Studies on Amino Acids and Peptides. Part 6.[1] Methods for Introducing Thioamide Bonds into the Peptide Backbone: Synthesis of the Four Monothio Analogues of Leucine Enkephalin †

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A methodology for preparing peptide analogues in which a thioamide bond replaces the normal amide bond is described. Thus, the synthesis of the three leucine enkephalin analogues [Phet[4]]-, [Glyt[2]]-, and [Tyrt[1]]-leucine enkephalin and the attempted synthesis of [Glyt[3]]-leucine enkephalin is reported. The replacement of an amide group in position 4 is most conveniently achieved by thionation of Boc–Phe–Leu–OBzl using Lawesson's Reagent (LR), followed by deprotection of the Boc-group and segment coupling with Boc–Tyr(Bzl)–Gly–Gly–OH. Final deprotection is accomplished by using liquid HF–anisole. Single thioamino acid residues are introduced in positions 1, 2, and 3, respectively, by using protected amino acid dithio esters, which are prepared in high yields in a four-step reaction sequence starting from the *N*-protected amino acids.

Among peptide backbone modifications the replacement of an amide bond by a thioamide bond has until recently attracted relatively little attention.[1-12] This may be explained by the lack of general synthetic methods for the preparation of endothiopeptides. ‡ Actually, only one other report describing the synthesis of fully deprotected endothiopeptides has appeared so far; [11] Jones *et al.*[5] synthesized an analogue of deamino-oxytocin with the terminal amide replaced by a thioamide group, Ried *et al.*,[2-4] Mock *et al.*,[6] Campbell and Nashed,[9] and Bartlett *et al.*[10] have reported methods leading to N^{α}-protected endothiopeptides, and we [7,8] have reported a method leading to N^{α}-protected endothiodipeptide esters and endothiodipeptide ester salts; the latter method has been adopted by Brown *et al.*[11] in the synthesis of endothiodipeptide salts and protected endothiotripeptides.

As has been shown by an *X*-ray crystallographic investigation [12] of the protected endothiodipeptide Z–Glyt–Gly–OBzl §·¶ the replacement of an amide bond by a thioamide bond does not change the geometry of that particular bond. However, it is expected that the presence of a thioamide bond will influence the formation of secondary structure of the polypeptide chain due to the lowered tendency of sulphur [13,14] as compared to oxygen to participate in conformation-stabilizing hydrogen bonding. One field of peptide chemistry where the incorporation of thioamide bonds might be of special interest is in connection with structure-function studies of biologically active peptides, *e.g.* the enkephalins.[15]

This paper will report on a general and efficient method for preparing free endothiopeptides applied to the synthesis of the four possible monothio analogues of leucine-enkephalin: [Leu[5], Phet[4]]-, [Leu[5], Glyt[3]]-, [Leu[5], Glyt[2]]-, and [Leu[5], Tyrt[1]]-enkephalin.

Results and Discussion

The proposed synthesis of the four monothio leucine enkephalin analogues, (9),~~(31),~~(37), and (41), was mostly based on well-established methods of peptide synthesis, with due regard to the inevitable amendments that are caused by the introduction and presence of a thioamide group.

The [Leu[5], Phet[4]]-enkephalin analogue, (9), was prepared by the route shown in Scheme 1. The protected dipeptide Boc–Phe–Leu–OBzl, (1), was thionated by using 2,4-bis(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane 2,4-disulphide (Lawesson's Reagent) (LR), as described previously [7,8] to give the corresponding protected endothiodipeptide Boc–Phet–Leu–OBzl (2), in high yield. In analogy with the method (HBr–AcOH) used for removal of Z groups from protected endothiodipeptides,[7] the Boc group was easily removed from (2) by using 4M-HCl in dioxane to give the HCl salt (3). Next the fully protected monothio pentapeptide analogue (8) was obtained by a (3 + 2) segment condensation of the protected tripeptide Boc-Tyr(Bzl)–Gly–Gly–OH, (7), and (3) by using the DCC method [for details concerning the preparation of intermediate (7) see Scheme 1 and Experimental section]. The structural proofs of (1)—(8) were based on ^1H and ^{13}C n.m.r., i.r., and mass spectra (see section on spectroscopy). The known compounds were furthermore identified by their m.p.s and specific optical rotations, the unknown by microanalyses (Table 1). For final removal of the protecting groups a preliminary experiment with Z–Glyt–Gly–OBzl as substrate revealed that the HF method as developed and recently re-evaluated by Sakakibara *et al.*[16-19] indeed effected removal of the Z- and OBzl-protecting groups without affecting the thioamide bond. Compound (10), a colourless crystalline com-

$$\text{Z–Glyt–Gly–OBzl} \xrightarrow[0\ ^{\circ}\text{C, 30 min.}]{\text{HF–anisole}} \text{H–Glyt–Gly–OH} \quad (10)$$

pound, was characterized by ^1H and ^{13}C n.m.r., i.r., u.v., and mass spectra, and microanalysis (Experimental section). The

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‡ The name endothiopeptide is used for peptide derivatives containing one or more –C(S)NH– function(s) in the peptide backbone.

§ Abbreviations for the amino acids and protecting groups are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature, *Pure Appl. Chem.*, 1974, **40**, 317. Chiral amino acids are of the L-configuration.

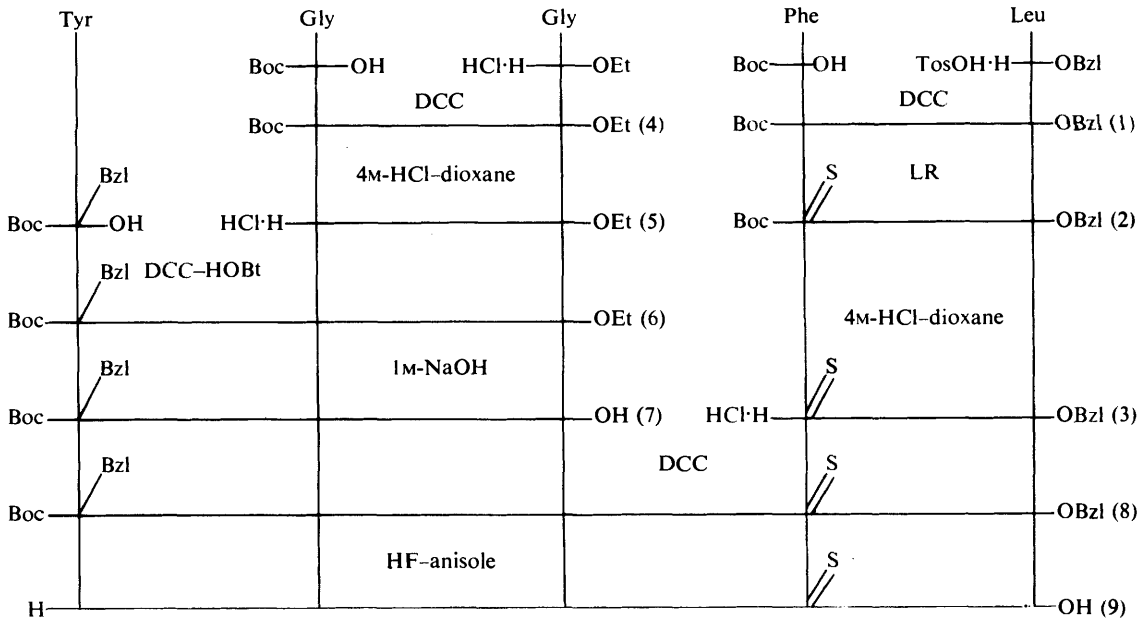
¶ The -t added to the amino acid symbol designates the thiocarbonyl analogue of the respective amino acid residue as proposed by Jones *et al.*[5] Alternatively, thiodipeptide analogues may be named as amide bond replacements (*e.g.* [Tyrψ[CSNH]Gly[1-2]]-leucine enkephalin) as recently proposed (A. F. Spatola, *et al.* in 'LHH–R Peptides as Female and Male Contraceptives,' eds. G. I. Zatuchni, J. Shelton, and J. J. Sciarra, Harper and Row, Philadelphia, 1981, p. 24).

Table 1. Physical and analytical data for peptide fragments and derivatives used in the synthesis of the four monothioleucine enkephalin analogues, (9), (31), (37), and (41)

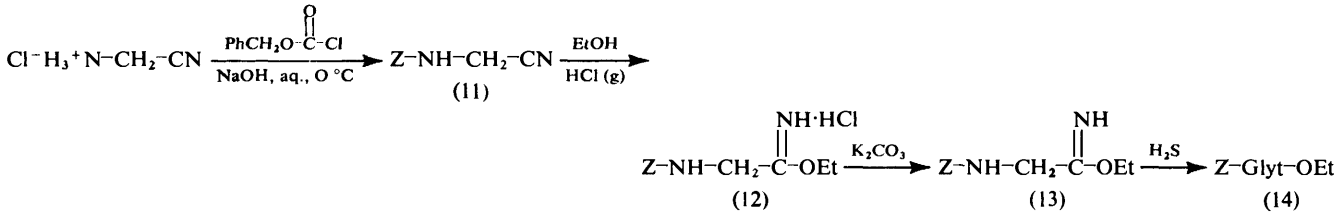
	M.p. (°C)		$[\alpha]_D$		Analysis Calc. / Found (%)				
	Found	Reported	Found	Reported	C	H	N	S	Cl
Boc–Phe–Leu–OBzl (1)	84—86	85—85.5 [a]	−24.2 (*c* 1.01, MeOH)	−27.2 [a] (*c* 1.01, MeOH)					
Boc–Phet–Leu–OBzl (2)	Oil		−14.1 (*c* 1.00, MeOH)		66.91 66.45	7.49 7.45	5.78 5.7	6.62 6.5	
HCl·Phet–Leu–OBzl (3)	166—168		+6.6 (*c* 1.00, MeOH)		62.76 62.35	6.94 7.1	6.65 6.75	7.62 7.05	8.42 8.05
Boc–Gly–Gly–OEt (4)	60—62	60—62 [31]							
HCl·Gly–Gly–OEt (5)	182—184	181 [b]							
Bzl | Boc–Tyr–Gly–Gly–OEt (6)	105—106		+13.6 (*c* 1.00, MeOH)		63.14 63.1	6.87 6.9	8.18 8.0		
Bzl | Boc–Tyr–Gly–Gly–OH (7)	155—157	No data [c]	+13.0 (*c* 1.00, MeOH)	No data [c]					
Bzl | Boc–Tyr–Gly–Gly–Phet–Leu–OBzl (8)	172—174 (decomp.)		−4.5 (*c* 1.00, MeOH)		66.25 66.25	6.74 7.0	8.22 8.5	3.76 3.5	
Z–Glyt–Phe–Leu–OBzl (23)	Foam		−2.9 (*c* 1.00, MeOH)		66.76 66.4	6.49 6.5	7.30 7.2	5.57 5.65	
Z–Glyt–Gly–Phe–Leu–OBzl (24)	Foam		−15.5 (*c* 1.00, MeOH)		64.54 64.45	6.37 6.55	8.85 8.75	5.07 4.95	
HCl·Phe–Leu–OBzl (25)	161—163	159—160 [d]	−21.0 (*c* 1.00, MeOH)	No data [d]					
Boc–Glyt–Phe–Leu–OBzl (26)	Foam		−3.1 (*c* 1.00, MeOH)		64.30 63.55	7.26 7.3	7.76 7.5	5.92 5.85	
HCl·Glyt–Phe–Leu–OBzl (27)	Foam		+12.0 (*c* 1.00, MeOH)		60.30 58.4	6.75 6.95	8.79 8.2	6.71 6.45	7.42 8.25

