ORGANIC LETTERS

2007
Vol. 9, No. 24
5011−5014

Click Chemistry as a Macrocyclization Tool in the Solid-Phase Synthesis of Small Cyclic Peptides

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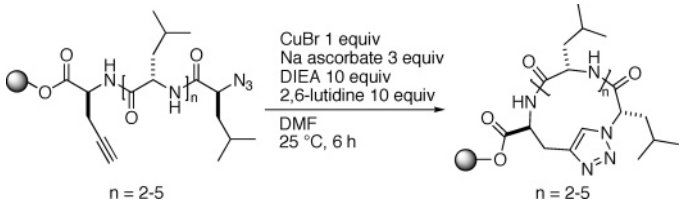
Received September 11, 2007

ABSTRACT



Despite the vast number of techniques developed for the cyclization of small peptides, cyclization efficiency remains problematic in peptides that lack turn-promoting structures. Here we demonstrate the utility of click chemistry as a macrocyclization tool in the solid-phase synthesis of cyclic tetra-, penta-, hexa-, and heptapeptides. On-resin cyclization is completed at room temperature within 6 h, resulting in predominantly monomer with small amounts of cyclomultimer byproducts.

Although peptides can be potent and selective as ligands in vitro, poor bioavailability often places severe limits on their utility as drugs. Cyclization is one approach to solving this problem, wherein the removal of ionizable C- and N-termini renders cyclic peptides more permeable and less prone to in vivo enzymatic degradation than their linear counterparts.[1−3] Cyclization can also constrain a bioactive peptide in its active conformation, thus lowering the entropic cost of binding to its biological target.[4−7] These factors, along with the wealth of biologically active cyclic peptides from natural sources, underscore the importance of developing new synthetic methods for accessing cyclic peptide scaffolds and their analogs.[8−12]

Many reagents and techniques have been developed to facilitate the combinatorial synthesis of cyclic peptide libraries,[13−15] for which the yield-limiting step is generally the cyclization reaction. Cyclization of tetra-, penta-, and hexapeptides in the all-L-configuration can be problematic, particularly in the absence of β-turn promoting structures such as glycine, proline, or a D-amino acid.[16−18] Despite the wide variety of reagents that promote efficient cyclization

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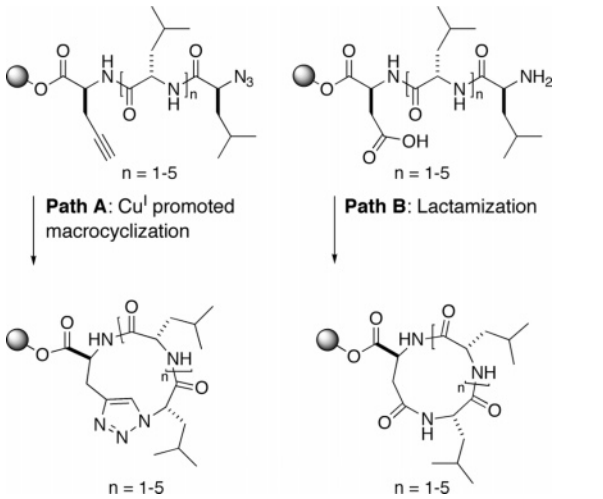
10.1021/ol702228u CCC: $37.00
Published on Web 10/23/2007

in general,[19−22] there remains a need for general and mild techniques that are effective in the cyclization of these difficult sequences.

The triazole moiety has been utilized in β-turn mimetic peptides[23] and as an isostere of the amide bond.[24−28] Recently, a Huisgen-type 1,3-dipolar cycloaddition reaction generating a 1,4-disubstituted-1,2,3-triazole—the so-called "click reaction"—was used as an amide bond surrogate and cyclization aid in the solution-phase synthesis of a cyclic tetrapeptide analogue.[29] Previous attempts to synthesize cyclo[L-Pro-L-Val-L-Pro-L-Tyr] via lactamization proved unfruitful, despite the presence of two turn-promoting structures in the sequence.[30] The "ring contraction" mechanism of the Cu^{I}-catalyzed alkyne/azide cycloaddition reaction, together with the increased ring size of the triazole analog, was proposed to promote cyclization more efficiently than macrolactamization.[31−34] The target tetrapeptide analogue was achieved with 70% yield by refluxing the linear peptide with a copper(I) catalyst at 110 °C for 16 h. This solution-phase macrocyclization demonstrated the proficiency of the 1,3-dipolar cycloaddition reaction under conditions where lactamization failed, but there have been no reports on the applicability of the click reaction as a macrocyclization tool in the solid-phase synthesis of small cyclic peptides, or its potential for use in the synthesis of cyclic peptide libraries.

