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MALEIMIDE-ASSISTED ON-RESIN MACROCYCLIZATION

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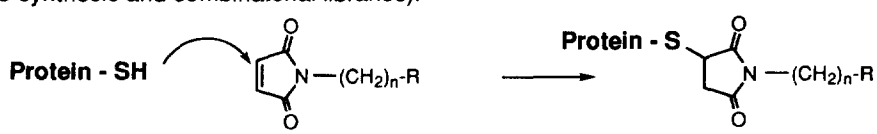
Summary: The facile addition of a sulfhydryl group to a maleimide moeity has been exploited for on-resin macrocyclization of peptides. This methodology can be easily extended to multiple synthesis of peptide/peptide-like molecules. Copyright © 1996 Elsevier Science Ltd

In general, linear peptides are conformationally flexible and susceptible to proteolytic cleavage. In addition, poor solubility, bioavailability, biodegradation and bioselectivity have limited the use of unmodified peptides as drugs to a large extent. Modifications in peptides aim not only to overcome these limitations but also offer a means to investigate factors that are important in determining the flexibility of peptides and their bioactive conformations.[1] One of the ways to restrict peptide conformation is to present the sequence in cyclic format. The first step in this process is to make cyclic disulfide bridged peptide by synthesizing the linear sequence flanked by two cysteine residues followed by cyclization by oxidation. While this leads to some stabilization of conformation, this does not always improve the serum stability of a peptide. As a result, other methods of cyclization like end-to-end and side chain-to-side chain linked cyclic lactams[2], monocarba[3] and dicarba analogs[4] have been employed with considerable success. However, these were prepared mainly by solution phase procedures. Replacement of Cys-Cys disulfide bridge by a thioether group is another way to stabilize cyclic peptides.

Maleimide based reagents have been used extensively in solution phase chemistry for cross-linking and labelling of peptides and proteins through cysteine[5] (Scheme 1) and histidine residues.[6] The sulfhydryl group adds readily to the activated double bond of maleimido group. This reaction approach can be extended to the intramolecular cyclization of peptides and peptide-like molecules containing sulfhydryl and maleimido groups. Here, we report a simple method of replacing Cys-Cys bridge by a S-Maleimido group using commercially available maleimidopropionic acid (N-maleoyl-β -alanine) linker on the solid support. The method is highly suited for automation (especially for multiple synthesis and combinatorial libraries).



