Electronic Supporting Information

Water-soluble squaramide functionalised peptides for sulfate recognition in aqueous media

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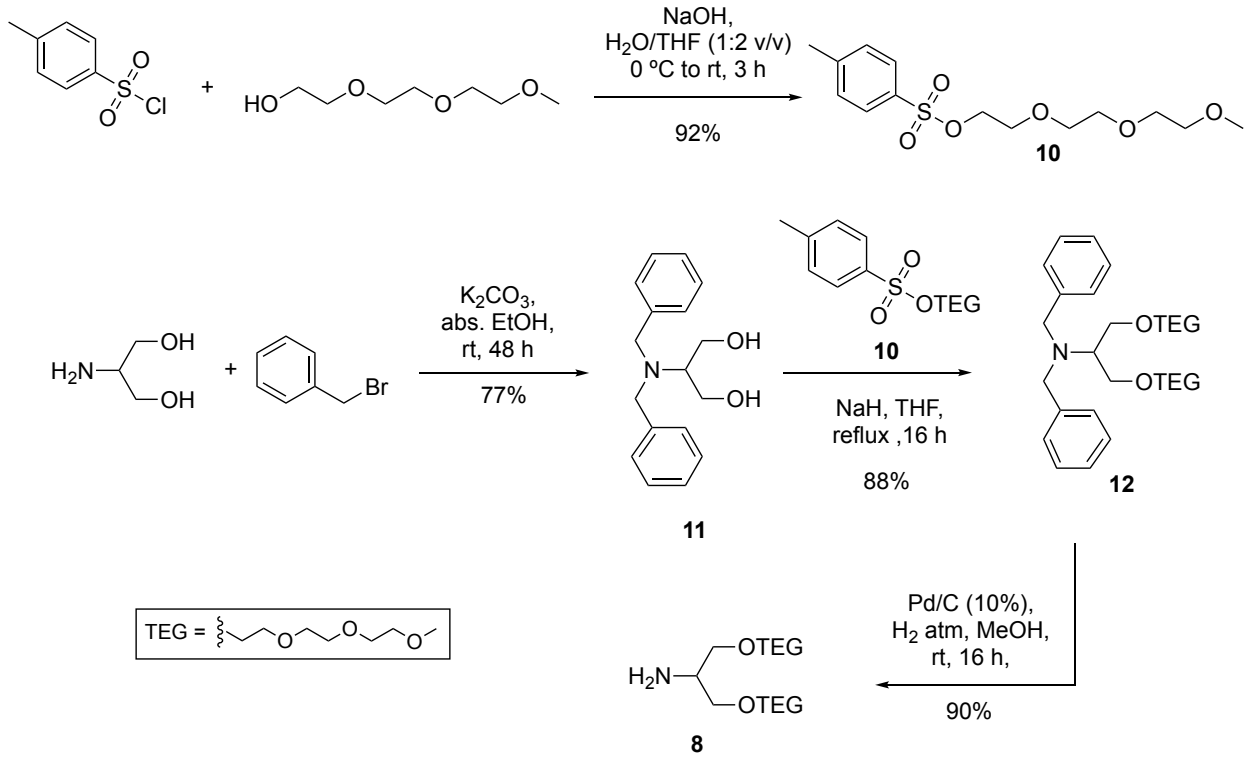
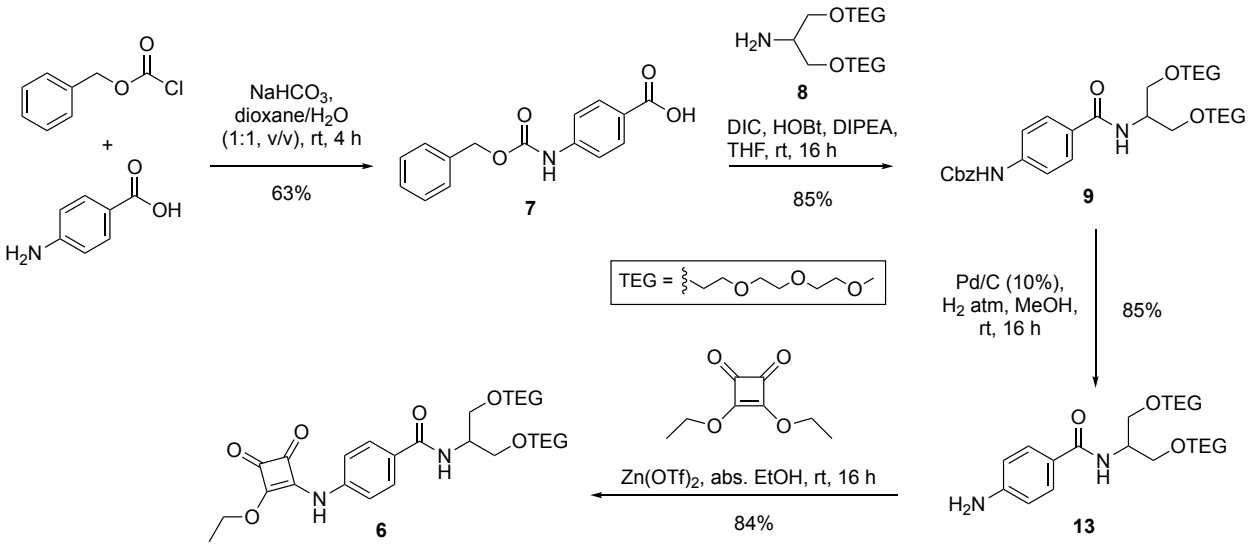


Scheme S1: Synthesis of **8.**



Scheme S2: Synthesis of **6**

Experimental

General Remarks

Melting points (**m.p.**) were observed manually using a Stanford Research Systems Optimelt apparatus and are uncorrected. Optical rotations (**$[\alpha]_D^{20}$**) were obtained using a Perkin Elmer model 341 polarimeter at 589 nm (sodium D line) with a cell path length of 2 cm, using the indicated spectroscopic grade solvent. Nuclear magnetic resonance (NMR) spectroscopy was conducted at 300 K in deuterated dimethyl sulfoxide (DMSO-d_6) using a Bruker Avance DPX 500 spectrometer, a Bruker Avance DPX 400 or a Bruker Avance DPX 300. Proton NMR (**^{1}H NMR**) was conducted at the indicated frequency with chemical shifts (δ) recorded as parts per million (ppm) using chloroform (7.26 ppm) dimethyl sulfoxide (2.50 ppm) as an internal reference. Multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant (*J* Hz) and relative integral (nH) are reported. Carbon-13 NMR (**^{13}C NMR**) spectroscopy was conducted at the indicated frequency and chemical shifts (δ) are reported as parts per million (ppm) using chloroform (77.16 ppm) dimethyl sulfoxide (39.52 ppm) as an internal reference. Infrared (**IR**) absorption spectra were recorded on a Bruker Alpha-E FT–IR spectrometer using attenuated total reflection (ATR) of a thin film or solid. Notable vibrational wavenumbers are recorded in ν_{max} cm^{-1}. Low resolution mass spectra (**MS**) were recorded on either a Thermo Finnigan LCQ Deca Ion Trap mass spectrometer or Shimadzu 2020 mass spectrometer using electrospray ionisation (ESI, in positive mode). High resolution mass spectra (**HRMS**) using ESI were obtained using a Bruker BioApex Fourier Transform Ion Cyclotron Resonance mass spectrometer (FTICR) operating at 4.7 T. Commercial materials were used as received. Amino acids, coupling reagents and resins were obtained from Novabiochem or GL biochem. Solid phase peptide synthesis was performed in polypropylene syringes equipped with Teflon filters and the HPLC grade dimethylformamide (DMF) used for peptide synthesis was obtained from LabScan and used without further purification.

Triethylene glycol monomethyl ether (TEG) tosylate (10)

Triethylene glycol monomethyl ether (13.2 g, 82.8 mmol) and sodium hydroxide (12.8 g, 320 mmol) were dissolved in H_2O/THF (100 mL, 1:2 *v/v*) and the mixture was cooled to 0 ºC. Tosyl chloride (1.6 g, 8.6 mmol) in THF (10 mL) was then added dropwise, the solution was stirred at 0 ºC for 3 hours and then allowed to come to room temperature. The mixture was diluted with H_2O (100 mL) and the product was extracted with iPr-OH/$CHCl_3$ (1:3 *v/v*, 3 × 30 mL). The combined organic phase was washed with aqueous hydrochloric acid (50 mL, 3M) and dried over magnesium sulfate. The solvent was removed under reduced pressure to give **10** as a colourless oil (24.2 g, 91%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81 – 7.71 (m, 2H), 7.36 – 7.26 (m, 2H), 4.16 – 4.07 (m, 2H), 3.75 – 3.46 (m, 10H), 3.33 (s, 3H), 2.41 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 144.8, 133.0, 129.8, 127.9, 71.9, 70.7, 70.5, 70.2, 69.2, 68.6, 59.0, 21.6; MS (ESI) *m/z* = 319 $[M + H]^+$. These data are in agreement with those previously reported.[2]

2-(Dibenzylamino)propane-1,3-diol (11)

Serinol (4.0 g, 44 mmol) and potassium carbonate (16.6 g, 121 mmol) were dissolved in absolute EtOH (100 mL). Benzyl bromide (11.6 mL, 92 mmol) was added dropwise and the mixture was then stirred at room temperature for 48 h. The excess potassium carbonate was filtered off and the solvent was removed under reduced pressure to give a white solid. This was treated with hot toluene and the soluble portion was purified by recrystallization (hexane/toluene, 1:1 *v/v*, 50 mL) to give **11** as white needles (9.22 g, 77%); m.p. 96-99 ˚C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.16 (d, *J* = 7.5 Hz, 4H), 7.08 (t, *J* = 7.4 Hz, 4H), 6.98 (t, *J* = 7.0 Hz, 2H), 4.15 (s, 2H), 3.54 (s, 4H), 3.39 (q, *J* = 10.8 Hz 4H), 3.17 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 140.6, 128.4, 128.0, 126.5, 60.5, 59.5, 54.1; MS (ESI) *m/z* = 272 $[M + H]^+$. This data is in agreement with those previously reported.[1]

2-(Dibenzylamino)propane-1,3-diTEG (12)

Sodium hydride (2.1 g of a 60% dispersion in mineral oil, 54 mmol) was added to anhydrous THF (100 mL) and the resulting mixture was stirred to make a suspension. Compound **11** (4.8 g, 17.3 mmol) in anhydrous THF (50 mL) was added dropwise under nitrogen. The mixture was stirred for 30 minutes at room temperature and **10** (12.1 g, 38.2 mmol) in anhydrous THF (50 mL) was added dropwise. The mixture was stirred for 2 hours and the temperature was slowly increased to reflux and stirred for 16 h. The reaction was allowed to cool to room temperature and was carefully quenched with methanol until effervescence had ceased. The reaction was filtered and the solvent

was removed and the resulting brown oil was purified by reversed-phase column chromatography (100 g C8-reversed phase silicagel; H_2O/MeCN, 0 to 100% MeCN, 50 mL min^{-1}) to give **12** as a pale yellow oil (8.6 g, 88%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39 – 7.37 (m, 4H), 7.29 – 7.15 (m, 6H), 3.78 (s,4H), 3.65 – 3.53 (m, 28H), 3.37 (s, 6H), 3.13 – 3.05 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 140.8, 128.6, 128.0, 126.5, 71.8, 70.6, 70.5, 70.4, 70.3, 58.9, 56.2, 55.0 - two signals overlapping or obscured; MS (ESI) *m/z* = 565 $[M + H]^+$. These data are in agreement with those previously reported.[2]

2-Aminopropane-1,3-diTEG (8)

Compound **12** (10.7 g, 19 mmol) was dissolved in MeOH (200 mL). Pd/C (10%, 0.5 g) was added under nitrogen. The mixture was stirred at room temperature under an atmosphere of H_2 gas and monitored via TLC. Once conversion was complete, the reaction mixture was filtered through celite and the solvent was removed to give **8** as a pale yellow oil (6.6 g, 90%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.64 – 3.61 (m, 21H), 3.54 – 3.47 (m, 7H), 3.39 – 3.33 (m, 8H), 3.20 – 3.16 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 73.6, 71.9, 70.6, 70.56, 70.53, 70.4, 58.9, 50.7 - one signal overlapping or obscured; MS (ESI) *m/z* = 384 $[M + H]^+$. These data are in agreement with those previously reported.[2]

4-(Cbz-amino)benzoic acid (7)

4-Aminobenzoic acid (4.1 g, 30 mmol) and sodium hydrogen carbonate (20 g, 250 mmol) were dissolved in dioxane/H_2O (1:1, *v/v*, 200 mL). Benzyl chloroformate (6.6 mL, 33 mmol) was added dropwise and the mixture was stirred at room temperature for 4 h. Water (40 mL) was added and the mixture was stirred at room temperature for a further 16 h. The mixture was carefully acidified to pH 3 with aqueous hydrochloric acid (3 M) and the resulting precipitate was filtered and washed with water (3 × 20 mL). The product was purified via recrystallisation (EtOAc/hexane, 1:1 *v/v*) to give **7** as a white solid (7.28 g, 89%); m.p. 211-218 ºC; ^{1}H NMR (200 MHz, DMSO-d_6) δ 12.63 (s, 1H), 10.13 (s, 1H), 7.94 – 7.81 (m, 2H), 7.65 – 7.52 (m, 2H), 7.42 – 7.34 (m, 5H), 5.18 (s, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.6, 153.8, 143.9, 137.0, 131.1, 129.1, 129.0, 128.7, 125.1, 118.0, 66.7; MS (ESI) *m/z* = 272 $[M + H]^+$. These data are in agreement with those previously reported.[3]

4-Cbz-amino-*N*-(1,3-diTEGpropyl)benzamide (9)

Compound **7** (2.6 g, 9.4 mmol) was dissolved in THF (200 mL) and DIPEA (2.23 mL, 12.9 mmol) were added. The mixture was stirred at room temperature for 15 mins before adding HOBt (1.74 g, 12.9 mmol) and DIC (2.02 mL, 12.9 mmol). The mixture was stirred at room

temperature for a further 10 mins before adding **8** (3.29 g, 8.58 mmol). The reaction was stirred overnight at room temperature, the solvent was removed under reduced pressure. The resulting pale brown oil was purified by reversed-phase chromatography (25 g C8-reversed phase silica gel; H_2O/MeCN, 0 to 100% MeCN, 25 mL min^{-1}) to give **9** as a pale yellow oil (0.76 g, 85%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.01 (s, 1H), 8.03 (d, J = 8.1 Hz, 1H), 7.83 – 7.75 (m, 2H), 7.57 – 7.49 (m, 2H), 7.46 – 7.30 (m, 5H), 5.17 (s, 2H), 4.29 – 4.17 (m, 1H), 3.62 – 3.31 (m, 28H), 3.21 (s, 6H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.6, 153.2, 141.8, 136.4, 128.5, 128.2, 128.14, 128.10, 117.1, 71.2, 69.9, 69.79, 69.75, 69.64, 69.56, 65.9, 58.0, 49.1 - two signals overlapping or obscured.

4-Amino-*N*-(1,3-diTEGpropyl)benzamide (13)

Compound **9** (0.7 g, 1.1 mmol) was dissolved in MeOH (25 mL). Pd/C catalyst (10%, 70 mg) was added and the solution was stirred under hydrogen atmosphere for 16 h at room temperature. The reaction mixture was filtered through Celite and the solvent was removed under reduced pressure to give **13** as a pale yellow oil (0.56 g, 85%); 1H NMR (300 MHz, $CDCl_3$) δ 7.66 – 7.56 (m, 2H), 6.82 (d, J = 8.3 Hz, 1H), 6.69 – 6.59 (m, 2H), 4.48 – 4.25 (m, 1H), 3.85 – 3.46 (m, 28H), 3.34 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.1, 149.4, 128.8, 123.9, 114.2, 71.7, 70.3, 70.0, 58.9, 48.7, 46.3, 46.2 - two signals overlapping or obscured; IR (thin film) ν_{max} 2879, 1692, 1606, 1510, 1309, 1200, 1102 cm^{-1}; MS (ESI) *m/z* = 503 $[M + H]^+$; HRMS (ESI) *m/z* = 525.2775 $[M + Na]^+$, 525.2783 calcd. for $C_{24}H_{42}N_2O_9Na^+$.

3-Ethoxy-4-(*N*-(1,3-DiTEGpropyl)benzamide)squaramate (6)

Diethyl squarate (1.0 mL, 6.94 mmol) was dissolved in absolute EtOH (20 mL) and zinc trifluoromethane sulfonate (0.50 g, 1.26 mmol) was added and the reaction mixture was cooled to 0 ºC. Compound **13** (3.17 g, 6.31 mmol) was dissolved in absolute EtOH (200 mL) and added to the reaction mixture dropwise and was stirred for 18 h at room temperature. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography (10% EtOH/CH_2Cl_2) to give **6** as a yellow oil (3.30 g, 84%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.92 (s, 1H), 8.13 (d, J = 8.0 Hz, 1H), 7.89 – 7.80 (m, 2H), 7.48 – 7.39 (m, 2H), 4.79 (q, J = 7.1 Hz, 2H), 4.35 – 4.10 (m, 1H), 3.60 – 3.37 (m, 28H), 3.22 (s, 6H), 1.43 (t, J = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 184.1, 178.8, 169.4, 165.4, 140.5, 129.5, 128.4, 118.7, 71.2, 69.9, 69.7, 69.6, 69.5, 58.0, 49.2, 15.6 - four signals overlapping or obscured; IR (thin film) ν_{max} 2886, 1712, 1524, 1407, 1248, 1104, 1031 cm^{-1}; MS (ESI) *m/z* = 649 $[M + Na]^+$; HRMS (ESI) *m/z* = 649.2943 $[M + Na]^+$, 649.2943 calcd. for $C_{30}H_{46}N_2O_{12}Na^+$.

SPPS General Procedures

Rink Amide Resin Loading:

Rink amide resin was swollen in DMF for 30 minutes at room temperature. The resin was then washed with DMF (× 5), CH_2Cl_2 (× 5) and DMF (× 5). This was followed by treatment with 20% *v/v* piperidine/DMF (2 × 5 min) to remove the Fmoc-group. The resin was then washed with DMF (× 5), CH_2Cl_2 (× 5) and DMF (× 5). A solution of Fmoc-Gly-OH (3 eq.), PyBOP (3 eq.) and DIPEA (6 eq.) in DMF was pre-activated for 5 min. After pre-activation, the solution was added to the resin and agitated for 2 h. The resin was drained then washed with DMF (× 5), CH_2Cl_2 (× 5) and DMF (× 5).

Estimation of Resin Loading:

The resin was treated with 20% *v/v* piperidine/DMF (2 × 5 min) and the combined deprotection solution was made up to 10 mL 20% *v/v* with piperidine/DMF. The solution was diluted with 20% *v/v* piperidine/DMF, until the concentration of the fulvene-piperidine adduct was in the range of 2.5–7.5 × 10^{-5} M, and the UV absorbance was measured (λ = 301 nm, ε = 7800 $M^{-1}cm^{-1}$) to estimate the amount of amino acid loaded onto the resin.

