A convergent synthesis of new β-turn mimics by click chemistry†

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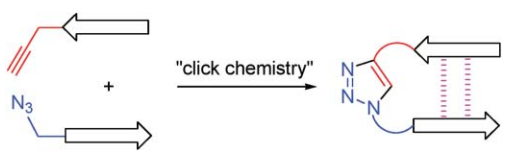
Alkyne–azide cycloaddition ("click" chemistry) between two peptide strands derivatized with terminal azide and alkyne, respectively, provides an efficient convergent synthesis of triazole ring-based new β-turn mimics.

Turns are important secondary structures of polypeptides along with α-helices and β-strands. Most protein foldings require reversals in the direction of their polypeptide backbones, which are often accomplished by a β-turn.[1] For gaining insight into the factors that affect the folding, stability, and interactions of β-sheets and proteins, and also for developing basic structural motifs for supramolecular and materials chemistry, a number of β-turn mimics employing various templates have been developed.[2] Despite these elegant studies, only relatively few examples of convergent synthesis of β-turn motifs have been reported and they are mostly limited to intramolecular cyclization reactions.[3] For many applications, it is highly desirable to develop efficient convergent syntheses of β-turn mimics through *intermolecular* ligation of preformed building blocks because they will provide us fast access to structurally diverse β-turn containing compounds.

In our search of a convergent synthesis of β-turn mimics, an efficient Cu(I)-catalyzed azide–alkyne cycloaddition reported recently in the literature attracted our attention.[4] This 1,3-dipolar cycloaddition is fast, simple to perform, compatible with many solvents and functional groups; and the formed triazoles can be used as peptide surrogates.[5] Our molecular modeling shows that a 1,4-connected 1,2,3-triazole ring might be commensurate with the required geometry for β-turns. This led us to propose that cycloaddition between peptide strands derivatized with azides and terminal alkynes may provide an efficient convergent synthesis of β-turn units based on the triazole ring. Presumably, simple mixing of two peptide modules derivatized with terminal azide and alkyne, respectively, should lead to formation of a triazole ring which may induce the formation of a β-turn (Scheme 1). In this communication, we report the demonstration of this concept using simple model peptide systems.



Scheme 1 "Click" to form turn

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To test our concept, we first examined a system in which two dipeptides are connected to a 1,2,3-triazole ring. Molecular modeling indicates that the propensity to form intramolecular amide–amide hydrogen bonding depends on the length of the spacers that connect the two amides to the triazole ring. Therefore, a series of 1,4-disubsituted 1,2,3-triazole-based tetrapeptides with various spacer lengths (**1–4**) were synthesized by cycloaddition between the corresponding alkynes and azides. Compounds **1–4** were characterized by NMR, FT-IR and mass spectrometry (Fig. 1; see ESI† for synthesis and characterization details). Comparison of ^{1}H NMR and FT-IR data for **1–4** reveals that the three-carbon linker in **3** is optimal for β-turn formation. An energy minimized conformation of **3** is shown in Fig. 1(c). The minimal β-turn unit in **3** is a 15-membered ring, which is reminiscent of the size for the dibenzofuran-based β-turn unit reported by Kelly and coworkers.[2e]

Some key ^{1}H NMR data for **3** are summarized in Fig. 1. The NOE cross peaks between the two amide proton H_C and H_V, and

