

Urea-cored peptides for anion binding and vesicle formation

Ishanki Bhardwaj, Rachit Sapra, and V. Haridas*

*Department of Chemistry, Indian Institute of Technology Delhi (IITD), Hauz Khas,
New Delhi-110016, India*

E-mail: haridasv@chemistry.iitd.ac.in

DOI: <https://doi.org/10.24820/ark.5550190.p009.876>

Abstract

Urea and urea-triazole cored peptides were synthesized and characterized. These compounds were analyzed for their potency to sense anions such as F^- , Cl^- , Br^- , I^- , $H_2PO_4^-$ and HSO_4^- by UV-vis and 1H NMR spectroscopy. Urea-cored compounds showed very weak binding, while the urea-triazole containing compound showed high binding towards $H_2PO_4^-$ and HSO_4^- . The urea-triazole containing compound also acts as colorimetric sensor for F^- and $H_2PO_4^-$. Both classes of compounds favored vesicular assembly in 1:1 $CH_3OH/CHCl_3$.

Keywords: Urea, anion recognition, colorimetric sensor, self-assembly, vesicles

Introduction

Anion sensing has attracted considerable attention due to the important roles of anions in chemistry, catalysis, medicine, biology, and environmental science.¹⁻² Therefore, considerable effort has been devoted to the design of receptors for anion binding and sensing. Anion binding is a great challenge because anions have different size, shape and charge and anion complexation is also pH-dependent.¹ Anion receptors can be positively charged or neutral; they bind anions mainly through hydrogen bonding and/or electrostatic interactions. Various neutral anion receptors which include scaffolds bearing amide, urea, pyrrole and triazole groups as binding sites have been reported.³⁻¹⁰

The pioneering work of Wilcox¹¹ and Hamilton¹² showed that urea moiety can bind anions, in particular anion such as acetate. A variety of receptors containing a urea motif have been developed and studied for their anion sensing.¹³⁻¹⁷ Therefore, the urea unit has been taken as an excellent motif for the construction of anion receptors. The success of urea as a binding motif is due to the fact that it possesses two close N-H units that can chelate a spherical anion in bifurcate hydrogen bonding mode, giving rise to a six-membered ring (Figure 1a). The two parallel H-bonds with two oxygen atoms of a carboxylate results in an eight-membered ring (Figure 1b). The high affinity of urea towards anion is due to spatially close H-bond donor sites.¹² Appending amino acids on urea-

based core can introduce additional H-bonding centers which can help in enhancing binding affinity towards anions. Amino acids containing functionalizable side chains provide an opportunity to design versatile receptors. Peptides with a urea core could be considered as a mimic of anion binding proteins.¹⁸

