Supplementary Information for

A luminogenic lanthanide-based probe for the highly selective detection of nanomolar sulfide levels in aqueous samples

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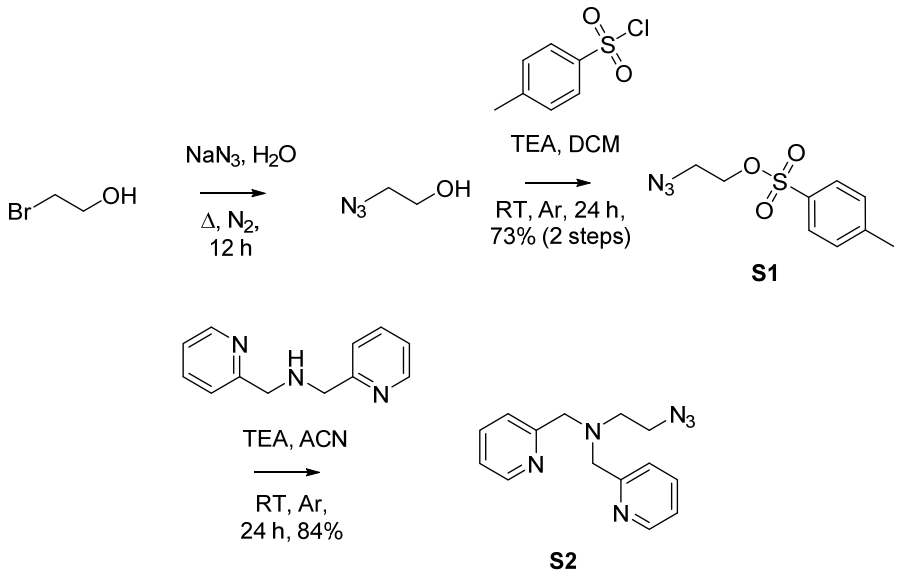
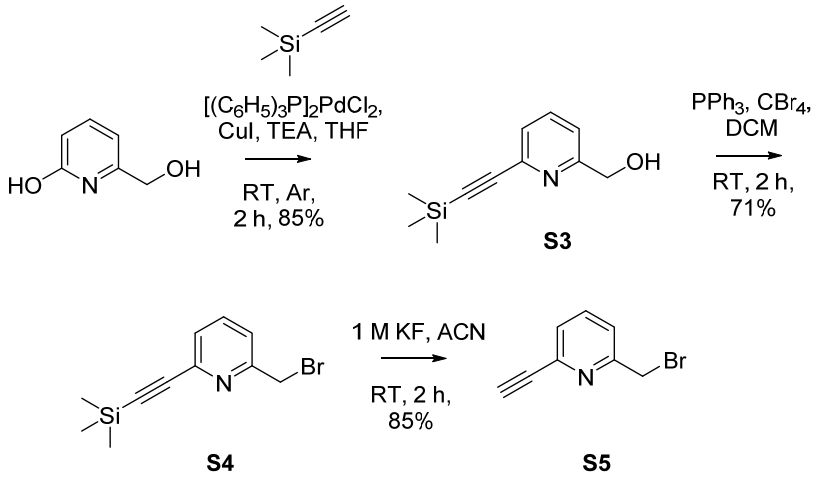


Scheme S1. Synthesis of 2-azido-*N*,*N*-*bis*((pyridin-2-yl)methyl)ethanamine (**S2**).



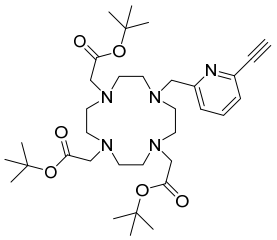
Scheme S2. Synthesis of 2-(bromomethyl)-6-ethynylpyridine (**S5**).

Experimental section

General considerations

Preparative HPLC was performed on an Agilent 1260 Infinity Prep LC controller with an Agilent 1260 Infinity Absorbance detector using a Phenomenex Luna C8 column (21.2 x 150 mm, 5 micron) with a flow rate of 10 mL min^{-1}. The elution method used for HPLC purification for ligand and complex was; 100% buffer A for 4 min, then gradient from 100% solvent A to 97% solvent A/3% solvent B over 40 min (solvent A = 0.1% formic acid in MilliQ water, solvent B = 0.1% formic acid in 80% ACN/20% MilliQ water). Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker DRX400 spectrometer operating at 400 MHz, as solutions in deuterated solvents as specified. Each resonance was assigned according to the following convention: chemical shift; multiplicity; observed coupling constants (*J* in Hz) and number of protons. Chemical shifts (δ), measured in parts per million (ppm), are reported relative to the residual proton peak in the solvent used as specified. Carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra were recorded on a Bruker DRX400 spectrometer operating at 100 MHz, as solutions in deuterated solvents as specified. Chemical shifts, measured in ppm, are reported relative to the residual proton peak in the solvent used as specified. Assignments were determined from J-Modulated Spin Echo experiments showing quaternary and methylene signals in the opposite phase to those of methine and methyl resonances. Correlation spectroscopy (COSY) was used to correlate chemical shifts of protons coupled to one another. Heteronuclear Multiple Quantum Correlation (HMQC) spectroscopy was used to correlate directly bonded ^{13}C-^{1}H nuclei. High resolution mass spectrometry (HRMS) was conducted using a Bruker BioApex 47e FTMS fitted with an analytical electrospray source using NaI for accurate mass calibration (ESI). Low resolution mass spectrometry (LRMS) was conducted using a Micromass Platform II QMS (ESI). Infrared spectra (IR) were recorded on an Agilent Technologies Cary 630 FTIR as thin films of compressed powders. UV-Visible absorption spectrum was recorded at room temperature using a Varian Cary 1E UV-Visible spectrophotometer. A cell with a path length of 10 mm was used. Luminescence emission spectra were recorded at room temperature using a Varian Cary-Eclipse fluorescence spectrophotometer set to phosphorescence mode. A quartz cell with a path length of 10 mm and a volume of 500 µL was used. The instrument excitation and emission slit widths were both set at 5 nm. The delay time used was 0.1 msec and the gate time was 1 msec. The concentration of sulfide in the sour water samples was measured in black, 96-well plates using a SpectraMax M5 instrument using an excitation wavelength of 260 nm and monitoring emission at 545 nm. After a 500 µs delay, the emission was integrated over 1000 µs. All starting materials and solvents were of reagent or analytical grade and used as purchased.

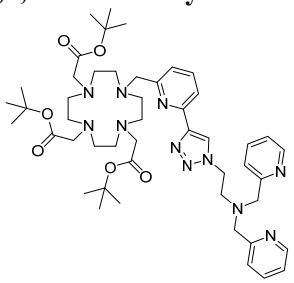
Tri-*tert*-butyl 2,2',2''-(10-((6-ethynylpyridin-2-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (2).



Compound **S5** (150 mg, 0.733 mmol), tri-*tert*-butyl 2,2',2''-(1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (397 mg, 0.667 mmol) and K_2CO_3 (289 mg, 2.10 mmol) were dissolved in CH_3CN (ACN, 6 mL) and refluxed with stirring overnight. The solution was evaporated under reduced pressure and purified via silica gel chromatography (5% MeOH/1% Et_3N in

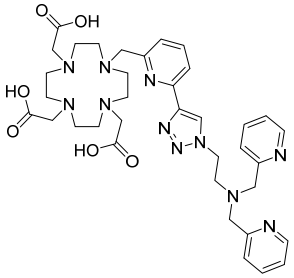
CH_2Cl_2 (DCM), R_f = 0.6) to yield the title compound as an orange oil (272 mg, 65%): ^{1}H NMR ($CDCl_3$) δ: 1.34 (s, 27H), 2.00 – 3.92 (broad m, 25H), 7.28 (m 2H), 7.63 (m, 1H); ^{13}C NMR ($CDCl_3$) δ: 27.9, 28.0, 28.1, 46.5, 49.2, 50.5, 50.7, 51.3, 52.1, 56.0, 56.3, 58.0, 59.7, 77.1, 82.1, 123.8, 126.3, 137.2, 142.4, 158.6, 170.5, 172.1, 172.5; IR (ATR): 2975, 2933, 2828, 1719, 1446, 1367, 1153 cm^{-1}; LRMS (ESI+): *m/z* $[M+H]^+$ 630.4 (100%); HRMS (ESI+): calcd. for $[M+H]^+$: 630.4225, found: 630.4229.

Tri-*tert*-butyl 2,2',2"-(10-((6-(1-(2-(*bis*(pyridin-2-ylmethyl)amino)ethyl)-1H-1,2,3-triazol-4-yl)pyridin-2-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (3).



