Pergamon

Tetrahedron Letters 40 (1999) 8197–8200

TETRAHEDRON LETTERS

Solid phase synthesis of 'head-to-tail' cyclic peptides using a sulfonamide 'safety-catch' linker: the cleavage by cyclization approach

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Received 1 July 1999; revised 7 September 1999; accepted 8 September 1999

Abstract

A new approach to the synthesis of cyclic peptides using Kenner's 'safety-catch' sulfonamide linker is described. After the linear peptide is assembled by Fmoc peptide synthesis, trityl is adopted as a temporary protecting group for the N-terminus amine while activating the acyl sulfonamide linker. Head-to-tail cyclization–cleavage of the amine to the acyl sulfonamide ensured final product purity. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: cyclization; peptides; polypeptides; solid-phase synthesis; combinatorial chemistry.

Cyclic peptides are important targets in peptide synthesis for their interesting biological properties. Constraining highly flexible linear peptides by cyclization is one of the mostly widely used approaches to define the bioactive conformation of peptides.[1] In addition, cyclic peptides often have increased receptor affinity/selectivity and metabolic stability over their linear counterparts. Among the three ways of cyclization, sidechain-to-sidechain, sidechain-to-backbone, and head-to-tail, the latter has been investigated extensively. Two general approaches are used for synthesis: (1) classical solution-phase linear peptide cyclization under high dilution conditions; and (2) resin-based cyclization.[2] Two distinct protocols are employed in the solid phase synthesis. One extensively studied approach is the on-resin cyclization of a peptide anchored via a sidechain functional group such as imidazole,[3] acid,[4] amine[5] or alcohol.[6] The peptide is orthogonally protected as an ester at the C-terminus, and the peptide is assembled through regular Boc or Fmoc synthesis followed by saponification, cyclization and cleavage. The other is the cyclization–cleavage approach, in which the cyclic peptide was synthesized by cyclization after stepwise linear peptide synthesis.[7] One major advantage to this method is that the sidechain does not need to be anchored, making the approach more general than the first one. The published methods use either the Kaiser oxime resin[7a–c] or a thioester resin[7d] employing a Boc/benzyl-based protocol. Special precautions must be taken to avoid premature cyclization during the synthesis. The sidechain protecting

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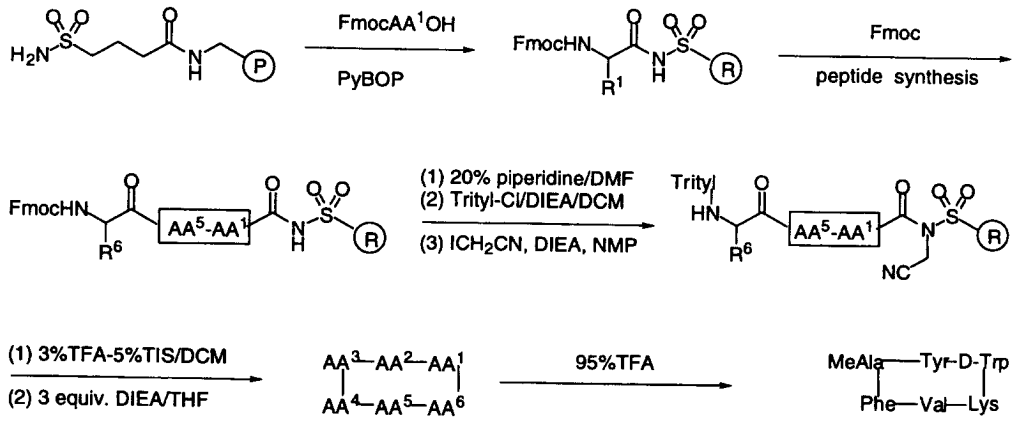
PII: S0040-4039(99)01701-3

groups were subsequently cleaved by HF. Herein we report a new method in the cyclization–cleavage synthesis of cyclic peptides using an Fmoc/Boc-based protocol.

Deprotection of Fmoc involves the use of strongly basic and nucleophilic piperidine, which makes any activated ester approach incompatible. In fact, diketopiperazine formation is one of the most common problems facing peptide acid synthesis. Therefore, the linker employed has to be stable to piperidine, while still allowing nucleophilic attack by the terminal amine after the assembly of the linear peptide. We applied Kenner's 'safety-catch' sulfonamide linker, which is stable to nucleophilic attack, yet can be activated when required.[8] The approach was systematically studied with the known cyclic hexapeptide MK-678 ([MeAla-Tyr-D-Trp-Lys-Val-Phe]).[9] The syntheses of MK-678 and its analogs have been investigated extensively in solution,[10] thus providing a comparison between solution and solid phase synthesis (Scheme 1).



