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Synthesis of ω-Amino Acid Peptides Related to Leucine-Enkephalin

Frederick H. C. Stewart

Division of Protein Chemistry, CSIRO,
Parkville, Vic. 3052.

Abstract

Syntheses are described of four peptides with modified leucine-enkephalin sequences in which the native glycylglycyl segment is replaced by an ω-amino acid residue. *o*-Nitrophenylthio-ω-amino acids were used as intermediates, and it was found that the derivatives of 4-aminobutyric and 5-aminovaleric acids undergo facile intramolecular cyclization under the influence of *N,N'*-dicyclohexylcarbodiimide.

Introduction

Since the isolation of the endogenous opiate agonists leucine- and methionine-enkephalin Tyr-Gly-Gly-Phe-(Leu,Met)-OH from brain tissue,[1] numerous analogues of these interesting pentapeptides have been synthesized.[2–4] In order to obtain peptides with enhanced resistance to enzymic degradation a recent approach involves replacement of peptide bonds by sterically similar but more stable hydrocarbon systems such as CH_2CH_2[5] and *trans* CH=CH.[6,7] Several of these isosteric analogues have been reported, including a methionine-enkephalin amide with the Gly-Gly unit replaced by a 5-aminovaleric acid residue.[5] Syntheses of some peptides similarly related to leucine-enkephalin, and represented by the general formula

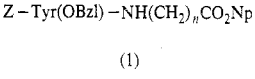
$$\text{Tyr-NH(CH}_2)_{2-5}\text{CO-Phe-Leu-OH}$$

are described in the present paper.

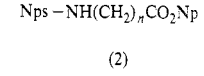
[1] Hughes, J., Smith, T. W., Kosterlitz, H. W., Fothergill, L. A., Morgan, B. A., and Morris, H. R., *Nature (London)*, 1975, **258**, 577.
[2] Terenius, L., Wahlström, A., Lindeberg, G., Karlsson, S., and Ragnarsson, U., *Biochem. Biophys. Res. Commun.*, 1976, **71**, 175.
[3] Agarwal, N. S., Hruby, V. J., Katz, R., Klee, W., and Nirenberg, M., *Biochem. Biophys. Res. Commun.*, 1977, **76**, 129.
[4] Ling, N., Minick, S., Lazarus, L., Rivier, J., and Guillemin, R., *Proc. Fifth Am. Pept. Symp.*, 1977, 96.
[5] Hudson, D., Kenner, G. W., Sharpe, R., and Szelke, M., *Int. J. Pept. Protein Res.*, 1979, **14**, 177.
[6] Hann, M. M., Sammes, P. G., Kennewell, P. D., and Taylor, J. B., *J. Chem. Soc., Chem. Commun.*, 1980, 234.
[7] Cox, M. T., Gormley, J. J., Hayward, C. F., and Petter, N. N., *J. Chem. Soc., Chem. Commun.*, 1980, 800.

Discussion

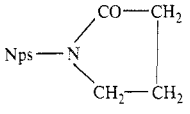
The scheme adopted initially for synthesis of all members of the projected series of ω-amino acid peptides was to prepare the corresponding protected intermediates Z-Tyr(OBzl)-NH$(CH_2)_{2-5}$CO-Phe-Leu-OBzl* by active ester coupling from a homologous set of *p*-nitrophenyl esters (1; $n = 2$–5) and the known dipeptide ester hydrochloride Phe-Leu-OBzl,HCl.[8] Successful application of this approach is illustrated in the case of the β-alanine derivative (1; $n = 2$), which was readily obtained by mixed carbonic anhydride coupling from Z-Tyr(OBzl)-OH and β-Ala-ONp,HBr according to the Goodman–Stueben 'backing-off' procedure.[9] Subsequent active ester coupling with Phe-Leu-OBzl,HCl gave the crystalline protected tetrapeptide Z-Tyr(OBzl)-β-Ala-Phe-Leu-OBzl in high yield.



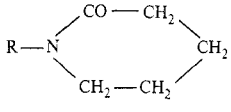
(1)



(2)



(3)



(4)

The β-Ala-ONp,HBr used in this synthesis was conveniently available by the action of hydrogen bromide in acetic acid on the benzyloxycarbonyl derivative Z-β-Ala-ONp.[10,11] For the other homologues (1; $n = 3$–5), however, it was expected that suitable amino acid active ester precursors would be easily obtained from the *p*-nitrophenyl esters (2; $n = 3$–5) of the corresponding *o*-nitrophenylthioamino acids by selective cleavage of the acid-labile amino-protecting group. This has recently proved to be an expeditious route to such compounds.[8,12]

The necessary *o*-nitrophenylthioamino acids were obtained by the standard preparative procedure.[13] Attempted conversion of the protected 4-aminobutyric and 5-aminovaleric acids into *p*-nitrophenyl esters (2; $n = 3$ and 4) by the usual *N,N'*-dicyclohexylcarbodiimide method,[14] however, resulted in facile intramolecular cyclization with formation of the pyrrolidinone (3) and piperidinone (4; R = Nps), respectively. This failure of the protective capacity of *o*-nitrophenylthio under typically mild peptide coupling conditions is paralleled by similar behaviour of the corresponding benzoyl derivatives in much more rigorous circumstances (boiling acetic anhydride) reported many years ago.[15,16] In contrast to the marked tendency

* Abbreviations used are: Z, benzyloxycarbonyl; Nps, *o*-nitrophenylthio; Pht, phthaloyl; Ts, *p*-toluenesulfonyl; Bzl, benzyl; Np, *p*-nitrophenyl; Su, 1-succinimidyl.

