CHAPTER 2

Design, Synthesis and Evaluation of Tryptophan-based Urea Derivatives as Receptors for Fluoride Anion

ABSTRACT

Three new tryptophan-derived isomeric urea ligands (UT1–UT3) are synthesized in moderate yields. UV-visible and fluorescence studies of UT1–UT3 with halide anions reveal that UT1 display excellent selectivity and sensitivity towards fluoride anion, compared to UT2 and UT3. Addition of TBAF triggered dramatic enhancement of fluorescence for UT1, which emanated from intramolecular charge transfer between urea and the indole group following fluoride-induced deprotonation of the latter. In contrast, the interaction of fluoride anion with UT2 and UT3 were minor, as evident from the UV–visible and fluorescence spectra. The variations in the coordination of fluoride to the urea-motif were elucidated using detailed $^1$H NMR titration analyses, which indicated distinct hydrogen bonding interactions between the anion and UT1, followed by deprotonation.
2.1 Introduction

Based on the X-ray crystallographic studies of several biological enzymes and their complexes with anionic species, it has been proposed that hydrogen-bonding interactions play critical role in the anion binding, recognition and transport processes [1,10,25,102,103]. For instance, intra-cellular transport of sulphate anions involves enzymes featuring Tryptophan residues at the active site. X-ray crystal structure of sulphate-binding protein (SBP) found in Salmonella typhimurium show that the sulphate anion is bonded to neutral amino acid residues including tryptophan, through seven hydrogen bonds [102].

Discovery of bacterial enzyme haloalkane dehalogenase, was quite significant because it demonstrated how hydrolytic cleavage of carbon-halogen bonds of short chain haloalkanes could occur under relatively mild conditions [103].

![Figure 2.1 Enzymatic active site of bacterial enzyme haloalkane dehalogenase](image)

This enzyme prominently featured two tryptophan residues in the active site that coordinated to halide, mainly chloride, through the indole NH protons [103]. This example demonstrated the hitherto unknown chemistry of this aromatic amino acid residue in the binding and recognition of halide anions.
The discovery of tryptophan in the active part of *haloalkane dehalogenase* generated renewed interest in the design of biomimetic anion receptors based on pyrrole, indole and carbazole moieties [104,105]. Since then, several amide-based ligands containing the pyrrole, indole and pyridine motifs have been examined as receptors for halide anions [106,107]. In an earliest instance, amide derivatives of 3,4-disubstituted pyrrole-2,5-dicarboxylic acid (68, Scheme 2.1) were evaluated as acyclic halide receptors, wherein the pyrrole NH and amide NH groups adopted *syn-syn* conformation to facilitate anion complexation. [22]

Scheme 2.1 Conformational changes observed for 68 upon anion binding

In another report, a bis-pyrrole system based on a 2,6-dicarboxamidopyridyl framework, 69, was shown capable of binding fluoride with multiple hydrogen bonding interactions. Moreover, the anion binding affinity of receptor 69 was relatively higher compared 70 due to pre-organisation emanating from the presence of pyridine ring. [23]
In several instances, the indole motif formed an integral part of the design of anion binding receptors, as a surrogate for tryptophan [45,103-106]. The indole motif could be further derivatised with amide or urea groups to provide additional hydrogen bond donor sites. The cleft-like anion receptor, 71, was constructed using 7-aminooindole with amide and indole NH groups as hydrogen bond donors. Crystallographic studies show receptor 71 to prefer twisted conformation while binding to fluoride, such that the small anion becomes encapsulated within the cleft due to the formation of four hydrogen bonds. On the other hand, the chloride anion, due to its relatively larger size, perches over only one face of the receptor 71 [104]. Subsequent studies revealed that bis-indole containing urea and amide derivatives, 72 and 73, exhibited higher selectivity to oxoanions [105].

The conformational flexibility of pyrrole and indole-derived receptors upon anion binding could be avoided in analogous carbazole derivatives because of their structural rigidity [45,105,110,111]. Introducing an electron-withdrawing substituent at the 3- and 6-positions of a carbazole motif (74) further increases the acidity of the NH group, making it a suitable receptor for chloride anion [110]. Again, carbazole based bis-urea derivative 75 was found to afford complexes with acetate anions, stabilised through four urea NH···O interactions and one bifurcated hydrogen bond from the carbazole. [111a] In a related example, it was shown that a bis-urea receptor containing
two dichlorocarbazole motifs could bind to oxalate, malonate and succinate anions through multiple hydrogen bonding interactions [111b].

