Thioesters of Amino Acid Derivatives as Thioacylating Agents in Thiopeptide Synthesis

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Thioesters, mainly of *N*-substituted glycine, have been synthesized and examined for their ability to thioacylate amino acids, peptides, and esters and amides of amino acids and peptides. Methyl, ethyl, and benzyl thioesters have been obtained in high yield by thiohydrolysis of the corresponding imino esters. All were found to be excellent thioacylating agents for amino acids, though under the conditions used, they reacted much less readily with amino acid esters and amides including peptides, except where the reacting nucleophile was the amino group of Gly or the imino group of Pro.† Attempts to improve the leaving properties of the alkoxy group (OR′) in *O*-alkyl thioesters (RCSOR′) by introduction of a 2-nitro substituent result in poorer yields of the thioester, probably because of competing elimination between the iminium group and OR′ on treatment of the imino ester with H_2S, although the nitro substituted thioesters are slightly more reactive than simple alkyl derivatives. The reaction of *N*-benzyloxycarbonylaminoacetonitrile with phenol gives a 30% yield of a product which failed to yield any thioester on reaction with H_2S.

There has recently been a growth of interest in the synthesis and properties of thiopeptides,[1–10] compounds in which the –CSNH– group replaces one or more peptide bonds. Synthetic routes employed for these modified peptides include (*a*) direct replacement of oxygen by sulphur using P_4S_{10}[1] or phosphothietanes such as 2,4-bis(*p*-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulphide (Lawesson's reagent)[2–7,11,12] and (*b*) the use of thioesters[10,13–15] or dithioesters[3,7] of *N*-protected amino acids. A drawback of the first method is that it is not usually possible to thiocarbonylate selectively a particular peptide bond; this limits the procedure to dipeptides, although Lajoie *et al.*[5] have reported that the bis-(*p*-phenoxyphenyl)-phosphothietane selectively thiocarbonylates Gly^2 of Met-enkephalin. Dithioesters offer a potentially more useful method, but yields are variable. *O*-Alkyl thioesters were first used for the preparation of thiopeptides by Reid and co-workers.[13–15] Recently, Clausen *et al.*[7] reported that Z-Glyt-OEt reacted with Gly-OEt to give a moderate yield of Z-Glyt-Gly-OEt but failed to give the required products on reaction with either Phe-Leu-CH_2Ph or Gly-Phe-Leu-CH_2Ph. We have recently examined some thioesters of amino acids and peptides as possible intermediates in the synthesis of thiopeptides and we now report the results.

Experimental

Benzyloxycarbonylaminoacetonitrile (72%) was prepared by the method of Mengelberg,[16] m.p. 62 °C [light petroleum (b.p. 60—80 °C)]. L-2-(*N*-Benzyloxycarbonyl)amino-3-phenylpropionitrile was prepared by treatment of Z-$PheNH_2$ (3.8 g, 13.4 mmol) with $SOCl_2$ (4.2 g, 35 mmol) in a mixture of *N*,*N*-dimethylformamide (DMF) (5 ml) and tetrahydrofuran (THF) (30 ml) by the method of Banerjee and Ressler,[17] (1.67 g, 51% from ethanol).

Fmoc-Aminoacetonitrile.—A solution of Fmoc chloride (3.9 g, 16 mmol) in dry THF (70 ml) was added over 15 min to a stirred solution of aminoacetonitrile·HCl (1.5 g, 16 mmol) and Na_2CO_3 (4 g) in water at 2 °C (50 ml). The resulting suspension was stirred at room temperature for a further 3 h. The THF was removed by evaporation and the aqueous residue was brought to pH 3 with dilute HCl before being extracted with ethyl acetate (2 × 60 ml). The organic phase was washed with water, dried ($MgSO_4$), and evaporated. The residue (4 g) was crystallised from MeCN–ether (2.8 g, 67%), m.p. 166—167 °C (Found: C, 73.7; H, 5.2; N, 9.8. $C_{17}H_{14}N_2O_2$ requires C, 73.4; H, 5.1; N, 10.1%).

