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Crystal-to-Crystal Synthesis of Triazole-Linked Pseudo-proteins via Topochemical Azide−Alkyne Cycloaddition Reaction

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Supporting Information

ABSTRACT: Isosteric replacement of amide bond(s) of peptides with surrogate groups is an important strategy for the synthesis of peptidomimetics (pseudo-peptides). Triazole is a well-recognized bio-isostere for peptide bonds, and peptides with one or more triazole units are of great interest for different applications. We have used a catalyst-free and solvent-free method, viz., topochemical azide−alkyne cycloaddition (TAAC) reaction, to synthesize pseudo-proteins with repeating sequences. A designed β-sheet-forming L-Ala-L-Val dipeptide containing azide and alkyne at its termini (N_3-Ala-Val-NHCH$_2$C≡CH, **1**) was synthesized. Single-crystal XRD analysis of the dipeptide **1** showed parallel β-sheet arrangement along the *b*-direction and head-to-tail arrangement of such β-sheets along the *c*-direction. This head-to-tail arrangement along the *c*-direction places the complementary reacting motifs, viz., azide and alkyne, of adjacent molecules in proximity. The crystals of dipeptide **1**, upon heating at 85 °C, underwent crystal-to-crystal polymerization, giving 1,4-triazole-linked pseudo-proteins. This TAAC polymerization was investigated by various time-dependent techniques, such as NMR, IR, DSC, and PXRD. The crystal-to-crystal nature of this transformation was revealed from polarizing microscopy and PXRD experiments, and the regiospecificity of triazole formation was evidenced from various NMR techniques. The MALDI-TOF spectrum showed the presence of pseudo-proteins >7 kDa.

Peptides constitute one of the most important building blocks of the biosphere, and many peptide-based natural biomaterials, such as silk, wool, etc., have instilled interest in development of synthetic peptide mimics with attractive properties.[1] Peptidomimetics, synthetic molecules that mimic the structure and often the function of peptides, attract much attention due to their easy synthesis, improved stability, and attractive properties compared to traditional peptides and find use in many fields from medicine to materials.[2] Isosteric replacement of the amide bond constitutes an important strategy for peptidomimetics synthesis, and such peptides with amide surrogates are called pseudo-peptides.[3] Triazole is a widely accepted isostere for peptide bonds due to its robust and biocompatible nature.[4] Polytriazolylpeptides are attractive polymers in the field of material science. However, the traditional synthesis of such polymers would involve Cu(I)-catalyzed click polymerization of monomers having azide and alkyne functionalities.[5] Apart from the unavoidable use of solvents, the purification of oligopeptides is cumbersome, as the metal catalyst can bind to the polymer formed. Topochemical reactions, proximity-driven reaction in a crystal lattice,[6] represent a plausible alternative that avoids catalysts, solvents, and purification. We have developed a topochemical azide−alkyne cycloaddition (TAAC) reaction for the synthesis of triazole-linked oligomers/polymers of carbohydrates and nucleosides.[7] We herein report the synthesis of triazole-linked pseudo-proteins or pseudo-polypeptides.

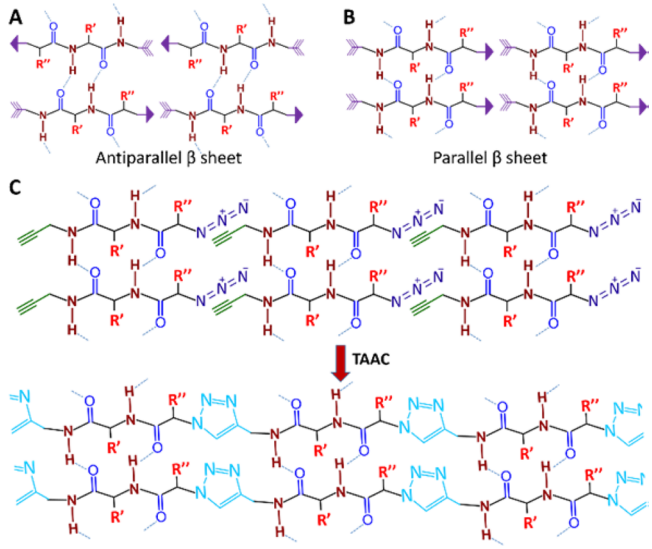

Figure 1. (A) Antiparallel and (B) parallel β-sheet arrangement of peptides. (C) Proposed head-to-tail arrangement of β-sheet-forming dipeptide mimic with complementary reacting motifs and their topochemical reaction.

