Electronic Supplementary Information

Squaramide-based tripodal receptors for selective recognition of sulfate anion

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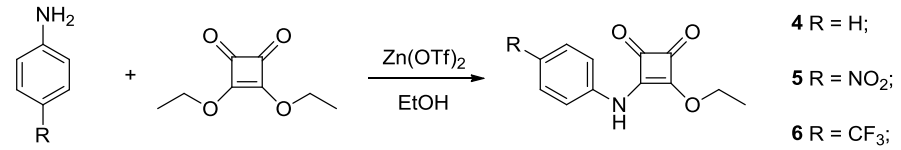
1. *Materials and methods*

All reagents were commercially available and used as supplied without further purification. NMR spectra were recorded with a Bruker Advance DMX 300 spectrophotometer or a Bruker Advance DMX 400 spectrophotometer with use of the deuterated solvent as the lock and the residual solvent or TMS as the internal reference. Low-resolution electrospray ionization mass spectra (LR-ESI-MS) were obtained on Finnigan Mat TSQ 7000 instruments. High-resolution electrospray ionization mass spectra (HR-ESI-MS) were recorded on an Agilent 6210 TOF LCMS equipped with an electrospray ionization (ESI) probe.

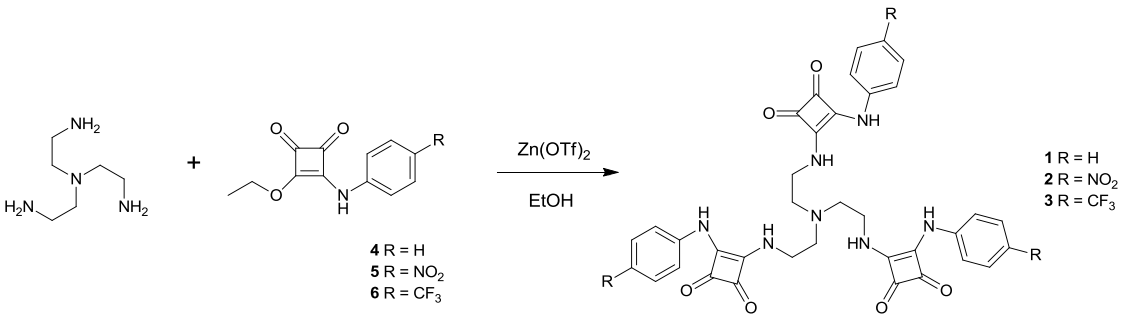
2. *General procedure for the synthesis of squaramide derivatives **4**-**6**.*



Scheme S1. Synthesis of squaramide derivatives **4**-**6**.

To a stirred solution of 3,4-diethoxycyclobut-3-ene-1,2-dione (diethyl squarate, 0.94 g, 5.5 mmol) and zinc trifluoromethanesulfonate (0.36 g, 1.0 mmol) in EtOH solution (40 mL) was added phenylamine (5.0 mmol). The solution was stirred for 10 h at room temperature. Isolated by filtration, the filtrate solvent was removed under reduced pressure to give the resulting residue as solid. **4**-**6** were obtained by column chromatography using PE/EtOAc (6/1, v/v) as an eluent in good yield (80-94%). **4**-**6** were performed as previously reported.[S1]

3. *General procedure for the synthesis of receptors **1**-**3**.*



Scheme S2. Synthesis of receptors **1**-**3**.

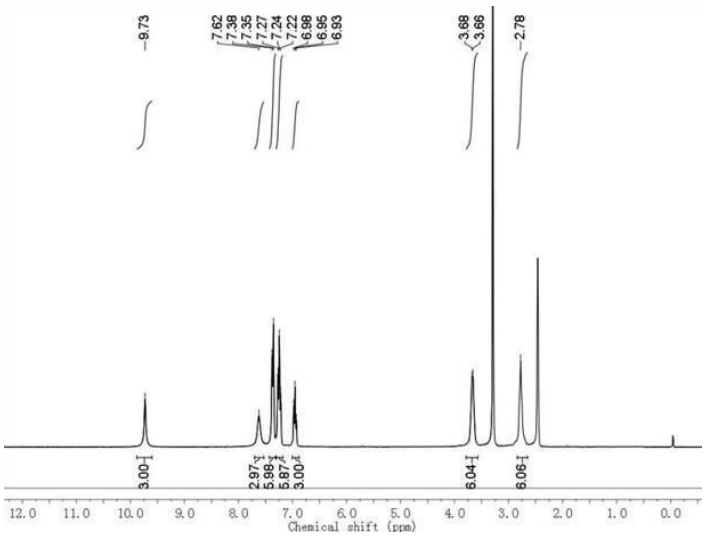
The relevant squaramide derivative, **4**-**6**, and $Zn(OTf)_2$ as a catalyst were dissolved in dry EtOH, tris(2-aminoethyl)amine was then added. The reaction mixture was then stirred over 24 h at room temperature. The resulting precipitate was then filtered and washed with EtOH and EtOAc for many times.

Compound **1**: **1** was synthesized according to general procedure using squaramide derivative **4** (325 mg, 1.5 mmol), $Zn(OTf)_2$ (22 mg, 0.06 mmol) and tris(2-aminoethyl)amine (49 mg, 0.3 mmol) in 20 mL dry EtOH. The product was got as a yellow solid (159 mg, 80% yield). m.p. above 300 °C. ^{1}H NMR (300 MHz, DMSO-d_6) 9.73 (s, 3 H, N*H*), 7.62 (s, 3 H, N*H*), 7.40 (d, 6 H, Ar-*H*, *J* = 7.5 Hz), 7.24 (t, 6 H, Ar-*H*, *J* = 7.5 Hz), 6.95 (t, 3 H, Ar-*H*, *J* = 7.5 Hz), 3.67 (m, 6 H, CH_2), 2.78 (brs, 6 H, CH_2); ^{13}C NMR (75 MHz, DMSO-d_6) 184.21, 180.08, 169.06, 163.81, 138.94, 129.24, 122.57, 118.07, 54.24, 41.75; LRESIMS (m/z): 660.17 $[M + H]^+$, 682.25 $[M + Na]^+$. HRESIMS (m/z): 658.2436 $[M - H]^-$.

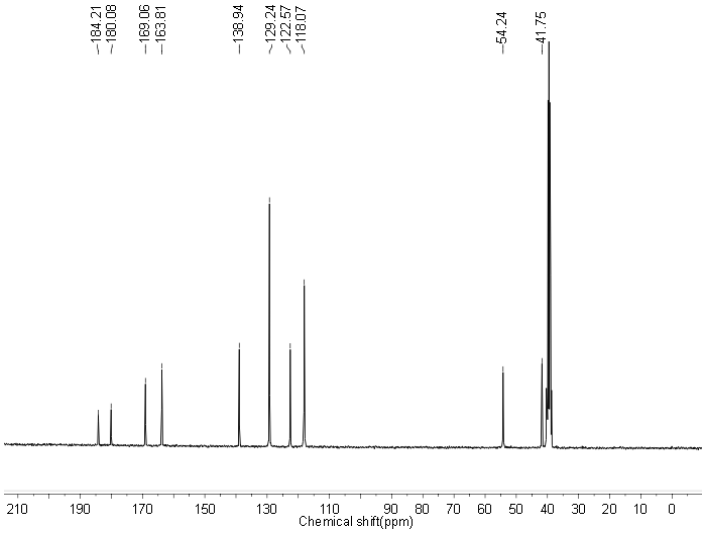
Compound **2**: **2** was synthesized according to general procedure using squaramide derivative **5** (428 mg, 1.5 mmol), $Zn(OTf)_2$ (22 mg, 0.06 mmol) and tris(2-aminoethyl)amine (49 mg, 0.3 mmol) in 20 mL dry EtOH. The product was got as a red solid (220 mg, 85% yield). m.p. above 300 °C. ^{1}H NMR (300 MHz, DMSO-d_6) 10.28 (s, 3 H, N*H*), 8.02 (d, 6 H, Ar-*H*, *J* = 9.0 Hz), 7.81 (s, 3 H, N*H*), 7.48 (d, 6 H, Ar-*H*, *J* = 9.0 Hz), 3.73 (brs, 6 H, CH_2), 2.82 (brs, 6 H, CH_2); ^{13}C NMR (75 MHz, DMSO-d_6) 185.97, 180.21, 170.42, 162.68, 145.78, 141.55, 125.70, 118.06, 54.26, 42.26; LRESIMS (m/z): 793.33 $[M - H]^-$. HRESIMS (m/z) 793.1979 $[M - H]^-$.

Compound **3**: **3** was synthesized according to general procedure using squaramide derivative **6** (393 mg, 1.5 mmol), $Zn(OTf)_2$ (22 mg, 0.06 mmol) and tris(2-aminoethyl)amine (49 mg, 0.3 mmol) in 20 mL dry EtOH. The product was got as a white solid (198 mg, 83% yield). m.p. above 300 °C. ^{1}H NMR (300 MHz, DMSO-d_6) 9.97 (s, 3 H, N*H*), 7.66 (s, 3 H, N*H*), 7.49 (brs, 12 H, Ar-*H*), 3.73 (brs, 6 H, CH_2), 2.82 (brs, 6 H, CH_2); ^{13}C NMR (75 MHz, DMSO-d_6) 184.94, 179.82, 169.51, 162.97, 142.46, 126.31, 122.36, 117.79, 53.53, 41.68. LRESIMS (m/z): 862.10 $[M - H]^-$. HRESIMS (m/z): 862.2048 $[M - H]^-$.

(a)



(b)



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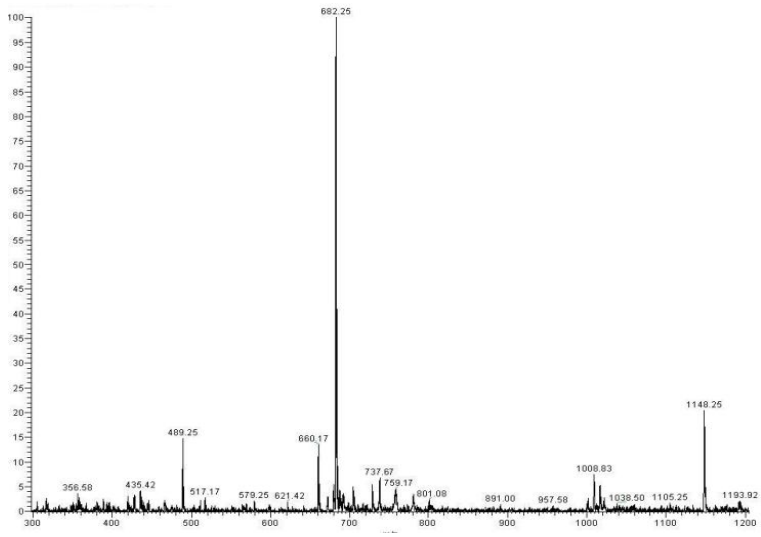
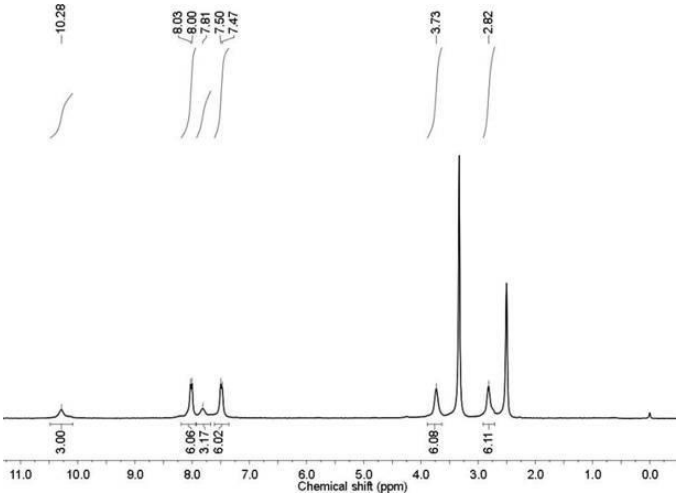


Figure S1. (a) ^{1}H-NMR spectra of **1** (300 MHz, DMSO-d_6). (b) ^{13}C-NMR spectra of **1** (75

MHz, DMSO-d_6). (c) LRESIMS spectra of **1**.