We examined the utility of the Cu^{I}-promoted alkyne/azide cycloaddition reaction in the macrocyclization of resin-bound tri-, tetra-, penta-, hexa-, and heptapeptides. We were interested in the cyclization efficiency of linear peptides that might present challenging cyclizations under traditional lactamization conditions. We chose a leucine-rich sequence that lacked β-turn-promoting structures, such as proline or glycine, and which contained only L-amino acids. We envisioned the use of L-propargyl glycine as a C-terminal, aspartic acid surrogate in a side-chain cycloaddition/macrocyclization with an N-terminal L-azido-leucine (Scheme 1).

Scheme 1. Click Macrocyclization of Propargyl Glycine Azido Leucine (Path A) Is an Analogue of Aspartic Acid Side-Chain Macrocyclization (Path B)



Since any amino acid with a primary amine can be converted into the corresponding azido-acid,[35] this C-terminal alkyne/N-terminal azide motif would maximize the potential size of a cyclic peptide library by allowing the N-terminal position at which macrocyclization occurs to be a point of diversity in a parallel split-pool synthesis.

We investigated the general feasibility of this macrocyclization technique under the standard solid-phase peptide synthesis conditions used to generate peptide libraries, employing the acid labile 2-chlorotrityl chloride resin to perform a parallel split−split synthesis (Scheme 2). Each amino acid coupling was verified by the bromophenol blue test,[36] and the final macrocyclization step was carried out at room temperature using commercially available Cu^{I} salts, sodium ascorbate, DIEA, 2,6-lutidine, and DMF.

Initially, we investigated the efficiency of the macrocyclization using catalytic CuBr (0.2 equiv). We found that the long reaction times of 24−72 h were impractical and fostered a mixture of cyclic and linear products, regardless of the presence or absence of other additives such as sodium ascorbate or nitrogenous bases. We were pleased to find that

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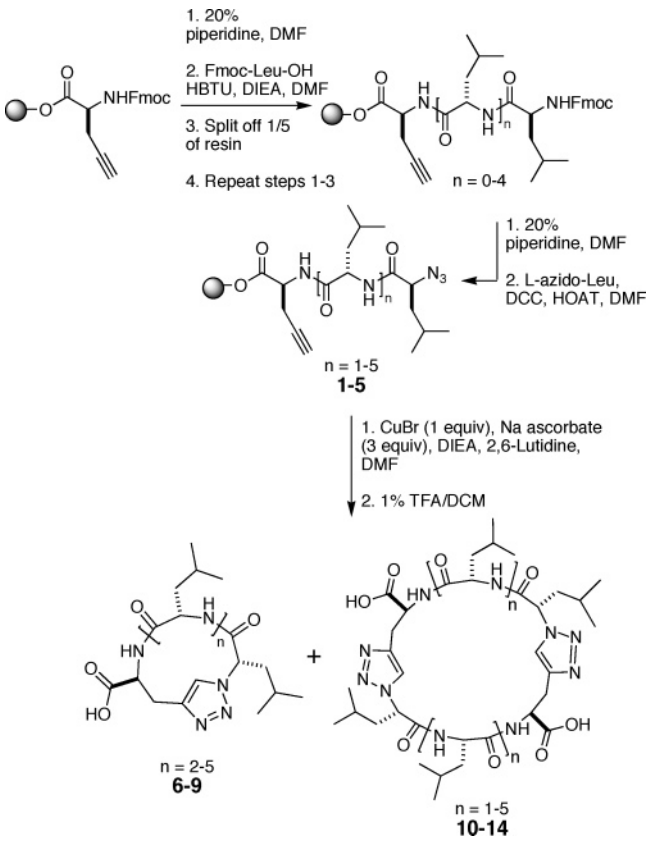
Scheme 2. Synthetic Route to On-Resin, Cu(I)-Promoted Click Macrocyclization