Iterative Peptide Assembly (Fmoc-SPPS):

The swollen resin was treated with 20% *v/v* piperidine/DMF (2 × 5 min) to remove the *N*-terminal Fmoc group. The resin was then washed with DMF (× 5), CH_2Cl_2 (× 5) and DMF (× 5). The appropriate protected amino acid (2.5 - 3 equiv.), PyBOP (2.5 - 3 equiv.) and *N*-methylmorpholine (8 equiv.) were dissolved in DMF and allowed to pre-activate for 5 min. The resin was treated with the coupling mixture and agitated for 1 hr. The resin was then washed with DMF (× 5), CH_2Cl_2 (× 5) and DMF (× 5).

N-Terminal Fmoc deprotection:

The swollen resin was treated with 20% *v/v* piperidine/DMF (2 × 5 min) to remove the *N*-terminal Fmoc group. The resin was then washed with DMF (× 5), CH_2Cl_2 (× 5) and DMF (× 5).

Acetylation:

After removal of the Fmoc protecting group the resin was treated with 20% *v/v* Ac_2O/pyridine (2 × 30 min) followed by washing with DMF (× 5), CH_2Cl_2 (× 5), DMF (× 5) and CH_2Cl_2 (× 5) and dried overnight *in vacuo*.

Allyloxycarbonyl (Alloc) deprotection:

The resin was swollen in CH_2Cl_2 for 30 minutes, then drained and treated with $Pd(PPh_3)_4$ (1.05 eq.) in CH_2Cl_2/N-methylmorpholine/AcOH (90:5:5, *v/v/v*).164 The syringe was shielded from light and agitated at room temperature for 2 h. The resin was drained and washed with CH_2Cl_2 (× 5) and a palladium chelating cocktail (DMF/diethyldithiocarbamic acid-3-water/Et_3N 25 mL: 0.25 g: 0.25 mL). The resin was washed with 0.5% *v/v* Et_3N/DMF (× 5), to remove the last traces of the palladium chelating cocktail. The resin was washed with DMF (× 5), CH_2Cl_2 (× 5) and DMF (× 5).

Squaramide functionalisation:

Following Alloc deprotection the resin was treated with **6** (2.5 equiv.) and Et_3N (6 equiv.) in DMF. The suspension was agitated at room temperature overnight, drained and then washed with DMF (× 5), CH_2Cl_2 (× 5) and DMF (× 5).

Cleavage from Resin:

The resin was washed with CH_2Cl_2 (× 5) and dried *in vacuo*. Once dry the resin was treated with a solution of TFA/TIS/H_2O (95:2.5:2.5, *v/v/v*) and agitated at room temperature for 1 h. The resin was drained and then washed with TFA (× 3). The cleavage solution and acid washes were combined and the solvent was removed under a stream of nitrogen until the volume was reduced to 1-2 mL and then triturated with ether.

Figure S1: ^{1}H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz) spectra of **6**.

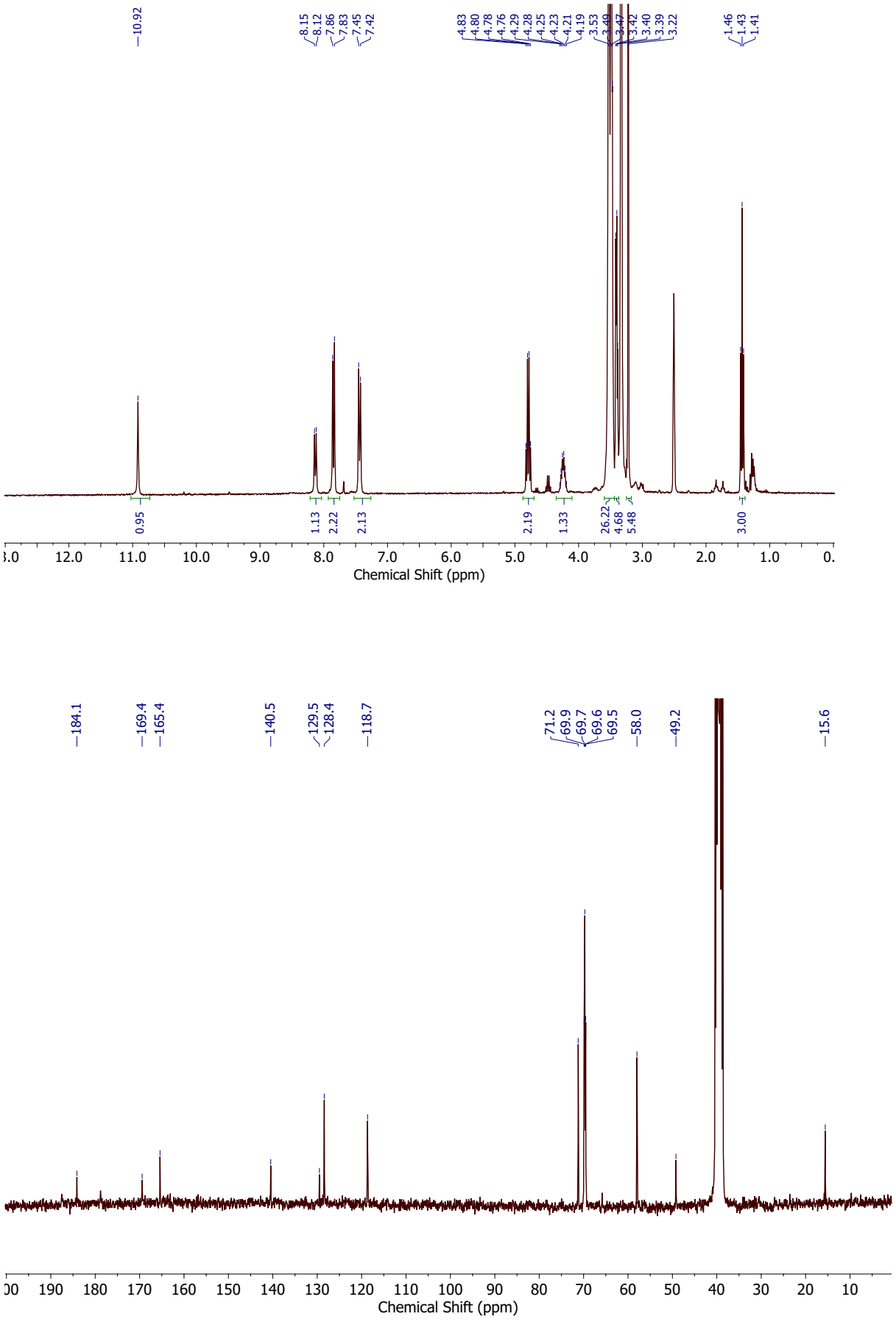


Figure S2: ^{1}H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz) spectra of **2**.

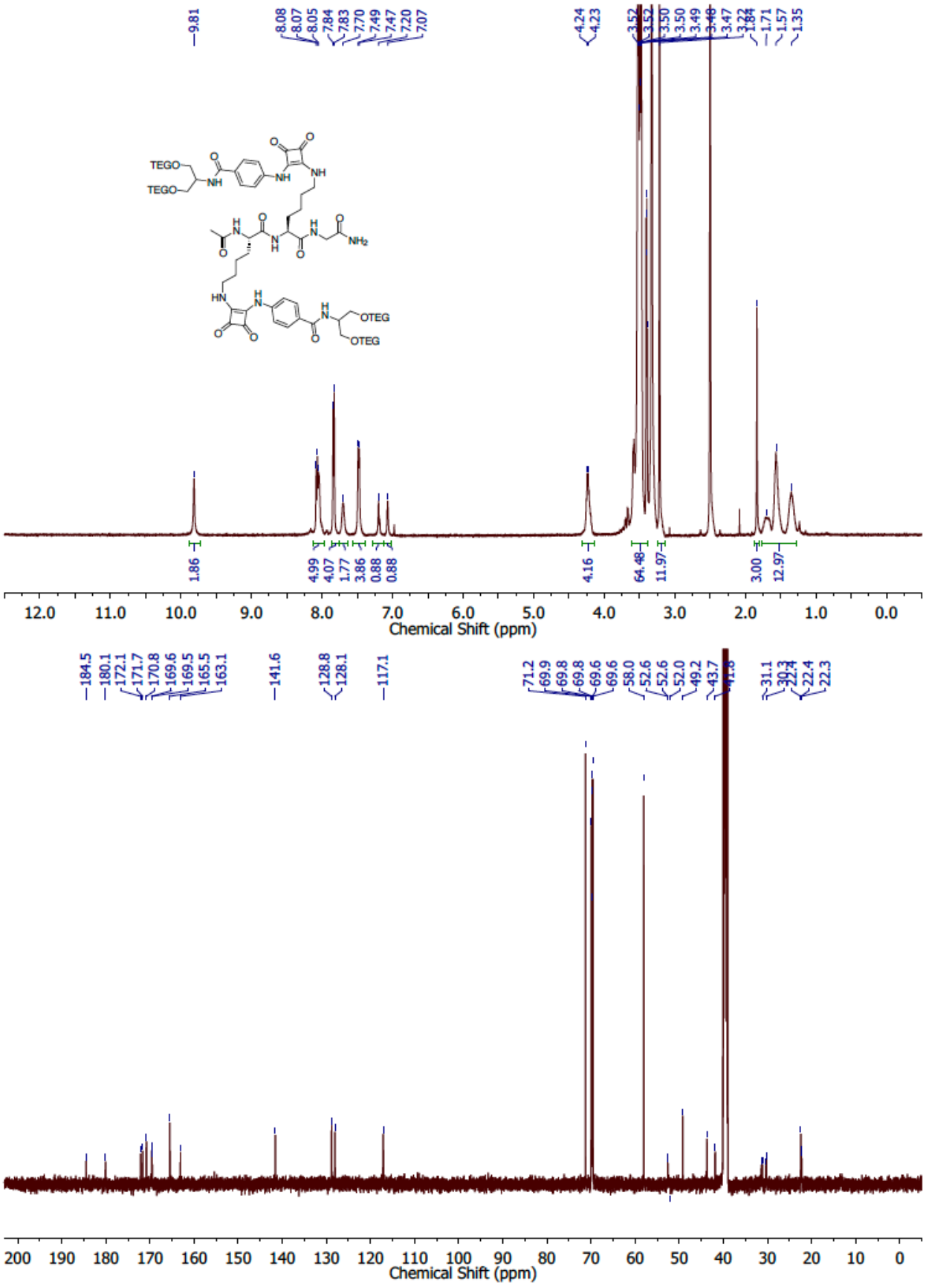


Figure S3: ^{1}H NMR (DMSO-d_6, 400 MHz) and ^{13}C NMR (DMSO-d_6, 100 MHz) spectra of **3**.

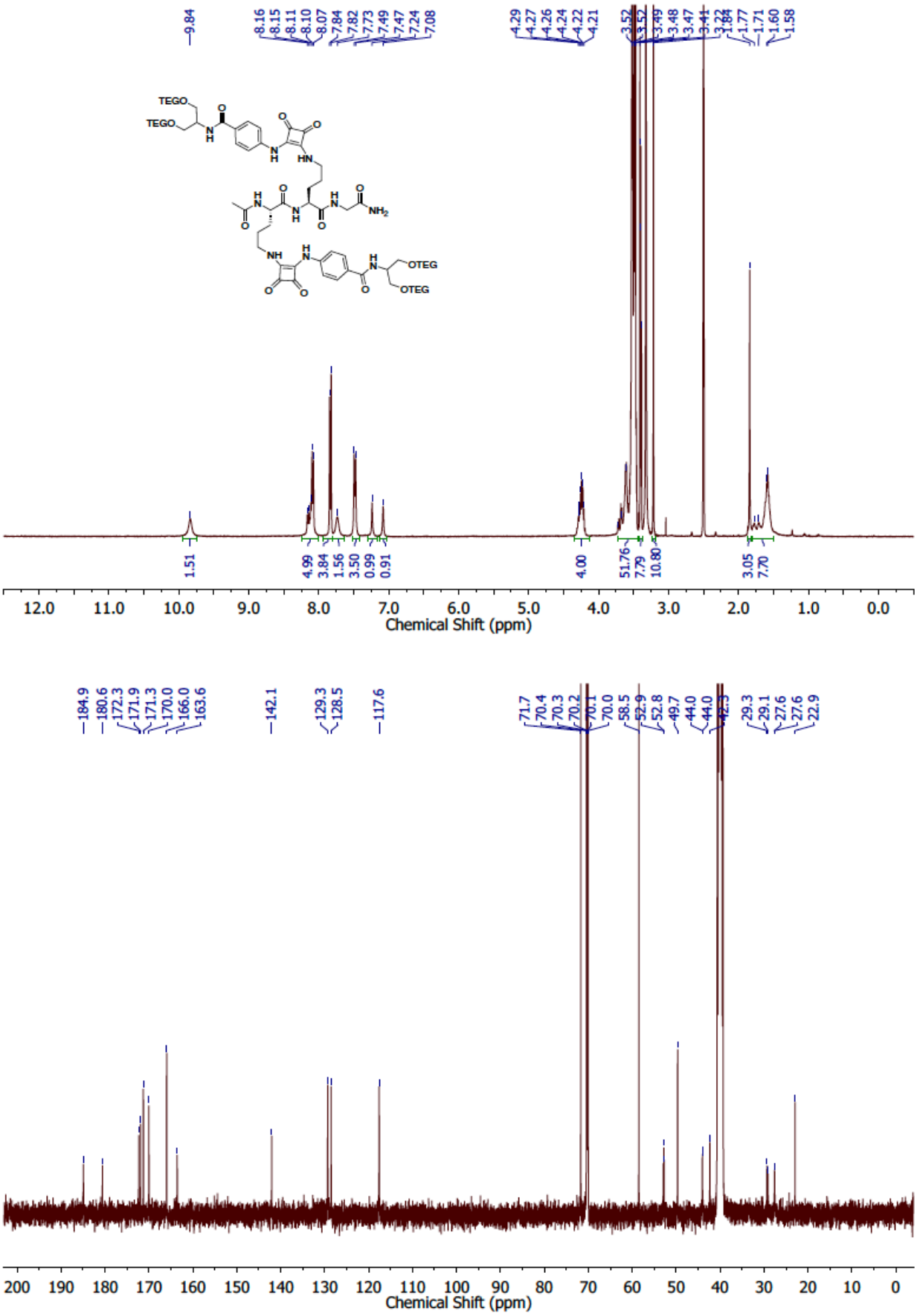


Figure S4: ^{1}H NMR (DMSO-d_6, 400 MHz) and ^{13}C NMR (DMSO-d_6, 100 MHz) spectra of **4**.

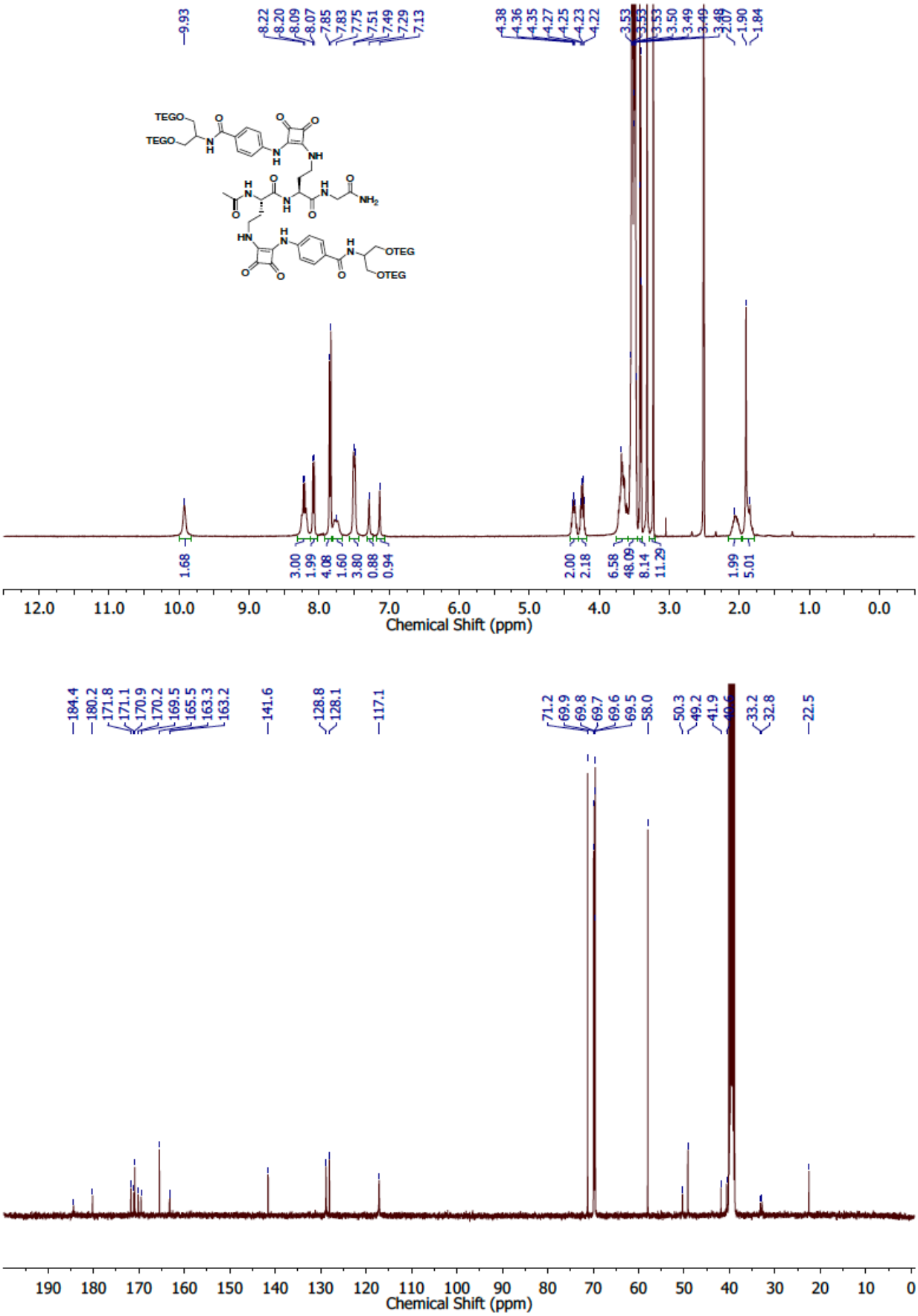
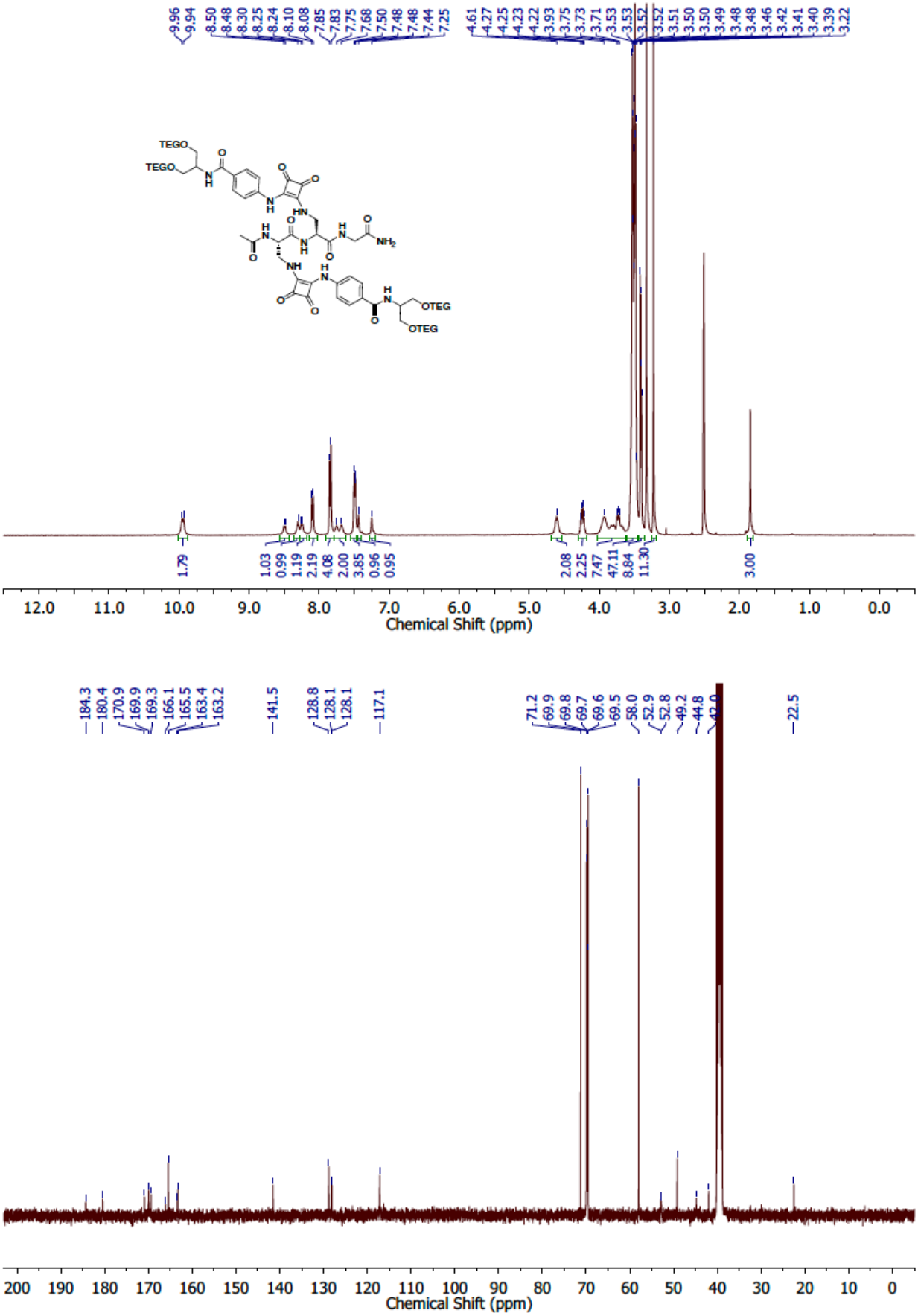


