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Head-to-Tail Dimerization and Organogelating Properties of Click Peptidomimetics

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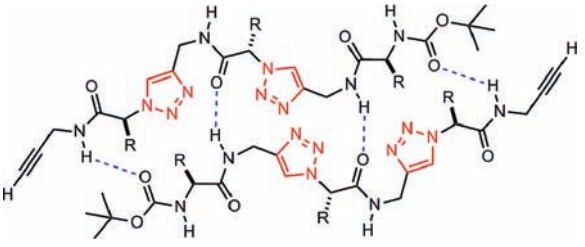
ABSTRACT



Click triazole-based oligopeptides 1−3 were found to self-dimerize ($K_{dim} \approx 10-680\ M^{-1}$) in a head-to-tail fashion based on ^1H variable concentration, 2D, and H/D exchange NMR, VPO, CD, FT-IR studies and Gaussian 03 simulations. The dimerization constant K_{dim} was shown to increase with increasing number of the amino acid units. Within the same oligomeric series, the K_{dim} value is strongly affected by the size of the *C*-terminal end group. The tripeptides 2 are also excellent organogelators of aromatic solvents.

The copper(I)-catalyzed cycloaddition between alkynes and azides (CuAAC),[1] a subclass of facile chemistry also known as click chemistry, has proven to be a highly effective synthetic tool to link two chemical entities together. Among the many CuAAC reaction partners, amino acids are of special interest because click linkage of them produces peptide-liked molecules that may possess interesting conformational and biological properties.[2] Many research efforts have been made to replace the peptide bond with the triazole unit as the latter has been considered as a nonclassical bioisostere of the former.[3] Although successes had been reported on using a single triazole unit to act as a helical component,[4] a β-turn unit,[5] and a *cis*/*trans*-prolyl ratio modifier[6] in linear peptidomimetics, only very limited examples have been disclosed on the conformational and supramolecular properties of oligo- and polytriazole analogs. Notably, Hecht reported the solvent dependent conformational properties of triazole-linked polypseudopeptides.[7] Other related findings had been reported by Arora[8] and Hughes,[9] but these are purely oligotriazole compounds without the peptide functionality.

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In Arora's studies,[8] the neighboring triazole units were found to align in an *anti*-fashion in acyclic triazolamers; however, such conformation preference was not found in the elongated triazolamers reported by Hughes.[9] Herein, we report the synthesis of a new class of click peptidomimetics **1**−**3** and their self-assembling properties (Figure 1). Notably, these peptides **1**−**3** self-dimerized ($K_{dim} \approx 10-680\ M^{-1}$) in a head-to-tail fashion in nonpolar solvents, and tripeptides **2** also form strong organogels with low minimum gelation concentrations (0.5−1% w/v) in aromatic solvents. The head-to-tail dimerization phenomenon is similar to the formation of antiparallel β-strand or β-turn structures in peptides, and hence this finding is of great importance in the design of new structural motifs in peptidomimetic chemistry.

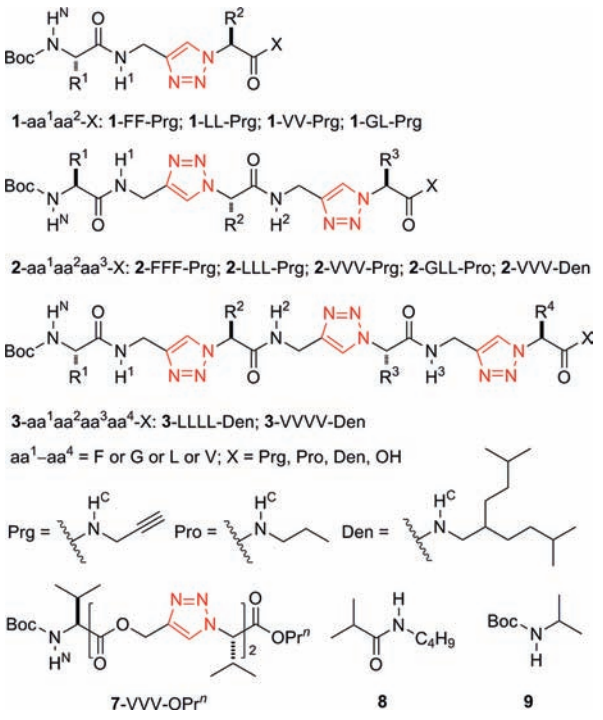
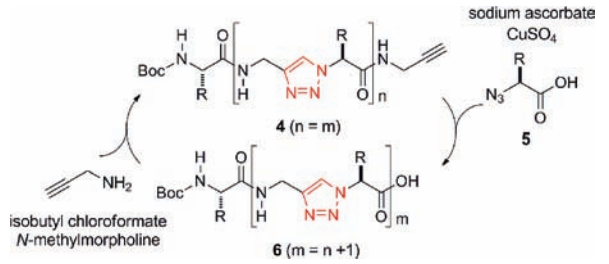


Figure 1. Structure of click di- **1**, tri- **2**, and tetrapeptides **3** with different *C*-end groups, triester **7**, amide **8**, and carbamate **9**.

