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Efficient Construction of Proline-Containing β-Turn Mimetic Cyclic Tetrapeptides via CuAAC Macrocyclization

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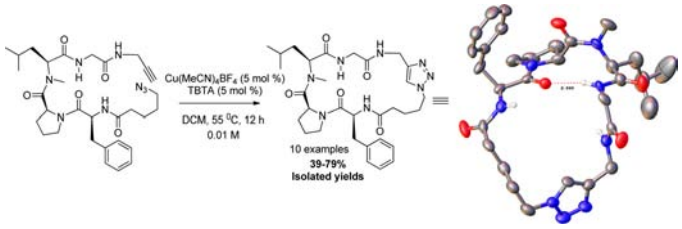
ABSTRACT



A range of macrocyclic β-turn mimetic tetrapeptides was prepared by efficient copper-tris(triazole) ligand complex catalyzed azide–alkyne "click" macrocyclizations in good to high yields. Preliminary conformational studies using X-ray crystallography and NMR spectroscopy demonstrated the presence of intramolecular H-bonds characteristic of β-turns in these cyclic tetrapeptides.

Peptide-based molecules are of considerable interest within the pharmaceutical industry as potential therapeutic agents,[1] particularly for the modulation of challenging molecular targets such as protein–protein interactions (PPIs).[2] However, the conformational flexibility of simple, linear peptides can limit their target selectivity and render them susceptible to degradation by peptidases/proteases.[3] Consequently, embedding bioactive peptide sequences within a macrocyclic framework represents an attractive strategy for circumventing these issues, provided that the bioactive conformation of the peptide is present among the low energy conformations of the macrocycle.[4] Such macrocyclic peptides may possess fewer rotatable bonds, lack charged termini, and, in some cases, can conceal their polarity in hydrophobic environments through the ability to establish intramolecular H-bonds, thereby enabling them to partition across cell membranes.[5] Not surprisingly therefore, in light of these potential pharmacological and physicochemical advantages, there has been considerable

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interest in new methods for the efficient construction of macrocyclic peptidomimetic motifs.[6]

β-Turns represent a particularly important element of secondary structure in the recognition of bioactive peptides and proteins by their cognate receptors, and so the ability to successfully stabilize this motif is a key strategy in the design of peptidomimetics.[7] Notably, β-turns are also the most common type of reverse turn found in many naturally occurring bioactive cyclic peptides, such as the clinically important cyclosporin A and gramicidin S.

Given the importance of both naturally occurring[8] and synthetic cyclic peptides in the design and development of new peptide-based theraputics,[6,9] many approaches have been developed to generate turn mimetic cyclic peptides.[6,9,10] Of these, the Cu-catalyzed azide−alkyne cycloaddition (CuAAC) reaction has proved to be especially popular in the construction of peptide-based macrocyles.[11]

However, most of the methods described to date require high dilution conditions, stoichiometric amounts of copper catalyst and/or additives (bases), harsh reaction conditions, and, in some cases, syringe pump addition, while providing only low to moderate yields of the desired cyclic peptides.[12] Thus, these methods are both inefficient and impractical in a drug discovery and development setting in terms of solvent consumption, reaction rate, and catalyst loading. Therefore, to fully exploit the potential of this approach to the construction of macrocyclic turn-mimetic peptides, a general, mild, and catalytic method is required which can generate these systems in high yield. In this regard, we have recently reported a very efficient method for the synthesis of diversely functionalized triazole-containing macrocyles using the CuAAC reaction,[13] and here we further describe the application of the methodology to the efficient construction of a series of turn-mimetic cyclic tetrapeptides.

Linear tetrapeptidic macrocyclic precursors were synthesized using standard peptide coupling conditions via a fragment condensation strategy (for full synthetic details, see Supporting Information (SI)). The optimal length of alkynylamide and azidoalkanoyl groups at the C- and N-termini of the tetrapeptide precursors was selected after computational analysis of the product macrocycles for the likely presence of a turn structure and characteristic intramolecular H-bond. The azido-alkyne containing linear tetrapeptide FPFG **1** was used as a model system for comparison of available macrocyclization methodologies, the results of which are outlined in Table 1.

