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Click Chemistry as a Route to Cyclic Tetrapeptide Analogues: Synthesis of *cyclo*-[Pro-Val-ψ(triazole)-Pro-Tyr]

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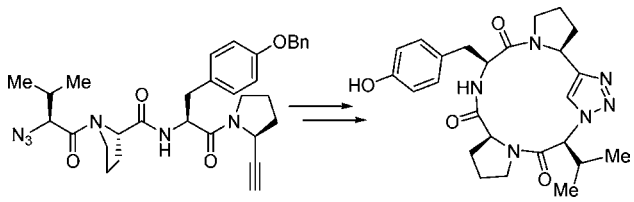
ABSTRACT



Despite the plethora of techniques to cyclize small peptides, a synthesis of *cyclo*-[(L)Pro-(L)Tyr-(L)Pro-(L)Val], a potent tyrosinase inhibitor, remains elusive because of the unfavorable transition state leading to the cyclic product. Herein, we report the successful synthesis of its triazole analogue, *cyclo*-[(L)Pro-(L)Val-ψ(triazole)-(L)Pro-(L)Tyr]. Attempted cyclization via peptide bond formation at room temperature fails to provide the desired product, but Cu^{I}-catalyzed alkyne-azide coupling at 110 °C affords the triazole tetrapeptide in 70% yield, demonstrating the utility of "click" chemistry.

Considering the vast number of biologically active cyclic peptides in nature and their potential as drugs in vivo,[1] the synthesis of cyclic peptides and their analogues remains an important goal for both academic and industrial laboratories. Head-to-tail cyclization of tetrapeptides in particular poses a significant challenge: not only is oligomerization a possible side reaction, but ring strain in the transition state leading to the cyclic product may prohibit cyclization altogether.[2] A number of strategies to facilitate the cyclization of strained peptides have been developed,[3] but a general, high-yielding route to cyclotetrapeptides has yet to be reported.

One route that we have chosen to explore involves the use of a 1,4-disubstituted 1,2,3-triazole as an amide bond surrogate and cyclization aid. These triazoles have atom placement and electronic properties similar to those of a peptide bond (Figure 1)[4] and are accessible in one step via Cu^{I}-catalyzed alkyne-azide cycloaddition.[5] Further, the increased ring size of triazole analogues and the apparent "ring contraction" mechanism of Cu^{I}-catalyzed alkyne-azide cycloaddition[6] may help to promote cyclization.[7]

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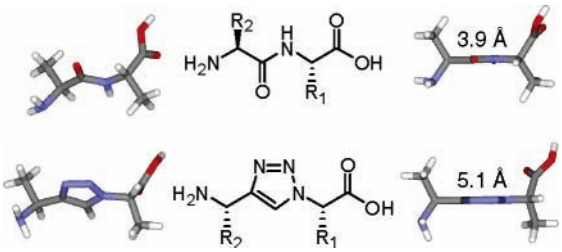


Figure 1. Topological similarities of amides (top) and 1,2,3-triazoles (bottom).

To demonstrate the cyclization potential of click chemistry on peptides, we have synthesized *cyclo*-[Pro-Val-ψ(triazole)-Pro-Tyr] (**2**), the triazole analogue of *cyclo*-[(L)Pro-(L)Val-(L)Pro-(L)Tyr] (**1**, Figure 2). First isolated from bacterium