Figure S5: ^{1}H NMR (DMSO-d_6, 400 MHz) and ^{13}C NMR (DMSO-d_6, 100 MHz) spectra of **5**.



NMR Binding Studies

^{1}H NMR spectroscopic titrations were performed on a Bruker Avance DPX 400 spectrometer at a frequency of 400.13 MHz and calibrated to the residual proton solvent peak in DMSO-d_6 ($\delta = 2.50$ ppm). These were performed by adding aliquots of the respective anion (as their TBA salts), in a solution containing the receptor (2.5 mM), to a solution of the receptor (500 µL, 2.5 mM), in the indicated solvent. Non-linear curve fitting of the titration isotherms (equivalents of anion vs. chemical shift of the signal attributable to the amide and/or aromatic protons), and the corresponding association constants (Ka, M^{-1}) were obtained using the commercially available software program HypNMR (Hyper-quad package)[2] and/or bindfit v0.5 (http://supramolecular.org). The obtained Ka values represent the average of two or more independent titrations.

Figure S6: ^{1}H NMR spectra (400 MHz) from the anion screen of receptor **2** with ten equivalents of various anions as their TBA salts in 20% H_2O/DMSO-d_6.

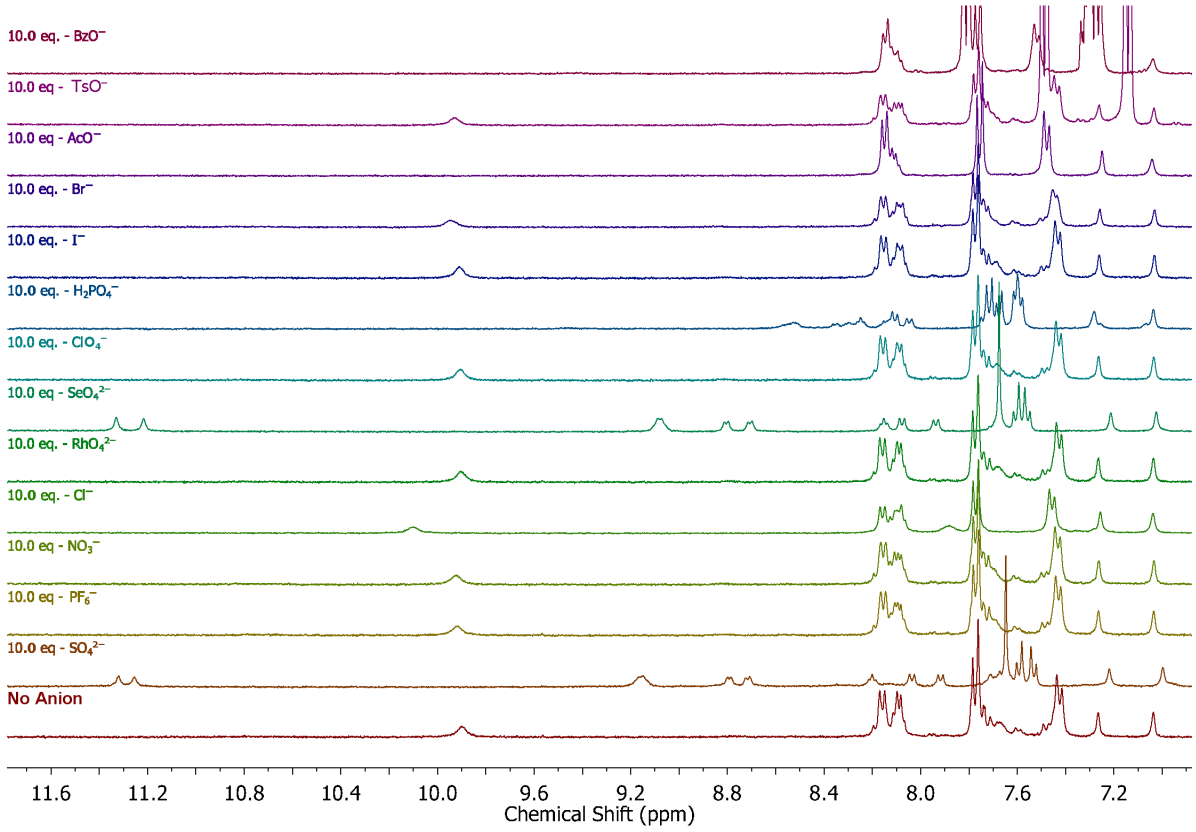
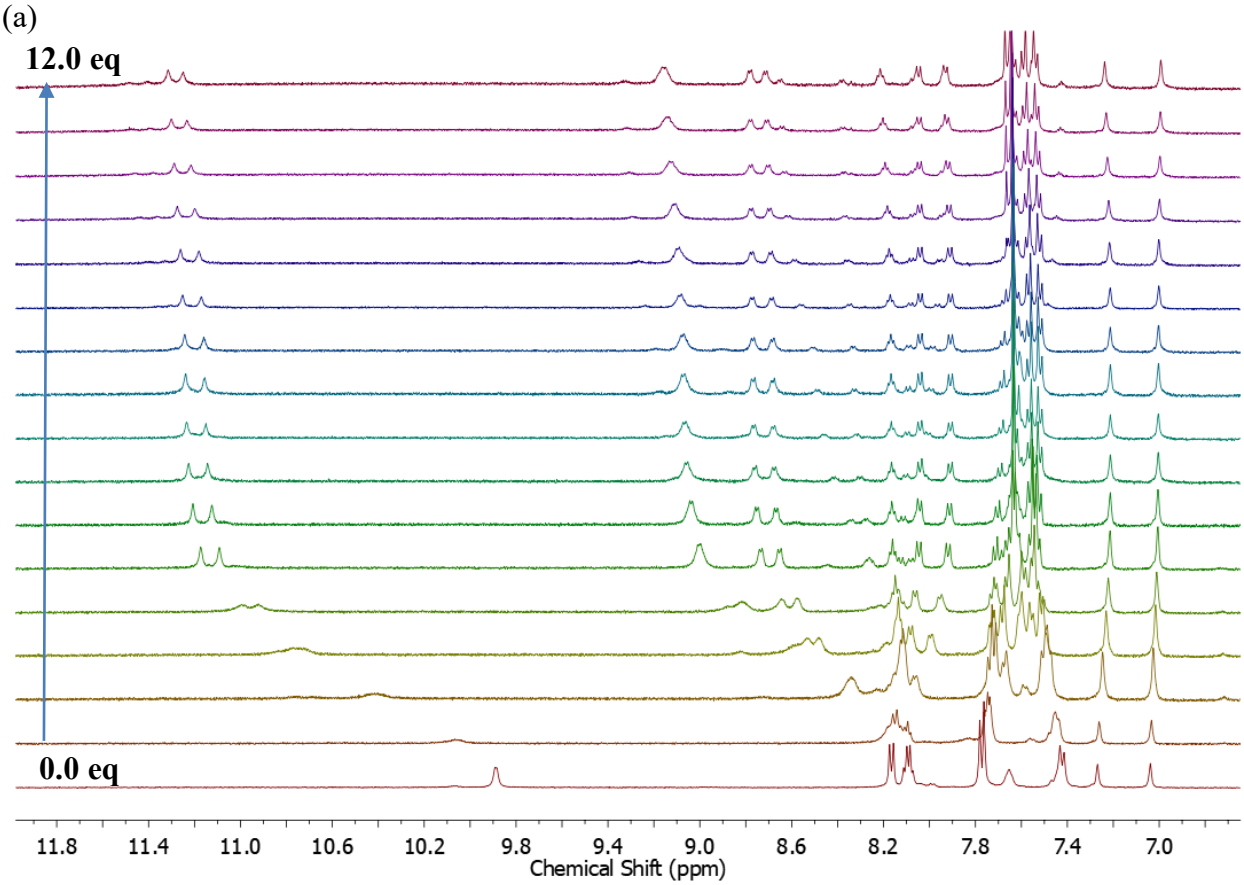


Figure S7: (a) ^{1}H NMR stack plot of **2** with TBA_2SO_4 in 20% H_2O in DMSO-d_6 at 300 K, (b) Titration isotherm for squaramide proton NH at δ = 9.89 ppm

(a)



(b)

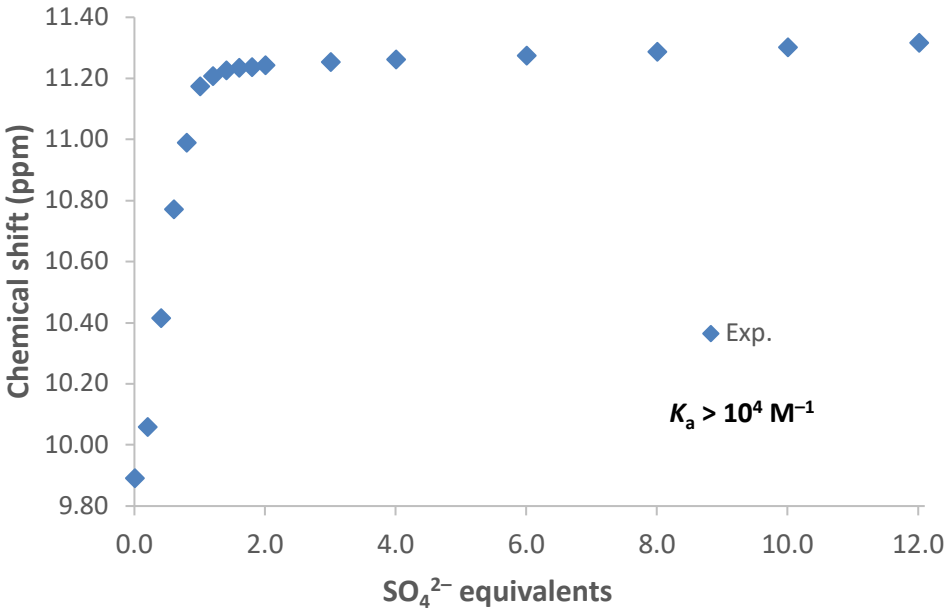
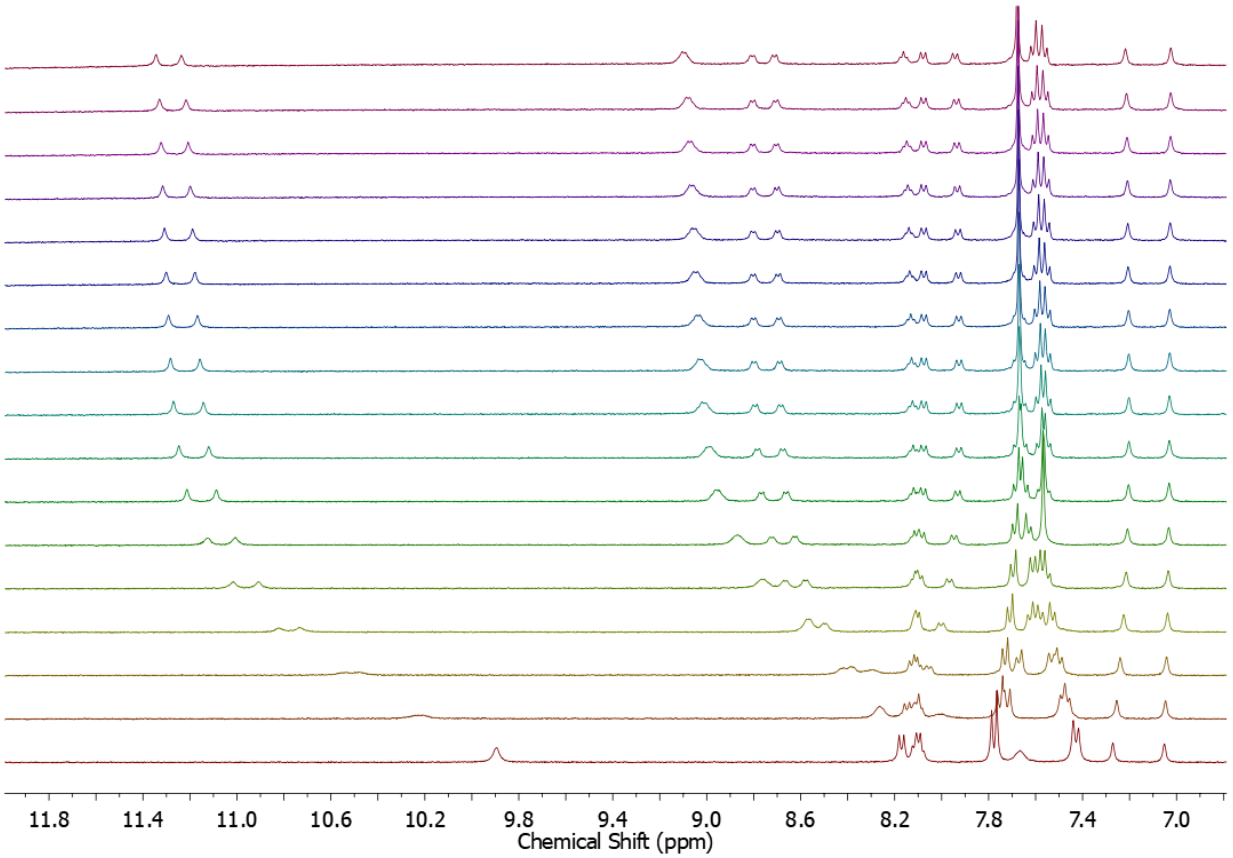


Figure S8: (a) ^{1}H NMR stack plot of **2** with TBA_2SeO_4 in 20% H_2O in DMSO-d_6 at 300 K, (b) Titration isotherm for squaramide proton NH at δ = 9.89 ppm

(a)



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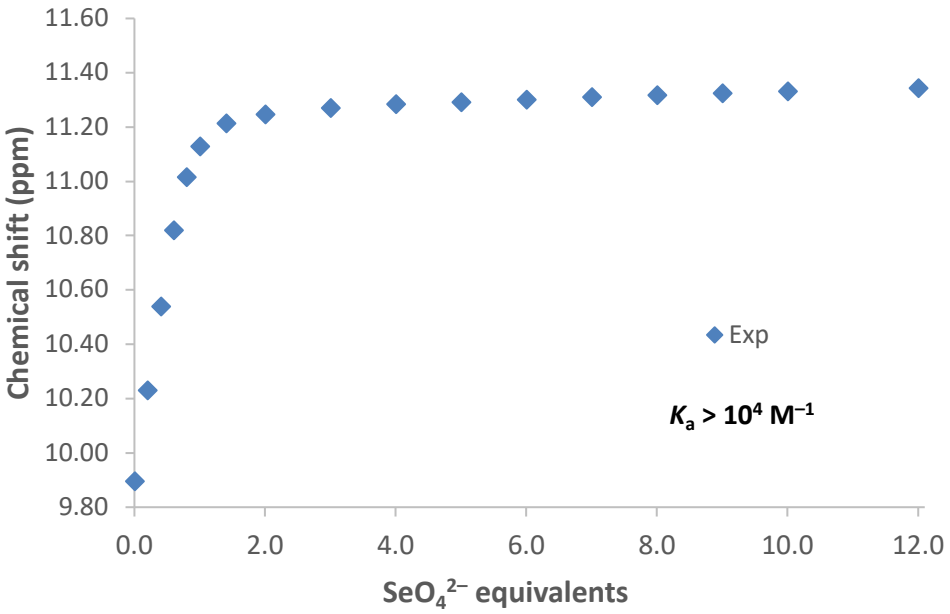
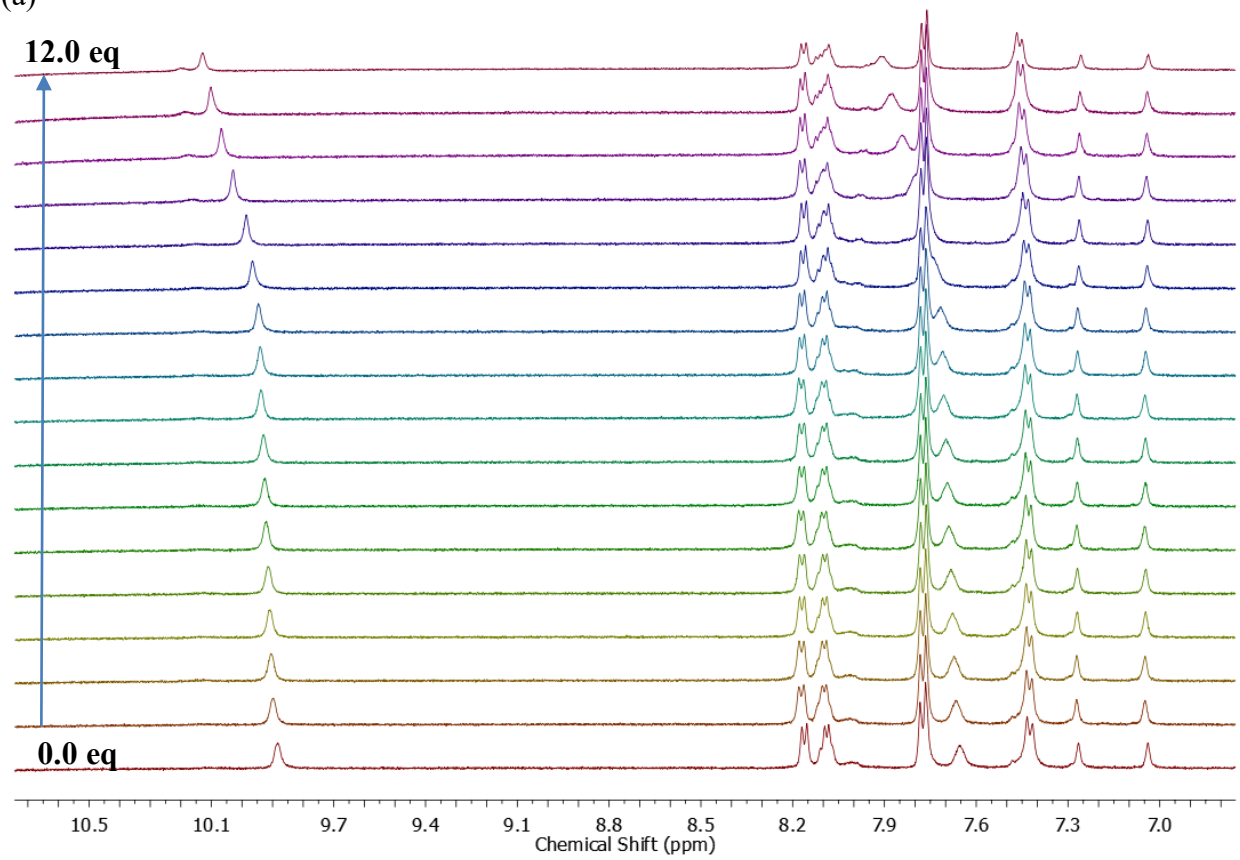