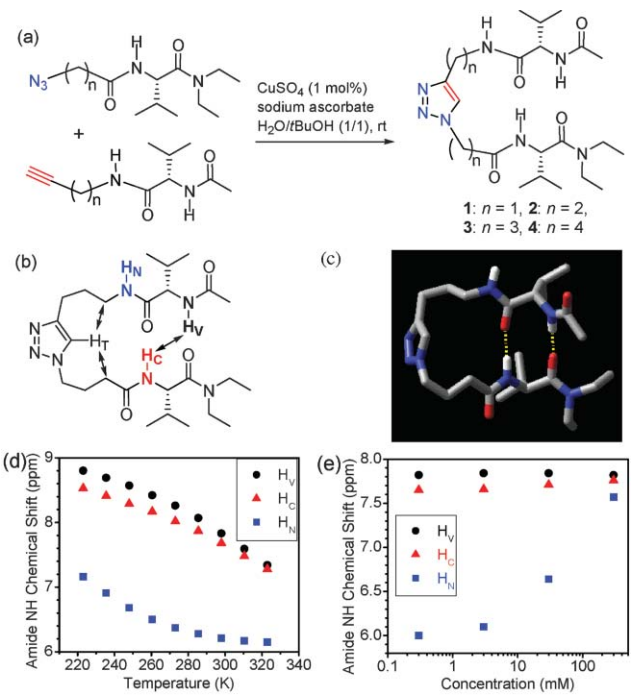

Fig. 1 (a) Synthesis of tetrapeptides **1–4** by azide–alkyne cycloaddition; (b) characteristic cross-peaks observed in ^{1}H–^{1}H NOESY spectrum of peptide **3** solution in $CDCl_3$ (3.0 mM); (c) an energy-minimized conformation for **3** (MacroModel 6.0 Amber* force field); (d) temperature dependence of the amide proton chemical shifts for peptide **3** solution in $CDCl_3$ (3.0 mM); and (e) concentration dependence of the amide NH protons chemical shifts of peptide **3** in $CDCl_3$ at 298 K.

between the bridgehead methylene protons and H_T in the 2D NOESY experiments agree with the formation of a β-turn structure (see Fig, S1 in ESI†). The resonance peaks of two amide NH protons, H_V and H_C, appeared at 7.83 and 7.68 ppm at 298 K, indicating strong hydrogen bonding. On the other hand, the chemical shift of H_N was 6.21 ppm, which is close to solvent exposed free amide NH. The concentration dependence of amide NH protons strongly supports the proposed conformation (Fig. 1(e)). Whereas H_V and H_C did not show any appreciable change in chemical shifts as the concentration was increased from 0.3 to 300 mM, H_N showed a significant downfield shift ($\Delta\delta$ 1.57 ppm) within the same concentration range. In the proposed conformation, only H_N is exposed to the solvent. As the concentration increases, H_N presumably could form an increasing extent of intermolecular hydrogen bonding which should result in a downfield shift. On the contrary, H_V and H_C form stable intramolecular hydrogen bonds that are insensitive to concentration change. As reported previously by other researchers,[2e,6] the temperature dependence of amide chemical shifts also reveals peptide conformation. Therefore, we carried out temperature dependence studies for the model peptide **3**. The temperature dependence of H_V and H_C were estimated to be 0.015 and 0.013 ppm K^{-1}, respectively, which supports these being hydrogen-bonded NH protons (>0.01 ppm K^{-1}).[2e,6] The temperature dependence of H_N was initially small but increased at lower temperature, which was attributed to an increased amount of intermolecular hydrogen bonding at lower temperatures.

Furthermore, FT-IR spectra of triazole amide **3** revealed two peaks at 3290 and 3435 cm^{-1} which correspond to hydrogen bonded and free amides NH, respectively (Fig. 2).[2e,g,7] The ratio of the two peaks was estimated to be *ca.* 2 : 1 (by height) favoring hydrogen bonded amide NH, which agrees with the proposed conformation. The ratio of hydrogen-bonded and free amide NH are significantly smaller for peptides **1**, **2** and **4**.

^{1}H NMR data revealed hydrogen-bond formation involving H_N and H_C in triazole **2**, suggesting a "misfolded" turn conformation (see Fig. S2, ESI†). In triazoles **1**, we observed both broadening of ^{1}H NMR peaks at low temperature and significant concentration dependence in the chemical shift of all NH protons, indicating intermolecular hydrogen bonding (see Fig. S3, ESI†). In triazole **4**, the amide protons have more upfield chemical shifts and only showed a small temperature dependence (see Fig. S4, ESI†). Thus, both NMR and FT-IR data confirm that triazole **3** has the optimal length of linkers for stable β-turn formation.