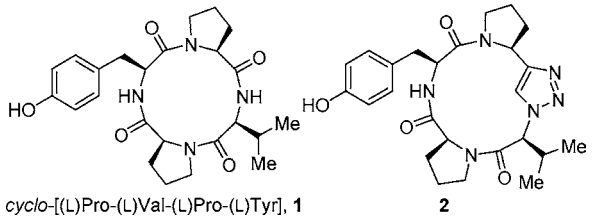


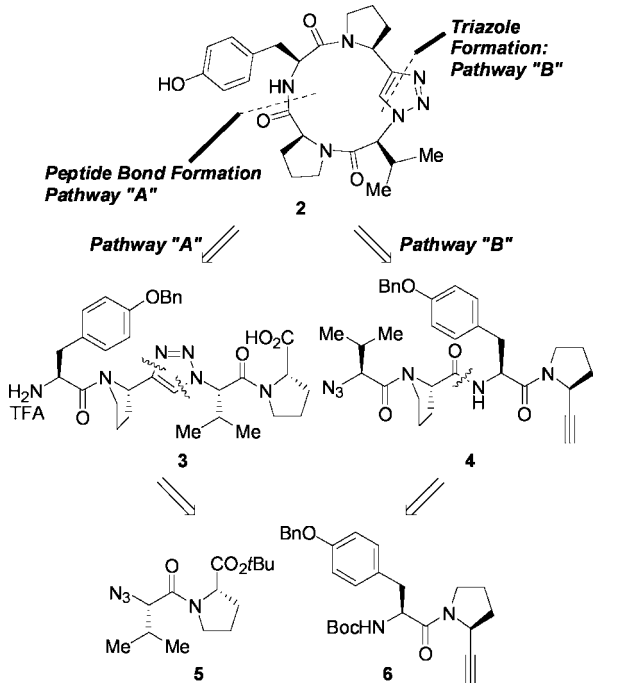
Figure 2. Tyrosinase inhibitor **1** and its triazole analogue *cyclo*-[Pro-Val-ψ(triazole)-Pro-Tyr].

L. helveticus in 1993,[8] cyclotetrapeptide **1** is a potent inhibitor of tyrosinase, a key enzyme involved in the browning of plant-derived food products and in various human dermatological disorders.[9] To date, no synthesis of all-L cyclotetrapeptide **1** has been reported; Schmidt and Langner attempted its synthesis in 1997 only to obtain the tyrosine epimer, *cyclo*-[(L)Pro-(L)Val-(L)Pro-(D)Tyr], in low yield.[3c]

We envisioned two pathways to triazole analogue **2** (Scheme 1): disconnecting the lone secondary amide bond retrosynthetically to give triazole-substituted tetrapeptide **3** and cyclization via Cu^{I}-catalyzed alkyne-azide coupling leading back to azide peptide analogue **4**. Conveniently, both linear precursors disconnect back to the same dipeptide coupling fragments: azido ester **5** and *N*-protected amino alkyne **6**.

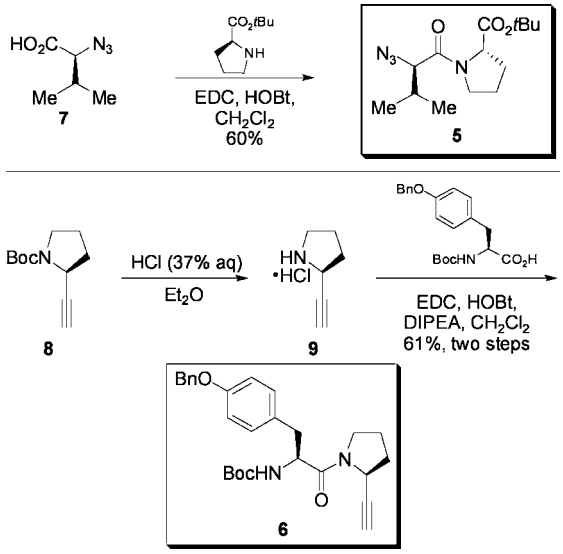
Synthesis of Val-Pro dipeptide coupling fragment **5** was achieved via EDC/HOBt-mediated peptide coupling of azido

Scheme 1. Retrosynthetic Analysis of Triazole Analogue **2**



valine **7**[10] with the *tert*-butyl ester of proline (Scheme 2).

Scheme 2. Synthesis of Dipeptide Coupling Fragments



Tyr-Pro coupling fragment **6** was obtained after HCl deprotection of known Boc-protected alkyne **8**[11] and subsequent peptide coupling with Boc-protected benzyl tyrosine (Scheme 2).

