Pergamon

Tetrahedron Letters, Vol. 35, No. 31, pp. 5547-5550, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $7.00+0.00

0040-4039(94)01104-4

Peptide-Cyclizations on Solid Support: A Fast and Efficient Route to Small Cyclopeptides

Lutz S. Richter*‡, Jeffrey Y.K. Tom and John P. Burnier

Department of Bioorganic Chemistry
Genentech, Inc.
390 Point San Bruno Boulevard
South San Francisco, CA 94080

Abstract: A series of cyclic penta-, hexa- and heptapeptides was synthesized using a novel cyclization strategy. After attachment of the first amino acid to the solid support with a thioester-linkage, the linear peptides were synthesized using Boc-chemistry. Head-to-tail cyclizations were performed on the solid support and provided the desired cyclopeptides in high purity and good yields.

Key words: peptide cyclizations, solid support, Boc-chemistry

Small, cyclic peptides show increased resistance to enzymatic degradation[1] and constrained flexibility[2] as compared to their linear analogs. Consequently, they frequently exhibit higher biological selectivity and activity.[3] Since they often favor one well-defined conformation in aqueous solution, they allow highly resolved conformational analyses using modern NMR and molecular modelling techniques. Recently, this strategy was used for the identification of the bioactive conformation of a small, highly potent cyclopeptide that inhibits platelet aggregation at a concentration of 150 nm.[4]

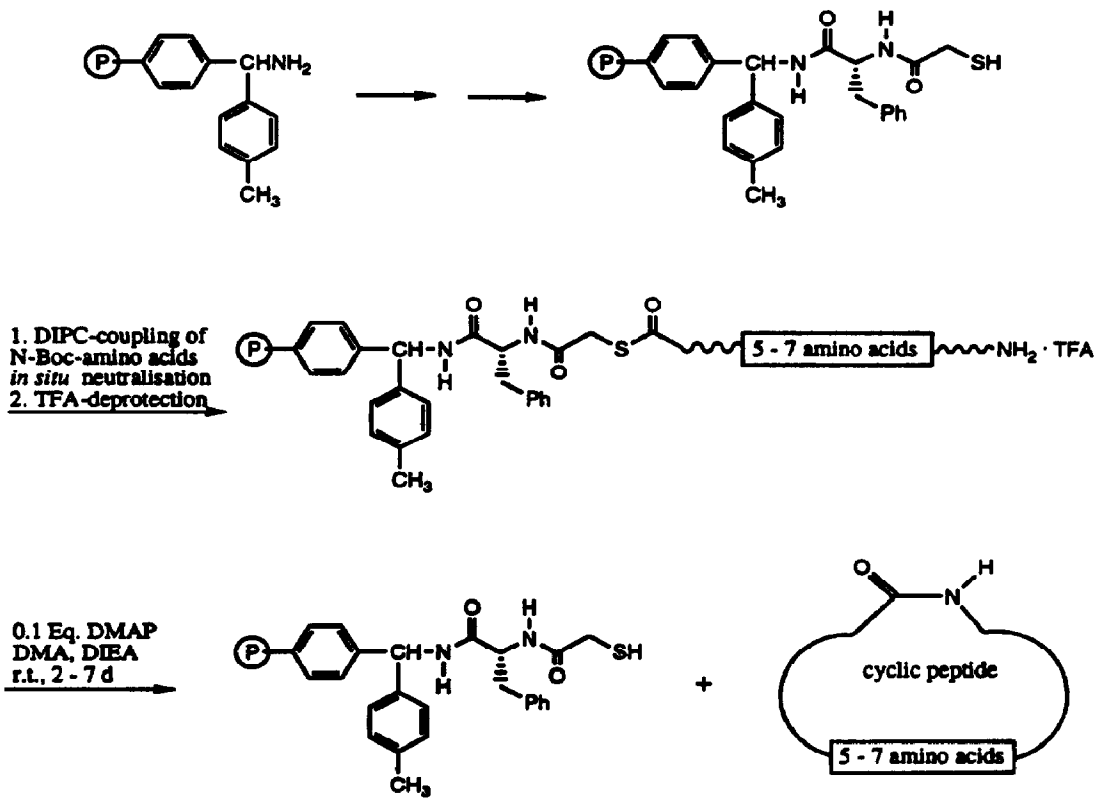
Generally, cyclizations on solid support give more favorable results than solution based methods. Due to the "pseudo-dilution-phenomenon",[5] the target cyclopeptides are accessible in higher purity using only small amounts of solvents. Recently, a number of resin-based techniques have been reported.[5–10]

