Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Modular 'Click' Sensors for Zinc and their Application *In Vivo*

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1. General Methods

Commercially available reagents were used as supplied. To remove traces of water, Et_3N was distilled over CaH_2 under N_2. Solvents (CH_2Cl_2, Et_2O and THF) were supplied dry from an MBRAUN MB SPS-800 solvent purification system. All reactions were carried out under a N_2 atmosphere unless otherwise stated. All glassware was oven-dried and cooled under an inert N_2 atmosphereprior to use.

Fluorescence measurements were performed using Jobin Yvon Horiba FlouroMax®-3 in a 1-cm-pathlength cell without an incident ray filter. Infrared spectra were recorded in the range 4000-600 cm^{-1}, obtained directly as either solids or neat liquids on a Bruker Tensor 37 FTIR machine using a PIKE MIRacle ATR accessory. ^{1}H spectra and ^{13}C NMR spectra were recorded on a Bruker AMX400 or a Bruker AV400 NMR spectrometer. Chemical shifts (δ) are reported in (ppm) and multiplicities of signals are reported using standard abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad. Coupling constants (J) are reported in Hertz.

Low resolution masses were recorded at Queen Mary, University of London, on MS Agilent 6890gc and 5973 msd (EI mode) and the LC-MS Agilent '1100 LC/MSD Trap' incorporating a SL Ion Trap. High resolution mass spectra were recorded at the National Mass Spectrometry Service, University of Wales, Swansea, using a Thermofisher LTQ Orbitrap XL with resolution up to 100,000 (FWHM).

Flash chromatography was performed using WVR silica gel (40µm – 63µm). Thin layer chromatography (TLC) was carried out using pre-coated aluminium backed plates with Merck Kieselgel 60 F254 and the plates were visualized under an ultraviolet lamp or by

staining with basified aqueous $KMnO_4$ solution followed by gentle heating. Petrol refers to the fraction of petroleum ether boiling in the range 40-60 °C.

2. Experimental procedure

Tri-*tert*-butyl 1,4,7,10-tetraazacyclododecane-1,4,7-carboxylate[1] **(S2)**. Cyclen **S1** (4.00 g, 23.2 mmol) was dissolved in CH_2Cl_2 (800 mL) and Et_3N (16.2 mL, 25.0 mmol). The reaction mixture was stirred for 10 minutes before a solution of $(Boc)_2O$ (10.2 g, 46.5 mmol) in CH_2Cl_2 (100 mL) was added dropwise. After cooling the reaction mixture to –15 ºC, a second portion of $(Boc)_2O$ (5.10 g 23.2 mmol) in CH_2Cl_2 (100 mL) was added, and the mixture stirred at rt for 16 h. The solution was treated with a 1 M solution of Na_2CO_3 (1 L), the organic phase separated and dried over $MgSO_4$. The solvent and Et_3N were removed *in vacuo*. The residue was purified by flash column chromatography (SiO_2, EtOAc) to give tri-boc cyclen **S2** (9.0 g, 82 %) as a white solid; mp. 64 – 67 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.65 (s, 1H, H_u), 3.15 – 3.06 (m, 12H, $H_{k,l,m,n,o,p}$), 2.77 – 2.64 (m, 4H, $H_{q,j}$), 1.38 (s, 27H, $H_{r,s,t}$); ^{13}C NMR (100 MHz, $CDCl_3$,) δ 155.8 (3 × C), 79.3, 79.1, 50.8, 49.5, 45.4, 28.6. 28.5; υ_{max} cm^{-1} 2975, 1686, 1464, 1407, 1364, 1245, 1159, 1102, 776; MS (ESI): *m*/*z* 473.4 [M + H]$^+$.

Tri-*tert*-butyl 10-(prop-2-yn-1-yl)-1,4,7,10-tetraazacyclododecane-1,4,7-carboxylate[2] **(1b)**. To a stirred solution of tri-boc cyclen **S2** (2.00 g, 4.20 mmol) in CH_3CN (50 mL) was added Na_2CO_3 (0.897 g, 8.50 mmol) and propargyl bromide (80% PhMe; 0.45 mL, 5.1 mmol). The mixture was heated at reflux for 16 h. The insoluble residues were separated by filtration and the solvent removed *in vacuo*. The residue was purified by flash column chromatography (SiO_2, 1:1 EtOAc : Petrol) to give acetylene **1b** as a white solid (1.68 g, 78%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.60 – 3.15 (m, 14H, $H_{k,l,m,n,o,p,i}$), 2.80 – 2.70 (m, 4H, $H_{j,q}$), 2.22 – 2.02 (m, 1H, H_h), 1.44 (br s, 27H, $H_{r,s,t}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.8, 155.5, 79.5, 77.9, 73.2, 53.0, 51.9, 50.7, 48.0, 47.5, 46.9, 44.8, 41.9, 28.5, 25.5; υ_{max} cm^{-1} 2977, 2150 1687, 1466, 1410, 1366, 1249, 1160, 1104; MS (ESI): *m*/*z* 511.4 $[M + H]^+$.

6-Bromo-2-ethyl-1*H*-benzo[*de*]isoquinoline-1,3(*2H*)-dione[3] **(S4)**. 4-Bromo-1,8-naphthalic anhydride (2.0 g, 7.2 mmol) and $EtNH_2$ (70 % solution in H_2O, 0.490 mL, 8.60 mmol) were heated under reflux in dioxane (80 mL) for 7 h. The reaction mixture was cooled to rt, a second aliquot of $EtNH_2$ (70 % solution in H_2O, 0.330 mL, 5.80 mmol) was added and the mixture heated at reflux for a further 7 h. The solution was cooled to rt and poured into H_2O

(800mL) and the precipitate collected by suction filtration. The residue was washed with H_2O (500 mL) and the solvent removed *in vacuo* to give amide **S4** (2.0 g, 95%) as cream solid. mp. 162 – 164 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (d, J = 7.2, 1H, H_e), 8.33 (d, J = 8.4, 1H, H_c), 8.19 (d, J = 7.8, 1H, H_g), 7.85 (d, J = 7.8, H_f), 7.76 – 7.57 (m, 1H, H_d), 4.14 (q, J = 7.1, 2H, H_b), 1.28 (t, J = 7.1, 3H, H_a); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.1 (2 × C), 132.7, 131.6, 130.7 (2 × ^{13}C), 130.1, 129.8, 128.5, 127.7, 122.8, 121.9, 35.5, 13.13; υ_{max} cm^{-1} 1693, 1659, 1339, 1243, 1060, 776, 748; MS (ESI): *m*/*z* 304 and 306 $[M + H]^+$.

6-Azido-2-ethyl-1*H*-benzo[*de*]isoquinoline-1,3(*2H*)-dione[4] (2). Sodium azide (1.3 g, 6.9 mmol) was stirred in *N*-methylpyrrolidinone (30 mL) for 24 h at 50 °C to dissolve the salt. Bromide **S4** (2.0 g, 6.6 mmol) was added and mixture was stirred for another 24 h at rt. The reaction mixture was diluted with H_2O (60 mL) and extracted with EtOAc (3 × 60 mL). The organic phase was washed with brine (3 × 30 mL) dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash chromatography (SiO_2, EtOAc/ Petrol) to give the desired azide **2** (1.56 g, 89%) as a dark brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (d, J = 7.2, 1H, H_e), 8.50 (d, J = 8.4, 1H, H_c), 8.34 (d, J = 7.8, 1H, H_g), 7.70 (d, J = 7.8, H_f), 7.38 – 7.57 (m, 1H, H_d), 4.22 (q, J = 7.1, 2H, H_b), 1.31 (t, J = 7.1, 3H; H_a); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.8, 163.4, 143.4, 132.2, 131.6, 129.2, 128.7, 126.9, 124.4, 122.8, 119.0, 114.7, 35.5, 13.4; υ_{max} cm^{-1} 2130, 1693, 1658, 1590, 1346, 1289, 1244, 776; MS (ESI): *m*/*z* 267.0 $[M + H]^+$.

Tri-*tert*-butyl 10-((1-(2-ethyl-1,3-dioxo-2,3-dihydro-1*H*-benzo[*de*]isoquinolin-6-yl)-1*H*-1,2,3-triazol-4-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylate (S5). To a stirred solution of acetylene **1b** (3.01 g, 5.90 mmol) and azide **2** (1.62 g, 5.90 mmol) in H_2O/THF (3:7, 40 mL), was added sodium ascorbate (0.230 g, 1.21 mmol) followed by $CuSO_4 \cdot 5H_2O$ (0.591 g, 0.190 mmol). The yellow solution was stirred at rt for 16 h. THF was removed *in vacuo* and the residue taken up in CH_2Cl_2 (100 mL), H_2O (80 mL) and basified with saturated $NaHCO_3$ (5 mL). The organic phase was separated and the aqueous phase extracted with CH_2Cl_2 (2 × 100 mL). The organic phases were combined and washed with basified 1 M EDTA (aq) then brine (25 mL), dried over $MgSO_4$ and concentrated *in vacuo*. This residue was purified by flash column chromatography (SiO_2, EtOAc/CH_2Cl_2 - gradient 1:6 to 4:6) to give triazole **S5** as a pale yellow solid (4.5 g, 92%); m.p. 90 – 93 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 – 8.60 (m, 2H, $H_{c,g}$), 8.20 (d, J = 8.5, 1H, H_e), 7.97 (s, 1H, H_h), 7.93 – 7.67 (m, 2H, $H_{d,f}$), 4.24 (q, J = 7.0, 2H, H_b), 4.16 – 3.90 (m, 2H, H_i), 3.69 – 3.21 (m, 12H, $H_{k,l,m,n,o,p}$), 2.78 – 2.50 (m, 4H, $H_{j,q}$), 1.53 – 1.25 (m, 30H, $H_{a,r,s,t}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.3, 162.8, 156.1, 155.8, 155.4, 138.1, 132.0, 130.5, 129.3, 128.9, 128.6, 126.3, 125.5, 123.8, 123.4, 123.0, 79.6, 79.5, 79.3, 54.4, 53.4, 50.0, 47.8, 46.5, 45.3, 35.7, 28.4, 13.2; υ_{max} /cm^{-1} 2975, 1685, 1465, 1159; MS (ESI): *m*/*z* 777.5 $[M + H]^+$. HRMS (ESI) calcd for $C_{40}H_{57}N_8O_8$, $[M + H]^+$: 777.4221. Found: 777.4292.