Compound **2** (0.15 g, 0.23 mmol) and 2-azido-*N*,*N*-*bis*((pyridin-2-yl)methyl)ethanamine (see supplementary information for synthesis) (0.18 mg, 0.67 mmol) was dissolved in a degassed (freeze-pump-thaw technique) 1:1 (v/v) ^{t}BuOH/H_2O mixture (12 mL) under a N_2 atmosphere. To this, a premixed solution of $CuSO_4$ (10 mg, 0.065 mmol) and *tris*(3-hydroxypropyltriazolylmethyl)amine (THTPA, 38 mg, 0.081 mmol) in H_2O (0.5 mL) was then added, followed by sodium ascorbate (0.2 g, 0.7 mmol). The mixture was kept under an Ar atmosphere and heated to 40 ˚C for 24 h.[†] Following removal of the solvent under reduced pressure, the crude product was purified via basic alumina chromatography (5% MeOH in DCM, R_f = 0.2) to yield the title compound as an orange oil (151 mg, 74%); ^{1}H NMR ($CDCl_3$) δ: 1.30 – 1.47 (br m, 27H), 2.23 – 3.39 (br m, 26H), 3.89 (s, 4H), 4.54 (t, J = 6.4 Hz, 2H), 7.10 (m, J = 6.14 Hz, 2H), 7.24 (d, J = 7.2 Hz, 1H), 7.31 (d, J = 7.7 Hz, 2H), 7.54 (td, J_1 = 1.6 Hz, J_2 = 7.7 Hz, 2H), 7.78 (m, 2H), 8.49 (d, J_1 = 4.2 Hz, 2H), 8.76 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 28.0, 28.3, 47.6, 48.5, 49.3, 51.4, 54.0, 55.9, 58.2, 60.2, 81.9, 119.3, 121.8, 122.4, 122.6, 123.3, 123.4, 123.9, 127.9, 136.7, 138.1, 146.9, 149.2, 149.4, 158.0, 158.7, 170.6, 171.9; IR (ATR): 2976, 2932, 2829, 1724, 1671, 1367, 1151 cm^{-1}; LRMS (ESI+): *m/z* $[M+H]^+$ 898.5 (30%), $[M+H]^{2+}$ 449.9 (100%), HRMS (ESI+): calcd. for $[M+H]^+$: 898.5662, found: 898.5671.

2,2',2"-(10-((6-(1-(2-(*bis*(Pyridin-2-ylmethyl)amino)ethyl)-1H-1,2,3-triazol-4-yl)pyridin-2-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (1).

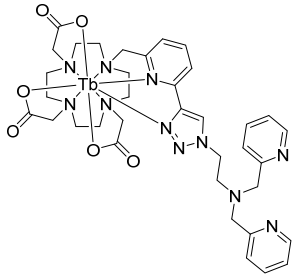


Compound **3** was dissolved in a 1:1 (v/v) DCM/CF_3COOH (TFA) mixture (3 mL). The reaction was allowed to stir for two days at room temperature (RT). The volatiles were then removed

[†] Poor reaction yields were obtained if the ^{t}BuOH/H_2O mixture was not degassed, using the freeze-pump-thaw technique, as well as performing the reaction under inert conditions.

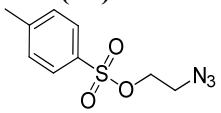
via a stream of N_2. The crude mixture was dissolved in H_2O and purified using preparative HPLC. Fractions were analyzed by analytical HPLC and LC-MS and only pure fractions were lyophilised to afford the title compound as a white solid (29 mg, 72%); mp: 109.4 – 110.9 ˚C; ^{1}H NMR (D_2O) δ: 2.99 – 3.80 (br m, 24H), 4.13 (s, 2H), 4.19 (s, 4H), 4.55 (t, *J* = 5.6 Hz, 2H), 7.5 (m, *J* = 6.4 Hz, 2H), 7.60 (d, *J* = 8.0 Hz, 2H), 7.85 (t, *J* = 6.4 Hz, 2H), 8.01 (m, *J* = 7.8 Hz, 1H), 8.09 (td, J_1 = 1.7 J_2 = 7.8 Hz, 2H), 8.12 (s, 1H), 8.49 (m, *J* = 5.2, 2H); ^{13}C NMR (D_2O) δ: 48.1, 48.4, 49.0, 50.4, 51.3, 54.2, 56.2, 57.6, 58.2, 120.6, 124.1, 124.5, 124.9, 125.2, 140.3, 142.4, 144.4, 146.6, 147.9,, 155.6; IR (ATR): 3405, 2843, 1686, 1637, 1437, 1380, 1197, 1121, 1086 cm^{-1}; LRMS (ESI+): $[M+H]^+$ 729.3 (100%), HRMS (ESI+): calcd. for $[M+Na]^+$: 752.3603, found: 752.3602.

Terbium (III), [10-((6-(1-(2-(*bis*(pyridin-2-ylmethyl)amino)ethyl)-1H-1,2,3-triazol-4-yl)pyridin-2-yl-κN)methyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triacetato(3$^-$)-κN^1,κN^4,κN^7,κN^{10},κO^1,κO^4,κO^7] (Tb-1).



A solution of ligand **1** (40 mg, 0.055 mmol) in H_2O (3 mL) was adjusted to pH 8 using 0.25 M NaOH and then $Tb(CF_3SO_3)_3$ (36 mg, 0.060 mmol) added. The reaction mixture was allowed to stir at 70 ˚C for 2 d, then purified directly using preparative HPLC. Fractions were analyzed by analytical HPLC and LC-MS and only pure fractions were lyophilised to afford the title complex as a white solid (31 mg, 61%); IR (ATR): 3305, 2861, 1686, 1602, 1368, 1081 cm^{-1}; LRMS (ESI+): *m/z* $[M+H]^+$ 886.3 (30%) $[M+Na]^+$ 908.2 (100%); HRMS (ESI+): calcd. for $[M+Na]^+$: 908.2622, found: 908.2616. Analytical HPLC: > 95% purity (254 nm). See Fig S1 for HPLC chromatogram.

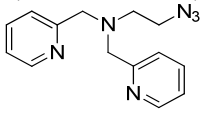
2-Azidoethyl-4-methylbenzenesulfonate (S1)



[***Warning***: small molecule organic azides are potentially-explosive and complete removal of solvent from such molecules should be avoided.] NaN_3 (0.7 g, 10 mmol) was added to a solution of bromoethanol (0.8 g, 6 mmol) in H_2O (10 mL). The mixture was refluxed overnight under a nitrogen atmosphere. After this time, 1 M NaOH (3 mL) was added and the aqueous solution extracted with Et_2O (3 × 20 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under a N_2 stream (the solvent was not fully removed due to hazardous nature of small azides). A solution of 2-azidoethanol in Et_2O (0.6 g, 6 mmol) was added to an ice-cooled solution of tosylchloride (1.5 g, 7.9 mmol) and EtN_3 (TEA, 0.9 g, 10 mmol) in CH_2Cl_2 (DCM, 50 mL). The reaction was stirring under Ar for 24 h. After this time, the mixture was washed with $NaHCO_3$ (20 mL), H_2O (20 mL) and brine (20 mL). The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was purified via silica gel chromatography (10% EtOAc in petroleum spirits (PET), R_f = 0.3) to yield the title compound as a clear oil (1.1 g, 73%): ^{1}H NMR ($CDCl_3$): 2.45 (s, 3H), 3.47 (t, *J* = 5.1 Hz, 2H), 4.15 (t, *J* = 5.1 Hz, 2H), 7.36 (d, *J* = 8.0 Hz, 2H), 7.80 (d, *J* = 8.0 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ: 21.8, 49.7, 68.2, 128.1, 130.1, 132.7, 145.4; IR (ATR): 2954, 2107, 1734, 1597, 1360, 1172,

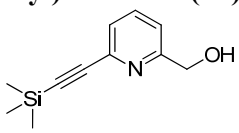
908 cm^{-1}; LRMS (ESI+): *m/z* $[M\text{-}C_2H_4N_3]^+$ 171.0 (100%); HRMS (ESI+): calcd. for $[M+H]^+$: 242.0594, found: 242.0594.

2-Azido-N,N-bis((pyridin-2-yl)methyl)ethanamine (S2)



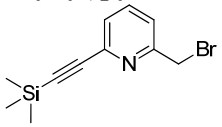
Compound **S1** (1.2 g, 5.0 mmol) and di(2-picolyl)amine (1.0 g, 5.0 mmol) were dissolved in dry CH_3CN (ACN, 5 mL) under an Ar atmosphere and TEA (0.8 g, 7.5 mmol) was then added. The stirred mixture was brought to reflux for 24 h. After this time, the solvent was removed under reduced pressure and the residue dissolved in saturated aqueous K_2CO_3 (40 mL) and extracted with DCM (3 × 30 mL). The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. The resultant crude product was purified via basic alumina chromatography (1% MeOH in DCM, R_f = 0.3) to yield the title compound as an orange oil (1.1 g, 84%): ^{1}H NMR ($CDCl_3$) δ: 2.83 (t, *J* = 6.0 Hz, 2H), 3.32 (t, *J* = 6.0 Hz, 2H), 3.87 (s, 4H), 7.14 (t, *J* = 5.7 Hz, 2H), 7.53 (d, *J* = 7.5 Hz, 2H), 7.66 (t, *J* = 7.5 Hz, 2H), 8.51 (d, *J* = 5.7 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ: 49.2, 53.5, 60.7, 122.3, 123.1, 136.7, 149.2, 159.2 ; IR (ATR): 3064, 2851, 2105, 1665, 1628, 1175, 1121 cm^{-1}; LRMS (ESI+): *m/z* $[M+H]^+$ 269.1 (100%); HRMS (ESI+): calcd. for $[M+H]^+$: 269.1509, found: 269.1511.

