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β-Turn Mimics by Chemical Ligation

Selma Crecente-Garcia, Albane Neckebroeck, J. Stephen Clark, Brian O. Smith, and Andrew R. Thomson*

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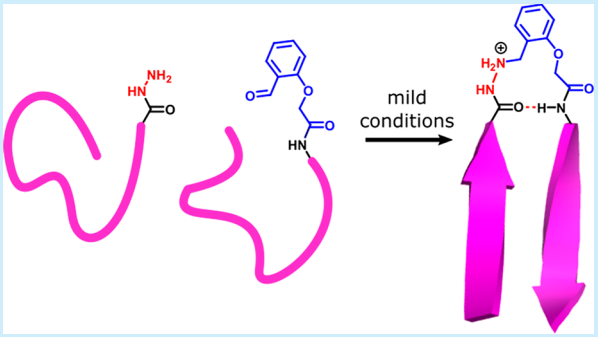
ABSTRACT: We report a simple reductive amination protocol to ligate two peptides, while simultaneously installing a β-turn mimic at the ligation junction. This strategy uses commercially available materials, mild chemical conditions, and a chemoselective ligation reaction of unprotected peptide substrates accessed through standard solid phase methods. This system was implemented in a designed β-hairpin system, and biophysical analysis demonstrates effective mimicry of the β-turn.



Chemical ligation methods have proven to be an enabling technology in chemical biology.[1] However, with the notable exception of native chemical ligation,[2] these chemistries typically leave behind a residual chemical functionality that serves no purpose beyond connecting the two ligation partners. In some cases it has been suggested that the residual functionality can act as a mimic of an amide bond;[3] however, there are as yet no reports in the literature of a ligation junction being designed to act as a mimic of a larger element of protein structure. Herein we present a simple and synthetically accessible ligation junction that enables the rapid and high-yielding chemical ligation of two peptide fragments, and which in doing so forms a mimic of a β-turn.

β-Turns are ubiquitous protein structural elements that play a key role in protein folding and function.[4,5] β-Turns comprise four amino acid residues, labeled *i* to *i*+3, and are generally defined by the presence of a hydrogen bond between the amide carbonyl of residue *i* and the amide NH of residue *i*+3 (Figure 1a).[6] This leads to a structure in which the protein backbone is folded back on itself. These turns are often found connecting secondary structure elements that interact with each other, such as in β-hairpins, where the two strands of an antiparallel β-sheet are linked by a β-turn. Modification of these turns has been proven key for the development of antimicrobial peptides[7] and protein−protein interaction inhibitors.[8] β-Turns have therefore been the focus of previous efforts at developing chemical mimics of their three-dimensional structure.[9,10] Previous examples of β-turn mimics either have been accessed through multistep organic syntheses[11] or are based on simple building blocks[12] that lack structural preorganization, limiting their utility in peptide and protein science.

We have made use of the established β-hairpin tryptophan zipper (TrpZip1)[13] as a model system for these studies. TrpZip1 is a designed 12 residue amino acid sequence that folds into a soluble, monomeric hairpin structure in solution. Importantly, TrpZip1 has been extensively characterized in terms of its 3D structure and thermodynamics of folding, and has been shown to contain a β-turn, modification of which can alter the stability of the peptide fold. Therefore, the TrpZip1 scaffold is an ideal base structure through which to investigate the conformational and thermodynamic effects of alteration to the β-turn.

In considering mimics of the β-turn structure, we reasoned that ortho-substituted aromatic systems would be a good starting point to approximate the geometry of the peptide chain and facilitate a hydrogen bond analogous to that between the *i* and *i*+3 positions in a canonical β-turn. We further reasoned that a reductive amination reaction between an acylhydrazine and an aldehyde could be effected under mild, chemoselective conditions and would yield an acylhydrazine moiety capable of forming hydrogen bonding interactions. These considerations led us to investigate the non-natural amino acid residue present in peptide **1** (magenta region, labeled "BTM", Figure 1b). Importantly, the precursors to this unit are synthetically accessible through standard peptide chemistry from commercially available materials. Simple

