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Tetrahedron Letters 47 (2006) 665–669
Tetrahedron Letters

# Solid phase synthesis of peptidotriazoles with multiple cycles of triazole formation

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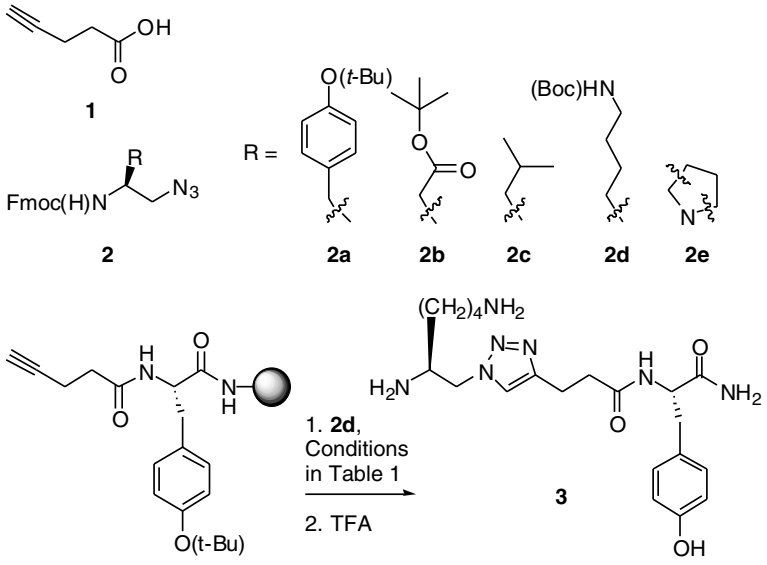
Received 2 November 2005; revised 18 November 2005; accepted 22 November 2005
Available online 9 December 2005

**Abstract**—Peptidotriazoles, unnatural oligomers with alternating amide and triazole linkages, are synthesized efficiently on solid support. The key transformations involve multiple cycles of 1,2,3-triazole formation, using soluble Cu(I) catalyst and conditions that do not generate precipitate on solid support nor require exclusion of oxygen. Our synthetic protocol will enable the preparation of other unnatural oligomers with multiple triazoles using solid phase methodologies.


Recently the synthesis of peptidomimetic unnatural oligomers—oligomers with peptide side chains built on alternative backbones—has attracted increasing attention for the design of biological effectors or foldamers.[1–6] Among these peptidomimetic oligomers are the oligo-ureas[7–11] and oligo-guanidines,[12–14] for which the preparation of monomers sometimes produces N-Fmoc protected amino azides as key intermediates.[11,13,15,16] With those amino azides in hand, we sought to further explore the utility of these compounds in peptidomimetics by introducing 1,2,3-triazole moiety in peptide backbone to form peptidotriazoles.[16] The




**Scheme 1.** Testing solid phase triazole formation conditions.

*Keywords*: Peptidotriazole; Peptidomimetics; Unnatural oligomer; Solid phase.
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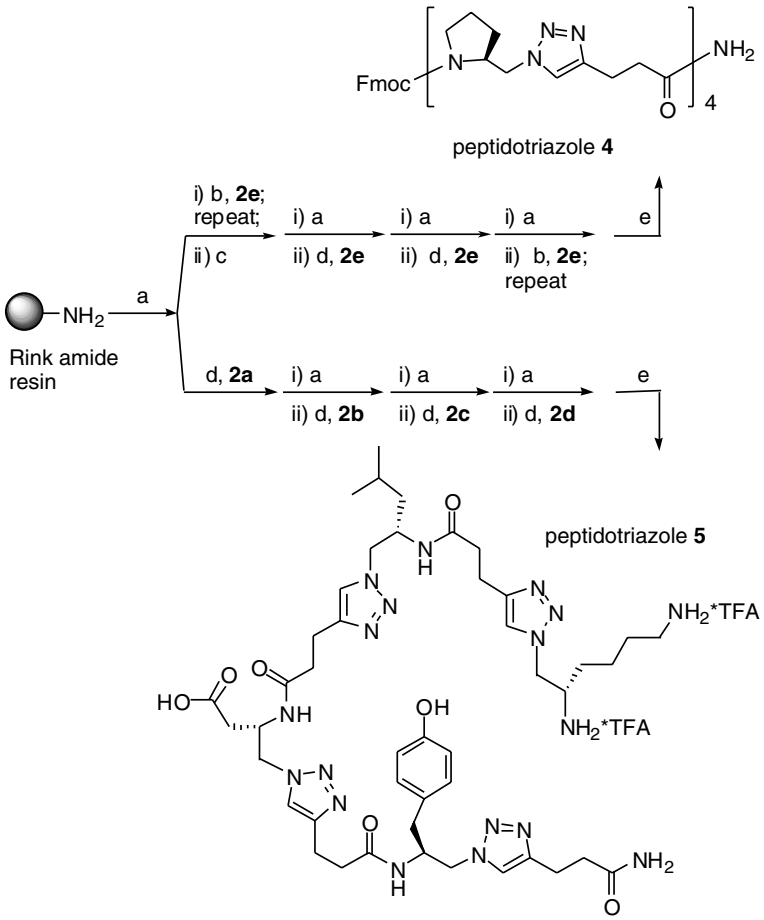
doi:10.1016/j.tetlet.2005.11.111

