

A Novel One-Pot Synthesis Strategy for Bicyclic Peptide Assembly

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Introduction

Bicyclic peptides are polypeptides forming two circular units. The cyclic structures often exhibit improved stability, higher potency and bioavailability. Therefore, bicyclic peptides are considered a novel therapeutic class, which lies between the small molecule and monoclonal antibody drug classes. [1-3]

Bicyclic peptides can be prepared by both solution phase and solid phase synthesis; however, their synthesis remains a challenge. The multiple steps of synthesis, cyclization, and purification often result in low overall yield. Therefore, optimizing the synthesis strategy plays a critical role in improving the final overall yield.

We recently synthesized a 13-mer bicyclic peptide, containing one disulfide bridge and one triazole bridge. Here we report the comparison of using a multi-step synthesis strategy and a one-pot synthesis.

Experimental Procedures

Peptides were synthesized on MBHA resin using Fmoc chemistry. Fmoc-deprotection was carried out using 20 % piperidine in DMF. Peptides were cleaved from resin using TFA cocktail (TFA / TIS / H₂O / Thioanisole = 90 : 2.5 : 2.5 : 5).

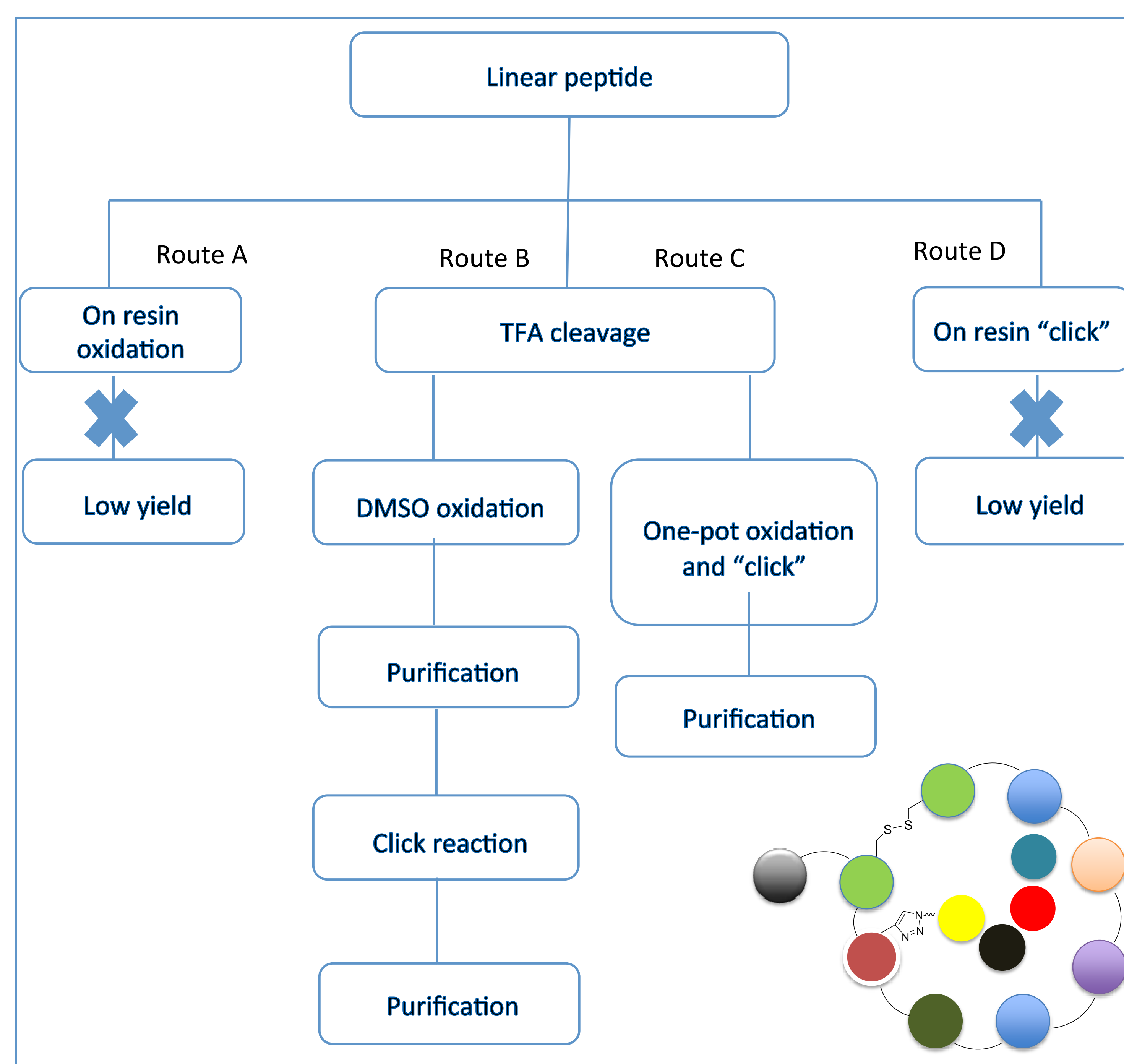
The peptides were characterized using analytical HPLC (Agilent 1200 with an Eclipse XBD-C18, 4.6 x 150 mm, 5 μm, 130 Å); gradient of ACN in 0.1 % TFA, flow 1 mL / min.

