Selectivity in the Trimethylsilylation and Acylation of Peptide Bonds, and its Application to Modification of the Enkephalins

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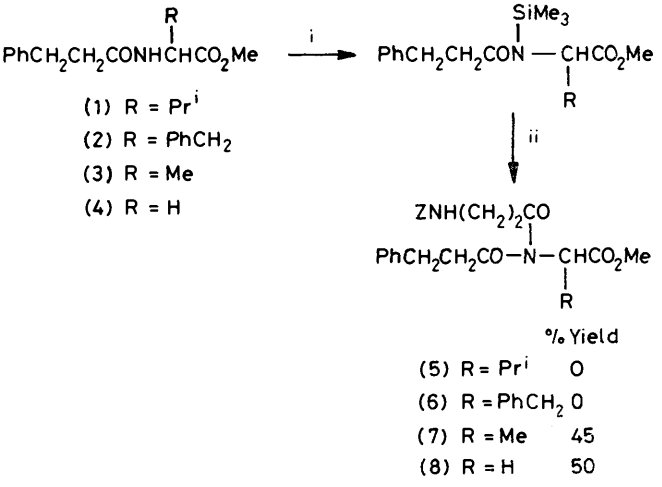
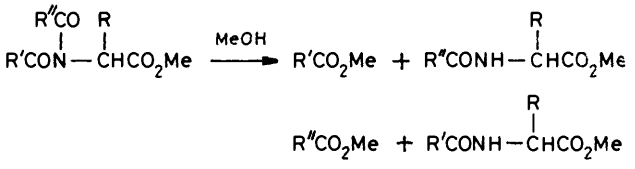
N.m.r. spectra of *N*-acylated peptides, formed by reaction of protected peptides with silylating agents followed by acylation, have provided a means for assessing selectivity in the acylation of amide bonds. Amino-acids such as valine and phenylalanine prevent significant acylation on neighbouring amide bonds while *N*-acylation occurs readily at glycyl amide bonds, one residue away from a hindered centre. Selective *N*-acylation of the Gly-Gly bond in enkephalin derivatives has been carried out using this methodology. Trimethylsilylation of Z-protected enkephalin derivatives provide a mild method for conversion into hydantoin (2,4-dioxoimidazoline) analogues of the enkephalins.

The selective acetylation of an amide bond in a model dipeptide derivative after treatment with trimethylchlorosilane–triethylamine and acetyl chloride [1,2] has prompted a broader investigation of the reactivity towards acylation of trimethylsilylated amide bonds in the peptide context. In the initial stages of the work it proved to be advantageous to use the 3-phenylpropionyl group for *N*-protection, as it could be considered as a deamino-analogue of phenylalanine as well as being a useful internal standard for n.m.r. spectral analysis.

The effect of the side-chain of the amino-acid on the ease of acylation of the *N*-terminal amide bond can be deduced from the results recorded in Scheme 1. No acylated products corresponding to structures (5) and (6) could be isolated, but compounds (7) and (8) were obtained in reasonable isolated yields, thus confirming that bulky side-chains hinder the silylation/acylation process. The *N*-acylated products (7) and (8) belong to a series of compounds which have been shown [1] to be difficult to purify, but in this work a short column of Kieselgel G (t.l.c. grade) proved successful. Addition of magnesium sulphate to the reaction mixture (probably functioning as a drying agent) also improved the yield.



Scheme 1. *Reagents*: i, Me_2SiCl–Et_3N; ii, $ZNH(CH_2)_2COCl$

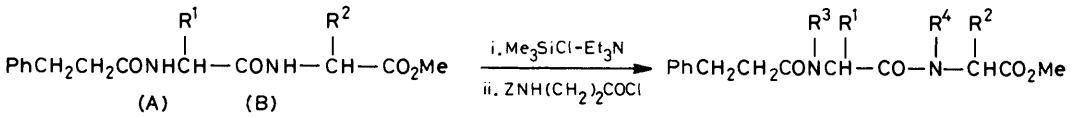


Scheme 2.

A detailed study of the changes in the n.m.r. characteristics of the compounds during the *N*-acylation process showed that compounds (7) and (8) had reduced signal multiplicity for the protons in the α-position and a chemical shift to lower field (*ca.* 0.4 p.p.m.). A similar deshielding effect was observed in the phenylpropionyl protons in the *N*-terminal position. These deshielding effects proved to be very useful in identifying the position of *N*-acylation in compounds containing more than one amide bond. Confirmation of the reliability of the n.m.r. effects was also obtained using product analysis on the methanolysis of *N*-acylated amides [1,2] as outlined in Scheme 2. This mild methanolysis furnishes product combinations which can pinpoint the original position of *N*-acylation. It also provides useful peptide derivatives for mass spectrometry.

Studies on the series of dipeptide ester derivatives listed in Scheme 3 provided further evidence for the influence of the side-chain on *N*-acylation reactions. Using de-shielding effects in the n.m.r. spectra for the identification of the products, the results show that in general the side-chains of phenylalanine and valine inhibit acylation at neighbouring amide groups. Only in the glycylphenylalanyl derivative (16) and the glycylvalyl derivative (18), where the amide bonds acylated are one glycine residue away from bulky side-chains was a significant amount of *N*-acylation recorded. The structural information obtainable by n.m.r. is best illustrated by an example, namely compound (21). The small difference in the chemical shifts of the phenyl groups of the substrate and acyl group allowed a quantitative integration, which indicated the incorporation of one acyl residue. The multiplicity of the glycyl CH_2 changed from a doublet in the starting material to a singlet in the product. A downfield chemical shift for the glycyl CH_2 protons from τ 6.2 to 5.8 was also observed. The *N*-terminal propionyl group was also affected, with the chemical shifts of the methylene protons merging to a singlet at τ 7.5 where in the starting material they were separate multiplets.

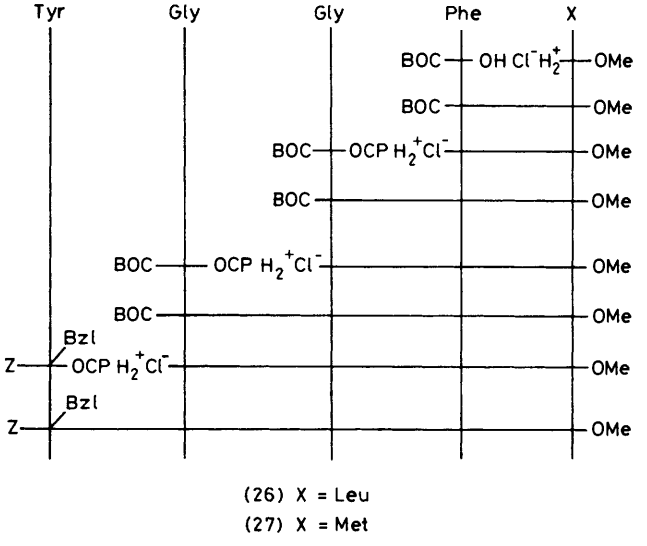
In a further study of selectivity in *N*-acylation, the naturally occurring opiate pentapeptides,[3] Leu-enkephalin (24) and Met-enkephalin (25) provided a useful template. The centrally situated Gly-Gly bond in these molecules seemed a prime candidate to check the selectivity of the silylation/acylation process. The research on these molecules could also be justified



