"Off-On" Aggregation-Based Fluorescent Sensor for the Detection of Chloride in Water

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Supporting Information

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Synthesis

General Methods. ^{1}H and ^{13}C NMR spectra were obtained on a Varian 300 MHz (^{1}H 299.95 Hz, ^{13}C 75.43 Hz), Inova 500 MHz (^{1}H 500.10 MHz, ^{13}C 125.75 MHz) or Bruker Avance III HD 600 MHz NMR spectrometer with Prodigy multinuclear broadband BBO CryoProbe (^{1}H: 600.02 MHz, ^{13}C: 150.89 MHz). Chemical shifts (δ) are expressed in ppm from solvent signal using non-deuterated solvent present in the bulk deuterated solvent ($(CD_3)_2SO$: ^{1}H 2.5 ppm, ^{13}C 39.52 ppm). Unless otherwise specified, solvents were distilled using published literature procedures directly before use. Mass spectra were acquired Waters LCT Premier ESI-MS in positive mode using acetone as a solvent. UV-Vis spectra were acquired with a Hewlett-Packard 8453 UV-Visible spectrophotometer equipped with a 250 nm cutoff filter. Fluorescence data was acquired with a Horiba Jobin-Yvon FluoroMax-4 fluorescence spectrophotometer.

Compound 3. To a stirred degassed solution of 2,6-dibromo-4-nitropyridine (0.298 g, 1.06 mmol) in 1:1 THF/DIPA (70 mL), CuI (0.020 g 0.11 mmol) and $Pd(PPh_3)_4$ (0.073 g, 0.064 mmol) were added at room temperature. The solution was degassed for an additional 30 min after which a degassed solution of alkyne **2** (0.373 g, 2.33 mmol) in THF (50 mL) was added via cannulation, and the reaction mixture was stirred for 3 h at room temp. Upon completion the crude reaction mixture was run through a pad of silica and eluted with MeCN to remove the organic byproducts. Compound **3** was eluted with DMF and precipitated via the addition of water. The precipitated product was filtered, rinsed thoroughly with water and dried via high vacuum. No further purification was needed. Product was a bright orange solid (0.426 g, 91% yield). mp 198 °C (decomp.). λ_{max}(DMSO)/nm 297 (ε□dm^3 mol^{-1} cm^{-1} 17100), 379 (7230). ν_{max}/cm^{-1} 3475, 3285, 2210 (C≡C), 1651 (C=O), 1570, 1520, 1451, 1364, 1335, 1152, 1104, 753. δ_H(300 MHz, DMSO-d_6) 8.55 (2H, s), 8.19 (2H, s), 8.13 (2H, d, J = 8.3 Hz), 7.60 (2H, d, J = 7.4 Hz), 7.41 (2H, t, J = 7.8 Hz), 7.04 (2H, t, J = 7.4 Hz), 6.50 (4H, s). δ_C(126 MHz, DMSO-d_6) 155.55, 154.32, 144.85, 141.95, 132.97, 130.94, 121.82, 119.88, 119.53, 109.22, 92.71, 88.18. HRMS (EI+) calcd for $C_{23}H_{17}N_6O_4$ [MH^+] 441.1311, found 441.1307.

Receptor 1. A suspension of **3** (0.408 g, 0.926 mmol) and potassium carbonate (0.640 g, 4.63 mmol) in *N*,*N*-dimethylethanolamine (9.28 mL, 92.6 mmol) was stirred at room temperature overnight. The mixture was concentrated under reduced pressure and purified by chromatography using a mixture of 7:2:1 MeCN:H_2O:TEA as eluent. The product was concentrated under reduced pressure affording an off-white solid (0.300 g, 67% yield). ν_{max}/cm^{-1}

3349, 3176, 2201 (C≡C), 1726, 1694, 1574, 1515, 1447, 1325, 1297, 1136, 1041, 748. δ_H(300 MHz, DMSO-d_6) 8.11 (2H, d, J = 8.4 Hz), 8.03 (2H, s), 7.52 (2H, d, J = 7.6 Hz), 7.45 (2H, s), 7.36 (2H, t, J = 7.9 Hz), 7.01 (2H, t, J = 7.6 Hz), 6.49 (4H, s), 4.25 (2H, t, J = 5.7 Hz), 2.67 (2H, t, J = 5.4 Hz), 2.23 (6H, s). HRMS (EI+) calcd for $C_{27}H_{27}N_6O_3^+$ [MH^+] 483.2145, found 483.2164.

Formation of 1^+. Compound **1** was protonated through the addition of trifluoroacetic acid to a suspension in MeCN and solvent removed via reduced pressure and high vacuum. On small scale a film is typically formed with MeCN. Dissolving the film in acetone followed by addition of hexanes to precipitate $\mathbf{1}^+$ with subsequent removal of solvent provides a dark yellow solid. λ_{max}(DMSO)/nm 292 (ε□dm^3 mol^{-1} cm^{-1} 20296), 331 (15982). δ_H(600 MHz, DMSO-d_6) 9.63 (1H, s), 8.10 (2H, d, J = 5.8 Hz), 8.09 (2H, s), 7.52 (2H, dd, J = 7.7, 1.6 Hz), 7.46 (2H, s), 7.37 (2H, ddd, J = 8.8, 7.4, 1.6 Hz), 7.02 (2H, td, J = 7.5, 1.2 Hz), 6.47 (4H, s), 4.53 (2H, t, J = 5.0 Hz), 3.58 (2H, m), 2.88 (6H, s). δ_C(151 MHz, DMSO) 163.97, 155.62, 143.89, 141.62, 132.66, 130.28, 121.67, 119.85, 114.22, 109.80, 93.58, 85.51, 63.05, 54.92, 42.81.

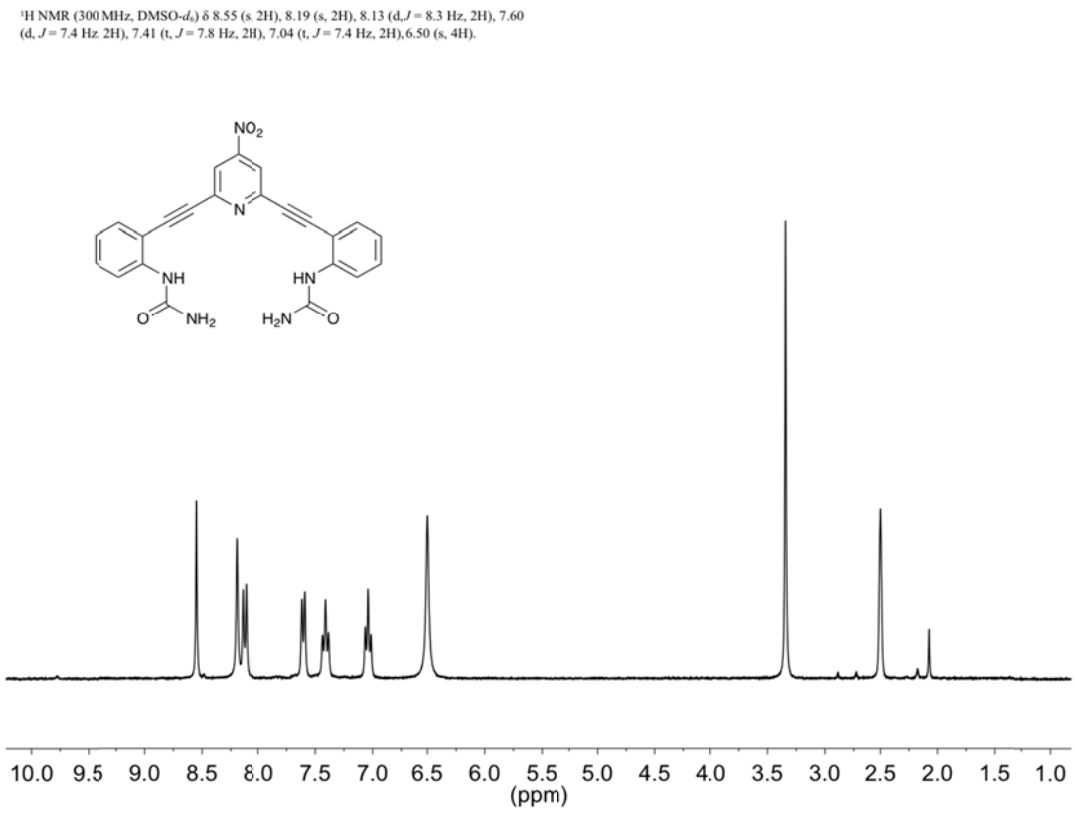

Figure S1. ^{1}H NMR spectrum of **3** in $CDCl_3$, 300 MHz. Impurities visible at 2.07, 2.73, and 2.89 can be attributed to DMF and acetonitrile.

^{13}C NMR (126 MHz, DMSO-d5) δ 155.55, 154.32, 144.85, 141.95, 132.97, 130.94, 121.82, 119.88, 119.53, 109.22, 92.71, 88.18.

