Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

Biotin-tagged fluorescent sensor to visualize ‘mobile’ Zn^{2+} in cancer cells

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General experimental

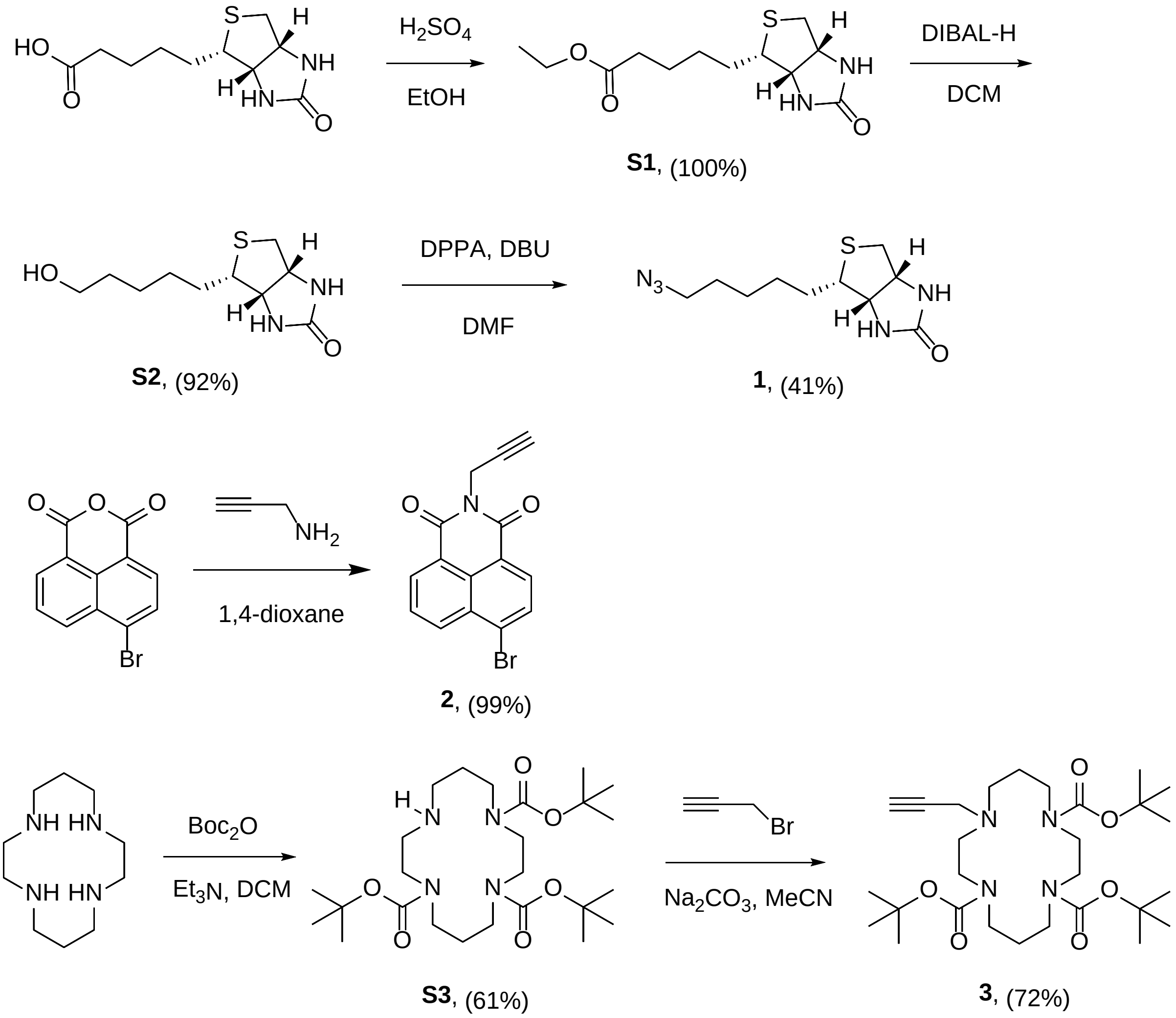
Unless otherwise stated, reactions were carried out using commercially available reagents, used as supplied from Sigma Aldrich, Acros Organics, or Alfa Aesar, in combination with solvents from Honeywell Solvents. Anhydrous DCM and DMF were obtained from an MBRAUN MB SPS-800 solvent purification system. "H_2O" refers to deionized water. The N-TERT keratinocyte cell line was provided by Prof. Michael P. Philpott and the MCF-7 cell line was sourced from American Type Culture Collection (ATCC Middlesex, UK).

For syntheses requiring anhydrous conditions, reactions were carried out under a nitrogen atmosphere using oven-dried glassware. Solvents were removed *in vacuo* using a Heidolph Hei-VAP Value G1 rotary evaporator with a water condenser. Due to the potentially explosive nature of small molecule azides, compound **1** was handled with extreme care, including storage at -18 °C, and removal of high-boiling point solvents by aqueous workup rather than rotary evaporation at high temperatures. Analytical thin layer chromatography was carried out on Merck Kiesel gel 60 aluminium-backed silica plates, with visualization using short-wave ultraviolet light (254 nm) or staining with iodine or PMA stain. Column chromatography was carried out using BDH (40-60 µm) silica.

Infrared spectra were obtained on a Bruker Tensor 37 FTIR spectrometer with the range 4000-600 cm^{-1}. UV-Vis spectra were obtained on a PerkinElmer Lambda 35 UV/Vis spectrometer. Fluorescence spectra were measured on an Agilent Cary eclipse fluorescence spectrophotometer with a 1 cm pathlength cell. The fluorescence microscope images were obtained from a CELENA S digital imaging system. ^1H NMR spectra were recorded at 400 MHz on a Bruker Avance I or Bruker Avance III spectrometer. Chemical shifts are reported in δ (ppm) and referenced to the residual solvent peak used as the internal standard: $CHCl_3$ (7.26 ppm), MeOH (3.31 ppm). Coupling constants (J) are measured in Hertz (Hz) and reported to 1 d.p. Peak multiplicities for resonances are noted as standard abbreviations: s, singlet; d, doublet; dd, double doublet; dt, doublet of triplets, t, triplet; q, quartet; m, unresolved multiplet; br, broad. ^{13}C NMR spectra were recorded at 101 MHz on a Bruker Avance III spectrometer. Chemical shifts (δ) are quoted to the nearest 0.1 ppm, with reference to the given solvent $CDCl_3$ (77.0 ppm), MeOD (49.00 ppm) as the internal standard. Electrospray ionization 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.

Experimental procedures

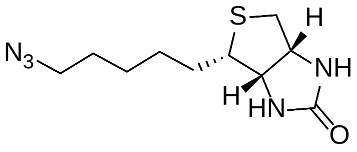
The synthetic route towards **1**,[1,2] **2**[3], and **3**[4,5] is shown in Scheme S1. The reported procedure for the synthesis of **1**[6] was ineffective in our hands. However, the azide was successfully prepared in improved yield using an adapted procedure for an analogous compound.[2]



Scheme S1 The synthetic route towards **1-3**.

Experimental procedures

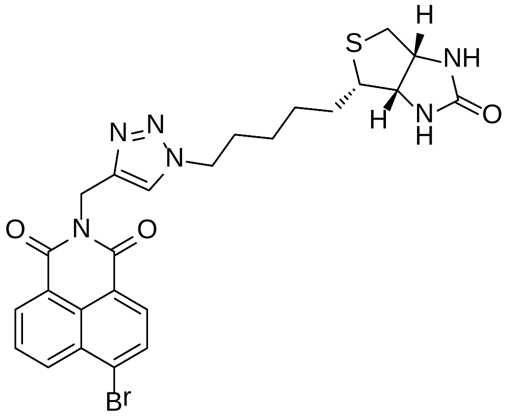
(3a*S*,4*S*,6a*R*)-4-(5-Azidopentyl)tetrahydro-1*H*-thieno[3,4-*d*]imidazol-2(3*H*)-one (**1**)



1

Under an atmosphere of nitrogen, biotinol **S2** (240 mg, 1.04 mmol, 1.0 eq.) was dissolved in anhydrous DMF (10.4 mL) and the colourless solution was cooled to 0 °C. DPPA (0.448 mL, 2.08 mmol, 2.0 eq.) was added followed by DBU (0.310 mL, 2.08 mmol, 2.0 eq.). The solution was stirred at 0 °C for 15 min, then heated to 65 °C, covered and stirred for 18 h. Then the reaction was cooled to room temperature and the resulting red-orange solution was diluted with H_2O (200 mL) and extracted with EtOAc (2 × 100 mL). The combined organic phase was washed with brine (2 × 100 mL) and saturated aqueous $NaHCO_3$ (2 × 100 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to give a pale yellow solid residue. This crude material was purified by flash chromatography (eluent: DCM/MeOH 20:1, R_F = 0.2), and recrystallized from toluene to give **1** as a white solid (104 mg, 41%). ^1H NMR (400 MHz, $CDCl_3$) δ_H 5.13 (s, 1H), 4.90 (s, 1H), 4.56-4.48 (m, 1H), 4.35-4.29 (m, 1H), 3.28 (t, 2H, *J* = 6.8), 3.19-3.15 (m, 1H), 2.94 (dd, 1H, *J* = 12.8, 5.1), 2.74 (d, 1H, *J* = 12.8), 1.72-1.40 (m, 8H). ^{13}C NMR: (100 MHz, $CDCl_3$): δ_C 164.1, 62.2, 60.2, 55.8, 51.5, 40.7, 28.7 (overlapping signals), 28.6, 26.7. All other spectroscopic data were consistent with those previously reported.[6]

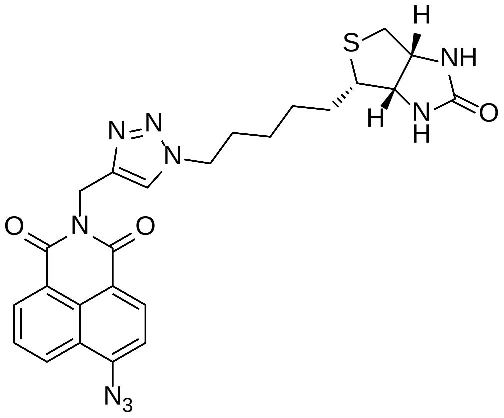
6-Bromo-2-((1-(5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentyl)-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**4**)



4

Biotin azide **1** (25 mg, 0.10 mmol, 1.0 eq.) and **2** (31 mg, 0.10 mmol, 1.0 eq.) were dissolved in anhydrous DCM (0.50 mL) under a nitrogen atmosphere. To this solution, tetrakis(acetonitrile)copper(I) hexafluorophosphate (7.5 mg, 0.020 mmol, 20 mol%) and DIPEA (10 µL, 0.057 mmol, 0.57 eq.) were added and the flask was covered with aluminium foil and stirred at room temperature for 48 h. Then the yellow solution was washed with a saturated solution of EDTA in 17% NH_3/H_2O (5 mL), and the aqueous layer was extracted with DCM (3 × 15 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo* to give **4** as a white solid (54 mg, 95%, m.p. 104-107 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.67 (d, 1H, *J* = 6.8), 8.57 (d, 1H, *J* = 8.4), 8.43 (d, 1H, *J* = 8.0), 8.04 (d, 1H, *J* = 8.0), 7.88-7.81 (m, 1H), 7.66 (s, 1H), 5.49 (s, 2H), 5.29 (s, 1H), 4.76 (s, 1H), 4.52-4.46 (m, 1H), 4.36-4.24 (m, 3H), 3.12-3.06 (m, 1H), 2.89 (dd, 1H, *J* = 13.0, 5.0), 2.70 (d, 1H, *J* = 13.0), 1.88-1.35 (m, 8H). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 163.9, 163.4 (overlapping signals), 143.2, 133.6, 132.3, 131.5, 131.1, 130.6, 129.0, 128.1, 123.4, 122.7, 121.8, 61.9, 60.0, 55.4, 54.2, 50.1, 42.3, 40.2, 35.1, 29.8, 28.3, 26.1. IR: (υ_{max}/cm^{-1}) 3231, 2934, 2680, 1698, 1657, 1463, 1343, 1235, 1046, 951. HR-ESI MS (*m/z*) $[M+H]^+$ calcd. for $C_{25}H_{26}BrN_6O_3S$ 569.0965, found 569.0962.

