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Supporting Information

Squaramide-Based Self-Associating Amphiphiles for Anion Recognition

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Materials and Methods:

Commercial materials were supplied by TCI Europe or Sigma Aldrich and were used without further purification. HPLC grade solvents were used as received. ^1H NMR spectra were recorded using a Bruker Avance III 500 at a frequency of 500.13 MHz, and are reported as parts per million (ppm) with $CDCl_3$ (δH 7.26 ppm) or DMSO-d_6 (δH 2.50 ppm) as an internal reference. The data are reported as chemical shift (δ), multiplicity (br = broad, s = singlet, d = doublet, t = triplet, m = multiplet), coupling constant (*J*, Hz) and relative integral. ^{13}C NMR spectra were recorded using a Bruker Avance III 500 at a frequency of 125 MHz and are reported as parts per million (ppm) with $CDCl_3$ (δH 77.1 ppm) or DMSO-d_6 (δH 39.5 ppm) as an internal reference. High resolution ESI spectra were recorded on an Agilent 6310 LCMS TOF. Analytical TLC was performed using pre-coated silica gel plates (Merck Kieselgel 60 F254). Flash chromatography was performed using silica gel 40-63 µM, 60 Å. Infrared absorption spectra were recorded on a Perkin Elmer Spectrum 100 FT-IR spectrometer using KBr disks. FT-IR are reported in wavenumbers (cm^{-1}). Diethyl squarate, 3-ethoxy-4-((4-nitrophenyl)amino)cyclobut-3-ene-1,2-dione and 3-((3,5-bis(trifluoromethyl)phenyl)amino)-4-ethoxycyclobut-3-ene-1,2-dione were synthesised as previously described.[1,2]

Spectroscopic Binding Studies: Spectroscopic titrations were performed by additions of aliquots of the putative anionic guest as the tetrabutylammonium (TBA) salt solution (20 mM) in DMSO to a solution of the receptor in DMSO. After each addition, the resulting solution was stirred for at least 30 seconds and the absorbance was recorded. Both salt and receptor were dried under high vacuum prior to use. Absorbance titrations were carried out using an Agilent Cary 50 spectrophotometer.

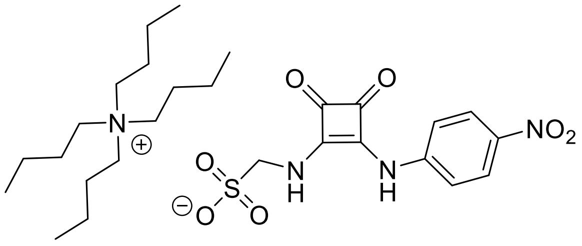
Determination of pK_a values: pK_a values were experimentally determined using the wavelength of maximum difference in absorbance between the UV/Vis spectra of the anionic and the neutral species. For **SQS-1** the absorbance was measured in a solution in DMSO/H_2O (1:1) containing 0.1M $TBAPF_6$ with pH values between 3.5 and 13.5. The solution was adjusted to acidic pH using an excess of HNO_3 before being slowly basified by small aliquots of an aqueous 0.1 M NaOH solution. The absorbance values at the wavelength of maximal difference were plotted against the pH values. A four parameter sigmoid curve using Sigma Plot (Systat Software Inc., Chicago, IL, USA) was fitted through the data points with the point of inflexion corresponding to the pKa value.

NMR Binding Studies: NMR titrations were performed by additions of aliquots of the putative anionic guest as the tetrabutylammonium (TBA) salt, to a solution of the receptor in DMSO-d_6. Typically, up to 30 equivalents of the anion were added to the solution. Both salt and receptor were dried under high vacuum prior to use. ^1H NMR spectra were recorded on a Bruker Avance III 500 spectrometer at a frequency of 500.13 MHz and calibrated to the residual protio solvent peak in DMSO-d_6 ($\delta = 2.50$ ppm). Stack plots were made using TopSpin 3.5. The calculation of association constants (K_a/M^{-1}) was carried out using the open access BindFit software program.[3-5]

Dynamic Light Scattering (DLS) measurements: DLS measurements were performed on an ALV/CGS-3 goniometer instrument, equipped with a HeNe laser operating at a wavelength of 632.8 nm, an optic fiber based detector and an ALV/LSE-5004 Light Scattering Electronics and Multiple Tau Digital Correlator. The sample temperature was maintained at 20°C using a Thermo Scientific DC30-K20 water bath and measured with a Pt-100 probe immersed into the index matching fluid vat. DLS measurements were carried out for 20 seconds, 50 times per sample, at a scattering angle of 90°. Samples were filtered through 0.2µm PTFE filters. Samples of compound **SQS1** were dissolved in either DMSO, DMSO and water mixtures or EtOH and water mixtures and stored at room temperature before measurements.

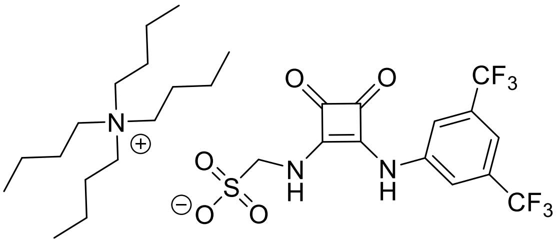
X-ray Crystallography: Structural and refinement parameters are presented in **Table S1**. The diffraction data for **SQS-1** were collected using a Bruker APEX-II Duo dual-source instrument using graphite-monochromated Mo Kα (λ = 0.71073 Å) radiation. The dataset was collected using ω and φ scans with the sample immersed in oil and maintained at a constant temperature of 100 K using a Cobra cryostream. The diffraction data for **SQS-2** were collected using a Bruker D8 Quest ECO diffractometer using graphite-monochromated Mo Kα (λ = 0.71073 Å), with the sample temperature maintained at 150 K using an Oxford Cryosystems Cryostream 800. The data for both datasets were reduced and processed using the Bruker APEX-3 suite of programs.[4] Multi-scan absorption corrections were applied using SADABS.[5] The diffraction data were solved using SHELXT and refined by full-matrix least squares procedures using SHELXL-2015 within the OLEX-2 GUI.[6-8] The functions minimized were $\Sigma w(F^2_o - F^2_c)$, with $w=[\sigma^2(F^2_o)+aP^2+bP]^{-1}$, where $P=[\max(F_o)^2+2F^2_c]/3$. All non-hydrogen atoms were refined with anisotropic displacement parameters. All carbon-bound hydrogen atoms were placed in calculated positions and refined with a riding model, with isotropic displacement parameters equal to either 1.2 or 1.5 times the isotropic equivalent of their carrier atoms. The positions of hydrogen atoms involved in hydrogen bonding interactions were refined to provide the best fit for the residual Fourier peaks and assigned a U_{iso} value equal to 1.5 times that of the nearest associated atom, with the appreciation that the exact positions of these atoms cannot be meaningfully inferred from X-ray diffraction data. Particular refinement strategies, including the specific use of restraints, are provided in the crystallographic information file. CCDC 2043909, 2087917.

Synthesis:



SQS - 1

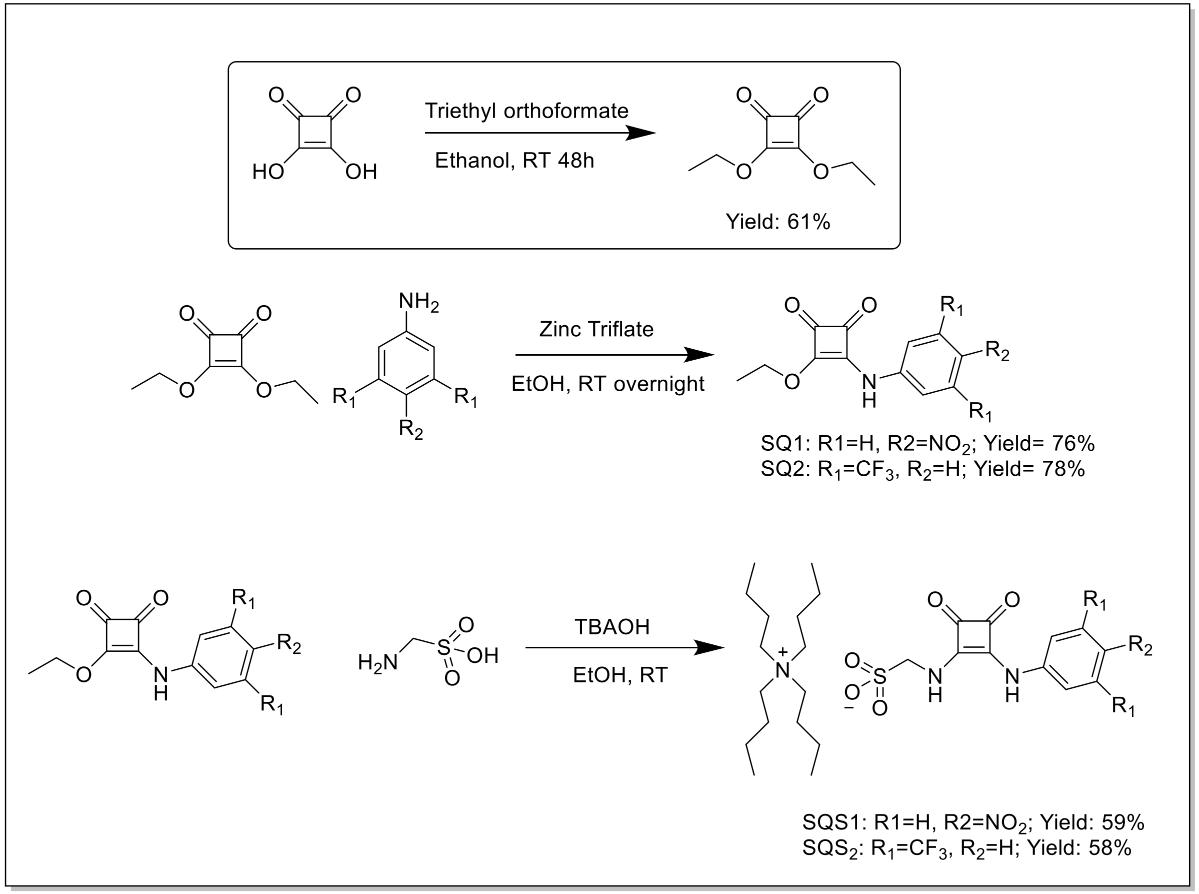
3-(4-nitrophenylamino)-4-ethoxy-cyclobut-3-ene-1,2-dione (500 mg, 1.9 mmol) was dissolved in EtOH (10 mL), followed by the addition of tetrabutylammonium hydroxide (500 mg, 1.92 mmol). In a separate vial, aminomethanesulfonic acid (237 mg, 1.9 mmol) was dissolved in MeCN (6 mL), with heating to assist solubilisation. This solution was added dropwise to the reaction vessel over 10 mins. The reaction mixture was stirred at room temperature for 18 hrs before being concentrated *in-vacuo* to afford an orange solid. The crude product was redissolved in DCM (20 mL) and washed with H_2O (3 x 30 mL) & dried over anhydrous $MgSO_4$ before being concentrated *in-vacuo* to afford the desired product as an orange solid (634 mg, 1.12 mmol, 59%). **M.P.** 238°C-242°C. **^{1}H NMR** (500 MHz, DMSO-*d*$_6$, 298 K, ppm) δ 10.38 (s, NH, 1H), 8.36 (s, NH, 1H), 8.22 (d, *J* = 8.0 Hz, CH, 2H), 7.63 (d, *J* = 8.5 Hz, CH, 2H), 4.33 (d, *J* = 4.0 Hz, CH_2, 2H), 3.15 (t, *J* = 8.5 Hz, CH_2, 8H), 1.55 (q, *J* = 7.0 Hz, CH_2, 8H), 1.32 (q, *J* = 7.0 Hz, CH_2, 8H), 0.93 (q, *J* = 7.5 Hz, CH_3, 12H). **^{13}C NMR** (126 MHz, DMSO-*d*$_6$, 298 K, ppm) 184.8, 180.5, 170.1, 162.2, 145.4, 141.5, 117.7, 59.5, 57.5, 23.1, 19.2, 13.5. **IR** (Film) ν_{max} (cm^{-1}): 3742, 3205, 2959, 2875, 1788, 1698, 1632, 1598, 1562, 1509, 1493, 1437, 1425, 1329, 1286, 1259, 1281, 1192, 1164, 1110, 1033, 984, 860, 848, 773,754, 714, 692, 657, 615, 578, 532, 520.



SQS - 2

3-(3,5-bis-trifluoromethyl-phenylamino)-4-ethoxy-cyclobut-3-ene-1,2-dione (707 mg, 1 mmol) was dissolved in EtOH (15 mL), followed by the addition of tetrabutylammonium hydroxide (545 mg, 2.1 mmol). In a separate vial aminomethanesulfonic acid (245 mg, 2.2 mmol) was dissolved in MeCN (10 mL), with heating to assist solubilisation. This solution was added dropwise to the reaction vessel over 10 mins. The reaction mixture was stirred at room temperature for 18 hrs before being concentrated *in-vacuo* to afford a white solid. The crude product was redissolved in DCM (30 mL) and washed with H_2O (3 x 30 mL) & dried over anhydrous $MgSO_4$ before being concentrated *in-vacuo* to afford the desired product as a white solid (343 mg, 0.52 mmol, 52%). **M.P.** 184-186°C (decomposition). **^{1}H NMR** (500 MHz, DMSO-*d*$_6$, 298 K, ppm) δ 8.30 (br, NH, 1H), 8.08 (s, ArH, 2H), 7.86 (br, NH, 1H), 7.66 (s, ArH, 1H), 3.15 – 3.19 (m, CH_2, 8H), 1.54 – 1.61 (m, CH_2, 8H), 1.32 (sx, *J* = 7.1 Hz, CH_2, 8H), 0.94 (t, *J* = 7.1 Hz, CH_3, 12H). **^{13}C NMR** (126 MHz, DMSO) δ 184.6, 181.2, 170.1, 163.0, 141.7, 131.8 (q, *J* = 33.3 Hz), 123.6 (q, *J* = 272.6 Hz), 118.4, 115.1, 59.8, 58.0, 23.5, 19.7, 13.9. **IR** (Film) ν_{max} (cm^{-1}): 3214, 3033, 2970, 2879, 1793, 1702, 1609, 1565, 1499, 1477, 1434, 1412, 1377, 1338, 1296, 1275, 1255, 1230, 1115, 1031, 1000, 932, 922, 908, 876, 849, 830, 767, 733, 701, 679.

Reaction Scheme:



Scheme S1: Synthesis of **SQS1** and **SQS2**.