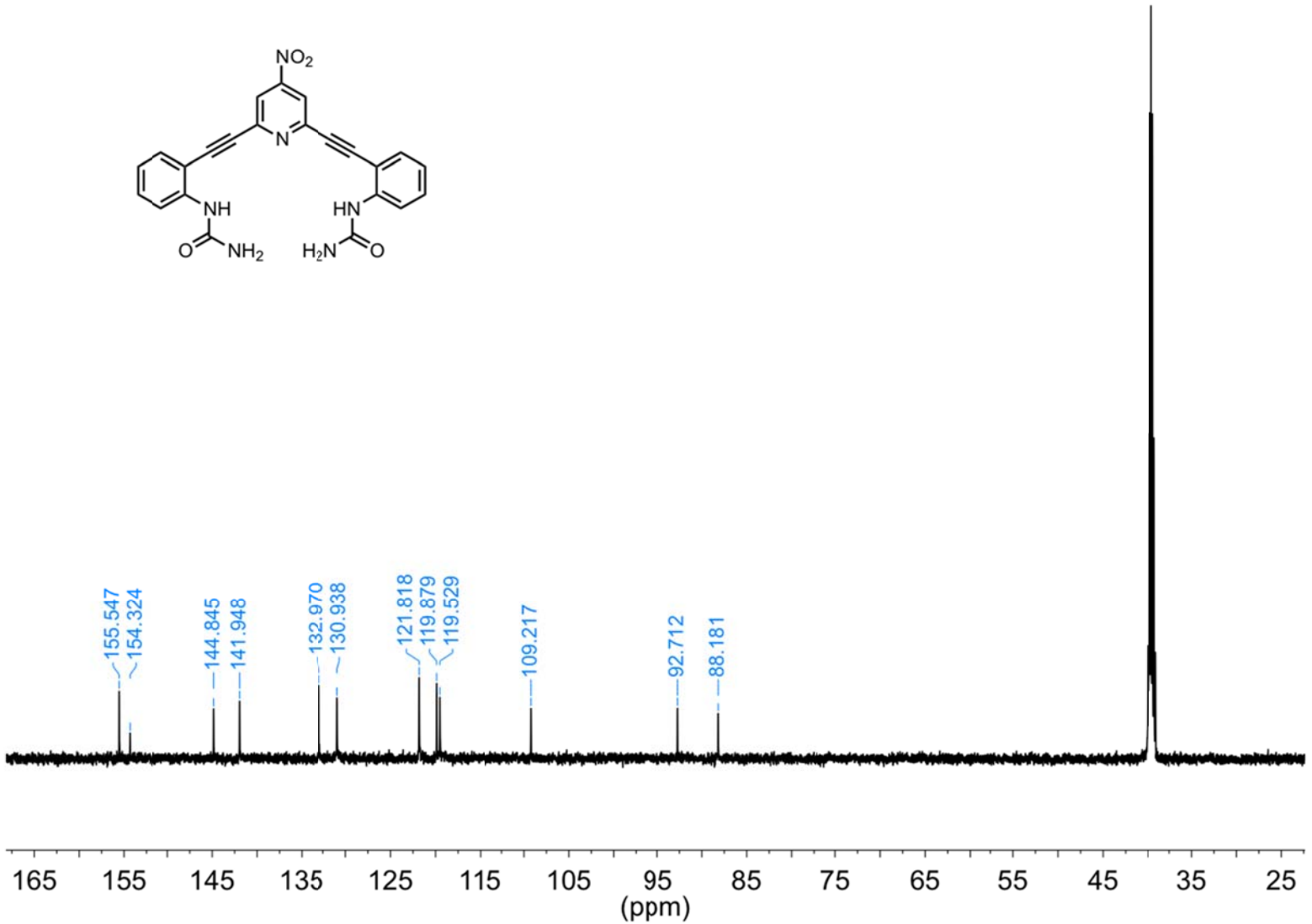

Figure S2. ^{13}C NMR spectrum of **3** in $CDCl_3$, 126 MHz.

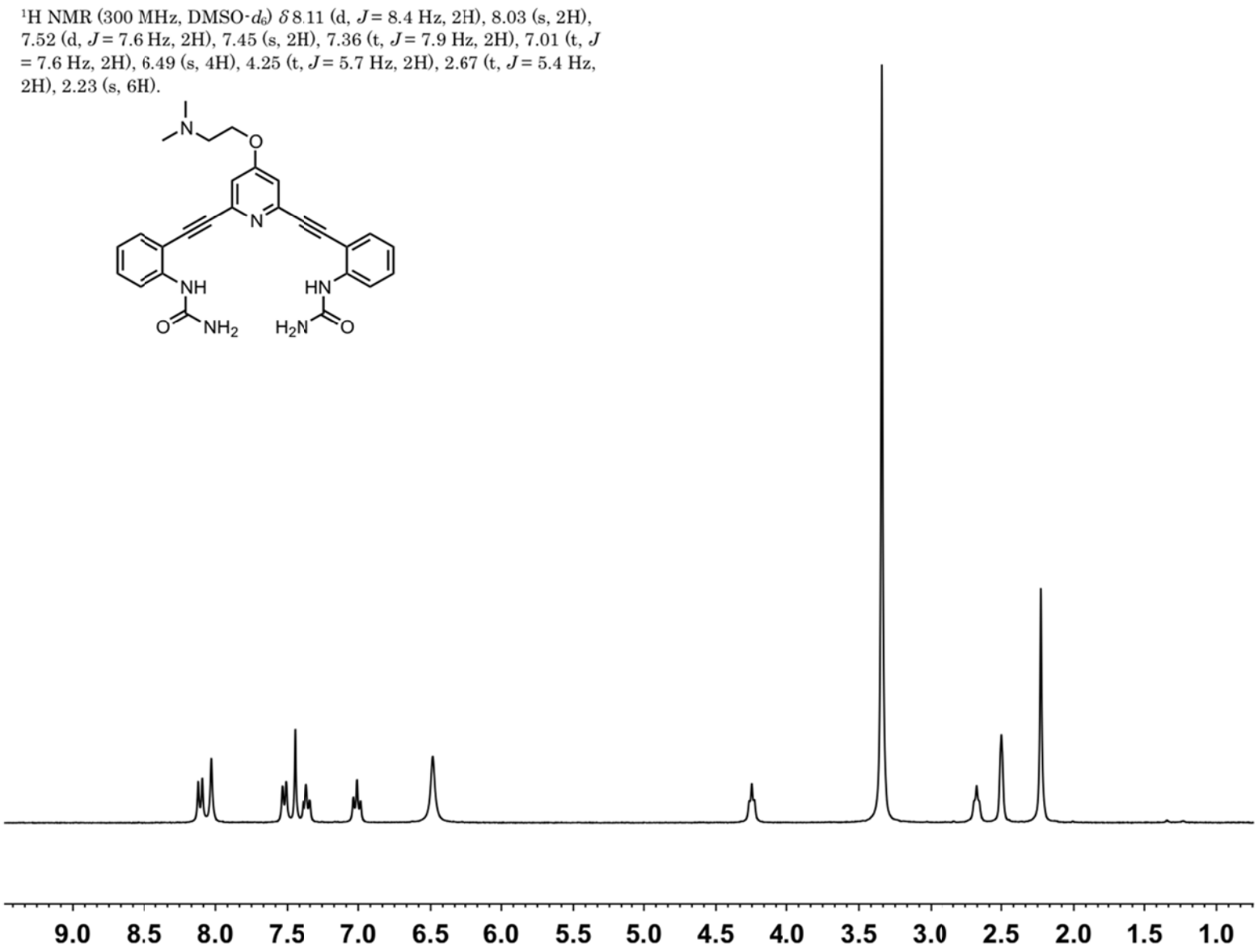

Figure S3. [1]H NMR spectrum of **1** in $CDCl_3$, 300 MHz.

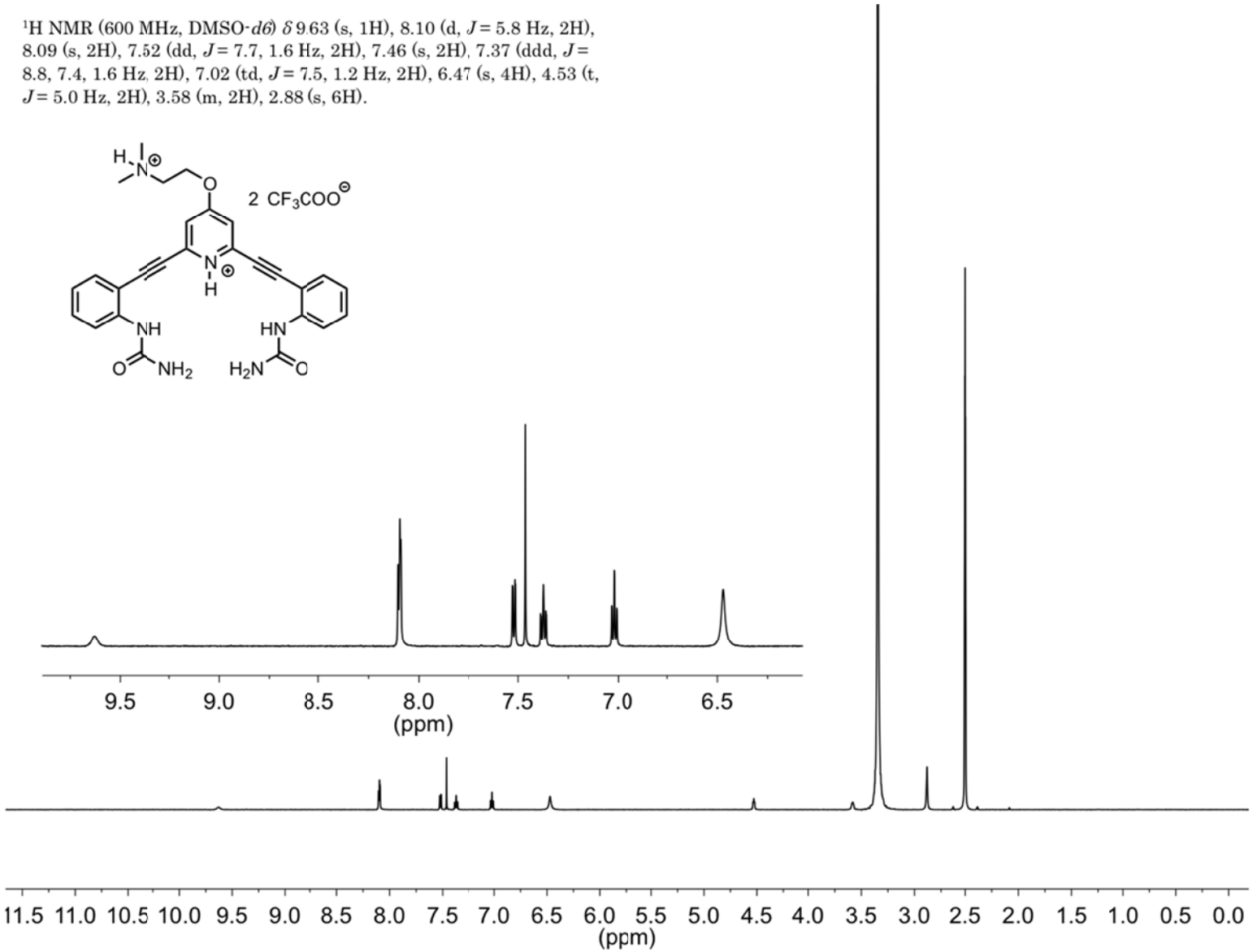

Figure S4. ^{1}H NMR spectrum of $\mathbf{1}^{+}$ in $CDCl_3$, 600 MHz.

^{13}C NMR (151 MHz, DMSO) δ 163.97, 155.62, 143.89, 141.62, 132.66, 130.28, 121.67, 119.85, 114.22, 109.80, 93.58, 85.51, 63.05, 54.92, 42.81.