6-(4-((1,4,7,10-tetraazacyclododecan-1-yl)methyl)-1*H*-1,2,3-triazol-1-yl)-2-ethyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (3b). Triazole **S5** (0.102 g, 0.12 mmol) was dissolved in a solution of TFA (20%) in CH_2Cl_2 (5 mL), and the yellow solution was stirred for 10 h at rt The solvent was removed *in vacuo*. The residue was taken up in $CHCl_3$ (10 mL) and washed with NaOH (1 M, 5 mL). The aqueous phase was extracted with $CHCl_3$ (3 × 10 mL). The organic phases were combined, dried over $MgSO_4$ and concentrated *in vacuo* to give sensor **3b** (0.046 g, 86%) as a yellow solid. m.p. 88 – 91 °C; ^{1}H NMR (400 MHz, CD_3CN) δ 8.61 – 8.50 (m, 2H, $H_{g,c}$), 8.24 (d, J = 8.5, 1H, H_h), 8.14 (s, 1H, H_e), 7.93 – 7.67 (m, 2H, $H_{d,f}$), 4.24 (q, J = 7.0, 2H, H_b), 4.16 – 3.90 (m, 2H, H_i), 3.69 – 3.21 (m, 19H, $H_{j,k,l,m,n,o,p,q,}$ 3 × N$\underline{H}$), 1.26 (m, 3H, H_a). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.4, 162.9, 146.3, 138.3, 132.0, 130.6, 129.6, 128.9, 128.4, 126.3, 124.7, 123.7, 123.5, 122.9, 51.3, 49.5, 47.0, 46.7, 45.0, 35.7, 13.24; υ_{max} /cm^{-1} 2975, 2361, 1700, 1658, 1589, 1437, 1343, 1244, 1064; MS (ESI): *m*/*z* 477.4, [M + H$^+$]. HRMS (ESI) calcd for $C_{25}H_{33}N_8O_2$, [M + H]$^+$: 477.2648. Found: 477.2721.

[Zn(3b)](ClO$_4$)$_2$. To a stirred solution of **3b** (0.150 g, 0.29 mmol) in MeOH (6 mL) was added Zn(ClO$_4$)$_2$·6H$_2$O (0.110 g, 0.29 mmol). A pale yellow precipitate was formed almost immediately. The mixture was stirred for 16 h at rt and the solid formed was then filtered, washed with cold MeOH (5 mL), and dried *in vacuo* to give the desired Zn^{2+} complex **S6** (0.13 g, 65%) as pale yellow solid. ^{1}H NMR (400 MHz, CD_3CN) δ 8.75 – 8.65 (m, 2H, H$_{g,c}$), 8.43 (s, 1H, H$_h$), 8.19 (d, J = 8.5, 1H, H$_e$), 8.01 (d, J = 7.8, 1H, H$_f$), 8.05 – 7.97 (m, 1H, H$_d$), 4.33 – 4.22 (m, 4H, H$_{b,i}$), 3.50 (s, 3H, H$_{r,s,t}$), 3.28 – 2.84 (m, 16H, H$_{j,k,l,m,n,o,p,q}$), 1.33 – 1.25 (t, 3H, J = 6.0, H$_a$); ^{13}C NMR (100 MHz, CD_3CN) 164.1, 163.6, 146.5, 137.7, 132.6, 131.0, 129.8, 129.2, 127.1, 127.0, 126.0, 125.9, 125.7, 124.0, 53.2, 49.4, 45.5, 45.6, 44.7, 35.2, 13.3; υ_{max} /cm^{-1} 3295, 2975, 2361, 1700, 1647, 1621, 1592, 1437, 1349, 1248, 1079, 959, 784, 621; MS (ESI): *m*/*z* 639.3, [M$^+$]. HRMS (ESI) calcd for [$C_{25}H_{33}N_8O_6Zn^{35}Cl]^+$, [M – ClO$_4$]$^+$: 639.1430. Found: 639.1419.

A specimen of $C_{25}H_{32}N_8O_2Zn·2(ClO_4)·C_2H_3N$, approximate dimensions 0.10 mm × 0.16 mm × 0.24 mm, was used for the X-ray crystallographic analysis. The X-ray intensity data were measured. The integration of the data using a monoclinic unit cell yielded a total of 69974 reflections to a maximum θ angle of 67.27° (0.84Å resolution), of which 5633 were independent (average redundancy 12.422, completeness = 97.5%, R_{int} = 4.32%, R_{sig} = 1.77%) and 5562 (98.74%) were greater than 2σ(F^2). The final cell constants of a = 12.2193(2) Å, b = 12.3857(2) Å, c = 42.4545(8) Å, β = 90.6920(10)°, volume = 6424.79(19) Å^3, are based upon the refinement of the XYZ–centroids of reflections above 20 σ(I). The

calculated minimum and maximum transmission coefficients (based on crystal size) are 0.5125 and 0.7393.

The structure was solved and refined using the Bruker SHELXTL Software Package, using the space group C 1 2/c 1, with Z = 8 for the formula unit, $C_{27}H_{35}Cl_2N_9O_{10}Zn$. The final anisotropic full-matrix least-squares refinement on F^2 with 464 variables converged at R1 = 6.37%, for the observed data and wR2 = 14.22% for all data. The goodness-of-fit was 1.322. The largest peak in the final difference electron density synthesis was 1.035 $e^-/Å^3$ and the largest hole was -0.732 $e^-/Å^3$ with an RMS deviation of 0.093 $e^-/Å^3$. On the basis of the final model, the calculated density was 1.617 g/cm^3 and F(000), 3232 e^-.

3. Fluorescence and ^{1}H NMR titration data

Fig. S1. ^{1}H NMR spectra of sensor **3b** in CD_3CN

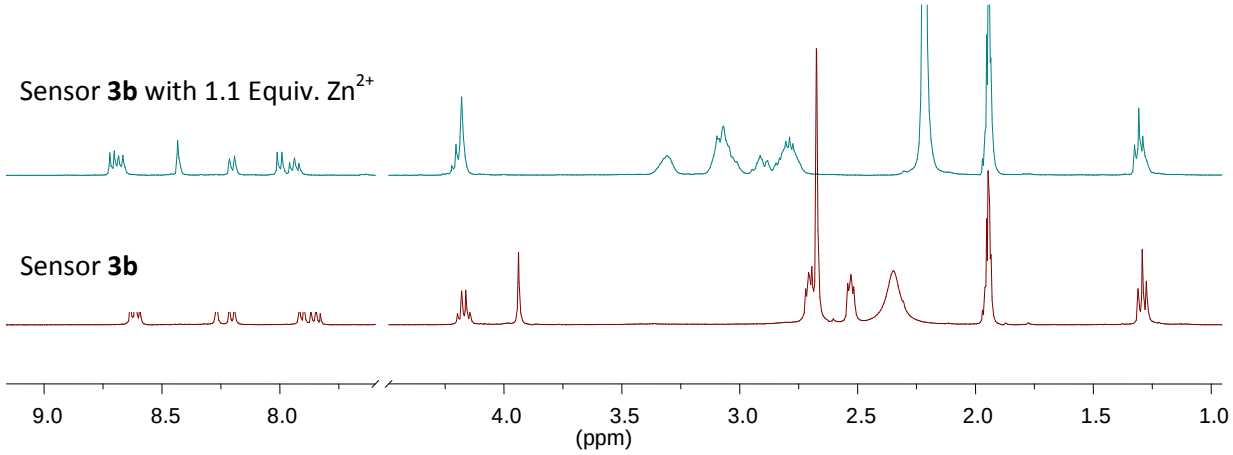


Fig. S2. pH dependent fluorescence emission of sensor **3b** in the presence and absence of Zn^{2+}

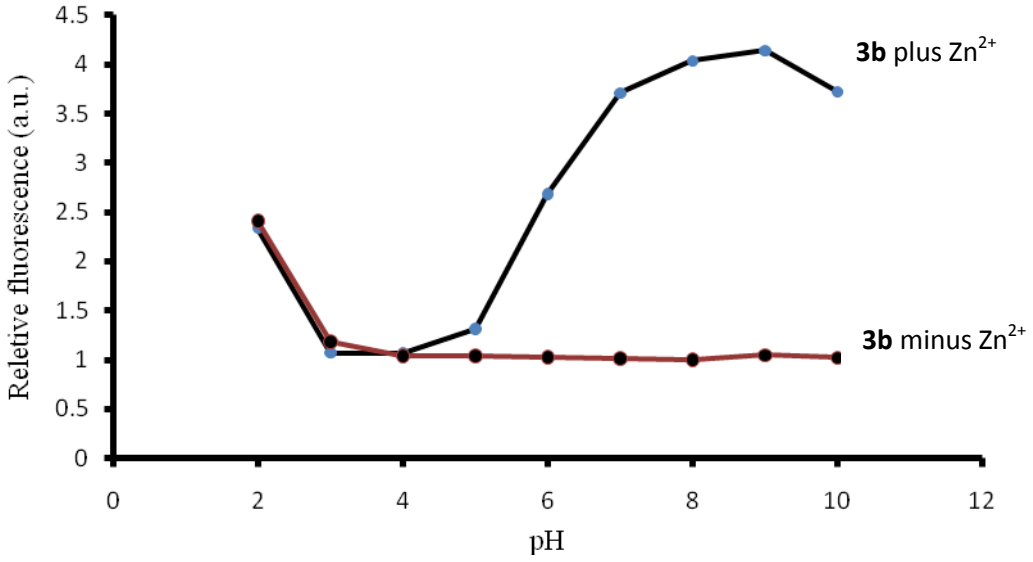
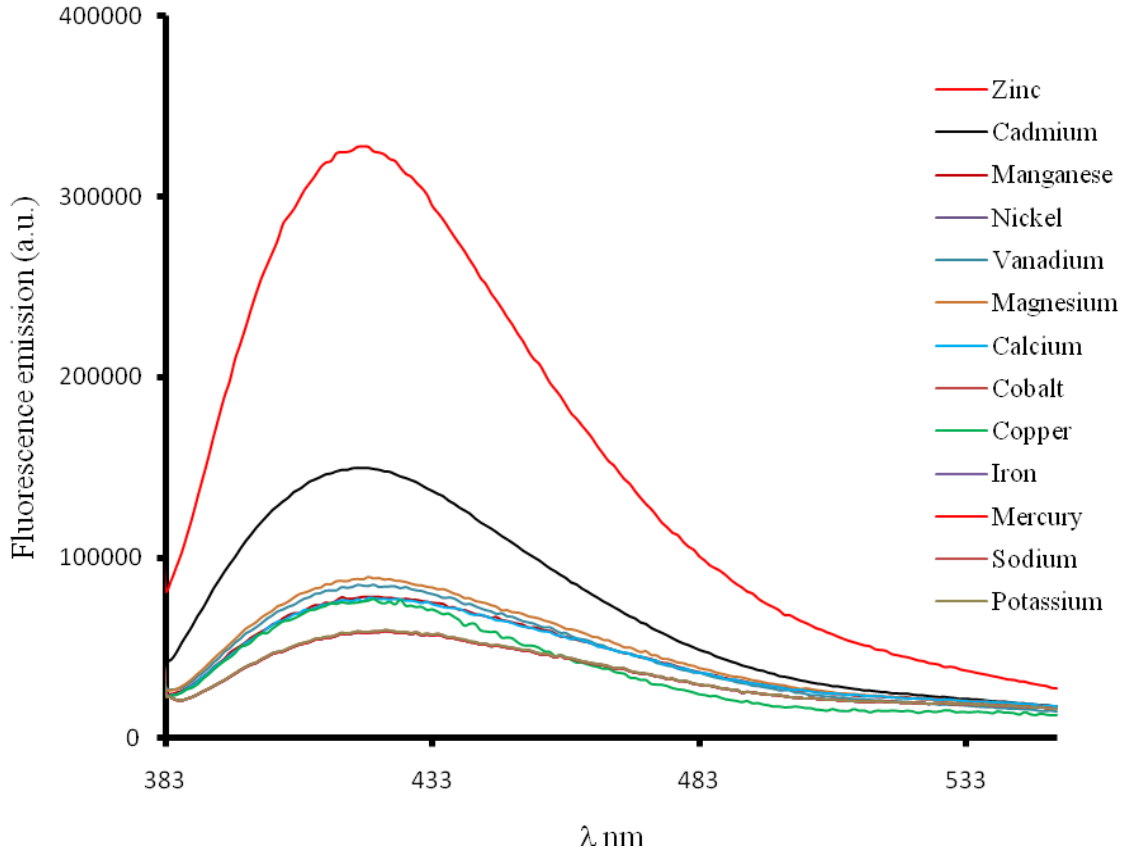


