Pergamon

Tetrahedron Letters, Vol. 37, No. 5, pp. 591-594, 1996
Copyright © 1996 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0040-4039/96 $15.00 + 0.00

0040-4039(95)02269-4

An Approach to Cyclic Peptide Libraries: Reducing Epimerization in Medium Sized Rings During Solid Phase Synthesis

Arno F. Spatola, Krzysztof Darlak, and Peteris Romanovskis

Department of Chemistry
University of Louisville
Louisville, KY 40292 USA

Abstract. Using an amino acid linked by its side chain to a solid support, head-to-tail cyclic peptide libraries of varying ring size have been prepared via resin-bound cyclization. These mixtures complement the use of linear peptide libraries for drug lead discovery and extend molecular diversity to conformationally constrained systems. During our synthesis of cyclic peptide mixtures, C-terminal epimerization was identified as a problem during chain elongation. This was significantly overcome by coupling with preactivated amino acid pentafluorophenyl esters.

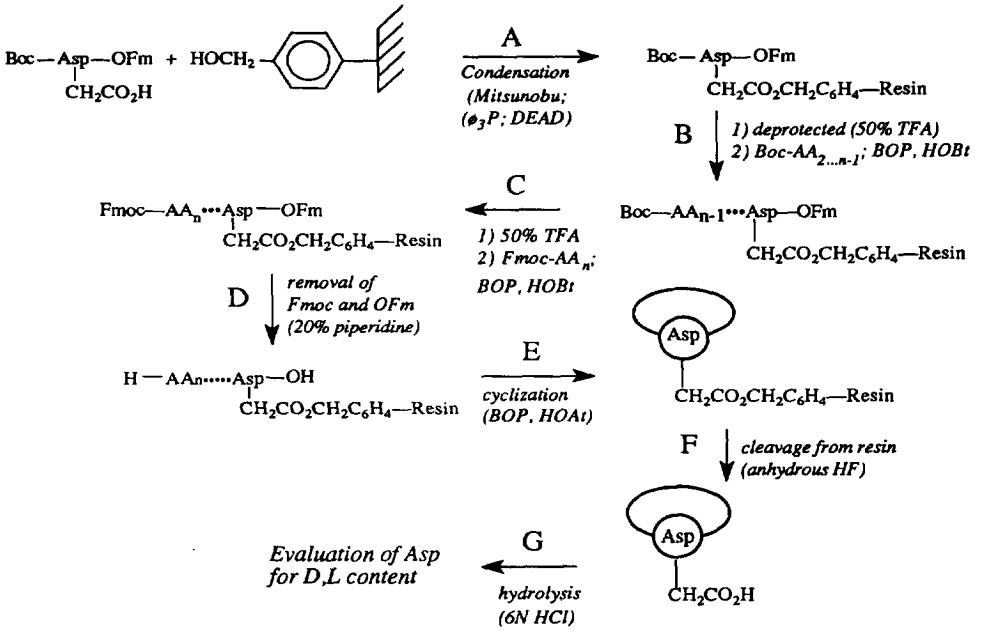
Linear peptide libraries have emerged as a powerful approach for new drug lead discovery.[1] But conformationally constrained cyclic peptides and related macrocycles represent better prospects in view of their reduced degrees of freedom and improved chemical and biochemical stabilities. We report the preparation of cyclic peptide mixtures on a solid support using side chain attachment and head-to-tail cyclization. While cyclic peptides have been prepared by Merrifield solid phase methods,[2] using resin bound cyclization,[3,4] we have for the first time adopted these methods for the preparation of cyclic peptide mixtures.[5] A recent report by Mihara et al.[6] describes the preparation of cyclic peptide mixtures with cyclization and concomitant cleavage from oxime resin, but most reported compounds contained Aca (ε-aminocaproic acid) as a flexible cyclization inducing C–terminal residue. Our synthetic approach (Scheme) uses the one-bead one-peptide strategy of Furka[7] and Lam.[8]

It has been noted that, even when using resin-bound cyclizations, oligomer formation can be a problem[9] (probably dependent on chain length) and epimerization during synthesis should be controllable. We have chosen a systematic approach to these problems by synthesizing a variety of head-to-tail cyclic peptides containing 5-7 amino acid residues and with aspartic acid or asparagine as the side-chain linked residue. Our approach permits a virtually limitless array of diverse cyclic structures, especially if other side-chain linked amino acids are used, or if various unusual amino acids or other backbone modified peptides (pseudopeptides) are substituted.[10]

