Exploring Urea-Fluoride Interactions in the Vicinity of a Tryptophan Residue

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Details of synthesis, characterization of the ligands **UT1**–**UT3** (Figures S1 to S6, copies of the ^{1}H & ^{13}C NMR spectra), ES-MS (Figure S7-S9), along with the relevant fluorescence spectral data for the various experiments (Figures S10-S11) and ^{1}H NMR titration results (Figures S12-S14).

1. General experimental techniques.

All chemicals were commercially available from Sigma-Aldrich or Spectrochem (India) and used as received. Solvents for spectroscopic experiments were distilled under nitrogen atmosphere before use. All ^{1}H and ^{13}C NMR were measured on a 300 MHz Bruker spectrometer, and reported in δ/ppm. The electronic absorption spectra were recorded on a Shimadzu UV-VIS spectrophotometer.

2. NMR experiments:

All the ^{1}H NMR experiments of **UT1**–**UT3** with acetate anions were performed in CD_3CN, with specific receptor concentration. In each case, the concentrations of TBAF were varied from 0-5.0 equiv.

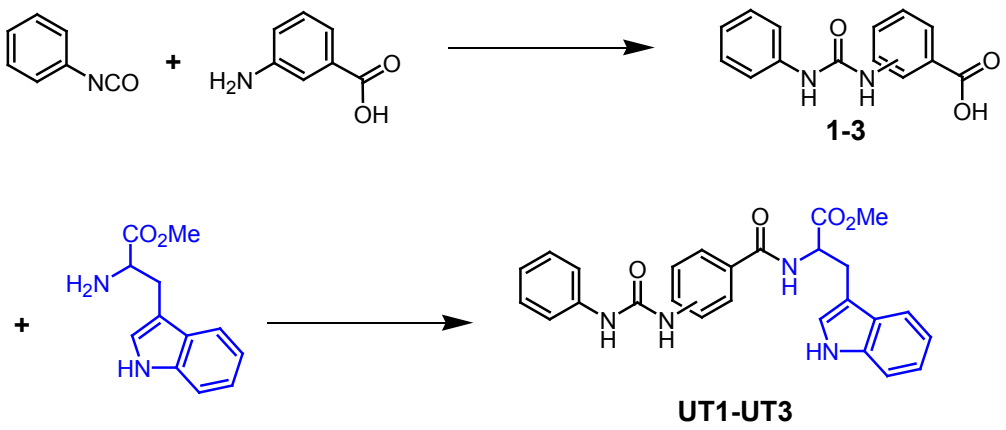
3. Calculation of association constants (*Ka*). Stock solutions of TBAF (50mM) were prepared in CD_3CN. The association constants in each case were obtained from the titration of ligands **UT2** and **UT3** (concentration: 4.0×10^{-5}M) with the salts, following Benesi-Hilderbrand analysis. Changes observed in the chemical shifts $1/(\delta_{max}-\delta_{obs})$ were plotted as function of reciprocal of guest concentration [S], and the values of association constants (*Ka*) were calculated according to Benesi-Hildebrand formulation:

$$1/(\delta_{max} - \delta_{obs}) = 1/(\delta_{max} - \delta_{min}) + [S]/Ka(\delta_{max} - \delta_{min})$$

Here δ_{max} is the fluorescence intensity of free receptor (for **UT2**, **UT3**), δ_{obs} is the observed fluorescence intensity at respective wavelengths in the presence of various guest anions (e.g. acetate at 382nm) and δ_{min} is the fluorescence intensity at saturation. The linear relationship between $1/(\delta_{max} - \delta_{obs})$ and the reciprocal of the guest concentration indicates the formation of a 1:1 complex between guest and the receptor.

4. Synthetic Procedures

Synthesis of ligands **UT1** – **UT3**: The compounds were synthesized according to the following reaction scheme.



Preparation of Urea 1-3:

Urea 1: In a typical experiment, 2-aminobenzoic acid (0.137g, 1 mmol) was dissolved in dry THF (10mL), and triethylamine (0.200g, 2mmol) was added. To this mixture, phenyl isocyanate (0.120g, 1 mmol) was added using a micropipette and the reaction was stirred at 60°C for 24h. Subsequently, the solvents were evaporated on the rotavapor, and residue was diluted with aqueous $NaHCO_3$ solution and filtered. The filtrate was acidified with hydrochloric acid, and was recrystallised from hot acetone which afforded the compound **1** as a white amorphous solid. Yield 55%; ^{1}H NMR (300 MHz, $CDCl_3/CD_3SOCD_3$): δ 10.37 (1H, s, urea NH), 9.51 (1H, s, urea NH), 8.35 (1H, d, aromatic CH, J = 8.4 Hz), 7.89 (1H, d, aromatic CH, J = 8.4 Hz), 7.46 (3H, m, aromatic CH), 7.17 (2H, t, aromatic CH, J = 4.8 Hz), 6.90 (2H, m, aromatic CH).

Urea 2: Same procedure as above provided **Urea 2** as a white amorphous solid. Yield 68%; ^{1}H NMR (300 MHz, CD_3SOCD_3): δ 7.88 (1H, s, urea NH), 7.68 (1H, d, aromatic CH, J = 4.8 Hz), 7.31 (1H, s, urea NH), 7.15 (1H, s, aromatic CH), 6.98 (1H, d, aromatic CH, J = 7.2 Hz), 6.80 (2H, d, aromatic CH, J = 7.8 Hz), 6.68-6.60 (3H, m, aromatic CH), 6.34 (1H, t, aromatic CH, J = 4.2 Hz), 2.89 (bs, -CO_2H merged with residual H_2O signal).

Urea 3: Same procedure as above provided **Urea 3** as a white crystalline solid. Yield 80%; ^{1}H NMR (300 MHz, $CDCl_3/CD_3SOCD_3$): δ 8.35(1H, s, urea NH), 8.06 (1H, s, urea NH), 7.51 (2H, d, aromatic CH, J = 8.4 Hz), 7.14 (2H, d, aromatic CH, J = 8.4 Hz), 7.06 (2H, d, aromatic CH, J = 7.5 Hz), 6.89 (2H, m, aromatic CH), 6.58 (1H, m, aromatic CH), 2.88 (bs, -CO_2H merged with residual H_2O signal).

Preparation of Ligands UT1-UT3:

In a typical experiment, urea **1** (0.255g, 1 mmol) was suspended in dry dichloromethane (2mL) and BOP (0.450g, 1 mmol) added under nitrogen atmosphere. The reaction mixture was stirred at 0°C for 2h which produced a pale yellow solution. To this solution, tryptophan methyl ester hydrochloride (0.254g, 1 mmol) was added in portions, followed by triethylamine (0.2g, 2mmol). The reaction was allowed to continue overnight, after which the solvents were removed under vacuum. The residue was rinsed with water, dilute $NaHCO_3$ (5% in water) solutions and finally with warm water. The product **UT1** was obtained as white solid, which was recrystallised from acetone.

