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ENDOTHIOPEPTIDES

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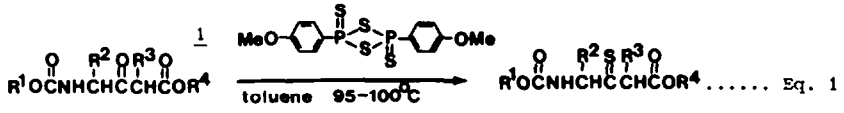
Abstract - N-protected dipeptide esters have been converted into the corresponding protected endothiopeptides[1†] using Lawesson's reagent,[2] 1. Methods of amino and carboxyl deprotection, and coupling of the thiopeptides with amino acids have been defined and used to prepare two examples of novel N-protected endothiotripeptide esters with a single thioamide link. Possible effects of the thioamide bond on the conformation of the dipeptide esters are considered.

Until recently there were very few reports in the literature concerning the synthesis of endothiopeptides.[1,3] The introduction of Lawesson's reagent by which amides are readily converted in high yields into thioamides[2] has given new impetus[3a,b] to this synthetic objective. We are prompted by these recent disclosures[3] to report our interest in sulphur analogues of biologically important peptides. Our complementary studies in this area led us to examine the scope of this reagent and to determine whether the existing protection, deprotection, and coupling procedures used in conventional peptide synthesis are generally applicable to these new systems.

It has been shown that both the amino and carboxyl functions react with Lawesson's reagent[2] 1 and so N-protected dipeptide esters, 2 were used. The thiopeptides, 3 shown in Table 1 were readily prepared from the corresponding peptides, 2 in high yields according to the generalised equation 1.

The same reaction conditions have been employed for each substrate but no difference in rate of thionation of the different dipeptide amide funtions has been detected, thus it is improbable that selective thionation of tri- and higher peptides will easily be achieved. High resolution mass measurements (Table 1) correspond to the expected molecular formulae; the fragmentation patterns conform to those previously described.[3a] All compounds were homogeneous by TLC and NMR.

The ^{13}C NMR, ^{1}H NMR and UV data on these compounds are presented in Table 2 and the linear relationship described by Clausen et al[3a] whereby $\delta(C{=}S)=1.62$. $\delta(C{=}O)-74.15$ used to obtain the calculated ^{13}C chemical shift values quoted. All data are consistent with N-Z-endiothiodipeptide esters, 3.



2 → 3 ..... Eq. 1

† The name endothiopeptide has been used previously for peptides with the thioamide link in the backbone.[1,2]

The protective benzyloxycarbonyl (Z) group was removed from the doubly-protected endothiopeptides, 3 by hydrogen bromide in acetic acid at room temperature[4] and the tert-butoxycarbonyl (Boc) group similarly using trifluoroacetic acid.[5] In each case very good yields of N-deprotected compounds were obtained in the former as the hydrobromide and in the latter as the trifluoroacetate salt.

The NMR spectral date of the N-deprotect-compounds, 4 are collected in Table 3. Both 4a and 4b have prominent ions in their mass spectra at M-81 due to their parent amines. Fragmentation in 4a occurs via the loss of methanol and then CO and analogously in 4b where the tropylium ion also figures prominently. In each case the base peak is at 30 mass units $[CH_2{=}NH_2]^+$.

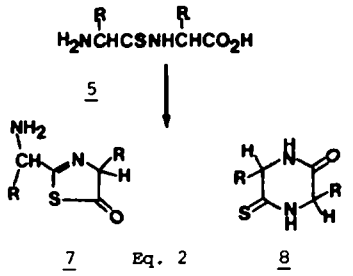
Attempts to remove the carboxyl protecting groups from the doubly-protected peptide, 3a, selectively using alkaline hydrolysis methods[3b] result in deep orange/red coloured mixtures (by TLC). Only poor yields of the free acid were obtained and so this deprotection method was not pursued.

Removal of both amino and carboxyl protecting groups of 3a, 3b and 3d by HBr/AcOH at 60° gave the endothiopeptides, 5, representing for the first time the preparation of totally deprotected systems.

Surprisingly, double deprotection of the ester 3d takes place much more rapidly than 3a in the presence of hydrogen bromide in acetic acid.

The ^1H- and ^{13}C- NMR spectra of 5 are consistent with those expected for the endothiodipeptides (see Table 3).