Figure S9: (a) ^{1}H NMR stack plot of **2** with TBACl in 20% H_2O in DMSO-d_6 at 300 K, (b) Titration isotherm for squaramide proton NH at δ = 9.89 ppm

(a)



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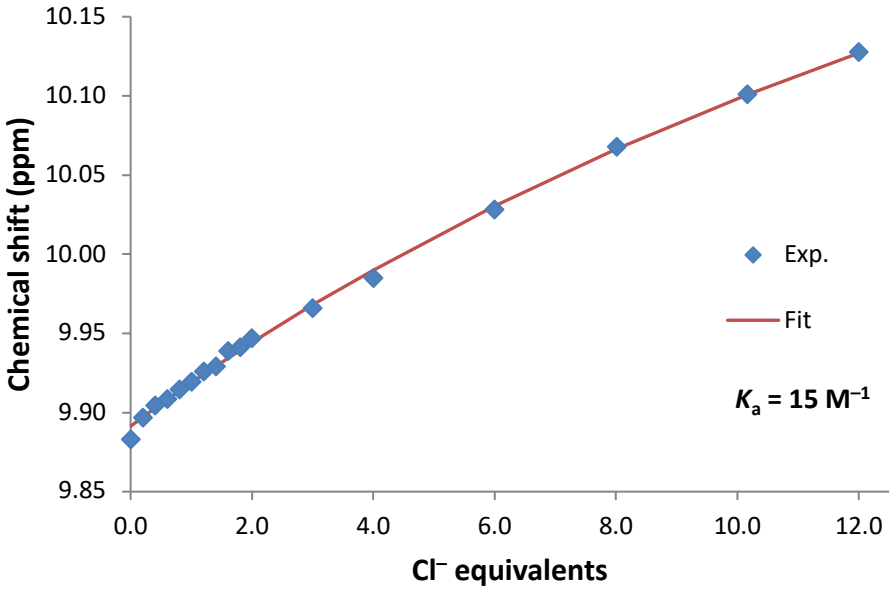
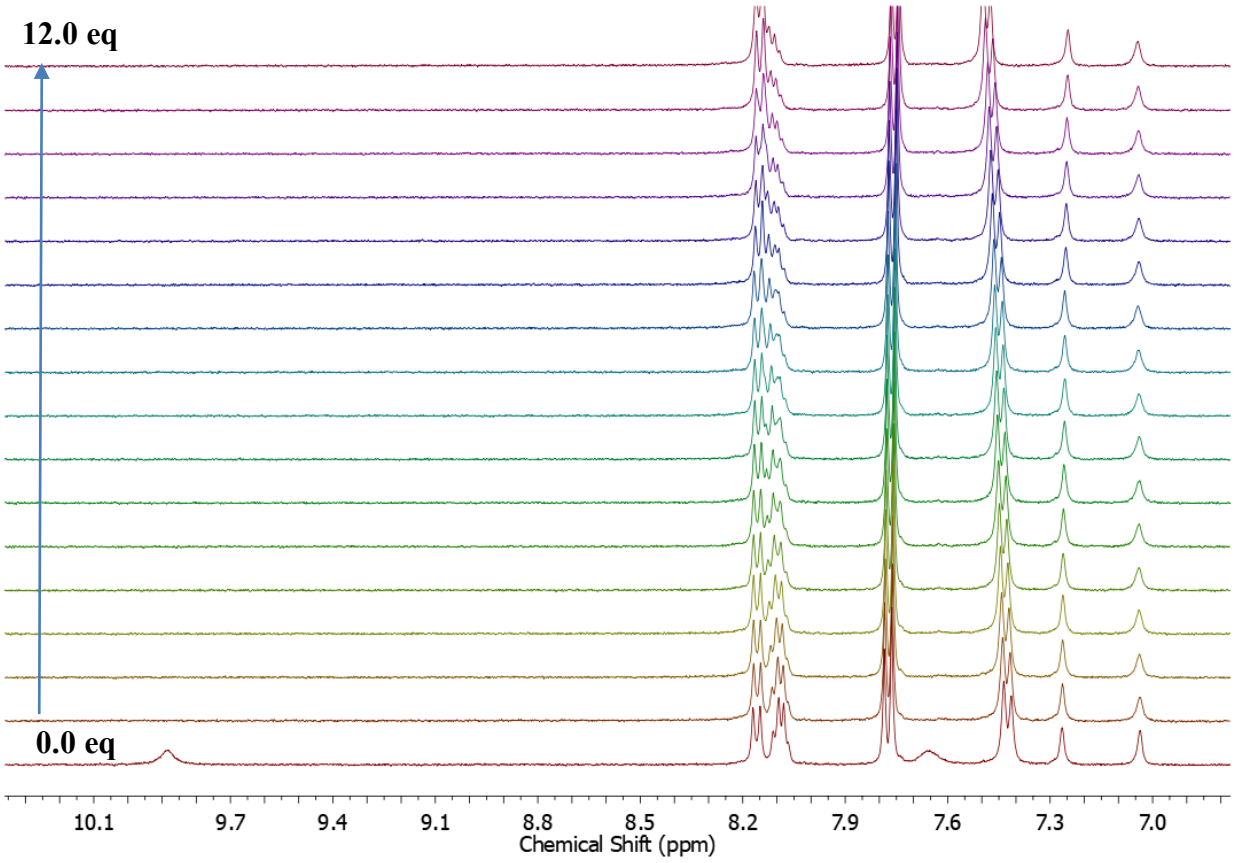


Figure S10: (a) ^{1}H NMR stack plot of **2** with TBAAcO in 20% H_2O in DMSO-d_6 at 300 K, (b) Titration isotherm for aromatic proton CH at δ = 7.44 ppm

(a)



(b)

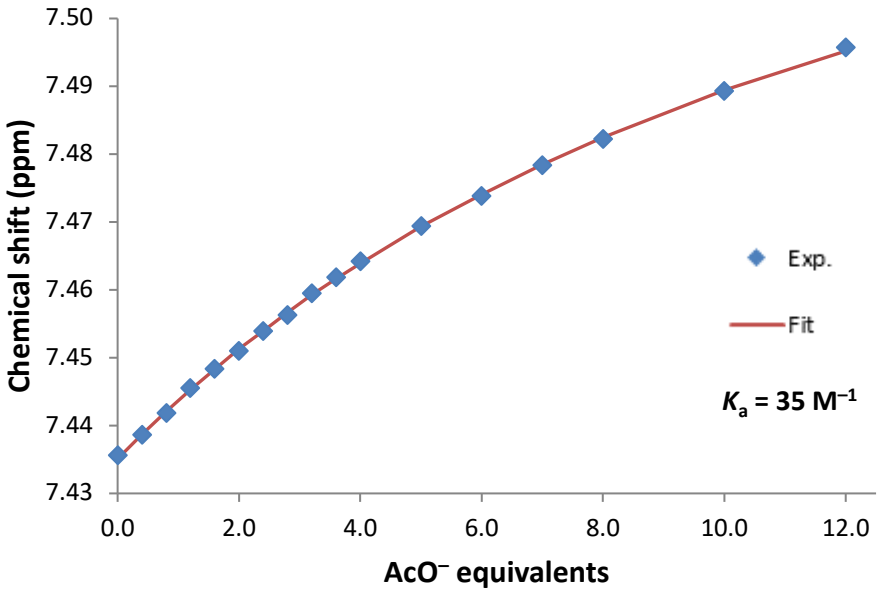
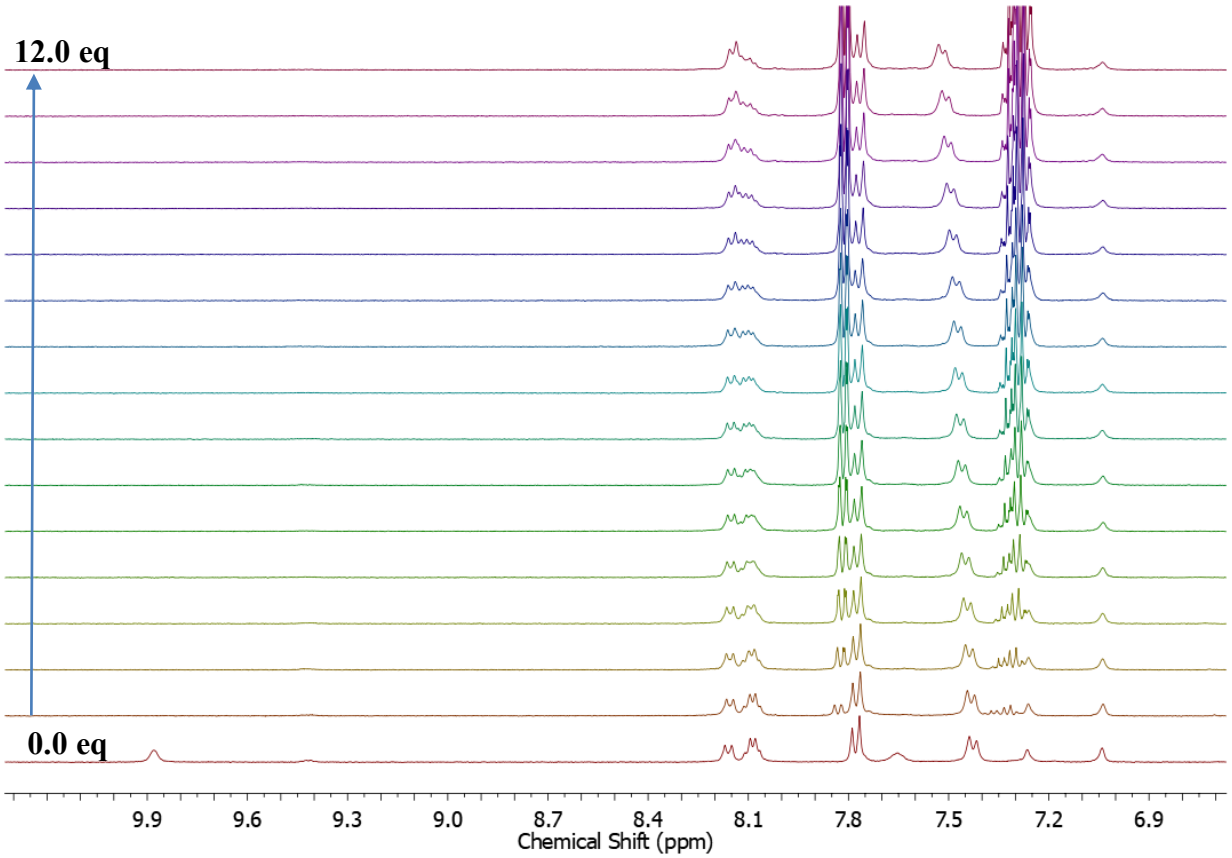


Figure S11: (a) ^{1}H NMR stack plot of **2** with TBABzO in 20% H_2O in DMSO-d_6 at 300 K, (b) Titration isotherm for aromatic proton CH at δ = 7.44 ppm

(a)



(b)

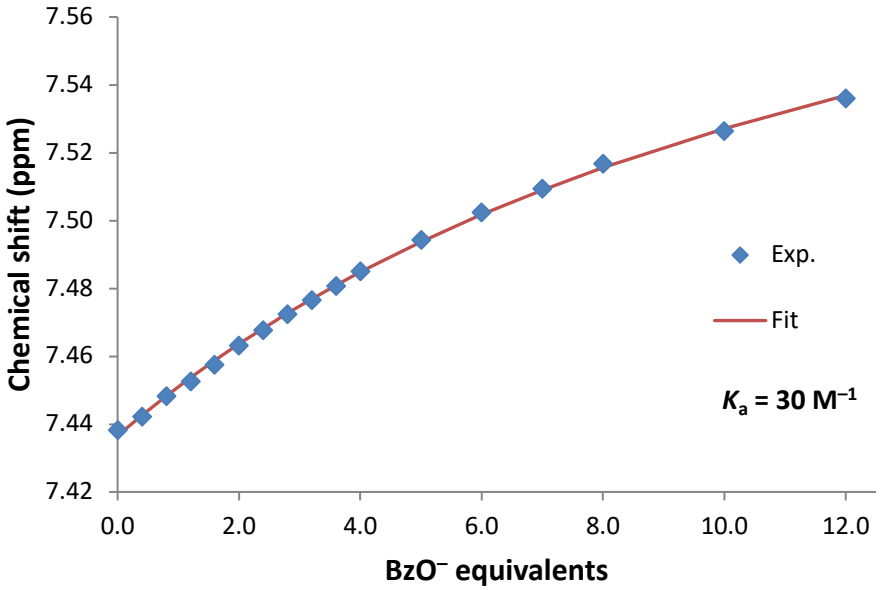
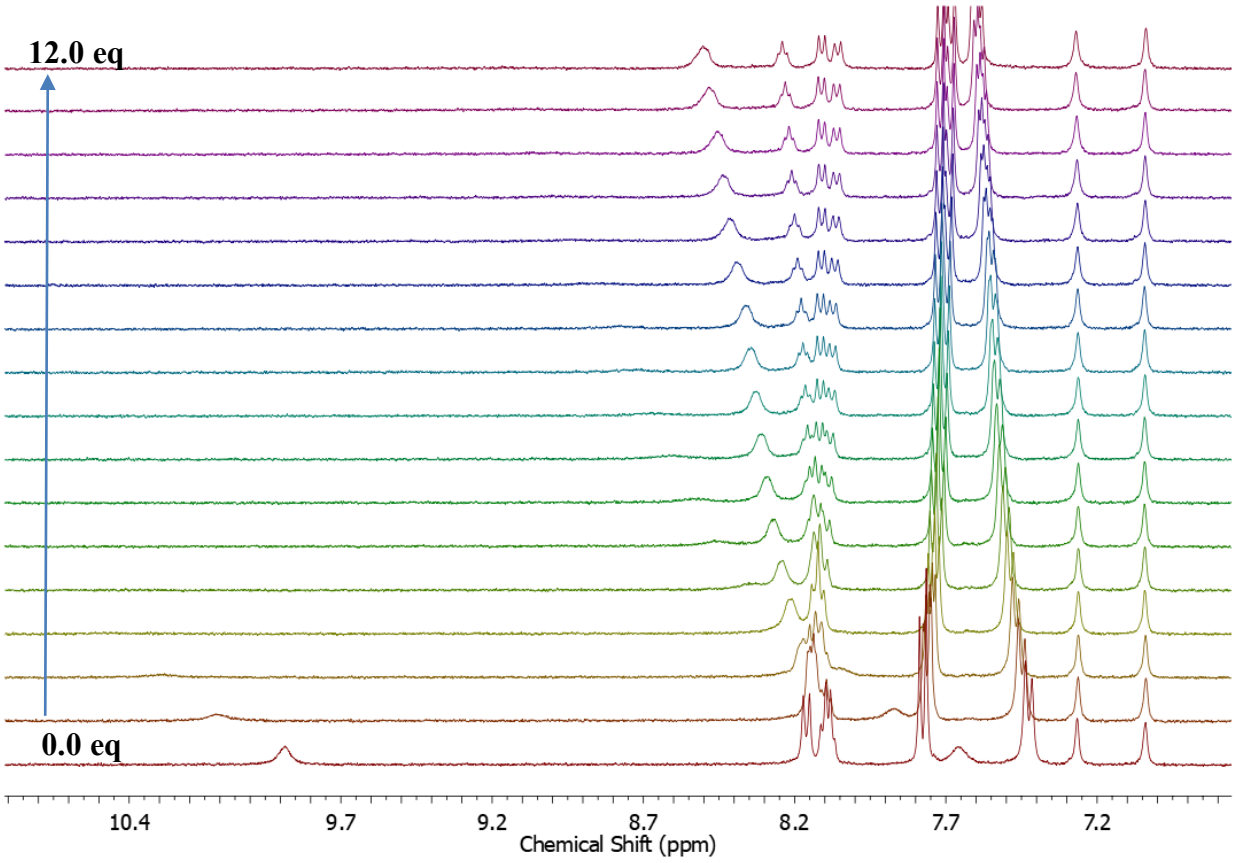


Figure S12: (a) ^{1}H NMR stack plot of **2** with $TBAH_2PO_4$ in 20% H_2O in DMSO-d_6 at 300 K, (b) Titration isotherm for aromatic proton CH at δ = 7.44 ppm

(a)



(b)