Boc-Gly-$NHCH_2CN$.—Finely powdered aminoacetonitrile·HCl (1.5 g, 16 mmol) in dry THF (20 ml) was treated with *N*-methylmorpholine (1.63 g, 16 mmol) and the suspension was stirred vigorously for 10 min. Boc-Gly (2.8 g, 16 mmol) and *N*-methylmorpholine (1.62 g, 16 mmol) in dry THF (10 ml) were stirred in an ice–salt bath; isobutyl chloroformate (1.91 g, 14 mmol) was added and the nitrile–*N*-methylmorpholine suspension was added in one portion after 2 min. The mixture was stirred at room temperature for 14 h. The solvent was removed by evaporation, water (50 ml) was added, and the brown solution was extracted with ethyl acetate (2 × 100 ml). The ethyl acetate was dried ($MgSO_4$) and evaporated to a brown gum which solidified on trituration with light petroleum (b.p. 60—80 °C), m.p. 109—111 °C [ethyl acetate–light petroleum (b.p. 60—80 °C)] (2.1 g, 70%) (Found: C, 51.0; H, 7.3; N, 19.4. $C_9H_{15}N_3O_3$ requires C, 50.7; H, 7.1; N, 19.7%).

Z-Tyr(CH_2Ph)-*Gly*-$NHCH_2CN$.—To Boc-Gly-$NHCH_2CN$ (0.5 g, 2.3 mmol) in a mixture of dichloromethane (20 ml) and dry THF (5 ml) was added $MeSO_3H$ (0.25 g, 2.6 mmol) in order to remove the Boc group. After 4 h, the suspension was evaporated under vacuum and the solid residue was stirred with ether and filtered off (0.45 g, 92%). The product had strong i.r. peaks at 1 160 cm^{-1} (SO_3^-) and 3 000 cm^{-1} (NH_3^+) but no urethane carbonyl band. It was used without further characterisation. *N*-Methylmorpholine (0.2 g, 2 mmol) followed by isobutyl chloroformate (0.27 g, 2 mmol) were added to *O*-CH_2Ph-Z-Tyr (0.8 g, 1.9 mmol) in dry THF stirred at −10 °C. After 2 min, the amino nitrile sulphonate (0.38 g, 1.8 mmol), suspended in dry THF (10 ml) containing *N*-methylmorpholine (0.18 g, 1.8 mmol) was added. The mixture was stirred for a further 5 h during which time it was allowed to warm to room

† Abbreviations for amino acids, peptides, and their protecting groups are as recommended in *Biochem. J.*, 1984, **219**, 345—373; thiopeptides are named by adding the letter t to the three-letter code for the amino acid carrying the S atom (Jones *et al.*, *J. Am. Chem. Soc.*, 1973, **95**, 5 677—5 679).

temperature. The solvent was evaporated and the residue was partitioned between ethyl acetate and aqueous $NaHCO_3$. The ethyl acetate layer was dried ($MgSO_4$) and evaporated to give a solid (0.75 g), m.p. 145—150 °C. Crystallisation of the solid from ethyl acetate–light petroleum (b.p. 60—80 °C) gave the pure product (0.5 g, 58%), m.p. 160—162 °C (Found: C, 67.2; H, 5.7; N, 11.2. $C_{28}H_{28}N_4O_5$ requires C, 67.2; H, 5.6; N, 11.2%).

Fmoc-Gly-$NHCH_2CN$.—This was prepared from Fmoc-Gly (4.4 g, 14.8 mmol) as described for the Boc analogue except that the nitrile was added in DMF (20 ml). After the THF had been evaporated off, the residual DMF solution was poured into water (200 ml) and the product was collected by filtration (3.8 g), m.p. 168 °C (decomp.) (MeCN) (Found: C, 67.8; H, 4.8; N, 12.3. $C_{19}H_{17}N_3O_3$ requires C, 68.0; H, 5.1; N, 12.5%).