The click peptidomimetics **1**−**3** with different amino acid compositions were prepared by an iterative synthetic procedure (Scheme 1). Hence, an *N*-Boc-α-amino acid *C*-terminal propargyl amide **4** was coupled to an α-azido acid **5** to afford the triazole linked peptide **6**, which was then converted to the corresponding *C*-terminal propargyl amide for further click coupling in the next cycle (Scheme 1). The overall yields of each cycle were 70−85% (see Supporting Information (SI) for details). It was noted that the solubility of the click peptides decreased gradually with increasing number of triazole units. Hence, while the dipeptides **1**-aa¹aa²-Prg and tripeptides **2**-aa¹aa²aa³-Prg with a *C*-terminal propargyl amide group (Prg) possess good solubility in organic solvents, tetrapeptides **3**-aa¹aa²aa³aa⁴-Prg are poorly soluble (except in DMF and DMSO) and require the introduction of a bulky hydrophobic dendritic[10] *C*-end group (Den = dendritic) to confer better solubility in nonpolar organic solvents. Three model compounds, a triester analog **7** with all three amide bonds replaced by ester linkages, an amide **8**, and a carbamate **9**, were also prepared for comparison studies. The structures of all compounds were confirmed by a ^1H, ^{13}C, and/or 2D NMR spectroscopy, mass spectral, and/or elemental analyses.

Scheme 1. Iterative Synthesis of Click Peptidomimetics



The self-assembling properties of the click peptidomimetics **1**−**3** were first analyzed using variable concentration ^1H NMR spectroscopy. It was noted that the signals of all amide NH protons of the di- **1**, tri- **2**, and tetrapeptides **3** exhibited concentration dependent shifts, suggesting the presence of intermolecular H-bonding (Figure 2; also see p S24 of SI for details). Furthermore, it was found that the amide NH chemical shift values in the homotripeptides **2**-aa¹aa¹aa¹-Prg were consistently larger than those of the corresponding NH in the homodipeptides **1**-aa¹aa¹-Prg at the same sample concentration (also at the same NH concentration). A similar trend was also observed when comparing the tetrapeptides **3**-aa¹aa¹aa¹aa¹-Den and the tripeptides **2**-aa¹aa¹aa¹-Den. This suggested that the strength of intermolecular H-bonding increased with increasing number of amides and triazole units. Incidentally, the amide NH chemical signals of the peptide with a *C*-Den group shifted to the upfield region as compared to the corresponding ones of the same peptide but carrying a *C*-Prg group, showing that the strength of the intermolecular H-bonding was impeded by the larger size of the *C*-Den end group.

The NH concentration dependence ^1H NMR data were then fitted into a dimerization model as reported by Hunter.[11] All data fitted perfectly well with a good correlation factor ($r^2 > 0.99$). For the dipeptide **1**-VV-Prg, a binding constant of $10 \pm 3\ M^{-1}$ was obtained. The K_{dim}

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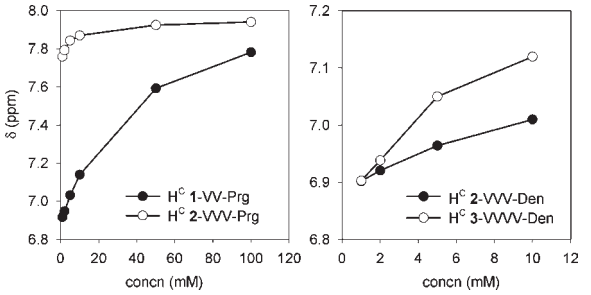

Figure 2. Dependence of ^{1}H NMR (700 MHz, $CDCl_3$, 25 °C) amide NH^c chemical shift values of **1**-VV-Prg, **2**-VVV-Prg, **2**-VVV-Den, and **3**-VVVV-Den on sample concentration. Note that the *x*-axis scales are different in the two figures.

