Amino-acids and Peptides. Part XV.[1] Syntheses of Four Tetrapeptides containing Tyrosine and Glycine Residues

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Four tetrapeptides, Tyr-Gly-Gly-Tyr, Tyr-Gly-Tyr-Gly, Gly-Tyr-Gly-Tyr, and Gly-Tyr-Tyr-Gly, together with intermediate dipeptides, have been synthesised using the *o*-nitrophenylthio-group for *N*-protection and *NN'*-dicyclohexylcarbodi-imide–2,4,5-trichlorophenol for coupling reactions.

MAJOR changes in the structures of proteins which have been subjected to photo-irradiation have been attributed to specific photolytic degradations at tyrosyl and other aromatic residues in the proteins,[2] but little is known about the mechanism of such processes. Some physicochemical studies have been carried out, but evidence of the nature of products and intermediates is lacking. We have synthesised four tetrapeptides (1)—(4), each with two glycyl and two tyrosyl residues, to enable an investigation of the photochemical degradation of tyrosyl residues in peptides.

The four peptides have been prepared through combination of appropriate derivatives of the dipeptides Gly-Tyr(Bzl) and Tyr(Bzl)-Gly. *N*-Protection used *o*-nitrophenylthio- (Nps) groups[3] and *C*-protection employed alkyl esters. The Nps group was removed with hydrochloric acid in acetone.

Side-chain protection of tyrosine with benzyl groups[4] was employed throughout. Subsequently, products were debenzylated with hydrobromic acid–acetic acid. Our results with hydrogen bromide–acetic acid confirm the observations of Trudelle and Spach[5] in that we did not detect any significant rearrangement of the *O*-benzyltyrosine residues under these conditions, in contrast to the conditions surveyed by Erickson and Merrifield[6] which give significant amounts of 3-benzyltyrosine.

After trials with several conventional coupling procedures, we found that the dipeptides (5) and (6) could be prepared most conveniently and in excellent yield by the use of a mixture of dicyclohexylcarbodi-imide (DCCI) and 2,4,5-trichlorophenol (TCP). An equimolar mixture of the dicyclohexylammonium salt of the Nps-amino-acid, the ester hydrochloride of the second amino-acid, and TCP, all in ethyl acetate at −10 to −15°, was treated with 1 mol. equiv. of DCCI. The products were obtained from the solution after removing the dicyclohexylurea and dicyclohexylammonium chloride by filtration. This procedure gave very much better yields than reactions in which DCCI alone, or previously prepared TCP esters of the Nps-amino-acids were employed. The four tetrapeptides were prepared from the appropriate dipeptides (7)—(10) by a similar coupling procedure.

The method described here avoids difficulties which have been observed[7] when the synthesis of tyrosyl peptides is attempted using the *p*-nitrophenyl ester, the imidazolide and, occasionally, the azide procedures. The results of experiments on the photochemical degradation of the new tetrapeptides (1)—(4) will be reported elsewhere.

H-Tyr-Gly-Gly-Tyr-OH (1)

H-Tyr-Gly-Tyr-Gly-OH (2)

H-Gly-Tyr-Gly-Tyr-OH (3)

H-Gly-Tyr-Tyr-Gly-OH (4)

R^1Gly-Tyr(Bzl)-R^2	R^1Tyr(Bzl)-Gly-R^2
(5) R^1 = Nps, R^2 = OEt	(6) R^1 = Nps, R^2 = OMe
(7) R^1 = Nps, R^2 = OH	(8) R^1 = Nps, R^2 = OH
(9) R^1 = H, R^2 = OEt	(10) R^1 = H, R^2 = OMe

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$R^1Tyr(R^2)$-Gly-Gly-$Tyr(R^2)$-R^3