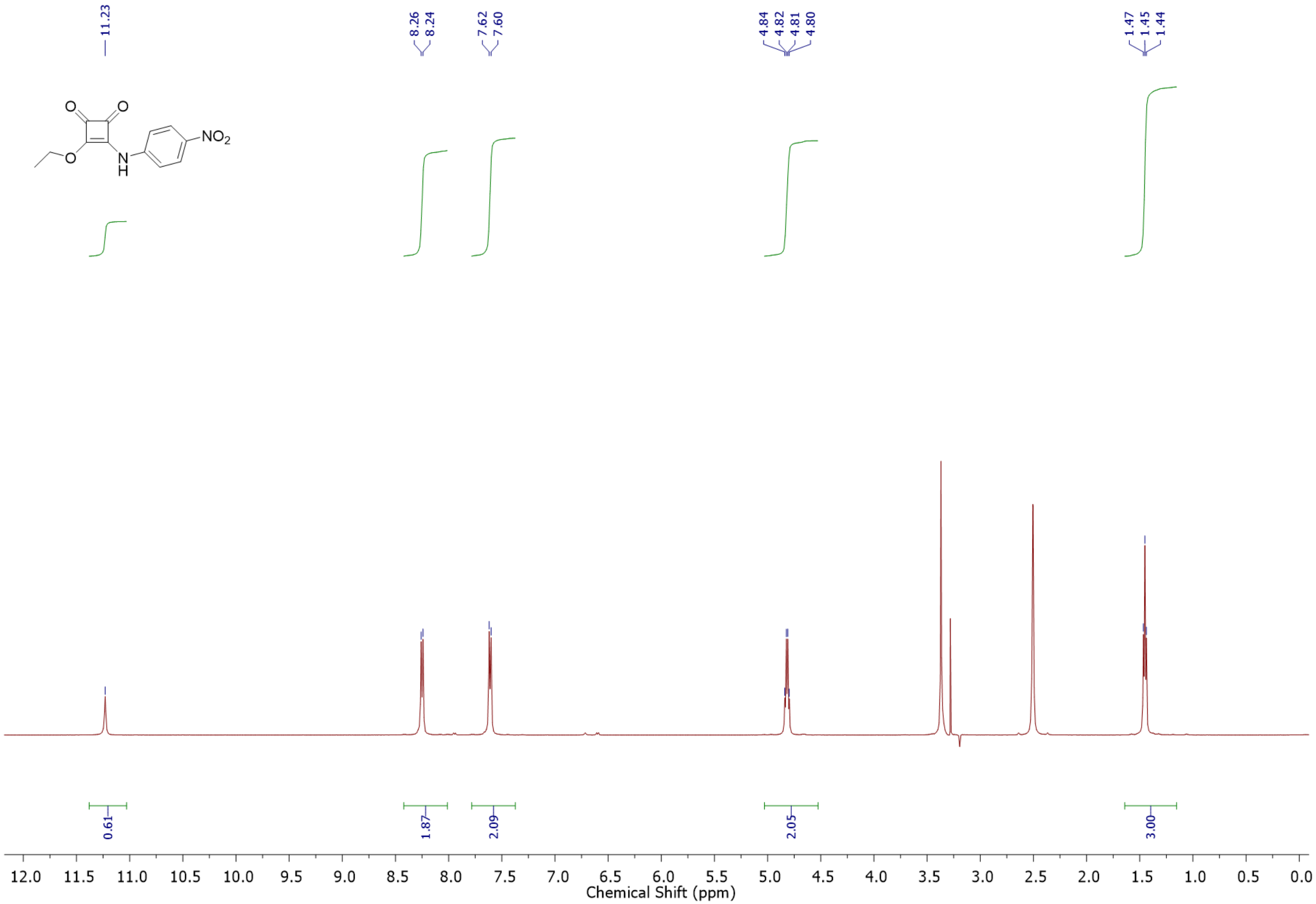


Figure S1: *The ^{1}H NMR spectra of* ***SQ-1*** *at 298K (500 MHz, DMSO-$_{d6}$)*

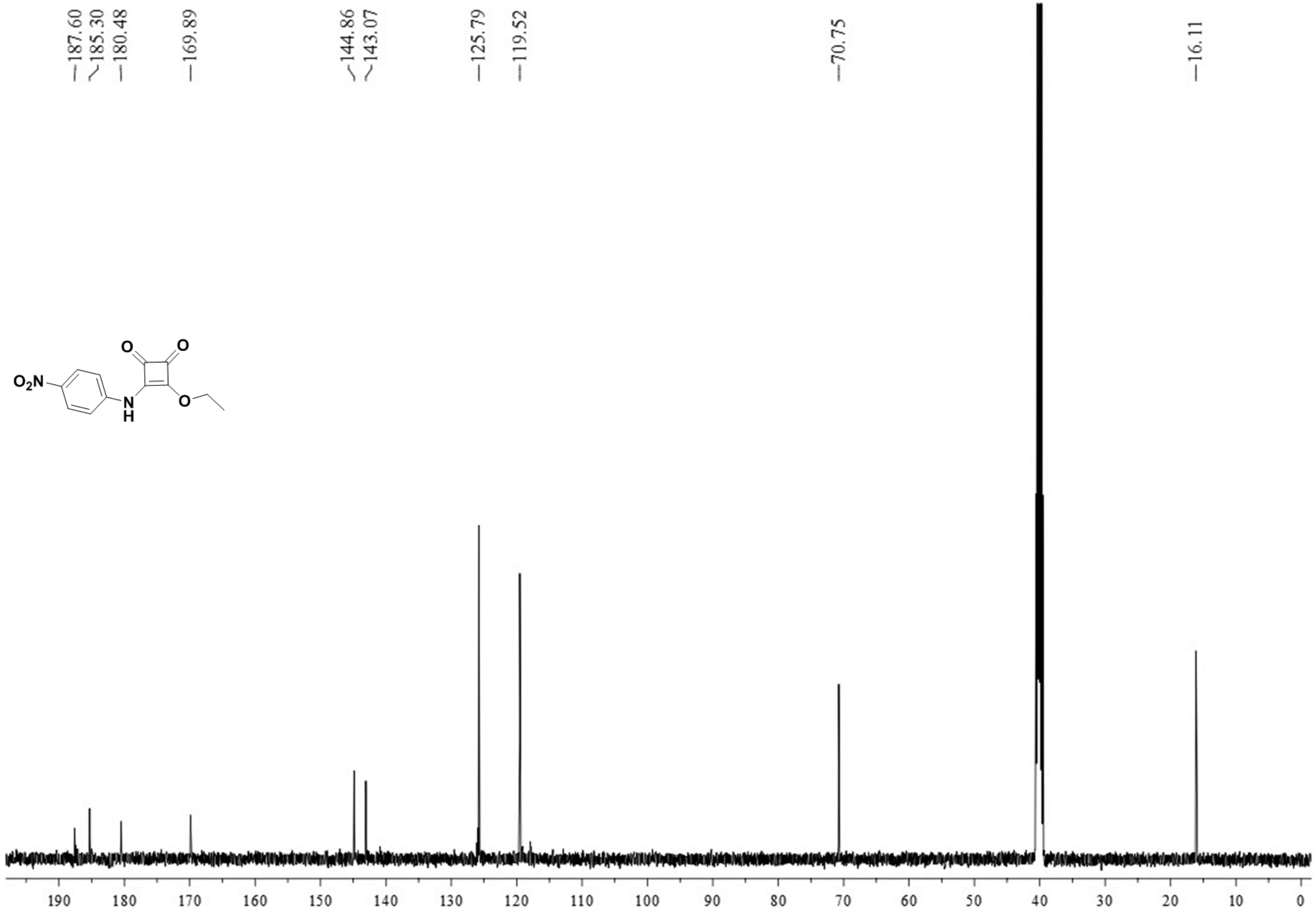


Figure S2: *The ^{13}C NMR spectra of* ***SQ-1*** *at 298K (125 MHz, DMSO-$_{d6}$)*

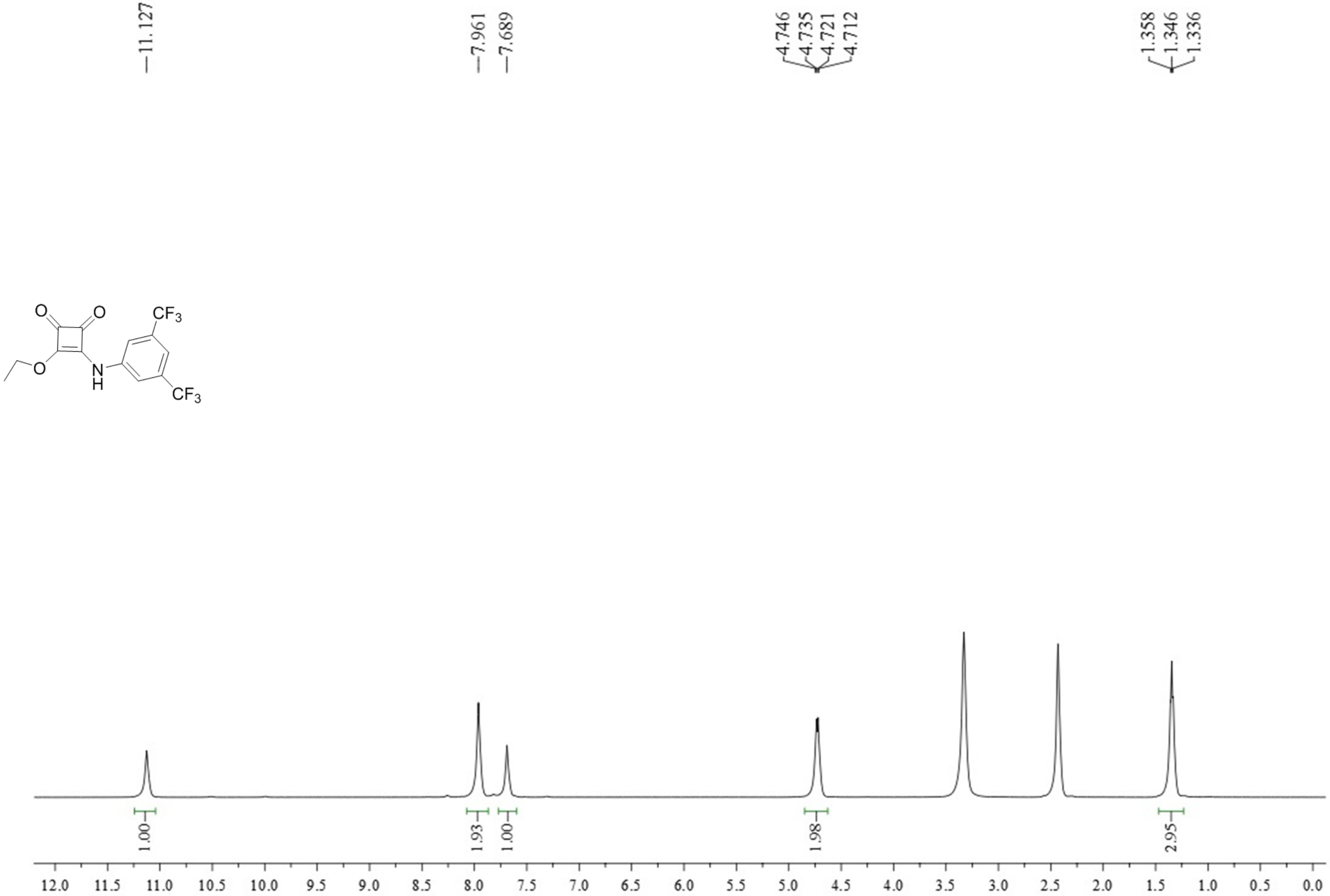


Figure S3: *The ^{1}H NMR spectra of* ***SQ-2*** *at 298K (500 MHz, DMSO-$_{d6}$)*

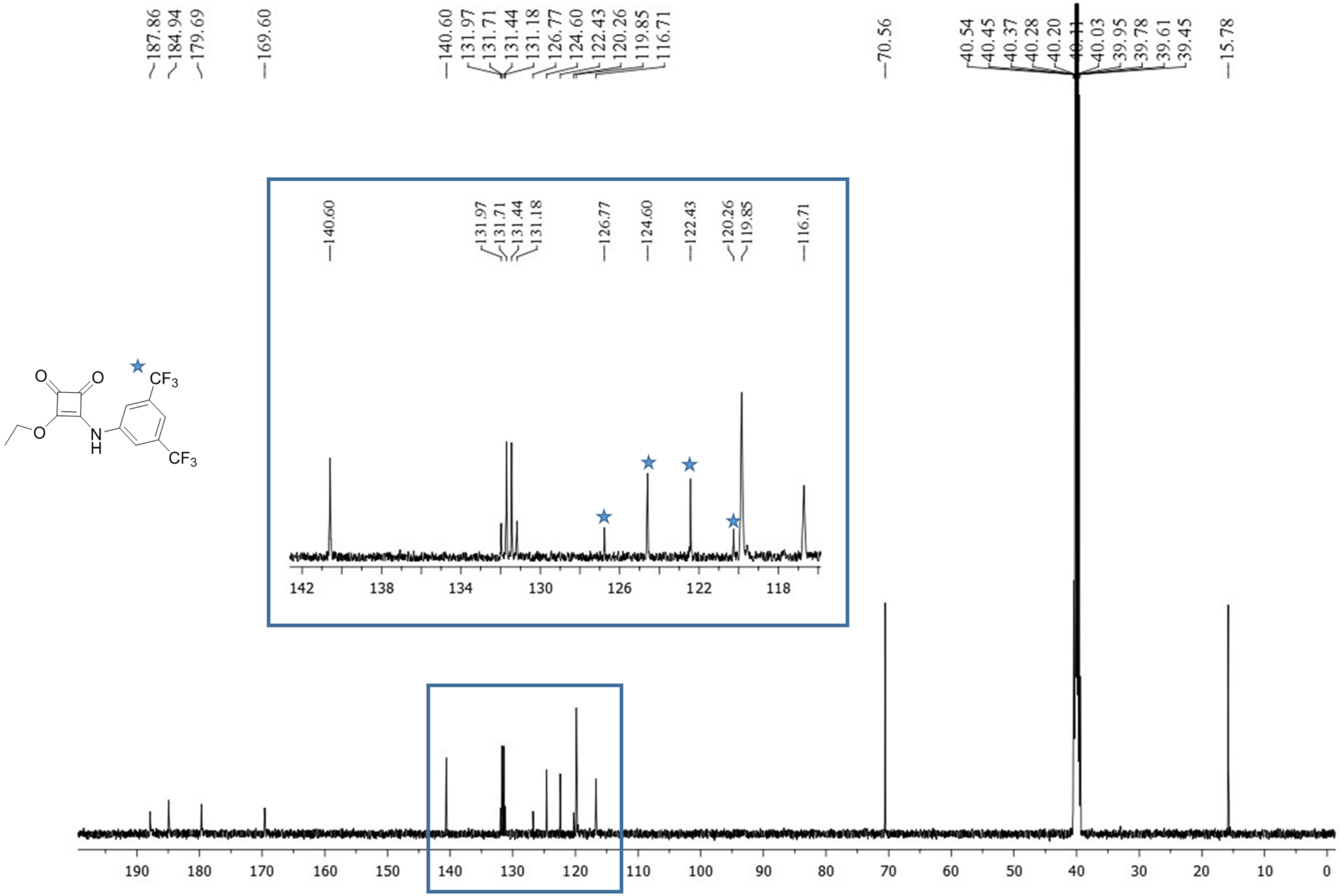


Figure S4: *The* ^{13}*C NMR spectra of* ***SQ-2*** *at 298K (125 MHz, DMSO-*$_{d6}$*)*

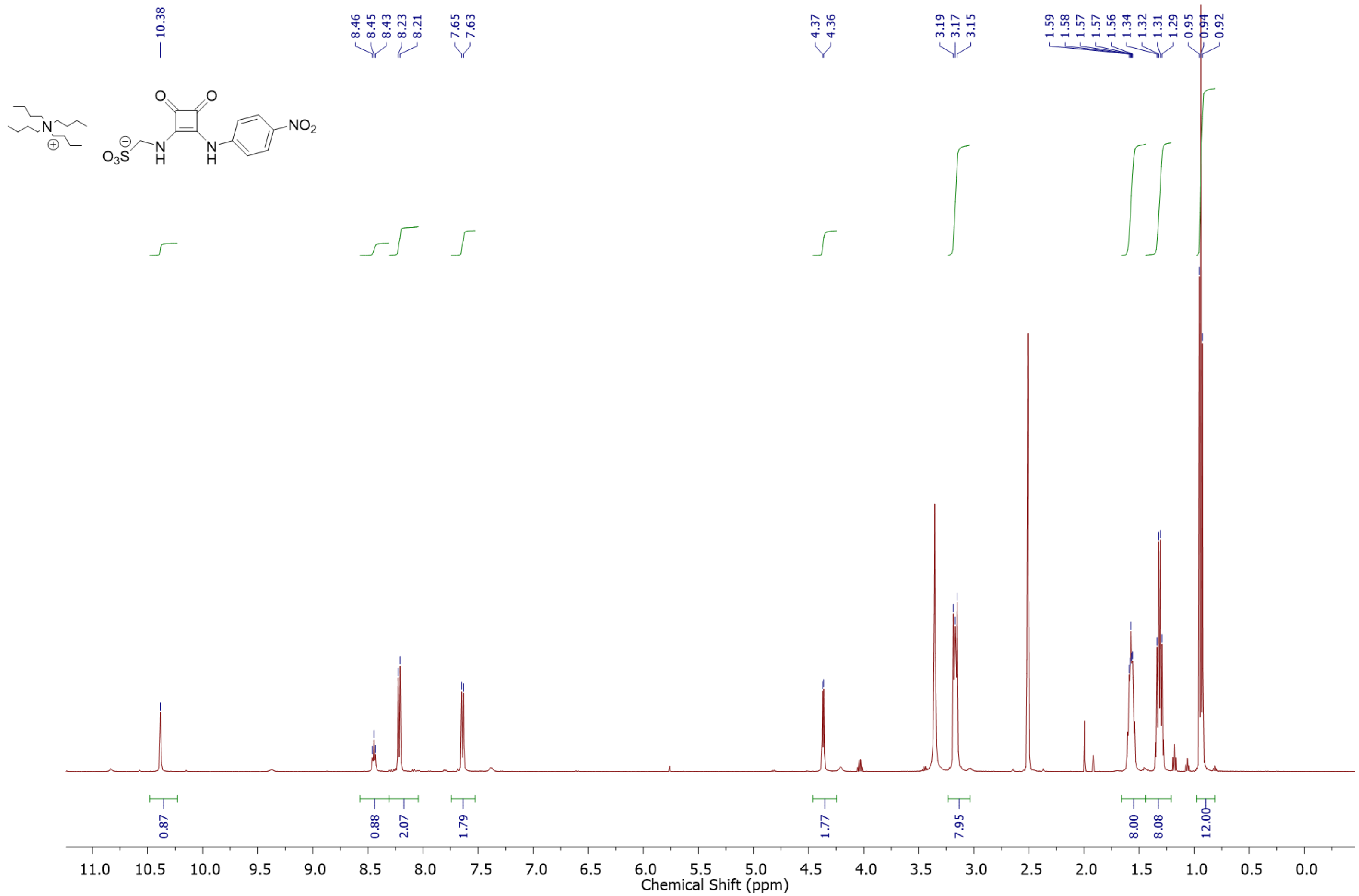


Figure S5: *The ^{1}H NMR spectra of* ***SQS-1*** *at 298K (500 MHz, DMSO-$_{d6}$)*