values of the various tripeptides **2** were higher (25–280 M^{-1}) but were strongly dependent on the *C*-end group. Hence, for **2**-VVV-Prg, the $K_{dim}$was about 240 ± 30 M^{-1} in $CDCl_3$, but it dropped to 25 ± 5 M^{-1} for **2**-VVV-Den. Vapor pressure osmometry (VPO) analysis of a $CHCl_3$ solution of **2**-VVV-Prg using concentrations at the higher end range (50–133 mM) gave an apparent molecular weight (MW) corresponding to that of the dimeric species, while using concentrations at the lower end range (5–16 mM) produced an apparent MW of the monomeric species. These results were consistent with a monomer–dimer equilibrium with a K_{dim} of 240 M^{-1}. Incidentally, the apparent MW determined by VPO at the 50–133 mM concentration range in *n*-PrOH was about 1.8 times the theoretical value, suggesting it also exists predominately in dimeric form even in protic solvents. For the soluble tetrapeptide **3**-VVVV-Den, the K_{dim} was found to be 680 ± 100 M^{-1}. Interestingly, the triester analog **7** was found to exist as a monomer according to VPO studies, suggesting the importance of the amide units in the association binding process. Hence, both ^{1}H NMR and VPO studies confirmed that the larger the number of amide units, the stronger the K_{dim} value. The enhanced self-association with increasing number of amide units had been attributed to a zip effect reported earlier by us.[12] Similar findings had also been demonstrated by Hunter[11] in studies of aromatic amide oligomers.

2D NOESY ^{1}H NMR studies were then conducted on **2**-LLL-Prg and **2**-VVV-Prg in $CDCl_3$ to probe their solution structure. At low concentrations (1–5 mM), no long-range cross peaks could be identified. At 100 mM, NOE cross peaks were noted between the protons of the Boc group and N–H^C, protons of the Boc group and the acetylenic H, and α C–Hs of the first (H$^{\alpha 1}$) and the third (H$^{\alpha 3}$) amino acids (see p S31 of SI for details). Hence, the *N*-end Boc moiety of one molecule is in close proximity to the *C*-terminal acetylene group of another molecule. Unfortunately, for the dipeptide **1**-VV-Prg (small K_{dim}) and tetrapeptide **3**-VVVV-Den (limited solubility ~10 mM), we were unable to identify any long-range cross peaks in their 2D NOESY and ROSEY spectra.

To determine the binding mode of the tripeptides, hydrogen/deuterium (H/D) exchange experiments were performed according to the method described by Linton.[13] It was found that N–H^1 and N–H^C exchanged more slowly than the NH of amide **8**, indicating they acted as H-bond donors, while N–H^2 served as a H-bond acceptor (see p S29 of SI for details). For the carbamate N–H^N, it possessed a significant shorter exchange half-life in comparison to that of carbamate **9** and, hence, served as a H-bond acceptor.

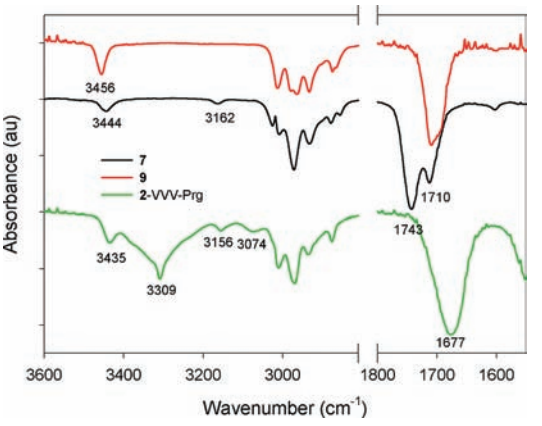

Figure 3. Stacked solution FT-IR spectra (30 mM in $CHCl_3$) of compounds **2**-VVV-Prg, **7**, and **9**.

Data from the N—H and C=O stretching regions in FT-IR spectra (at 2 cm^{-1} spectral resolution) provided additional information into the degree of H-bonding of these click peptides in nonpolar solvents (Figure 3). For the ester analog **7**, three major absorption peaks, attributed to the stretching frequencies of the carbamate N—H (3444 cm^{-1}), the ester C=O (1743 cm^{-1}), and the carbamate C=O (1710 cm^{-1}) bonds were found. These values were closely related to the corresponding absorption frequencies of non-H-bonded esters and carbamates. For tripeptide **2**-VVV-Prg, four absorption bands, due to the stretching frequencies of the carbamate N—H (3435 cm^{-1}), the H-bonded amide N—H (3309 and 3074 cm^{-1}), and the H-bonded C=O (1677 cm^{-1}) were found. There was also a small peak at 3162 cm^{-1} in the IR spectra of non-H bonded triester **7**, which could be assigned to the stretching frequencies of the triazole C—H. For the self-associating **2**-VVV-Prg, this signal was red-shifted to 3156 cm^{-1}. This finding suggested that the triazole C—H may function as a H-bond donor.[14]