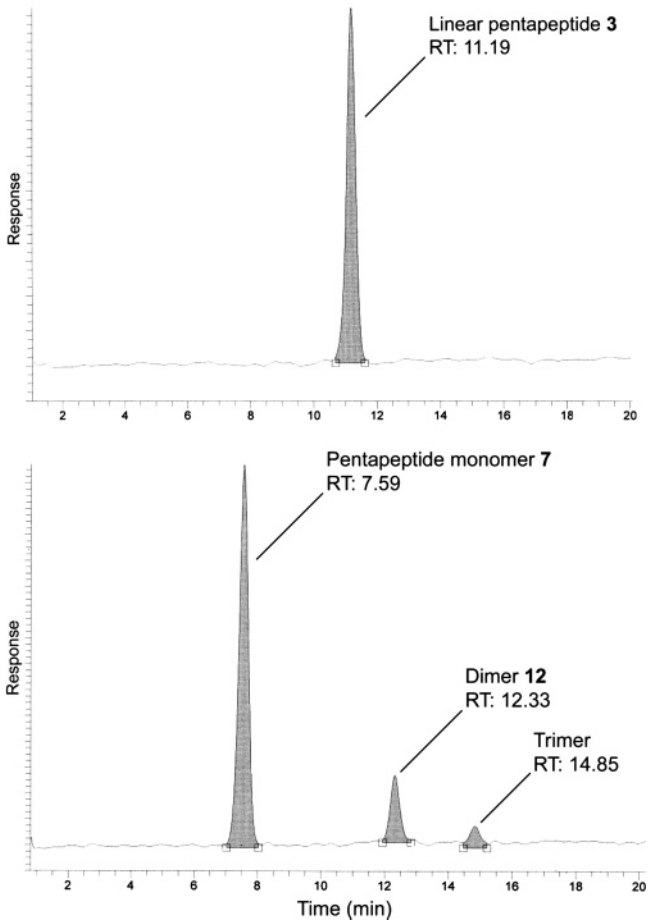


Figure 1. ELSD traces of crude linear pentapeptide **3** (top), and crude cyclization mixture (bottom) showing the cyclomonomer **7**, cyclodimer, **12**, and trace cyclotrimer.

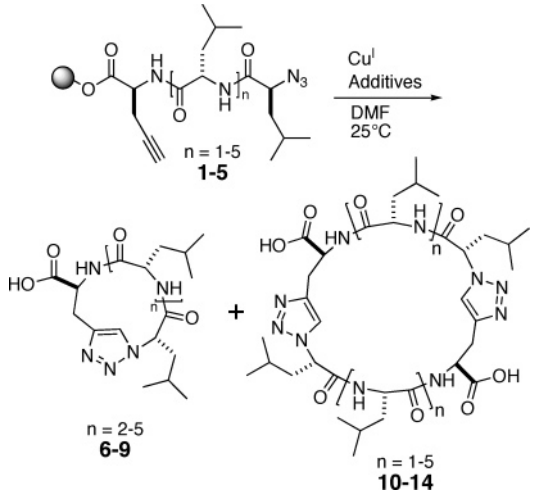
using stoichiometric CuBr (1 equiv) and an excess of sodium ascorbate reduced the reaction time to 4−6 h and resulted in a clean mixture of cyclomonomer and cyclodimer products, and, in some cases, trace amounts of trimer product. Increasing CuBr to three equivalents had no effect on cyclization efficiency, reaction time, or product ratios.

Despite having the same molecular weight, in the series of peptides that we examined, each of the azide-terminated linear peptides had a longer retention time than did the cyclic product. We used the evaporative light-scattering detector (ELSD) trace to determine the optimal reaction time and to establish the cleanliness of the crude reaction mixture (Figure 1). We determined the cyclomonomer/cyclodimer product ratios by integrating the peaks from the photodiode array detector (PDA) trace (Table 1). All peptide products were purified by preparative HPLC and their identities verified by ^{1}H NMR.

Among the peptides examined, the click macrocyclization seems best suited for peptides 4−6 amino acids in length. In light of the difficulty associated with lactamization of tri- and tetrapeptides, we were not surprised to find that the tripeptide formed almost exclusively cyclodimer product, with a small amount of cyclotrimer product as well. We were pleased to find, however, that the click macrocyclization proceeded smoothly with the tetrapeptide, producing cyclomonomer and cyclodimer products with a ratio of 3.5:1, with only trace amounts of trimer product. The click macrocyclization shows particular potential for the library synthesis of cyclic penta- and hexapeptides. The pentapeptide yielded a cyclomonomer/cyclodimer ratio of 5.5:1, with only trace amounts of trimer product. The click macrocyclization of the hexapeptide was nearly as efficient, resulting in only cyclomonomer and cyclodimer products with a ratio of 5.4:1. With the reputed ease of macrolactamization among heptapeptides, we were surprised that our heptapeptide produced a lower, but still useful, ratio of 3.6:1.

