Adamantane appended antimicrobial supramolecular self-associating amphiphiles

Andzelika Rutkauskaite, ‡ Lisa J.White, ‡ Jessica E. Boles, Kira L. F. Hilton, Melanie Clifford, Bethany Patenall, Bree R. Streather, Daniel P. Mulvihill, Samantha A. Henry, Mark Shepherd, J. Mark Sutton,* Charlotte K. Hind,* and Jennifer R. Hiscock*

Contents

Experimental

General remarks: A positive pressure of nitrogen and oven dried glassware were used for all reactions. All solvents and starting materials were purchased from known chemical suppliers or available stores and used without any further purification unless specifically stipulated. The NMR spectra were obtained using a Burker AV2 400 MHz or AVNEO 400 MHz spectrometer. The data was processed using MestReNova software. NMR Chemical shift values are reported in parts per million (ppm) and calibrated to the centre of the residual solvent peak set (s = singlet, br = broad, d = doublet, t = triplet, q = quartet, m = multiplet). Tensiometry measurements were undertaken using the Biolin Scientific Theta Attension optical tensiometer. The data was processed using Biolin OneAttension software. A Hamilton (309) syringe was used for the measurements. The melting point for each compound was measured using Stuart SMP10 melting point apparatus. High resolution mass spectrometry was performed using a Bruker microTOF-Q mass spectrometer and spectra recorded and processed using Bruker's Compass Data Analysis software. Infrared spectra were obtained using a Shimadzu IR-Affinity-1 model Infrared spectrometer. The data are analysed in wavenumbers (cm^{-1}) using IRsolution software. DLS and Zeta Potential studies were carried out using Anton Paar Litesizer™ 500 and processed using KalliopeTM Professional.

^{1}H NMR Diffusion Ordered Spectroscopy (DOSY): The hydrodynamic diameter was derived from diffusion rates obtained from ^{1}H NMR DOSY measurements using the Stokes-Einstein equation. The viscosity value used for the calculation was 0.00199 mPa (DMSO).

Mass Spectrometry: Approximately 1 mg of each compound was dissolved in 1 mL of methanol. This solution was further diluted 100-fold before undergoing analysis where 10 µL of each sample was then injected directly into a flow of 10 mM ammonium acetate in 95% water (flow rate = 0.02 mL/min).

Self-association constant calculation: Self-association constants were determined using Bindfit v0.5 (http://app.supramolecular.org/bindfit/).[1] All the data can be accessed online using the hyperlinks provided.

Tensiometry Studies: All samples were prepared in an EtOH/H_2O 1:19 solution. All samples underwent an annealing process in which the various solutions were heated to approximately 40 °C before being allowed to cool to room temperature, allowing each sample to reach a thermodynamic minimum. All samples were prepared through serial dilution of the most concentrated sample. Three surface tension measurements (n=3) were obtained for each sample at a given concentration, using the pendant drop method. The average values were then used to calculate the critical aggregation concentration (CAC).

DLS Studies: All vials used for preparing the samples were clean dry. All solvents used were filtered to remove any particulates that may interfere with the results obtained. Samples of differing concentrations were obtained through serial dilution of a concentrated solution. All samples underwent an annealing process, in which they were heated to 40 °C before being allowed to cool to 25 °C. A series of 10 runs were recorded at 25 °C.

Zeta Potential Studies: All vials used for preparing the samples were clean dry. All solvents used were filtered to remove any particulates that may interfere with the results obtained. All samples underwent an annealing process in which the various solutions were heated to approximately 40 °C before cooling to room temperature, allowing each sample to reach a thermodynamic minimum. The final zeta potential value given is an average of the number of experiments conducted at 25 °C.

Biological experimental

Preparation of Luria Broth media (LB): Yeast extract (5 g), tryptone (10 g) and sodium chloride (10 g) were dissolved in dH_2O (1 L) then divided into bottles and autoclaved.

Preparation of Luria Broth (LB) agar plates: Agar (6 g) was added to LB (400 mL) and autoclaved. Once cool, the LB agar was poured into sterile petri dishes under sterile conditions and allowed to set. LB plates were stored at 4 °C until use.

Preparation of antimicrobial compounds for screening: Compounds were dissolved in 5% ethanol in water to make up 10 or 20 mM solutions on the day of experiment.

Preparation of antimicrobial compounds for MIC_{50} calculations: Compounds were dissolved into 5% ethanol in water to make up solutions to the desired concentration on the day of experiment.

Preparation of bacterial plates: Sterile LB agar plates were streaked using the desired bacteria (either DH10β *Escherichia coli*, USA300 Methicillin-Resistant *Staphylococcus aureus* or PA01 *Pseudomonas aeruginosa*) then incubated at 37 °C overnight.

Preparation of Inoculum: An initial culture was made up by inoculating LB media (5 mL) with 3 single colonies of the desired bacteria under sterile conditions and incubated at 37 °C overnight. The following day, a subculture was made up using LB (5 mL) and the initial culture (50 µL), then incubated at 37 °C until the culture had reached an optical density (OD) of 0.4 at 600 nm. Optical Density was adjusted using sterile dH2O to equal the 0.5 McFarland standard (107 – 108 cfu/mL), then a 1:10 dilution was carried out using sterile dH_2O (900 µL) and the McFarland adjusted suspension (100 µL). A final dilution (1:100) was carried using the 1:10 suspension (150 µL) and LB (14.85 mL) before use to achieve a final cell concentration of 105 cfu/mL.

Preparation of 96 well microplate for screening: Compounds at a concentration of 10 mM or 20 mM were made up using 5% ethanol. The 1:100 cell suspension (150 µL) was pipetted into the wells. Compound solutions (30 µL) were added into 3 wells on the plate. The final screening concentration for each compound was 1.65 or 3.33 mM in the well. The plate was incubated for 20 hours in a plate reader, with optical density readings being taken at 600 nm every 15 minutes. Optical density readings were plotted against time to produce growth curves. Compounds that inhibited growth by 10% or more were taken forward for MIC_{50} calculations.

Preparation of 96 well microplate for MIC_{50}: The 1:100 cell suspension (150 µL) was dispensed into individual wells under sterile conditions. Compounds at different concentrations (30 µL) were added to the wells to equal a total volume of 180 µL in the well and carried out in triplicate. The plates were sealed using parafilm and incubated at 37 °C. An optical density reading was taken at 600 nm at 900 minutes.

Calculation of MIC_{50}: OD_{600} readings were taken at 900 minutes for each concentration of drug and plotted in Microsoft® Excel® 2013. These readings were then plotted in Origin® 2015 and the resultant curve was normalized and fitted using the Boltzmann fit to define the MIC_{50} values for each drug.

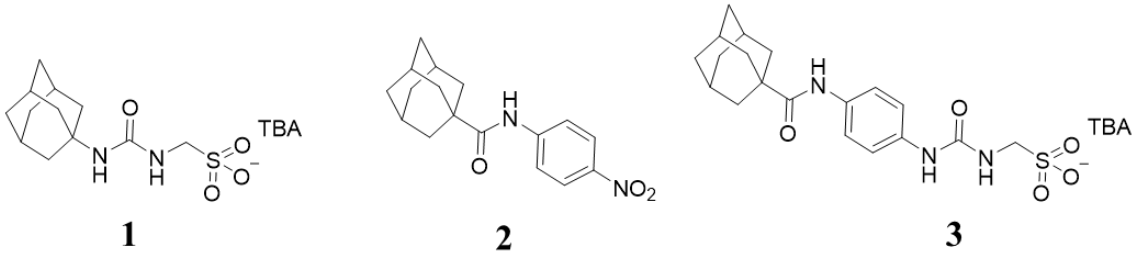
Haemolysis assay: Protocol modified from Travis et al.[2] Heparinised human red blood cells collected from a volunteer and washed three times in phosphate-buffered saline (PBS) (pH 7.4). SSAs were serial diluted using PBS buffer across a 96-well V bottom Greiner plate. Negative control (100 µL PBS), and positive control (100 µL 0.1% (v/v) Triton-X-100) were added to the plate. 100 µL of 10% (v/v) of blood suspension added to all wells. Plates were incubated for 60 minutes at 37 °C. After incubation, plates were centrifuged for 15 mins at 4680 rpm. The resultant supernatant was then transferred to a 96-well flat bottom plate and absorbance read at 540 nm (Fluostar Omega). Percent haemolysis was calculated using the formula as shown in Equation S1.

Equation S1 - Percentage haemolysis calculation.

$$\text{Haemolysis (\%)} = \frac{A_{sample} - A_{negative}}{A_{Triton} - A_{negative}} X\ 100$$

***G. mellonella* treatment assay**: *G. mellonella* larvae were purchased from Livefood UK Ltd. (Rooks Bridge, UK) and maintained on wood chips in the dark at 15 °C until use. *Galleria* larvae were injected with 10 µL of SSA in 5% ethanol in dH_2O and incubated at 37 °C for 5 days and the deaths counted. Groups of 10 *Galleria* were injected per compound.

Chemical structures



1 **2** **3**

Figure S1 – Chemical structures of SSAs **1** and **3** and synthetic intermediate **2**. TBA = tetrabutylammonium.

Chemical synthesis

Compound 1: A solution of 1-adamantyl isocyanate (0.35 g, 2.00 mmol) and tetrabutylammonium aminomethane sulfonate (0.70 g, 2.00 mmol) in ethyl acetate (30 mL) was heated at reflux under N_2 overnight. Ethyl acetate was decanted, and residual oil was sonicated in ethyl acetate (50 mL) for 30 minutes, after which ethyl acetate was decanted once again. The residual oil was then dissolved in minimal, hot methanol and allowed to cool. The resultant crystals were then removed by filtration and the filtrate taken to dryness, dissolved in DCM (30 mL) and washed with water (30 mL) to give the final product as a yellow oil (0.88 g, 1.68 mmol). Yield: 84%; Melting point: 83 °C; ^{1}H NMR: (400 MHz, 298 K, DMSO-d_6): δ: 5.95 (s, 1H), 5.91 (t, *J* = 5.72 Hz, 1H), 3.71 (d, *J* = 5.8 Hz, 2H), 3.19-3.14 (m, 8H), 1.98 (br s, 3H), 1.84-1.84 (m, 6H), 1.59-1.53 (m, 14H), 1.36-1.26 (m, 8H), 0.94 (t, *J* = 7.28 Hz, 12H); ^{13}C{^{1}H} NMR: (100 MHz, 298 K, DMSO-d_6): δ: 156.1 (CO), 58.0 (CH_2), 56.9 (CH_2), 42.5 (CH_2), 36.6 (CH_2), 29.4 (CH), 23.5 (CH_2), 19.7 (CH_2), 14.0 (CH_3); IR (film): ν = 3315 (NH stretch), 1670 (C=O stretch), 1168 (C-O stretch), 1035 (S=O stretch); HRMS for anionic component ($C_{12}H_{19}N_2o_4S^-$): m/z: act: 287.1071 $[M]^-$, cal: 287.0195$[M]^-$.

Compound 2: Adamantane-1-carbonyl chloride (1.45 g, 7.25 mmol) was added to a stirring solution of 4-nitroaniline (1.00 g, 7.25 mmol) in a mixture of dry pyridine (10 mL) and chloroform (40 mL) and heated at 60 °C under N_2 overnight. The solution was then washed with water (50 mL), taken to dryness, and further refluxed in methanol (50 mL) for 2 hours. The solution was then allowed to cool to room temperature, filtered, and the resultant solid was washed with cold methanol (10 mL), producing Compound **2** as a light pink solid (1.67 g, 5.52 mmol). Yield: 76%. Melting point: > 200 °C; ^{1}H NMR: (400 MHz, 298 K, DMSO-d_6): δ: 9.70 (s, 1H), 8.20 (d, *J* = 9.32 Hz, 2H), 7.96 (d, *J* = 9.32 Hz, 2H), 2.03 (br s, 3H), 1.92-1.93 (m, 6H), 1.71 (br s, 6H); ^{13}C{^{1}H} NMR: (100 MHz, 298 K, DMSO-d_6): δ: 177.3 (CO), 146.2 (ArC), 142.5 (ArC), 125.1 (ArCH), 120.0 (ArCH), 41.8 (CH_2), 38.4 (CH_2), 36.4 (CH_2), 28.0 (CH); IR (film): ν = 3310 (NH stretch), 1666 (C=O stretch), 1176 (C-O stretch); HRMS for $C_{17}H_{20}N_2O_3$: m/z: act: 299.1401 $[M-H]^-$, cal: 299.1395 $[M-H]^-$.

