Tetrahedron Letters, Vol.31, No.20, pp 2837-2840, 1990
Printed in Great Britain

0040-4039/90 $3.00 + .00
Pergamon Press plc

L-2-THIOL-HISTIDINE: INTRODUCTION OF CONFORMATIONAL CONSTRAINTS INTO PEPTIDES VIA THIOETHER LINKAGE

Linda L. Maggiora*, Clark W. Smith and Alex Hsi
Biopolymer Chemistry, The Upjohn Company, Kalamazoo, MI 49001

Summary: A synthetic strategy has been developed for a new type of conformational constraint in peptides, whereby two L-2-thiol-histidine (HisS) residues are bridged by a bis-thioether alkane linkage. The specificty of S-alkylation *vs.* N-alkylation in liquid ammonia would allow cyclization in the presence of nitrogen containing functional groups.

Structural features important to the interactions of a biologically active ligand with its receptor can often be evaluated through the use of conformationally constrained analogs. Studies to determine the importance of specific conformational properties of peptide ligands have commonly been facilitated by the use of cyclic peptide analogs generated by formation of disulfide or thioether linkages between the side chains of two cysteine residues[1,2]. A limitation of this approach is that the requisite two cysteine amino acid residues may not mimic the biologically active pharmacophore. Therefore, we have studied L-2-thiol-histidine (HisS), 1, as a synthon for forming bis-thioether bridged peptides, and found it to be a useful sulfur-containing amino acid with different electronic and steric properties than cysteine.

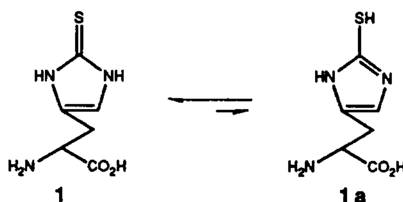
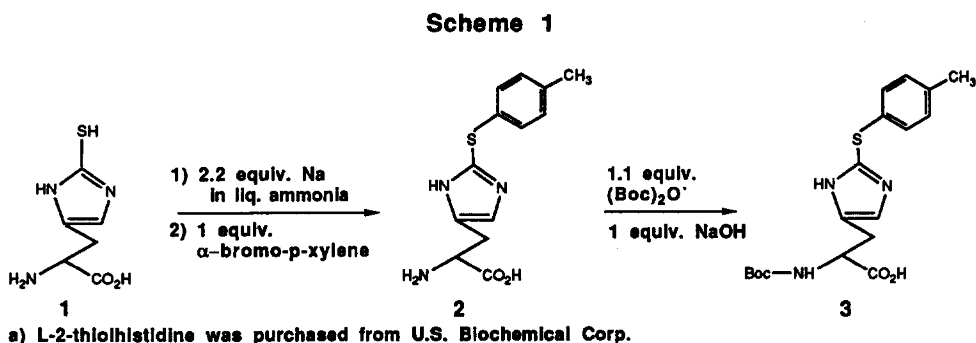


Figure 1.

While the properties of 1 are very similar to histidine in many respects, 1 has a strong absorbance at 257nm (ε 19,370), providing a convenient marker for spectrophotometric monitoring; in addition, the pK of the imidazole nitrogen of 1 is 2 pH units higher than that of histidine[3] (pK 8.5 *vs.* pK 6.5). Thus 1 could be a surrogate for a histidine residue that is protonated in its biologically active state, or could represent a conformationally constrained lysine residue. Furthermore, in analogy to 2-mercaptoimidazole[4,5], 1 exists predominantly (Figure 1) in the keto form as shown in figure 1 and would not be expected to be susceptible to oxidization. In fact, we have found that 1 does not react with 5,5-dithio-bis(2-nitrobenzoic acid) in the Ellman's test for free thiol[6] and does not form a disulfide dimer in the presence of 1, 2-diiodoethane. This property of 1 offers an advantage over the use of cysteine residues, since disulfide or polymer formation can be avoided during peptide synthesis and purification manipulations, but confers the disadvantage that disulfide linkages cannot be used directly to form constrained peptides.

The incorporation of 1 into a peptide using the Merrifield solid phase synthesis strategy (SPPS)[7] requires the temporary protection of the α-amino group and the permanent protection of the sulfur on the imidazole ring, which would otherwise be susceptible to modification during the repetitive cycles of SPPS. In addition, a lipophilic side-chain protecting group imparts significantly improved solubility properties in organic solvents to 2 or 3 compared to 1 (Scheme 1).