Route B: DMSO oxidation of linear peptide was carried out in DMSO, ACN and water (5 : 10 : 85) for two days followed by preparative RP-HPLC peptide purification. The purified peptide was redissolved in water and IPA (4 : 1). CuSO₄ (4 equiv.) in 1 mL water was added to the peptide solution, followed by dropwise addition of L-Ascorbic acid (4 equiv.) in 2mL water. The click reaction was monitored by HPLC and finished in 1-2 hours. The reaction solution was loaded to prep. HPLC for a second round of purification.

Route C (One-pot synthesis): DMSO oxidation of linear peptide was performed in DMSO, ACN and water (5 : 10 : 85) for two days and the reaction was monitored using LCMS. Then CuSO₄ (4 equiv.) in 1 mL water was added to the above solution. After that, L-Ascorbic acid (4 equiv.) in 2mL water was added dropwise to the reaction solution. The click reaction was complete in 4 hours, as monitored by analytical HPLC.

Results and Discussion

Multiple synthesis routes have been tested (Scheme 1). On resin oxidation and on resin click reaction (route A and D) gave low yields. Therefore cyclization in solution (route B and C) was performed. Using Route B, the crude linear peptide was oxidized in solution, purified and lyophilized. Then the oxidized, purified peptide was redissolved in water and IPA solution to perform the click reaction. Route B presents a common synthetic strategy for bicyclic peptide synthesis. However, it is a time consuming process, and multiple synthesis and purification steps may decrease the overall yield.



Scheme 1. Synthesis routes tested for a 13-mer bicyclic peptide.