Compound 3: Intermediate (**2**) (0.60 g, 2.00 mmol) was dissolved in a mixture of DMSO (1 mL) and ethanol (10 mL) to which activated palladium on carbon 10% (0.06 g) was suspended. The mixture was then stirred under a hydrogen atmosphere overnight. The palladium on carbon 10% was then removed by filtration, the ethanol within the filtrate was then removed under reduced pressure, and the resultant DMSO solution added to water (30 mL). The resultant precipitate was then removed by filtration and dried under reduced pressure to give a white solid, assumed yield 100%. The crude white solid was dissolved in a mixture of dry dimethylformamide (3 mL) and ethyl acetate (25 mL). This mixture was heated to 65 °C and then triphosgene (0.64 g, 2.16 mmol) was then added and heated for a further 1.5 hours. To this solution was then added tetrabutylammonium aminomethane sulfonic acid (0.70 g, 2.00 mmol) and the solution heated at 65 °C overnight. The solution was then allowed to cool to room temperature and washed with water (2 × 20 mL) and the organic phase taken to dryness. The resultant oil was then dissolved in chloroform (30 mL) and a precipitate removed via filtration. The resultant filtrate was then taken to dryness to give compound **3** as a white solid (0.80 g, 1.24 mmol). Yield: 62%; Melting point: 147 °C; ^{1}H NMR: (400 MHz, 298 K, DMSO-d_6): δ: 8.94 (s, 1H), 8.66 (br s, 1H), 7.46 (d, *J* = 8.88 Hz, 2H), 7.27 (d, *J* = 8.88 Hz, 2H), 6.41 (br s, 1H), 3.85 (d, *J* = 5.8 Hz, 2H), 3.18-3.12 (m, 8H), 2.00 (br s, 3H), 1.89-1.89 (m, 6H), 1.69-1.52 (m, 14H), 1.35-1.26 (m, 8H), 0.93 (t, *J* = 7.32 Hz, 12H); ^{13}C{^{1}H} NMR: (100 MHz, 298 K, DMSO-d_6): δ: 175.8 (CO), 155.0 (CO), 136.4 (ArC), 133.3 (ArC), 121.4 (ArCH), 117.9 (ArCH), 58.0 (CH_2), 56.6 (CH_2), 38.9 (CH_2), 36.5 (CH_2), 28.2 (CH), 23.5 (CH_2), 19.7 (CH_2), 14.0 (CH_3); IR (film): ν = 3298 (NH stretch), 1683 (C=O stretch), 1176 (C-O stretch), 1037 (S=O stretch); HRMS for anionic component ($C_{19}H_{24}N_3O_5S^-$): m/z: act: 406.1442 [M]$^-$, cal: 406.1418 [M]$^-$.

NMR

NMR characterisation data

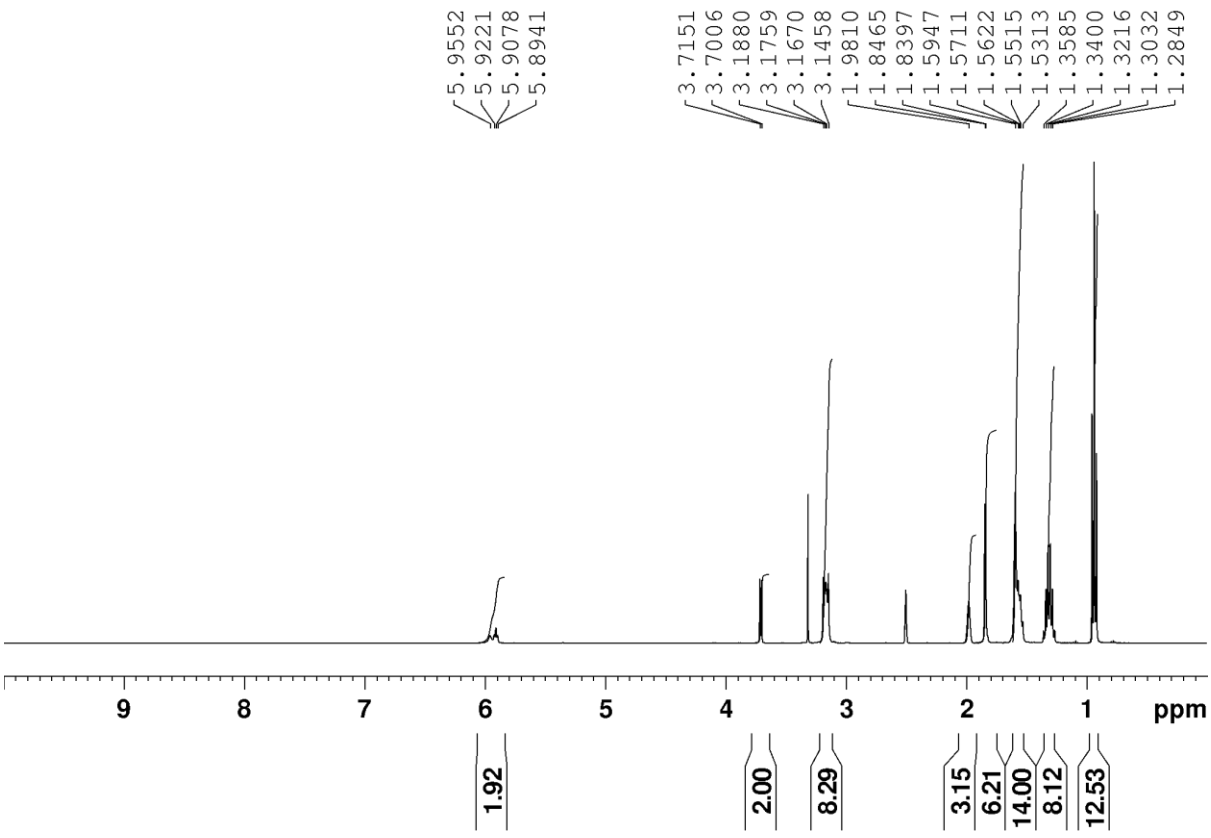


Figure S2 – ^{1}H NMR of compound **1** in DMSO-d_6 conducted at 298 K.

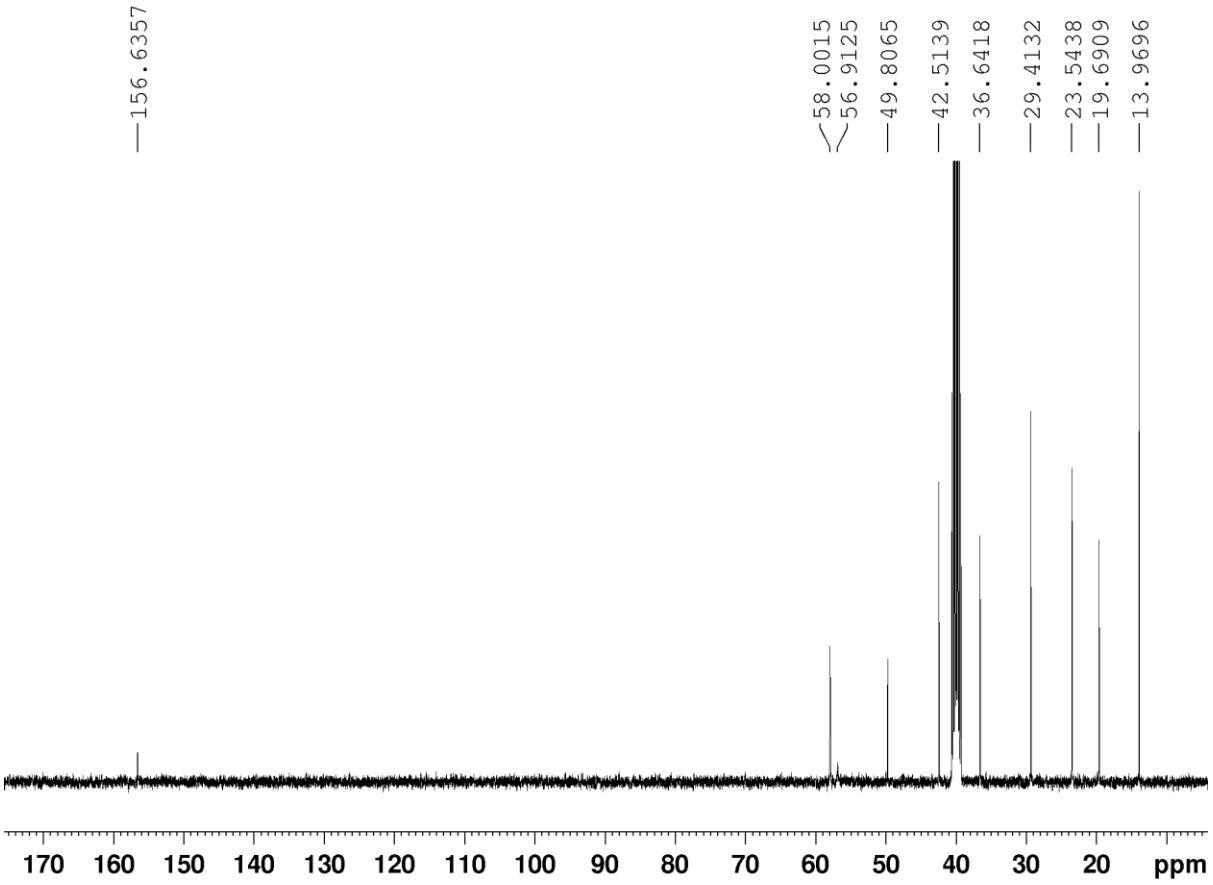


Figure S3 - $^{13}C\{^{1}H\}$ NMR of compound **1** in DMSO-d_6 conducted at 298 K.

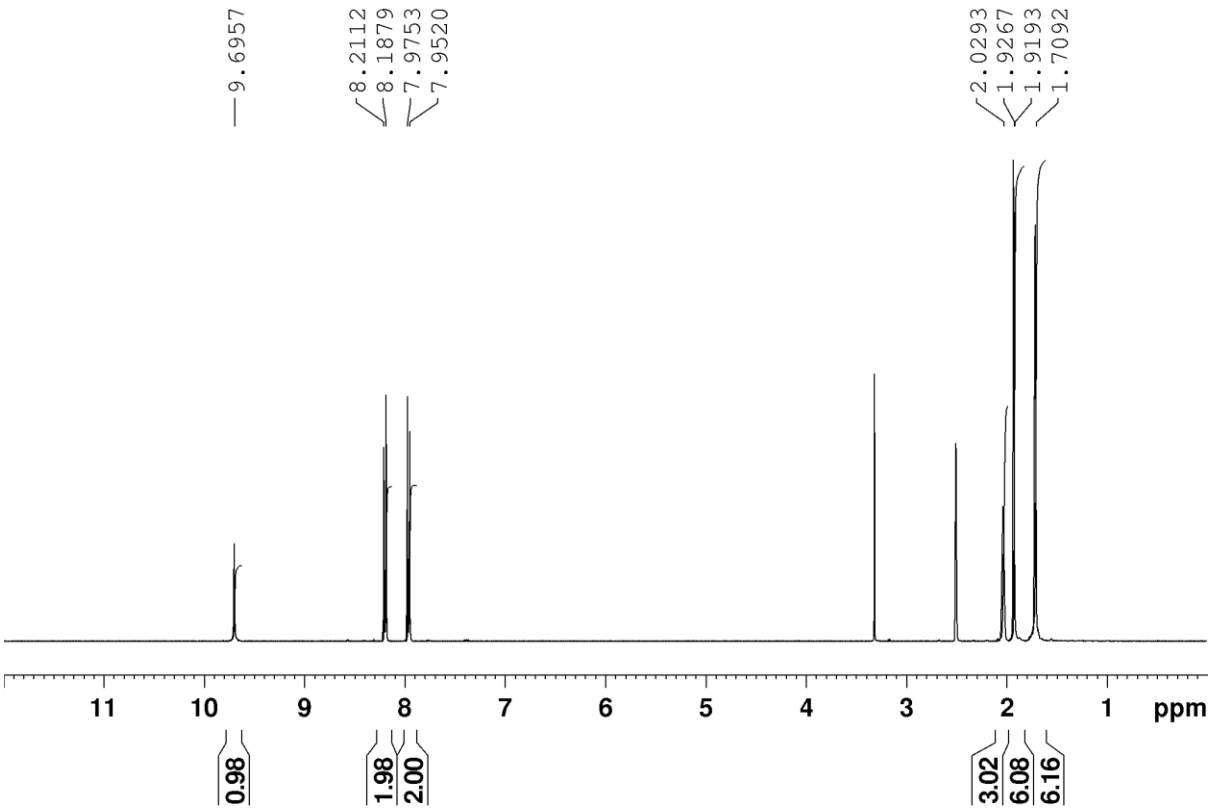


Figure S4 – ^{1}H NMR of compound **2** in DMSO-d_6 conducted at 298 K.