To further test the inherent propensity of the triazole ring for β-turn formation, a series of simple diamide compound **5–8** were synthesized. The extent of intramolecular amide–amide hydrogen bonding was evaluated by monitoring the temperature dependence of chemical shifts for the two amide NH protons (Fig. 3).[2e,6] Comparison of the data for **5–8** again indicates that the three-carbon linker in triazole amide **7** is optimal for the turn formation. The H_C in triazole **7** was more downfield shifted and showed a relatively large temperature dependence (0.012 ppm K^{-1})[2e,6] (Fig. 3), suggesting intramolecular hydrogen bonding. The H_N of **7** and all amide NH protons in **5**, **6** and **8** have more upfield chemical shifts and showed only moderate temperature dependence (Fig. 3 and Fig. S5a, ESI†). Not surprisingly, the single intramolecular hydrogen bonding is not sufficiently robust to completely inhibit intermolecular association at high monomer concentrations. Both H_C and H_N showed an appreciable increase in chemical shift at relatively high monomer concentration, which presumably resulted from some extent of intermolecular hydrogen bonding at high concentration range (see Fig, S5b, ESI†).

To exclude the possibility that amide NH protons are hydrogen bonded to nitrogen atoms on the triazole ring, two control compounds (**9** and **10**) were prepared by substituting either side of the amide groups of **7** with simple alkyl groups. The amide NH protons in both **9** and **10** showed very small temperature dependence (<0.006 ppm K^{-1}) and upfield chemical shifts (<6.0 ppm at 298 K). These data suggest that there is no appreciable intramolecular hydrogen bonding between amide NH protons and nitrogen atoms on the triazole ring. This further supports that the observed temperature dependence of H_C in **7** was due to intramolecular amide–amide hydrogen bonding.

In conclusion, we have developed an efficient convergent strategy for constructing a new β-turn mimicking unit through intermolecular alkyne–azide cycloaddition. We discovered that the tendency of β-turn formation for the triazole system strongly depends on the linker length and three-carbon linker is optimal for

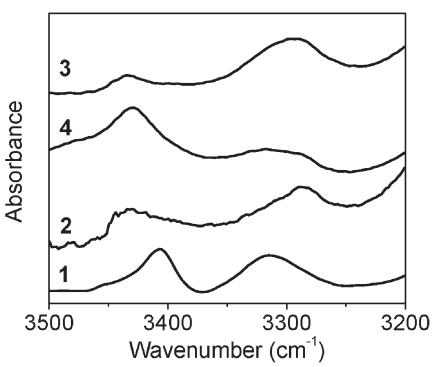

Fig. 2 Amide NH stretch region of the FT-IR spectra for 3.0 mM solutions of peptides **1–4** in $CDCl_3$ at rt.

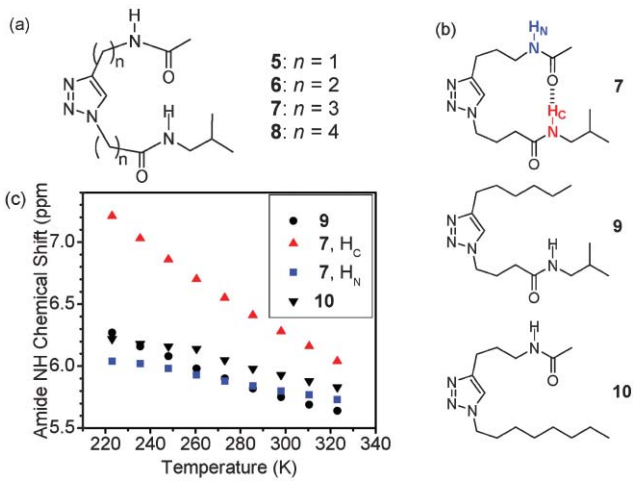

Fig. 3 (a) Chemical structures of peptides **5–8**; (b) comparison of peptide **7** and control compounds **9** and **10**; and (c) temperature dependence of the amide NH proton chemical shifts for peptide **7**, **9** and **10**, respectively (3.0 mM solutions in $CDCl_3$).

stable β-turn formation. Cycloaddition of peptide alkynes to peptide azides affords quick access to triazole-based β-turn units. This convergent synthesis is mild, efficient, and potentially tolerant to functional groups. These unique features make this strategy attractive for applications in areas including bioorganic, supramolecular, combichem, and materials chemistry. We are currently applying this strategy to both small molecule and polymer synthesis with the goal to control molecular architecture and high-order structures.

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