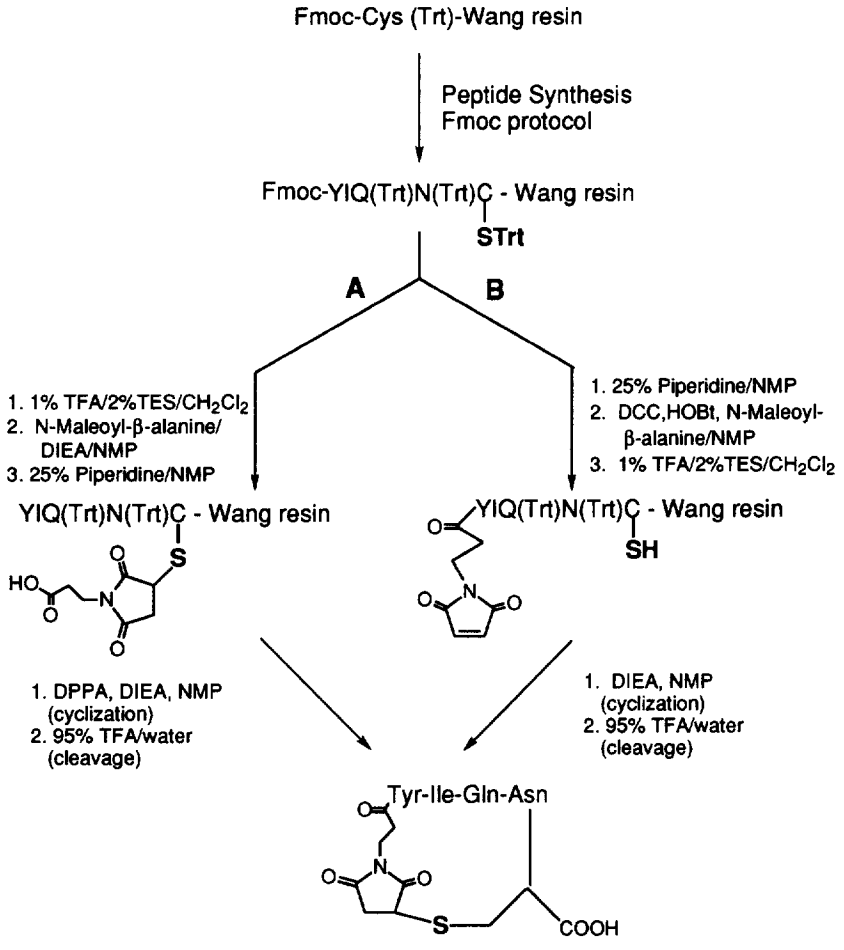
Scheme 1: Maleimide addition reaction

We prepared a protected peptide, a sequence derived from Tocinoic acid, YIQ(Trt)N(Trt)C(Trt)-resin on a commercially available Wang resin using standard Fmoc-protocol (Scheme 2). The peptide was assembled on an Applied Biosystems 430A peptide synthesizer. Two pathways were followed, one in which the maleimido linker was first added to the sulfhydryl of the Cys residue (Pathway A) and the second in which maleoyl-β- alanyl linker was put on the peptide at N-terminal (Pathway B). Pathway A involved the removal of the S-trityl group of Cys with 1% TFA/2%TES in methylene chloride (2x10 min, 1x 20 min).[7] Following removal of the Cys trityl group from peptide resin, a solution containing N-maleoyl-β -alanine (3 eq, Aldrich) and DIEA (3 eq) in NMP was added. The maleimide addition on resin is usually complete within 16-24 hours. Further, the peptide was cyclized on resin using the free carboxylic end of Mal with the free amino N-terminus using diphenylphosphoryl-azide (3 eq)/DIEA (9 eq) in NMP. In general, the cyclization is complete within 16 hours. Finally, the peptide was cleaved from the resin with 95% TFA in water to give a single cyclic product.[8] The desired peptide was purified by reverse phase HPLC (Shimadzu RP-HPLC; 5 - 95% acetonitrile, 0.1% aq TFA, 30 min). The resulting cyclic peptide provided correct mass (ESI, m/z 790.3) in 42% overall yield.

In Pathway B, N-maleoyl-β-alanine was acylated to the Cys-Trt protected peptide resin on a peptide synthesizer followed by the removal of S-Trt group with 1% TFA. The resulting peptide resin was cyclized with DIEA (3 eq) in NMP. However, unlike Pathway A, the cleavage of the cyclic peptide with 95% TFA/water provided two cyclic products with the same mass (ESI m/z 790.3) and close retention times by RP-HPLC (overall yield 43%). These two products were isolated in a 1:1 ratio (RP-HPLC, C_{18}, 36% isolated yield) and one of them corresponded (more hydrophilic product) to the main product from Pathway A. The preliminary NMR experiments suggest that the two cyclic products (with similar mass and close retention times on RP-HPLC) is a racemic mixture resulting from sulfydryl addition to the maleimide moeity. Further NMR studies are in progress.

We have developed a new and efficient method for on-resin macrocyclization of peptides which may find its application in replacing the disulfide bridge in peptides. Several macrocyclic peptides have been prepared in our laboratory using this methodology. Currently, we are further optimizing the on-resin sulfhydryl addition conditions and will investigate the scope of maleimide-assisted macrocyclization of peptides and non-peptides of different ring sizes.

Acknowledgements. We thank John S. Gounarides for initial NMR experiments.



Scheme 2: Macrocyclization of maleimidopeptides: Both pathways **A** and **B** lead to the same macrocyclized product.