The design and development of such synthetic receptors was an important step towards appreciating aspects related to selective binding and recognition of anions. In particular, the various aspects related to design of supramolecular receptors for coordination, binding and the recognition of fluoride anion have attracted a great deal of attention [106]. Recent studies on fluoride recognition have brought to light various types of interactions between indole derivatives and the fluoride, including anion induced deprotonation of NH groups [19,45,46,112-114]. An important challenge has been to understand the nature of interactions of bioactive molecules such as tryptophan with fluoride anions, due to the unique and complicated biological functions associated with this particular anion [45].

Previous studies have shown that judicious insertion of urea and thiourea motifs into a receptor can facilitate selective binding and recognition of anions [20,99,107,108]. In fact, urea-based receptors have been developed which can preferentially bind to halides, acetates, and sulphate and phosphate anions [46,109]. It has been found that the urea-anion binding interactions could be affected by the presence of substituents.
Moreover, incorporating chromomeric and fluorogenic moieties to the urea motifs can provide access to direct monitoring of urea-anion interactions [46].

We observed that the nature of interactions between the urea motif and fluoride anions in the presence of tryptophan residues remains relatively unexplored. [45,103] In an effort to understand how the presence of a tryptophan residue in close proximity could affect urea–fluoride interactions, we synthesized and characterized isomeric tryptophan derived urea ligands UT1–3 (Scheme 2.2) wherein the fluoride anion could potentially interact at the urea motif, the indole group or both. Earlier, the utility of urea-derived tryptophan ligands has been illustrated using N-substituted urea-derivatives of L-tryptophan which behave as antagonists for Neurokinin 1 (NK1) and Neurokinin 2 (NK2) receptors [115].

2.2 Results and Discussions

Tryptophan derived isomeric urea based ligands UT1, UT2 and UT3 were synthesized in moderate yield by condensation of three isomeric urea carboxylic acids with tryptophan methyl ester hydrochloride using EDC-HOBT coupling strategies (Scheme 2.2). The purity of ligands, UT1-UT3, was fully confirmed by $^1$H, $^{13}$C NMR and E1-MS analyzes.

Each of the receptors UT1, UT2 and UT3 contain a phenyl substituted urea group connected to a tryptophan residue at the 1,2-, 1,3- and 1,4-linkages respectively.
As shown in Scheme 2.4, the possible interactions between the indole moiety of tryptophan and the urea groups for the ligands UT1–UT3 could be regulated by the ureido-benzamide ‘hinge’ group. Given the geometrical restraints of the ‘hinge’ group in UT1 and UT2, the tryptophan residues could be expected to reside close to the urea motif as compared to UT3. Thus, depending on the proximity of the pendant tryptophan residue to the urea motif, we hypothesized that coordination of fluoride to the ligands at the urea NH could also invoke participation of the indole NH groups. Further, we anticipated that the interactions of fluoride with the ligands and the tryptophan residues could trigger changes in the absorption and fluorescence behaviour.
Scheme 2.4 Possible coordination of tryptophan during the interaction of fluoride anion and urea motif of UT1

2.2.1 UV-visible spectroscopic investigations

The UV-visible spectra of UT1-3 are recorded in acetonitrile at conc. 3.0 \times 10^{-5} \text{ M}. As shown in Figure 2.2, UT1 produced distinct absorptions at \( \lambda_{\text{max}} = 219 \) nm, 264 nm and weak band at 320 nm; in comparison, ligands UT2 and UT3 exhibited absorptions in the 270–300 nm range, as expected for tryptophan derivatives. Fluoride receptor ability of the ligands UT1-3 was investigated using UV-visible and fluorescence spectroscopy.

Figure 2.3a, shows the UV-visible titration spectra of UT1 upon addition of fluoride anions (in the form of tetrabutyl ammonium fluoride, TBAF) recorded in acetonitrile. Incremental addition of fluoride (as TBAF) to UT1 led to gradual decrease in the absorption at 264 nm and the appearance of two new absorption bands at 283 and 357 nm (\( \lambda_{\text{max}} \)), with an isobestic point at 272 nm. The appearance of the red shifted absorption at 357 nm was indicative of extended conjugation in the ligand system caused apparently by the coordination of fluoride anion to UT1. Again the occurrence of isobestic point at 272 nm could indicate the presence of two species in equilibrium.
However, addition of other anions like chloride, bromide, iodide and acetate did not produce any red shifted absorption band in the UV-visible spectra of UT1 (Figure 2.3b).