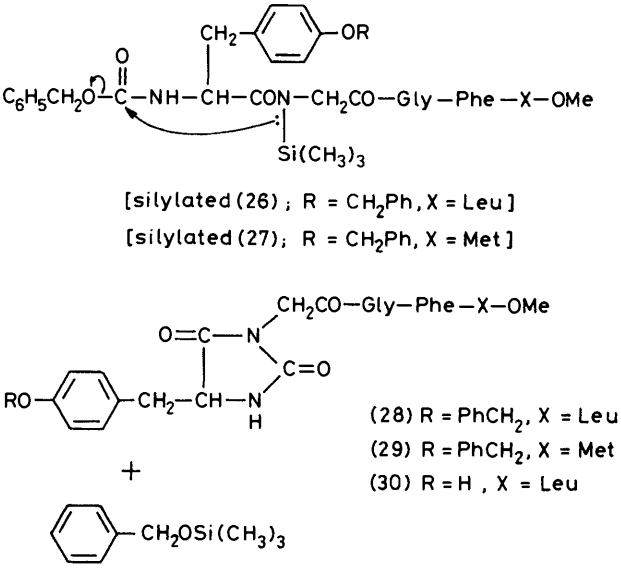
	R^1	R^2	Yield (%)	Bond acylated
(9)	Pr^i	Pr^i	0	—
(10)	$PhCH_2$	Pr^i	0	—
(11)	Pr^i	Me	0	—
(12)	Me	Pr^i	10	(A)
(13)	$PhCH_2$	Me	0	—
(14)	Me	$PhCH_2$	10	(A)
(15)	$PhCH_2$	H	—	—
(16)	H	$PhCH_2$	50	(A)
(17)	Pr^i	H	10	(B)
(18)	H	Pr^i	60	(A)

	Structure			
	R^1	R^2	R^3	R^4
(19)	Me	Pr^i	$ZNH(CH_2)_2CO$	H
(20)	Me	$PhCH_2$	$ZNH(CH_2)_2CO$	H
(21)	H	$PhCH_2$	$ZNH(CH_2)_2CO$	H
(22)	Pr^i	H	H	$ZNH(CH_2)_2CO$
(23)	H	Pr^i	$ZNH(CH_2)_2CO$	H

Scheme 3.



Scheme 4.



Scheme 5.

H-Tyr-Gly-Gly-Phe-Leu-OH (24)

H-Tyr-Gly-Gly-Phe-Met-OH (25)

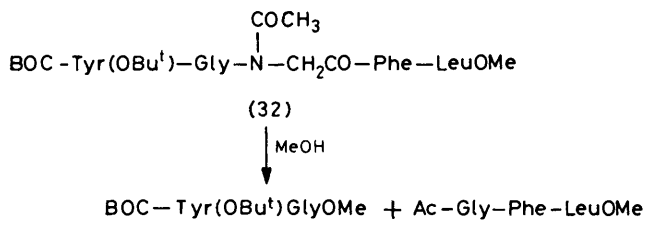
in the pharmacological context, since carefully chosen acyl groups could improve lipid/water partition coefficients, and could affect the 'blood-brain barrier' transport characteristics of the enkephalins. Choosing *N*-acylation as a peptide bond modification could also be beneficial since the resulting imidic system produced could be slowly hydrolysed under physiological conditions to re-form the free enkephalins. For these studies enkephalin derivatives (26) and (27) were synthesised using the methods summarised in Scheme 4. Extension of the

Z-Tyr(OBzl)-Gly-Gly-Phe-Leu-OMe (26)

Z-Tyr(OBzl)-Gly-Gly-Phe-Met-OMe (27)

silylation studies to the enkephalins necessitated the application of more volatile silylating agents to improve the separation of products, and with these larger peptides a suitable solvent had to be found. Bistrimethylsilyltrifluoroacetamide (BSTFA)[4] in acetonitrile proved to be the best combination. In line with the pharmacological interest, the ultimate aim was to study 2-ethylhexanoyl chloride as *N*-acylation agent to increase lipophilicity. On subjecting both peptide (26) and peptide (27) to these conditions it became evident that the usual silylation/acylation reactions were not taking place. Instead trimethylsilylation alone caused a side reaction which was identified as a nucleophilic displacement of the benzyloxycarbonyl group according to Scheme 5 to give 82% yields of the hydantoins (28) and (29). There have been previous examples[5-7] of peptides containing glycine as the penultimate *N*-terminal residue undergoing this type of reaction, although usually the vigour of the conditions has resulted in the hydantoins being converted into urea derivatives.[7]

The availability of the hydantoin derivatives (28) and (29) provided a means of testing and augmenting the results of Tomatis and Salvadori[7] who had reported some depressant effects for hydantoin (2,4-dioxoimidazoline) derivatives of enkephalin. For a direct comparison with the enkephalins it was necessary to remove the protecting benzyl groups prior to testing. Debenzylation of (28) using Pd/C in aqueous dioxan–acetic acid proved unsuccessful. Removal of the benzyl group was achieved in good yield on treatment with HBr–AcOH to give the deprotected hydantoin [(30; R = H, X = Leu) in



Scheme 6.

Scheme 5]. Bio-assay of (30) *in vitro* using a mouse vas deferens assay showed that (30) possessed a weak depressant effect, unlike that induced by morphine. The effect was not reversed by the opiate antagonist naloxone and thus it is reasonable to assume that this activity is not opiate-receptor mediated but the depressant effect confirms the results of Tomatis and Salvadori [7] for an analogous derivative. Hydantoin (30) showed no antagonist activity.

The loss of the benzyloxycarbonyl group as depicted in Scheme 5 could be prevented by replacing the Gly [2] residue with D-Ala. In fact when Z-Tyr(Bzl)-D-Ala-Gly-Phe-Leu-OMe was subjected to the silylation conditions there was no spectroscopic evidence of silylation having occurred at any position along the peptide backbone. This result confirmed the previous model studies that efficient *N*-silylation/acylation is restricted to the availability of non-hindered Gly-Gly amide bonds.

It has also been shown that t-butoxycarbonyl used for *N*-protection is stable to the silylating/acylating conditions. Thus BOC-Tyr(OBu^t)-Gly-Gly-Phe-Leu-OMe (31) (synthesised using Scheme 4 but with BOC-Tyr(OBu^t)OH used in the last coupling step) could be successfully silylated and when this silylated derivative was treated with acetyl chloride–triethylamine a mono-*N*-acetylated derivative of (31) could be characterised as having structure (32). N.m.r. evidence indicated the incorporation of one acetyl group (singlet at τ 7.95), but the exact position of his group could not be unambiguously assigned from n.m.r. data alone, although the α-proton region between τ 5 and 6 showed an increase in the number of protons indicating the possible deshielding of the glycine methylene groups. The methanolysis sequence outlined in Scheme 6 with the identification of the products by t.l.c. and mass spectrometry however proved conclusive, thus confirming the selectivity of the acylation reactions.

