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# MONO- AND DITHIONOPEPTIDE SYNTHESIS

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Abstract: Syntheses of an endothioenkephalin are described. Additionally the first dithionopeptide has been prepared, and its oxidative transformation into a thiazole has been observed.

Endothioenkephalins such as (2), in which a specific peptide bond in the natural enkephalin (1) is replaced by a thioamide unit, are potentially important inhibitors of enkephalinases for the following reasons:

(a) they may be isosteric analogues of the natural substrates,

(b) following attack by an enzymically directed nucleophile, at the active site of the enzyme, the endothiono group should afford a relatively stable tetrahedral transition state surrogate for the cleavage process, and

(c) if metal ion catalysis is involved in the cleavage process then the vicinal thiolamino intermediate should further be stablized.

Evidence has emerged that endothionopeptides are in fact substrates for carboxypeptidase A, and that they are more slowly hydrolysed (over 1000 times) than the amide analogues.[1]

In devising methods to inhibit enkephalinases, and thus to derive new approaches to analgesia, we therefore targeted systems such as (2). The synthesis of endothionopeptides[2] has received increasing attention, and a range of approaches has emerged. Thioesters[2] and dithioesters[3] have been employed in coupling reactions with α-aminocarboxylates. A particularly important development, however, has been the introduction of Lawesson's phosphetane disulphide as a direct thionating agent for suitably protected dipeptides.[4] More recently, specific monothionation of the less-hindered glycyl amide sites in tri-, tetra- and pentapeptides has been described.[5] We now extend our earlier

contribution[4] in this area and describe synthetic approaches to a representative endothioenkephalin, complementing and extending other methods and describing problems of protection and deprotection of intermediates and products. The synthesis of analogue (2) of Leu-enkephalin (1) is illustrative (Schemes 1 and 2).

Endothionodipeptide ZGly$^{t}$GlyOPh (3) is readily available.[4c] Direct coupling of the phenyl ester, typically (2.0mmol) with phenylalanyl leucine methyl ester hydrochloride salt (2.5mmol) in the presence of triethylamine (2.75mmol) by heating (2.5h) under reflux in isopropanol (50cm$^{3}$), (see Scheme 1) exemplifies the effectiveness of this particular method, giving tetrapeptide (4) in yields up to 70%. N.m.r. Analysis at 400 MHz showed no detectable racemization. Deprotection of (4) under a range of conditions was complicated by participation of the thiono group.[6] However, HBr-HOAc gave H-Gly$^{t}$GlyPhe-LeuOMe in 47% yield, together with H-PheLeuOMe. Coupling with Z-Tyr(Bzl)OH was best effected by the diphenylphosphinyl chloride method,[7] giving, in 50% yield, the fully protected Leu-enkephalin analogue (5).

An alternative and better approach (Scheme 2) was to couple (6) with Z-Tyr(Bzl)OH (DPPCl/NMM)[7], giving tripeptide (7) (92%), and thence the fully protected pentapeptide, by coupling with H-PheLeuOMe, although in low yield. The reaction was conducted as above for (4). In this case the combined, like fractions from medium pressure chromatography (Sigel 60F$_{254}$; EtOAc/DCM) gave on evaporation a homogeneous pale oil (45%) which on trituration with diethyl ether yielded (5) as a white solid (27%) mp 161-162/5°C. The pentapeptides formed by each route were identical, and free from racemization (400 MHz n.m.r.).

Final deprotection was problematical because of the presence of three different protecting groups, each of which caused complications during attempted removal. This problem was resolved by repeating the above route and employing BOC and $^{t}$Bu protecting groups. Thus (8) was synthesized as above (62%), coupled with H-PheLeuO$^{t}$Bu, and endothionopentapeptide (9) (68%) isolated. This represents a 28% overall yield from ZGlyGlyOPh and is an improvement on each of the other routes. Deprotection was achieved [typically TFA (0.3cm$^{3}$), DCM (2.7cm$^{3}$), 9 (0.11mmol), anisole (0.1ml), RT] giving (2) as the TFA salt (20%), together with the monoester (10)(31%), separation being achieved by reversed phase flash

chromatography. The salt of (2) can be obtained from (10) using identical deprotection conditions in 50% yield. Elemental analysis, FAB MS and 400 MHz n.m.r. spectroscopy[8] were in accord with the desired structure.

The synthesis of dithionopeptides is an interesting and natural extension of these studies. Thus, ZGlyGlyPheOMe was reacted with 1.1 molar equivalents of Lawesson's reagent, to give (11) (65%). Attempted selective oxidations of the Gly$^{t}$Gly thiono group to the amide with $AgNO_3$/dioxan[4a] or with $AgNO_3$/dioxan/$H_2O$[2] were unsuccessful, but peracid (MCPBA) gave the thiazole (12) (50%). This new thiazole is an interesting structural variant of the increasingly important family of peptide thiazole antibiotics.