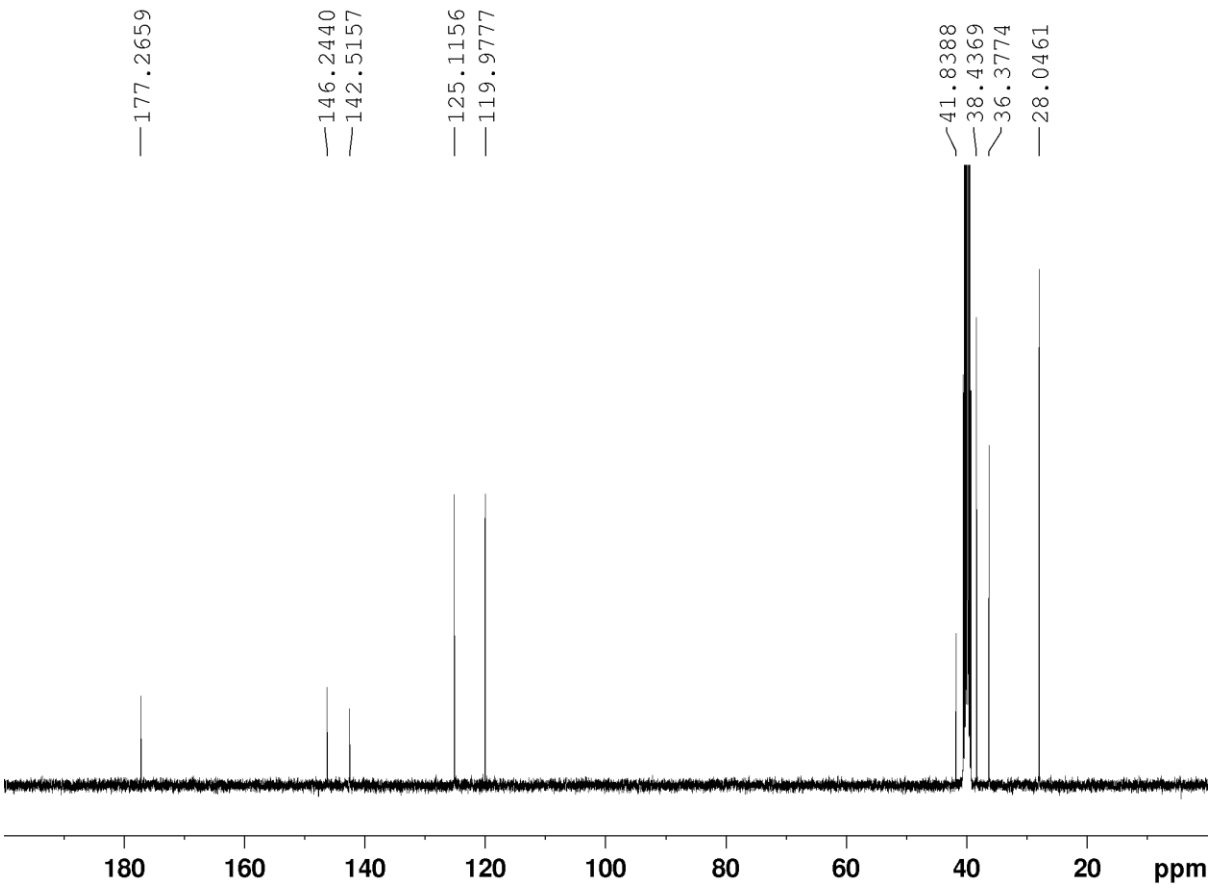


Figure S5 - $^{13}C\{^{1}H\}$ NMR of compound **2** in DMSO-d_6 conducted at 298 K.

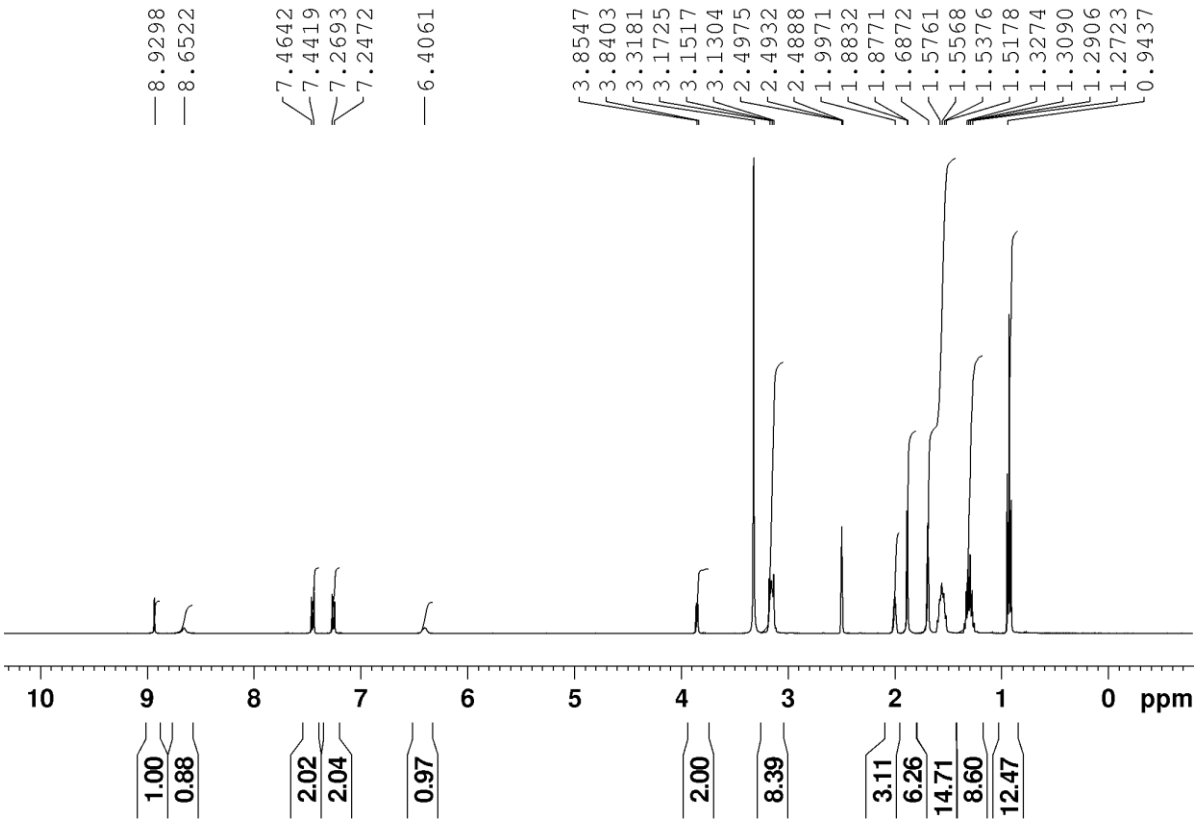


Figure S6 – ^{1}H NMR of compound **3** in DMSO-d_6 conducted at 298 K.

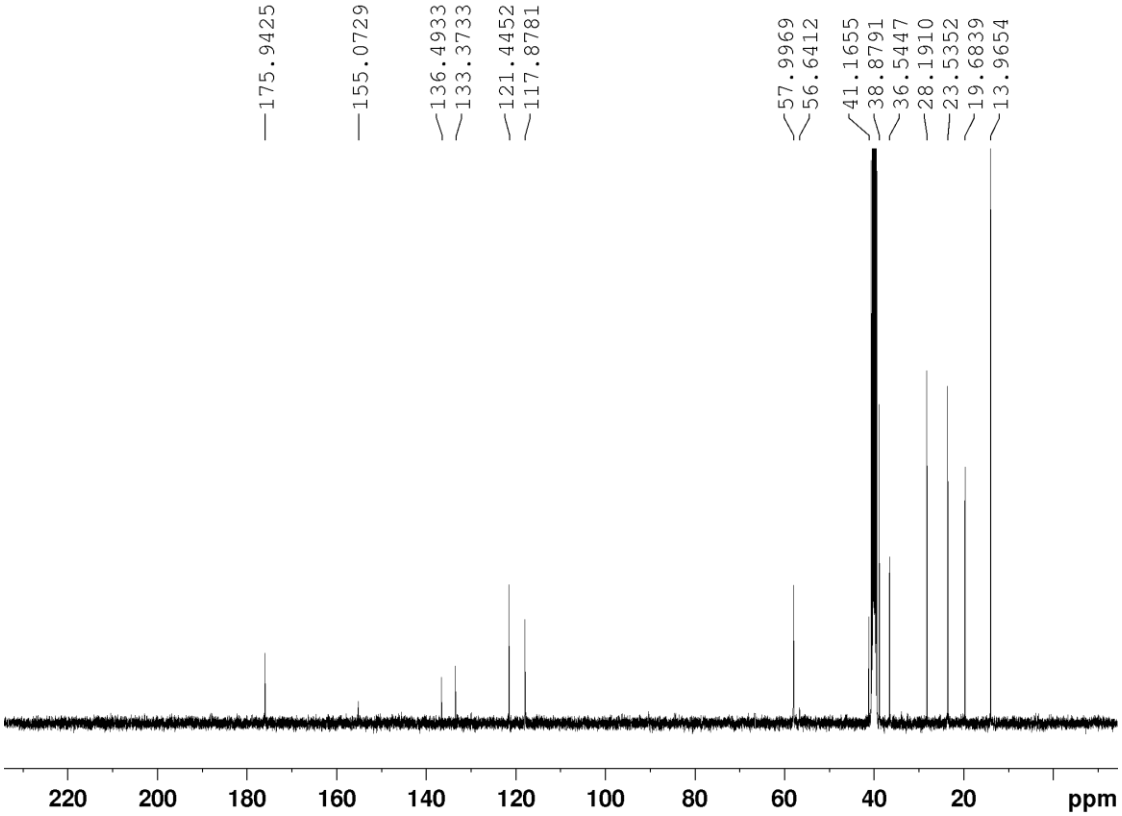


Figure S7 – ^{13}C{^{1}H} NMR of compound **3** in DMSO-d_6 conducted at 298 K.

