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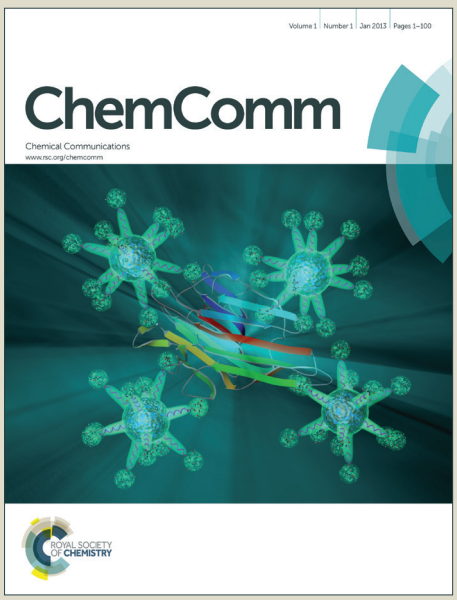

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Triazolo-β-Aza-ε-Amino Acid and Its Aromatic Analogue as Novel Scaffolds for β-turn Peptidomimetics

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Subhendu Sekhar Bag,* Subhasish Jana, Afsana Yashmeen and Suranjan De

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Triazolo-β-aza-ε-amino acid and its aromatic analogue (^{Al}TAA/^{Ar}TAA) in peptide backbone mark a novel class of conformationally constrained molecular scaffolds to induce β-turn conformations. This was demonstrated for ^{Al}TAA in a Leu-enkephalin analogue and in a designed pentapeptide wherein FRET process was established. Restricted rotation induced chirality and turn conformation into the achiral aromatic scaffold, ^{Ar}TAA, which in a short tripeptide backbone acted as a β-turn mimic as a β-sheet nucleator.

Introduction of a conformationally constrained nonpeptide isostere into peptide backbones in order to achieve desirable secondary structures along with pharmacologically viable peptide-based drug candidates is of great interest in recent time.[1] Among the various secondary structures,[2-3] β-turns[3] are important targets for mimicry, both because they serve as recognition sites in peptides and proteins as well as they allow a protein chain to fold back upon itself to form a compact structure.[1c] Considerable efforts have thus been invested in delineating the impact of appended molecular scaffolds in one hand and nucleating turn mimics on the other hand, on the conformational preferences of proteins and peptides in solution.[1c] Despite an exponential growth on the development of constrained non-peptidic molecular scaffolds, very few peptidomimetic drugs have been developed, necessitating an overhaul in the existing design principles.[1-3]

As a part of our ongoing research efforts on the design of unnatural biomolecular building blocks[4] via click chemistry and β-turn peptidomimetics,[4c] we report herein on the simple synthesis and application of triazolo-β-aza-ε-amino acid (**1**, ^{Al}**TAA**) and its aromatic analogue (**2**, ^{Ar}**TAA**) as new and novel constrained molecular scaffolds (Fig. 1). Similar to the sugar amino acids,[3j-k] the rigid frameworks of pseudo-aromatic triazole units prompted us to use triazolo amino acids as novel molecular scaffolds in peptidomimetic studies.[5] These two molecules with constrained backbone angles, ω(i) and ϕ(i+1), are expected to induce folded conformations in linear peptides. The triazolo amino acids are advantageous with respect to their metabolically inertness, easy associability with biological targets and tolerance to various reaction conditions used in peptide synthesis. Moreover, the triazolo unit acts as *trans*-amide mimetic which makes the scaffolds more prone to nucleate β-turn structure while present in a short peptide backbone. Though the click chemistry has been utilised in mimicking the protein's secondary structures, to the best of our knowledge, the triazolo amino acids as scaffold has not been explored.[6] We envisioned that upon incorporation of ^{Al}**TAA**/^{Ar}**TAA** into backbone a linear peptide, such as, Leu-enkephalin analogue, might adopt β-turn conformation.[3,7]