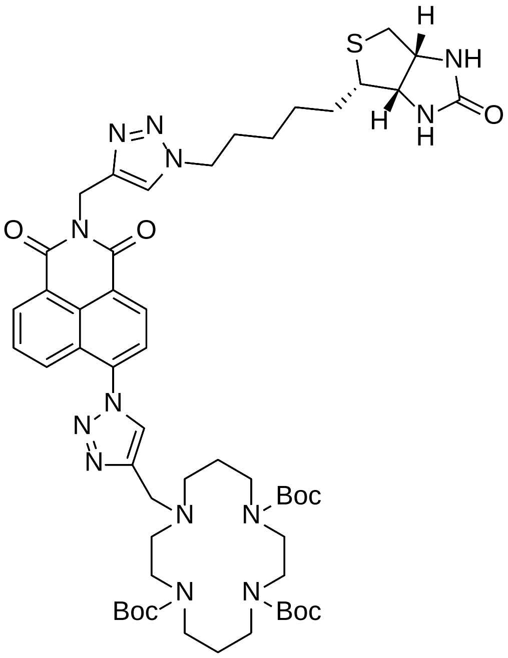
6-Azido-2-((1-(5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentyl)-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**5**)



5

Bromide **4** (38 mg, 0.067 mmol, 1.0 eq.) and NaN_3 (6.5 mg, 0.10 mmol, 1.5 eq.) were combined in DMF (2.0 mL) and the mixture was stirred at room temperature for 3 days. Then the mixture was diluted with H_2O (20 mL) and extracted with EtOAc (3 × 15 mL). The organic layers were combined and washed with brine (2 × 30 mL), dried over $MgSO_4$, and concentrated *in vacuo* to give **5** as yellow solid (33 mg, 93%, m.p. 154-157 °C). ^1H NMR (400 MHz, $CDCl_3$) δ_H 8.68 (d, 1H, *J* = 7.4), 8.63 (d, 1H, *J* = 8.0), 8.47 (d, 1H, *J* = 8.6), 7.80-7.73 (m, 1H), 7.66 (s, 1H), 7.50 (d, 1H, J = 8.0), 5.51 (s, 2H), 4.95 (s, 1H), 4.62 (s, 1H), 4.54-4.49 (m, 1H), 4.35-4.28 (m, 3H), 3.16-3.09 (m, 1H), 2.93 (dd, 1H, *J* = 12.9, 5.0), 2.72 (d, 1H, *J* = 12.9), 1.93-1.37 (m, 8H). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 168.0, 167.8, 167.3, 147.9, 136.4, 136.0, 133.1, 133.0, 130.8, 128.2, 127.5, 126.1, 122.3, 118.7, 65.9, 64.0, 59.5, 54.1, 44.2, 39.0, 33.8, 33.5, 32.3, 32.2, 30.1. IR: (υ_{max}/cm^{-1}) 3083, 2098, 1733, 1649, 1494, 1374, 1268, 1082, 1017, 809, 658. HR-ESI MS (*m/z*) $[M+H]^+$ calcd. for $C_{25}H_{26}N_9O_3S$ 532.1847, found 532.1869.

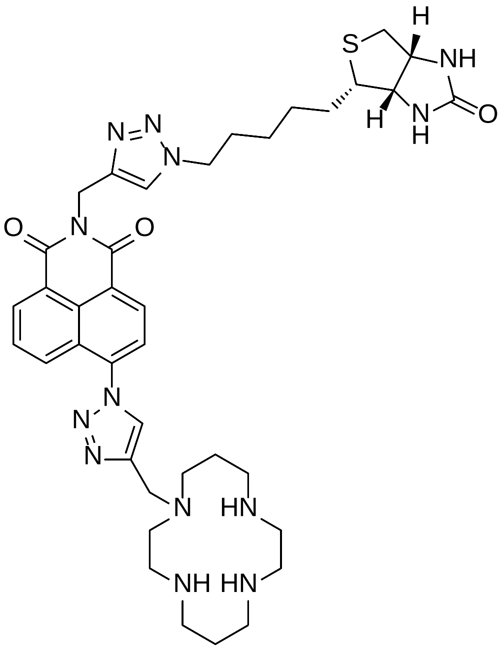
tri-*tert*-Butyl 11-((1-(1,3-dioxo-2-((1-(5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2,3-dihydro-1*H*-benzo[*de*]isoquinolin-6-yl)-1*H*-1,2,3-triazol-4-yl)methyl)-1,4,8,11-tetraazacyclotetradecane-1,4,8-tricarboxylate (**6**)



6

The azide **5** (32 mg, 0.060 mmol, 1.0 eq.) and acetylene **3** (33 mg, 0.060 mmol, 1.0 eq.) were dissolved in NMP (1.0 mL), followed by CuI (2.3 mg, 0.012 mmol, 20 mol%), NaOAc (1.0 mg, 0.012 mmol, 20 mol%), and EtOH (1.0 mL). The mixture was stirred at room temperature for 3 days under a nitrogen atmosphere. After that time a saturated solution of EDTA in 17% NH_3/H_2O (5 mL) was added and the yellow precipitate was collected by filtration, washed with H_2O and dried *in vacuo*. The crude product was dissolved in DCM (2 mL) and poured into petroleum ether (20 mL) to give **6** as a yellow powder (36 mg, 56%, m.p. 148-151 °C). ^1H NMR (400 MHz, $CDCl_3$) δ_H 8.74-8.69 (m, 2H), 8.27 (d, 1H, *J* = 7.6), 8.04 (s, 1H), 7.90-7.83 (m, 2H), 7.70 (s, 1H), 5.50 (s, 2H), 5.41 (s, 1H), 4.97 (s, 1H), 4.45-4.40 (m, 1H), 4.31 (t, 2H, *J* = 4.1), 4.25-4.20 (m, 1H), 3.96 (s, 2H), 3.42-3.30 (m, 12H), 3.10-3.05 (m, 1H), 2.87 (dd, 1H, *J* = 12.9, 4.9), 2.73 (t, 2H, *J* = 5.4), 2.67 (d, 1H, *J* = 12.9), 2.58 (t, 2H, *J* = 5.6), 1.94-1.86 (m, 4H), 1.83-1.75 (m, 4H), 1.65-1.61 (m, 4H), 1.42 (s, 27H). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 163.5, 162.9, 143.1, 138.4, 132.4, 131.0, 129.9, 129.1, 128.6, 126.5, 123.6, 122.8, 79.7, 61.8, 60.0, 55.6, 50.2, 47.3, 40.6, 35.4, 29.9, 28.4, 27.9, 26.4 (overlapping signals). IR: (υ_{max}/cm^{-1}) 3016, 2970, 1741, 1729, 1435, 1366, 1229, 1217. HR-ESI MS (*m/z*) [M+H]$^+$ calcd. for $C_{53}H_{76}N_{13}O_9S$ 1070.5604, found 1070.5606.

6-(4-((1,4,8,11-Tetraazacyclotetradecan-1-yl)methyl)-1*H*-1,2,3-triazol-1-yl)-2-((1-(5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentyl)-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**7**)



7

The tri-Boc protected compound **6** (31 mg, 0.029 mmol) was dissolved in a solution of 20% TFA in DCM (1.2 mL) and stirred for 10 h at room temperature. The solvent was then removed *in vacuo* and the residue dissolved in $CHCl_3$ (10 mL) and washed with 1 M aqueous NaOH solution (2 mL). The aqueous layer was extracted with $CHCl_3$ (3 × 10 mL) and the organic layers were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give **7** as a brown solid (13 mg, 58%, m.p. 197-201 °C). ^{1}H NMR (400 MHz, MeOD) δ_H 8.66 (d, 1H, *J* = 7.8), 8.63 (d, 1H, *J* = 7.3), 8.56 (s, 1H), 8.25 (d, 1H, *J* = 8.6), 8.05 (s, 1H), 7.99 (d, 1H, *J* = 7.3), 7.89-7.83 (m, 1H), 5.45 (s, 2H), 4.48-4.43 (m, 1H), 4.41 (t, 2H, *J* = 6.9), 4.27-4.22 (m, 1H), 4.01 (s, 2H), 3.29-3.23 (m, 4H), 3.21-3.16 (m, 4H), 3.16-3.09 (m, 1H), 3.05-3.01 (m, 4H), 2.97-2.92 (m, 4H), 2.87 (dd, 1H, *J* = 12.8, 5.8), 2.64 (d, 1H, *J* = 12.8), 2.18-2.11 (m, 2H), 1.95-1.89 (m, 2H), 1.70-1.33 (m, 8H). ^{13}C NMR (101 MHz, MeOD) δ_C 164.6, 164.1 (overlapping signals), 146.3, 144.6, 139.4, 133.2, 131.9, 130.7, 130.0, 129.7, 127.5, 127.5, 125.4, 125.2, 124.9, 123.9, 63.4, 61.6, 57.0, 55.4, 54.0, 51.3, 51.1, 50.5, 49.5, 49.3, 49.1, 48.1, 46.8, 46.3, 41.0, 36.3, 31.0, 29.6, 27.4, 26.5, 24.6. IR: (υ_{max}/cm^{-1}) 2921, 2851, 1662, 1587, 1427, 1233, 1199, 952, 785. HR-ESI MS (*m/z*) $[M+H]^+$ calcd. for $C_{38}H_{52}N_{13}O_3S$ 770.4031, found 770.4024.

Photophysical properties

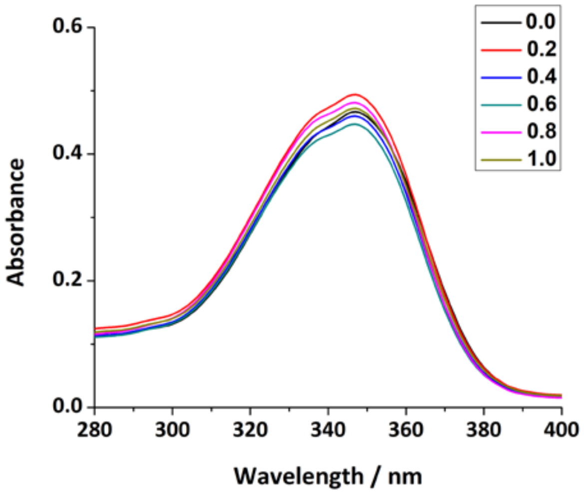


Fig. S1 The UV-Vis spectra of sensor **7** (50 µM) with different equivalents of Zn^{2+} in 0.01 mM HEPES buffer at pH 7.4.

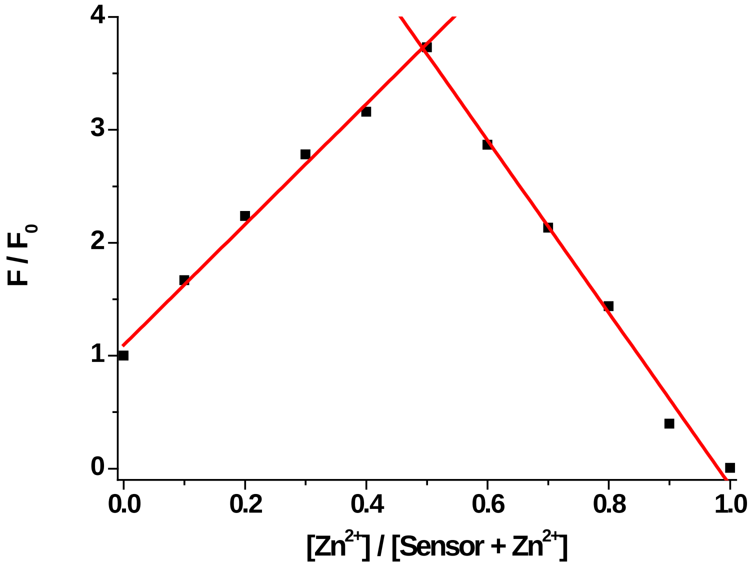


Fig. S2 The Job plot to determine the stoichiometry of the complex formed between sensor **7** and Zn^{2+}.