In a preliminary study involving replacement of the acetyl chloride with 2-ethylhexanoyl chloride, it has been shown that a mono-2-ethylhexanoyl derivative is obtained but in reduced yields, probably due to the increasing steric bulk of the acylating group. The deprotection of this derivative and an assessment of its stability and biological activity is in hand.

In summary, therefore the non-hindered glycyl-glycyl amide bonds in quite complicated peptides when 'activated' by silylation, do provide access to *N*-acyl peptide derivatives, provided other *N*-protecting groups are also carefully chosen. This confirms the general trend deduced from other model peptides.[2]

Experimental

I.r. spectra were determined as KBr discs or liquid films on Perkin-Elmer 257 or Pye-Unicam SP 1050 spectrophotometers. ^{1}H N.m.r. spectra were determined at 100 MHz with both Varian HA 100 and XL-100 instruments. Tetramethylsilane was used as internal standard, except in the case of certain trimethylsilyl derivatives when dichloromethane was used. Mass spectra were obtained with an AEI MS 9 mass spectrometer. T.l.c. plates were prepared from Kieselgel GF_{254} or used as pre-coated plates (Merck). The solvent systems, $CHCl_3$–MeOH (9 : 1), EtOAc–Me_2CO (2 : 1), EtOAc–C_6H_6 (1 : 1), and BuOH–AcOH–H_2O (4 : 1 : 5) proved to be useful developing systems and visualisation of the plates was carried out by (i) GF_{254} plates under u.v. light or (ii) exposure to I_2 vapour.

In general, organic extracts were dried over anhydrous sodium sulphate and evaporated under reduced pressure. Acetonitrile for silylation reactions was distilled from phosphorus pentoxide and used immediately. Apparatus for silylation reactions was dried in an oven prior to use.

N-(3-*Phenylpropionyl*) *Derivatives of selected Amino-acids.*—The amino-acid (0.1 mol) in 2M-sodium hydroxide (100 cm^3, 0.2 mol) was cooled in ice. 3-Phenylpropionyl chloride [8] (0.11 mol) and 2M sodium hydroxide (100 cm^3, 0.2 mol) were added in ten equal and alternate portions with thorough mixing. After acidification (5M-HCl) and cooling, crystals separated which were recrystallised from the solvents listed in Table 1.

N-(3-*Phenylpropionyl*)*amino-acid Methyl Esters.*—The amino-acid methyl ester hydrochloride * (0.05 mol) suspended in ether (200 cm^3) containing triethylamine (0.05 mol) was treated with 3-phenylpropionyl chloride [8] with cooling. After extraction of the ether successively with, 1M-HCl, 0.5M-$NaHCO_3$, and water the dried ether layer yielded products as follows.

N-(3-*Phenylpropionyl*)-L-*valine methyl ester* (1) (70% yield) was a clear non-crystallisable oil, b.p. 150 °C at 0.05 mmHg, $[\alpha]_D^{20}$ −32° (*c* 2, MeOH) (Found: C, 68.2; H, 7.5; N, 5.2. $C_{15}H_{21}NO_3$ requires C, 68.4; H, 8.0; N, 5.3%), $\nu_{max.}$ 1 730 (ester) and 1 650 cm^{-1} (amide); τ($CDCl_3$) 2.78 (5 H, s, ArH), 3.86 (1 H, d, NH), 5.52 (1 H, dd, α-CH), 6.39 (3 H, s, OCH_3), 7.07 (2 H, t, PhCH_2CH$_2$), 7.50 (2 H, t, PhCH$_2$CH_2), 7.95 (1 H, m, CH_3CHCH_3), 9.19 (3 H, d, CH_3CH), and 9.22 (3 H, d, CH_3CH).

N-(3-*Phenylpropionyl*)-L-*phenylalanine methyl ester* (2) (70% yield) had m.p. 74—75 °C from ether, $[\alpha]_D^{20}$ −1.7° (*c* 4.7, MeOH) (Found: C, 73.2; H, 6.6; N, 4.5. $C_{19}H_{21}NO_3$ requires C, 73.3; H, 6.8; N, 4.5%), $\nu_{max.}$ 1 750 (ester) and 1 650 cm^{-1} (amide); τ($CDCl_3$) 2.9 (10 H, br, s, 2ArH), 3.97 (1 H, d, NH), 5.18 (1 H, m, α-CH), 6.4 (3 H, s, OCH_3), 7.01 (2 H, d, PhCH_2CH), 7.12 (2 H, t, PhCH_2CH$_2$), and 7.58 (2 H, t, PhCH$_2$-CH_2).

N-(3-*Phenylpropionyl*)-L-*alanine methyl ester* (3) (80% yield) had m.p. 86—87 °C from ether, $[\alpha]_D^{20}$ −43.0° (*c* 0.4 MeOH) (Found: C, 66.2; H, 6.9; N, 6.0. $C_{13}H_{17}NO_3$ requires C, 66.4; H, 7.3; N, 6.0%), $\nu_{max.}$ 1 740 (ester) and 1 640 cm^{-1} (amide); τ($CDCl_3$), 2.84 (5 H, s, ArH), 4.0 (1 H, br, s, NH), 5.47 (1 H, m, α-CH), 6.35 (3 H, s, OCH_3), 7.07 (2 H, t, PhCH_2CH$_2$), 7.54 (2 H, t, PhCH$_2$CH_2), and 8.70 (3 H, d, CH_3CH).

N-(3-*Phenylpropionyl*)*glycine methyl ester* (4) (75% yield) had m.p. 71—73 °C from ether (Found: C, 65.3; H, 7.0; N, 6.3. $C_{12}H_{15}NO_3$ requires C, 65.1; H, 6.8; N, 6.3%), $\nu_{max.}$ 1 740 (ester) and 1 675 cm^{-1} (amide); τ($CDCl_3$), 2.84 (5 H, s, ArH), 3.97 (1 H, t, NH), 6.05 (2 H, d, NHCH_2CO), 6.34 (3 H, s, OCH_3), 7.06 (2 H, t, PhCH_2CH$_2$), and 7.50 (2 H, t, PhCH$_2$-CH_2).