[8] Stewart, F. H. C., *Aust. J. Chem.*, 1980, **33**, 121.
[9] Goodman, M., and Stueben, K. C., *J. Am. Chem. Soc.*, 1959, **81**, 3980.
[10] Manning, M., and du Vigneaud, V., *Biochemistry*, 1965, **4**, 1884.
[11] Stewart, F. H. C., *Aust. J. Chem.*, 1971, **24**, 2193.
[12] Stewart, F. H. C., *Aust. J. Chem.*, 1981, **34**, 2431.
[13] Zervas, L., Borovas, D., and Gazis, E., *J. Am. Chem. Soc.*, 1963, **85**, 3660.
[14] Bodanszky, M., and du Vigneaud, V., *Biochem. Prep.*, 1962, **9**, 110.
[15] Schotten, C., *Ber. Dtsch. Chem. Ges.*, 1888, **21**, 2239.
[16] Kanewskaja, S. J., *Ber. Dtsch. Chem. Ges.*, 1936, **69**, 266.

of these compounds to cyclize, the higher homologue from 6-aminohexanoic acid formed the *p*-nitrophenyl ester (2; $n = 5$) normally, and it was also established that this was the case for the β-alanine derivative (2; $n = 2$).

Synthesis of a suitable protected enkephalin sequence containing 4-aminobutyric acid was approached instead by way of the phthaloylamino acid Pht>$N(CH_2)_3CO_2H$. Cyclization is prevented here by the prior displacement of both amino protons by the protecting group, and the compound is known to form active esters without difficulty by the *N,N'*-dicyclohexylcarbodiimide method.[17,18] Accordingly, the previously described 1-succinimidyl ester Pht>$N(CH_2)_3CO_2Su$[18] was coupled with Phe-Leu-OTmb,HOTs[12] to give the protected tripeptide Pht>$N(CH_2)_3$CO-Phe-Leu-OTmb. Acidic cleavage of the 2,4,6-trimethylbenzyl ester group with trifluoroacetic acid[19] produced Pht>$N(CH_2)_3$CO-Phe-Leu-OH. The same intermediate was obtained alternatively by catalytic hydrogenation of Pht>$N(CH_2)_3$CO-Phe-Leu-OBzl (from Phe-Leu-OBzl,HCl [8]). Treatment with aqueous methylamine[20] gave the free peptide $NH_2(CH_2)_3$CO-Phe-Leu-OH, which was homogeneous by electrophoresis and thin-layer chromatography. In terms of ease of isolation of the product, this depthaloylation method was superior to the more frequently cited hydrazinolysis. Finally, active ester coupling with Z-Tyr(OBzl)-OSu in dimethylformamide in the presence of triethylamine gave the protected tetrapeptide Z-Tyr(OBzl)-$NH(CH_2)_3$CO-Phe-Leu-OH.

A different route was used for the preparation of a protected intermediate incorporating 5-aminovaleric acid. The *o*-nitrophenylthioamino acid was converted into the 2,4,6-trimethylbenzyl ester Nps-$NH(CH_2)_4CO_2$Tmb by condensation with 2,4,6-trimethylbenzyl chloride and triethylamine in dimethylformamide.[8,21] Selective removal of the amino-protecting group with hydrogen chloride in methanol[13,21] gave the hydrochloride $NH_2(CH_2)_4CO_2$Tmb,HCl. This compound, or the corresponding *p*-toluenesulfonate prepared from it by metathesis, was subjected to active ester coupling with Z-Tyr(OBzl)-ONp [22] to give the protected dipeptide Z-Tyr(OBzl)-$NH(CH_2)_4CO_2$Tmb. Acidolysis of the ester group with trifluoroacetic acid[19] produced Z-Tyr(OBzl)-$NH(CH_2)_4CO_2H$. Attempted direct coupling of this compound with Phe-Leu-OBzl by the *N,N'*-dicyclohexylcarbodiimide/benzotriazol-1-ol procedure,[23] usually very successful for joining peptide fragments, proved to be unsatisfactory, and crystalline Z-Tyr(OBzl)-$NH(CH_2)_4$CO-Phe-Leu-OBzl was isolated in poor yield only. This result could be partly due to cyclization of the type observed with 5-(*o*-nitrophenylthio)aminovaleric acid. Treatment of Z-Tyr(OBzl)-$NH(CH_2)_4CO_2H$ with *N,N'*-dicyclohexylcarbodiimide gave the corresponding *N*-acyl-*N,N'*-dicyclohexylurea as the major product (*c.* 45% yield), but an appreciable amount of a more sparingly soluble *N*-substituted piperidinone [4; R = Z-Tyr(OBzl)] was also formed (*c.* 19%). It is evident that use of *N,N'*-dicyclohexylcarbodiimide with this protected dipeptide, as in attempted preparation of active esters such as (1; $n = 4$), should be avoided. Pure crystalline (1; $n = 4$) was readily obtained in

[17] Bailin, G., and Lukton, A., *J. Org. Chem.*, 1962, **27**, 684.
[18] Stewart, F. H. C., *Aust. J. Chem.*, 1978, **31**, 1861.
[19] Stewart, F. H. C., *Aust. J. Chem.*, 1966, **19**, 1511.
[20] Wolfe, S., and Hasan, S. K., *Can. J. Chem.*, 1970, **48**, 3572.
[21] Stewart, F. H. C., *Aust. J. Chem.*, 1967, **20**, 365.
[22] Bodanszky, M., and du Vigneaud, V., *J. Am. Chem. Soc.*, 1959, **81**, 5688.
[23] König, W., and Geiger, R., *Chem. Ber.*, 1973, **106**, 3626.

good yield, however, by the less commonly employed bis-*p*-nitrophenyl sulfite exchange method,[24] and active ester coupling with Phe-Leu-OBzl, as in the original plan, produced Z-Tyr(OBzl)-NH$(CH_2)_4$CO-Phe-Leu-OBzl without contaminating by-products.