Fig. S3. Fluorescence emission of sensor **3b** with different metals



4. Quantum yield calculation of 3b and Zn(3b)](ClO$_4$)$_2$

The quantum yield of fluorescence was determined by comparison of the integrated area of the corrected emission spectrum of the [Zn(**3b**)](ClO$_4$)$_2$] to that of 2-amino pyridine[5] and anthracene[6] as standard fluorescence references. The quantum yield of sensor **3b** was calculated using the following equation:

$$\Phi_x = \Phi_{St} \left(\frac{\mathbf{Gradient_x}}{\mathbf{Gradient_{St}}}\right) \left(n_1 x^2 / n_1 st^2\right)$$

Φ is the quantum yield and n is the refractive index of the sample (H_2O) and standard (EtOH).

First the two standards were used to cross-calibrated the instrument. The above equation employed in calculating the quantum yields of each standard sample relative to each other. The concentration of **3b** and [Zn(**3b**)](ClO$_4$)$_2$ was adjusted to match the absorbance of the anthracene at the wavelength of excitation. The Emission for **3b** and [Zn(**3b**)](ClO$_4$)$_2$ was

integrated from 381 to 550 nm with excitation at 375 nm. The quantum yield of [Zn(**3b**)]$(ClO_4)_2$ was calculated against anthracene as follows:

$$Ø_{[Zn(\mathbf{3b})]} = \left[\frac{0.27 \times 923879}{5434791}\right] \times (1.33^2/1.36^2)$$

$$Ø_{[Zn(\mathbf{3b})]} = 0.045898 \times 0.956$$

$$Ø_{[Zn(\mathbf{3b})]} = 0.044$$

The quantum yield of **3b** was calculated against anthracene as follows:

$$Ø_{\mathbf{3b}} = \left[\frac{0.27 \times 105234}{5434791}\right] \times (1.33^2/1.36^2)$$

$$Ø_{\mathbf{3b}} = 0.005228 \times 0.956$$

$$Ø_{\mathbf{3b}} = 0.005$$

Fig. S4. Standard 1 = anthracene

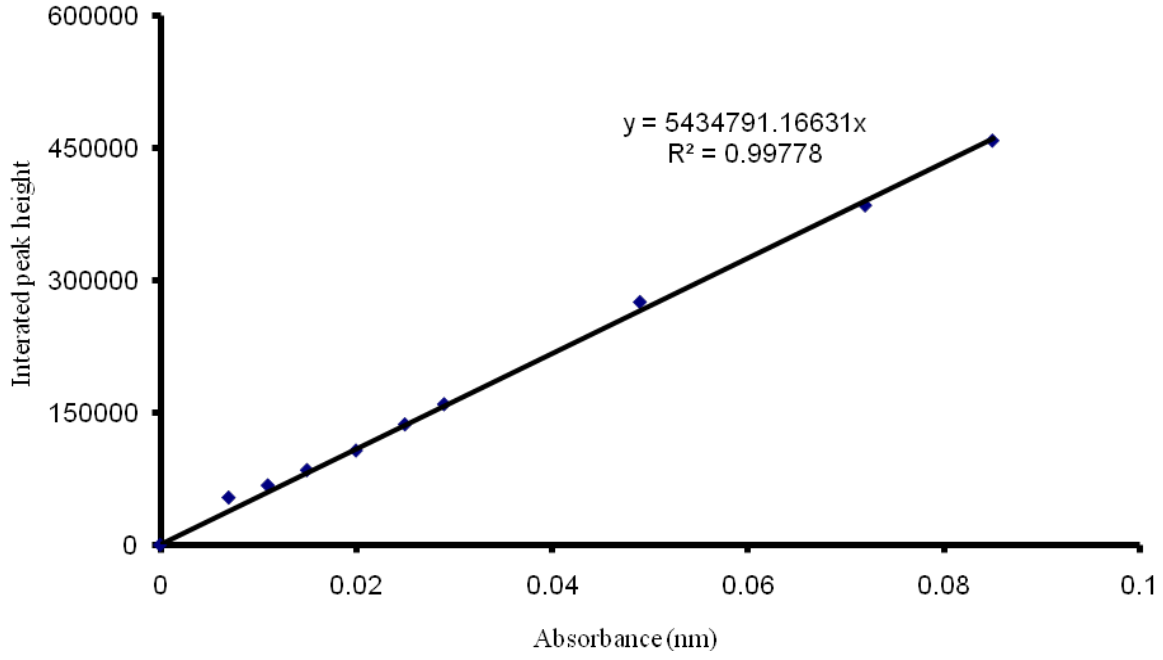


Fig. S5. Standard 2 = 2-amino pyridine

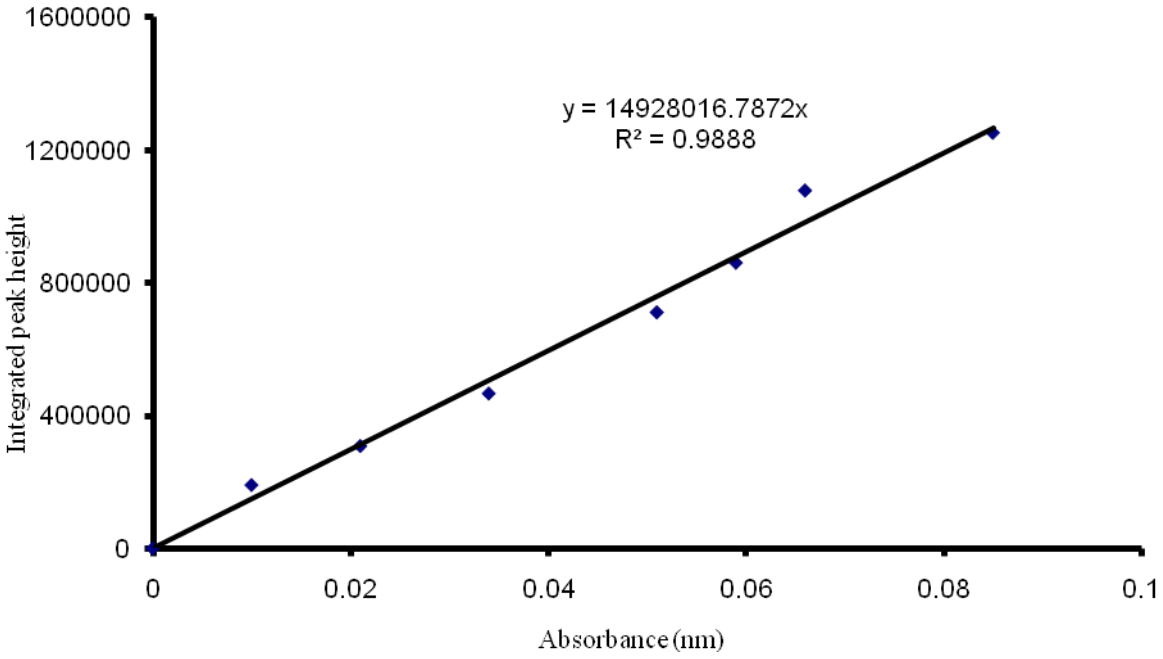
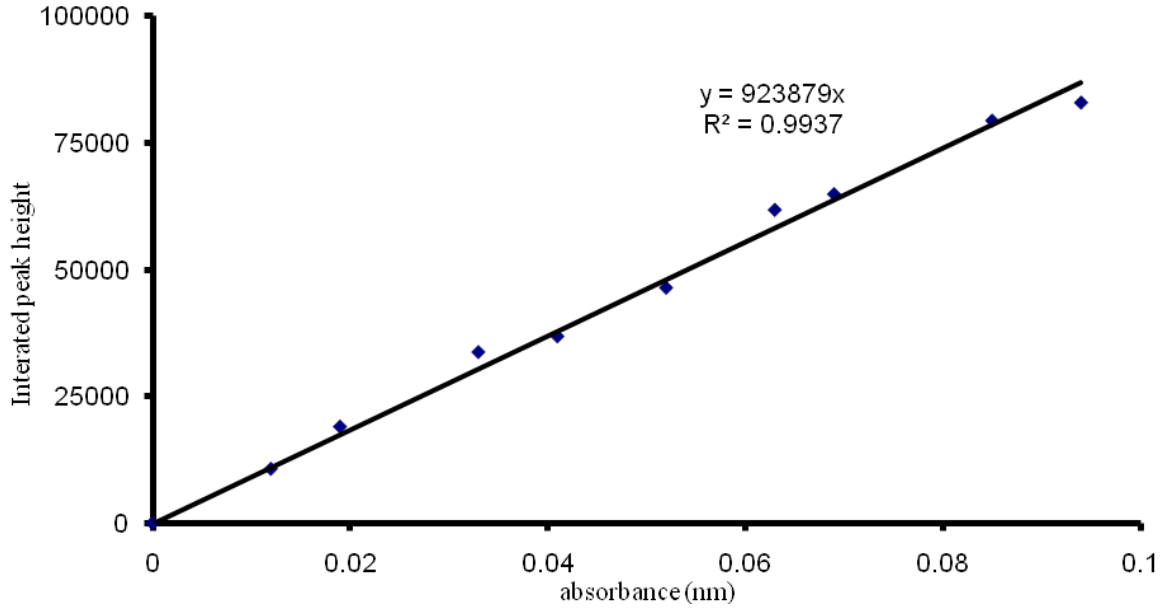