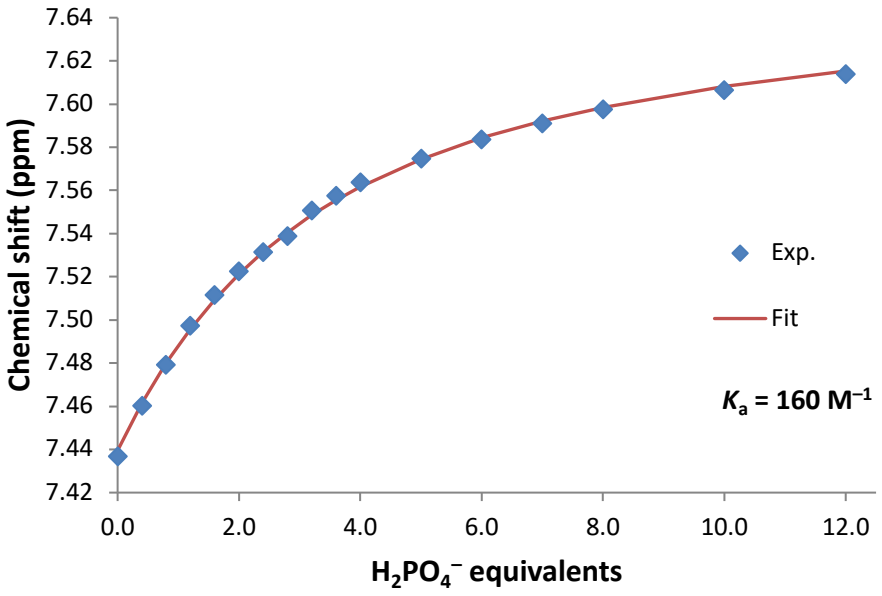
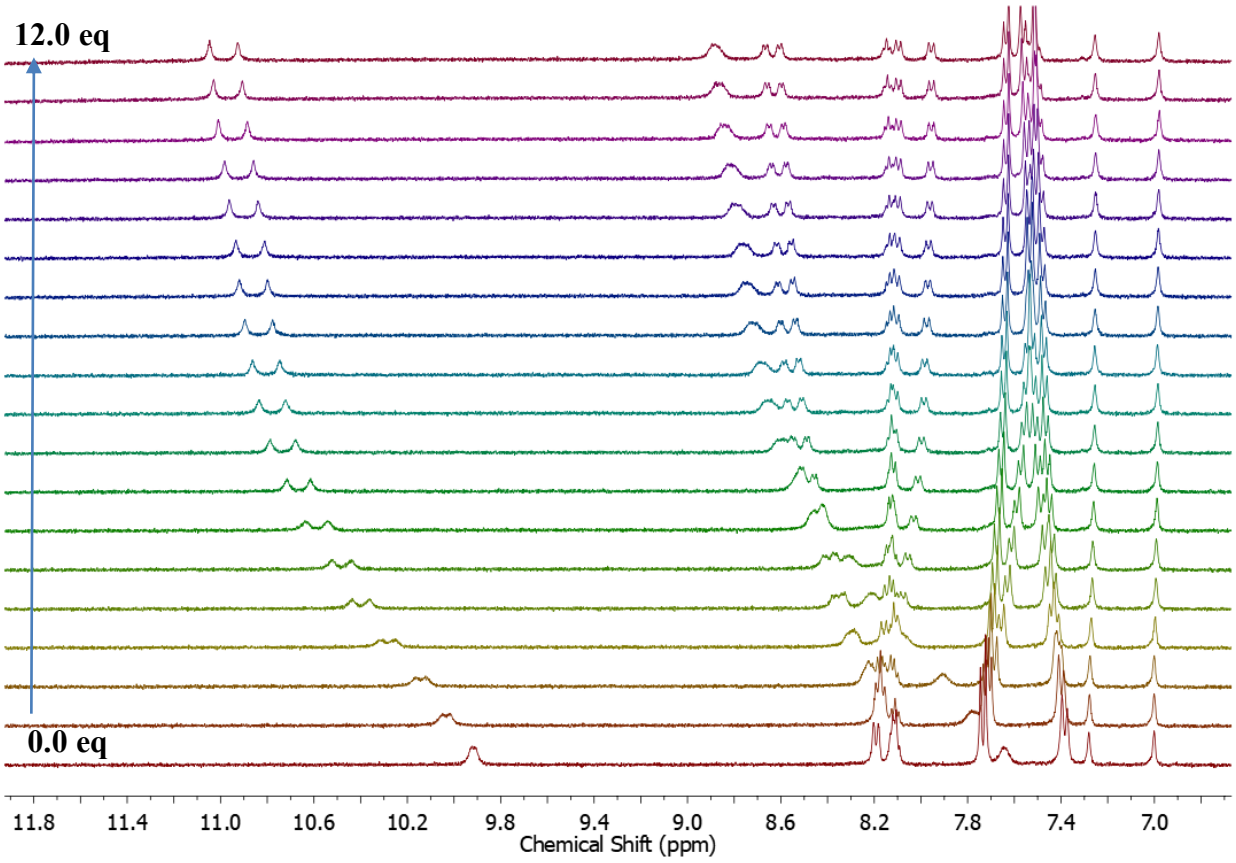


Figure S13: (a) ^{1}H NMR stack plot of **2** with TBA_2SO_4 in 35% H_2O in DMSO-d_6 at 300 K, (b) Titration isotherm for squaramide proton NH at δ = 9.92 ppm

(a)



(b)

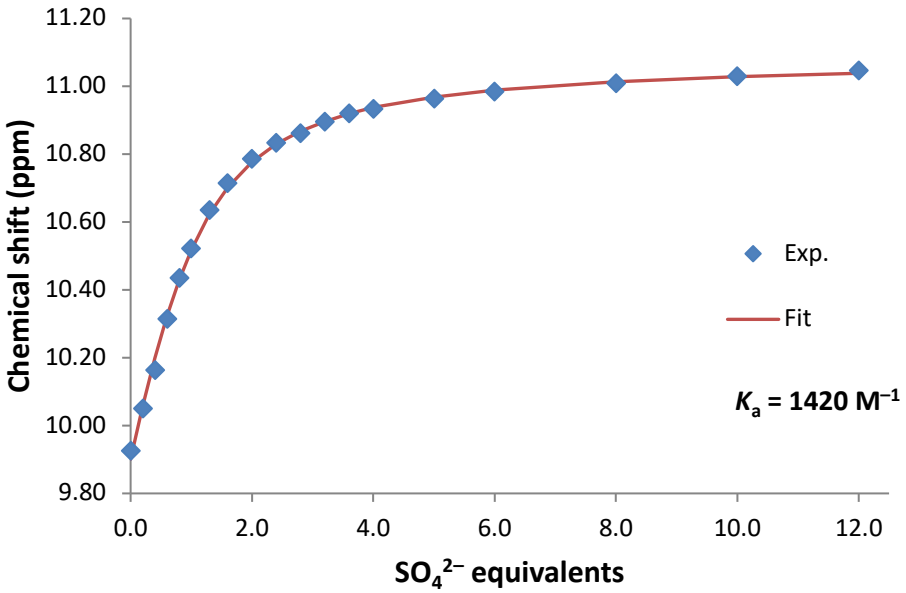
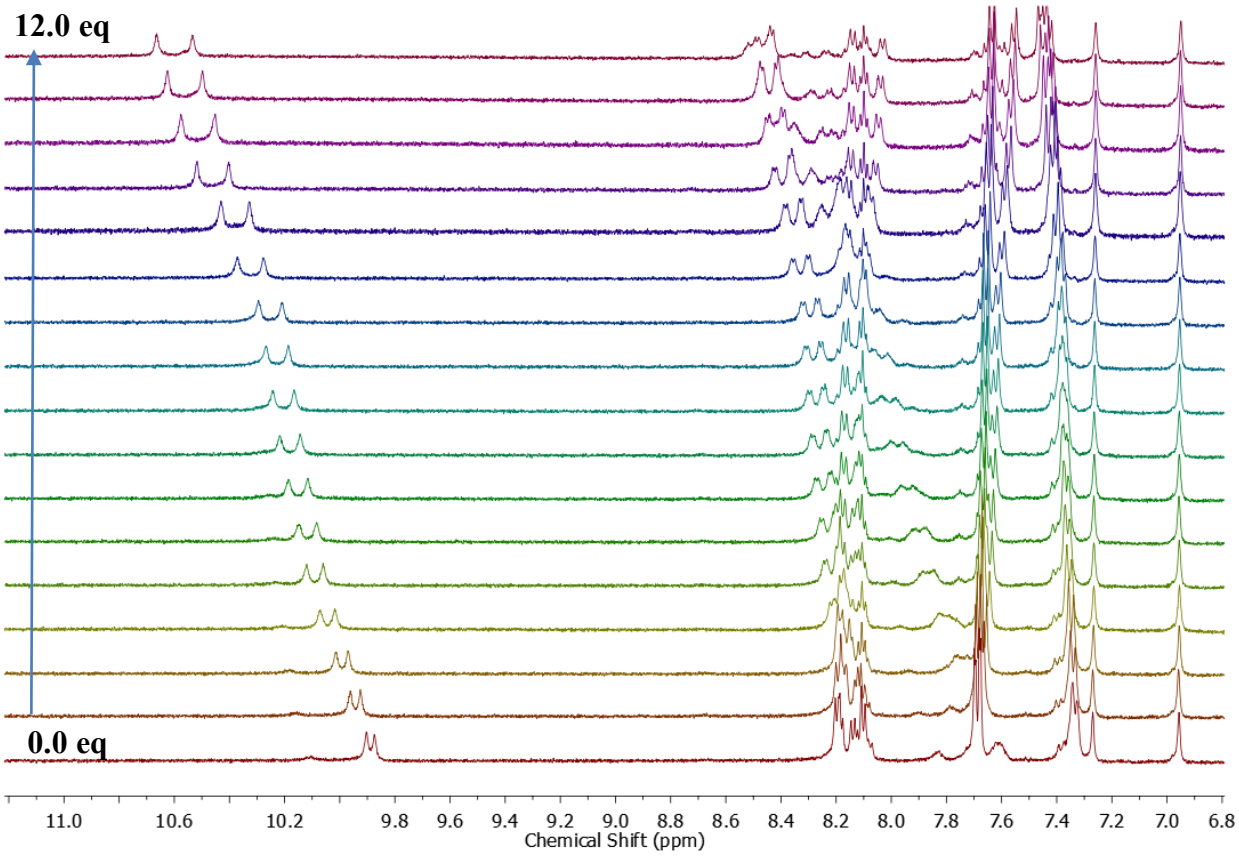


Figure S14: (a) ^{1}H NMR stack plot of **2** with TBA_2SO_4 in 50% H_2O in DMSO-d_6 at 300 K, (b) Titration isotherm for squaramide proton NH at δ = 9.90 ppm

(a)



(b)

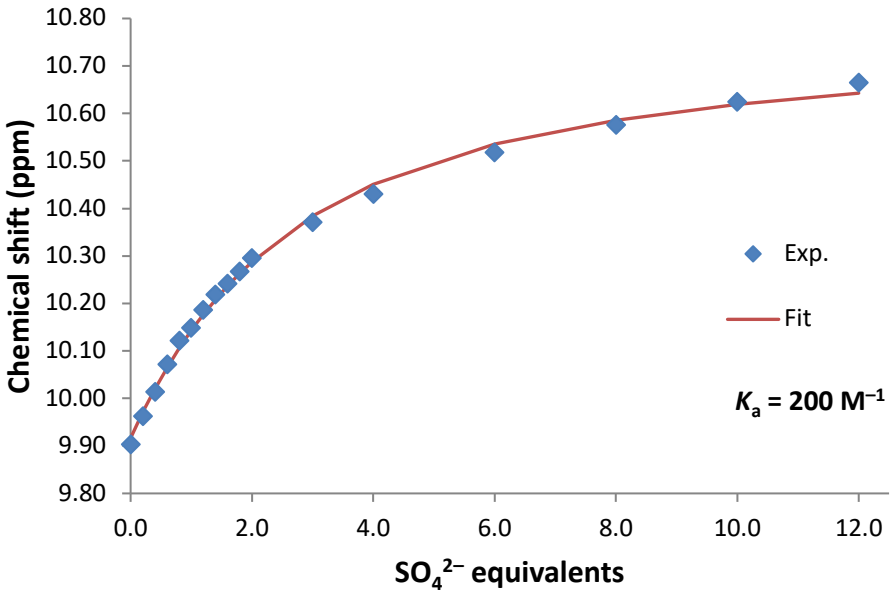
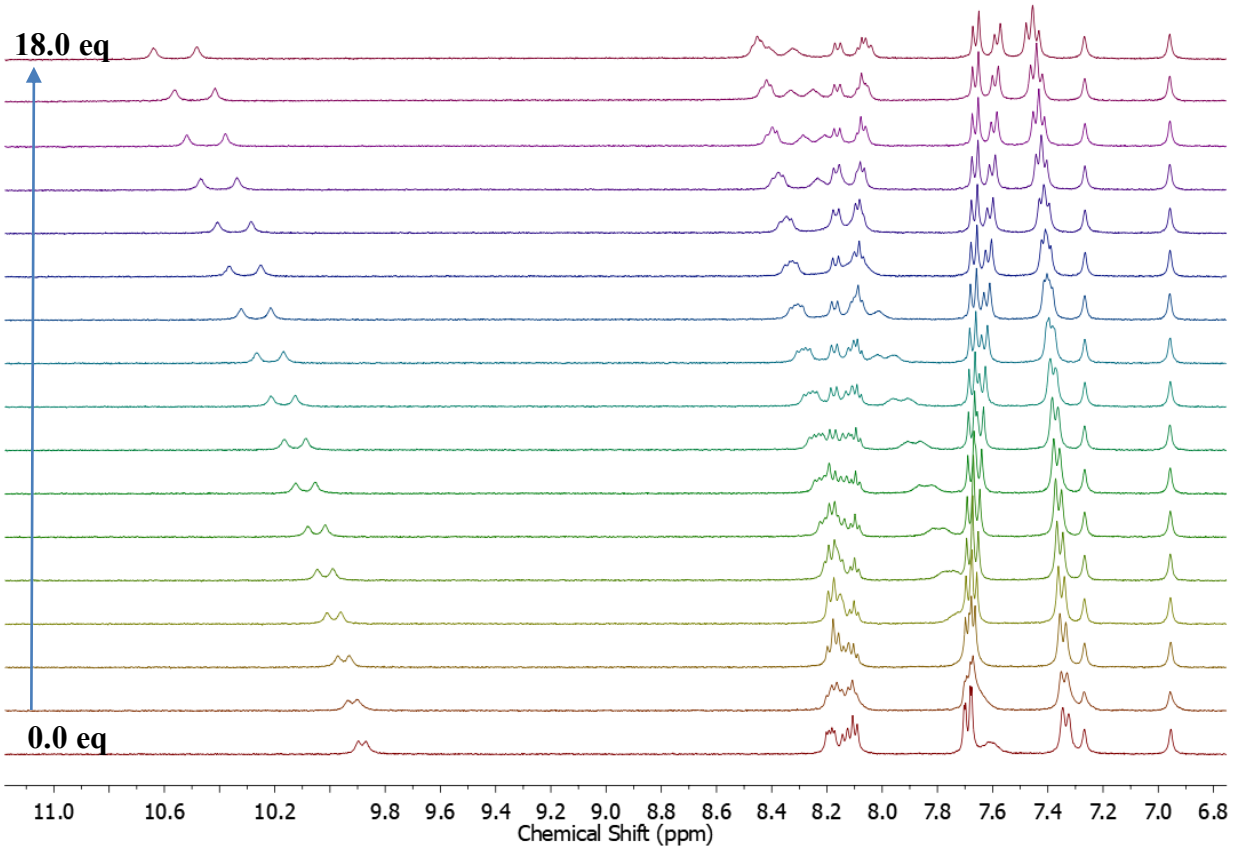


Figure S15: (a) ^{1}H NMR stack plot of **2** with TBA_2SeO_4 in 50% H_2O in DMSO-d_6 at 300 K, (b) Titration isotherm for squaramide proton NH at δ = 9.90 ppm

(a)



(b)

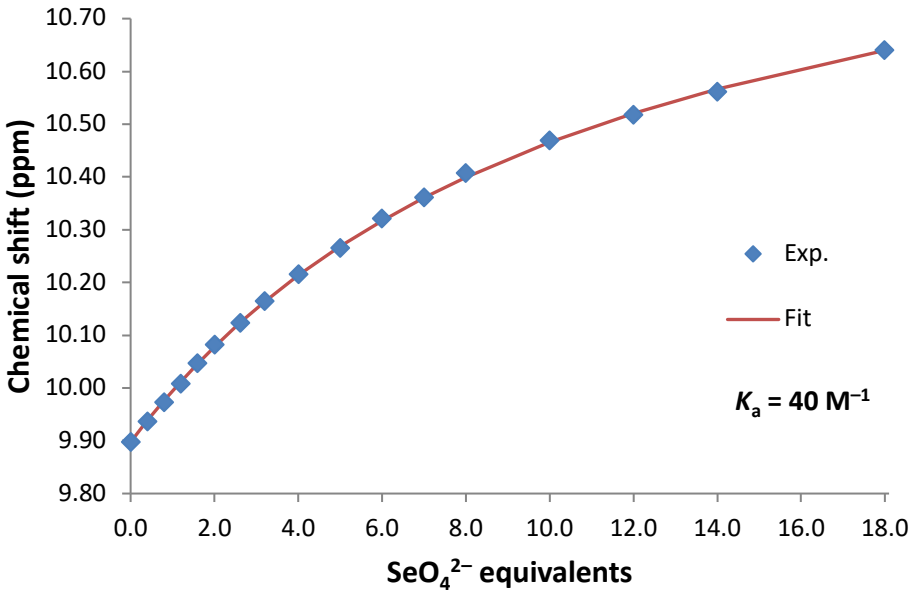
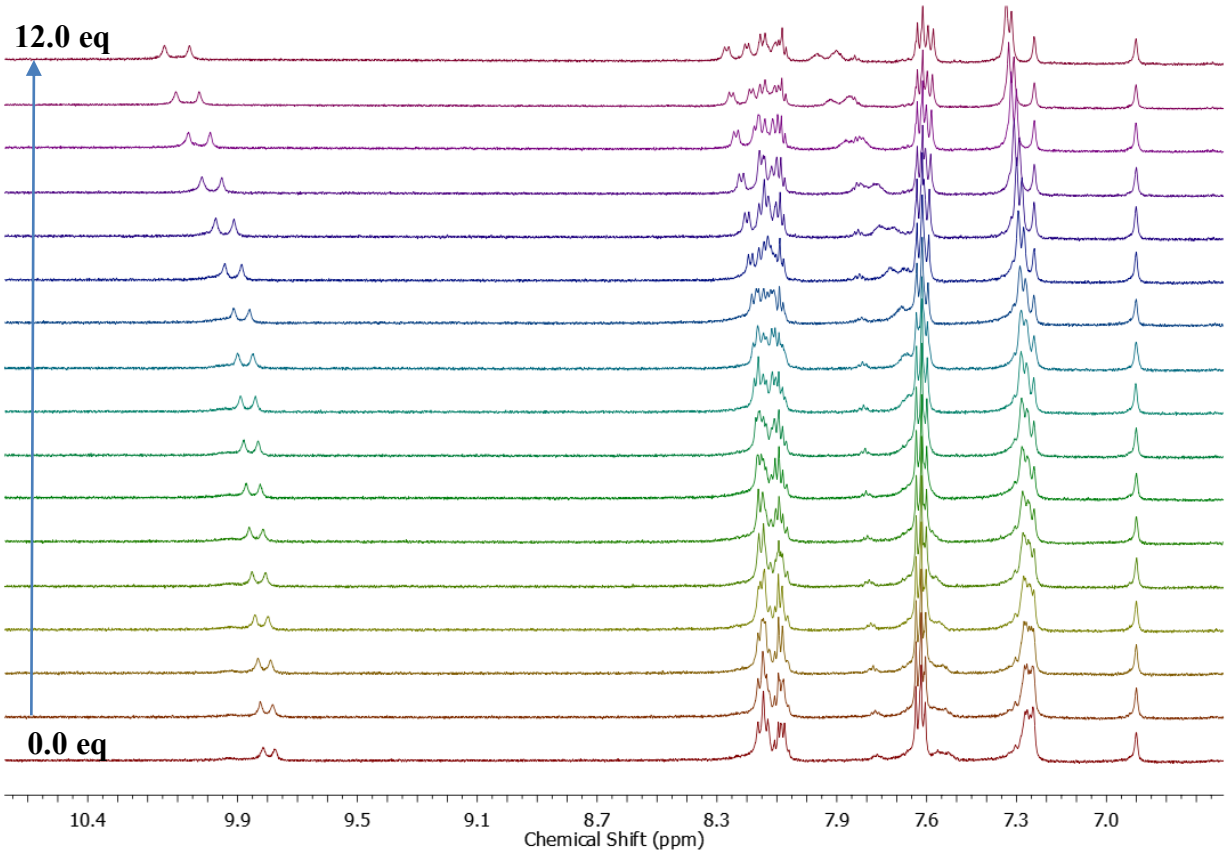