Peptide-cyclizations on solid support have been performed using side-chain-attachments of Asp or Glu where the α-carboxylate was protected as an Fmoc[6], trimethylsilyl ethyl[7] or allyl ester.[8] This efficient strategy is compatible either with the Boc[6]- or Fmoc[7,8]-protocol, but it is restricted to peptides that contain side-chains like Asp or Glu which can be attached to the solid support. Other authors used Kaiser's oxime resin[9] for peptide cyclizations;[10] however, the preparation of cyclic tetrapeptides using Kaiser's oxime resin suffered from production of considerable amounts of the cyclic dimers.[11]

Described herein is a general and efficient route to small, cyclic peptides using solid phase peptide synthesis (Scheme 1). The starting resin is easily prepared from commercially available MBHA-resin.[12] We obtained best results when we used Phenylalanine as a spacer before attaching S-trityl protected thioglycolic acid. After removal of the trityl group with 50% TFA in CH_2Cl_2 in the presence of

5 equivalents triethylsilane,[13] the first amino acid was attached to the free thiol with DIPC in CH_2Cl_2 in the presence of a tertiary amine.[14] The linear peptide was built up using the standard Boc-protocol. In order to minimize premature cyclization reactions, we neutralized the TFA-salts of the N-terminal amino acids *in situ* with either NMM or DIEA during the coupling of the subsequent amino acid.

Scheme 1



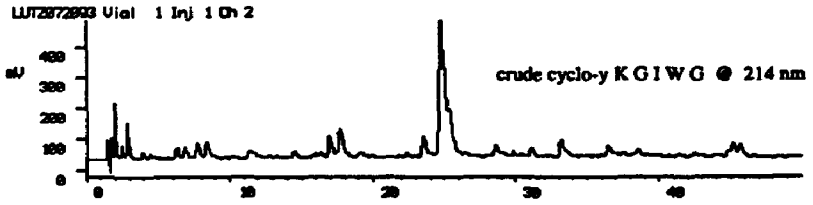
Cyclizations were accomplished at 25°C in DMA with 3 equivalents of DIEA and 0.1 equivalent of DMAP as an acyl activating agent. The side-chain protected cyclic peptides were precipitated with water after 2-7 days. Table 1 shows yields and reaction times for cyclization of selected peptides. Figure 1 shows a typical HPLC-chromatogram of a crude cyclopeptide (cyclo - y K G I W G, entry 2) after cleavage of the side-chain protecting groups with HF.

Table 1

Entry	peptide sequence	cyclization yield[a)]	reaction time
1	cyclo - y R G I W G[b)]	32%[a)]	96 h
2	cyclo - y K G I W G	35%	96 h
3	cyclo - p R S I W G	26%	36 h
4	cyclo - w d P v L	29%	7 d
5	cyclo - G H w AW f K	25%	48 h
6	cyclo - (β-A)[c)]H w AW f K	15%	48 h
7	cyclo - A H w A W f K G	70%[d)]	48 h
8	cyclo - G y R G D F	15%	48 h

a) Yields are based on the weight of the side-chain protected, crude cyclopeptides; typically 70-95% content of the target cyclopeptide. b) The amino acid attached to the thiol is printed bold, D-amino acids are printed lower case. c) beta-alanine d) only 40% content of the target cyclopeptide.

Figure 1



Epimerization at the thioester coupled C-terminal amino acid was no more than 15% using this method. Using glycine thioester-coupled peptides, entries 1,2,3, and 7, obviates this reaction and, in general, provides the desired cyclized products in higher yield.

We experienced only few examples where our cyclization method failed to provide the desired cyclic peptide. For example, attempts to cyclize IWSpRG failed under the standard conditions described above. The cyclized product could be isolated, however, under forcing conditions in either DMA or pyridine at 40°C in the absence of DMAP. After a reaction time of 7 days, the fairly pure product (approx. 50% content of the desired cyclopeptide) was isolated in 18% yield.

