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Self-assembling asymmetrical tripodal-like peptides as anion receptors

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ABSTRACT

Aspartic and glutamic-acid based molecules **T1a**–**b**–**T5a**–**b** appended with different moieties were designed and synthesized. These molecules displayed unique self-assembly behavior dependent upon the nature of appended units. Trp favored vesicles, while phenyl unit favored fibrillar assembly. These molecules also showed binding towards anions.

1. Introduction

Molecular self-assembly is a powerful method for fabricating supramolecular structures.[1] The formation of well-ordered supramolecular structures through self-assembly of diverse organic and inorganic building blocks has drawn much attention owing to their potential applications in biology and chemistry.[2] Peptides possess hydrogen bonding capability; hence adopt specific structures through self-assembly. The intermolecular non-covalent interactions are utilized in designing diverse supramolecular structures such as fibers, vesicles, spheres, rods and helical ribbons. The potential applications of these assemblies involve their use in drug delivery, tissue engineering and wound healing.[3–10] External stimuli such as temperature, pH, electric field, chemicals and ions can bring changes in the structure and solubility characteristics of assembly.[11,12] Such self-assembling molecules have several applications in the design of stimuli responsive materials.[13–15]

Designed peptides can act as good receptors for anions as they have ability to bind guest through hydrogen bonds.[16] The binding efficiency of a host depends upon its geometry and the cavity size. Designing tripodal receptors containing asymmetrical binding arms is an interesting area of host-guest chemistry research.[17,18] Amino acids with functionalizable side chain seem to be ideal candidates for the design of tripodal receptors. The arms of the tripodal receptors could offer more binding sites for guest and thereby achieve high binding affinity and selectivity. Appending with fluorophore can help in fluorescence detection of guests. Amino acids such as threonine, serine, tyrosine, histidine and tryptophan have OH or NH groups in their side chains, hence are useful models for designing receptors for anions.[19]

2. Results and discussion

We report aspartic acid and glutamic acid-cored peptides **T1a**–**b**–**T5a**–**b** with and without tryptophan residues (Fig. 1). **T1a**–**b** contain benzyl amine appended on Boc-L-Asp and Boc-L-Glu amino acid. **T2a**–**b** and **T3a**–**b** are synthesized by coupling p-nitrobenzoic acid and Boc-L-Tryptophan to the N-terminal of **T1a** and **T1b** respectively. **T4a**–**b** and **T5a**–**b** constitute another class of scaffolds where Boc-Asp and Boc-Glu amino acid are partially or fully functionalized with tryptophan residues (Scheme 1, SI, Schemes S1 and S2).

These diverse classes of molecules with amide functionalities provide an interesting set of molecules for anion binding and self-assembling studies. The three arms of aspartic and glutamic acid serve as branches to functionalize with different moieties to generate molecules with tripodal-like topology. Tryptophan provides indole NH as H-bond donor for binding to the guest and also helps in fluorescent detection of guests.

Keeping in mind this concept, the binding efficiencies of **T1a**–**b**–**T5a**–**b** towards anions such as F^-, Cl^-, Br^-, I^-, $H_2PO_4^-$ and HSO_4^- were analyzed by using spectroscopic techniques such as

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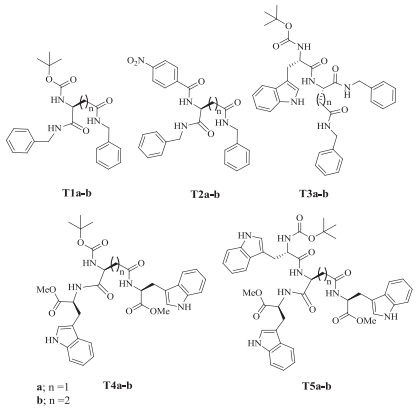
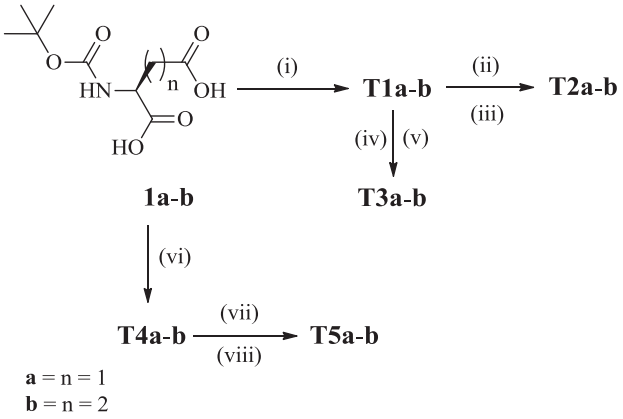


