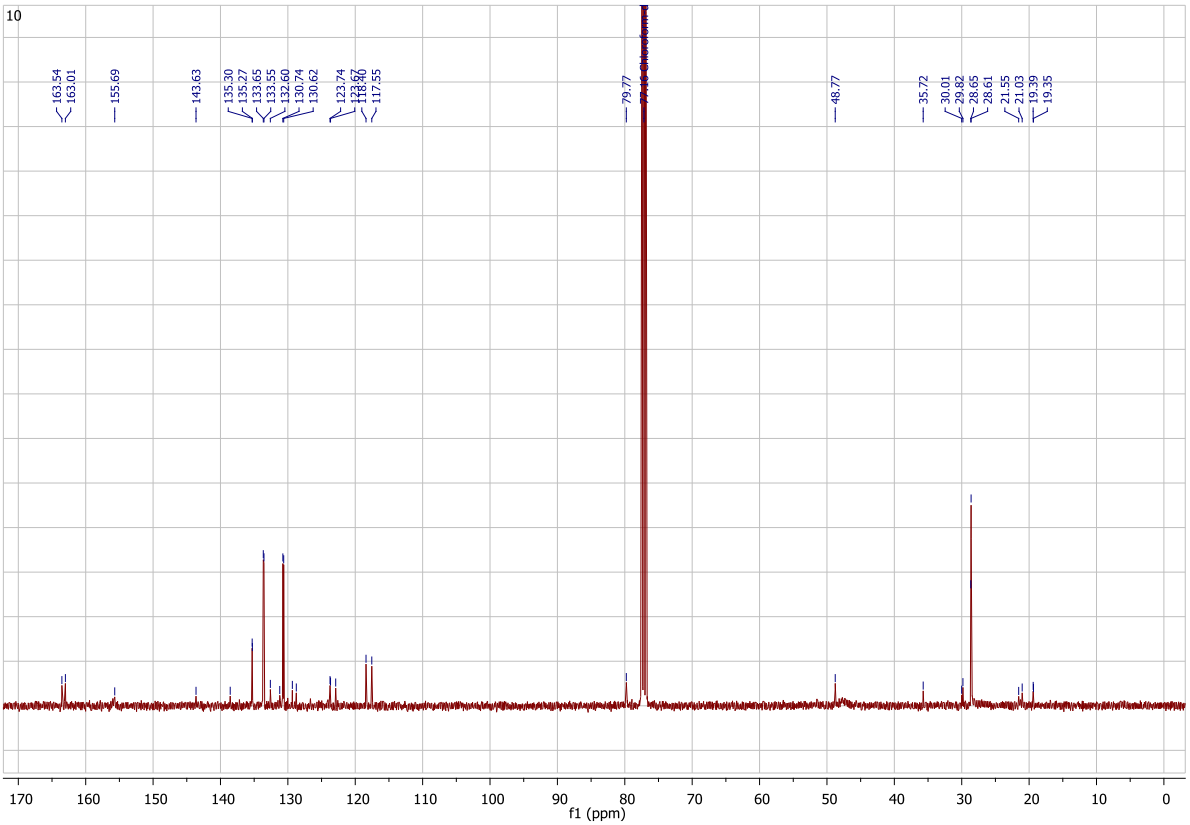
^{13}C NMR of **8a**



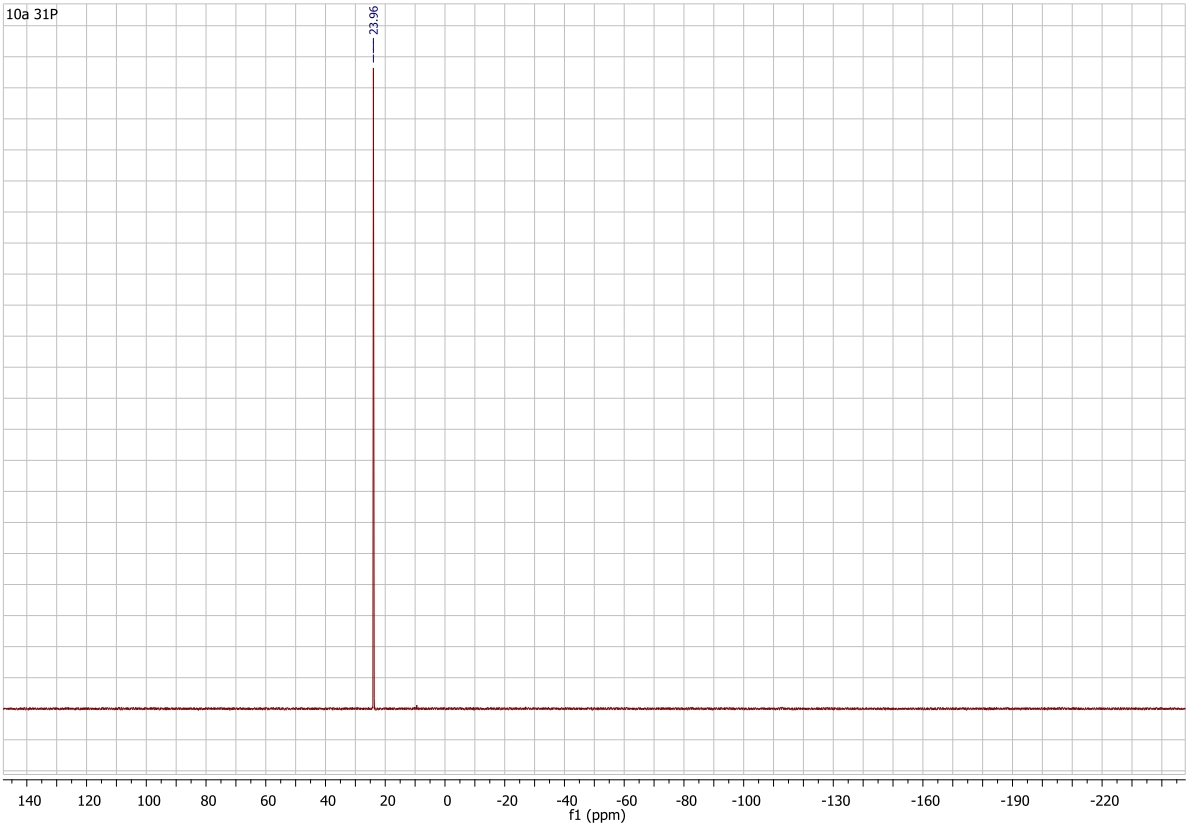
^{1}H NMR of **9a**



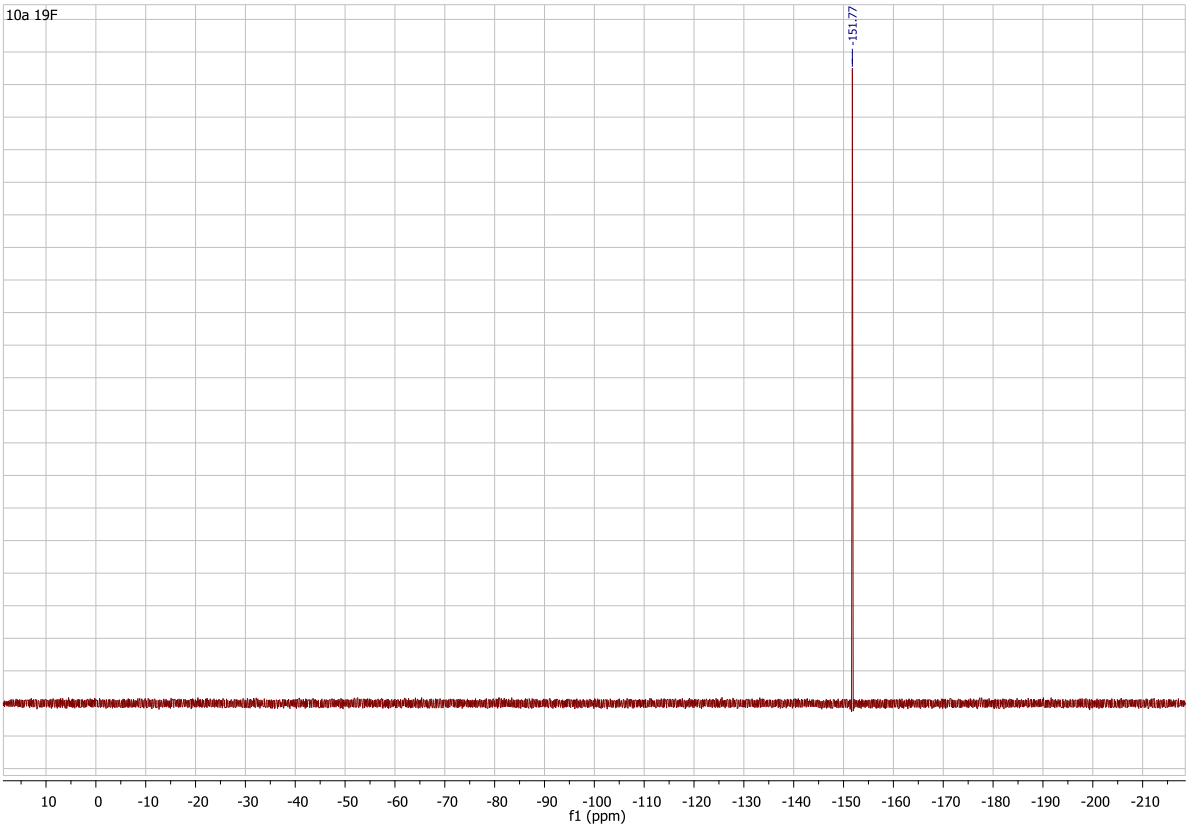
^{13}C NMR of **9a**



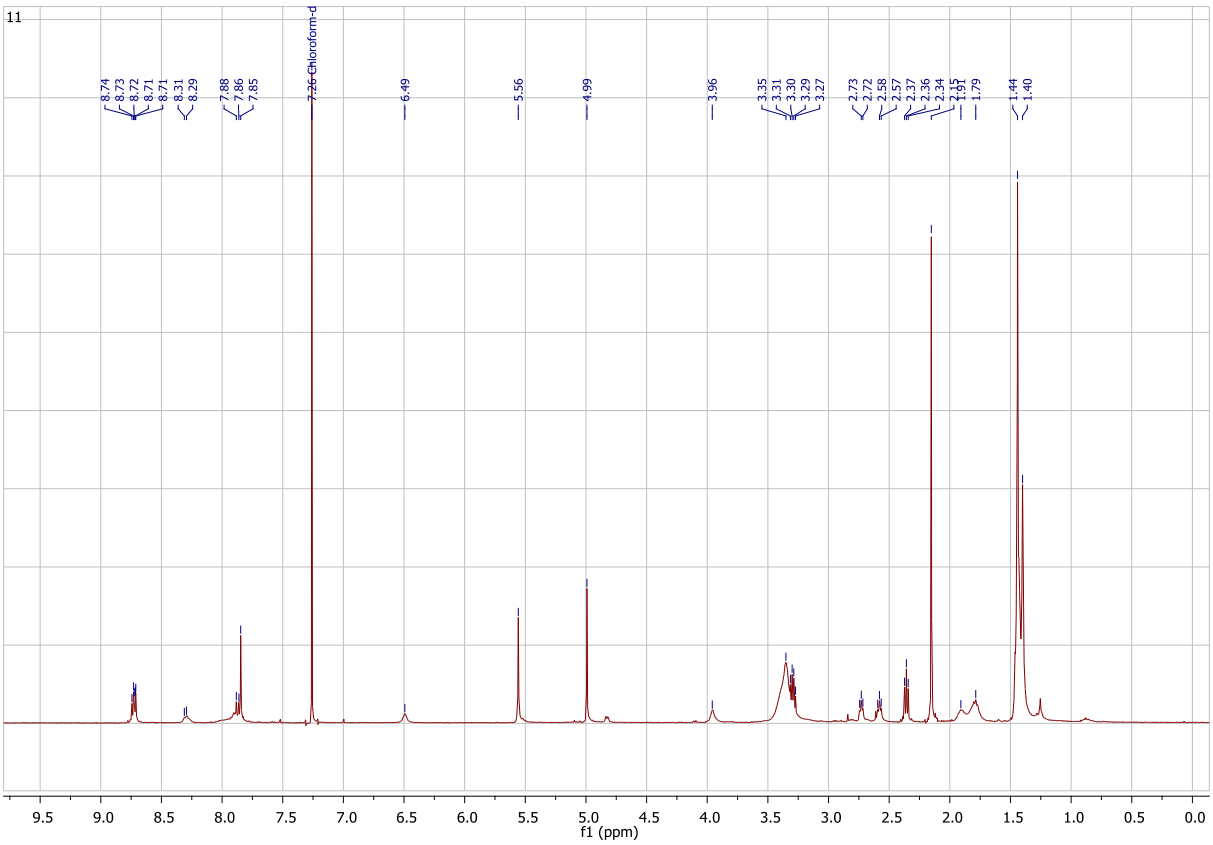
^{31}P of **9a**:



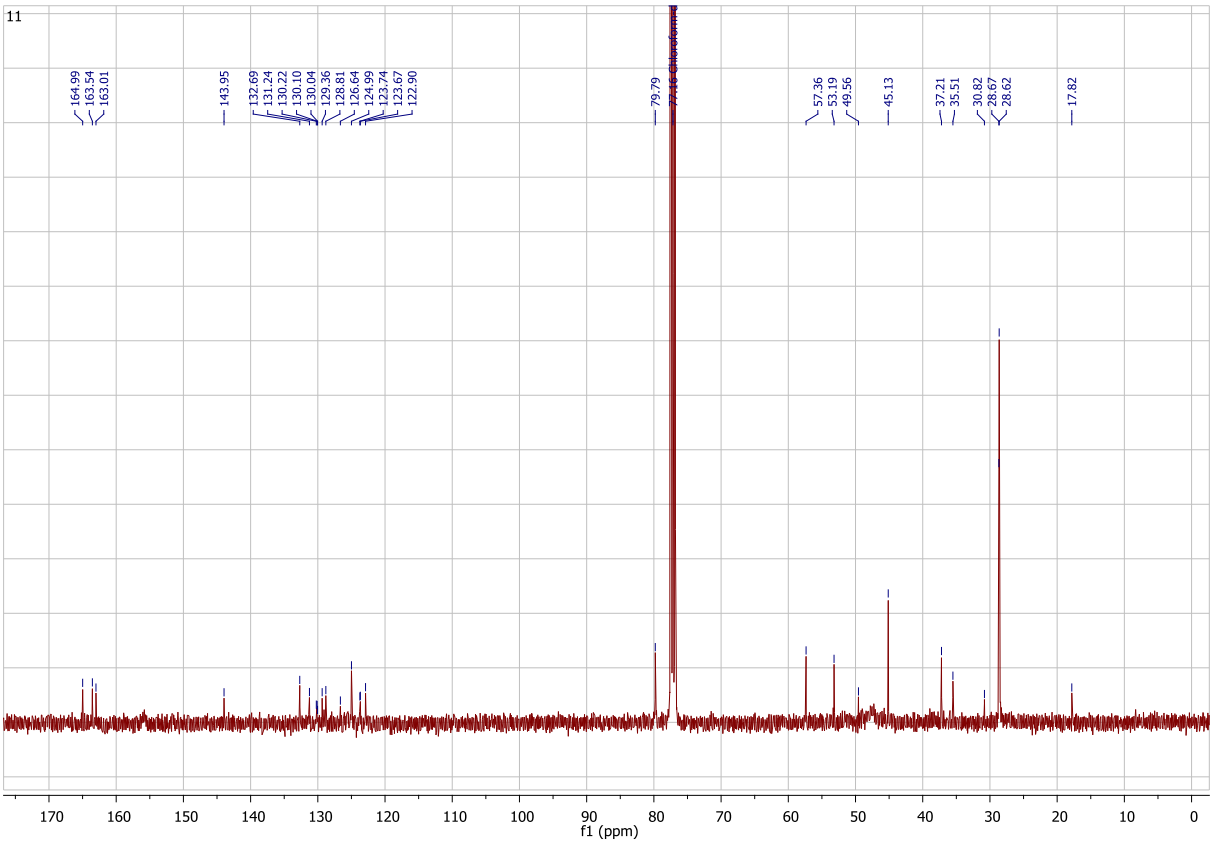
^{19}F of **9a**:



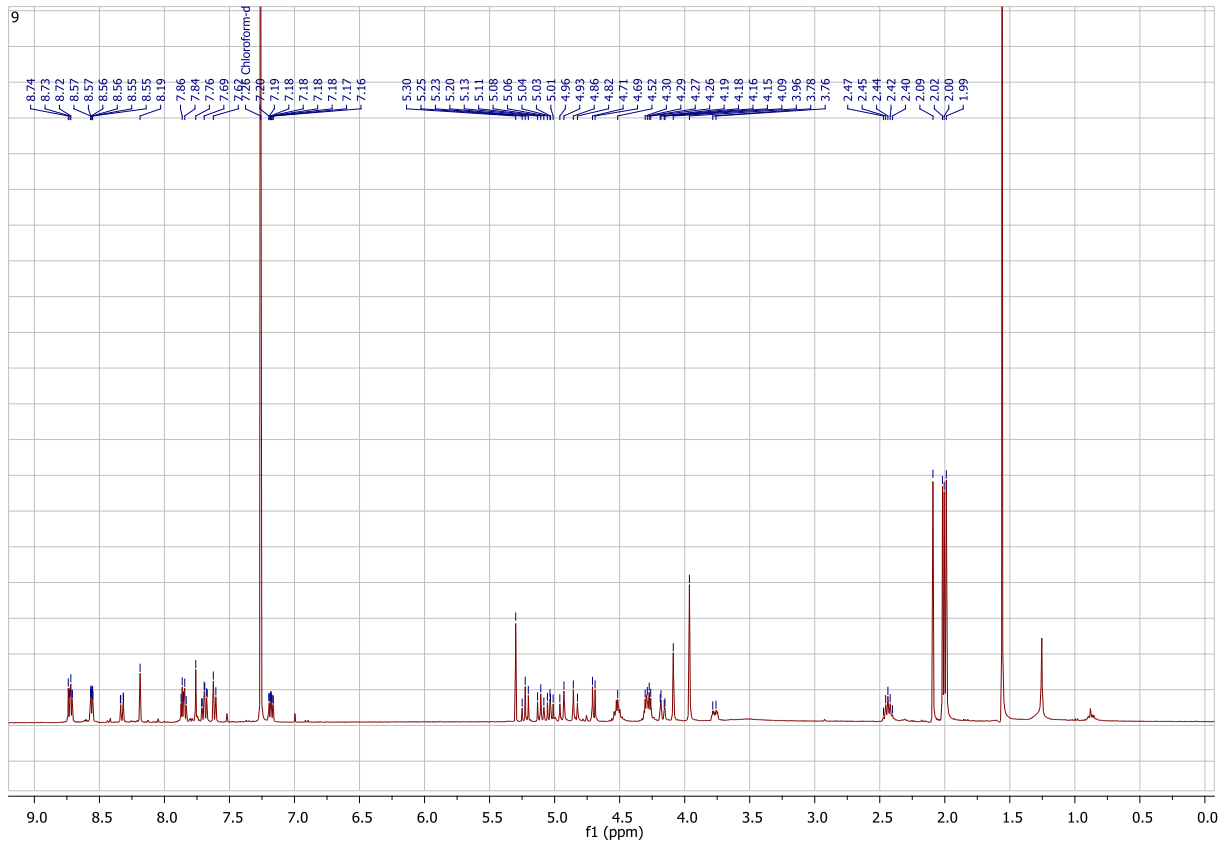
^{1}H NMR of **10a**



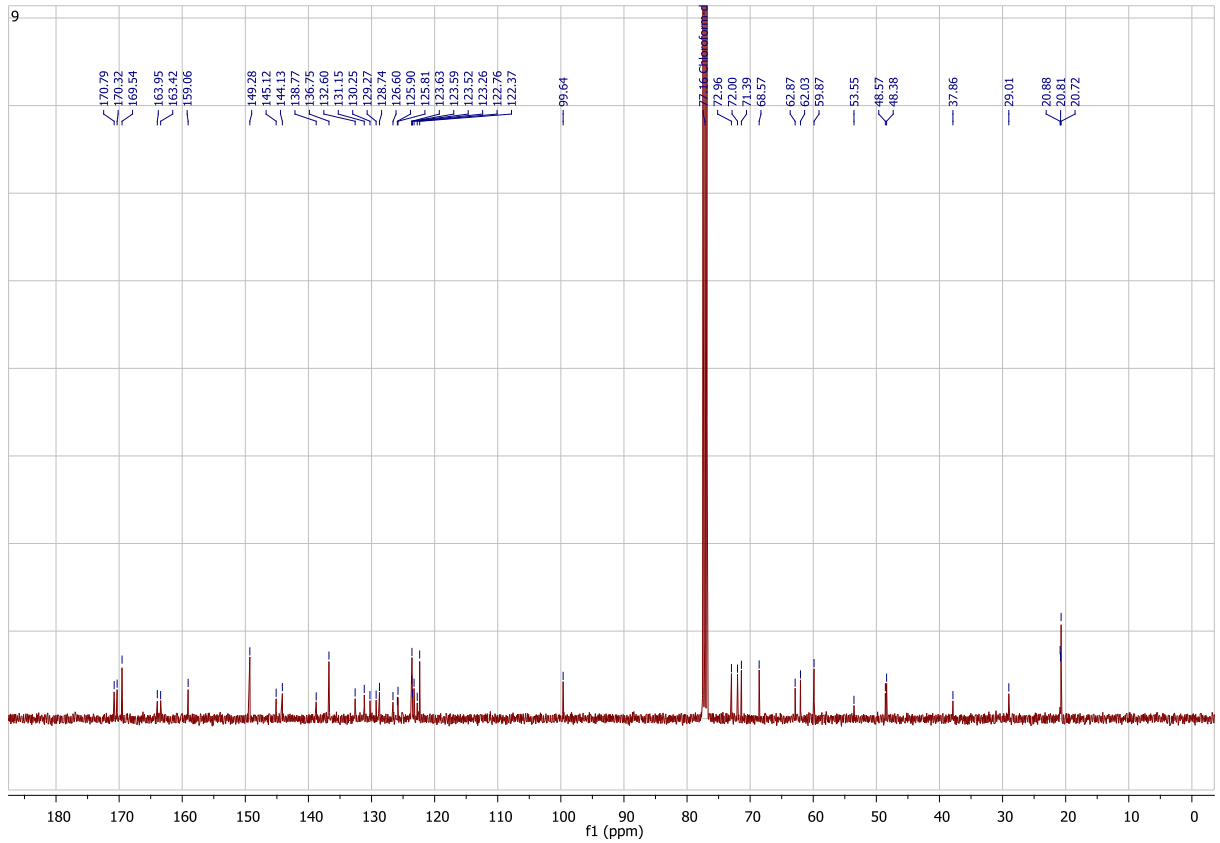
^{13}C NMR of **10a**



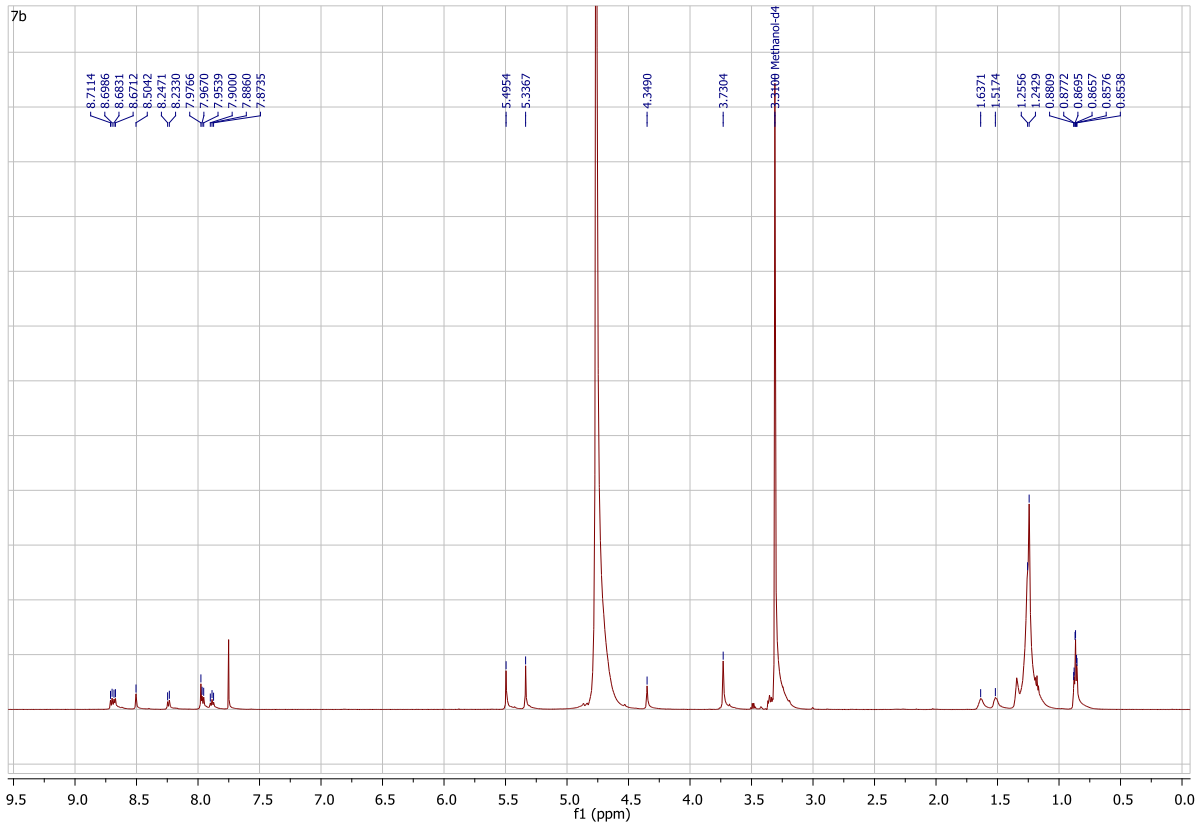
1H NMR of **11a**



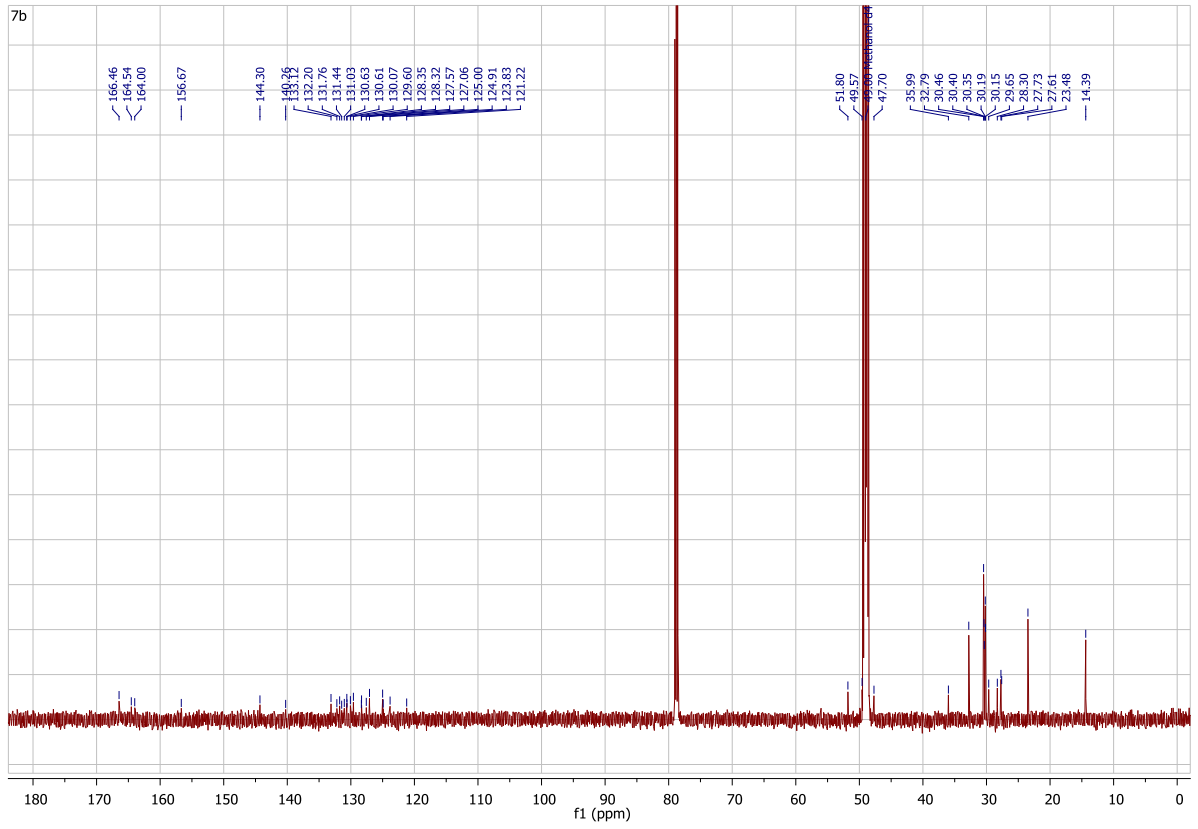
13C NMR of **11a**