Figure 2.2 UV-visible spectra of UT1–UT3 (conc. = 3.0 x 10^{-5} M in CH₃CN)

Figure 2.3 Changes in the UV-visible spectra of UT1 (conc. = 3.0 x 10^{-5} M ) following the addition of (a) fluoride anions (as TBAF in CH₃CN); (inset) plots of absorbance of UT1 vs. [TBAF] at the wavelength specified; (b) different anions as tetrabutyl ammonium salt in CH₃CN (20 equiv)
Similar red-shifted absorptions were also observed in the UV-visible spectra of UT2 and UT3 upon addition of fluoride. For UT2, the UV-visible spectra in acetonitrile revealed a gradual shift of the absorption at 258nm to 271nm ($\lambda_{\text{max}}$) upon addition of fluoride solution (Figure 2.4a). In this case, the changes in absorbance at 258nm and 271nm corresponding to increasing fluoride anion concentration produced an isobestic point at 262nm. Similarly, the addition of fluoride anions (>10 equiv.) to UT3 also caused shifting of the absorption at 268 nm ($\lambda_{\text{max}}$) to 291 nm, along with a broad absorption at 350 nm (Figure 2.4b).

![Figure 2.4](image)

**Figure 2.4** Changes in the UV-visible spectra of (a) UT2 (b) UT3 following the addition of fluoride anions (as TBAF in CH$_3$CN); (inset) plots of absorbances (UT2–UT3) vs. [TBAF] at the wavelength specified.

### 2.2.2 Fluorescence spectroscopic investigations

The fluorescence emissions of tryptophan have been known to be environment-sensitive [116]. Often this property of tryptophan has been used to study protein structures and
for probing enzyme activity [117]. In this perspective, we undertook fluorimetric measurements of UT1-UT3 in acetonitrile to gain insight into the nature of anion receptor interactions.

As shown in Figure 2.5, the fluorescence emission spectra of the three isomer UT1-UT3 are notably different. Ligand UT1 produced two distinct emissions, one at 357 nm and a broad emission at 490 nm. Ligand UT2 and UT3 produced slightly red shifted emission compared to UT1. For UT2 a distinct emission was observed at 364 nm (Δλmax=10 nm), whereas UT3 produced a weak emission near 418 nm (Δλmax= 50 nm). Red-shifted emissions may be originated from the extended conjugations arising from the enhanced linearity of the molecules from UT1 to UT3.

![Fluorescence spectra of UT1-UT3](image)

Figure 2.5 Fluorescence spectra of UT1-UT3 (conc. = 3.0×10⁻⁵ M in CH₃CN) recorded in CH₃CN; λex =300 nm

Next, we examined the changes in the fluorescence behaviour of the ligands upon titration with fluoride anion in acetonitrile. As shown in figure 2.6a, gradual addition of TBAF to UT1 (3.0×10⁻⁶ M) caused dramatic enhancement of fluorescence at
438nm, while the initial emission at 357 nm almost disappeared. The latter was coincident with the TBAF-induced absorption at 357 nm in the UV-visible spectrum. The variation of fluorescence at 357nm and 438nm as function of fluoride anion concentration has been shown in Figure 2.6b

![Image of Figure 2.6](image)

Figure 2.6 (a) Addition of TBAF to UT1 causes fluorescence enhancement at 438 nm (conc. $3.0 \times 10^{-6}$ M in CH$_3$CN; $\lambda_{ex}$ 295 nm); (inset) pictures of UT1 after addition of TBAF, taken in the dark under 365 nm UV-illumination, which show intense blue emission; (b) Plot of fluorescence intensity Vs. fluoride concentration at 438nm and 357nm.

The new red-shifted emission at 438nm ($\Delta \lambda_{max} = 80$ nm) was attributed to the formation of UT1-fluoride complex with possible charge-transfer emissions from indole to the urea motif. As shown in Figure 2.7, the interaction of fluoride with urea could be favoured by the proximal indole NH, leading to the formation of strong hydrogen
bonds. Because fluoride anion is known to be strong bronsted base, the formation of strong hydrogen bonds can provide the basis for an incipient proton-transfer process, which subsequently results in deprotonation of the acidic NH protons. This was reasonable because in protic solvents, especially MeOH and water, the greater solvation of fluoride anions affected the formation of urea–fluoride anion complex, and hence the fluorescence changes were subdued. Further, it was observed that the addition of protic solvents such as methanol reduced the emission at 438nm, while the addition of water (> 25% v/v) quenched the fluorescence completely.