The essential criterion for topochemical reaction is the proximity of reacting motifs in the crystal lattice, and design of molecules that, upon crystallization, place the reacting partners at distances suitable for their topochemical reaction is one of the major challenges in this field. It is well known that valine- and alanine-rich small peptides (di- or tripeptides) adopt β-sheet packing in their crystals (Figure 1A,B and Supporting Information (SI)). Also, the adjacent sheets arrange in such a way that peptides form head-to-tail arrangements in the direction perpendicular to the direction of the sheet formation. We envisioned that peptides with L-Ala and L-Val and modified with azide and alkyne, two complementary reacting motifs (CRMs),

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at their termini would crystallize via β-sheet packing. This would bring the azide and alkyne motifs of adjacent molecules in close proximity, enabling TAAC reaction between them, forming oligo/poly-triazolylpeptides (Figure 1C).

Dipeptide **1** was synthesized from L-valine and L-alanine (see the SI) and crystallized from a dichloromethane/toluene mixture. The Fourier transform infrared (FTIR) spectrum of crystals of the dipeptide **1** showed the CO stretching band at 1630 cm^{-1}, indicating formation of the β-parallel assembly.[8] Single-crystal X-ray analysis revealed that dipeptide **1** crystallizes in the monoclinic C_2 space group (Figure 2B). As anticipated,

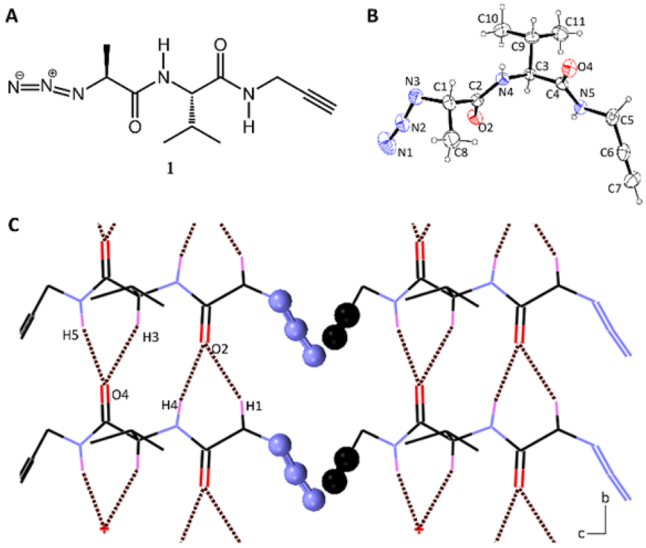

Figure 2. (A) Chemical structure and (B) ORTEP diagram of the dipeptide **1**. (C) Packing arrangement of dipeptide **1** along the *bc*-plane, showing the proximal placement of azide and alkyne motifs (highlighted in ball-and-stick model). The brown dotted lines represent the hydrogen bonds, N5—H5···O4 and N4—H4···O2, along the *b*-direction.

dipeptide **1** adopts parallel β-sheet packing along the *b*-direction via N5—H5···O4 (2.10 Å) and N4—H4···O2 (2.13 Å) hydrogen bonds (Figure 2C). In addition, two C—H···O hydrogen bonds, viz., C1—H1···O2 (2.31 Å, 150.8°) and C3—H3···O4 (2.44 Å, 149.8°), also help in stabilization of the β-sheet assembly. The shortest distance between the azide and alkyne is 3.7 Å (N1···C6), and the distances between the termini of the azide and alkyne are 4.1 and 4.7 Å. Though these distances satisfy the proximity criterion, the azide and alkyne motifs are not in a parallel orientation—the arrangement necessary for their cycloaddition—but at an angle of 72.2°. However, it would be possible to attain the essential parallel geometry by minor conformational changes of the flexible propargyl and the azide groups.