UT1: White solid. Yield 55%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.18 (1H, s, indole NH), 8.41 (1H, d, urea NH), 8.16 (1H, d, urea NH), 7.56-6.98 (14H, m, aromatic & indole CH merged with amide NH), 6.76 (1H, d, indole CH, J = 4.8 Hz), 5.05 (1H, m, CH), 3.77 (3H, s, ester-OCH_3), 3.42 (2H, m, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) : δ 172.2, 168.8, 152.6, 140.1, 138.4, 136.0, 132.6, 128.9, 126.8, 123.3, 122.8, 122.3, 121.6, 121.1, 119.8, 119.7, 118.3, 111.4, 109.5, 53.3, 52.7, 27.6.

UT2: White solid. Yield 70%; ^{1}H NMR (300 MHz, $CDCl_3$/ CD_3SOCD_3): δ 9.90 (1H, s, indole NH), 8.44 (1H, s, urea NH), 8.21 (1H, s, urea NH), 7.78 (1H, d, aromatic CH, J = 6.9 Hz), 7.56 (1H, s, indole CH), 7.45-7.34 (3H, m, aromatic CH), 7.25-7.15 (6H, m, indole & aromatic CH), 7.02-6.89 (4H, m, indole CH), 4.92 (1H, m, CH), 3.61 (3H, s, ester-OCH_3), 3.31 (2H, m, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$/CD_3SOCD_3) : δ 171.5, 165.9, 152.0, 138.9, 138.3, 135.3, 133.5, 128.0, 127.8, 126.4, 122.4, 121.3, 120.6, 119.6, 118.0, 117.7, 117.3, 116.2, 110.5, 108.3, 75.9, 52.2, 51.3, 26.5.

UT3: White solid. Yield 80%; ^{1}H NMR (300 MHz, $CDCl_3$/ CD_3SOCD_3): δ 9.89 (1H, s, indole NH), 8.45 (1H, s, urea NH), 8.22 (1H, s, urea NH), 7.52 (2H, d, aromatic CH, J = 8.4 Hz), 7.46-7.26 (5H, m, aromatic & indole CH), 7.19 (1H, d, indole CH, J = 7.8 Hz), 7.15-6.90 (7H, m, indole & aromatic CH), 4.92 (1H, m, CH), 3.61 (3H, s, ester-OCH_3), 3.31 (2H, m, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$/CD_3SOCD_3) : δ 171.6, 165.5, 151.7, 141.9, 138.1, 135.3, 127.8, 127.3, 126.4, 125.9, 122.3, 121.4, 120.6, 118.0, 117.7, 117.3, 116.4, 110.5, 108.3, 76.0, 52.4, 51.2, 26.4.