(6-(Trimethylsilyl)ethynyl)pyridin-2-yl)methanol (S3)



(6-Bromopyridin-2-yl)methanol (1.06 g, 5.36 mmol), trimethylsilyl acetylene (784 mg, 1.20 mL, 8.46 mmol), $Pd(PPh_3)_2Cl_2$ (172 mg, 0.282 mmol), CuI (101 mg, 0.564 mmol) and TEA (1.6 g, 2.2 mL, 16.0 mmol) were dissolved in dry tetrahydrofuran (THF, 12.5 mL) and stirred at room temperature (RT) under an Ar atmosphere for 2 h. The solvent was removed under reduced pressure, H_2O (25 mL) was added, and the crude product extracted with EtOAc (2 × 20 mL). The combined organic players were dried over $MgSO_4$ and evaporated under reduced pressure. The resultant brown solid was purified by silica gel chromatography (30% EtOAc in PET, R_f = 0.2) to yield the title compound as an off-white solid (930 mg, 85%): ^{1}H NMR ($CDCl_3$) δ: 0.27 (s, 9H), 3.50 (t, *J* = 5.2 Hz, 1H), 4.73 (d, *J* = 5.2 Hz, 2H), 7.22 (d, *J* = 7.7 Hz, 1H), 7.36 (d, *J* = 7.7 Hz, 1H), 7.62 (t, *J* = 7.7 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ: 0.2, 64.5, 95.3, 103.6, 120.1, 126.3, 136.8, 142.1, 160.0; IR (ATR): 3298, 2959, 2159, 1568, 1445, 1249 cm^{-1}; LRMS (ESI+): *m/z* $[M+H]^+]^+$ 206.1 (55%), $[M+Na]^+$ 228.2 (100%) ; HRMS (ESI+): calcd. for $[M+H]^+$: 206.0996, found: 206.0997.

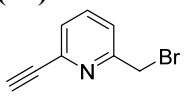
2-(Bromomethyl)-6-(trimethylsilyl)ethynyl)pyridine (S4)



Compound **S3** (930 mg, 4.53mmol) and PPh_3 (2.4 g, 9.1 mmol) were dissolved in DCM (25 mL) and the solution cooled in an ice-bath. A solution containing CBr_4 (2.3 g, 6.8 mmol) in DCM (15 ml) was then added. The reaction mixture was warmed to RT and allowed to stir for 2 h. The solvent was removed under reduced pressure and the crude product purified by silica gel chromatography (10% EtOAc in PET, R_f = 0.6) to yield the title compound as a white solid (853 mg, 71%): mp: 57.2 – 58.8 °C; ^{1}H NMR ($CDCl_3$) δ: 0.27 (s, 9H), 4.53 (s, 2H), 7.38 (dd,

J_1 = 1.0 Hz, J_2 = 7.7 Hz, 1H),7.41 (dd, J_1 = 1.0 Hz, J_2 = 7.8 Hz, 1H), 7.65 (t, J = 7.9 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ: 0.1, 33.5, 95.6, 103.3, 123.2, 126.9, 137.3, 142.8, 157.4; IR (ATR): 2956, 2160, 1563, 1450, 1250, 1210, 840 cm^{-1}; LRMS (ESI+): *m/z* $[M+H]^+$ 268.1 (100%); HRMS (ESI+): calcd. for $[M+H]^+$: 270.0131, found: 270.0132 .

2-(Bromomethyl)-6-ethynylpyridine (S5)



Compound **S4** (323 mg, 1.20 mmol) was dissolved in ACN (10 mL), followed by the addition of 1 M KF (105 g, 1.8 mL, 1.8 mmol), and the mixture allowed to stir for 2 h at RT. The solvent was then removed under reduced pressure and the residue redissolved in H_2O (5 mL) and extracted with DCM (2 × 5 mL). The combined organic layers were dried over $MgSO_4$ and evaporated under reduced pressure to yield the title compound as a pale red solid (201 mg, 85%): mp: 56.3 – 57.8 °C; ^{1}H NMR ($CDCl_3$) δ: 3.19 (s, 1H), 4.54 (s, 2H), 7.43 (dd, J_1 = 0.8 Hz, J_2 = 8.0 Hz, 2H), 7.69 (t, J = 8.0 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ: 33.0, 78.0‡, 82.3†, 123.4, 126.6, 137.6, 142.0†, 157.4†; IR (ATR): 3175, 2917, 2103, 1581, 1446, 1202 cm^{-1}; LRMS (ESI+): *m/z* $[M+H]^+$ 196.0 (100%); HRMS (ESI+): calcd. for $[M+H]^+$: 195.9756, found: 195.9757.

***In situ* preparation of Tb-1.Cu^{2+}probe. Tb-1.Cu^{2+}** was prepared by combining equal volumes of 1 mM aqueous stock solutions of **Tb-1** and $Cu(NO_3)_2$.

Sulfide-dependent luminescence spectra. Time-gated luminescence spectra of **Tb-1.Cu^{2+}** (5 μM) were measured in the presence of 0–30 μM Na_2S[1] in 10 mM HEPES buffer (pH 7.4) with λ_{ex} = 260 nm. Spectra were acquired with a 500 μs delay, integrating over 1000 μs.

Quantum yield determinations. Quantum yields (ϕ) were determined, using tryptophan in water (pH 7.2, 25 °C) as the reference compound (ϕ = 0.14),[2] according to the following equation:

$$\phi_X = \phi_{ST}(Grad_X/Grad_{ST})\times(\eta_X/\eta_{ST})^2$$

where the subscripts X and ST denote sample and standard respectively, Grad is the gradient of plotted integrated luminescence intensity *vs* absorbance, and η is the refractive index of the solvent.

Effect of metal ions. The time-gated luminescence of **Tb-1.Cu^{2+}** (3 μM) at 545 nm (λ_{ex} = 260 nm) was measured in 10 mM HEPES buffer (pH 7.4), both in the absence and presence of 1.0 or 10.0 molar equivalents of various metal ions (added in the form of NaI, $HgCl_2$, $CoCl_2$, $CaCl_2$, KCl, $CuCl_2$, $MgCl_2$, $NiCl_2$, $Pb(OAc)_2.3H_2O$ $FeCl_3.H_2O$, $Zn(NO_3)_2.6H_2O$ and $Cd(NO_2)_2$). The emission from solutions additionally containing 2.0 molar equivalents of Na_2S was also measured.

Effect of anions and sulfurous compounds. The time-gated luminescence of **Tb-1.Cu^{2+}** (3 μM) at 545 nm (λ_{ex} = 260 nm) was measured in 10 mM HEPES buffer (pH 7.4), both in the absence and presence of 50.0 molar equivalents of various anions (added as NaCl, NaI, $NaHCO_3$, Na_2CO_3, NaClO, $NaNO_2$, NaOAc, Na_2SO_3, Na_2SO_4, $Na_2S_2O_3$, $Na_2S_2O_4$, $Na_2S_2O_5$)

‡ Denotes that signals were observed in the 2D NMR spectra.

and sulphurous compounds (lipoic acid and cysteamine hydrochloride). The emission from solutions additionally containing 2.0 molar equivalents of Na_2S was also measured.

Limit of detection for sulfide. Solutions of **Tb-1.Cu^{2+}** (5 μM) in 10 mM HEPES buffer (pH 7.4) were incrementally spiked with a standard solution of Na_2S over the concentration range of 0–30 μM, with the luminescence at 545 nm recorded after each addition (λ_{ex} = 260 nm). From the measured data, the LOD was calculated from the linear range of the curve (0-10 μM) using 3sB/sensitivity, where sB corresponds to the standard deviation of the blank and the sensitivity is the slope of the least-squares linear fitted luminescence signal *vs* [Na_2S] calibration curve (r^2 = 0.9812).[3,4]

Detection of sulfide in sour water sample. Known concentrations of NaHS (0–300 μM) were used to make standard curves for all three assays. The sour water (obtained by Water and Energy Systems Technology, Inc., Kaysville, UT, United States, from a local refinery) was diluted sequentially to create a dilution within the workable range of all assays. The luminescence of the resulting solution was then measured at 545 nm (λ_{ex} = 260 nm) and the concentration of sulfide determined using the relevant standard curve. Standard methylene blue protocols, as well as a coumarin-based probe developed in our laboratory (AzMC),[5] were used for validation. For the methylene blue assay, a 20 μL aliquot of known NaHS concentration was incubated with 30 μL of a 10% (w/v) trichloroacetic acid solution, 30 μL of a 1% (w/v) aqueous $Zn(OAc)_2$ solution, and 10 μL of a 30 mM solution of $FeCl_3$ in 1.2 M HCl. The addition of a 10 μL aliquot of 20 mM *N*,*N*-dimethyl-*p*-phenylenediamine sulfate in 7.2 M HCl yielded a blue colour, which was detected after 30 min at 670 nm.