Figure S16: (a) ^{1}H NMR stack plot of **2** with TBA_2SO_4 in 75% H_2O in DMSO-d_6 at 300 K, (b) Titration isotherm for squaramide proton NH at δ = 9.82 ppm

(a)



(b)

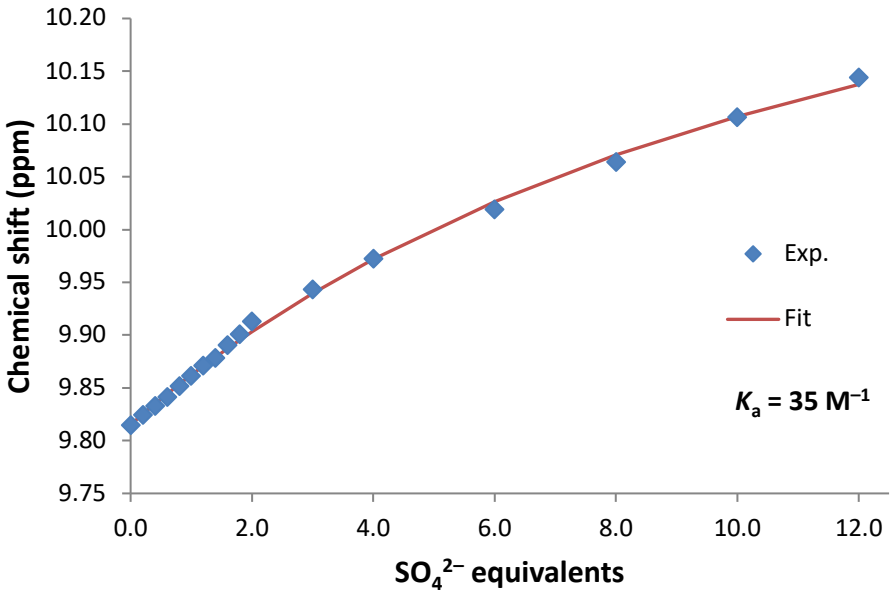
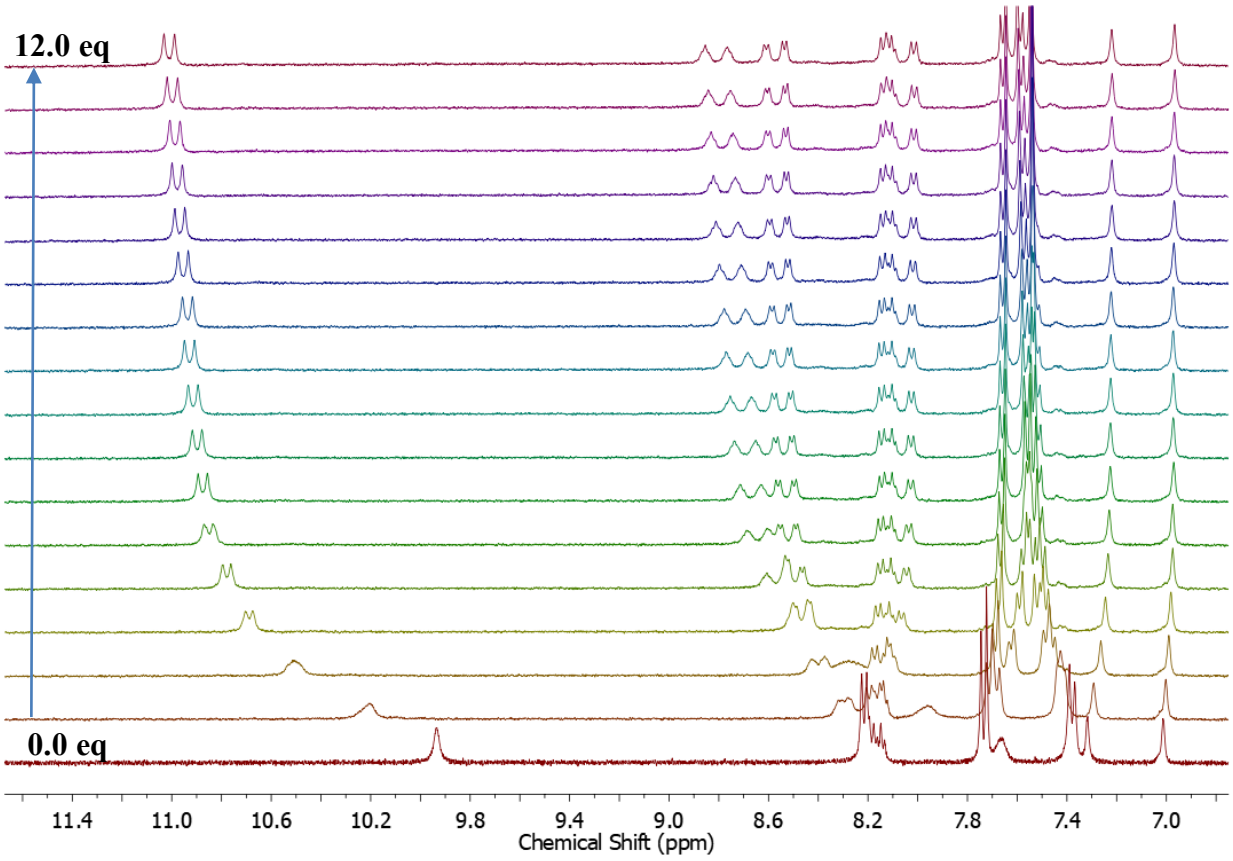


Figure S17: (a) ^{1}H NMR stack plot of **3** with TBA_2SO_4 in 35% H_2O in DMSO-d_6 at 300 K, (b) Titration isotherm for squaramide proton NH at δ = 9.93 ppm

(a)



(b)

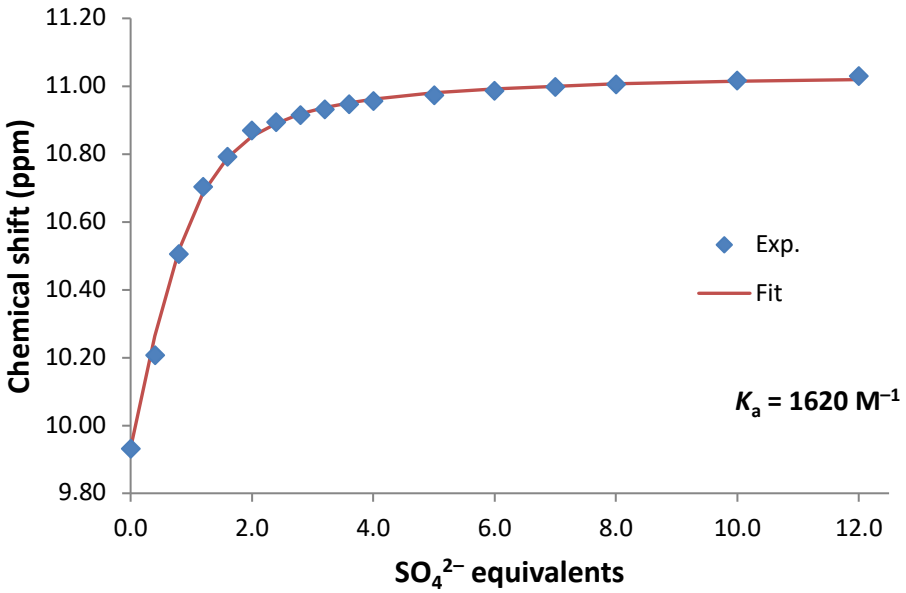
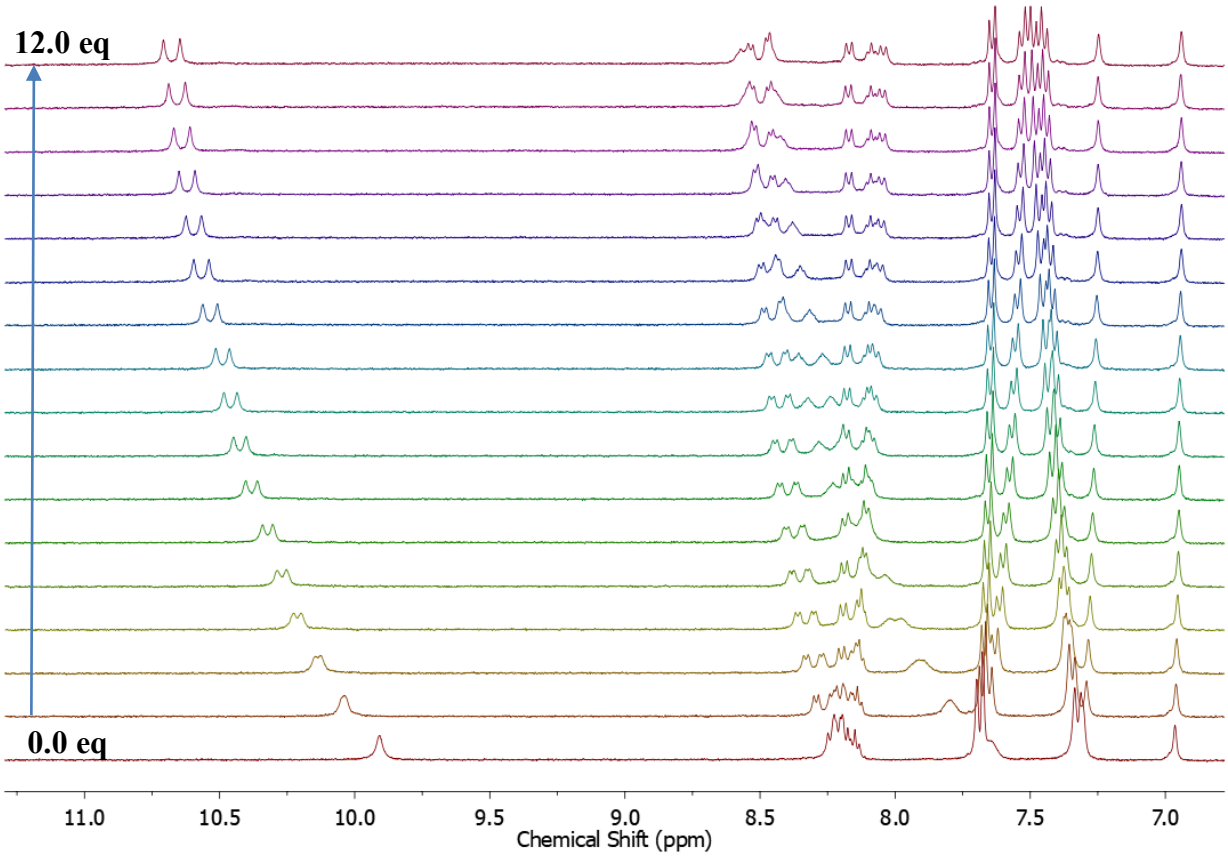


Figure S18: (a) ^{1}H NMR stack plot of **3** with TBA_2SO_4 in 50% H_2O in DMSO-d_6 at 300 K, (b) Titration isotherm for squaramide proton NH at δ = 9.90 ppm

(a)



(b)

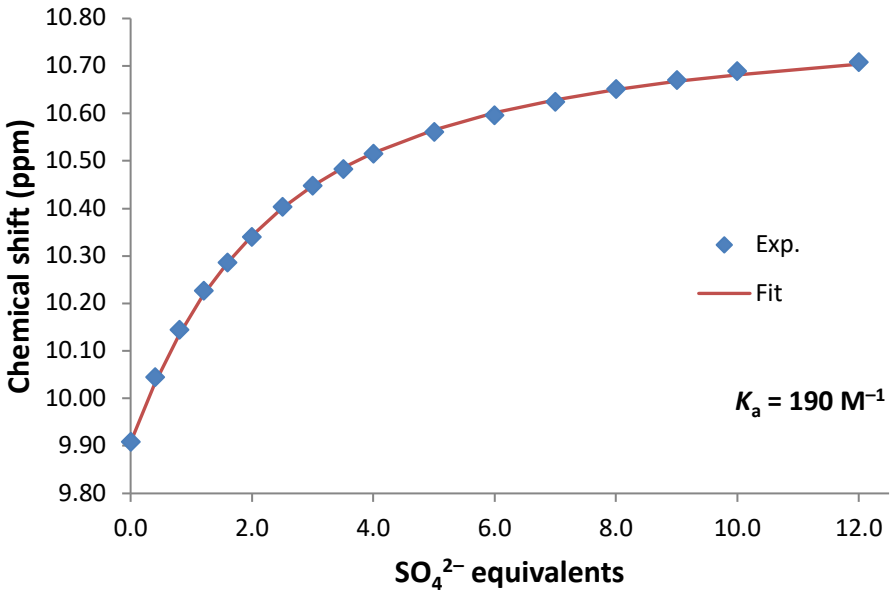
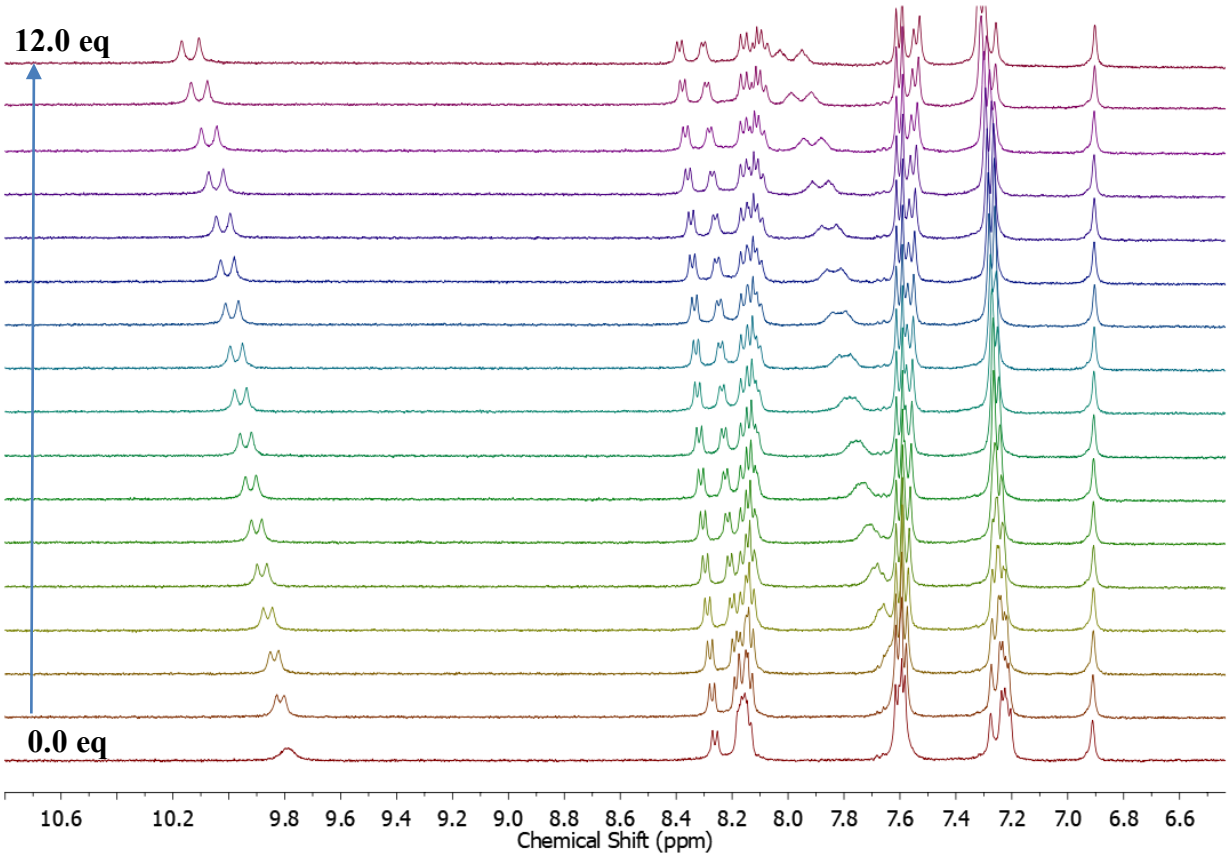


Figure S19: (a) ^{1}H NMR stack plot of **3** with TBA_2SO_4 in 75% H_2O in DMSO-d_6 at 300 K, (b) Titration isotherm for squaramide proton NH at δ = 9.79 ppm

(a)



(b)