Coupling of dipeptides **5** and **6** via Cu^{I}-catalyzed alkyne-azide cycloaddition yielded triazole-substituted tetrapeptide **3** cleanly in 74% yield (Scheme 3). As expected, however,

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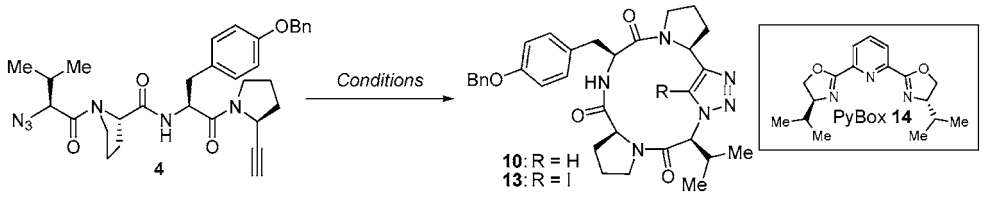
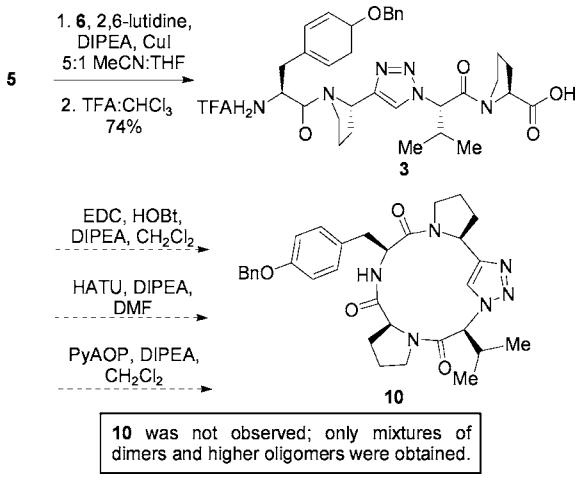
Table 1. Optimization of Cu^{I}-Catalyzed Alkyne-Azide Cycloaddition Conditions



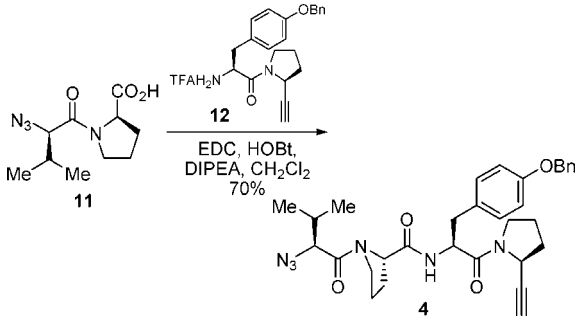
	Cu salt (equiv)	additives (equiv)	solvent	time	temperature	yield [a]	ratio [b] 10 : 13 : 4
1	$CuSO_4$ (0.03)	Na ascorbate (0.3)	4:1 *t*BuOH:H_2O	8 days	rt	0%	-
2	CuI (2)	2,6-lutidine (2) DIPEA (2)	5:1 MeCN:THF	3 days	rt	33%	1.4 : 1 : 0
3	CuI (0.3)	DBU (20)	toluene	7 days	50 °C	43%	24 : 1 : 4
4	$Cu(PPh_3)_3Br$ (0.2)	DBU (3)	toluene	16 h	110 °C	58% [c]	1 : 0 : 0
5	CuBr (0.2)	DBU (3) PyBox **14** (0.4)	toluene	16 h	110 °C	57% [d]	1 : 0 : 0
6	CuBr (0.2)	DBU (3)	toluene	16 h	110 °C	70%	1 : 0 : 0

[a] Isolated yield of **10**, **13**, and **4**. [b] Ratio after column chromatography. [c] Yield includes 11% (w/w) PPh_3 byproducts. [d] Yield includes 10% (w/w) PyBox **14**.

Scheme 3. Synthesis and Attempted Cyclization of Triazole-Substituted Tetrapeptide **3**



Scheme 4. Synthesis of Linear Tetrapeptide Analogue **4**



cyclization proved difficult: peptide couplings mediated by EDC/HOBt, HATU, and PyAOP all failed to provide even trace quantities of benzyl-protected triazole **10**.