To check the feasibility of topochemical reaction of dipeptide **1**, its crystals were heated. The crystals did not melt, even at very high temperature (300 °C), suggesting that the crystals may be undergoing polymerization upon heating. Differential scanning calorimetry (DSC) analysis of the crystals of **1** also did not show any endothermic peak that can be ascribed to melting. On the other hand, the DSC profile showed an exothermic peak at ~150 °C, which could be due to the exothermic dipolar cycloaddition reaction between azide and alkyne (Figure 3A). The IR spectrum of heated crystals also suggested that the azide and the alkyne reacted during heating; while the signals due to the azide and alkynyl groups were sharp and strong in the starting crystals, the

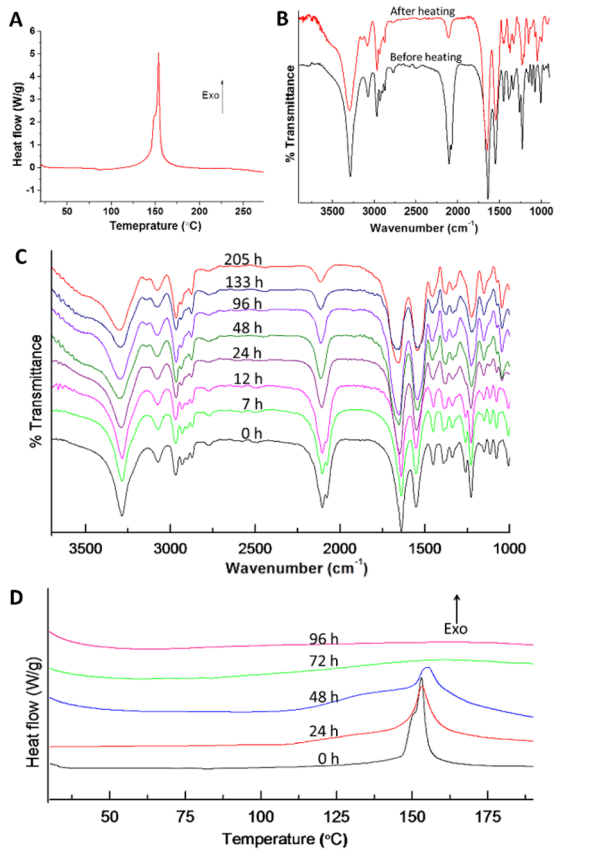

Figure 3. (A) DSC profile of crystals of dipeptide **1**. (B) IR spectra of crystals before and after heating at 153 °C. (C) Time-dependent IR spectra during the course of TAAC reaction of dipeptide **1**. (D) Time-dependent DSC analyses during TAAC reaction of dipeptide **1**.

intensity and sharpness of these peaks reduced to an insignificant level after heating, suggestive of their consumption (Figure 3B). Also, the solubility of the heated crystal was very low in common solvents (SI), ascribable to the formation of large polymers. The ^{1}H NMR spectrum of the crystals heated at 153 °C for 1 h showed a complex mixture of products having both 1,4- and 1,5-triazolyl linkages (Figure S2, SI), suggesting uncontrolled and non-selective polymerization.

To do a systematic study of this reaction, we have kept crystals of dipeptide **1** at 85 °C and withdrawn small amounts of them at regular intervals for various time-dependent studies. Time-dependent FTIR spectroscopy with these crystals revealed that the azide stretching band at 2103 cm^{-1} gradually decreased with time of heating. This suggests that the azide gets consumed gradually with time, probably by reacting with the alkyne (Figure 3C). As stated earlier, DSC analysis of the peptide showed an exothermic peak at 153 °C, ascribable to the sudden, uncontrolled reaction between azide and alkyne. DSC studies with the crystals kept at 85 °C for different durations revealed that the intensity of the exothermic peak due to uncontrolled thermal reaction at ~150 °C decreased with increase in duration of pre-heating at 85 °C (Figure 3D). This also suggests that some of the azide and alkyne groups are gradually reacting during the pre-heating, thereby reducing the numbers of azide and alkyne left for their uncontrolled reaction at high temperature. The extent of reaction also depends on the duration of pre-heating.

