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Severe Side-Reaction in the Acidolytic Cleavage of a C-Terminal Met-containing Peptide from the Solid Support. Formation of the Homoserine Lactone Peptide[1]

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Abstract: *Acidolytic cleavage of C-terminal Met-containing peptides from the solid support can lead to the formation of the homoserine lactone peptide as the major product. Formation of such undesired peptides may be avoided by removal of all tert-butyl groups from Met-containing peptides before cleavage from the resin.*

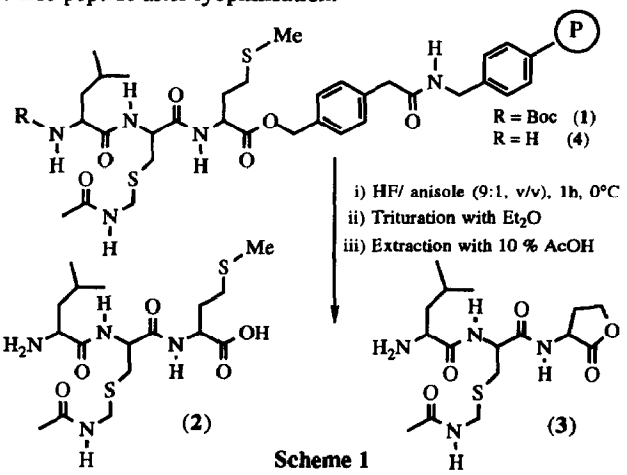
Although methionine can undergo unwanted oxidation or alkylation during peptide synthesis there is no clear consensus on whether or not this amino acid should be used in a protected form.[2] Oxidation of Met to the sulfoxide has been observed under a variety of conditions[3] and alkylation of the thioether is also extensively documented.[4] Isolated examples of the cyclisation of the amino acid methionine to homoserine lactone have been reported[5] but this side-reaction has not, to our knowledge, been described in the solid-phase synthesis of a peptide.

In order to characterise the tripeptide H-Leu-Cys(Acm)-Met-OH (**2**), corresponding to the Cys-protected *C*-terminal 68-70 sequence of the Uteroglobin monomer,[6] the peptide resin[7] Boc-Leu-Cys(Acm)-Met-OCH_2-PAM-Resin (**1**) was treated with HF/anisole (9:1). Analytical HPLC of the crude showed two major peaks (**2**) and (**3**) in a ratio of 3:7 respectively (Figure 1 a). Cleavage of peptide-resin (**1**) using HF/*p*-cresol (9:1) or by treatment with HF alone, gave crudes which showed similar analytical HPLC profiles (Figure 1 b and c).

ES-MS of product (**2**) showed a molecular ion consistent with the mass of the desired peptide H-Leu-Cys(Acm)-Met-OH. This structure was confirmed by high field ^{1}H-NMR. Amino acid analysis of the major product (**3**) indicated an absence of Met. High field ^{1}H-NMR of this compound indicated that the methyl group of Met was absent and that a downfield shift of the α, β and γ-protons had occurred. These data led us to identify compound (**3**) as H-Leu-Cys(Acm)-homoSer lactone. Confirmation of this structural assignment was provided by ES-MS data which showed a molecular ion 48 daltons less than the mass of (**2**) (See Scheme 1).

Alkylation with carbocations under acidic conditions is one of the major side reaction of unprotected Met.[3] The formation of the S-*tert*-butylsulfonium salts of Met during deprotection of *tert*-butyl-based protecting groups is well known in peptide synthesis, but cyclisation of Met as a consequence of their formation is much less common. Regeneration of free Met from the S-*tert*-butylsulfonium salt has been described.[4b] The method involves either i) removal of the Boc group after solid-phase peptide synthesis and heating of the peptide-resin

before acidolytic cleavage with HF, or alternatively, ii) acidolysis of the Boc-protected peptide-resin with HF, followed by heating of the free peptide after lyophilisation.