(a)



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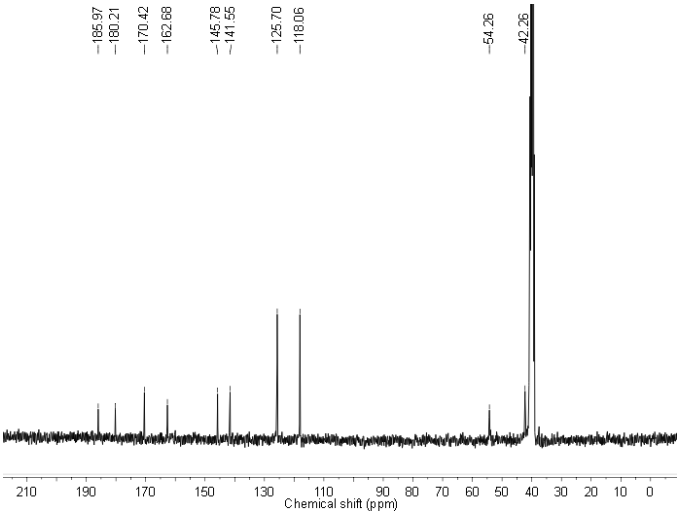

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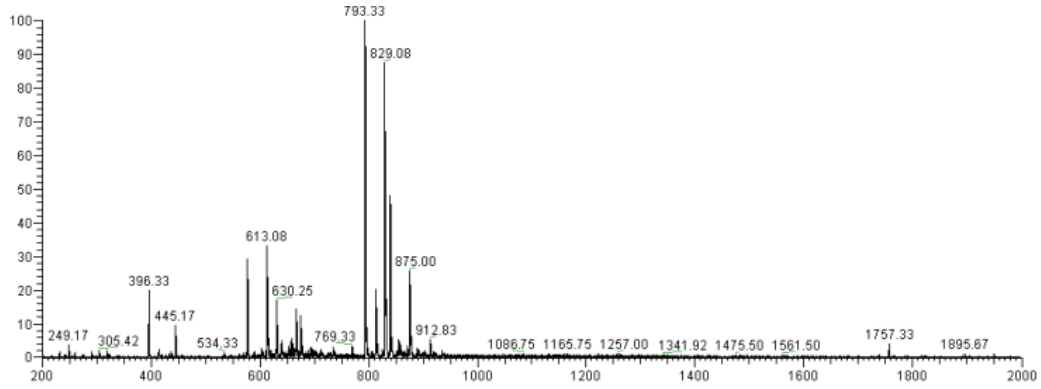
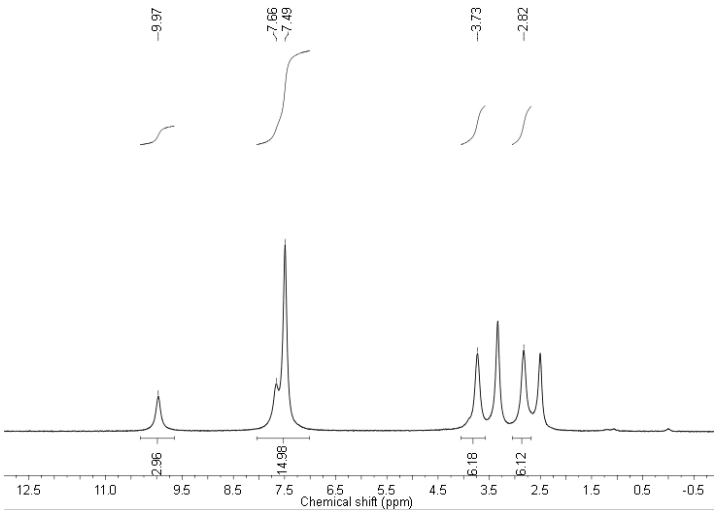
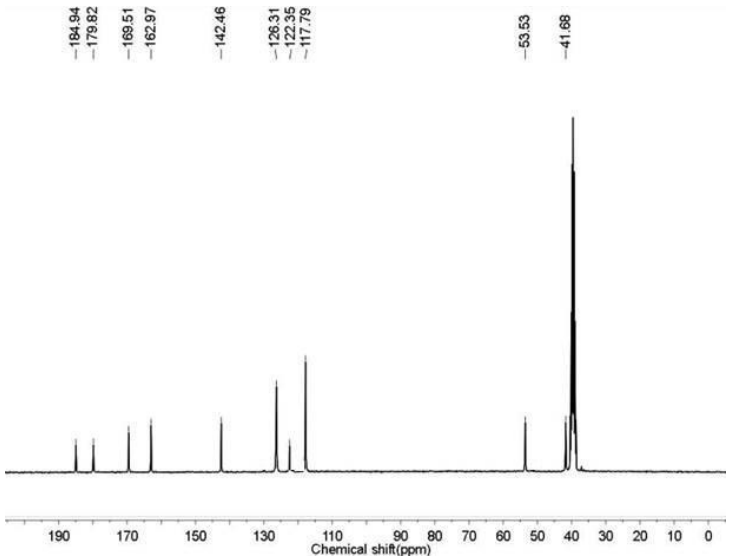


Figure S2. (a) ^{1}H-NMR spectra of **2** (300 MHz, DMSO-d_6). (b) ^{13}C-NMR spectra of **2** (75 MHz, DMSO-d_6). (c) LRESIMS spectra of **2**.

(a)



(b)



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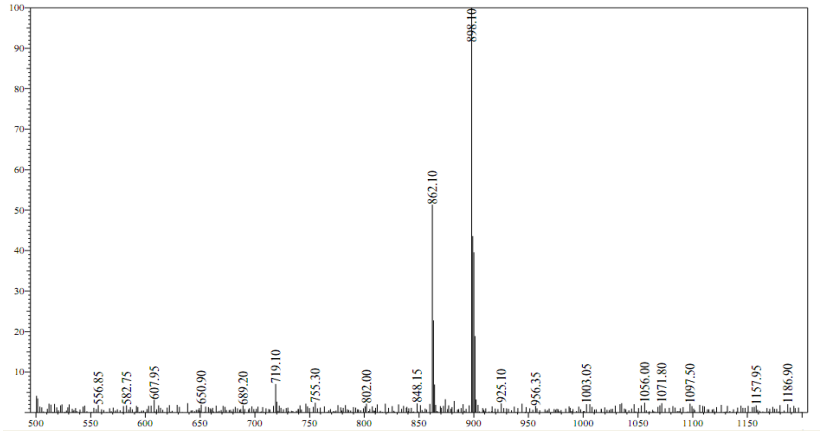


Figure S3. (a) ^{1}H-NMR spectra of **3** (300 MHz, DMSO-d_6). (b) ^{13}C-NMR spectra of **3** (75 MHz, DMSO-d_6). (c) LRESIMS spectra of **3**.

4. NMR binding studies.

Binding constants were obtained by ^{1}H NMR titrations of receptors with different anions in DMSO-d_6. All measurements were carried out on a Bruker Advance DMX 300 spectrophotometer at 298 K. The anionic guests were used as TBA salts (10 mmol) in DMSO-d_6 to a solution of the receptor in DMSO-d_6. The data analysis was conducted with WinEQNMR2[S2] software (using a 1:1 model unless otherwise stated) using the most upfield squaramide NH.[S3] Stock plots or fitplots can be found in Figures S5-S27.

Job plot experiments were performed in a separate experiment. 10 NMR tubes were filled with 0.4 mL of a DMSO-d_6 solution containing 2.0 mM of an anion-receptor mixture in different rations (receptor/anion): 10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9. ^{1}H NMR spectra were recorded on Bruker Advance DMX 300 spectrophotometer at 298 K. Job plots were obtained by plotting the molar fraction of the receptor as a function of the relative change in chemical shift.

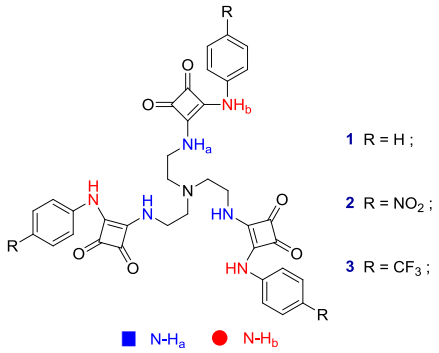


Figure S4. Structures of receptors **1-3** and signs of N-H$_a$, N-H$_b$.

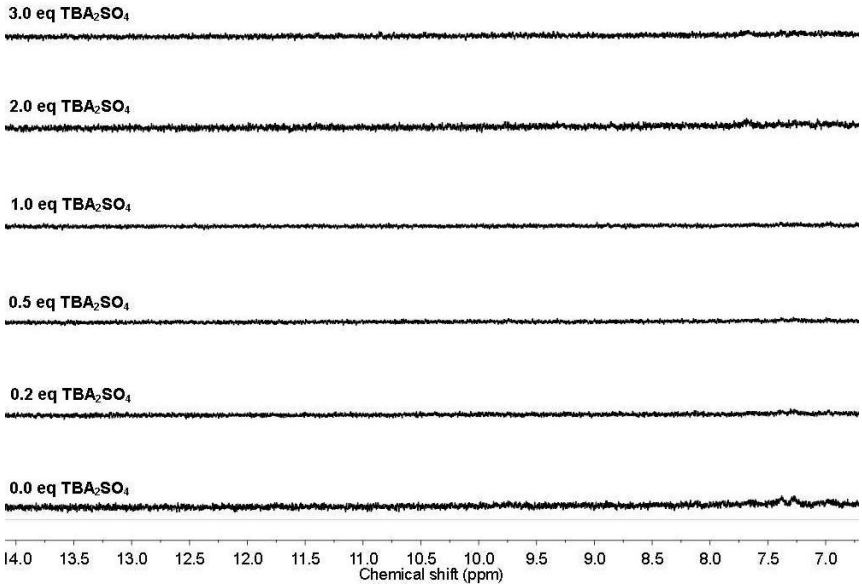


Figure S5. ^{1}H NMR titration of compound **1** (0.01 mM) with TBA_2SO_4 in DMSO-d_6 at 298 K. Stack plot.

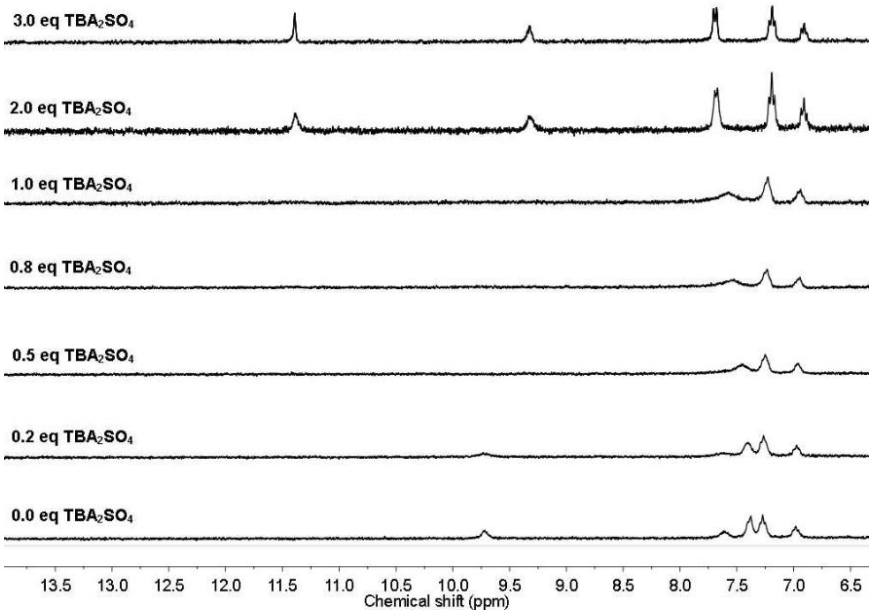
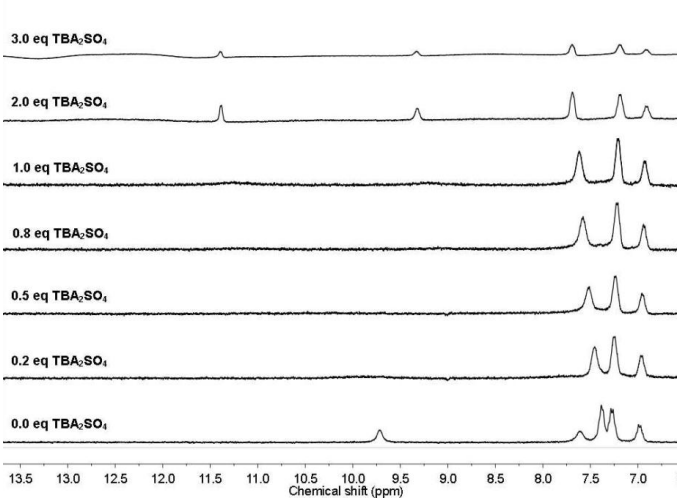


Figure S6. ^{1}H NMR titration of compound **1** (0.1 mM) with TBA_2SO_4 in DMSO-d_6 at 298 K. Stack plot.