In the case of 6-(*o*-nitrophenylthio)aminohexanoic acid *p*-nitrophenyl ester (2; $n = 5$), the amino-protecting group was cleaved with hydrogen chloride in methanol to give the hydrochloride $NH_2(CH_2)_5CO_2Np$,HCl, which served as the precursor of (1; $n = 5$), and hence Z-Tyr(OBzl)-NH$(CH_2)_4$CO-Phe-Leu-OBzl, exactly as for the β-alanine homologue already mentioned.

The four protected tetrapeptides were hydrogenated in methanol over 10% palladium on charcoal catalyst to form free peptides Tyr-NH$(CH_2)_{2-5}$CO-Phe-Leu-OH. Amino acid analysis of acid hydrolysates gave the correct ratio of constituents for each peptide. Electrophoresis and thin-layer chromatography indicated that the products were homogeneous except for a trace of fast-running ninhydrin-positive contaminant in the 4-aminobutyric acid peptide. The possibility was considered that slight loss of the amino-terminal residue in $NH_2(CH_2)_3$CO-Phe-Leu-OH might have occurred by peptide bond cleavage assisted by intramolecular lactamization under the mildly basic conditions used in the coupling with Z-Tyr(OBzl)-OSu. Such a reaction has been demonstrated with a model dipeptide 4-aminobutyrylglycine which gave pyrrolidin-2-one and glycine.[25] The protected tripeptide Z-Tyr(OBzl)-Phe-Leu-OH formed after excision of 4-aminobutyric acid in this way would, if present as an impurity during the hydrogenation, lead to Tyr-Phe-Leu-OH, which was synthesized for comparison, but did not correspond to the observed trace spot on thin-layer chromatography.

Experimental

Microanalyses were carried out by the Australian Microanalytical Service, Melbourne. Melting points are uncorrected. Infrared spectra were obtained with KBr discs. ^{1}H n.m.r. spectra were run in ($^2H_6$)dimethyl sulfoxide with a Varian A-60D spectrometer. Thin-layer chromatography was performed in the solvent systems (A) butan-1-ol/acetic acid/water (3:1:1) and (B) propan-1-ol/25% ammonium hydroxide (2:1).

(*a*) o-*Nitrophenylthio ω-Amino Acids*

The standard preparative method described by Zervas, Borovas and Gazis[13] was used.

(i) *Nps-β-Ala-OH* formed an oil in quantitative yield. The product failed to crystallize, and was characterized as the *dicyclohexylammonium salt*, which was recrystallized from methanol, m.p. 170·5–172·5° (Found: C, 59·7; H, 7·8; N, 9·7. $C_{21}H_{33}N_3O_4S$ requires C, 59·6; H, 7·8; N, 9·9%).

(ii) *Nps-NH$(CH_2)_3CO_2H$* (93% yield) was recrystallized from ethyl acetate/cyclohexane, m.p. 92·5–94° (Found: C, 46·9; H, 4·8; N, 11·0. $C_{10}H_{12}N_2O_4S$ requires C, 46·9; H, 4·7; N, 10·9%).

(iii) *Nps-NH$(CH_2)_4CO_2H$* (92% yield) was an amorphous solid, m.p. 81–82° (Found: C, 48·7; H, 5·5; N, 10·1; S, 12·0. $C_{11}H_{14}N_2O_4S$ requires C, 48·9; H, 5·2; N, 10·4; S, 11·9%).

(iv) *Nps-NH$(CH_2)_5CO_2H$* (93% yield) was recrystallized from ethyl acetate/cyclohexane, m.p. 131–132° (Found: C, 50·7; H, 5·8; N, 9·6. $C_{12}H_{16}N_2O_4S$ requires C, 50·7; H, 5·6; N, 9·9%).

(*b*) p-*Nitrophenyl Esters of* o-*Nitrophenylthio ω-Amino Acids*

These compounds were prepared in ethyl acetate solution by the normal *N,N'*-dicyclohexylcarbodiimide procedure.[14]

[24] Iselin, B., Rittel, W., Sieber, P., and Schwyzer, R., *Helv. Chim. Acta*, 1957, **40**, 373.
[25] Poduška, K., Katrukha, G. S., Silaev, A. B., and Rudinger, J., *Collect. Czech. Chem. Commun.*, 1965, **30**, 2410.

(i) *Nps-β-Ala-ONp* (2; $n = 2$) was obtained as an oil which was repeatedly treated with cold ethyl acetate to remove *N,N'*-dicyclohexylurea, and then crystallized on trituration with ethanol (85% yield), m.p. 63–65° (Found: C, 49·5; H, 3·7; N, 11·4; S, 9·0. $C_{15}H_{13}N_3O_6S$ requires C, 49·6; H, 3·6; N, 11·6; S, 8·8%). ^{1}H n.m.r. δ 7·45, 8·33, AA'BB' pattern, J 9 Hz, Np arom. H.

(ii) *Nps-NH(CH$_2$)$_5$CO$_2$Np* (90% yield) was recrystallized from ethanol, m.p. 84–85° (Found: C, 53·1; H, 4·9; N, 10·3. $C_{18}H_{19}N_3O_6S$ requires C, 53·3; H, 4·7; N, 10·4%). ^{1}H n.m.r. δ 7·42, 8·29, AA'BB' pattern, J 9 Hz, Np arom. H.