Trimethylsilylation of Amide Bonds.—All glassware was carefully dried for these experiments. Samples (500 mg) of compounds (1)—(4), individually dissolved in dry chloroform (10 cm^3) were treated with triethylamine (2 cm^3), followed by trimethylchlorosilane (2 cm^3). The solution was left to mix

* Amino-acid methyl esters hydrochloride were prepared using $SOCl_2$–MeOH by a literature method.[9]

Table 1. *N*-3-Phenylpropionyl amino-acids

Compd.	Amino-acid	M.p. (°C)	Crystallisation solvent	$[\alpha]^{20}$	Elemental analysis (%) Found C	H	N		Calc. for C	H	N
(1)	L-Valine	171—173	MeOH–H_2O	−20.5° (*c* 2 in MeOH)	67.2	7.6	6.0	$C_{14}H_{19}NO_3$	67.4	7.8	5.6
(2)	L-Phenylalanine	165—167	MeOH–H_2O	+9.7° (*c* 2 in MeOH)	72.7	6.1	4.9	$C_{18}H_{19}NO_3$	72.7	6.4	4.7
(3)	L-Alanine	108—109	H_2O	?	65.5	6.8	6.5	$C_{11}H_{15}NO_3$	65.1	6.8	6.3
(4)	Glycine	116—118	H_2O	—	64.2	6.3	6.5	$C_{11}H_{13}NO_3$	63.75	6.3	6.8

Table 2.

Compd.	% Silylation (based on disappearance of ν_{NH}
(1)	0
(2)	40
(3)	90
(4)	100

for a few minutes, before the reaction mixture was analysed in the solution cell of an infrared spectrometer. An approximate estimation of the degree of trimethylsilylation of the amide bond is recorded in Table 2.

The trimethylsilylated amide derivatives used for the acylation step were obtained by evaporating the chloroform solution under reduced pressure and using the resulting residue directly.

Acylation of Trimethylsilylamide Derivatives.—The trimethylsilylated derivatives (0.02 mol) prepared as above were dissolved in dry toluene to which *N*-benzyloxycarbonyl-β-alanyl chloride (prepared from *N*-benzyloxycarbonyl-β-alanine [10] and phosphorus(v) chloride) (0.02 mol) in toluene was added together with magnesium oxide (0.5 g) and magnesium sulphate (0.5 g). The solution was stirred for 2 h at 0 °C followed by removal of toluene under reduced pressure. The resulting oil was purified by rapid separation on a short column (25 cm × 3 cm) of Kieselgel G (t.l.c. grade) with dichloromethane–ethyl acetate (50 : 50). The relevant fractions containing *N*-acylated material were evaporated to dryness to give residual gums, which were immediately subjected to n.m.r. and mass spectral analysis.

Using the above methodology no *N*-acylated products were identified for the esters (1) and (2). The ester (3) gave N-(3-*phenylpropionyl*)-N-(N′-*benzyloxycarbonyl*-β-*alanyl*)*alanine methyl ester* (7) (45% yield), τ($CDCl_3$) 2.74 (5 H, s, ArH), 2.83 (5 H, s, ArH), 4.7 (1 H, t, NH), 4.98 (2 H, s, PhCH_2O), 5.57 (1 H, q, α-CH), 6.42 (3 H, s, OCH_3), 6.60 [2 H, m, CH_2CO(β-Ala)], 7.08 (4 H, s, PhCH_2CH_2), 7.16 (2 H, t, CH_2NH), and 8.85 (3 H, d, CH_3CH); M^+, 440. Methanolysis of this compound using methanol under reflux for 2 h gave methyl 3-phenylpropionate, *N*-(3-phenyl propionyl)alanine methyl ester, and *N*-benzyloxycarbonyl-β-alanine methyl ester.

The ester (4) under the same conditions gave N-(3-*phenylpropionyl*)-N-(N′-*benzyloxycarbonyl*-β-*alanyl*)*glycine methyl ester* (8) (60% yield), ($CDCl_3$) 2.80 (5 H, s, Ar*H*), 2.88 (5 H, s, Ar*H*), 4.54 (H t, NH, β-Ala), 5.01 (2 H, s, PhCH_2O), 5.76 (2 H, s, CH_2 of Gly), 6.61 (2 H, m, CH_2 of β-Ala), 7.13 (4 H, s, PhCH_2CH_2), 7.20 (2 H, t, $HNCH_2$ of β-Ala); M^+, 426. Methanolysis of (8) gave methyl 3-phenylpropionate, *N*-(3-phenylpropionyl)glycine methyl ester, and *N*-benzyloxycarbonyl-β-alanine methyl ester.

Preparation of Model Dipeptides listed in Scheme 3.—Using the appropriate combination of *N*-terminal and *C*-terminal residues, the dipeptides (9)—(18) were prepared using the following general method.

The *N*-(3-phenylpropionyl)amino-acid (prepared as above) (0.05 mol) in dry benzene (50 cm^3) was treated with *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline, EEDQ [11] (0.05 mol) and the amino-acid methyl ester (0.05 mol) dissolved in benzene (100 cm^3). The mixture was stirred at room temperature for 2 h after which ethanol (10 cm^3) was added to dissolve the suspension.[11] The solvent was removed and the resultant oil dissolved in chloroform (200 cm^3) and extracted successively with 1M-HCl, 0.5M-$NaHCO_3$, and water before being dried over $MgSO_4$. The chloroform was removed and the products in most cases yielded crystalline material with satisfactory elemental analysis. Physical data for compounds (9)—(18) appear in Table 3.

Trimethylsilylation/Acylation of Compounds (9)—(18).—In a typical experiment the dipeptide ester (1.0 g) was trimethylsilylated using the same conditions as described earlier, *i.e.* trimethylchlorosilane and triethylamine in dry chloroform solution. The solvent mixture was evaporated under reduced pressure and *N*-benzyloxycarbonyl-β-alanine acid chloride (0.5 g) dissolved in toluene (50 cm^3) containing suspended magnesium oxide (0.5 g) and magnesium sulphate (0.5 g) was added. The mixture was stirred at 0 °C for 2 h, filtered, and the solvents removed under reduced pressure. Purification was carried out by fast elution on a small Kieselgel G column eluted with dichloromethane–ethyl acetate (50/50). Only the dipeptides (12), (14), (16), (17), and (18) yielded new products.

The peptide ester (17) yielded an oil (109 mg, 10% yield) identified as N-[N′-(3-*phenylpropionyl*)-L-*valyl*]-N-(N-*benzyloxycarbonyl*-β-*alanyl*)*glycine methyl ester* (22), τ($CDCl_3$) 2.75 (10 H, s, 2ArH), 4.80 (1 H, br, NH), 5.00 (2 H, s, ArCH_2O), 5.58 (2 H, s, GlyCH_2), 5.70 (H, q, CH), 6.40 (3 H, s, OCH_3), 6.60 (4 H, m, β-AlaCH_2), 7.10 (2 H, t, PhCH_2CH_2), 7.42 (2 H, t, PhCH_2CH_2), 8.00 [1 H, m, $(CH_3)_2CH$], 9.12 [3 H, d, $(CH_3)_2CH$], and 9.28 [3 H, d, $(CH_3)_2CH$]. The other NH signal could not be located; M^+, 526.