Abbreviations: DPPA, diphenylphosphorazide; Mal, N-maleoyl-β-alanyl; TES, triethylsilane; TFA, trifluoroacetic acid; DIEA, N,N'-Diisopropylethylamine; NMP, N-methylpyrrolidinone; DCC, N,N'-dicyclohexylcarbodiimide;Y, Tyr; I, Ile; Q, Gln; N, Asn; C, Cys; Trt, trityl. HOBt, 1-hydroxybenzotriazole

References and Notes:

1. H. Kessler, *Angew. Chem. Int. Ed. Engl.* **21**, 512 (1982).
2. (a) S. Chen, M. Yang, C. Wu, K. Wang, *Bioorg. Med. Chem. Lett.* **4**, 2123 (1994). (b) Y. Hitotsuyanagi, J.Suzuki, K. Takeya, H. Itokawa, *Bioorg. Med. Chem. Lett.* **4**, 1633 (1994).(c) R.M. Friedinger, P.D. Williams, R.D. Tung, M.G. Bock, D.J. Pettibone, B.V. Clineschmidt, R.M. DiPardo, J.M. Erb, V.M. Garski, N.P. Gould, M.J. Kaufman, G.F. Lundell, D.S. Perlow, W.L. Whitter, D.F. Veber, *J. Med. Chem.* **33**, 1845 (1990). (d) F. Al-Obeidi, M.E. Hadley, B.M. Pettitt, V.J. Hruby, *J. Am. Chem. Soc.* **111**, 3413 (1989).(e) P. Rovero, L. Quartara, G. Fabbri, *Tetrahedron Lett.* 32, 2639 (1991). (f) A. Trzeciak, W. Bannwarth, *Tetrahedron Lett.* **33**, 4557 (1992). (g) C.K. Marlow, *Bioorg. Med. Chem. Lett.* **3**, 437 (1993).
3. (a) K. Jost, *Collect. Czech. Chem. Commun.* **31**, 234 (1971). (b) M. Lebl, T. Barth, K. Jost, ibid, **43**, 1538 (1978). (c) Z. Prochazka, M. Lebl, L. Servitova, T. Barth, K. Jost, *Collect. Czech. Chem. Commun.*, **46**, 947 (1981).
4. (a) D.F. Veber, R.G. Strachan, S.J. Bergstrand, F.W. Holly, C.F. Homnick, R. Hirschmann,
M. Torchiana, R. Saperstein, *J. Am. Chem. Soc.* **98**, 2367 (1976). (b) R.F. Nutt, D.F. Veber, R. Saperstein, *J. Am. Chem. Soc.* **102**, 6359 (1980). (c) M.L. Moore, C. Albrightson, B. Brickson, H.G. Bryan, N. Caldwell, J.F. Callahan, J. Foster, L.B. Kinter, K. Newlander, D.B. Schmidt, E. Sorenson, F.L. Stassen, N.C. Yim, W.F. Huffman, *J. Med. Chem.* **31**, 1489 (1988).
5. (a) M.D. Partis, D.G. Griffiths, G.C. Roberts, *J. Prot. Chem.* **2**, 263-277 (1983). (b) N. Haugaard, J. Cutler, M.R. Ruggieri, *Anal. Biochem.* **116**, 341-343 (1981).
6. A. Papini, S. Rudolph, G. Siglmuller, H.-J. Musiol, W. Gohring, L. Moroder, *Int. J. Peptide Protein Res.* **39**, 348 (1992).
7. In a separate experiment, we observed that these deprotection conditions are suitable for removal of the trityl group off Cys, whereas, only up to 4-5% of the trityl group is lost from Asn and Gln side chains.
8. The sulfhydryl addition was monitored by RP-HPLC (C_{18}, 5-95% acetonitrile, 0.1% aq TFA, 30 min). Cyclic and non-cyclic products have different retention times. Also, cyclic product gave negative Ellman's test.

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