(*c*) *Action of* N,N'-*Dicyclohexylcarbodiimide on the* o-*Nitrophenylthio Derivatives of 4-Aminobutyric and 5-Aminovaleric Acids*

Attempted preparation of *p*-nitrophenyl esters as in (*b*) resulted in intramolecular cyclization with the *p*-nitrophenol remaining unchanged as indicated by ^{1}H n.m.r. spectroscopy. For preparation and characterization of the cyclization products the *p*-nitrophenol was omitted from the reaction mixture, and the usual work-up procedure followed.

(i) *1*-o-*Nitrophenylthiopyrrolidin-2-one* (3) (86% yield) was recrystallized from ethanol, m.p. 138–141° (Found: C, 50·6; H, 4·9; N, 12·1; S, 13·7. $C_{10}H_{10}N_2O_3S$ requires C, 50·4; H, 4·2; N, 12·1; S, 13·4%). M^+ 238. ν_{max} 1710 cm^{-1} (amide I). ^{1}H n.m.r. δ 7·20–8·40, m, 4H, Nps arom. H; 3·65, t, J 7 Hz, 2H, 3-CH$_2$; 2·00–2·70, m, 4H, 4,5-CH$_2$CH$_2$.

(ii) *1*-o-*Nitrophenylthiopiperidin-2-one* (4; R = Nps) (91% yield) was recrystallized from ethyl acetate/light petroleum, m.p. 148–151° (Found: C, 52·6; H, 5·2; N, 10·9; S, 12·6. $C_{11}H_{12}N_2O_3S$ requires C, 52·4; H, 4·8; N, 11·1; S, 12·7%). M^+ 252. ν_{max} 1665 cm^{-1} (amide I). ^{1}H n.m.r. δ 7·19–8·30, m, Nps arom. H; 3·55–3·76, m, 2H, 3-CH$_2$; 2·43–2·67, m, 2H, 6-CH$_2$; 1·80–2·00, m, 4H, 4,5-CH$_2$CH$_2$.

(*d*) *β-Alanine* p-*Nitrophenyl Ester Hydrobromide*

Benzyloxycarbonyl-β-alanine *p*-nitrophenyl ester[10,11] (1·0 g) was treated with 2 M hydrogen bromide in acetic acid (10 ml) for 1 h at room temperature. Ether was added, and the *hydrobromide* (739 mg, 87%) recrystallized from methanol/ethyl acetate, m.p. 210–211·5° (Found: C, 37·2; H, 3·9; Br, 27·5; N, 9·7. $C_9H_{11}BrN_2O_4$ requires C, 37·1; H, 3·8; Br, 27·5; N, 9·6%). ν_{max} 1750 cm^{-1} (Np ester CO). ^{1}H n.m.r. δ 7·53, 8·33, AA'BB' pattern, J 9 Hz, Np arom. H.

(*e*) *Benzyloxycarbonyl*-O-*benzyl*-L-*tyrosyl-β-alanine* p-*Nitrophenyl Ester*

The mixed carbonic anhydride from Z-Tyr(OBzl)-OH (405 mg) and isobutyl chloroformate (0·14 ml) was prepared in acetonitrile (5 ml) containing *N*-methylmorpholine[26] (0·12 ml) at −15° for 10 min. β-Ala-ONp,HBr (291 mg) was added, and then *N*-methylmorpholine (0·12 ml) in acetonitrile (1·0 ml) whereupon rapid solidification ensued. After 2–3 h the mixture was diluted with water, and the product washed with water and ether (567 mg, 95%). The *compound* was recrystallized from methanol, m.p. 148·5–149·5° (Found: C, 66·0; H, 5·2; N, 6·9. $C_{33}H_{31}N_3O_8$ requires C, 66·3; H, 5·2; N, 7·0%). $[\alpha]_D^{19}$ −12·6° (*c*, 0·5 in HCONMe$_2$). ν_{max} 1755 (Np ester CO), 1690 (Z CO), 1655 cm^{-1} (amide I). ^{1}H n.m.r. δ 7·46, 8·30, AA'BB' pattern, J 9 Hz, Np arom. H; 7·40, s, Z arom. H; 7·29, s, Bzl arom. H; 7·05, AA'BB' pattern, J 8·5 Hz, Tyr arom. H; 4·96, 5·06, s, Z and Bzl CH$_2$.

(*f*) *Benzyloxycarbonyl*-O-*benzyl*-L-*tyrosyl-β-alanyl*-L-*phenylalanyl*-L-*leucine Benzyl Ester*

Active ester coupling with Z-Tyr(OBzl)-β-Ala-ONp from (*e*) and Phe-Leu-OBzl, HCl[8] in dimethylformamide (0·25 M) in the presence of triethylamine (1 equiv.) for 2–3 days at room temperature, and dilution with water, gave the protected tetrapeptide in 98% yield. The *compound* was recrystallized from ethanol, m.p. 208–209° (Found: C, 71·0; H, 6·5; N, 6·8. $C_{49}H_{54}N_4O_8$ requires C, 71·2; H, 6·5; N, 6·8%). $[\alpha]_D^{20}$ −20·8° (*c*, 0·5 in HCONMe$_2$). ν_{max} 1730 (ester CO), 1690 (Z CO), 1640 cm^{-1} (amide I). ^{1}H n.m.r. δ 7·38, s, Z arom. H; 7·35, s, Bzl ester arom. H; 7·27, s, Bzl ether arom. H; 7·20, s, Phe arom. H; 7·03, AA'BB' pattern, J 8·5 Hz, Tyr arom. H; 5·12, s, Bzl ester CH$_2$; 4·94, 5·04, s, Z and Bzl ether CH$_2$; 0·75–0·93, m, Leu CH$_3$.

[26] Anderson, G. W., Zimmerman, J. E., and Callahan, F. M., *J. Am. Chem. Soc.*, 1967, **89**, 5012.

