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# Intrinsically Photoswitchable α/β Peptides Toward Two-State Foldamers

Giulia Marafon,[a] Marco Crisma,[b] Alessandro Moretto*[a,b]

**Abstract:** A simple, unsaturated, *E*-*Z* photoisomerizable β-amino acid, (*Z*)-3-aminoprop-2-enoic acid, has been introduced into peptide foldamers via a one-pot chemical coupling, based on Pd/Cu-catalyzed olefin oxidative amidation, between two peptide segments carrying, respectively, a -Gly-$NH_2$ residue at the C-terminus and an acryloyl group at the N-terminus. Reversible conversion between the *Z* and *E* configurations of the 3-aminoprop-2-enoic linkage was achieved photochemically. A crystallographic analysis on two model compounds shed light on the consequences, in terms of 3D-structure and self-association properties, brought about by the different configuration of the unsaturated linkage. As a proof of concept, *E*-*Z* photoisomerization of a 3-aminoprop-2-enoic acid residue, inserted as the junction between two conformationally distinct peptide domains (one helical while the other β-sheet promoter), allowed supramolecular self-association to be reversibly turned on/off.

The field of foldamers[1] encompasses a growing set of conformationally controlled, oligomeric molecules able to develop well defined 3D-architectures. Examples include peptides based on non-coded α-amino acids, β- and γ-peptides, azapeptides, oligoureas, aromatic oligoamides.[2-9] Typically, a foldamer is designed to highly populate a single conformation. Development of foldamers able to switch their 3D-structure between two states in a controlled way, although amenable in principle to interesting applications, is particularly challenging.[10-14] To this end, an appealing potential is offered by *E*-*Z* photoisomerization of double-bond containing molecules. We have recently shown that incorporation of a fumaramide or maleamide unit at the N-terminus of a peptidomimetic foldamer allows this latter to exhibit a functional response as a result of photoisomerization.[15] In this work, we expanded the photo-induced control of conformational switches to appropriately designed foldamers incorporating at an internal position an unsaturated β-amino acid residue, namely (*E*/*Z*)-3-aminoprop-2-enoic acid [or (*E*/*Z*)-3-aminoacrylic acid], which can be viewed as the $C^{\alpha,\beta}$-unsaturated analog of β-alanine (β-Ala). By combining this latter abbreviation with the $\Delta^E/\Delta^Z$ terminology commonly used for $C^{\alpha,\beta}$-didehydro analogs of protein amino acids, the *E* and *Z* isomers of 3-aminoprop-2-enoic acid are herewith abbreviated as $\Delta^E$βAla and $\Delta^Z$βAla, respectively. The instability of $\Delta^E$βAla / $\Delta^Z$βAla derivatives carrying the free amino group hampers their use as amino component in standard peptide coupling reactions. Not surprisingly, peptides containing this residue have not been reported yet. A way around the synthetic problem may be offered by an approach based on the oxidative amidation of conjugated olefins, reported by Kim, Chang and coworkers, who efficiently prepared simple *Z*-configured enamides by reacting primary amides with conjugated olefins in the presence of a Pd/Cu co-catalyst system.[16] By adapting this strategy, starting from Z-Gly-$NH_2$ (Z, benzyloxycarbonyl) and ethyl acrylate we successfully synthesized the (*Z*) dipeptide Z-Gly-$\Delta^Z$βAla-OEt **1**, which was subsequently quantitatively photo-converted to its *E*-isomer **2** by irradiation at 290-320 nm (Figure 1A). Compounds **1** and **2** were crystallographically characterized. In the structure of **1** (Figure 1B) Gly(1) is nearly fully-extended ($\phi_1,\psi_1$ = 156.1°,-176.0°).[6] The conformation of $\Delta^Z$βAla(2) is described by three torsion angles, related to rotations about the N-$C^\beta$ ($\phi_2$ = -176.2°), $C^\beta$-$C^\alpha$ ($\theta_2$ = 1.5°), and $C^\alpha$-C ($\psi_2$ = 176.9°) bonds. This arrangement allows formation of an N-H···O=C intra-residue H-bond which closes a 6-atom *pseudo*cycle. In the structure of **2** (Figure 1C), not only Gly(1) is fully extended ($\phi_1,\psi_1$ = 178.5°,-178.8°), but also all of the φ, θ, and ψ backbone torsion angles of $\Delta^E$βAla(2) are within -179.2° and 173.2°. The packing modes of **1** and **2** are in part similar, being characterized by layers of antiparallel molecules (Figures 1B and 1C). However, they differ by the number of intermolecular H-bonds. Specifically, each molecule of **1** makes two N-H···O=C intermolecular H-bonds on one side only, whereas on the opposite side only C-H···O and van der Waals contacts are observed (Figure 1B; see also *SI*, Table S5) because the intramolecularly H-bonded conformation adopted by $\Delta^Z$βAla prevents its N-H group from being approached within H-bonding distance by any other potential H-bond acceptor of the flanking molecule. Conversely, for **2**, each molecule is connected to its neighbors by four intermolecular H-bonds, two on each side (Figure 1C), giving rise to a flattened β-sheet.