Scheme 1



a) L-2-thiolhistidine was purchased from U.S. Biochemical Corp.

The sodium and liquid ammonia reaction conditions in Scheme 1 effected alkylation of the sulfur, as determined by ^{13}C NMR for 3. The C_2 carbon resonance of the imidazole was shifted upfield to 134 ppm as compared to 160 ppm for N-alkylated 2-mercaptoimidazole[8]. No alkylation of either the imidazole or α-amino nitrogen of 2 was detected. This specificity reportedly could not be achieved with 2-mercaptoimidazole using phase transfer catalysis and a base such as potassium t-butoxide[9].

Compound 3 was then incorporated into peptide 4 (Table I), a model peptide designed in our laboratory as a substrate-based inhibitor of the aspartyl protease renin. The synthesis was accomplished on 4-methylbenzhydrylamine polystyrene resin using standard SPPS conditions[10]. The completed peptide was then cleaved from the supporting resin by reaction with HF-anisole (90/10) at 0°C for one hour. Surprisingly, we did not obtain concommitant cleavage of the 4-methylbenzyl (Meb) protecting group from the mercaptoimidazole, even though these conditions do cleave the Meb group from S-protected cysteine. We speculate that the electron withdrawing effect of the HF-protonated imidazole moiety renders the sulfur of the 2-thiol-histidine(Meb) a weaker base than that of cysteine (Meb) ($pK = -6.5$), and thus the sulfur is not protonated by HF ($pK = -10$) in the requisite first step of the SN1 reaction mechanism for cleavage[15].

To determine the feasibility of a one-pot synthesis to remove the sulfur protecting group and form a thioether, model reactions were performed with peptide 4 using either bromomethane or 1-bromopropane as the alkylating agent to form peptide 5 or 6[16]. When the peptide was treated with sodium in liquid ammonia followed by a 45-fold excess of bromomethane, bis-alkylated product was obtained. When the same reaction was carried out with a 10-fold excess of 1-bromopropane, a mixture of mono- and di-alkylated products was obtained. The reaction to

form a dithioether cyclic derivative of peptide 4 was then explored using the same high dilution reaction conditions as for the model reactions. With 1.5 equivalents of 1,6-dibromohexane peptide 7 was obtained. Only minor amounts of any side products were observed by HPLC of the crude reaction mixture. As compared to the intermolecular reaction with 1-bromopropane to form a dialkylated derivative, the intramolecular reaction to displace the second bromine in the cyclization reaction appears to be highly favored.

Table I. L-2-thiol-histidine-containing peptides

Ac—NH—CH(CH₂-imidazole-X)—C(=O)—Pro—Phe—NH—CH(CH₂-imidazole-Y)—C(=O)—Pheψ[CH₂NH]Phe—NH₂

Cmpd	X	Y
4	$-S-CH_2(C_6H_4)CH_3$	$-S-CH_2(C_6H_4)CH_3$
5	$-S-CH_3$	$-S-CH_3$
6	$-S-CH_2CH_2CH_3$	$-S-CH_2CH_2CH_3$
7	$-S-(CH_2)_6-S-$	

Liquid ammonia proved to be an excellent solvent for the peptides and can impart the necessary specificity to allow the alkylation of sulfur in 2-thiol-histidine even in the presence of primary amines such as lysine or a free N-terminal amine. However, reduction with sodium in liquid ammonia reportedly can cause cleavage of susceptible X–Pro amide bonds[17,18]. Thr–Pro or Arg–Pro amide is especially labile. In our hands, this side reaction was not a problem, when we controlled the addition of the sodium metal by careful titration to a faint blue endpoint using a glass encased stick of sodium[19]. After exactly one minute of reaction time the bromoalkane was added. In only one experiment did we detect a minor product that resulted from His–Pro cleavage. Additionally, we characterized a product from incomplete removal of the sulfur protecting group in an experiment that we quenched in less than one minute of reaction time.

The ease with which HisS residues can be incorporated into a peptide, and then further elaborated into a bis-thioether derivative constrained by an alkane bridge, thus provides a new method for forming constrained peptide analogs.