Bzl Boc–Tyr–Gly–OEt (28)	127—128	123—126 [e] 130 [f]	+1.4 (*c* 2.00, MeOH)	+1.2 [e] (*c* 2, MeOH) +1.5 [f]					
Bzl Boc–Tyr–Gly–OH (29)	149—151	149—150 [f]	+1.45 (*c* 2.00, MeOH)	+1.5 [f] (*c* 2, MeOH)					
Bzl Boc–Tyr–Gly–Glyt–Phe–Leu–OBzl (30)	121—123		+3.5 (*c* 1.00, MeOH)		66.25 66.05	6.74 6.9	8.22 8.2	3.76 3.75	
Boc–Gly–Phe–Leu–OBzl (32)	Foam	Oil [g]	−24.4 (*c* 1.00, MeOH)	No data [g]					
HCl·Gly–Phe–Leu–OBzl (33)	Foam	No data [g]	−12.9 (*c* 1.00, MeOH)	No data [g]					
Boc–Glyt–Gly–Phe–Leu–OBzl (34)	Foam		−24.0 (*c* 1.00, MeOH)		62.19 61.7	7.07 7.1	9.36 9.2	5.36 5.25	
HCl·Glyt–Gly–Phe–Leu–OBzl (35)	148—(decomp.)		−27.2 (*c* 1.00, MeOH)		58.36 57.6	6.59 6.5	10.47 10.15	5.99 5.85	6.33 6.55
Bzl Boc–Tyr–Glyt–Gly–Phe–Leu–OBzl (36)	164—167 (decomp.)		−9.3 (*c* 1.00, MeOH)		66.25 66.1	6.74 6.85	8.22 8.2	3.76 3.25	
Boc–Gly–Gly–Phe–Leu–OBzl (38)	161—162	162.5—163.5 [h]	−16.8 (*c* 2.00, MeOH)	−18.1 [h] (*c* 2.00, MeOH)					
HCl·Gly–Gly–Phe–Leu–OBzl (39)	Foam	Amorph. [i]	−18.0 (*c* 1.00, MeOH)	No data [i]					
Bzl Boc–Tyrt–Gly–Gly–Phe–Leu–OBzl (40)	184—188		+2.2 (*c* 1.00, MeOH)		66.25 66.0	6.74 6.85	8.22 8.2	3.76 3.7	

[a] M. M. Sarasua, M. E. Scott, J. A. Helpern, P. B. W. TenKartenaar, N. T. Boggs, L. G. Pedersen, K. A. Koehler, and R. G. Hiskey, *J. Am. Chem. Soc.*, 1980, **102**, 3404. [b] H. N. Rydon and P. W. G. Smith, *J. Chem. Soc.*, 1955, 2542. [c] C.-H. Wong, S. T. Chen, and K.-T. Wang, *Biochem. Biophys. Acta*, 1979, **576**, 247. [d] L. Zervas, D. Borovas, and E. Gazis, *J. Am. Chem. Soc.*, 1963, **85**, 3660. [e] R. Matsueda, H. Maruyama, E. Kitazawa, H. Takahagi, and T. Mukaiyama, *Bull. Chem. Soc. Jpn.*, 1973, **46**, 3240. [f] B. Rzestotarska and H. Kmiecik-Chmura, *Rocz. Chem.*, 1977, **51**, 1523. [g] F. H. C. Stewart, *Aust. J. Chem.*, 1980, **33**, 121. [h] E. R. Stimson, Y. C. Meinwald, and H. A. Scheraga, *Biochemistry*, 1979, **18**, 1661. [i] F. H. C. Stewart, *Aust. J. Chem.*, 1979, **32**, 661.



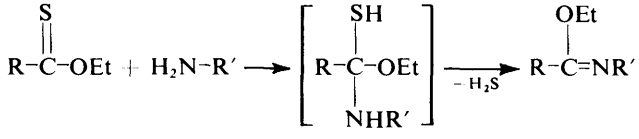
Scheme 1. Preparation of [Leu^5, Phet^4]-enkephalin



Scheme 2.

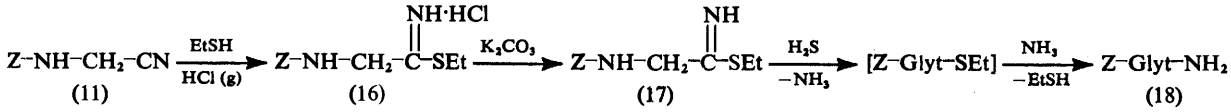
^{1}H n.m.r. spectrum in D_2O showed only two singlets assignable to the two methylene groups (all other hydrogens are exchanged with the solvent). The ^{13}C spectrum showed four lines assignable to the thiocarbonyl, carbonyl, and two methylene carbons. In the u.v. spectrum characteristic $\pi \rightarrow \pi^*$ absorption was observed. Mass spectroscopy gave the molecular ion $M^{+\cdot}$ and a fragment corresponding to loss of H_2O from the molecule. The Boc-, Bzl-, and OBzl-protecting groups of (8) were cleaved simultaneously by using liquid HF with anisole added as a scavenger to avoid Tyr ring benzylation, and the free monothiopentapeptide (9) was subjected to Sephadex gel filtration and was further purified to homogeneity by reverse-phase high-performance liquid chromatography (h.p.l.c.). The purity and structural proof of compound (9) was established by and based on t.l.c., analytical h.p.l.c., amino acid analysis, and FAB mass spectrometry [20] (see Experimental and spectroscopy sections).

For the preparation of the three remaining monothiopentapeptides two different approaches were examined. From the literature it is known that simple thionoesters and dithioesters are thioacylating reagents.[21-25] Ried *et al.*[2-4] used N$^{\alpha}$-protected amino acid thionoesters of the type XNHCHRC(S)OEt (X = Tos$^-$, Z$^-$, Pht<) to prepare N$^{\alpha}$-protected endothiodipeptides. We synthesized Z–Glyt–OEt (14) as described before [2,3] starting from aminoacetonitrile hydrochloride through the sequence shown in Scheme 2 (the Pinner synthesis [26]). Physical properties of the known compounds (11)—(14) are given in Table 2. In addition, spectroscopic data for compound (14) are given in the Experimental section. As a test for its thioacylating ability (14) was allowed to react with HCl·Gly–OEt to give Z–Glyt–Gly–OEt, (15), in 35—58% yield depending on the reaction conditions. However, when (14) was allowed to react with HCl·Phe–Leu–OBzl (25), and HCl·Gly–Phe–Leu–OBzl (33), complex reaction mixtures were formed with evolution of H_2S and it was not possible to isolate the desired Z–Glyt–Phe–Leu–OBzl (23), and Z–Glyt–Gly–Phe–Leu–OBzl (24). The evolution of H_2S can be explained by the formation of an imino ester:

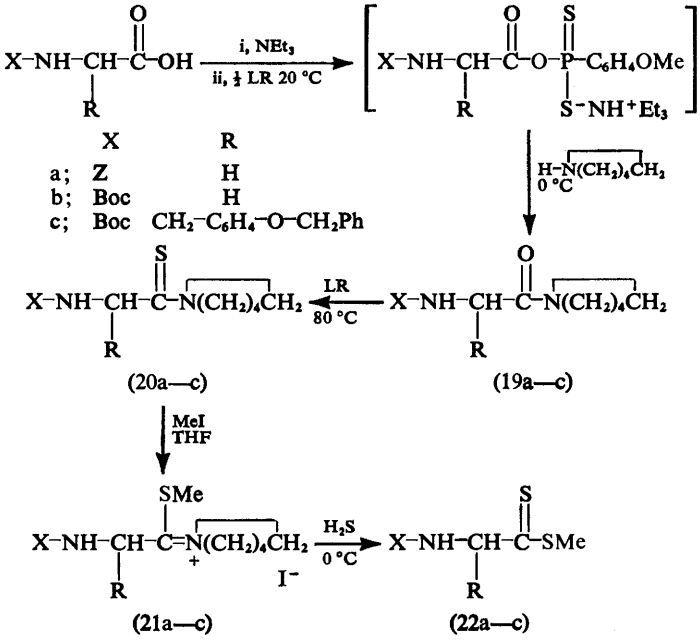


Because of these unpromising results with amino acid thionoesters we turned to N-protected amino acid dithioesters. Mock *et al.*[6] used Bz–Gly–Glyt–SEt to prepare Bz–Gly–Glyt–Phe–OH; but no details with respect to the synthesis of the dipeptide dithioester were reported. Similarly, Campbell and Nashed [9] used Bz–Glyt–SEt and Bz–Gly–Glyt–SEt to prepare Bz–Glyt–Phe–OMe and Bz–Gly–Glyt–Phe–OH, respectively.

Foye and Kauffman [27] synthesized Z–Glyt–SEt (yield 22%), Hartmann *et al.*[28] synthesized amino acid dithioesters of the type XNHCHRC(S)SEt (X = Bz, Ac; yield, 14.5—27%), and recently Storer *et al.*[29] have reported the synthesis of a series of protected amino acid dithioesters of the type X–Glyt–SEt [X = Bz, Ac, Bzl, $PhCH_2CH_2C(O)$, Z; yield, 10—25%]. In all cases the reactions started from the corresponding nitriles,



Scheme 3.



Scheme 4.

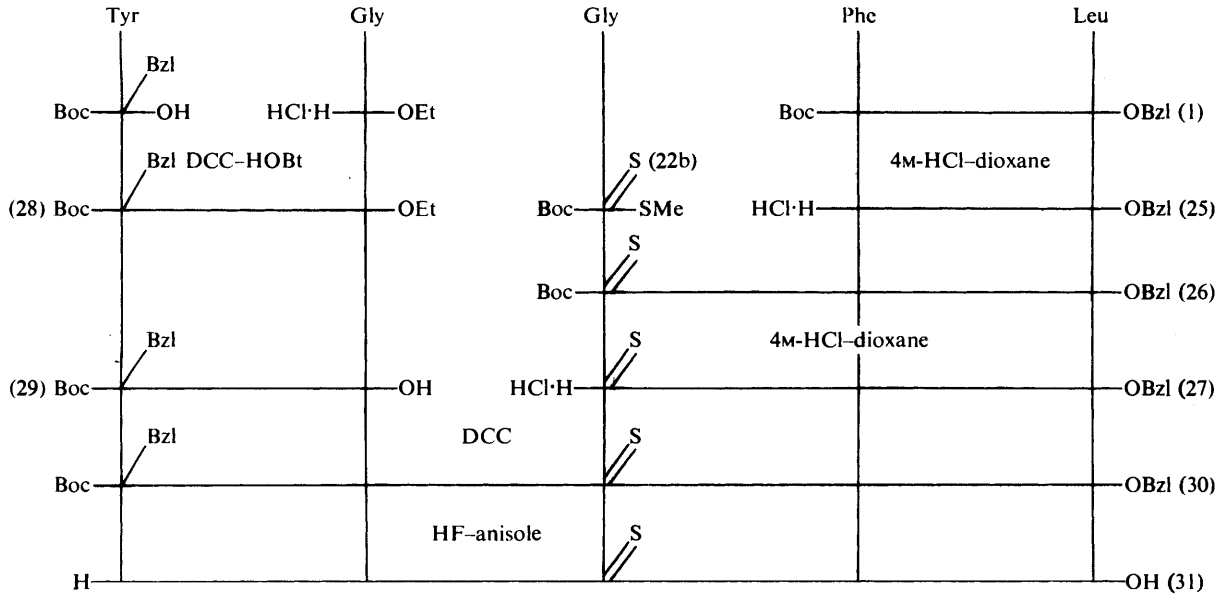
which upon reaction with ethanethiol and hydrogen chloride gave the imino thioester salts (the Pinner synthesis [26]), which were converted into the dithioesters with hydrogen sulphide in the presence of base (Na_2CO_3 or pyridine). By using a procedure analogous to the procedure we used for the preparation of Z-Glyt-OEt (14) (Scheme 2) we tried to prepare Z-Glyt-SEt (Scheme 3).