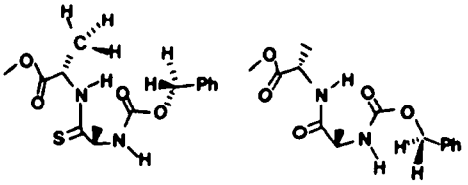
In the case of 5d and 3d, the main difference in the ^{13}C-NMR spectrum is the absence of resonance at about 52.0 ppm for 5d, the characteristic position of the methoxyl carbon. Spectroscopic data were in accord with endothioaminocarboxylic acids. The mass spectra of these compounds give highest mass ions of 100 mass units (-HBr-H_2O) lower than that expected and may be due to ions corresponding to thiazolin-5-one, 7, or thioketopiperazine structures, 8.



5 Eq. 2 7 8

In an initial study of the conformational consequences of the introduction of the thioamide group into petides, differential nOe spectra were taken on compounds 2e and 3e. Each spectrum was consistent with favoured conformations 9 and 10 resulting from hydrogen bonding between the amide N-H and carbamate carbonyl oxygen atom. In the case of the thioamide 3e a strong (33%) enhancement of the methylene protons was observed when the Ala-methyl group was continuously irradiated. There was no corresponding enhancement in 2e, but instead an enhancement (14%) of the signal due to the carbamate N-H proton when the methylene protons were irradiated. These observations may correspond to different preferred conformations about the carbamate C-O bond.

The thioamide proton is more acidic than the amide one and this could bring about stronger hydrogen bonding in 3e. The resulting



10 9

depletion in electron density in the carbamate C=O might result in more double bond character in its C-O bond favouring the conformation about the carbamate bond shown in 3e. In conclusion, difference nOe at 400 MHz indicates a significantly populated conformation (10) for the endothiopeptide 3e.

It has been reported that thionation of peptides with Lawesson's reagent takes place with no appreciable degree of racemization. This claim is based on a comparison of optical rotations of samples of N-protected dipeptide

Table 1: Yields and Physical Data for N-protected Endothiodipeptide esters, 3.

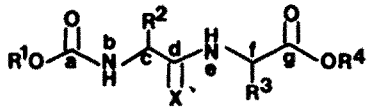
Substituent					Product (3)[a]	Yield	mp	Mol.formula	Accurate mass	
	R^1	R^2	R^3	R^4		%	°C		Calc.	Obs.
3a	$PhCH_2$	H	H	CH_3	Z-Glyt-Gly-OMe	92	89.0- 90.0	$C_{13}H_{16}N_2O_4S$	296.0827	296.0833
b	$(CH_3)_3C$	H	H	CH_2Ph	BOC-Glyt-Gly-OBzl	89	103.5-105.0	$C_{16}H_{22}N_2O_4S$	338.1295	338.1302
c	$PhCH_2$	$PhCH_2$	H	CH_3	Z-Phet-Gly- OMe	83	68.0- 69.5	$C_{20}H_{22}N_2O_4S$	386.1295	338.1301
d	$PhCH_2$	$CH_3CH_2CH(CH_3)$	H	CH_3	Z-iLet-Gly-OMe	86	-[b]	$C_{17}H_{24}N_2O_4S$	352.1451	352.1459
e	$PhCH_2$	CH_3	CH_3	CH_3	Z-Alat-Ala-OMe	100	-[b]	$C_{15}H_{20}N_2O_4S$	324.1139	324.1151
f	$PhCH_2$	H	H	Ph	Z-Glyt-Gly-OPh	70	92.5- 94.0	$C_{18}H_{18}N_2O_4S$	264.0557[c]	264.0557

a The symbols for the thiocarbonyl derivatives are those used by du Vigneaud et al[8].

b Products obtained as colourless gums.

c M-PhOH, molecular ion not observed.

Table 2: ^{13}C-NMR, ^{1}H-NMR[a] and UV Data for N-Z-dipeptide esters, 2 and N-Z-endothiodipeptide esters, 3.