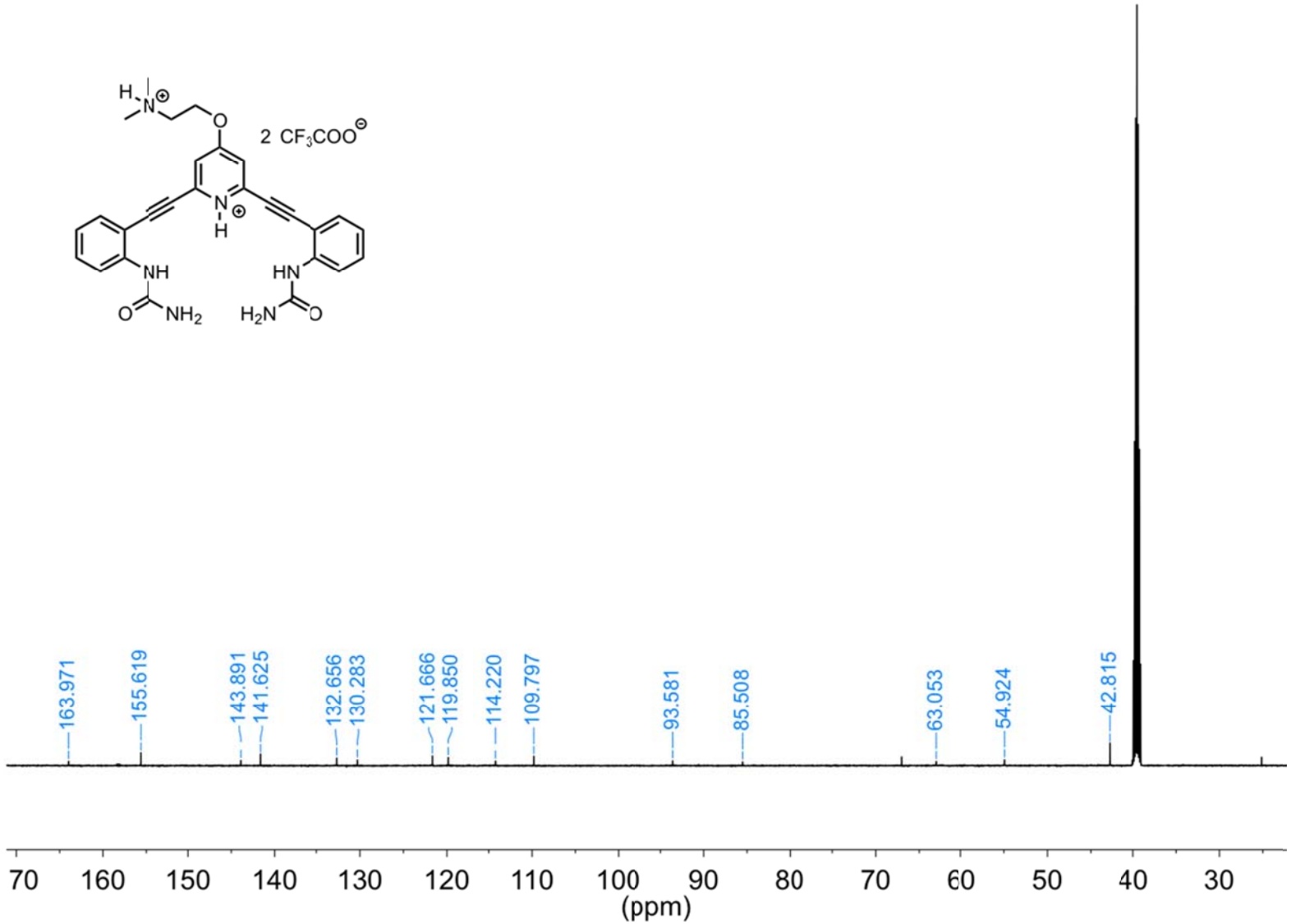

Figure S5. ^{13}C NMR spectrum of **1**$^{+}$ in $CDCl_3$, 151 MHz.

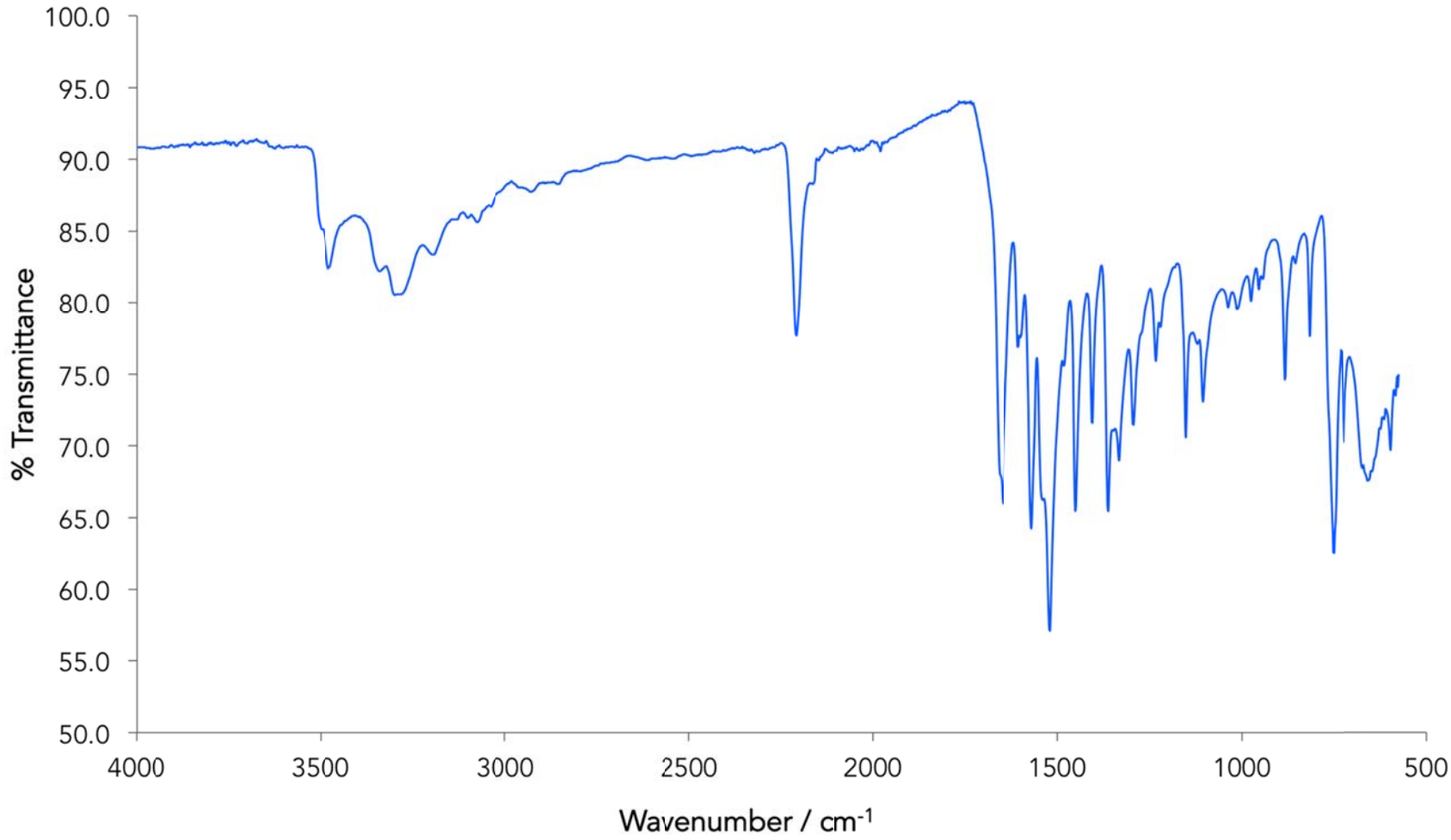

Figure S6. ATIR spectrum of **2**(neat).

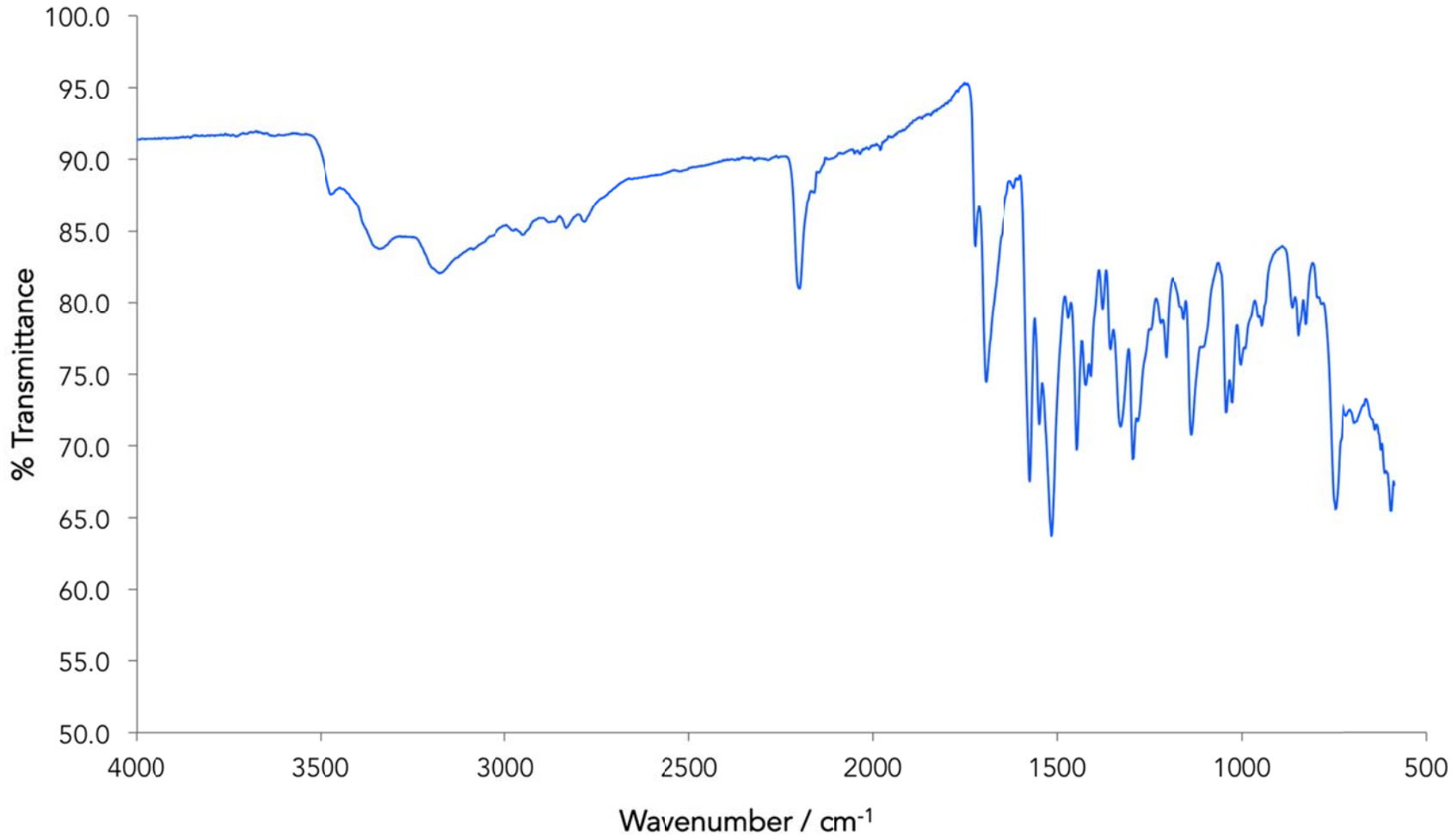

Figure S7. ATIR spectrum of **1**(neat)

^{1}H NMR Titrations

Tetrabutylammonium salts were dried at 50 °C under vacuum and stored in a calcium carbonate filled desiccator. In all titrations the receptor concentration was kept constant during the titration. Stock solutions of receptor were prepared in either DMSO-d_6 containing 0.5% H_2O or a 50:50 mixture of DMSO-d_6 and MeCN-d_3. Host-guest solutions were prepared in the stock receptor solution. All additions were performed with a Hamilton µL syringe. Representative data are provided for each set. ^{1}H NMR titrations in DMSO-d_6 were carried out on a Bruker Avance III HD 600 MHz NMR Spectrometer with Prodigy multinuclear broadband BBO CryoProbe (^{1}H: 600.02 MHz, ^{13}C: 150.89 MHz). ^{1}H NMR titrations in 50:50 DMSO-d_6:MeCN-d_3 were carried out on an Inova 500 MHz spectrometer (^{1}H 500.10 MHz). Chemical shifts (δ) are expressed in ppm downfield from tetramethylsilane (TMS) using non-deuterated solvent present in the bulk deuterated solvent (DMSO: ^{1}H 2.50 ppm). Chemical shifts of urea protons broadened into the baseline were determined using line broadening.