References


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2. For early studies see (a) W. Ried and W. von der Emden, Annalen, 1961, 642, 128; (b) W. Ried and E. Schmidt, ibid., 1966, 695, 217.
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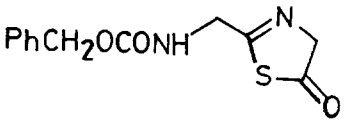
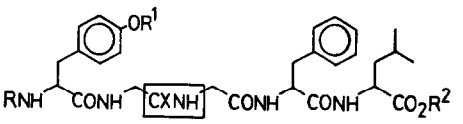
   (b) K. Clausen, M. Thorsen and S-O. Lawesson, Chemica Scripta, 1982, 20, 14;
   (c) D.W. Brown, M.M. Campbell and C.V. Walker, Tetrahedron, 1983, 39, 1075.
5. G. Lajoie, F. Lepine, L. Maziak and B. Belleau, Tetrahedron Letters, 1983, 3815.
6. It should be noted that problems were encountered when attempting to hydrolyse the esters ZGly$^{t}$GlyOPNB or ZGly$^{t}$GlyOMe, prior to coupling as activated esters, because of the formation of thiazolinone (A).




(A)

7. R. Ramage, D. Hopton, M.J. Parrott, R.S. Richardson, G.W. Kenner and G.A. Moore, J.Chem.Soc.Perkin I, 1985, 461. Note that other activated ester methods gave thiazolinone (A).
8. The endothionopentapeptide (2) trifluoroacetate salt was obtained as a white powder. $^{1}$H-NMR ($D_2O$,MeOH.$d_4$) 0.95 and 0.98 (3H,d,6.0Hz,2x$CH_3$-,Leu);1.65 (2H,br.t,7.0Hz,-$CH_2$CHMe$_2$)

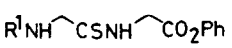
1.72 (1H,br.m,-CHMe$_2$,Leu); 2.98 and 3.01 (1H,dd,14.0,9.0Hz and 14.0, 10.0Hz,X protons of Tyr and Phe); 3.21 and 3.24 (1H,dd,14.0,6.0Hz and 14.0,4.5Hz, M protons of Tyr and Phe); 4.11, 4.18, 4.48 and 4.58 (1H,d,16.0Hz,-CH$_2$-,Gly and Glyt); 4.15 (1H,dd,9.0 and 6.0Hz, A proton of Tyr or Phe); 4.35 (1H,t,7.0Hz,backbone-CH-Leu); 4.71 (1H,dd,10.0Hz, 4.5Hz, A proton of Tyr or Phe); 6.85 and 7.17 (1H,d,8.5Hz,Tyr aromatics); 7.25 (5H,m,Phe aromatics).



(1) R = R$^1$ = R$^2$ = H, X = O

(2) R = R$^1$ = R$^2$ = H, X = S

SCHEME 1



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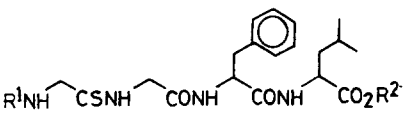
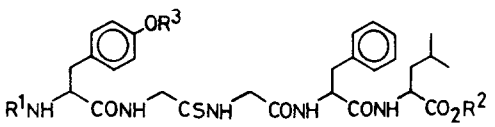
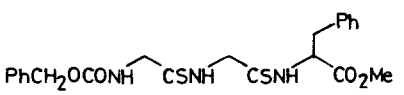


(4) R$^1$ = PhCH$_2$OCO, R$^2$ = Me



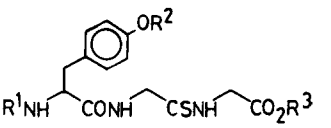
(5) R$^1$ = PhCH$_2$OCO, R$^2$ = Me, R$^3$ = PhCH$_2$



(11)

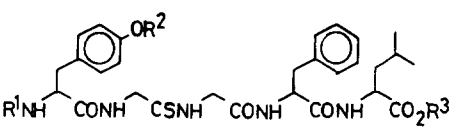
SCHEME 2

(6)



(7) R$^1$ = PhCH$_2$OCO, R$^2$ = PhCH$_2$, R$^3$ = Ph

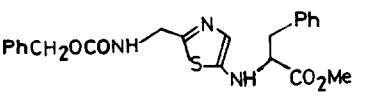
(8) R$^1$ = R$^2$ = BOC, R$^3$ = $^t$Bu



(9) R$^1$ = R$^2$ = BOC, R$^3$ = $^t$Bu

(2) R$^1$ = R$^2$ = R$^3$ = H

(10) R$^1$ = R$^2$ = H, R$^3$ = $^t$Bu



(12)

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