Notably, attempts to carry out the olefin oxidative amidation by replacing either the benzyloxycarbonyl $N^\alpha$-protecting group (with Fmoc or Boc), or Gly with other amino acids (e.g., Ala, Leu, Val) did not afford any product, in all probability owing to the different stability of the protecting groups close to the reaction site and to the steric hindrance exerted by the side chains, respectively.

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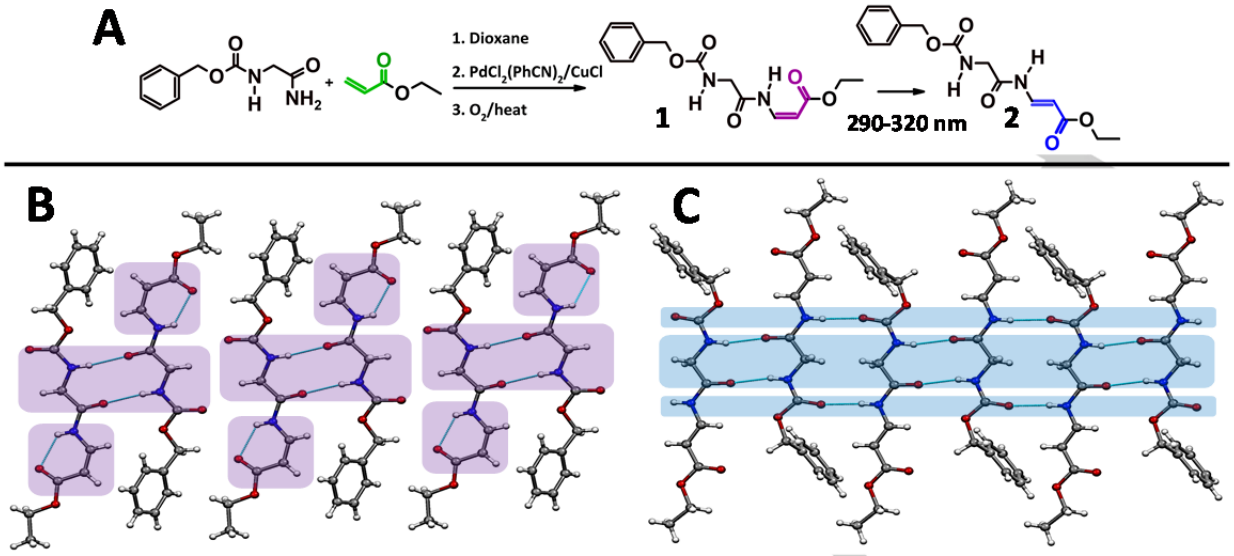


**Figure 1.** (**A**): Synthesis of Z-Gly-$\Delta^{Z}$βAla-OEt (**1**) and its photo-conversion to Z-Gly-$\Delta^{E}$βAla-OEt (**2**). (**B**) and (**C**): Packing modes in the crystal structures of **1** and **2**, respectively. Intra- and intermolecular H-bonds are indicated by dashed lines.

Next, we explored the introduction of the unsaturated β-amino acid *internally* to the peptide backbone. To this aim, a set of $N^{\alpha}$-acrylamide-functionalized α-amino acid ester derivatives of the type Acr-Aaa-OR [Acr = acryloyl; Aaa = Gly, Ala, Leu, Aib (α-amino-isobutyric acid); R = methyl or ethyl] were prepared and placed to react with Z-Gly-$NH_2$ through olefin oxidative amidation to afford the corresponding tripeptides of general formula Z-Gly-$\Delta^{Z}$βAla-Aaa-OR. Yields decreased from excellent to poor with increasing bulk of Aaa (Gly: 88%; Ala: 53%; Leu: 28%; Aib: 8%). However, the *Z* / *E* stereoselectivity of product formation was comparable in all cases (≅ 15:1). A representative example of the photo-conversion of these *Z*-configured tripeptides to the corresponding *E*-isomers and back to their *Z*-form is illustrated in Figure 2 in the case of the Z-Gly-$\Delta^{Z}$βAla-Leu-OMe (**3**) / Z-Gly-$\Delta^{E}$βAla-Leu-OMe (**4**) pair. We found by HPLC monitoring (Figure 2A, right panel) that the **3**-to-**4** conversion occurs quantitatively within 20 min by irradiation at 290-320 nm, whereas the reverse process, carried out by irradiation at 254 nm (to exploit a slight difference in the UV-Vis absorption profiles of **3** and **4**; see *SI, Figure S37*), reaches a photo-stationary equilibrium in which (*E*) **4** and (*Z*) **3** are present in a 30% : 70% molar ratio. Photo-conversions (*Z* to *E* and *E* to *Z*) were also monitored by $^{1}$H-NMR spectrometry (Figure 2B).