Table 1. Thioesters, $RCSOR^1$, of amino acid derivatives

R	R^1	Yield (%)	M.p. (°C)	Found (%) (required) C	H	N
Z-$NHCH_2$	Me	92	Oil		*a*	
Z-$NHCH_2$	$(CH_2)_2NO_2$	47	Oil	48.3 (47.8)	4.7 (4.9)	9.4 (9.0)
Z-$NHCH_2$	CH_2Ph	90	56—58	64.7 (64.5)	5.4 (5.6)	4.4 (4.3)
Fmoc-$NHCH_2$	Me	89	105	66.0 (65.8)	5.2 (4.9)	4.3 (4.4)
Z-Gly-$NHCH_2$	Me	90	83	52.7 (52.9)	5.4 (5.2)	9.5 (9.6)
Fmoc-$NHCH_2$	$CH_2CHMeNO_2$	40	Gum	60.0 (59.5)	5.0 (4.7)	7.0 (6.7)
Z-Tyr(Bzl)-Gly-$NHCH_2$	Me	78	141—143	63.4 (63.0)	5.7 (5.3)	7.6 (7.3)
Z-NHCH(CH_2Ph)	Me	60	77—80		*b*	

[a] Not analysed. I.r. and ^2H n.m.r. data were consistent with assigned structure. [b] No satisfactory analysis, but was converted into Z-Phet-Gly in 89% yield (see Table 2).

Table 2. Thiopeptides from thioesters

Product	Thioester	Yield (%)	M.p. (°C)	Found (%) (required) C	H	N
Z-Glyt-Gly	Z-Glyt-OMe	76	140	51.0 (51.1)	4.9 (5.0)	9.8 (9.9)
Z-Glyt-Phe[a,b]	Z-Glyt-OMe	82	182	67.6 (67.2)	8.0 (7.8)	7.5 (7.6)
Z-Glyt-Leu[b]	Z-Glyt-OMe	62	125	65.0 (64.7)	8.8 (8.7)	7.9 (8.1)
Z-Gly-Glyt-Phe[b]	Z-Gly-Glyt-OMe	90	166	64.9 (64.9)	7.6 (7.6)	9.2 (9.2)
Z-Glyt-Phe-Leu[c]	Z-Glyt-$O(CH_2)_2NO_2$	40	62	60.8 (60.4)	7.1 (7.0)	10.7 (10.8)
Z-Glyt-Gly-Gly[d]	Z-Glyt-OMe	73	127—129	49.2 (49.5)	5.3 (5.0)	12.1 (12.4)
Z-Glyt-Pro-Gly[b]	Z-Glyt-OMe	25	162	62.0 (62.1)	7.7 (7.9)	10.1 (10.0)
Z-Phet-Gly[b]	Z-Phet-OMe	89	149—151	67.3 (67.2)	7.9 (7.8)	7.6 (7.6)
Fmoc-Glyt-Phe	Fmoc-Glyt-OMe	55	88—90	68.2 (67.8)	5.2 (5.3)	6.0 (6.1)

[a] Also obtained from *O*-2-nitroethyl- and *O*-benzyl-thioesters in yields of 72 and 77%, respectively. [b] Dicyclohexylamine salt. [c] Methylamine salt. [d] Also obtained in 80% yield from the *O*-benzyl thioester.

Table 3. N.m.r. data for representative thioxo compounds[a]

