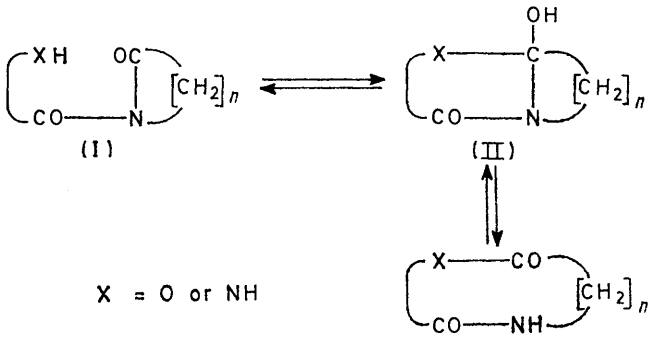
Amino-acids and Peptides. Part XIV.[1] Modification of Peptides by Amino-acid Insertion [2]

By **John S. Davies, Cedric H. Hassall,*† and Keith H. Hopkins,** Department of Chemistry, University College of Swansea, Singleton Park, Swansea SA2 8PP

Amide bonds in peptide derivatives have been silylated with chloro(trimethyl)silane and the products have been acylated readily to form *N*-acyl peptides. Deprotection of the derivatives of *N*-α- or -β-aminoacyl peptides so formed was followed by rearrangement, with incorporation of the aminoacyl residue into the peptide chain. The inserted optically active amino-acid residues were not racemised.

NEIGHBOURING group interactions of amino- and hydroxy-groups with amide bonds [3] have been employed in an interesting manner for the synthesis of cyclopeptides [4] and -depsipeptides [5] by insertion reactions. The method (Scheme 1) involves rearrangement of an



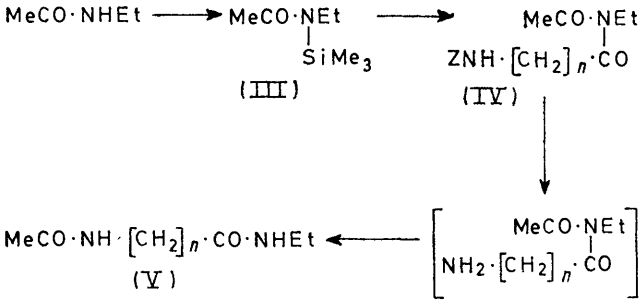
SCHEME 1

N-acylamide (I) to a carbinolamide (II), which in favourable cases gives rise to an enlarged ring resulting from insertion of the original *N*-acyl residue.

To our knowledge there has been only one report [6] of an attempt to use this principle for the modification of linear peptides. In this case there was evidence of insertion but vigorous conditions were required for acylation, which probably accounted for the racemisation of the inserted aminoacyl residue. We have investigated the acylation of various amides and peptides by use of their *N*-silyl derivatives [7] as intermediates, and have studied the rearrangement of the products.

In preliminary experiments we used *N*-ethylacetamide. Treatment with chloro(trimethyl)silane in the presence of triethylamine at room temperature gave the silylated derivative (III). Other reagents such as bistrimethylsilylacetamide proved less efficient. The formulation of (III) as the *N*-silyl rather than the *O*-silyl form was based on i.r. spectral evidence [8] and the ready conversion into *N*-acyl compounds such as (IV).

The trimethylsilyl group in the derivative (III) was readily displaced in the presence of acid chlorides of acetic acid, benzyloxycarbonylglycine, and benzyloxycarbonyl-β-alanine to give the corresponding *N*-acyl derivatives in almost quantitative yields. *N*-Acylation resulted in the deshielding of the CH_3·CO protons (τ −0·33) and the methylene protons (τ −0·42) of the *N*-ethyl group of *N*-ethylacetamide. This deshielding effect has subsequently proved useful in identifying the position of *N*-acylation in peptides.[9] Strong electrophilic acylating agents such as acid chlorides and acid anhydrides ‡ are favoured for this process. There was very little acylation of the silylated amide bond when *p*-nitrophenyl esters, 2,4,5-trichlorophenyl esters, or imidazolides were used although the last-named have



been shown to be effective for making *N*-silyl-lactams.[4] Treatment of the model amide and *N*-benzoylglycylglycine methyl ester (VI) with acetyl chloride without preliminary formation of their silyl derivatives as expected resulted in no acylation of the amide bonds.