The peptide ester (18) yielded (650 mg, 60% yield) of an oil identified as N-(3-*phenylpropionyl*)-N-(N-*benzyloxycarbonyl*-β-*alanyl*)*glycyl*-L-*valine methyl ester* (23), τ($CDCl_3$) 2.80 (5 H, s, ArH), 2.88 (5 H, s, ArH), 3.20 (1 H, m, NH), 4.45 (1 H, t, NH), 5.01 (ArCH_2O), 5.56 (H, q, α-CH), 5.71 (2 H, s, GlyCH_2), 6.41 (3 H, s, OCH_3), 6.60 (2 H, d, β-AlaCH_2), 7.09 (6 H, s, β-AlaCH_2N and PhCH_2CH_2), 7.90 [H, m, $(CH_3)_2CH$], 9.13 [3 H, d, $(CH_3)_2CH$], and 9.16 [3 H, d, $(CH_3)_2CH$]; M^+, 526.

The peptide ester (16) yielded an oil (595 mg, 50% yield) identified as N-(3-*phenylpropionyl*)-N-(N-*benzyloxycarbonyl* β-*analyl*)*glycyl*-L-*phenylalanine methyl ester* (21), τ($CDCl_3$) 2.80 (10 H, s, ArH), 2.89 (5 H, s, ArH), 4.40 (1 H, t, NH), 4.98 (2 H, s, ArCH_2O), 5.25 (1 H, m, α-CH), 5.80 (2 H, s, GlyCH_2), 6.44 (3 H, s, OCH_3), 6.61 (2 H, m, β-AlaCH_2), 7.00 (2 H, t, β-AlaCH_2N), and 7.16 (6 H, m, PhCH_2CH_2 and PhCH_2). The other NH signal could not be located; M^+, 573.

The peptide ester (12) yielded an oil (112 mg, 10% yield) identified as N-(3-*phenylpropionyl*)-N-(N-*benzyloxycarbonyl*-β-*alanyl*)-L-*alanyl*-L-*valine methyl ester* (19), τ($CDCl_3$) 3.77 (5 H, s, ArH), 2.85 (5 H, s, ArH), 3.42 (H, br, NH), 4.56 (H, br, NH), 5.00 (2 H, s, PhCH_2O), 5.39 (H m, Ala-α-C*H*), 5.59 (H, q, Val-α-C*H*), 6.38 (3 H, s, OCH_3), 6.56 (2 H, m, β-AlaCH_2), 7.09 (4 H, s, PhCH_2CH_2), 7.18 (2 H, t, β-AlaCH_2), 7.90 [1 H, m, $(CH_3)_2CH$], 8.53 (3 H, d, AlaCH_3), 9.13 [3 H, d, $(CH_3)_2$-CH], and 9.16 [3 H, d, $(CH_3)_2CH$]; M^+, 539.

The peptide ester (14) yielded (125 mg, 11% yield) of an oil, N-(3-*phenylpropionyl*)-N-(N-*benzyloxycarbonyl*-β-*analyl*)-L-*alanyl*-L-*phenylalanine methyl ester* (20), τ($CDCl_3$) 2.78 (10 H, s, 2 × Ar*H*), 2.87 (5 H, s, Ar*H*), 3.48 (H, br, NH), 4.62 (H, br, NH), 5.00 (2 H, s, PhCH_2O), 5.34 (2 H, m, α-CH's), 6.40 (3 H, s, OCH_3), 6.60 (2 H, m, β-AlaCH_2), 7.00 (4 H, m, β-AlaCH_2N, PhCH_2), 7.25 (4 H, t, PhCH_2CH_2), and 8.65 (3 H, d, CH_3CH); M^+, 587.

Synthesis of Enkephalin Derivatives.—Z-Tyr(OBzl)-Gly-Gly-Phe-Leu-OMe (26). *N*-Benzyloxycarbonyl-glycyl-L-phenylalanyl-L-leucine methyl ester [12] (1.45 g, 3 mmol) in methanol–water and 10% palladium/charcoal (150 mg) was

Table 3. Physical data on compounds (9)—(18)

Compd.	M.p. (°C)	Cryst. solvent	$[\alpha]_D^{20}$ (MeOH) (°C)	M^+ ion	100 MHz ^{1}H N.m.r. in $CDCl_3$							OCH_3	Side-chains
					Ph-CH_2-CH_2			α-CH		NH			
(9)	150—152	Et_2O	−43.4 *c* 2	362	2.86 (s)	7.08 (t)	7.5 (t)	5.6 (m)		3.1 (d)	3.5 (d)	6.35 (s)	9.13 [2 × $(CH_3)_2CH$] 8.0 [2 × $(CH_3)_2CH$]
(10)	138—140	Et_2O	−20.9 *c* 2	410	2.88 (s)	7.16 (t)	7.58 (t)	5.1 (m)	5.62 (dd)			6.38 (s)	7.95 $(CH_3)CH$ 2.9 (Ar*H*), 7.06 ($ArCH_2$) 9.2 [$(CH_3)_2CH$], 9.18 [$(CH_3)CH$]
(11)	210—213	Et_2O	−28.6 *c* 2	334	2.87 (s)	7.10 (t)	7.52 (t)	5.49 (m)		3.4 (d)		6.36 (s)	8.02 [m, $(CH_3)_2CH$], 8.64 (d, CH_3CH) 9.14 [' t,' $(CH_3)_2CH$]
(12)	124—126	Et_2O	−59.4 *c* 2	334	2.86 (s)	7.08 (t)	7.52 (t)	5.16 (m)	5.58 (dd)	2.24 (d)	2.58 (d)	6.38 (s)	7.90 [m, $(CH_3)_2CH$], 8.71 (d, CH_3CH) 9.12 [d, $(CH_3)_2CH$]
(13)	171—173	Et_2O	−22.4 *c* 1.4	382	2.88 (s)	7.18 (t)	7.60 (t)	5.26 (m)	5.60 (m)	3.12 (d)	3.45 (d)	6.38 (s)	2.91 (s, ArH), 7.05 (d, $ArCH_2$) 8.74 (d, CH_3CH)
(14)	140—141	Et_2O	−17.2 *c* 2	382	2.89 (s)	7.13 (t)	7.58 (t)	5.30 (m)		2.43 (d)		6.44 (s)	2.9 (s, ArH), 7.0 (d, $ArCH_2$) 8.76 (d, CH_3CH)
(15)	133—135	EtOAc	−19.2 *c* 2	368	2.86 (s)	7.15 (t)	7.69 (t)	5.27 (m)	6.14 (d)	2.38 (d)	3.74 (d)	6.37 (s)	2.9 (s, ArH), 7.02 (d, $ArCH_2$)
(16)	Oil		−39.2 *c* 2	368	2.88 (s)	7.12 (t)	7.56 (t)	5.25 (m)	6.20 (d)	3.22 (t)		6.46 (s)	2.89 (s, ArH), 7.00 (d, $ArCH_2$)
(17) *	172—175	$CHCl_3$-LP †	−0.1 *c* 0.8	320	2.85 (s)	7.08 (t)	7.50 (t)	5.62 (dd)	6.04 (t)	2.56 (t)	3.22 (s)	6.32 (s)	8.02 [m, $CH(CH_3)_2$], 9.12 [t, $(CH_3)_2CH$]
(18)	Oil		−16.7 *c* 2	320	2.90 (s)	7.09 (t)	7.50 (t)	5.58 (dd)	6.07 (d)	2.52br	2.33br	6.42 (s)	7.90 [$(CH_3)_2CH$], 9.11 [$(CH_3)_2CH$]