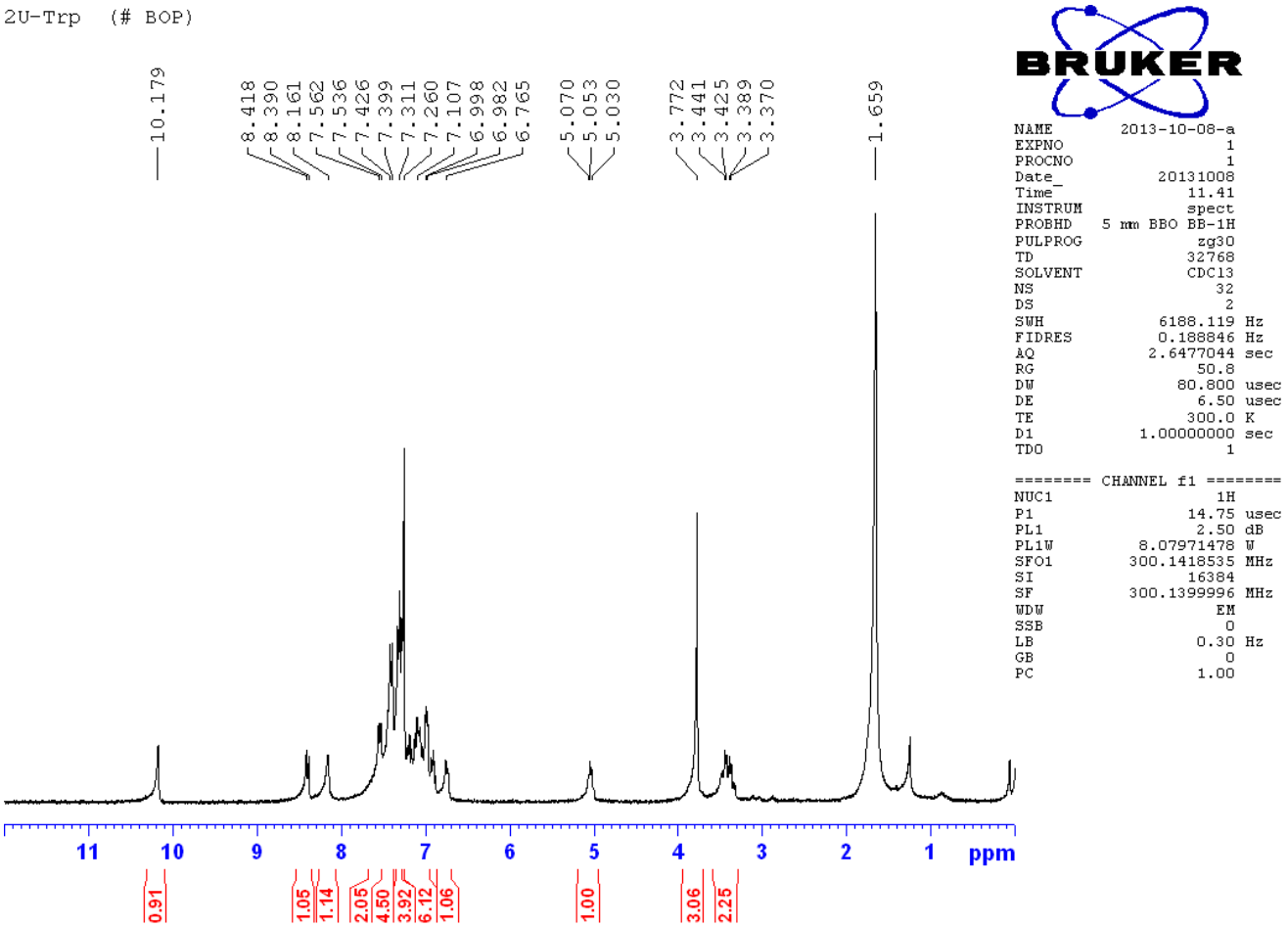


Fig. S1. ^{1}H NMR of **UT1** in $CDCl_3$

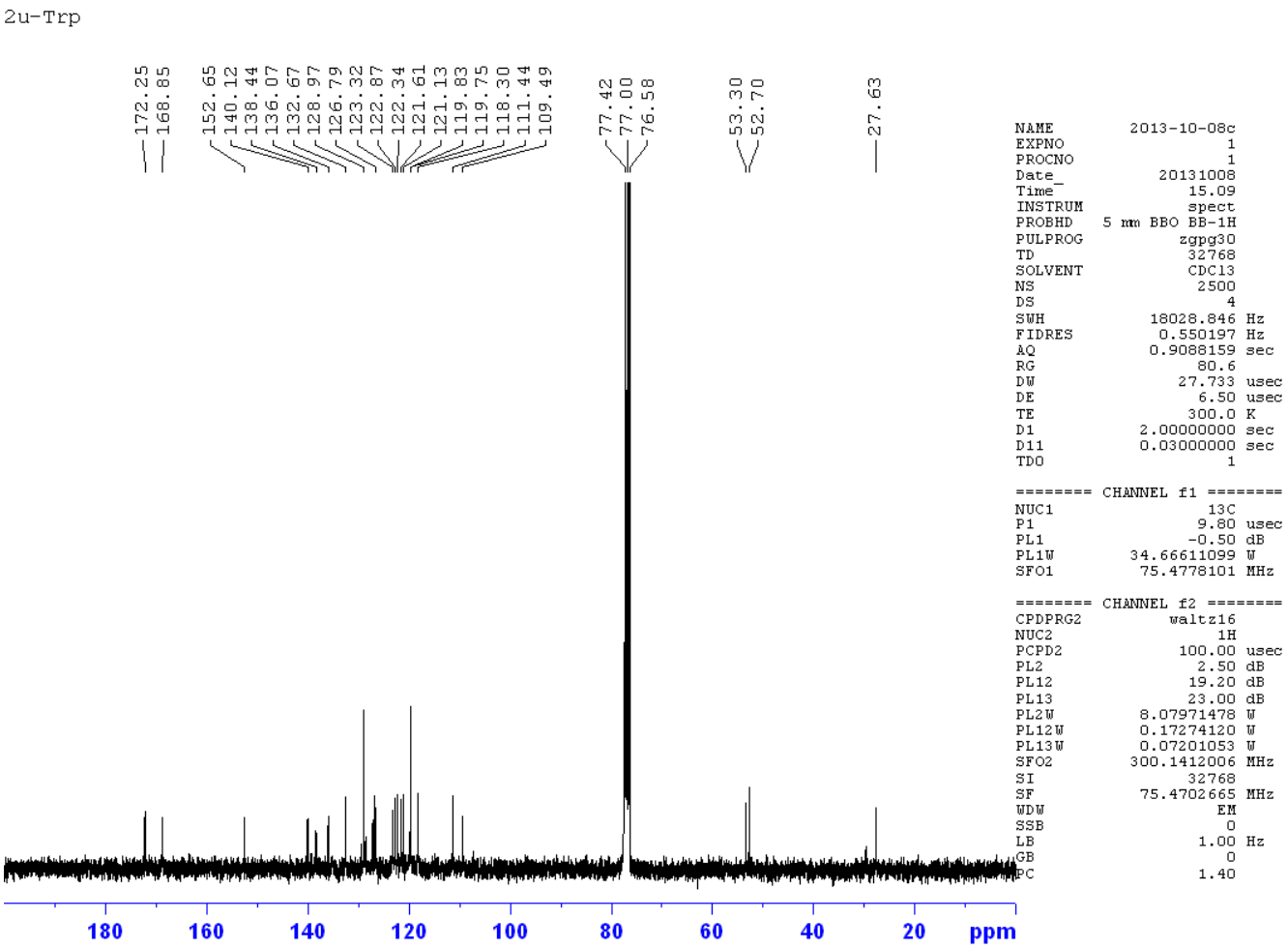


Fig. S2. ^{13}C NMR of **UT1** in $CDCl_3$.