recently discovered coupling reaction of terminal alkyne and azide under Cu(I) catalysis yielding 1,4-substituted 1,2,3-triazole[16,17] with exclusive regioselectivity, mild conditions, and high yields has prompted its application in bioconjugation[18–21] including macrocyclization.[22–28] These examples usually involve only one or two triazoles. Multiple triazole linkages can be found in polymers,[29–31] dendrimers,[32–35] and cyclodextrins[36] prepared from solution phase synthesis. For peptidotriazoles, it was reported that 1,2,3-triazole can act as peptide surrogate[26] and as an effective replacement for a peptide portion in HIV-1 protease inhibitors.[37] Peptides containing the 1,2,3-triazole moiety in the backbone inhibited the growth of *Leishmania mexicana*.[38] Triazole(s) in peptide terminal,[16] center,[38] or side chain positions linking to sugars[39] or other peptides[40] have been reported. However, there is still the lack of solid phase synthetic methods for peptidotriazoles with multiple triazole units in the backbone. Herein, we report the solid phase synthesis of peptidotriazoles with alternating triazole and amide linkages in the backbone via the coupling between pentynoic acid **1** and amino azides **2**.

**Table 1.** Yield of **3** from triazole formation described in Scheme 1

| Entry | Conditions | Yield[a] |
|---|---|---|
| 1 | $CuSO_4$ (0.5 equiv), ascorbate (1.0 equiv), DMF/$H_2O$ (95:5), 17 h | 8% |
| 2 | CuI (0.5 equiv), DIPEA (10 equiv), THF, 17 h | 0% |
| 3 | CuI (5 equiv), DIPEA (10 equiv), THF, 17 h | 41% |
| 4 | $Cu(PPh_3)_3Br$ (5 equiv), DIPEA (10 equiv), THF, 17 h | 45% |
| 5 | CuI (5 equiv), DIPEA (10 equiv), ascorbic acid (5 equiv), DMF/pyridine (7:3), 17 h | 0% |
| 6a | CuI (5 equiv), DIPEA (10 equiv), ascorbic acid (5 equiv), DMF/2,6-lutidine (7:3), 17 h | 82% |
| 6b | Performing 6a twice | Quant. |
| 7 | CuI (5 equiv), DIPEA (10 equiv), ascorbic acid (5 equiv), DMF/piperidine (8:2), 5 h | Quant. |
| 8 | CuI (5 equiv), ascorbic acid (5 equiv), DMF/piperidine (8:2), 5 h | Quant. |

[a] All reactions were performed at room temperature with no special oxygen exclusion beyond capping the polypropylene columns. The resin bound **3** was treated with 20% piperidine in DMF to remove Fmoc group if necessary, then cleaved with TFA/$H_2O$/TIS (94:3:3). Yield was calculated based on the relative UV intensity (from tyrosine) of **3** and unreacted dipeptide in LC–MS analysis.