The combination of dichloromethane as solvent and $Cu(CH_3CN)_4BF_4$ as a Cu source had proven optimal in our earlier study of CuAAC macrocyclizations, and so we chose to examine reaction with $Cu(CH_3CN)_4BF_4$ (5 mol %) alone at rt for 24 h. This provided a low yield of macrocycle **2** and gave primarily dimers/oligomers (Table 1, entry 1). As anticipated from our earlier studies,[13] the yield of **2** was improved dramatically by heating and addition of a catalytic amount of the ligand tris((1-benzyl-1*H*-1,2,3-triazolyl)-methyl)amine (TBTA) (Table 1, entry 2). Although we had previously examined systems containing one or two amino acid residues,[13] we were extremely gratified to demonstrate the efficient macrocyclization of this more challenging tetrapeptide substrate.

We compared this reaction outcome with several other known literature methods employing stoichiometric amounts of copper. None of these conditions offered comparable yields (Table 1, entries 3−5).[12a−c]

To demonstrate the scope of this peptide CuAAC-macrocyclization protocol, a series of azido-alkyne containing linear tetrapeptides were synthesized and subjected

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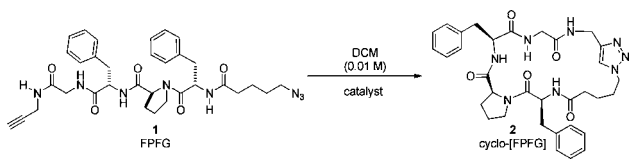
Table 1. Optimization of CuAAC Macrocyclizations[14]



entry	condition (mol %)	t (°C)	time (h)	yield (%)	**2**/dimer/ oligomer[a]
1	$Cu(CH_3CN)_4BF_4$ (5)	rt	24	38	1.5:1.4:1
2	$(Cu(CH_3CN)_4BF_4$ (5), TBTA (5)	55	12	80	13:2.3:1
3	$Cu(CH_3CN)_4PF_6$ (100)[b,12a]	rt	24	53	2:0.74:1
4	Amberlite-21-$CuPF_6$ (100)[c,12b]	55	17	67	5.2:1.5:1
5	CuBr (100), DBU (300)[12c]	rt	7	59	3.3:1.3:1

[a] Ratio determined by integration of peaks in HPLC-MS trace (λ = 210 nm, see SI). [b] Reaction run in MeOH:toluene (4:1). [c] Reaction run in toluene.

to the optimized conditions. Results are summarized in Figure 1. A small library of proline-based cyclic tetrapeptides, incorporating alternative amino acids at residues i, $i + 3$, and $i + 4$, were generated in good to high yields. Notably, >200 mg of macrocyclic product could readily be generated in a 0.01 M scale reaction, employing just 50 mL of solvent. Further diversity could be introduced via incorporation of D-amino acids (Figure 1, **4**, **9**, **10**, and **11**).

It was also possible to prepare N-methylated cyclic tetrapeptides, since this maneuver is known to improve membrane permeability by the reduction of polar surface area (PSA) (Figure 1, **6**–**10**).[15]

To examine whether these systems show lower PSA scores, we calculated this property for the lowest energy conformations (generated using Schrodinger's MacroModel software; see SI) of several members of the library, using the X-ray structure of cyclosporin A (CsA) as a reference point (PSA = 74 Å^2), since this N-methylated cyclic peptide is known to cross cell membranes. Whereas **2**, **3**, and **6** exhibit PSA scores in the 99–114 Å^2 range, example **10**, containing two N-methylated amide residues, begins to approach the PSA score for CsA (PSA = 81 Å^2). When a D-proline residue is employed at $i + 1$ (Figure 1, **9**, establishing a clear trans-amide bond within the turn, according to modeling studies; cf. Figure1, **6**–**8** showing