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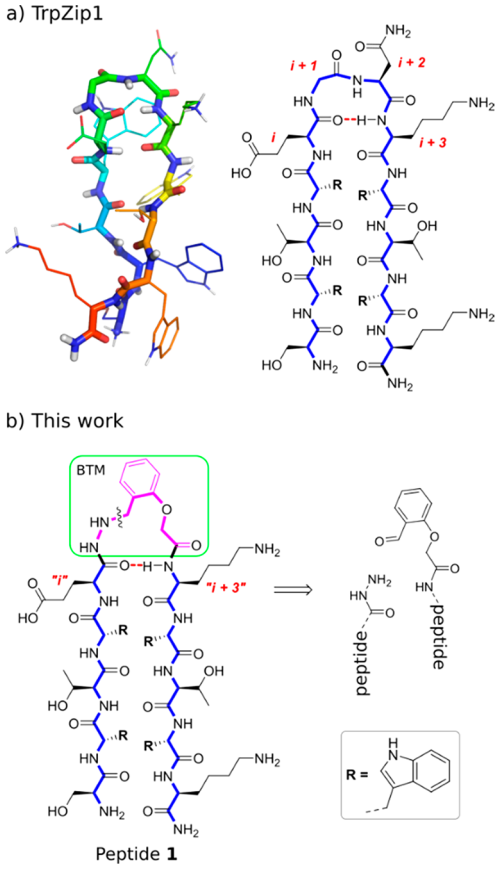

Figure 1. Original TrpZip1 peptide and peptide **1**. (a) 3D and structural view of TrpZip1 (PDB: 1le0), showing residue numbering convention and key hydrogen bond for the β-turn. (b) TrpZip1 sequence containing the β-turn mimic (BTM, in magenta) and indicating key retrosynthetic disconnection.

atomistic models of BTM indicated that it is geometrically compatible with a β-turn-like structure.

In order to test the potential of BTM to function as a β-turn mimic, we carried out molecular dynamics (MD) simulations of the original TrpZip1 and our modified peptide system, **1**. After position restrained energy minimization, simulations were run for 100 ns. Overall the structures were similar, with peptide **1** exhibiting a comparable hydrogen bonded β-hairpin, with the same pattern of aromatic stacking between tryptophan residues. Importantly, the β-turn-like hydrogen bond is maintained in the simulation for **1** and, in fact, is present for a greater proportion of the trajectory than the comparable hydrogen bond in the parent TrpZip1 structure. The carbonyl oxygen to amide NH distance was less than 3 Å for 90% of frames in the trajectory for peptide **1**, compared to 63% of frames for the TrpZip1 trajectory (Figure 2).

In order to test our design, and to establish a ligation protocol, the peptide fragments **2** and **3** were synthesized using standard solid-phase peptide synthesis protocols (Figure 3). Ligation reactions were conducted at 1.5 mM concentration in 1:1 methanol/acetic acid mixtures using sodium cyanoborohydride as reductant[10c,14] and were found to be complete after 15 min. The reaction proceeded cleanly, with no evidence of competing reductive amination reactions involving lysine side chains or the N-terminal amine of **1** or **2**. The ligated peptide was purified by RP-HPLC. Using this protocol,

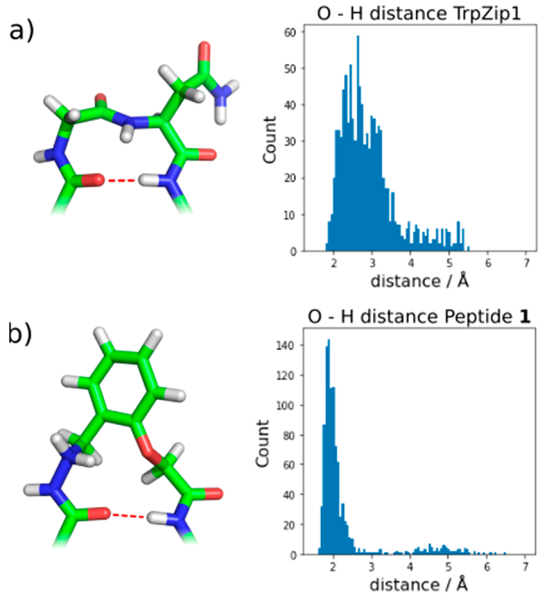

Figure 2. MD Analysis of mimic and control, showing the key region of a representative frame from the MD trajectory and a histogram of the O−H distance for the β-turn hydrogen bond. (a) TrpZip1. (b) Peptide **1**.