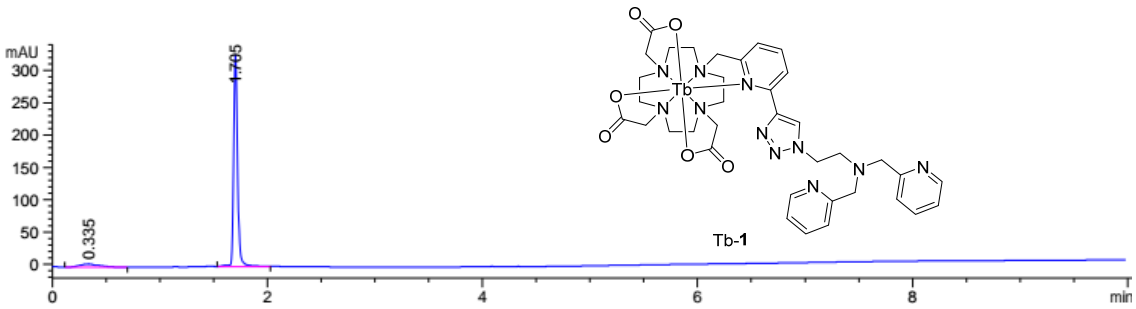


Fig. S1. HPLC chromatogram of **Tb-1**, with detection at 254 nm. Elution method: 100% solvent A for 4 min, then linear gradient from 100% solvent A to 3% solvent B/97% solvent A over 40 min (solvent A = 0.1% TFA in MilliQ water, solvent B = 0.1% TFA in 80% ACN/20% MilliQ water).

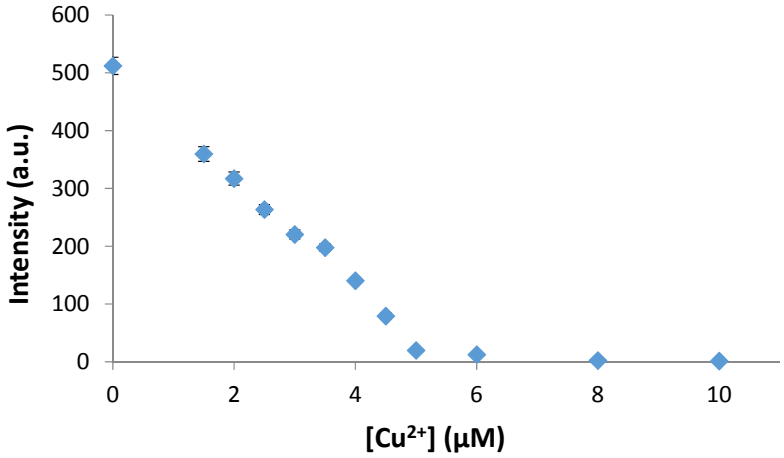


Fig. S2. Changes in the luminescent intensity of **Tb-1** (5 μM) detected at 545 nm upon the addition of Cu^{2+} (0–10 μM); spectra measured in 10 mM HEPES buffer (pH 7.4) with λ_{ex} = 260 nm.

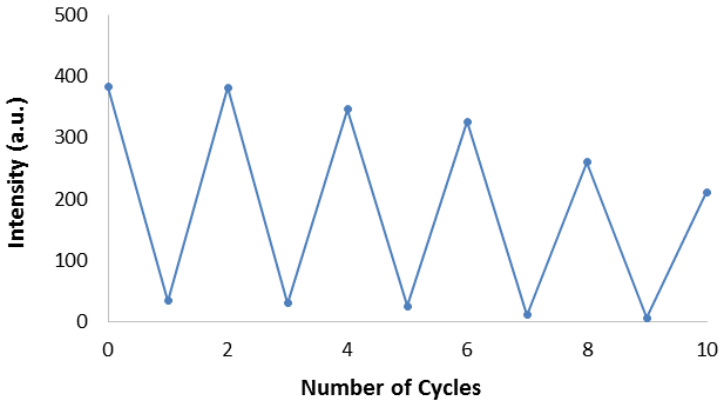
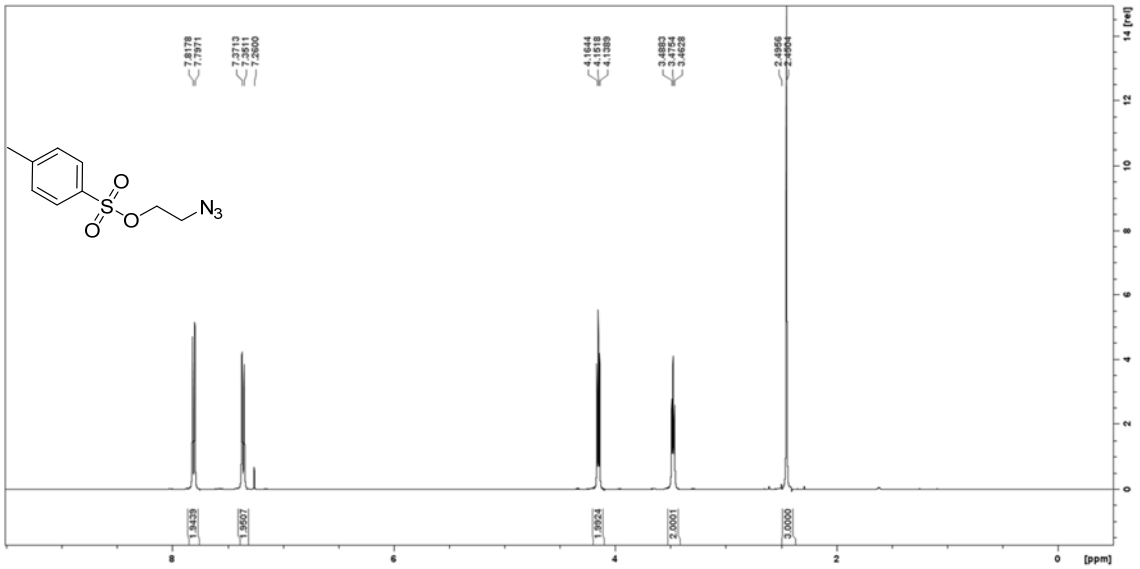


Fig. S3. Luminescence intensity of **Tb-1** (3 μM) in 10 mM HEPES buffer (pH 7.4) upon the alternate addition of Cu^{2+} (3 μM) ions and Na_2S (6 μM). Measurements were made directly after each addition, with a 10 mins interval between additions. No change in the luminescence occurred during this period.