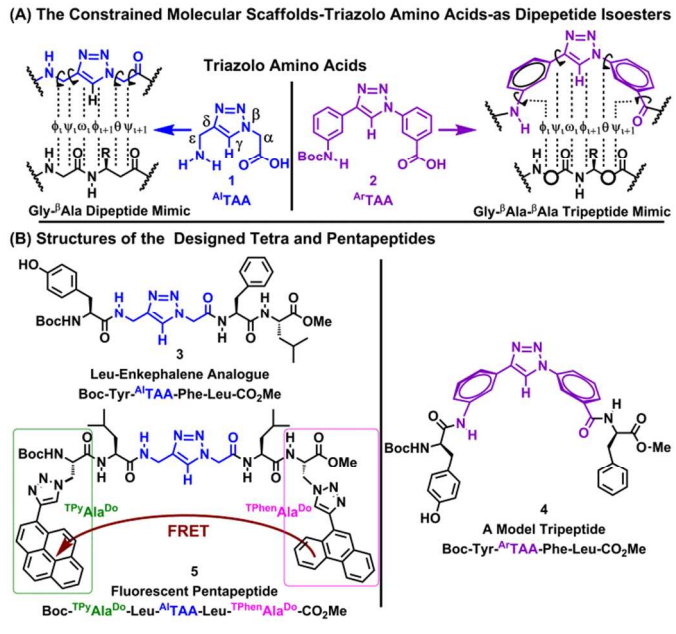
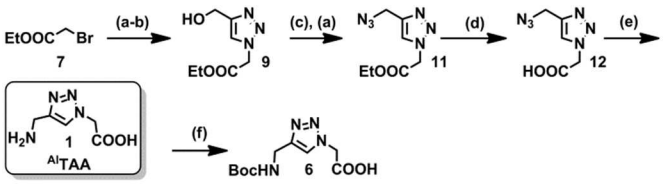


Figure 1. (A) The constrained molecular scaffolds-triazolo amino acids (**1**, ^{Al}**TAA** and **2**, ^{Ar}**TAA**) and (B) structures of the designed peptidomimetics **3-5**.

We, therefore, demonstrate the synthesis of triazolo amino acids, ^{Al}**TAA** (**1**) and ^{Ar}**TAA** (**2**), and their effect on the structural properties of linear peptides wherein the scaffolds present in a peptide backbone (Fig. 1). The peptides **3** and **5** with ^{Al}**TAA** in the backbone adopted purely β-turn structures as were determined by CD, NMR and modelling studies. A sheet like structure with 20% turn was the result of induction by ^{Ar}**TAA** in the backbone of a designed tripeptide **4**. Interestingly, the β-turn peptide **5**, (Boc-^{TPy}**Ala**^{Do}-Leu-^{Al}**TAA**-Leu-^{TPhen}**Ala**^{Do}-CO_2Me), showed a Förster resonance energy transfer (FRET) process between the terminal unnatural fluorescent triazolyl amino acids, ^{TPhen}**Ala**^{Do} and ^{TPy}**Ala**^{Do}.

The synthesis of the aliphatic triazolo amino acid, ^{Al}**TAA** (**1**, Fig. 1) proceeded through a novel click reaction path followed by hydrolysis of azido ester (**11**) and then reduction of the azide (**12**) (Scheme 1). The aromatic triazolo amino acid (**2**, ^{Ar}**TAA**, Fig. 1) was synthesized in a similar way via a click chemistry protocol (ESI†, Scheme S2). The amino acid scaffolds were characterised by NMR, mass spectrometry, and single crystal X-ray diffraction analysis for scaffold **2**.



Reagents and Condition: (a) NaN_3, DMF; (b) Propargyl alcohol, CuI, DIPEA; (c) MsCl , Et_3N, DCM; (d) 2.5 (M) NaOH, MeOH; (e) H_2/Pd-C, MeOH; (f)($Boc)_2O$, NaOH, H_2O-Dioxane.

