Pergamon

Tetrahedron Letters 39 (1998) 6633–6636

TETRAHEDRON LETTERS

A Simple and Efficient Method for the Syntheses of Thioether Cyclic Peptides

Lin Yu*, Yonghong Lai, Janice V. Wade, and Stephen M. Coutts

La Jolla Pharmaceutical Company, 6455 Nancy Ridge Drive, San Diego, California 92121

Received 6 May 1998; revised 23 June 1998; accepted 25 June 1998

Abstract: A general method is described for the synthesis of thioether cyclic peptides. The thioether linkage of cyclic peptides was formed in moderate to high yield through an intramolecular substitution of the chloro group of β-chloroalanine with the thiol group of cysteine. The peptides containing β-chloroanaline were readily prepared on resin by converting a TBDMS-protected hydroxyl group of homoserine to a chloro group with triphenylphosphine dichloride. © 1998 Elsevier Science Ltd. All rights reserved.

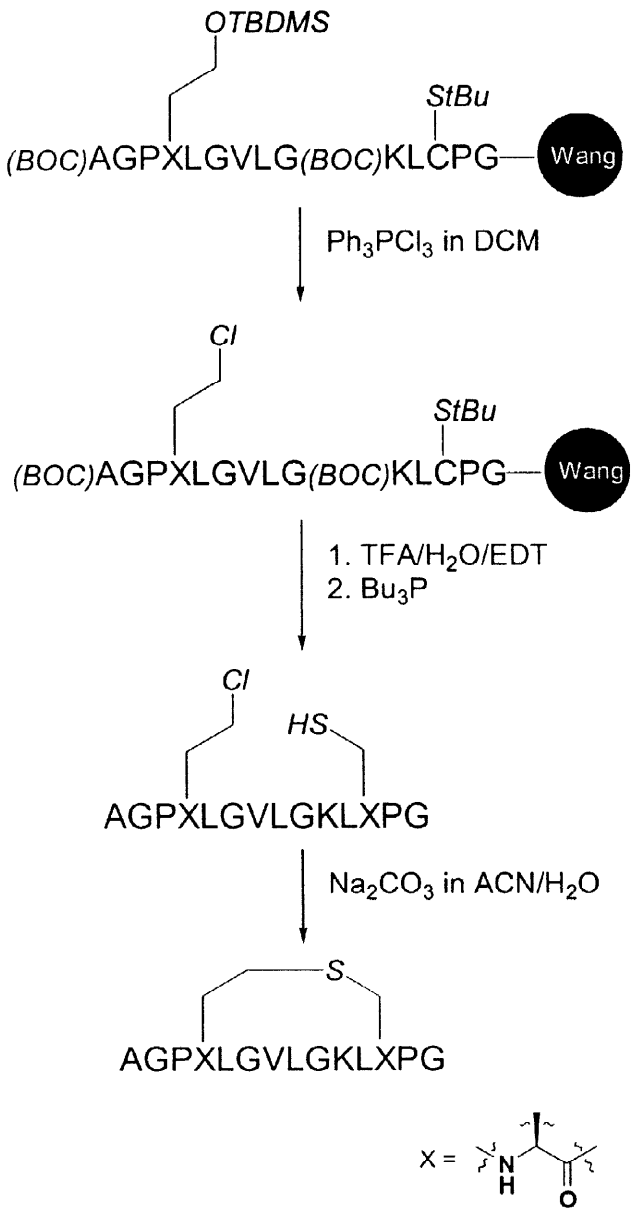
The thioether linkage has been widely utilized as a stable disulfide surrogate to replace native disulfide bridges of bioactive cyclic peptides, such as hormones, neurotransmitters and neuromodulators, to prolong their biological activities.[1-3] The thioether linkage has also been used to prepare cyclic analogues of normally acyclic polypeptides to restrict their conformational mobility and thus to increase their biological activity and stability against biodegradation.[4-7]

Additionally, thioether-linked cyclic peptides have been found in nature, especially in a family of polycyclic peptide antibiotics, lantibiotics, including nisin, an important food preservative, epidermin, a therapeutic agent against acne, as well as enzyme inhibitors and immunologically active peptides.[8-10] Prominent structural features of all lantibiotics are intrachain sulfide bridges formed by thioether diaminodicarboxylic acids, lanthionines.