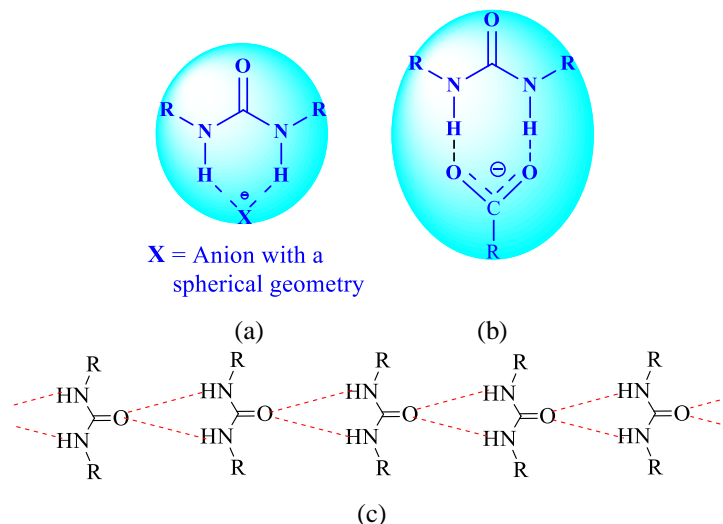


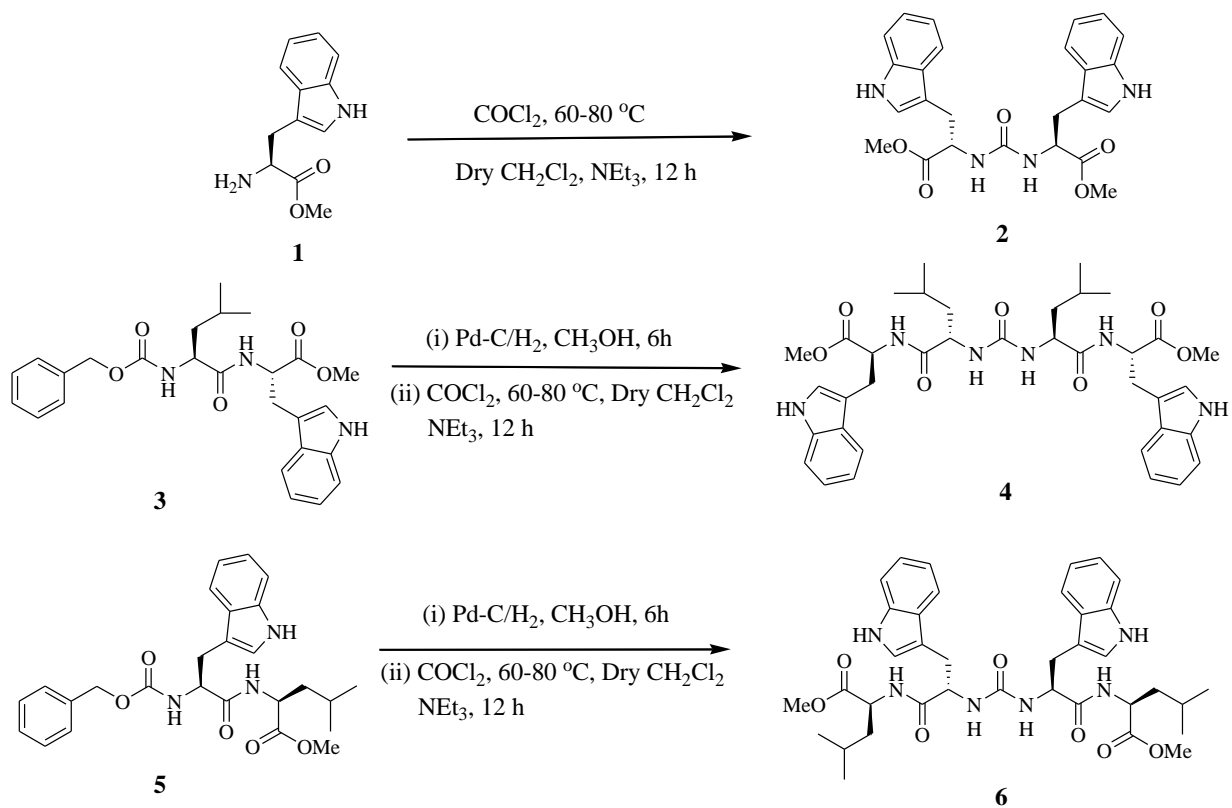
Figure 1. Mode of binding in urea-based molecules by formation of two parallel H-bonds (a) forming six-membered ring with spherical anion (b) forming eight-membered ring with a compound containing a carboxylate group ($R-COO^-$) (c) Intermolecular hydrogen bonds in urea-based molecules.

In addition to providing NHs for binding to anions, the urea motif can form a supramolecular arrangement as a result of the urea α -type hydrogen bonding pattern (Figure 1c) leading to distinct self-assembling features. Incorporation of amino acid residues can result in diverse supramolecular structures through additional non-covalent interactions. The hydrogen bonding capability of peptides can assist in forming specific supramolecular structures such as spheres, vesicles, fibers, helical ribbons and rods.^{19,20} The structurally modified urea derivatives have been extensively explored in organocatalysis,^{21,22} crystal engineering,²³ supramolecular gelation,²⁴⁻²⁶ and as membrane transporters for anions.²⁷

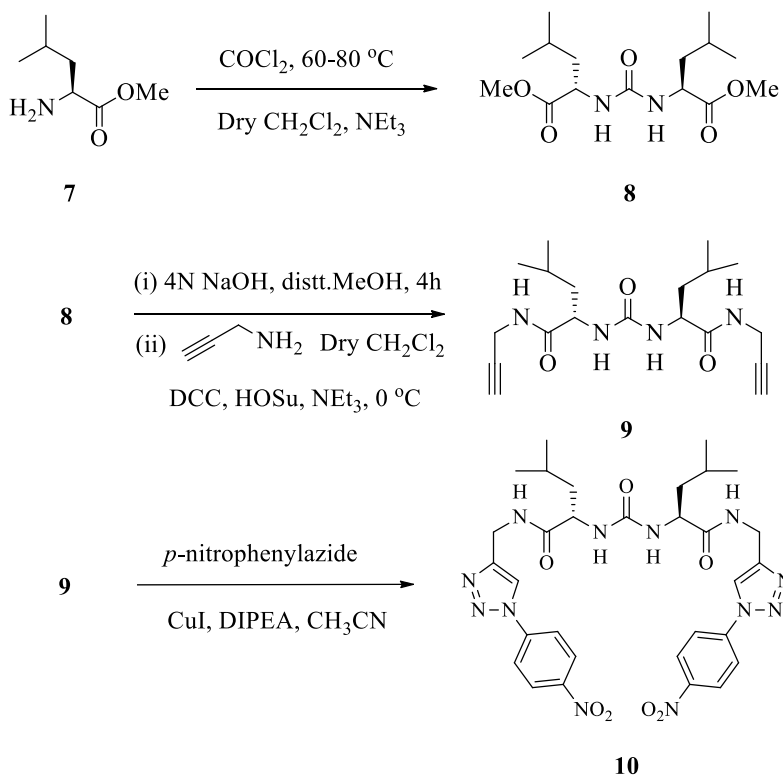
Results and Discussion

Synthesis

Herein, we report urea-cored and urea-triazole containing molecules **2**, **4**, **6** and **10**. Compound **2** is based on L-tryptophan amino acid; **4** and **6** are based on dipeptide comprising L-tryptophan and L-leucine; **10** is a urea functionalized peptide consisting of additional triazole rings. Compound **2** was synthesized by reacting tryptophan methyl ester **1** with phosgene at 70-80 °C. Similarly, **4** and **6** were synthesized by reacting the corresponding dipeptides such as NH_2 -Leu-Trp-OMe (**3**) and NH_2 -Trp-Leu-OMe (**5**) with phosgene (Scheme 1).



