Fully Automated Synthesis of Oxopiperazine Helix Mimics on Prelude® X

Application Note 25 D0032255/A

Introduction

This application note was authored by Professor Paramjit Arora and Dr. Ganesh Jedhe in the Department of Chemistry at New York University.

Protein–protein interactions are often mediated by amino acid side chain functionality organized on secondary structures. Small molecule scaffolds that reproduce the array of protein-like functionality at interfaces offer an attractive approach to target therapeutically important interactions. We have described the design, synthesis and biological potential of small molecule helix mimetics derived from an oxopiperazine scaffold to target protein complexes in which binding is largely dictated by one face of the interfacial helix.[1-6] Here we describe a fully automated solid phase synthesis of oxopiperazine helix mimics (OHMs) from α-amino acids using a standard Fmoc solid-phase peptide synthesis methodology, enabling rapid diversification of the scaffold and discovery of ligands for protein targets. In this application note, an OHM dimer is synthesized on Prelude® X peptide synthesizer using Rink Amide resin. The key step involves formation of six-membered rings via Mitsunobu chemistry. We tested the optimized conditions on the OHM dimer with the sequence LLAE. The structure of the dimer is shown in Figure 1.

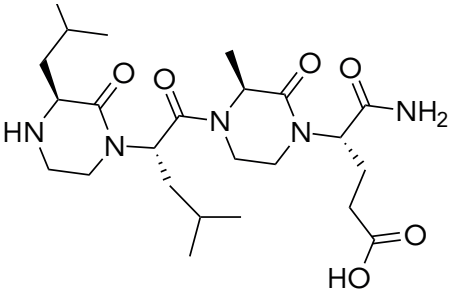
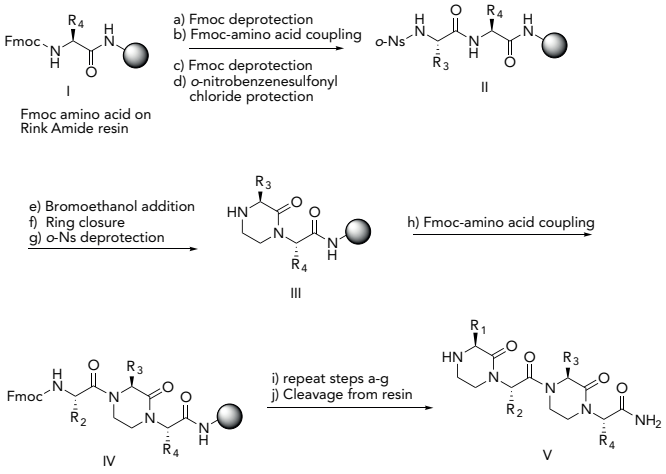
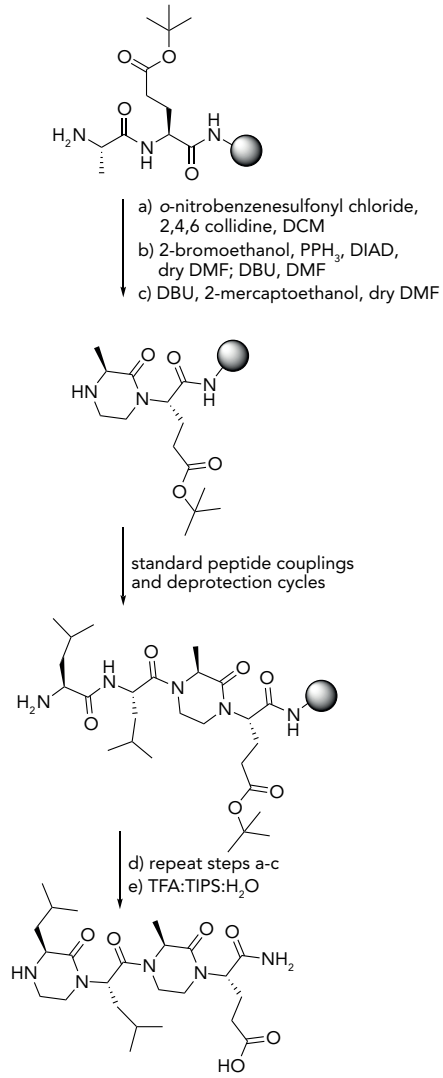


Figure 1. OHM dimer with sequence LLAE structure.

Scheme 1.



Scheme 2.