As a further step, we placed a $\Delta^{Z}$βAla residue as the junction between two conformationally distinct peptide domains. Specifically, Z-$(Ala\text{-}Aib)_2$-Ala-Gly-$NH_2$ **5** (highly folded, $3_{10}$-helical)[18-21] was combined through olefin oxidative amidation with the β-amyloid[22] 16-20 segment derivative Acr-$Lys^{(Boc)}$-Leu-Val-Phe-Phe-OH **6**, giving Z-$(Ala\text{-}Aib)_2$-Ala-Gly-$\Delta^{Z}$βAla-$Lys^{(Boc)}$-Leu-Val-Phe-Phe-OH **7** in 64% yield (Figure 3A). Again, photo-conversion from (*Z*) **7** to (*E*) **8** was achieved quantitatively.

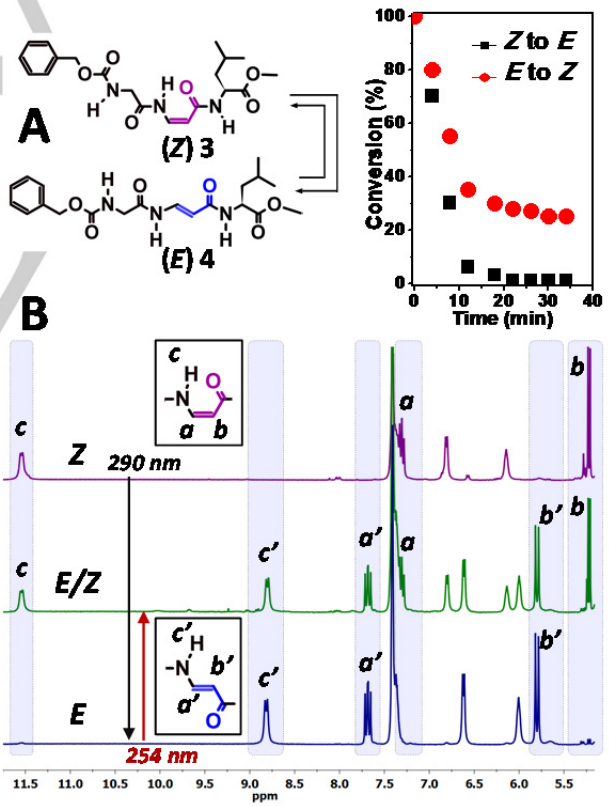


**Figure 2.** (**A**) Left: formulas of Z-Gly-$\Delta^{Z}$βAla-Leu-OMe (**3**) and Z-Gly-$\Delta^{E}$βAla-Leu-OMe (**4**). Right: time evolution of their photo-conversions monitored by HPLC. (**B**) $^{1}$H NMR spectra ($CD_3CN$) of (*Z*) **3** (top), its (*E*) isomer **4** obtained by irradiation at 290-320 nm (bottom), and the mixture of *E*/*Z* isomers resulting as photo-stationary equilibrium upon irradiation of the *E* isomer at 254 nm (center). Diagnostic signals are marked. $^{3}$J coupling constants between olefinic protons are 9 Hz for (*Z*) **3** and 14 Hz for (*E*) **4**.