Quantitative ^{1}H NMR studies

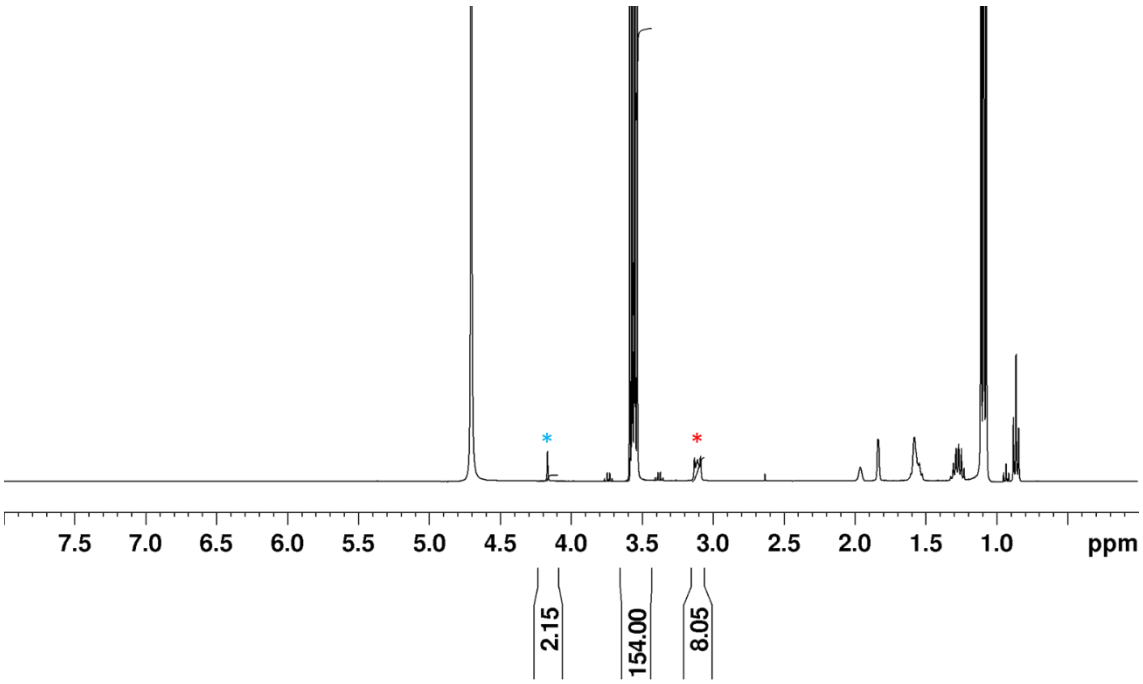


Figure S8 – Quantitative ^{1}H NMR spectrum with a delay (d_1 = 60 s) of Compound **1** (5.56 mM) in D_2O/ 5.0% EtOH. Comparative integration indicated 0% of the anionic component of SSA and 0% of TBA counter cation has become NMR silent (anion*, TBA*).

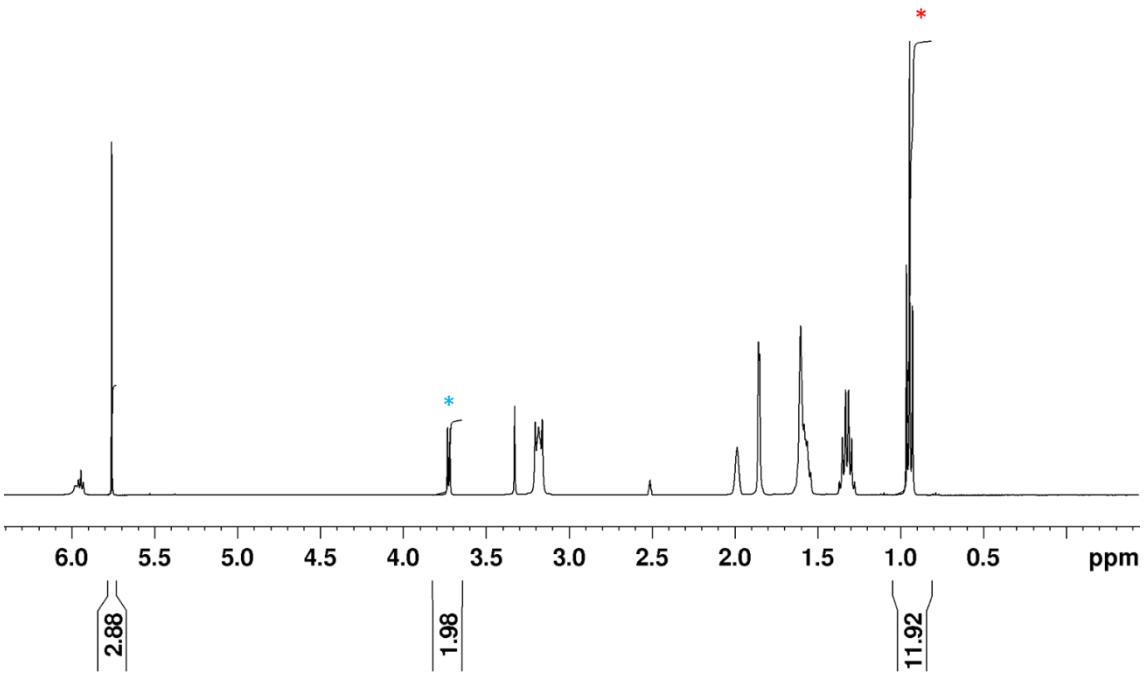


Figure S9 - Quantitative ^{1}H NMR spectrum with a delay (d_1 = 60 s) of compound **1** (111.2 mM) in DMSO-d_6/ 1.0% DCM. Comparative integration indicated 0% of the anionic component of SSA and 0% of TBA counter cation has become NMR silent (anion*, TBA*).

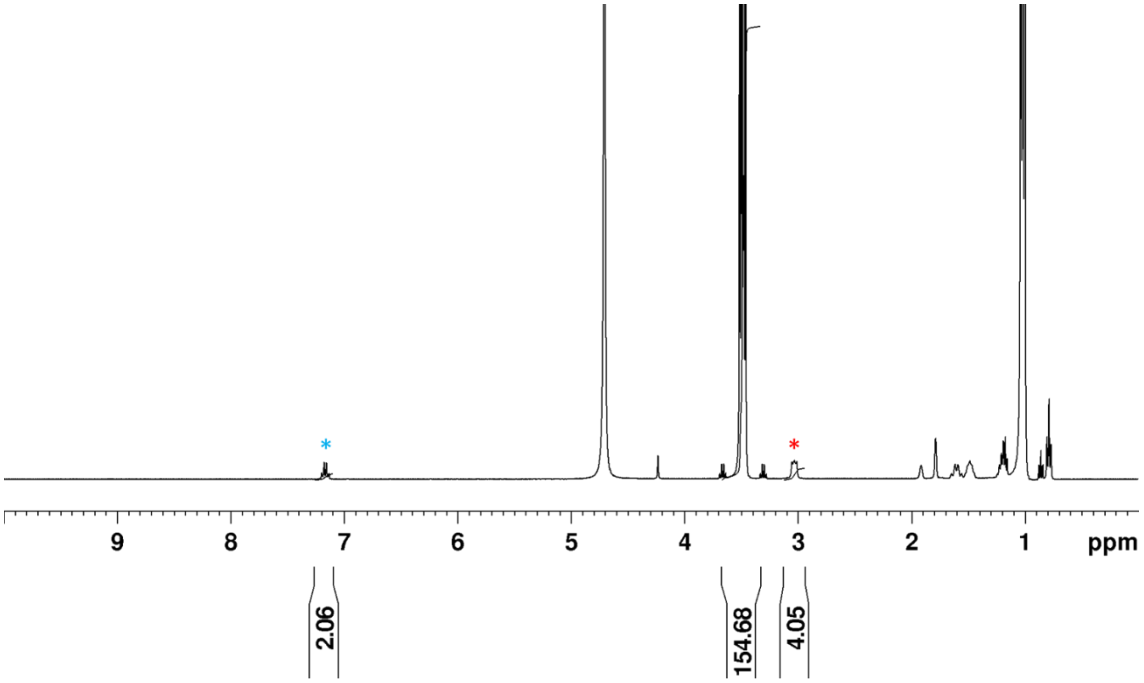


Figure S10 - Quantitative ^{1}H NMR spectrum with a delay (d_1 = 60 s) of Compound **3** (5.56 mM) in D_2O/ 5.0% EtOH. Comparative integration indicated 48% of the anionic component of SSA and 49% of TBA counter cation has become NMR silent (anion*, TBA*).

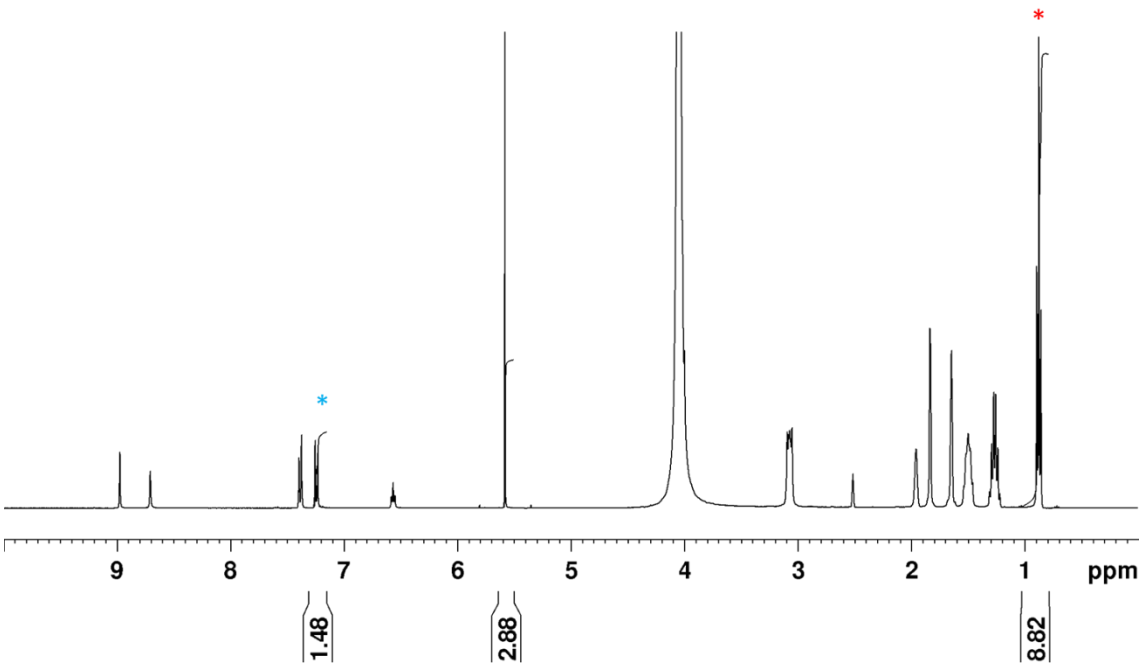


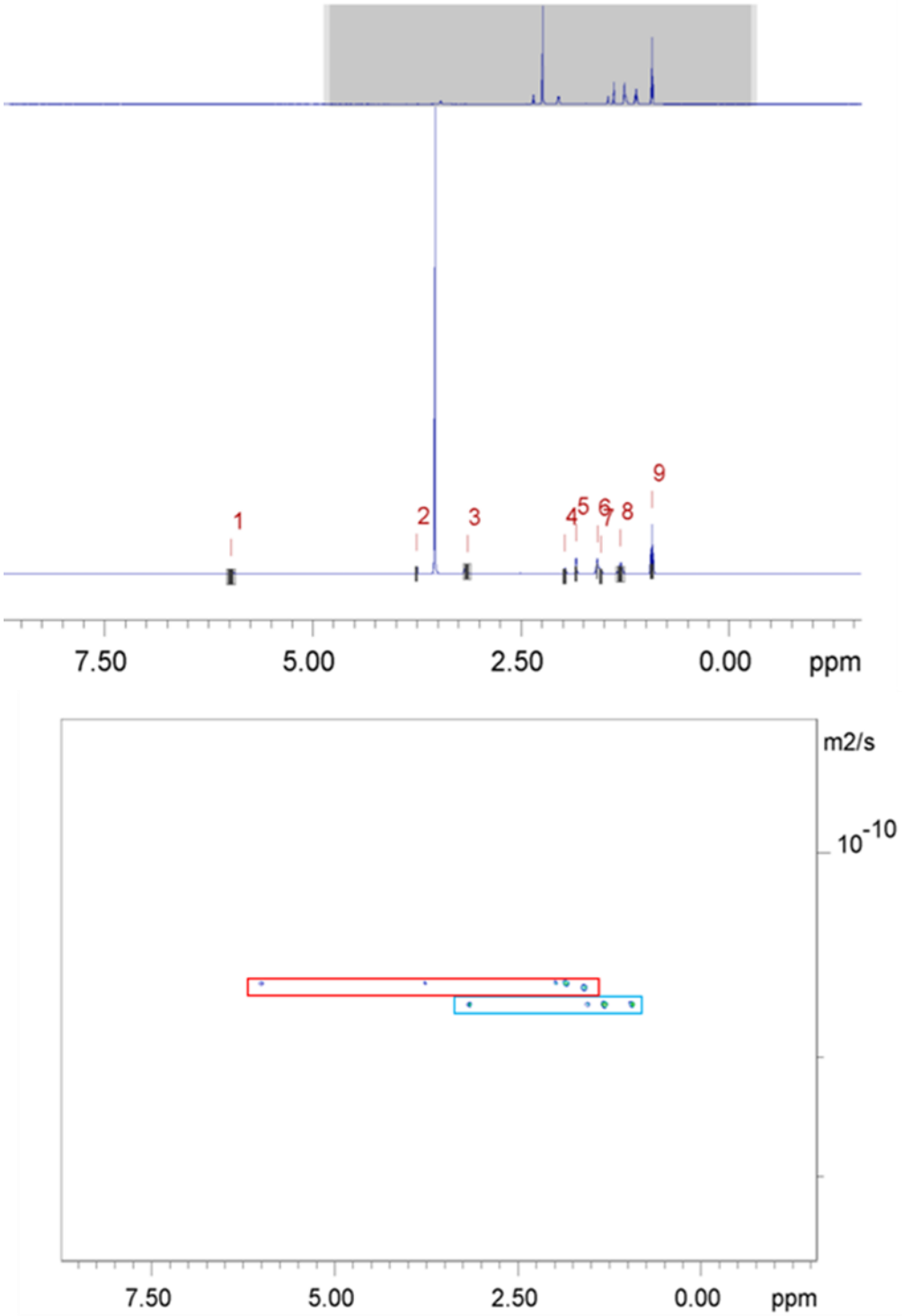
Figure S11 - Quantitative ^{1}H NMR spectrum with a delay (d_1 = 60 s) of compound **3** (111.8 mM) in DMSO-d_6/ 1.0% DCM. Comparative integration indicated 26% of the anionic component of SSA and 27% of TBA counter cation has become NMR silent (anion*, TBA*).