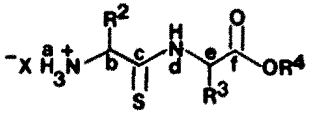


2 : X = O
3 : X = S

COMPOUND		^{13}C-NMR Chemical shift (ppm)					^{1}H-NMR Chemical shift (ppm)				UV ($CHCl_3$) λmax (ε)(nm)	
		a	c	d(calc.)	f	g	b	c	e	f		
2a	Z-Gly-Gly-OMe	156.7	44.5	169.7(-)	41.2	170.3	5.90	3.90	7.00	4.00	-	-
b	BOC-Gly-Gly-OBzl	156.2	44.3	169.7(-)	41.3	170.2	5.65	3.95	7.15	4.05	-	-
c	Z-Phe-Gly-OMe	156.0	56.3	171.3(-)	41.2	169.8	5.50	4.50	6.60	3.95	-	-
d	Z-iLe-Gly-OMe	156.5	59.8	172.0(-)	41.1	170.2	5.80	4.20	7.10	4.00	-	-
e	Z-Ala-Ala-OMe	155.8	50.8	173.2(-)	48.3	172.3	5.60	4.40	6.90	4.40	-	-
f	Z-Gly-Gly-OPh[b]	156.7	43.8	168.4(-)	41.1	170.1	8.15	3.80	7.00	4.20	-	-
3a	Z-Glyt-Gly-OMe	156.8	52.4	200.5(200.8)	46.7	168.8	5.90	4.25	8.60	4.40	266	(12,700)
b	Z-Glyt-Gly-OBzl	156.3	52.1	201.0(200.8)	46.5	168.4	5.45	4.20	8.70	4.40	266	(12,800)
c	Z-Phet-Gly-OMe	155.9	62.6	204.1(203.4)	46.8	168.5	5.80	4.80	8.30	4.20	269	(12,300)
d	Z-iLet-Gly-OMe	156.5	65.3	205.8(204.5)	46.6	168.7	5.95	4.45	9.20	4.30	269	(11,300)
e	Z-Alat-Ala-OMe	155.9	56.3	205.5(206.4)	53.4	172.2	5.90	5.10	8.70	4.70	270	(10,900)
f	Z-Glyt-Gly-OPh	156.8	52.0	201.0(198.7)	47.0	167.2	5.80	4.20	8.70	4.55	265	(16,060)

a The spectra were determined in $CDCl_3$ at 30°C unless otherwise stated.
b The spectra of 2f were obtained in $CDCl_3$: DMSO-d_6, 1:1 at 60°.

Table 3: Spectral and Physical Data for Endothiodipeptide esters, 4 and Endthiodipeptides, 5.

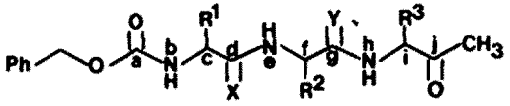


4: R^2, R^3, R^4 as for compounds: 2 and 3
5: R^2, R^3 as for compounds: 2 and 3, R^4=H
4a, 5a, 5d: X=Br
4b: X=CF_3CO_2

Compound	^{1}H-NMR					^{13}C-NMR					mp
	Solvent	Chemical Shift (ppm)				Chemical Shift (ppm)					°C
		a	b	d	e	b	c	e	f	g[a]	
4a	DMSO-d^6	8.25	3.95	11.00	4.40	42.7	198.0	47.8	169.5	-	171-173(d)
4b	DMSO-d^6	8.80	3.95	-	4.50	45.8	196.8	46.5	167.5	66.1	129-131(d)
5a	D_2O	-	3.90	-	4.10					-	91- 94
5d	D_2O	-	4.05	-	4.50	63.5	200.8	47.6	172.1	-	175-179(d)

a where R =$PhCH_2$, g is shift for methylene carbon.

Table 4: Spectral and Physical Properties of N-Z-endothiotripeptide esters, 6.



6a : X = O, Y = S
6b : X = S, Y = O

	Substituent			Yield	mp	Chemical shift (ppm)[c] ^{1}H-NMR						^{13}C-NMR						
	R^1	R^2	R^3	%	°C	b	c	e	f	h	i	a	c	d	f	g	j	i
6a	CH_3	H	H	79	108.0-109.0	5.9	4.3	7.55	4.3	9.00	4.3	156.5	51.2	173.6	50.1	200.3	46.9	169.0
6b	H	H	H	47	130.5-132.5	7.3	4.2	9.6	4.3	8.25	3.9	156.7	48.1	200.2	40.9	169.9	52.0	167.8

a Acc. mass = 367.1215, calc. for $C_{16}H_{21}N_3O_5S$ = 367.1197

b Acc. mass = 353.1044, calc. for $C_{15}H_{19}N_3O_5S$ = 353.1041

c The spectra were determined in $CDCl_3$.