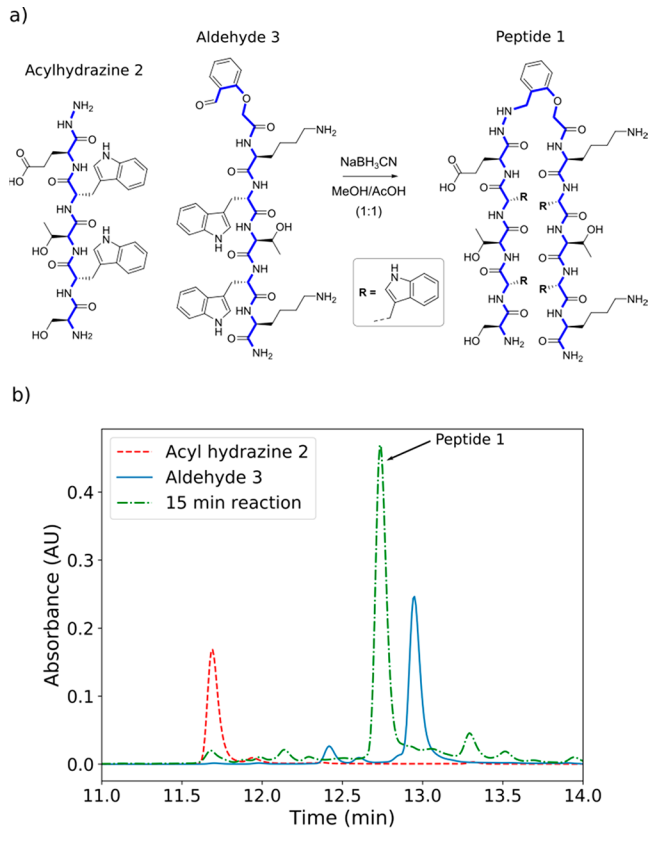

Figure 3. Synthetic conditions and representative HPLC traces. (a) Conditions for the ligation and product. (b) HPLC trace of the ligation progress.

multimilligram quantities of peptide were readily accessible for further biophysical characterization.

We characterized the peptide conjugate using circular dichroism (CD) spectroscopy in order to determine its secondary structural content and thermal stability. The CD

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spectrum of peptide **1** was directly comparable to that of the parent TrpZip1 peptide. Both peptides display far-UV signals commensurate with β-strand structure (Figure 4a), as well as

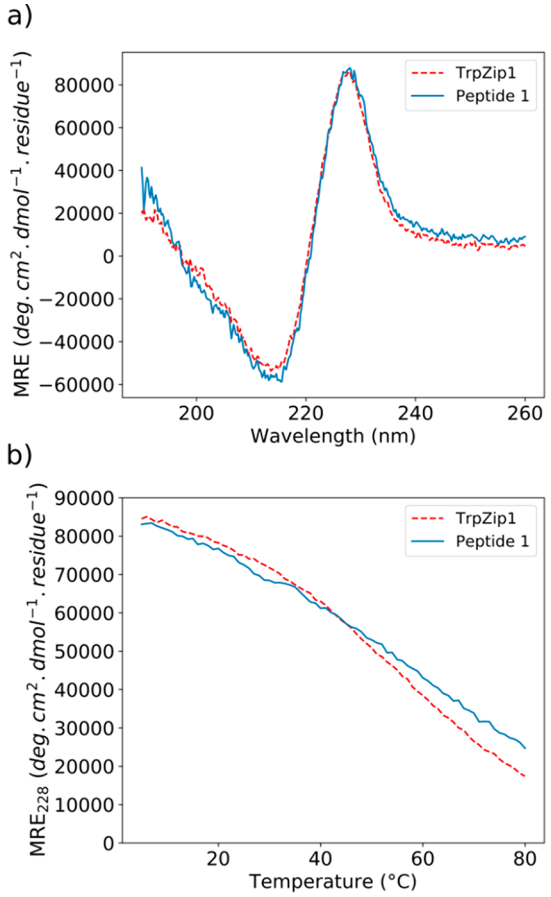

Figure 4. CD analysis of the peptide conjugate **1** and control. (a) Far-UV CD at 5 °C. (b) Thermal denaturation monitored at 228 nm.

characteristic near- and far-UV signals indicative of aromatic stacking of the Trp indole side chains (Figure S12). The thermal stability of peptide **1** was assessed by monitoring the CD signal at 228 nm as a function of temperature.[15,16] Peptide **1** exhibited a thermal denaturation and spectra of the pre- and postmelt samples at 5 °C were fully superimposable, indicating that the thermal unfolding was completely reversible (Figure S10). The melt curve for **1** is slightly shallower than that for TrpZip1 and, in contrast to the TrpZip1 curve, does not exhibit a clear transition midpoint from the first or second derivative of the curve. Taken together, these data indicate that the β-hairpin structure is maintained in peptide **1**, the structure melts and refolds reversibly, and the β-turn mimic maintains the overall stability of the hairpin structure compared to the glycine-asparagine turn of TrpZip1.