Comparative overview

Table S1 – Overview of results of quantitative ^{1}H NMR (qNMR) studies. Values given in % represent the observed proportion of compound that has become NMR silent. Studies performed at concentrations of ≈ 112 mM in DMSO-d_6 and 5.56 mM in D_2O.

Compound		DMSO-d_6 1% DCM (%)	D_2O 5% EtOH (%)
1	Anion	0	0
	Cation	0	0
3	Anion	26	48
	Cation	27	49

^{1}H NMR DOSY studies



Peak name	F2 [ppm]	Io	error	D [m2/s]	error
1	5.982	2.87e+08	1.138e+04	1.55e-10	1.362e-14
2	3.754	3.31e+08	6125	1.55e-10	6.373e-15
3	3.148	9.86e+08	1.077e+04	1.68e-10	4.049e-15
4	1.974	5.30e+08	7191	1.55e-10	4.684e-15
5	1.839	8.98e+08	5623	1.55e-10	2.159e-15
6	1.586	8.36e+08	4627	1.58e-10	1.941e-15
7	1.541	5.37e+08	6755	1.67e-10	4.633e-15
8	1.308	1.43e+09	1.098e+04	1.67e-10	2.839e-15
9	0.927	2.25e+09	7642	1.67e-10	1.251e-15

Figure S12 - ^{1}H DOSY NMR spectrum of compound **1** (112.0 mM) in DMSO-d_6 at 298 K and a table reporting the diffusion constants calculated for each peak used to determine the hydrodynamic diameter of the anionic components of **1** (d_H = 1.41 nm). Peaks 1, 2 and 4-6 correspond to the anionic component of **1** and peaks 3, 7-9 correspond to the cationic component of **1**.

Comparative overview

Table S2 - Overview of diffusion coefficients (m^2s^{-1}) for compound **1**, in DMSO-d_6 at 298 K. Errors for diffusion constants are no greater than $\pm$ 1 x 10^{-13} m^2s^{-1}.

	Diffusion Coefficient (m^2s^{-1})	
Compound	Anion	TBA
1	1.55 x 10^{-10}	1.67 x 10^{-10}

Table S3 - Overview of hydrodynamic diameters (nm) for compound **1** in DMSO-d_6 at 298 K.

Compound	Anion	Cation
1	1.41	1.31

^{1}H NMR self-association (dilution) studies

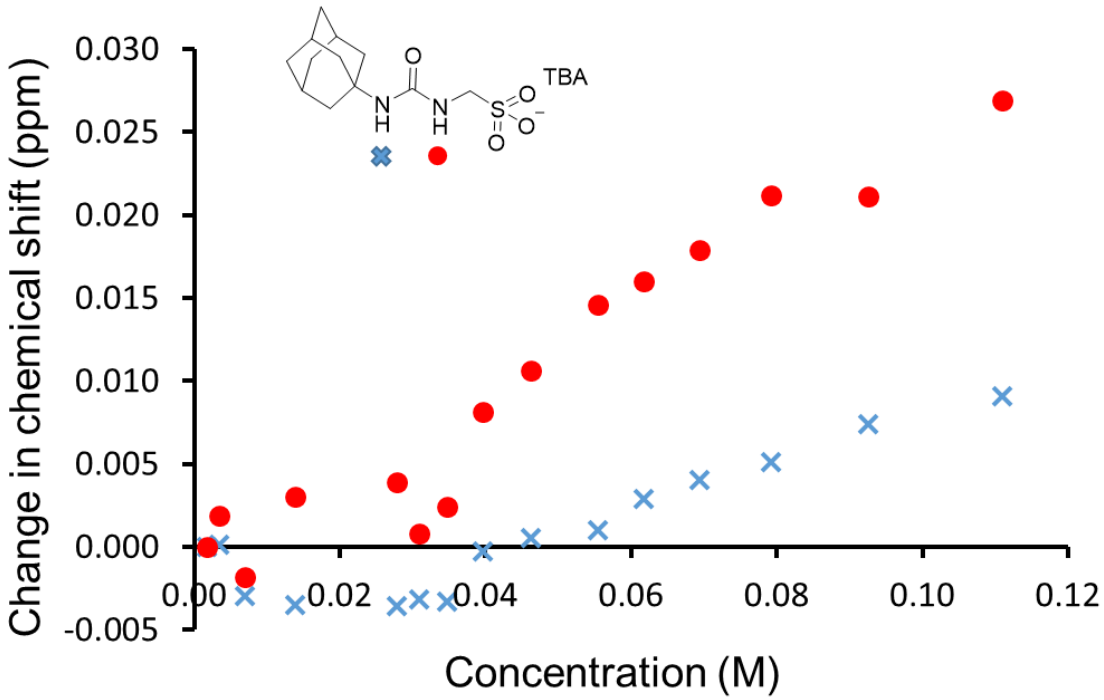


Figure S13 - Graph illustrating the ^{1}H NMR down-field change in chemical shift of urea NH resonances with increasing concentration of compound **1** in DMSO-d_6 0.5% H_2O (298 K).

Self-association constant calculation

Compound **1** - Dilution study in DMSO-d_6 0.5% H_2O. Values calculated from data gathered from both NH 1 and 2

Note - Data could not be fitted to bindfit Equal K/Dimerization model

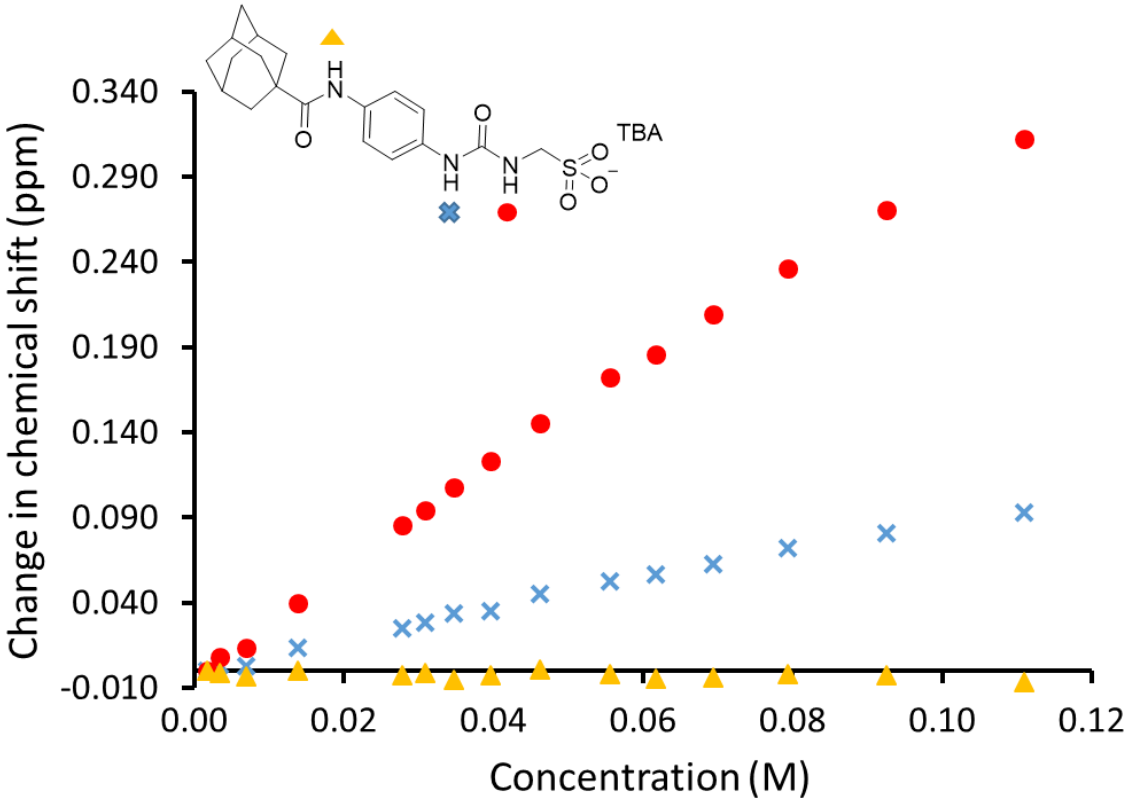


Figure S14 - Graph illustrating the ^{1}H NMR down-field change in chemical shift of urea NH resonances with increasing concentration of compound **3** in DMSO-d_6 0.5% H_2O (298 K).

Self-association constant calculation

Compound **3** - Dilution study in DMSO-d_6 0.5% H_2O. Values calculated from data gathered from NH 1, 2 and 3

Note - Data could not be fitted to bindfit Equal K/Dimerization model

Dynamic light scattering (DLS) data

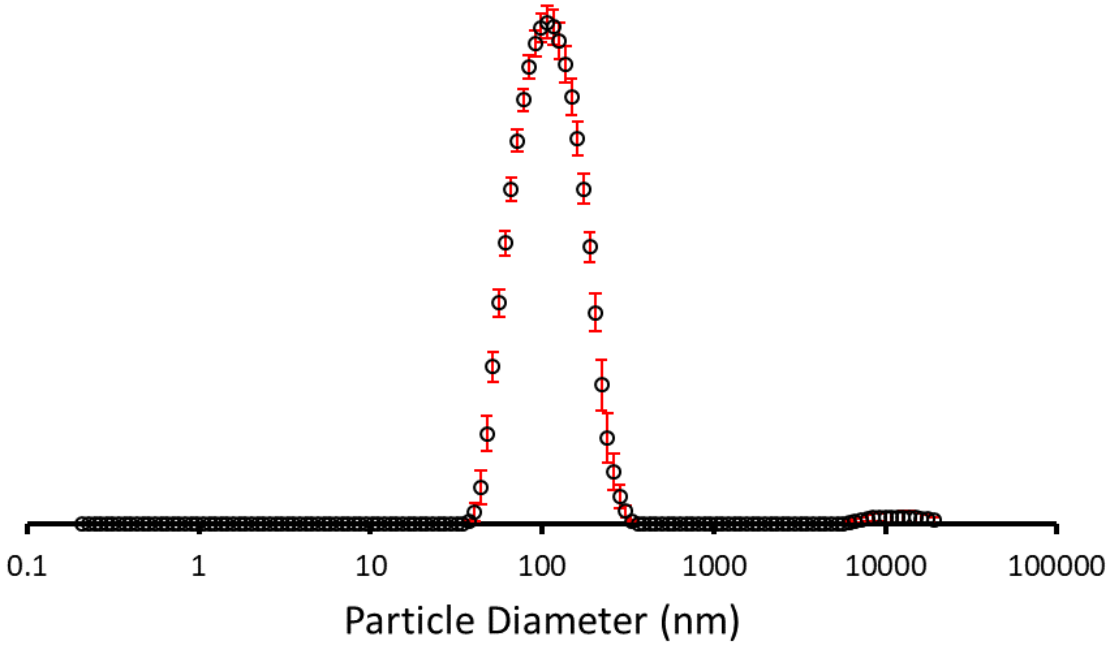


Figure S15 - The average intensity particle size distribution calculated using 10 DLS runs for compound **3** (5.56 mM) in an EtOH/H_2O 1:19 solution at 298 K.