Figure 2.7 Proposed hydrogen bonding interactions in urea–fluoride complexes for UT1, UT2 and UT3, indicating the role of the indole groups (the cation not shown).

We have also studied the nature of interactions of ligand UT1 with chloride, bromide, iodide, acetate, nitrate, bicarbonate and hydrogen phosphate anions. But none of these anions did cause any significant change to the fluorescence spectra of UT1 (Figure
2.8a). Figure 2.8b shows the fluorescence titration of UT1 with hydroxide anions (upto 4.0 equiv. in CH₃CN); the changes with fluoride were comparable with that for hydroxide anions which produced similar fluorescence enhancement at 438 nm. On this basis, we inferred that fluorescence enhancement of UT1 due to addition of hydroxide could result from the fluoride induced deprotonation of indole-NH group.

![Fluorescence spectra](image)

Figure 2.8 (a) Changes observed in the fluorescence spectra of UT1 following the addition of fluoride, chloride, bromide, iodide, acetate, nitrate, bicarbonate and hydrogen phosphate anions (upto 4.0 equiv.); (b) Fluorescence titration of UT1 with hydroxide anions (upto 4.0 equiv. in CH₃CN).

In a competition experiment, we analysed the fluorescence profiles of the UT1–fluoride complex in the presence of chloride, bromide, iodide, acetate, nitrate, bicarbonate and hydrogen phosphate anions (upto 4.0 equiv., Figure 2.9). We found that the presence of acetate anions did not affect the fluorescence of the UT1-fluoride complex, while the addition of chloride, bromide, and iodide slightly decreased the fluorescence.
Figure 2.9 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 emission of the UT1-fluoride complex (conc. 3.0×10^{-6} M, λ_{ex} 295 nm, in CH_{3}CN).

Figure 2.10 Addition of TBAF to (a) UT2 and (b) UT3 leads to quenching of fluorescence (conc. 3.0×10^{-6} M) λ_{ex} =295 nm, in CH_{3}CN.
Figure 2.10a shows the spectra obtained during the titration of UT2 (3.0×10⁻⁶ M) with TBAF (stock = 15mM) in acetonitrile. In contrast to UT1, the addition of incremental amounts of fluoride to UT2 caused quenching of fluorescence at 356 nm. Similar situation occurred with UT3, wherein the addition of fluoride anions produced reduction in the fluorescence emission of the ligand at 374 nm (Figure 2.10b). In this case, the presence of fluoride also caused a 34 nm (Δλ_max) red-shift in the fluorescence emission to 408 nm. Similar quenching of fluorescence could be observed for UT2 and UT3 in the presence of chloride, bromide, iodide, acetate, nitrate, bicarbonate and hydrogen phosphate anions.

Figure 2.11 Fluorescence changes observed for UT1-UT3 (conc. 3.0×10⁻⁶ M) after addition of TBAF (8 equiv.) under 365 nm UV-illumination.

Figure 2.11 shows the variation in the fluorescence emissions of UT1, UT2 and UT3 upon addition of fluoride in the solution phase under 365 nm UV-illumination. As such, the fluorescence studies clearly demonstrated that the urea and the indole groups of UT1 were significantly affected by the fluoride coordination compared to UT2 and UT3. As depicted in Scheme 2.4, we propose that the ligand geometry adopted by UT1 could be reminiscent of anthranilic foldamers featuring intra-molecular hydrogen bonding interactions. [118,119]
In this situation, the indole motif of tryptophan (of **UT1**) would further assist the formation of urea-fluoride complex. Accordingly, intramolecular charge transfer (ICT) processes become favourable for **UT1**, which account for the changes observed in the absorbance and fluorescence spectra. In contrast, the orientations of the indole motifs in **UT2** and **UT3** would have limited influence on the complexation of fluoride at the urea-motif; such variations in the nature of urea-fluoride complexation, with possible anion coordination at indole site could account for PET-induced quenching of ligand fluorescence in **UT2** and **UT3**. [120]

Further, we evaluated the practical application for **UT1** by preparing sensor-paper for the detection of fluoride. As shown in Figure 2.12, the addition of TBAF enhanced the emission of the strip of paper coated with **UT1**, which could be observed under 365nm UV-illumination.