Tetrabutylammonium Chloride in 0.5 % H_2O/d_6-DMSO. A 3 mL stock solution of **1**$^+$ (3.18 mg, [R] = 1.49 mM) in 0.5 % H_2O/DMSO-d_6 was prepared and used in the preparation of a 2 mL TBACl guest solution (21.43 mg, [G] = 47.0 mM). Starting volume of 500 µL.

Table S1. Example titration for the addition of tetrabutylammonium chloride to **1**$^+$ in 0.5% H_2O/d_6-DMSO.

Addition (µL)	Total Volume Anion (µL)	[TBACl] (M)	Equiv. Cl^-	$\delta(R_3HN^+)$ (ppm)	$\delta(C_{Py}H)$ (ppm)	$\delta(HN_{urea})$ (ppm)	$\delta(H_2N_{urea})$ (ppm)
0	0	0.00E+00	0.00	9.625	7.462	8.096	6.474
10	10	9.22E-04	0.62	9.742	7.491	8.110	6.491
10	20	1.81E-03	1.21	9.846	7.517	8.123	6.506
15	35	3.08E-03	2.06	9.956	7.545	8.137	6.521
15	50	4.27E-03	2.87	10.053	7.569	8.151	6.535
20	70	5.77E-03	3.87	10.143	7.594	8.164	6.551
20	90	7.17E-03	4.81	10.209	7.612	8.174	6.562
25	115	8.79E-03	5.89	10.284	7.632	8.185	6.573
35	150	1.09E-02	7.27	10.361	7.654	8.197	6.586
50	200	1.34E-02	9.01	10.431	7.674	8.208	6.599
75	275	1.67E-02	11.18	10.519	7.701	8.224	6.617
150	425	2.16E-02	14.48	10.609	7.730	8.242	6.634

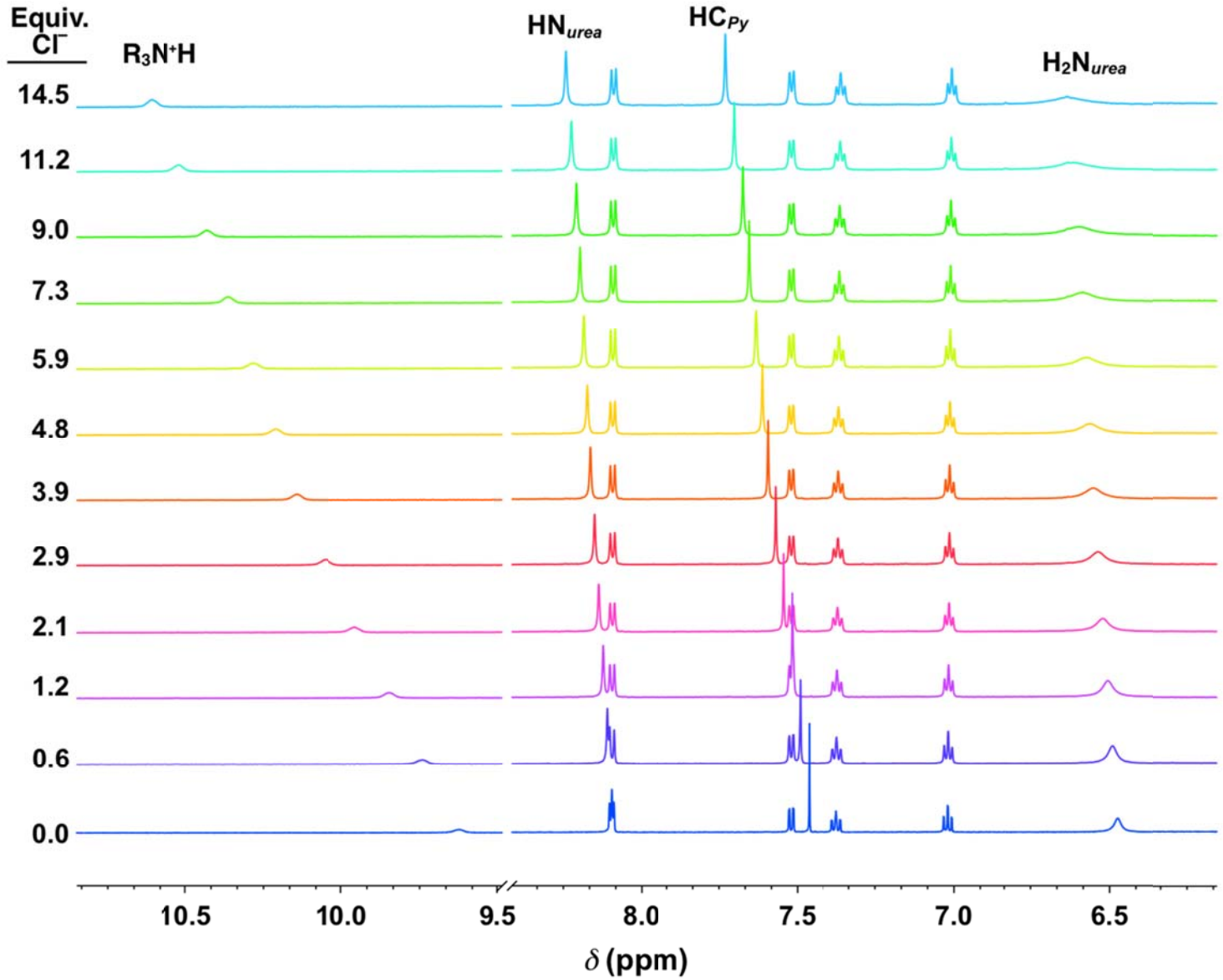

Figure S8. Example stacked spectra from the titration for the addition of tetrabutylammonium chloride to into $\mathbf{1}^{+}$ in 0.5% H_2O/d_6-DMSO.

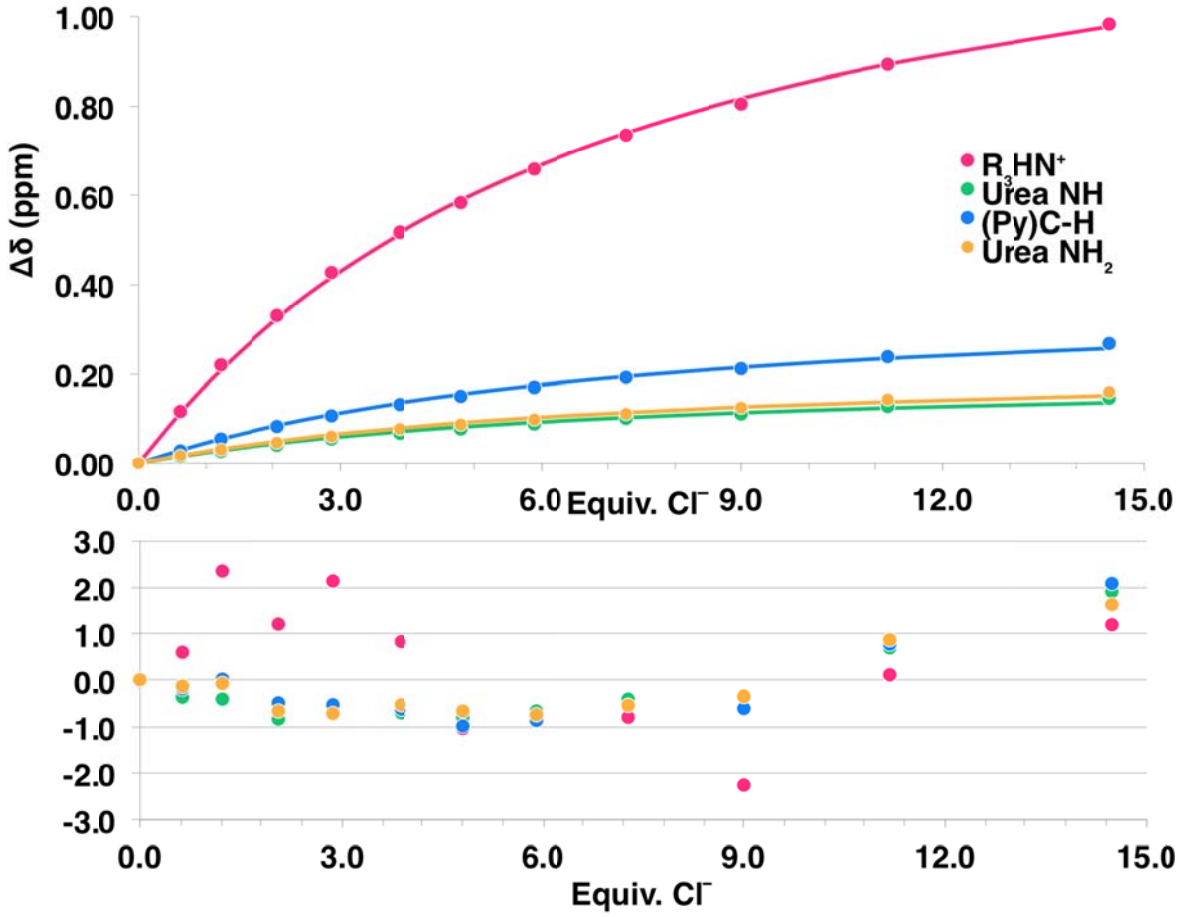

Figure S9. Example 1:1 fitting using non-linear regression from the data obtained for the titration of tetrabutylammonium chloride into $\mathbf{1}^+$ in 0.5 % H_2O/d_6-DMSO.

Tetrabutylammonium Bromide in 0.5 % H_2O/DMSO-d_6. A 3 mL stock solution of $\mathbf{1}^+$ (1.00 mg, [R] = 0.47 mM) in 0.5 % H_2O/DMSO-d_6 was prepared and used in the preparation of a 2 mL TBABr guest solution (0.151 g, [G] = 0.235 M). Starting volume of 500 µL.