Scheme 1. Synthesis of the aliphatic triazolo amino acid scaffold (**1**, ^{Al}**TAA**).

The crystal structure analysis of scaffold **2** (mp 168 °C; chiral space group $P2_12_12_1$) revealed that the aminophenyl unit was 25.1 ° out of plane with respect to triazole unit. Overall the scaffold adopted a hairpin shape wherein the two hairpins packed face-to-face via weak H-bonding interaction leading to "S"-shaped structure which were linked each other via ArCH….$N_{Triazole}$, ArCH-π bonding (side way) and hydrophobic interaction through ^{t}Butyl-units to link "S"- units linearly leading to a helical like construct (Fig. 2A-B and ESI†, Section 7). More interestingly, the solid state chirality was well maintained in methanol solution as was revealed from the appearance of two distinct UV-visible absorption bands at 234 and 297 nm, specific rotation ($\alpha_D^{20} + 1.5$) and the CD spectra with a signature of turn conformation (Fig. 2C). Therefore, the origin of chirality was the restricted rotation of out-of-plane aminophenyl unit about $C_{(aminophenyl)}$-$C_{(triazole)}$ bond both in solid and solution. The maintenance of the chiral turn conformation in both the solid and solution phase motivated us to use the triazolo amino acid **2** as a scaffold for peptidomimetic study.

A comparison of the backbone bonds and the dihedral angles of the dipeptide isostere **1** (^{Al}**TAA**) with those of a natural dipeptide showed that the triazole unit of **1** fixes ω_i (*trans*-amide bond) and ϕ_{i+1} angles at about 180°. Accordingly, peptidomimetic **1** could be used to replace two adjacent amino acids (Gly-β^3Ala) having conformational restriction at ω_i and ϕ_{i+1} angles. On the other hand, ψ_i angle is restricted by 25.1° out-of-plane in addition to the fixed ω_i and ϕ_{i+1} angles in scaffold **2** (Fig. 1). Therefore, both the scaffolds would expect to show high propensity to induce short linear peptide chain to fold back upon itself resulting in a compact conformation.

To explore the constrained property and to determine the effect that scaffold **1** and **2** exert on the conformation of a peptide, we, next, incorporated them into (a) a linear Leu-enkephalin analogue (for ^{Al}**TAA**) (**3**), (b) a designed tripeptide (**4**) (for ^{Ar}**TAA**) and (c) a designed pentapeptide (for ^{Al}**TAA**) containing two of our triazolyl fluorescent UAAs into two termini (**5**) (Fig. 1). All the peptides were synthesized by solution phase peptide coupling protocol. The peptide **5** was synthesized via a two steps coupling of amine of dipeptide, BocNH-^{Tphen}**Ala**^{Do}-CO(NMe)OMe (**34**) and acid of dipeptide, BocNH-^{TPy}**Ala**^{Do}-Leu-CO_2Me (**40**) containing unnatural fluorescent triazolyl amino acids [^{Tphen}**Ala**^{Do} and ^{TPy}**Ala**^{Do}, respectively] with triazolo amino acid, ^{Al}**TAA** (**1**) (ESI†, Section 2-6).

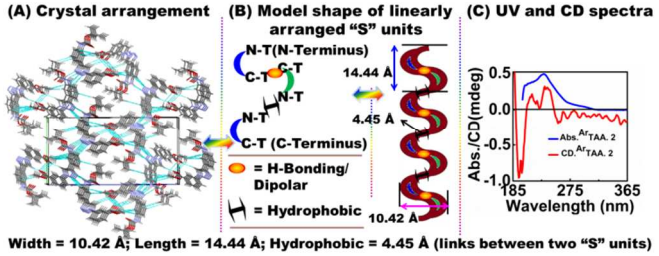


Figure 2. (A) Molecular arrangement; (B) Model shape of linearly arranged "S" units (two molecules of unit cell) [CCDC 997089] and (E) UV-visible and CD spectra of aromatic triazolo amino acid scaffold **2** (^{Ar}**TAA**).