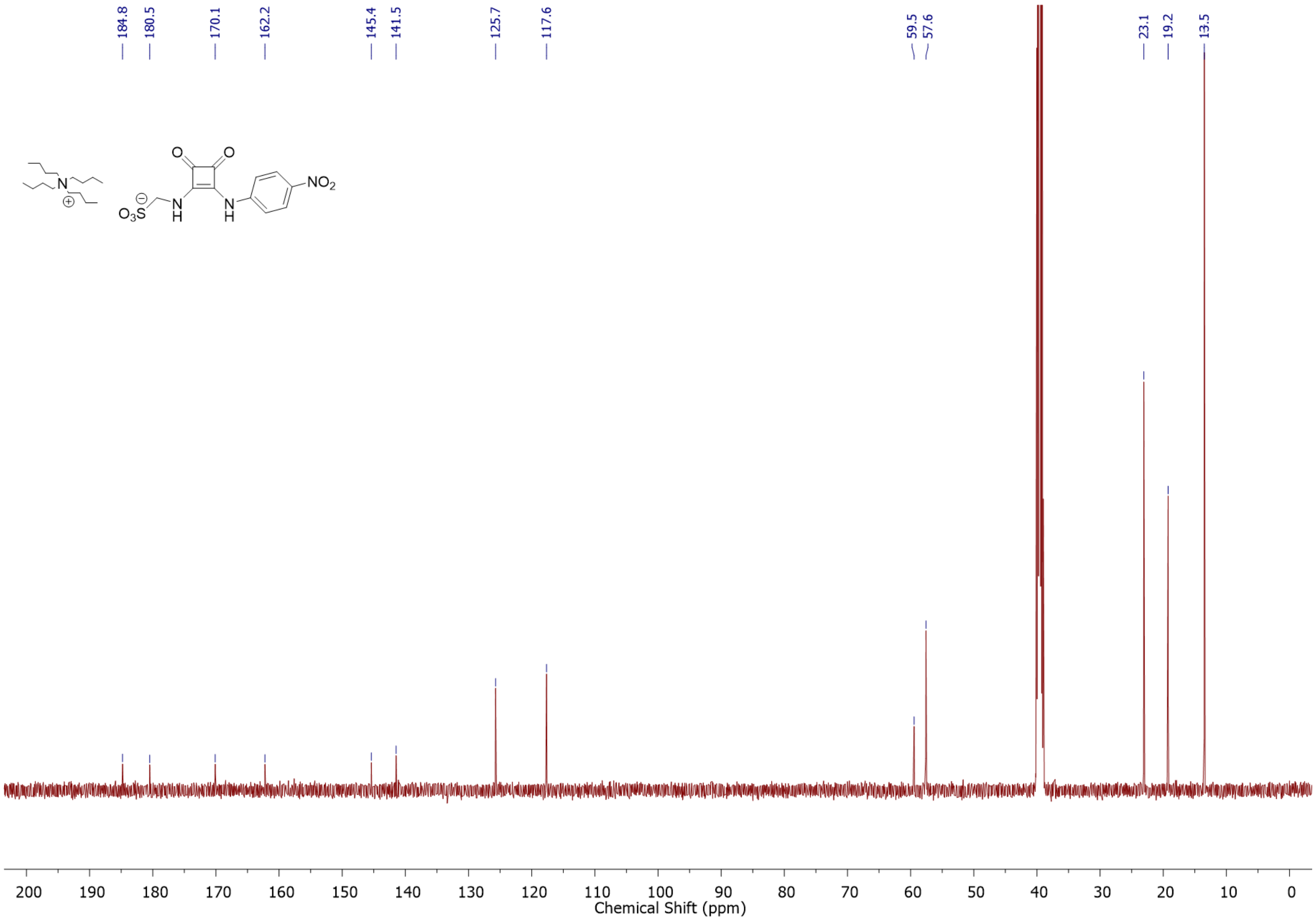


Figure S6: *The ^{13}C NMR spectra of* ***SQS-1*** *at 298K (125 MHz, DMSO-$_{d6}$)*

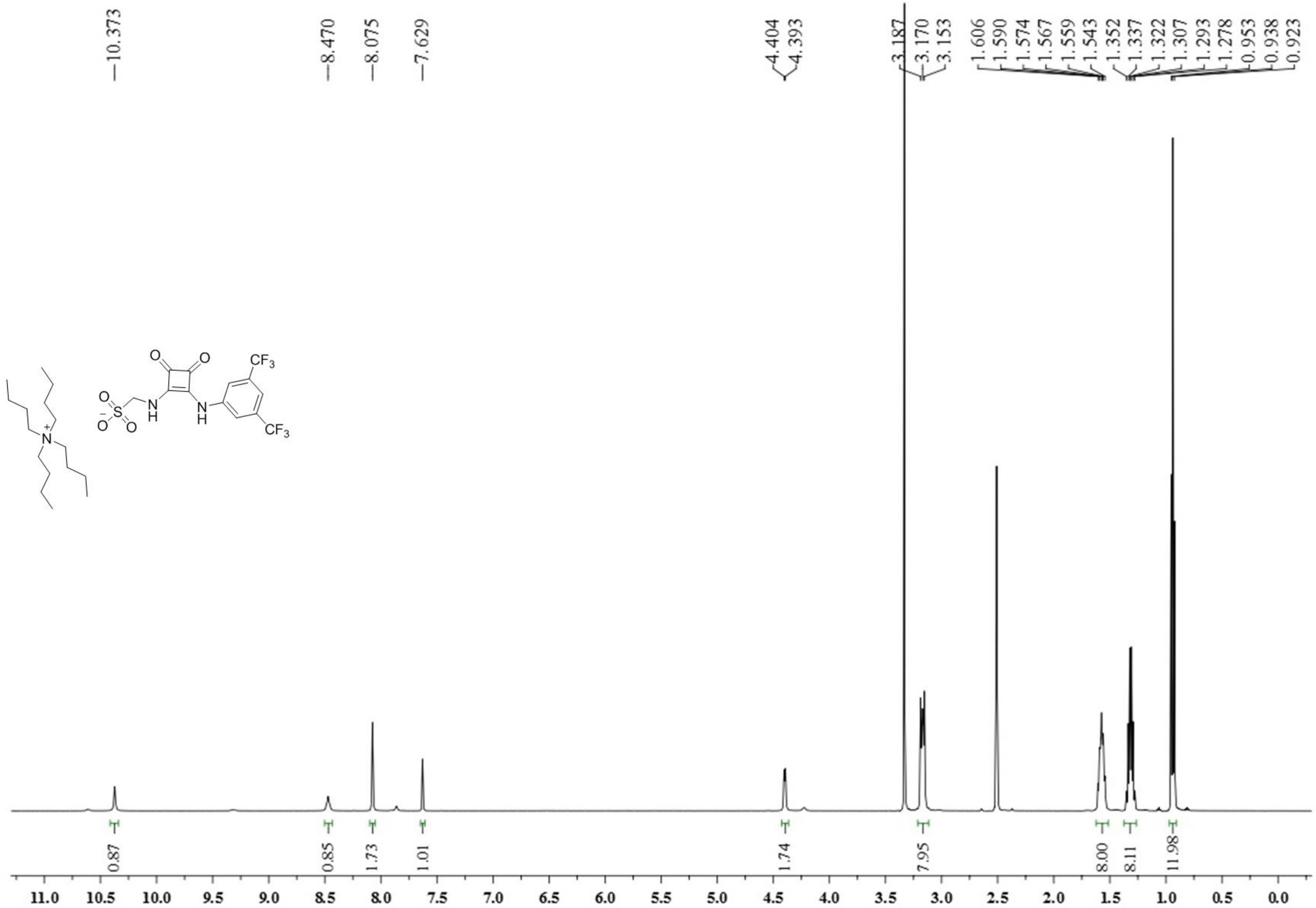


Figure S7: *The* ^{1}*H NMR spectra of* ***SQS-2*** *at 298K (500 MHz, DMSO-*$_{d6}$*)*

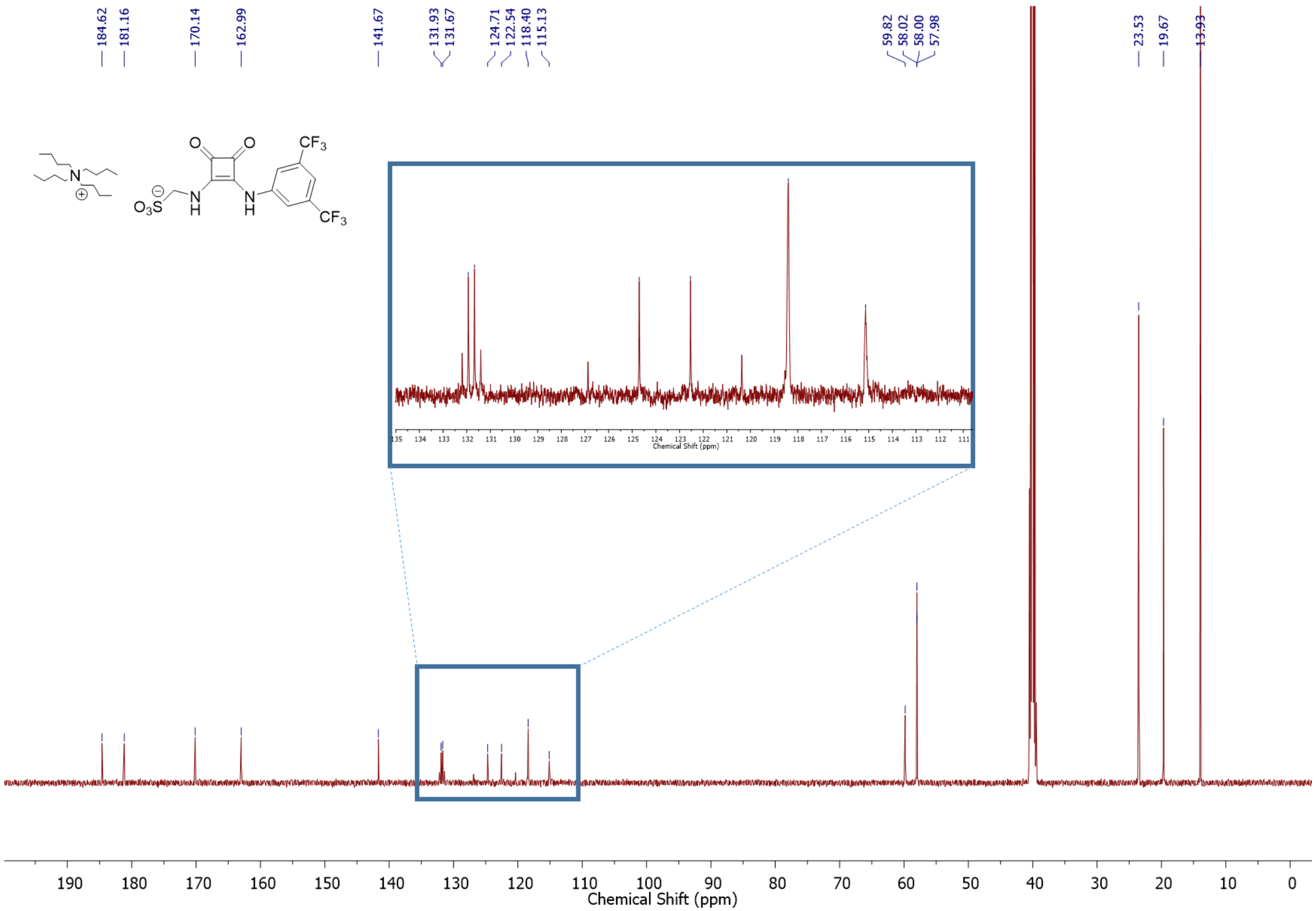


Figure S8: *The ^{13}C NMR spectra of* ***SQS-2*** *at 298K (125 MHz, DMSO-$_{d6}$)*

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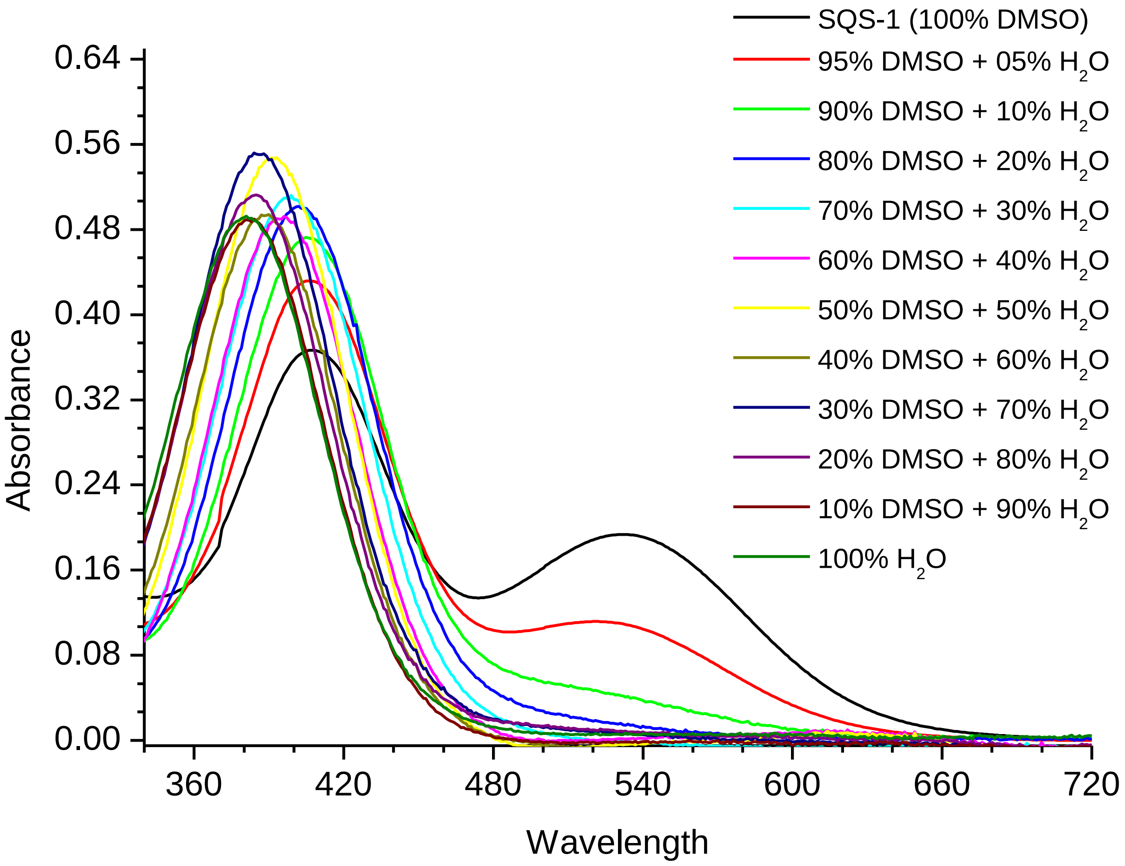

Figure S9: Absorbance spectra of probe **SQS-1** (20 x 10^{-6} M) at different ratios of DMSO/H_2O (0.5% Et_3N) (v/v). Absorption at 408 nm underwent a hypsochromic shift while the shoulder at 540 nm underwent a hypochromic shift.