* Racemic form—synthesised by DCCI coupling. † LP = Light petroleum.

hydrogenated overnight. After removal of catalyst and evaporation of solvent, the oily residue was dissolved in dimethylformamide (DMF) (10 cm^3) and treated with a solution of *N*-Boc-glycine 2,4,5-trichlorophenyl ester [13] (1.07 g, 3 mmol) in DMF (5 cm^3). The reaction mixture was stirred at 0 °C for 1 h and then at room temperature overnight. The evaporated product was extracted with ethyl acetate (30 cm^3) and washed with water (3 × 15 cm^3). Evaporation of the dried extract gave *N-t-butoxycarbonylglycylglycyl-L-phenylalanyl-L-leucine methyl ester* (1.18 g, 77% yield) from ethyl acetate–light petroleum, m.p. 125—127 °C (Found: C, 59.6; H, 7.8; N, 10.9. $C_{25}H_{38}N_4O_7$ requires C, 59.3; H, 7.6; N, 11.1%), $\nu_{max.}$ 3 310 (NH), 1 750 (ester), 1 725 (urethane), 1 700, and 1 645 cm^{-1} (amide); τ[$(CD_3)_2SO$] 1.10 (1 H, d, NH), 2.05 (2 H, m, 2 × NH), 2.82 (5 H, s, ArH), 3.12 (1 H, m, NH), 5.45 [1 H, m, C*H*(Phe)], 5.74 (1 H, m, C*H*Leu), 6.44 (7 H, m, OCH_3 and 2 × CH_2), 7.10 (2 H, m, PhCH_2), 8.45 [3 H, m, $CH_2CH(CH_3)_2$], 8.65 [9 H, s, $(CH_3)_3C$], and 9.14 (6 H, t, 2 × CH_3). This t-Boc derivative (2.02 g, 4 mmol) in trifluoroacetic acid–dichloromethane (1 : 1, 40 cm^3) was left at room temperature for 1 h. The product after solvent evaporation was dried *in vacuo* over NaOH for 16 h to remove an excess of acid. It was then dissolved in DMF (10 cm^3) and added to a stirred solution of *N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosine 2,4,5-trichlorophenyl ester [14] (2.34 g, 4 mmol) and triethylamine (0.56 cm^3, 4 mmol) in DMF (10 cm^3). The reaction was stirred overnight at 5 °C and evaporated. The residue on work up yielded *N-benzyloxycarbonyl-O-benzyl-L-tyrosyl-glycylglycyl-L-phenylalanyl-leucine methyl ester* (26) after gradient elution on Kieselgel 60 with $CHCl_3$–MeOH (95 : 5), yield 2.57 g (81%), m.p. 144—145 °C (Found: C, 66.4; H, 6.1; N, 8.8. $C_{44}H_{51}N_5O_9$ requires C, 66.6; H, 6.5; N, 8.8%), $\nu_{max.}$ 3 310 (NH), 1 750 (ester), 1 710 (urethane), and 1 660 cm^{-1} (amide); τ[$(CD_3)_2SO$] 1.70 (2 H, m, 2NH), 2.00 (2 H, m, 2NH), 2.65 (5 H, d, $C_6H_5CH_2O$), 2.77 (5 H, s, $OCOCH_2C_6H_5$), 2.80 (5 H, s, $CHCH_2C_6H_5$), 2.70—3.20 [4 H, q (partially hidden), C_6H_4 of tyrosyl], 5.00 (2 H, s, PhCH_2O), 5.09 (2 H, s, PhCH_2O), 5.30—5.85 (3 H, m, 3-α-C*H*), 6.30 [4 H, t (overlapping d), 2 × α-CH_2], 6.42 (3 H, s, OCH_3), 6.8—7.35 (4 H, m, 2 × β-CH_2), 8.44 [3 H, m, $CH_2CH(CH_3)_2$], and 9.14 (6 H, t, 2 × CH_3).

Z-Tyr(OBzl)Gly-Gly-Phe-Met-OMe (27). *N*-t-Butoxycarbonyl-L-phenylalanyl-L-methionine methyl ester [14] (2.05 g, 0.5 mmol) in a 50% solution of 4M-HCl in dioxan (30 cm^3) was left at room temperature for 30 min. After evaporation of the solvent the residue was dissolved in DMF (10 cm^3) and added to a stirred solution of *N*-t-butoxycarbonylglycine 2,4,5-trichlorophenyl ester [13] (1.77 g, 5 mmol) and triethylamine (0.7 cm^3, 5 mmol) in DMF (10 cm^3). The reaction mixture was stirred overnight at 5 °C and then evaporated; the residue was dissolved in ethyl acetate (50 cm^3) and the solution filtered. The filtrate was then washed with 10% citric acid (2 × 10 cm^3) and water (10 cm^3). The residue obtained on drying and evaporating the solution was eluted from a Kieselgel 60 (230—400 mesh) column by elution with a gradient of chloroform with methanol (0—10%). *N*-t-Butoxycarbonylglycyl-L-phenylalanyl-L-methionine methyl ester (1.69 g, 72%), obtained from ether–light petroleum, had m.p. 117—118 °C (lit.,[15] 69—71 °C) (Found: C, 57.0; H, 7.2; N, 9.1. Calc. for $C_{22}H_{33}N_3O_6S$: C, 56.6; H, 7.4; N, 8.8%), $[\alpha]_D^{20}$ −16.3° (*c* 1.94 in DMF) {lit.,[15] $[\alpha]_D^{20}$ −15.5° (*c* 2 in DMF)}; τ($CDCl_3$) 2.82 (5 H, s, ArH), 2.96 (2 H, s, 2NH), 4.62 (1 H, t, N*H*CH_2), 4.15—4.60 (2 H, m, 2 CH, 6.26 (2 H, d, NHCH_2), 6.36 (3 H, s, OCH_3), 6.96 (2 H, d, CHCH_2Ph), 7.64 (2 H, t, CH_2CH_2S 8.00 (3 H, s, SCH_3), 8.05 (2 H, m, $CHCH_2CH_2$), and 8.63 [9 H, s, $(CH_3)_3C$].

The protected ester above (2.34 g, 5 mmol) was deprotected by HCl–dioxan as described above and coupled with *N*-t-butoxycarbonylglycine 2,4,5-trichlorophenyl ester (1.77 g, 5 mmol) as described to yield *N*-t-butoxycarbonylglycylglycyl-L-phenylalanyl-L-methionine methyl ester (2.12 g, 81% yield), m.p. 102—104 °C (which again did not correspond to a literature value [15] of 78—80 °C although all elemental and spectral data were consistent with the structure).