† *Present address:* Roche Products Ltd., Welwyn Garden City, Hertfordshire.

‡ After the completion of this work similar results were reported by H. R. Kricheldorf and G. Greber, *Chem. Ber.*, 1971, **104**, 3131.

[1] Part XIII, C. H. Hassall, D. G. Sanger, and B. K. Handa, *J. Chem. Soc.* (*C*), 1971, 2814.

[2] Preliminary communication, J. S. Davies, C. H. Hassall, and K. H. Hopkins, *Chem. Comm.*, 1971, 1118.

[3] J. A. Davies, C. H. Hassall, and I. H. Rogers, *J. Chem. Soc.* (*C*), 1969, 1358; M. Brenner, J. P. Zimmerman, J. Wehrmuller, P. Quitt, and I. Photaki, *Experientia*, 1955, **11**, 397; R. W. Holley and A. D. Holley, *J. Amer. Chem. Soc.*, 1952, **74**, 3069, 5445; Th. Wieland and H. Urbach, *Annalen*, 1958, **613**, 84; P. L. Russell, R. M. Topping, and D. E. Tutt, *J. Chem. Soc.* (*B*), 1971, 657.

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[5] Yu. A. Ovchinnikov, in 'XXIIIrd International Congress of Pure and Applied Chemistry,' Butterworths, 1971, vol. 2, p. 121.

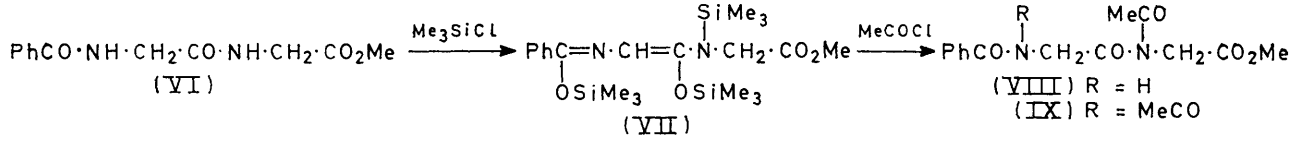
[6] T. R. Telesnina, V. K. Antoniov, and M. M. Shemyakin, *Zhur. obshchei Khim.*, 1968, **38**, 1691.

[7] M. Rothe and T. Toth, *Chem. Ber.*, 1966, **99**, 3820.

[8] U. Wallwitz, H. Schmidt, and W. Gosda, *J. prakt. Chem.*, 1966, **32**, 274.

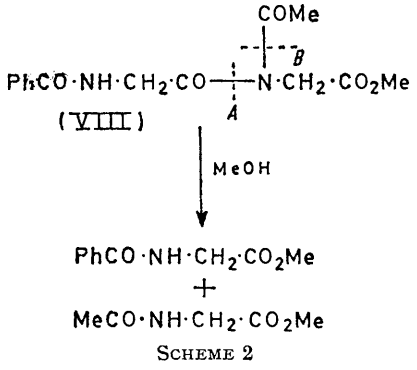
[9] J. S. Davies, C. H. Hassall, and R. K. Merritt, unpublished results.

Hydrogenolysis of the *N*-(*N*-benzyloxycarbonyl-β-alanyl)-*N*-ethylacetamide (IV; $n = 2$) or its glycyl analogue (IV; $n = 1$) in an inert solvent containing a mild base (triethylamine) resulted in removal of the protecting group and spontaneous rearrangement to the corresponding amino-acid derivative (V) (85% yield). It was found that use of alcohols as solvents caused



cleavage of the *N*-acyl intermediate; this ready alcoholysis[10] has been used to advantage in determining the position of acylation in the model peptide systems described later.