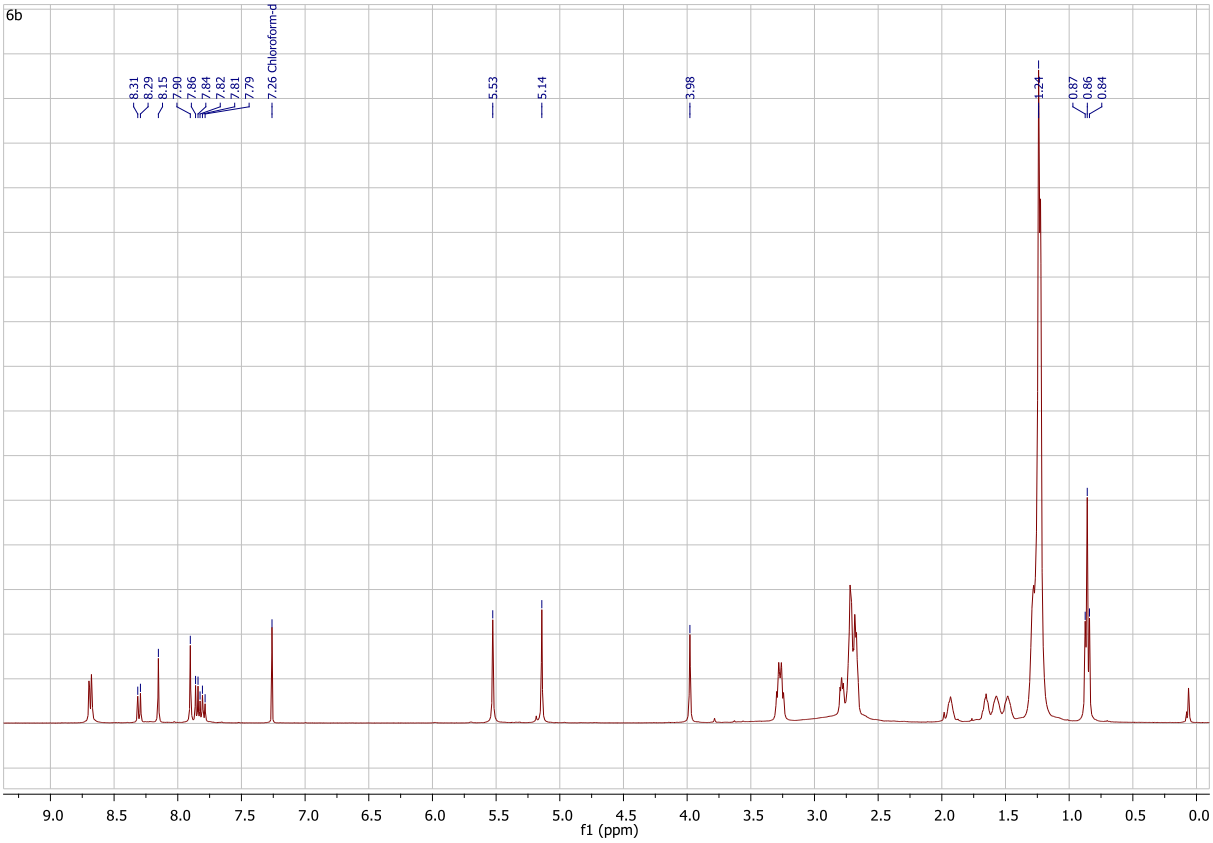
^{1}H NMR of **7b**



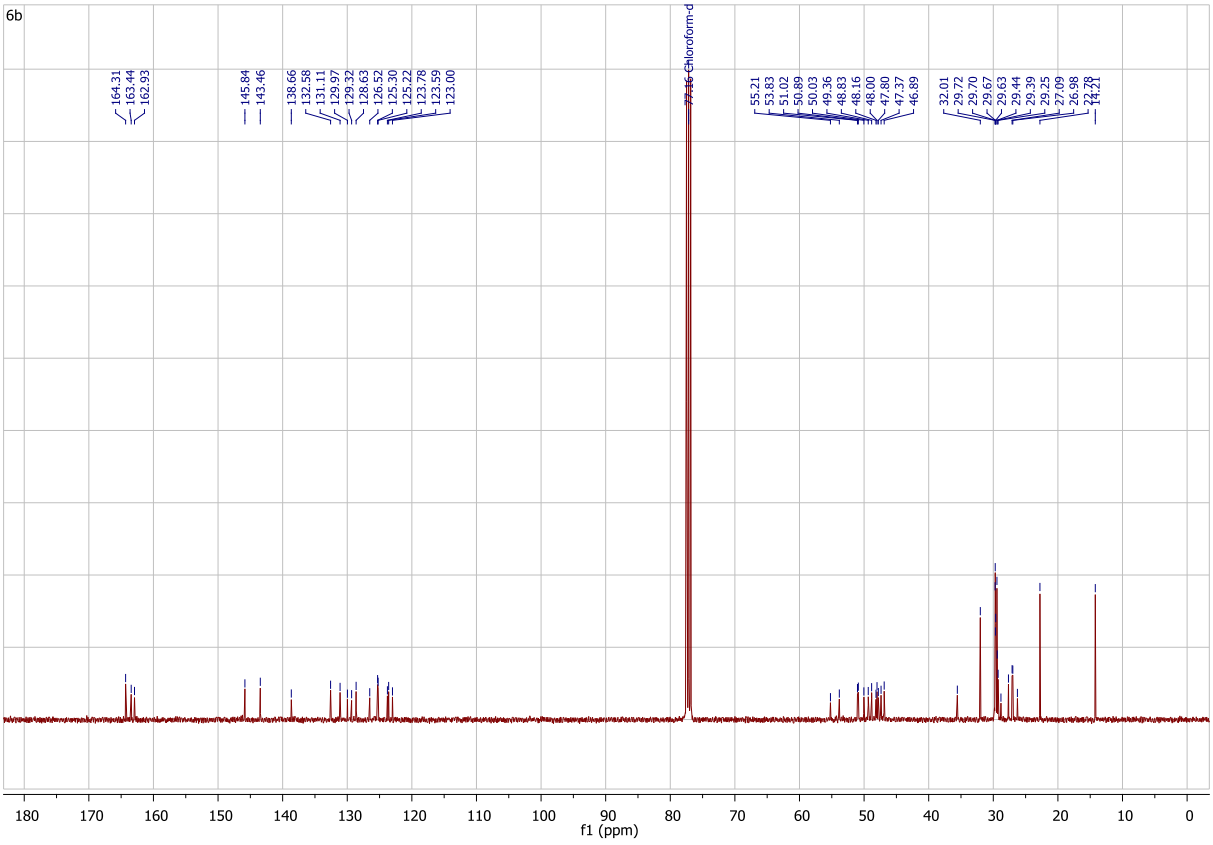
^{13}C NMR of **7b**



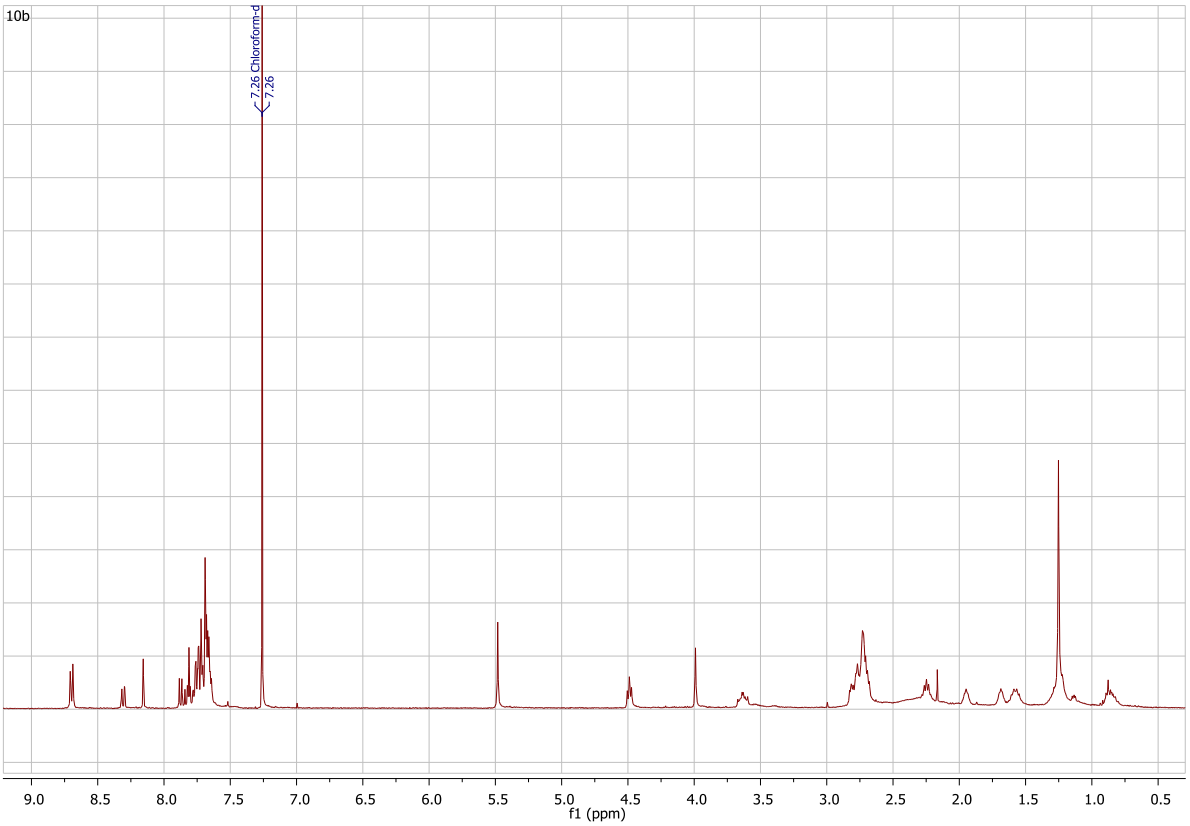
^{1}H NMR of **8b**:



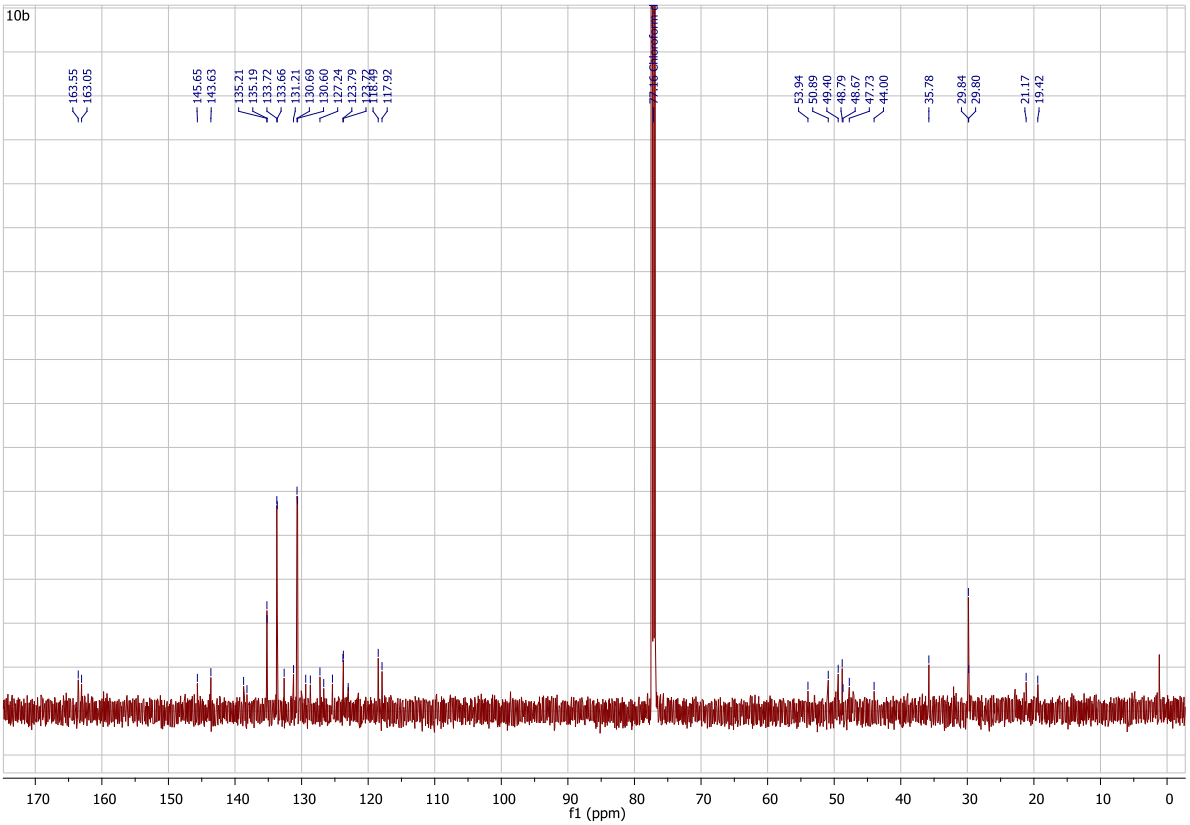
^{13}C NMR of **8b**:



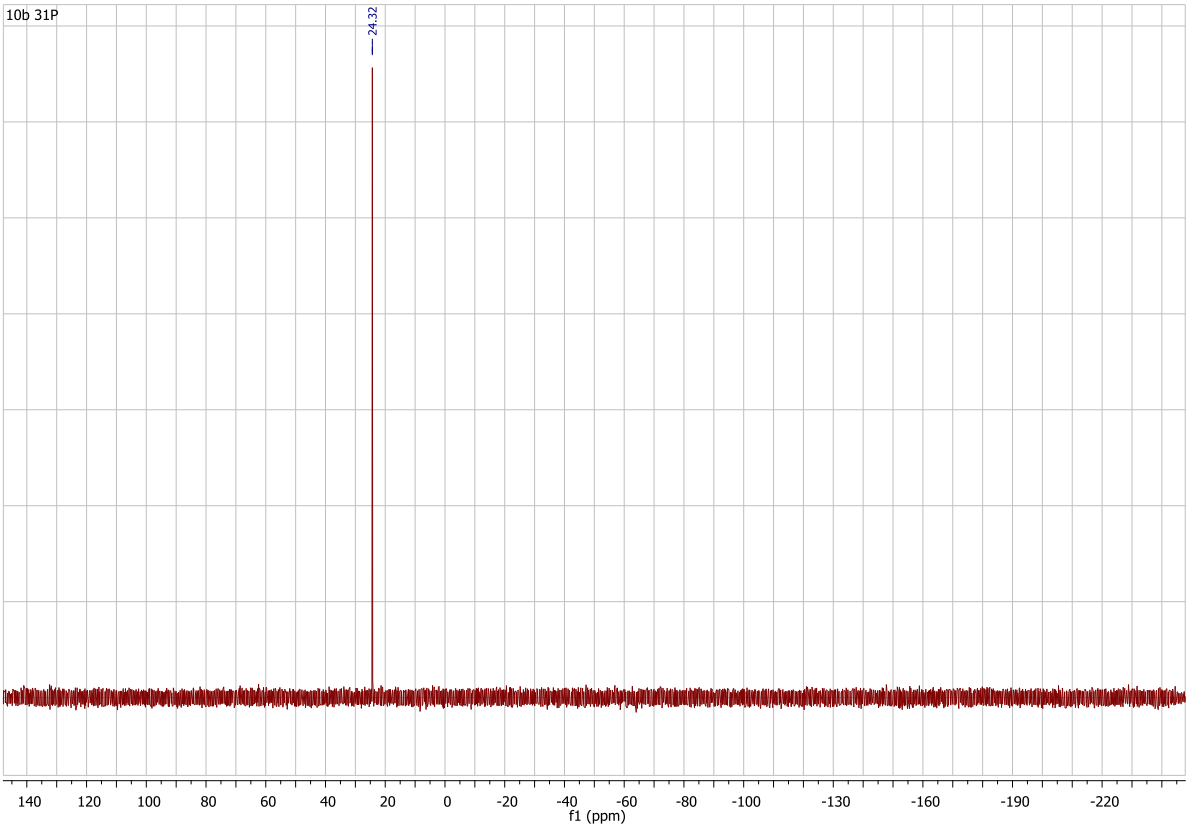
^{1}H NMR of **9b**



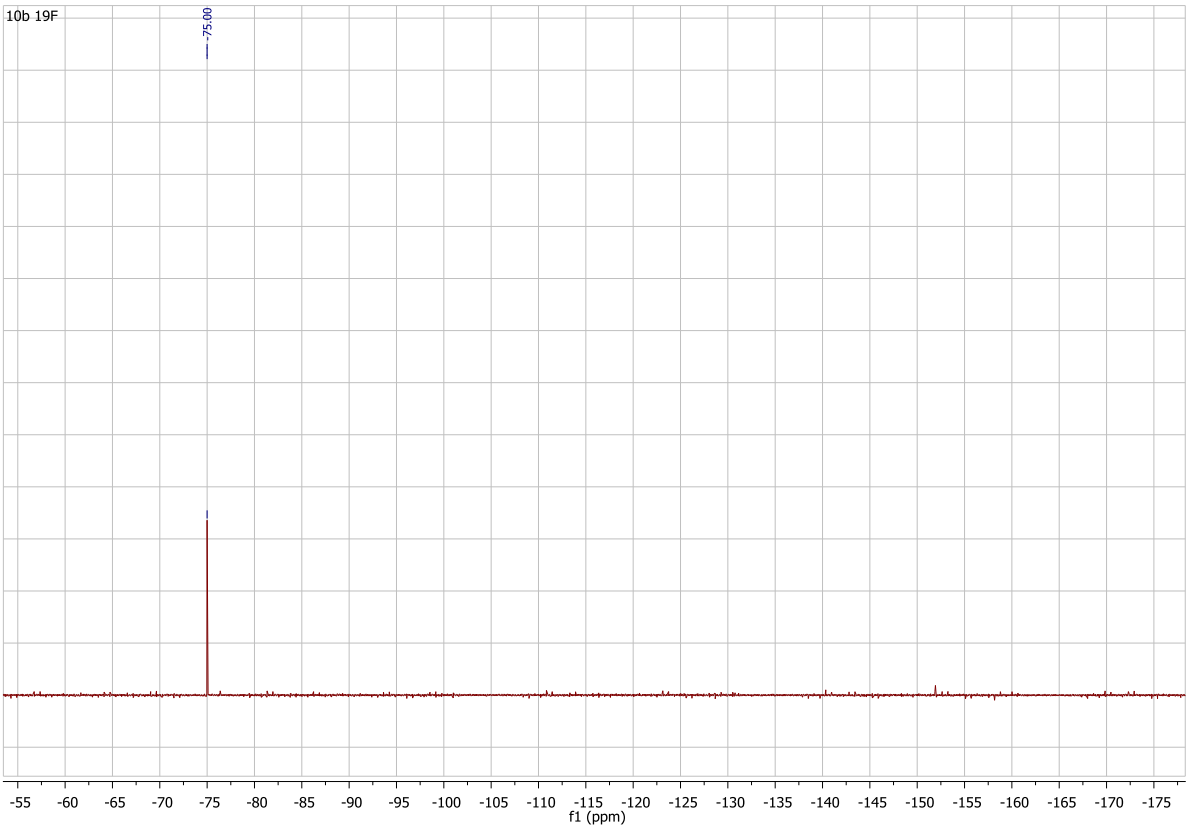
^{13}C NMR of **9b**



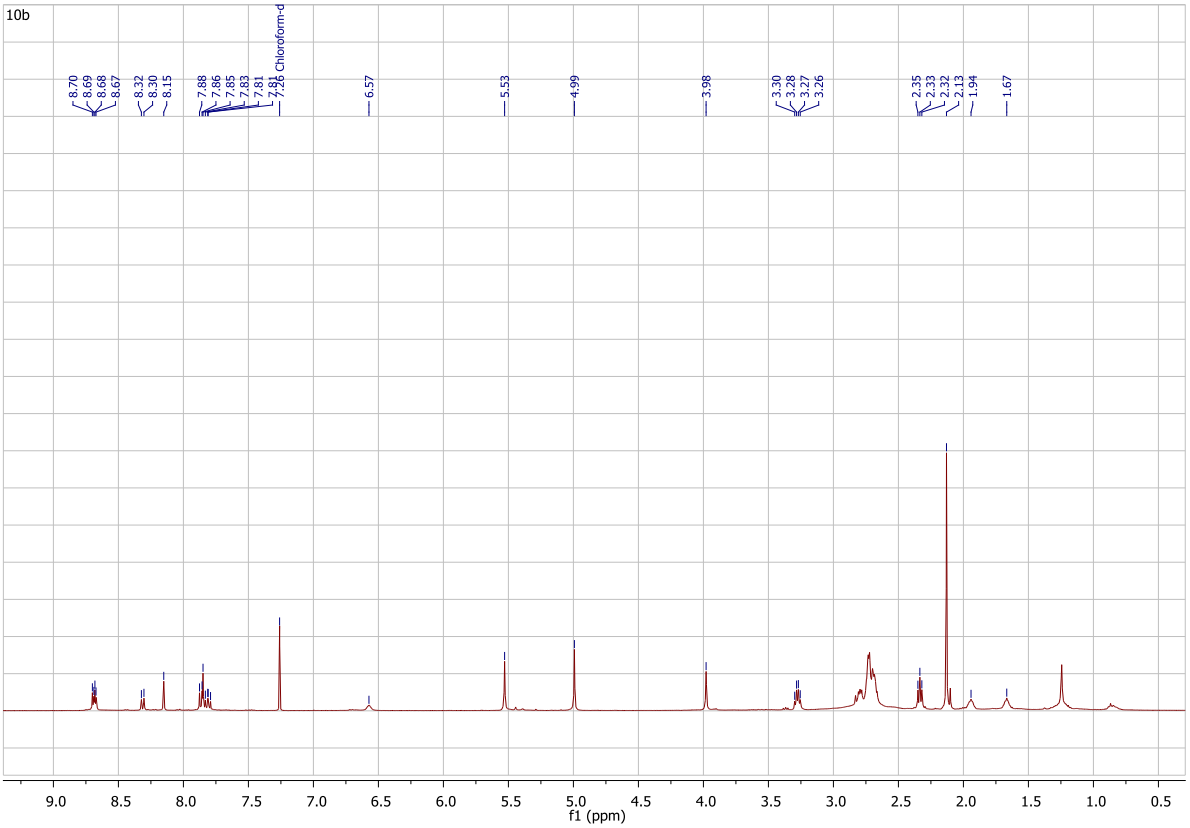
^{31}P of **9b**:



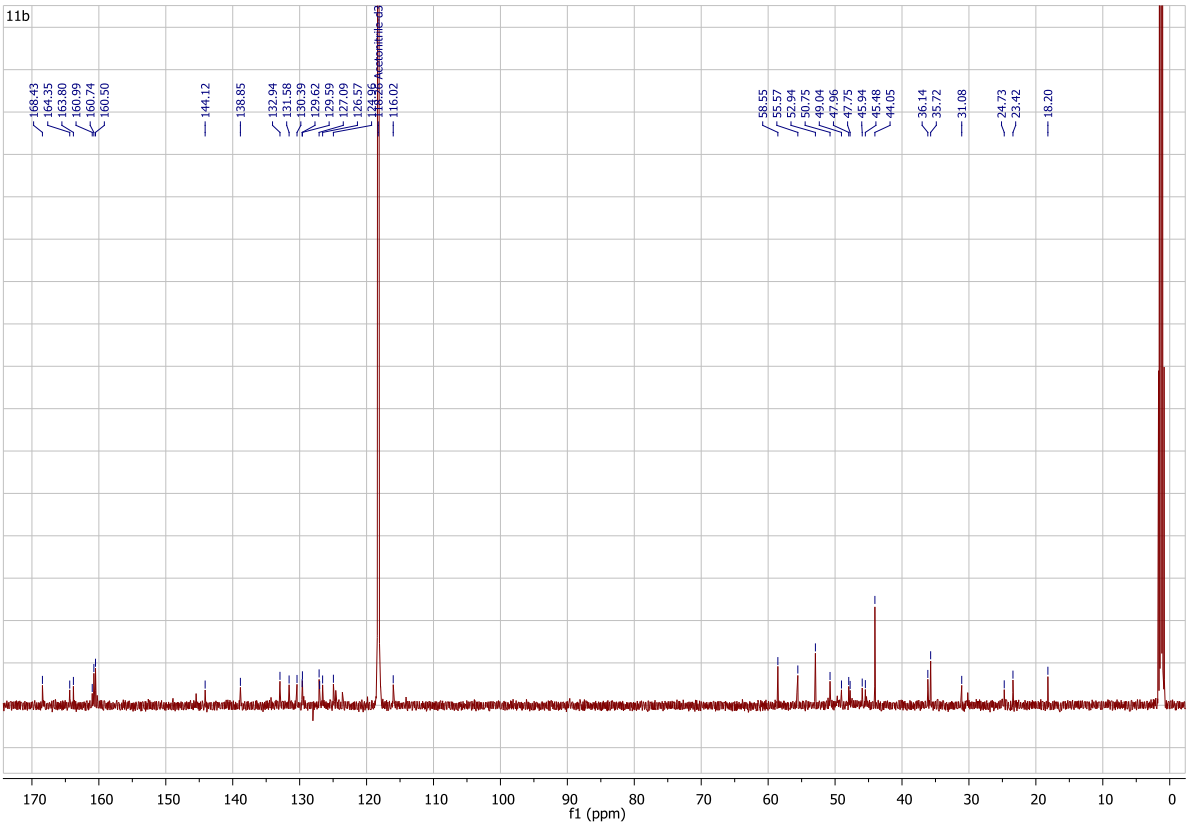
^{19}F of **9b**:



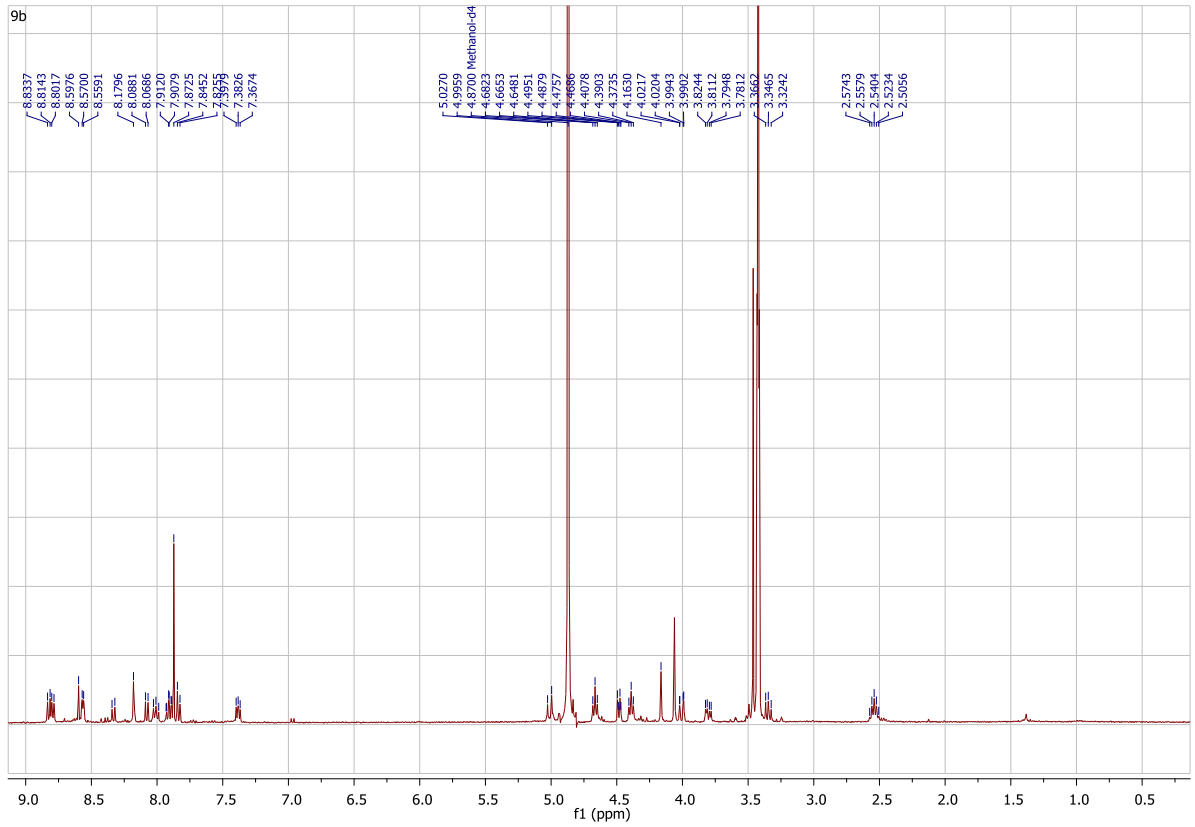
^{1}H NMR of **10b**:



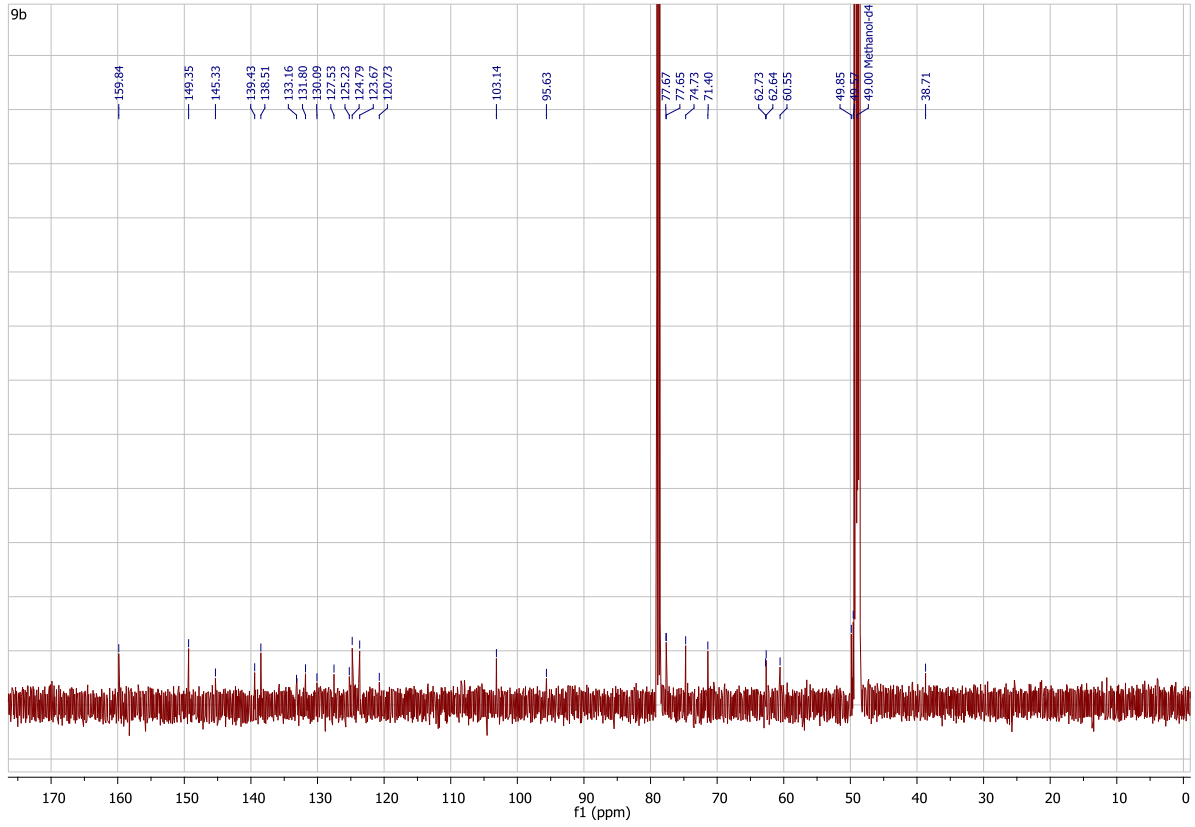
^{13}C NMR of**10b**:



1H NMR of **11b**:

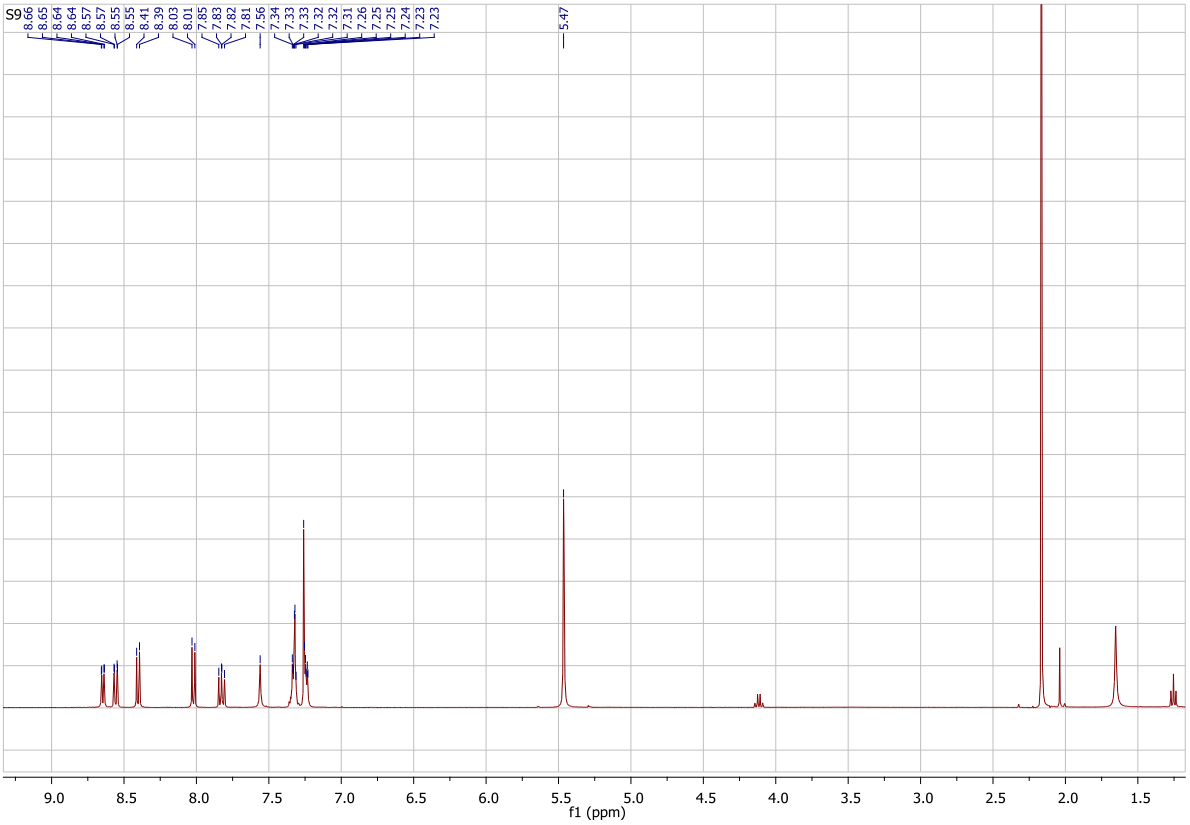


13C NMR of **11b**:

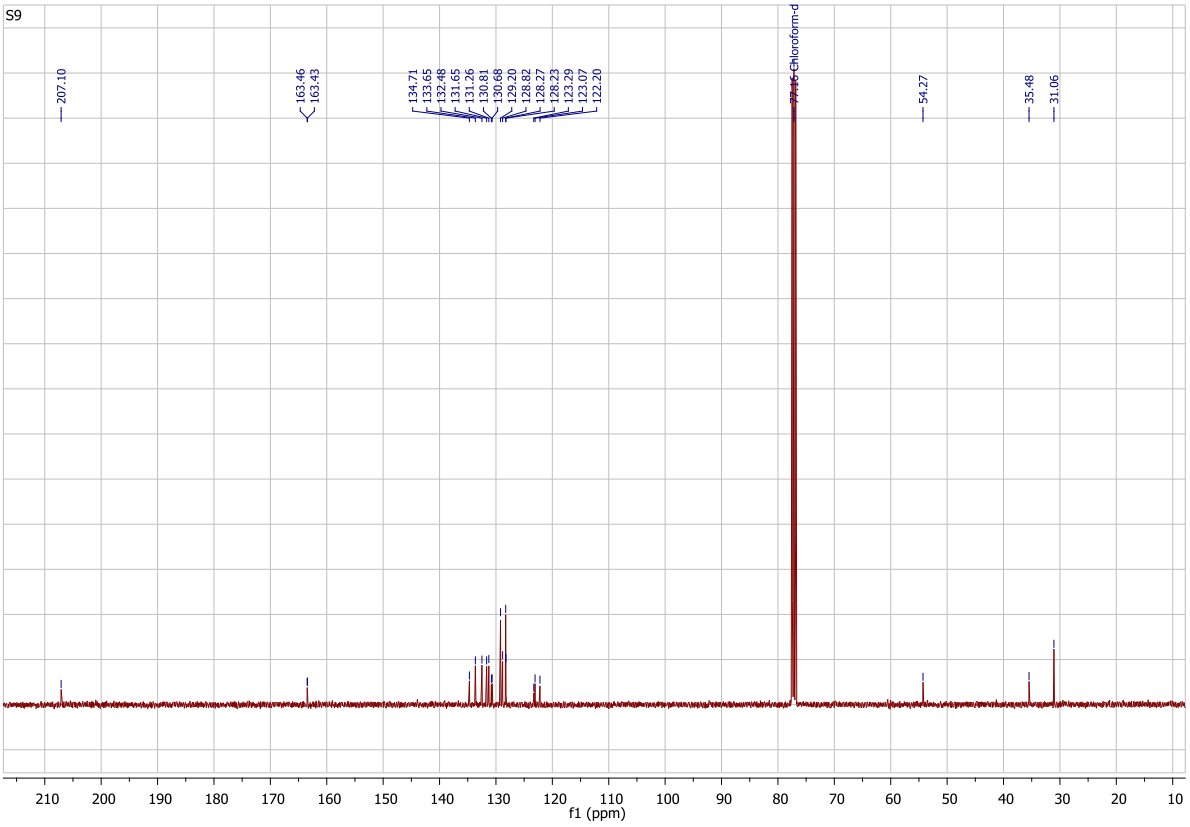


b) Starting materials, intermediates and other compounds

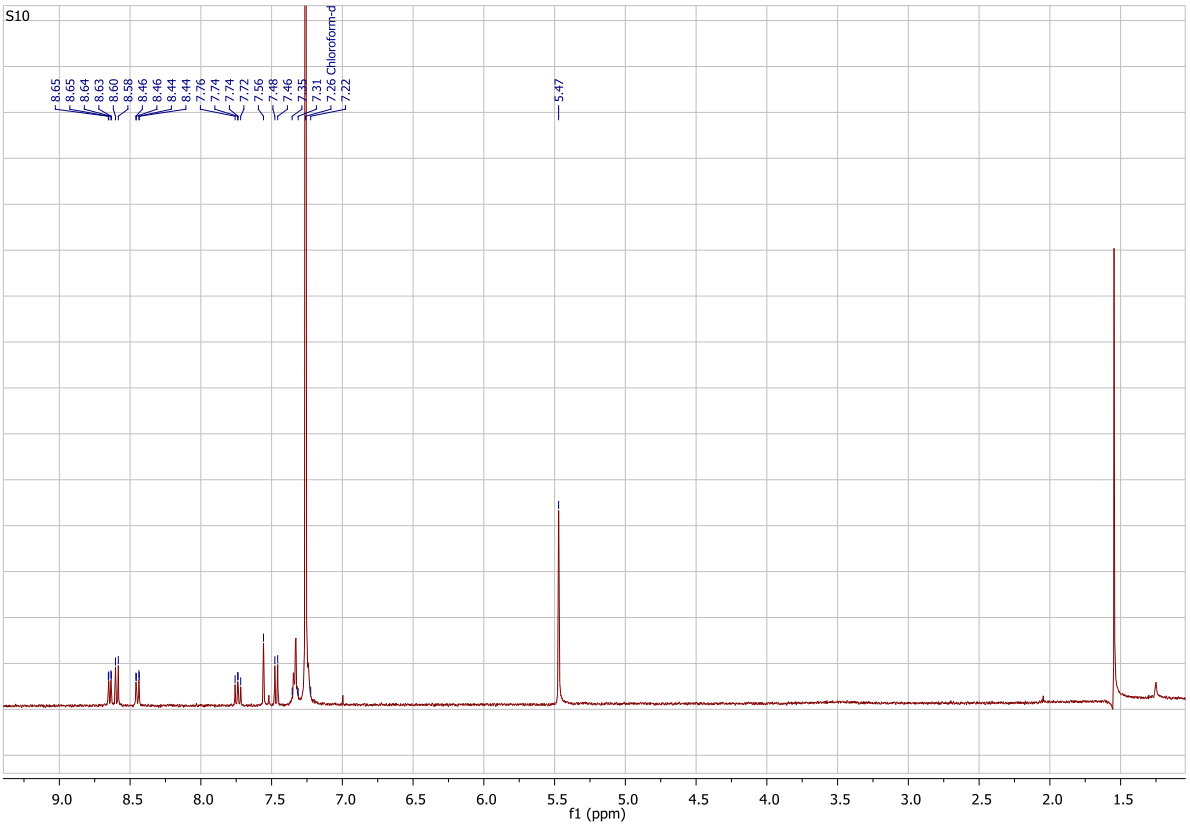
^{1}H NMR of **S9**



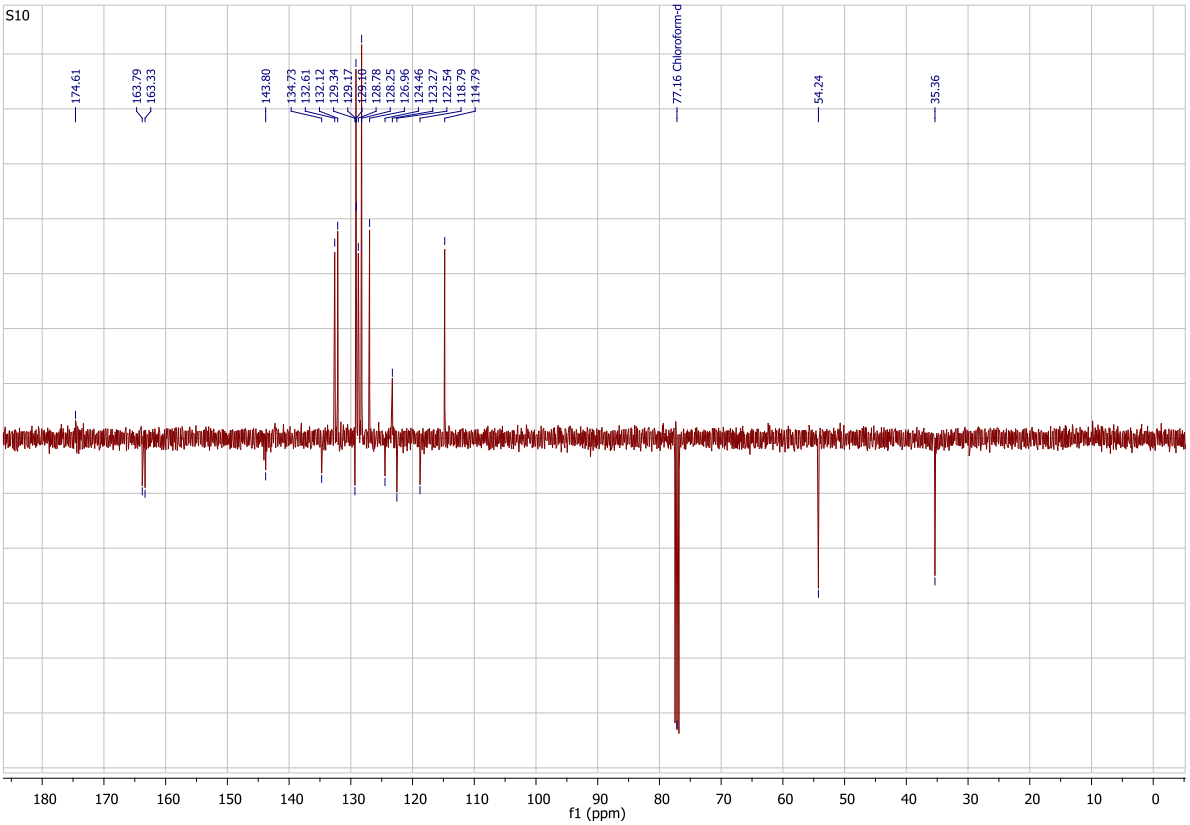
^{13}C NMR of **S9**



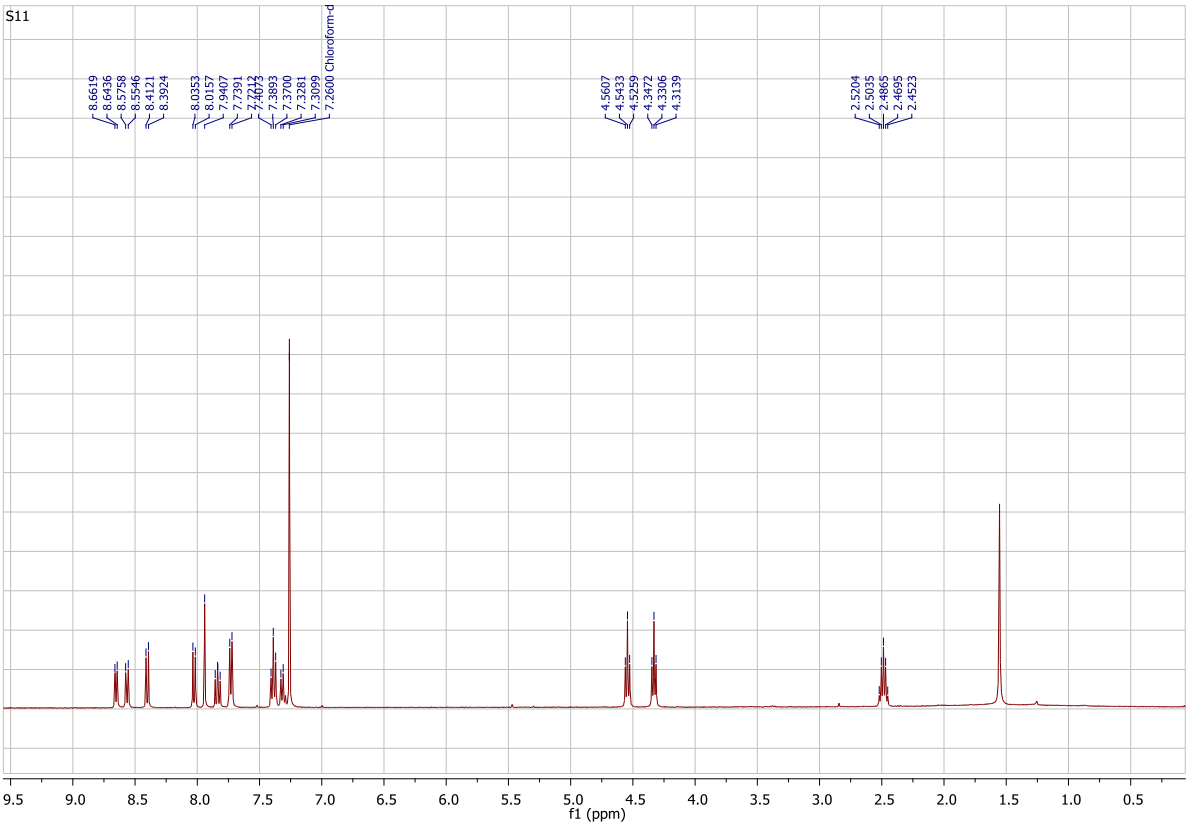
^{1}H NMR of **S10**



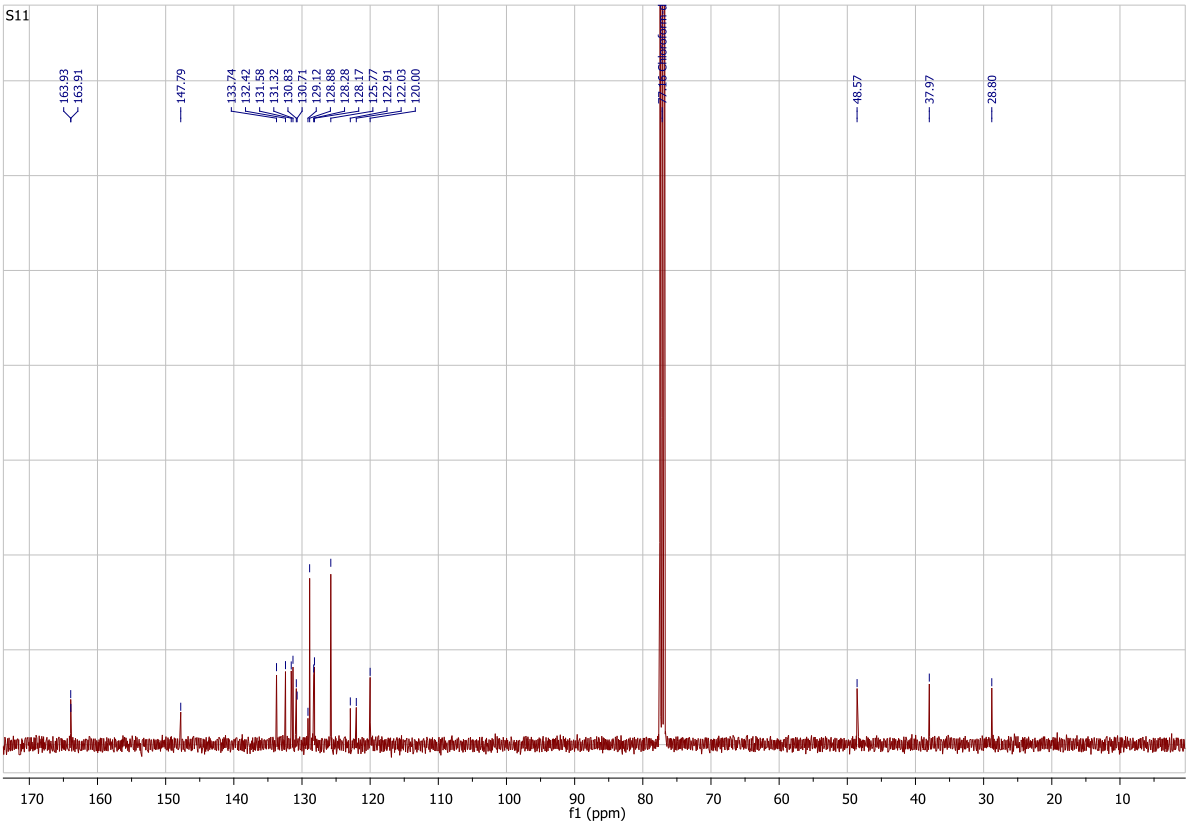
^{13}C NMR of **S10**



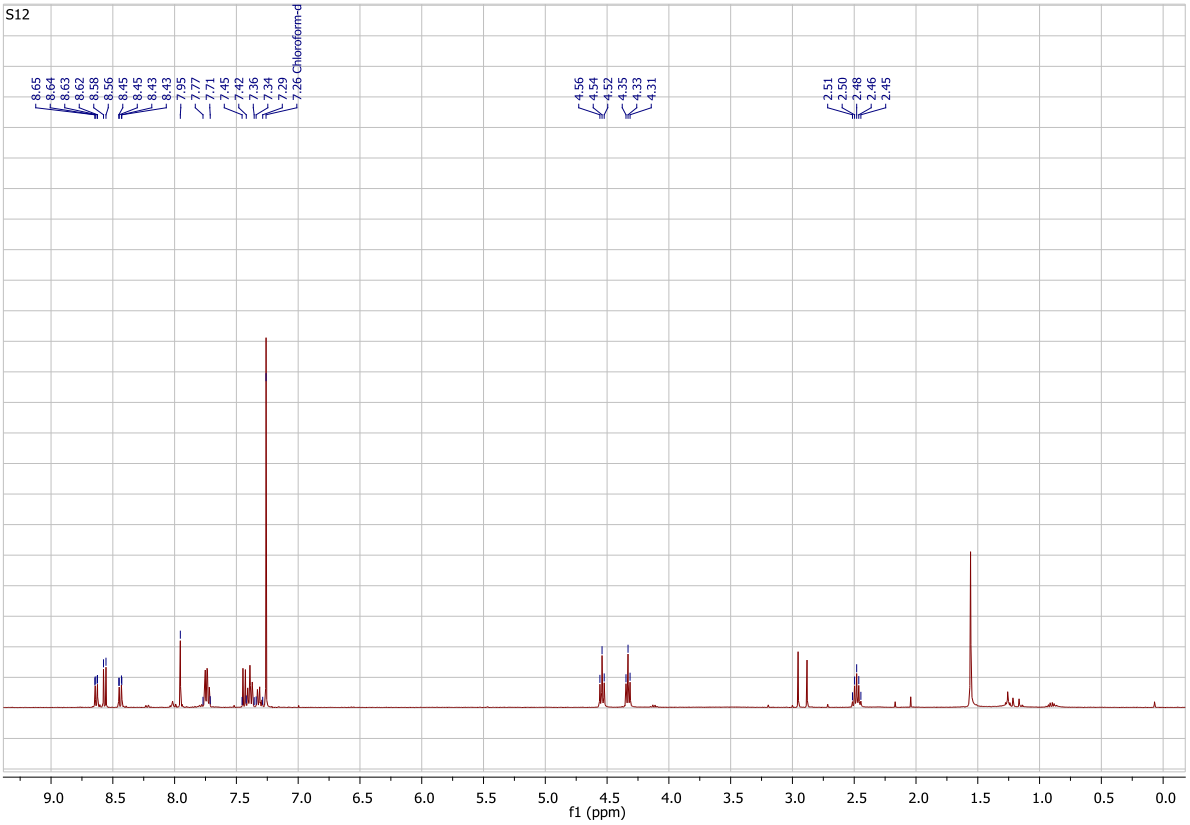
^{1}H NMR of **S11**



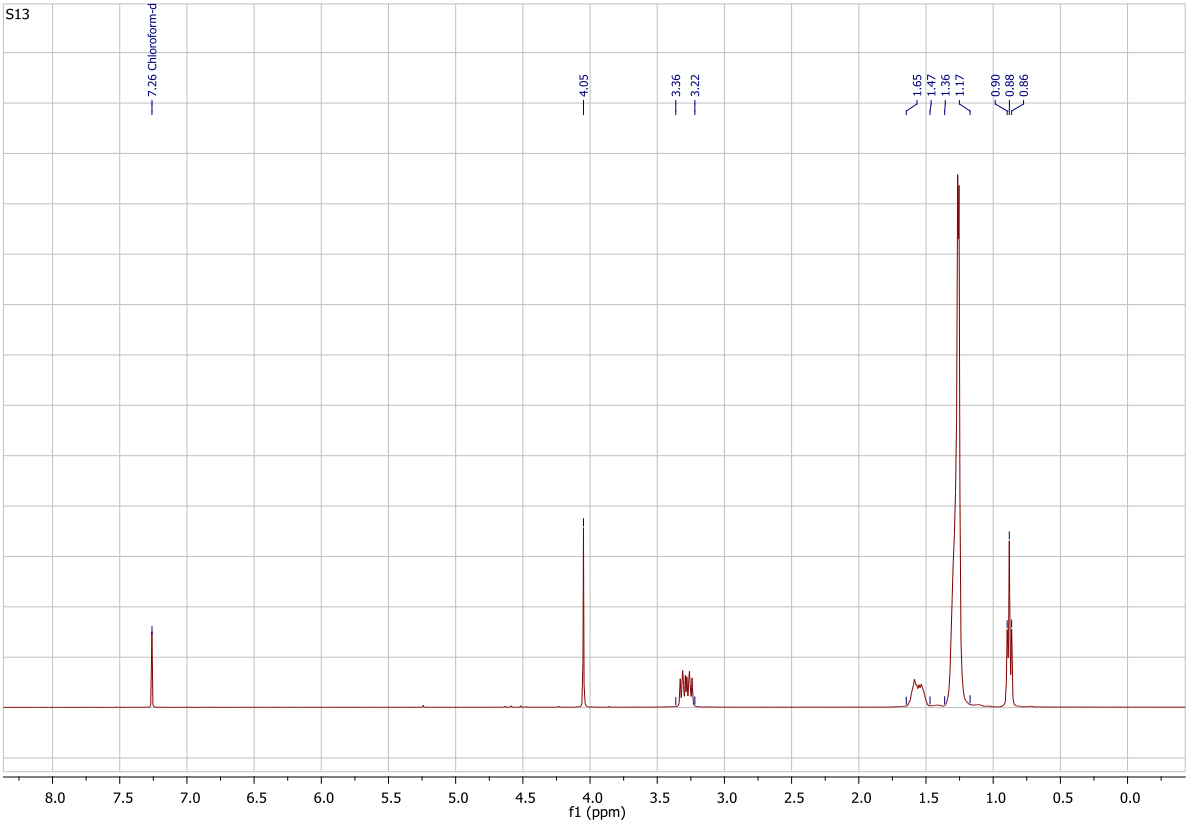
^{13}C NMR of **S11**



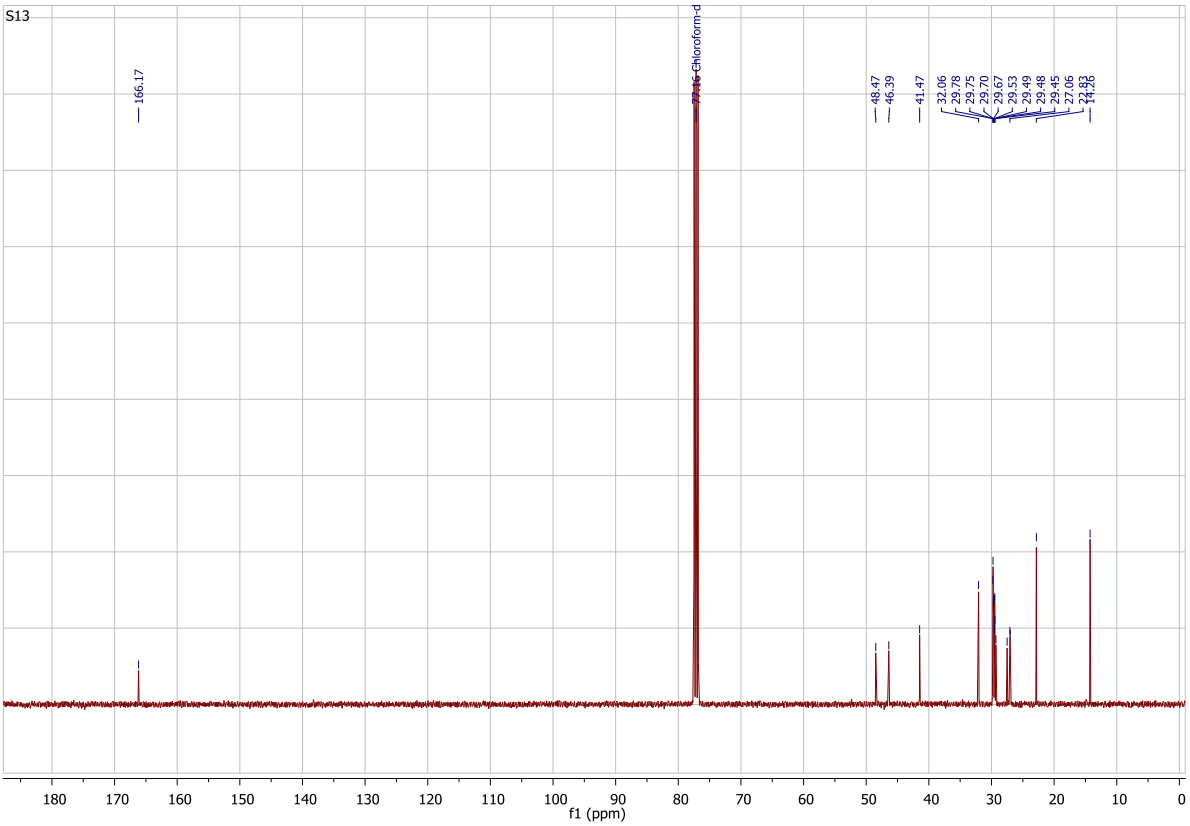
^{1}H NMR of **S12**


S12
8.65
8.64
8.63
8.62
8.58
8.56
8.45
8.45
8.43
8.43
7.95
7.77
7.71
7.45
7.42
7.36
7.34
7.29
7.26 Chloroform-d
4.56
4.54
4.52
4.35
4.33
4.31
2.51
2.50
2.48
2.46
2.45
9.0
8.5
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)