Fig. S6. Correlation of absorbance and fluorescence output of sensor **3b**



5. K_d Calculations[7] for sensor 3b

Fluorescence spectroscopy was used to determine the dissociation constant of sensor **3b**. 0.5mM of **3b** in pH 7, (0.1 mM HEPES buffer) was titrated with increasing concentration of Zn^{2+} and fluorescence measured. At 1:1 complexation, the fluorescence plateaus and no increase in fluorescence measured (**Fig. S7** and **S8**). Hence, $K_d = 1/2F_{max}$. Using this principle and applying it to the equation below used in Maruyama *et al.*,[7] the dissociation constant was calculated as follows;

$$F = \frac{F_{max}[Zn^{2+}] + K_d F_{min}}{K_d - [Zn^{2+}]}$$

$$[F(K_d - [Zn^{2+}]) = (F_{max}[Zn^{2+}] + K_d F_{min})$$

$$K_d(F - F_{min}) = [Zn^{2+}](F_{max} - F)$$

$$K_d = [Zn^{2+}]\frac{(F_{max} - F)}{(F - F_{min})}$$

At $\frac{F_1}{2}$

$$\frac{(F_{max} - F)}{(F - F_{min})} = 1$$

Therefore

$$K_d = [Zn^{2+}]$$

But at $\frac{F_1}{2}$

$$[Zn^{2+}] = 2.8 \times 10^{-4}\ M$$

Therefore

$$K_d = 2.8 \times 10^{-4}\ M$$

Fig. S7. Fluorescence intensity as a function of $[Zn^{2+}]$

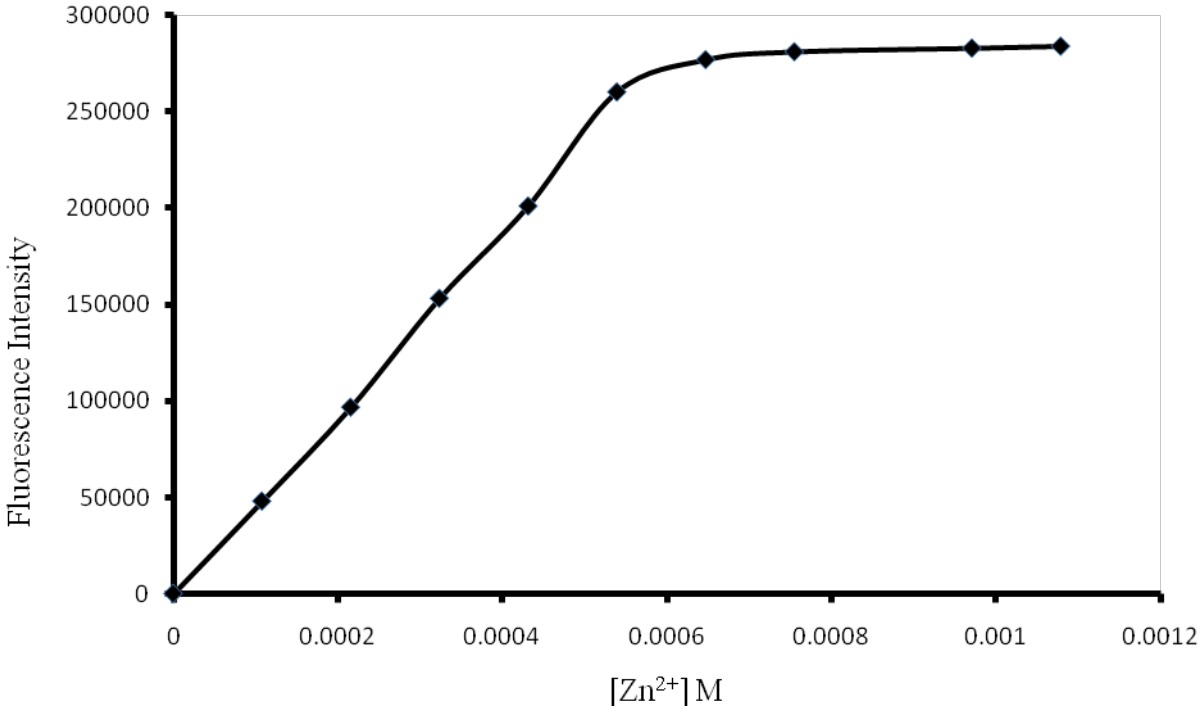
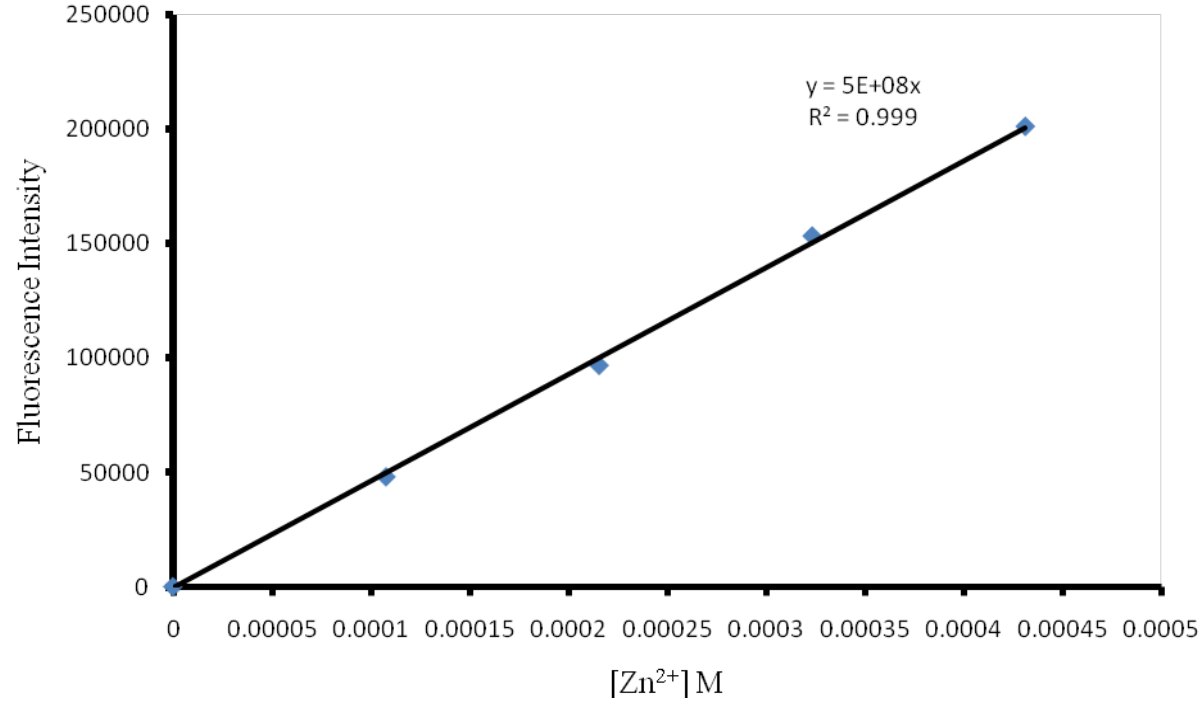


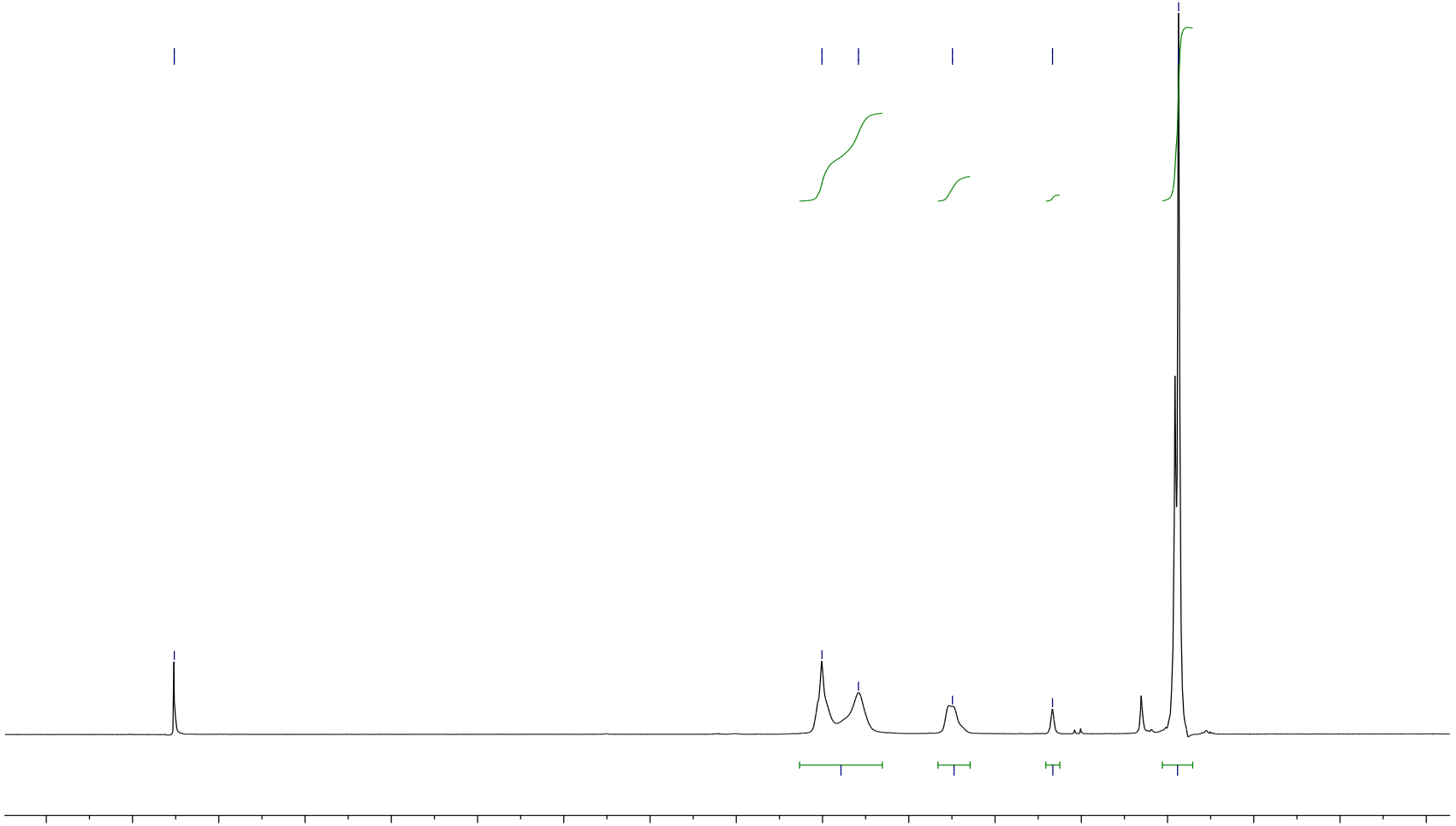
Fig. S8. Linear relationship between fluorescence intensity **3b** and $[Zn^{2+}]$