An X-ray crystal structure of the pentapeptide cyclomonomer revealed an interesting and novel type-I′/-II′ β-turn structure (Figure 2). Within the macrocycle, the triazole unit essentially acts as a spacer between the i and $i + 3$ positions of a β-turn. Interestingly, the crystal contains two distinct molecules in the asymmetric unit with virtually identical backbone conformations, in which one of the molecules has a type-II′ β-turn, while the other has a type-I′ β-turn. As in proteins, type-I′ and -II′ turns are also rare in cyclic peptides.[37] A survey of the Cambridge Crystallographic Database revealed 33 cyclic peptides that contain a type-II′ β-turn, and 12 cyclic peptides with a type-I′ β-turn (out of a total of ∼570 cyclic peptides in the database). Among these, most contain proline and/or D-amino acid residues at

Table 1. Results of Cu^{I}-Promoted Cyclization Reactions



cmpd	n	monomer:dimer[a]	isolated yield[b] (%)
10	1	0:1	75[c]
6	2	3.5:1	23[d]
7	3	5.5:1	25[d]
8	4	5.4:1	27[d]
9	5	3.6:1	20[d]

[a] Ratio by integration of peaks in PDA trace (λ = 220−240 nm). [b] Products isolated by prep HPLC. [c] Yield of cyclodimer product. [d] Yield of cyclomonomer product. The majority of the product is lost on HPLC purification, as crude yields for all sequences (monomer plus multimers) were >95%.

the *i* or *i* + 2 position, while none have standard L-amino acids at both the *i* and *i* + 2 positions. Thus, the triazole spacer acts to stabilize an unusual turn type and provides access to type-I′/-II′ turns for "all L" sequences.

Despite the availability of reagents and methods to facilitate the cyclization of linear peptides,[38] there remains a need for mild techniques that enable the cyclization of small peptides that are inherently difficult due to an absence of turn-promoting structures. We have demonstrated the usefulness of click chemistry in the on-resin cyclization of a series of leucine rich tetra-, penta-, hexa-, and heptapeptides. The cyclization is complete within 6 h, utilizing mild conditions that are orthogonal to the protection groups common to SPPS. Furthermore, because these mild conditions are unreactive to the majority of amino acid side chains, it is possible, in principle, to carry out the cyclization in the absence of side-chain protection groups.

The Fmoc strategy of solid-phase peptide synthesis makes it possible to generate vast libraries of cyclic peptides for high throughput screening and lead discovery. The efficiency of cyclization, however, is highly dependent on the length, stereochemistry, and sequence of the linear peptide.[39] Inefficiencies in the cyclization process can result in byproducts that compromise yield and purity requirements for library synthesis. The ease and reliability of on-resin click cyclization demonstrated here indicates the potential to carry out the library synthesis in a one-bead−one-compound format and bioassay directly from the crude products. Deconvolution of hit compounds would simply entail one additional purification step to separate cyclomonomer and multimer products for individual screening.

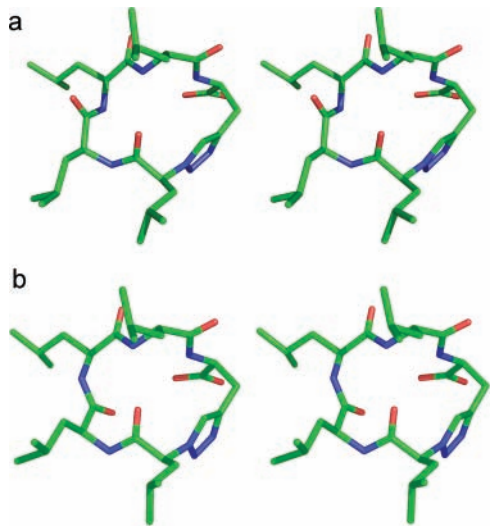


Figure 2. Stereoscopic views of the two molecules in the unit cell from the crystal structure of the pentapeptide **7**, exhibiting a type-I′ β-turn (A) and a type-II′ β-turn (B).

Acknowledgment. We gratefully acknowledge the National Institutes of Health for funding (CA104569-03). We also thank Roche (Palo Alto, CA) for donation of the LC/MS system used in this work. The single-crystal X-ray diffraction data in this work was obtained on an instrument supported by the National Science Foundation's Major Research Instrumentation (MRI) Program under Grant No. CHE-0521569.

Note Added after ASAP Publication. While this manuscript was in press, a similar on-resin 1,3-dipolar azide-alkyne cycloaddition reaction was reported in the synthesis of vascular endothelial growth factor I ligands: Goncalves, V.; Gautier, B.; Regazzetti, A.; Coric, P.; Bouaziz, S.; Garbay, C.; Vidal, M.; Inguimbert, N. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5590−5594. The original version was published ASAP October 23, 2007; the corrected version was published on November 2, 2007.

Supporting Information Available: Detailed synthetic procedures; full X-ray coordinates for **7**; method for calculating monomer:dimer ratios; ^{1}H and ^{13}C NMR spectra, LC/MS traces, and tabulated IR data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL702228U

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