Fluorescence response to Zn^{2+}

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

Equation S1 Equation used to determine K_d for sensor **7**. *CM* and *CL* are concentrations of Zn^{2+} and sensor respectively, *F* is the observed fluorescence intensity, *F(0)* is the observed fluorescence of the sensor alone (i.e. *CM* = 0). Non-linear curve fitting analysis was used to determine *F(max)*/*F(0)* and K_d.

Quantum yield

$$\Phi_X = \Phi_{ST}\left(\frac{Grad_X}{Grad_{ST}}\right)\left(\frac{\eta_X}{\eta_{ST}}\right)^2$$

Equation S2 Equation used to calculate fluorescence quantum yield.[7] Where the subscripts *ST* and *X* denote standard and test respectively, Φ is the fluorescence quantum yield, *Grad* the gradient from the plot of integrated fluorescence intensity *vs* absorbance, and η the refractive index of the solvent.

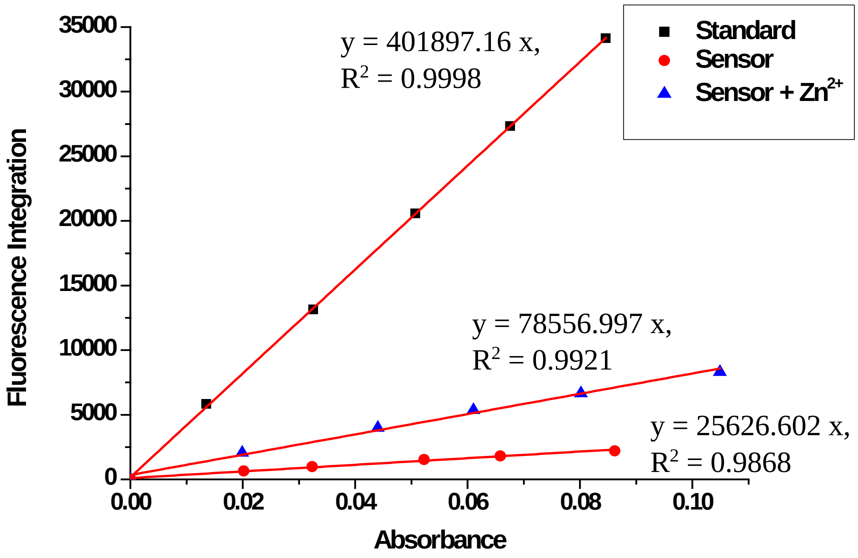


Fig. S3 Linear plots for standard sample, **7**, and **7** + Zn^{2+} to obtain quantum yields.

pH profile to establish pK_a values

$$\frac{F - F_0}{F_{max} - F_0} = \frac{\Delta F_{1max}}{(1 + 10^{(pH - pK_{a1})})} + \frac{\Delta F_{2max}}{(1 + 10^{(pH - pK_{a2})})} + \frac{\Delta F_{3max}}{(1 + 10^{(pH - pK_{a3})})} + \frac{\Delta F_{4max}}{(1 + 10^{(pH - pK_{a4})})}$$

Equation S3 The equation used to calculate pK_a values. F_{max} is the maximum normalized emission integration (F_{max} = 1), F_0 is the minimum normalized emission integration obtained for pH = 12.84, and

ΔF_{1max}, ΔF_{2max}, ΔF_{3max} and ΔF_{4max} are the maximum fluorescence integration changes associated with the corresponding pK_a values.

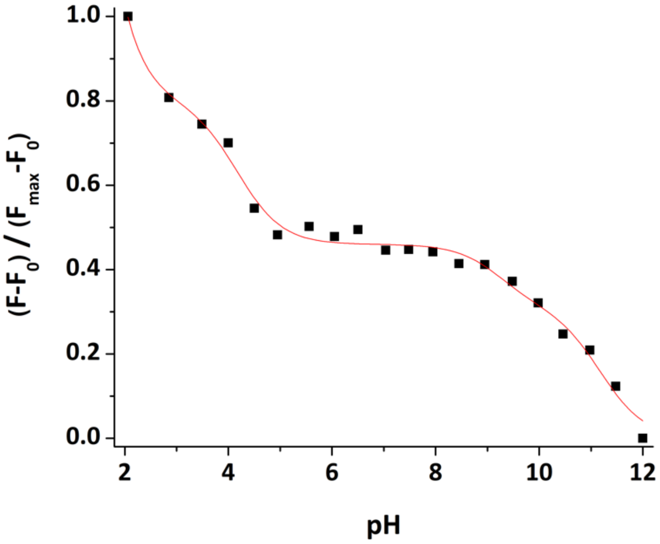


Fig. S4 Normalized integrated fluorescence emission *vs* pH, the nonlinear curve fitting used to determine the apparent pK_a values of **7**.

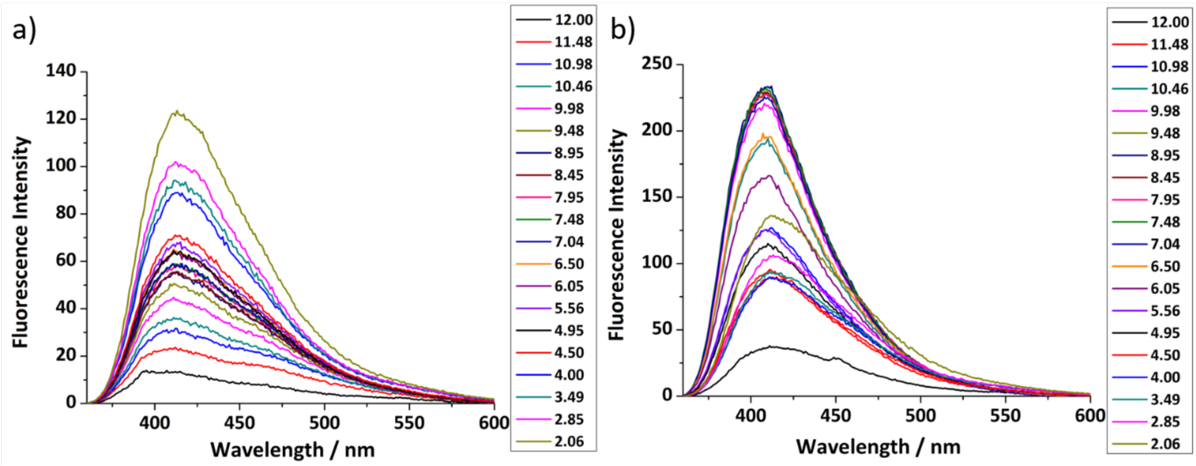


Fig. S5 Fluorescence emission spectra of sensor (a) **7** (50 μM) and (b) **7** + Zn^{2+} (50 μM) at different pH in 0.01 mM HEPES buffer.

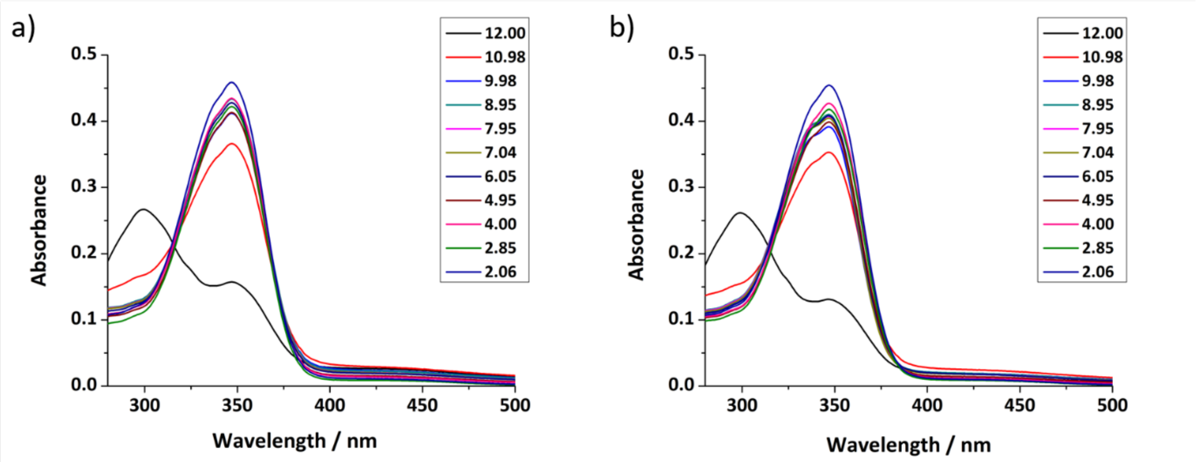


Fig. S6 The UV-Vis spectra of sensor (a) **7** (50 μM) and (b) **7** + Zn^{2+} (50 μM) at different pH in 0.01 mM HEPES buffer.

Biological applications

Toxicity test of sensor **7** to cells

Table S1 The fluorescence reading of alamerBlue indicating that cells are alive

	MCF-7						NTERT					
Sensor concentration (μM)	0	10	30	50	70	100	0	10	30	50	70	100
Fluorescence reading	5255	5037	5050	5335	5454	5239	7631	7606	7517	7053	6874	8911
	5207	4920	4990	4922	5519	5133	7753	7324	7233	7349	9130	8404
	5197	4809	4422	5151	5268	5453	8800	6909	7214	7347	7110	8226
	5317	5207	4620	4902	5177	5340	7924	7515	7195	7189	7928	7999
	5558	5490	4861	5262	5001	5493	7017	7583	7896	7567	8536	8227
average	5260	5055	4824	5112	5230	5344	7769	7474	7321	7295	7858	8286
percent	100%	96%	92%	97%	99%	102%	100%	96%	94%	94%	101%	107%