The kinetics of the reaction was studied by time-dependent ^{1}H NMR spectroscopy (Figure 4A). One portion of the pre-heated

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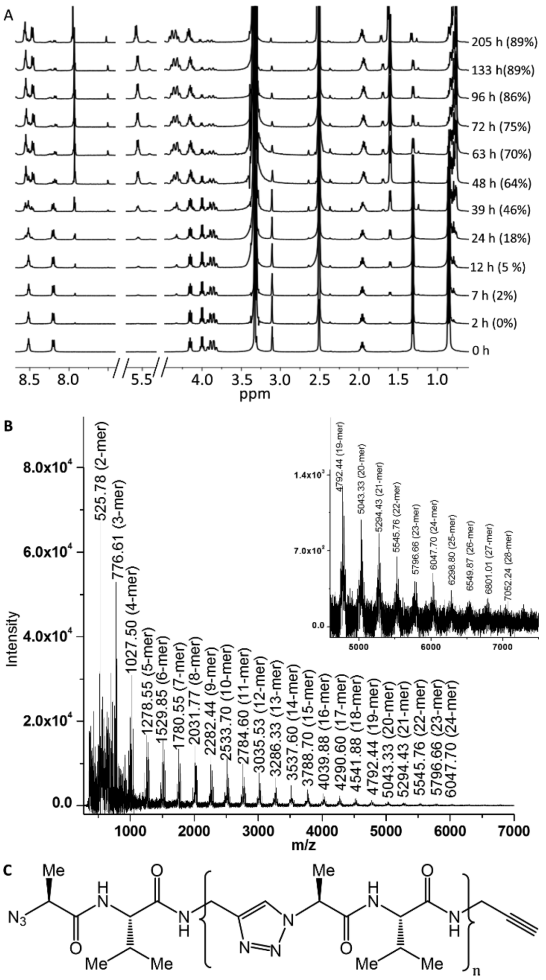

Figure 4. (A) Kinetics of the TAAC reaction at 85 °C monitored by ^{1}H NMR. (B) MALDI-TOF spectrum of polymerized crystals. (C) Chemical structure of oligopeptides obtained by TAAC.

crystals withdrawn at each time was analyzed by ^{1}H NMR spectroscopy after being dissolved in DMSO-d_6. It was observed that the reaction started at 7 h of heating, as evident from the emergence of new signals, notably the ones due to the triazolyl proton at δ 7.93, the methyne proton connected to triazole at δ 5.56, and methylene protons as dd at δ 4.42. The intensities of these signals increased gradually with time, along with concomitant reduction in the intensities of signals due to the dipeptide **1**. This trend continued until the reaction was 89% complete in 6 days, after which the reaction reached a state of stagnancy. A plot of % of the reaction against time revealed that the reaction followed a sigmoidal kinetics, as anticipated for a topochemical reaction (Figure S1B, SI).

The MALDI-TOF mass spectrum of the soluble fraction of the heated crystals showed peaks corresponding to oligomers from dimer to 28-mers (similar size to a 7 kDa protein) with a gradual decrease in their intensities. This decrease in intensities with size suggests that the solubility decreases with increase in size of the polymer (Figure 4B), and this is in line with the trend of other triazole-based polymers.[7a−c] Many natural proteins are as small as 7 kDa.[9] It is interesting to note that we could make pseudopeptides of sizes similar to small functional proteins.

While the reaction under high temperature proceeded nonselectively, distinct and clear ^{1}H NMR signals were observed for each proton throughout the reaction at 85 °C, suggesting the regiospecific formation of only one kind of triazole linkages (linkage homogeneity) in all the oligomers formed (Figure 4C). To know which of the two possible regiomeric triazoles is formed in the reaction, we have chromatographically isolated a few oligomers (trimer and tetramer) and characterized them extensively using various spectroscopic techniques (SI). In both cases, it was found that 1,4-triazole is formed in the reaction. The very similar NMR patterns of trimer, tetramer, and even higher oligomers suggest that, in all the oligomers/polymers, the 1,4-triazolyl linkage is conserved.