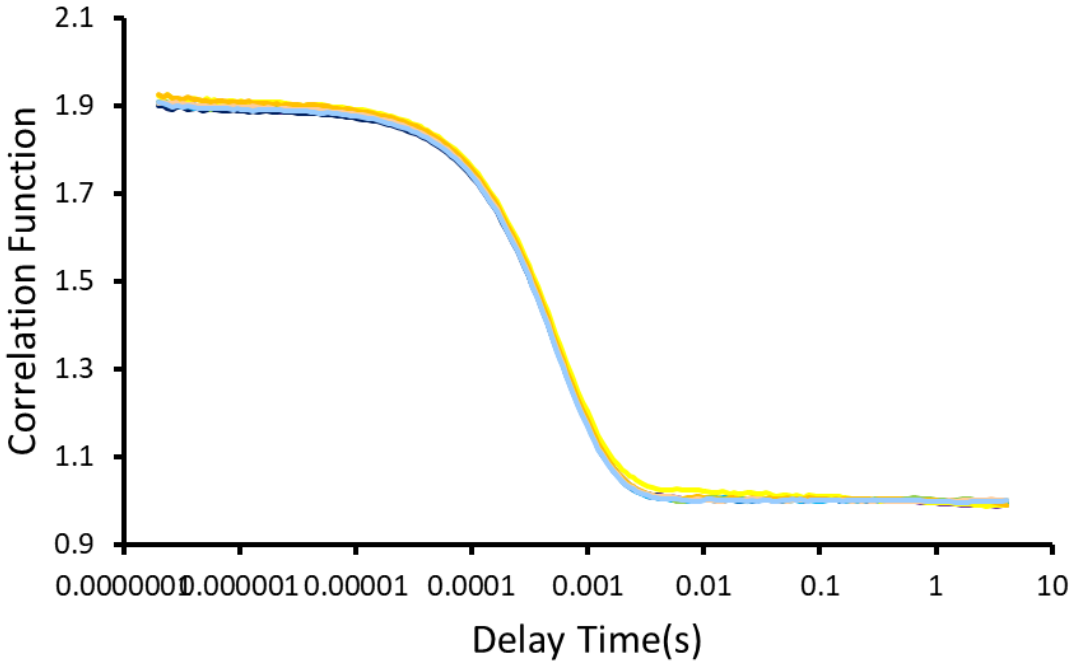


Figure S16 - Correlation function data for 10 DLS runs of compound **3** (5.56 mM) in an EtOH/H_2O 1:19 solution at 298 K.

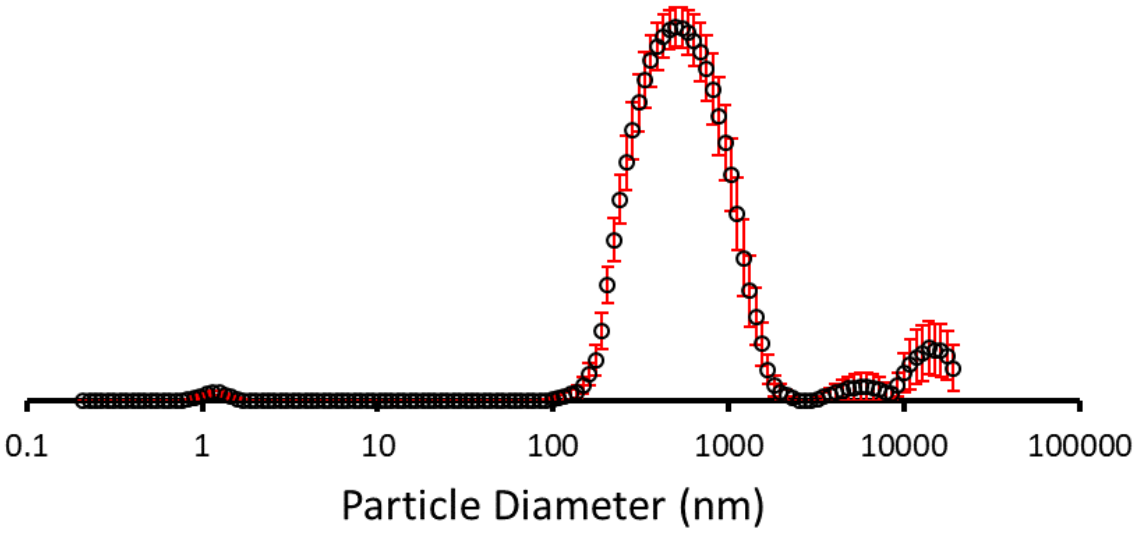


Figure S17 - The average intensity particle size distribution calculated using 10 DLS runs for compound **3** (111.2 mM) in DMSO at 298 K.

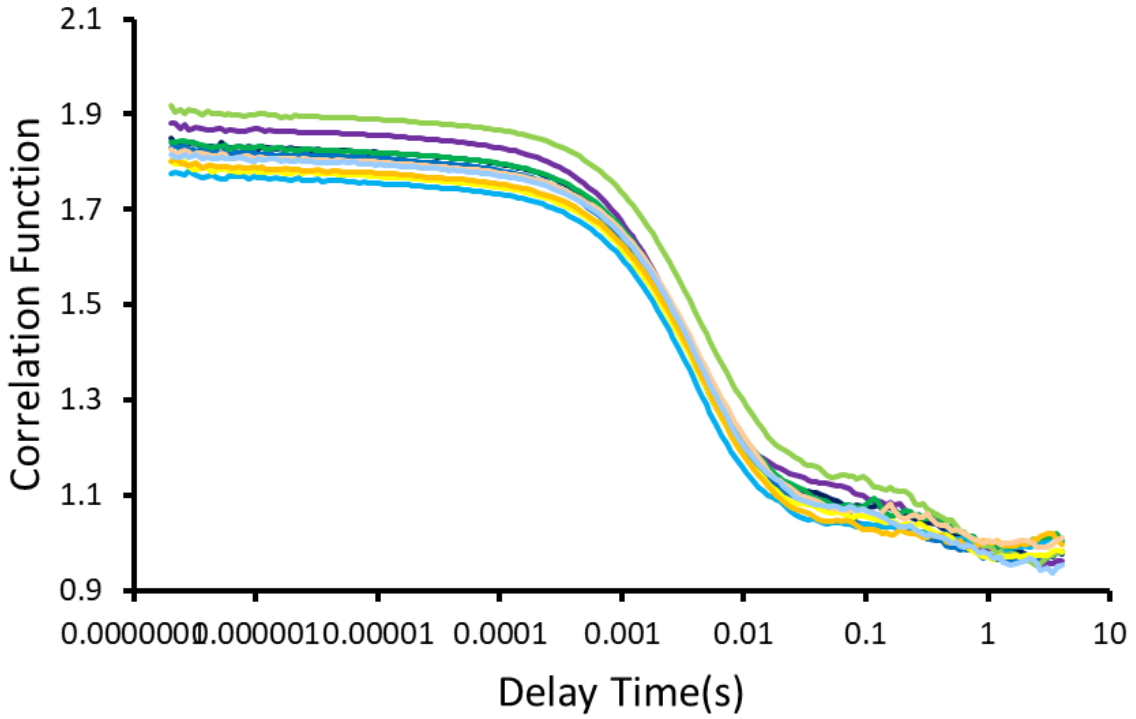


Figure S18 - Correlation function data for 10 DLS runs of compound **3** (111.2 mM) in DMSO at 298 K.

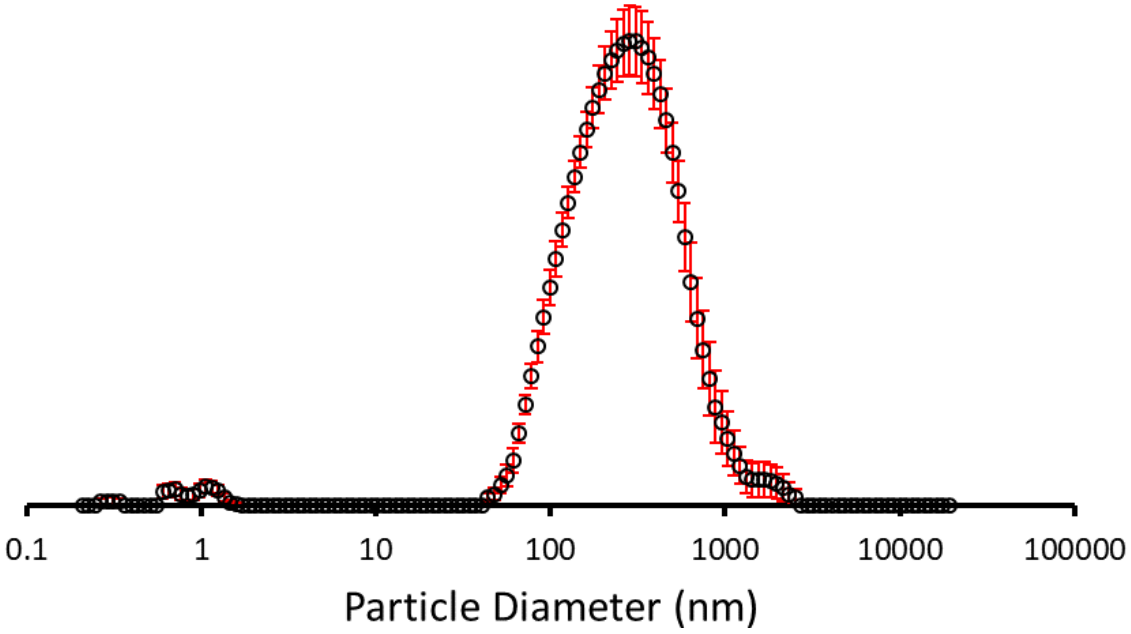


Figure S19 - The average intensity particle size distribution calculated using 10 DLS runs for compound **3** (11.2 mM) in DMSO at 298 K.

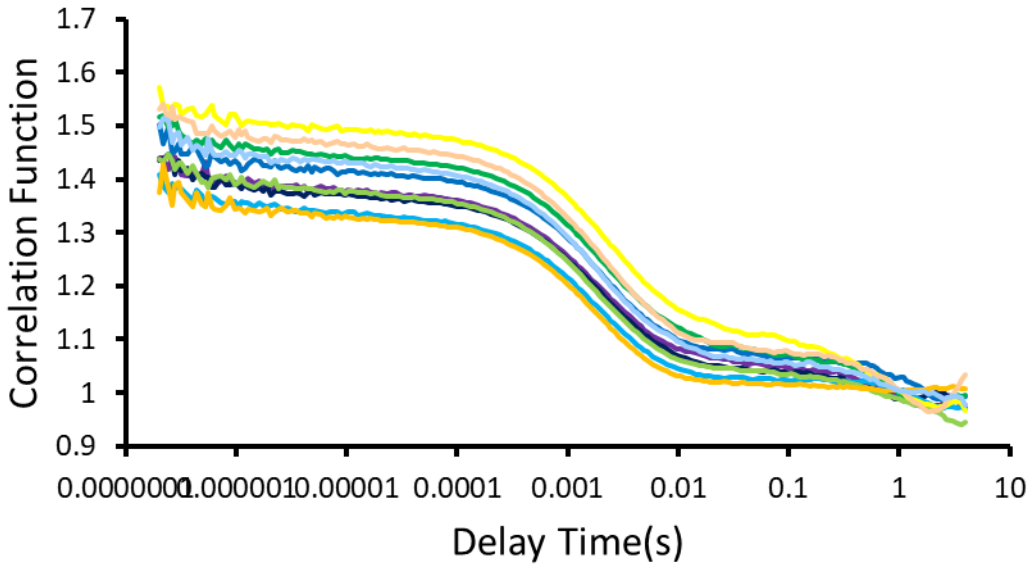


Figure S20 - Correlation function data for 10 DLS runs of compound **3** (11.2 mM) in DMSO at 298 K.

Comparative overview

Table S4 - Peak maxima obtained from an average intensity particle size distribution of **3** obtained at 0.56 mM and 5.56 mM in a solution of EtOH/H_2O 1:19 and at 111.2 mM and 11.2 mM in DMSO by DLS. Hydrodynamic aggregate diameter is given in nm. Polydispersity index (PDI) given in % to 1 dp. Conc. = concentration. Error = standard error of the mean to 1 dp.