The same reaction sequence has been applied to derivatives of glycylglycine. However, the amide



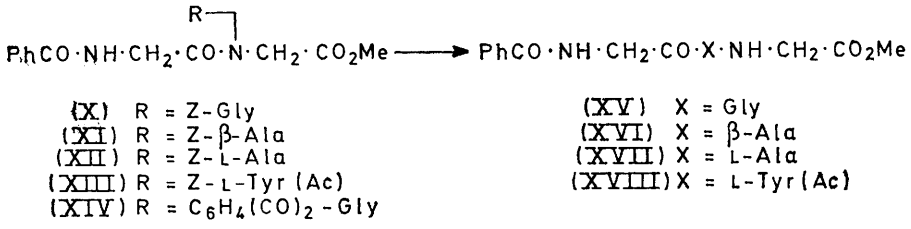
SCHEME 2

groups in these derivatives showed different reactivities towards the reagents. Thus the product of the reaction of *N*-benzoylglycylglycine methyl ester (VI) with chloro(trimethyl)silane and triethylamine was shown by mass spectral (*m/e* 466) and ^{1}H n.m.r. evidence to have three trimethylsilyl residues. The silylated peptide, which was tentatively assigned[11] structure (VII), reacted with acetyl chloride at room temperature to form the monoacetyl derivative (VIII) in 75% yield; the diacetyl derivative (IX) (15% yield) was obtained when the reaction time was prolonged. Confirmation of the structure of the derivative (VIII) was obtained from ^{1}H n.m.r. evidence and through methanolysis. The ^{1}H n.m.r. spectrum revealed a three-proton singlet at τ 7·72 (Ac) and a reduced multiplicity in one of the methylene peaks (singlet, τ 5·55) while the other methylene peak remained as a doublet (τ 5·39). A downfield shift occurred in the signal due to the methylene protons adjacent to the acylated amide bond. Methanolysis of the *N*-acetylated peptide (VIII) gave *N*-benzoylglycine methyl ester and *N*-acetylglycine methyl ester, resulting from cleavage at positions *A* and *B* (Scheme 2), respectively.

A similar selective *N*-acylation occurred when the dipeptide (VI) was treated with acid chlorides of the *N*-benzyloxycarbonyl derivatives of glycine, β-alanine, L-alanine, *O*-acetyl-L-tyrosine, and *N*-phthalylglycine to give the corresponding derivatives (X)—(XIV). Hydrogenolysis of these compounds in toluene–triethylamine led to removal of the benzyloxycarbonyl groups and rearrangement to the products (XV)—(XVIII), the result of (formal) amino-acid insertion. Yields greater than 70% were obtained for the rearrangement reaction.

In the examples of acylation for which optically active forms of the amino-acids were used, the products (XVII) and (XVIII) of the insertion reaction had the same optical rotation as those synthesised by conventional methods involving no significant degree of racemisation.

The insertion of amino-acid residues, without racemisation, may prove applicable to the synthesis of particular linear peptides. The potential of the method will be related, particularly, to the variation in the reactivity towards acylation of peptide bonds in different environments. This is being investigated.



EXPERIMENTAL

M.p.s were determined with a Kofler hot-stage apparatus. I.r. spectra were determined for potassium bromide discs, or chloroform solutions, unless otherwise stated, with a Perkin-Elmer 257 spectrophotometer. Optical rotation measurements were obtained with a Perkin-Elmer 141 automatic polarimeter (10 cm cells). ^{1}H N.m.r. spectra

[10] F. Weygand, R. Geiger, and U. Glockler, *Chem. Ber.*, 1956, **89**, 1543.

[11] S. I. Lur'e, E. S. Chaman, and G. A. Ravdel, *Zhur. obshchei Khim.*, 1953, **23**, 1392.

were determined with a Varian HA-100 instrument (tetramethylsilane as internal standard, except for certain trimethylsilyl derivatives for which dichloromethane was employed). Mass spectra were obtained with an A.E.I. MS9 spectrometer. Accurate mass measurements were performed by peak matching with fragment ions of heptacosafluorotributylamine. Amino-acid analyses were carried out on a Beckman 120C Analyser with Beckman custom spherical resin and elution with buffer pH 3·25—4·30. G.l.c. analysis was carried out on an F and M 810 instrument equipped with a flame ionisation detector; column packings were 10% w/w polyphenyl ether or silicone gum rubber on Diatoport S. T.l.c. was carried out with plates coated with Kieselgel G and GF_{254} (Merck) in the solvent systems (A) benzene–ethyl acetate (1 : 1), (B) n-butanol–acetic acid–water (12 : 3 : 5), (C) acetone–benzene (1 : 1), and (D) acetone–benzene–methanol (9 : 9 : 2). Compounds were identified by their iodine-positive reactivity or by observation of the GF_{254} plates under an u.v. lamp.