Compound	Nucleus	Field (MHz) (Solvent)	δ (p.p.m.)	Assignment
$RCH_2C(=S)OMe$	^1H	90 ($CDCl_3$)	4.2 (5 H, s)	Gly α-CH_2 and OMe
			5.01 (2 H, s)	PhCH_2O
			5.50 (1 H, br s)	NH
			7.2—7.3 (5 H, m)	Ph
$RCH_2C(=S)OCH_2Ph$	^{13}C	22.7 ($CDCl_3$)	156.1	O(CO)NH
			218.1	(CS)OMe
	^1H	250 ($CDCl_3$)	4.16 (2 H, d)	Gly α-CH_2
			5.12 (2 H, s)	PhCH_2O
			5.53 (2 H, s)	PhCH_2OC=S
			5.57 (1 H, br t)	Gly NH
			7.2—7.38 (10 H, m)	2 × Ph
$RCH(CH_2Ph)C(=S)OMe$	^1H	250 ($CDCl_3$)	3.05—3.16 (2 H, m)	Phe β-CH_2
			4.02 (3 H, s)	OMe
			4.85 (1 H, q)	Phe α-CH
			5.08 (2 H, s)	PhCH_2O
			5.57 (1 H, br d)	NH
			7.1—7.37 (10 H, m)	2 × Ph
$RCH_2C(=S)NHCH_2CO_2H$	^1H	250 [$(CD_3)_2SO$]	4.18 (2 H, d)	Gly2 α-CH_2
			4.31 (2 H, d)	Gly1 α-CH_2
			5.10 (2 H, s)	PhCH_2O
			7.33—7.40 (6 H, m)	Ph and Gly1 NH
			9.6 (1 H, br s)	Gly2 NH
$RCH_2C(=S)NHCH_2C(=O)NHCH_2CO_2H$		250 [$(CD_3)_2SO$]	3.80 (2 H, d)	Gly3 α-CH_2
			4.04 (2 H, d)	Gly2 α-CH_2
			4.26 (2 H, d)	Gly1 α-CH_2
			5.06 (2 H, s)	PhCH_2O
			7.31—7.36 (5 H, m)	Ph
			7.73 (1 H, br t)	Gly1 NH
			8.37 (1 H, br t)	Gly3 NH
			9.85 (1 H, br t)	Gly2 NH
$RCH_2C(=S)NHCH(CH_2Ph)C(=O)NHCH(CH_2CHMe_2)CO_2H$			10.1—10.4 (1 H, br)	CO_2H
	^1H	250 [$(CD_3)_2SO$]	0.84 (6 H, t)	Leu δ-Me × 2
			1.4—1.6 (3 H, m)	Leu β-CH_2, γ-CH
			2.98—3.29 (2 H, m[b])	Phe β-CH_2
			3.82—4.06 (3 H, m)	Gly α-CH_2, Leu α-CH
			5.02 (2 H, s)	PhCH_2O
			5.16 (1 H, br s)	Phe α-CH
			7.16—7.24 (5 H, m)	Ph
			7.34 (5 H, s)	Ph
			7.85 (1 H, br t)	Gly NH
			8.01 (1 H, d)	Leu NH
			8.1—8.9 (2 H, br)	CO_2H and Phe NH
	^{13}C	91.5 [$(CD_3)_2SO$]	156.0	O(CO)NH
			168.2	CO_2H
$FmocNHCH_2C(=S)NHCH(CH_2Ph)CO_2H$			175.2	(CO)NH
			199.0	(CS)NH
	^1H	250 [$(CD_3)_2SO$]	3.05—3.28 (2 H, m[c])	Phe β-CH_2
			3.90—4.10 (2 H, m)	Gly α-CH_2
			4.23—4.27 (3 H, m)	Fluorenyl $CHCH_2$
			5.10 (1 H, br q)	Phe α-CH
			7.21 (5 H, s)	Ph
			7.33 (2 H, t)	Fluorene ring-H
			7.43 (2 H, t)	Fluorene ring-H
			7.72 (3 H, m)	Gly NH and fluorene ring-H
			7.90 (2 H, d)	Fluorene ring-H
			9.85 (1 H, d)	Phe NH

[a] Relative to $SiMe_4$; R = $PhCH_2O(CO)NH$–. [b] Part of ABX spin system; ν_A 3.03, ν_B 3.26; J_{AB} 13.9, J_{AX} 3.2, J_{BX} 2.7 Hz. [c] Part of ABX spin system: ν_A 3.10, ν_B 3.24; J_{AB} 14.03, J_{AX} 7.75, J_{BX} 5.21 Hz.

Z-Gly-$NHCH_2CN$.—This was prepared from Z-Gly (5.2 g, 25 mmol) as described for the Boc analogue (2.6 g), m.p. 105—107 °C [EtOAc–light petroleum (b.p. 60—80 °C)] (Found: C, 58.5; H, 5.5; N, 16.7. $C_{12}H_{13}N_3O_3$ requires C, 58.3; H, 5.3; N, 17.0%).

Preparation of Imino Esters.—The appropriate dry alcohol (at least 100 mmol) was added to the *N*-protected amino nitrile (20 mmol) in dry ether or dry THF (100 ml). Dry HCl was passed into the reaction mixture for 1—2 h which was then left at 4 °C to allow complete precipitation of the product. If

necessary, dry ether was added to assist this. The suspension was quickly filtered, washed with dry ether, and dried briefly at 40 °C before final drying *in vacuo* over P_2O_5. All the imino esters were used without further purification.