(a)



(b)

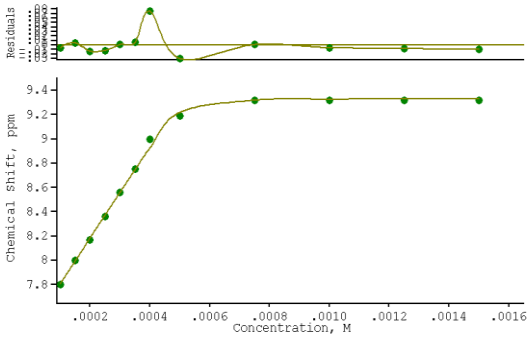
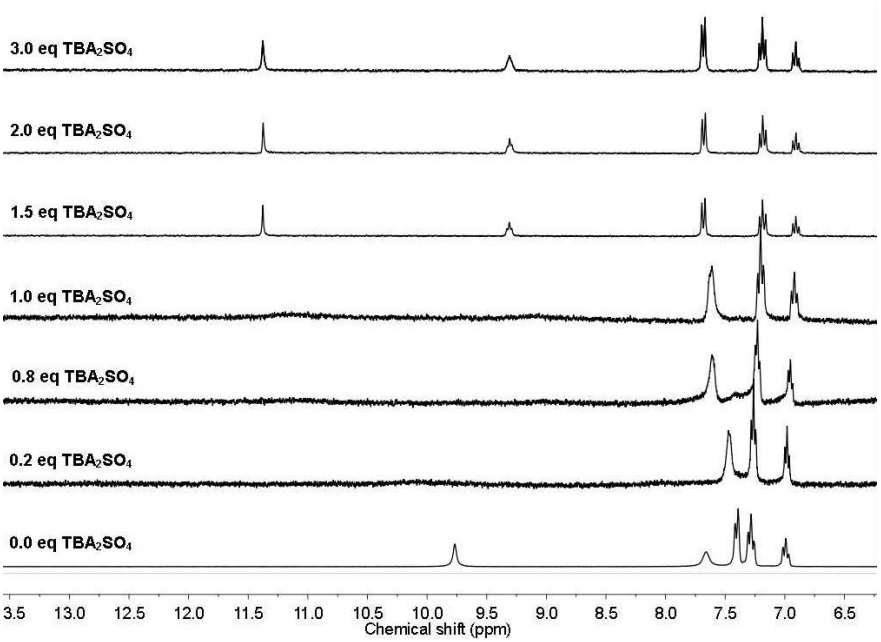
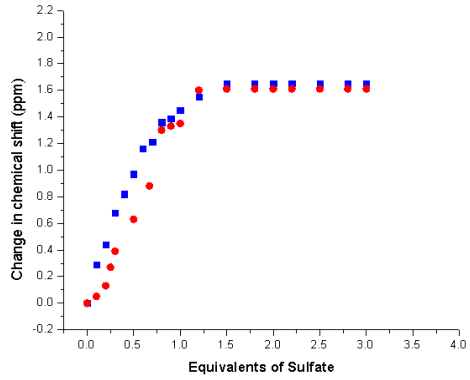


Figure S7. ^{1}H NMR titration of compound **1** (0.5 mM) with TBA_2SO_4 in DMSO-d_6 at 298 K. (a) Stack plot. (b) Fitplot for NH proton at δ = 7.62 ppm. Log K_a = 4.73 ± 0.11.

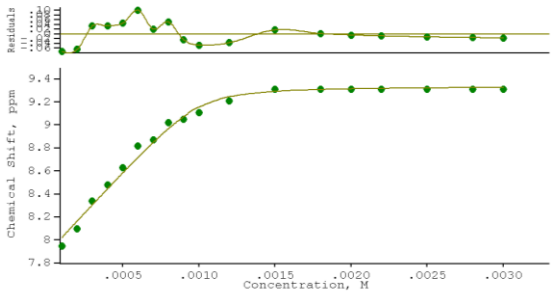
(a)



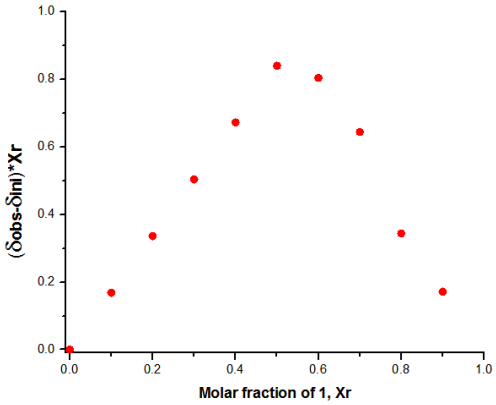
(b)



(c)



(d)



(e)

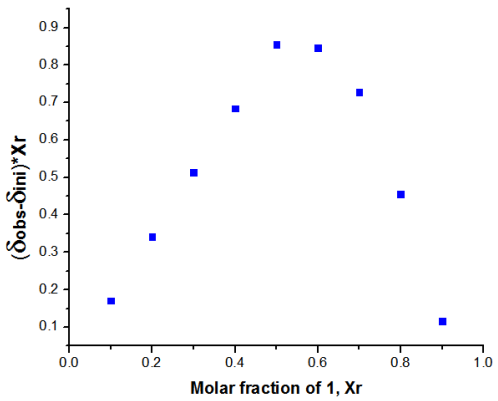
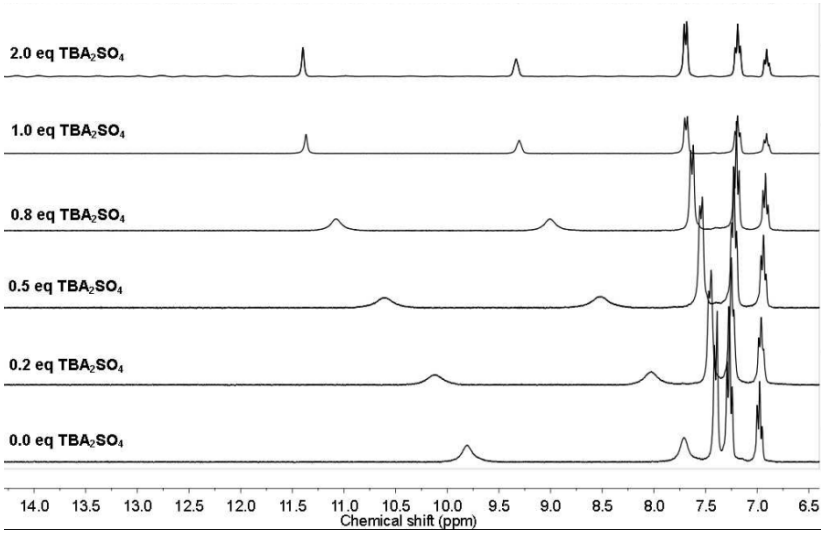


Figure S8. ^{1}H NMR titration of compound **1** (1.0 mM) with TBA_2SO_4 in DMSO-d_6 at 298 K. (a) Stack plot. (b) Chemical shift of N-H resonances. (c) Fitplot for NH proton at δ = 7.62 ppm. Log K_a = 4.75 ± 0.11. (d) Job plot analysis for NH proton at δ = 9.73 ppm. (e) Job plot analysis for NH proton at δ = 7.62 ppm.

(a)



(b)

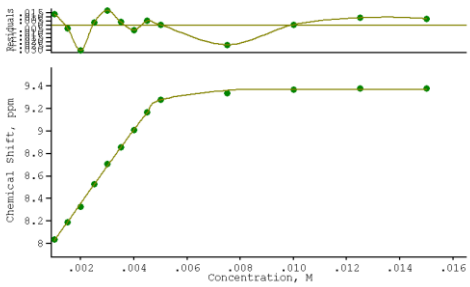
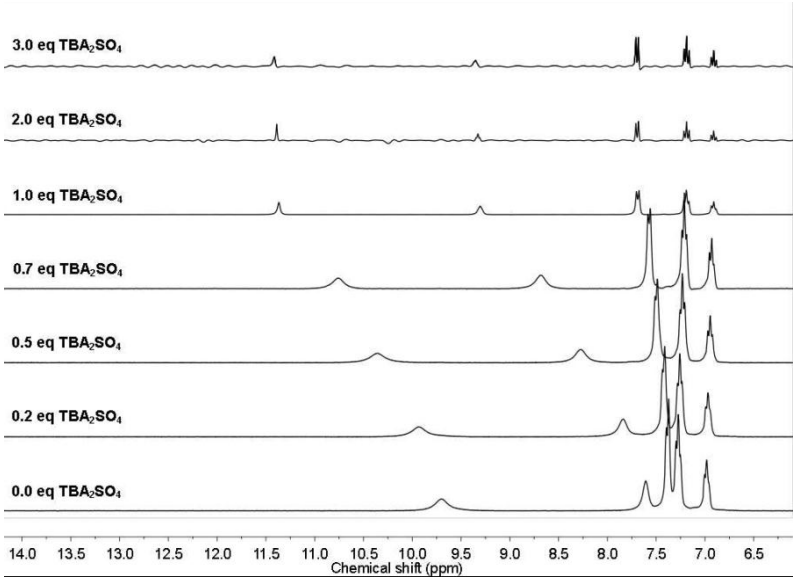


Figure S9. ^{1}H NMR titration of compound **1** (5.0 mM) with TBA_2SO_4 in DMSO-d_6 at 298 K. (a) Stack plot. (b) Fitplot for NH proton at δ = 7.62 ppm. Log K_a = 4.77 ± 0.08.

(a)



(b)

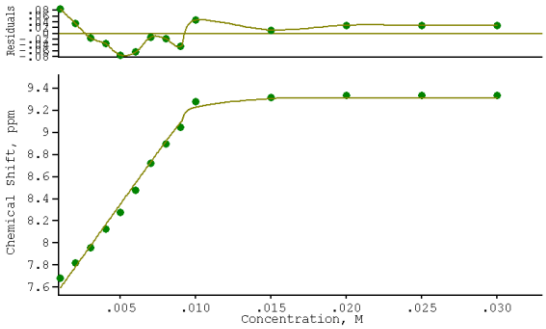
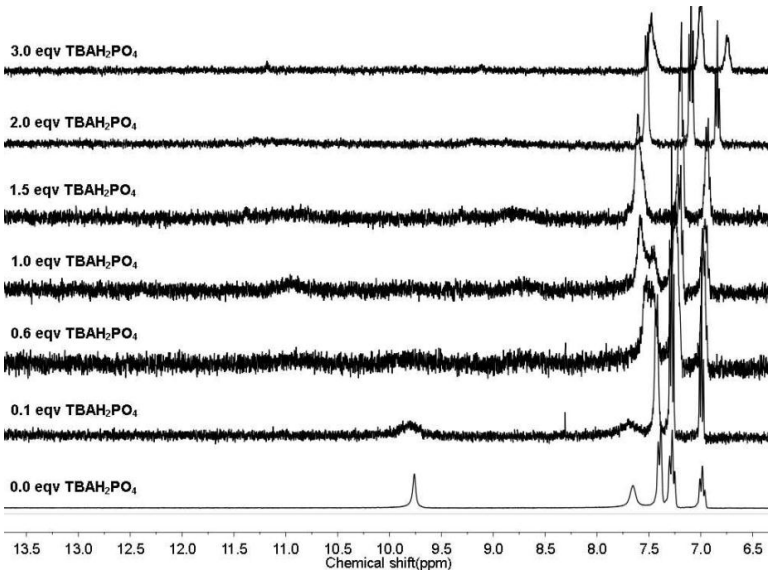
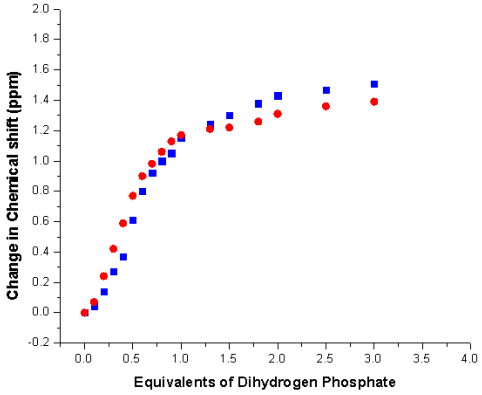


Figure S10. ^{1}H NMR titration of compound **1** (10.0 mM) with TBA_2SO_4 in DMSO-d_6 at 298 K. (a) Stack plot. (b) Fitplot for NH proton at δ = 7.62 ppm. Log K_a = 4.73 ± 0.16.