N.B. In all the reactions involving silylating reagents, dry and purified solvents were always used and dry-box techniques employed wherever practicable.

Trimethylsilylation of N-*Ethylacetamide*.—*N*-Ethylacetamide [12] (9·3 ml, 0·10 mol), triethylamine (20·3 ml, 0·15 mol), and toluene (30 ml) were heated gently under reflux, and chloro(trimethyl)silane (19·1 ml, 0·15 mol) was added dropwise during 20 min. Heating was continued for a further 20 min and the mixture was cooled to room temperature. (A variation of this method involved mixing the reactants in ether or toluene as solvent and stirring at room temperature for 4 h.) Removal of the precipitated triethylamine hydrochloride followed by fractional distillation of the filtrate yielded N-*ethyl*-N-*trimethylsilylacetamide* (III) (14·3 g, 90%) as a pale yellow liquid, b.p. 50—52° at 18 mmHg, 152—154° at 760 mmHg, $\nu_{max.}$ (film) 1640 (CO), 1395, 1250 ($SiMe_3$), and 1170 (SiN) cm^{-1}, τ ($CDCl_3$) 9·83 (9H, s, $SiMe_3$), 8·94 (3H, t, J 7·2 Hz, $CH_3{\cdot}CH_2$), 8·04 (3H, s, $CH_3{\cdot}CO$), and 6·86 (2H, q, J 7·2 Hz, $CH_3{\cdot}CH_2$). Characteristic peaks at *m/e* 159 (M) and 158 ($M - 1$) confirmed the identity of the product, but owing to the extreme susceptibility of the compound to moisture, no satisfactory elemental analysis was obtained.

Compound (III) was also obtained in 50% yield by the reaction of *N*-ethylacetamide with *NO*-bistrimethylsilylacetamide at room temperature.

Acylation of N-*Ethyl*-N-*trimethylsilylacetamide* (III).—(a) *With acetyl chloride*. The silylamide (III) (1·05 g) at 0° in an ice-bath was treated with acetyl chloride (1·25 g). G.l.c. of the products showed that the strongly exothermic reaction was complete in 2 min. Fractional distillation of the mixture at atmospheric pressure yielded *N*-ethyldiacetamide (0·78 g, 93%), b.p. 192° at 760 mmHg (lit.,[13] 183—185° at 633 mmHg) as a liquid, $\nu_{max.}$ (film) 1695 cm^{-1} (imide), τ (CCl_4) 8·93 (3H, t, J 7·0 Hz, $CH_3{\cdot}CH_2$), 7·75 (6H, s, $CH_3{\cdot}CO$), and 6·36 (2H, q, J 7·0 Hz, $CH_3{\cdot}CH_2$).

(b) *With* N-*benzyloxycarbonylglycyl chloride*. *N*-Benzyloxycarbonylglycyl chloride (2·10 g, 0·0092 mol) (freshly prepared from *N*-benzyloxycarbonylglycine [14] and phosphorus pentachloride) in toluene (6 ml) was added to the silylamide (III) (1·34 g, 0·0084 mol) in toluene (5 ml). The mixture was stirred for 6 h at 0—5 °C then added to chloroform (ethanol-free) (200 ml), and the solution was washed with aqueous sodium hydrogen carbonate, dried, and evaporated to yield N-(N-*benzyloxycarbonylglycyl*)-N-*ethylacetamide* (IV; $n = 1$) (1·86 g, 80%), a white crystalline solid, m.p. 107—108° (from toluene–petroleum) (Found: C, 60·6; H, 6·6; N, 10·5. $C_{14}H_{18}N_2O_4$ requires C, 60·4; H, 6·5; N, 10·1%), $\nu_{max.}$ 3260 (NH), 1735 and 1640 (imide CO), and 1700 cm^{-1} (urethane CO), τ ($CDCl_3$) 8·86 (3H, t, J 6·8 Hz, $CH_3{\cdot}CH_2$), 7·71 (3H, s, $CH_3{\cdot}CO$), 6·36 (2H, q, J 6·8 Hz, $CH_3{\cdot}CH_2$), 5·68 (2H, d, J 4·7 Hz, $NH{\cdot}CH_2{\cdot}CO$), 4·93 (2H, s, $ArCH_2{\cdot}O$), 4·24br (1H, NH), and 2·72 (5H, s, ArH), *m/e* 278 (M^+).