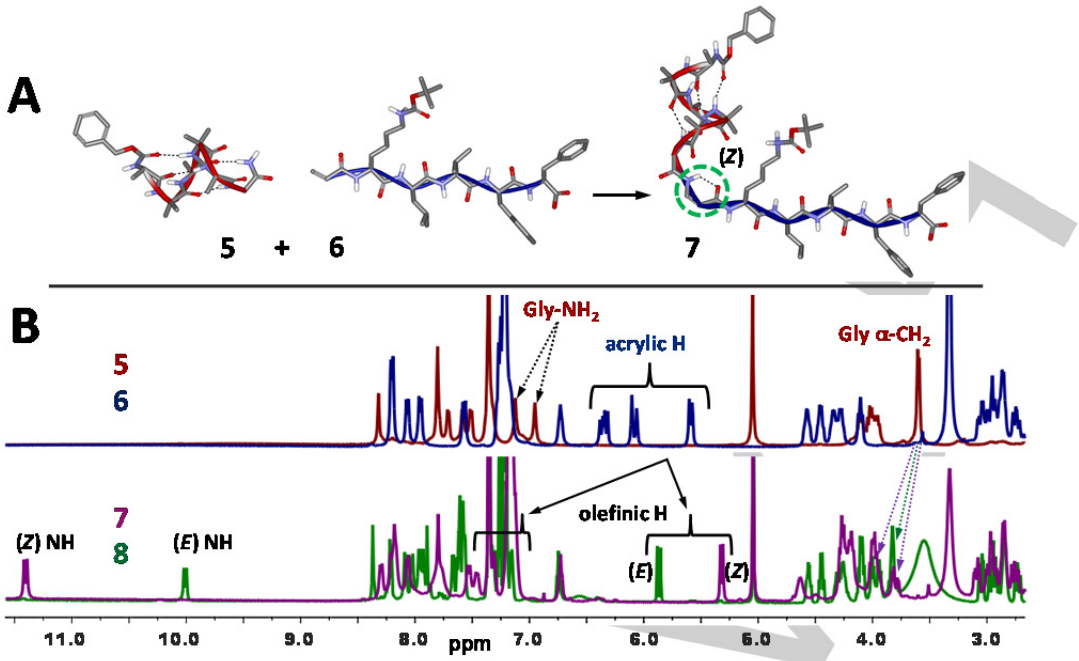


**Figure 3.** (**A**) Computer-generated model of Z-(Ala-Aib)$_2$-Ala-Gly-$\Delta^Z$βAla-Lys$^{(Boc)}$-Leu-Val-Phe-Phe-OH (**7**), which combines a helical domain (from **5**) with a β-sheet promoting segment (from **6**) The -$\Delta^Z$βAla- junction is circled. (**B**) Overlap of the $^1$H NMR spectra (DMSO-$d_6$) of the two reactants (upper part, **5**, red trace, and **6,** blue trace) and (lower part) of the resulting product (*Z*) **7** (violet) and its *E* isomer **8** (green). Diagnostic signals are marked.

Figure 3B compares the $^1$H NMR spectra of the two reactants **5** and **6** with that of the resulting product (*Z*) **7**, highlighting the disappearance of the acrylate proton signals belonging to **6** and of the Gly-NH$_2$ signals belonging to **5**, accompanied in **7** by a downfield shift of the Gly α-CH$_2$ signals and the onset of the olefinic signals and of the low-field $\Delta^Z$βAla NH. The latter signal moves upfield upon photoisomerization to (*E*) **8**.

Notably, the Lys side-chain Boc-protection survived in **7**. Its subsequent removal afforded Z-(Ala-Aib)$_2$-Ala-Gly-$\Delta^Z$βAla-Lys-Leu-Val-Phe-Phe-OH **9** which, in turn, was photo-converted to its $\Delta^E$βAla-containing isomer **10**. Upon standing, the 5 mM water solution of (*E*) **10** turned into a stable hydrogel, suggesting that the *E*-isomeric state of the olefinic junction allows efficient β-sheet formation involving the β-amyloid-derived segment of the foldamer without interference from the helical domain (Figure 4B). Hydrogelation is not observed in the case of **9**, in all probability because the *Z* configuration of the olefinic junction, in addition to sequestering the $\Delta^Z$βAla NH group from intermolecular H-bonding, makes a kink in the overall shape of the foldamer (Figure 4A).

According to our model (Figure 4B), **9** may thus self-associate at best into dimers (as indirectly supported by the crystal structure of **2**), but extensive β-sheet formation does not occur. Interestingly, the hydrogel formed by **10**, upon 15 min irradiation at 254 nm, returns to a fluid state, in which the (*E*) **10** and (*Z*) **9** isomers are present in nearly equimolar amounts.