The protected tetrapeptide ester above (1.57 g, 0.003 mol) was deprotected by the HCl–dioxan method and coupled to *N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosine 2,4,5-trichlorophenyl ester [14] (1.75 g, 3 mmol) by the method described for the leucine analogue above. After work-up *N-benzyloxycarbonyl-O-benzyl-L-tyrosylglycylglycyl-L-phenylalanyl-L-methionine methyl ester* (27) (1.18 g, 36% yield) was obtained as a white solid from ethyl acetate–light petroleum and had m.p. 143—145 °C (Found: C, 63.1; H, 6.3; N, 8.4. $C_{43}H_{49}N_5O_9S$ requires C, 63.6; H, 6.1; N, 8.6%), $\nu_{max.}$ 1 745 (ester), 1 710 (urethane) or 1 655 and 1 645 cm^{-1} (peptide CO); τ[$(CD_3)_2SO$] 1.70 (2 H, m, 2 NH), 2.00 (2 H, m, 2 × NH), 2.65 (5 H, d, $C_6H_5CH_2O$), 2.77 (5 H, s, $C_6H_5CH_2OCO$), 2.80 (5 H, s, $CHCH_2C_6H_5$), 2.70—3.2 [4 H, q (partially hidden), C_6H_4 of tyrosyl], 5.00 (2 H, s, $C_6H_5CH_2O$), 5.09 (2 H, s, $OCOCH_2C_6H_5$), 5.30—5.85 (3 H, m, 3 × CH), 6.30 [4 H, t (overlapping doublet), 2 × $CH_2(\alpha)$], 6.42 (3 H, s, OCH_3), 6.80—7.35 [4 H, m, $2CH_2(\beta)$], 7.54 (2 H, m, CH_2CH_2S), 8.00 (3 H, s, SCH_3), and 8.10 (2 H, m, $CHCH_2CH_2$); one NH signal not located.

Rearrangement of Peptide Derivatives (26) *and* (27) *on Trimethylsilylation.*—(a) *Peptide derivative* (27). The derivative (27) (406 mg, 0.5 mmol) was silylated with BSTFA [4] (1.35 cm^3, 5 mmol) in acetonitrile (37 cm^3). The mixture was shaken overnight and refluxed gently for 4 h. The solvent was removed under reduced pressure and the oily residue extracted with toluene and stirred at room temperature. Elution from a Kieselgel 60 (230—400 mesh) column with ethyl acetate (with an acetone 0—33% gradient) gave pure 5-(p-*benzyloxybenzyl*)-2,4-*dioxoimidazolin*-3-*ylacetylglycyl*-L-*phenylalanyl*-L-*methionine methyl ester* (29) (290 mg, 82%), m.p. 205—206 °C from trituration with ether (Found: C, 61.2; H, 6.3; N, 9.9; S, 4.3. $C_{36}H_{41}N_5O_8S$ requires C, 61.4; H, 5.9; N, 9.9; S, 4.6%), $\nu_{max.}$ 1 780 (hydantoin CO) 1 745 (ester), 1 720 (hydantoin CO), and 1 650 cm^{-1} (peptide); τ[$(CD_3)_2SO$] 1.5—2.0 (4 H, m, 4 × NH), 2.64 (5 H, d, $OCH_2C_6H_5$), 2.8 (5 H, s, $CHCH_2C_6H_5$), 2.80—3.20 [4 H, q, C_6H_4 (Tyr)], 5.0 (2 H, s, $OCH_2C_6H_5$), 5.2—5.8 (3 H, m, 3 × CH), 6.12 (2 H, s, NCH_2CO), 6.40 (5 H, s, OCH_3, $NHCH_2$), 6.8—7.3 [4 H, m, 2 × $CH_2(\beta)$], 7.5 (2 H, m, CH_2CH_2S), 8.0 (3 H, s, SCH_3), and 8.10 (2 H, m, $CHCH_2CH_2$). Analysis of a sample of the crude oil before purification confirmed that the by-product of the reaction was benzyl alcohol trimethylsilyl ether.[16]

(b) *Peptide derivative* (26). Treatment of the derivative (26) as for (27) above yielded 5-(p-*benzyloxybenzyl*)-2,4-*dioxoimidazolin*-3-*ylacetylglycyl*-L-*phenylalanyl*-L-*leucine methyl ester* (28) (82% yield), m.p. 190—191 °C (Found: C, 64.6; H, 6.3; N, 9.9. $C_{37}H_{43}N_5O_8$ requires C, 64.8; H, 6.3; N, 10.2%), $\nu_{max.}$ 1 785 (hydantoin CO), 1 745 (ester CO), 1 730 (hydantoin CO), and 1 650 cm^{-1} (peptide CO); τ[$(CD_3)_2SO$] 1.60—2.0 (4 H, m, 4 × NH), 2.65 (5 H, d, $OCH_2C_6H_5$), 2.8 (5 H, s, $C_6H_5CH_2CH$), 2.85—3.2 [4 H, q, C_6H_4(Tyr)], 5.0 (2 H, s, $OCH_2C_6H_5$), 5.3—6.0 (3 H, m, 3 × CH), 6.18 (2 H, s, NCH_2CO), 6.44 (5 H, s, OCH_3 and $NHCH_2$), 6.90—7.40 [4 H, m, 2 × $CH_2(\beta)$], 8.45 [3 H, m, $CH_2CH(CH_3)_2$], and 9.15 (6 H, t, 2 × CH_3)$_2$.

Deprotection (*De-benzylation*) *of the Hydantoin* (2,4-*Dioxoimidazoline*) *Derivatives* (28) *and* (29.)—The protected hydantoin substrate (28) (137 mg, 2 mmol) in glacial acetic acid (2 cm^3) was treated with a 4M solution of hydrogen bromide in glacial acetic acid (0.5 cm^3). The reaction mixture was

left at room temperature for 4 h and then evaporated under reduced pressure. The dried residue was chromatographed on Kieselgel 60 (chloroform–methanol 0—10% gradient). Fractions containing the required product were combined and after evaporation and trituration with ether yielded 5-(p-*hydroxybenzyl*)-2,4-*dioxoimidazolin*-3-*ylacetylglycyl*-L-*phenylalanyl*-L-*leucine methyl ester* (30) (97 mg, 81%), m.p. 190—192 °C (Found: C, 57.7; H, 6.4; N, 10.4. $C_{30}H_{37}N_5O_8 \cdot 3/2H_2O$ requires C, 57.8; H, 6.5; N, 11.2%), τ[$(CD_3)_2SO$], 0.8 (1 H, s, OH), 1.6—2.0 (4 H, series, 4 × NH), 2.8 (5 H, s, C_6H_5), 3.0—3.4 (4 H, q, C_6H_4), 5.4—5.9 (3 H, m, 3 × C*H*), 6.2 (2 H, s, NCH_2CO), 6.44 (5 H, s, OCH_3, $NHCH_2$), 6.9—7.25 [4 H, series, 2 × $CH_2(\beta)$], 8.45 (3 H, m, CH_2CH), 9.14 (6 H, t, 2 × CH_3). This product was used by the Central Toxicology Lab. at I.C.I. Ltd., Alderley Park, Cheshire for agonist and antagonist tests using a mouse vas deferens.