Scheme 1. Synthesis of urea functionalized molecules **2**, **4** and **6**.



Scheme 2. Synthesis of triazole-based urea functionalized molecule **10**.

Compound **10** was synthesized by a different scheme (Scheme 2). First, urea derivative of L-leucine methyl ester (**8**) was prepared from NH₂-Leu-OMe (**7**) using Scheme 1. Compound **8** was subjected to basic hydrolysis followed by reaction with propargylamine to give the dialkyne **9**. Compound **9** was then reacted with *p*-nitrophenyl azide in the presence of CuI to give 1,2,3-triazole containing compound **10**. Introduction of Trp in place of Leu in **10** resulted in an insoluble compound.

Anion binding studies

These urea-cored and urea-triazole compounds provide an interesting class of molecules for anion binding and self-assembling studies. The scaffolds are functionalized with amino acid residues leucine and tryptophan. Tryptophan and triazole provide additional H-bond donors in the form of indole NH and CH respectively. Keeping in mind this concept, the binding efficiencies of the designed receptors were determined by UV-vis. and NMR spectroscopic techniques. UV-vis. and NMR titrations of **2** showed a very weak binding towards biphosphate with 1:1 stoichiometry (Figures S1 and S2). Compound **2** did not show much affinity towards other anions due to steric crowding by two indole units of Trp. The steric crowding around urea NHs is reduced in **4**, in which Trp was replaced by the dipeptide L-Leu-Trp-OMe. UV-vis. and ¹H NMR titrations showed that **4** binds to acetate anion in 1:1 fashion (Figure 2, Figure S1) and for other anions, no observable changes in spectra were seen. The binding constant with CH₃COO⁻ in CHCl₃ was calculated using 1:1 non-linear fit analysis²⁸ (<http://www.supramolecular.org>) and was found out to be 115.53 M⁻¹ (Figure S2). ¹H NMR titration of **4** with acetate was carried out in CDCl₃ (Figure 2). Addition of tetrabutylammonium acetate to **4** resulted in downfield shift of urea protons (2.4 ppm), while the

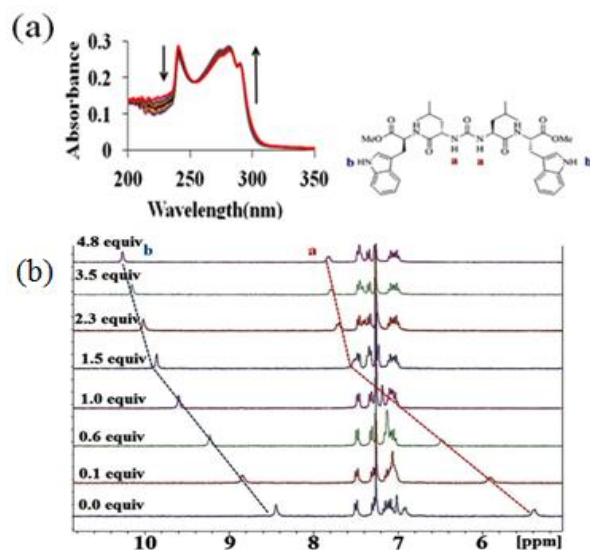


Figure 2. (a) UV-vis. titration profile for **4** (2.1×10^{-5} M) with CH₃COO⁻ (3.3×10^{-2} M) (0.0-216.0 equiv) in CHCl₃ (b) Partial ¹H NMR (300 MHz) titration profile for **4** (4.6×10^{-3} M) with CH₃COO⁻ (0.33 M) in CDCl₃ (Temperature 296-298 K).

indole NH showed a downfield shift of 1.78 ppm (Table S1) indicating the binding of **4** to acetate. The amide protons did not take part in binding as evident from negligible changes in the chemical shift values upon addition of anion salt, this could be due steric hindrance by Leu-CH₃ groups. Compound **6** also surprisingly did not show considerable affinity towards anions which could be due to steric crowding around urea along with distantly placed Trp side chain.

Urea-triazole containing scaffold **10** shows highest affinity amongst all of the compounds tested. Compound **10** forms 1:1 complex with the anions (Figure S1). UV-vis. titration carried out with fluoride, biphosphate and bisulfate showed one isosbestic point at 300 nm (Figure 3, Figure S2). The binding constants (calculated by performing 1:1 non-linear fit analysis)²⁸ for fluoride, biphosphate and bisulfate ions are reported in Table 1. ¹H NMR titration of **10** showed that upon slot-wise addition of TBAH₂PO₄ (Figure 3), the urea NHs shifted downfield (~ 2.0 ppm) and amide NH shifted downfield by approximately 1.0 ppm. The triazole CH protons (c) showed a downfield shift of ~ 0.80 ppm,^{10, 29-30} while aromatic protons showed a small downfield shift (~ 0.30 ppm). ¹H NMR titration of **10** with TBAHSO₄ showed that the urea NHs and amide NHs shifted downfield by ~0.80 ppm and ~0.35 ppm, respectively (Figure S3).