Tetrabutylammonium Nitrate, Hydrogen Sulfate, Iodide, Perchlorate in 0.5 % H_2O/DMSO-d_6. 1.5 mM $\mathbf{1}^+$ in 0.5 % H_2O/DMSO-d_6 was titrated with a $\mathbf{1}^+$•Guest solution up to 21.4 equivalents of guest for $TBANO_3$, $TBAHSO_4$, TBAI, and $TBAClO_4$. No changes in chemical shift were observed throughout the titrations.

Tetrabutylammonium Dihydrogen Phosphate in 0.5 % H_2O/DMSO-d_6. 1.5 mM $\mathbf{1}^+$ in 0.5 % H_2O/DMSO-d_6 was titrated with a $\mathbf{1}^+$•$H_2PO_4^-$ solution up to 21.4 equivalents.

Table S2. Example titration for the addition of tetrabutylammonium bromide to $\mathbf{1}^+$ in 0.5% H_2O/d_6-DMSO.

Addition (µL)	Total Volume Anion (µL)	[TBABr] (M)	Equiv. Br^-	$\delta(R_3HN^+)$ (ppm)	$\delta(C_{Py}H)$ (ppm)	$\delta(HN_{urea})$ (ppm)	$\delta(H_2N_{urea})$ (ppm)
0	0	0.00E+00	0	9.775	7.481	8.108	6.485
25	25	1.12E-02	2.38	9.806	7.521	8.121	6.505
25	50	2.14E-02	4.54	9.831	7.547	8.130	6.516
30	80	3.24E-02	6.89	9.854	7.570	8.138	6.526
35	115	4.39E-02	9.33	9.875	7.589	8.144	6.536
40	155	5.56E-02	11.81	9.892	7.606	8.150	6.545
50	205	6.83E-02	14.52	9.909	7.622	8.156	6.554
70	275	8.33E-02	17.71	9.928	7.639	8.162	6.562
100	375	1.01E-01	21.39	9.948	7.656	8.169	6.568
150	525	1.20E-01	25.57	9.965	7.671	8.174	6.576
200	725	1.39E-01	29.55	9.986	7.687	8.180	6.589

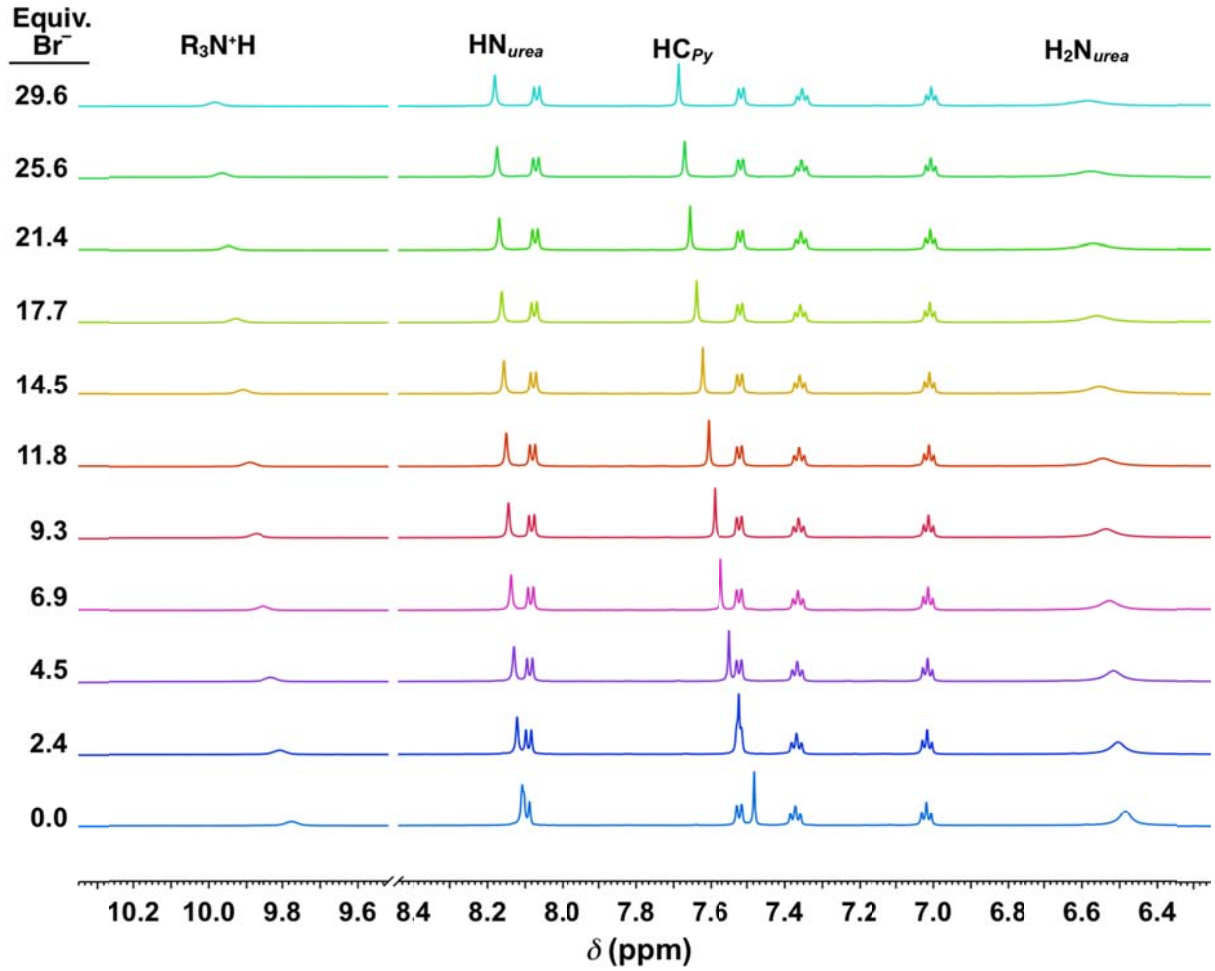

Figure S10. Example stacked spectra from the titration for the addition of tetrabutylammonium bromide to into $\mathbf{1}^+$ in 0.5 % H_2O/d_6-DMSO.

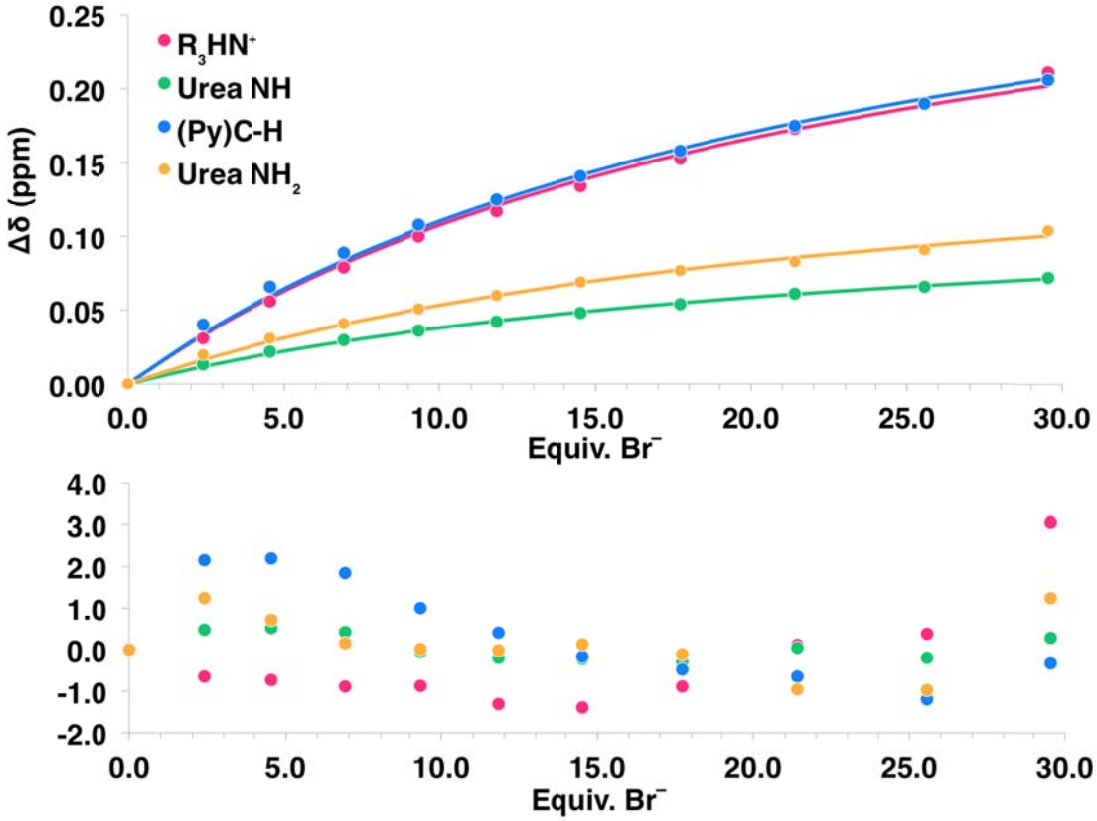

Figure S11. Example 1:1 fitting using non-linear regression from the data obtained for the titration of tetrabutylammonium bromide into $\mathbf{1}^+$ in 0.5 % H_2O/d_6-DMSO.