(*g*) *4-(Phthaloyl)aminobutyryl-L-phenylalanyl-L-leucine 2,4,6-Trimethylbenzyl Ester*

The *compound* was obtained in quantitative yield from Pht>$N(CH_2)_3CO_2Su$ [18] and Phe-Leu-OTmb,HOTs [12] as in (*f*), and recrystallized from methanol, m.p. 192–193° (Found: C, 71·2; H, 7·0; N, 6·9. $C_{37}H_{43}N_3O_6$ requires C, 71·0; H, 6·9; N, 6·7%). $[\alpha]_D^{20}$ −15·2° (*c*, 0·5 in $HCONMe_2$). ν_{max} 1770 (Pht CO), 1720 (combined Pht and ester CO), 1640 cm^{-1} (amide I). ^1H n.m.r. δ 7·86, s, Pht arom. H; 7·19, s, Phe arom. H; 6·84, s, Tmb arom. H; 5·11, s, Tmb CH_2; 2·16, 2·27, s, Tmb CH_3; 0·76–0·92, m, Leu CH_3.

(*h*) *4-(Phthaloyl)aminobutyryl-L-phenylalanyl-L-leucine Benzyl Ester*

Prepared in 97% yield from Phe-Leu-OBzl,HCl [8] as in (*g*). The *protected peptide* was recrystallized from ethanol, m.p. 146–148° (Found: C, 70·5; H, 6·6; N, 7·4. $C_{34}H_{37}N_3O_6$ requires C, 70·0; H, 6·3; N, 7·2%). $[\alpha]_D^{20·5}$ −14·4° (*c*, 0·5 in $HCONMe_2$). ν_{max} 1770 (Pht CO), 1715 (combined Pht and ester CO), 1640 cm^{-1} (amide I). ^1H n.m.r. δ 7·86, s, Pht arom. H; 7·36, s, Bzl arom. H; 7·22, s, Phe arom. H; 5·13, s, Bzl CH_2; 0·78–0·95, m, Leu CH_3.

(*i*) *4-(Phthaloyl)aminobutyryl-L-phenylalanyl-L-leucine*

(i) A methanol solution of the benzyl ester from (*h*) was hydrogenated over 10% palladium on charcoal (Fluka) for 2 h. Filtration and evaporation gave the *product*, which was triturated with ether (91% yield), and recrystallized from ethyl acetate, m.p. 128° (Found: C, 65·9; H, 6·5; N, 8·5. $C_{27}H_{31}N_3O_6$ requires C, 65·7; H, 6·3; 8·5%). $[\alpha]_D^{20}$ −11·0° (*c*, 0·5 in $HCONMe_2$).

(ii) The compound was obtained in quantitative yield by cleavage of the ester group in Pht>$N(CH_2)_3CO$-Phe-Leu-OTmb with trifluoroacetic acid in the presence of anisole.[19] Complete reaction was established by ^1H n.m.r. spectroscopy, and the product used directly for the next step (δ 7·85, s, Pht arom. H; 7·21, s, Phe arom. H; 0·78–0·95, m, Leu CH_3).

(*j*) *Benzyloxycarbonyl-O-benzyl-L-tyrosine 1-Succinimidyl Ester*

The *active ester* was obtained in 91% yield from Z-Tyr(OBzl)-OH and 1-hydroxysuccinimide by the *N,N'*-dicyclohexylcarbodiimide method in dioxan solution,[27] and recrystallized from propan-2-ol, m.p. 142° (Found: C, 66·9; H, 5·3; N, 5·6. $C_{28}H_{26}N_2O_7$ requires C, 66·9; H, 5·2; N, 5·6%). $[\alpha]_D^{21}$ −49·2° (*c*, 0·5 in $HCONMe_2$). ν_{max} 1810, 1795, 1750 (Su ester CO), 1695 cm^{-1} (Z CO). ^1H n.m.r. δ 7·39, s, Z arom. H; 7·30, s, Bzl arom. H; 7·10, AA'BB' pattern, *J* 8·5 Hz, Tyr arom. H; 5·03, 5·07, s, Z and Bzl CH_2; 2·84, s, Su CH_2CH_2.

(*k*) *4-(Benzyloxycarbonyl-O-benzyl-L-tyrosyl)aminobutyryl-L-phenylalanyl-L-leucine*

The phthaloyl derivative from (*i*) (736 mg) was dissolved in 40% aqueous methylamine (7·4 ml) and the solution kept for 2 days at room temperature. Evaporation and addition of ethanol and acetic acid (*c*. 0·1 ml) gave the chromatographically homogeneous free tripeptide (440 mg, 76%), m.p. 257·5–258° (Found: C, 62·6; H, 8·3; N, 11·5. $C_{19}H_{29}N_3O_4$ requires C, 62·8; H, 8·0; N, 11·6%). R_F (A) 0·49, (B) 0·41. The amino acid composition of an acid hydrolysate (6 M HCl for 24 h at 110° under vacuum) was $NH_2(CH_2)_3CO_2H_{1·00}Phe_{1·01}Leu_{1·00}$.

A mixture of the tripeptide (121 mg), triethylamine (0·047 ml) and Z-Tyr(OBzl)-OSu (167 mg) in dimethylformamide (3 ml) was stirred for 2–3 days at room temperature, acidified with 0·1 M HCl, and the solid washed with water (246 mg, 98%). The amorphous *protected tetrapeptide* was precipitated from ethanol/ether, m.p. 176–178° (Found: C, 68·5; H, 6·9; N, 7·7. $C_{43}H_{50}N_4O_8$ requires C, 68·8; H, 6·7; N, 7·5%). $[\alpha]_D^{19·5}$ −19·2° (*c*, 0·5 in $HCONMe_2$). ^1H n.m.r. δ 7·41, s, Z arom. H; 7·31, s, Bzl arom. H; 7·25, s, Phe arom. H; 7·05, AA'BB' pattern, *J* 8·5 Hz, Tyr arom. H; 4·98, 5·07, s, Z and Bzl CH_2; 0·78–0·95, m, Leu CH_3.