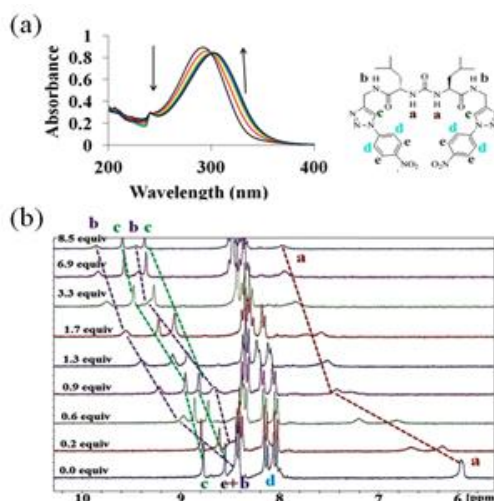


Figure 3. (a) UV-vis. titration profile for **10** (4.3×10^{-5} M) with H_2PO_4^- (2.8×10^{-2} M) (0.0-9.5 equiv) in acetone (b) Partial ¹H NMR (300 MHz) titration profile for **10** (4.3×10^{-5} M) with H_2PO_4^- (4.1×10^{-1} M) in acetone-*d*₆ (Temperature 296-298 K).

The triazole protons shifted downfield (~ 0.20 ppm), while the aromatic protons showed a slight downfield shift (~ 0.10 ppm) (Figure S3). The stronger binding of **10** towards anions as compared to other receptors can be correlated to its structure. The *p*-nitrophenyl group enhances the acidity of triazole CH, which strengthens the hydrogen bonding. The less steric crowding around urea unit allows all NHs to take part in binding along with triazole CHs, hence increases the binding. Compound **10** also showed colorimetric response towards anions in acetone. The color change was monitored by adding 10.0 equiv. of TBA salts of anions to 4.34×10^{-3} M solution of

10 in acetone. A dark brown color was observed in the case of F^- and $H_2PO_4^-$ (Figure 4). Thus, **10** acts as colorimetric sensor for anions and enable naked eye detection of anions. The binding constants of **2**, **4**, **6** and **10** are summarized in Table 1.

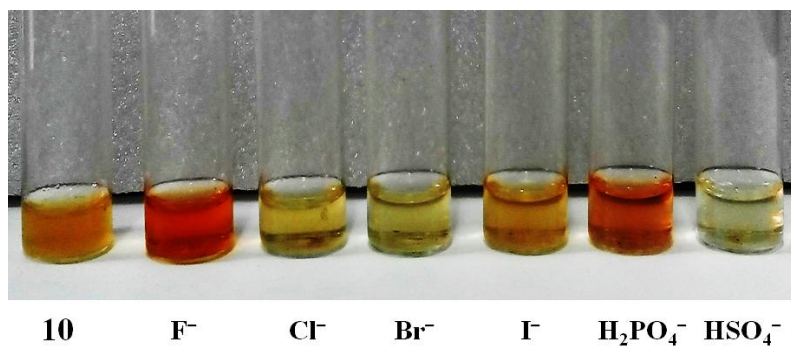


Figure 4. Images of vials showing colorimetric response of **10** (4.34×10^{-3} M) after addition of 10.0 equiv of TBA anions in acetone

Table 1. Binding constants for **2**, **4**, **6** and **10** with TBA anions calculated from UV-vis titrations.

| Anion | Receptors | | | |
|-------------|-----------|---------------------|----------|------------------------|
| | 2 | 4 | 6 | 10 |
| F^- | ND | ND | ND | $50130.86 \pm 3.32\%$ |
| $H_2PO_4^-$ | ND | ND | ND | $653911.93 \pm 9.67\%$ |
| HSO_4^- | ND | ND | ND | $41912.44 \pm 2.54\%$ |
| CH_3COO^- | ND | $115.53 \pm 0.63\%$ | ND | ND |

ND: not determined

Self-assembly studies

Compounds **2**, **4**, **6** and **10** contain amino acid as well as urea linkage, hence possess excellent capability for self-assembly. Therefore, self-assembling features of **2**, **4**, **6** and **10** were studied by using several microscopic techniques. Scanning electron microscopic (SEM) images of **2**, **4**, **6** and **10** in 1:1 $CH_3OH/CHCl_3$ showed vesicular morphology (Figure 5). The morphological features of **2**, **4**, **6** and **10** were also confirmed by transmission electron microscopy (TEM) (stained with 0.2 % phosphotungstic acid) and atomic force microscopy (AFM) (tapping mode) (Figure S4).

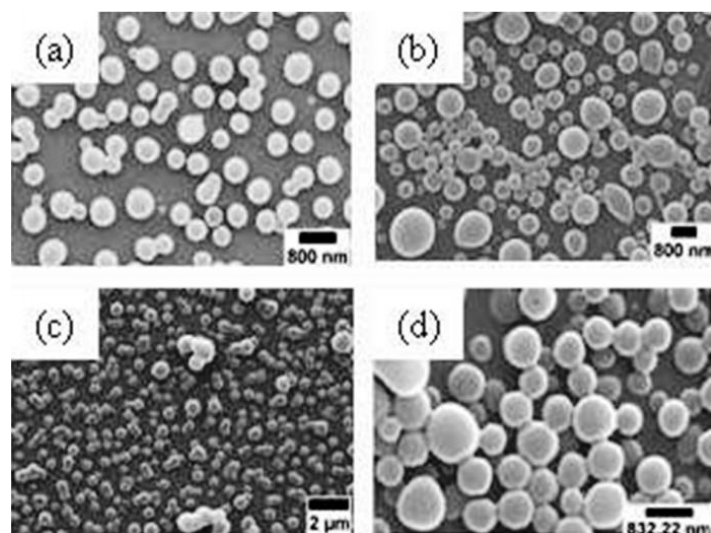


Figure 5. SEM images of (a) **2** (3.2 mM) (b) **4** (2.9 mM) (c) **6** (2.8 mM) (d) **10** (2.8 mM) in 1:1 CH₃OH/CHCl₃ respectively.