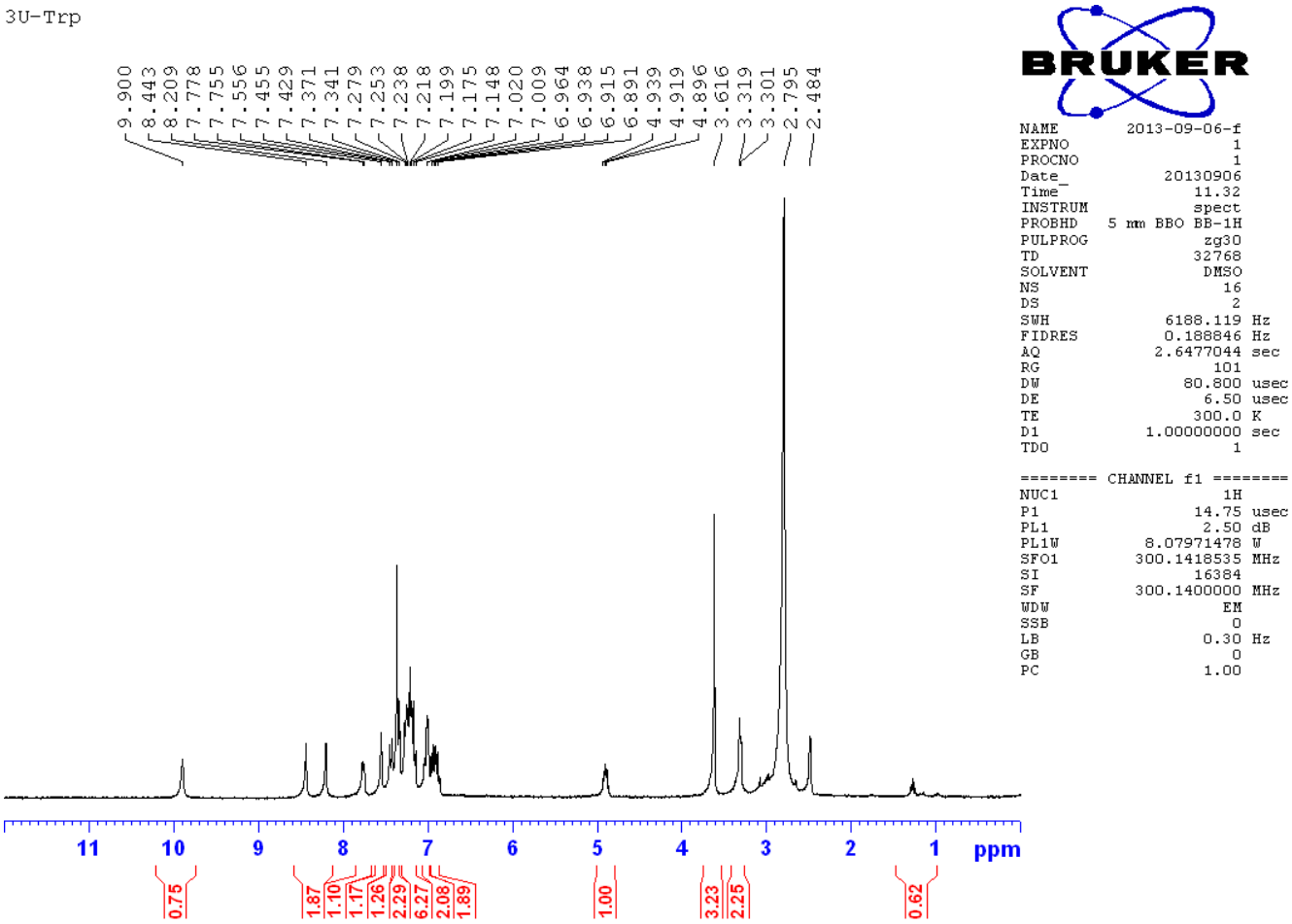


Fig. S3. ^{1}H NMR of **UT2** in $CD_3SOCD_3/CDCl_3$

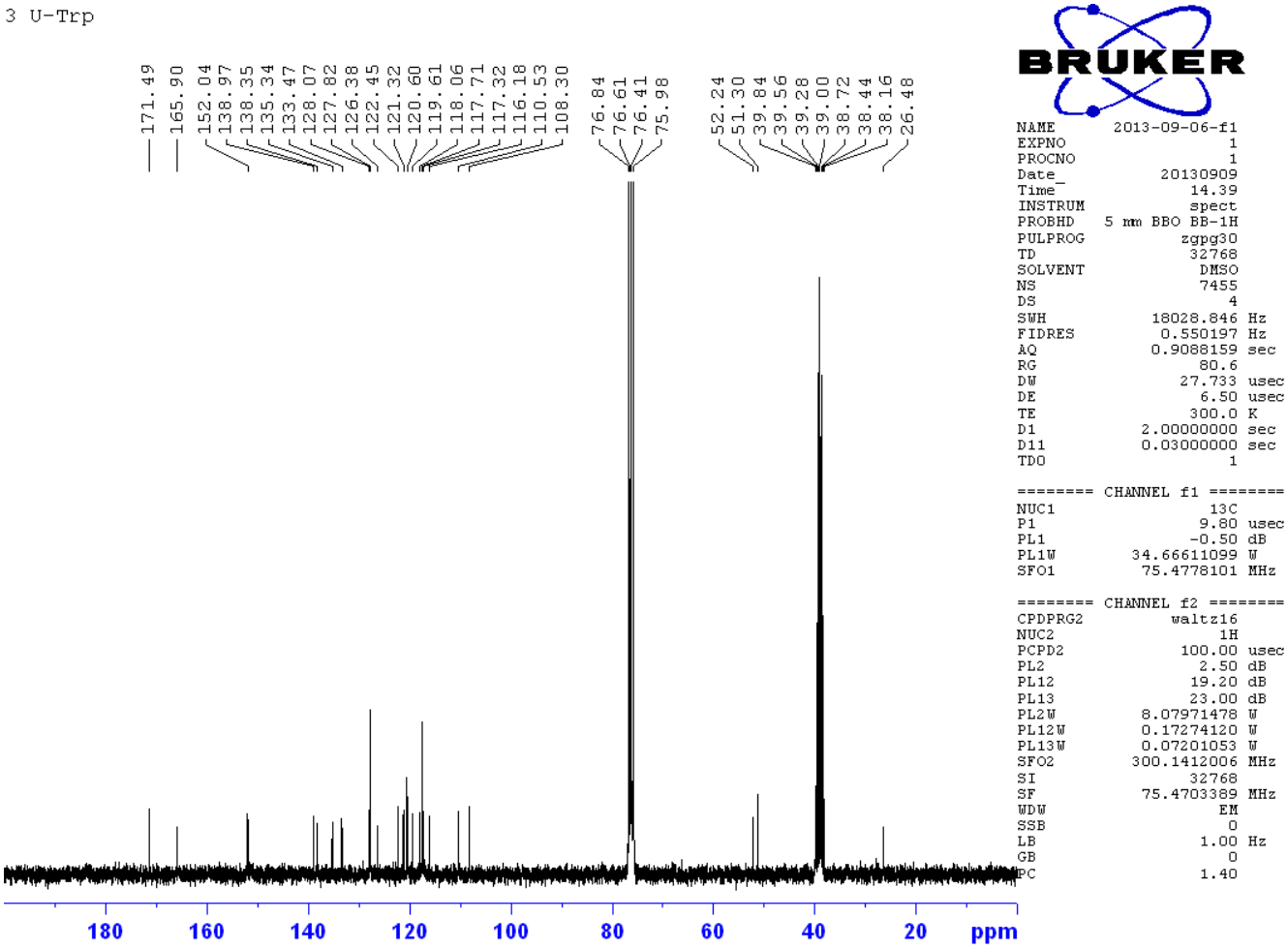


Fig. S4. ^{13}C NMR of **UT2** in CD_3SOCD_3-$CDCl_3$