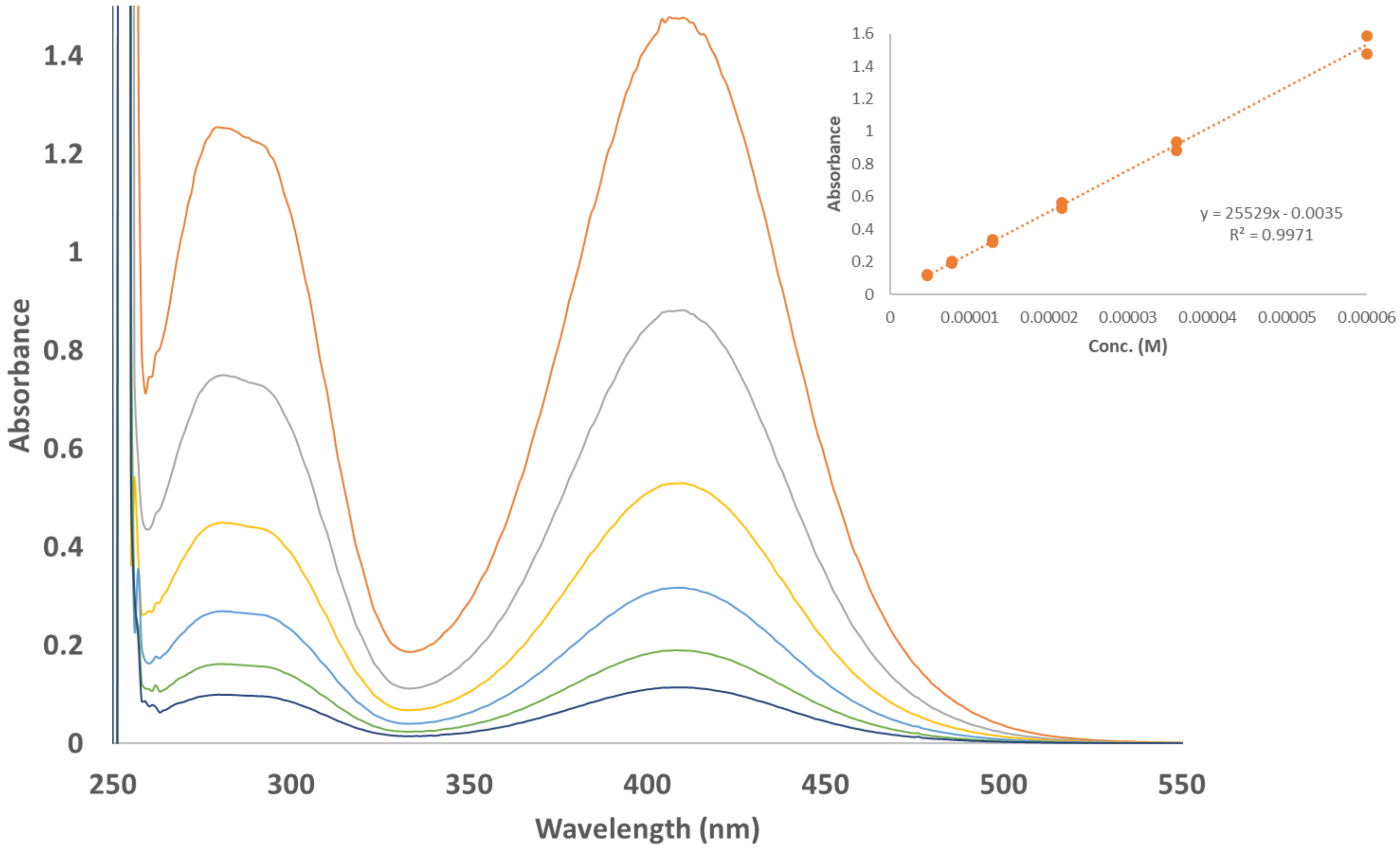

Figure S10: Absorbance spectra of probe **SQS-1** at various concentrations (5 – 60 µM) in in DMSO/H_2O (99.5:0.5, v/v). **Inset:** The absorbance values at 409 nm as a function of concentration and the linear regression line of best fit.

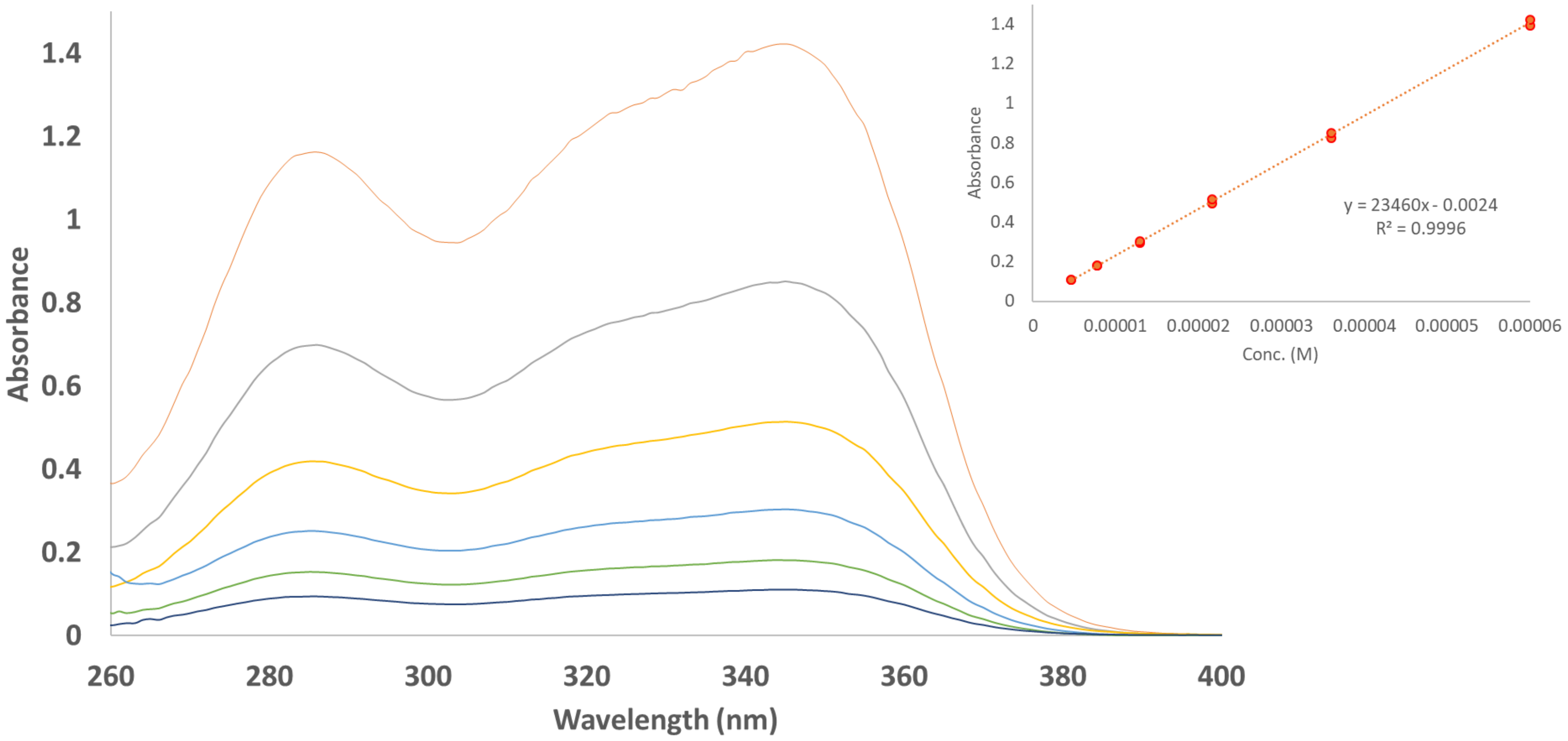

Figure S11: Absorbance spectra of probe **SQS-2** at various concentrations (5 – 60 µM) in in DMSO/H_2O (99.5:0.5, v/v). **Inset:** The absorbance values at 345 nm as a function of concentration and the linear regression line of best fit.

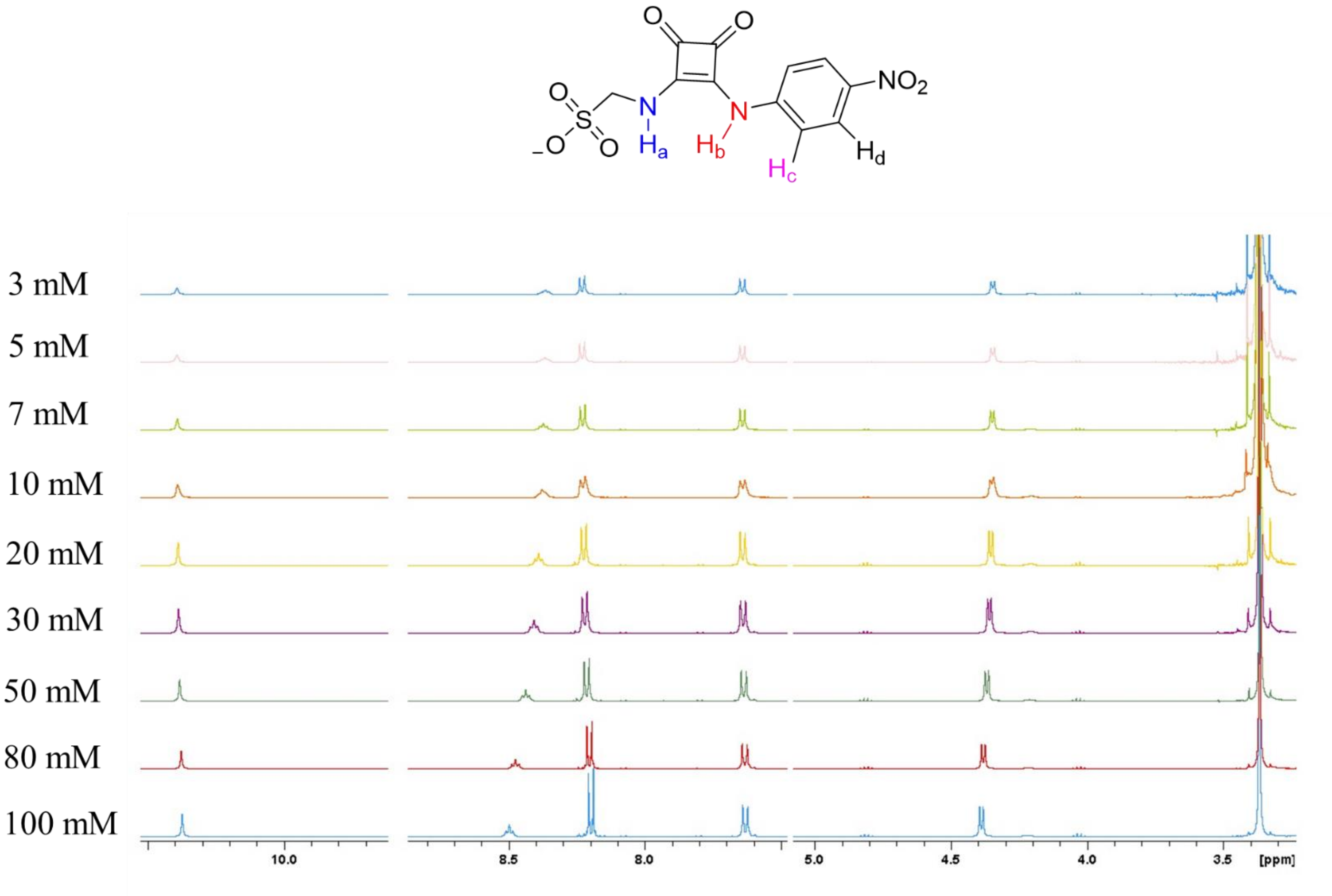

Figure S12: The NMR spectra of **SQS-1** in DMSO-*d6* (0.5 % H_2O) at various concentrations.

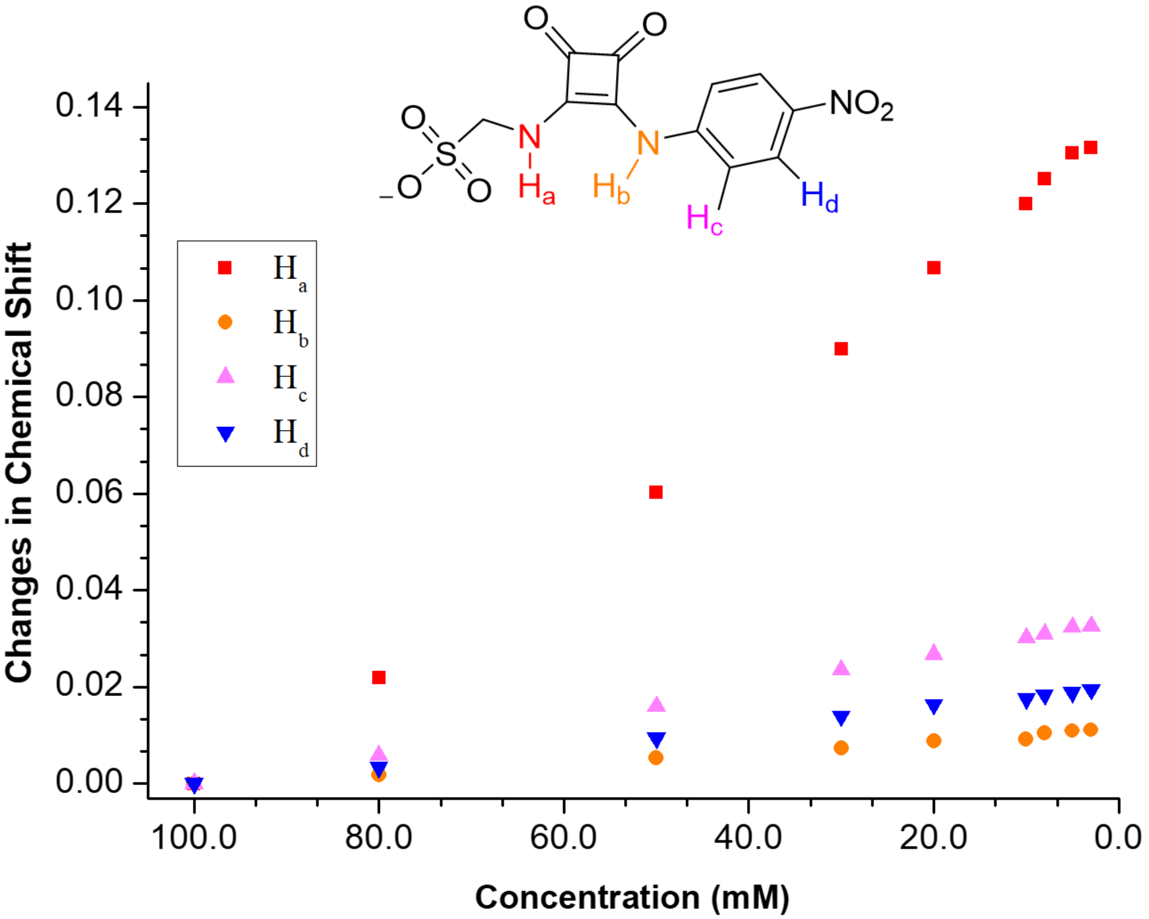

Figure S13: Changes in chemical shift in NMR spectra of **SQS-1** in DMSO-d_6 (0.5 % H_2O) as function of concentration.