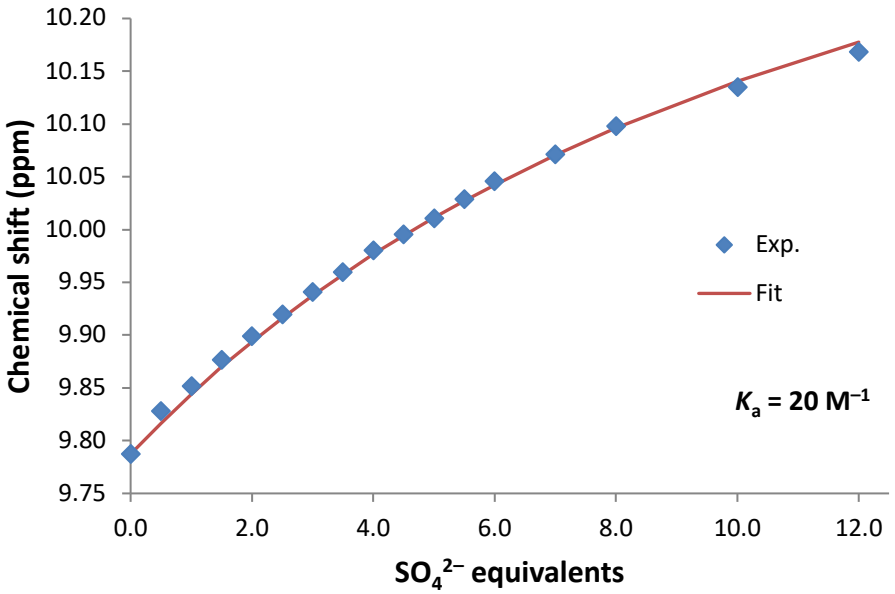
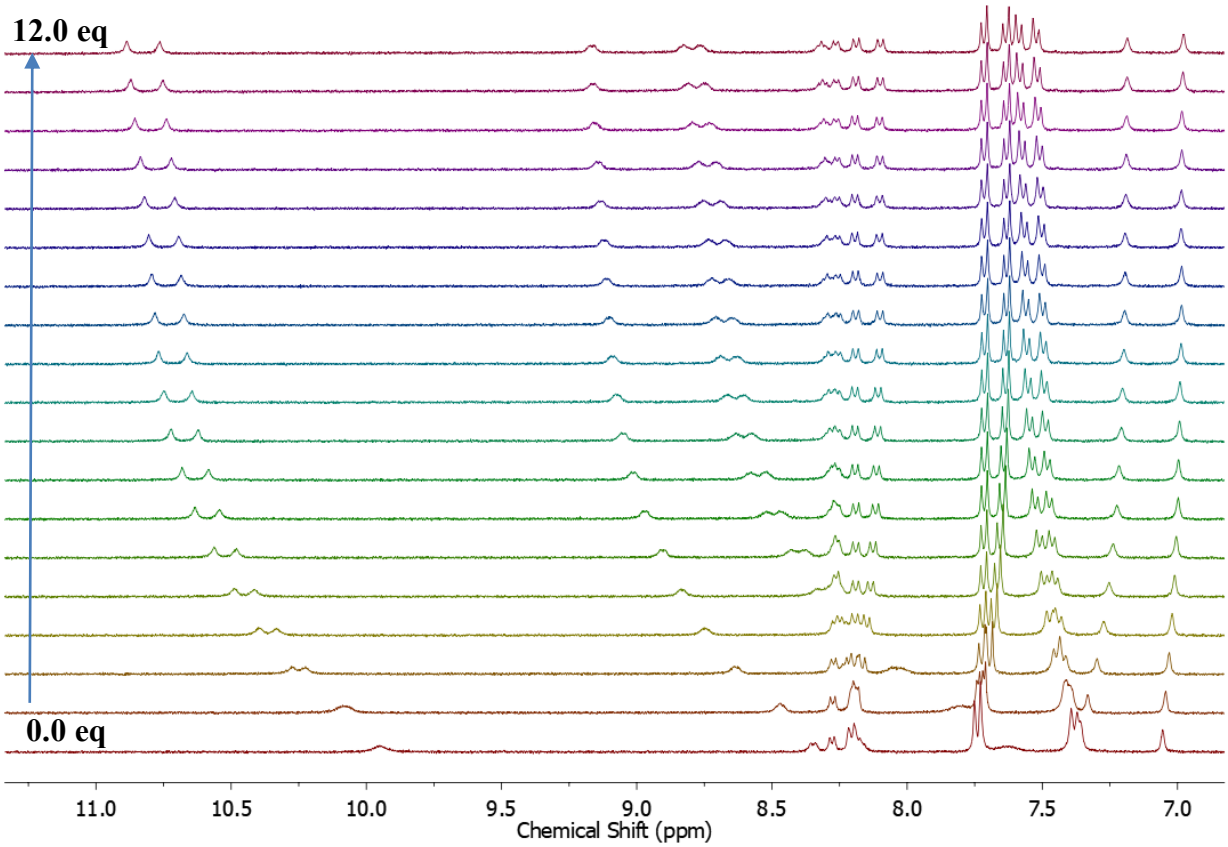


Figure S20: (a) ^{1}H NMR stack plot of **4** with TBA_2SO_4 in 35% H_2O in DMSO-d_6 at 300 K, (b) Titration isotherm for squaramide proton NH at δ = 9.93 ppm

(a)



(b)

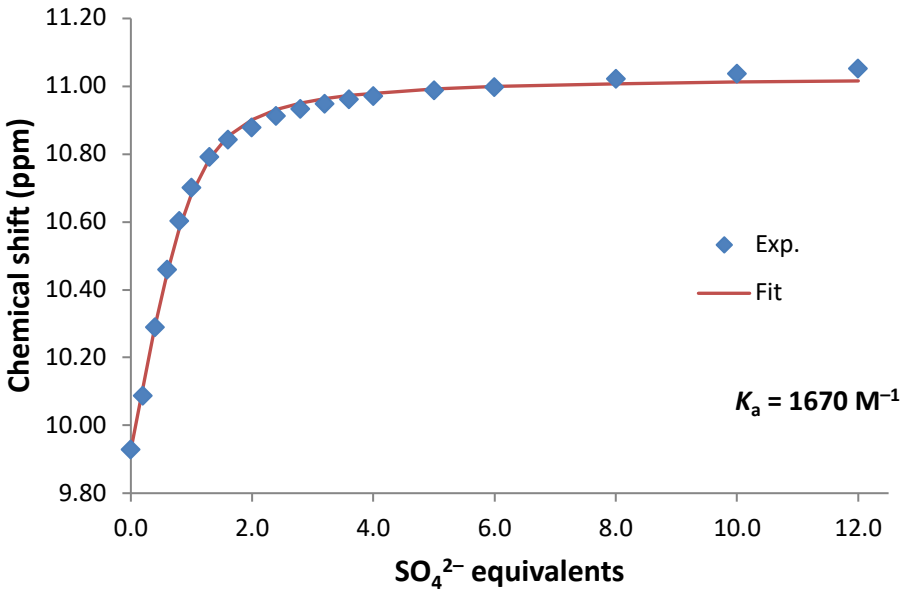
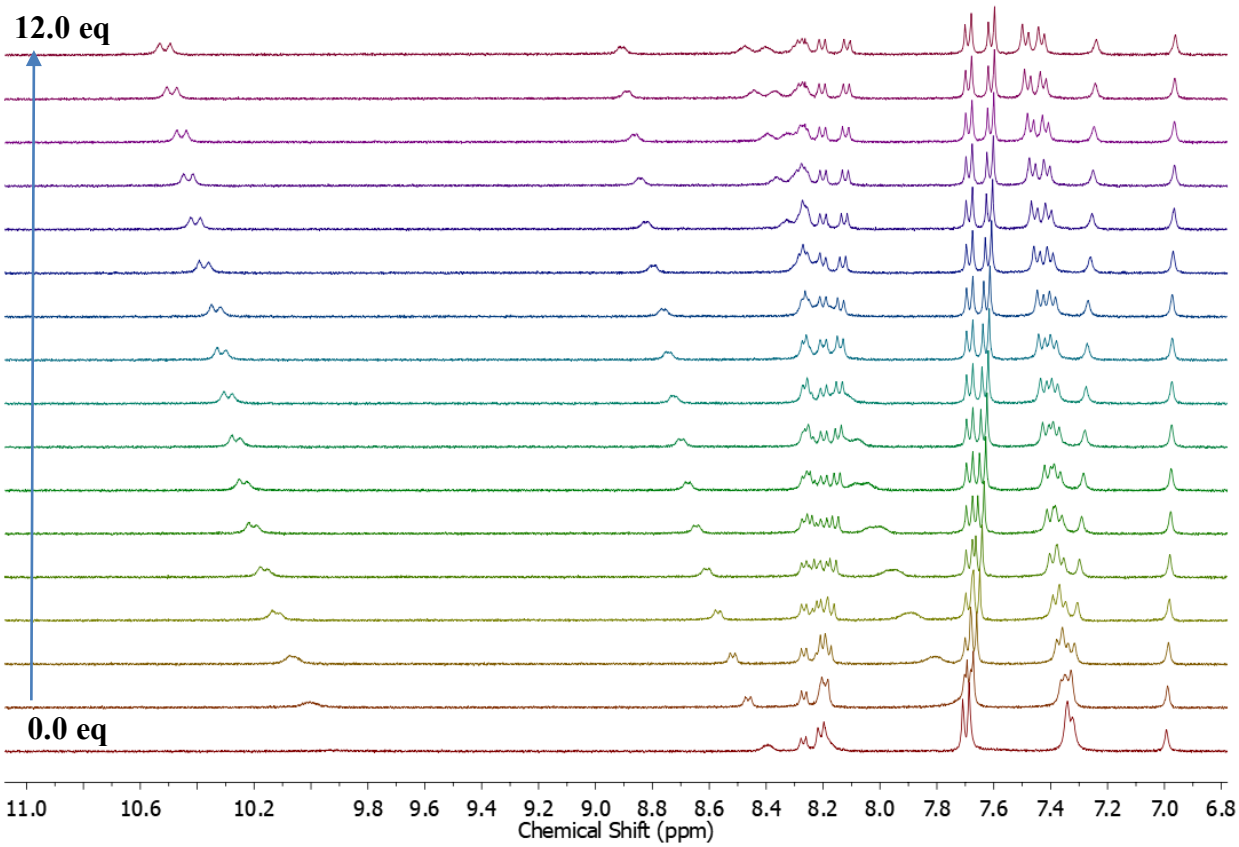


Figure S21: (a) ^{1}H NMR stack plot of **4** with TBA_2SO_4 in 50% H_2O in DMSO-d_6 at 300 K, (b) Titration isotherm for squaramide proton NH at δ = 9.93 ppm

(a)



(b)

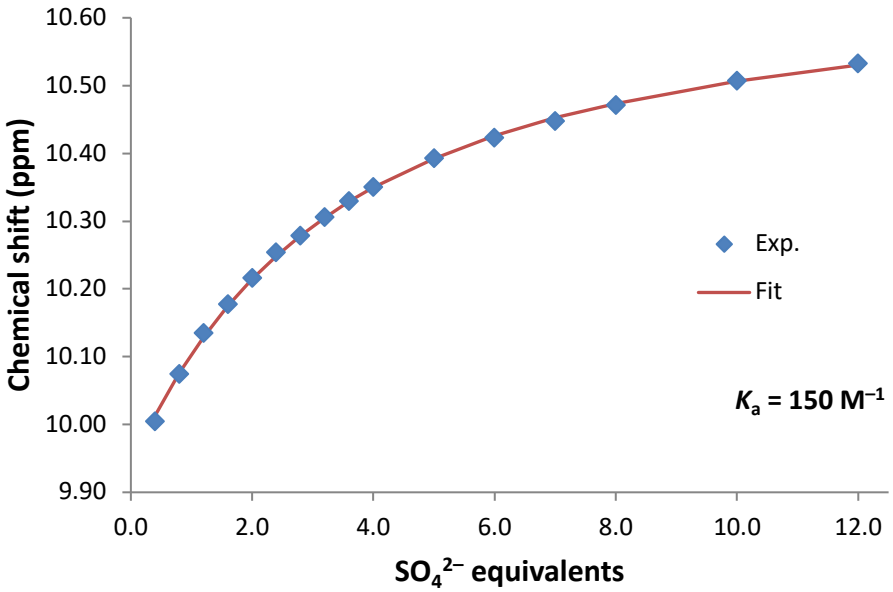
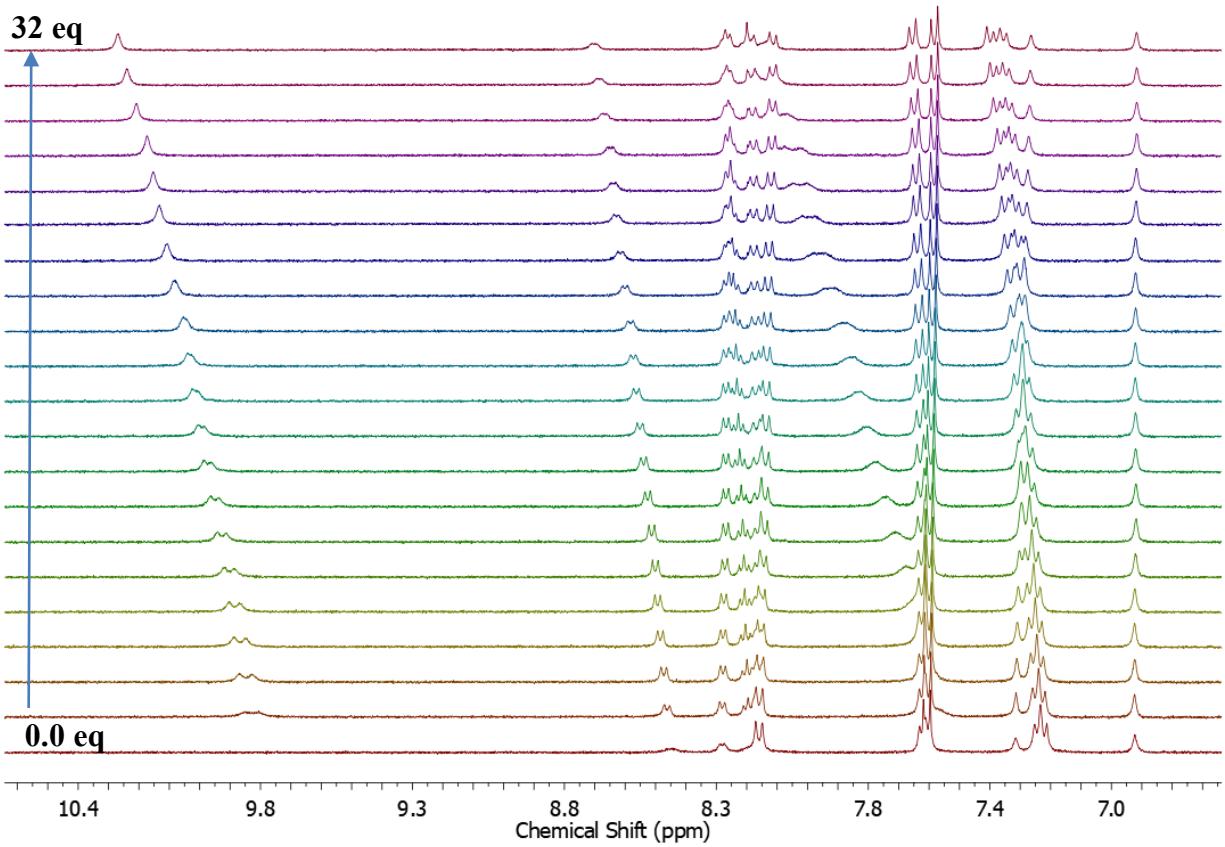


Figure S22: (a) ^{1}H NMR stack plot of **4** with TBA_2SO_4 in 75% H_2O in DMSO-d_6 at 300 K, (b) Titration isotherm for squaramide proton NH at δ = 9.85 ppm

(a)



(b)

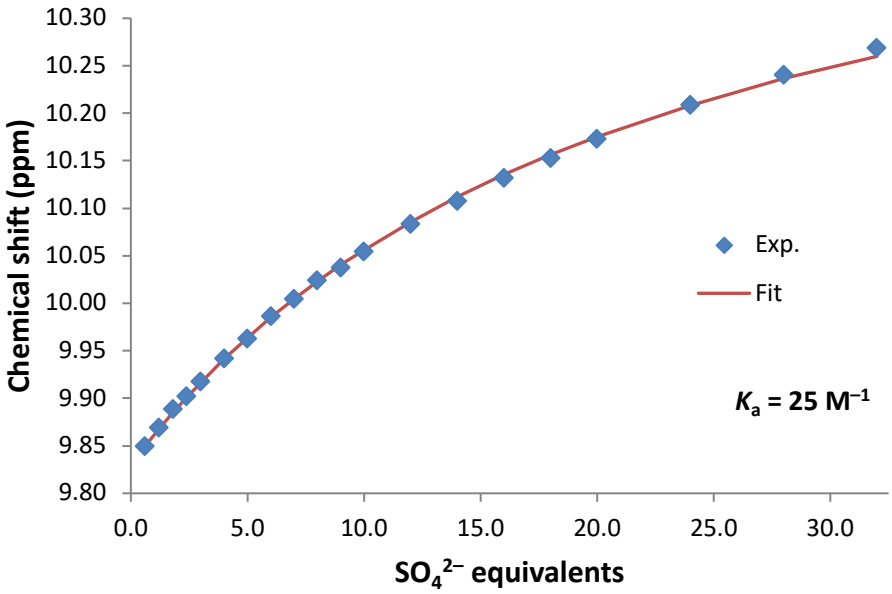
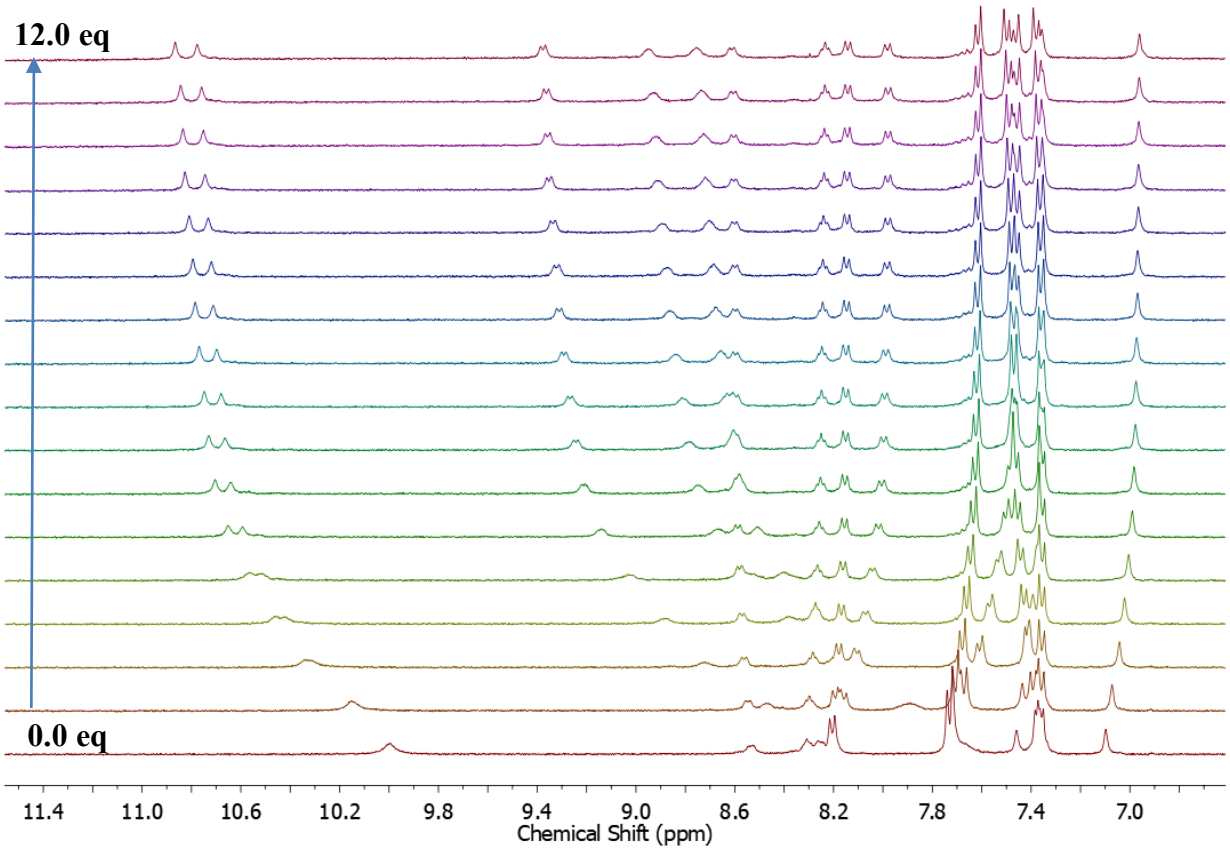