(*l*) *5-Aminovaleric Acid 2,4,6-Trimethylbenzyl Ester Hydrochloride and* p-*Toluenesulfonate*

5-(*o*-Nitrophenylthio)aminovaleric acid from (*a*) was converted into the 2,4,6-trimethylbenzyl ester by a standard method,[8,21] and the resultant oil treated directly with 1 M hydrogen chloride in methanol.[13] Evaporation and addition of ether gave crude hydrochloride (72% yield), which was

[27] Anderson, G. W., Zimmerman, J. E., and Callahan, F. M., *J. Am. Chem. Soc.*, 1964, **86**, 1839.

not homogeneous by thin-layer chromatography, and was purified by treatment with aqueous sodium bicarbonate and extraction with ether. Addition of methanolic hydrogen chloride to the dried extract gave homogeneous *hydrochloride*, which was recrystallized from ethanol/ether, m.p. 105·5–106·5° (Found: C, 62·2; H, 8·5, Cl, 12·5; N, 4·9. $C_{15}H_{24}ClNO_2$ requires C, 63·0; H, 8·4; Cl, 12·4; N, 4·9%). ν_{max} 1720, 1730 cm^{-1} (ester CO). ^1H n.m.r. δ 6·83, s, Tmb arom. H; 5·08, s, Tmb CH_2; 2·20, 2·27, s, Tmb CH_3.

A solution of the crude hydrochloride in water was treated with concentrated aqueous sodium *p*-toluenesulfonate to give the sparingly soluble *p*-toluenesulfonate in 86% yield. The chromatographically homogeneous *compound* was recrystallized from ethanol, m.p. 165–167° (Found: C, 62·8; H, 7·7; N, 3·5. $C_{22}H_{31}NO_5S$ requires C, 62·7; H, 7·4; N, 3·3%). ^1H n.m.r. δ 7·34, AA′BB′ pattern *J* 8 Hz, Ts arom. H; 6·87, s, Tmb arom. H; 5·12, s, Tmb CH_2; 2·23, 2·29, s, 12H, Tmb and Ts (2·29) CH_3.

(m) 5-(Benzyloxycarbonyl-O-benzyl-L-tyrosyl)aminovaleric Acid 2,4,6-Trimethylbenzyl Ester

The *compound* was obtained in 95% yield by active ester coupling from Z-Tyr(OBzl)-ONp [22] and either the foregoing hydrochloride or *p*-toluenesulfonate in dimethylformamide (0·5 M) in the presence of *N*-methylmorpholine (1 equiv.), and recrystallized from ethanol, m.p. 155–156·5° (Found: C, 73·5; H, 7·0; N, 4·4. $C_{39}H_{44}N_2O_6$ requires C, 73·6; H, 6·9; N, 4·4%). $[\alpha]_D^{19·5}$ −12·2° (*c*, 0·5 in $HCONMe_2$). ν_{max} 1725 (ester CO), 1690 (Z CO), 1650 cm^{-1} (amide I). ^1H n.m.r. δ 7·36, s, Z arom. H; 7·28, s, Bzl arom. H; 7·01, AA′BB′ pattern, *J* 8·5 Hz, Tyr arom. H; 6·82, s, Tmb arom. H; 5·07, s, Tmb CH_2; 4·94, 5·04, s, Z and Bzl CH_2; 2·21, 2·28, s, Tmb CH_3.

(n) 5-(Benzyloxycarbonyl-O-benzyl-L-tyrosyl)aminovaleric Acid

Treatment of the 2,4,6-trimethylbenzyl ester from (*m*) with trifluoroacetic acid in the presence of anisole[19] as in (*i*) gave the product in 91% yield. The *protected dipeptide* was recrystallized from ethyl acetate/ether, m.p. 150–152° (Found: C, 68·8; H, 6·6; N, 5·9. $C_{29}H_{32}N_2O_6$ requires C, 69·0; H, 6·3; N, 5·6%). ν_{max} 1710 (sh; CO_2H), 1690 (Z CO), 1645 cm^{-1} (amide I). ^1H n.m.r. δ 7·36, s, Z arom. H; 7·27, s, Bzl arom. H; 7·01, AA′BB′ pattern, Tyr arom. H; 4·94, 5·03, s, Z and Bzl CH_2.

(o) Action of N,N-*Dicyclohexylcarbodiimide on Z-Tyr(OBzl)-NH(CH₂)₄CO₂H*

A solution of Z-Tyr(OBzl)-$NH(CH_2)_4CO_2H$ (168 mg) in dimethylformamide (1·5 ml) was stirred with *N,N′*-dicyclohexylcarbodiimide (69 mg) for 12 h at room temperature. *N,N′*-Dicyclohexylurea (31 mg, 41%) was separated by filtration and washing with ethyl acetate. The ethyl acetate solution was washed with aqueous sodium bicarbonate, dried and evaporated, and the residue washed with ether and light petroleum (163 mg); ν_{max} 2930, 2860, 1810, 1690 and 1655 cm^{-1}. This material was precipitated from hot ethanol to give the sparingly soluble *piperidin-2-one* [4; R = Z-Tyr(OBzl)] (31 mg, 19%), which was washed with hot methanol, m.p. 168–169° (Found: N, 5·6. $C_{29}H_{30}N_2O_5$ requires N, 5·8%). ν_{max} 2930, 2865 [ring $(CH_2)_4$], 1805 (CONCO system), 1690 (Z CO), 1655 cm^{-1} (amide I). ^1H n.m.r. δ 7·38, s, Z arom. H; 7·27, s, Bzl arom. H; 7·01, AA′BB′ pattern, *J* 8 Hz, Tyr arom. H; 4·94, 5·04, s, Z and Bzl CH_2; 1·30–1·58, m, 4H, piperidinone CH_2CH_2.