esters, obtained by dethiation of the corresponding endothiopeptides prepared using the reagent, with samples of original starting N-protected dipeptide esters. Recent studies[10] have shown that N-alkyloxycarbonyl protected peptides are much more resistant to racemization than N-acyl protected peptides under reaction conditions appropriate for new peptide bond formation. As a consequence of the above we expected our peptides to retain their stereo-chemistry and used high resolution (400 MHz) ^{1}H-NMR spectroscopy to confirm this expectation as follows. The methyl ester 3e, Z-Alat-Ala-OMe contains two chiral centres and so racemization at either centre would give rise to a mixture of diastereoisomers. The ^{1}H-NMR spectrum shows overlapping doublets (1.46 and 1.47, 3H, J=7Hz) due to the methyl groups and two pentets (4.60 and 5.05, 1H, J=7Hz) due to the two methine protons further coupled (J=7Hz) to N-H. Decoupling from N-H reduced each pentet to a quartet. The ^{13}C-NMR spectrum (Table 2) contains thirteen lines only. The foregoing data can only be explained in terms of one diastereoisomer. The high signal to noise ratio and the sharpness of the signals in the ^{1}H-NMR spectrum would allow the detection of isomeric impurities down to perhaps 2-3%; only signals due to 3e were present in its NMR spectra.

The feasibility of synthesising higher endothiopeptides by coupling with N-protected amino acids at the terminal amino group was confirmed by the reaction of the hydrobromide salt of the endothiopeptide ester 4a with Z-Ala-OH to give Z-Ala-Glyt-Gly-OMe 6a. This preparation was carried out using standard conditions[6] whereby the salt was neutralised with triethylamine and the coupling promoted by dicyclohexylcarbodiimide (DDC). This is represented in Equation 3.

Z-Ala-OH + HBr.Glyt-Gly-OMe 4a $\xrightarrow{\text{DCC, 20hr.}}$

Z-Ala-Glyt-Gly-OMe 6a .... Eq. 3

Having branched from the amino terminal, we investigated the alternative coupling at the C-terminal end of an N-protected endothiopeptide ester. This was achieved by utilizing a phenyl ester protecting group (first proposed by Galpin et al[7]). Thus Z-Glyt-Gly-Gly-OMe, 6b, has been prepared from Z-Glyt-Gly-OPh, 3f, and glycine methyl ester according to Equation 4.

Z-Glyt-Gly-OPh 3f **+ HCl.Gly-OMe** $\xrightarrow[\text{reflux 8hr.}]{\text{TEA/IPA}}$

Z-Glyt-Gly-Gly-OMe 6b ... Eq. 4

Attempts to improve the relatively poor yield (47%) using an ester group which was more susceptible to nucleophilic displacement, eg p-nitrophenyl, by a free amino group have been unsuccessful. Thionation of Z-Gly-Gly-OnPh with 1 afforded several inseparable, unidentified products. Significantly p-nitrophenol was also obtained from the reaction so that thionation possibly led to an intramolecular cyclization whereby the nitrophenate anion was displaced by the thiono group.

To sum up, we have extended the range of doubly protected dipeptide substrates for which thionation using Lawesson's reagent proceeds in high yield, defined a major conformer of an N-Z-endothiopeptide ester and shown that the conventional peptide deprotection and coupling procedures are applicable to the synthesis of endothiopeptides from smaller units containing the thioamide bond.

EXPERIMENTAL

The abbreviations used are those in common use and all the amino-acids have the L-configuration. Other abbreviations are as follows* TEA. triethylamine; DCM, dichloromethane; DCC, N,N'-dicyclohexylcarbodiimide; nPh, 4-nitrophenyl; DCU, N,N'-dicyclohexylurea; IPA, isopropanol. Silica gel 60 (Merck No: 7747) was used. The ^{1}H-NMR spectra were determined at 100 MHz using a Jeol PS100 spectrometer, unless otherwise stated, and ^{13}C-NMR spectra at 22.5 MHz using a Jeol FX90Q spectrometer. The ^{1}H-NMR spectra of 2e and 3e were determined on A Brucker WH400 with a pulse length of 4μs at an angle of 45-50°. The differential nOe spectra were run at 310 K with GM (LB=0.85) with 40 scans accumulated for each spectrum. Tetramethylsilane was used as internal standard. Mass spectra were recorded by PCMU (Harwell) at 70eV. Lawesson's reagent, 1, was either prepared by the literature procedure[2] or a commercial sample (Aldrich Ltd) was used.

Z-Ala-Ala-OMe, 2e, was prepared from the free acid by a standard esterification method[9] ($SOCl_2$/MeOH). Z-Gly-Gly-OPh, 2f, was prepared using the method of Galpin et al[7], from the free acid (Z-Gly-Gly-OH).

Preparation of N-protected endothiopeptide esters, 3.