However, the only product isolated in the last step, in which the imino thioester (17) was treated with H_2S, was Z-Glyt-NH_2 (18). The formation of this product is obviously due to the fact that the dithioester formed in the reaction of (17) with H_2S is a much stronger thioacylating reagent than the corresponding thionoester (14) and, therefore, it reacts immediately with the ammonia, which is eliminated during the thiolysis. Physical properties of the known compounds (16) and (18) are presented in Table 2. Preparation of the N-Z-protected amino acid dithioester Z-Glyt-SMe (22a), was achieved by a new and efficient method starting from the readily available Z-protected amino acid (Scheme 4). First the N-protected amino acid was transformed to the corresponding piperidide (19). This was most effectively obtained through the mixed anhydride formed by reaction of the amino acid NEt_3-salt with LR; this is a quite new and promising mixed anhydride method, which has been dealt with in a recent paper.[30] Next LR was used as thionation reagent to form the corresponding thiopiperidide (20). The thiopiperidide was S-methylated by reaction with an excess of MeI in THF at 20 °C for 12—24 h. In the last step the dithioester (22) was obtained by thiolysis of (21). As a test for its thioacylating ability Z-Glyt-SMe (22a), was allowed to react with HCl·Glyt-OEt giving 97% of the expected product Z-Glyt-Gly-OEt (15). When Z-Glyt-SMe was allowed to react with HCl·Phe-Leu-OBzl (25), and HCl·Gly-Phe-Leu-OBzl (33), respectively, the expected products Z-Glyt-Phe-Leu-OBzl (23), and Z-Glyt-Gly-Phe-Leu-OBzl (24), were formed in 93 and 89% yield. The structural proofs of (23) and (24) are based on ^1H and ^{13}C n.m.r., i.r., u.v., and mass spectra (see spectroscopic section). Their physical data are summarized in Table 1. On trying to remove the Z groups by using 20 and 36% HBr–AcOH, decomposition of the monothio tri- and tetra-peptide occurred. In analogy with the successful strategy used in preparing [Leu^5, Phet^4]-enkephalin (Scheme 1), we then turned to the Boc-protected amino acid dithioesters, which were prepared as described above for Z-Glyt-SMe. To our knowledge no Boc-protected amino acid dithioesters have ever been described in the literature before. All the glycine and tyrosine derivatives, (19)—(22), excepting compound (19a) are new compounds, which were characterized on the basis of ^1H and ^{13}C n.m.r., i.r., u.v., mass spectral evidence, and microanalyses (see section on spectroscopy). The physical properties of compounds (19)—(22) are summarized in Table 2. By using the Boc-protected amino acid dithioesters we succeeded in preparing the three remaining fully protected monothioleucine enkephalin derivatives, the [Glyt^3]-analogue by a (2 + 3) segment coupling (Scheme 5) and the [Glyt^2]- and [Tyrt^1]-analogues by stepwise procedures (Schemes 6 and 7) using DCC and DCC–HOBt mediated coupling. Compounds (25)—(30), (32)—(36), and (33)—(40), were characterized as described for compounds (1)—(8) (see section on spectroscopy and Table 1). The free enkephalin analogues (37) and (41) were obtained by using liquid HF–anisole as described for the preparation of (9). The purity and structural proofs of compounds (37) and (41) were established by, and based on, the methods used for compound (9) (Experimental and spectroscopy sections). When the fully protected [Glyt^3]-analogue (31), was treated with HF–anisole as described above

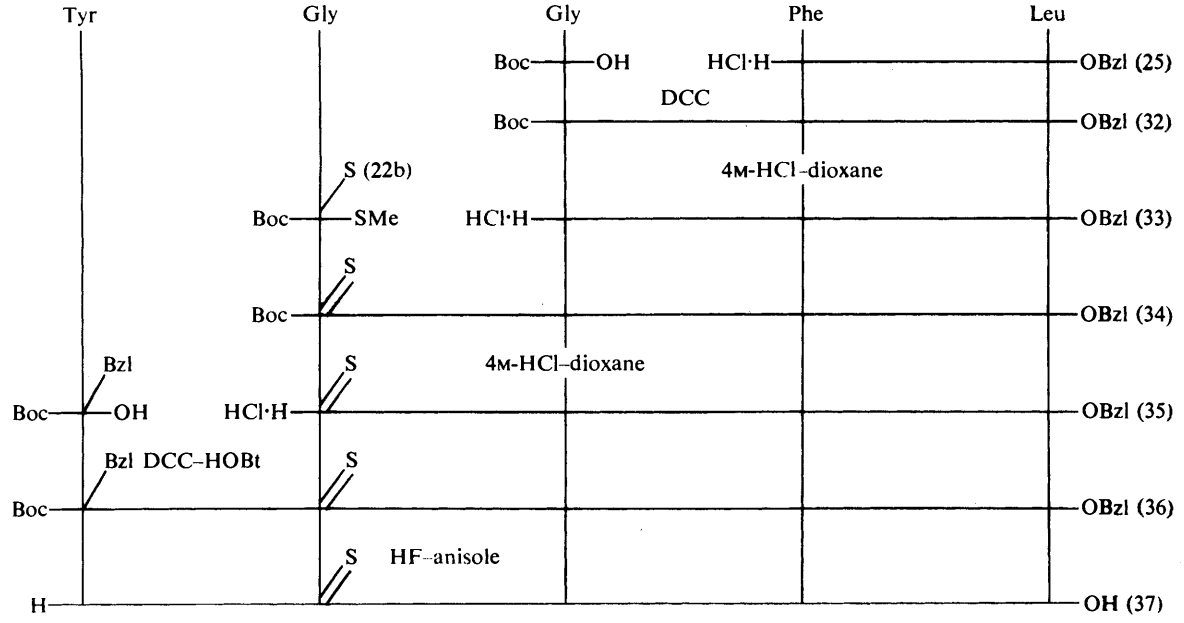
Table 2. Physical and analytical data for amino acid derivatives (11)—(14) and (16)—(22c)

	M.p. (°C)		Analysis Calc. / Found (%)				
	Found	Reported	C	H	N	S	I
Z-NH-CH_2-CN (11)	59—62	64 [a]					
Z-NH-CH_2-C(=NH·HCl)-OEt (12)	118—120 (decomp.)	118—119 [34]					
Z-NH-CH_2-C(=NH)-OEt (13)	55—56	58—59 [35]					
Z-Glyt-OEt (14)	30—31	27 [4]					
Z-NH-CH_2-C(=NH·HCl)-SEt (16)	142—144 (decomp.)	138—142 [27]					
Z-NH-CH_2-C(=NH)-SEt (17)	Not characterized, homogenous by t.l.c.						
Z-Glyt-NH_2 (18)	141—143	141—143 [6]					
Z-Gly-N(CH_2)$_4$$CH_2$ (19a)	108—110	110—112 [b]					
Boc-Gly-N(CH_2)$_4$$CH_2$ (19b)	42—44		59.48 59.3	9.15 9.0	11.56 11.5		
Boc-Tyr(Bzl)-N(CH_2)$_4$$CH_2$ (19c)	Foam *		71.21 71.05	7.81 7.9	6.39 6.2		
Z-Glyt-N(CH_2)$_4$$CH_2$ (20a)	73—74		61.62 61.4	6.89 6.9	9.58 9.65	10.97 10.9	
Boc-Glyt-N(CH_2)$_4$$CH_2$ (20b)	68—70		55.78 55.85	8.58 8.6	10.84 10.85	12.41 12.45	
Boc-Tyrt(Bzl)-N(CH_2)$_4$$CH_2$ (20c)	Foam †		68.69 68.05	7.54 7.5	6.16 5.95	7.05 6.9	
Z-NH-CH_2-C(SMe)=$^+$N(CH_2)$_4$$CH_2$ I^- (21a)	116—118 (decomp.)		44.25 44.2	5.34 5.4	6.45 6.5	7.38 7.5	29.22 29.25
Boc-NH-CH_2-C(SMe)=$^+$N(CH_2)$_4$$CH_2$ I^- (21b)	134—135 (decomp.)		39.00 39.0	6.29 6.4	7.00 6.95	8.01 8.25	31.70 31.9
Boc-NH-CH(-CH_2-C_6H_4-OCH$_2$Ph-*p*)-C(SMe)=$^+$N(CH_2)$_4$$CH_2$ I^- (21c)	140—142 ‡		54.36 53.95	6.25 6.25	4.70 4.6	5.37 5.5	21.27 21.1
Z-Glyt-SMe (22a)	60—61		51.74 52.1	5.13 5.15	5.49 5.45	25.11 24.85	
Boc-Glyt-SMe (22b)	74—75		43.41 43.45	6.83 6.65	6.33 6.25	28.97 29.0	
Boc-Tyrt(Bzl)-SMe (22c)	121—122 §		63.28 63.3	6.52 6.5	3.35 3.3	15.36 15.3	

* $[\alpha]_D^{22}$ = +2.5 (*c* 1.00, MeOH). † $[\alpha]_D^{22}$ = +41.7 (*c* 1.00, MeOH). ‡ $[\alpha]_D^{22}$ = +161.0 (*c* 1.00, MeOH). § $[\alpha]_D^{22}$ = −6.2 (*c* 1.00, MeOH).
[a] A. H. Cook, G. Harris, and A. L. Levy, *J. Chem. Soc.*, 1949, 3227. [b] D. M. Brunwin, G. Lowe, and J. Parker, *J. Chem. Soc. C*, 1971, 3756.



Scheme 5. Attempted preparation of [Leu^5, Glyt^3]-enkephalin



Scheme 6. Preparation of [Leu^5, Glyt^2]-enkephalin

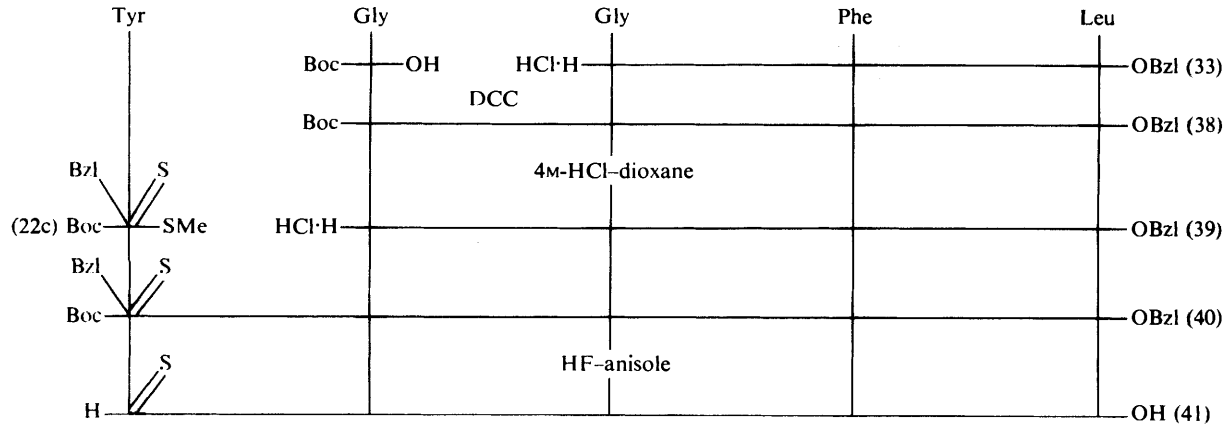
we were unable to isolate any compound with an amino acid analysis compatible with structure (31). Other methods for the preparation of compound (31) are now under investigation.

Spectroscopic Section.—Spectroscopy on peptide segments and peptide derivatives. All the peptide segments and derivatives in Schemes 1, 5—7 showed the expected signals in their ^{1}H n.m.r. spectra (Experimental section). Upon converting C=O into C=S the amide NH proton shifted *ca.* 1.7 p.p.m. downfield. The ^{13}C n.m.r. chemical shifts of diagnostic value are given in Table 3. For the protecting groups the shifts are as follows: Boc, tertiary carbon *ca.* 79.5 p.p.m., Me carbon *ca.* 28.0 p.p.m., carbonyl carbon *ca.* 155.3 p.p.m.; Z, methylene carbon *ca.* 66.8 p.p.m.; Bzl, methylene carbon *ca.* 69.7 p.p.m.; OEt, methylene carbon *ca.* 61.0 p.p.m., Me carbon *ca.* 13.9 p.p.m.; OBzl methylene carbon *ca.* 66.8 p.p.m. As expected the carbonyl carbon shifted *ca.* 30—34 p.p.m. downfield upon conversion into C=S. The assignments are in accordance with those published for segments of [Leu^5]-enkephalin [31] and those reported for protected endothiodipeptide esters.[7,8] I.r. spectroscopy showed the urethane band at 1 690—1 705 cm^{-1}, amide I at *ca.* 1 670 cm^{-1}, thioamide II at 1 485—1 525 cm^{-1}, and ester at 1 720—1 750 cm^{-1}. The u.v. spectra of all thioamide-containing peptides showed the characteristic $\pi\rightarrow\pi^*$ absorption in the range 265—270 nm with ε values in the range 8.8 × 10^3—1.7 × 10^4. In the mass spectra the molecular ion $[M]^{+\cdot}$ was only observed in a few cases [with the protected dipeptides (1), (2), (4), and with the protected tri- and tetra-peptides (26) and (34)]. For the Boc-protected compounds a general trend was the loss from the molecular ion of 56 ($M^{+\cdot}$ − $Me_2C{=}CH_2$) and 73 ($M^{+\cdot}$ − $Me_3CO^{\cdot}$).