Confocal microscopy images of N-TERT cells

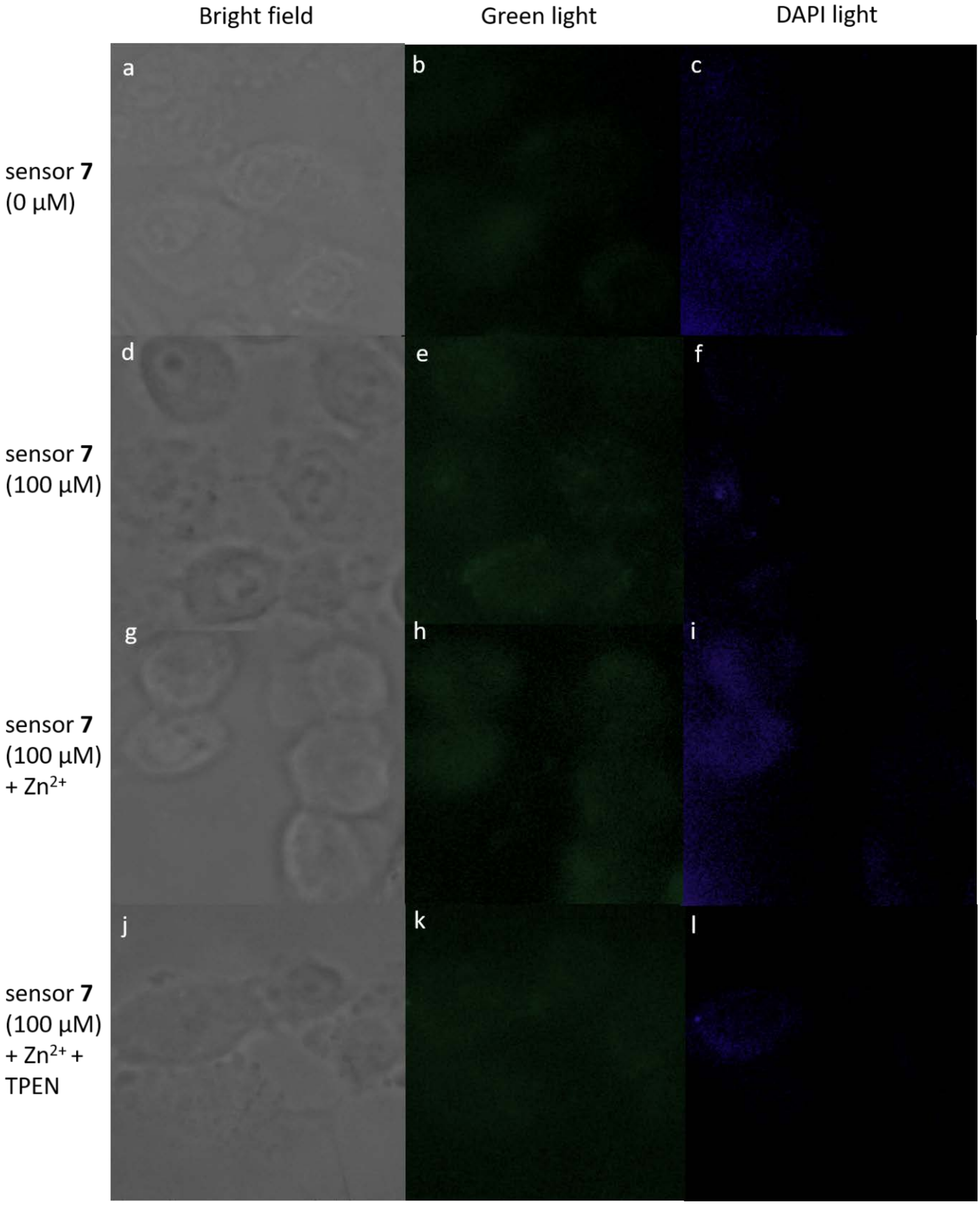


Fig. S7 Confocal microscopy images of N-TERT keratinocytes cells treated with no sensor (a-c), 100 µM sensor **7** solution (d-f), sensor **7** (100 µM) with saturated zinc pyrithione (g-i), and sensor **7** (100 µM) after loading the cells with zinc pyrithione, then TPEN (2 µM) was added (j-l).

Theoretical calculations

To understand the binding behaviour of sensor **7** with Zn^{2+} and its fundamental electronic structure, DFT and TDDFT studies were performed. The structure of sensor **7** and its complex with Zn^{2+} were first optimized using B3LYP function and 6-31G* basic set in Gaussian 09, followed by calculation of excited states at CAMB3LYP/6-31G* level of theory. The structures of the S_1 minima were also obtained at CAMB3LYP/6-31G* level of theory. All calculations considered H_2O as solvent using a continuum model (PCM). The vertical excitations and emission energies are shown in Table S2 and Fig S6.

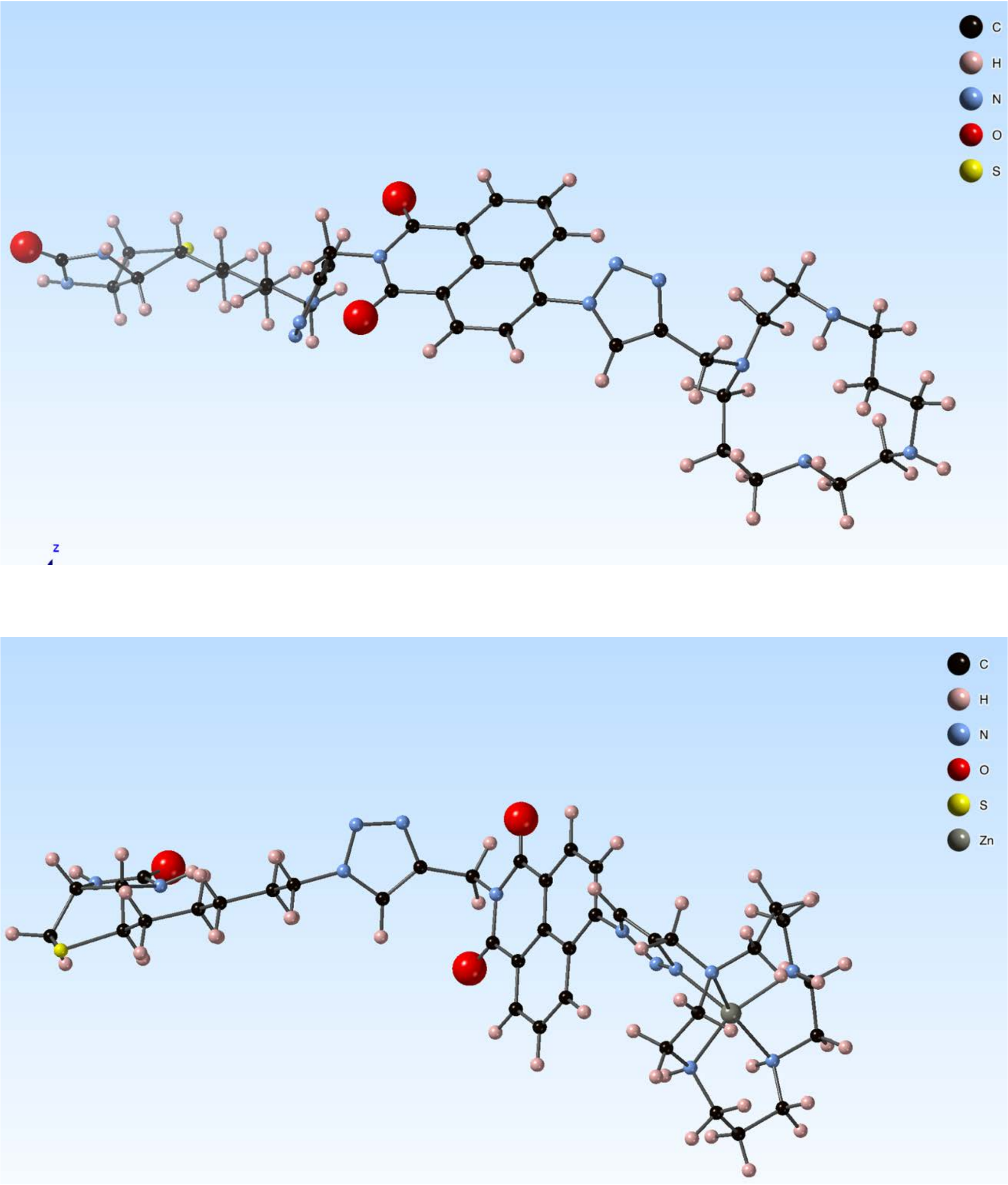


Fig. S8 The optimized structures of sensor **7** and its complex with Zn^{2+}

Cartesian coordinates of sensor **7**:

0 1

C	0.655314	-1.80133	2.666483
C	1.998198	-1.490319	2.953816
C	2.983085	-1.680282	2.005632
C	2.663554	-2.194827	0.720516
C	1.295216	-2.489168	0.423583
C	0.304726	-2.290397	1.420145
C	3.62148	-2.417063	-0.316404
C	3.23795	-2.874669	-1.563974
C	1.886237	-3.140518	-1.838733
C	0.924631	-2.96154	-0.860979
N	5.002968	-2.154472	-0.093314
C	-0.490329	-3.272439	-1.174088
N	-1.421255	-3.079544	-0.143872
C	-1.115313	-2.59559	1.134016
O	-0.845328	-3.685853	-2.272571
O	-2.007805	-2.441345	1.963503
C	-2.839975	-3.402101	-0.418394
C	-3.670657	-2.205082	-0.773472
C	-4.25537	-1.256721	0.044245
N	-4.884694	-0.413219	-0.805452
N	-4.71462	-0.810175	-2.084996
N	-3.976252	-1.892501	-2.067001
C	-5.730598	0.741486	-0.49661
C	-7.189252	0.34636	-0.239627
C	-8.063933	1.563157	0.087406
C	-9.529806	1.185287	0.338953
C	-10.403755	2.397701	0.694163
C	-11.867908	2.024387	0.955821
C	-12.657839	1.570391	-0.303166
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C	5.867628	-1.47951	-0.899665
C	8.44102	-1.02907	-0.676904
N	8.794058	0.292606	-0.135182
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C	8.840967	3.197703	-2.302837
N	9.964329	3.448898	-1.409343

C	8.882136	0.355711	1.328266
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C	10.519407	4.794612	-1.400327
C	11.545648	4.935984	-0.267194
N	12.675365	3.991716	-0.38276
C	12.066608	0.77064	1.699098
C	12.836567	3.041585	0.733096
C	11.953395	1.800505	0.56548
H	-0.119421	-1.655796	3.411335
H	2.259414	-1.094783	3.930039
H	4.01309	-1.444067	2.241078
H	3.988583	-3.049016	-2.326905
H	1.588264	-3.50405	-2.815578
H	-2.843665	-4.10197	-1.251837
H	-3.232888	-3.892017	0.472538
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H	-5.298282	1.241546	0.374551
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H	-7.225951	-0.372002	0.589526
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H	6.899642	1.15168	-0.6436

Cartesian coordinates of the complex of **7** with Zn^{2+}:

2 1

C	0.454277	1.483893	1.039157
C	1.737004	1.95698	0.698754
C	2.691376	1.100556	0.18962
C	2.39687	-0.27526	-0.00573
C	1.087395	-0.74946	0.319383
C	0.130525	0.151919	0.852377
C	3.317496	-1.23108	-0.52666
C	2.964419	-2.54703	-0.7458
C	1.664925	-2.98585	-0.43436
C	0.742061	-2.10736	0.101384
N	4.659881	-0.83678	-0.84108
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C	-12.5673	1.636208	-0.48318
C	-13.348	0.329154	-0.1885
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C	-15.1308	2.130196	-0.29559
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C	9.380477	0.19551	3.077424
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H	3.696133	-3.24713	-1.13383
H	1.38433	-4.02042	-0.59426
H	-2.74534	-3.23785	1.59084
H	-3.07123	-1.67226	2.371934
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H	-8.15678	-1.26179	-0.49769
H	-7.58658	0.203156	0.303527
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H	10.54662	2.376666	2.161684
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H	9.482014	4.651513	1.9652
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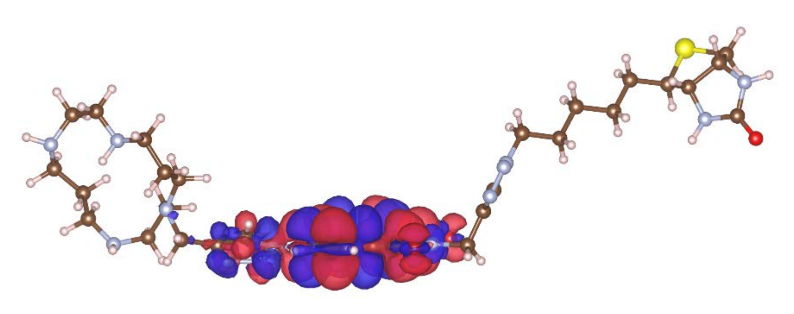
H	9.378918	-2.8303	-1.9094
H	10.93196	-2.60912	-1.14379
H	7.30323	2.926142	-0.28007
H	7.608793	0.199541	-3.50714
Zn	8.393813	0.671664	0.294078

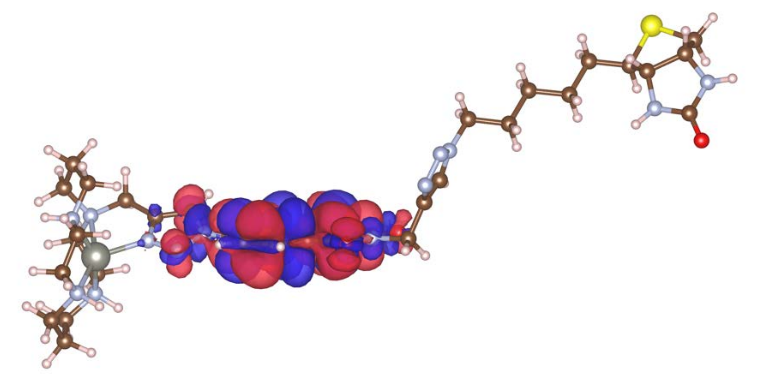
Table S2. Comparison of the experimental data with the calculated result of vertical electronic excitation energies (eV) and the emission from S_1 to ground state of **7** and its Zn^{2+} complex.