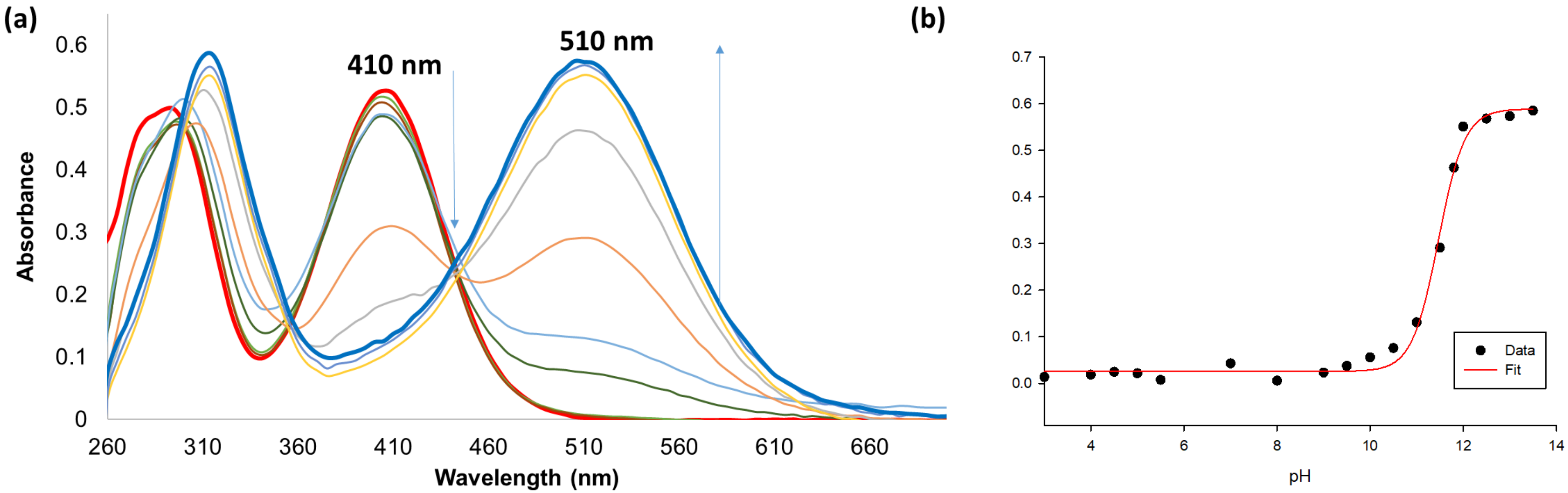

Figure S14: Absorbance spectra of **SQS-1** (20 x 10^{-6} M) at different pH values in DMSO/H_2O (1:1) (0.1 M $TBAPF_6$) and the corresponding four parameter sigmoid curve fit to the data. The pH of the solution was increased by addition of TBAOH. The pKa value of 11.47 was determined by fitting a four parameter sigmoidal curves through the data points using Sigma Plot (Systat Software Inc., Chicago, IL, USA) with the point of inflexion corresponding to the pKa.

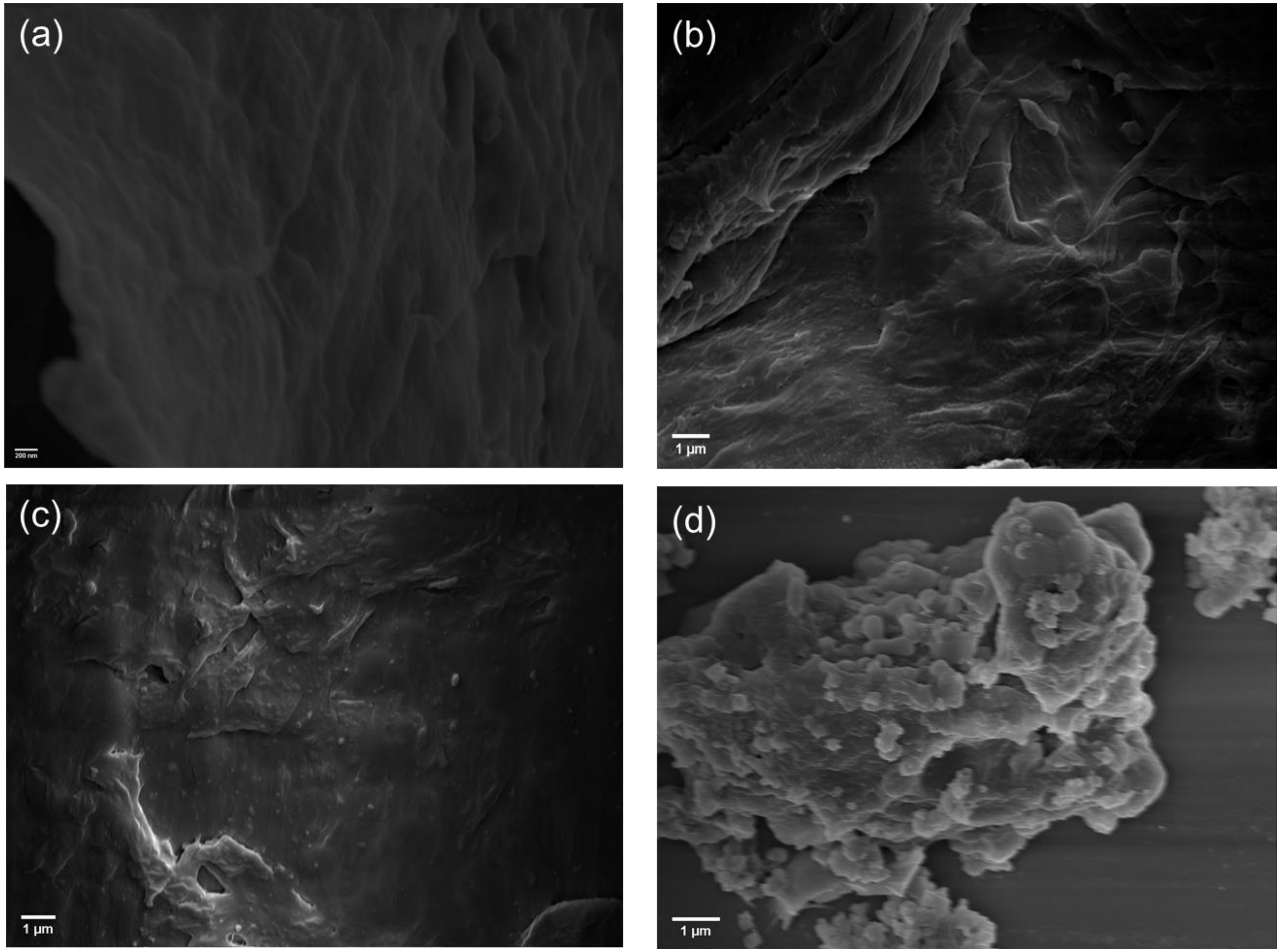

Figure S15: SQS-1 spotted from 1 μM solutions in EtOH (a), H2O (b), DMSO (c) and 1:1 DMSO:H2O mixture (d)**.**

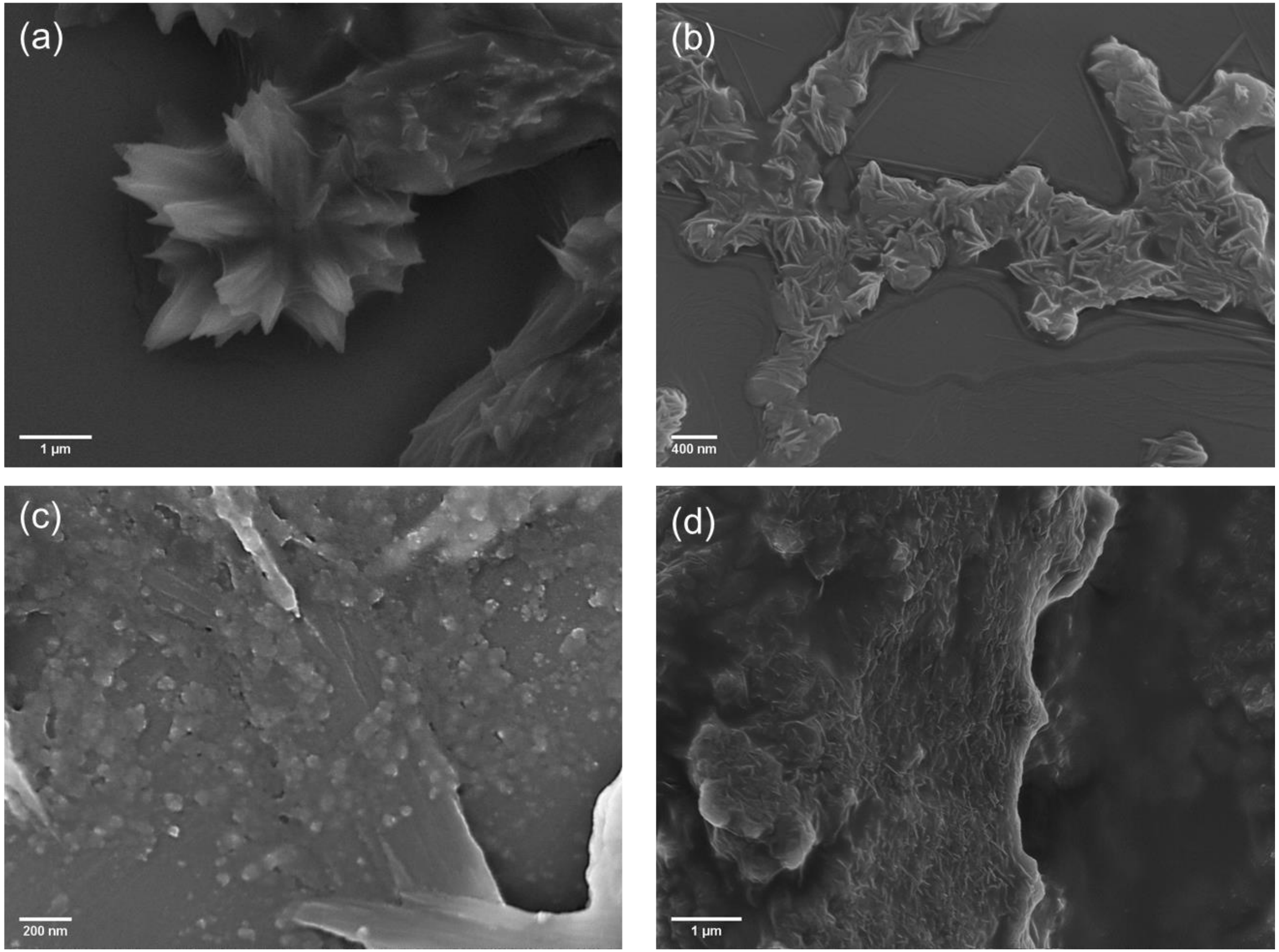

Figure S16: SQS-2 spotted from 1 µM solutions in EtOH (a), H_2O (b), DMSO (c) and 1:1 DMSO:H_2O mixture (d).

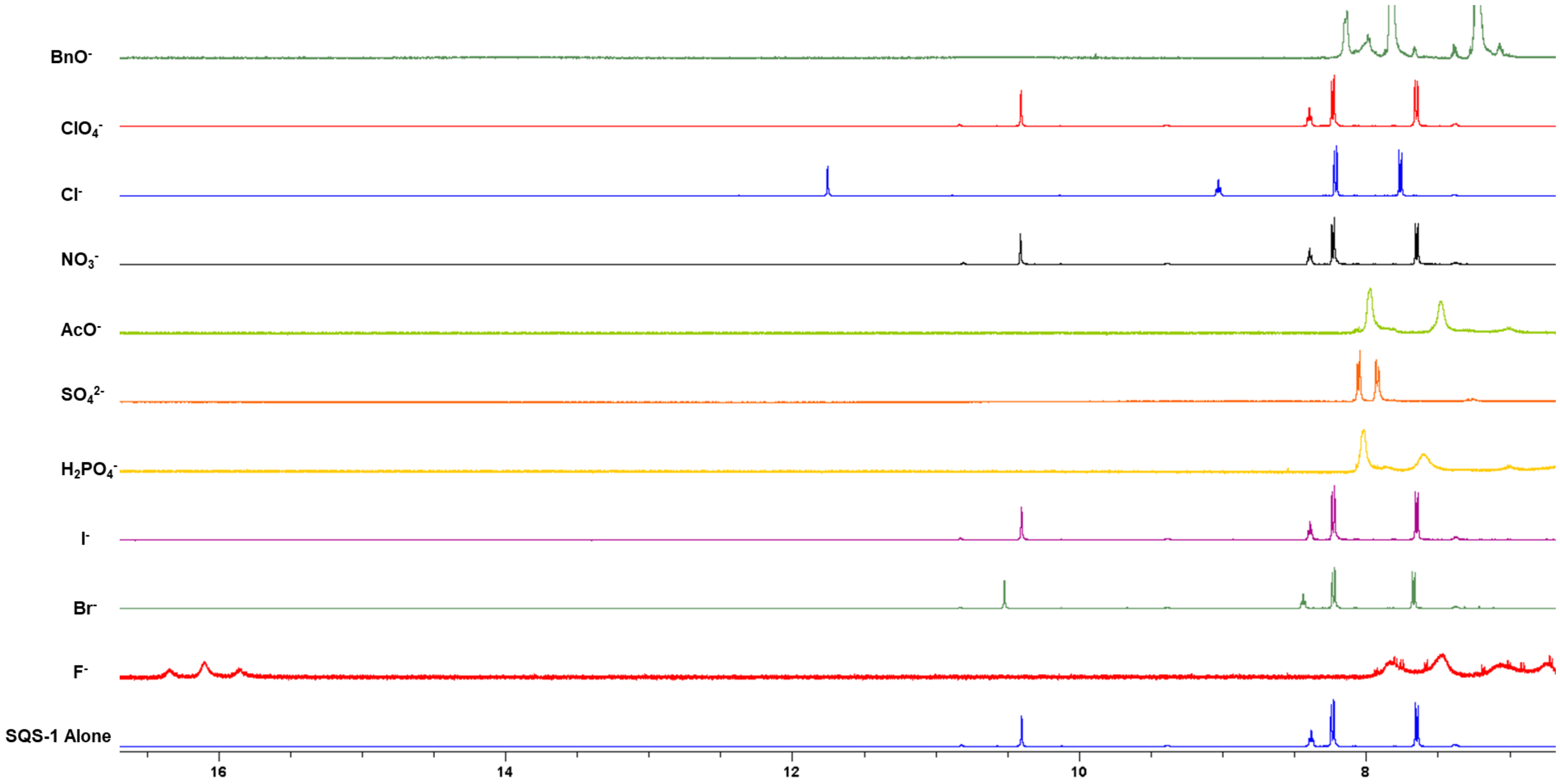

Figure S17: Changes in the ^{1}H NMR spectrum of **SQS-1** (5 mM) upon the addition of 20 equiv. of various anions as their TBA salts in DMSO-d_6.