The conventional approach to the synthesis of thioether-linked cyclic peptides utilizes thioether diamino acids lanthionines and cystathionines as building blocks. The peptide cyclization is accomplished through the formation of an amide bond rather than a thioether bond.[1, 11, 12] This approach requires tedious and extensive synthesis of orthogonally protected lanthionine and cystathionine derivatives.[13-17] Recently, Rolinsky and co-workers reported a synthetic approach which features an intramolecular Michael addition of the thiol group of a cysteine residue to an activated olefin to yield a lanthionine-containing peptide.[2] However, this approach often yields two diastereomeric products due to the lack of stereospecificity of the Michael addition reaction.[16] Mayer and co-workers have described a route which relies upon an intramolecular substitution reaction of bromide by the thiol group of a cysteine residue to provide a cystathionine-containing peptide.[3] This approach is limited by the low coupling efficiency of the bromo amino acid in the peptide synthesis due to competing intramolecular cyclization reaction. The thioether bridge can also be formed through reversible sulfur extrusion with tris(dialkyamino)phosphine from the disulfide peptides in moderate yields.[18] We describe here a

PII: S0040-4039(98)01397-5

Scheme 1



novel general approach to the synthesis of a thioether cyclic peptide and its key chloropeptide intermediate (Scheme 1).

The three model peptides used here for developing this methodology are from our internal autoimmune recurrent thrombosis project and their sequences are shown in Table 1. These peptides were synthesized using standard Fmoc strategy.[19, 20] Two different resins were employed in the peptide synthesis: Wang resin for peptide acid and Rink amide MBHA resin for peptide amide. During each cycle, the Fmoc group was removed with 20% piperidine in DMF. The amino acid was activated with the DIC/HOBt and coupled to the resin using a three-molar excess. Completion of the coupling was ensured by a negative ninhydrin test.[21] The α-amino group of the last amino acid coupled was protected with BOC.

The side-chain functional groups of amino acids were protected with common protecting groups used in Fmoc chemistry, *i.e.* BOC for lysine, Pmc for arginine, *t*-butyl for aspartic acid. The thiol group of cysteine was protected with either *t*-butylthio or trityl groups. The homoserine hydroxyl was specially protected with TBDMS. After completion of all coupling, the peptide resin was washed thoroughly with methylene chloride and dried *in vacuo* before chlorination.

Table 1. Synthesis of Chloropeptides and Thioether Cyclic Peptides

	Sequence	Yield (HPLC Purity)	
		Chlorination	Cyclization
1	AGPHsLGVLGKLCPG	22% (89%)	95%
2	HsLGVLGKLC-amide	55% (84%)	42%
3	GPHsILLARDRCG	48.5% (77%)	36%