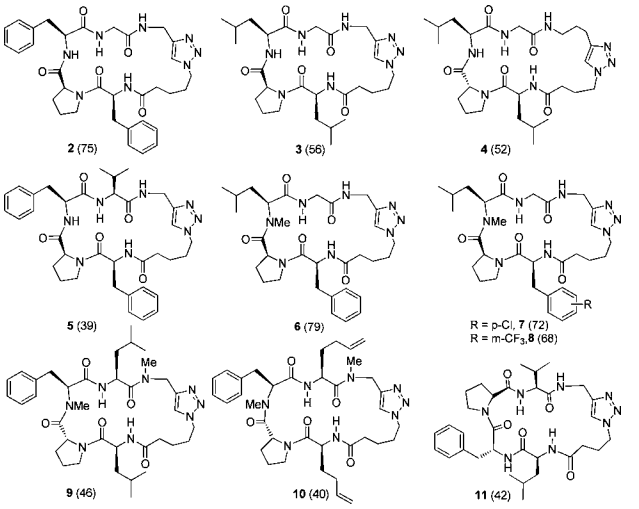


Figure 1. Substrate scope for CuAAC macrocyclization of tetrapeptides. Numbers in parentheses correspond to isolated yields after column chromatography.

cis-amides) a PSA score of 62 Å^2 is achieved, suggesting that this system has the potential to show membrane permeability, despite a molecular weight of 678.

Additionally, 3-butenyl glycine was incorporated at positions i and $i + 3$ for eventual generation of the corresponding macrobicyclic peptide via a ring closing metathesis reaction (Figure 1, **10**).[16]

Finally, although most of the macrocyclic peptides were bridged via cycloaddition between a C-terminal N-propargyl group and an N-terminal azido-pentanoyl group, we were able to demonstrate that the methodology was equally applicable to alternative bridge configurations (Figure 1, **4**). We were able to grow crystals of several of these cyclic tetrapeptides and determine their structure by X-ray crystallography (Figure 2 A and B), which clearly illustrated the presence of a β-turn secondary structure, with a H-bond between the carbonyl of phenylalanine at i and the NH of glycine at $i + 3$. It is noteworthy that two intermolecular H-bonds were also observed in the unit cell of **6** (Figure 2C), one of which involved a triazole N-atom.

We had included a mimic of the turn portion of the naturally occurring antimicrobial cyclic decapeptide Gramicidin S in our substrate scope (Figure 1, **11**), and its conformation was studied using 1D, 2D, and variable temperature (VT)-^{1}H NMR spectroscopy (see SI for additional details).[17] The VT experiments show the presence of secondary structure in **11** which is stabilized by H-bonds involving amide protons of Val and Phe (low $\Delta\delta_{NH}/\Delta T$)

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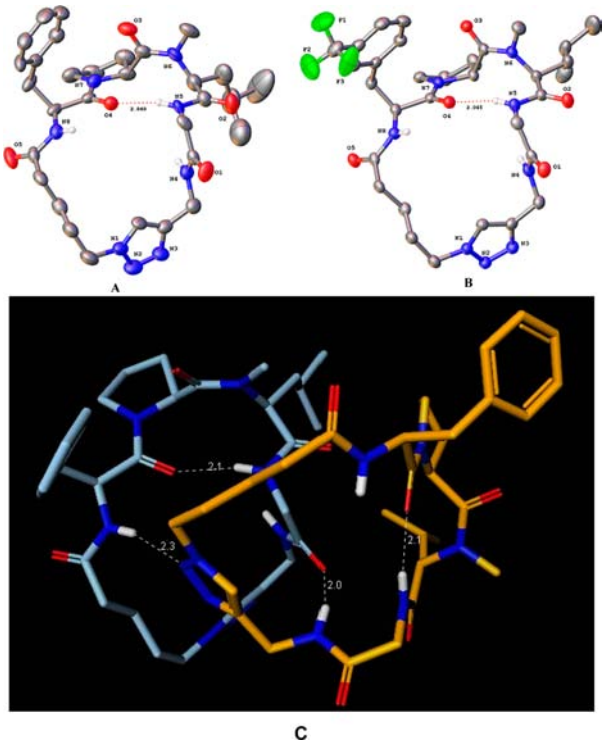

Figure 2. X-ray crystal structure of macrocyclic tetrapeptide **6** (A) and **8** (B) and the two molecules in the unit cell in the crystal structure of the cyclic tetrapeptide **6** showing inter- and intramolecular H-bonding (C, dashed line).