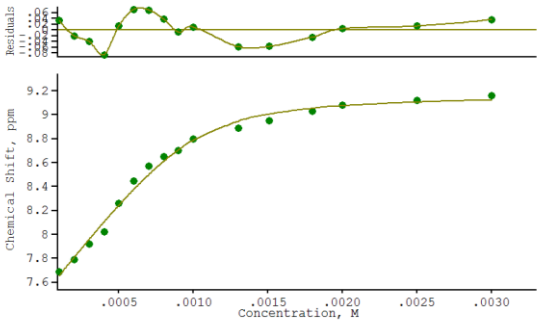
(a)



(b)



(c)



(d)

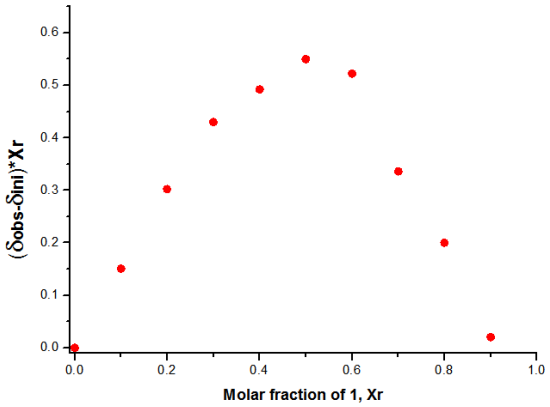
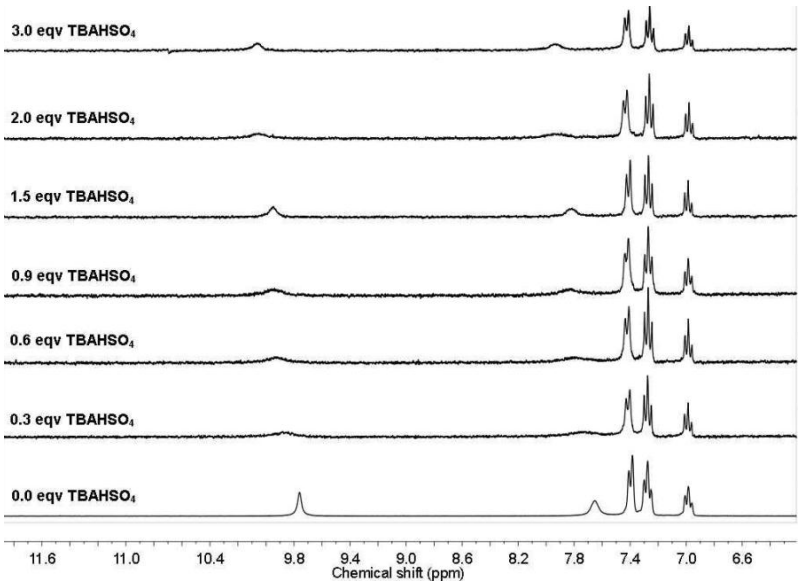


Figure S11. ^{1}H NMR titration of compound **1** (1.0 mM) with $TBAH_2PO_4$ in DMSO-d_6 at 298 K. (a) Stack plot. (b) Chemical shift of N-H resonances. (c) Fitplot for NH proton at δ = 7.62 ppm. Log K_a = 4.15 ± 0.14. (d) Job plot analysis for NH proton at δ = 9.73 ppm.

(a)



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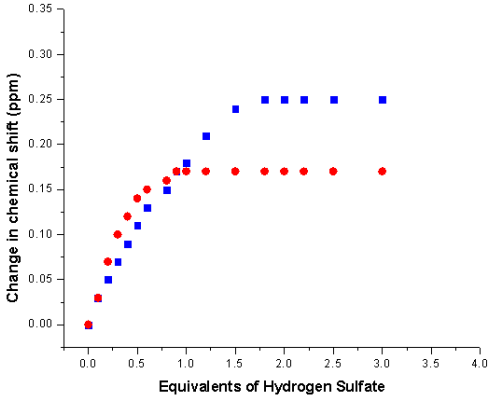


(c)

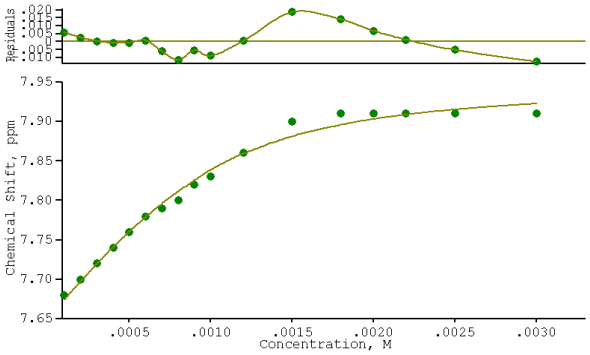
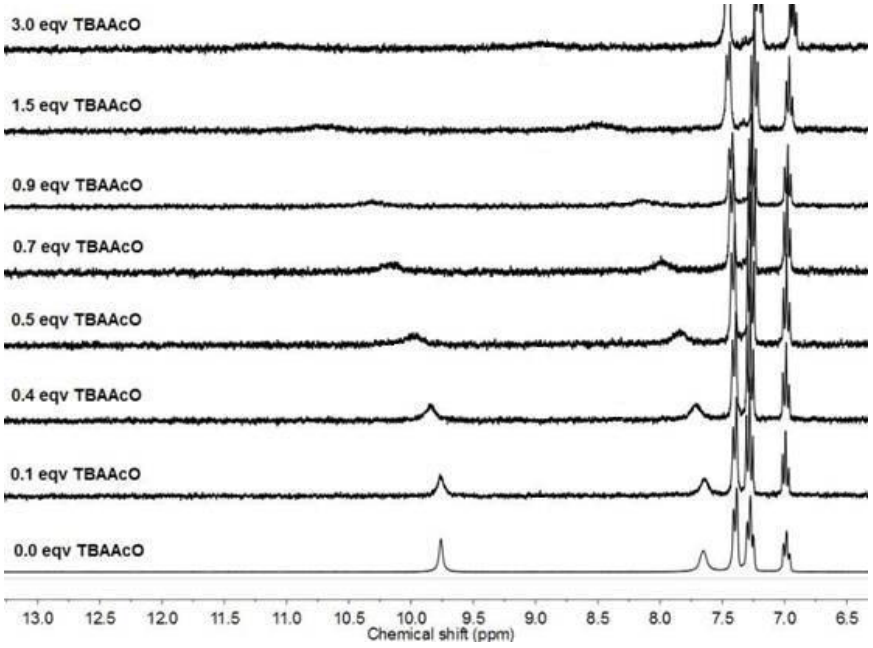
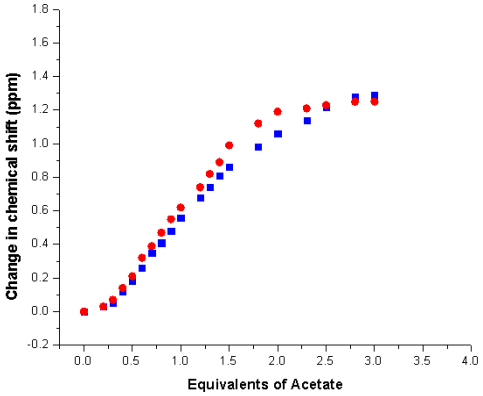


Figure S12. ^{1}H NMR titration of compound **1** (1.0 mM) with $TBAHSO_4$ in DMSO-d_6 at 298 K. (a) Stack plot. (b) Chemical shift of N-H resonances. (c) Fitplot for NH proton at δ = 7.62 ppm. Log K_a = 3.65 ± 0.12.

(a)



(b)



(c)

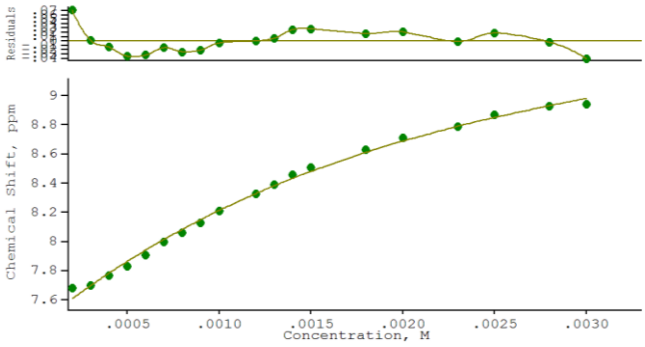
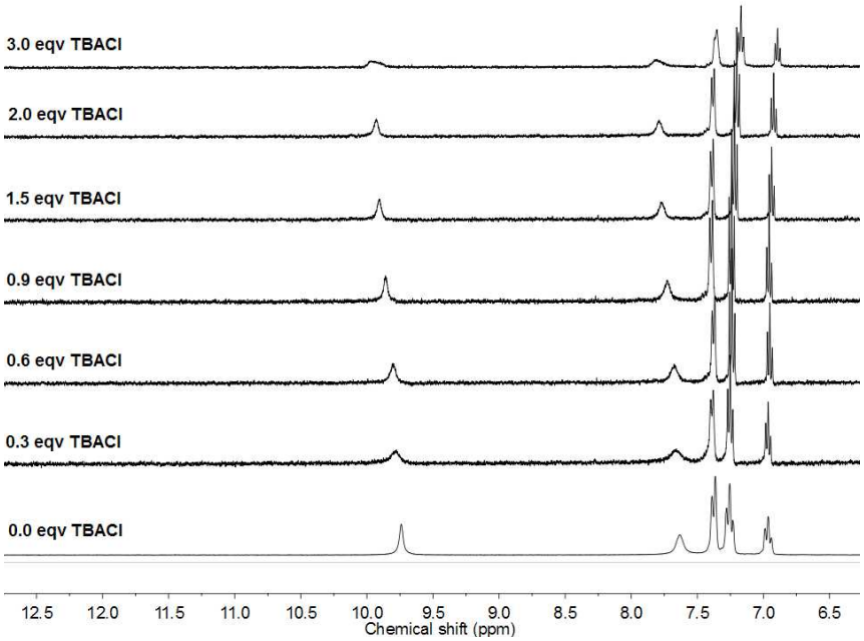


Figure S13. ^{1}H NMR titration of compound **1** (1.0 mM) with TBAAcO in DMSO-d_6 at 298 K. (a) Stack plot. (b) Chemical shift of N-H resonances. (c) Fitplot for NH proton at δ = 7.62 ppm. Log K_a = 2.82 ± 0.07.

(a)



(b) (c)

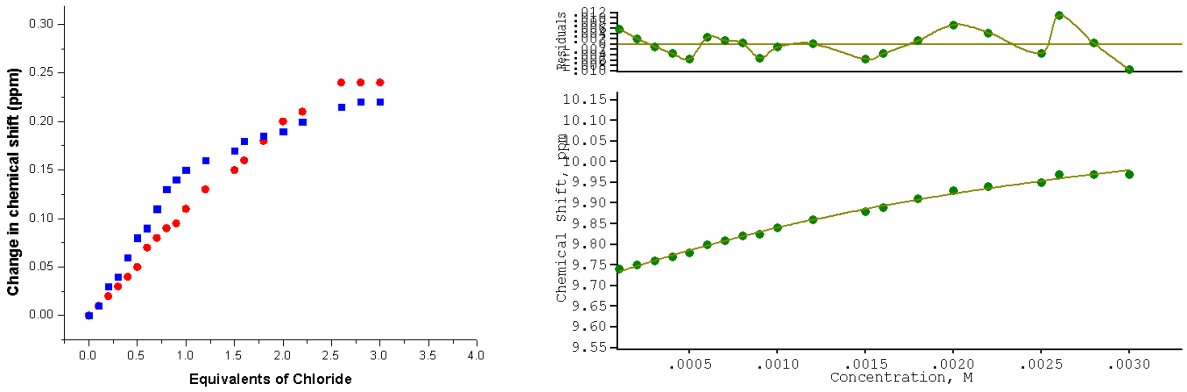
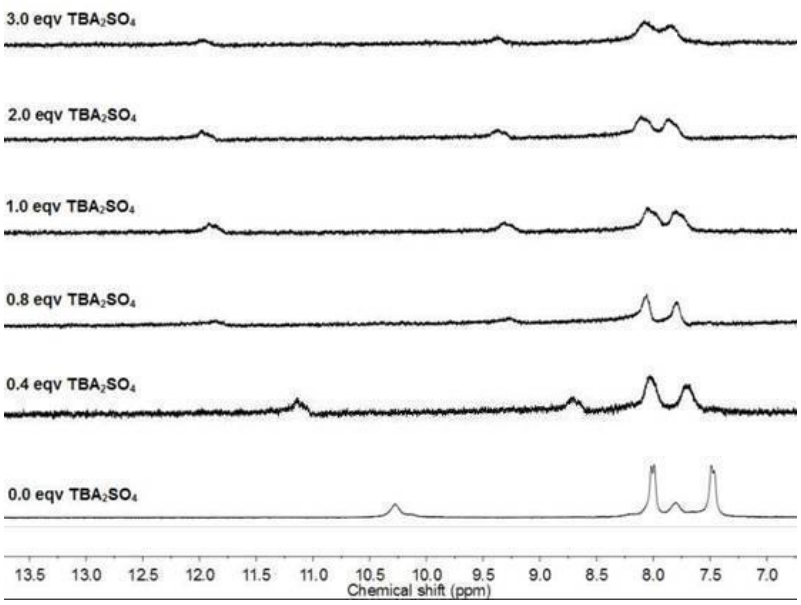


Figure S14. ^{1}H NMR titration of compound **1** (1.0 mM) with TBACl in DMSO-d_6 at 298 K. (a) Stack plot. (b) Chemical shift of N-H resonances. (c) Fitplot for NH proton at δ = 9.73 ppm. Log K_a = 2.58 ± 0.08.