**Scheme 2.** Synthesis of two peptidotriazole sequences **4** and **5**. Reagents and conditions: (a) PyBop/HOBt, DIPEA, **1**, DMF; (b) CuI, ascorbic acid, DIPEA, 30% 2,6-lutidine/DMF; (c) 20% piperidine/DMF; (d) CuI/ascorbic acid, 20% piperidine in DMF; (e) TFA/$H_2O$/TIS (94:3:3).

In solution phase synthesis of 1,2,3-triazole from terminal alkyne and azide, the Cu(I) catalyst usually is produced in situ from the reduction of Cu(II) salts with sodium ascorbate,[17] tris(carboxyethyl)phosphine,[41–43] or Cu(0) and Cu(II).[44] Direct utility of Cu(I) salts such as CuI requires exclusion of oxygen to avoid oxidizing Cu(I) to Cu(II), and the assistance of stabilizing agents to avoid the breakdown of Cu(I) to Cu(0) and Cu(II). For stabilization of Cu(I), tris-triazole ligands[45,46] and bipyridine[46] were developed to protect Cu(I) from both oxidation and breakdown. With those Cu(I) complexes, the triazole formation can be performed without special exclusion of oxygen. The addition of ascorbic acid to Cu(I) has the same effect to protect Cu(I) from oxidation.[17] In solid phase synthesis, even though some reactions directly employed CuI as catalyst,[38,47–49] it is desirable for peptidotriazoles with multiple triazole units in the backbone that the Cu(I) used is non-precipitating, stable, and efficient in organic solution and can be washed away after each coupling reaction. In order to find efficient protocols with easy handling in solid phase synthesis, we examined the Cu(I)-catalyzed reaction conditions for coupling the Fmoc protected lysine azide (**2d**) and Rink amide resin bound dipeptide as shown in Scheme 1.

As shown in Table 1, Cu(I) catalyst source from $CuSO_4$ and ascorbate in DMF/$H_2O$ gave low yield in the solid phase reaction (entry 1). Employing 0.5 equiv of CuI with DIPEA (entry 2) did not work either. When the amount of CuI was increased to 5 equiv (entry 3), there was 41% triazole formation, but excess of CuI caused the copper species deposited on the resin. Changing of CuI to organic soluble $Cu(PPh_3)_3Br$[33,50] produced moderate yield (entry 4). Excess CuI dissolved in DMF/pyridine with DIPEA and ascorbic acid (entry 5) did not produce the product. However, when the pyridine in entry 5 was replaced with 2,6-lutidine, 82% triazole formation was found (entry 6a). Repetition of this treatment (entry 6b) gave almost quantitative yield. This is a good condition for solid phase triazole formation with the Fmoc group remained intact after the reaction. When the resin from the entry 3 reaction was treated with 20% piperidine in DMF to remove the Fmoc group, it was found that the copper species deposited on the resin were dissolved. This prompted us to investigate if piperidine could replace 2,6-lutidine in entry 6 to help dissolve and stabilize CuI. The results were exciting. With or without DIPEA (entry 7 or 8), 20% piperidine in DMF in place of 2,6-lutidine gave almost quantitative yields and the reaction time was also shortened from 17 to 5 h. It was possible that piperidine as a secondary amine can form complex with CuI to enhance its stability. In conclusion of this test of solid phase reaction, two conditions in Table 1 (entries 6b and 8) can be used for triazole formation. If the Fmoc group is desired to be remained after reaction, the condition in entry 6b is a good choice. Otherwise the condition in entry 8 is preferred since it requires shorter time and the Fmoc is removed at the same time.

With those two conditions in hand for triazole formation on solid support, we started to investigate the synthesis of peptidotriazole sequences **4** and **5** in solid phase as shown in Scheme 2.[51] The first sequence **4** is a 4-unit repetition of pentynoic acid and Fmoc-proline azide with alternating amide bond and triazole linkage. The condition of entry 6b in Table 1 was used for the first and the last triazole formation to keep the Fmoc group for loading determination and as the chromophore for HPLC purification. The reaction was clean and only one desired product peak appeared in LC–MS analysis (Fig. 1) after resin cleavage and ether wash. The final isolated yield of **4** after HPLC purification was 62% based on the first loading.