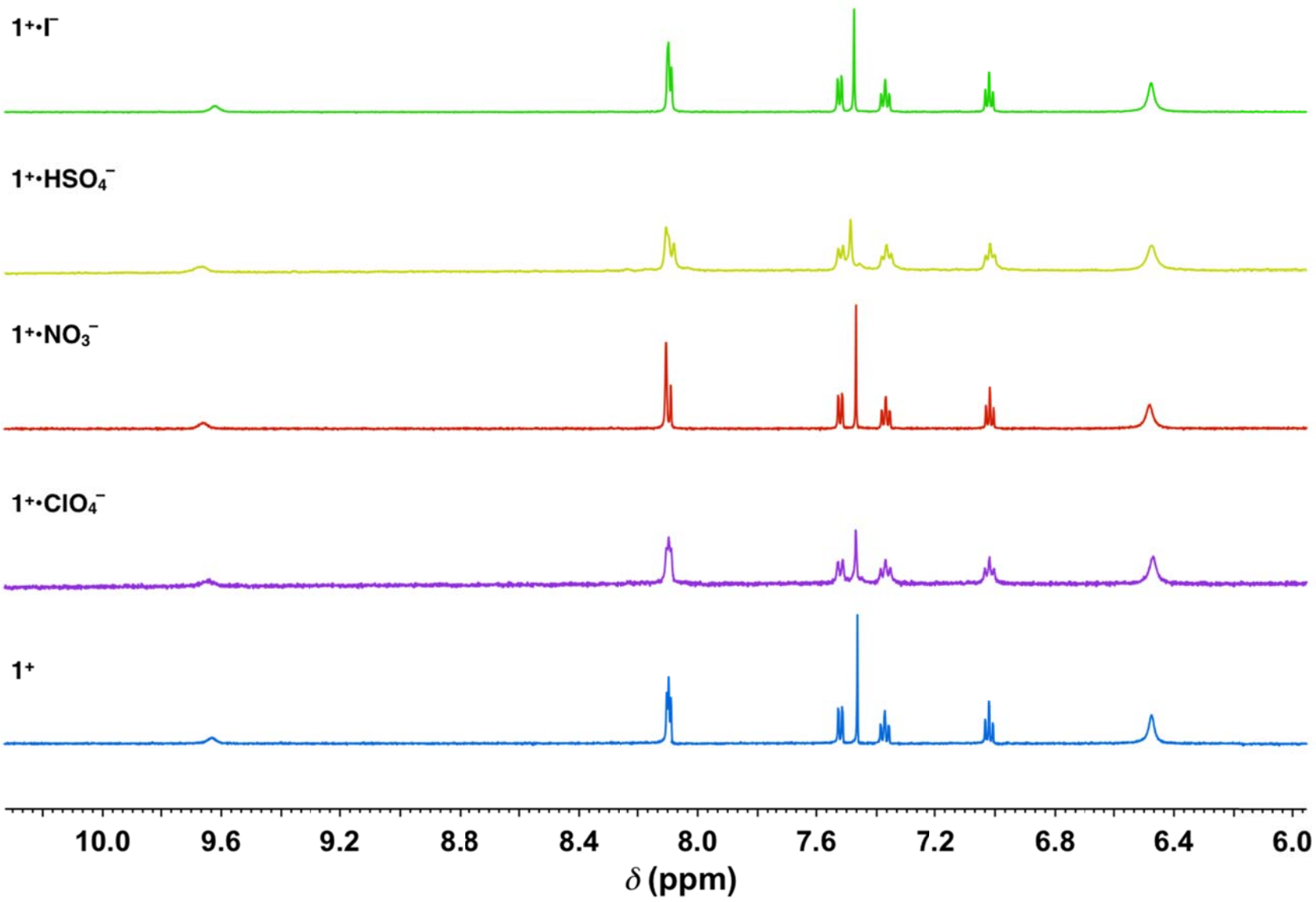

Figure S12. ^{1}H NMR spectra of $\mathbf{1}^{+}$ and 21.4 equivalents of TBA salts of perchlorate, nitrate, hydrogen sulfate, and iodide. Minimal to zero change in chemical shifts is observed throughout their titrations.

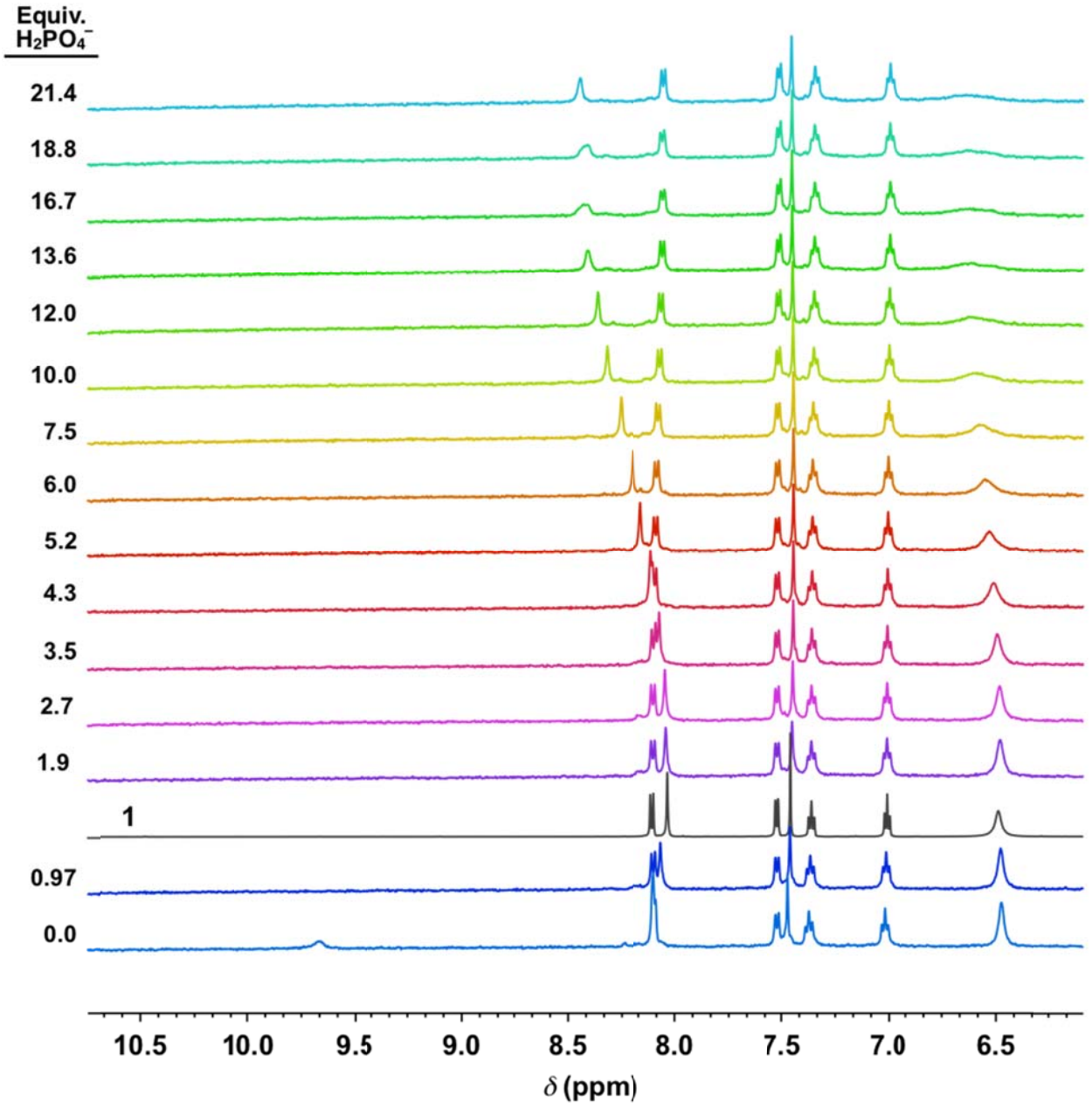

Figure S13. Stacked ^{1}H NMR spectra for the titration of tetrabutylammonium dihydrogen phosphate into $\mathbf{1}^{+}$. After the addition of 1 equivalent of guest, the $R_3N^{+}H$ proton resonance disappears and the other resonances return to the approximate shifts of unprotonated **1** (shown for comparison).

Tetrabutylammonium Chloride in 50% d_6-DMSO/50% d_3-MeCN. A fresh 3 mL stock solution of $\mathbf{1}^+$ (2.22 mg **1**, [H] = 1.53 mM) in 50% d_6-DMSO/50% d_3-MeCN was used in the preparation of a 2 mL TBACl guest solution (13.19 mg, [G] = 28.9 mM). Starting volume of 500 μL.

Tetrabutylammonium Bromide in 50 % d_6-DMSO/50 % d_3-MeCN. A fresh 3 mL stock solution of $\mathbf{1}^+$ (2.42 mg **1**, [H] = 1.67 mM) in 50% d_6-DMSO/50% d_3-MeCN was used in the preparation of a 2 mL TBABr guest solution (0.133 g, [G] = 0.206 M). Starting volume of 500 μL.

Table S3. Example titration for the addition of tetrabutylammonium chloride to $\mathbf{1}^+$ in 50% d_6-DMSO/50% d_3-MeCN.

Addition (μL)	Total Volume Anion (μL)	[TBACl] (M)	Equiv. Cl^-	$\delta(R_3HN^+)$ (ppm)	$\delta(C_{Py}H)$ (ppm)	$\delta(HN_{urea})$ (ppm)	$\delta(H_2N_{urea})$ (ppm)
0	0	0.00E+00	0.00	9.799	7.396	8.044	6.266
10	10	5.67E-04	0.37	9.976	7.433	8.061	6.287
10	20	1.11E-03	0.73	10.124	7.466	8.077	6.306
10	30	1.64E-03	1.07	10.240	7.493	8.089	6.322
10	40	2.14E-03	1.40	10.342	7.516	8.101	6.333
15	55	2.87E-03	1.87	10.459	7.546	8.116	6.354
15	70	3.55E-03	2.32	10.551	7.568	8.128	6.365
15	85	4.21E-03	2.74	10.618	7.586	8.138	6.375
20	105	5.02E-03	3.28	10.691	7.607	8.149	6.387
20	125	5.79E-03	3.77	10.753	7.623	8.159	6.397
25	150	6.68E-03	4.36	10.812	7.641	8.169	6.410
30	180	7.66E-03	5.00	10.866	7.660	8.180	6.425
40	220	8.84E-03	5.77	10.921	7.678	8.191	6.431
50	270	1.01E-02	6.62	10.976	7.697	8.202	6.436

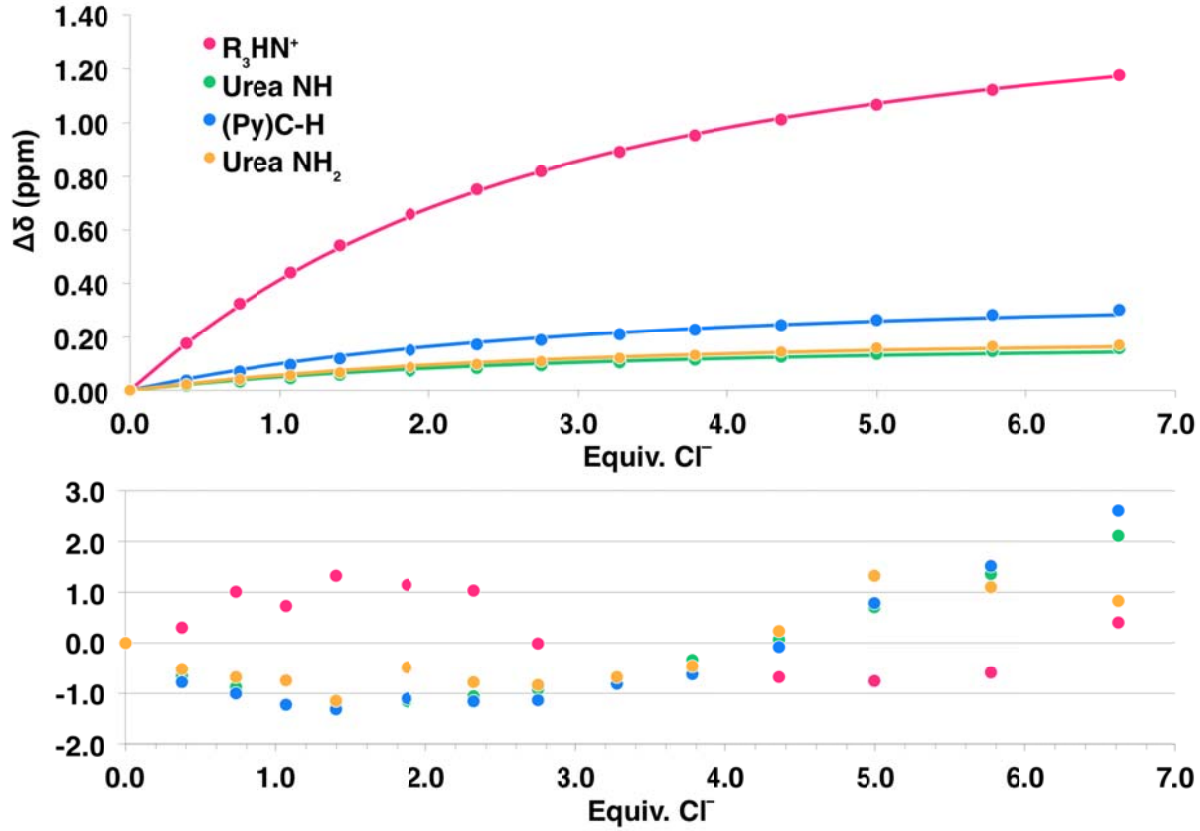

Figure S8. Example 1:1 fitting using non-linear regression from the data obtained for the titration of tetrabutylammonium chloride into $\mathbf{1}^+$ in 50 % d_6-DMSO/50 % d_3-MeCN.