	Electronic transition	Energy (eV)	Wavelength (nm)	Oscillator strengths (f)	Experimental data
Sensor **7**					
Absorption	$S_0 \rightarrow S_1$	3.88	320	0.5493	3.58 eV/346 nm
	$S_0 \rightarrow S_2$	4.27	290	0.0438	
	$S_0 \rightarrow S_3$	4.37	284	0.0001	
	$S_0 \rightarrow S_4$	4.39	282	0.0002	
	$S_0 \rightarrow S_5$	4.67	265	0.0004	
	$S_0 \rightarrow S_6$	4.72	262	0.0011	
Emission	$S_1 \rightarrow S_0$	3.18	390	0.7172	3.01 eV/412 nm
Complex of **7** with Zn^{2+}					
Absorption	$S_0 \rightarrow S_1$	3.95	314	0.5023	3.58 eV/346 nm
	$S_0 \rightarrow S_2$	4.26	291	0.0521	
	$S_0 \rightarrow S_3$	4.34	286	0.0003	
	$S_0 \rightarrow S_4$	4.62	268	0.0002	
	$S_0 \rightarrow S_5$	4.94	251	0.0319	
	$S_0 \rightarrow S_6$	5.29	234	0.0055	
Emission	$S_1 \rightarrow S_0$	3.22	385	0.6672	3.01 eV/412 nm

The calculated vertical excitations are in good agreement with the experimental values. The deviations from the experimental values are due to the lack of explicit consideration of vibrational effects and the solvent molecules in our calculations. In line with the experimental measurements, the predicted emission energy for the Zn^{2+} complex is very similar to the obtained for sensor **7** (See explanation below).



Sensor **7**

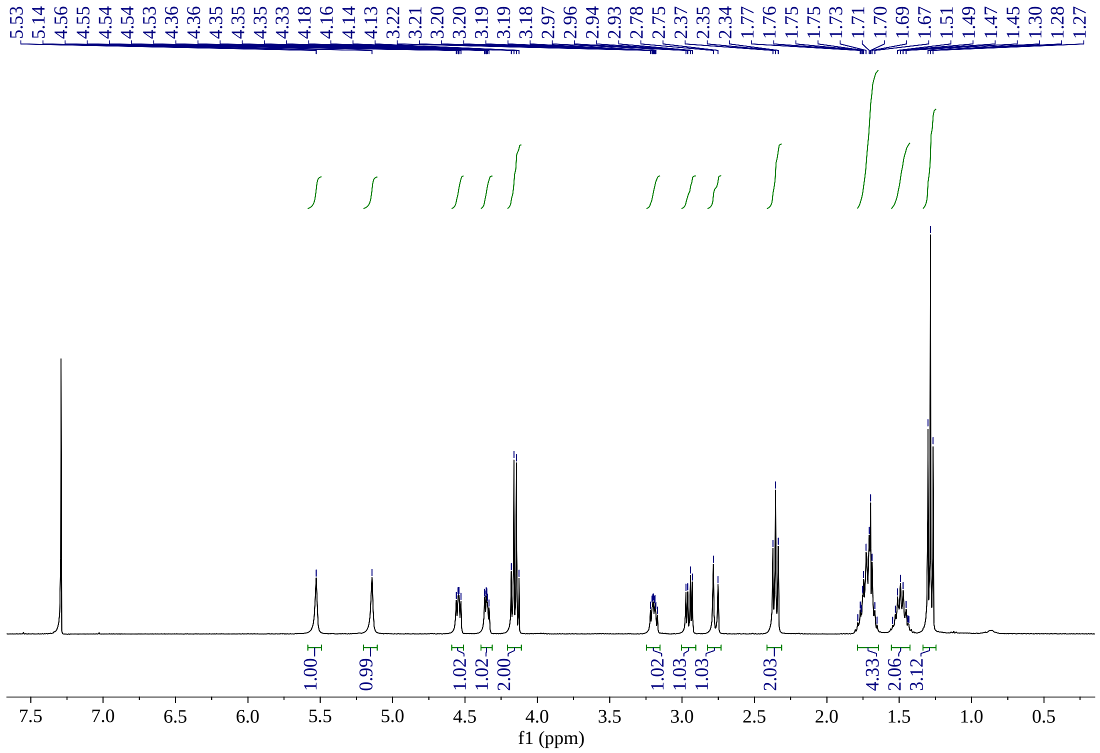


Complex with Zn^{2+}

Fig. S9. S_1-S_0 electron density map for sensor **7** and its complex with Zn^{2+} (S_1 minima). Red represents positive densities and blue negative values.

The S_1-S_0 electron density plots (Fig. S9) show that the electron transition is localised on naphthalimide moiety and the vicinal triazole for both sensor **7** and its complex with Zn^{2+}. These calculations show that cyclam does not contribute to the process. The formation of the complex has a minor impact on the electronic densities. There is a slight decrease of the electron density on the nitrogen from the triazole involved in the complex formation and the oscillator strength is not significantly affected. Consequently, this does not justify the enhancement of emission observed experimentally. Based on these results, the mechanism of enhancement of fluorescence seems to be consistent with the restriction of the intramolecular rotations due to complexation with Zn^{2+}. This mechanism is normally used to explain aggregation induced emission[8] but it can also operate in other systems where vibrational modes are restricted (in this case due to the formation of a stable complex). The stabilisation of the complex hampers large amplitude vibrations in the vicinity of the fluorophore reducing the possibility of relaxation through nonradiative pathways and increasing the quantum yield of emission. The still small quantum yield can be associated with other nonradiative mechanisms that are not affected by complexation with Zn^{2+} including intramolecular rotations and energy transfer involving the second triazole and biotin.

Compound **S1**:



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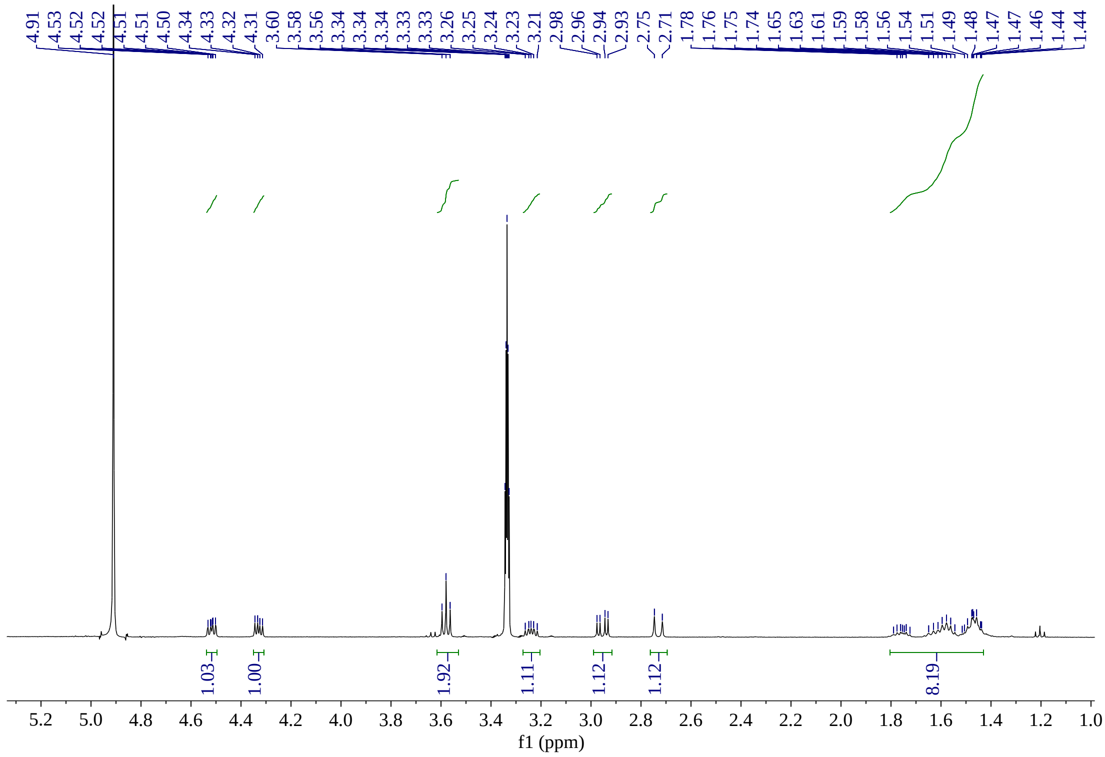