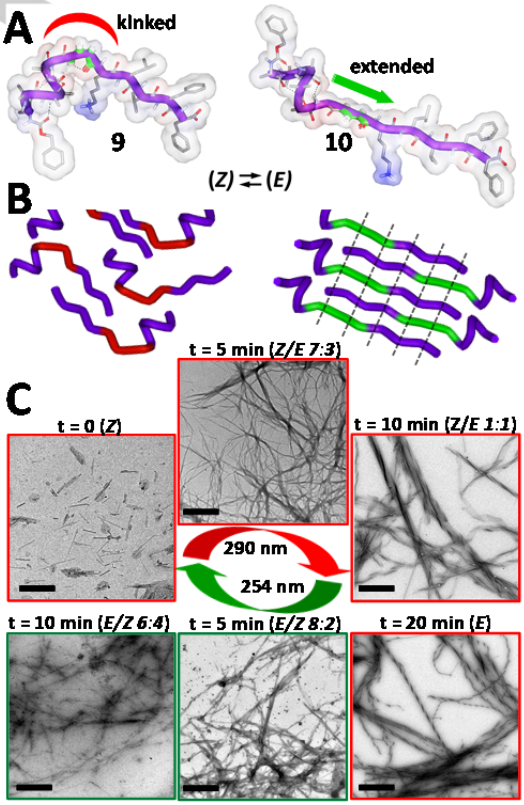


**Figure 4.** (**A**) Models of the two-domain foldamers Z-(Ala-Aib)$_2$-Ala-Gly-$\Delta^Z$βAla-Lys-Leu-Val-Phe-Phe-OH (*Z*) **9** and its *E*-isomer **10**. (**B**) Schematic representation of the self-assembly modes of (*Z*) **9** and (*E*) **10**. (**C**) TEM images taken at time intervals monitoring the photo-conversion of (*Z*) **9** to (*E*) **10** and back from (*E*) **10** to a 6:4 (*E*) **10** / (*Z*) **9** mixture (scale bars: 500 nm).

Morphological insight on these phenomena was provided by TEM analysis (Figure 4C). Specifically, we recorded a set of TEM images, beginning with a sample cast from a 5 mM water solution of **9** and then analyzing samples taken after 5, 10 and 20 min of irradiation at 290-320 nm. The increasing (*E*) **10** / (*Z*) **9** molar ratio (from 0 to 95%) resulting from the photo-conversion gives rise to formation of fibers of increasing size. Such fibers significantly dissociate upon partial back photo-conversion (from 95% **10** to a 6:4 mixture of **10** and **9**) promoted by 254 nm irradiation (see *SI, p. S37-S40*).

To summarize, we succeeded in introducing the simplest unsaturated (*E*-*Z* photoisomerizable) β-amino acid, namely (*Z*)-3-aminoprop-2-enoic acid ($\Delta^Z$βAla), into peptide foldamers, *via* a one-pot chemical coupling based on Pd/Cu-catalyzed olefin oxidative amidation. A limitation to such synthetic approach, at least in our hands, is that among protein amino acids only Gly seems to be allowed at the position preceding the *in situ* generated $\Delta^Z$βAla unit. Higher versatility is tolerated about the nature of the following residue, although yields decrease with increasing bulk of its side chain. This novel type of ligation gives access to peptidomimetics and foldamers, some properties of which, including 3D-structure and self-association tendency, can be tuned photochemically. This view is supported by our crystallographic analyses on two model compounds. As a specific case, the switch between the *Z* and *E* isomers of a 3-aminoprop-2-enoic acid residue, inserted as the junction between two different peptide domains (one helical while the other β-sheet promoter), allows supramolecular self-association to be reversibly turned on/off. We are confident that the photo-switchable $\Delta^Z$βAla / $\Delta^E$βAla system may provide a valuable structural element in the design of two-state functional foldamers for biomimetic and nanotechnological applications.[10-14,23,24]

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## Conflict of interest

The authors declare no conflict of interest.

**Keywords:** chemical ligation • foldamers • peptidomimetics • photoisomerization • supramolecular chemistry

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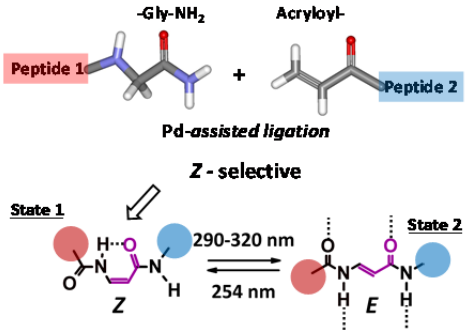
# COMMUNICATION

A simple unsaturated β-amino acid was introduced into peptide foldamers via a one-pot chemical coupling between two peptide segments carrying, respectively, a primary amide at the C-terminus and an acryloyl group at the N-terminus. This novel type of ligation gives access to peptidomimetics and foldamers with photochemically tunable 3D-structure and self-association tendency.

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