Next, the secondary structure of peptide **3**, a Leu-enkephalin analogue, was estimated by recording its CD spectrum in methanol which showed a strong positive band at 206 nm and a negative band at 191 nm indicating a type II β-turn conformation (Fig. 3A).[8] The peptide secondary structure estimation using CD estima program[8b] showed 100% β-turn structure in peptide **3** the existence of which implied the possible presence of intramolecular H-bonds between the peptide strands. CD spectrum also indicated a predominantly type II β-turn conformation in fluorescent pentapeptide **5** (Fig. 3A).[8] Moreover, the signature of aromatic π-π stacking interaction between Phe and Tyr in peptide **3** was also evident from the appearance of a positive band at 217 nm.[8a,c] The tripeptide **4** containing the aromatic scaffold in the backbone showed a broad negative peak at 221 nm and a positive peak at 238 (characteristic of tyrosine) indicating a β-sheet like structure with 20% turn conformation (Fig. 3A).[8b] The less propensity of the tripeptide **4** for adopting a fully turn structure might be because of short peptide chain length and high rigidity of the scaffold **2** which in the turn conformation might be acting as a β-turn mimic as β-sheet folding nucleator.[3g-h]

To probe the intramolecular H-bonding in all peptides we used IR and variable temperature NMR (VT NMR)

spectroscopy. Thus, the presence of intramolecular H-bonded and free amide -NH stretching absorptions at 3281-3313 and 3409-3440 cm^{-1}, respectively, in the IR spectra of all the peptides also supported the turn structures (ESI†, Section 8).[9] From the VT-NMR experiment, strong intramolecular H-bonding involving the amide NH (of Phe) at i+3 was observed in peptide **3** supporting the type II β-turn structure in peptide **3** (ESI†, Section 8).[10] The amide NHs of tripeptide **4** containing the aromatic amino acid scaffold **2** showed medium to weak intramolecular H-bonding ability. The amide NH (of scaffold) and Leu-NH of both the termini in peptide **5** showed strong intramolecular H-bonding supporting the β-turn structure.

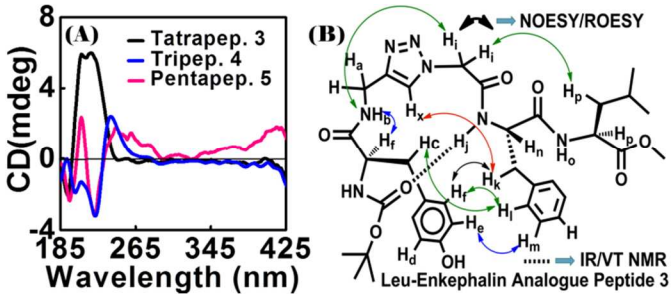


Figure 3. (A) CD spectra of peptides **3-5** in methanol. (B) Representative example of some important interactions observed in tetrapeptide **3**.

Next, the solution conformations of β-turn peptides **3-5**, were determined by studying, in detail, their ^{1}H NMR spectra in DMSO-*d*6. Thus, from the coupling of side chain protons of peptide **3** in ^{1}H NMR spectra it was observed that the Leu- side chain was very rigid and adopted predominantly one single conformation about χ_1 and χ_2 with an *anti*-relationship between βH(*pro-S*) and γH. Strong ROESY interaction between Leuβ-H/$CH2_{(C\text{-terminal})}$ of scaffold also supported this observation. The cross peak in the ROESY spectrum between LeuCαH and LeuδCH_3(pro-S) provided additional evidence of the rigidity of the Leu side-chain. The Phe and Tyr-side chains also showed their rigidity as well as the presence of one single conformation about χ_1. The NOESY and ROESY spectra of peptide **3** revealed the interactions among the aromatic hydrogens of Tyr and Phe suggesting their close proximity as well as rigidity in one single conformation. The presence of NOESY cross peak between $H_\alpha(i^{th})$/NH (i+1) also revealed a type-II β-turn conformation (Fig. 3B, and ESI†, Section 8).