Table 4. Example titration for the addition of tetrabutylammonium bromide to $\mathbf{1}^+$ in 50% d_6-DMSO/50% d_3-MeCN.

Addition (µL)	Total Volume Anion (µL)	[TBABr] (M)	Equiv. Br^-	$\delta(R_3HN^+)$ (ppm)	$\delta(C_{Py}H)$ (ppm)	$\delta(HN_{urea})$ (ppm)	$\delta(H_2N_{urea})$ (ppm)
0.0	0.0	0.00E+00	0.00	9.807	7.394	8.040	6.258
5.0	5.0	2.04E-03	1.22	9.845	7.426	8.056	6.274
10.0	15.0	6.01E-03	3.59	9.903	7.468	8.068	6.293
10.0	25.0	9.82E-03	5.87	9.939	7.496	8.075	6.305
10.0	35.0	1.35E-02	8.07	9.968	7.517	8.083	6.318
12.5	47.5	1.79E-02	10.70	10.001	7.538	8.092	6.328
15.0	62.5	2.29E-02	13.71	10.026	7.557	8.099	6.339
17.5	80.0	2.84E-02	17.01	10.050	7.574	8.106	6.347
20.0	100.0	3.44E-02	20.56	10.079	7.591	8.113	6.356
22.5	122.5	4.06E-02	24.27	10.100	7.605	8.120	6.367
25.0	147.5	4.70E-02	28.10	10.118	7.619	8.125	6.372
30.0	177.5	5.40E-02	32.32	10.133	7.633	8.130	6.376

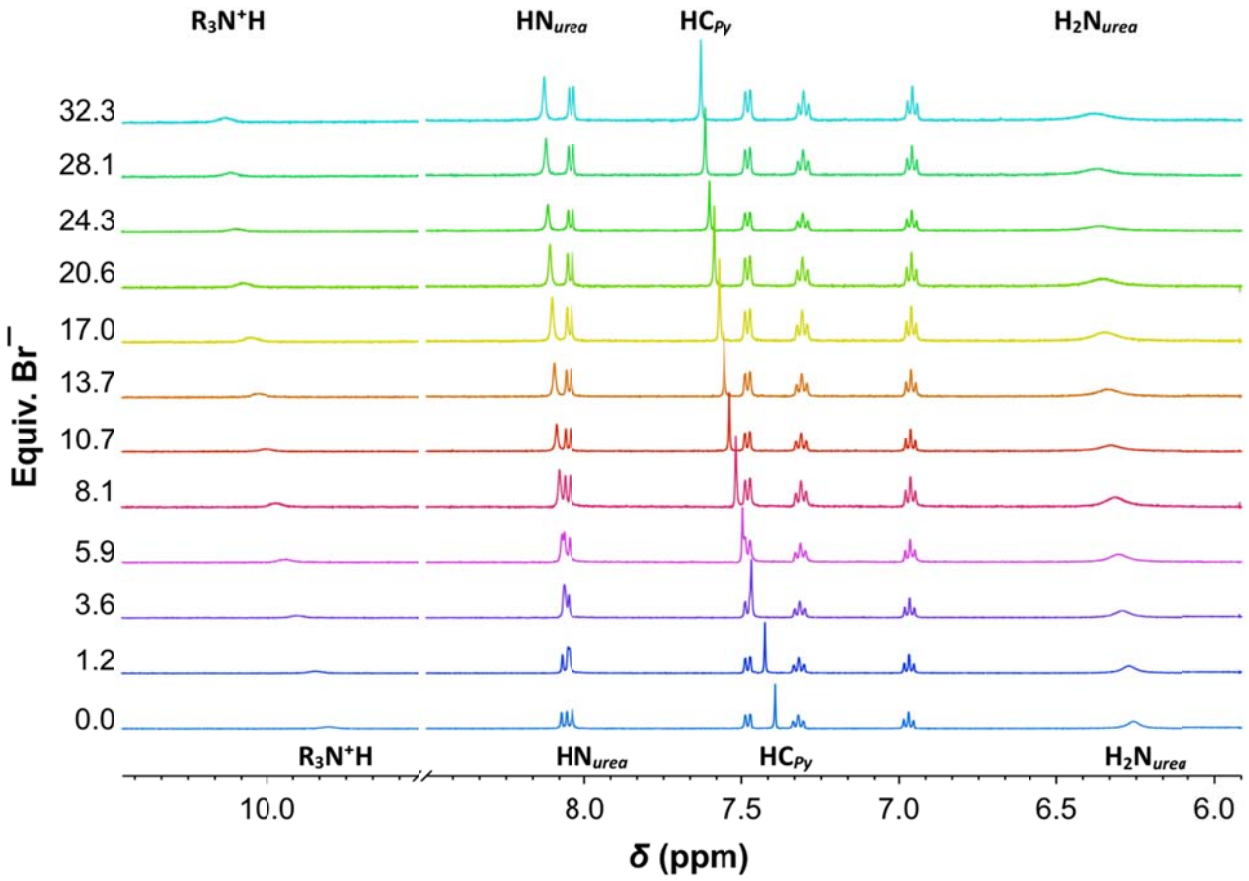

Figure S15. Example stacked spectra from the titration for the addition of tetrabutylammonium bromide to into $\mathbf{1}^+$ in 50% d_6-DMSO/50% d_3-MeCN.

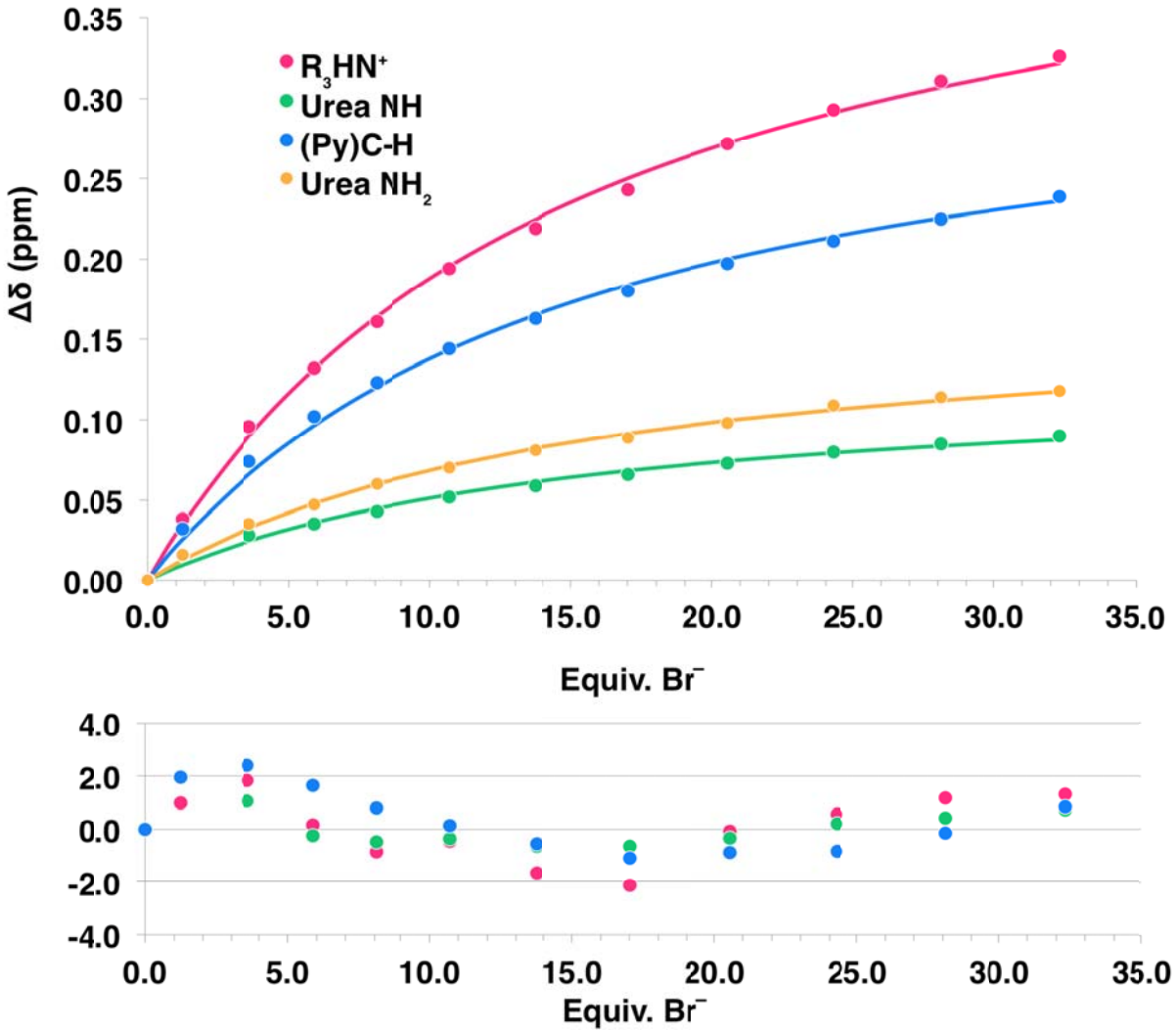

Figure S16. Example 1:1 fitting using non-linear regression from the data obtained for the titration of tetrabutylammonium bromide into $\mathbf{1}^+$ in 50 % d_6-DMSO/50 % d_3-MeCN.