Even though, there is a high propensity for urea-based molecules to form fibrous or sheet-like morphology, yet it is surprising to observe that **2**, **4**, **6** and **10** formed vesicles. It is noteworthy that **1** (precursor of **2**) shows fiber-like morphology, while **3** and **5** (precursors of **4** and **6** respectively) formed vesicles (Figure S5). Compound **1** contains lesser number of amide bonds and aromatic rings, compared to **2**, **4**, **6** and **10**. This might be the reason for fibrous morphology of **1**. Therefore, it shows the importance of number of amide bonds as well as aromatic rings in the molecule. Compounds **8** and **9**, which are precursors of **10**, formed fibrous morphology (Figure S5), thus underlining the importance of triazole and aromatic rings present in **10** to give vesicular structure. Aromatic rings may facilitate π - π stacking, while urea and amide NHs can form hydrogen bonding which results in this vesicular morphology. The size of vesicles obtained from microscopic studies range from 0.3-1.3 μ m (Figure S6). This size approximately matches with the size obtained from dynamic light scattering (DLS) measurements (Figure S7). The DLS studies show the size of the vesicles ranges from 0.3-0.8 μ m.

The effect of addition of anions on vesicles of **10** was studied by SEM and DLS. Addition of 5.0 equiv. of TBA anions (biphosphate/bisulfate) to a solution of **10** (Figure 6) resulted in the formation of flattened vesicles with increased size. Addition of biphosphate salt resulted in the formation of pores on the surface of vesicles (Figure 6). The increase in the average size of vesicles upon addition of 5.0 equiv. of anion salt is also supported by DLS studies (Figure S7). The increase in the size upon addition of anions is attributed to the binding of molecules to anions.^{8, 31-32} The presence of vesicular structure even after addition of 5.0 equiv. of anion salt demonstrates the robust nature of the vesicles.

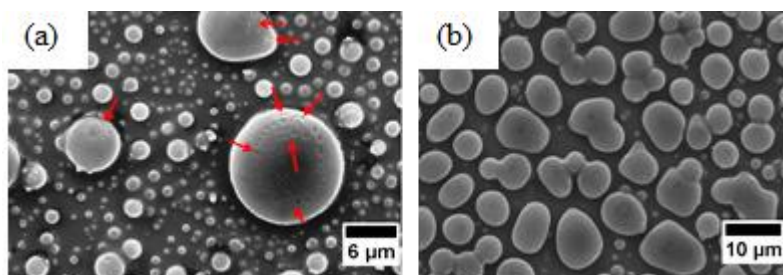


Figure 6. SEM images of (a) **10** + 5.0 equiv. H_2PO_4^- (arrows show position of small pores on the surface of vesicles) (b) **10** + 5.0 equiv. HSO_4^- in 1:1 $\text{CH}_3\text{OH}/\text{CHCl}_3$ respectively.

Conclusions

In summary, we have designed and synthesized urea functionalized peptides **2**, **4**, **6** and **10**. The binding property of these peptides towards tetrabutylammonium salts of different anions was analyzed in detail. It was found that urea-cored compounds **4** and **6** have very weak affinity, while urea-triazole containing **10** turned out to be the most efficient receptor. Compound **10** acts as a colorimetric sensor for fluoride and biphosphate. The self-assembling studies on these urea-cored and urea-triazole containing peptides revealed that **2**, **4**, **6** and **10** self-assembled into vesicles. Addition of anions to the self-assembled vesicles of **10** resulted in the change of vesicles size. The unison of Trp or triazole with urea enabled strong bias towards vesicular self-assembly. Incorporation of triazole improved the anion binding ability of the urea-cored amino acid appended molecule. These findings will help in the design of new generation of self-assembling and anion binding molecules.

Experimental Section

General information

All organic solvents employed in the synthesis were distilled and dried using appropriate drying agents. Reactions were monitored by silica gel-based thin layer chromatography (TLC). Silica gel (100-200 mesh) was used for purification by column chromatography. A Fisher-Scientific apparatus was used for recording melting points. FT-IR spectra were measured using a Nicolet Protégé 460 spectrometer using KBr pellets. ^1H NMR spectra were recorded on Bruker-DPX-300 spectrometer. Tetramethylsilane (^1H) was used as an internal standard. Coupling constants are reported in Hz and data are reported as s (singlet), d (doublet), br (broad), t (triplet), dd (double doublet) and m (multiplet). High resolution mass spectra (HRMS) were recorded in Bruker MicrO-TOF-QII model using ESI technique. UV-visible spectra were recorded in Shimadzu double beam spectrophotometer, UV-2450.

