

On-Resin Head-To-Tail Peptide Cyclization on the Prelude®

Application Note 18

D0029178/C

Introduction

Cyclic peptides have interesting biological properties. They are increasingly being used as drug targets as they often are more metabolically stable and have greater affinity and selectivity to biological receptors than their linear counterparts. Commonly, peptide cyclization has been performed in diluted solution. In 1999, Yang and Morriello¹ reported solid phase synthesis of 'head-to-tail' cyclic peptides using a sulfonamide 'safety-catch' linker. This application note describes the synthesis of naturally occurring heptapeptide, Agardhiptin A (c[LHGWPWG]) (Figure 1). In 1996, Agardhiptin A was isolated from the cyanobacterium *Oscillatoria agardhii*.² It shows inhibition of plasmin with an IC₅₀

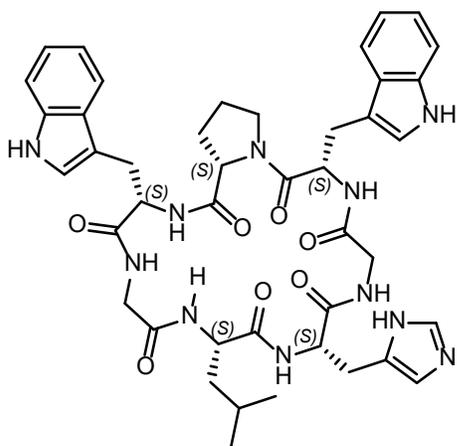


Figure 1: Structure of Agardhiptin A.

of 65 g/mL. In this application note, the peptide Agardhiptin A is synthesized and cleaved on the Prelude® peptide synthesizer using the 'safety-catch' sulfonamide linker. In this method, the linker was activated by cyanomethylation and deprotected N-terminus is able to form cyclic peptide while simultaneously cleaving peptide from the resin.

Method

Linear Peptide Synthesis: The peptide was synthesized on a Prelude peptide synthesizer at the 20 µmol scale on H-Gly-Sulfamyl Butyryl NovaSyn TG resin (0.24 mmol/g) from Novabiochem. The resin was swelled in DMF 3 x 10 minutes prior to synthesis. Deprotection was performed with 20% piperidine in DMF for 3 x 5 minutes. Coupling was performed with 10:10:10:15 amino acid/PyBOP/DIPEA/HOBt in DMF for 2 x 30 minutes. The Fmoc group was removed from the completed linear peptide prior to tritylation. The bottle configuration on the Prelude was as follows:

Solvent 1: DMF

Solvent 3: 20% Piperidine/DMF

Solvent 5: 200 mM PyBOP/DIPEA in DMF

Amino acids were dissolved at 200 mM with 300 mM HOBt in DMF.

Tritylation: A trityl protecting group was added to the N-terminus of the peptide by adding 3.5 equivalents of trityl chloride and 6 equivalents of DIPEA in DCM to the resin and allowing it to react overnight. The resin was then washed 5 times with DCM and 2 times with DMF. The bottle configuration on the Prelude was as follows:

Solvent 1: DMF

Solvent 2: DCM

AA21: Tr-Cl/DIPEA/DCM

Cyanomethylation: Activation of the Kenner 'safety-catch' sulfonamide linker was accomplished by treating the resin with 10 equivalents of iodoacetonitrile and 10 equivalents of DIPEA in NMP overnight followed by washing 4 times with DMF and 4 times with DCM. The bottle configuration on the Prelude was as follows:

Solvent 1: DMF

Solvent 2: DCM

AA22: IMeCN/DIPEA/NMP

Selective Trityl Group Removal: Removal of the N-terminal trityl protecting group was accomplished

¹ L. Yang and G. Morriello, *Tetrahedron Letters*, 40, 8197-8200 (1999).

² H. J. Shin, H. Matsuda, M. Murakami, K. Yamaguchi, *Tetrahedron*, 52(41), 13129-13136 (1996).

by adding 3% TFA and 5% TIS in DCM to the resin for 2 hours followed by washing 5 times with DCM, 5 times with THF, once with 1% DIPEA in THF, and once with THF. The bottle configuration on the **Prelude** was as follows:

Solvent 4: THF

AA23: TFA/TIS/DCM

AA24: 1% DIPEA/THF

Cyclization and Cleavage: The peptide was cyclized and simultaneously cleaved from the resin overnight with 3 equivalents of DIPEA in THF. The peptide solution was collected as well as 1 volume of THF used to rinse the resin following the initial collect. The bottle configuration on the **Prelude** was as follows:

Solvent 4: THF

AA25: DIPEA/THF

Side-Chain Deprotection: Following cleavage, the THF was removed with dry N₂ gas. The side chain protecting groups were removed from the peptide by treatment with 2 mL of 92.5:2.5:2.5:2.5 TFA/water/EDT/TIS for 4 hours. Peptide was precipitated from the solution with ice-cold ether that was then removed by centrifugation. The peptide was rinsed three more times with ether and allowed to air-dry.

HPLC Analysis: The peptide was analyzed on a Varian Microsorb C18 column (4.6 x 250 mm) on a Rainin Dynamax HPLC using an aqueous acetonitrile, 0.1% TFA buffer system with an increasing gradient of 5-60% acetonitrile over 60 minutes. Detection was at 214 nm.

Results/Discussion

The HPLC of the cyclized peptide is shown in Figure 2.

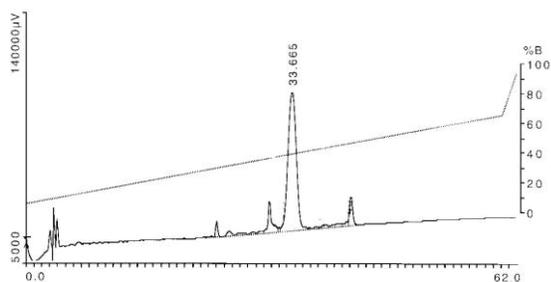


Figure 2: HPLC of cyclized Agardhipeptin A.

Conclusion

The cyclic peptide Agardhipeptin A was synthesized successfully on the **Prelude** using Fmoc chemistry and the Kenner 'safety-catch' sulfonamide linker.