Preparation of O-Alkyl Thioesters.—For *O*-methyl or *O*-ethyl thioesters the imino ester hydrochloride (10 mmol) was dissolved in the corresponding dry alcohol (4 ml g^{-1}), cooled in an ice bath, and treated with dry pyridine (10 mmol). In other cases dry THF replaced the alcohol. Dry H_2S was passed into the mixture for 15 min. The solvent was removed by evaporation under reduced pressure and the residue was partitioned between ethyl acetate and ice-cold dilute HCl. The ethyl acetate layer was washed with water, dried, and evaporated. *O*-Methyl, *O*-ethyl, and *O*-benzyl thioesters were pure enough to use directly. T.l.c. of the product in ether showed in all cases one major component and a very minor, slower running impurity, both of which contained sulphur. Purification could be effected by either flash chromatography on silica gel eluting with dichloromethane–light petroleum (b.p. 60—80 °C) or crystallisation from light petroleum (b.p. 60—80 °C) in the case of the higher melting esters. Details of the thioesters are given in Table 1.

Thioacylation of Amino Acids or Peptides.—The amino acid or peptide (15 mmol) was stirred in a solution of Na_2CO_3 (25 mmol) in water (40 ml) and treated with the thioester (10 mmol) in THF. The apparent pH was adjusted to between 9 and 9.2 and the mixture was stirred for 18—24 h at room temperature. The THF was removed by evaporation under reduced pressure, the aqueous residue was diluted with water to *ca.* 75 ml, and the pH was adjusted if necessary to between 8.5 and 9. It was then washed with ethyl acetate, brought to pH 3 by addition of dilute H_2SO_4, and extracted with ethyl acetate. The extract was washed with water, dried, and evaporated under reduced pressure. The residue was purified either by crystallisation or by conversion into a suitable salt (Table 2). ^1H N.m.r. data for some of the thioesters and thiopeptides are given in Table 3.

Discussion

O-Alkyl thioesters have been prepared previously from imino esters under a variety of conditions. Pyridine has frequently been added in excess, sometimes serving as the solvent.[18] Reaction times have varied from 20 min to 18 h either at room temperature or at 0 °C. In some cases the imino ester has been freed from its salt before reaction with H_2S. We found that in the presence of 1 equiv. of pyridine, either at 0 °C or at room temperature, H_2S reacted rapidly and efficiently with imino esters to give high yields of thioesters. Reaction times were 15 min or less and were probably limited by the rate at which the gas could be bubbled into the reaction mixture. Omission of pyridine under these conditions led to recovery of more than 80% of starting material. The successful thiohydrolysis of the imidate Z-$NHCH_2C(OEt)$=NH in the absence of base, reported by Clausen *et al.*[7] may be attributed to its prior liberation from the iminium salt. It was found necessary, when preparing the thioesters, to use, as the solvent, either the alcohol corresponding to the alkoxy group of the imino ester or an inert solvent such as THF because of the likelihood of ester exchange. For example, when Z-$NHCH_2C(OCH_2Ph)$=NH_2^+ was treated with H_2S in methanol, h.p.l.c. of the product showed that the major component was Z-Glyt-OMe, together with benzyl alcohol. Similarly, the same ester was the only product obtained when Z-$NHCH_2C(SCH_2CO_2H)$=NH_2^+ was treated with H_2S in methanol.

The reaction of Z-Glyt-OMe with Phe is typical of the reactions of the thioesters described. Under the same conditions however, its reaction with Phe-Leu produced only a trace of thiotripeptide. This may be partly due to the weaker nucleophilic character of the amino group in peptides as compared with amino acids, but steric factors also seem to be involved because in a similar reaction Gly-Gly yielded 70% of Z-Glyt-Gly-Gly. Attempts to increase the reactivity of the thioesters by introduction of a 2-nitro substituent into the alkoxy portion produced some improvement. Reaction of the *O*-2-nitropropyl thioester or the *O*-2-nitroethyl thioester of Z-Gly for 24 h at room temperature with Phe-Leu produced 28 and 20%, respectively, of Z-Glyt-Phe-Leu. Reaction of the latter ester with Phe was complete in 8 h, compared to a reaction time of 18—20 h required when Z-Glyt-OMe was used. Similarly, Z-Glyt-OCH_2Ph reacted with Phe to give a very high yield of Z-Glyt-Phe but failed to react to any extent with Phe-Leu. Although the nitroalkyl esters showed slightly enhanced reactivity, they could not be obtained in the high yields seen for the alkyl or benzyl esters because of the formation of the amides and thioamides as by-products.