Our first synthetic target involved a small mixture of cyclic pentapeptides based on a *retro-inverso* version of the recently described cyclic pentapeptide endothelin antagonist,[11] cyclo(D-Trp-D-Asp-Pro-D-Val-Leu) known as BQ–123. During the syntheses of the four analogs cyclo[Xxx-D-Leu-Val-D-Pro-Asp], where Xxx = Ala, Phe, Tyr, and Trp, we noted two major synthetic problems. The first involved unusually long cyclization times using BOP/HOBt, as monitored by the ninhydrin test. However upon FABMS analysis, this problem was attributed to formation of a linear piperidide as a minor side product as previously reported by others.[12] This difficulty was solved by more complete washing with tertiary amine to remove residual piperidine salts following N- and C-

terminal deprotection (see Scheme, step D). Most cyclizations are now routinely completed in 2 hours or less, especially if a potent condensing agent such as Carpino's recently described aza analog of HBTU (known as HATU) is used,[13] in which case the macrocycles form in as little as 30 minutes.

The second problem involved unacceptable levels of racemization of the C-terminal aspartic acid. This was discovered both through routine analysis of the stereochemistry of hydrolyzed amino acids using Marfey's reagent, N_{α}-(2,4-dinitro-5-fluorophenyl)-L-alaninamide),[14] as well as by identification of a minor diastereomeric by-product accompanying each member of the cyclic peptide library when analyzed by LC-MS. Although epimerization or racemization could have occurred in at least seven major steps of the synthetic process (including racemization during amino acid analysis), the most likely step was initially thought to be the activation of the linear C-terminus and transformation to a cyclic product. However, detailed quantification of relative D- vs. L-



Scheme. Generalized protocol for synthesis of cyclic peptide libraries using aspartic acid (Asp) as the example of a side chain-bound trifunctional amino acid. Steps A-G represent seven loci where racemization of aspartic acid could theoretically occur.

aspartic acid content of each step (Scheme, A-G) using Marfey's reagent revealed that *epimerization accompanied chain growth* and was therefore most likely caused by premature removal of the C-terminal OFm ester (as suggested by Bednarak et al.[15]) and inappropriate activation by the condensing agent during stepwise N-terminal elongation.

This problem should be reduced by using the active ester method of peptide coupling. Accordingly a synthesis of an identical peptide mixture was undertaken using preformed Boc-protected amino acid pentafluorophenyl esters.[16] This time the amount of D-Asp observed following hydrolysis was about 11% (Table I, entry 3) as opposed to 30% (entry 1). As a control, 6N HCl hydrolysis of L or D-aspartic acid led to 6.3 and 3.7% levels of racemization (entries 4 and 5). Alternatively, using BOP/HOBt and reducing the amount of base during couplings was actually equally effective (entry 9).

Table I. Cyclic Peptide Mixtures, Aspartic Acid Analogs, and Their Analysis by Marfey's Test

	Amino Acid or Peptide	Cmpds	Mixture	Coupling reagent; Cyclization reagent	AAA	Mass Spectra FABMS; LCMS	D-Asp (%) Marfey's Test (L-Asp)
1	Xxx–D-Leu–Val–D-Pro–Asp	4	Xxx = Ala, Phe, Tyr, Trp	BOP/HOBt; BOP/HOBt	+	+;+	30.0
2	Xxx–D-Leu–Yyy–D-Pro–Asp	16	Xxx = Phe, Tyr, Trp, Nal Yyy = Gly, Ala, Val, Leu	BOP/HOBt; BOP/HOBt	+	+; +	26.5
3	Xxx–D-Leu–Val–D-Pro–Asp	4	Xxx = Ala, Phe, Tyr, Trp	Active ester; HATU/ HOAt	+	+; +	10.7
4	L-Asp		—				6.3
5	D-Asp		—				96.3 (3.7±1.0)
6	Boc-Asp-OFm		—				7.4
7	Boc-Asp(OCH_2-Resin)-OFm		—		+		8.8
8	Aaa–Bbb–Ccc–Ddd–Asp	1296	Aaa = Ala, Phe, Tyr, Ile, His, Gly Bbb = Lys, Ala, Thr, Phe, Gly, Pro Ccc = Arg, Gly, Trp, Phe, Met, Gln Ddd = Gly, Tyr, Val, Leu, Ser, Glu	BOP/HOBt; HATU/ HOAt	+ +	+; ND +; ND	30.3 27.8
9	Trp–D-Leu–Val–D-Pro–Asp	1	—	BOP/HOBt (only eq DIEA); HATU/ HOAt	+	+; ND	7.4