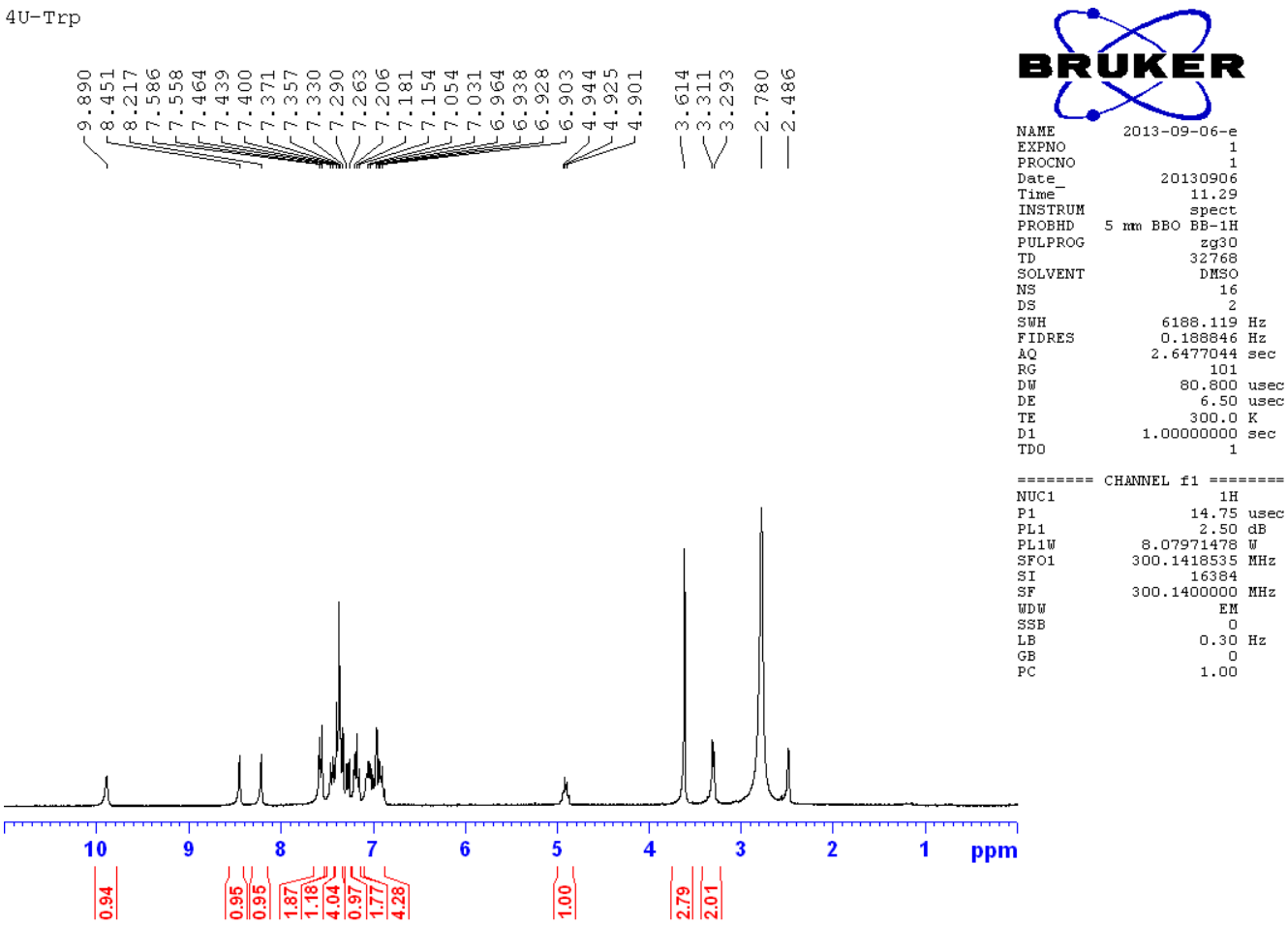


Fig. S5. ^{1}H NMR of **UT3** in CD_3SOCD_3-$CDCl_3$

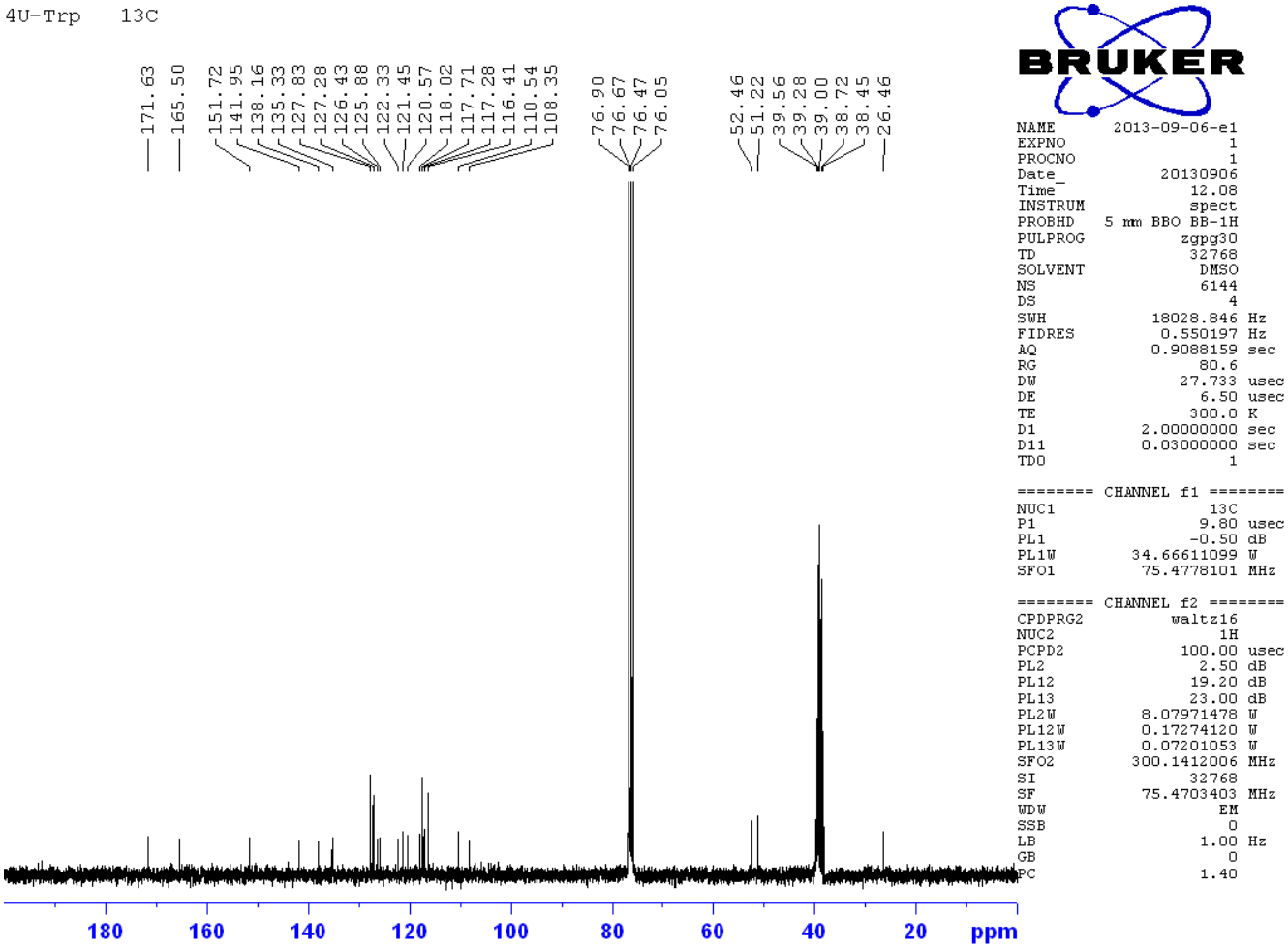


Fig. S6. ^{13}C NMR of **UT3** in CD_3SOCD_3-$CDCl_3$