Table 3. ^{13}C N.m.r. chemical shifts of the peptide fragments and derivatives used in the synthesis of the four monothioleucine enkephalin analogues, (9), (31), (37), and (41)

	Tyr[1]			Gly[2]		Gly[3]		Phe[4]			Leu[5]				
	C_1^{α}	C_1^{β}	C_1	C_2^{α}	C_2	C_3^{α}	C_3	C_4^{α}	C_4^{β}	C_4	C_5^{α}	C_5^{β}	C_5^{γ}	C_5^{δ}	C_5
(1) $CDCl_3$								55.5	38.2	171.4	50.7	40.9	24.4	22.5 21.7	172.2
(25) DMSO								53.3	36.7	168.3	50.8	39.0	24.1	22.7 21.5	171.7
(2) $CDCl_3$								61.6	40.0	204.6	56.4	41.7	24.5	22.1 22.0	171.0
(3) DMSO								57.9	39.1 *	199.2	56.6	39.7 *	24.2	22.6 21.4	170.1
(32) $CDCl_3$						44.1	169.6	54.1	38.3	170.9	50.9	40.9	24.6	22.5 21.8	172.1
(33) DMSO						40.0	165.6	54.1	37.8	171.1	50.7	(DMSO)	24.3	22.8 21.4	172.1
(26) $CDCl_3$						51.7	199.7	59.0	36.7	169.2	51.0	40.9	24.4	22.4 21.6	171.8
(23) $CDCl_3$						51.5	199.3	59.1	36.7	169.3	51.0	40.8	24.4	22.4 21.6	171.8
(27) DMF						45.8	195.2	60.3	36.2	169.1	50.4	39.1	23.5	21.8 20.3	171.6
(38) $CDCl_3$				43.8	169.7	42.9	168.5	54.2	39.2	171.3	51.0	41.0	24.8	22.7 21.9	172.4
(39) DMSO				40.2	166.2	41.9	168.0	54.0	37.7	171.4	50.7	39.0	24.3	22.7 21.4	172.1
(34) $CDCl_3$				49.7	199.2	48.7	167.2	54.1	39.1	171.6	51.2	40.5	24.8	22.7 21.8	172.2
(24) $CDCl_3$				50.0	198.6	49.1	167.2	54.3	39.5	171.5	51.2	40.8	24.8	22.7 21.8	172.3
(35) DMF				46.3	195.8	47.8	166.0	54.2	37.0	170.8	50.4	39.4	23.8	21.8 20.4	171.6
(4) $CDCl_3$				43.8	169.5	40.9	170.2								
(5) DMSO				40.1	166.7	40.8	169.5								
(6) $CDCl_3$	56.1	37.5	172.4	42.8	169.3 *	41.1	169.5 *								
(7) DMSO	56.2	36.9	172.3	42.1 *	169.3	42.0 *	171.2								
(28) $CDCl_3$	55.7	37.5	172.0	41.1	169.4										
(29) DMSO	55.9	37.1	172.4	40.9	171.3										
(8) $CDCl_3$	55.8	40.1	171.7	42.7	168.2	42.7	168.2	60.4	40.1	204.9	55.8	~41	25.0	22.7 22.1	171.2
(30) $CDCl_3$	56.6	36.8	172.9	43.4	169.7 *	50.7	199.1	59.6	36.8	169.4	51.3	41.1	24.7	22.7 21.8	172.0
(36) DMF	56.0	37.2	170.6	49.4 *	199.8	49.7 *	166.3	53.6	39.5	171.6	50.3	39.5	23.8	21.9 20.5	172.2
(40) $CDCl_3$	62.0	41.7	204.1	48.6	167.8	43.2	168.3	54.2	39.4	170.7	51.2	41.7	24.9	22.8 22.4	173.0

* Interchangeable.



Scheme 7. Preparation of [Leu^5, Tyrt^1]-enkephalin

Table 4. ^{1}H and ^{13}C N.m.r. chemical shifts and u.v. absorptions of compounds (19a)—(22c)

	^{1}H N.m.r.*			^{13}C N.m.r.*			U.v.†	
	H_L	H_L^{α}	H_L^{β}	C^{α}	C^{β}	C	$\lambda_{max.}$	ε
(19a)	5.85	3.95		44.8		165.7		
(19b)	5.55	3.95		44.9		166.0		
(19c)	5.45	4.85	2.90	50.7	38.9	169.3		
(20a)	6.45	4.10		51.5		194.4	278	1.47×10^4
(20b)	6.05	4.05		51.5		195.1	278	1.48×10^4
(20c)	5.85	~5	3.00	54.1	42.4	200.8	284	1.32×10^4
(21a)	7.3	4.70		41.1		188.1		
(21b)	6.75	4.60		40.6		188.2		
(21c)	7.3	~5	3.6	55.9	35.2	191.0		
(22a)	5.80	4.40		56.4		234.4	305	1.19×10^4
(22b)	5.45	4.30		56.4		235.5	308	1.01×10^4
(22c)	5.25	~5	3.05	66.3	42.7	239.2	308	1.13×10^4

* All n.m.r. spectra were obtained in $CDCl_3$. † In each case u.v. spectra were obtained in EtOH.

Spectroscopy on Gly *and* Tyr *derivatives* (19)—(22). In the ^{1}H n.m.r. spectra the signals belonging to the Z, Boc, and Bzl groups were found where expected and in the ^{13}C n.m.r. spectra the shifts for the protecting groups were as described above. The ^{1}H n.m.r. chemical shifts of H, H^{α}, and H^{β}, and the ^{13}C n.m.r. chemical shifts of C^{α}, C^{β}, and the carbonyl carbons are given in Table 4. Upon conversion of the piperidide (19) into thiopiperidide (20) the amide carbonyl carbon shifted *ca.* 30 p.p.m. downfield (from the amide region 165.7—169.3 p.p.m. to the thioamide region 194.4—200.8 p.p.m.). The carbons of the imino thioester groups in (21a—c) resonated in the region 188.1—191.0 p.p.m. Finally, the dithioester carbonyl carbons of (22a—c) were found in the range 234.4—239.2 p.p.m. typical for dithioesters.[32] In the i.r. spectra the urethane band was observed at 1 685—1 715 cm^{-1}, the amide I (19a—c) at *ca.* 1 625 cm^{-1}, the thioamide I (20a—c) at *ca.* 1 290 cm^{-1}, the thioimidate C=N (21a—c) at *ca.* 1 590 cm^{-1}, and the dithioester C=S band at *ca.* 1 160 cm^{-1}. Compounds (20a—c) and (22a—c) showed expected $\pi \rightarrow \pi^*$ transitions (Table 4). In their mass spectra compounds (19), (20), and (22) gave molecular ions $[M]^{+\cdot}$ (possibly $[M + 1]^{+\cdot}$), with typical fragments ($M^{+\cdot} - PhCH_2^{\cdot}$), ($M^{+\cdot} - PhCH_2OH$) (Z-protected compounds), ($M^{+\cdot} - Me_2C{=}CH_2$), ($M^{+\cdot} - Me_3CO^{\cdot}$) (Boc-protected compounds).

FAB mass spectrometry on the thiopeptides (9), (37), *and* (41). In accordance with the findings of Barber *et al.*,[20] who have described the positive-ion FAB mass spectra of leucine enkephalin, the FAB mass spectra of all three thioenkephalins (9), (37), and (41) show the protonated molecular ion $[M + H]^+$ and intense diagnostic peaks at *m/z* values 136, 120, 107, 91, and 86 corresponding to the following ions: $H_2\overset{+}{N}{=}CHCH_2C_6H_4OH$-*p* (from Tyr), $H_2\overset{+}{N}{=}CHCH_2Ph$ (from Phe), $\overset{+}{C}H_2C_6H_4OH$-*p* (from Tyr), $\overset{+}{C}H_2Ph$ (from Phe), and $H_2\overset{+}{N}{=}CHCH_2CHMe_2$ (from Leu). Also some of the sequence ions arising from C-terminal and N-terminal cleavages are observed (see Experimental section).

In conclusion we have developed a general and efficient method for the incorporation of thioamide linkages in peptides, the method of choice in the general case being the use of Boc-protected amino acid dithioesters, which are easily accessible from the amino acid precursors, and in the special case where the replacement is required at the C-terminal peptide bond an alternative route has been devised. The potential value of this method has been illustrated by the facile synthesis of the four monothioleucine enkephalin analogues, and it is expected that the method will gain use for the facile incorporation of thioamide linkages into a wide variety of peptides of biological interest.

The results of biological testing of the three leucine enkephalin analogues (9), (37), and (41) will be published elsewhere.

Experimental

^{1}H N.m.r. spectra were recorded at 60 MHz on a Varian EM-360 spectrometer. ^{13}C N.m.r. spectra were recorded at 20 MHz on a Varian CFT-20 spectrometer. $SiMe_4$ was used as internal standard and chemical shifts are expressed in δ values. $CDCl_3$, $(CD_3)_2SO$, $(CD_3)_2N{\cdot}CDO$, or D_2O were used as solvents. I.r. spectra were recorded on a Beckman IR-18 spectrophotometer. U.v. spectra were recorded on a Perkin-Elmer 402 spectrophotometer. Mass spectra were recorded on a Micromass 7070 F spectrometer operating at 70 eV using direct inlet, and FAB mass spectra were recorded on a Varian MAT CH-5-DF mass spectrometer, with samples introduced in glycerol and with an accelerating voltage of 3.000 V.

H.p.l.c. was performed with a Du Pont 850 Liquid Chromatograph using C-18 reversed-phase Zorbax ODS columns; for preparative purposes (250 × 6.2 mm i.d.) and for analytical purposes (250 × 4.5 mm i.d.). Operating conditions were as follows: MeOH–0.25M AmOAc (pH 4.1), gradient 30—40% over 20 min, flow rate 2 ml/min, column temperature 50 °C, u.v. detection at 270 nm. R_t of leucine enkephalin reference sample (analytical), 8.46 min. Elemental analyses were carried out by Løvens Kemiske Fabrik, DK-2750 Ballerup. Optical rotations were measured in a Perkin-Elmer 241 polarimeter. Samples for amino acid analysis were prepared by hydrolysis with 6M-HCl at 110 °C for 22—24 h and analysed on a Dionex D-300 instrument. Silica gel 60 (Merck) was used for chromatography. M.p.s are uncorrected.

Lawesson's Reagent (*LR*).—This was prepared as described earlier.[33]

Boc–Phe–Leu–OBzl (1).—To a mixture of Boc–Phe–OH (26.53 g, 0.1 mol) TosOH·Leu–OBzl (39.35 g, 0.1 mol), and TEA (10.1 g, 0.1 mol) in CH_2Cl_2 (200 ml) was added DCC (20.6 g, 0.1 mol) at −10 °C; the mixture was stirred for 1 h and then kept at room temperature for 10 h. The solution was filtered, the filtrate evaporated, and the residue redissolved in AcOEt (400 ml), filtered, and then extracted with 0.1M-HCl (2 × 100 ml), 0.5M-$NaHCO_3$ (2 × 100 ml), and water (100 ml) (aq. NaCl). The organic phase was dried with $MgSO_4$, the solvent evaporated, and the residue recrystallized from Et_2O (50 ml)–light petroleum (150 ml); yield 41.54 g (89%); δ ($CDCl_3$) 7.30 [5 H, s, Ph(OBzl)], 7.15 [5 H, s, Ph(Phe)], 6.55 (1 H, d, *J* 8 Hz, H_2), 5.15 (1 H, m, H_1), 5.10 [2 H, s, CH_2(OBzl)], 4.4 (2 H, m, H_1^{α}, H_2^{α}), 3.00 (2 H, d, *J* 6 Hz, H_1^{β}), 1.5 (3 H, m, H_2^{β}, H_2^{γ}), 1.35 [9 H, s, Me(Boc)], 0.85 (6 H, m, H_2^{δ}); $\nu_{max.}$ ($CHCl_3$) 1 700 (urethane), 1 675 (amide I), and 1 735 cm^{-1} (ester); *m/z* 468 ($M^{+\cdot}$), 412 ($M^{+\cdot}$ − $Me_2C{=}CH$), 395 ($M^{+\cdot}$ − $Me_3CO^{\cdot}$).