(c) *With* N-*benzyloxycarbonyl*-β-*alanyl chloride*. The acid chloride (2·41 g, 0·01 mol) (freshly prepared from *N*-benzyloxycarbonyl-β-alanine [14] and phosphorus pentachloride) in toluene (8 ml) was added to the silylamide (III) (1·44 g, 0·009 mol) in toluene (5 ml). Work-up as in (b) yielded N-(N-*benzyloxycarbonyl*-β-*alanyl*)-N-*ethylacetamide* (IV; $n = 2$) (2·20 g, 84%), a non-crystallisable gum (Found: C, 61·4; H, 7·0; N, 9·4. $C_{15}H_{20}N_2O_4$ requires C, 61·6; H, 6·9; N, 9·6%), $\nu_{max.}$ (film) 3340 (NH) and 1690br (urethane and imide CO) cm^{-1}, τ (CCl_4) 8·93 (3H, t, J 7·0 Hz, $CH_3{\cdot}CH_2$), 7·74 (3H, s, $CH_3{\cdot}CO$), 7·20 (2H, t, J 5·1 Hz, $CO{\cdot}CH_2{\cdot}CH_2{\cdot}NH$), 6·65 (2H, m, $CO{\cdot}CH_2{\cdot}CH_2{\cdot}NH$), 6·41 (2H, q, J 7·0 Hz, $CH_3{\cdot}CH_2$), 5·02 (2H, s, $ArCH_2{\cdot}O$), 4·45br (1H, NH), and 2·77 (5H, s, ArH), *m/e* 292 (M^+).

Hydrogenolysis of the N-*Acyl Compounds* (IV; $n = 1$ or 2).—Both *N*-acyl compounds (0·034 mol) were hydrogenolysed in toluene (10 ml) and triethylamine (50 ml), over palladium–charcoal (0·10 g). After removal of catalyst the solution evaporated to dryness.

Compound (IV; $n = 1$) gave a gum which sublimed (115 °C at 0·5 mmHg) to give N-(N-*acetylglycyl*)*ethylamine* (V; $n = 1$) (0·45 g, 87%), m.p. 139—141° (Found: C, 49·6; H, 7·9; N, 19·4. $C_6H_{12}N_2O_2$ requires C, 50·0; H, 8·4; N, 19·4%), $\nu_{max.}$ 3280 (NH) and 1645 (amide CO) cm^{-1}, τ ($CDCl_3$) 8·90 (3H, t, J 7·0 Hz, $CH_3{\cdot}CH_2$), 8·0 (3H, s, $CH_3{\cdot}CO$), 6·78 (2H, m, $J_{CH_3{\cdot}CH_2}$ 7·0, $J_{CH_2{\cdot}NH}$ 6·0 Hz, $CH_3{\cdot}CH_2$), 6·10 (2H, d, J 5·5 Hz, $NH{\cdot}CH_2{\cdot}CO$), and 3·0br (2H, NH), *m/e* 144 (M^+). The compound was also unambiguously synthesised from *N*-(*N*-benzyloxycarbonylglycyl)ethylamine by hydrogenolysis in the presence of acetic anhydride.

Compound (IV; $n = 2$) gave a compound which sublimed (110° at 0·5 mmHg) to give N-(N-*acetyl*-β-*alanyl*)-*ethylamine* (V; $n = 2$) (0·38 g, 71%), m.p. 136—137° (Found: C, 53·1; H, 9·3; N, 17·7. $C_7H_{14}N_2O_2$ requires C, 53·1; H, 8·9; N, 17·7%), $\nu_{max.}$ 3300 (NH) and 1645 (amide CO) cm^{-1}, τ ($CDCl_3$) 8·88 (3H, t, J 7·3 Hz, $CH_3{\cdot}CH_2$), 8·04 (3H, s, $CH_3{\cdot}CO$), 7·60 (2H, t, J 6·5 Hz, $CH_2{\cdot}CO$), 6·77 (2H, m, $J_{CH_2{\cdot}CH_2}$ 7·3, $J_{NH{\cdot}CH_2}$ 5·8 Hz, $CH_3{\cdot}CH_2$), 6·54 (2H, q, $J_{CH_2{\cdot}CH_2} = J_{NH{\cdot}CH_2} = 6·5$ Hz, $NH{\cdot}CH_2{\cdot}CH_2$), and 3·76br and 3·32br (2H, NH), *m/e* 158 (M^+). Unambiguous synthesis of the compound was carried out by hydrogenolysis of *N*-(*N*-benzyloxycarbonyl-β-alanyl)ethylamine in the presence of acetic anhydride.