Scheme 1. Synthesis of cyclic peptide MK-678

The 4-sulfamylbutyrylaminomethyl polystyrene resin[8c,11] was used in our synthesis. The first Fmoc protected amino acid was loaded using benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP) and *N,N*-diisopropylethylamine (DIEA) in DMF, first at −50°C and then at room temperature. Double coupling ensures complete loading as indicated by Fmoc analysis. The remaining linear peptide synthesis follows the standard Fmoc-protocol. At this point, the N-terminal amine has to be reprotected before activating the acyl sulfonamide linker since Fmoc is unstable under the cyanomethylation conditions. Trityl was chosen for its bulkiness to prevent cyanomethylation of the terminal amine, yet it can be removed selectively by very mild acidic conditions. Thus, after Fmoc removal, the resin-bound amine was treated with trityl chloride and DIEA in dichloromethane (DCM) to furnish the trityl intermediate. After cyanomethylation, the resin was treated with 3% trifluoroacetic acid (TFA) and 5% triisopropylsilane (TIS) in DCM to remove the trityl-protecting group. Cyclization and cleavage was accomplished by treatment with DIEA (3 equiv.) in THF at room temperature. The sidechain protected cyclic peptide was passed through a short flash column eluting with hexane and ethyl acetate to remove the non-polar impurities. Cleavage of the sidechains by treatment with 95% TFA and 2.5% TIS in water gave the cyclic peptide.[12]

We studied the dependence of yield on sequence of the linear peptides. The results are consistent with results obtained in solution synthesis. The best yield and purity for the cyclic peptide were obtained with the D-Trp to Tyr cyclization (52%) (Table 1), as observed in the original MK-678 synthesis. On the other hand, cyclization from N-Me-Ala to Phe gave no desired product. Steric hindrance by the secondary

Table 1

Sequence	Crude yield	Purity[13]
D-Trp(Boc)-Lys(Boc)-Val-Phe-NMeAla-Tyr(ᵗBu)-Resin	52%	79%
Lys(Boc)-Val-Phe-NMeAla-Tyr(ᵗBu)-D-Trp(Boc)-Resin	17%	44%
Val-Phe-NMeAla-Tyr(ᵗBu)-D-Trp(Boc)-Lys(Boc)-Resin	42%	55%
Phe-NMeAla-Tyr(ᵗBu)-D-Trp(Boc)-Lys(Boc)-Val-Resin	39%	71%
NMeAla-Tyr(ᵗBu)-D-Trp(Boc)-Lys(Boc)-Val-Phe-Resin	0	-
Tyr(ᵗBu)-D-Trp(Boc)-Lys(Boc)-Val-Phe-NMeAla-Resin	36%	74%

amine offers a plausible explanation. Other cyclization results are Lys to D-Trp (17%), Tyr to N-Me-Ala (36%), Phe to Val (39%) and Val to Lys (42%).

In order to study the methodology in another sequence, we chose to synthesize a head-to-tail cyclic hexapeptide c(Pro-D-Phe-His-D-Phe-Arg-Trp). His-D-Phe-Arg-Trp has been identified as the essential pharmacophore of the melanocortin agonist MT-II,[13] which is a sidechain-to-sidechain cyclic peptide. The incorporation of Pro-D-Phe was to form two β-turns in the cyclic peptides as observed with MK-678. His and Trp were orthogonally protected with Boc, and Arg with Pbf (2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl). Trp to Pro cyclization gave the desired peptide in 30% yield and 75% purity after sidechain deprotection. The compound is identical to a sample prepared by solution phase chemistry in terms of NMR, MS and biological activities.[14]

In summary, we have developed a novel method for the synthesis of cyclic peptides using Kenner's 'safety-catch' sulfonamide linker using the Fmoc-protocol. One major advantage to this method is that the sidechain does not need to be anchored, making the approach general for peptides with or without sidechain handles.

Acknowledgements

We would like to thank Drs. Christopher Willoughby of Molecular Design and Diversity and Stephen F. Brady of Medicinal Chemistry at Merck West Point for helpful discussions. We are also grateful to Dr. Arthur A. Patchett for his support of this effort.

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11. Purchased from Calbiochem-Novabiochem Corp, loading 1 mmol/g.
12. Typical procedure: (a) After the completion of the linear peptide synthesis, the resin (200 mg) was treated with DIEA (6 equiv.) and trityl chloride (3.5 equiv.) overnight while agitated. The resin was drained and washed with DCM (×5) and NMP. (b) The resin was treated with DIEA (10 equiv.) and iodoacetonitrile (10 equiv.) in NMP and agitated at room temperature overnight. The resin was drained and washed with NMP (×4) and DCM (×4). (c) Add 2 mL of a stock solution of 3% TFA, 5% TIS, 92% DCM. The mixture was agitated at room temperature for 2 h. The resin was drained and washed with DCM (2×10 mL), THF (2×10 mL), and 1% DIEA/THF (1×3 mL). (d) Immediately to the resin was added 2 mL THF and 38 μl DIEA (3 equiv.) and agitated overnight. The solution was collected and the resin washed with THF, and the combined solution was concentrate to dryness. The residue was purified by a short silica gel column, removing both polar and non-polar impurities. (e) The protected cyclic peptide was treated with 2 mL of a solution of 95% TFA, 2.5% water, 2.5% TIS for 4 h. The solution was then concentrated to dryness and precipitated with ether.
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