^{1}H NMR spectrum of 2-azidoethyl-4-methylbenzenesulfonate (**S1**) in $CDCl_3$

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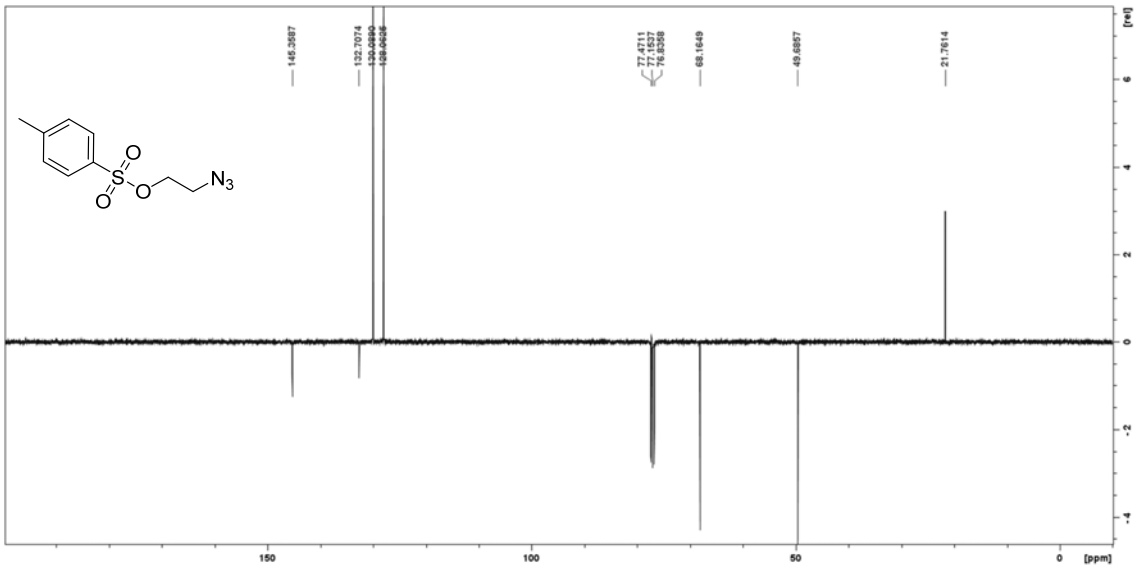
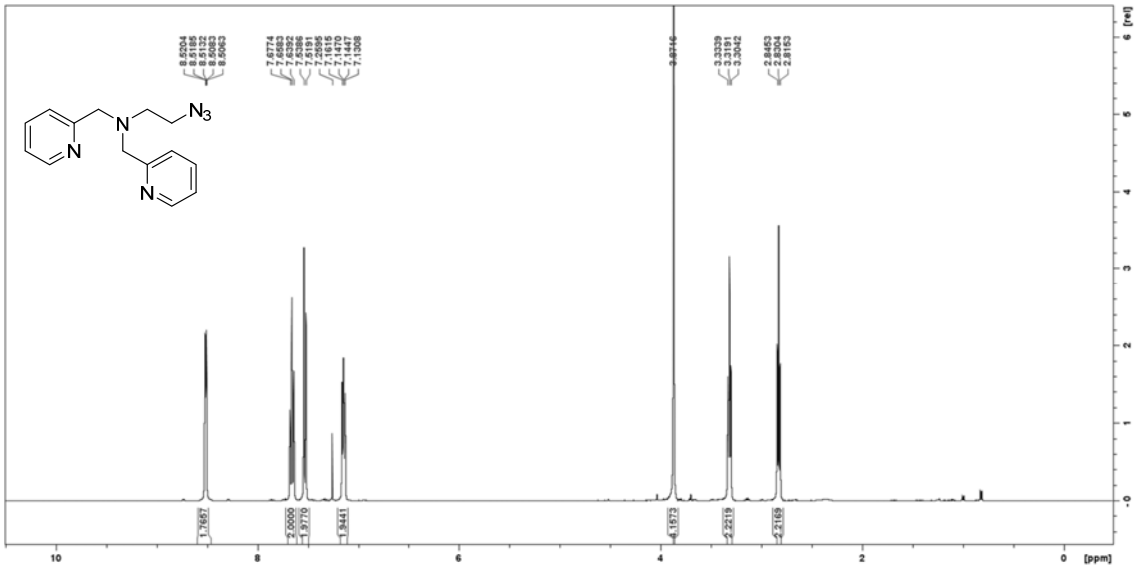
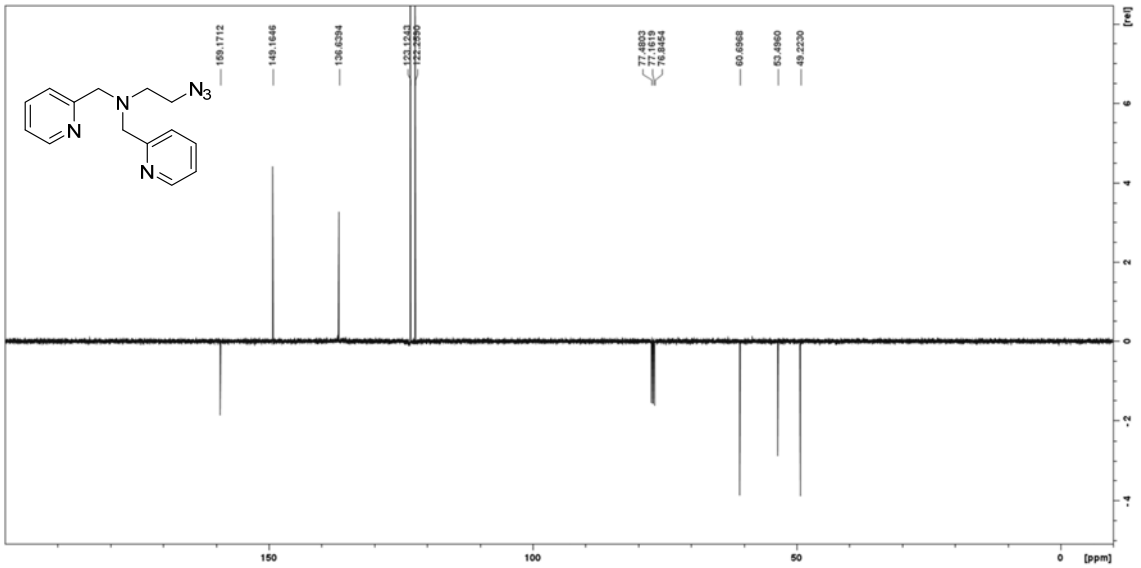


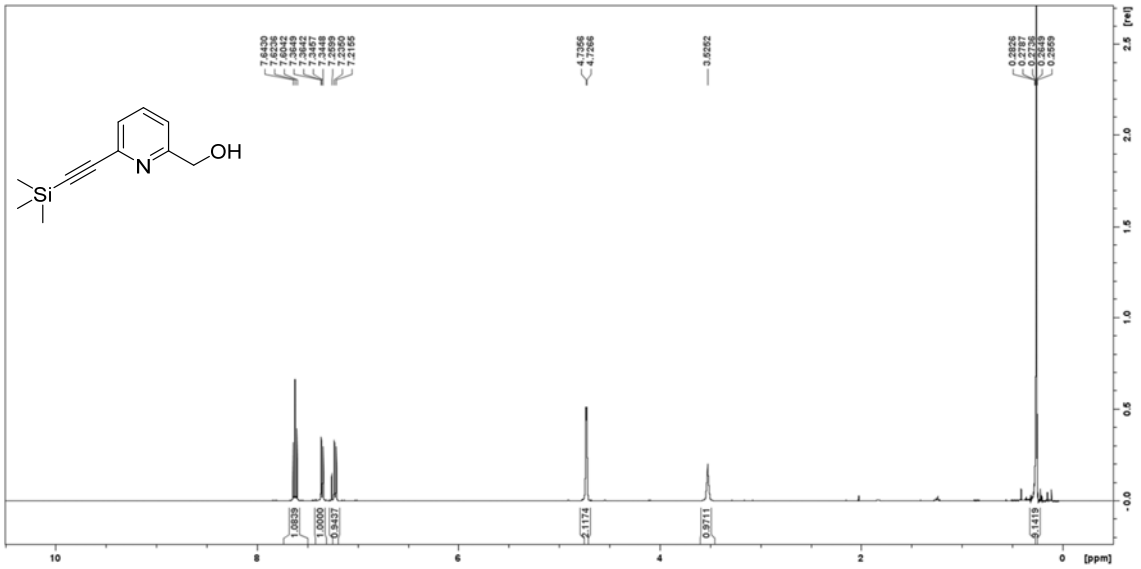
^{13}C-JMOD NMR spectrum of 2-azidoethyl-4-methylbenzenesulfonate (**S1**) in $CDCl_3$



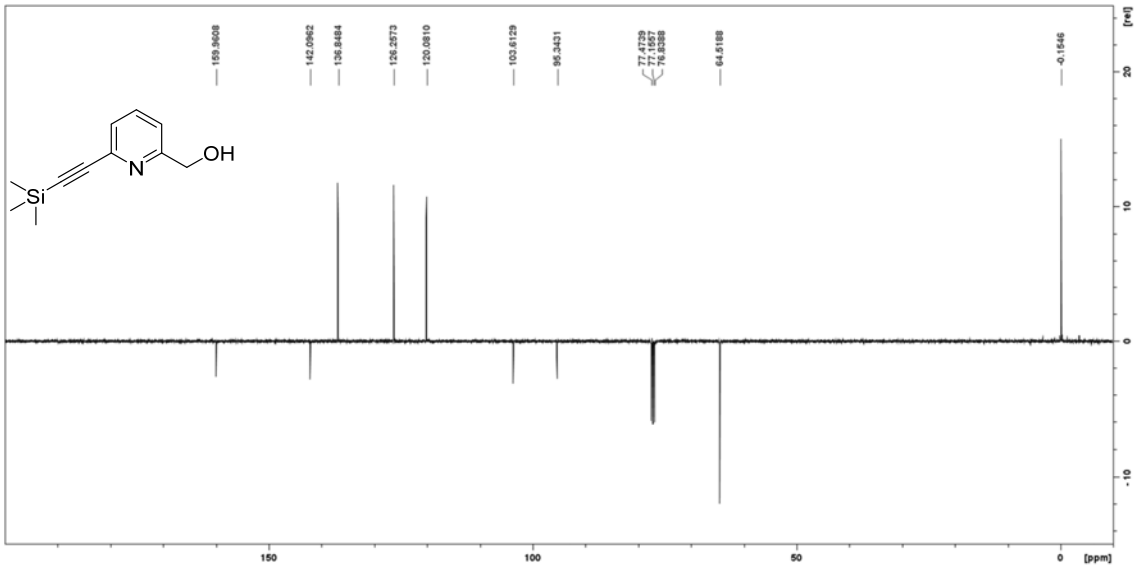
^{1}H NMR spectrum of 2-azido-*N*,*N*-bis((pyridin-2-yl)methyl)ethanamine (**S2**) in $CDCl_3$