(a)



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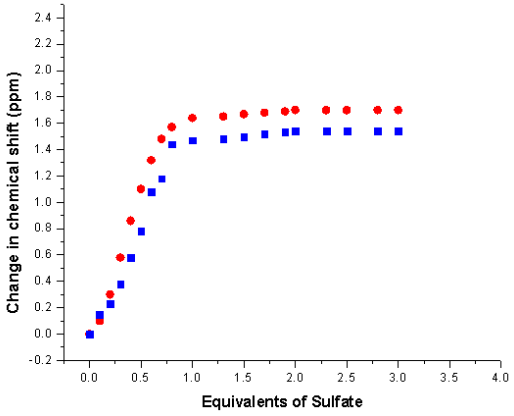


(c)

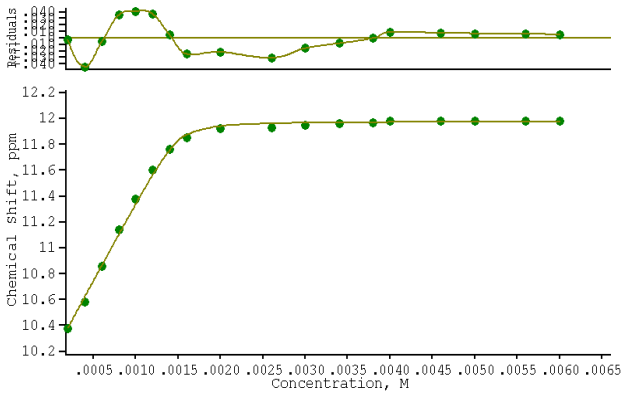


Figure S15. ^{1}H NMR titration of compound **2** (2.0 mM) with TBA_2SO_4 in DMSO-d_6 at 298 K. (a) Stack plot. (b) Chemical shift of N-H resonances. (c) Fitplot for NH proton at δ = 10.28 ppm. Log K_a = 4.95 ± 0.12.

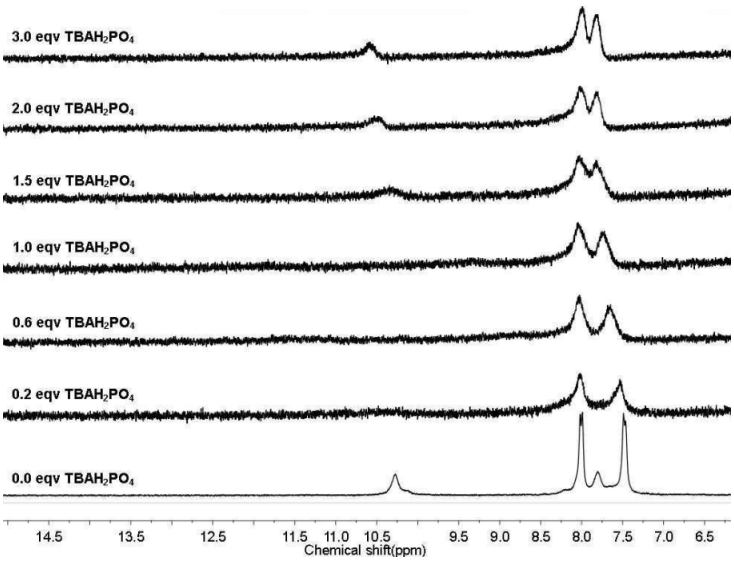
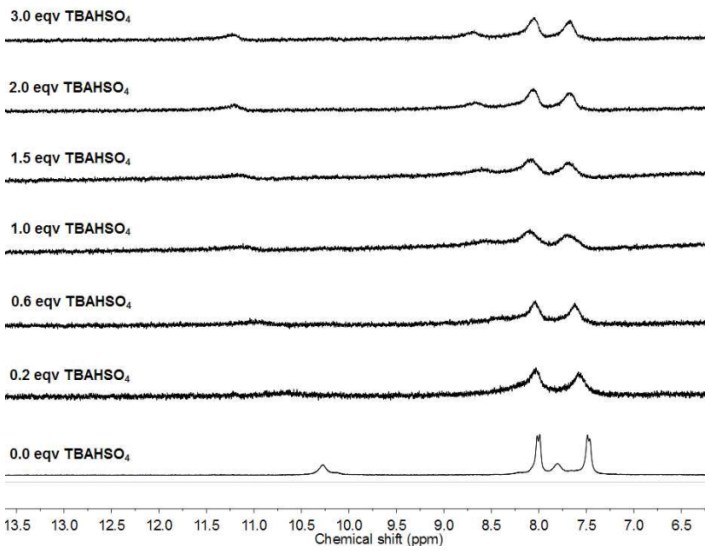
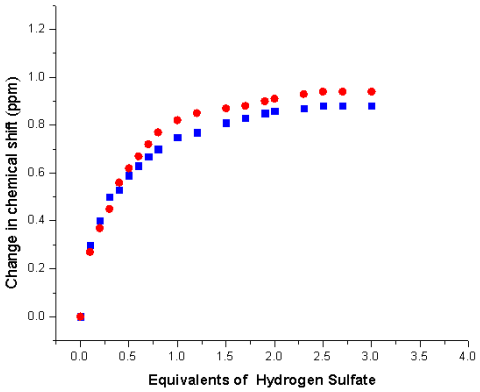


Figure S16. ^{1}H NMR titration of compound **2** (2.0 mM) with $TBAH_2PO_4$ in DMSO-d_6 at 298 K. Stack plot.

(a)



(b)



(c)

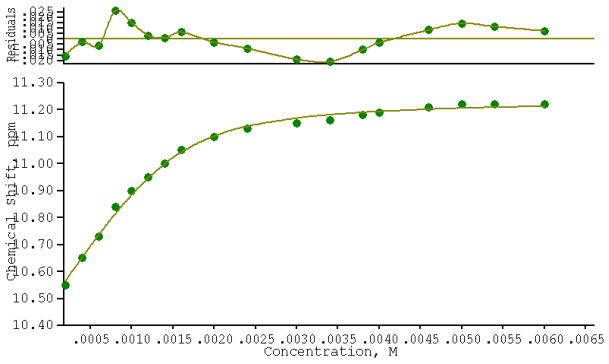


Figure S17. ^{1}H NMR titration of compound **2** (2.0 mM) with $TBAHSO_4$ in DMSO-d_6 at 298 K. (a) Stack plot. (b) Chemical shift of N-H resonances. (c) Fitplot for NH proton at δ = 10.28 ppm. Log K_a = 3.78 ± 0.05.

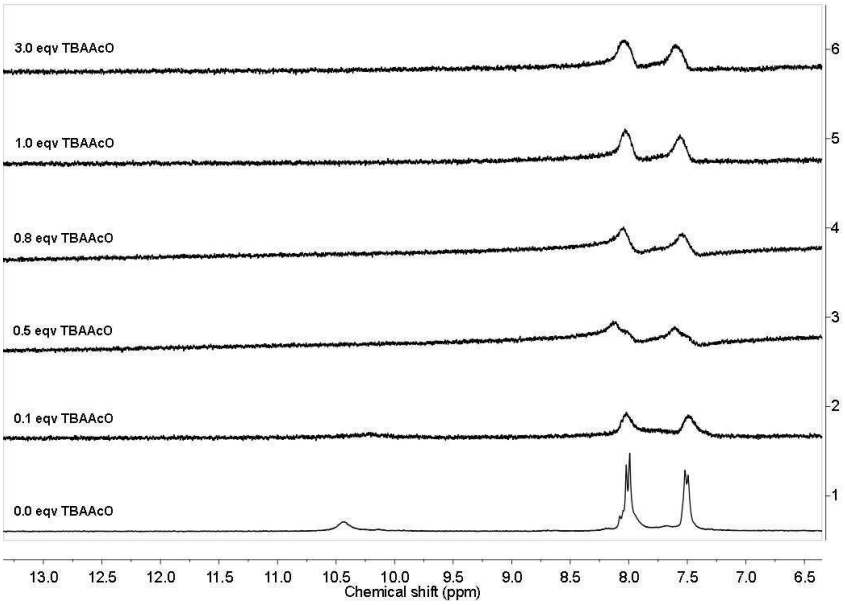
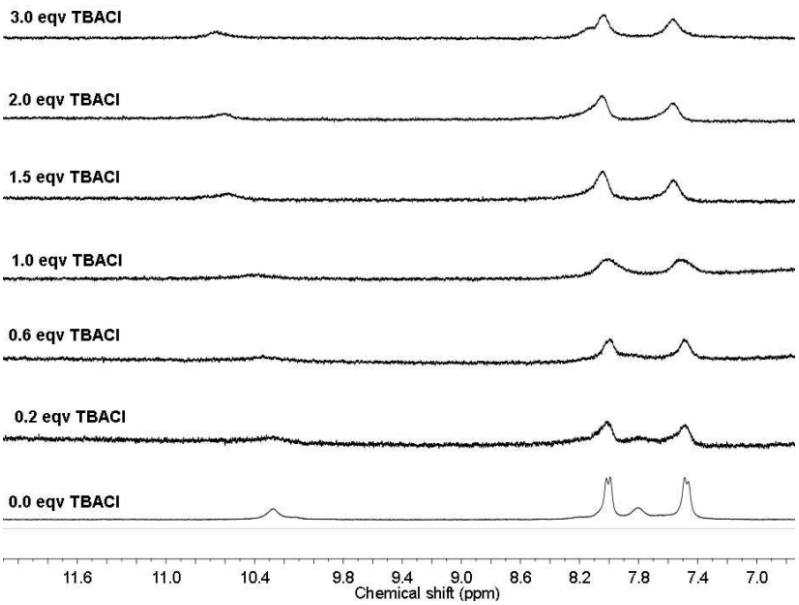
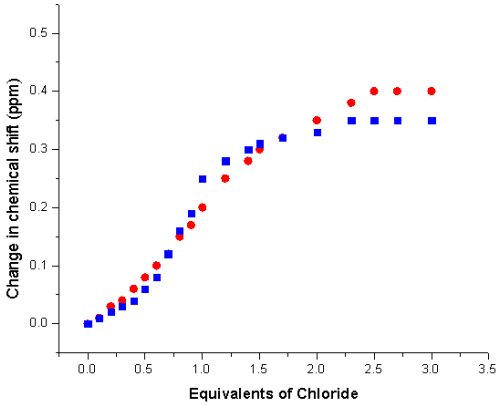


Figure S18. ^{1}H NMR titration of compound **2** (2.0 mM) with TBAAcO in DMSO-d_6 at 298 K.