Boc–Phet–Leu–OBzl (2).—Compound (1) (9.36 g, 0.02 mol) and LR (4.04 g, 0.01 mol) were heated in anhydrous benzene (50 ml) at 80 °C until the starting material was consumed (40 min, as monitored by t.l.c. in 50% Et_2O–light petroleum). After evaporation of the solvent the residue was chromatographed on a silica gel column (4.5 × 20 cm) in 30% Et_2O–light petroleum which yielded the product (2) as a colourless oil; yield 8.91 g (92%); δ ($CDCl_3$) 8.25 (1 H, d, *J* 8 Hz, H_2), 7.25 [5 H, s, Ph(OBzl)], 7.15 [5 H, s, Ph(Phe)], 5.15 (1 H, m, H_1), 5.10 [2 H, s, CH_2(OBzl)], 4.65 (2 H, m, H_1^{α}, H_2^{α}), 3.05 (2 H, d, *J* 6 Hz, H_1^{β}), 1.6 (3 H, m, H_2^{β}, H_2^{γ}), 1.35 [9 H, s, Me(Boc)], 0.85 (6 H, m, H_2^{δ}); $\nu_{max.}$ ($CHCl_3$) 1 690 (urethane), 1 485 (thioamide II), and 1 730 cm^{-1} (ester); $\lambda_{max.}$ (EtOH) 270 nm (ε 1.0 × 10^4); *m/z* 484 ($M^{+\cdot}$), 428 ($M^{+\cdot}$ − $Me_2C{=}CH_2$), 411 ($M^{+\cdot}$ − $Me_3CO^{\cdot}$).

HCl·Phet–Leu–OBzl (3).—To compound (2) (7.26 g, 0.015 mol) was added 4M-HCl–dioxane (80 ml) and the mixture was stirred for 30 min. The solvent was evaporated and the residue recrystallized from MeOH (20 ml)–Et_2O (150 ml); yield 5.35 g (85%); δ [$(CD_3)_2SO$] *ca.* 11 (1 H, br, H_2), *ca.* 9 (3 H, br, H_3N^+), 7.30 [10 H, m, Ph(OBzl, Phe)], 5.15 [2 H, s, CH_2(OBzl)], 4.8 (2 H, m, C_1^{α}, C_2^{α}), 3.2 (2 H, m, H_1^{β}), 1.8 (3 H, m, H_2^{β}, H_2^{γ}), 0.9 (6 H, m, H_2^{δ}); $\nu_{max.}$ (KBr) 1 490 (thioamide II) and 1 750 cm^{-1} (ester); $\lambda_{max.}$ (EtOH) 268 nm (ε 1.04 × 10^4).

Boc–Gly–Gly–OEt (4).—This was prepared as described for (1) from Boc–Gly–OH (17.52 g, 0.1 mol), HCl·Gly–OEt (13.96 g, 0.1 mol), TEA (10.1 g, 0.1 mol), CH_2Cl_2 (200 ml), and DCC (20.6 g, 0.1 mol); reaction time 21 h. It was recrystallized from Et_2O (100 ml)–light petroleum (75 ml); yield: 23.55 g (90%); δ ($CDCl_3$) 7.15 (1 H, t, *J* 6 Hz, H_2), 5.65 (1 H, t, *J* 6 Hz, H_1), 4.20 [2 H, q, *J* 7 Hz, CH_2(OEt)], 4.00 (2 H, d, *J* 6 Hz, H_2^{α}), 3.85 (2 H, d, *J* 6 Hz, H_1^{α}), 1.45 [(9 H, s, Me(Boc)], 1.25 [3 H, t, *J* 7 Hz, Me(OEt)]; $\nu_{max.}$ ($CHCl_3$) 1 700 (urethane), 1 675 (amide I), and 1 730 cm^{-1} (ester); *m/z* 260 ($M^{+\cdot}$), 204 ($M^{+\cdot}$ − $Me_2C{=}CH_2$), 187 ($M^{+\cdot}$ − $Me_3CO^{\cdot}$).

HCl·Gly–Gly–OEt (5).—This was prepared as described for (3) from (4) (22.12, 0.085 mol) and 4 M-HCl–dioxane (450 ml); it was recrystallized from EtOH (200 ml)–Et_2O (50 ml); yield 15.83 g (95%); δ [$(CD_3)_2SO$] 9.1 (1 H, t, *J* 6 Hz, H_2), 8.3 (3 H, br, H_3N^+), 4.05 [2 H, q, *J* 6 Hz, CH_2(OEt)], 3.85 (2 H, d, *J* 6 Hz, H_2^{α}), 3.55 (2 H, s, H_1^{α}), and 1.15 [3 H, t, *J* 6 Hz, Me(OEt)]; $\nu_{max.}$ (KBr) 1 675 (amide I) and 1 730 cm^{-1} (ester).

Boc–Tyr(Bzl)–Gly–Gly–OEt (6).—To a mixture of Boc–Tyr(Bzl)–OH (9.29 g, 0.025 mol), (5) (4.92 g, 0.025 mol), and *N*-ethylmorpholine (2.88 g, 0.025 mol) in dioxane (50 ml) was added HOBt (3.65 g, 0.025 mol) (incl. 8% water) at −10 °C followed by DCC (5.15 g, 0.025 mol); the mixture was stirred thus for ½ h, and then kept at room temperature for 16 h. The solution was filtered, the residue washed with AcOEt, and the combined filtrate evaporated. The residue was taken up in AcOEt (150 ml), and then washed with 0.5M-$NaHCO_3$ (4 × 25 ml), 0.1M-HCl (2 × 25 ml), and water (10 ml). The organic phase was dried with $MgSO_4$, the solvent evaporated, and the residue crystallized from AcOEt (50 ml)–light petroleum (100 ml); yield 12.06 g (94%); δ ($CDCl_3$) 7.25 [5 H, s, Ph(Bzl)], 6.90 [6 H, m, H_2, H_3, C_6H_4(Tyr)], 5.35 (1 H, d, *J* 8 Hz, H_1), 4.90 [2 H, s, CH_2(Bzl)], 4.30 (1 H, m, H_1^{α}), 4.10 [2 H, q, *J* 7 Hz, CH_2(OEt)], 3.9 (4 H, d, *J* 6 Hz, H_2^{α}, H_3^{α}), 2.95 (2 H, m, H_1^{β}), 1.35 [9 H, s, Me(Boc)], and 1.20 [3 H, t, *J* 7 Hz, Me(OEt)], $\nu_{max.}$ ($CHCl_3$) 1 700 (urethane), 1 680 (amide I), and 1 750 cm^{-1} (ester).

Boc–Tyr(Bzl)–Gly–Gly–OH (7).—To compound (6) (6.16 g, 0.012 mol) dissolved in MeOH (50 ml) was added 1M-NaOH (13 ml, 0.013 mol), and the mixture was stirred for 1 h. The mixture was then neutralized by addition of 1M-HCl (3 ml), and evaporated. Water (50 ml) and 1M-NaOH (20 ml) were then added, and the mixture was extracted with AcOEt (3 × 25 ml). By addition of 1M-HCl (35 ml) the mixture was acidified to pH 2—3, and extracted with AcOEt (4 × 50 ml). The combined extracts were washed with water (10 ml), and dried ($MgSO_4$), evaporated, and solidified by addition of AcOEt (20 ml) followed by Et_2O (20 ml); yield 4.80 g (82%); δ [$(CD_3)_2SO$] 8.15 (2 H, br, H_2, H_3), 7.35 [5 H, s, Ph(Bzl)], 7.05 [5 H, m, C_6H_4(Tyr), H_1], 5.05 [2 H, s, CH_2(Bzl)], 4.15 (1 H, m, H_1^{α}), 3.8 (4 H, d, *J* 6 Hz, H_2^{α}, H_3^{α}), 2.90 (2 H, m, H_1^{β}), and 1.3 [9 H, s, Me(Boc)]; $\nu_{max.}$ (KBr) 1 690 (urethane), 1 670 (amide I), and 1 725 cm^{-1} (CO_2H); *m/z* 440 (M − $CO_2H^{\cdot}$).

Boc–Tyr(Bzl)–Gly–Gly–Phet–Leu–OBzl (8).—This was prepared as described for (1) from compound (7) (2.43 g, 0.005

mol), compound (3) (2.11 g, 0.005 mol), TEA (0.51 g, 0.005 mol), CH_2Cl_2 (50 ml), and DCC (1.03 g, 0.005 mol); reaction time 22 h. Because of the low solubility of this compound in AcOEt, the CH_2Cl_2 was not evaporated, but after filtration the CH_2Cl_2 phase was extracted with water (4 × 25 ml), 0.1M-HCl (2 × 25 ml), $NaHCO_3$ (satd.) (2 × 25 ml), and water (25 ml); it was recrystallized from AcOEt (150 ml), yield 4.17 g (98%); δ ($CDCl_3$) 9.75 (1 H, br, H_5), 7.9 (3 H, m, H_2, H_3, H_4), 6.8—7.4 [19 H, Ph(OBzl, Phe, Bzl), C_6H_4(Tyr)], 5.9 (1 H, br, H_1), 5.2 (2 H, m, H_4^{α}, H_5^{α}), 5.15 [2 H, s, CH_2-(OBzl)], 4.80 [2 H, s, CH_2(Bzl)], 4.2 (5 H, m, H_1^{α}, H_2^{α}, H_3^{α}), 2.9 (4 H, br, C_1^{β}, C_4^{β}), 1.6 (3 H, m, C_5^{β}, C_5^{γ}), 1.40 [9 H, s, Me(Boc)], and 0.85 (6 H, m, H_5^{δ}); $\nu_{max.}$ ($CHCl_3$) 1 700 (urethane), 1 650 (amide I), 1 520 (thioamide II), and 1 740 cm^{-1} (ester); $\lambda_{max.}$ (EtOH) 270 nm (ε 1.13 × 10^4).

H-Tyr-Gly-Gly-Phet-Leu-OH (9).—To a mixture of compound (8) (0.852 g, 0.001 mol) and anisole (1.5 ml, *ca.* 0.015 mol) in a 100-ml polyethylene bottle was added anhydrous HF (20 ml) at 0 °C; the mixture was then kept at 0 °C for 30 min. The HF was removed by using a plastic water-suction pump. The oily residue was treated with Et_2O (50 ml), whereupon the product solidified. The Et_2O was decanted and the residue washed with Et_2O (2 × 25 ml). The solid material was transferred to a 100-ml round-bottomed flask by using MeOH, and the solvent evaporated; yield 0.68 g. A portion of this material (350 mg) was dissolved in 30% AcOH–H_2O (5 ml) and subjected to a Sephadex G-15 column (100 × 2.8 cm), flow rate 20 ml/h, monitored by u.v. at 270 nm. The fractions eluting from 375—575 ml were pooled and lyophilized; yield 305 mg. A portion of this material (20 mg) was chromatographed by reverse-phase h.p.l.c. Final lyophilization from 20% AcOH–H_2O yielded 11.2 mg (53% *). Homogeneous by t.l.c. in Bu^nOH–AcOH–H_2O (5 : 10 : 1), R_F 0.61; analytical h.p.l.c. R_t 16.61 min; amino acid analysis: Tyr 1.01, Gly 2.08, Phe 1.01, Leu 1.00; FAB mass spec.: *m/z* 572 ([*M* + H]$^+$, 5%), 352 ([Gly-Phet-Leu]$^+$, 2.4) 295 ([Phet-Leu]$^+$, 11), 278 ([Tyr-Gly-Gly]$^+$, 16), 221 ([Tyr-Gly]$^+$, 12), 136 (78), 120 (100), 107 (31), 91 (100), and 86 (28).