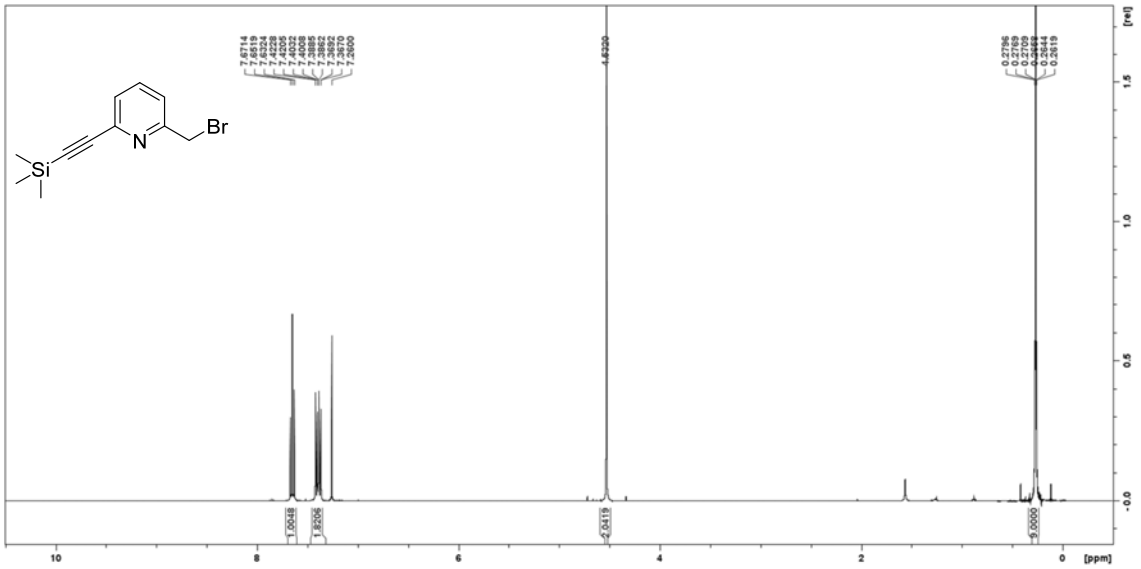
^{13}C-JMOD NMR spectrum of 2-azido-*N*,*N*-bis((pyridin-2-yl)methyl)ethanamine (**S2**) in $CDCl_3$



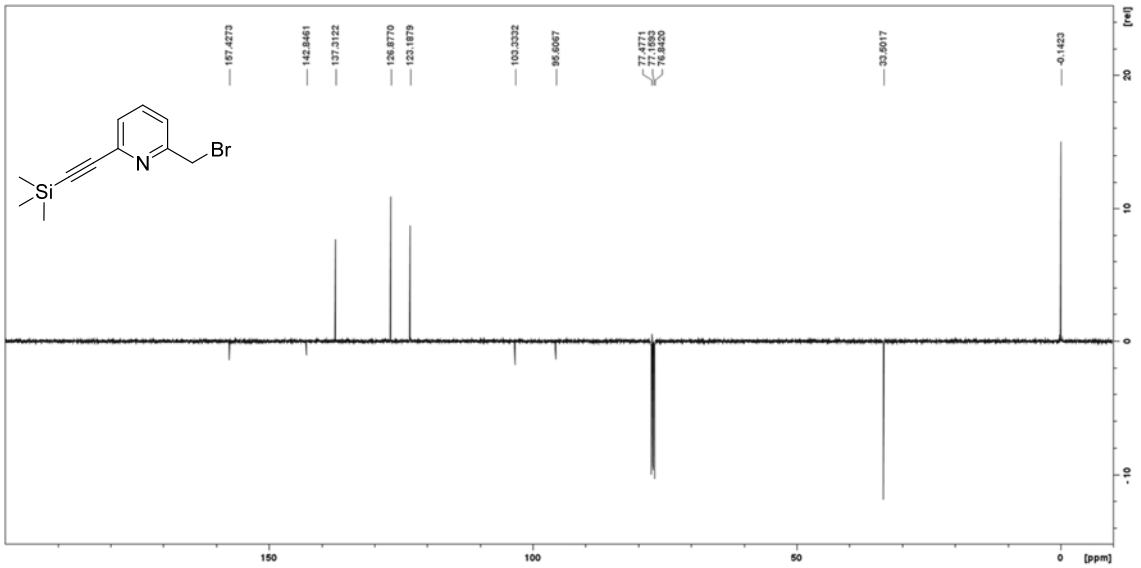
^{1}H NMR spectrum of (6-(trimethylsilyl)ethynyl)pyridin-2-yl)methanol (**S3**) in $CDCl_3$



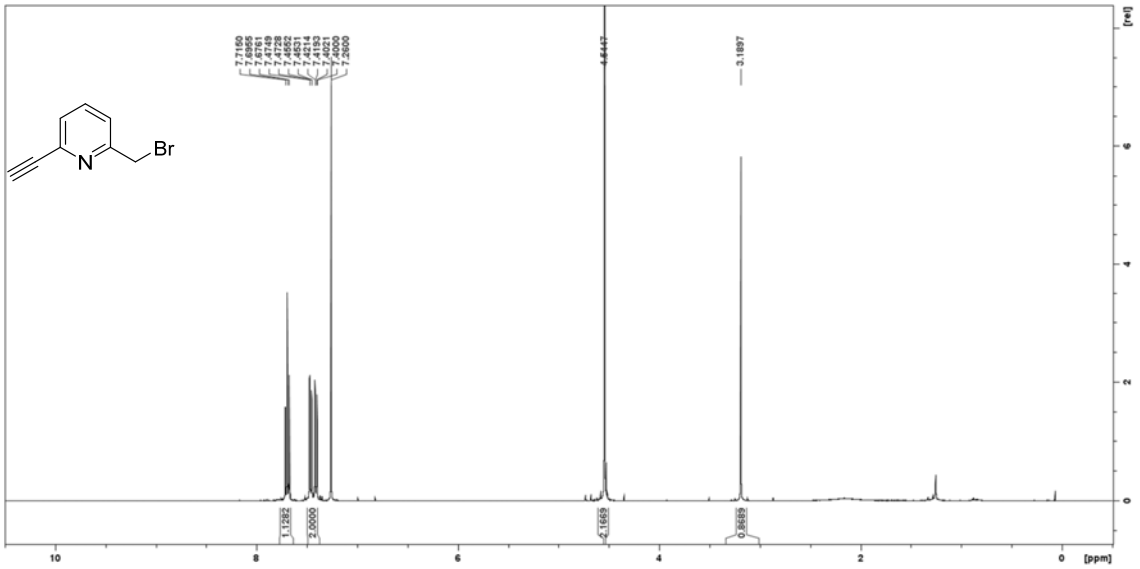
^{13}C-JMOD NMR spectrum of (6-(trimethylsilyl)ethynyl)pyridin-2-yl)methanol (**S3**) in $CDCl_3$



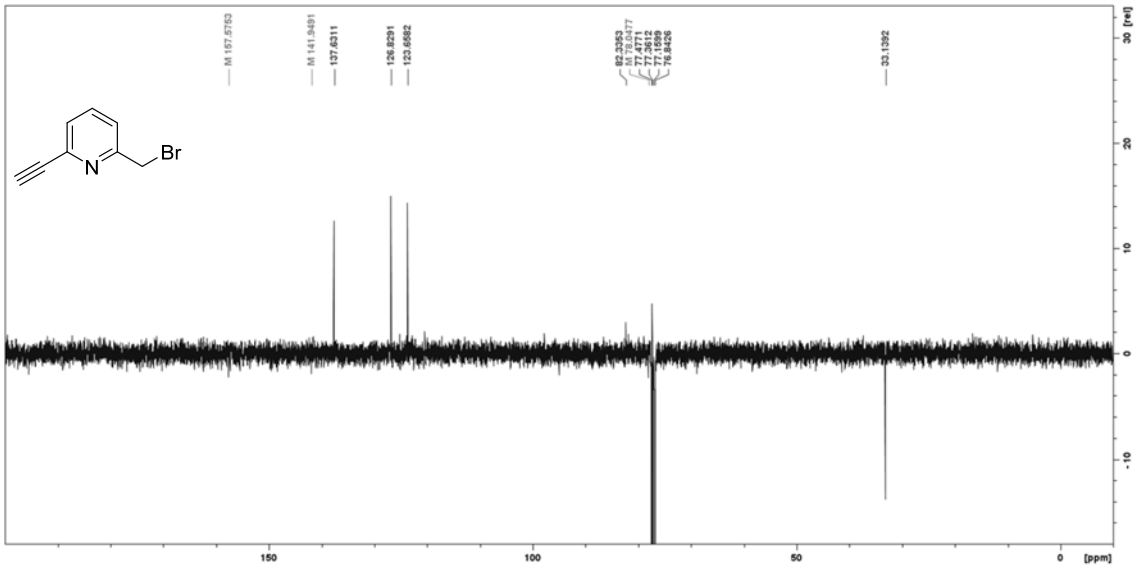
^{1}H NMR spectrum of 2-(bromomethyl)-6-(trimethylsilyl)ethynyl)pyridine (**S4**) in $CDCl_3$