(a)



(b)



(c)

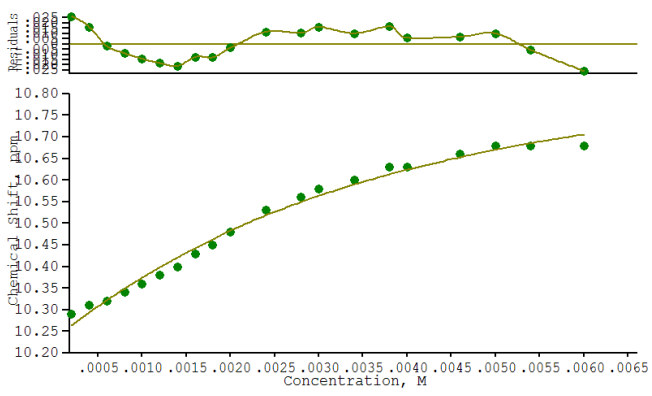
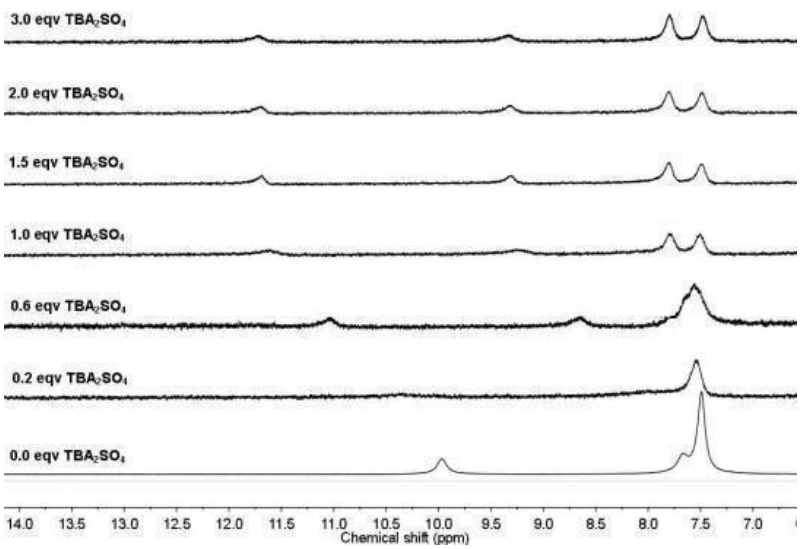
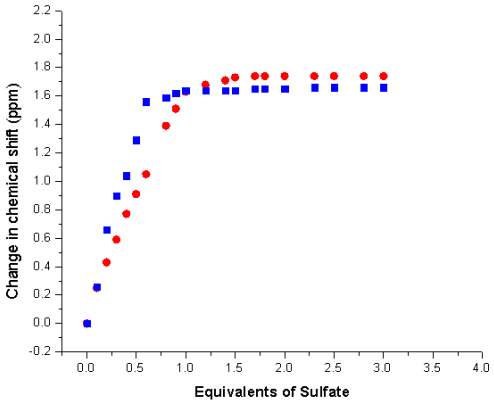


Figure S19. ^{1}H NMR titration of compound **2** (2.0 mM) with TBACl in DMSO-d_6 at 298 K. (a) Stack plot. (b) Chemical shift of N-H resonances. (c) Fitplot for NH proton at δ = 10.28 ppm. Log K_a = 2.61 ± 0.11.

(a)



(b)



(c)

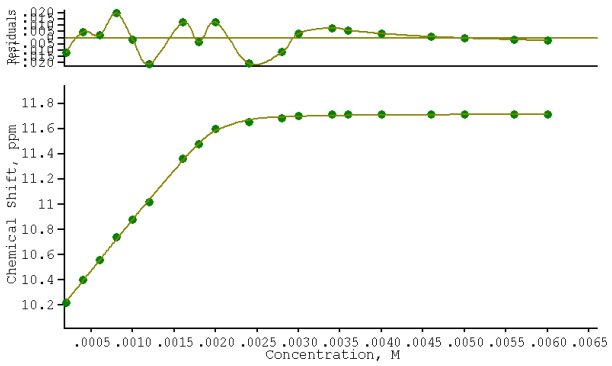


Figure S20. ^{1}H NMR titration of compound **3** (2.0 mM) with TBA_2SO_4 in DMSO-d_6 at 298 K. (a) Stack plot. (b) Chemical shift of N-H resonances. (c) Fitplot for NH proton at δ = 9.97 ppm. Log K_a = 4.87 ± 0.07.

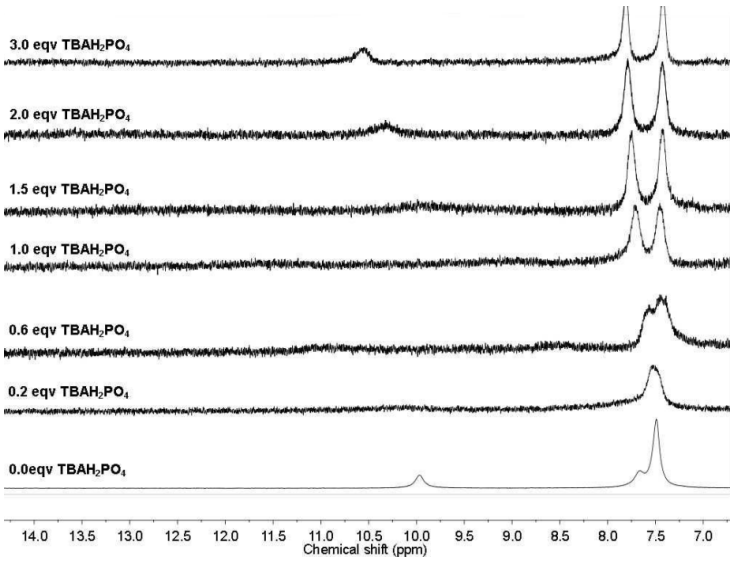
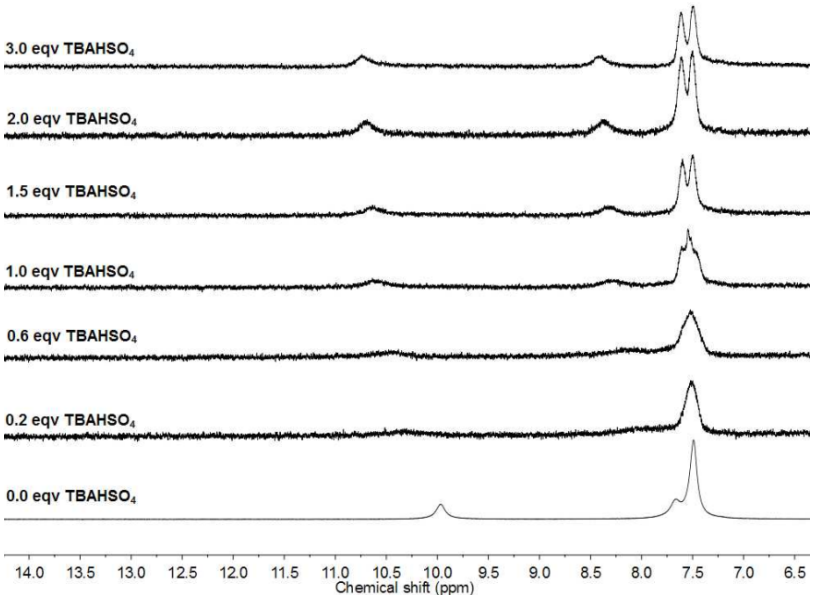
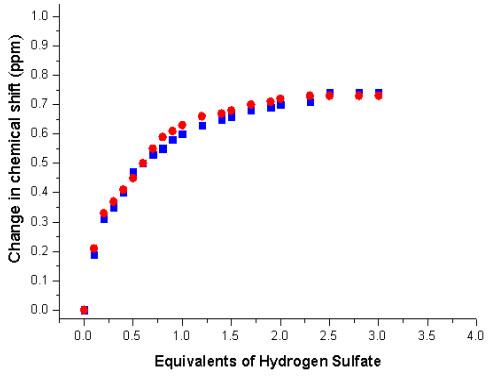


Figure S21. ^{1}H NMR titration of compound **3** (2.0 mM) with $TBAH_2PO_4$ in DMSO-d_6 at 298 K. Stack plot.

(a)



(b)



(c)

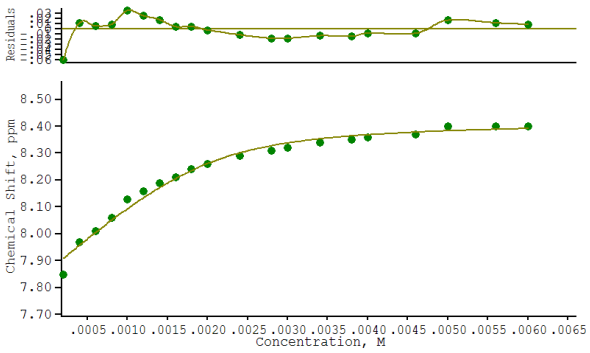


Figure S22. ^{1}H NMR titration of compound **3** (2.0 mM) with $TBAHSO_4$ in DMSO-d_6 at 298 K. (a) Stack plot. (b) Chemical shift of N-H resonances. (c) Fitplot for NH proton at δ = 7.66 ppm. Log K_a = 3.65 ± 0.14.

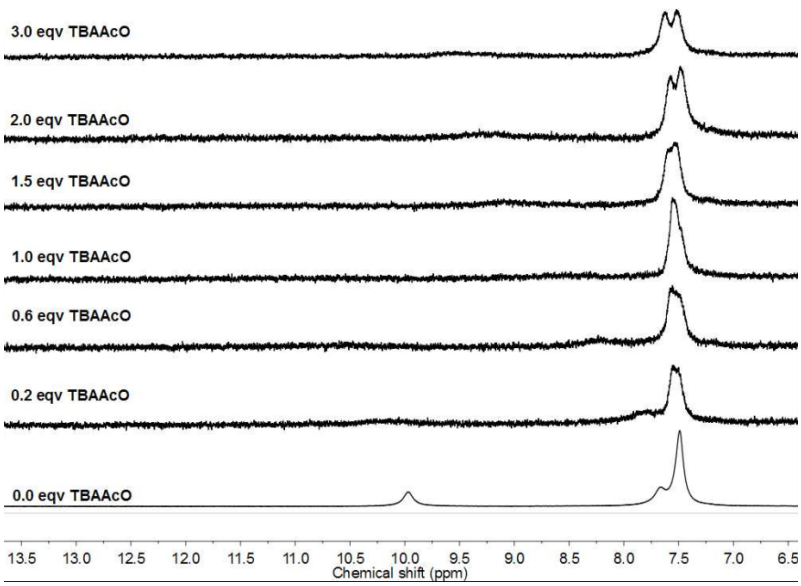
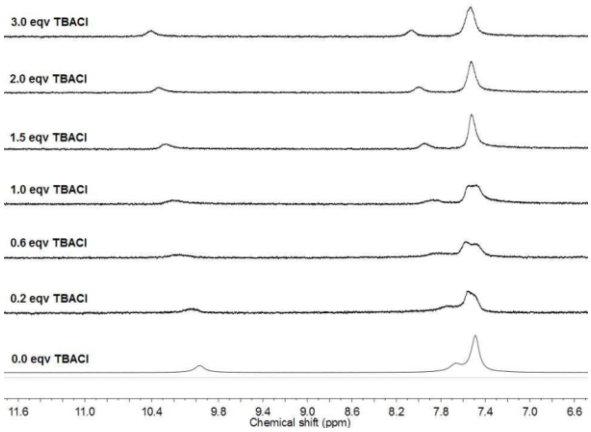


Figure S23. ^{1}H NMR titration of compound **3** (2.0 mM) with TBAAcO in DMSO-d_6 at 298 K.

(a)



(b) (c)

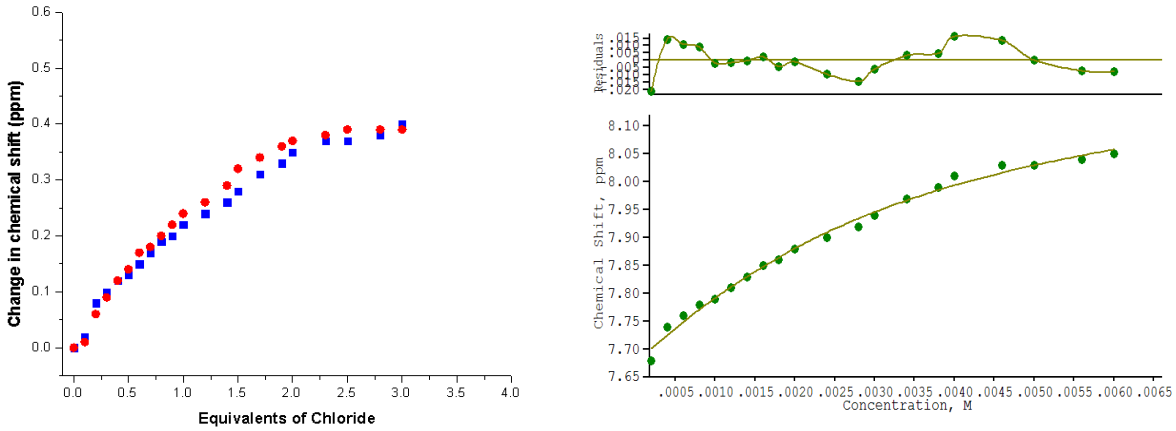


Figure S24. ^{1}H NMR titration of compound **3** (2.0 mM) with TBACl in DMSO-d_6 at 298 K. (a) Stack plot. (b) Chemical shift of N-H resonances. (c) Fitplot for NH proton at δ = 7.66 ppm. Log K_a = 2.65 ± 0.09.