Trimethylsilylation of N-*Benzoylglycylglycine Methyl Ester* (VI).—The dipeptide ester [15] (VI) (0·5 g, 0·002 mol) in chloroform (10 ml) and toluene (5 ml) was warmed slightly to dissolve the ester. Triethylamine (5 ml) was added to the mixture in a stoppered flask, followed by chloro(trimethyl)silane, giving an exothermic reaction. After 5 min the solvent and the excess of reagents were evaporated off. The residue was triturated several times with toluene (10 ml portions) and the precipitated triethylamine hydrochloride

[12] A. W. Titherley, *J. Chem. Soc.*, 1901, 401.
[13] J. D. Park, R. D. Englert, and J. S. Meek, *J. Amer. Chem. Soc.*, 1952, **74**, 1010.
[14] M. Bergmann and L. Zervas, *Ber.*, 1932, **65**, 1192.
[15] E. Fischer, *Ber.*, 1905, **1**, 605.

was removed; evaporation of the filtrate *in vacuo* yielded the trimethylsilylated product (VII) as a viscous gum (0·76 g, 82%) which could not be purified further owing to its high reactivity; $\nu_{max.}$ 1760 (ester CO) and 1660br cm^{-1} (C=N and C=C bonds or *N*-silylated amide bonds), τ (CCl_4 with CH_2Cl_2 internal standard) 9·82 and 9·6 (m) (27H, $SiMe_3$), 6·18 (3H, s, CH_3·O), 5·43 (2H, s, N·C*H*$_2$·CO_2Me), 3·70 (1H, s, =N·C*H*=C), and 2·56 (5H, m, Ar*H*), *m/e* 466 (M^+, indicating incorporation of three trimethylsilyl groups).

Acylation of the Trimethylsilylated Derivative (VII).—(a) *With acetyl chloride.* Acetyl chloride (0·4 g, 0·005 mol) was added to the silylated derivative (VII) (1·0 g, 0·002 mol) in toluene (10 ml) and the mixture was shaken for 3 h at room temperature. T.l.c. [system (A)] revealed two products, R_F 0·22 and 0·48. A solution of the mixture in chloroform was extracted with aqueous sodium hydrogen carbonate and evaporated; the residue was chromatographed on a silica gel (60—120 mesh) column with ethyl acetate–benzene (1 : 1) as eluant. Fractions containing the product of R_F 0·48 gave NN′-*diacetyl*-N-*benzoylglycylglycine methyl ester* (IX) as a pale yellow gum (0·084 g, 12%) (Found: M^+, 334·1165 ± 0·0016. $C_{16}H_{18}N_2O_6$ requires *M*, 334·1165), $\nu_{max.}$ 1750 (ester CO) and 1705 cm^{-1} (imide CO). Fractions containing the product of R_F 0·22 gave N-*acetyl*-N-(N-*benzoylglycyl*)*glycine methyl ester* (VIII) (0·46 g, 75%), m.p. 95—97° (from benzene) (Found: C, 57·6; H, 6·1; N, 9·7. $C_{14}H_{16}N_2O_5$ requires C, 57·5; H, 5·5; N, 9·6%), $\nu_{max.}$ 3320 (NH), 1740 (ester CO), 1705 and 1670 (imide CO), and 1650 cm^{-1} (amide), τ ($CDCl_3$) 7·72 (3H, s, CH_3·CO), 6·25 (3H, s, CH_3·O), 5·55 [2H, s, (CO)$_2$N·C*H*$_2$], 5·39 (2H, d, *J* 4·5 Hz, NH·C*H*$_2$·CO), 3·0br (1H, NH), and 2·4 (5H, m, Ar*H*).