H-Glyt-Gly-OH (10).—To a mixture of Z-Glyt-Gly-OBzl[7] (0.744 g, 0.002 mol) and anisole (1 ml, *ca.* 0.009 mol) in a 100-ml polyethylene bottle was added anhydrous HF (20 ml) at 0 °C, and the mixture was kept at 0 °C for 30 min. The HF was removed by using a plastic water-suction pump. The oily residue was treated with anhydrous Et_2O (25 ml), whereupon the product solidified. The Et_2O was decanted and the residue washed with anhydrous Et_2O (3 × 25 ml). The material (0.34 g) was recrystallized from water (2 ml)–EtOH (20 ml); yield 0.273 g (92%); m.p. 191 °C (decomp.) (Found: C, 31.45; H, 5.2; N, 18.05; S, 20.35. Calc. for: C, 32.42; H, 5.44; N, 18.90; S, 21.64%) δ (D_2O/DDS) 4.7 (s, H_2O), 4.25 (2 H, s, H_2^{α}), and 3.95 (2 H, s, H_1^{α}); δ_C (D_2O) 197.72 (C_1), 176.64 (C_2), 51.45 (C_2^{α}), and 48.64 (C_1^{α}); $\nu_{max.}$ 1 510 cm^{-1} (thioamide II); $\lambda_{max.}$ (H_2O) 265 nm (ε: 8.69 × 10^3); *m/z* 148 ($M^{+\cdot}$), 130 (NH-CH_2-CS-NH-CH_2-CO).

Phenylmethyl Cyanomethylcarbamate (11).—To a mixture of aminoacetonitrile hydrochloride (46.25 g, 0.5 mol) and 50% benzyl chloroformate–toluene (170.5 g, 0.5 mol) was added 4M-aqueous NaOH (250 ml, 1.0 mol) at 0 °C during 20 min. The toluene phase was separated and the water phase extracted with Et_2O (2 × 400 ml). The combined organic phases were dried ($MgSO_4$), evaporated, and crystallized from Et_2O (100 ml)–light petroleum (25 ml); yield 72.7 g (77%).

Ethyl 2-Phenylmethoxycarbonylaminoethanimidate Monohydrochloride (12).—This compound was prepared according to the method of Mengelberg;[34] yield (93%).

Ethyl 2-Phenylmethoxycarbonylaminoethanimidate (13).—This compound was prepared according to the method of Hirotsu *et al.*;[35] yield (91%).

Z-Glyt-OEt (14).—To a solution of compound (13) (20.8 g, 0.088 mol) in Et_2O (250 ml) was bubbled a stream of H_2S at 0 °C for 2 h. The resulting yellow solution was filtered, the filtrate evaporated, and the residue was chromatographed on a silica gel column (7 × 15 cm) in CH_2Cl_2. Crystallization from Et_2O (50 ml)–light petroleum (50 ml); yield 17.12 g (77%); δ ($CDCl_3$) 7.3 [5 H, s, Ph(Z)], 5.55 (1 H, br, H_1), 5.05 [2 H, s, CH_2(Z)], 4.55 [2 H, q, *J* 7 Hz, CH_2(OEt)], 4.05 (2 H, d, *J* 6 Hz, H_1^{α}), 1.35 [3 H, t, *J* 7 Hz, Me(OEt)]; δ_C ($CDCl_3$) 217.5 [C(S)O], 156.0 [O-C(O)-NH], 68.6 [CH_2(OEt)], 66.6 [CH_2-(Z)], 51.7 (C_1^{α}), 13.3 [Me(OEt)]; $\nu_{max.}$ ($CHCl_3$) 1 715 (urethane) and 1 050 (thionoester); $\lambda_{max.}$ (EtOH) 243 nm (ε 5.9 × 10^3); *m/z* 253 ($M^{+\cdot}$), 192 ($M^{+\cdot}$ − ·SEt), 146 ($M^{+\cdot}$ − $PhCH_2O$·).

Z-Glyt-Gly-OEt (15).[7]—(a) *From* Z-Glyt-OEt (14). To a solution of HCl·Gly-OEt (0.70 g, 0.005 mol) and TEA (0.51 g, 0.005 mol) in AcOEt (10 ml) was added compound (14) (1.27 g, 0.005 mol) and the mixture was stirred for 28 h at 20 °C. The reaction was monitored by t.l.c. in 10% AcOEt–CH_2Cl_2. The solvent was evaporated and the residue chromatographed on a silica gel column (2 × 15 cm) in 10% AcOEt–CH_2Cl_2 to give starting Z-Glyt-OEt (0.40 g) and compound (15) (0.64 g, 41%), m.p. 82—84 °C (lit.,[7] 82—84 °C). By using 2 equiv. of TEA and a reaction time of 21 h, at 20 °C, 0.24 g of starting Z-Glyt-OEt and 0.54 g (35%) of (15) were obtained; by using 10 equiv. of TEA, a reaction time of 23 h, at 20 °C, 0.30 g of starting Z-Glyt-OEt and 0.90 g (58%) of (15) were obtained; by using 5 equiv. of TEA and 10 ml of toluene, a reaction time of 4 h, at 80 °C, 0.16 g of starting Z-Glyt-OEt and 0.90 g (58%) of (15) were obtained.

(b) *From* Z-Glyt-SMe (22a). To a solution of HCl·Gly-OEt (0.140 g, 0.001 mol) and TEA (0.101 g, 0.001 mol) in Et_2O (10 ml) was added (22a) (0.255 g, 0.001 mol), and the mixture was stirred for 28 h at 20 °C. The reaction was monitored by t.l.c. in 10% AcOEt–CH_2Cl_2. After addition of AcOEt (10 ml) the mixture was filtered, the solvent evaporated, and the residue chromatographed on a silica gel column (2 × 15 cm) in 20% AcOEt–CH_2Cl_2; yield 0.30 g (97%).

Ethyl 2-Phenylmethoxycarbonylaminoethaneimidothioate Monohydrochloride (16).—Compound (11) (34.2 g, 0.18 mol) and ethanethiol (16.8 g, 0.27 mol) in dry Et_2O (300 ml) were treated with HCl(g) at −5 °C for 25 min. After 5 min the solution became clear and after 10 min a white material started to precipitate. Stirring was continued at 20 °C for 1 h. The mixture was filtered and the precipitate was washed with dry Et_2O (2 × 25 ml) and then dried in a desiccator and crystallized from $CHCl_3$–light petroleum (1 : 1); yield 42.1 g (81%).

Ethyl 2-Phenylmethoxycarbonylaminoethanimidothioate (17).—Compound (16) (42.1 g, 0.146 mol) in Et_2O (500 ml) was stirred vigorously with 4M-K_2CO_3 (40 ml; 0.16 mol) for ½ h. The Et_2O phase was separated, dried ($MgSO_4$), and then evaporated to give (17) (36 g, 98%), which was used without

* Based on the assumption that the product exists as the monoacetate salt.

further purification or characterization in the following reaction.

Z-Glyt-NH_2 (18).—To a solution of compound (17) (17.71 g, 0.07 mol) in dry Et_2O (200 ml) was bubbled a stream of H_2S at 0 °C for 1 h. The solvent was evaporated and the residue chromatographed on a silica gel column (4.5 × 15 cm) starting with CH_2Cl_2 (500 ml) and then with Et_2O to give (18) as the sole product. Crystallization from $CHCl_3$ (500 ml) yielded pure Z-Glyt-NH_2 (7.86 g, 50%).

General Method for the Preparation of the Piperidides (19a—c).—N-Protected amino acid (0.1 mol), TEA (10.1 g (0.1 mol) and LR (20.2 g, 0.05 mol) in CH_2Cl_2 (100 ml) were stirred for 5 min at 20 °C. The reaction mixture was then cooled to −15 °C and piperidine (17.03 g, 0.2 mol) was added dropwise (strongly exothermic) for ½ h. The temperature was raised to 20 °C and the reaction mixture stirred for 4 h. The solvent was evaporated and AcOEt (400 ml) added. The organic phase was extracted with water (4 × 100 ml), 4M-$NaHCO_3$ (4 × 25 ml), 0.1M-HCl (4 × 25 ml), and water (25 ml). The organic phase was dried ($MgSO_4$) and evaporated. The residue was chromatographed on a silica gel column (7 × 20 cm): (19a) (eluted with Et_2O), yield 21.0 g (76%), recrystallized from AcOEt–Et_2O (1 : 1); (19b) (eluted with 2% MeOH–CH_2Cl_2—5% MeOH/CH_2Cl_2), yield 19.7 g (81%); recrystallized from light petroleum; *m/z* 243 (M + 1), 186 ($M^{+\cdot}$ − Me_2C=CH), and 169 ($M^{+\cdot}$ − $Me_3CO\cdot$); (19c) eluted with 5% MeOH–CH_2Cl_2, yield 30.87 g (70%), *m/z* 438 ($M^{+\cdot}$) and 365 ($M^{+\cdot}$ − $Me_3CO\cdot$).

General Procedure for Preparation of the Thiopiperidides (20a—c).—Compound (19) (0.05 mol) was heated with LR (10.1 g, 0.025 mol) in benzene at 80 °C for 20—30 min. The benzene was evaporated and the residue subjected to column chromatography (7 × 20 cm) starting with CH_2Cl_2 until a P,S by-product [2,4,6-tris(4-methoxyphenyl)-1,3,5,2,4,6-trioxatriphosphorinane 2,4,6-trisulphide [8]] was eluted. Compound (20a) was eluted with Et_2O and crystallized from AcOEt (15 ml)–Et_2O (300 ml); yield 12.77 g (87%); *m/z* 292 ($M^{+\cdot}$), 201 ($M^{+\cdot}$ − $PhCH_2\cdot$), and 184 ($M^{+\cdot}$ − $PhCH_2OH$). Compound (20b) was eluted with Et_2O, and crystallized from light petroleum; yield 10.96 g (85%); *m/z* 258 ($M^{+\cdot}$), 202 ($M^{+\cdot}$ − Me_2C=CH), 185 ($M^{+\cdot}$ − $Me_3CO\cdot$). Compound (20c) was eluted with 5% AcOEt–CH_2Cl_2; yield 12.36 g (56%); *m/z* 455 ($M^{+\cdot}$ + 1), 421 ($M^{+\cdot}$ − $SH\cdot$), 399 ($M^{+\cdot}$ − Me_2C=CH), and 365 ($M^{+\cdot}$ − Me_2C=CH − H_2S).

General Procedure for Preparation of Compounds (21a—c).—Compound (20) (0.04 mol) and MeI (28.39 g, 0.20 mol) in THF (25 ml) was stirred for 12 h at 20 °C. The excess of MeI was evaporated at 20 °C to give a thick slurry, which was crystallized from the following: (21a) EtOH (100 ml)–Et_2O (200 ml), yield 14.14 g (81%); (21b), EtOH–Et_2O (1 : 6), yield 13.76 g (86%); (21c), EtOH–Et_2O (1 : 3), yield 20.88 g (88%).

General Procedure for Preparation of the Dithioesters (22a—c).—Into compound (21) (0.03 mol) in EtOH (50 ml) was bubbled a stream of H_2S at 0 °C for 40 min, during which time the solution turned orange-red. The solvent was evaporated and the residue chromatographed on a silica gel column (4.5 × 20 cm): (22a) eluted with 10% AcOEt–CH_2Cl_2, and recrystallized from Et_2O (50 ml)–light petroleum (50 ml), yield, 7.15 g (93%), *m/z* 255 ($M^{+\cdot}$), 208 ($M^{+\cdot}$ − $SMe\cdot$), 164 ($M^{+\cdot}$ − $PhCH_2\cdot$) and 147 ($M^{+\cdot}$ − $PhCH_2OH$); (22b) eluted with 5% AcOEt–CH_2Cl_2, and recrystallized from Et_2O–light petroleum (1 : 5), yield 5.52 g (83%), *m/z* 221 ($M^{+\cdot}$), 206 ($M^{+\cdot}$ − $CH_3\cdot$), 165 ($M^{+\cdot}$ − Me_2C=CH_2), and 148 ($M^{+\cdot}$ − $Me_3CO\cdot$); (22c) eluted with 5% AcOEt–CH_2Cl_2, and recrystallized from Et_2O, yield 11.4 g (91%), *m/z* 417 ($M^{+\cdot}$).