McElvain and Fajardo-Pinzon[18] reported that reaction of equimolar amounts of phenol and acetonitrile in a Pinner reaction produced 27% yield of the phenyl iminium ester after 15 days at 4 °C. Because *O*-aryl thioesters should be much more reactive than alkyl derivatives we attempted to prepare Z-Glyt-OPh. When a 5-fold excess of phenol was allowed to react with Z-$NHCH_2CN$ in dry ether for 2 days at room temperature in the presence of anhydrous HCl a crystalline precipitate was obtained in 30% yield. Attempts to convert this material into a thioester by the method described above gave a product which appeared, from n.m.r. data, to be a mixture of phenol and Z-Glyt-NH_2.

In conclusion, for preparation of *N*-protected peptides having a *C*-terminal thiopeptide bond, thioesters may be the reagents of choice since they can be obtained in good yields by simple means. Other cases where thioesters may be useful are in the thioacylation of peptides having an *N*-terminal Gly or Pro residue. Attempts to increase their reactivity by incorporation of a better leaving group may not be fruitful because of the increased possibility of such a group leaving the imino ester on reaction with H_2S.

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References

1 P. A. Bartlett, K. L. Spear, and N. E. Jacobsen, *Biochemistry*, 1982, **21**, 1608.
2 K. Clausen, M. Thorsen, and S.-O. Lawesson, *Tetrahedron*, 1981, **37**, 3635.
3 M. Thorsen, B. Yde, U. Pedersen, K. Clausen, and S.-O. Lawesson, *Tetrahedron*, 1983, **39**, 3429.
4 B. Yde, I. Thomsen, M. Thorsen, K. Clausen, and S.-O. Lawesson, *Tetrahedron*, 1983, **39**, 4121.
5 G. Lajoie, F. Lepine, L. Maziak, and B. Belleau, *Tetrahedron Lett.*, 1983, **24**, 3815.
6 D. W. Brown, M. M. Campbell, and C. V. Walker, *Tetrahedron*, 1983, **39**, 1075.
7 K. Clausen, M. Thorsen, S.-O. Lawesson, and A. F. Spatola, *J. Chem. Soc., Perkin Trans. 1*, 1984, 785.
8 W. L. Mock, J. T. Chen, and J. W. Tseng, *Biochem. Biophys. Res. Commun.*, 1981, **102**, 389.
9 T. F. M. La Cour, H. A. S. Hansen, K. Clausen, and S.-O. Lawesson, *Int. J. Pept. Protein Res.*, 1983, **22**, 509.
10 D. J. S. Guthrie, C. H. Williams, and D. T. Elmore, *Int. J. Pept. Protein Res.*, 1986, **28**, 208.
11 R. A. Cherkasov, G. A. Kutyrev, and A. N. Pudovik, *Tetrahedron*, 1985, **41**, 2567.

12 M. P. Cava and M. I. Levinson, *Tetrahedron*, 1985, **41**, 5061.
13 W. Reid and W. von der Emden, *Angew. Chem.*, 1960, **72**, 268.
14 W. Reid and W. von der Emden, *Justus Liebigs Ann. Chem.*, 1961, **642**, 128.
15 W. Reid and E. Schmidt, *Justus Liebigs Ann. Chem.*, 1966, **695**, 217.
16 M. Mengelberg, *Chem. Ber.*, 1956, **89**, 1185.
17 S. N. Banerjee and C. Ressler, *Int. J. Pept. Protein Res.*, 1979, **14**, 234.
18 S. M. McElvain and B. Fajardo-Pinzon, *J. Am. Chem. Soc.*, 1945, **67**, 690.

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