![Figure 2.12 Sensor-papers prepared with UT1 when exposed to TBAF (~1.0 mM), in daylight and under UV-illumination, which illustrates the fluoride sensitivity.](image)

**2.2.3 NMR Investigation**

In order to substantiate the arguments made in Scheme 2.4, and examine the nature of interactions between **UT1-UT3** and fluoride in detail, $^1$H NMR investigations were
undertaken. As shown in Figure 2.13, the addition of fluoride (as TBAF) anion to UT1 (4.3mM) in acetonitrile-d₃ caused distinct changes in the proton resonances due to the indole and amide NH groups. Addition of 1.0 equiv. of fluoride ions to UT1 induced deprotonation of the urea NH groups, as indicated by distinct broadening and disappearance of the proton resonances at 10.03 and 9.32 ppm. At this point, we found the indole NH resonance broadened and shifted downfield from 9.23 ppm to 9.36 ppm.

Figure 2.13  Partial ¹H NMR of UT1 (4.3mM) showing the effect of TBAF on the urea NH resonances (●), indole NH (▲), amide NH (▼) and the aromatic CH (■) resonances in acetonitrile-d₃ at 25°C
ppm. Further addition of fluoride (> 2.0 equiv.) to the ligand was marked by the broadening of indole NH resonance at 9.77 ppm, which seemed to indicate rapid exchange of the same. Similar broadening was also observed for the amide NH resonance at 7.91 ppm, which initially shifted downfield with broadening. Apart from these changes, subsequent deprotonation of the urea NH group produced a shielding effect, and consequently the resonances of the aromatic CH groups shifted upfield reflecting increase of electron density in the phenyl rings.

In addition, the interactions of UT2 and UT3 with fluoride anions were investigated using $^1$H NMR spectroscopy to elucidate the nature of urea-fluoride interactions and the role of tryptophan residues. In both UT2 and UT3, distinct complexation-induced shifts could be seen for the urea and indole NH groups, upon incremental addition of TBAF. The results indicated that both UT2 and UT3 could interact with two fluoride anions, invoking the urea and the indole NH groups as potential hydrogen-bond donors.

As shown in Figures 2.14 and 2.15, the family of spectra obtained during the addition of TBAF to UT2 and UT3 presented a different picture of the urea-fluoride interactions. Similar to UT1, in this case also the addition of incremental amounts of fluoride (upto 5.0 equiv) caused the urea NH and indole NH resonances to progressively shift downfield. However unlike UT1, the downfield shifting for urea NH are more prominent in comparison to indole NH at low anion concentration. For instance, the stoichiometry of UT2-fluoride complex was dependent on the anion concentration. As calculated by the Job’s method (based on the urea NH resonances), the formation of 1:1 complex was preferred at low anion concentrations; the apparent association constants ($K_a$) was found to be 220 (± 24) M$^{-1}$. 56
Figure 2.14 (a) 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; (b) Changes in the chemical shifts of urea NH; (c) Jobs Plot for UT2-Fluoride system indicates 1:1 complexation.

At higher concentrations of TBAF (> 2.0 equiv), the indole NH resonances also exhibited complexation-induced downfield shifts from 9.65 ppm to 10.47 ppm, while the aromatic CH resonances were shifted upfield, indicating multiple anion binding events. This indicated complexation of fluoride initially occurred at the urea motif (at low anion concentration regime), and when the concentration of the anion was high, the indole NH group also coordinated to the anion.

a)
Figure 2.15 (a) Partial $^1$H NMR of UT3 (4.3mM) upon addition of fluoride anion (as TBAF) in CD$_3$CN as observed at 25°C; (b) Changes in the chemical shifts of urea NH (c) Jobs plot which indicates 1:2 stoichiometry for UT3-F complex.

Similarly, the changes in indole and urea NH resonances for UT3 upon addition of fluoride were consistent with multiple anion binding events (Figure 2.15).