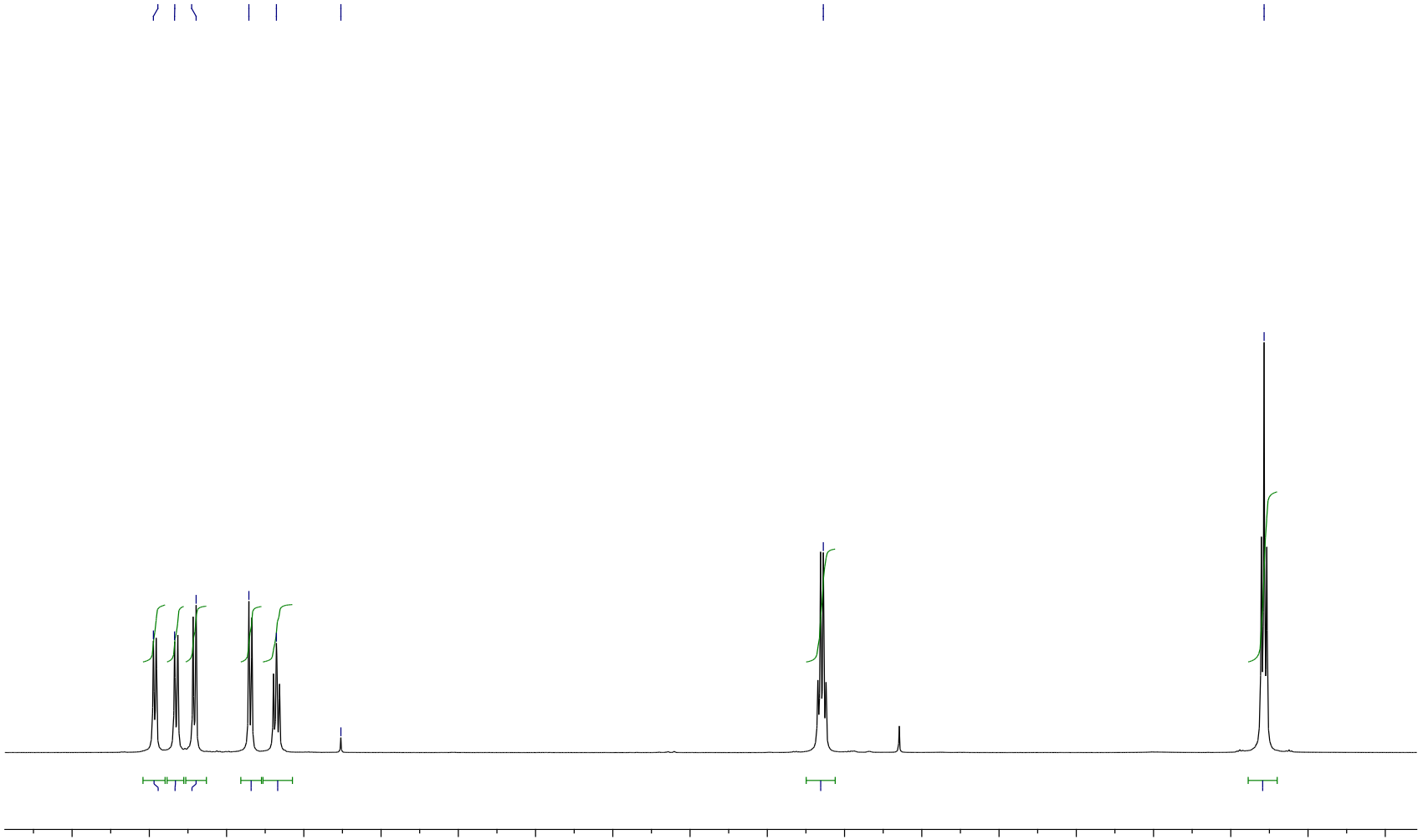
6. *In Vivo* Studies

Zebra fish eggs, 12 hpf, were incubated in respective 25mL petri dishes with sensors **3a** and **3b** (20 mL fish water, 50 µM). These embryos were then kept under standard conditions[8] at 28 °C for 5 days in agreement with Home Office regulations on a 12 h light / 12 h dark cycle. Tricaine methane sulphonate (MS222) was used to anesthetise the fish before any fluorescence microscopy. Fluorescence images were obtained by means of a Leica DC300F digital camera equipped with a GFP excitation filter of 425/60nm and barrier filter set at 480nm.

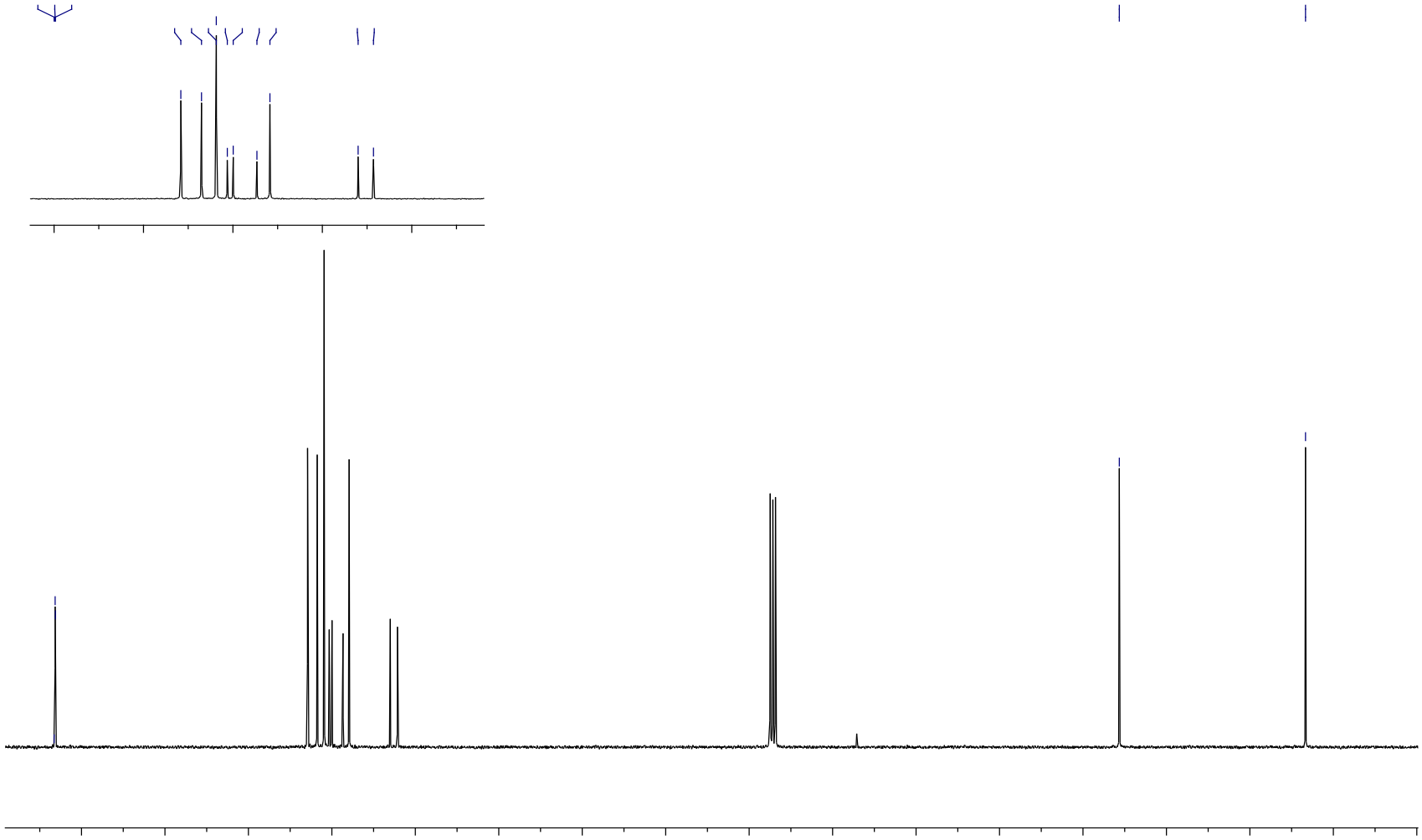
1b; ^{1}H NMR (400 MHz, $CDCl_3$)



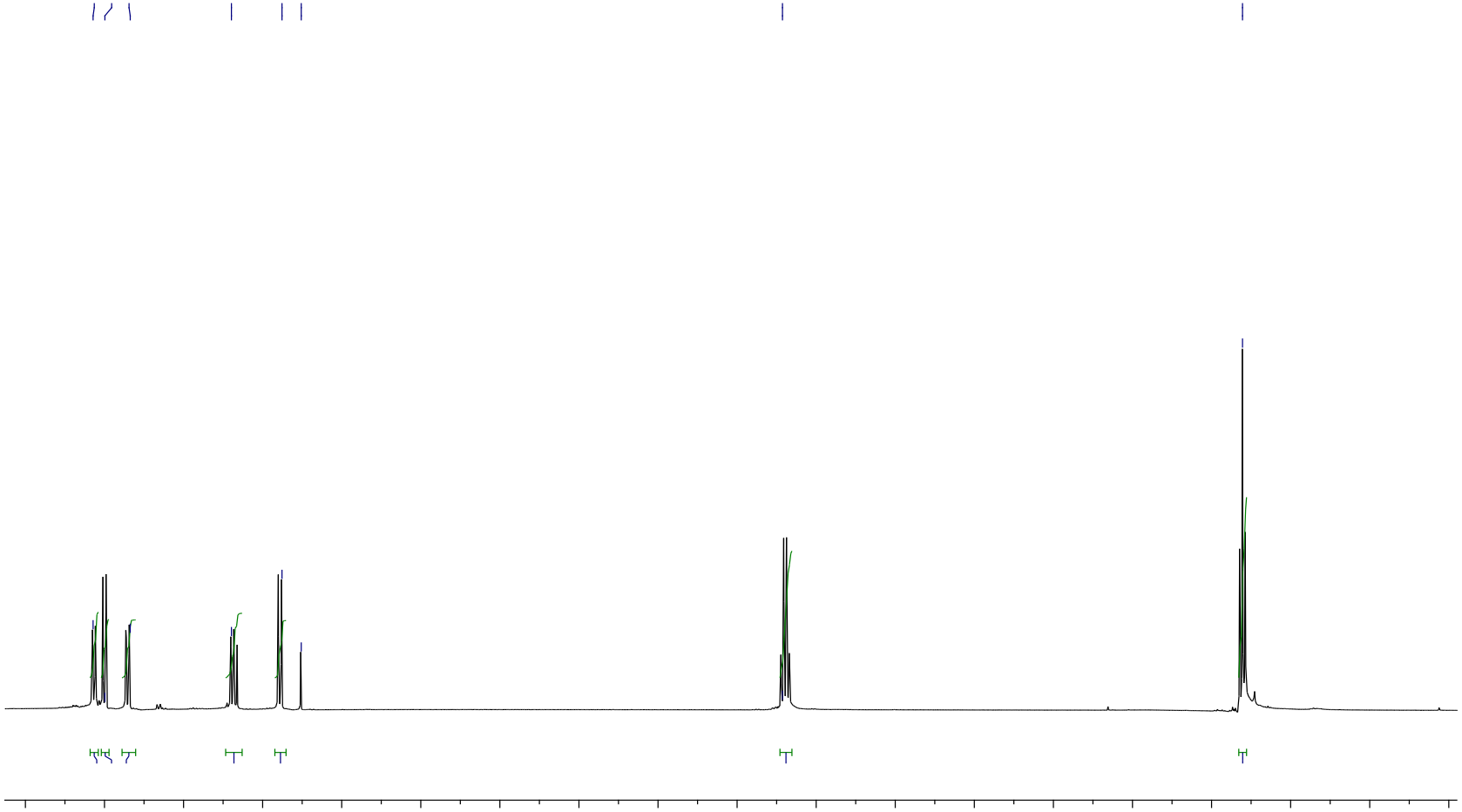
S4; ^{1}H NMR (400 MHz, $CDCl_3$)