To demonstrate the broad compatibility of our protocol to different monomers of amino azides, the Fmoc-proline-azide **2e** used in the synthesis of **4** was substituted by side chain-protected Tyr, Asp, Leu, and Lys amino azides for sequence **5** as shown in Scheme 2. These amino azide monomers contain side chains bearing hydrophilic, hydrophobic, negatively charged, and positively charged groups. The condition of entry 8 in Table 1 was used for efficient triazole formation and concomitant removal of the Fmoc group. Again, the reaction was clean and only one desired product peak appeared in LC–MS analysis of the crude product cleaved from the resin (Fig. 2). The isolated yield of **5** after HPLC purification was 56% yield.

In summary, we have developed protocols for efficient 1,2,3-triazole formation on solid phase that use soluble Cu(I) and do not require special oxygen exclusion. These protocols enable multiple cycles of triazole formation to be performed. They should offer opportunities for the synthesis of peptidomimetics with triazole-mixed backbones.

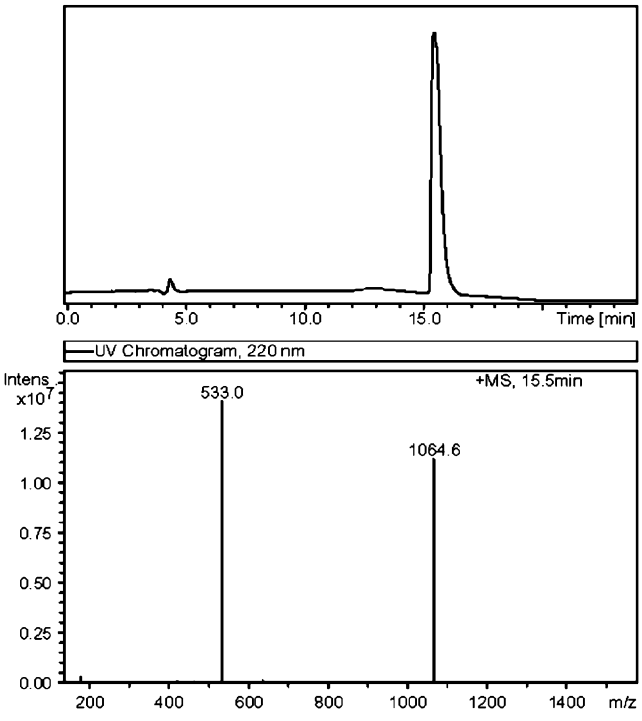



**Figure 1.** LC–MS analysis of crude peptidotriazole **4**. Top: LC trace ($\lambda$ 220 nm); Bottom: mass at $t = 15.5$ min.

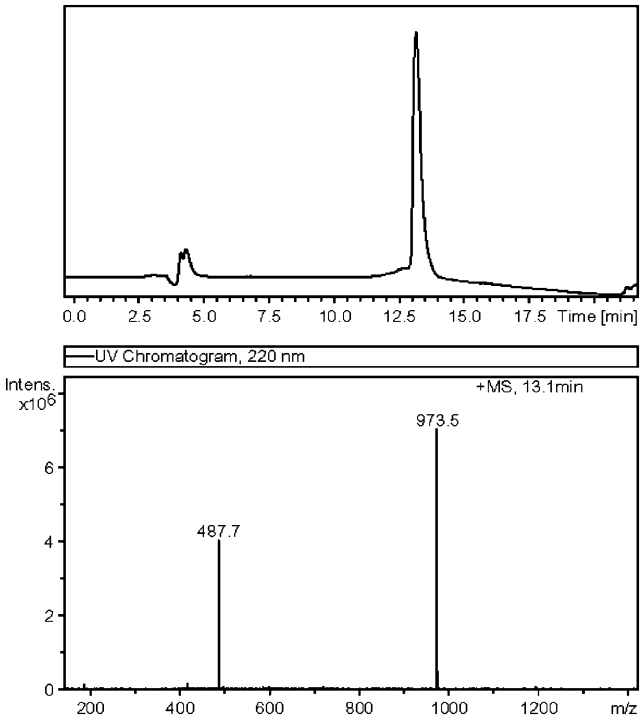



**Figure 2.** LC–MS analysis of crude peptidotriazole **5**. Top: LC trace ($\lambda$ 220 nm); Bottom: mass at $t = 13.1$ min.