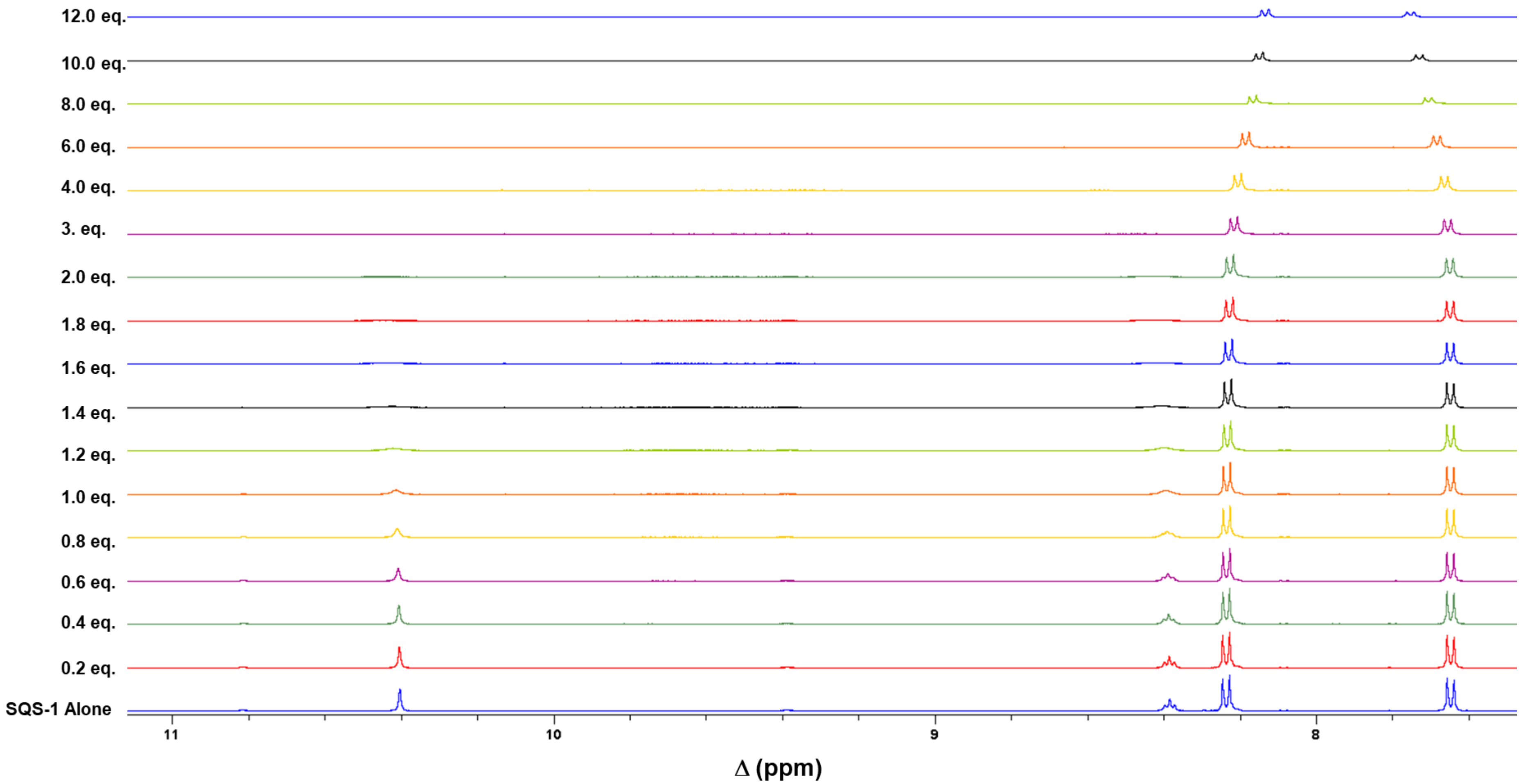

Figure S18: Changes in the ^{1}H NMR spectrum of **SQS-1** (5 mM) upon increasing TBA sulfate concentration

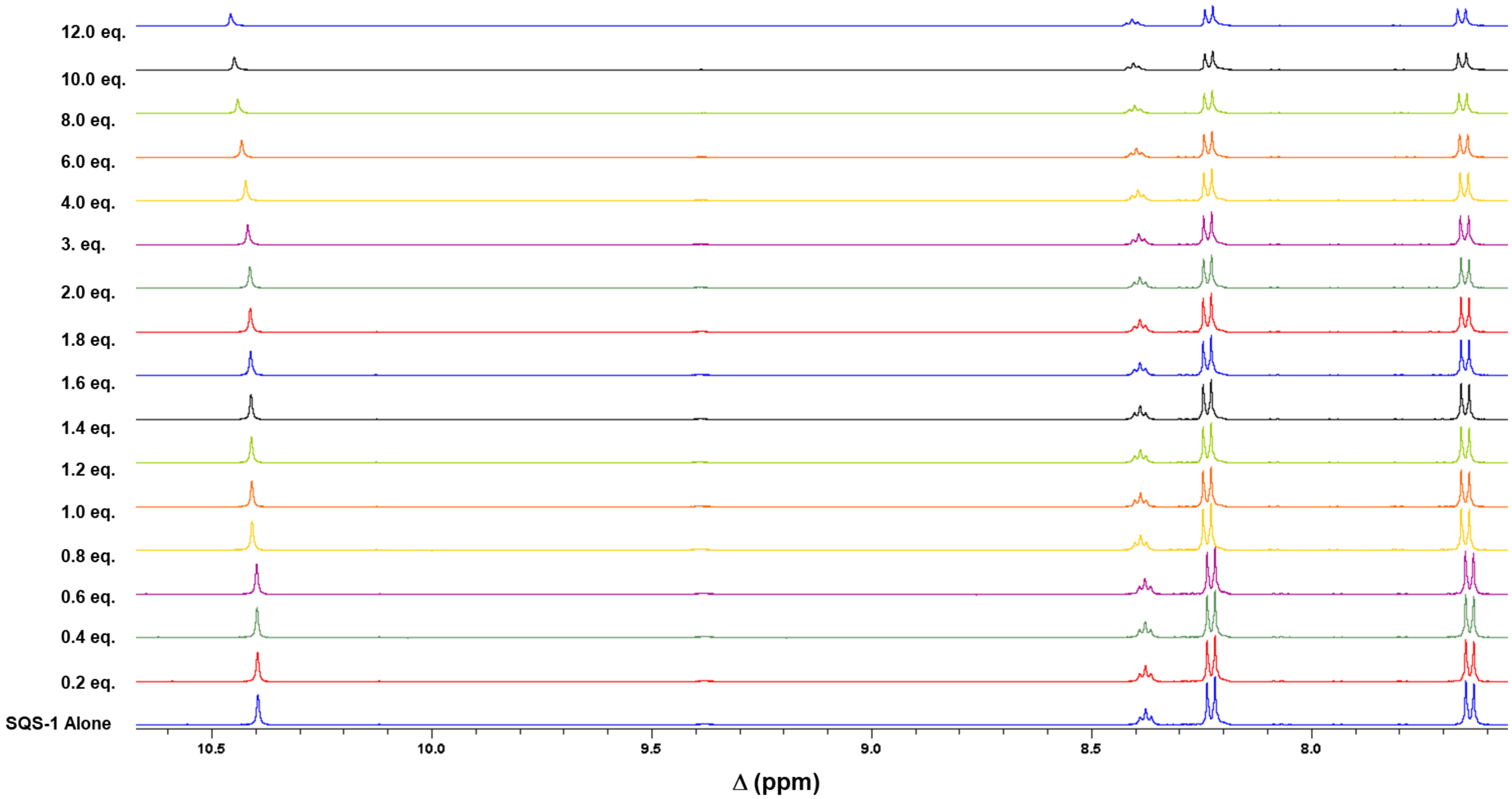

Figure S19: Changes in the ^{1}H NMR spectrum of **SQS-1** (5 mM) upon increasing TBA bromide concentration

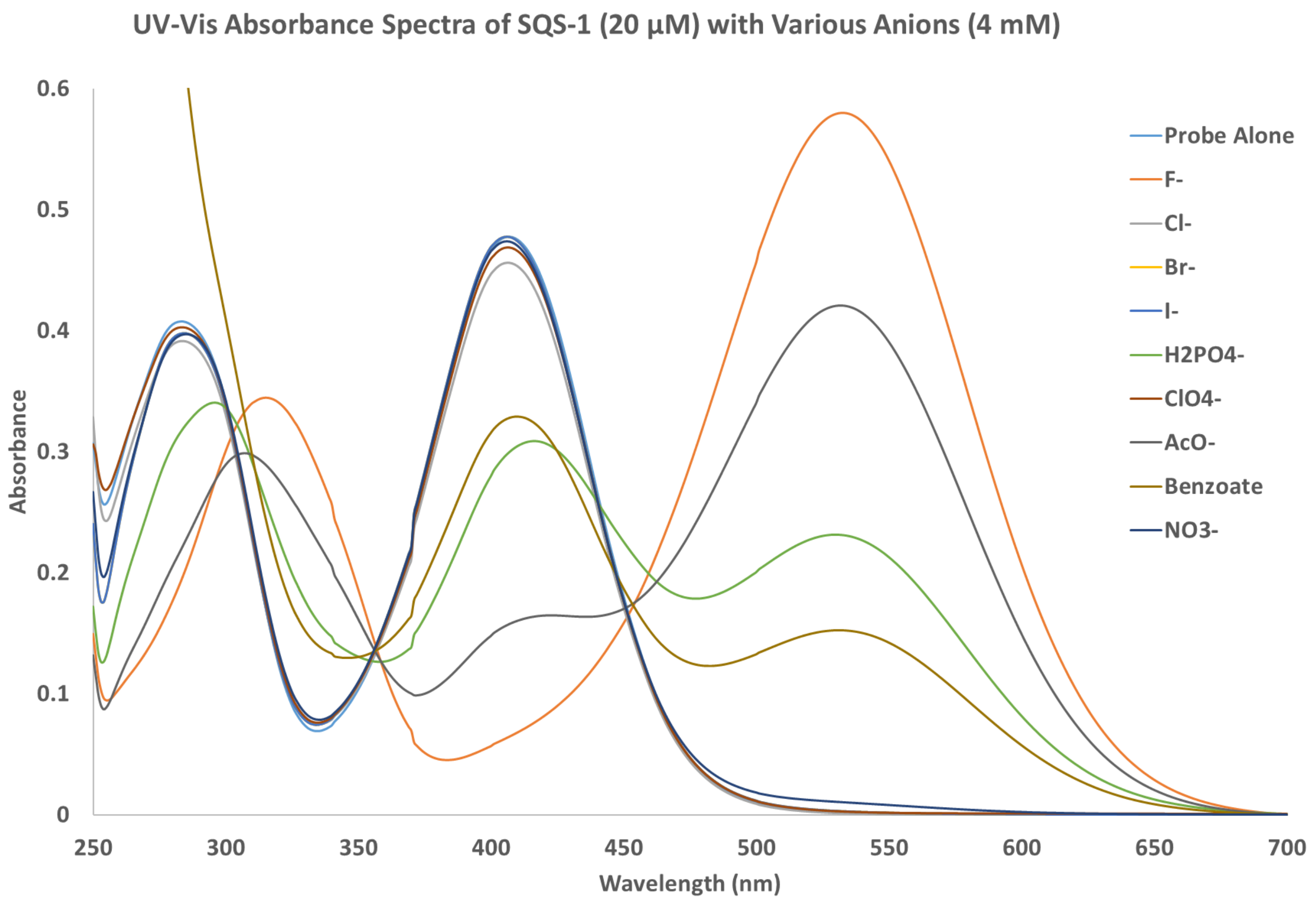

Figure S20: Absorbance spectra of **SQS-1** (20×10^{-6} M) with different anions (as their TBA salts, 4 mM) in DMSO (0.5 % H_2O).

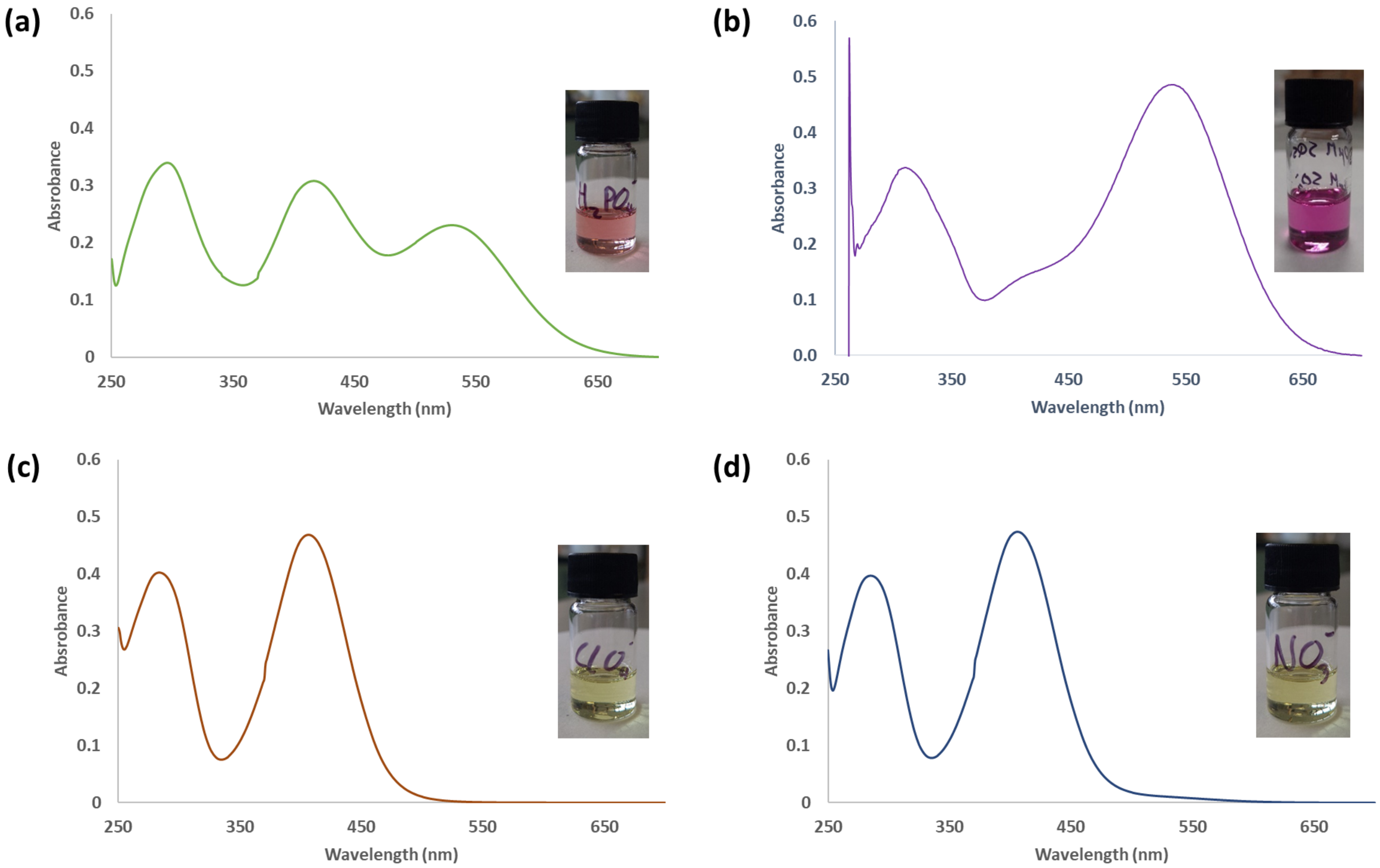

Figure S21: Absorbance spectra and associated colour change observed for **SQS-1** (20 x 10^{-6} M) with (a) $H_2PO_4^-$ (b) SO_4^{2-} (c) ClO4$^-$ (d) NO_3^- (as their TBA salts, 4 mM) in DMSO (0.5 % H_2O).