Detailed structural insights into peptide **1** were gained by NMR spectroscopy (Figure 5). All resonances were assigned using 2D NOESY, COSY, and TOCSY spectra. The chemical shifts of the HN and Hα protons of peptide **1** are consistent with a β-strand secondary structure, which is supported by intense H$\alpha(i)$−HN$(i+1)$ crosspeaks.[17,18] More significantly, cross-sheet NOE interactions are observed between the amide NH groups at hydrogen-bonding positions of the hairpin (Thr3 to Thr9 and Glu5 to Lys7). Similarly, Hα to Hα through-space interactions are evident for the non-hydrogen

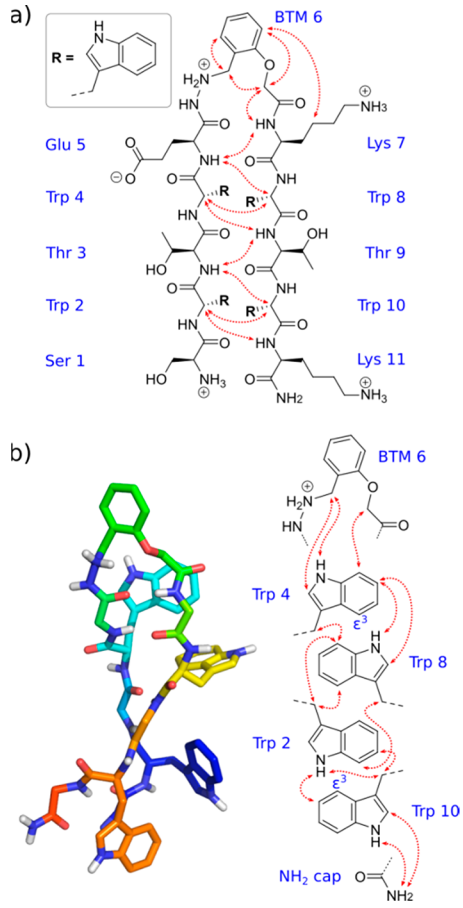

Figure 5. Key NOE interactions for peptide **1**. (a) Across-chain and BTM NOEs. (b) Representative frame from MD simulation (left) and through-space interactions confirming Trp stacking (right).

bonding positions (Trp2 to Trp10 and Trp4 to Trp8). Within the BTM unit, through-space interactions are visible between the benzylic and OCH_2 protons, indicating a compact structure. The presence of a cross peak between the Glu5 NH and Lys7 NH confirms the presence of the key β-turn-like hydrogen bond. Furthermore, the dispositions of the side chains are conserved between peptide **1** and TrpZip1, the two peptides having the same ordering of side chains within the aromatic stack. Consistent with what is observed for TrpZip1, Trp4 and Trp10 in peptide **1** have lower than usual chemical shifts for their ε^3 protons (5.9 and 5.4 ppm, respectively), indicating their participation in an edge-to-face stacking interaction. These data show that the BTM unit operates as designed, resulting in a hydrogen bonded β-turn-like structure that does not perturb either the backbone or side chain conformations.

In conclusion, we have demonstrated a readily accessible ligation protocol that installs a β-turn mimic at the same time as ligating together two peptide fragments under mild conditions. The β-turn mimic is constructed using established peptide synthesis methods and commercially available materials. This mimic has been shown to replicate key contacts present in a model β-hairpin structure, indicating that it mimics the key hydrogen bond and subsequent geometry of a β-turn. We anticipate that this mimic will be useful in the development of peptidomimetic systems, and chemical biology more generally.

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Experimental section: Initial computational models were prepared with Avogadro[19] and minimized using the MMFF94 force field and gradient descent minimization. Molecular dynamics simulations were prepared using ambertools[20] and run using OpenMM.[21] MD trajectories were analyzed using the MDanalysis python module.[22] Peptides were synthesized on a CEM Liberty Blue peptide synthesis instrument, using DIC/OxymaPure activation and rink amide and 2-chlorotrityl chloride polystyrene resins. Peptides were purified on a semipreparative scale on a Phenomenex, Gemini C18, 5 μm, 250 mm × 21.2 mm column. CD spectra were acquired on a Jasco J-810 spectropolarimeter using a 20 mM sodium phosphate buffer (pH 7). NMR spectra were collected on a Bruker AVANCE 600 MHz spectrometer equipped with a TCI cryoprobe, at 288 K in an AcOD buffer with 5% D_2O (pH 5.5), and the data were analyzed using CCPN analysis.[23] Full experimental details are given in the Supporting Information.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.0c01427.

Full experimental and biophysical characterization (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Andrew R. Thomson − *School of Chemistry, University of Glasgow, Glasgow G12 8QQ, U.K.*; orcid.org/0000-0002-1066-1369; Email: drew.thomson@glasgow.ac.uk

Authors

Selma Crecente-Garcia − *School of Chemistry, University of Glasgow, Glasgow G12 8QQ, U.K.*

Albane Neckebroeck − *School of Chemistry, University of Glasgow, Glasgow G12 8QQ, U.K.*

J. Stephen Clark − *School of Chemistry, University of Glasgow, Glasgow G12 8QQ, U.K.*; orcid.org/0000-0003-3935-0377

Brian O. Smith − *Institute of Molecular Cell & Systems Biology, University of Glasgow, Glasgow G12 8QQ, U.K.*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.orglett.0c01427

Notes

The authors declare no competing financial interest.

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