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To further understand the dimerization features, a theoretical calculation on (**2**-VVV-Prg)$_2$ was performed using the Gaussian 03 software package.[15] Starting from a head-to-tail dimeric structure, the dimerization geometry was fully optimized using the B3LYP[16,17]/6-31G(d,p) method followed by harmonic vibration frequency calculations. In the optimized dimeric structure (Figure 4), two molecules were held together by four intermolecular H-bonds via C═O···N—H interactions and the H-bonding acceptor/donor characteristics were consistent with results obtained from the H/D exchange experiments. In addition, the *N*-terminal Boc moiety of one molecule was also found in close proximity to the *C*-end terminal acetylene group of another molecule.

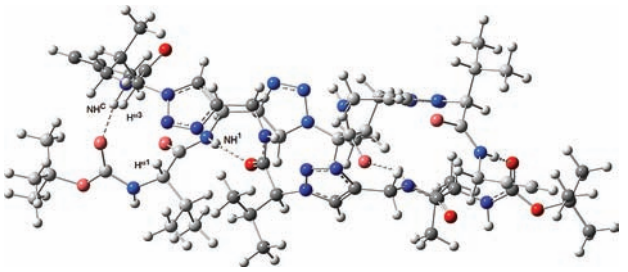

Figure 4. B3LYP/6-31G(d,p) optimized dimeric structure of (**2**-VVV-Prg)$_2$. For C═O···N—H^C (H-bond length: 1.91 Å; H-bond angle: 168.4°); for C═O···N—H^1: (H-bond length: 2.16 Å; H-bond angle: 153.6°).

The head-to-tail dimer structure is reminiscent of the antiparallel β-strand conformation that exists in many peptides. Although the correlation between the circular dichroism (CD) pattern and secondary structures for this new type of click peptidomimetics has not been fully established, CD analysis may provide useful information when used together with other spectroscopic techniques. The CD spectra of the tripeptide **2**-VVV-Prg (8.2 mM) and tetrapeptide **3**-VVVV-Den (5.0 mM) in *n*-PrOH both showed a positive band at 213 nm and a negative one at 230 nm (see p S36 of SI for details). This CD pattern was very similar to that of a typical β-sheet structure[18] except both peaks experienced a red shift of about 15 nm. The shift could be attributed to a difference in the solvent used (*n*-PrOH vs H_2O) and also to a potential aromatic stacking interaction[19] of the triazole moieties. Upon addition of 5% DMSO into the solution of tripeptide **2**-VVV-Prg, the CD β-sheet profile disappeared, suggesting the principal driving force for the dimerization was intermolecular H-bond interaction.

The trimeric compounds **2**-aa^{1}aa^{2}aa^3-Den and **2**-aa^{1}aa^{2}aa^3-Prg were found to be good organogelators in organic solvents. Their minimum gelation concentrations were in the range of 0.5−1% w/v in aromatic solvents. The gel-to-sol transition temperature (T_{gel}) of **2**-aa^{1}aa^{2}aa^3-Den is 25 °C lower than that of **2**-aa^{1}aa^{2}aa^3-Prg at the same gelator concentration, consistent with the weaker self-association power of the former. Their unique gelation properties in aromatic solvents suggested that the triazole units might also be responsible for the gelation due to $\pi-\pi$ interaction with the aromatic solvents. Scanning electron microscopic analysis of a freeze-dried sample of **2**-aa^{1}aa^{2}aa^3-Prg showed the existence of long fibrous structures with a diameter in the range of 30−60 nm, suggesting that these were supramolecular bundles resulting from the further self-assembly of individual dimers (see p S41 of SI for details).

In summary, a new class of click peptidomimetics **1**−**3** was prepared in good yields by an iterative synthetic procedure. These peptidomimetics were found to form head-to-tail dimers in both nonpolar and protic organic solvents. The formation of such head-to-tail dimeric structures is very unique and is reminiscent of the β-strand structure found in many peptides. Furthermore, the high functional group tolerance of the click coupling protocol allows their facile preparations without invoking protective group chemistry. Hence, these click peptidomimetics are potentially useful structural motifs in biomimetic chemistry.

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Supporting Information Available. Full synthesis details and characterization, and ^{1}H and ^{13}C NMR spectra for **1**−**4**; ^{1}H NMR variable concentration, VPO, and CD spectra of **1**−**3**, 2D NOSEY and H/D exchange data, SEM studies, gelating data and Gaussian 03 simulation results of **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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