We then turned our attention to the cyclization of linear tetrapeptide analogue **4** (Scheme 1). Following TFA deprotection of dipeptides **5** and **6** to yield **11** and **12**, respectively (Scheme 4), EDC/HOBt-mediated coupling proceeded smoothly in 70% yield to afford linear tetrapeptide analogue **4** as a single diastereomer. Although initial attempts at cyclization utilizing $CuSO_4$ with sodium ascorbate[5a] yielded no product after 8 days at room temperature (Table 1, entry 1), reaction with 2 equiv of CuI proceeded to completion in 3 days. To our surprise, we observed no byproducts from dimerization, a common side reaction in macrocycle formation via Cu^{I}-catalyzed alkyne-azide cycloaddition.[7c,f] Instead, the main side product, comprising more than 40% of the isolated product, was iodotriazole **13** (Table 1, entry 2). Recent reports indicate that I_2 contamination in CuI can generate 5-substituted iodotriazoles,[12] but in our hands, even high commercial grade CuI[13] gave substantial quantities of the iodotriazole. Conditions utilizing catalytic CuI and DBU at elevated temperatures failed to go to completion after 7 days and still afforded trace amounts of iodotriazole **13** (Table 1, entry 3).

As a result, we explored the use of sources of CuBr, which gratifyingly gave no 5-substituted side products (Table 1). Increasing the temperature succeeded in reducing the reaction time substantially, even under conditions of catalytic Cu^{I}. Both reaction with $Cu(PPh_3)_3Br$, a highly soluble, non-air-sensitive CuBr source,[14] and CuBr stabilized by pybox-type ligand **14**[15] went to completion and gave no side products, but chromatographic removal of both PPh_3 byproducts and ligand **14** proved difficult. Gratifyingly, however, reaction with CuBr and DBU in refluxing toluene afforded cyclic peptide **10** in 70% yield (Scheme 5). Subsequent deprotection yielded triazole analogue **2**.

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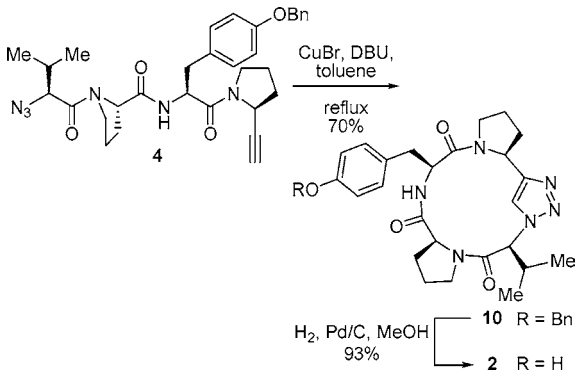
Scheme 5. Completion of the Synthesis of *cyclo*-[Pro-Val-ψ(triazole)-Pro-Tyr] (**2**)



Overall, the failed cyclization of triazole tetrapeptide **3** with traditional peptide coupling methodologies, in contrast to the successful Cu^{I}-catalyzed click chemistry cyclization, indicates that the mere presence of a triazole moiety in a tetrapeptide is not enough to overcome the barriers to cyclization. Although the triazole increases ring size and therefore reduces ring strain in the product, strain in the transition state may still prevent cyclization under conditions of peptide coupling. The transition state of Cu^{I}-catalyzed click chemistry, however, involves a binuclear Cu complex that brings the alkyne and azide moieties in close proximity before "ring contraction" provides the triazole.[6,16] Clearly, this reaction enables cyclization in a case where other reactions fail to give the desired product.

In summary, we have demonstrated the potential of click chemistry as a means of obtaining small peptide analogues too strained for ring closure via lactamization. While coupling via peptide methodologies failed to provide the product, the desired cyclic peptide was isolated in 70% yield after Cu^{I}-catalyzed cycloaddition. Triazole analogue **2** is now being tested for biological activity, and the synthesis of additional triazole analogues to biologically active cyclotetrapeptides[17] is underway.

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Supporting Information Available: Experimental methods and compound characterization data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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