	Conc. (mM)	Peak 1	PDI (%)
EtOH/H_2O 1:19	5.56	127	20.2 (*± 0.7)*
	0.56	121	25.3 (*± 0.4)*
DMSO	111.2	897	29.2 (*± 0.5)*
	11.2	437	28.7 (*± 1.3)*

Surface tension and stability data

Zeta potential

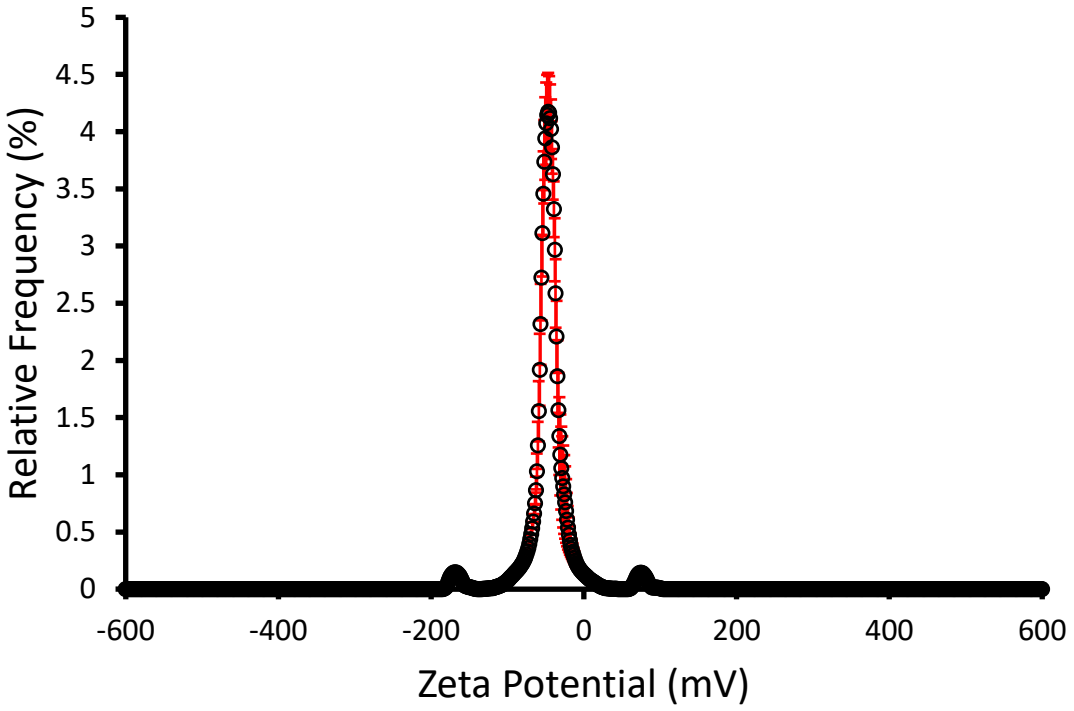


Figure S21 - The average zeta potential distribution calculated using 10 runs for compound **1** (90 mM) in an EtOH/H_2O 1:19 solution at 298 K. Average zeta potential value of -36 mV.

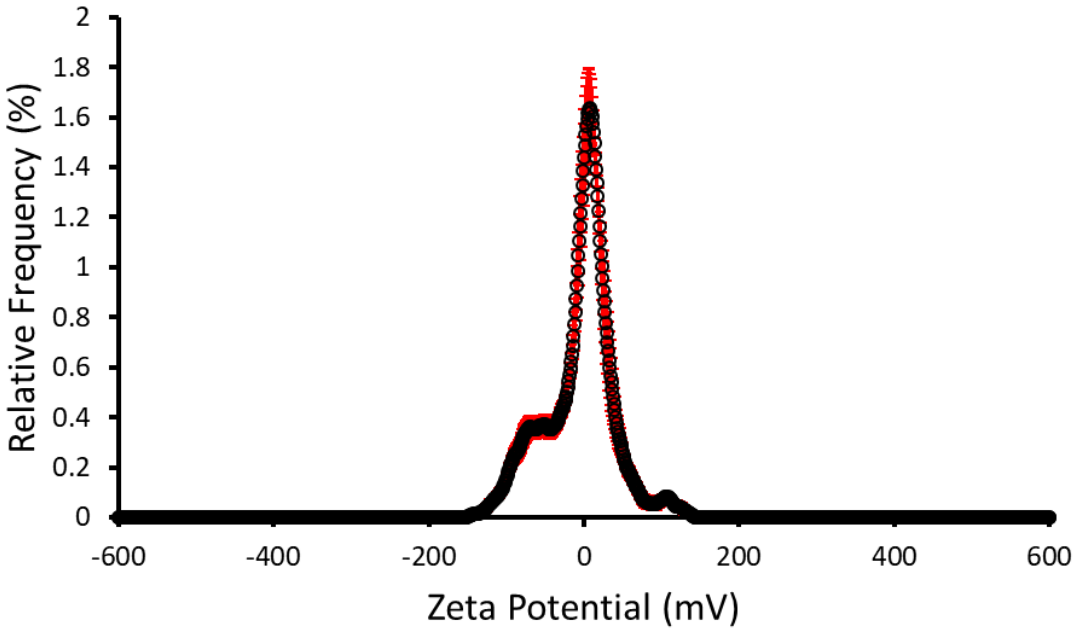


Figure S22 - The average zeta potential distribution calculated using 10 runs for compound **3** (5.56 mM) in an EtOH/H_2O 1:19 solution at 298 K. Average zeta potential values of +8 mV and -68 mV.

Surface tension measurements and critical aggregation concentration (CAC) determination

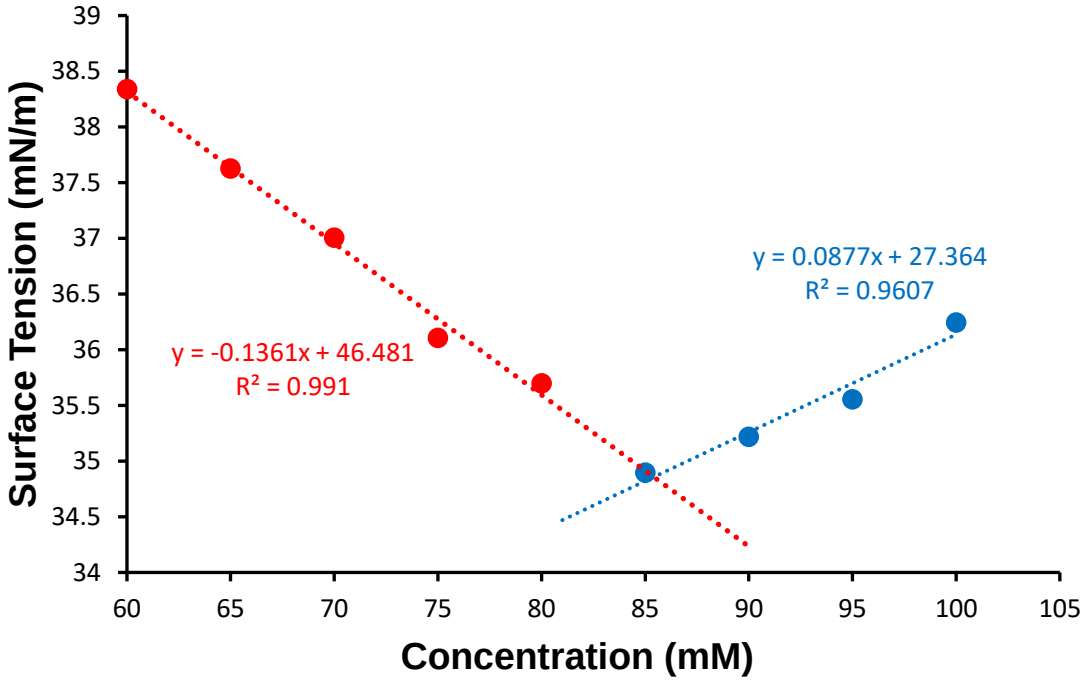


Figure S23 - Calculation of CAC (85.4 mM) for compound **1** in an EtOH/H_2O 1:19 mixture using surface tension measurements.

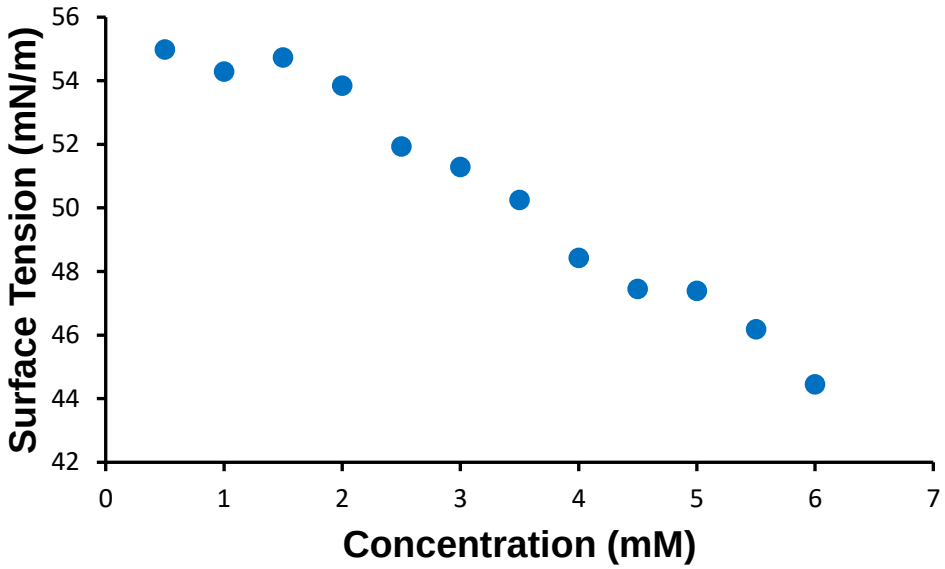


Figure S24 - Calculation of CAC for compound **3** could not be obtained due to the CAC being above the limit of solubility.

Comparative overview

Table S5 – Summary of zeta potential at 90 mM (**1**) and 5.56 mM (**3**), CAC and surface tension at CAC. Data obtained in an EtOH/H_2O 1:19 solution.

SSA	Zeta potential (mV)	CAC (mM)	Surface tension at CAC (mN/m)
1	-36	85.4	34.86
3	+8	*a*	*a*

a - CAC above the limit of solubility.

In-silico modelling

Computational calculations to identify primary hydrogen bond donating and accepting sites were conducted in line with studies reported by Hunter using Spartan 16''.[3] Calculations were performed using semi-empirical PM6 methods, after energy minimisation calculations, to identify E_{max}, E_{min} and LogP values. PM6 was used over AM1 in line with research conducted by Stewart.[4]

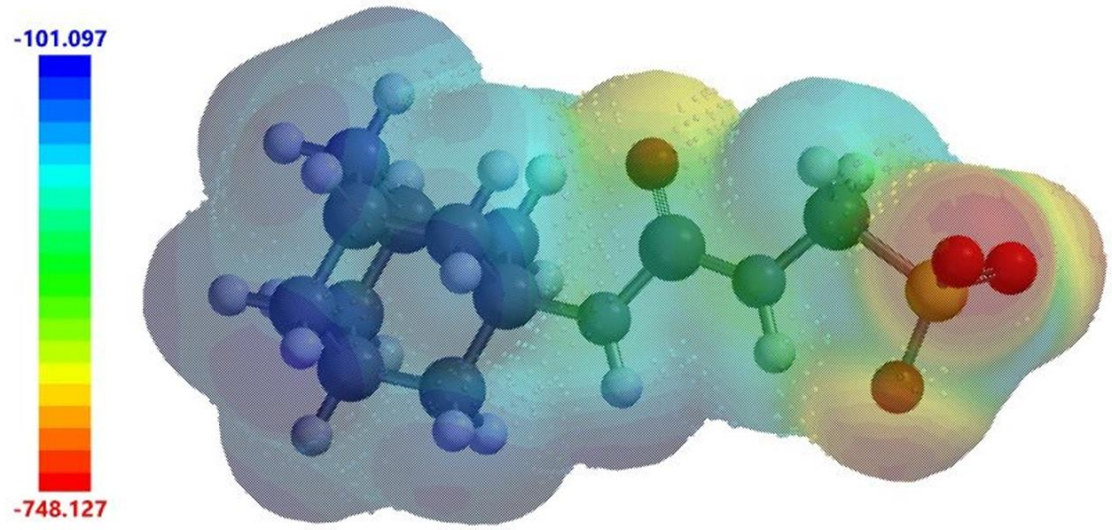


Figure S25 - Electrostatic potential map calculated for the anionic component of **1**. E_{max} and E_{min} values depicted in the figure legends are given in KJ/mol.