Determining the Fluorescent Character of $1^+ \bullet Cl^-$ Aggregates

Lithium chloride solutions were prepared by dissolving 1.74 g and 1.78 g in 1% TFA/DMSO (spectroscopic grade) and 1% TFA/H_2O (nanopure, 18 Ω) respectively (1.64 mM and 1.68 mM LiCl). Receptor **1** (11.9 mg) was protonated as previously noted and dissolved in 1% TFA/DMSO. In each of ten vials, 0.3 mL of the $\mathbf{1}^+$ solution was pipetted and diluted with volumes of LiCl solutions corresponding to 0, 10, 20, 30, 40, 50, 60, 70, 80, and 90% water with 0.49 mM $\mathbf{1}^+$ and 3000 equivalents of chloride. Fluorescence spectra were obtained from 440-835 nm by exciting at 425 nm with slit widths of 2 nm and 2 nm. Intensity values represent the sum of intensity from 440-740 nm. Beyond 740 nm the fluorescence of each sample is in the baseline.

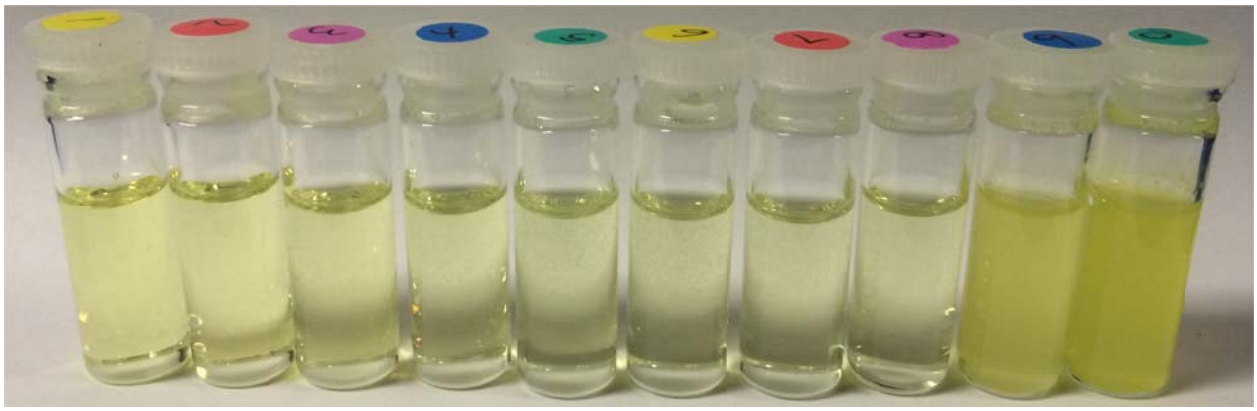

Figure S9. Observable color change based on the addition of chloride and variation in water percentage (0–90% from left to right). Aggregates are clearly visible in the 80 and 90% water/DMSO solutions.

SEM of $1^+ \bullet Cl^-$ Aggregates

Samples of $\mathbf{1}^+ \bullet \mathbf{Cl}^-$ were visualized under a Scanning Electron Microscope (SEM) to observe the aggregate structure (Figure S16). A $\mathbf{1}^+$ 0.29 mM solution was prepared by pre-protonating 0.69 mg of **1** and, after application of high vacuum, was dissolved in 1% TFA/H_2O (nanopure, 18 Ω) respectively. This solution was mixed NaCl salt corresponding ~1200 and 2000 equivalents of chloride. A 10 µl drop of each $\mathbf{1}^+ \bullet \mathbf{Cl}^-$ solution was pipette onto a 10 mm x 10 mm arsenic-doped silicon wafer and left to dry for 30 minutes. SEM images were taken using a Zeiss Ultra-55 SEM operating at 5 kV with a 20 µm aperture and an in-lens detector. In both samples, the excess NaCl produced crystalline structures on the surface of the dried $\mathbf{1}^+ \bullet \mathbf{Cl}^-$. The latter sample, with a larger concentration of chloride, only produced flat translucent $\mathbf{1}^+ \bullet \mathbf{Cl}^-$ sheets amidst the NaCl crystals. These sheets were previously observed during SEM on a treated silica

wafer on a sample prepared from a suspension of **1** in water diffused with HCl gas (no TFA present, Figure S18a). When the sample with 10^3 equivalents of chloride was visualized, a river delta-like structure or dendritic formation was observed (Figure S18b). Closer inspection of this pattern (Figure S18c) revealed non-crystalline nano-sized particles. This formation also terminated into the translucent sheets previously observed, which assembled into larger featureless clusters (Figure S18d).

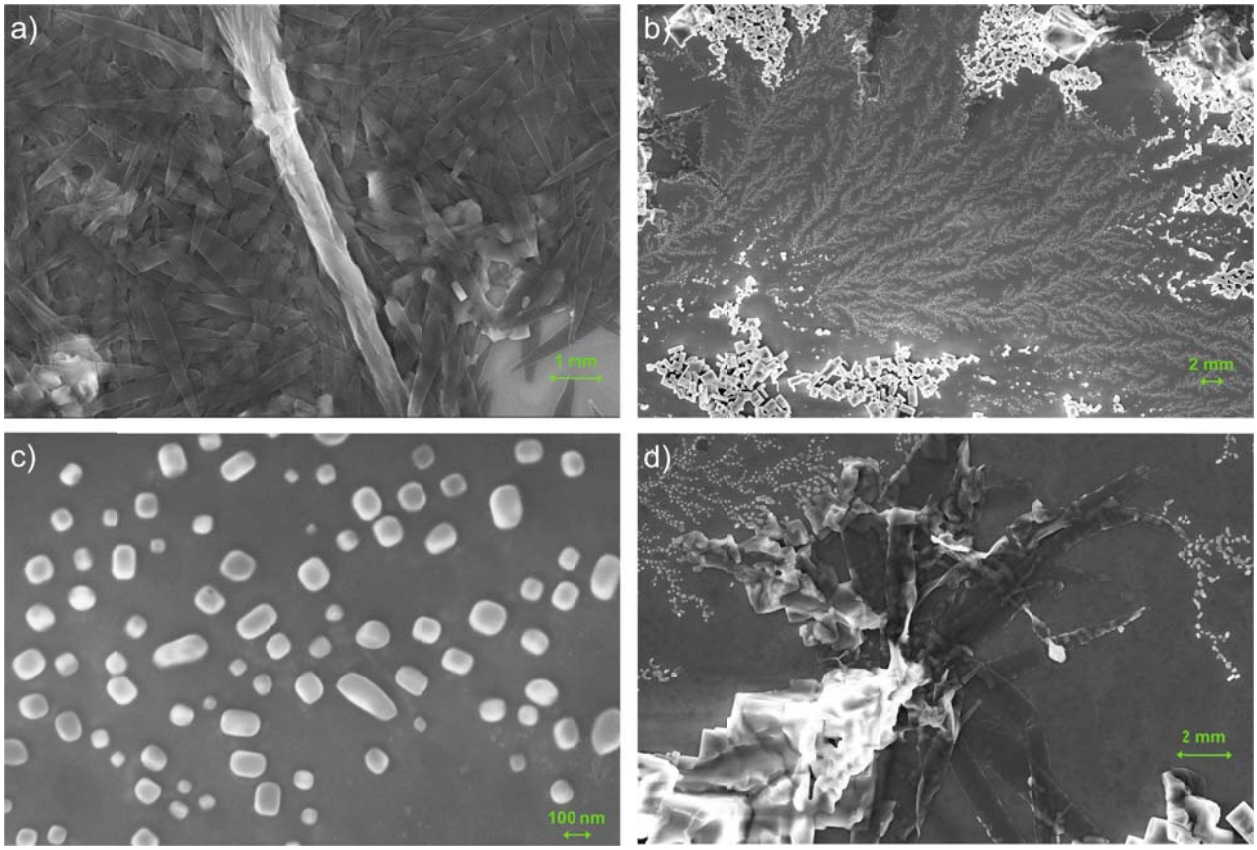

Figure S18. SEM images of aggregated samples evaporated onto silica wafers: (a) HCl gas bubbled through a suspension of $\mathbf{1}^+$ in water. (b-d) Addition of ~10^3 equiv. of NaCl to $\mathbf{1}^+$ in 1% TFA/water.

Dependence of $\mathbf{1}^+\bullet\mathbf{Cl}^-$ Aggregate Fluorescence on Concentration of $\mathbf{1}^+$

A 10 mL 0.36 mM $\mathbf{1}^+$ solution was made in 1% TFA/H_2O (nanopure, 18 Ω) by protonating 1.76 mg of **1**. This solution was used to prepare 0.17 M NaCl host•guest solution which demonstrated turn-on fluorescence with the presence of aggregates. Fluorescence spectra were obtained by excitation at 316 and 425 nm. Successive dilutions of the 500 equivalent $\mathbf{1}^+\bullet\mathbf{Cl}^-$ solution provided an assessment of turn-on fluorescence based on varying concentration

in comparison to the simultaneous dilutions of a 0.36 mM $\mathbf{1}^+$ solution without guest. Intensity ratios comparing $\mathbf{1}^+$ with and without the presence of 500 equivalents of chloride were determined for each set (Figure S19). A sharp transition of turn-on fluorescence is observed between 0.25 and 0.30 mM $\mathbf{1}^+$.

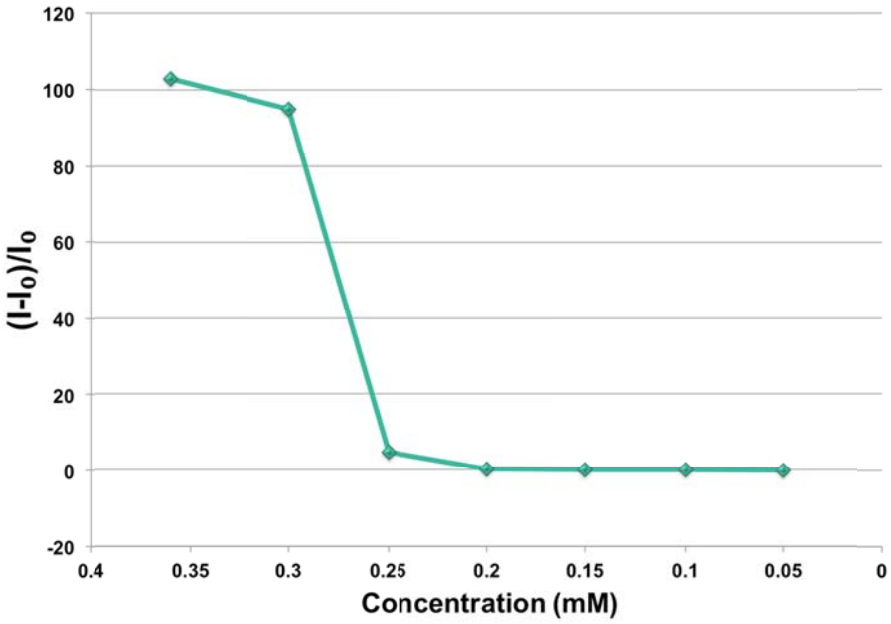

Figure S10. Plot of intensity ratios for fluorescence turn-on of $\mathbf{1}^+$ with 500 equivalents of chloride across a concentration range of 0.36 to 0.05 mM of $\mathbf{1}^+$.