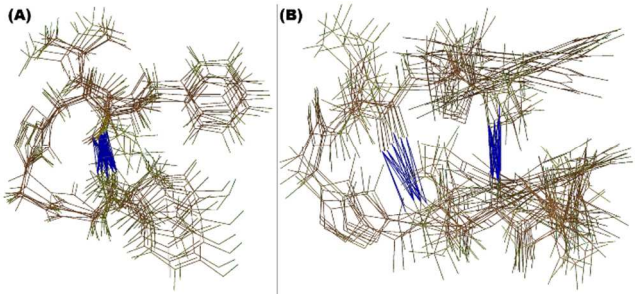


Figure 4. Molecular dynamics simulated clustering of conformers within 21 kJ/mole from the global minima of peptide **3** (A) and **5** (B).

The NOESY and ROESY spectra of peptide **4** revealed that the scaffold itself remained in a hairpin shape with interactions among the Triazole-CH/Aromatic-H; PheNH/ Aromatic-H; Aromatic-H/Tyr-OH leading to overall turn like shape. The NOESY and ROESY spectra of peptide **5** showed the interaction between H_α (of ^{TPy}**Ala**^{Do})/OMe-H (of ^{TPhen}**Ala**^{Do}); TPyAr-H (of ^{TPy}**Ala**^{Do})/$CH2_{(N\text{-terminal})}$ of scaffold, triazole-CH, LeuNH $_{(C\text{-terminal})}$; $CH2_{(N\text{-terminal})}$ of scaffold/LeuNH $_{(C\text{-terminal})}$ which supported the turn structure with the pyrene inside the loop closer to the triazole unit of the scaffold (ESI†, Section 8).

To gain insight into the conformation observed in peptide **3-5** molecular dynamics (MD) simulations were carried out using Schrodinger Macromodel (Maestro vs. 9.0) software package with OPLS 2005 force field.[9b, 11] The superimposition of eight minimum energy conformations out of 20 generated structures revealed that the peptide **3** took type II β-turn structure stabilized by backbone H-bonding involving the carbamate >CO at i and the amide NH (of Phe) at i+3 and aromatic−aromatic stacking which was also supported by CD, IR and VT-NMR study (Fig. 4A).[3] The folded conformation with close proximity of the parallelly oriented Phe and Tyr suggested that the peptide **3** might serve as a potential ligand for the δ-receptor.[12] The short chain tripeptide **4** showed some turn structure having backbone H-bond between carbamate >C=O at i and amide-NH (of scaffold) at i + 2 which is also possible as was revealed from a VT-NMR study reflecting turn structure. The existence of 20% turn structure in peptide **4** revealed from the CD spectroscopic study was thus supported by macromodel study. Moreover, it was stabilized by a second side chain H-bonding involving –OH of Tyr at N-terminus and amide NH of Phe at C-terminus (ESI†, Section 10). The type II β-turn structure as was evident from spectroscopic studies was also supported by a MD simulation for peptide **5** (Fig. 4B).

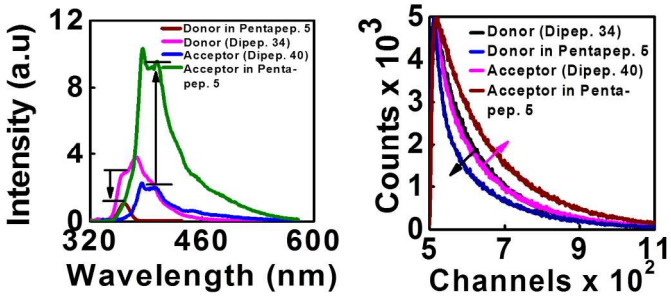


Figure 5. Steady state (A) and time resolved (B) fluorescence spectra of donor chromophore in absence or in presence of acceptor showing a FRET process in pentapeptide **5** in methanol.