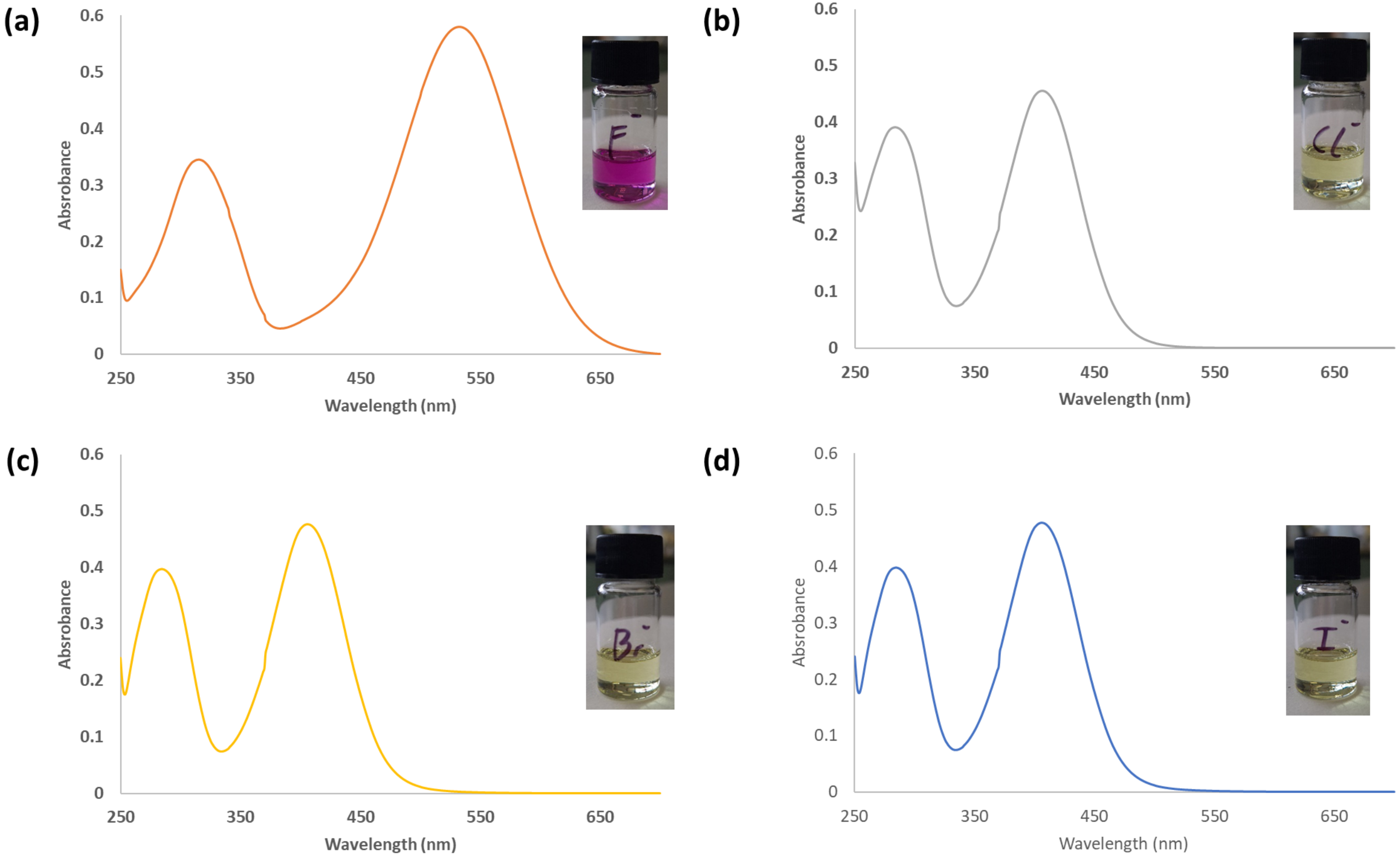

Figure S22: Absorbance spectra and associated colour change observed for **SQS-1** (20 x 10^{-6} M) with (a) F^- (b)Cl^- (c) Br^- (d) I^- (as their TBA salts, 4 mM) in DMSO (0.5 % H_2O).

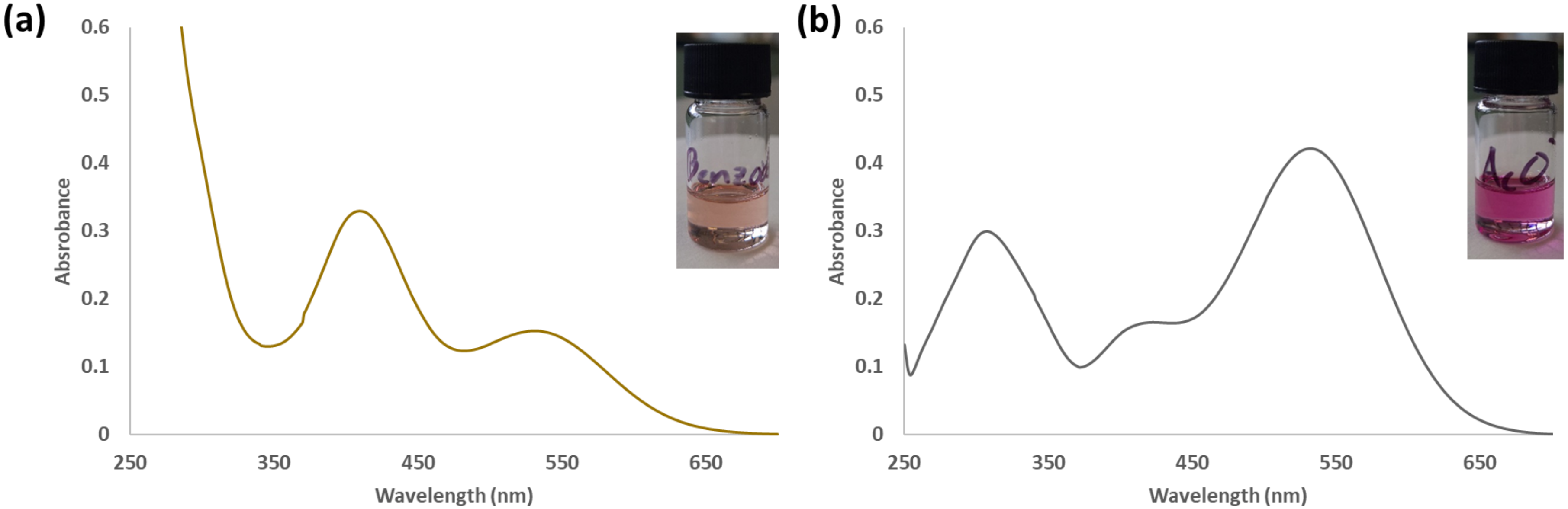

Figure S23: Absorbance spectra and associated colour change observed for **SQS-1** (20 x 10^{-6} M) with (a) benzoate (b) acetate (as their TBA salts, 4 mM) in DMSO (0.5 % H_2O).

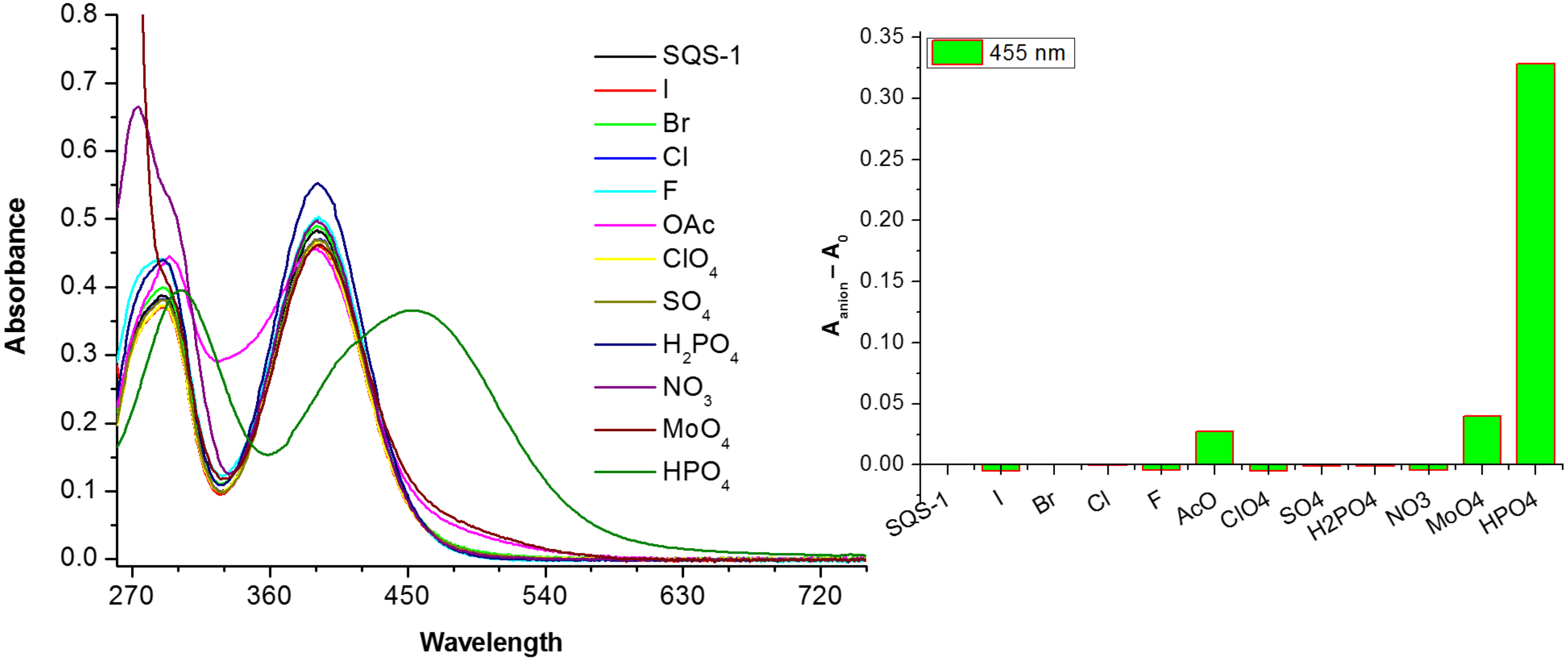

Figure S24: Absorbance spectra of **SQS-1** (20 x 10^{-6} M) with various anions (as their sodium salts) in DMSO/H_2O (1:1, v/v).

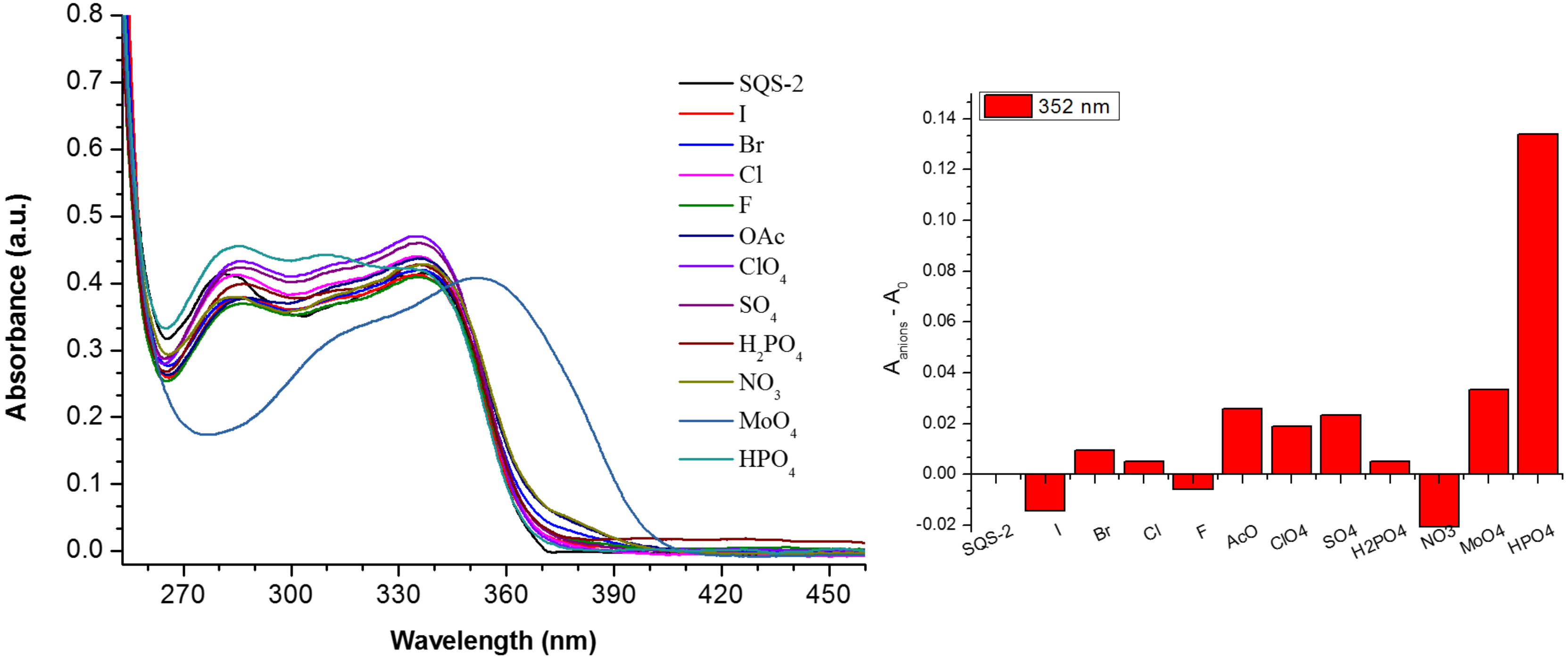

Figure S25: Absorbance spectra of **SQS-2** (20×10^{-6} M) with 20 mM different anions of sodium salts in Aq. DMSO/H_2O Solvent (1:1, v/v).

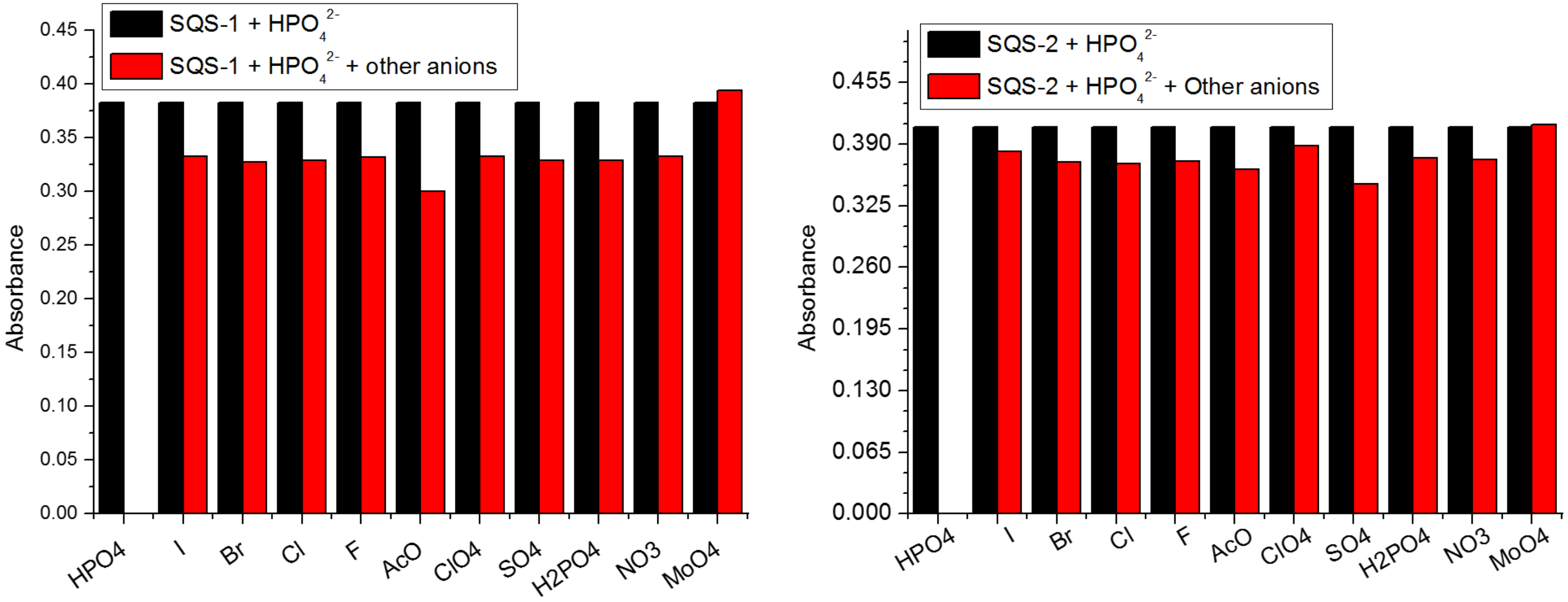

Figure S26: Absorbance spectra of SQS-1 & SQS-2 (20 x 10^{-6} M) with 20 mM different anions of sodium salts in Aq. DMSO/H_2O Solvent (1:1, v/v). Black bar (Probe with HPO_4^{2-}) and Red bar (Probe with HPO_4^{2-} + 10 equiv. of other anions). λ_{abs} = 455 nm.