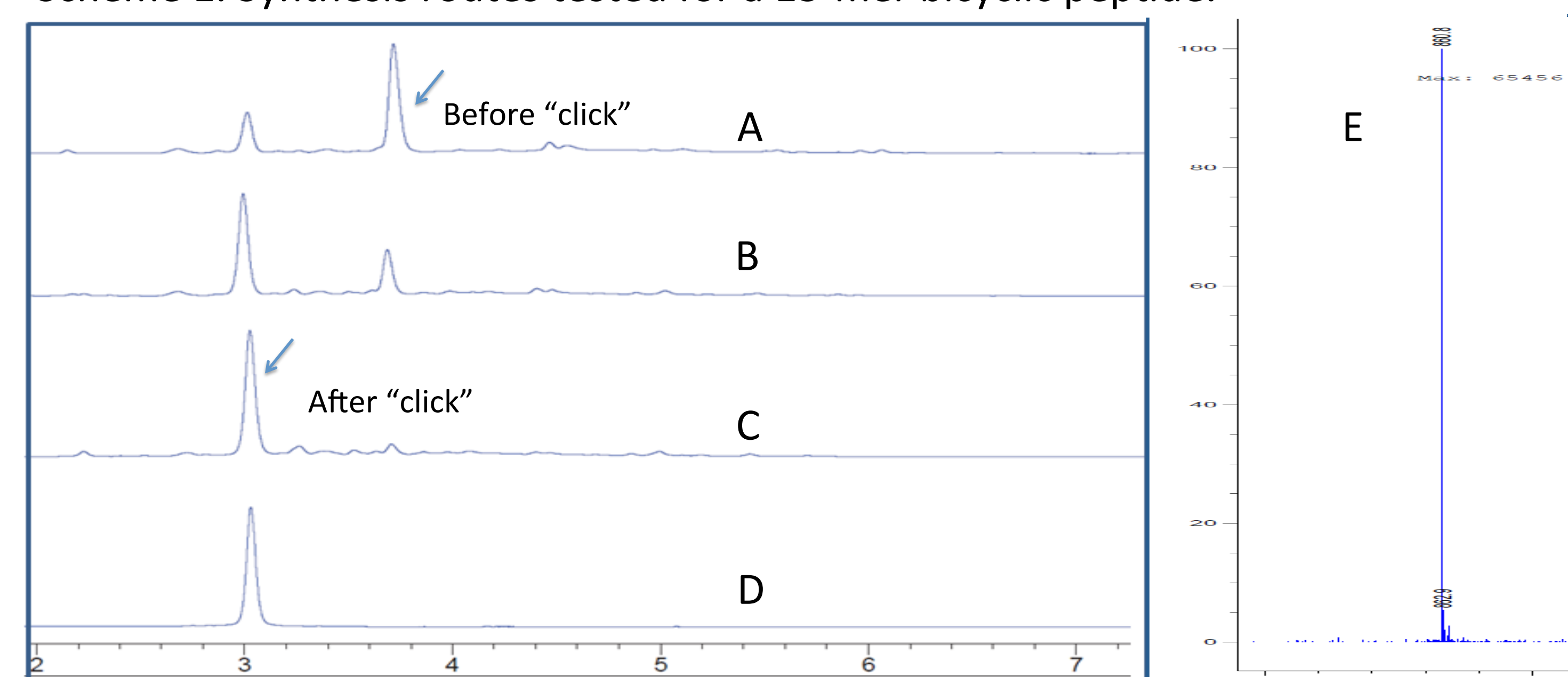


Figure 1. After air oxidation, Copper sulfate and L-ascorbic acid were added to the reaction solution directly. Click reaction was monitored using analytical HPLC at three time points. a) 0.5 h; b) 2.5 h; c) 4 h; d) purified final product; E) mass spectrogram of purified bicyclic peptide.

Table 1. Comparison of Route B and Route C.

	Route B	Route C (one-pot)
Synthesis scale (mmole)	0.2	0.2
Final peptide purity (%)	97.0 %	97.9%
Overall yield (%)	19.0 %	30.0%
Production time (days)	13	8

We optimized conditions for a novel one-pot synthesis strategy in order to facilitate the synthetic process, improve the yield and shorten synthesis time. The two cyclization reactions were carried out in one solution, without purification of the intermediate (oxidized peptide) (Scheme 1). The oxidation reaction was monitored by LCMS and the click reaction was followed using analytical HPLC (Figure 1). Then the reaction solution was loaded onto a prep. HPLC column directly for purification. The synthesis results comparison of Route B and Route C is shown in Table 1. The overall yield was improved by more than 10 %, and one week of production time was saved. Production time may be further reduced for larger scale syntheses. The elimination of extra purification and lyophilization steps also reduced the overall production cost.

Conclusion

We tested two sequences using a one-pot synthesis strategy (Route C). For both of the sequences, the overall yield for the one-pot reaction was increased by 10 % compared with the overall yield for route B; and the production time was at least one week shorter than that for Route B for small scale synthesis. This optimized one-pot synthesis strategy is also more cost effective due to the elimination of additional purification and lyophilization steps.

This novel strategy may be applicable to facilitate the synthesis of a broad range of bicyclic peptides when the preparation of a disulfide bridge and triazole bridge within a single sequence is required.

During the method transfer process of using the one-pot synthesis strategy on other bicyclic peptide sequences, some other factors may play important roles in the process, including the pH value for each step, oxidized peptide solubility and final peptide solubility. These factors should be carefully tested and evaluated.

References

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