The above procedure was repeated on the methionine analogue (29) (140 mg, 0.2 mmol) and the product 5-(p-*hydroxybenzyl*)-2,4-*dioxoimidazolin*-3-*ylacetylglycyl*-L-*phenylalanyl*-L-*methionine methyl ester* was obtained as a gum, τ[$(CD_3)_2SO$] spectrum similar to (30) except that signals at 7.5 (2 H, m, CH_2CH_2S), 7.95 (3 H, s, SCH_3), and 8.0 replaced the signals at 8.45 and 9.14.

N-*t-Butoxycarbonyl*-O-*t-butyl*-L-*tyrosylglycylglycyl*-L-*phenylalanyl*-L-*leucine Methyl Ester* (31).—A solution of N-t-butoxycarbonyl-*O*-t-butyl-L-tyrosine dicylohexylammonium salt [17] (1.55 g, 3 mmol) in DMF (30 cm^3) was treated with glycylglycyl-L-phenylalanyl-L-leucine methyl ester hydrochloride (formed as described previously) (3 mmol) in DMF (15 cm^3). The mixture was stirred at 0 °C for 5 min and then treated with DCCI (0.68 g, 3 mmol). The reaction was stirred at 0—3 °C overnight, filtered, and the filtrate evaporated to give a residue which after work-up, was purified on Kieselgel 60 (230—400 mesh) by elution with chloroform–methanol (0—10% gradient) to yield N-*t-butoxycarbonyl*-O-*t-butyl*-L-*tyrosylglycylglycyl*-L-*phenylalanyl*-L-*leucine methyl ester* (31) (1.11 g, 51% yield), m.p. 145—147 °C (Found: C, 62.7; H, 7.9; N, 9.6. $C_{38}H_{53}N_5O_9$ requires C, 62.9; H, 7.6; N, 9.6%), $\nu_{max.}$ 1 750 (ester), 1 700 (urethane), 1 665, 1 655, and 1 650 cm^{-1} (peptide CO); τ[$(CD_3)_2SO$] 1.50—2.10 (5 H, m, 5 × NH), 2.72 (5 H, s, $CHCH_2C_6H_5$), 2.75—3.2 (4 H, q, C_6H_4 Tyr), 5.25—5.9 (3 H, m, 3 × CH), 6.26 (4 H, m, 2 × $NHCH_2$), 6.38 (3 H, s, OCH_3), 6.8—7.4 [4 H, m, 2 × $CH_2(\beta)$], 8.45 [3 H, s, $CH_2CH(CH_3)_2$], 8.74 [18 H, s, $(CH_3)_3CO$ and $(CH_3)_3COCO$], and 9.14 (6 H, t, 2 × CH_3).

Trimethylsilylation and Acetylation of the Derivative (31).—The peptide derivative (31) (290 mg, 0.4 mmol) was treated with BSTFA [4] (1 cm^3) and after removal of excess of reagent the residue was dissolved in toluene and treated with acetyl chloride (0.14 cm^3, 2 mmol) and triethylamine (0.28 cm^3, 2 mmol). The reaction was stirred at room temperature for 24 h and then evaporated. The residue was extracted with ethyl acetate (40 cm^3) and filtered to remove the precipitated amine hydrochloride salt. Gradient elution on Kieselgel 60 (ethyl acetate–acetone as eluant) gave 21% of unchanged starting material and (82 mg) of the monoacetylated derivative of (31); τ[$(CD_3)_2SO$)] values were similar to those for (31) except that there was an additional signal at 7.95 (3 H, s, CH_3CO), loss of one NH signal, and increased integration in the τ 5—6 region.

Proof for the position of *N*-acetylation was obtained from methanolysis. Thus the mono-acetylated derivative of (31) (40 mg) was refluxed for 16 h in methanol (5 cm^3). The evaporated residue was analysed on t.l.c. and bands from the t.l.c. plates subjected to mass spectrometry, against the standards.

(i) Compound (31); (ii) Boc-Tyr(OBu^t)-GlyOMe; and (iii) Ac-Gly-Phe-LeuOMe. No evidence was found for the formation of (i) in the methanolysis mixture but (ii) and (iii) were positively identified as the only products. This confirmed that the *N*-acetyl group was present on the Gly-Gly bond.

N-t-Butoxycarbonyl-*O*-t-butyl-L-tyrosylglycine methyl ester used as the standard above was prepared from *N*-t-butoxycarbonyl-*O*-t-butyl-L-tyrosine dicyclohexylammonium salt [17] (1.55 g, 3 mmol) and glycine methyl ester hydrochloride (0.38 g, 3 mmol) in chloroform (20 cm^3) at 0 °C, after treatment with DCCI (0.62 g, 3 mmol) in chloroform (10 cm^3). On work-up in the usual way, *N*-t-*butoxycarbonyl*-O-*t-butyl*-L-*tyrosylglycine methyl ester* (0.61 g) was obtained from ether–light petroleum, m.p. 98—90 °C (Found: C, 61.7; H, 8.1; N, 7.1. $C_{21}H_{32}N_2O_5$ requires C, 61.7; H, 7.9; N, 6.9%), $\nu_{max.}$ 1 765 (ester), 1 690 (urethane), and 1 670 cm^{-1} (peptide); τ($CDCl_3$) 2.85—3.2 (4 H, q, C_6H_4Tyr), 3.4 (1 H, t, NH), 4.85 (1 H, d, NH), 5.65 (1 H, q, CH), 6.05 (2 H pair of d, $NHCH_2CO$), 6.32 (3 H, s, OCH_3), 6.9—7.1 [2 H, m, $(CH_2)\beta$], 8.64 [9 H, s, $(CH_3)_3CO$], and 8.72 [9 H, s ($CH_3)_3COCO$].

N-Acetylglycyl-L-phenylalanyl-L-leucine methyl ester standard was prepared by allowing *N*-acetylglycine [18] (0.35 g, 3 mmol), *N*-hydroxybenzotriazole (0.41 g, 3 mmol) in DMF (10 cm^3) at 0 °C and DCCI (0.68 g, 3 mmol), to react with L-phenylalanine-L-leucine methyl ester hydrochloride (3 mmol) and triethylamine (0.42 cm^3, 3 mmol) in DMF (5 cm^3). The N-*acetylglycyl*-L-*phenylalanyl*-L-*leucine methyl ester* was obtained after work-up from ethyl acetate–light petroleum as a white crystalline solid, m.p. 173—174 °C, $\nu_{max.}$ 1 760 (ester CO), 1 700 (amide CO), and 1 635 cm^{-1} (peptide); τ[$(CD_3)_2SO$] 2.00—2.30 (3 H, m, 3 × NH), 2.82 (5 H, s, C_6H_5), 5.3—5.8 (2 H, series of peaks, 2 × CH), 6.2—6.38 (5 H, m, $NHCH_2CO$ and OCH_3), 8.1 (3 H, s, CH_3CO), 8.35 [3 H, m, $CH_2CH(CH_3)_2$], and 9.1 (6 H, m, 2 × CH_3).

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