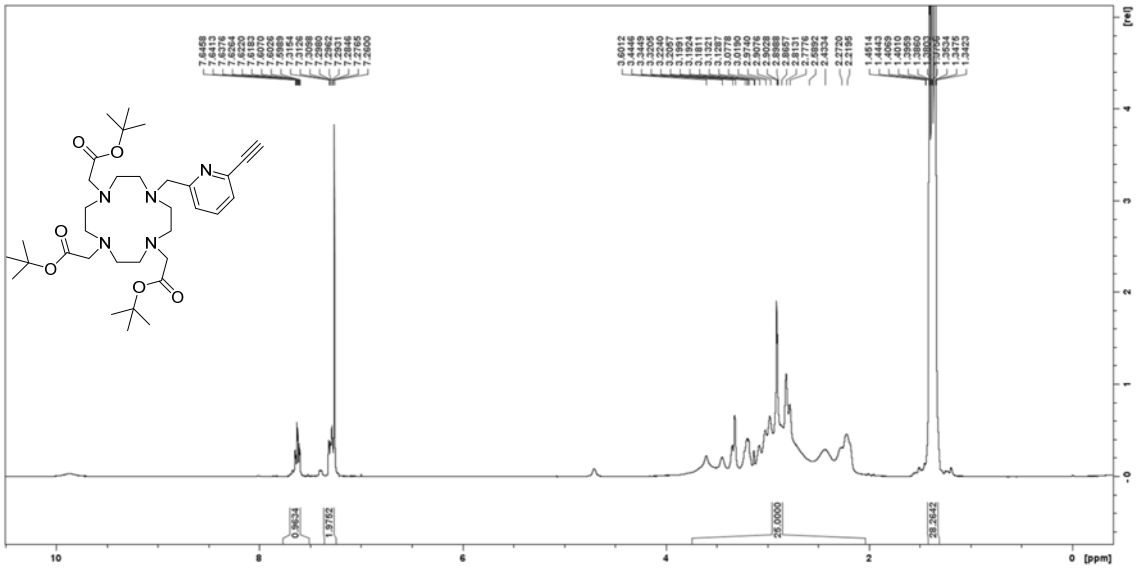
^{13}C-JMOD NMR spectrum of 2-(bromomethyl)-6-(trimethylsilyl)ethynyl)pyridine (**S4**) in $CDCl_3$



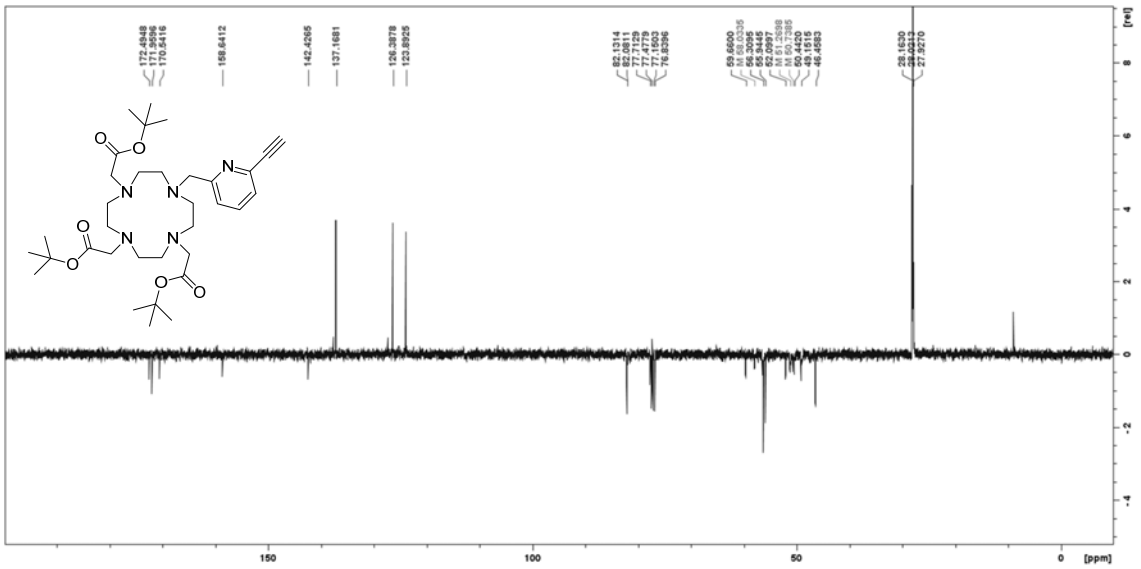
^{1}H NMR spectrum of 2-(bromomethyl)-6-ethynylpyridine (**S5**) in $CDCl_3$



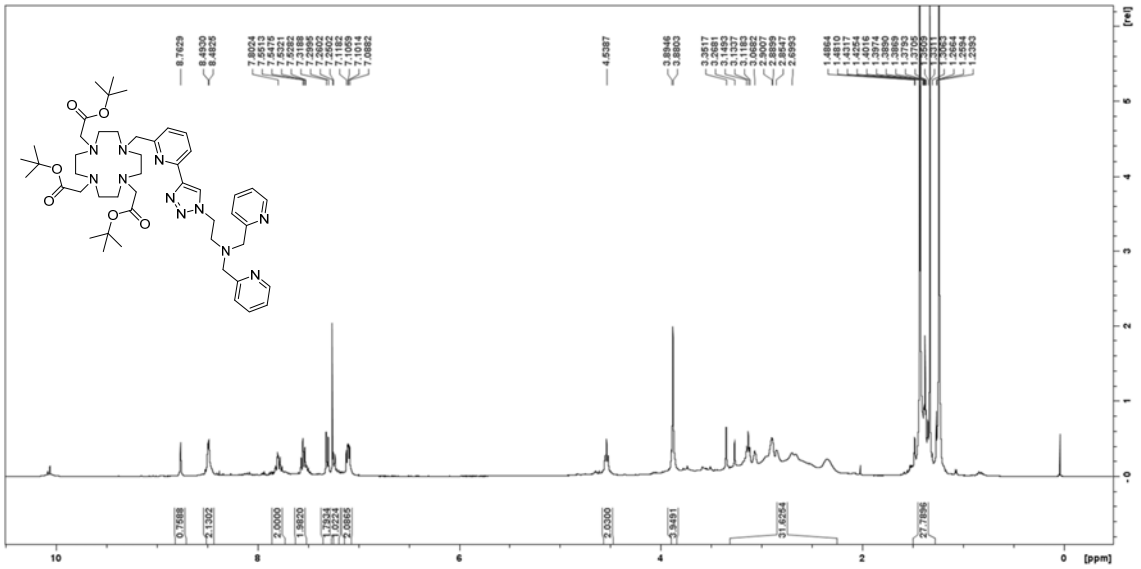
^{13}C-JMOD NMR spectrum of 2-(bromomethyl)-6-ethynylpyridine (**S5**) in $CDCl_3$



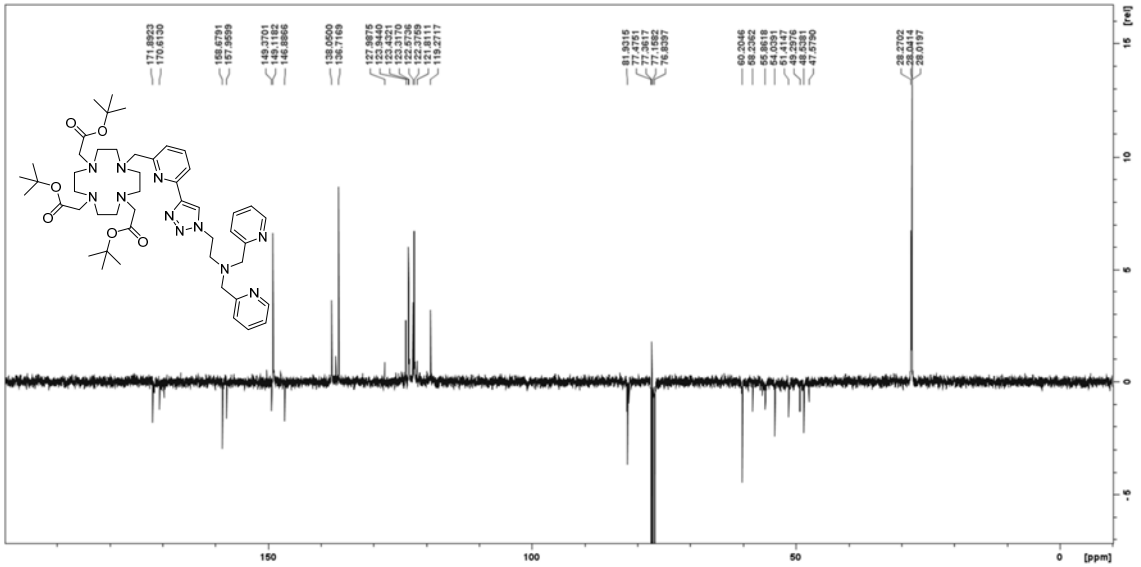
^{1}H NMR spectrum of tri-*tert*-butyl 2,2',2''-(10-((6-ethynylpyridin-2-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (**2**) in $CDCl_3$