Finally, we examined the possibility of photophysical interaction between the terminal triazolyl unnatural amino acids in fluorescent pentapeptide **5** in methanol. The UV-visible and fluorescence spectra of the ^{TPhen}**Ala**^{Do} containing dipeptide (**34**) and ^{TPy}**Ala**^{Do} containing dipeptide (**40**) revealed that the fluorescence spectrum of former overlapped significantly with the absorption spectrum of the latter (ESI†, Section 12). Moreover, the peptide **5** containing these two amino acids could selectively be excited at 300 nm (λ_{abs}^{max} of ^{TPhen}**Ala**^{Do}) where

there was very low absorbance of ^{TPy}**Ala**^{Do}. Therefore, these two triazolyl amino acids[4d] should form a FRET pair in our designed pentapeptide **5** where the conceptual donor amino acid ^{TPhen}**Ala**^{Do} and ^{TPy}**Ala**^{Do} acted as FRET donor and acceptor, respectively. With this observation we turned our attention to study the FRET process in detail.[13] Thus, when excited at absorption maximum of the donor, ^{TPhen}**Ala**^{Do} (λ_{ex} = 300 nm), it was observed that the fluorescence intensity of the acceptor, ^{TPy}**Ala**^{Do}, increased from that of the free acceptor emission by almost four-five times in presence of donor in peptide **5**. On the other hand, the fluorescence intensity of the donor, ^{TPhen}**Ala**^{Do}, in peptide **5** decreased almost three times of that of the free donor fluorescence in presence of acceptor, ^{TPy}**Ala**^{Do}. This ratiomatric change in fluorescence intensity of donor/acceptor revealed the visual evidence of FRET process from ^{TPhen}**Ala**^{Do} to ^{TPy}**Ala**^{Do} in peptide **5** (Fig. 5A).[13c] The calculated Förster radius (R_0), the efficiency of energy transfer (E) and the donor-acceptor distance (r) were found to be 31 Å, 85% and 28 Å, respectively. The occurrence of FRET process was also evident from a time resolved fluorescence study wherein we observed a decrease in donor life time (^{TPhen}**Ala**^{Do}; λ_{ex} = 293 nm, λ_{em} = 370 nm) from 13.7 ns to 2.0 ns. More interestingly, the lifetime of acceptor (^{TPy}**Ala**^{Do}; λ_{ex} = 293 nm, λ_{em} = 400 nm) in presence of donor was found to increase from 18.2 ns (in absence of donor) to 19.0 ns (in presence of donor) evidencing the FRET process (Fig. 5B, and ESI†, Section 13). [13d]

Conclusions

In conclusion, the easily accessible aliphatic and aromatic triazolo amino acids **1** and **2**, respectively, were introduced for the first time, as β-turn-mimetic constrained molecular scaffolds. The structural and conformational analysis of Leu-enkephalin analogue peptide **3** and fluorescent peptide **5** by various spectroscopic techniques and MD simulation studies established well-defined type II β-turn structure induced by the novel β-turn-mimetic constrained molecular scaffold, the triazolo amino acid **1**. Moreover, we established the FRET process in peptide **5** containing a new class of fluorescent unnatural triazolyl amino acids at the two termini. Under study are the explorations of turn mimetic **2** and other analogues in details and the sequence specific DNA binding event of tetra-amides of these molecular scaffolds which might lead to the generation of a new family of distamycin analogues.

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Notes and references

Bioorganic Chemistry Laboratory, Department of Chemistry, Indian Institute of Technology Guwahati, North Guwhati-781039, Assam, India. Fax: +91-361-258-2349; Tel: +91-361-258-2324.E-mail: ssbag75@iitg.ernet.in

Electronic Supplementary Information (ESI) available: [Synthesis, characterisation data, spectroscopic data, macromodel study and ^{1}H and ^{13}C NMR spectra]. See DOI: 10.1039/b000000x/

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