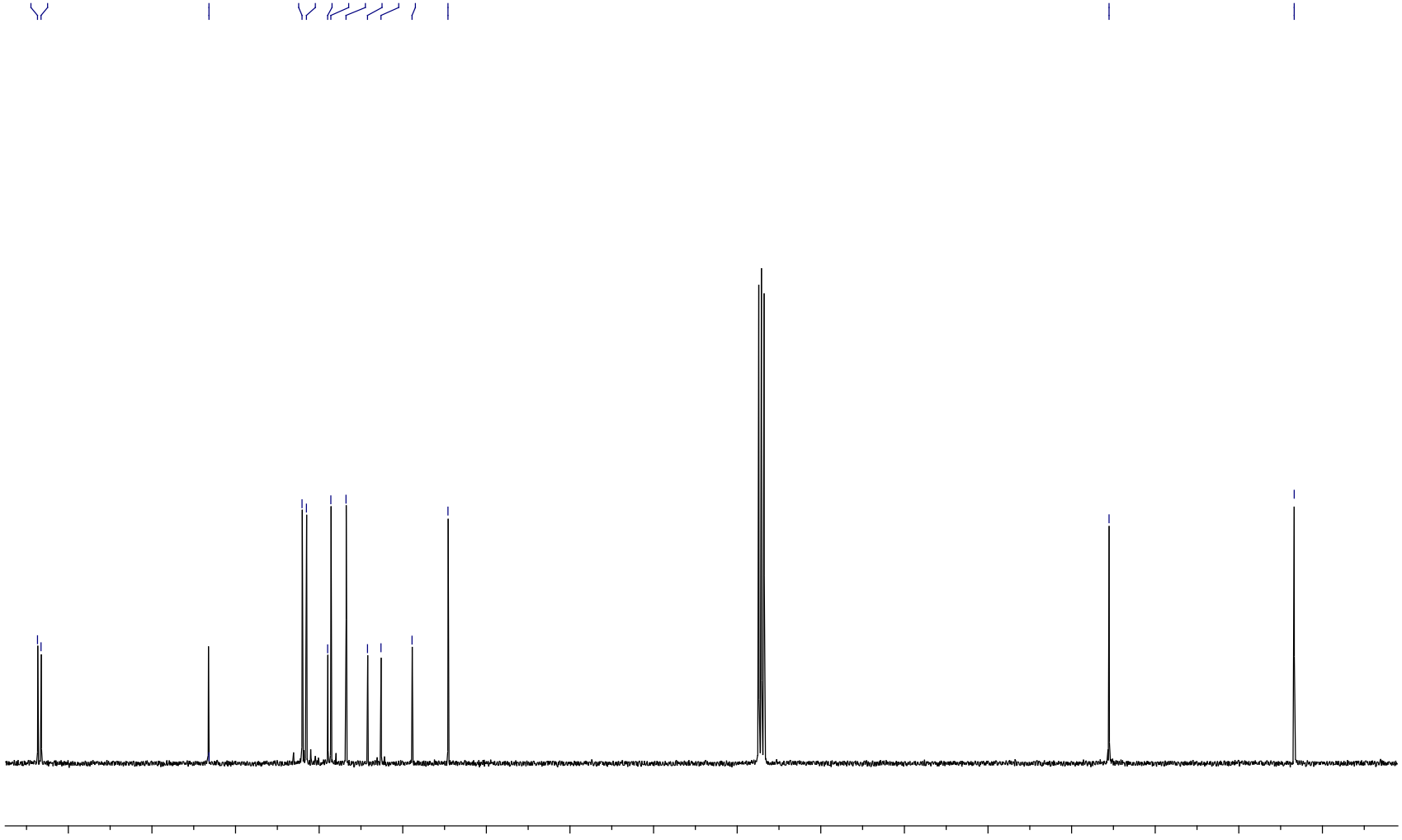
S4; ^{13}C NMR (400 MHz, $CDCl_3$)



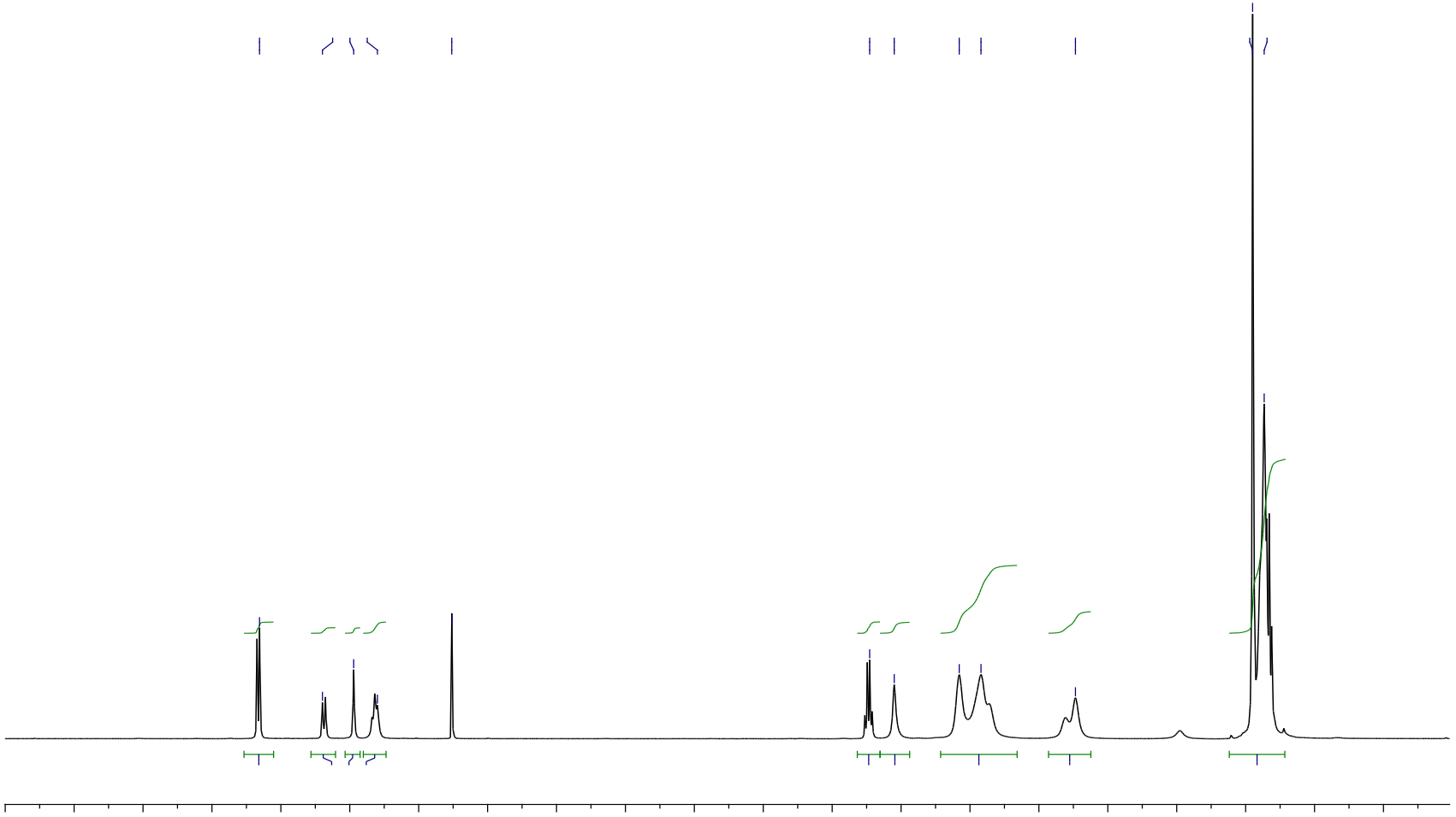
2; ^{1}H NMR (400 MHz, $CDCl_3$)