We have also carried out the synthesis of larger libraries (64-1296) of various ring sizes (5, 6, and 7 amino acid residues). These have been submitted for bioanalysis as unseparated mixtures. In these ring sizes, dimer formation has not been a significant problem, and in the preparation of a fairly large (1000+) mixture of cyclic pentapeptides containing only L-amino acids, cyclizations were complete in under 2 hours (entry 8) using either BOP/HOBt or HATU/HOAt for cyclization.

Acknowledgements

We are grateful for financial support from NIH (GM33376), and mass spectral data from the Universities of Nebraska and Michigan and Finnigan-MAT, and HOAt and HATU from Millipore.

Abbreviations used:

AA = amino acid fragment; DEAD = diethylazodicarboxylate; DIEA = diisopropylethylamine; DMF = dimethylformamide; Boc = tert-butyloxycarbonyl; FABMS = fast atom bombardment mass spectrometry; HOBt = N-hydroxybenzotriazole; HOAt = 1-hydroxy-7-azabenzotriazole; Bop = 1-benzotriazolyloxytris(dimethylamino)-phorphonium hexafluorophosphate; HATU = O-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; TFA = trifluoroacetic acid; ϕ_3P = triphenylphosphine.

References

1. Gordon, E. . ; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gallop, M. A. *J. Med. Chem.* **1994** *37*, 1385-1401 and references therein.
2. Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149-2154.
3. (a) Sklyarov, L. Y.; Shashkova, I. V. *J. Gen. Chem. (in Russian)* **1969**, *39*, 2778-2783 ; (b) Rovero, P.; Quartara, L.; Fabbri, G. *Tetrahedron Lett.*, **1991**, *32*, 263-2642.
4. Brugghe, H.F.; Timmermans, H. A. M.; Van Unen, L. M. A.; Jan Ten Hove, G.; Van De Werken, G. Poolman, J. T.; Hoogerhout, P. *Int J. Peptide Protein Res.* **1994**, *43*, 166-172.
5. Darlak, K.; Romanovskis, P.; Spatola, A. F. in "Peptides: Chemistry, Structure, and Biology," R. S. Hodges and J. A. Smith, eds., ESCOM, Leiden, 1994, pp. 981-983.
6. Mihara, H.; Yamabe, S.; Niidome, T.; Aoyagi, H.; Kumagai, H. *Tetrahedron Lett.* **1995**, *36*, 4837-4840.
7. Furka, A.; Sebestyen, F.; Asgendom, M.; Dibo, G. *Int. J. Peptide Protein Res.* **1991**, *37*, 487-493.
8. Lam, K. S.; Salmon, S. E.; Hersh, E. M.; Hruby, V. J.; *Nature* **1991**, *354*, 82-84.
9. McMurray, J. S. *Tetrahedron Lett.* **1991**, *32*, 7682.
10. Spatola, A. F. in *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*, Vol. VII, B. Weinstein, ed., Marcel Dekker, NY, 1983, pp. 267-357.
11. Ihara, M.; Noguchi, K.; Saeki, T.; Fukurode, T.; Tsuchido, S.; Kimura, S.; Jukami, T.; Ishikawa, K.; Nishibe, M.; Yano, M. *Life Sci.* **1992**, *50*, 247.
12. Kates, S. A.; Solé, N. A.; Johnson, C. R.; Hudson, D.; Barany, G.; Albericio, F. *Tetrahedron Lett.* **1993**, *34*, 1549-1552, see reference 17.
13. Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397-4398.
14. Marfey, P. *Carlsberg Res. Commun.* **1984**, 591-596.
15. Bednarek, M. I.; Bodanszky, M. *Int. J. Peptide Protein Res.* **1983**, *21*, 196-201.
16. (a) Kisfaludy, L.; Roberts, J. E.; Johnnson, R. H.; *J. Org. Chem.* **1970**, *35*, 3563-3564; (b) Kisfaludy, L.; Low, M.; Nyeki, O.; Szirtes, T.; Schoen, I.; *Liebigs Ann. Chem. 1973* **9**, 1421-1429.

(*Received in USA* 18 *October* 1995; *revised* 17 *November* 1995; *accepted* 20 *November* 1995)