Even after 89% of the reaction was over, the crystals were morphologically intact. Polarizing microscopic images of dipeptide **1** before and after the polymerization reaction show birefringence, suggesting that the crystallinity is maintained throughout the course of the reaction (Figure 5A,B).[6ad] This was

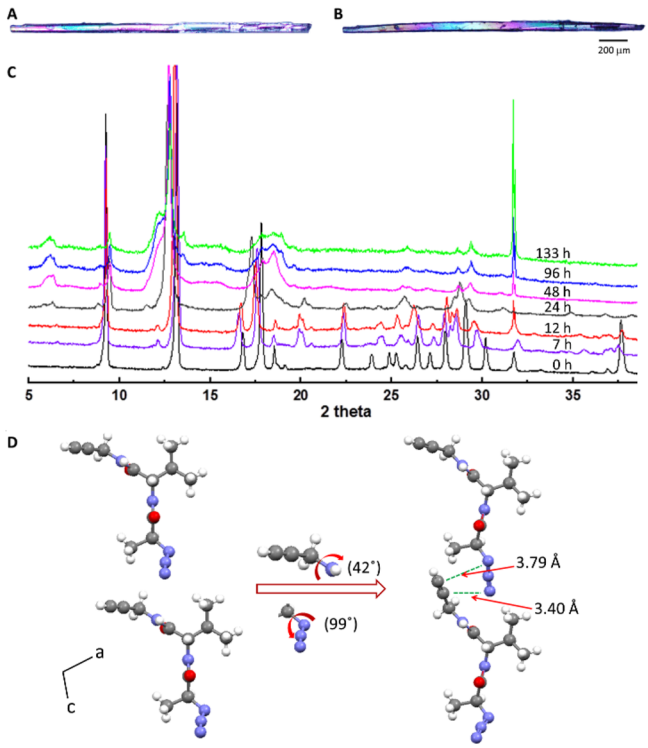

Figure 5. (A) Polarizing microscopic image of the crystals of dipeptide **1** (A) before and (B) after TAAC. (C) Time-dependent PXRD spectra of crystals of dipeptide **1** kept at 85 °C for various durations. (D) Plausible rotation of alkyne and azide, giving transition-state-like arrangement for the 1,4-regioisomer.

also evident from the time-dependent PXRD analysis. A comparison of the PXRD spectra of the crystals pre-heated for different durations suggested that the reaction occurred in a crystal-to-crystal fashion (Figure 5C). The diffraction pattern of polypeptide (a heated single crystal of dipeptide **1**) also suggested its crystalline nature, but we failed in solving its crystal structure (Figure S15, SI). It is clear that the crystals of the dipeptide **1** undergo crystal-to-crystal topochemical azide−alkyne cycloaddition upon mild heating, giving oligomers/polymers having only 1,4-triazolyl linkages. The regiospecificity is very interesting, as the parent crystal did not have any orientation of azide and alkyne that favored either of the two possible regioisomers. However, from a close look at the crystal structure, it is clear that both the propargyl and azide groups can rotate around the C−N single bond, without significant steric

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hindrance, to adopt a conformation wherein the azide and the alkyne orient in anti-parallel arrangement, favoring 1,4-triazole formation. Also in this conformation, the azide and alkyne are at much more favorable distances for their easy topochemical reaction (Figure 5D).

In summary, we have designed a dipeptide, decorated with two complementary reacting motifs, viz., azide and alkyne, which can self-assemble to form β-sheet structures in its crystal with proximally placed azide and alkyne from adjacent sheets. The crystals of this modified dipeptide underwent smooth topochemical azide−alkyne cycloaddition reaction upon heating, yielding triazole-linked polypeptides, which are otherwise difficult to synthesize using conventional solution-phase reactions. Though the azide and alkyne were not properly oriented for the topochemical reaction, in the crystal, the flexibility of the propargyl and azide groups and the empty space around them allowed their rotation, which led to a transition-state-like anti-parallel arrangement of azide and alkyne at short distance, and this led to the regiospecific formation of a 1,4-triazolyl-linked polypeptide in a crystal-to-crystal fashion. This is the first synthesis of a pseudo-protein or pseudo-polypeptide in the solid state. This proof-of-concept should generate interest in the topochemical synthesis of additional pseudo-polypeptides with repeating sequences for various applications.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.6b07538.

Synthesis and characterization of dipeptide **1** and oligomers (trimer **9** and tetramer **10**), including DSC, PXRD, SXRD, IR, NMR, TGA, and MALDI-TOF (PDF)
X-ray crystallographic data for **1** (CIF)

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Notes

The authors declare no competing financial interest.

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