Synthetic procedures

Synthesis of compound 3. To Z-Leucine-OH (Z = benzyloxycarbonyl, an amine-protecting group) (0.2 g, 0.75 mmol) in dry CH₂Cl₂ at 0 °C, added 0.5 mL dimethylformamide (DMF) followed by *N*-hydroxysuccinimide (HOSu) (0.11 g, 0.91 mmol) and *N,N'*-dicyclohexylcarbodiimide (DCC) (0.19 g, 0.91 mmol). To this was added a solution of L-Trp-OMe.HCl (0.23 g, 0.91 mmol) in dry CH₂Cl₂ and NEt₃ (0.13 mL, 0.91 mmol). The reaction mixture was stirred for 24 h. The organic layer was washed sequentially with 0.2 N H₂SO₄, sat. solution of NaHCO₃ and distilled water followed by drying on anhyd. Na₂SO₄. Then it was concentrated to give crude compound. The crude compound was purified by column chromatography using ethyl acetate-hexane as eluents to give **3** as white solid (59 %). Mp: 57-60 °C. ¹H NMR (300 MHz, CDCl₃): δ 0.89 (br s*, 6H, Leu (CH₃)₂), 1.34 (m, 3H, Leu (CH + CH₂)), 3.06 (br d, 2H, *J* 3.0 Hz, Trp (CH)₂), 3.43 (s, 3H, -OCH₃), 4.02 (m, 1H, α-CH), 4.67 (m, 1H, α-CH), 4.81 (m, 2H, Phe (CH₂)), 5.01 (d, 1H, *J* 9.0 Hz, NH), 6.45 (d, 1H, *J* 6.6 Hz, NH), 6.72 (s, 1H, ArH), 6.89 (m, 2H, ArH), 7.07 (m, 6H, ArH), 7.27 (d, 1H, *J* 7.5 Hz, ArH), 7.95 (br s, 1H, indole NH). ¹³C NMR (75 MHz, CDCl₃): δ 22.85, 24.92, 27.50, 33.94, 41.44, 49.18, 52.36, 52.79, 53.55, 66.97, 109.68, 111.23, 118.50, 119.65, 122.22, 123.05, 127.52, 128.03, 128.19, 128.55, 136.05, 156.03, 171.68, 171.96. IR (KBr): 1053, 1204, 1357, 1444, 1526, 1640, 1727, 2930, 3068, 3327, 3397 cm⁻¹. HRMS calcd for C₂₆H₃₁N₃O₅Na *m/z* 488.2155 found 488.2154.

Synthesis of compound 5. To Z-Tryptophan-OH (Z = benzyloxycarbonyl) (0.5 g, 1.47 mmol) in dry CH₂Cl₂ at 0 °C, added 0.5 mL DMF followed by HOSu (0.20 g, 1.76 mmol) and DCC (0.36 g, 1.76 mmol). To this was added solution of L-leu-OMe.HCl (0.32 g, 1.76 mmol) in dry CH₂Cl₂ and NEt₃ (0.25 mL, 1.76 mmol). The reaction mixture was stirred for 24 h. The organic layer was washed sequentially with 0.2 N H₂SO₄, sat. solution of NaHCO₃ and distilled water followed by drying on anhyd. Na₂SO₄. Then it was concentrated to give crude compound. The crude compound was purified by column chromatography using ethyl acetate-hexane as eluents to give **5** as white solid (46 %). Mp: 55-57 °C. ¹H NMR (300 MHz, CDCl₃): δ 0.85 (br s*, 6H, Leu (CH₃)₂), 1.46 (m, 3H, Leu (CH + CH₂)), 3.22 (m, 2H, Trp (CH)₂), 3.64 (s, 3H, -OCH₃), 4.52 (m, 2H, α-CH), 4.95 (s, 2H, Phe (CH₂)), 5.55 (d, 1H, *J* 6.0 Hz, NH), 7.19 (m, 10H, ArH), 7.69 (d, 1H, *J* 6.0 Hz, NH), 8.25 (br s, 1H, indole NH). ¹³C NMR (75 MHz, CDCl₃): δ 21.86, 22.55, 24.72, 27.29, 41.38, 50.80, 52.15, 55.41, 66.97, 110.23, 111.17, 118.76, 119.71, 122.16, 123.46, 127.31, 128.04, 128.47, 136.20, 155.95, 171.00, 172.78. IR (KBr): 1051, 1224, 1345, 1447, 1536, 1663, 1730, 2953, 3062, 3312, 3388 cm⁻¹. HRMS calcd for C₂₆H₃₁N₃O₅Na *m/z* 488.2156 found 488.2162.

General procedure for synthesis of compounds 2, 4, 6. To the amino acid methyl ester (0.21 g, 0.64 mmol) in dry CH₂Cl₂ and NEt₃ (0.08 mL, 0.16 mmol) at 60-70 °C, phosgene (0.07 mL, 0.64 mmol) in dry CH₂Cl₂ was added dropwise. The reaction mixture was refluxed with constant stirring for 12 h. The organic layer was washed with 0.2 N H₂SO₄, sat. NaHCO₃ and distilled water. Organic layer was dried over anhyd. Na₂SO₄, evaporated and weighed to give crude compound. The crude compound was purified by column chromatography using ethyl acetate-hexane as eluents to give desired pure urea-based compound.