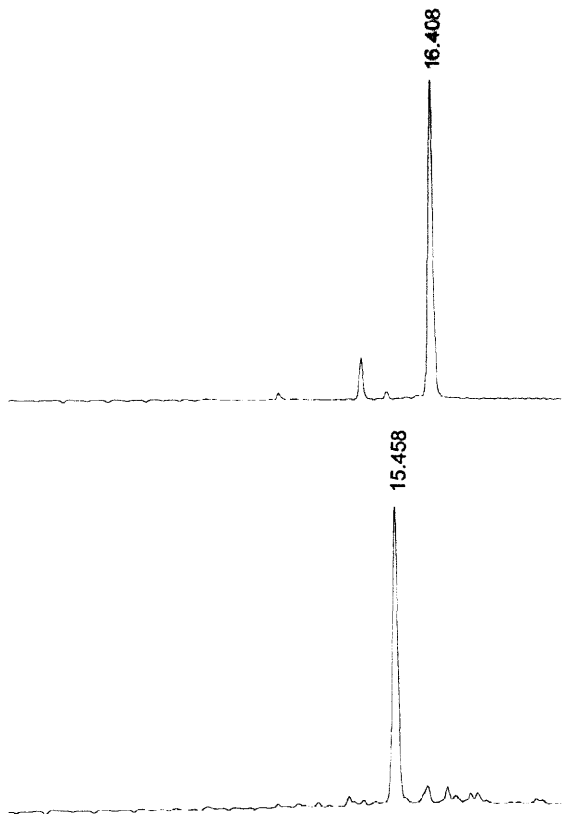


Figure 1. HPLC chromatography at 210 nm of
A) the crude chloropeptide **1** and
B) the crude thioether cyclic peptide **1**.

Conversion of the TBDMS-protected hydroxyl group of homoserine to chloro was carried out on the resin with triphenylphosphine dichloride. Typically, the chlorination of peptides is carried out using a solution of triphenylphosphine dichloride (0.50 M) in methylene chloride at room temperature overnight. This reaction is best conducted with freshly prepared triphenylphosphine dichloride, which is readily synthesized from triphenyl-phosphine and hexachloraethane in acetonitrile as described.[22] The chloropeptides were then deprotected and cleaved off the resin with 90% TFA, 5% water, 5% mercaptoethanol for three hours at room temperature. In the case of chloropeptide **1**, the *t*-butylthio protecting group on cysteine was removed with tributylphosphine. The chlorination reaction was highly efficient. Of the three peptides tested, all gave a single peak on HPLC analysis of the crude materials, as exemplified by chloropeptide **1** (Figure 1A). Details of the HPLC analysis and the preparative yields for each peptide are summarized in Table 1. The preparative yield was affected by the peptide sequence and the type of resin used. The crude peptide was purified by preparative HPLC and characterized by electrospray mass spectrometry.[23]

The thioether linkage was formed through intramolecular substitution of the chloride with the cysteine thiol. The cyclization reaction was carried out at room temperature under argon in a 1:1 mixture of acetonitrile and water, containing 1 mg/mL of chloropeptide and 10 mg/mL of sodium carbonate. The reaction was monitored by analytical HPLC and the cyclization typically took 24 hr to complete. The cyclization reaction of chloropeptide **1** was very efficient and yielded only one product as shown in Figure 1B. The crude peptide was purified by preparative HPLC and characterized by electrospray mass spectrometry.[24] The preparative yield for cyclic peptide **1** was 95%. The preparative yield for cyclic peptide **2** was 42%, although the cyclization reaction yielded only one product as determined by analytical HPLC. The cyclization of chloropeptide **3** took 36 hr to complete and the preparative yield was 36%. These results indicate that the efficiency of the cyclization is significantly dependent on the sequence of chloropeptide.

In conclusion, we have developed a facile and efficient method for the synthesis of thioether cyclic peptides. This simple and general strategy is comparable to existing literature methods with cyclization yields ranging from 36 to 95%. Further studies of the use of this method will be communicated in the near future.