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Method

The synthesis of oxopiperazine peptidomimetics is outlined in **Schemes 1 and 2.** For the target sequence, we began with the synthesis of Ala-Glu(OtBu) dipeptide on Rink Amide MBHA resin (0.8 mol/g) from Fmoc amino acids. The resin was swelled in DCM for 10 min on the synthesizer. The Fmoc group was removed using standard deprotection conditions with 20 % piperidine in DMF for 3 x 5 minutes. Coupling was performed with 3:3:5 amino acid/HBTU/N-methylmorpholine in DMF for 2 x 30 minutes at 50°C. The bottle configuration on Prelude X was as follows:

Solvent 1 DMF
Solvent 2 DCM
Solvent 5 NMM in DMF
Solvent 6 HBTU in DMF
Solvent 7 20% Piperidine/DMF
Amino acids were dissolved at 100 mM in DMF.

a) Nosyl protection: A nosyl protecting group was added to the N-terminus of the peptide by adding 2,4,6 collidine (6 eq) and o-nitrobenzenesulfonyl chloride (6 eq) in dry DCM to the resin and was allowed to react for 2 h. The resin was washed 5 times with DCM and DMF. The bottle configuration on Prelude X was as follows:

Solvent 1 DMF
Solvent 2 DCM
AA21 2,4,6-collidine in dry DCM
AA22 Nosylchloride in dry DCM

b) Fukuyama-Mitsunobu Reaction[7,8] Triphenylphosphine (PPh_3, 6 eq), diisopropyl azodicarboxylate (DIAD, 6 eq), and 2-bromoethanol (6 eq) solutions in dry DMF were sequentially added and the reaction was allowed to shake without heating on the synthesizer for 4 h. The resin was then washed 5 times with DCM and 5 times with DMF. DBU in dry DMF was added to the resin and the reaction was allowed to shake for another 2 h. The resin was then washed 5 times with DMF and DCM. This step was repeated twice to achieve quantitative cyclization. The bottle configuration on Prelude X was as follows:

Solvent 1 DMF
Solvent 2 DCM
Solvent 3 DBU in dry DMF
AA23 Triphenylphosphine(PPh3) in dry DMF
AA24 DIAD in dry DMF
AA25 2-bromoethanol in dry DMF

c) Nosyl deprotection: The nosyl protecting group was removed by adding 6 equivalents of both 2-mercaptoethanol and DBU in dry DMF to the resin and allowed to react for 2 h. The resin was sequentially washed 5 times with DMF and DCM. The bottle configuration on Prelude X was as follows:

Solvent 1 DMF
Solvent 2 DCM
Solvent 3 DBU in dry DMF
AA26 beta-mercaptoethanol in dry DMF

Cyclized secondary amine Ala-Glu(OtBu)-resin was coupled using standard couplings twice as above to obtain Leu-Leu-cyclic(Ala-Glu (OtBu))-Resin. This resin-bound sequence was reacted sequentially following the steps of Nosyl protection, Mitsunobu Reaction and Nosyl deprotection as described above. The final deprotection from the resin was performed manually using cleavage cocktail TFA:TIS:H_2O (95:2.5:2.5) for 2 h. Volatiles were evaporated under reduced pressure.

Results

The analytical HPLC trace of crude oxopiperazine dimer LLAE is shown below in Figure 2. The desired product was identified *via* LC-MS. The MS spectrum is shown in Figure 3.

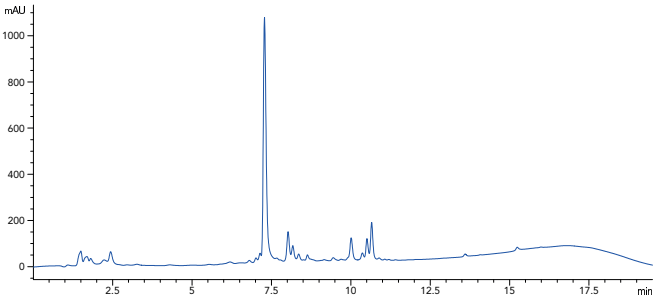

Figure 2. HPLC trace of crude oxopiperazine dimer LLAE.

OHMs have been successfully prepared using a fully automated method on Prelude X. Application of this method to the synthesis of diversified libraries will accelerate discovery of novel ligands for protein targets. Typical yields for the multi-step synthesis of oxopiperazine dimers are in the range of 5-15%. The product was synthesized with an overall yield of 12% over a total of 18 steps. Cyclization efficiency may vary depending on the individual amino acid sequence and may be monitored using micro-cleavage and LC-MS, if desired. Alternative routes for the synthesis of OHM scaffolds are currently being explored that may improve efficiency for some sequences.

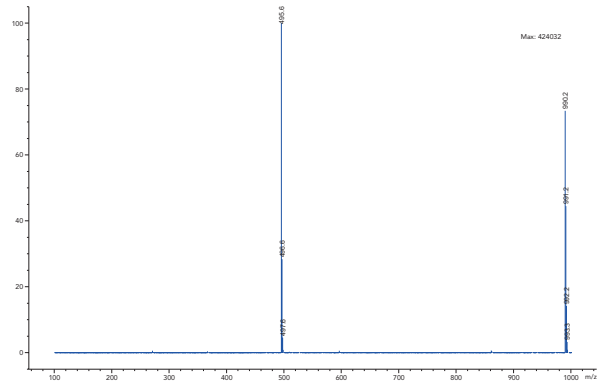

Figure 3. MS spectrum of oxopiperazine dimer LLAE.

References

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