The ester (VIII) (0·10 g) in absolute methanol (3 ml) was heated under reflux for 10 h. Isolation of the products by preparative t.l.c. [system (A)] showed that methanolysis yielded *N*-acetylglycine methyl ester, *N*-benzoylglycine methyl ester, and *N*-benzoylglycylglycine methyl ester, all identical (spectra and mixed m.p.) with authentic materials.

(b) *With* N-*protected aminoacyl chlorides* (*general method*). Freshly prepared acyl chloride (0·007 mol) in toluene (5 ml) was added to the silylated derivative (VII) (0·005 mol) in toluene (25 ml) at 0°. The mixture was stirred at 0° for 22 h and at room temperature for 4 h. Addition of chloroform (100 ml; ethanol-free), extraction of the solution successively with aqueous sodium hydrogen carbonate, dilute hydrochloric acid, and water, drying, and evaporation gave a pale yellow gum. In all cases t.l.c. indicated that the major components in the product mixture were unchanged starting material and the corresponding *N*-acylated product. Separation was carried out on a silica gel column by gradient elution with benzene–acetone mixtures.

The following *N*-acylated peptide derivatives were characterised (in general they decomposed readily and it was not possible in all cases to achieve sufficient purity for accurate elemental analyses): N-(N-*benzoylglycyl*)-N-(N-*benzyloxycarbonylglycyl*)*glycine methyl ester* (X) (20%), a yellow gum, $\nu_{max.}$ (film) 3330 (NH) and 1770—1660br cm^{-1} (imide, amide, urethane, and ester CO), *m/e* 441 (M^+); N-(N-*benzoylglycyl*)-N-(N-*benzyloxycarbonyl*-β-*alanyl*)*glycine methyl ester* (XI) (25%), a pale yellow gum (Found: M^+, 455·1685 ± 0·0023. $C_{23}H_{25}N_3O_7$ requires *M*, 455·1692), $\nu_{max.}$ ($CHCl_3$) 3430 (NH), 1750 (ester CO), 1730—1700 (imide and urethane CO), and 1670 cm^{-1} (amide CO); N-(N-*benzoylglycyl*)-N-(N-*benzyloxycarbonyl*-L-*alanyl*)*glycine methyl ester* (XII) (15%), a gum, $\nu_{max.}$ (film) 3320 (NH), 1750 (ester CO), 1740—1690 (imide and urethane CO), and 1660 cm^{-1} (amide CO), *m/e* 455 (M^+); N-(O-*acetyl*-N-*benzyloxycarbonyl*-L-*tyrosyl*)-N-(N-*benzoylglycyl*)*glycine methyl ester* (XIII) (45% from crystalline *O*-acetyl-*N*-benzyloxycarbonyl-L-tyrosine chloride [16]), $\nu_{max.}$ (film) 3300 (NH), 1760 (ester CO), 1715br (imide and urethane CO), and 1665 cm^{-1} (amide CO), *m/e* 589 (M^+); N-(N-*benzoylglycyl*)-N-(N-*phthaloylglycyl*)*glycine methyl ester* (XIV) (75%), a crystalline solid, m.p. 176—178° (from toluene) (Found: C, 60·4; H, 4·5; N, 9·6. $C_{22}H_{19}N_3O_7$ requires C, 60·4; H, 4·4; N, 9·6%), $\nu_{max.}$ 3270 (NH), 1770 (cyclic imide CO), 1755 (ester CO), 1715 (cyclic and acyclic imide), and 1655 cm^{-1} (amide CO), τ [$CDCl_3$–$(CD_3)_2SO$] 6·28 (3H, s, CH_3·O), 5·5 (2H, d, *J* 5·5 Hz, NH·C*H*$_2$·CO), 5·37 [2H, s, (CO)$_2$·N·C*H*$_2$·CO_2Me], 5·10 [2H, s, (CO)$_2$N·C*H*$_2$·CO·N], 2·54 and 2·20 (9H, m, ArH), and 1·38 (1H, t, *J* 5·5 Hz, NH), *m/e* 437 (M^+).

Hydrogenolysis of N-Acyl Compounds (X)—(XIII).—Each *N*-acyl compound (0·0005 mol) in toluene (10 ml) and triethylamine (60 ml) was hydrogenolysed for 48 h over Pd–C (10%; 0·01 g). After removal of the catalyst the mixture was concentrated and chromatographed on silica gel, with benzene–acetone (1 : 1) and benzene–acetone–methanol mixtures for elution.