Z-Glyt-Phe-Leu-OBzl (23).—To a mixture of compound (25) (4.05 g, 0.01 mol) and TEA (1.01 g, 0.01 mol) in Et_2O (50 ml) was added (22a) (2.56 g, 0.01 mol) at 20 °C and the mixture was stirred for 21 h (monitored by t.l.c. in 10% AcOEt–CH_2Cl_2). AcOEt (50 ml) was added and the mixture filtered. The solvent was evaporated and the residue chromatographed on a silica gel column (4.5 × 20 cm) in 10% AcOEt–CH_2Cl_2 to 20% AcOEt–CH_2Cl_2 to yield starting (22a) (0.21 g) and (23) (5.33 g, 93%); δ ($CDCl_3$) δ 8.6 (1 H, d, *J* 7 Hz, H_2), 7.25 [10 H, m, Ph(Z, OBzl)], 7.10 [5 H, s, Ph-(Phe)], 6.15 (2 H, m, H_1, H_3), *ca.* 5.5 (1 H, m, H_2^{α}), 5.10 [2 H, s, CH_2(OBzl)], 5.05 [2 H, s, CH_2(Z)], *ca.* 4.5 (1 H, m, H_3^{α}), 4.10 (2 H, d, *J* 6 Hz, H_1^{α}), 3.10 (2 H, m, H_2^{β}), 1.5 (3 H, m, H_3^{β}, H_3^{γ}), and 0.80 (6 H, m, H_3^{δ}); $\nu_{max.}$ ($CHCl_3$) 1 700 (urethane), 1 675 (amide I), 1 500 (thioamide II), and 1 740 cm^{-1} (ester); $\lambda_{max.}$ (EtOH) 268 nm (ε 1.07 × 10^4); *m/z* 541 ($M^{+\cdot}$ − H_2S).

Z-Glyt-Gly-Phe-Leu-OBzl (24).—To a mixture of (33) (4.62 g, 0.01 mol) and TEA (1.01 g, 0.01 mol) in Et_2O (50 ml) was added (22a) (2.56 g, 0.01 mol) at 20 °C; the mixture was stirred for 26 h (monitored by t.l.c. in 30% AcOEt–CH_2Cl_2). The mixture was worked up as above for (23); yield 5.66 g (89%), δ ($CDCl_3$) 9.1 (1 H, m, H_2), 7.6 (2 H, m, H_3, H_4), 7.25 [10 H, s, Ph(Z, OBzl)], 7.10 [5 H, s, Ph(Phe)], 6.25 (1 H, m, H_1), 5.10 [2 H, s, CH_2(OBzl)], 5.05 [2 H, s, CH_2(Z)], *ca.* 4.6 (2 H, m, H_3^{α}, H_4^{α}), *ca.* 4.2 (4 H, m, H_1^{α}, H_2^{α}), 3.00 (2 H, m, H_3^{β}), 1.6 (3 H, m, H_4^{β}, H_4^{γ}), and 0.8 (6 H, m, H_4^{δ}); $\nu_{max.}$ ($CHCl_3$) 1 705 (urethane), 1 670 (amide I), 1 500 (thioamide II), and 1 730 cm^{-1} (ester); $\lambda_{max.}$ 265 nm (ε 1.10 × 10^4); *m/z* 631 ($M^{+\cdot}$ − 1) and 598 ($M^{+\cdot}$ − H_2S).

HCl·Phe-Leu-OBzl (25).—This was prepared as described for (3) from (1) (28.08 g, 0.06 mol) and 4M-HCl–dioxane (320 ml); crystallization from MeOH (50 ml)–Et_2O (150 ml); yield 22.7 g (93%); δ [$(CD_3)_2SO$] 9.25 (1 H, d, *J* 8 Hz, H_2), 8.35 (3 H, br, H_3N^+), 7.30 [5 H, s, Ph(OBzl)], 7.15 [5 H, s, Ph(Phe)], 5.10 [2 H, s, CH_2(OBzl)], 4.25 (2 H, m, H_1^{α}, H_2^{α}), 3.05 (2 H, m, H_1^{β}), 1.60 (3 H, m, H_2^{β}, H_2^{γ}), and 0.85 (6 H, m, H_2^{δ}); $\nu_{max.}$ (KBr) 1 670 cm^{-1} (amide I) and 1 705 cm^{-1} (ester).

Boc-Glyt-Phe-Leu-OBzl (26).—This was prepared as described for (23) from compound (25) (2.03 g, 0.005 mol), TEA (0.51 g, 0.005 mol), Et_2O (25 ml), and compound (22b) (1.11 g, 0.005 mol); reaction time 22 h. Silica gel column (4.5 × 15 cm) in 5% AcOEt–CH_2Cl_2 to 20% AcOEt–CH_2Cl_2; yield 2.41 g (89%) δ ($CDCl_3$) 8.65 (1 H, d, *J* 7 Hz, H_2), 7.30 [5 H, s, Ph(OBzl)], 7.15 [5 H, s, Ph(Phe)], 6.15 (1 H, d, *J* 8 Hz, H_3), 5.25 (1 H, m, H_1), 5.15 (1 H, m, H_2^{α}), 5.10 [2 H, s, CH_2-(OBzl)], 4.4 (1 H, m, H_3^{α}), 4.05 (2 H, d, *J* 7 Hz, H_1^{α}), 3.15 (2 H, m, H_2^{β}), 1.5 (3 H, m, H_3^{β}, H_3^{γ}), 1.45 [9 H, s, Me(Boc)], 0.85 (6 H, m, H_3^{δ}); $\nu_{max.}$ ($CHCl_3$) 1 700 (urethane), 1 690 (amide I), 1 500 (thioamide II), and 1 730 cm^{-1} (ester); $\lambda_{max.}$ (EtOH) 270 nm (ε 1.1 × 10^4); *m/z* 541 ($M^{+\cdot}$), 507 ($M^{+\cdot}$ − H_2S), and 451 ($M^{+\cdot}$ − Me_2C=CH − H_2S).

HCl·Glyt-Phe-Leu-OBzl (27).—This was prepared as described for (3) from compound (26) (2.17 g, 0.004 mol) and 4M-HCl–dioxane (25 ml); yield 1.82 g (95%); δ [$(CD_3)_2NCDO$–$SiMe_4$] 11.35 (1 H, br, H_1), 8.95 (3 H, br, H_3N^+), 7.25 [10 H, m, Ph(OBzl, Phe)], 5.20 (1 H, m, H_2^{α}), 5.15 [2 H, s, CH_2-(OBzl)], 4.5 (1 H, m, H_2^{α}), 4.10 (2 H, m, H_1^{α}), 3.20 (2 H, m, H_2^{β}), 1.65 (3 H, m, H_3^{β}, H_3^{γ}), and 0.90 (6 H, m, H_3^{δ}); $\nu_{max.}$ (KBr) 1 660 (amide I), 1 520 (thioamide II), and 1 735 cm^{-1} (ester); $\lambda_{max.}$ (EtOH) 268 nm (ε 8.8 × 10^3).

Boc–Tyr(Bzl)–Gly–OEt (28).—This was prepared as described for compound (6) from Boc–Tyr(Bzl)–OH (9.29 g, 0.025 mol), HCl·Gly–OEt (3.49 g, 0.025 mol), *N*-ethylmorpholine (2.88 g, 0.025 mol), dioxane (50 ml), HOBt (3.65 g, 0.025 mol) (incl. 8% water), and DCC (5.15 g, 0.025 mol); crystallization from AcOEt (100 ml)–light petroleum (200 ml); yield 10.66 g (93%); δ ($CDCl_3$) 7.30 [5 H, s, Ph(Bzl)], 6.90 [5 H, m, H_2, C_6H_4(Tyr)], 5.2 (1 H, d, *J* 7 Hz, H_1), 5.00 [2 H, s, CH_2(Bzl)], 4.35 (1 H, d, *J* 7 Hz, H_1^{α}), 4.15 [2 H, q, *J* 7 Hz, CH_2(OEt)], 3.90 (2 H, d, *J* 5 Hz, H_2^{α}), 3.00 (2 H, m, H_1^{β}), 1.35 [9 H, s, Me(Boc)], and 1.25 [3 H, t, *J* 7 Hz, Me(OEt)]; $\nu_{max.}$ ($CHCl_3$) 1 700 (urethane), 1 670 (amide I), and 1 735 cm^{-1} (ester); *m/z* 383 ($M^{+\cdot}$ – $Me_3CO\cdot$).

Boc–Tyr(Bzl)–Gly–OH (29).—This was prepared as described for (7) from compound (28) (9.13 g, 0.02 mol), MeOH (75 ml), and 1M-NaOH (21 ml, 0.021 mol). Recrystallized from AcOEt (60 ml)–Et_2O (100 ml); yield 6.77 g (79%); δ [$(CD_3)_2SO$] 8.25 (1 H, t, *J* 6 Hz, H_2), 7.35 [5 H, s, Ph(Bzl)], 7.1 [5 H, m, H_1, C_6H_4(Tyr)], 5.05 [2 H, s, CH_2(Bzl)], 4.20 (1 H, m, H_1^{α}), 3.80 (2 H, d, *J* 5 Hz, H_2^{α}), 2.90 (2 H, m, H_1^{β}), and 1.30 [9 H, s, Me(Boc)]; $\nu_{max.}$ (KBr) 1 700 cm^{-1} (urethane), 1 650 (amide I), and 1 725 cm^{-1} (CO_2H); *m/z* 355 ($M^{+\cdot}$ – $Me_3CO\cdot$).

Boc–Tyr(Bzl)–Gly–Glyt–Phe–Leu–OBzl (30).—This was prepared as described for (8) from (29) (1.29 g, 0.003 mol), (27) (1.43 g, 0.003 mol), CH_2Cl_2 (30 ml), TEA (0.30 g, 0.003 mol), and DCC (0.62 g, 0.003 mol); reaction time 48 h. Because of the low solubility of the product in CH_2Cl_2, this solvent was evaporated and AcOEt (50 ml) was added. The mixture was filtered, the AcOEt evaporated, and the residue chromatographed on a silica gel column (4.5 × 15 cm) in 5% MeOH–CH_2Cl_2; yield 2.34 g (92%); crystallization from EtOH (10 ml)–Et_2O (100 ml); δ ($CDCl_3$) 8.85 (1 H, d, *J* 8 Hz, H_4), 7.1 [22 H, m, H_2, H_3, H_5, Ph(OBzl, Phe, Bzl), C_6H_4(Tyr)], 5.4 (2 H, m, H_1, H_4^{α}), 5.10 [2 H, s, CH_2(OBzl)], 4.95 [2 H, s, CH_2(Bzl)], 4.2 (4 H, m, H_1^{α}, H_2^{α}, H_3^{α}, H_5^{α}), 3.15 (4 H, m, H_2^{β}, H_4^{β}), 1.60 (3 H, m, H_5^{β}, H_5^{γ}), 1.40 [9 H, s, Me(Boc)], and 0.85 (6 H, m, H_5^{δ}); $\nu_{max.}$ ($CHCl_3$) 1 700 (urethane), 1 680 (amide I), 1 510 (thioamide II), and 1 740 cm^{-1} (ester); $\lambda_{max.}$ (EtOH) 268 nm (ε 1.7 × 10^4).

Attempted Synthesis of H-Tyr–Gly–Glyt–Phe–Leu–OH (31).—The procedure was as described for the preparation of (9), starting from (30) (0.852 g, 0.001 mol); yield after HF treatment 0.69 g. This material (350 mg) was subjected to a Sephadex G-15 column as described for (9). The fractions eluting from 340—610 ml were pooled and lyophilized; yield 295 mg. This material (20 mg) was chromatographed by reverse-phase h.p.l.c. and four main fractions with R_fs and amino acid analyses as follows were collected: (*a*) 8.81 min, Tyr 0.33, Gly 1.00, Phe 0.27; (*b*) 16.79 min, Tyr 0.26, Gly 0.72, Phe 0.27, Leu 0.19; (*c*) (main fraction) 17.93 min, Tyr 0.31, Gly 0.68, Phe 0.32; (*d*) 24.11 min, Tyr 0.22; Gly 0.53, Phe 0.22, Leu 0.16.

Boc–Gly–Phe–Leu–OBzl (32).—This was prepared as described for (1) from Boc–Gly–OH (5.26 g, 0.03 mol), (25) (12.15 g, 0.03 mol), CH_2Cl_2 (75 ml), TEA (3.03 g, 0.03 mol), and DCC (6.18 g, 0.03 mol); yield 15.6 g (99%); δ ($CDCl_3$) 7.30 [5 H, s, Ph(OBzl)], 7.15 [5 H, s, Ph(Phe)], 6.95 (2 H, m, H_2, H_3), 5.40 (1 H, t, *J* 6 Hz, H_1), 5.10 [2 H, s, CH_2(OBzl)], 4.8 (2 H, m, H_2^{α}, H_3^{α}), 3.80 (2 H, d, *J* 6 Hz, H_1^{α}), 3.00 (2 H, d, *J* 6 Hz, H_2^{β}), 1.60 (3 H, m, H_3^{β}, H_3^{γ}), 1.40 [9 H, s, Me(Boc)], 0.85 (6 H, m, H_3^{δ}); $\nu_{max.}$ ($CHCl_3$) 1 700 (urethane), 1 650 (amide I), and 1 740 cm^{-1} (ester); *m/z* 469 ($M^{+\cdot}$ – Me_2C=CH).