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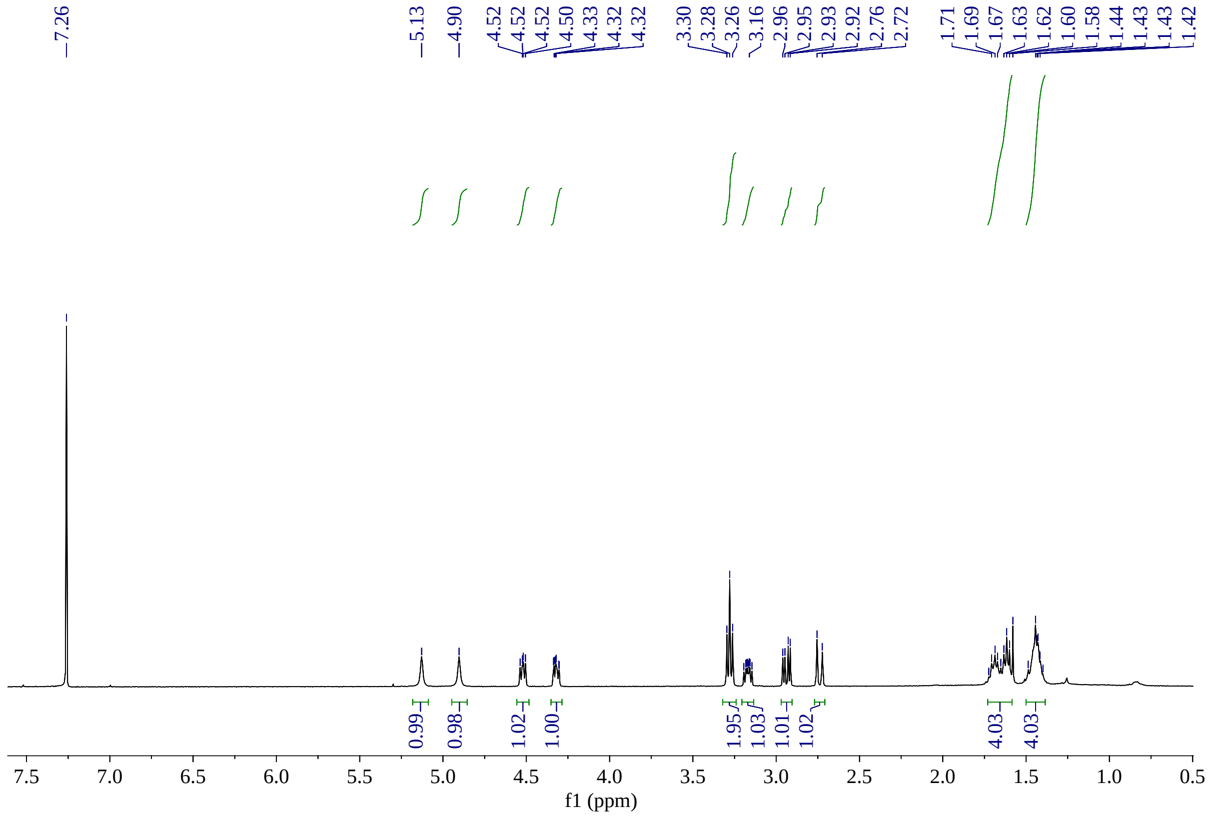
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3.16
2.96
2.95
2.93
2.92
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0.98
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1.95
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1.01
1.02
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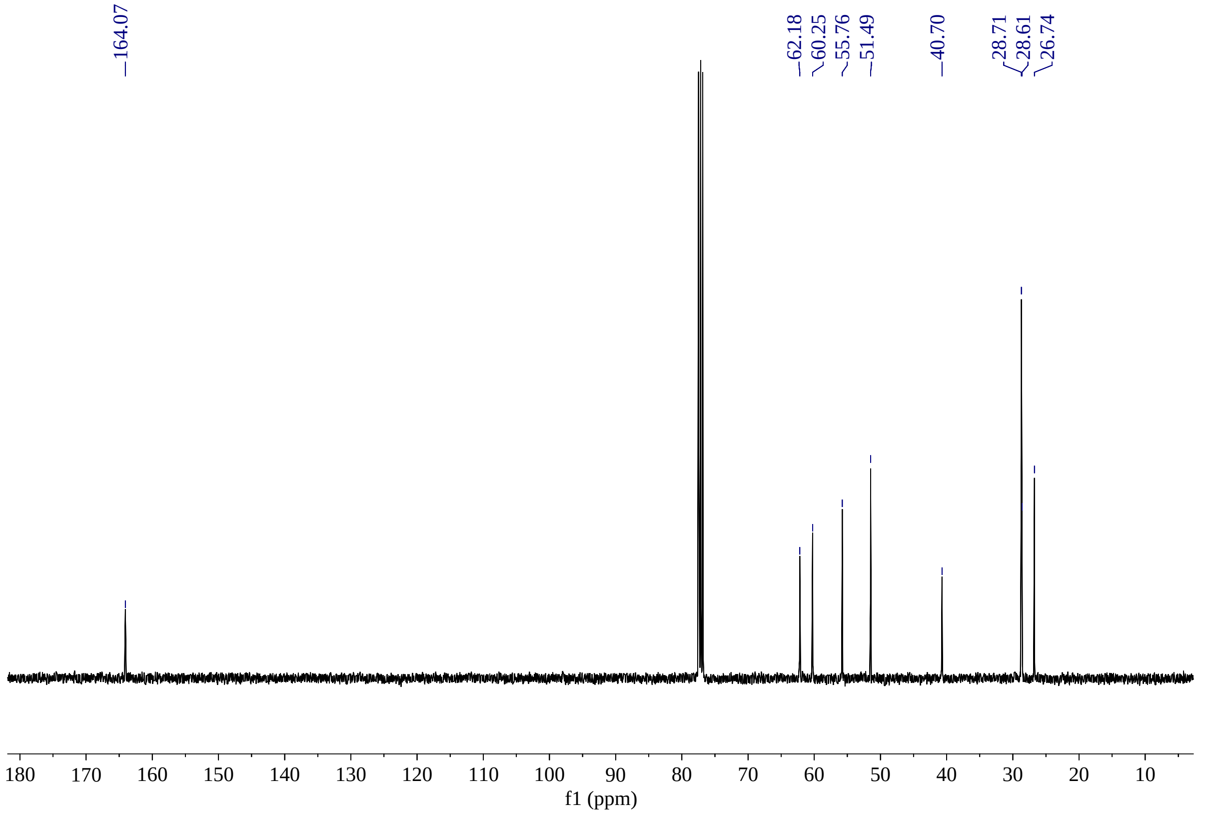
f1 (ppm)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f1 (ppm)

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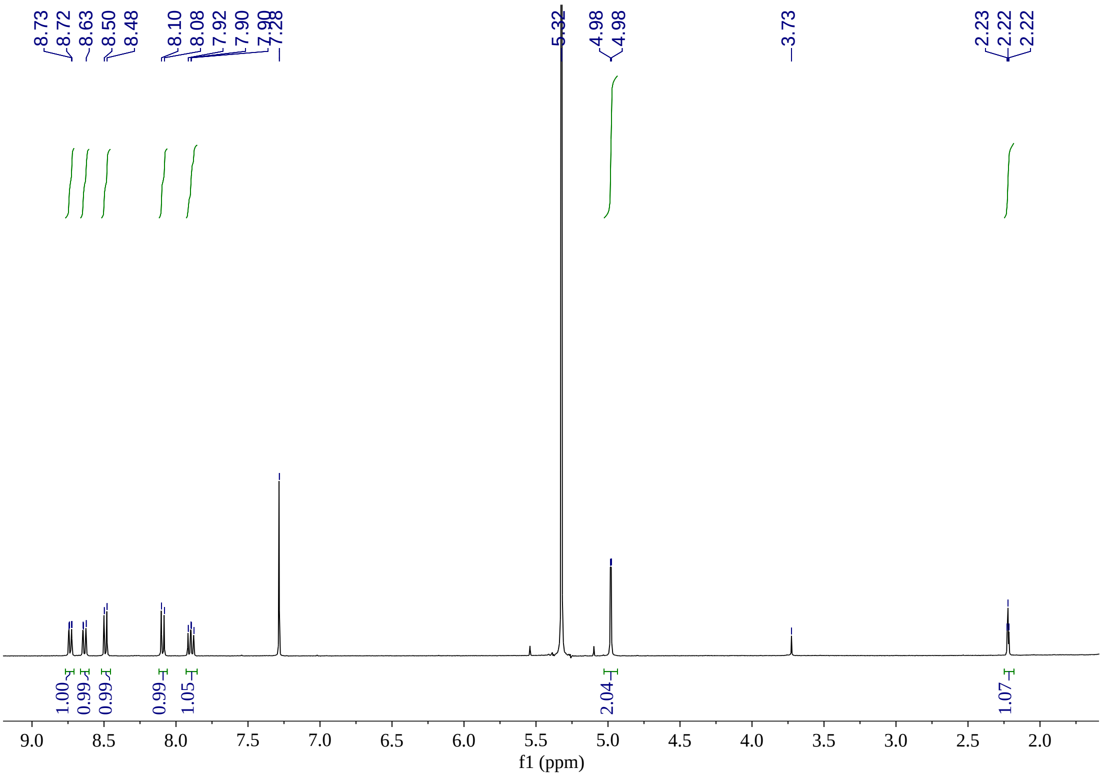

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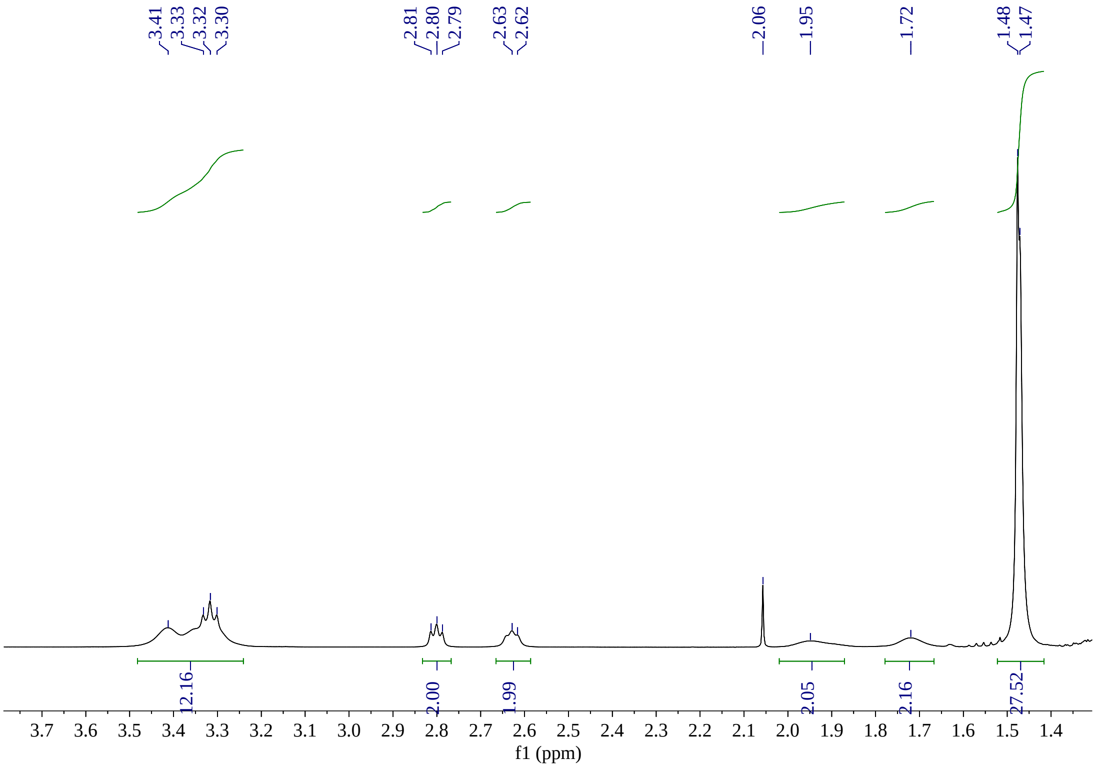

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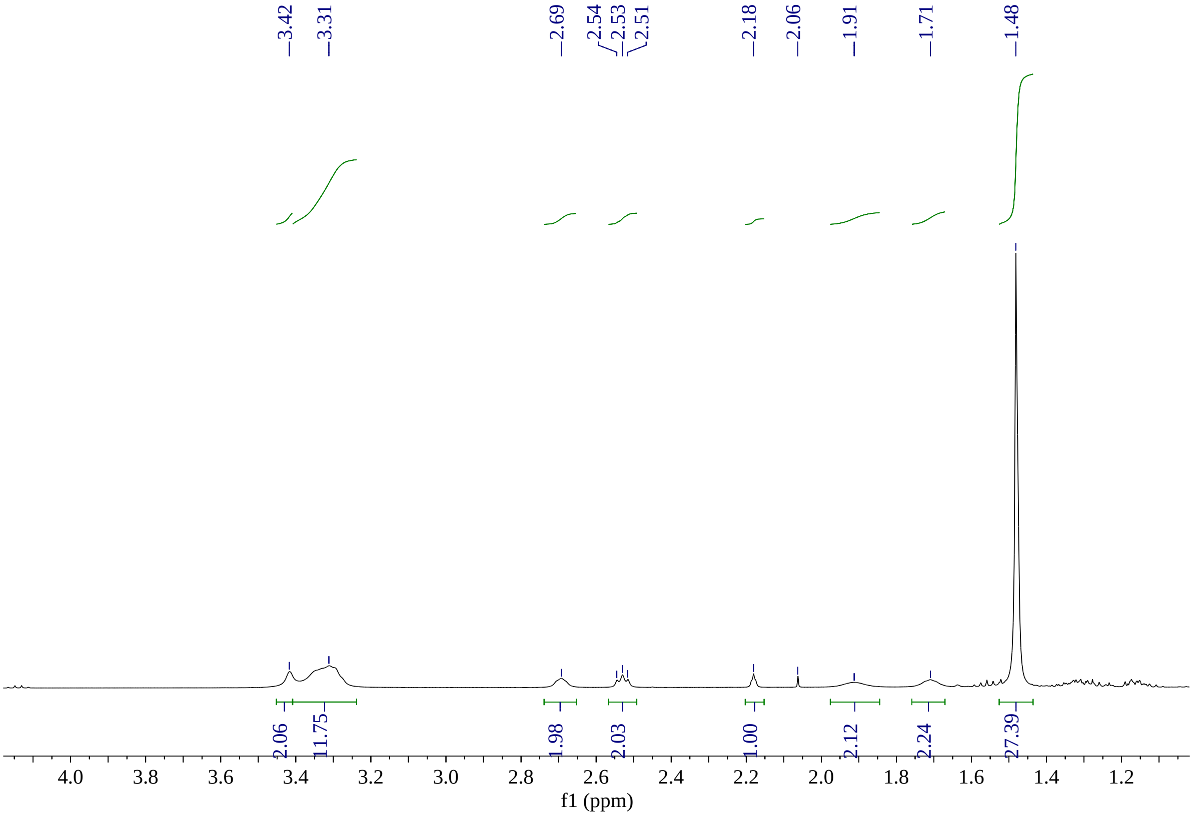