Data of 2. Appearance: Off-white solid. Yield: 88 %. Mp: 131-133 °C. ¹H NMR (300 MHz, CDCl₃): δ 3.10 (br d, 4H, *J* 5.4 Hz, Trp (CH₂)), 3.44 (s, 6H, -OCH₃), 4.84 (m, 2H, α-CH), 5.83 (d, 2H, *J* 8.4 Hz, NH), 6.74 (m, 2H, ArH), 7.10 (m, 4H, ArH), 7.30 (d, 2H, *J* 9.3 Hz, ArH), 7.45 (d, 2H, *J* 7.8 Hz, ArH), 8.37 (br s, 2H, indole (NH)). ¹³C NMR (75 MHz, CDCl₃): δ 28.05, 52.21, 53.10, 109.41, 111.24, 118.43, 119.33, 121.87, 123.38, 127.43, 135.94, 157.03, 173.88.

IR (KBr): 1015, 1104, 1213, 1351, 1440, 1554, 1651, 1734, 2854, 2924, 3397 cm⁻¹. HRMS calcd for C₂₅H₂₆N₄O₅Na *m/z* 485.1795 found 485.1794.

Data of 4. Appearance: White solid. Yield: 56 %. Mp: 65-67 °C. ¹H NMR (300 MHz, CDCl₃): δ 0.83 (br s*, 12H, Leu (CH₃)₂), 1.44 (m, 6H, Leu (CH + CH₂)), 3.20 (dd, 4H, *J*₁ 22.2 Hz, *J*₂ 10.5 Hz, Trp (CH)₂), 3.57 (s, 6H, -OCH₃), 4.40 (br s, 2H, α-CH), 4.86 (m, 2H, α-CH), 6.15 (m, 2H, *J* 24 Hz, NH), 7.12 (m, 10H, 8ArH + 2 NH), 7.47 (d, 2H, *J* 7.5 Hz, ArH), 8.59 (br s, 2H, indole (NH)). ¹³C NMR (75 MHz, CDCl₃): δ 21.90, 22.74, 24.64, 27.29, 41.68, 52.29, 52.73, 108.97, 109.27, 111.37, 111.64, 118.28, 119.25, 121.79, 122.07, 123.73, 127.31, 136.00, 158.14, 172.21, 173.72. IR (KBr): 1101, 1213, 1361, 1440, 1547, 1640, 1740, 2955, 3388 cm⁻¹. HRMS calcd for C₃₇H₄₈N₆O₇Na *m/z* 711.3477 found 711.3483.

Data of 6. Appearance: Off-white solid. Yield: 44 %. Mp: 67-70 °C. ¹H NMR (300 MHz, CDCl₃): δ 0.84 (br s*, 12H, Leu (CH₃)₂), 1.48 (m, 6H, Leu (CH + CH₂)), 3.15 (m, 4H, Trp (CH)₂), 3.57 (s, 6H, -OCH₃), 4.36 (m, 2H, α-CH), 4.74 (m, 2H, α-CH), 6.31 (d, 2H, *J* 6.6 Hz, NH), 7.23 (m, 10H, ArH), 8.10 (br s, 2H, NH), 8.20 (d, 2H, *J* 7.8 Hz, indole NH). ¹³C NMR (75 MHz, CDCl₃): δ 21.90, 22.74, 24.64, 27.29, 41.68, 52.29, 52.73, 108.97, 109.27, 111.37, 111.64, 118.28, 119.25, 121.79, 122.07, 123.73, 127.31, 136.00, 158.14, 172.21, 173.72. IR (KBr): 1210, 1447, 1655, 1739, 2934, 3388, 3411 cm⁻¹. HRMS calcd for C₃₇H₄₈N₆O₇Na *m/z* 711.3477 found 711.3477.

Synthesis of compound 9. Compound **8** (0.23 g, 0.73 mmol) in CH₃OH (4.0 mL) was hydrolysed using 4N NaOH (4.0 mL) at 0-4 °C to give diacid (0.2 g, 0.69 mmol). To the diacid (0.2 g, 0.69 mmol) in dry CH₂Cl₂ at 0-4 °C, was admixed, HOSu (0.175 g, 1.52 mmol) and DCC (0.31 g, 1.52 mmol) sequentially followed by propargyl amine (0.1 mL, 1.52 mmol). The reaction mixture was stirred for 24 h. The organic layer was washed sequentially with 0.2 N H₂SO₄, sat. solution of NaHCO₃ and distilled water followed by drying on anhyd. Na₂SO₄. Then it was concentrated to give crude compound. The crude compound was chromatographed on a column of silica gel by using ethylacetate-hexane as eluents to give pure **9** white solid (79.5 %). Mp: 123-125 °C. ¹H NMR (300 MHz, CDCl₃): δ 0.86 (br s*, 12H, Leu (CH₃)₂), 1.51 (m, 6H, Leu (CH + CH₂)), 2.28 (s, 2H, alkyne CH), 3.97 (dd, *J*₁ 17.1 Hz, *J*₂ 5.4 Hz, 4H, NH(CH₂)), 4.45 (m, 2H, α-CH), 6.34 (d, 2H, *J* 4.5 Hz, NH), 7.76 (br s, 1H, NH), 7.84 (br s, 1H, NH). ¹³C NMR (75 MHz, CDCl₃): δ 24.58, 25.57, 29.09, 33.89, 41.96, 49.18, 52.15, 71.35, 79.44, 156.74, 157.85, 173.70. IR (KBr): 1227, 1443, 1561, 1625, 2853, 2929, 3281, 3381 cm⁻¹. HRMS calcd for C₁₉H₃₀N₄O₃Na *m/z* 385.2210 found 385.2210.