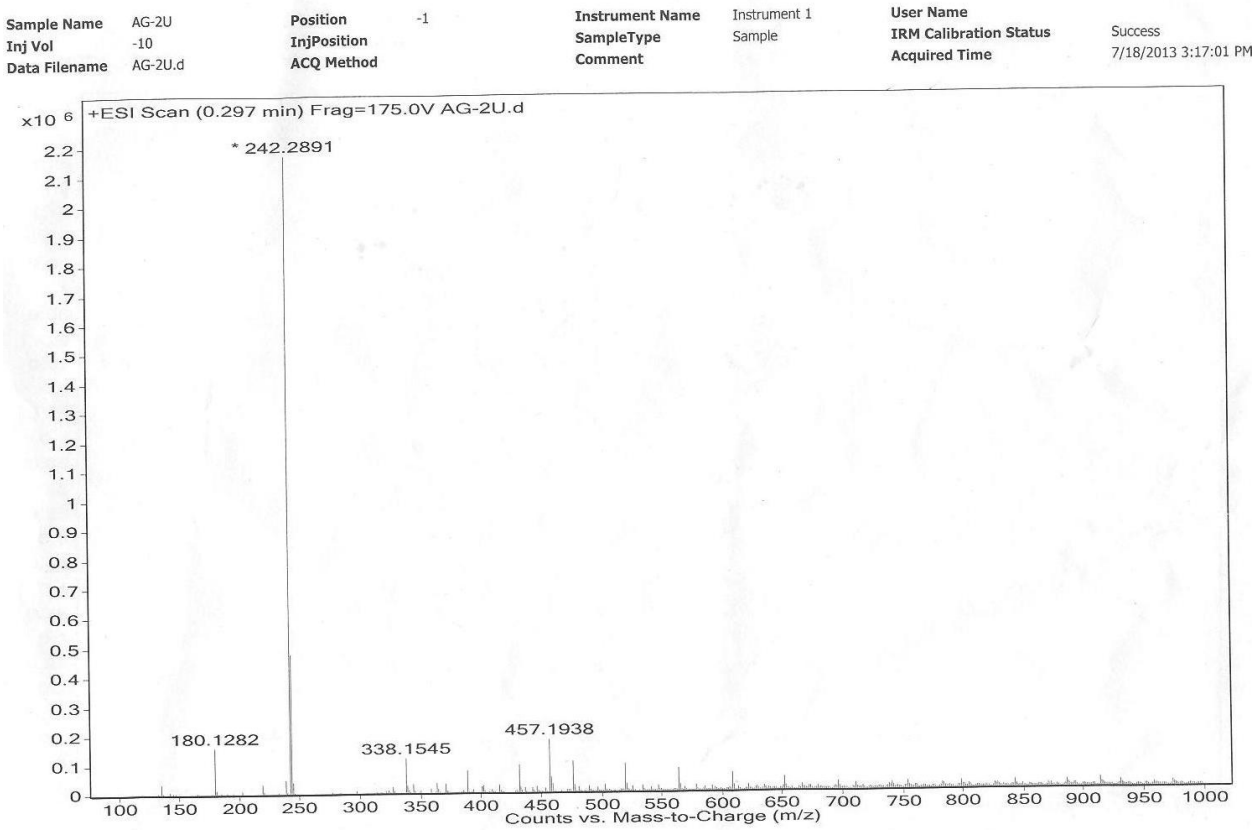


Fig. S7. ESI-.MS of **UT1** (m/z = 457.1938, M+1).

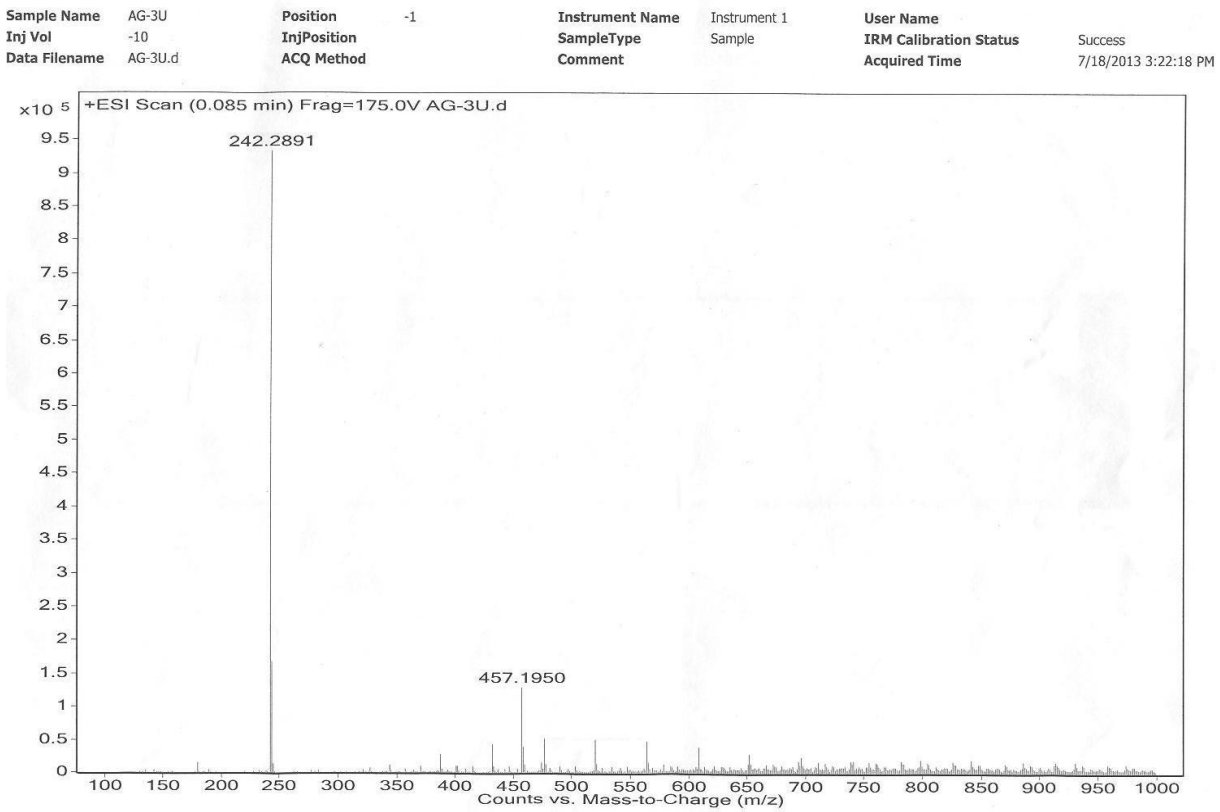


Fig. S8. ESI-MS of **UT2** (m/z = 457.1950, M+1).