Figure S23: (a) ^{1}H NMR stack plot of **5** with TBA_2SO_4 in 35% H_2O in DMSO-d_6 at 300 K, (b) Titration isotherm for squaramide proton NH at δ = 10.0 ppm

(a)



(b)

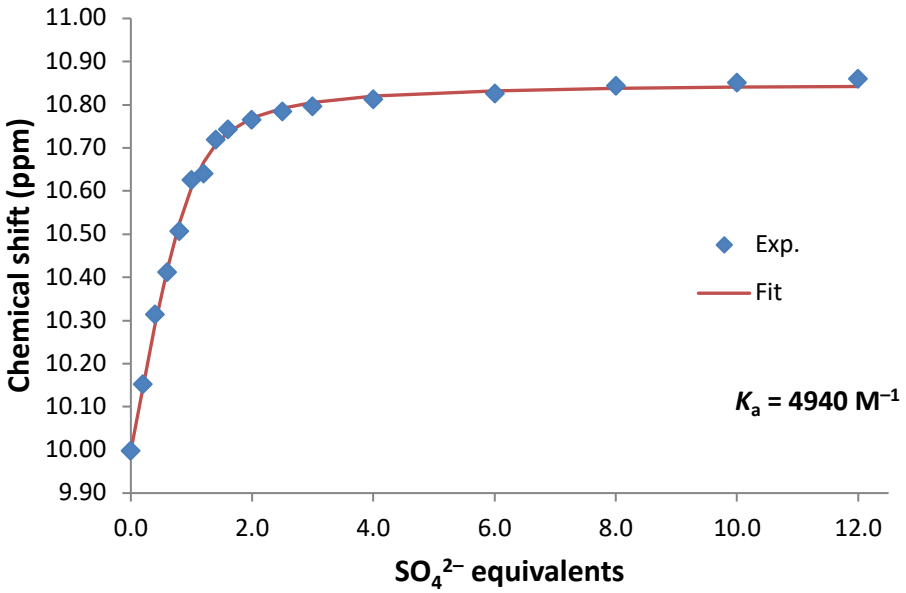
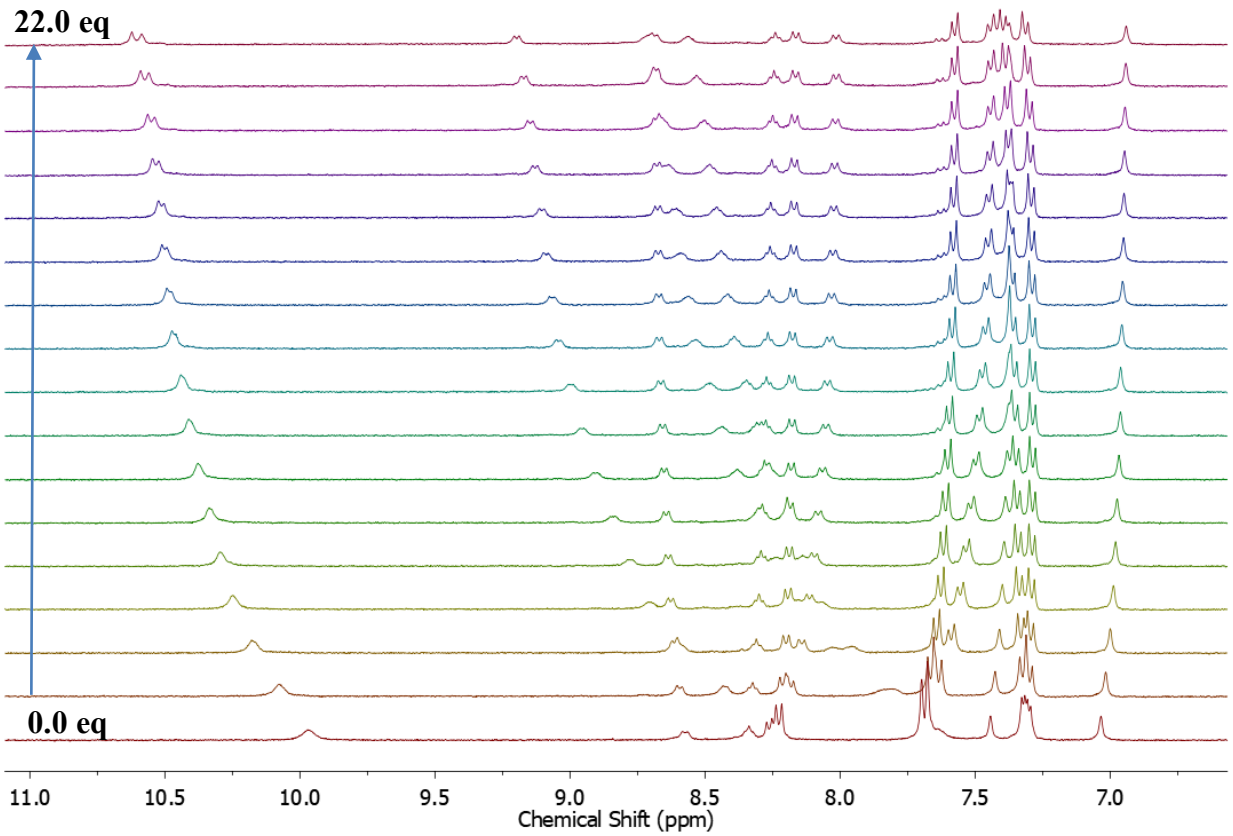


Figure S24: (a) ^{1}H NMR stack plot of **5** with TBA_2SO_4 in 50% H_2O in DMSO-d_6 at 300 K, (b) Titration isotherm for squaramide proton NH at δ = 9.95ppm

(a)



(b)

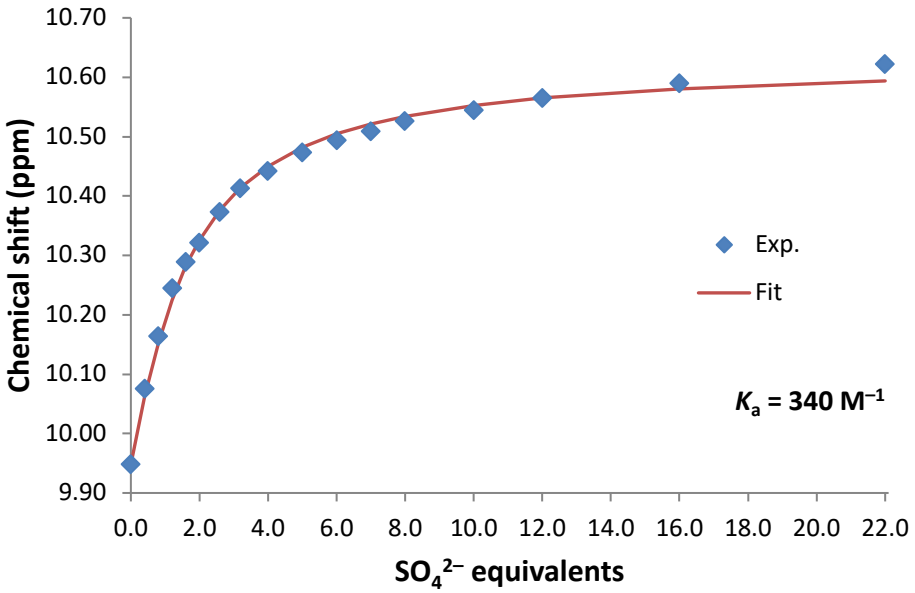
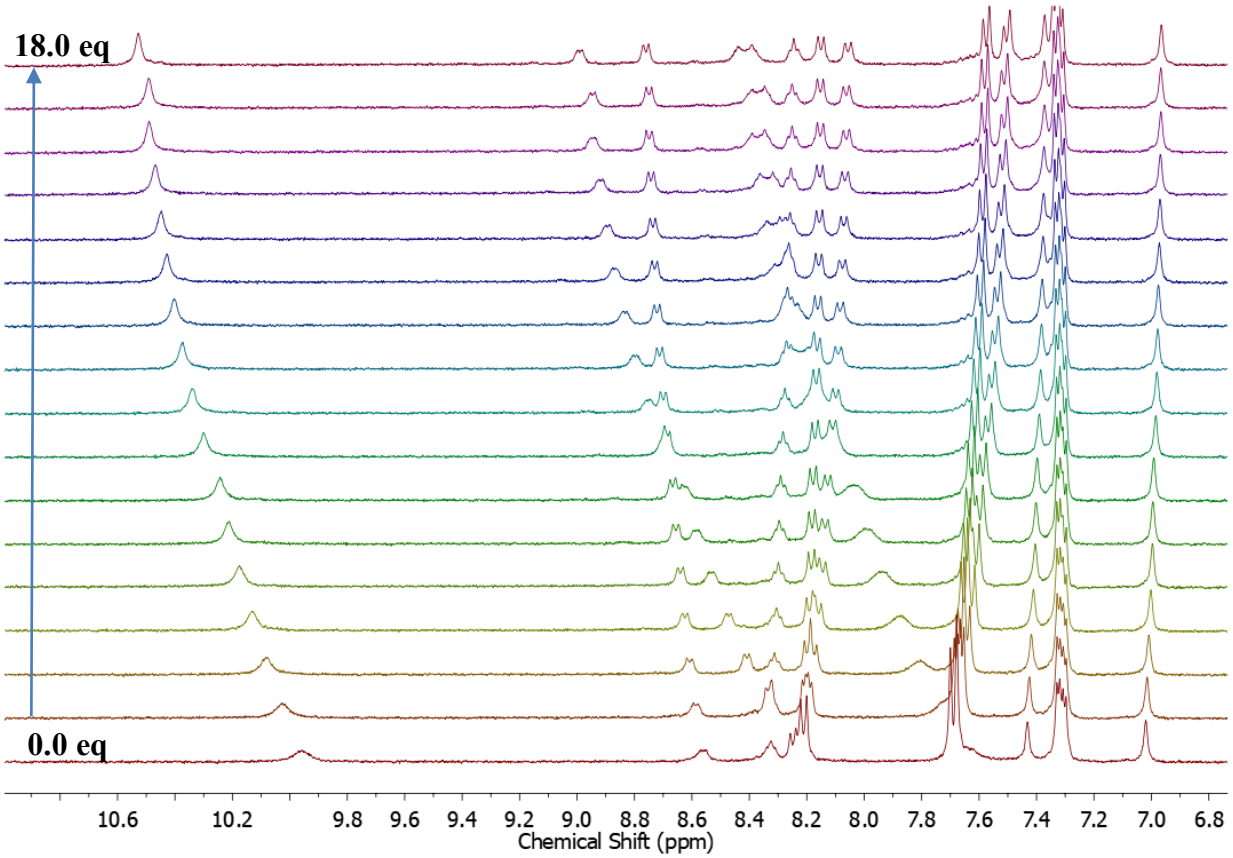


Figure S25: (a) ^{1}H NMR stack plot of **5** with TBA_2SeO_4 in 50% H_2O in DMSO-d_6 at 300 K, (b) Titration isotherm for squaramide proton NH at δ = 9.95ppm

(a)



(b)

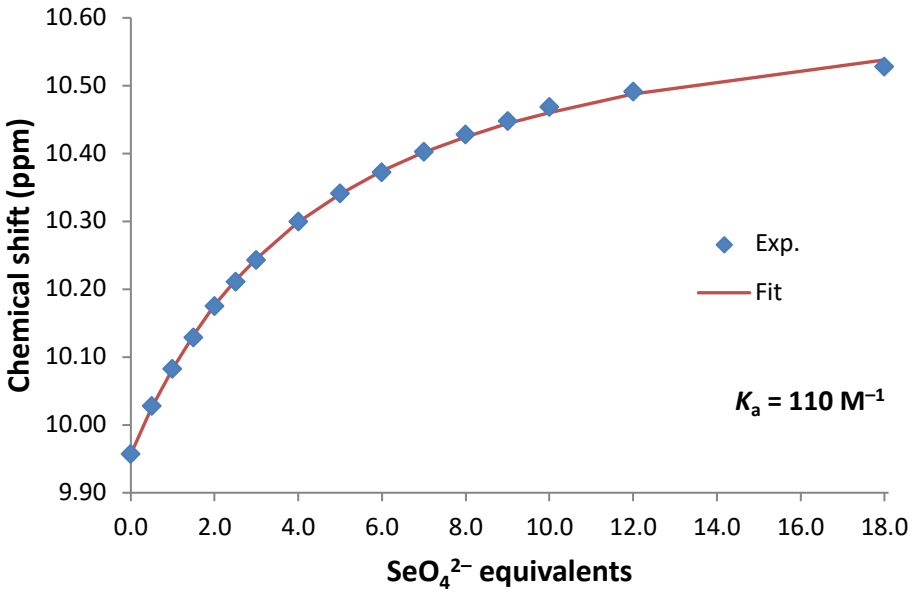
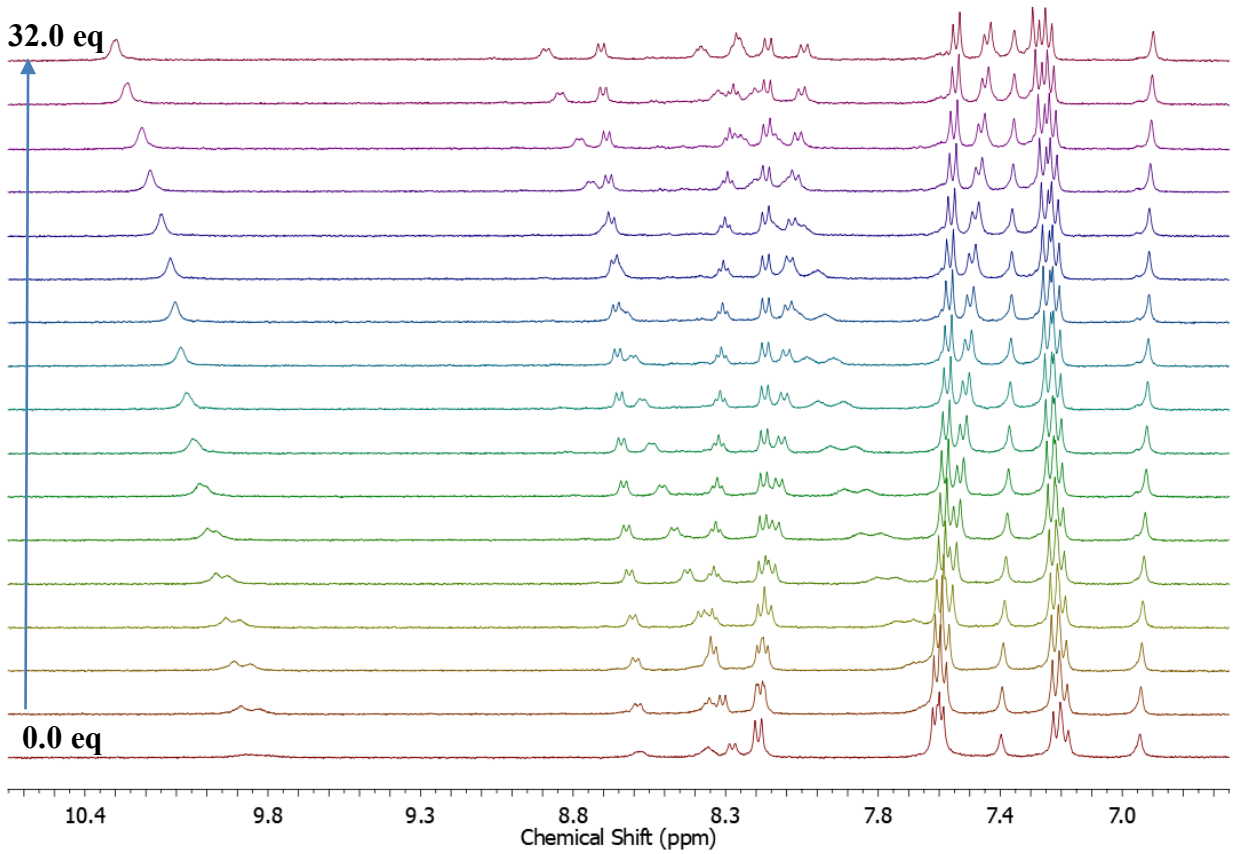
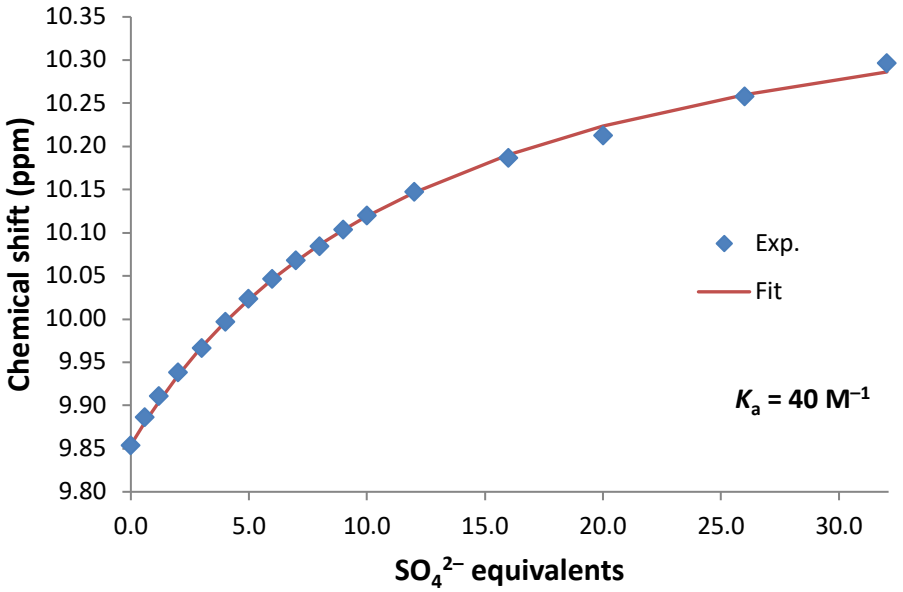


Figure S26: (a) ^{1}H NMR stack plot of **5** with TBA_2SO_4 in 75% H_2O in DMSO-d_6 at 300 K, (b) Titration isotherm for squaramide proton NH at δ = 9.85 ppm

(a)



(b)



Molecular Modelling

Molecular modelling of **2•SO_4^{2-}** and **5•SO_4^{2-}** was performed using Spartan 10 for Windows 7 (Wavefunction, Inc. Irvine, CA). The energy of the receptor-SO_4^{2-} complex was first minimized via molecular mechanics before optimisation with density functional theory (DFT) calculations were performed at the B3LYP/6-31G* level of theory.

References

1. R. Samudrala, X. Zhang, R. M. Wadkins and D. L. Mattern, *Bioorgan. Med. Chem.*, 2007, **15**, 186-193.
2. M. R. Hansen, T. Schnitzler, W. Pisula, R. Graf, K. Müllen and H. W. Spiess, *Angew. Chem. Int. Ed.*, 2009, **48**, 4621-4624.
3. A. J. Vernall, L. A. Stoddart, S. J. Briddon, S. J. Hill and B. Kellam, *J. Med. Chem.*, 2012, **55**, 1771-1782.
4. S. A. Kates, S. B. Daniels and F. Albericio, *Anal. Biochem.*, 1993, **212**, 303-310.