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1.00
2.12
2.24
27.39
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3.8
3.6
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f1 (ppm)

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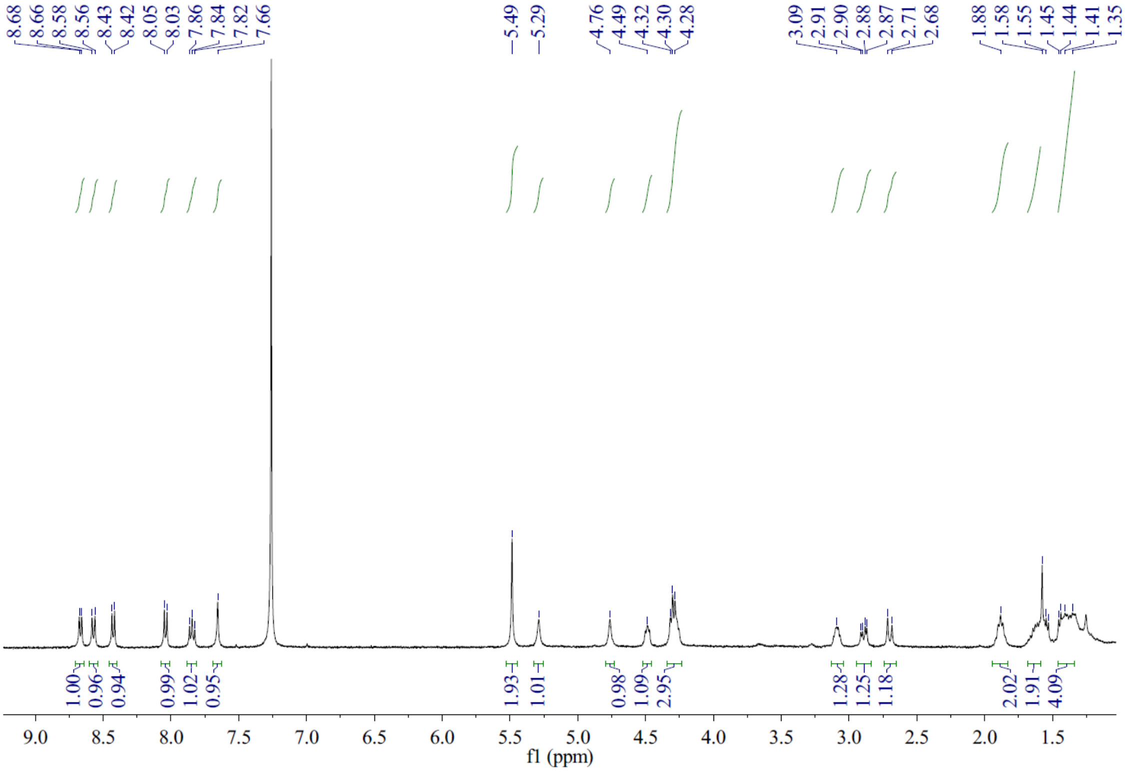
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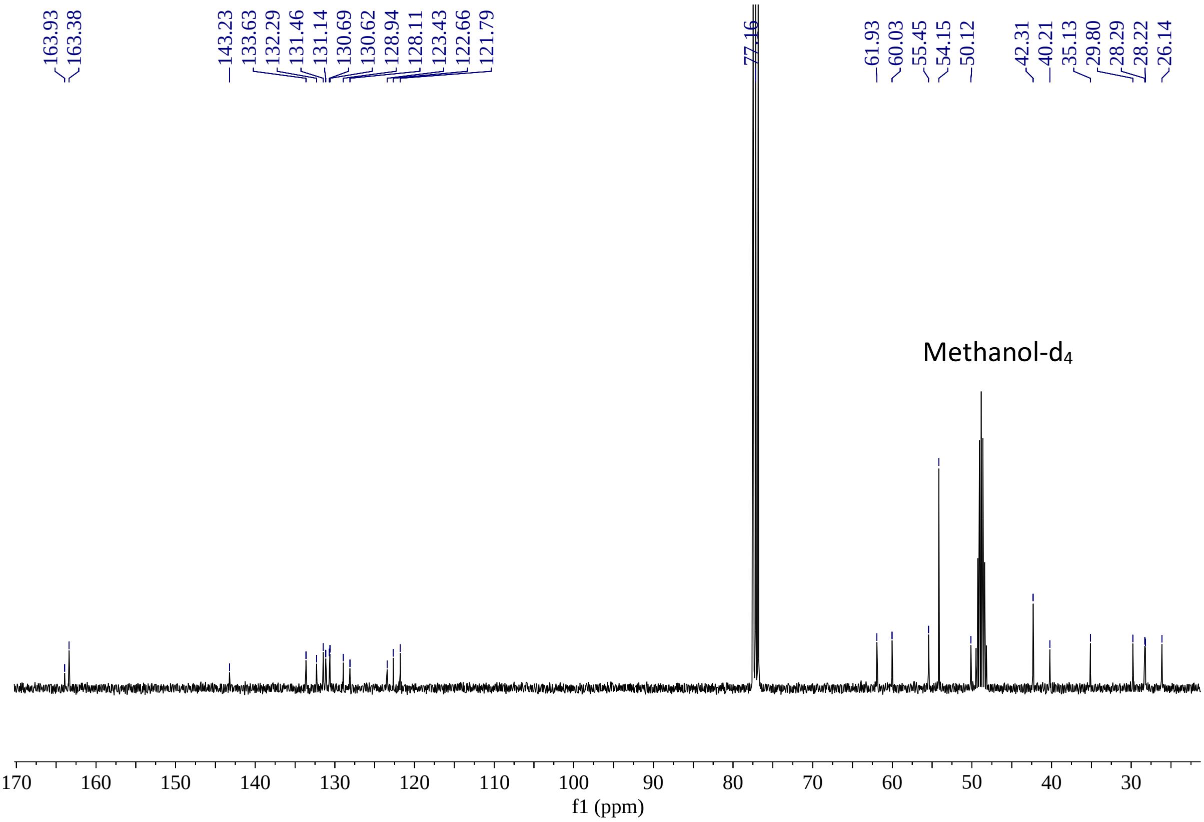
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28.22
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f1 (ppm)

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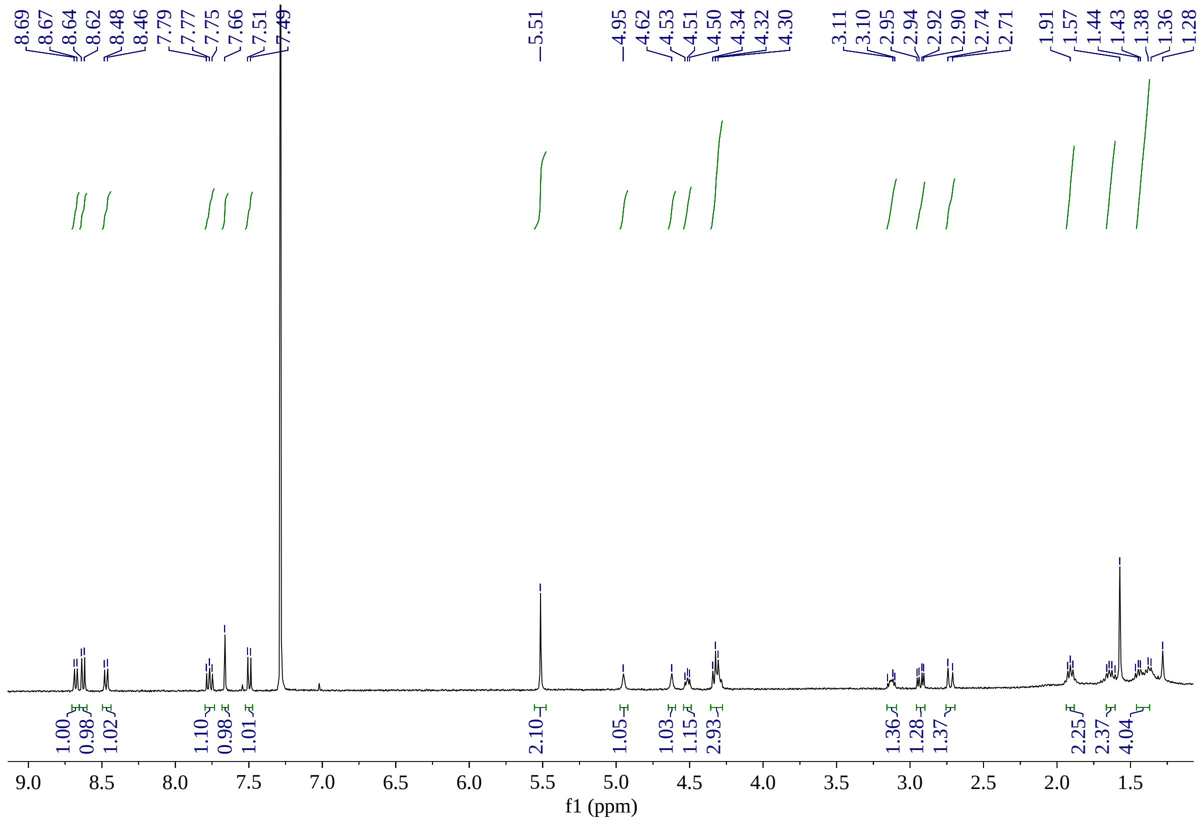
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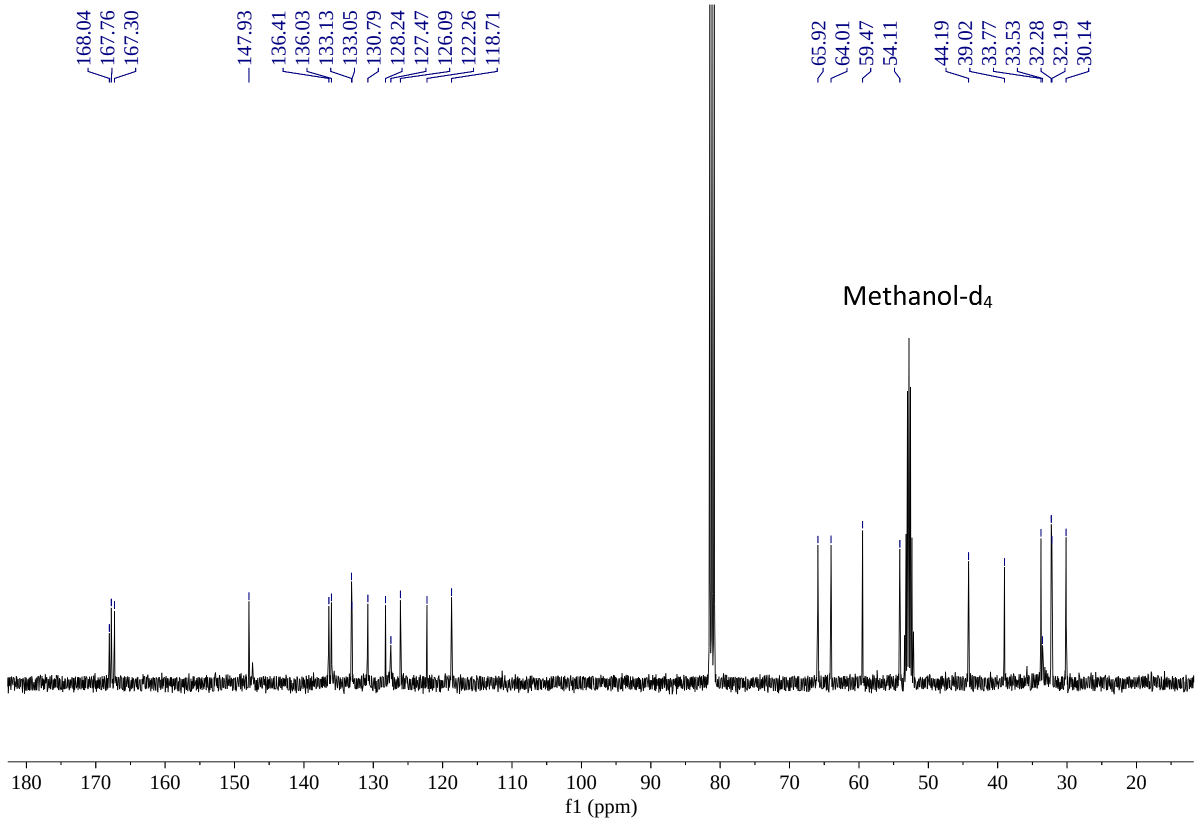
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Methanol-d₄
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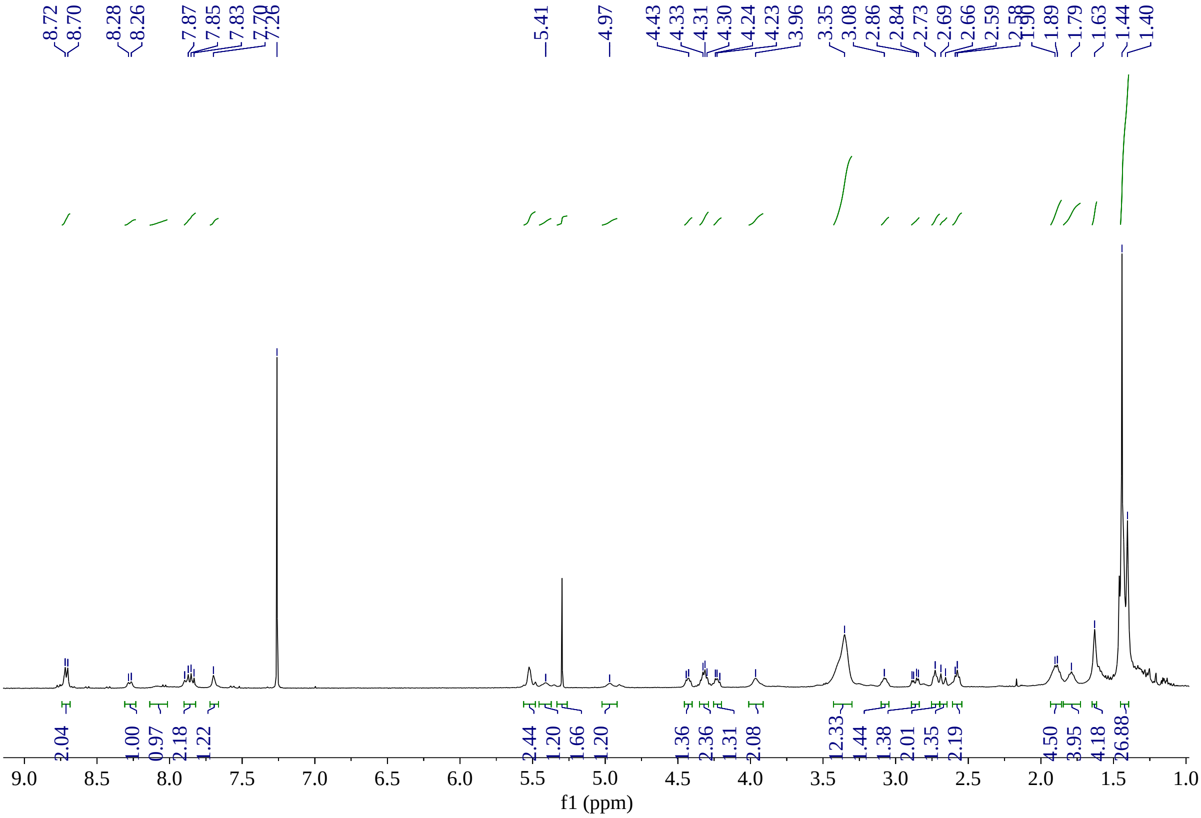
f1 (ppm)