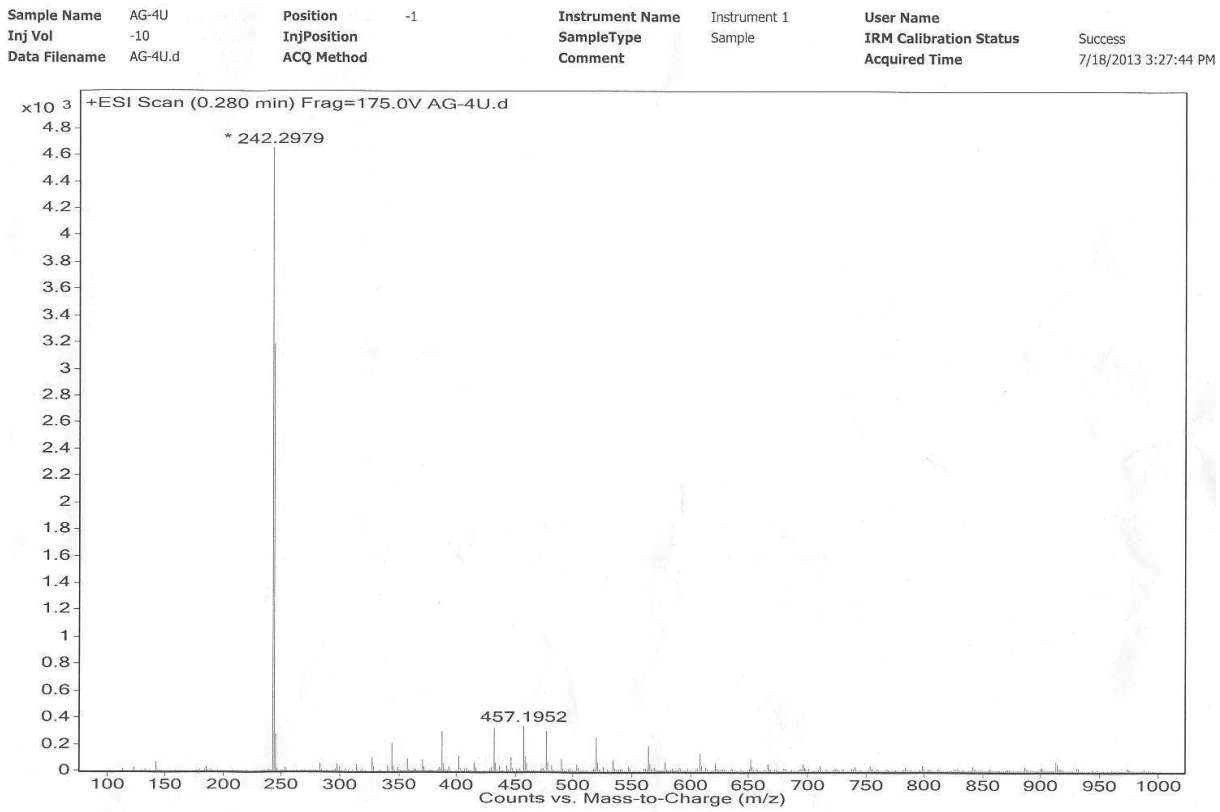


Fig. S9. ESI-MS of **UT3** (m/z = 457.1952, M+1).

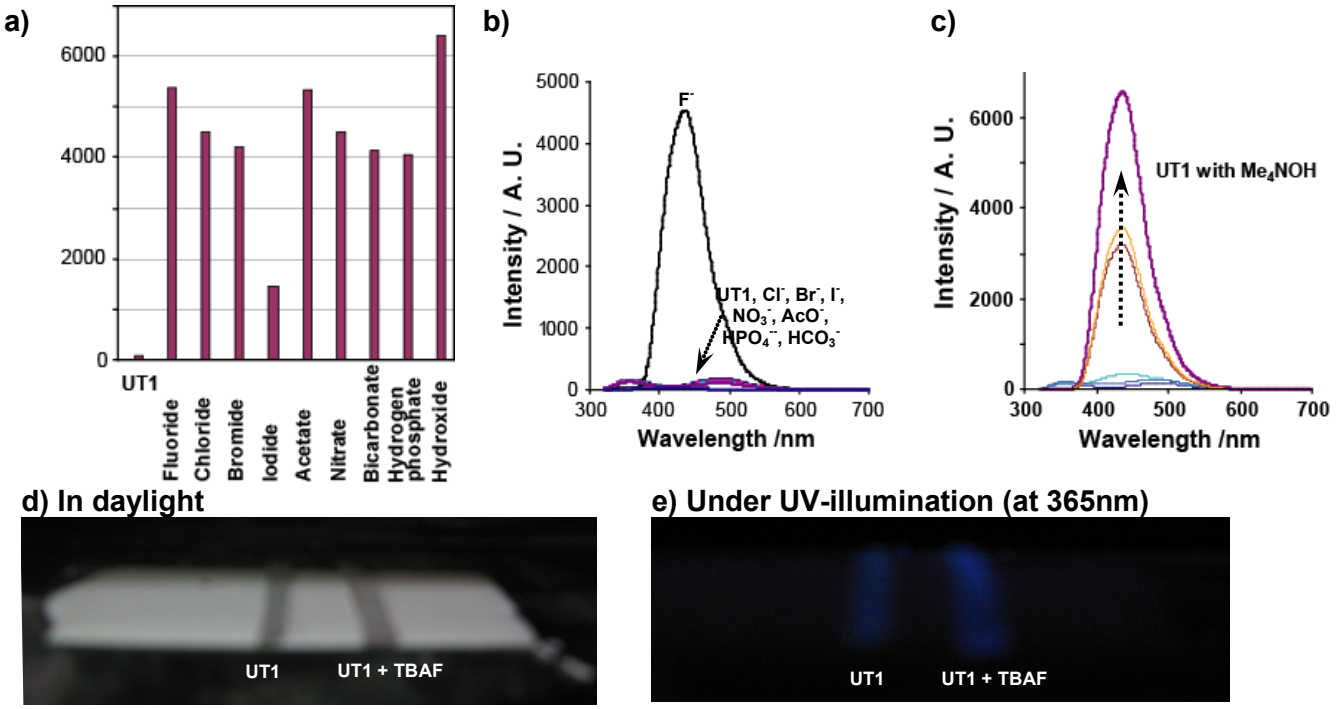


Fig. S10. (a) Bar diagram showing the relative effects of added chloride, bromide, iodide, acetate, nitrate, bicarbonate and hydrogen phosphate anions (upto 4.0 equiv.) on the fluorescence characteristics of the **UT1**-fluoride complex. (conc. $3.0X10^{-6}$M, λ_{ex} 295nm, in CH_3CN); (b) Fluorescence spectra of UT1 following the addition of fluoride, chloride, bromide, iodide, acetate, nitrate, bicarbonate and hydrogen phosphate anions (upto 4.0 equiv.); (c) Fluorescence titration of **UT1** with hydroxide anions (upto 4.0 equiv. in CH_3CN); the changes with fluoride were comparable with that for hydroxide anions which produced similar fluorescence enhancement at 438nm; (d), (e) Sensor-papers prepared with **UT1** when exposed to TBAF (~1.0 mM), in daylight and under UV-illumination, which illustrates the fluoride sensitivity.