## Acknowledgments

This work is supported in part by the W. M. Keck Foundation Center for Microbial Pathogens at the University of Washington.

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51. Experimental procedure: Rink amide MBHA resin (200 mg, loading 0.64 mmol/g) in polypropylene column was deprotected with 20% piperidine in DMF. After washing the resin thoroughly with DMF, solutions of DIPEA (10 equiv), pentynoic acid **1** (5 equiv), HOBt (5 equiv), and PyBop (5 equiv) in DMF were added to the resin. The reaction mixture was shaken gently overnight. After being washed with DMF and $CH_2Cl_2$, the resin was capped with acetic anhydride (10 equiv) and DIPEA (20 equiv) for 20 min and washed. The resin was then split into two equal parts for the synthesis of **4** and **5**. For the synthesis of **4**, the resin was first subjected to triazole formation under condition 6 in Table 1, using Fmoc-amino azide **2e** (5 equiv) and the solution of CuI (5 equiv), DIPEA (10 equiv), ascorbic acid (5 equiv) in 10 ml of 30% 2,6-lutidine in DMF. The reaction was allowed to proceed for 17 h at rt. This procedure was repeated once, after which the loading of the resin was determined to be 0.52 mmol/g (by monitoring the release of dibenzofulvene during Fmoc removal using UV at 290 nm). After Fmoc removal, the resin was subjected to acylation with **1** using the above condition for 2 h, followed by triazole formation under condition 8 in Table 1, using **2e** (5 equiv) and the solution of CuI (5 equiv), ascorbic acid (5 equiv) in 10 ml of 20% piperidine in DMF. The reaction was allowed to proceed for 5 h at rt. The resin was subjected to two more cycles of coupling with **1** (2 h) and triazole formation with **2e** (one using condition 8, one using condition 6b in Table 1) to obtain resin bound **4**. The resin was cleaved with 10 ml of TFA/$H_2O$/TIS (94:3:3) for 1 h. The crude product was concentrated and washed with ether, then purified by HPLC using a ZOBAX extended-C18 column to give the final product **4**: 34 mg (62%). $^1$H NMR (500 MHz, $CD_3OD$): $\delta$ 1.33–1.46 (m, 4H), 1.63–1.93 (m, 12H), 2.59–2.75 (m, 8H), 2.87–3.20 (m, 10H), 3.35–4.20 (m, 8H), 4.38–4.83 (m, 13H), 7.33–7.40 (m, 4H), 7.62–7.82 (m, 8H). ESI-MS ($m/z$): 1064.6 $[M+H]^+$ and 533.0 $[M+2H]^{2+}$.
For the synthesis of **5**, the resin was subjected to triazole formation under condition 8 in Table 1 using **2a**, followed by three more cycles of acylation with **1** (2 h) and triazole formation with **2b**, **2c**, and **2d**, respectively, under condition 8 in Table 1. Cleavage and purification followed procedures for compound **4**. Thirty five milligrams (56%) of **5** as TFA salt was obtained after HPLC purification. $^1$H NMR (500 MHz, $D_2O$): $\delta$ 0.63 (d, $J = 5.0$ Hz, 2H), 0.74 (d, $J = 5.0$ Hz, 2H), 1.20 (m, 3H), 1.42 (m, 2H), 1.60 (m, 4H), 2.27–2.91 (m, 28H), 4.10–4.38 (m, 6H), 6.65 (d, $J = 8.0$ Hz, 2H), 6.94 (d, $J = 8.0$ Hz, 2H), 7.20 (s, 1H), 7.53 (s, 1H), 7.64 (s, 1H), 7.68 (s, 1H). ESI-MS ($m/z$): 973.5 $[M+H]^+$ and 487.7 $[M+2H]^{2+}$.