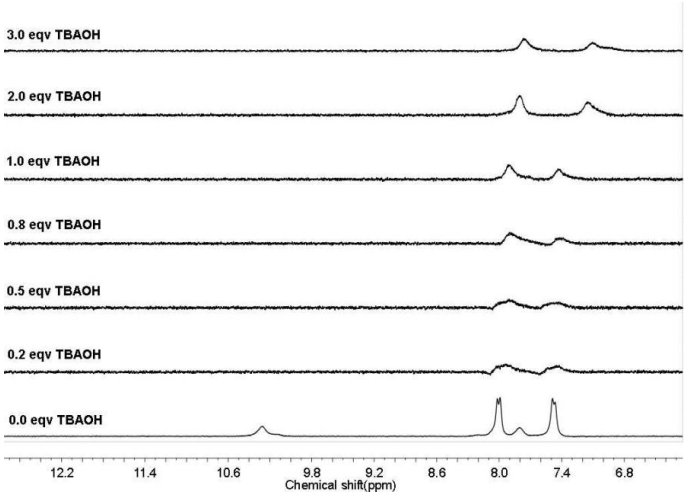


Figure S25. ^{1}H NMR titration of compound **2** (2.0 mM) with TBAOH in DMSO-d_6 at 298 K.

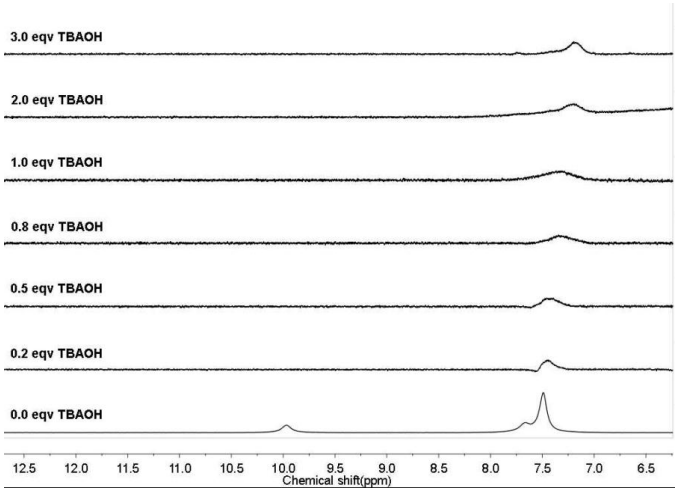


Figure S26. ^{1}H NMR titration of compound **3** (2.0 mM) with TBAOH in DMSO-d_6 at 298 K.

*5. Competition experiments of **1** with anions*

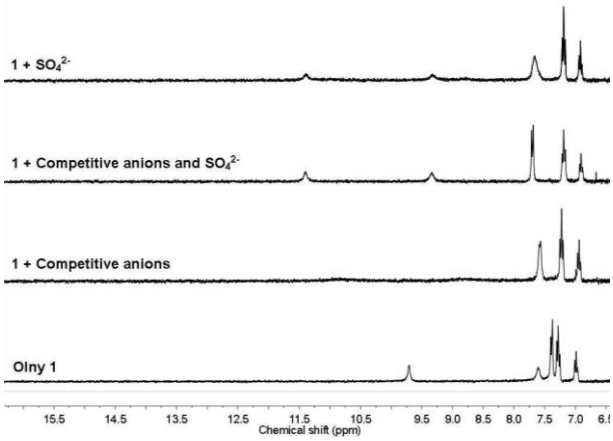


Figure S27. ^{1}H NMR (DMSO-d_6, 300 MHz) spectra of receptor **1** (1.0 mM) in the presence of one equivalent of various anions (added as TBA salts, competitive anions = Cl^-, Br^-, I^-, HSO_4^-, $H_2PO_4^-$, AcO^-, NO_3^- and ClO_4^-).

6. *NOESY and DOSY NMR spectra of **1** with SO_4^{2-} in DMSO-d_6*

NOESY: The pure receptor **1** showed two strong NOESY contacts between $H_a\cdots H_b$ and $H_b\cdots H_c$, attributed to the fact that the phenyl group in receptor **1** was in a coplanar form with the squaramide moiety which was also confirmed by the single crystal structure of 2TBA-[**1**·SO_4]. After the addition of SO_4^{2-}, the NOESY contact through $H_a\cdots H_b$ disappeared dramatically indicating the hydrogen bonding formation between N-$H_{a,\,b}$ of squaramide moieties and SO_4^{2-}, which was also supported by Das and Hossain who reported the similar formation of anion and tren-based (thio)urea receptors.[S4]

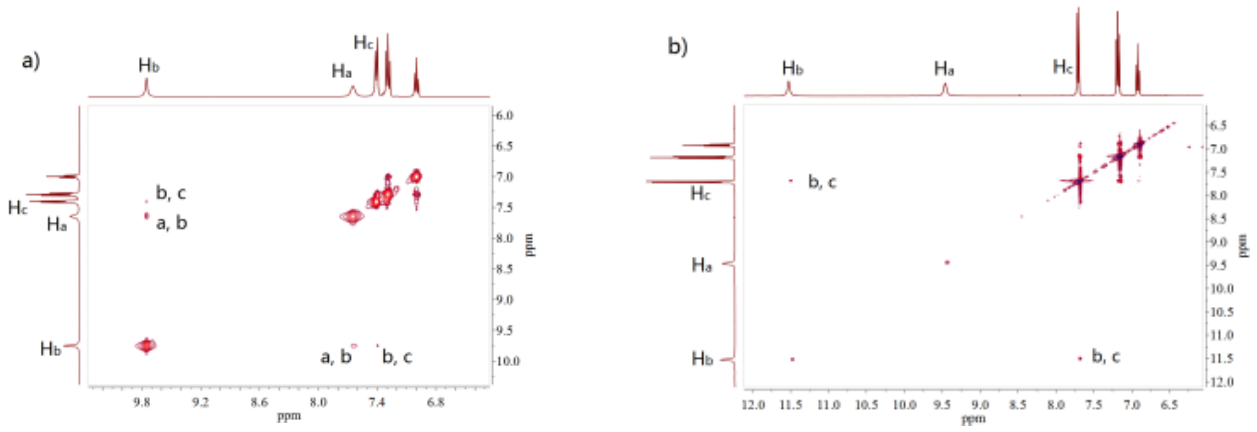


Figure S28. (a) 2D NOESY NMR experiment (400 MHz, 298 K) of only **1** (10 mM) in absence and (b) presence of 2.5 equiv. SO_4^{2-} (25 mM) in DMSO-d_6.

DOSY : The ledbpgp2s pulse sequence from Bruker Biospin was selected for the DOSY NMR by using gradients varied linearly from 5% up to 95% in 32 steps, with 16 scans per step. The diffusion time (Δ) was set at 20 ms, and the gradient length (δ) was set at 2 ms.[S5]
The diffusion coefficient of the mixture of **1** and 2.5 equiv SO_4^{2-} in DMSO-d_6 was found to be slightly larger than heptakis (2, 6-tri-O-methyl)-β-cyclodextrin (M = 1429 g/mol) as the internal reference, suggesting the dimeric size ($M_{\mathbf{1}@TBA2SO4}$ = 1239 g/mol) did not exist under these conditions.[S6]

Experiment	Ratio (Receptor **1**/ TBA_2SO_4)	Concentration of receptor **1**
NO. 1, ***Figure S29***	1:1	10 mM
NO. 2, ***Figure S30***	1:2.5	10 mM
NO. 3, ***Figure S31***	1:5	10 mM
NO. 4, ***Figure S32***	1:2.5	5 mM
NO. 5, ***Figure S33***	1:2.5	15 mM

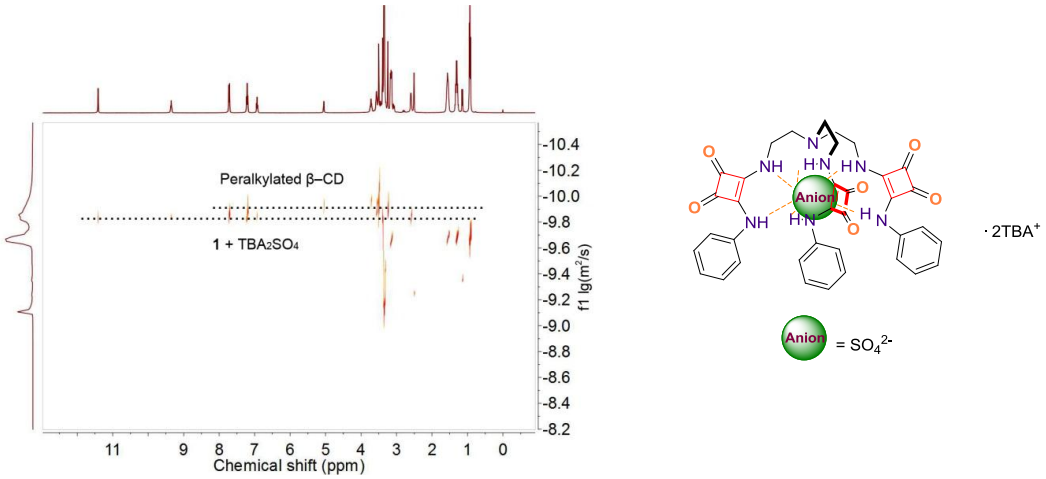


Figure S29. DOSY spectra (400 MHz, DMSO-d_6, 298 K) of the mixture of **1** (10 mM) and TBA_2SO_4 (10 mM).

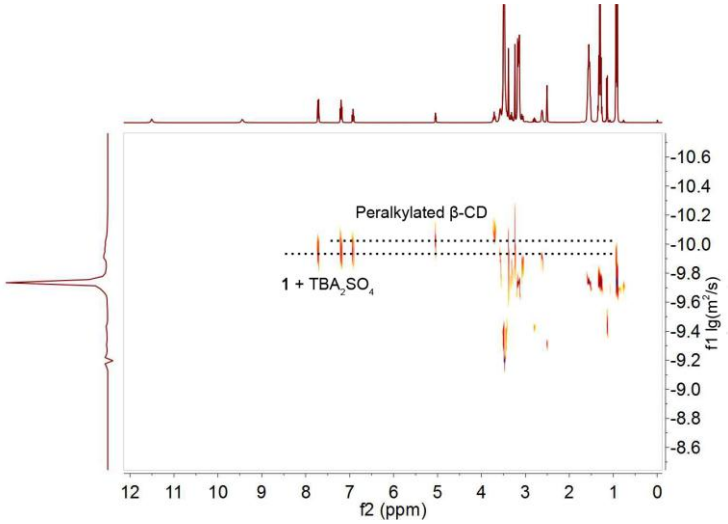


Figure S30. DOSY spectra (400 MHz, DMSO-d_6, 298 K) of the mixture of **1** (10 mM) and TBA_2SO_4 (25 mM).

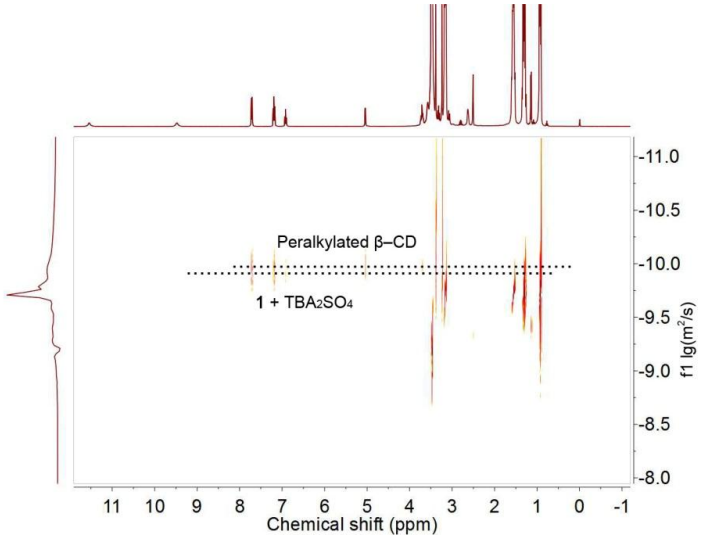


Figure S31. DOSY spectra (400 MHz, DMSO-d_6, 298 K) of the mixture of **1** (10 mM) and TBA_2SO_4 (50 mM).