REFERENCES

1. Lebl, M.; Hruby, V.J. *Tetrahedron Lett.* **1984,** *25*, 2067-2068.
2. Polinsky, A.; Cooney, M.G.; Toy-Palmer, A.; Osapay, G.; Goodman, M. *J. Med. Chem.* **1992,** *35*, 4185-4194.
3. Mayer, J.P.; Heil, J.R.; Zhang, J.; Munson, M.C. *Tetrahedron Lett.* **1995**, *36*, 7387-7390.
4. Mosberg H.I.; Omnaas, J.R. *J. Am. Chem. Soc.* **1985**, *107*, 2986-2987.
5. Hruby, V.J.; Al-Obeidi, F.; Kazmierski, W. *Biochem. J.* **1990**, *268*, 249-262
6. Kataoka, T.; Beusen, D.D.; Clark, J.D.; Yodo, M.; Marshall, G.R. *Biopolymers* **1992**, *32*, 1519-1533.
7. Hruby, V.J.; Bonner, G.G. *Methods in Molecular Biology* **1994**, *35*, 201-240.
8. Jung, G. *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1051-1192.
9. Jack, R.W.; Sahl, H.G. *Trends in Biotechnology* **1995**, *13*, 269-278.
10. Sahl, H.G.; Jack, R.W.; Bierbaum, G. *Eur. J. Biochem.* **1995**, *230*, 827-853.
11. Osapay, G.; Goodman, M.J. *Chem. Soc. Chem. Commun.* **1993**, 1599-1600.
12. Safar, P.; Slaninova, J.; Lebl, M. in *Peptides: Chemistry, Structure and Biology*; Hodges, R. S. and Smith, J. A., Eds.; Escom: Leiden, The Netherlands, 1994; pp. 119-120.
13. Jost, K.; Rudinger, J. *Collect. Czech. Chem. Commun.* **1967,** *32*, 2485-2490.
14. Cavelier-Frontin, F.; Daunis, J.; Jacquier, R. *Tetrahedron Asymmetry* **1992**, *3*, 85 -94.
15. Shao, H.; Wang, S.H.H.; Lee, C-W.; Osapay, G.; Goodman, M. *J. Org. Chem.* **1995,** *60*, 2956-2957.
16. Probert, J.M.; Rennex, D.; Bradley, M. *Tetrahedron Lett.* **1996**, *37*, 1101-1104.
17. Harpp, D.N.; Gleason, J.G. *J. Org. Chem.* **1971**, *36*, 73-80.
18. Fukase, K.; Kitazawa, M.; Sano, A.; Shimbo, K.; Fujita, H.; Horimoto, S.; Wakamiya, T.; Shiba, T. *Bull. Chem. Soc. Jpn.* **1985**, *59*, 2505-2508.
19. Stewart, J.M.; Young, J.D. Solid Phase Peptide Synthesis, 2nd, Pierce Chemical Co.: Rockford, IL., 1984; p. 82.
20. Fields, G.B.; Noble, R.L. *Int. J. Pept. Protein Res.* **1990**, *35*, 161-214.
21. Kaiser, E.; Colescott, R.L.; Bossinger, C.D.; Cook, P.I. *Anal. Biochem.* **1970**, *34*, 595-598.
22. Appel, R.; Scholer, H. *Chem. Ber.* **1977**, *110*, 2382-2384.
23. Chloropeptides: AGPHsLGVLGKLCPG: MS (ESI): m/e (M+1) calcd. for $C_{57}H_{99}N_{15}O_{15}SCl$: 1301; obsd.: 1301; HsLGVLGKLC-amide: MS (ESI): m/e (M+1) calcd. for $C_{40}H_{75}N_{11}O_9SCl$: 920; obsd.: 920; GPHsILLARDRCG: MS (ESI): m/e (M+1) calcd. for $C_{53}H_{94}N_{18}O_{15}SCl$: 1290; obsd.: 1290.
24. Cyclic peptides: AGPHsLGVLGKLCPG: HRMS (ESI): m/e ($M+Cs^+$) calcd. for $C_{57}H_{97}N_{15}O_{15}SCs$: 1396.6064; obsd.: 1396.6083; HsLGVLGKLC-amide: HRMS (ESI): m/e (M+1) calcd. for $C_{40}H_{74}N_{11}O_9S$: 885.5470; obsd.: 885.5491; GPHsILLARDRCG: MS (ESI): m/e (M+1) calcd. for $C_{53}H_{93}N_{18}O_{15}S$: 1254; obsd.: 1254.