HCl·Gly–Phe–Leu–OBzl (33).—This was prepared as described for (3) from (32) (13.13 g, 0.025 mol) and 4M-HCl–dioxane (150 ml). On evaporation a foam formed, which could not be crystallized; yield 11.05 g (96%); δ [$(CD_3)_2SO$] 8.85 (2 H, m, H_2, H_3), 8.35 (3 H, br, H_3N^+), 7.35 [5 H, s, Ph(OBzl)], 7.25 [5 H, s, Ph(Phe)], 5.20 [2 H, s, CH_2(OBzl)], 4.5 (2 H, m, H_2^{α}, H_3^{α}), 3.0 (2 H, m, H_2^{β}), 1.65 (3 H, m, H_3^{β}, H_3^{γ}), and 0.90 (6 H, m, H_3^{δ}); $\nu_{max.}$ ($CHCl_3$) 1 650 (amide I) and 1 725 cm^{-1} (ester).

Boc–Glyt–Gly–Phe–Leu–OBzl (34).—This was prepared as described for (23) from (33) (4.62 g, 0.01 mol), TEA (1.01 g, 0.01 mol), Et_2O (50 ml), and (22b) (2.21 g, 0.01 mol); reaction time 22 h; monitored by t.l.c. in 30% AcOEt–CH_2Cl_2; silica gel column (4.5 × 15 cm) in 10% AcOEt–CH_2Cl_2 to 50% AcOEt–CH_2Cl_2; yield 4.33 g (72%); δ ($CDCl_3$) 9.1 (1 H, br, H_2), 7.55 (3 H, br, H_3N^+), 7.25 [5 H, s, Ph(OBzl)], 7.10 [5 H, s, Ph(Phe)], 5.95 (1 H, br, H_1), 5.10 [2 H, s, CH_2(OBzl)], 4.6 (2 H, m, H_3^{α}, H_4^{α}), 4.15 (4 H, m, H_1^{α}, H_2^{α}), 3.00 (2 H, m, H_3^{β}), 1.6 (3 H, m, H_4^{β}, H_4^{γ}), 1.45 [9 H, s, Me(Boc)], 0.85 (6 H, m, H_4^{δ}); $\lambda_{max.}$ 268 nm (ε 9.20 × 10^3); $\nu_{max.}$ ($CHCl_3$) 1 700 (urethane), 1 670 (amide I), 1 500 (thioamide II), and 1 730 cm^{-1} (ester); *m/z* 598 ($M^{+\cdot}$), 542 ($M^{+\cdot}$ – Me_2C=CH_2).

HCl·Glyt–Gly–Phe–Leu–OBzl (35).—This was prepared as described for (3) from (34) (2.99 g, 0.005 mol) and 4M-HCl–dioxane (30 ml); yield 2.59 g (97%); crystallization from MeOH (2 ml)–Et_2O (30 ml); δ [$(CD_3)_2NCDO$–$SiMe_4$] *ca.* 11 (1 H, br, H_2), 8.4 (5 H, m, H_3, H_4, H_3N^+), 7.30 [5 H, s, Ph(OBzl)], 7.15 [5 H, s, Ph(Phe)], 5.15 [2 H, s, CH_2(OBzl)], 4.6 (2 H, m, H_4^{α}, H_5^{α}), 4.2 (4 H, m, H_1^{α}, H_2^{α}), 3.1 (2 H, m, H_3^{β}), 1.65 (3 H, m, H_4^{β}, H_4^{γ}), and 0.85 (6 H, m, H_4^{δ}); $\nu_{max.}$ ($CHCl_3$) 1 650 (amide I), 1 520 (thioamide II), and 1 750 cm^{-1} (ester); $\lambda_{max.}$ (EtOH) 268 nm (ε 9.97 × 10^3).

Boc–Tyr(Bzl)–Glyt–Gly–Phe–Leu–OBzl (36).—This was prepared as described for (6) from Boc–Tyr(Bzl)–OH (0.93 g, 0.0025 mol), (35) (1.34 g, 0.0025 mol), dioxane (20 ml), *N*-ethylmorpholine (0.29 g, 0.0025 mol), HOBt (0.36 g, 0.0025 mol) (incl. 8% H_2O), and DCC (0.52 g, 0.0025 mol); reaction time 48 h. Because of the low solubility of this compound in AcOEt, the reaction mixture was filtered, evaporated, and subjected to column chromatography on a silica gel column (4.5 × 20 cm) in 5% MeOH–CH_2Cl_2; yield 1.78 g (84%); crystallization from EtOH (25 ml)–Et_2O (100 ml); δ [$(CD_3)_2NCDO$–$SiMe_4$] 9.65 (1 H, br, H_3), 8.4 (3 H, m, H_2, H_4, H_5), 7.2 [19 H, m, Ph(OBzl, Phe, Bzl), C_6H_4(Tyr)], 6.25 (1 H, d, *J* 7 Hz, H_1), 5.2 [2 H, s, CH_2(OBzl)], 5.1 [2 H, s, CH_2(Bzl)], 4.4 (7 H, m, H_1^{α}, H_2^{α}, H_3^{α}, H_4^{α}, H_5^{α}), 3.1 (4 H, m, H_1^{β}, H_5^{β}), 1.65 (3 H, m, H_5^{β}, H_5^{γ}), 1.40 [9 H, s, Me(Boc)], and 0.90 (6 H, m, H_5^{δ}), $\nu_{max.}$ (KBr), 1 700 (urethane), 1 655 (amide I), and 1 740 cm^{-1} (ester); $\lambda_{max.}$ 266 nm (ε 1.23 × 10^4).

H–Tyr–Glyt–Gly–Phe–Leu–OH (37).—This was prepared as described for (9) from (36) (0.852 g, 0.001 mol); yield after HF treatment 0.44 g. This material (200 mg) was subjected to a Sephadex G-15 column, and the fractions eluting from 420—540 ml were pooled and lyophilized to yield 205 mg of material. This material (40 mg) was chromatographed by reverse-phase h.p.l.c. as described for (9); final lyophilization from 20% AcOH–H_2O yielded 31.2 mg (56%) of material. Homogeneous by t.l.c. in Bu^nOH–AcOH–H_2O (5 : 10 : 1), R_F 0.49; analytical h.p.l.c., R_t 14.76 min; amino acid analysis: Tyr 1.02, Gly 2.14, Phe 1.03, Leu 1.00 (Found: C, 51.7; H, 6.75; N, 9.45: S, 4.0. Calc. for $C_{28}H_{37}N_3O_6S$·3 AcOH·2 H_2O: C, 51.83; H, 6.78; N, 8.89; S, 4.07), FAB-MS: *m/z* 572 ([M + H]$^+$, 13%), 336 ([Gly–Phe–Leu]$^+$, 4.1), 294 ([Tyr-

Glyt–Gly]$^+$, 16), 279 ([Phe–Leu]$^+$, 34), 136 (72), 120 (100), 107 (12), 91 (38), and 86 (27).

Boc–Gly–Gly–Phe–Leu–OBzl (38).—This was prepared as described for (1) from (33) (4.62 g, 0.01 mol), Boc–Gly–OH (1.75 g, 0.01 mol), CH_2Cl_2 (25 ml), TEA (1.01 g, 0.01 mol), and DCC (2.06 g, 0.01 mol); recrystallized from EtOH (15 ml)–Et_2O (100 ml); yield 5.00 g (86%); δ ($CDCl_3$) 7.6 (3 H, m, H_2, H_3, H_4), 7.25 [5 H, s, Ph(OBzl)], 7.05 [5 H, s, Ph(Phe)], 5.8 (1 H, m, H_1), 5.15 [2 H, s, CH_2(OBzl)], 4.6 (2 H, m, H_4^{α}, H_5^{α}), 3.9 (4 H, m, H_1^{α}, H_2^{α}), 3.00 (2 H, m, H_3^{β}), 1.65 (3 H, m, H_4^{β}, H_4^{γ}), 1.4 [9 H, s, Me(Boc)], and 0.85 (6 H, m, H_4^{δ}); $\nu_{max.}$ ($CHCl_3$) 1 700 (urethane), 1 655 (amide I), and 1 740 cm^{-1} (ester).

HCl·Gly–Gly–Phe–Leu–OBzl (39).—This was prepared as described for (3) from (38) (5.00 g, 0.0086 mol) and 4M-HCl–dioxane (50 ml); yield 4.25 g (95%); δ [$(CD_3)_2SO$] 8.6 (3 H, m, H_2, H_3, H_4), 8.3 (3 H, br, H_3N^+), 7.35 [5 H, s, Ph(OBzl)], 7.20 [5 H, s, Ph(Phe)], 5.15 [2 H, s, CH_2(OBzl)], 4.5 (2 H, m, H_3^{α}, H_4^{α}), 3.7 (4 H, m, H_1^{α}, H_2^{α}), 3.00 (2 H, m, H_3^{β}), 1.65 (3 H, m, H_4^{β}, H_4^{γ}), and 0.9 (6 H, m, H_4^{δ}); $\nu_{max.}$ ($CHCl_3$) 1 640 (amide I) and 1 720 cm^{-1} (ester).

Boc–Tyrt(Bzl)–Gly–Gly–Phe–Leu–OBzl (40).—This was prepared as described for (23) from (39) (0.778 g, 0.0015 mol) TEA (0.15 g, 0.0015 mol), Et_2O (10 ml), AcOEt (10 ml), and (22c) (0.626 g, 0.0015 mol); monitored by t.l.c. in 5% MeOH–CH_2Cl_2. The solvent was evaporated and the residue chromatographed on a silica gel column (4.5 × 15 cm) in 10% MeOH–CH_2Cl_2; yield 1.23 g (96%); crystallization from EtOH (20 ml)–Et_2O (150 ml); δ ($CDCl_3$) 8.95 (1 H, br, H_2), 7.75 (3 H, br, H_3, H_4, H_5), 7.2 [19 H, m, Ph(OBzl, Bzl, Phe), C_6H_4(Tyr)], 5.8 (1 H, br, H_1), 5.15 [2 H, s, CH_2(OBzl)], 4.8 (3 H, m, H_1^{α}, H_2^{α}), 4.3 (3 H, m, H_3^{α}, H_4^{α}, H_5^{α}), 3.00 (4 H, m, H_1^{β}, H_4^{β}), 1.65 (3 H, m, H_5^{β}, H_5^{γ}), 1.4 [9 H, s, Me(Boc)], 0.85 (6 H, m, H_5^{δ}); $\nu_{max.}$ ($CHCl_3$) 1 700 (urethane), 1 650 (amide I), 1 500 (thioamide II), and 1 730 cm^{-1} (ester); $\lambda_{max.}$ (EtOH) 268 nm (ε 1.40 × 10^4).

H–Tyrt–Gly–Gly–Phe–Leu–OH (41).—This was prepared as described for (9) from (40) (0.852 g, 0.001 mol); yield after HF treatment 0.70 g. This material (350 mg) was subjected to a Sephadex G-15 column and the fractions eluting from 420—740 ml were pooled and lyophilized to yield 310 mg of material. This material (20 mg) was chromatographed by reverse-phase h.p.l.c. as described for (9); final lyophilization from 20% AcOH–H_2O yielded 14.2 mg (70%) of material; the product was homogeneous by t.l.c. in Bu^nOH–AcOH–H_2O (5 : 10 : 1), R_F 0.63; analytical h.p.l.c. R_t 12.59 min; amino acid analysis: Tyr 0.81, Gly 1.97, Phe 0.99, Leu 1.00; FAB-MS: *m/z* 572 ([M + H]$^+$, 2.1%), 336 ([Gly–Phe–Leu]$^+$, 2.2), 294 ([Tyrt–Gly–Gly]$^{\bullet}$, 2.5), 279 ([Phe–Leu]$^+$, 4.7), 182 ([$C_9H_{12}NOS$]$^+$, 9), 136 (21), 120 (100), 107 (46), 91 (50), and 86 (42).

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