2.3 Conclusions

In conclusion, we have synthesized and characterized three isomeric urea derivatives of tryptophan UT1-UT3 and evaluated their nature of interactions with the fluoride anion using UV-visible, fluorescence and $^1$H-NMR spectroscopy. UV-visible and fluorescence studies indicated distinct interactions between fluoride anions and the ligands; surprisingly, only UT1 exhibited enhancement of fluorescence in the presence fluoride anion, apparently via ICT process. However, fluorescent responses of UT2 and UT3 towards the fluoride anion were entirely different, and in both cases
quenching occurred. On the basis of $^1$H NMR evidence, we could establish that the observed changes in UV-visible and fluorescence spectra of ligand UT1 (compared to UT2 and UT3) were consequence of fluoride induced deprotonation of indole NH groups. Under the same conditions, the addition of fluoride to UT2 and UT3 afforded distinct complexation-induced shifts to the urea and the indole NH resonances, however, without deprotonation. We have thus demonstrated a molecular system in which the presence of tryptophan residue in the vicinity of urea motif dramatically changes the nature of fluoride coordination including the fluorescence characteristics. Further, the observations enumerated here illustrate how structural encoding may be an important determinant in molecular recognition and reactivity, which is reminiscent of ligand-induced activation or deactivation of biomolecular systems.

2.4 Experimental Section

2.4.1 Materials and Methods

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 Bruker 300 MHz spectrometer, and reported in $\delta$/ppm. The electronic absorption spectra were recorded on a Shimadzu UV-VIS spectrophotometer.

All the $^1$H NMR experiments of UT1–UT3 with acetate anions were performed in CD$_3$CN, with specific receptor concentration. In each case, the concentrations of TBAF were varied from 0-5.0 equiv.
2.4.2 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 (0.137 g, 1 mmol) was dissolved in dry THF (10 mL), and triethylamine (0.200 g, 2 mmol) was added. To this mixture, phenyl isocyanate (0.120 g, 1 mmol) was added using a micropipette and the reaction was stirred at 60°C for 24 h. Subsequently, the solvents were evaporated on the rotavapor, and residue was diluted with aqueous NaHCO₃ 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%; ¹H NMR (300 MHz): 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%; ¹H NMR (300 MHz): 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, -CO₂H merged with residual H₂O signal).

**Urea 3:** Same procedure as above provided Urea 3 as a white crystalline solid. Yield 80%; ¹H NMR (300 MHz, chloroform-d /DMSO-d₆): 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).
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%; \(^1\text{H NMR} \) (300 MHz, chloroform-d): \( \delta \) 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); \(^{13}\text{C NMR} \) (75 MHz, CDCl_3) : \( \delta \) 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%; \(^1\text{H NMR} \) (300 MHz, chloroform-d /DMSO-d_6): \( \delta \) 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, CH2); \(^{13}\text{C NMR} \) (75 MHz, chloroform-d /DMSO-d_6)
UT3: White solid. Yield 80%; 1H NMR (300 MHz, chloroform-d /DMSO-d$_6$): 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, CH2); $^{13}$C NMR (75 MHz, chloroform-d /DMSO-d$_6$) : 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.

Figure 2.16 $^1$H NMR of UT1 in chloroform-d
Figure 2.17 $^{13}$C NMR of UT1 in chloroform-d

Figure 2.18 $^1$H NMR of UT2 in chloroform-d /DMSO-d$_6$
Figure 2.19 $^{13}$CNMR of UT2 in chloroform-d /DMSO-d$_6$

Figure 2.20 $^1$H NMR of UT3 in chloroform-d /DMSO-d$_6$
Figure 2.21 $^{13}$C NMR of UT3 in chloroform-d / DMSO-d$_6$

Figure 2.22 ESI-.MS of UT1 (m/z = 457.1938, M+1).
Figure 2.23 ESI-MS of UT2 (m/z = 457.1950, M+1).

Figure 2.24 ESI-MS of UT2 (m/z = 457.1950, M+1).
2.4.3 Calculation of association constants ($K_a$) by fluorescence studies

Stock solutions of TBAF (50mM) were prepared in CD$_3$CN. The association constants in each case were obtained from the titration of ligands UT2 and UT3 (concentration: 4.0x10$^{-5}$M) with the salts, following Benesi-Hilderbrand analysis. Changes observed in the chemical shifts $1/(\delta_{\text{max}} - \delta_{\text{obs}})$ were plotted as function of reciprocal of guest concentration [S], and the values of association constants ($K_a$) were calculated according to Benesi-Hildebrand formulation:

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

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