Evaporation of the ethanol solution left a residue of N-[*5-(benzyloxycarbonyl-O-benzyl-L-tyrosyl)-aminovaleryl*]-N,N′-*dicyclohexylurea* (107 mg, 45%), which was precipitated from ethanol/light petroleum, m.p. 143–414° (Found: C, 71·0; H, 7·9; N, 7·4. $C_{42}H_{54}N_4O_6$ requires C, 71·0; H, 7·6; N, 7·9%). ν_{max} 2930, 2860 (cyclohexyl), 1690 (Z CO), 1650 cm^{-1} (amide I); no absorption at 1805 cm^{-1}. ^1H n.m.r. δ 7·38, s, Z arom. H; 7·28, s, Bzl arom. H; 7·03, AA′BB′ pattern, Tyr arom. H; 4·94, 5·05, s, Z and Bzl CH_2; 1·00–2·00, m, 24H, 4 aliphatic and 20 alicyclic CH_2.

(p) 5-(Benzyloxycarbonyl-O-benzyl-L-tyrosyl)aminovaleric Acid p-*Nitrophenyl Ester*

A solution of Z-Tyr(OBzl)-$NH(CH_2)_4CO_2H$ (325 mg) in dry pyridine (3 ml) was treated with bis-*p*-nitrophenyl sulfite[24] (418 mg, 2 equiv.) for 2 days at room temperature. Ethyl acetate was added, and the mixture washed with 1 M $NaHCO_3$, water, 1 M HCl and water. Evaporation of the dried extract gave the p-*nitrophenyl ester*, which was recrystallized from methanol twice (300 mg, 74%), m.p. 150–151·5° (Found: C, 67·4; H, 5·8; N, 6·8. $C_{35}H_{35}N_3O_8$ requires C 67·2; H, 5·6; N, 6·7%). $[\alpha]_D^{20}$ −11·2° (*c*, 0·5 in $HCONMe_2$). ν_{max} 1765 (Np ester CO), 1685 (Z CO), 1650 cm^{-1}.

^{1}H n.m.r. δ 7·42, 8·30, AA′BB′ pattern, J 9 Hz, Np arom. H; 7·39, Z arom. H; 7·29, s, Bzl arom. H; 7·05, AA′BB′ pattern, J 8 Hz, Tyr arom. H; 4·97, 5·06, s, Z and Bzl CH_2.

(*q*) *5-(Benzyloxycarbonyl-O-benzyl-L-tyrosyl)aminovaleryl-L-phenylalanyl-L-leucine Benzyl Ester*

(i) Triethylamine (0·07 ml) and benzotriazol-1-ol (68 mg) were added to a suspension of Phe-Leu-OBzl,HCl[8] (203 mg) in acetonitrile (3 ml) at 0°, followed by Z-Tyr(OBzl)-NH$(CH_2)_4CO_2$H (252 mg) and *N,N′*-dicyclohexylcarbodiimide (103 mg). The mixture was stirred for 3–4 h, filtered and extracted into warm ethyl acetate. The washed extract was evaporated to give crude product (352 mg), which was recrystallized from methanol, m.p. 178·5–179·5° (Found: C, 71·6: H, 6·9; N, 6·8. $C_{51}H_{58}N_4O_8$ requires C, 71·7; H, 6·8; N, 6·6%). $[\alpha]_D^{19·5}$ −21·6° (*c*, 0·5 in $HCONMe_2$). ^{1}H n.m.r. δ 7·39, s, Z arom. H; 7·36, s, Bzl ester arom. H; 7·28, s, Bzl ether arom. H; 7·21, s, Phe arom. H; 5·13, s, Bzl ester CH_2; 4·97, 5·07, s, Z and Bzl ether CH_2; 0·78–0·95, m, Leu CH_3.

The yield of pure *protected peptide* was 180 mg (42%). Evaporation of the mother liquor gave material which was evidently heavily contaminated with *N*-acyl-*N,N′*-dicyclohexylurea [cf. (*o*)].

(ii) The compound was also obtained by active ester coupling from Z-Tyr(OBzl)-NH$(CH_2)_4CO_2$Np and Phe-Leu-OBzl,HCl[8] as described in (*f*) (85% yield), and recrystallized from methanol, m.p. 181–182°; $[\alpha]_D^{19·5}$ −20·0° (*c*, 0·4 in $HCONMe_2$). The infrared and ^{1}H n.m.r. spectra of both products were identical.

(*r*) *6-Aminohexanoic Acid* p-*Nitrophenyl Ester Hydrochloride*

The *o*-nitrophenylthio derivative from (*b*) (ii) was treated with 1 M hydrogen chloride in methanol[13] to give the hydrochloride in 76% yield. The *compound* was recrystallized from ethanol/ether, m.p. 113–115° (Found: C, 49·9; H, 6·2; N, 9·6. $C_{12}H_{17}ClN_2O_4$ requires C, 49·9; H, 5·9; N, 9·7%). ν_{max} 1765 cm^{-1} (ester CO). ^{1}H n.m.r. δ 7·47, 8·31, AA′BB′ pattern, J 9 Hz, Np arom. H.