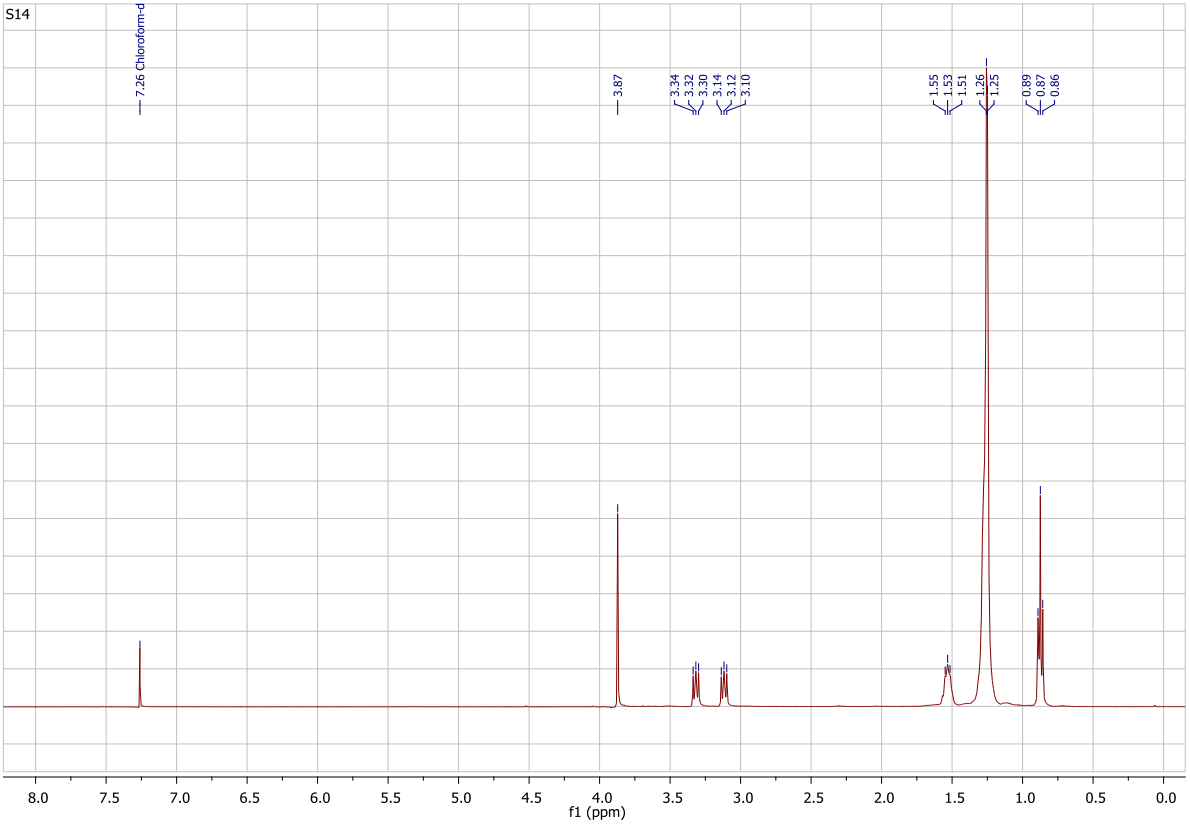
^{1}H NMR of **S13**



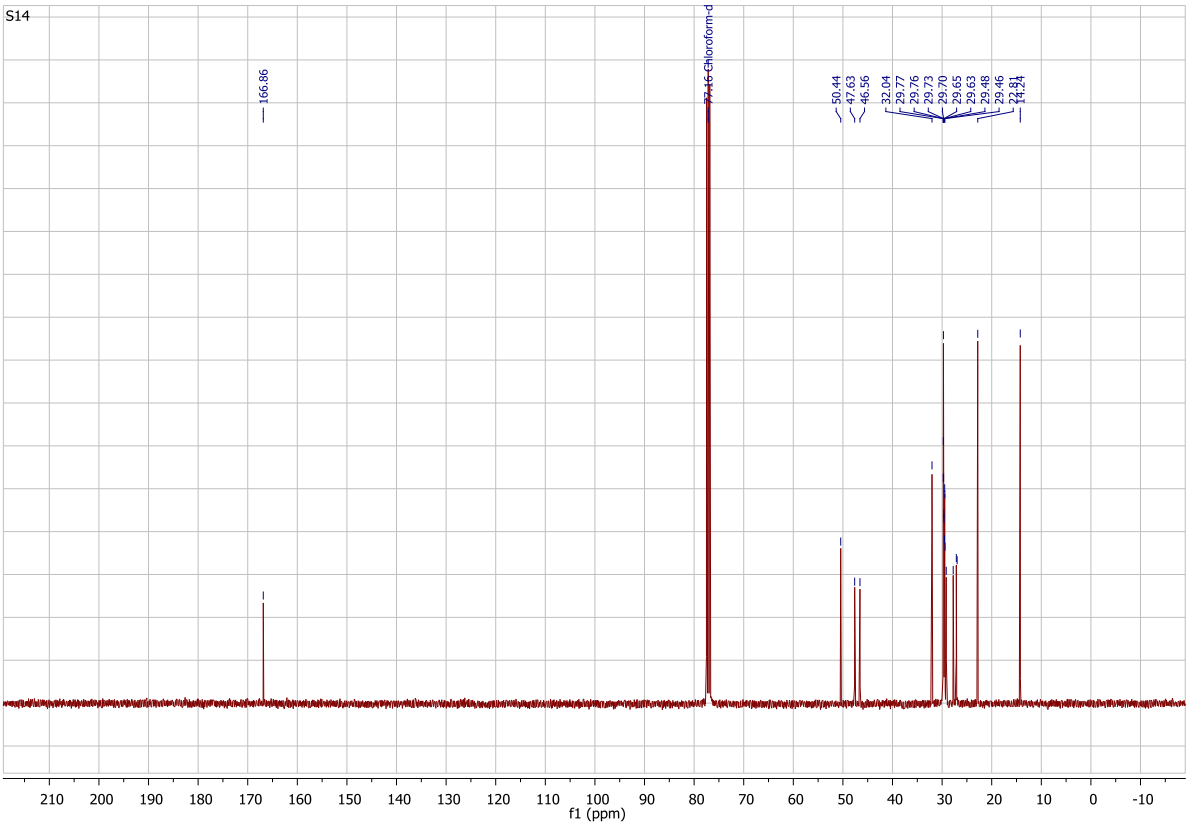
^{13}C NMR of **S13**



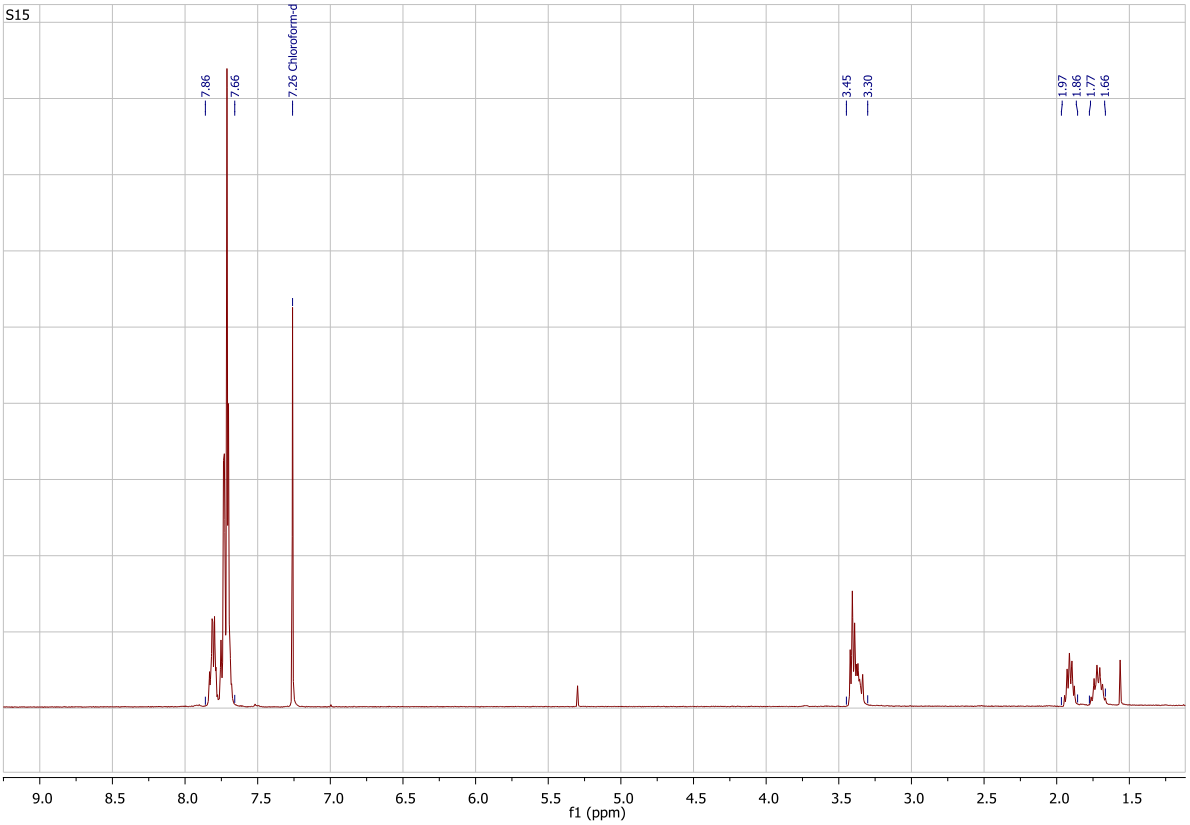
^{1}H NMR of **S14**



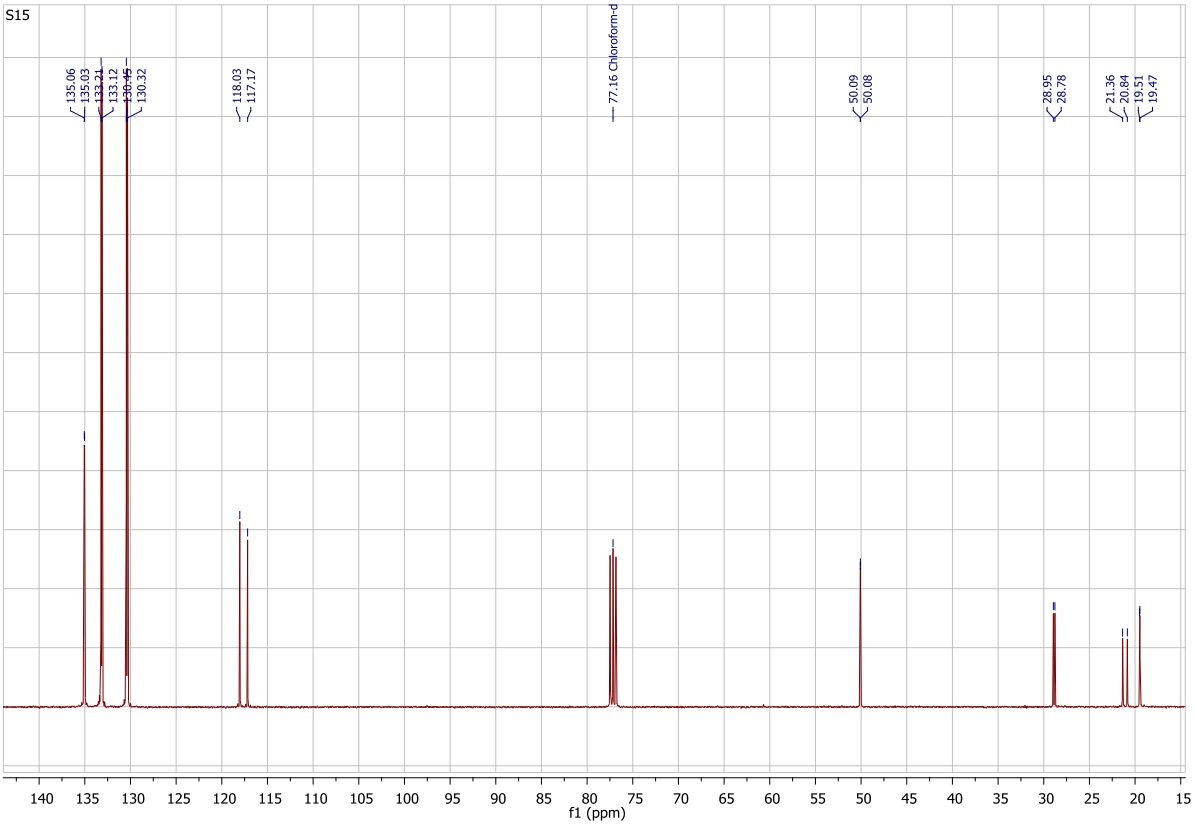
^{13}C NMR of **S14**



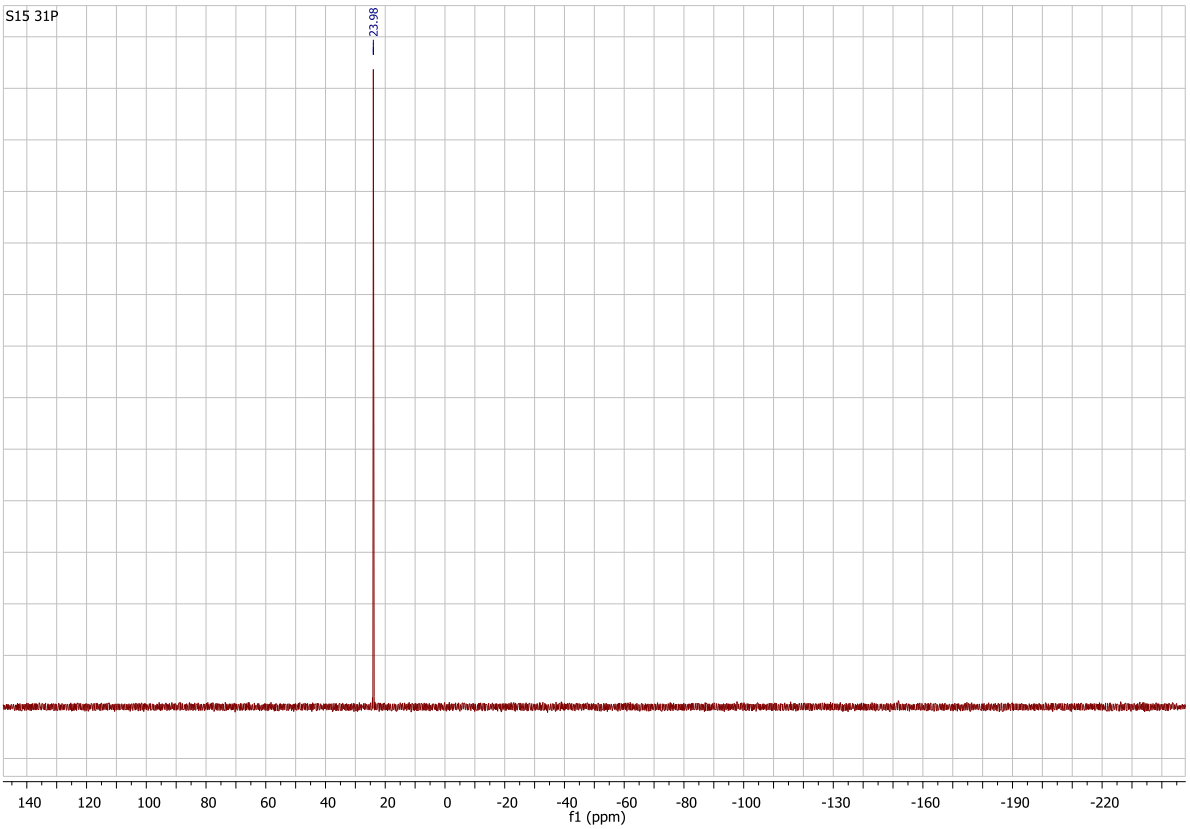
^{1}H NMR of **S15**



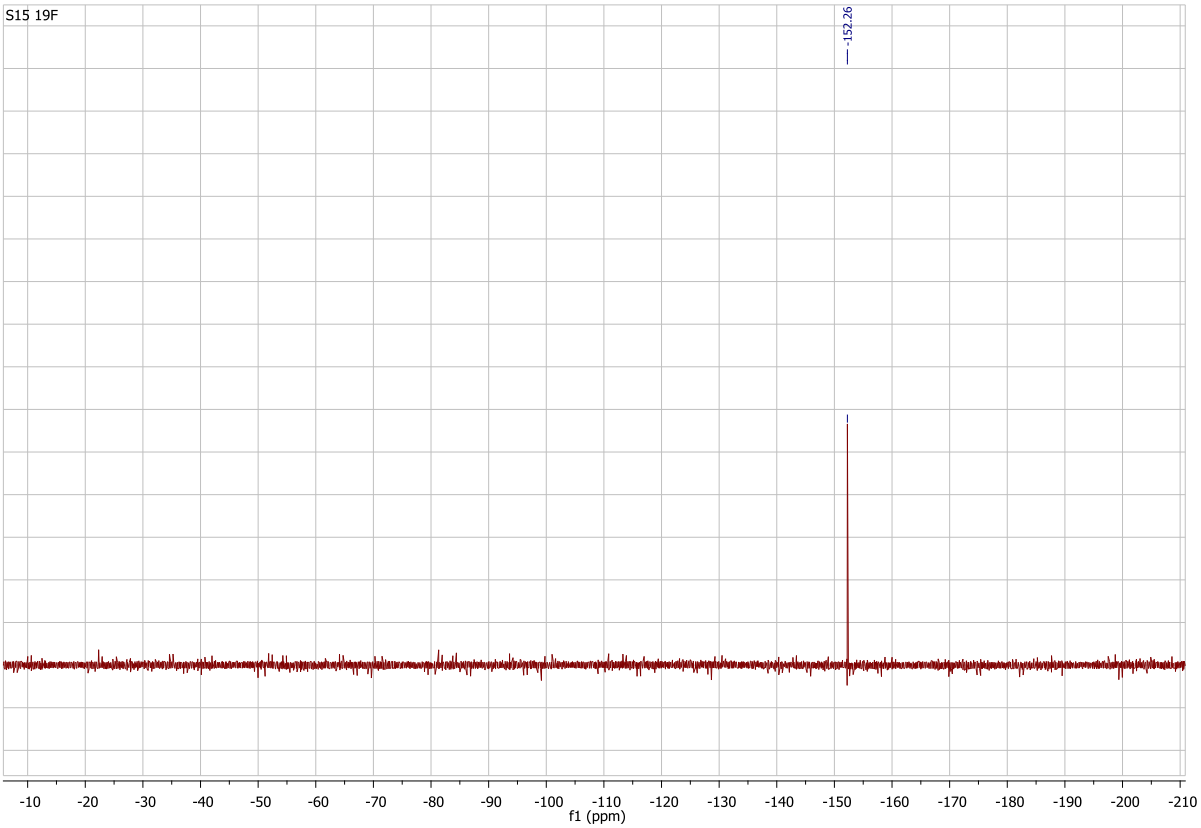