Scheme 1

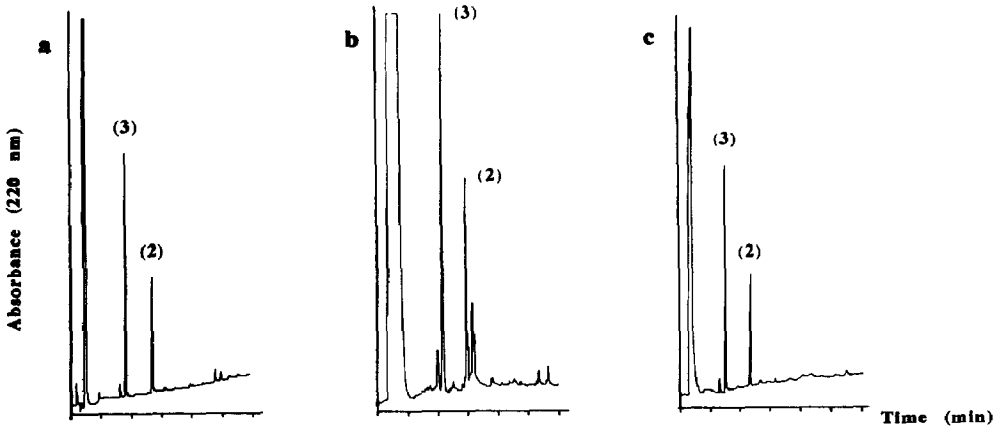


Figure 1. Analytical HPLC traces using a Nucleosil C_{18} reversed-phase column and eluting with a linear gradient from 5% to 65% of B in A over 30 min, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA at a flow rate of 1 $mlmin^{-1}$. Detection at 220 nm by UV absorbance. The large peak at the beginning of each chromatogram is due to 10% AcOH. (a) Cleavage with HF/anisole 9:1. (b) Cleavage with HF/*p*-cresol 9:1. (c) Cleavage with HF alone.

Accordingly, removal of the Boc group and heating H-Leu-Cys(Acm)-Met-OCH_2-PAM-Resin (**4**) at 85° C for 4 h followed by acidolytic cleavage with HF/anisole (9:1) or HF/*p*-cresol (9:1) (Figure 2 a and b respectively) gave the desired peptide (**2**) in high yield (> 90%). Less than 5% of (**3**) was detected under these conditions. Cleavage of the peptide from the resin followed by heating of the free peptide is not advisable in this case since the cleavage of the peptide from the resin leads to Met cyclisation.

These results lead us to speculate that lactone formation proceeds *via* the *tert*-butylsulfonium salt of Met and that cyclisation to give homoSer lactone takes place either during the acidolytic cleavage of the peptide from the resin or during the work-up (Scheme 2, path **A**).

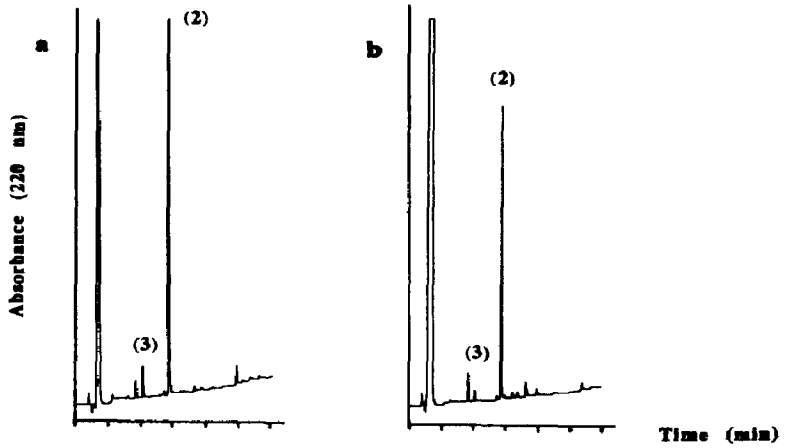
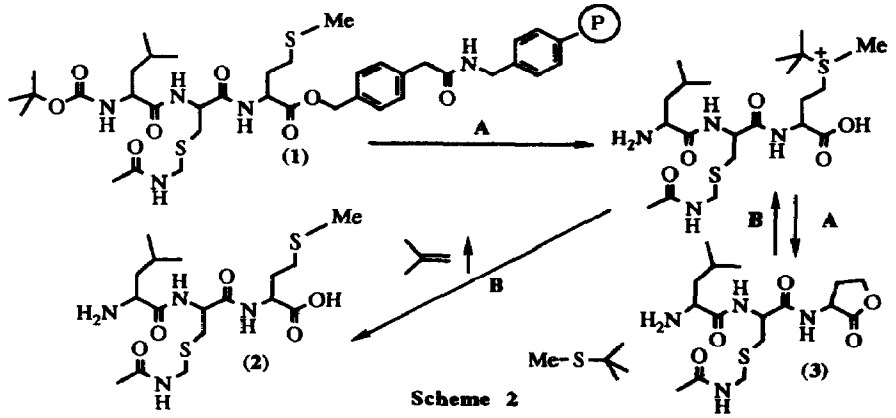


Figure 2. Analytical HPLC traces of crude cleavage products after prior removal of Boc group and heating of the peptide-resin at 85°C for 4 h. Conditions as for Figure 1. (a) Cleavage with HF/anisole 9:1. (b) Cleavage with HF/*p*-cresol 9:1.