Compound (X) gave N-*benzoylglycylglycylglycine methyl ester* (XV) (65%), m.p. 176—178° (from aqueous methanol) (Found: C, 54·6; H, 5·9; N, 13·8. $C_{14}H_{17}N_3O_5$ requires C, 54·7; H, 5·6; N, 13·7%), $\nu_{max.}$ 3300 (NH), 1740 (ester CO), and 1655—1635 cm^{-1} (amide CO), τ [$(CF_3)_2CO$–D_2O (2 : 1)] 6·21 (3H, s, CH_3·O), 5·85 (6H, d, NH·C*H*$_2$·CO), 4·81br (HOD), and 2·38 (5H, m, ArH), identical with material synthesised from *N*-benzoylglycylglycylglycine [17] and diazomethane. Compound (XI) gave N-*benzoylglycyl*-β-*alanylglycine methyl ester* (XVI) (71%), m.p. 158—160° (from aqueous methanol) (Found: C, 56·1; H, 6·1; N, 13·2. $C_{15}H_{19}N_3O_5$ requires C, 56·1; H, 6·0; N, 13·1%), $\nu_{max.}$ 3300 (NH), 1745 (ester CO), and 1645 cm^{-1} (amide CO); amino-acid analysis: β-Ala : Gly, 0·89 : 1·92 mol. The compound was identical with that synthesised from *N*-benzoylglycine and β-alanylglycine methyl ester [dicyclohexylcarbodi-imide (DCCI) as coupling reagent].

Compound (XII) gave N-*benzoylglycyl*-L-*alanylglycine methyl ester* (XVII) (65%), m.p. 161—163°, $[\alpha]_D^{24}$ −44° (*c* 0·05 in MeOH) (Found: C, 56·1; H, 5·5; N, 12·6. $C_{15}H_{19}N_3O_5$ requires C, 56·1; H, 6·0; N, 13·1%), $\nu_{max.}$ 3340 (NH), 1745 (ester CO), and 1660 cm^{-1} (amide CO), τ (CF_3·CO_2H) 8·43 (3H, d, *J* 6·5 Hz, C*H*$_3$·CH), 6·10 (3H, s, CH_3·O), 5·75 (2H, d, *J* 6·0 Hz, NH·C*H*$_2$·CO_2), 5·51br (2H, NH·C*H*$_2$·CO), 5·20 (1H, m, C*H*Me), 2·3 (5H, m, Ar*H*), and 2·06br and 1·90br (2H, NH); amino-acid analysis; Ala : Gly, 0·94 : 1·88 mol. Compound (XVII) synthesised from *N*-benzoylglycine and deprotected benzyloxycarbonyl-L-alanylglycine methyl ester [18] (with DCCI) had $[\alpha]_D^{24}$ −44·5° (*c* 0·16 in MeOH) and was identical with the sample just described.

Compound (XIII) rearranged to N-*benzoylglycyl*-L-O-*acetyltyrosylglycine methyl ester* (XVIII) (65%), m.p. 196—197°, $[\alpha]_D^{20}$ −4·95° (*c* 0·45 in AcOH) (Found: C, 60·3; H,

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5·2; N, 9·1. $C_{23}H_{25}N_3O_7$ requires C, 60·7; H, 5·5; N, 9·2%), $\nu_{max.}$ 3380 and 3280 (NH), 1760 and 1740 (ester CO), and 1650 cm^{-1} (amide CO), τ ($CDCl_3$–CF_3·CO_2H) 7·69 (3H, s, CH_3·CO), 6·95 (2H, m, ArCH_2), 6·26 (3H, s, CH_3·O), 6·06—5·96 (4H, m, NH·CH_2·CO), 5·16 [1H, q, NH·C*H*-(CH_2Ar)·CO], and 3·15—2·2 (9H, m, Ar*H*), identical (rotation, mixed m.p.) with a sample synthesised by coupling *N*-benzoylglycine with L-*O*-acetyltyrosylglycine methyl ester (DCCI). The dipeptide methyl ester in this case was obtained from benzyloxycarbonyl-L-*O*-acetyltyrosine and glycine methyl ester by coupling with DCCI.

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