(*s*) *6-(Benzyloxycarbonyl-O-benzyl-L-tyrosyl)aminohexanoic Acid* p-*Nitrophenyl Ester*

The *compound* was obtained in 91% yield from Z-Tyr(OBzl)-OH and $NH_2(CH_2)_5CO_2$Np,HCl by mixed carbonic anhydride coupling exactly as for the β-alanine analogue in (*e*), and recrystallized from methanol, m.p. 154–156° (Found: C, 68·0; H, 5·9; N, 6·7. $C_{36}H_{37}N_3O_8$ requires C, 67·6; H, 5·8; N, 6·6%). $[\alpha]_D^{20·5}$ −12·6° (*c*, 0·5 in $HCONMe_2$). ν_{max} 1755 (Np ester CO), 1690 (Z CO), 1650 cm^{-1} (amide I). ^{1}H n.m.r. δ 7·45, 8·30, AA′BB′ pattern, J 9 Hz, Np arom. H; 7·43, s, Z arom. H; 7·32, s, Bzl arom. H; 7·07, AA′BB′ pattern, J 8·5 Hz, Tyr arom. H; 4·99, 5·08, s, Z and Bzl CH_2.

(*t*) *6-(Benzylcarbonyl-O-benzyl-L-tyrosyl)aminohexanoyl-L-phenylalanyl-L-leucine Benzyl Ester*

The *protected tetrapeptide* was obtained in 94% yield by active ester coupling from Z-Tyr(OBzl)-NH$(CH_2)_5CO_2$Np and Phe-Leu-OBzl,HCl[8] as in (*f*), and recrystallized from methanol, m.p. 167–168° (Found: C, 72·1; H, 7·0; N, 6·6. $C_{52}H_{60}N_4O_8$ requires C, 71·9; H, 6·9; N, 6·5%). $[\alpha]_D^{19}$ −16·8° (*c*, 0·5 in $HCONMe_2$). ν_{max} 1740 (ester CO), 1690 (Z CO), 1640, 1655 cm^{-1} (amide I). ^{1}H n.m.r. δ 7·38, s, Z arom. H; 7·35, s, Bzl ester arom. H; 7·29, s, Bzl ether arom. H; 7·22, s, Phe arom. H; 7·04, AA′BB′ pattern, Tyr arom. H; 5·13, s, Bzl ester CH_2; 4·97, 5·06, s, Z and Bzl ether CH_2; 0·78–0·94, m, Leu CH_3.

(*u*) *Benzyloxycarbonyl-O-benzyl-L-tyrosyl-L-phenylalanyl-L-leucine Benzyl Ester*

Standard active ester coupling with Z-Tyr(OBzl)-ONp[22] and Phe-Leu-OBzl,HCl[8] gave the product in 96% yield. The *protected tripeptide* was recrystallized from ethyl acetate/light petroleum, m.p. 179–180° (Found: C, 73·1; H, 6·6; N, 5·5. $C_{46}H_{49}N_3O_7$ requires C, 73·1; H, 6·5; N, 5·6%). $[\alpha]_D^{20}$ −30·3° (*c*, 0·5 in $HCONMe_2$). ν_{max} 1735 (ester CO), 1690 (Z CO), 1640 cm^{-1} (amide I). ^{1}H n.m.r. δ 7·37, s, Z arom. H; 7·35, s, Bzl ester arom. H; 7·25, s, Bzl ether arom. H; 7·20, s, Phe arom. H; 5·12, s, Bzl ester CH_2; 4·93, 5·03, s, Z and Bzl ether CH_2.

(*v*) *Catalytic Hydrogenation of Protected Peptides*

The benzyloxycarbonyl peptide derivatives were hydrogenated in methanol over 10% palladium on charcoal catalyst (Fluka; *c.* 50 mg/100 mg) with addition of 1–2 drops of glacial acetic acid for

2–3 h. Electrophoresis and thin-layer chromatography were used to check the homogeneity of the free peptides. Samples for amino acid analysis were hydrolysed in 6 M HCl at 110° for 24 h under vacuum with addition of a crystal of phenol.

(i) *Tyr-Phe-Leu-OH* (89% yield of crystalline solid) had the composition $Tyr_{0.98}$ $Phe_{0.98}$ $Leu_{1.00}$; R_F (A) 0·74, (B) 0·69.

(ii) *Tyr-β-Ala-Phe-Leu-OH* (95% yield of amorphous solid) had the composition $Tyr_{0.99}$ β-$Ala_{0.97}$ $Phe_{0.99}$ $Leu_{1.00}$; R_F (A) 0·75, (B) 0·69.

(iii) *Tyr-NH(CH$_2$)$_3$CO-Phe-Leu-OH* (97% yield of amorphous solid) had the composition $Tyr_{0.95}$ $(NH_2(CH_2)_3CO_2H)_{1.01}$ $Phe_{0.99}$ $Leu_{1.00}$; R_F (A) 0·70, (B) 0·67. This peptide contained a trace of ninhydrin-positive impurity; R_F (A) 0·77, (B) 0·81.

(iv) *Tyr-NH(CH$_2$)$_4$CO-Phe-Leu-OH* (96% yield of amorphous solid) had the composition $Tyr_{0.99}$ $(NH_2(CH_2)_4CO_2H)_{1.01}$ $Phe_{0.99}$ $Leu_{1.00}$; R_F (A) 0·72, (B) 0·70.

(v) *TyrNH(CH$_2$)$_5$CO-Phe-Leu-OH* (96% yield of hygroscopic amorphous solid) had the composition $Tyr_{1.00}$ $(NH_2(CH_2)_5CO_2H)_{0.98}$ $Phe_{1.01}$ $Leu_{1.00}$; R_F (A) 0·71, (B) 0·69.

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