In order to compare peptide cyclizations using the thioester-linkage to existing methodology with Kaiser's oxime resin, we synthesized cyclo- y K G I W G (entry 2) and cyclo- p R S I W G (entry 3) on oxime resin using identical synthesis and cyclization conditions. After the usual work-up, we obtained the two cyclic peptides in 36% and 21% crude yield; however, the material obtained from Kaiser's oxime resin was less pure than the material that had been obtained using the thioester-linkage.

On the other hand, attempts to cyclize D Phg R G^{15} using a thioester-linkage failed to give the desired cyclic tetrapeptide.

In summary, we have shown that small cyclic peptides can be synthesized and cyclized on resin using a novel thioester-attachment of the first amino acid. This broadly applicable strategy is compatible with Boc-chemistry and allows efficient cyclizations on solid support.

Acknowledgement: We thank Martin Struble for HPLC purifications and Daniel Burdick for mass spectroscopic analyses.

References amd Notes:

‡Present address: Chiron Corporation, 4560 Horton Street, Emeryville, CA 94608

Abbreviations: DIEA, N,N-Diisopropylethylamine; DIPC, Diisopropylcarbodiimide; DMA, N,N-Dimethylacetamide; DMAP, 4-(N,N-Dimethylamino)pyridine; NMM, N-Methylmorpholine, TFA, Trifluoroacetic acid.

1. Plattner, J.J.; Norbeck, D.W. : Obstacles to drug development from peptide leads. In *Drug Discovery Technologies*, Clark, C.R.; Moos, W.H. (Editors), Ellis Horwood Ltd., Chichester, England 1990, 92.

2. Toniolo, C. *Int. J. Peptide Protein Res.* **1990**, *35*, 287.

3. a) Barker, P.L.; Bullens, S.; Bunting, S.; Burdick, D.J.; Chan, K.S.; Deisher, T.; Eigenbrot, C.; Gadek, T.R.; Gantzos, R.; Lipari, M.T.; Muir, C.D.; Napier, M.A.; Pitti, R.M.; Padua, A.; Quan, C.; Stanley, M.; Struble, M.; Tom, J.Y.K.; Burnier, J.P. *J. Med. Chem.* **1992**, *35*, 2040. b) Smith, D.D.; Slaninova, J.; Hruby, V.J. *J. Med. Chem.* **1992**, *35*, 1558.

4. McDowell, R.S.; Gadek, T.R. *J. Am. Chem. Soc.* **1992**, *114*, 9245.

5. Kates, S.A.; Solé, N.A.; Johnson, C.R.; Hudson, D.; Barany, G.; Albericio, F. *Tetrahedron Lett.* **1993**, *34*, 1549 and references cited herein.

6. a) Rovero, P.; Quartara, L.; Fabbri, G. *Tetrahedron Lett.* **1991**, *32*, 2639. b) see ref. 6.

7. a) Trzeciak, A.; Bannwarth, W. *Tetrahedron Lett.* **1992**, *33*, 4557. b) see ref. 6.

8. Marlowe, C.K. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 437.

9. a) DeGrado, W.F.; Kaiser, E.T. *J. Org. Chem.* **1980**, *45*, 1295. b) Nakagawa, S.H.; Kaiser, E.T. *J. Org. Chem.* **1983**, *48*, 678.

10. a) Ösapay, G.; Taylor, J.W. *J. Am. Chem. Soc.* **1990**, *112*, 6046. b) Ösapay, G.; Profit, A.; Taylor, J.W. *Tetrahedron Lett.* **1990**, *31*, 6121. c) see ref. 11.

11. Nishino, N.; Xu, M.; Mihara, H.; Fujimoto, T. *Tetrahedron Lett.* **1993**, *33*, 1479.

12. Watsueda, G.; Stewart, J.M. *Peptides* **1981**, *2*, 45

13. Pearson, D.A.; Blanchette, M.; Baker, M.L.; Guindon, C.A. *Tetrahedron Lett.* **1989**, *30*, 2739.

14. In the absence of a tertiary amine, we sometimes observed incorporation of two glycine residues instead of one when attaching the first amino acid to the thiol on resin. This undesired side-reaction can be suppressed by addition of three equivalents of base (NMM, DIEA).

15. Cyclization of this tetrapeptide (Phg, Phenylglycine) on Kaiser'r oxime resin has been reported to give a 1 : 1-mixture of the desired cyclopeptide and the cyclic dimer (see ref. 11).

(*Received in USA* 28 *March* 1994; *revised* 2 *June* 1994; *accepted* 7 *June* 1994)