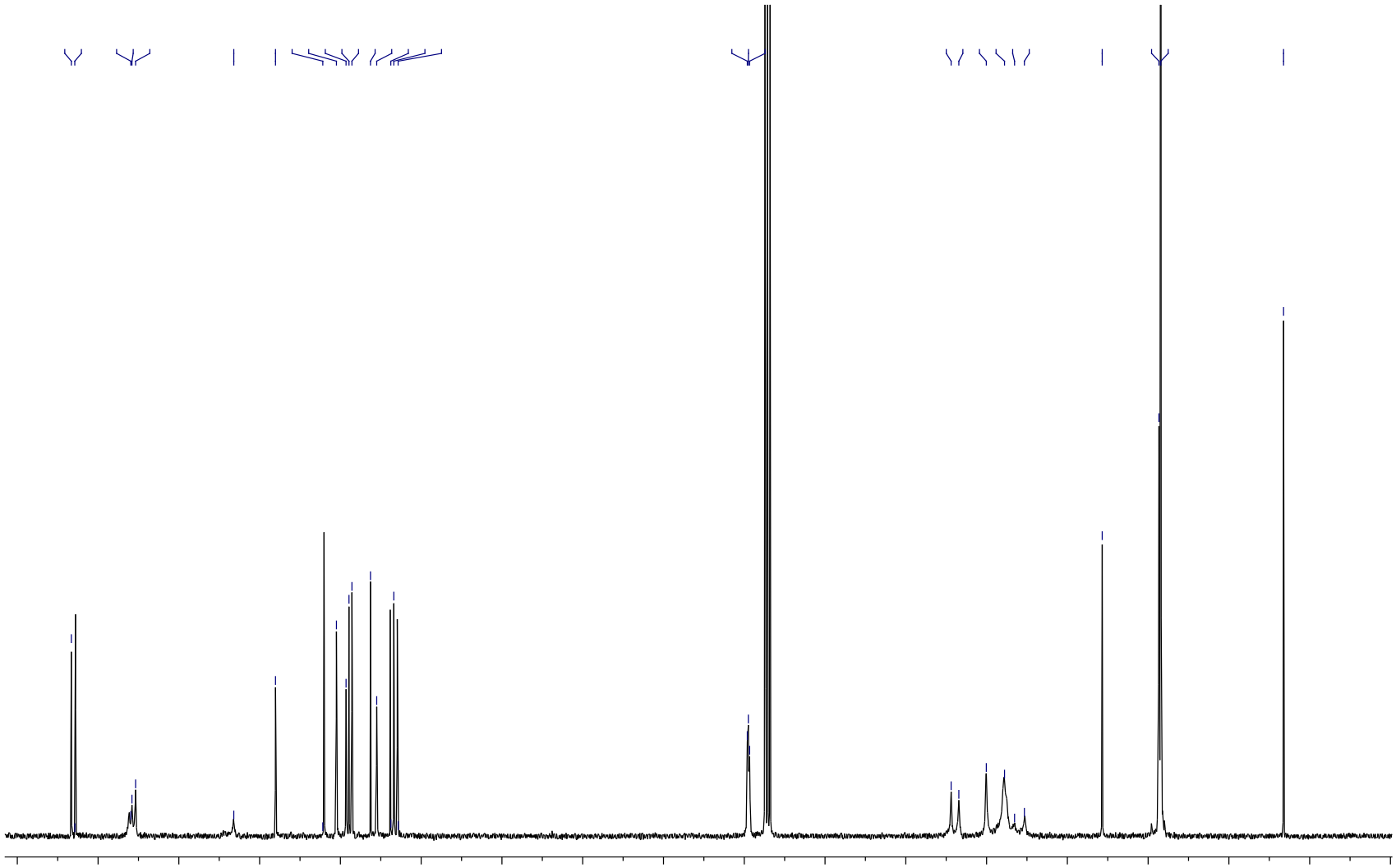
2, ^{13}C NMR (400 MHz, $CDCl_3$)



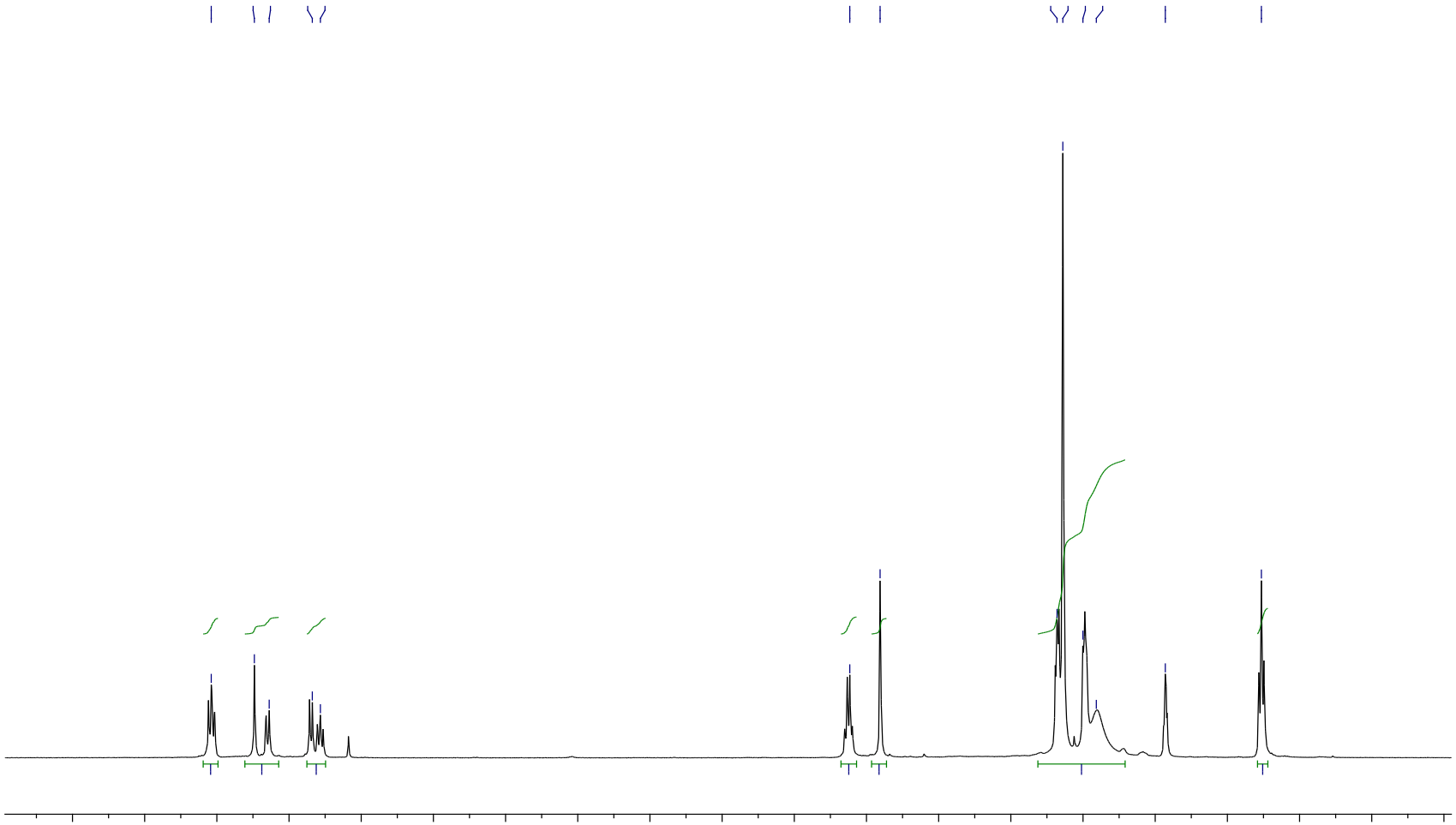
S5; ^{1}H NMR (400 MHz, $CDCl_3$)



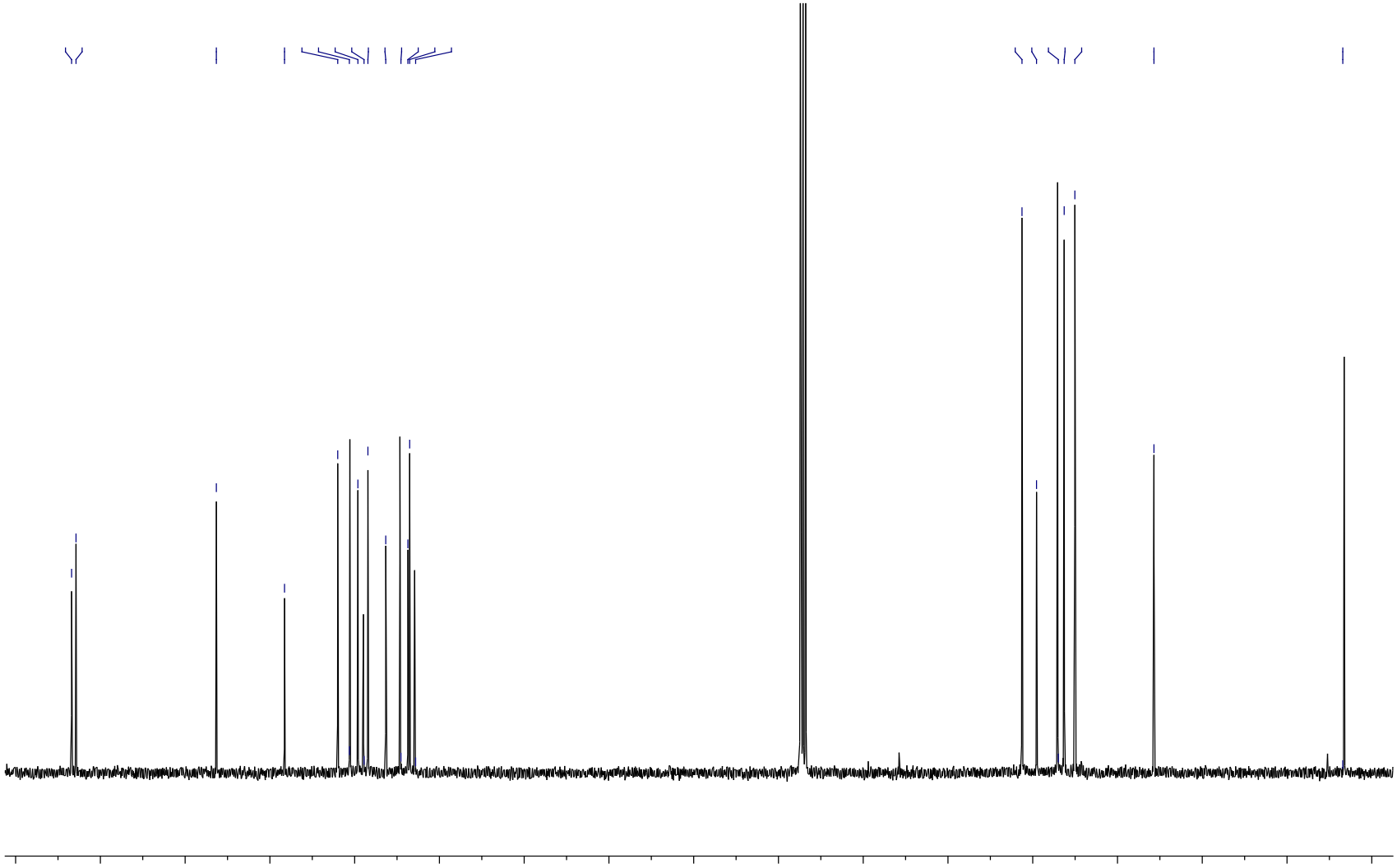
S5, ^{13}C NMR (400 MHz, $CDCl_3$)