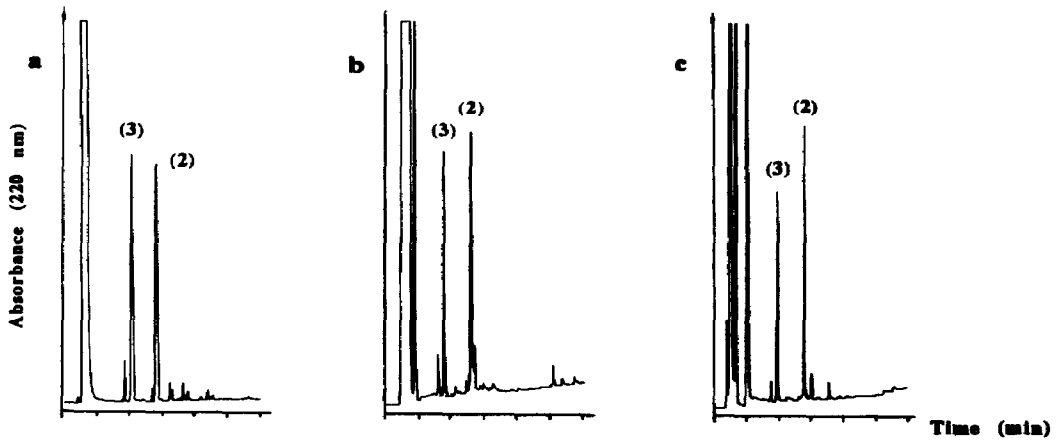


Figure 3. Analytical HPLC traces of crude cleavage products after lyophilisation. Conditions as for Figure 1. (a) Cleavage with HF/anisole 9:1. (b) Cleavage with HF/*p*-cresol 9:1. (c) Cleavage with HF alone.

Interestingly, it appears that conversion of lactone (**3**) to peptide (**2**) can take place during lyophilisation. We have noted that HPLC analysis of the lyophilised peptide crudes showed a different distribution of peaks due to (**2**) and (**3**). Comparison of the chromatograms of the crude cleavage products (Figure 1 a, b and c) with the same crudes after lyophilisation (Figure 3 a, b and c), showed that the proportion of peptide (**2**) relative to lactone (**3**) had increased.

This can be explained by assuming that the *tert*-butyl methyl sulfide formed in the cyclisation of the Met *tert*-butylsulfonium salt to homoSer lactone can, on lyophilisation of the crude mixture, re-open lactone (**3**) by nucleophilic attack at the γ-carbon. This leads to the formation of the Met *tert*-butylsulfonium salt, which under lyophilisation conditions loses isobutene, regenerating the Met derivative. (Scheme 2, path **B**).

As yet we have few data concerning the generality of this side reaction but these results do indicate that when Met is at the *C*-terminal in solid-phase peptide synthesis, alkylation of the thioether can, on acidolytic cleavage of the peptide from the resin, lead to cyclisation to give homoSer lactone. In the case of H-Leu-Cys(Acm)-Met-OH this can be avoided by removal of the *tert*-butyl group from the Met *tert*-butylsulfonium salt before cleavage from the resin. These side reactions can be avoided completely by using Met(O) for peptide synthesis.[6b]

REFERENCES

1 Abbreviations used in this paper for amino acids and for the designations of peptides follow the rules of the IUPAC-IUB Comission of Biochemical Nomenclature in *European J. Biochem.*, **1984**, *138*, 9-37 and *J. Biol. Chem.*, **1989**, *264*, 633-673. The following additional abbreviations are used: AcOH, acetic acid; Boc, *tert*-butyloxycarbonyl; DCC, *N,N'*-dicyclohexylcarbodiimide; DIEA, diisopropylethylamine; DMF, *N,N'*-dimethylformamide; ES-MS, electrospray mass spectrometry; HPLC, high-performance liquid chromatography; MeCN, acetonitrile; -resin, poly(styrene-*co*-1% divinylbenzene); NMR, nuclear magnetic resonance; PAM, phenylacetamidomethyl-; TFA, trifluoroacetic acid.

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3 See for example (a) Hofmann, K.; Haas, W.; Smithers, M. J.; Wells, R. D.; Wolman, Y.; Yanaihara, N.; Zanetti, G. *J. Am. Chem. Soc.*, **1965**, *87*, 620-631. (b) Norris, K.; Halstrom, J.; Brunfeldt, K. *Acta Chem. Scand.*, **1971**, *25*, 945-954. (c) Joppich-Kuhn, R.; Corkill, J. A.; Giese, R. W. *Anal. Biochem.*, **1982**, *119*, 73-77. (d) Hammer, R.; Albericio, F.; Gera, L.; Barany, G. *Int. J. Peptide Protein Res.*, **1990**, *36*, 31-45.

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7 Peptide synthesis was carried out manually with 300 mg of a commercial Boc-Met-OCH$_2$-Pam-resin, using the following protocol: 1) CH_2Cl_2, 4 x 0.5 min; 2) 33% TFA/CH_2Cl_2, 1 x 1 min, 1 x 30 min; 3) CH_2Cl_2, 3 x 0.5 min; 4) 5% DIEA/CH_2Cl_2, 3 x 0.5 min; 5) CH_2Cl_2, 4 x 0.5 min; 6) Boc-amino acid (3 eq) in CH_2Cl_2 or CH_2Cl_2/DMF if necessary, after 2 min the equivalent amount of DCC in CH_2Cl_2 was added and the resin allowed to stand 60 min at r.t. with occasional agitation; 7) CH_2Cl_2, 4 x 0.5 min; 8) DMF, 4 x 0.5 min; 9) CH_2Cl_2, 4 x 0.5 min. The qualitative ninhydrin test was used to monitor the synthesis. If the test was positive the protocol was repeated from step 4.

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