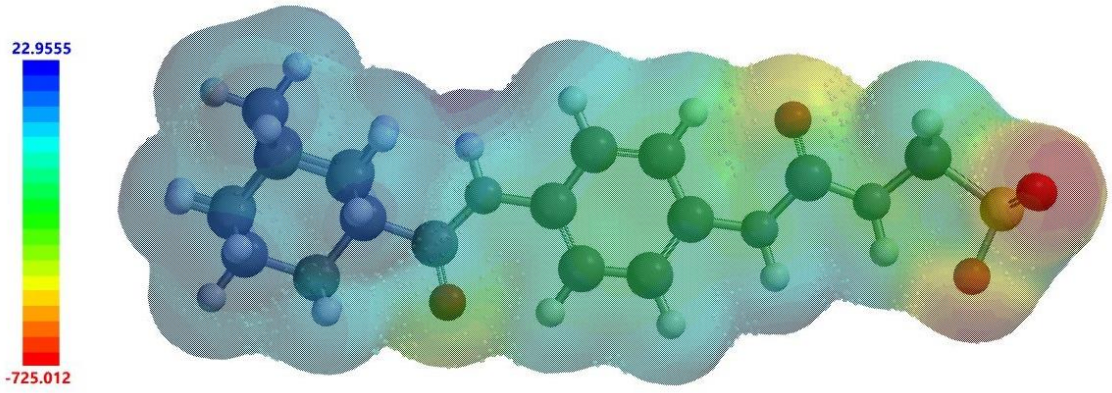


Figure S26 - Electrostatic potential map calculated for the anionic component of **3**. E_{max} and E_{min} values depicted in the figure legends are given in KJ/mol.

Overview

Table S6 - Summary of E_{max}, E_{min} and LogP values.

Compound	E_{min} (KJ/mol)	E_{max} (KJ/mol)	LogP
1	- 748.127	- 101.097	2.83
3	- 725.012	22.956	2.83

Antimicrobial screening

Methicillin-resistant *Staphylococcus aureus* USA300 (MRSA)

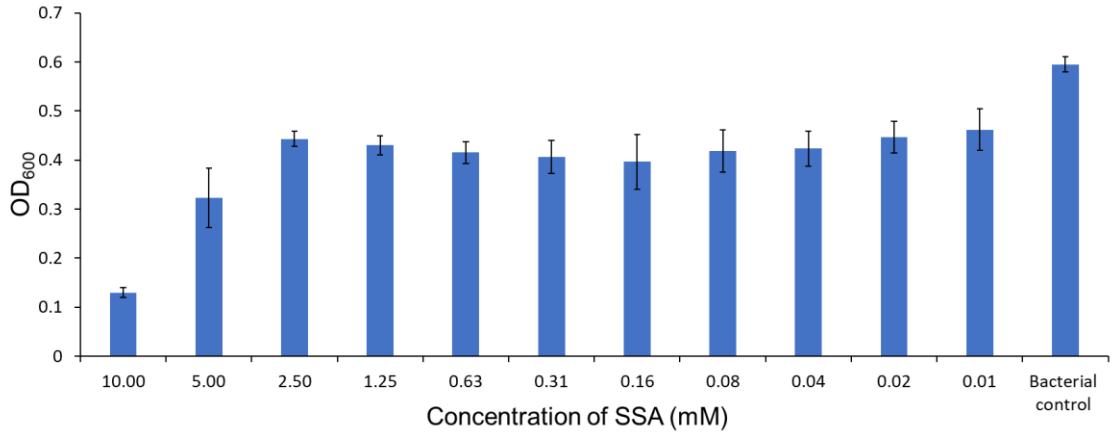


Figure S27 - MRSA USA300 microbial inhibition of **1** in an EtOH/H_2O 1:19 solution. OD_{600} measurements were taken at 900 minutes. Control data demonstrated normal bacterial growth in the absence of SSA.

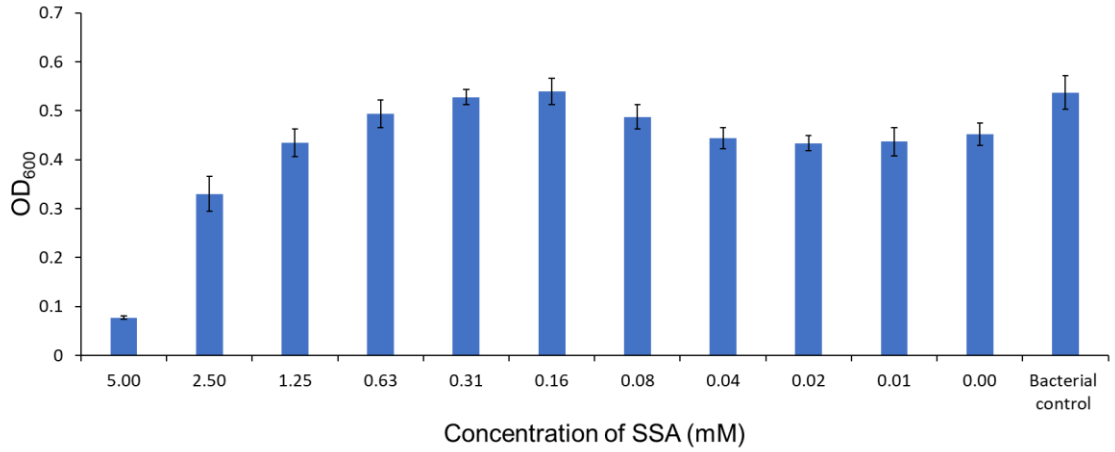


Figure S28 - MRSA USA300 microbial inhibition, of **3** in an EtOH/H_2O 1:19 solution. OD_{600} measurements were taken at 900 minutes. Control data demonstrated normal bacterial growth in the absence of SSA.

Escherichia coli (*E. coli*) DH10β

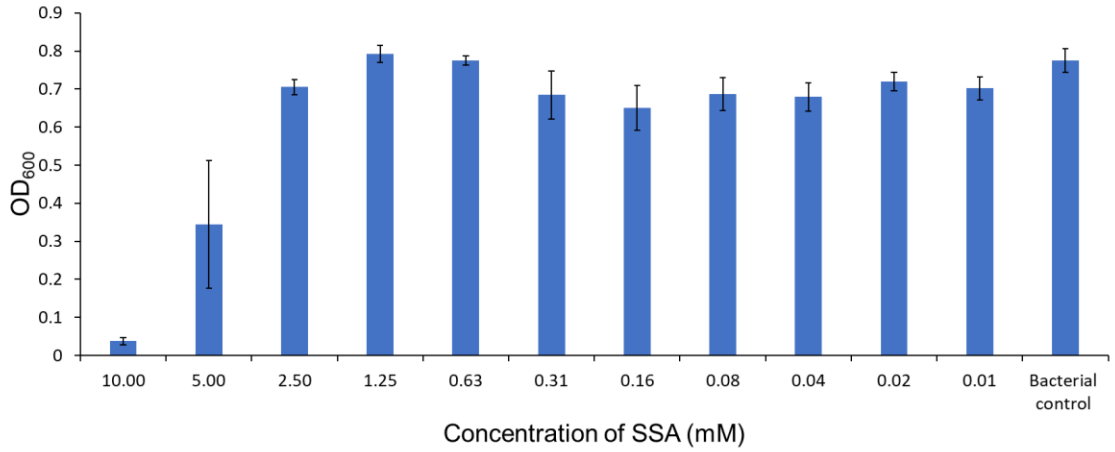


Figure S29 - *E. coli* DH10β microbial inhibition, of **1** in an EtOH/H_2O 1:19 solution. OD_{600} measurements were taken at 900 minutes. Control data demonstrated normal bacterial growth in the absence of SSA.

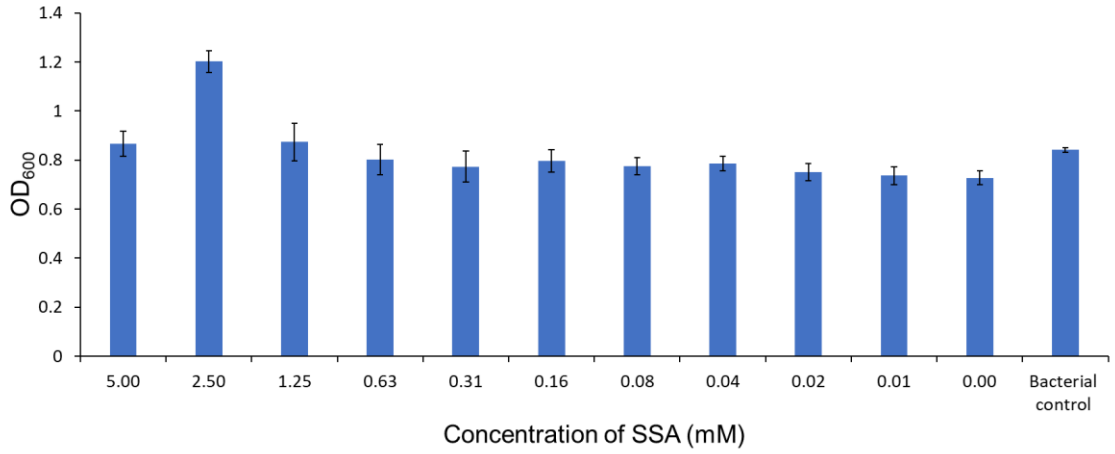


Figure S30 - *E. coli* DH10β microbial inhibition, of **3** in an EtOH/H_2O 1:19 solution. OD_{600} measurements were taken at 900 minutes. Control data demonstrated normal bacterial growth in the absence of SSA.

Galleria mellanella toxicity studies

Table S7 – Summary of the *Galleria mellanella* toxicity studies for **1** and **3** (5 mM, 10 μL), phosphate-buffered saline (PBS) and 5% EtOH, taken over a 5 day period.

	Quantity of *Galleria mellanella* lavae still living					
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
1	10	9	9	9	9	9
3	10	4	4	4	4	4
PBS	10	10	10	10	10	10
5% EtOH	10	10	10	10	10	10

Haemolysis assay results

Table S8 - Summary of % haemolysis results obtained for **1** and **3** at 1.39 mM and 5% EtOH control. Error = standard deviation (SD) to 2 dp.

	% haemolysis at 1.39 mM				
	rep 1	rep 2	rep 3	AVG	SD
1	0.5	0.5	1.0	0.7	0.29
3	1.0	0.8	1.0	0.9	0.11
5% EtOH	0.5	0.6	0.5	0.5	0.05

Table S9 – Summary of the Haemolytic Concentration (HC) results obtained for SSAs **1** and **3**. The HC_{10} and HC_{50} represent the concentration of SSA needed to lyse 10% and 50% of those red blood cells present respectively.

	HC_{10} (mM)			**HC_{50} (mM)**		
	rep 1	rep 2	rep 3	rep 1	rep 2	rep 3
1	>5	>5	>5	>5	>5	>5
3	>5	>5	>5	>5	>5	>5

Results summary: The HC_{10} and HC_{50} data show that these compounds are not haemolytic at this concentration

References

1 Supramolecular.org - Binding Constant Calculators | Supramolecular, http://app.supramolecular.org/bindfit/, (accessed 1 July 2020).

2 S. M. Travis, N. N. Anderson, W. R. Forsyth, C. Espiritu, B. D. Conway, E. P. Greenberg, P. B. McCray, R. I. Lehrer, M. J. Welsh and B. F. Tack, *Infect. Immun.*, 2000, **68**, 2748–2755.

3 C. A. Hunter, *Angew. Chemie Int. Ed.*, 2004, **43**, 5310–5324.

4 J. J. P. Stewart, *J. Mol. Model.*, 2007, **13**, 1173–1213.