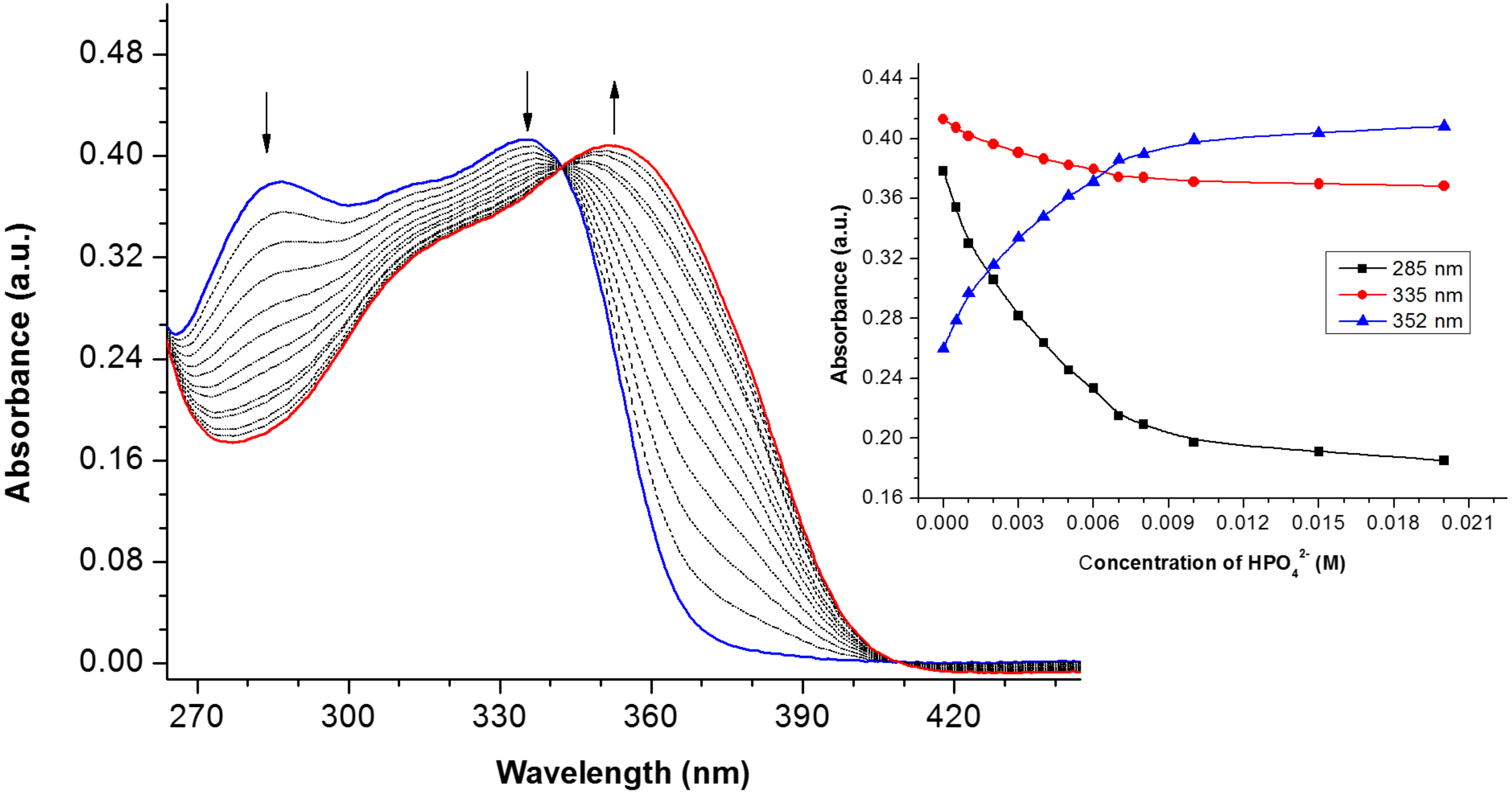

Figure S27: Absorbance spectra of **SQS-2** (20 x 10^{-6} M) at different concentration of HPO_4^{2-} (0-20 mM) in DMSO/H_2O Solvent (1:1, v/v). Band wavelength located at 335 nm shifted towards red shift and intensity of band located at 285 nm quenched with increment of anion concentration.

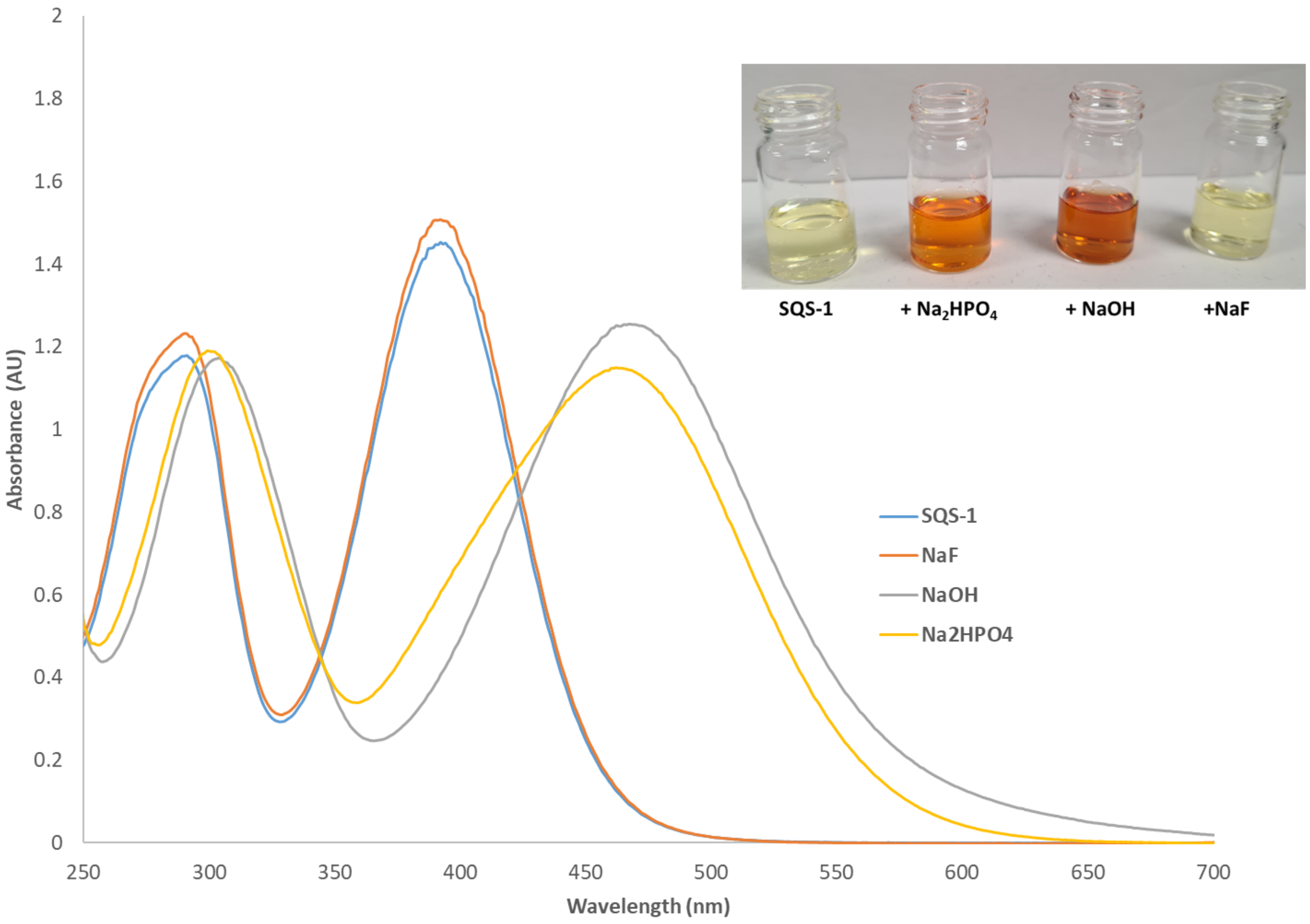

Figure S28: Absorbance spectra of **SQS-1** (20 x 10^{-6} M) upon addition of NaF, NaOH and Na_2HPO_4 (20 mM) in DMSO/H_2O (1:1, v/v). *Inset:* The corresponding colour change observed upon anion addition. The pH values of the corresponding solutions were measured as 12.8, 9.2, 11.3 respectively for OH^-, F^- and HPO_4^{2-}.

Table 1 Crystal data and structure refinement for SQS-1 and SQS-2

Identification code	SQS-1	SQS2
Empirical formula	$C_{27}H_{44}N_4O_7S$	$C_{29}H_{43}F_6N_3O_5S$
Formula weight	568.72	659.72
Temperature/K	100(2)	150(2)
Crystal system	monoclinic	monoclinic
Space group	$P2_1/n$	$P2_1/c$
a/Å	11.0801(4)	11.9487(7)
b/Å	11.1451(4)	28.0955(16)
c/Å	23.7179(8)	9.5951(6)
α/°	90	90
β/°	90.5600(10)	91.858(2)
γ/°	90	90
Volume/Å^3	2928.76(18)	3219.4(3)
Z	4	4
ρ_{calc}g/cm^3	1.290	1.361
μ/mm^{-1}	0.161	0.177
F(000)	1224.0	1392
Crystal size/mm^3	0.4 × 0.16 × 0.1	0.32 × 0.2 × 0.16
Radiation	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)
2Θ range for data collection/°	3.434 to 66.294	5.528 to 55.234
Index ranges	$-17 \le h \le 17$, $-17 \le k \le 17$, $-25 \le l \le 36$	$-15 \le h \le 15$, $-36 \le k \le 36$, $-12 \le l \le 12$
Reflections collected	61547	35194
Independent reflections	11160 [R_{int} = 0.0315, R_{sigma} = 0.0260]	7458 [R_{int} = 0.0726, R_{sigma} = 0.0530]
Reflections Obs. [I>=2σ (I)]	8804	4636
Data/restraints/parameters	11160/2/362	7458/51/428
Goodness-of-fit on F^2	1.018	1.009
Final R indexes [I>=2σ (I)]	R_1 = 0.0363, wR_2 = 0.0901	R_1 = 0.0543, wR_2 = 0.1189
Final R indexes [all data]	R_1 = 0.0529, wR_2 = 0.0984	R_1 = 0.1056, wR_2 = 0.1478
Largest diff. peak/hole / e Å^{-3}	0.48/-0.44	0.39/-0.37

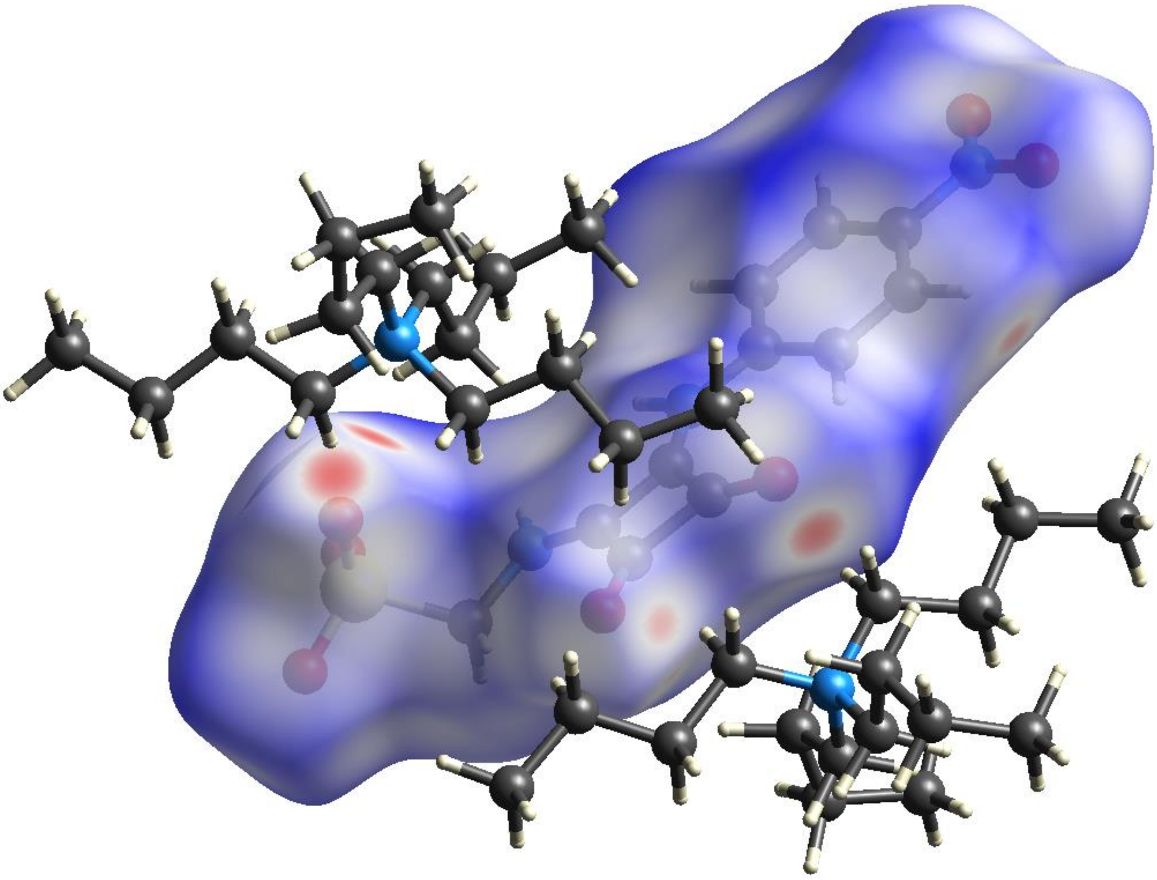

Figure S29: Interaction between NBu_4^+ cations and the **SQS-1** anion, with C-H···O contacts highlighted by visualisation on a Hirshfeld surface mapped with normalised contact distance (surface isovalue scaled to -0.3 / +1.2).[11]

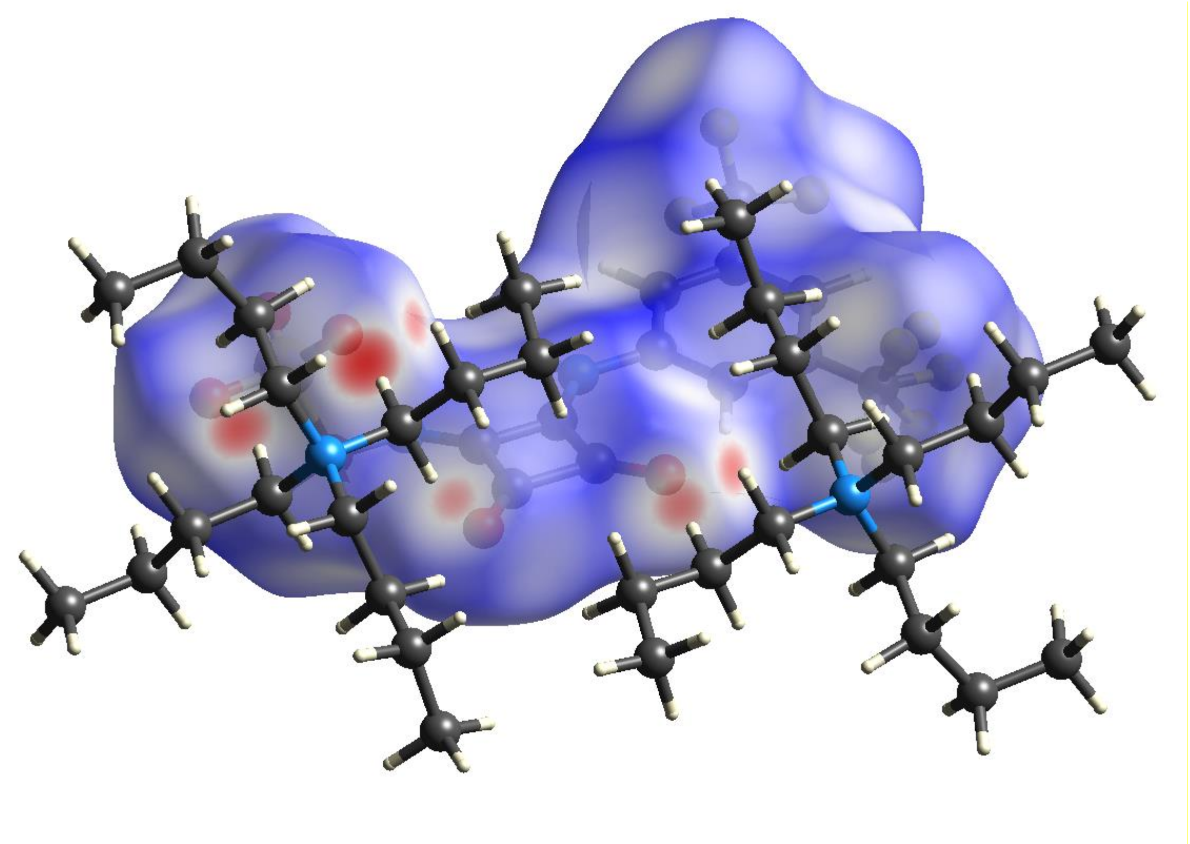

Figure S30: The interaction between the NBu_4^+ cations and the **SQS-2** anion, with C-H···O contacts highlighted by visualisation on the normalised contact distance mapping of the Hirshfeld surface (surface isovalues -0.3/+1.2).[11]

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