Z-Phet-Gly-OMe, 3c, Z-Phe-Gly-OMe, 2c (460 mg, 1.24 mM) and Lawesson's reagent[2], 1 (250 mg, 0.62 mM) were heated together at 95-100° in sodium dried toluene (20 cm^3) for 2 hr. The reaction was followed by TLC on silica gel (10%, ethyl acetate-dichloromethane). Evaporation *in vacuo* gave a pale yellow oil which was chromatographed (10% EtOAc-DCM, flash chromatography) giving 3c (400 mg, 1.04 mM, 84%), mpt: 68-69.5°C as a white solid which could be recrystallized from $MeOH/H_2O$. 3d And 3e were obtained as colourless oils.

Removal of the Z-group from the N-Z-endothiopeptide esters 3.

$Br^-H^+_2$-Glyt-Gly-OMe, 4a. 3a,(640 mg. 2.2 mM) was stirred for 1 hour at 20°C in HBr/AcOH (48% w/v, 10 cm^3) in a flask fitted with a $CaCl_2$ drying tube. Dry ether (50 cm^3) was then added to precipitate more white solid and the whole then filtered and the residue washed with ether. This gave 4a (500 mg, 2.1 mM, 95% yield) as a white solid, mpt: 171-173°C (decomp). (Found: $M^{+\cdot}$-HBr, 162.0475. $C_5H_{10}N_2O_2S$ requires 162.0461).

Simultaneous removal of the amino and carboxyl-protective groups.

$Br^-H_2^+$-Glyt-Gly-OH, 5a. Z-Glyt-Gly-OMe 3a (190 mg, 0.64 mM) and HBr/AcOH (48% w/v, 10 cm^3) were stirred at 55°C in a flask fitted with a $CaCl_2$ drying tube and a reflux condenser for 6 hr. (1.5 hr. for 3d). On cooling, ether (50 cm^3) was added to precipitate a white solid* which was filtered and washed with a small amount of ether. This gave 5a (140 mg, 0.61 mM, 95% yield) as a slightly coloured solid, mpt: 91-94°C. (Found: $M^{+\cdot}$-HBr-H_2O, 130.0201. $C_4H_6N_2O_2S$ requires 130.0200).

*For the deprotection of Z-Ilet-Gly-OMe, 3d, a reaction time of 1.5 hr at 20°C was sufficient. Addition of ether did not precipitate the hydrobromide 5d but the ether layer was extracted with H_2O (2 x 50 cm^3) and the aqueous extract evaporated under vacuum to give a pale orange solid (75 mg, 0.25 mM, 83% yield), mpt: 175-179°C (decomp.)

Removal of the Boc-group from 3.

$TFA^-H_2^+$-Glyt-Gly-OBzl, 4b, Boc-Glyt-Gly-OBzl, 3a (169 mg, 0.5 mM) was stirred in a solution of 90% TFA (10 cm^3) at RT for 0.5 hr. The excess TFA was removed by toluene azeotrope (2 x 20 cm^3) *in vacuo* to give a viscous oil which solidified on standing to an off-white solid, 4b, (140 mg, 0.47 mM, 94% yield), mpt: 129-131°C (decomp). (Found: $M^{+\cdot}$-TFA, 238.0769 $C_{11}H_{14}N_2O_2S$ requires 238.0773).

Preparation of N-Z-endothiotripeptide esters, 6

Z-Ala-Glyt-Gly-OMe, 6a. A suspension of the salt, 4a (242 mg, 1.0 mM) and N-Benzyloxycarbonylalanine (223 mg, 1.0 mM) in DCM (5 cm^3) was added over 5 min. and the suspension stirred for a further 5 min. and then allowed to reach RT. A solution of DCC (206 mg, 1 mM) in DCM (10 cm^3) was added slowly over 1.25 hr. After stirring at RT for a further 20 hr., the reaction mixture was filtered to remove DCU and the solvent evaporated under vacuum. The residue was subjected to medium pressure column chromatography (5% EtOH in $CHCl_3$), giving a white crystalline solid, 6a, (290 mg, 0.79 mM, 79% yield, mpt: 108-109°C.

Z-Glyt-Gly-Gly-OMe, 6b. Z-Glyt-Gly-OPh, 3f, (171 mg, 0.5 mM), glycine methyl ester hydrochloride (63 mg, 0.5 mM) and TEA (51 mg, 1 equiv) were heated to reflux in IPA (25 cm^3) for 8.5 hr. (No apparent reaction occurred at 20°C). After cooling, the solvent was evaporated *in vacuo* and the orange-brown residue subjected to medium pressure column chromatography (30% EtOAc/DCM) to remove impurities. This gave 6b as a white solid (83 mg, 0.24 mM, 47% yield), mpt: 130.5-132.5°C.

Acknowledgements

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