References and Notes

1. Schiller, P.W.; Eggiman, B.; DiMaio, J.; Lemieux, C.; Nguyen, T.M.-D. Biochem Biophys. Res. Commun. **1981**, 101, 337-343.
2. Mosberg, H.I.; Omnaas, J.R. J. Am. Chem. Soc. **1985**, 107, 2986.

3. Sober, H.H. ed., Handbook of Biochemistry; The Chemical Rubber Co., 1970 p.J-198.
4. Bojarska-Olejnik, E.; Stefaniak, L.; Witanowski, M.; Hamdi, B.T.; Webb G.A. Mag. Res. in Chem. **1985**, 23, 166-169.
5. Faure, R., Vincent E.-J.; Assef, G., Kister, J.; Metzger, J. J. Org. Mag. Res. **1977**, **9**, **688-694**.
6. Glasser, C.B.; Maeda, H.; Meinhofer, J. J. Chromatogr. **1970**, 50. 151.
7. For a review of solid-phase petide synthesis refer to: Stewart, J.M., Young, J.D. In Solid Phase Peptide Synthesis, Pierce Chemical Co.: Rockford, IL, 1984.
8. Faure, R.; Vincent, E.-J.; Assef, G.; Kister, J.; Metzger, J. Org. Mag. Res. **1977**, 9, 688- 694.
9. Hassanaly, P.; Dou, H.J.M.; Metzger, J.; Assef, G.; Kister, J. Syn. **1977**, 253-254.
10. SPPS of 4: The general procedure for Boc protected amino acids detailed in ref 11 was followed. Nα-Boc-Pheψ[CH_2NH]Phe was prepared according to the procedure in ref 12. The coupling of the pseudo-dipeptide and each Nα-Boc-amino acid was mediated by activation of 3 equivalents of amino acid with 3 equivalents of "Bop reagent" (benzotriazeol-1-yloxytris[dimethylamino]-phosphonium hexafluoro-phospate)[13] in the presence of 6 equivalents of N,N diisopropylethylamine. The coupling of 3 did require longer reaction times than the other amino acids, as judged by the ninhydrin assay[14].
11. Sawyer, T.K.; Pals, D.T.; Mao, B.; Maggiora, L.L.; Staples, D.J., deVaux, A.E.; Schostarez, H.J.; Kinner, J.H.; Smith C.W. Tetrahedron **1987**, 44, 661-671.
12. Perry, M.J.; Russell, A.B.; Szelke, M. In Chemistry and Biology of Peptides; Meienhofer, J., Ed.; Ann Arbor Science Publishers: Ann Arbor, MI; p541.
13. Fournier, A.; Wang, C-T.; Felix, A.M. Int. J. Peptide Protein Res. **1988**, 31, 86-97.
14. Savin, V.K.; Kent, S.B.H.; Tam, J.P.; Merrifield, R.B. In Peptides: Synthesis-Structure- Function; Rich, D.H., Gross, E., Eds; Pierce Chemical Co.: Rockford, IL, 1981; pp221-224.
15. Tam, J.P.; Heath, W.F.; Merrifield, R.B. J. Am. Chem. Soc. **1983**, 105, 6442-6455.
16. **General Procedure for thiol-alkylation reactions**: Peptide 4•TFA salt (50 mg, 0.034 mmole) was dissolved in liquid ammonia (200 mL) under a nitrogen atmosphere. A glass encased stick of sodium[19] was used to titrate the solution to a faint blue endpoint. The solution was stirred for exactly one minute and then quenched by the addition of a few crystals of NH_4Cl. The appropriate alkylbromide was then added, either as an ether solution or neat, and the reaction was refluxed for two hours. The ammonia was removed under a stream of nitrogen, 2 mL of 2M acetic acid were added, and the residue was lyophilized from 10% acetic acid in water. The crude peptides were purified by C18-reverse phase chromatography using a water/acetonitile gradient elution scheme with 0.1% trifloroacetic acid as a counter-ion. Each peptide was characterized by FAB mass spectroscopy, and was a single symmetrical peak by analytical reverse-phase chromatography.
17. Ramachandran, J. Nature **1965**, 206, 927-928.
18. Marglin, A.; Merrifield, R.B. J. Am. Chem. Soc. **1966**, **88**, 5051-5052.
19. Smith, C.W.; Ferger, M.F. J. Med. Chem. **1976**, 19, 250-254.

(Received in USA 31 January 1990)