Compound **6**: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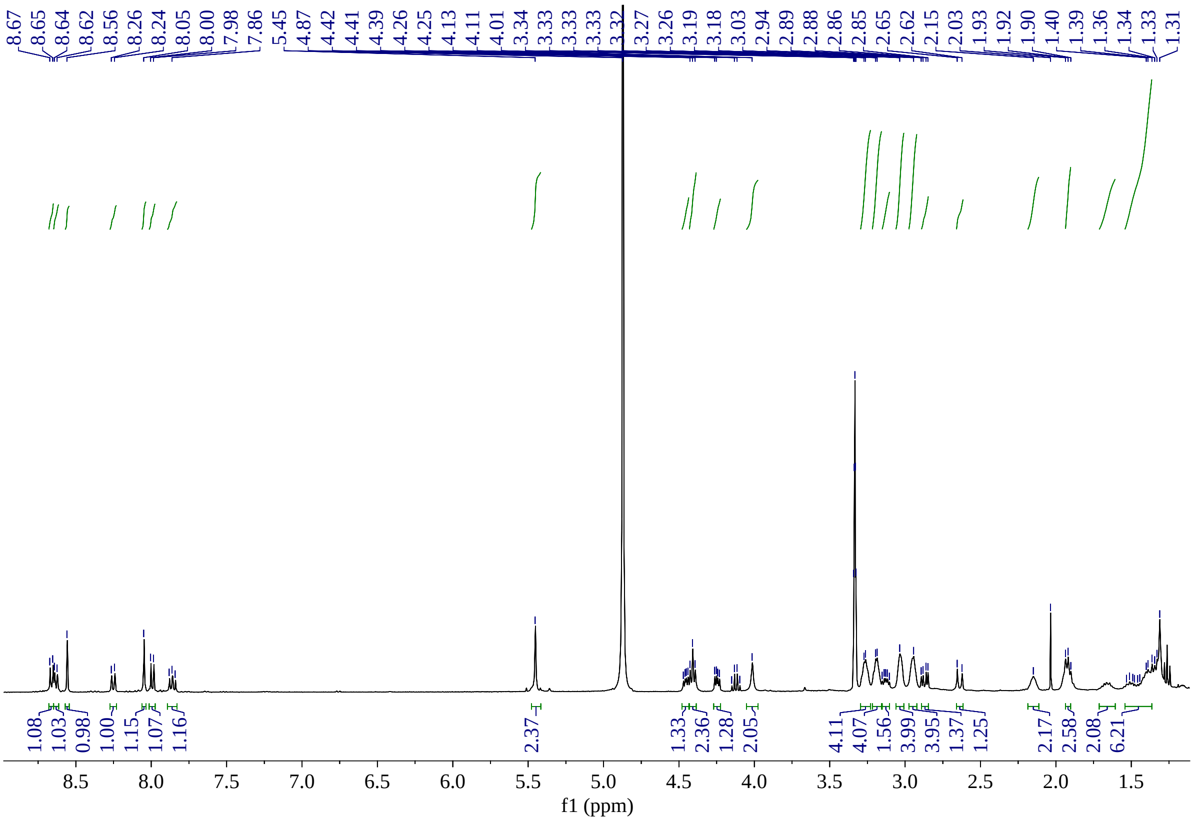
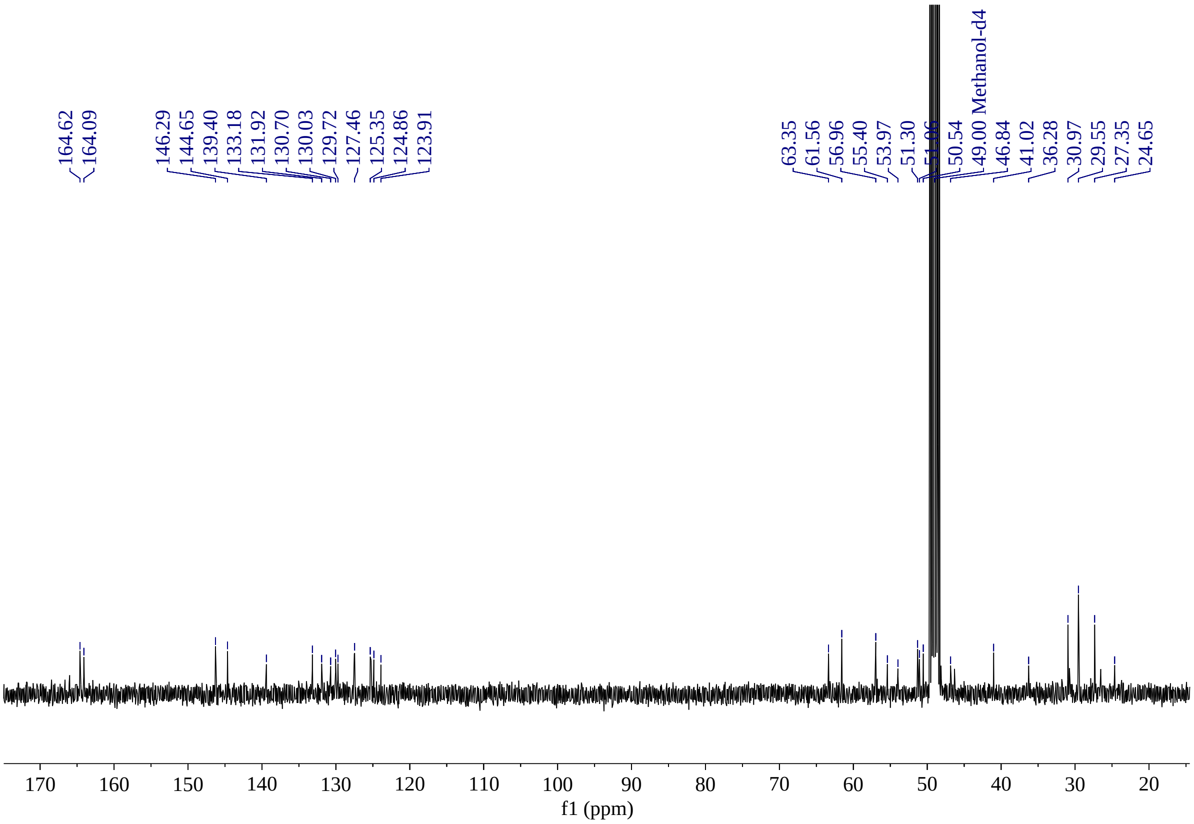
2.44
1.20
1.66
1.20
1.36
2.36
1.31
2.08
12.33
1.44
1.38
2.01
1.35
2.19
4.50
3.95
4.18
26.88
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

Compound **7**:


8.67 8.65 8.64 8.62 8.56 8.26 8.24 8.05 8.00 7.98 7.86 5.45 4.87 4.42 4.41 4.39 4.26 4.25 4.13 4.11 4.01 3.34 3.33 3.33 3.33 3.32 3.27 3.26 3.19 3.18 3.03 2.94 2.89 2.88 2.86 2.85 2.65 2.62 2.15 2.03 1.93 1.92 1.90 1.40 1.39 1.36 1.34 1.33 1.31
1.08 1.03 0.98 1.00 1.15 1.07 1.16 2.37 1.33 2.36 1.28 2.05 4.11 4.07 1.56 3.99 3.95 1.37 1.25 2.17 2.58 2.08 6.21
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
f1 (ppm)


164.62 164.09 146.29 144.65 139.40 133.18 131.92 130.70 130.03 129.72 127.46 125.35 124.86 123.91
63.35 61.56 56.96 55.40 53.97 51.30 51.06 50.54 49.00 Methanol-d4 46.84 41.02 36.28 30.97 29.55 27.35 24.65
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
f1 (ppm)

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