(Figure 3A).[18] The cross peaks in the ROESY spectrum of **11** clearly suggested the presence of β and γ turns (Figure 3B).[5b]

To gain additional insight into the conformation of these macrocyclic tetrapeptides, we performed VT and 2D NMR studies on cyclic peptide **3** in $CDCl_3$ (see SI). This agent differs from analogs **6** and **8**, for which we have X-ray crystallographic structures, by virtue of a leucine, as opposed to a phenylalanine derivative, at *i*. The characteristic ROE correlations in **3** are shown in Figure 4A.

NMR studies show the presence of only one conformer of **3** in $CDCl_3$ at rt (**2** and **5** show cis/trans amide mixtures on the NMR time scale), and the low temperature coefficients for NH12 and NH15 suggest intramolecular H-bonding, which is consistent with the lowest energy conformation generated via a MacroModel conformational search (Figure 4B).[19] Strong ROEs were observed between NH12 and NH7, NH15 and the α and β positions of the leucine at $i + 2$. These results suggest that **3** shows two consecutive β-turns in solution; in the first turn proline occupies the $i + 1$ position, and in the second, the leucine$_2$ occupies $i + 1$.

In conclusion, we have demonstrated the utility of a copper−tris(triazole) ligand complex in peptide CuAAC

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(19) We also performed conformational analysis by simulated annealing using the DISCOVER (Molecular Simulations, Inc.) program within the molecular modeling package Insight II (for details on computer simulations, see SI).

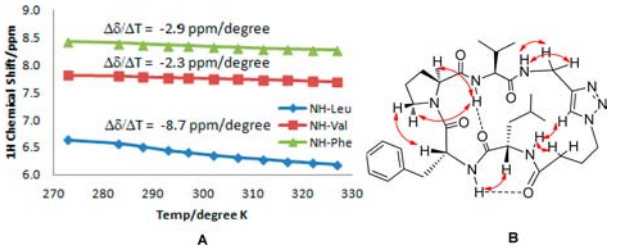

Figure 3. (A) Variable temperature NMR experiments of **11** in $CDCl_3$. (B) Schematic structure of **11** showing key $^1H-^1H$ ROESY cross-peaks (red arrows) and potential H-bonds from temperature coefficient experiments.

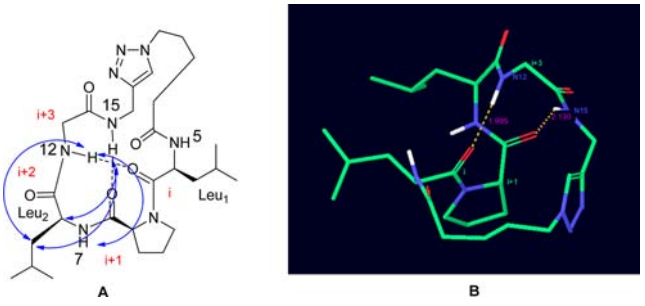

Figure 4. (A) Schematic diagram of **3** showing key $^1H-^1H$ ROESY cross-peaks (blue arrows) and potential H-bonds from temperature coefficient experiments. (B) Lowest energy conformer of **3** generated using the OPLS 2005 force field and the mixed-torsional/low-mode conformational search in MacroModel.

macrocyclizations to generate a library of β-turn mimetic tetrapeptides in good to high yields. We have demonstrated the presence of secondary structure in these CTPs by some preliminary solution NMR and conformational search studies. We are currently pursuing the detailed NMR conformational and membrane permeability properties of these systems and also preparing various other macrocycles/macrobicycles utilizing azide and alkyne derived α-amino acids.[20]

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Supporting Information Available. Experimental procedures, X-ray crystal structure (data and CIF files), HPLC and NMR data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.