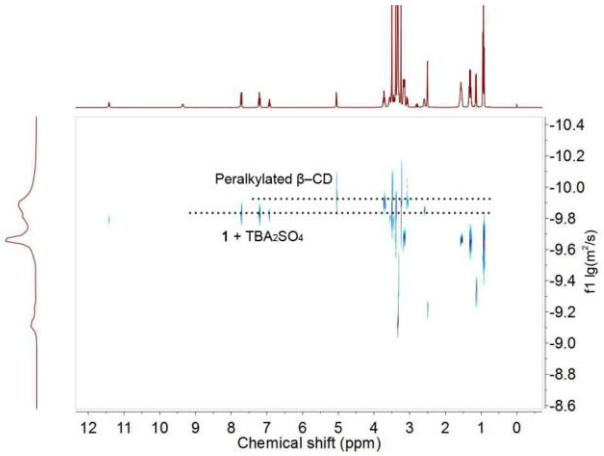


Figure S32. DOSY spectra (400 MHz, DMSO-d_6, 298 K) of the mixture of **1** (5 mM) and TBA_2SO_4 (12.5 mM).

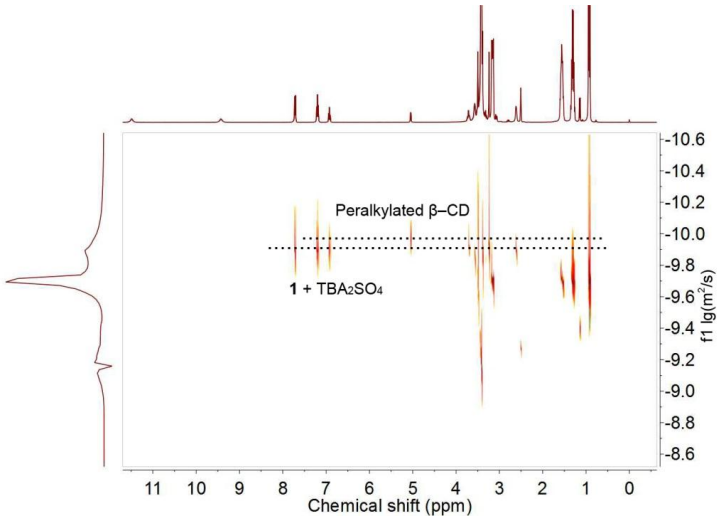


Figure S33. DOSY spectra (400 MHz, DMSO-d_6, 298 K) of the mixture of **1** (15 mM) and TBA_2SO_4 (37.5 mM).

*7. Proposed binding mode of receptor **1** and sulfate anion in solution*

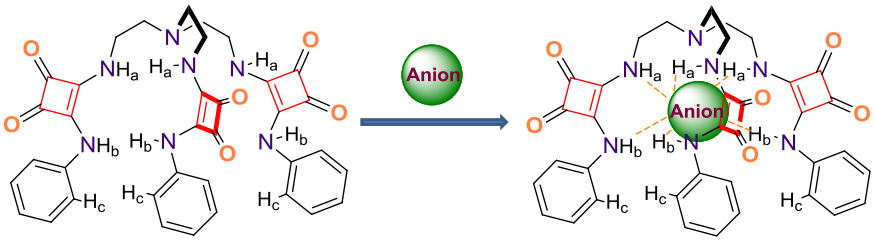


Figure S34. Proposed binding mode of receptor **1** and sulfate anion in solution

8. *HR-ESI-MS spectra of **1-3** with SO_4^{2-}*

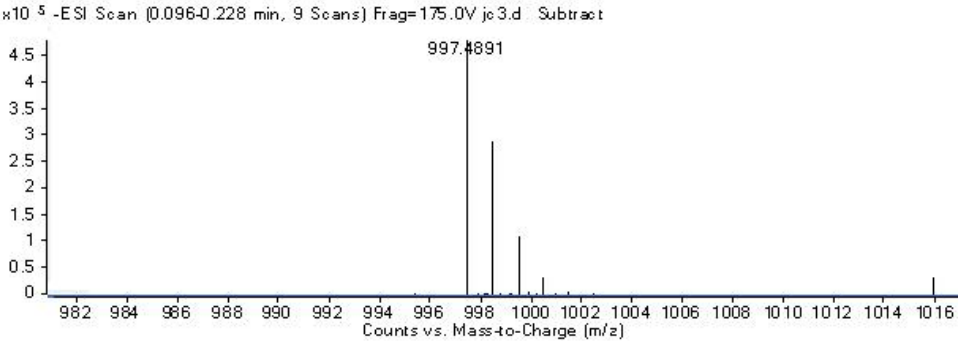


Figure S35. HR-ESI-MS spectra of complex **1** and TBA_2SO_4: *m/z* calcd for [**1** + $TBASO_4$]$^-$ $C_{52}H_{69}N_8O_{10}S$, 997.4857; found 997.4891.

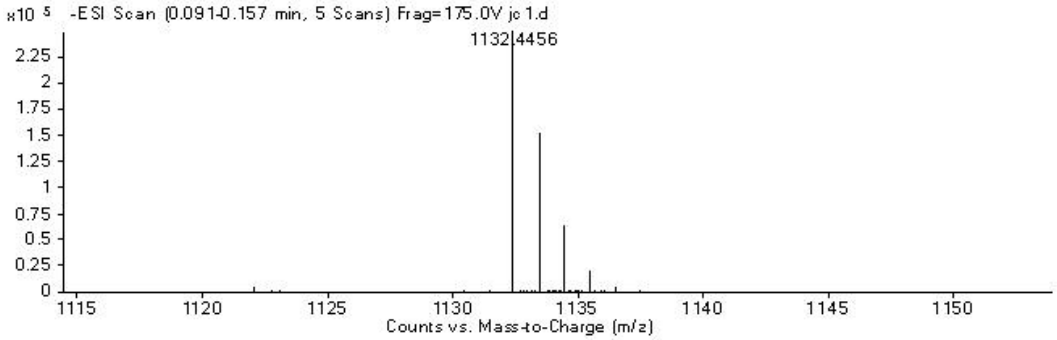


Figure S36. HR-ESI-MS spectra of complex **2** and TBA_2SO_4: *m/z* calcd for [**2** + $TBASO_4$]$^-$ $C_{52}H_{66}N_{11}O_{16}S$, 1132.4410; found 1132.4456.

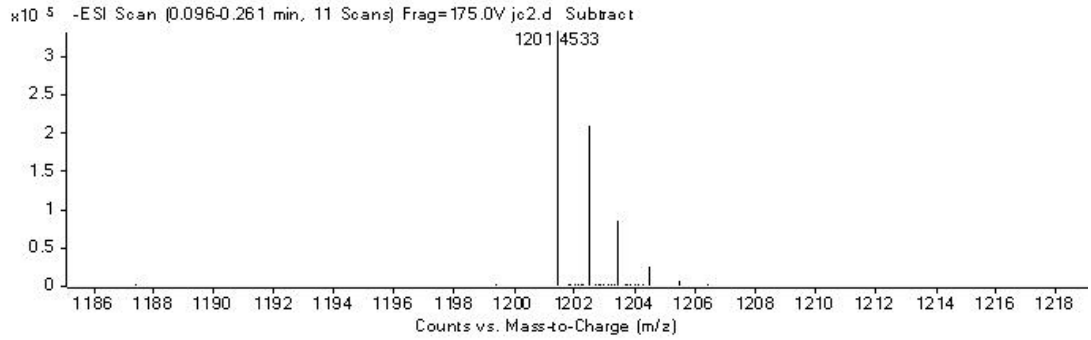


Figure S37. HR-ESI-MS spectra of complex **3** and TBA_2SO_4: *m/z* calcd for [**3** + $TBASO_4$]$^-$ $C_{55}H_{66}F_9N_8O_{10}S$, 1201.4479; found 1201.4533.

9. *X-ray crystallography data of 2TBA-[**1**·SO_4]*

Slow diffusion of a mixture of **1** with tetrabutylammonium sulfate (TBA_2SO_4) in DMSO/H_2O solution resulted in the single crystal formation of a sulfate-encapsulated complex 2TBA-[**1**·SO_4]. However, attempts to obtain single crystals of **2** and **3** with sulfate or other anions were unsuccessful.

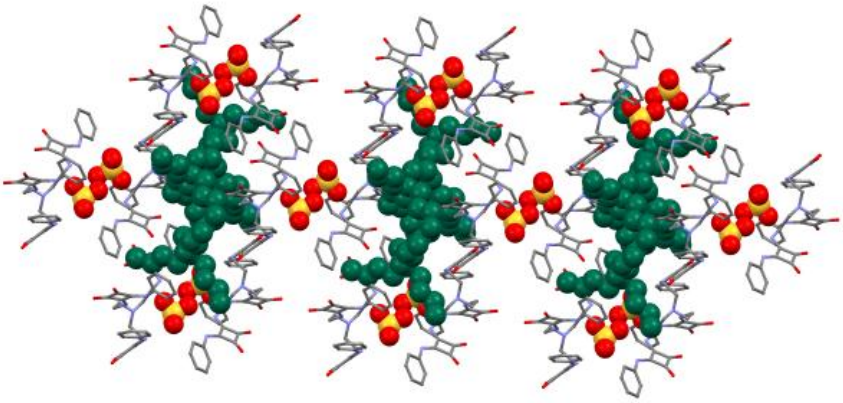


Figure S38. Lattice structure of 2TBA-[**1**·SO_4] viewed along the c axis. The TBA cations are drawn in space-filling style and colored green for clarity. All of H-bond donors of the squaramide units were directed towards the sulfate anions, and each set of the sulfate anion formed six hydrogen bonds with the H-bond donors of the squaramide units.

Table S1: hydrogen bonding parameters (Å, °) for 2TBA-[**1**·SO_4] and SO_4^{2-}

D-H···A	d (H···A)	d (D···A)	∠ D-H···A
N2-H2···O14	1.999	2.821	159.85
N2-H2···O22	2.193	2.971	150.46
N3-H3···O22	1.890	2.714	160.03
N3-H3···O12	2.169	3.006	164.12
N4-H4···O21	1.989	2.769	150.32
N4-H4···O11	2.526	3.301	150.44
N5-H5···O21	1.831	2.652	158.94
N5-H5···O11	1.937	2.781	166.72
N6-H6···O24[a]	1.956	2.809	171.16
N6-H6···O13[a]	2.077	2.838	147.21
N7-H7···O23[a]	1.942	2.795	171.33
N7-H7···O12[a]	2.272	3.023	145.93
N7-H7···O13[a]	2.569	3.364	154.19

[a] Symmetry code: -x, -y, -z+1

Table S2: Crystal data and structure refinement for 2TBA-[**1**·SO_4]

CCDC number	889595
Empirical formula	$C_{68}H_{105}N_9O_{10}S$
Formula weight	1240.67

Temperature	296(2)
Wavelength	0.71073 Å
Crystal system	Triclinic
Space group	$P\bar{1}$
a	12.9919(11)
b	14.4679(12)
c	19.3290(13)
α	94.8590(10)
β	96.631(2)
γ	94.2570(10)
Volume	3582.9(5) $Å^3$
Z	2
Density (calculated)	1.150
Absorption coefficient	0.105
F(000)	1344
Crystal size	0.26 × 0.22 × 0.20 mm^3
Theta range for data collection	2.21 to 27.99°
Index ranges	-15<=h<=14, -17<=k<=17, -19<=l<=22
Reflections collected	12092
Independent reflections	7282 [R(int) = 0.0245]
Completeness to theta = 24.71°	99.0 %
Absorption correction	multi-scan
Refinement method	Full-matrix least-squares on F^2
Goodness-of-fit on F^2	0.986
Final *R* indices [$I > 2\text{sigma}(I)$]	*R*1 = 0.0456, *wR*2 = 0.1071
R indices (all data)	*R*1 = 0.0741, *wR*2 = 0.1124
Largest diff. peak and hole	0.344 and -0.319 e·$Å^{-3}$

References and notes

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