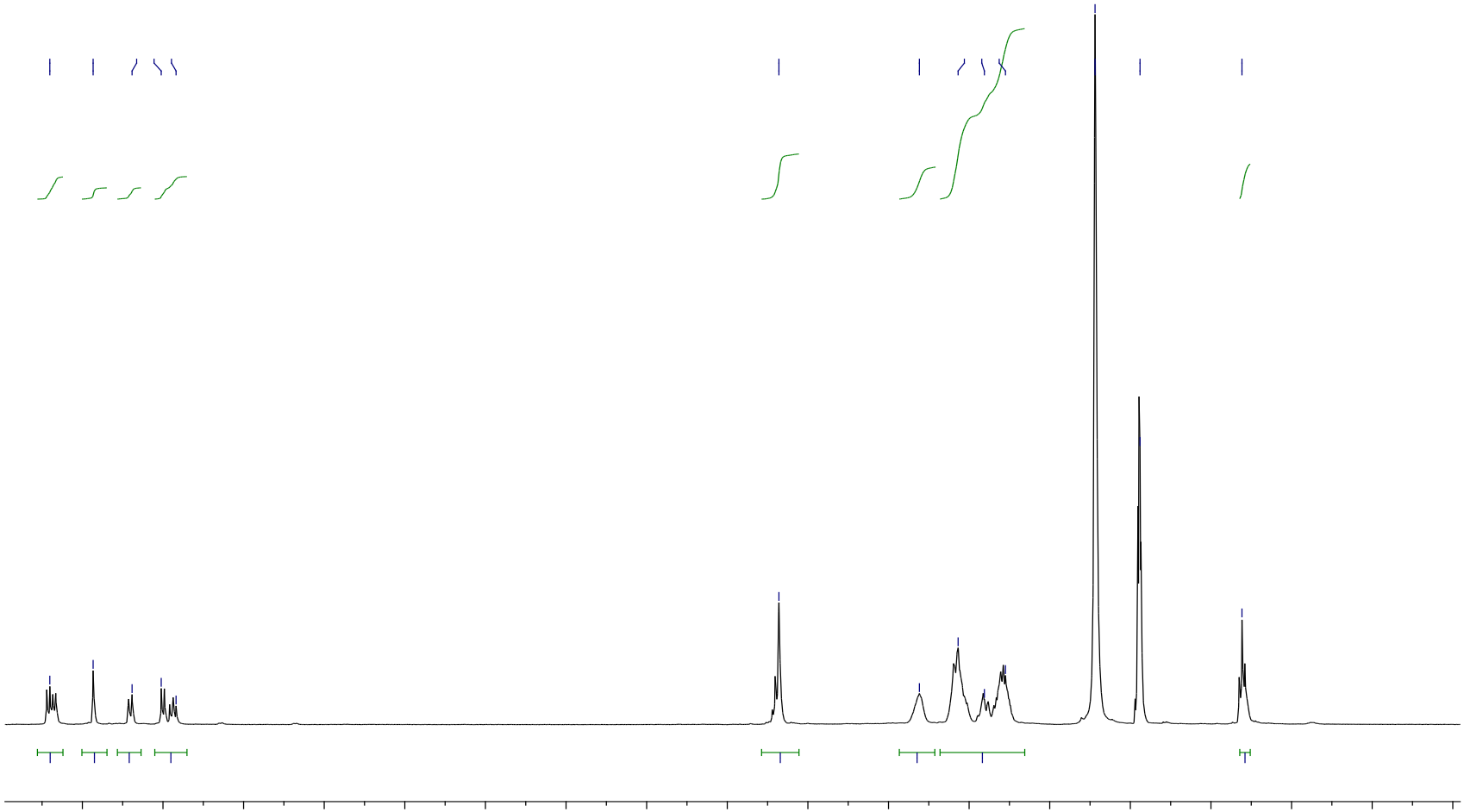
3b; ^{1}H NMR (400 MHz, CD_3CN)



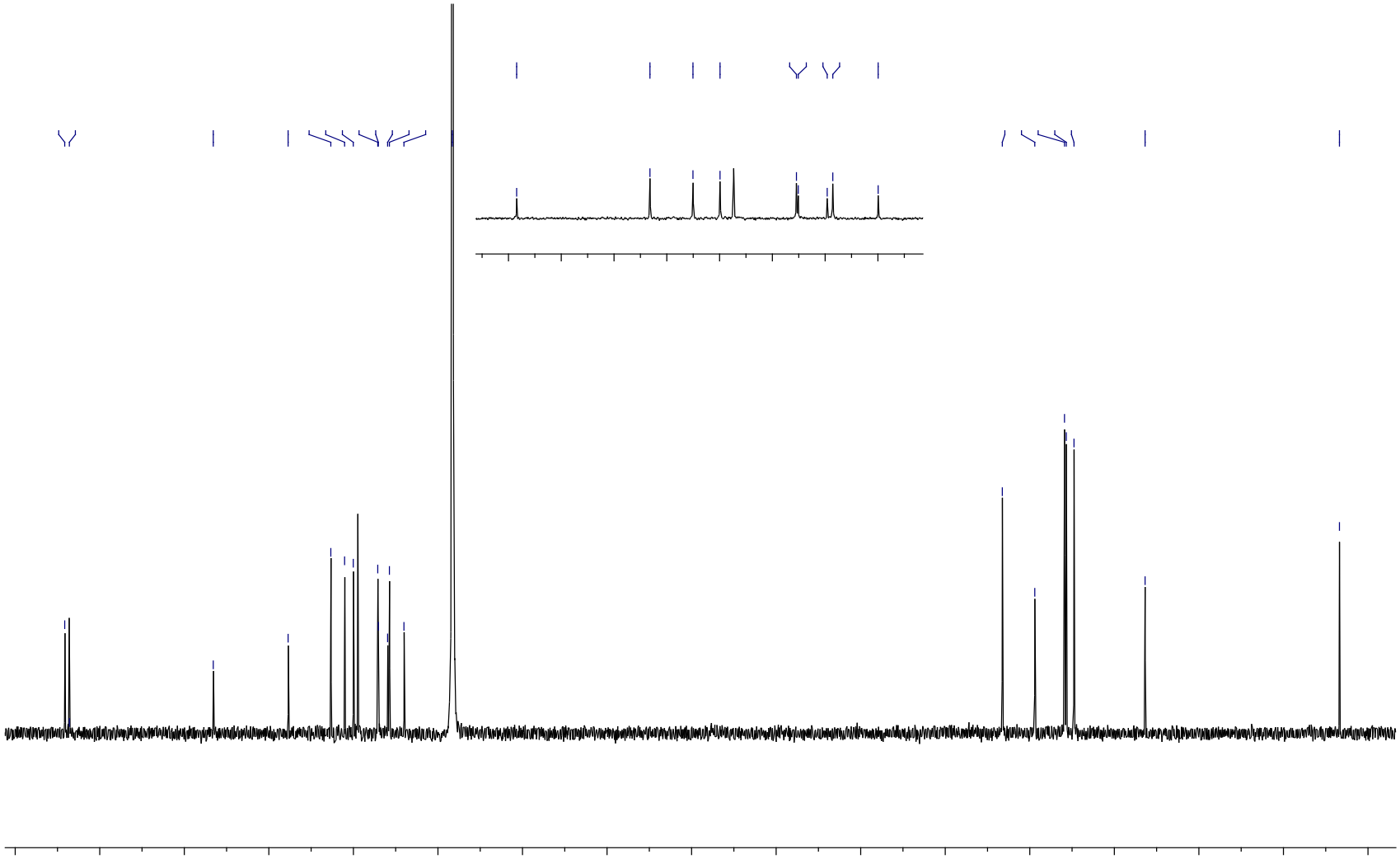
3b; ^{13}C NMR (400 MHz, $CDCl_3$)



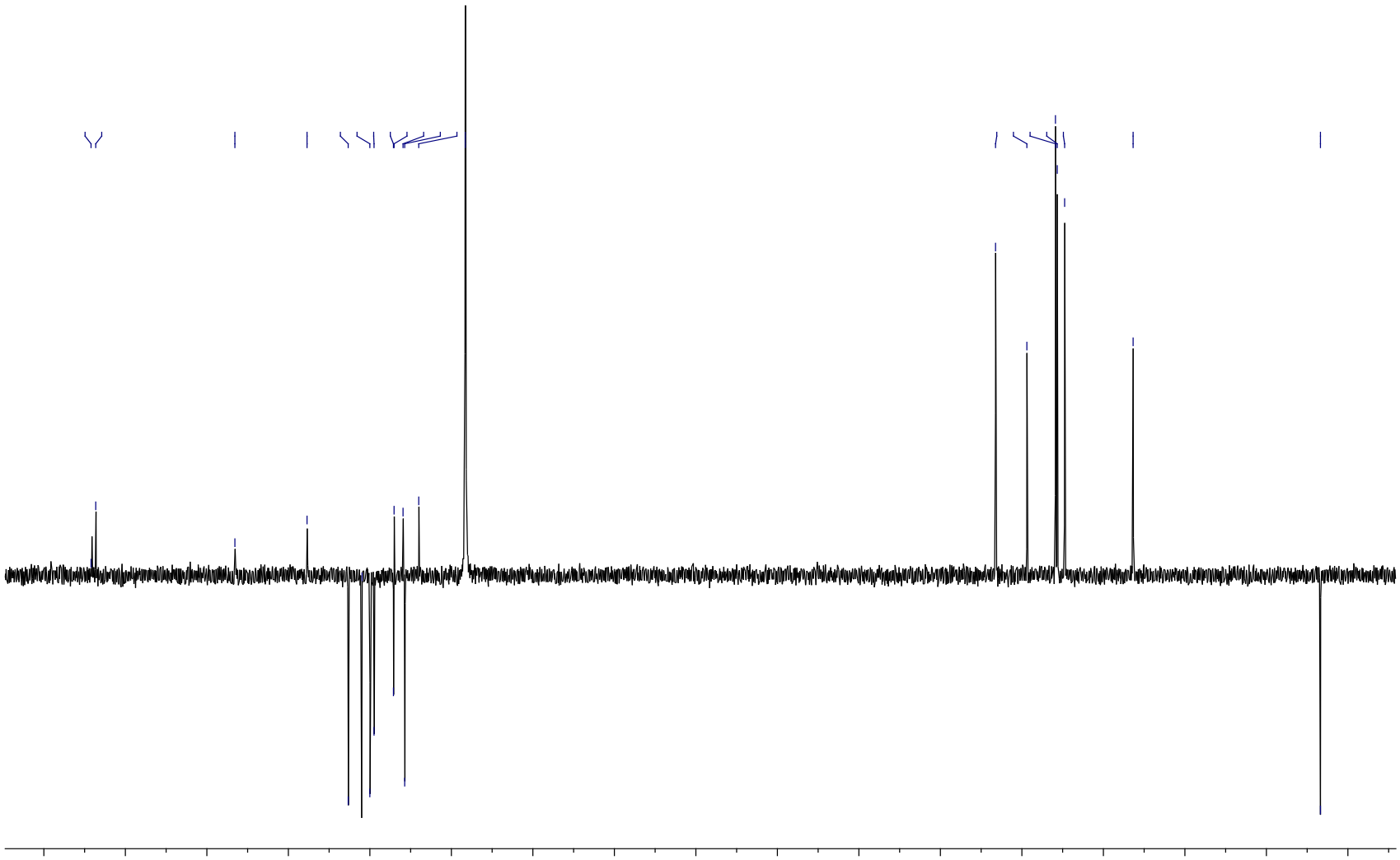
[Zn(3b)](ClO$_4$)$_2$; ^{1}H NMR (400 MHz, CD$_3$CN)



[Zn(3b)](ClO$_4$)$_2$; ^{13}C NMR (400 MHz, CD$_3$CN)



[Zn(3b)](ClO_4)$_2$; DEPT-Q135 (400 MHz, CD_3CN)



8. References

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