Fig. 1. Structural representation of tripodal receptors **T1a**–**b**–**T5a**–**b**.



Scheme 1. Synthesis of tripodal peptides **T1a**–**b**–**T5a**–**b**. (i) DCC, HOSu, NEt_3, Benzyl amine, dry CH_2Cl_2, 0 °C, 24 h (ii) Trifluoroacetic acid, dry CH_2Cl_2, 0 °C, 4 h (iii) p-nitrobenzoyl chloride, NEt_3, dry CH_2Cl_2, 0 °C, 12 h (iv) Trifluoroacetic acid, dry CH_2Cl_2, 0 °C, 4 h (v) Boc-L-Trp-OH, DCC, HOSu, NEt_3, dry CH_2Cl_2, 0 °C, 24 h (vi) DCC, HOSu, NEt_3, L-Trp-OMe, dry CH_2Cl_2, 0 °C, 24 h (vii) saturated soln. of HCl in ethyl acetate, 0 °C, 6 h (viii) Boc-L-Trp-OH, DCC, HOSu, NEt_3, dry CH_2Cl_2, 0 °C, 24 h.

UV–vis fluorescence and NMR (Figs. 2 and 3, SI, Figs. S1–S4). The anion binding studies were carried out in chloroform. UV–vis and NMR titrations showed that **T2b** acts as a receptor for phosphate and chloride ion (Table 1). The occurrence of one isosbestic point in UV titration profile of **T2b** (4.5×10^{-5} M) with phosphate (4.5×10^{-2} M) is a noteworthy observation (Fig. 2a). Surprisingly, **T2a** (with one methylene less than **T2b**) didn't show any changes in the UV spectra when titrated with $TBAH_2PO_4$, TBABr, TBAI and $TBAHSO_4$. This may be due to steric crowding around the two amide NHs in **T2a** as compared to **T2b**. Presence of one additional methylene in **T2b** relieves the steric strain and hence increases the binding. One of the three arms of **T1a**–**b** is not functionalized in comparison to **T2a**–**b** and **T3a**–**b**. Therefore, **T1a**–**b** show differences in binding selectivity compared to tripodal receptors **T2a**–**b** and **T3a**–**b** (Table 1). **T1b** showed higher binding compared to **T1a** due to same steric reasons. Changing the N-terminal substituent of **T2a**–**b** from p-nitrobenzoyl to Boc-L-Tryptophan provided **T3a**–**b**. The introduction of tryptophan residue was expected to increase the binding affinity as it can provide an additional NH for binding. Interestingly, the binding studies showed no considerable increase in binding affinity (Table 1). This may be due to orientation of tryptophan group away from the binding cavity, thereby not participating in the binding. The stoichiometry of binding for **T1a**–**b**–**T3a**–**b** and **T4b**–**T5a**–**b** with all anions is 1:1, while **T4a** binds to sulfate in 1:2 fashion (G:H 1:2) as evident from Job's plot (SI, Fig. S2).