Synthesis of compound 10. To the compound **9** (0.19 g, 0.54 mmol) in acetonitrile at 0 °C, was added DIPEA (0.38 mL, 1.18 mmol) followed by 4-nitrophenylazide (0.19 g, 1.18 mmol) in CH₃CN. To this reaction mixture was added CuI (0.020 g, 0.11 mmol) followed by stirring for 24 h under argon atmosphere. Reaction mixture was filtered and the filtrate was evaporated. The

residue obtained from evaporation of filtrate was insoluble in chloroform, ethyl acetate and dichloroform. Therefore, the residue was directly washed with 9:1 $\text{NH}_4\text{Cl}/\text{NH}_4\text{OH}$ solution ($50 \text{ mL} \times 3$) and distilled water ($50 \text{ mL} \times 3$) to give crude **10**. The crude product was purified by column chromatography with methanol-chloroform as eluents to give **10** as light yellow solid (89 %). Mp: 135-137 °C. ^1H NMR (300 MHz, Acetone- d_6): δ 0.87 (m, 12H, Leu (CH_3)₂), 1.65 (m, 6H, Leu ($\text{CH} + \text{CH}_2$)), 4.28 (m, 2H, $\alpha\text{-CH}$), 4.42 (br s, 2H, NHCH_2), 4.56 (m, 2H, NHCH_2), 6.13 (m, 2H, NH), 8.07 (m, 6H, 4 $\text{ArH} + 2\text{NH}$), 8.41 (d, 4H, J 8.7 Hz, ArH), 8.55 (br s, 1H, triazole- CH), 8.75 (br s, 1H, triazole- CH). ^{13}C NMR (75 MHz, Acetone- d_6): δ 22.01, 23.41, 26.00, 35.56, 42.17, 54.13, 120.88, 121.78, 126.30, 132.21, 142.07, 147.78, 159.35, 174.75. IR (KBr): 1046, 1256, 1344, 1525, 1642, 2953, 3301 cm^{-1} . HRMS calcd for $\text{C}_{31}\text{H}_{38}\text{N}_{12}\text{O}_7\text{Na}$ m/z 713.2879 found 713.2868.

* br s = broad singlet

UV-vis titration experiments

Stock solutions of compounds **2**, **4**, **6** and **10** were prepared in spectroscopy grade chloroform with concentration (10^{-4} - 10^{-5} M). Solutions of anion salts were prepared in chloroform with concentration (10^{-1} - 10^{-2} M). The absorbance of blank compound solution and solution after gradual addition of anion salt were recorded using Shimadzu double beam UV-vis. spectrophotometer model UV-2450.

NMR titration experiments

Stock solutions of compounds **2**, **4**, **6** and **10** were prepared in CDCl_3 with concentration 10^{-3} M. Solutions of anion salts were prepared in CDCl_3 with concentration 10^{-1} M. The spectra were collected on a Bruker DRX 300 (300 MHz) NMR spectrometer. Anion binding titrations were carried out by monitoring changes in the aromatic proton (ArH), amide NH and triazole CH signal of receptors as a function of added halide salt concentration.

Calculation of binding constants and stoichiometry

The binding constants were calculated by a non-linear fitting using Bindfit (<http://www.supramolecular.org>). UV fitting for 1:1 model was done using the Nelder-Mead method. Stoichiometry of binding was calculated by Job plot from UV-vis. absorbance data.

Microscopy methods

Optical microscopy: A drop of solution of the compound was put on a glass slide, the solvent was allowed to evaporate in air. It was then viewed using Nikon Eclipse TS100 optical microscope system.

Transmission electron microscopy: Around 3.0 mM solution of the sample in methanol-chloroform (1:1) was used for TEM. All the sample solutions were filtered through a nylon syringe filter (0.2 μm). About 2 μl aliquot of the sample solution was placed on a 200 mesh copper grid and stained with 0.2 % wt. phosphotungstic acid in water for 2 min. and the grid was allowed to dry in atmosphere. Samples were viewed using a Philips CM 12 transmission electron microscope.

Scanning electron microscopy: Around 3.0-4.0 mM solution of compound in 1:1 methanol-chloroform was prepared and one drop of the solution was put on the glass cover slip pasted on a carbon tape mounted on a stub, dried under sodium lamp and coated with ~ 10 nm of gold. Samples were analyzed using scanning electron microscope ZEISS EVO 50 SEM.

Atomic force microscopy: Around 3.0-4.0 mM solution of compound in 1:1 methanol-chloroform was prepared and one drop of the solution was put on silicon wafer and dried in atmosphere. Samples were analyzed using Dimension Icon AFM operating at tapping mode in air. Images were recorded in air at room temperature and data analysis was performed using nanoscope 5.31r software.

Acknowledgements

We thank DST [Grant No. SB/S1/OC-23/2014], New Delhi for financial assistance. We acknowledge the Department of Textile Engineering, IITD for SEM images. IB and RS thanks CSIR, New Delhi for the fellowship.

Supplemental material

¹H NMR, ¹³C NMR, HRMS spectral data, UV-vis. data and microscopic images are available in the supplementary material provided with this manuscript.

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