^{13}C NMR of **S15**



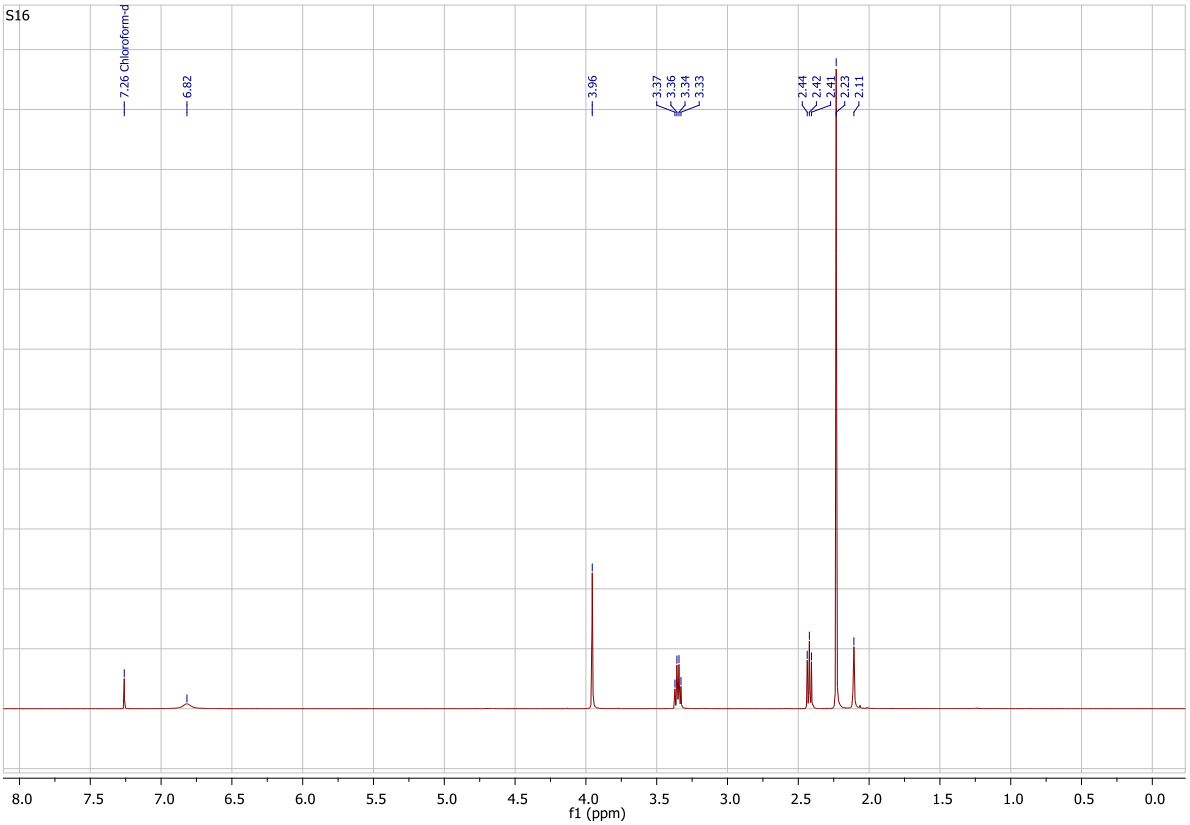
^{31}P of **S15**:



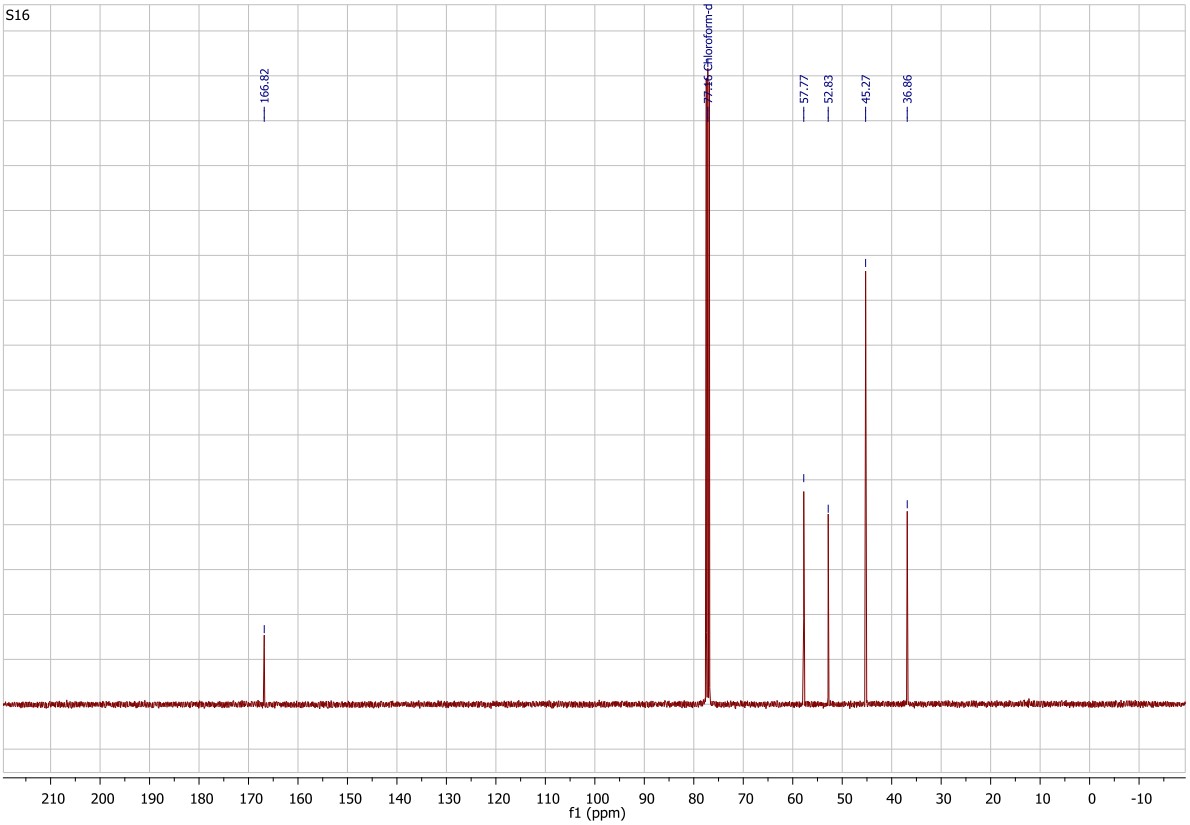
^{19}F of **S15**:



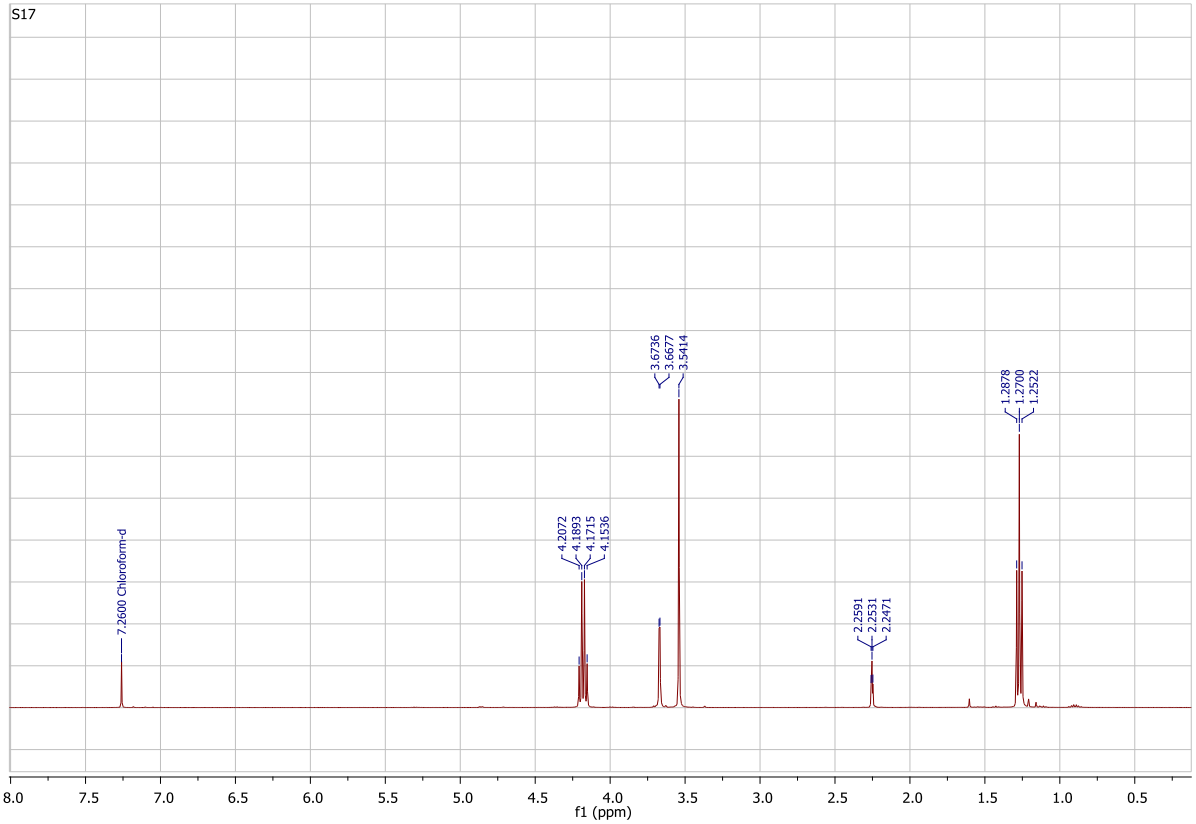
1H NMR of **S16**



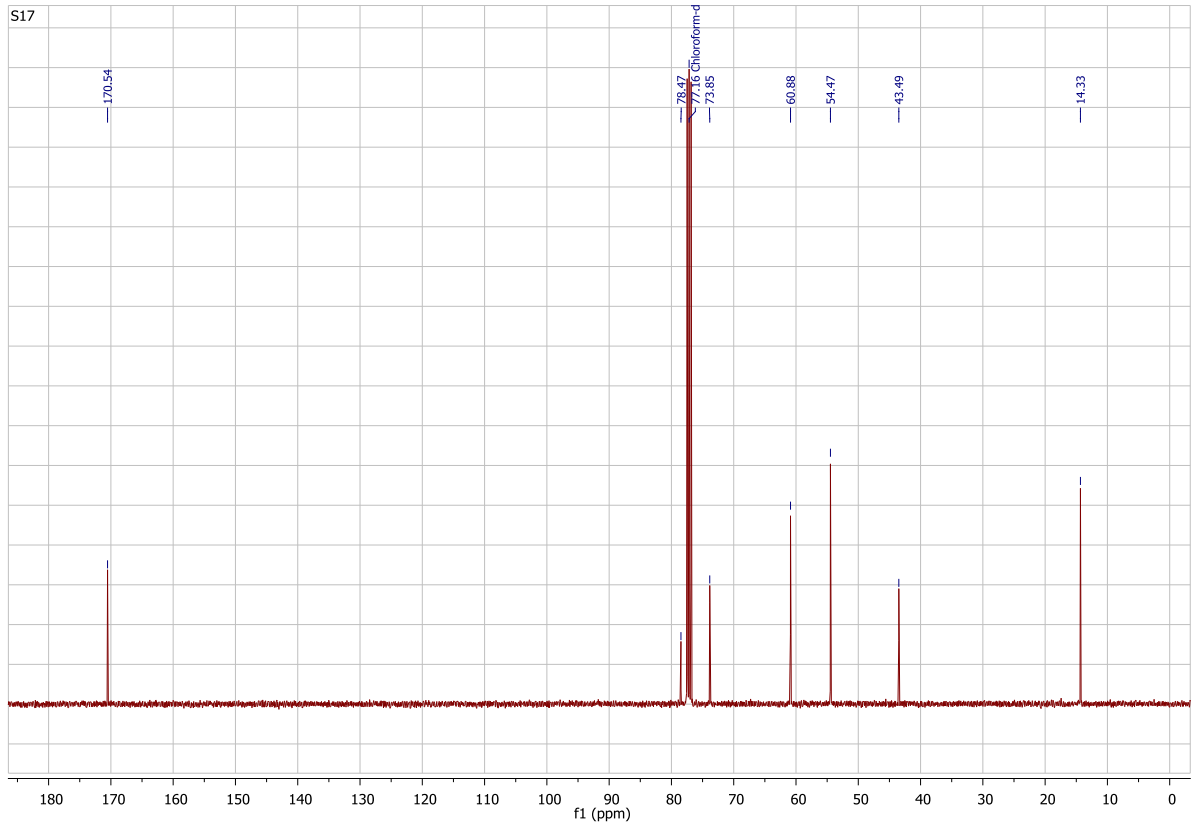
13C NMR of **S16**



^{1}H NMR of **S17**



^{13}C NMR of **S17**



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