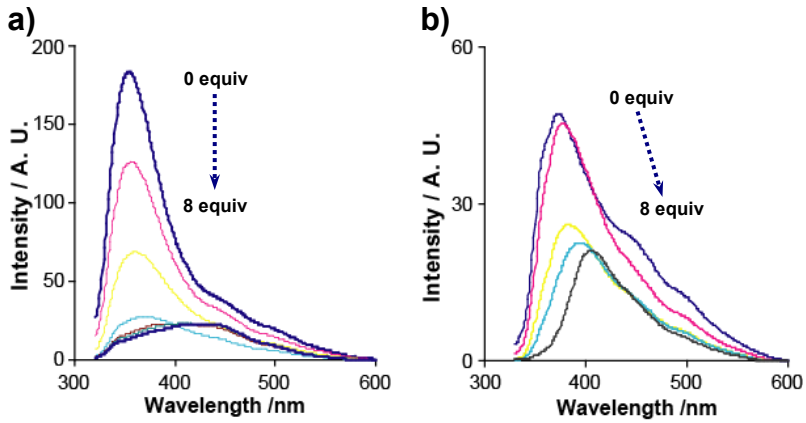


Fig. S11. Addition of TBAF to **UT2** and **UT3** leads to quenching of fluorescence (conc. $3.0X10^{-6}$M, i.e. 0.003mM; λ_{ex} 295nm, in CH_3CN).

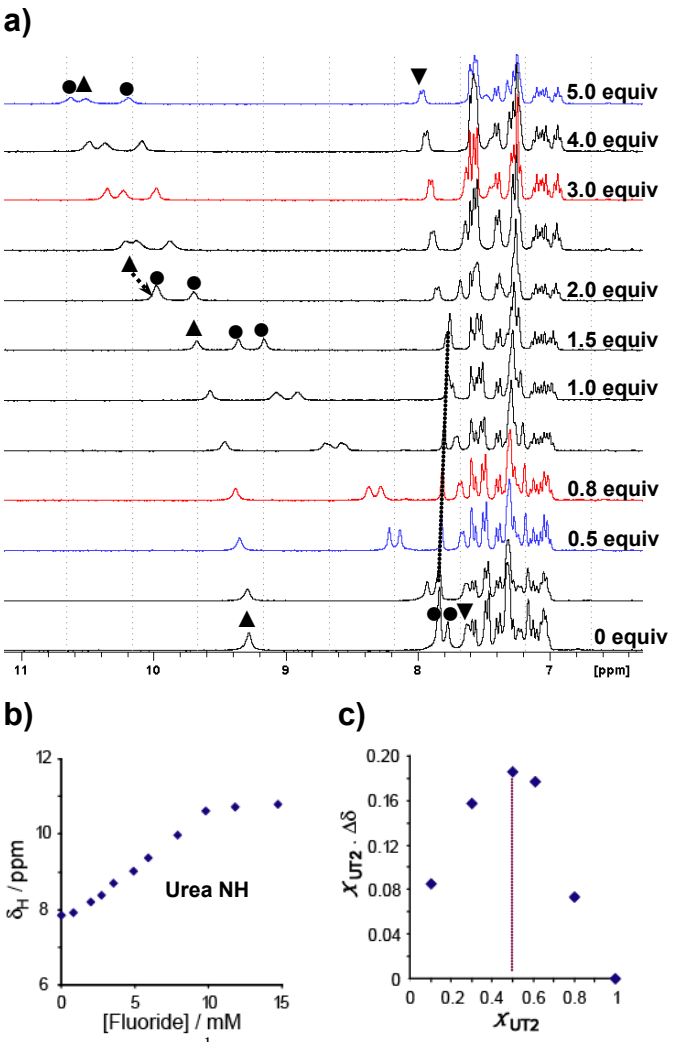


Fig. S12. Partial ^{1}H NMR of **UT2** (4.3mM) following the addition of TBAF: note the changes in the urea NH resonances (●), indole NH (▲), amide NH (▼) and the aromatic CH (■) resonances; Jobs Plot for **UT2**-Fluoride system indicates 1:1 complexation.

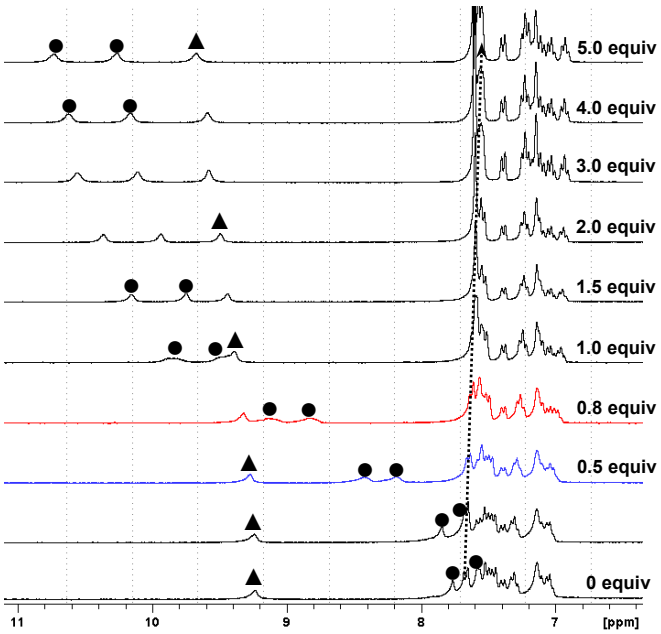


Fig. S13. Partial ^{1}H NMR of **UT3** (4.3mM) upon addition of TBAF in CD3CN as observed at 25°C.

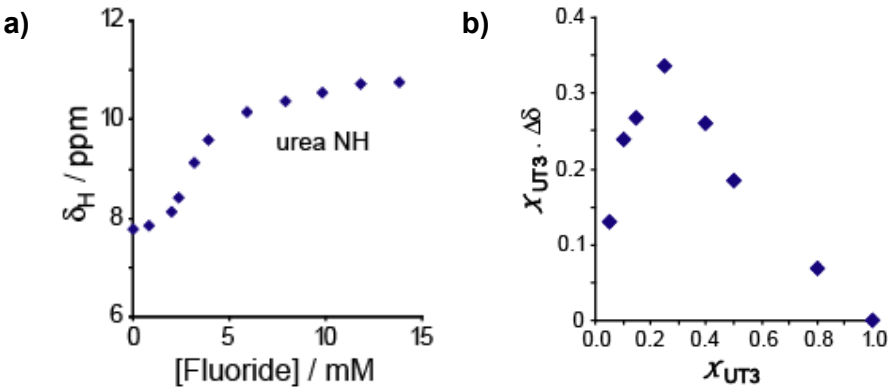


Fig. S14. (a) Complexation-induced chemical shifts for the urea NH resonances following the addition of various amounts of TBAF; (b) Jobs plot which indicates 1:2 stoichiometry for **UT3**-F^{-} complex.

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