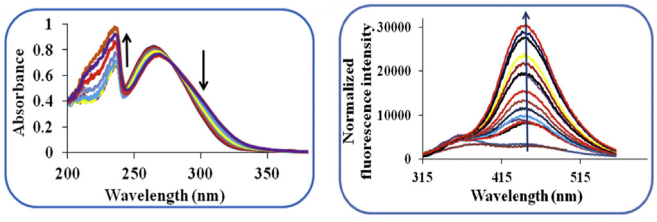


Fig. 2. (a) UV titration profile for **T2b** (4.5×10^{-5} M) with (0–40 equiv) $TBAH_2PO_4$ (4.5×10^{-2} M) in $CHCl_3$ (b) Fluorescence titration profile for **T4a** (1.2×10^{-4} M) with $TBAHSO_4$ (1.08×10^{-1} M) in $CHCl_3$.

In order to enhance the binding, **T4a**–**b** and **T5a**–**b** with two and three tryptophan residues were synthesized. UV–vis and fluorescence titration experiments carried out with anions revealed that **T4a** efficiently binds to phosphate and sulfate.

Fluorescence titration showed increase in fluorescence intensity at 445 nm upon slot-wise addition of $TBAHSO_4$ in chloroform (Fig. 2b). ^1H NMR titration showed the downfield shift of NHs upon addition of anion salt (Fig. 3). It is evident from Fig. 3, that amide NH(b) showed downfield shift from 6.32 ppm to 6.78 ppm while indole NH's (d and e) showed downfield shift from 8.11 ppm to 9.12 ppm indicating that these NHs are involved in binding with the guest. **T4b** has less binding affinity as compared to **T4a** (Table 1). The extra methylene group present in **T4b** moves the side chains away from each other, thus decreasing the binding. When the N-terminal of **T4a**–**b** was functionalized by another tryptophan residue to give **T5a**–**b**, the observed binding affinity is comparatively lesser or similar (Table 1). The contribution towards binding is majorly due to two tryptophan residues. None of these receptors showed binding towards Br^- and I^- ions.

As **T1a**–**b**–**T5a**–**b** contain amide bonds, they can associate by intermolecular hydrogen bonds. In addition, they contain amino acid residues with side chains capable of π–π interactions due to presence of aromatic groups. The presence of these moieties in the scaffolds can provide distinct self-assembling features. Hence, these peptides provide interesting candidates for self-assembly studies. The self-assembling features of **T1a**–**b**–**T5a**–**b** were studied in 1:1 $CH_3OH/CHCl_3$ by several microscopic techniques such as scanning electron microscopy (SEM), transmission electron microscopy (TEM) and atomic force microscopy (AFM). The studies revealed that **T1a**–**b** and **T2a**–**b** self-assembled into fibers, while **T3a**–**b**–**T5a**–**b** self-assembled into vesicles (Fig. 4, SI, Figs. S5–S6). The backbone of **T1a**–**b**–**T5a**–**b** consists of Asp and Glu amino acids, while the arms are functionalized with different units. Thus, the self-assembly can be controlled by appended units. A closer look at the SEM images showed that the average size of vesicles of **T3a**–**b**–**T5a**–**b** is 0.3–0.8 μm (SI, Fig. S7). TEM (stained with 0.2% phosphotungstic acid) revealed that **T3a**–**b**–**T5a**–**b** formed vesicles in the range of 0.3–0.9 μm.

After discovering the distinct morphological features of these peptides, we turned our attention to evaluate the encapsulating potency of the vesicles formed from self-assembly of **T3a**–**b**–**T5a**–**b**.

Fluorescence microscopic analysis of **T3a**–**b**–**T5a**–**b** revealed that the vesicles can be seen in blue color (λ_{ex}=380–450 nm), while the rhodamine B entrapped vesicles appeared red (λ_{ex}=510–560 nm) (Fig. 5a,b) clearly indicating the encapsulation potency of rhodamine B by the vesicles. Addition of 0.2 equiv of sulfate salt to the dye entrapped vesicles resulted in the disruption of vesicles (Fig. 5c), since we could observe the reappearance of blue fluorescence of **T3b** (Fig. 5d). The vesicular morphology is lost due to anion binding, thus disrupting the self-assembly.

The influence of anion binding on self-assembly of **T1a**–**b**–**T5a**–**b** was studied by microscopic imaging. Experiments were performed by adding the anion to the self-assembled system