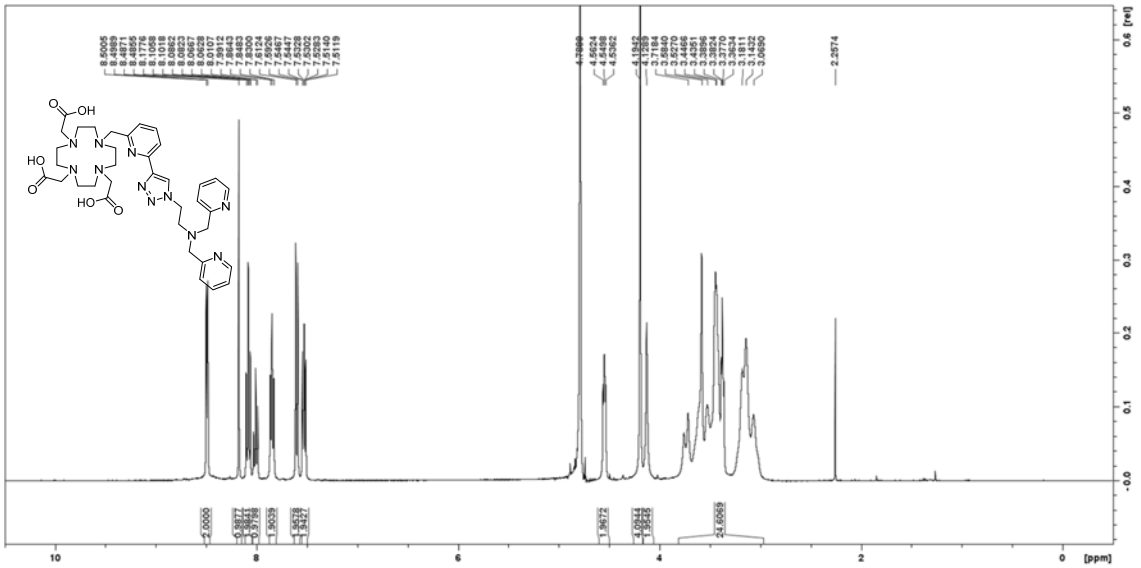
^{13}C-JMOD NMR spectrum of tri-*tert*-butyl 2,2',2''-(10-((6-ethynylpyridin-2-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (**2**) in $CDCl_3$



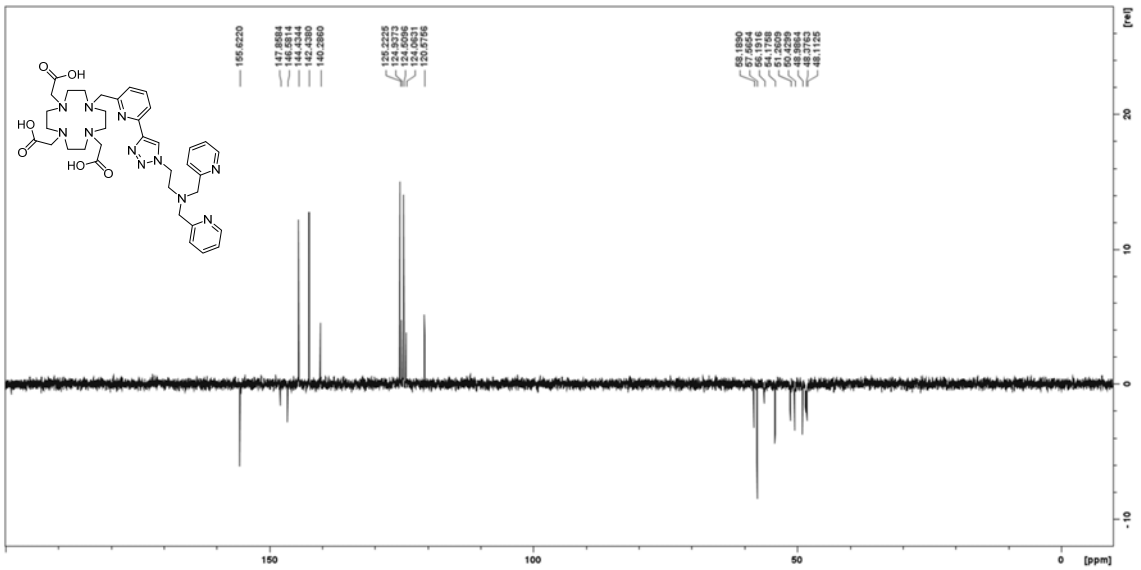
^{1}H NMR spectrum of tri-*tert*-butyl 2,2',2"-(10-((6-(1-(2-(bis(pyridin-2-ylmethyl)amino)ethyl)-1H-1,2,3-triazol-4-yl)pyridin-2-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (**3**) in $CDCl_3$



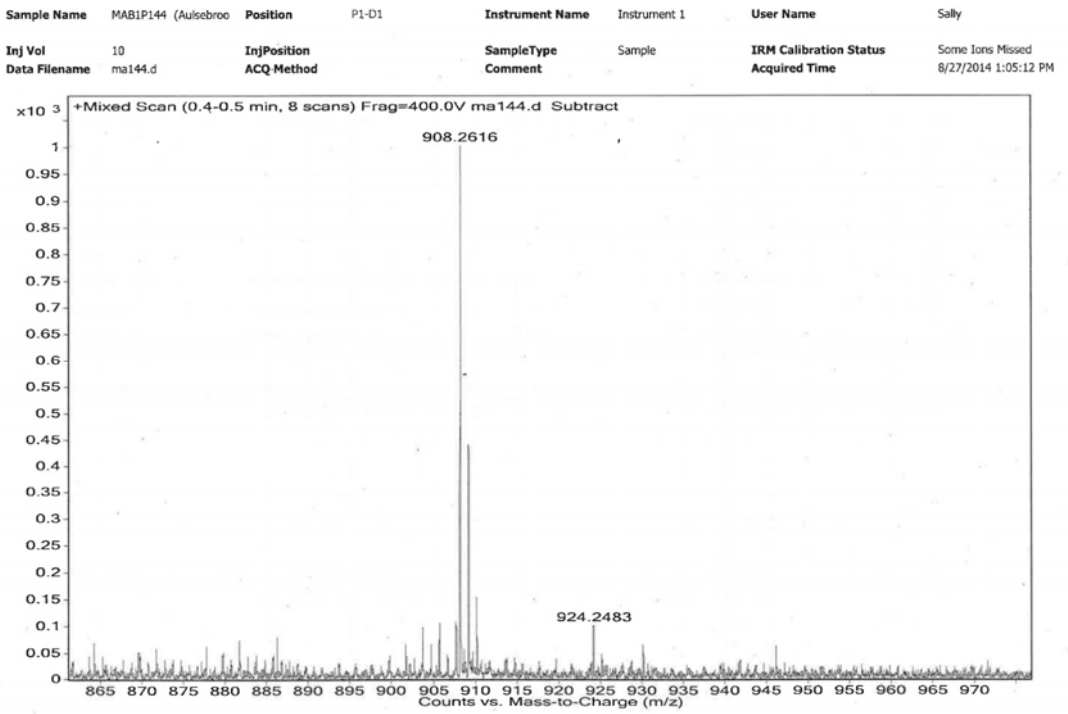
^{13}C-JMOD NMR spectrum of tri-*tert*-butyl 2,2',2"-(10-((6-(1-(2-(bis(pyridin-2-ylmethyl)amino)ethyl)-1H-1,2,3-triazol-4-yl)pyridin-2-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (**3**) in $CDCl_3$



^{1}H NMR spectrum of 2,2',2''-(10-((6-(1-(2-(bis(pyridin-2-ylmethyl)amino)ethyl)-1H-1,2,3-triazol-4-yl)pyridin-2-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (**1**) in D_2O



^{13}C-JMOD NMR spectrum of 2,2',2''-(10-((6-(1-(2-(bis(pyridin-2-ylmethyl)amino)ethyl)-1H-1,2,3-triazol-4-yl)pyridin-2-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (**1**) in D_2O



HRMS of terbium (III), [10-((6-(1-(2-(*bis*(pyridin-2-ylmethyl)amino)ethyl)-1H-1,2,3-triazol-4-yl)pyridin-2-yl- κ N)methyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triacetato(3^-)- κ N^1, κ N^4, κ N^7, κ N^{10}, κ O^1, κ O^4, κ O^7] (**Tb-1**)

References

1. P. Nagy, Z. Pálinkás, A. Nagy, B. Budai, I. Tóth and A. Vasas, *Biochim. Biophys. Acta, Gen. Subj.*, 2014, **1840**, 876-891.
2. W. H. Melhuish, *J. Phys. Chem.*, 1961, **65**, 229.
3. J. C. Miller and J. N. Miller, Statistics for Analytical Chemistry, 2nd ed., 1988.
4. D. A. Skoog, F. J. Holler and S. R. Crouch, *Principles of instrumental analysis*, Thomson Brooks/Cole, 2007.
5. M. K. Thorson, T. Majtan, J. P. Kraus and A. M. Barrios, *Angew. Chem. Int. Ed.*, 2013, **52**, 4641.