(11) R^1 = Nps, R^2 = $PhCH_2$, R^3 = OEt
(15) R^1 = R^3 = H, R^2 = $PhCH_2$

$R^1Tyr(R^2)$-Gly-$Tyr(R^2)$-Gly-R^3

(12) R^1 = Nps, R^2 = $PhCH_2$, R^3 = OMe
(16) R^1 = R^3 = H, R^2 = $PhCH_2$

R^1Gly-$Tyr(R^2)$-Gly-$Tyr(R^2)$-R^3

(13) R^1 = Nps, R^2 = $PhCH_2$, R^3 = OEt
(17) R^1 = R^3 = H, R^2 = $PhCH_2$

R^1Gly-$Tyr(R^2)$-$Tyr(R^2)$-Gly-R^3

(14) R^1 = Nps, R^2 = $PhCH_2$, R^3 = OMe
(18) R^1 = R^3 = H, R^2 = $PhCH_2$

Nps = p-NO_2·C_6H_4S

EXPERIMENTAL

Physical data reported in this paper were obtained as described in the experimental section of Part XIV.[1] Ethyl acetate–light petroleum (b.p. 40—60°) (1 : 4 v/v) was used with Kieselgel G t.l.c. plates for monitoring formation of the Nps-protected peptide esters. Spots on t.l.c. were identified by the yellow colour of the Nps-derivatives, or their iodine-positive reactivity, or by spraying with ninhydrin solution and developing the colour at 100 °C. The i.r. and n.m.r. spectra of new compounds are unexceptional, and are listed in Supplementary Publication No. SUP 21125 (8 pp.).*

N-o-*Nitrophenylthio-amino-acid Salts*.—These were prepared by the method of Zervas.[3,8] L-(*O*-Benzyl)tyrosine [4] gave N-o-*nitrophenylthio*-L-(*O-benzyl*)*tyrosine dicyclohexylammonium salt* (70% yield), m.p. 170—171 °C (Found: C, 66·9; H, 6·8; N, 7·0. $C_{34}H_{43}N_3O_5S$ requires C, 67·4; H, 7·15; N, 6·95%), $[\alpha]_D^{22}$ 28 ± 0·5° (*c* 0·5 EtOAc).

General Procedure for the Synthesis of Dipeptide Derivatives.—The Nps-amino-acid dicyclohexylammonium salt (0·1 mol), the amino-acid ester hydrochloride (0·1 mol), and 2,4,5-trichlorophenol (0·1 mol) were added to ethyl acetate (150 cm^3) at −40 °C. DCCI (0·1 mol) was added and the mixture was kept at −10 to −15 °C for 5 h. The liberated urea and dicyclohexylammonium chloride were removed by filtration, and the residue obtained after evaporation of the filtrate was washed repeatedly with ether to remove trichlorophenol. The residue was purified further by crystallisation. The following dipeptide derivatives were prepared: N-o-(*nitrophenylthio*)*glycyl*-L-(*O-benzyl*)*tyrosyl ethyl ester* (5) (60—80% yield) as yellow needles (from ethyl acetate–petroleum), m.p. 112 °C (Found: C, 61·35; H, 5·15; N, 7·9. $C_{26}H_{27}N_3O_6S$ requires C, 61·25; H, 5·3; N, 8·25%), $[\alpha]_D^{27}$ +35 ± 0·5° (*c* 0·5 EtOAc); N-o-*nitrophenylthio*-L-(*O-benzyl*)*tyrosylglycine methyl ester* (6) (70—75% yield), crystallised from ethyl acetate–petroleum as yellow needles, m.p. 143 °C (Found: C, 60·3; H, 5·1; N, 8·15. $C_{25}H_{25}N_3O_6S$ requires C, 60·65; H, 5·05; N, 8·5%), $[\alpha]_D^{26}$ +44·4 ± 0·5° (*c* 0·5 EtOAc).

Removal of Ester Groups.—A modified procedure to that described by Wunsch [4] was used. The dipeptide ester in dioxan–water (5 : 1 v/v) was treated with an equivalent amount of 1M-sodium hydroxide at room temperature with vigorous mixing. After 1 h the mixture was titrated with an equivalent quantity of 1M–hydrochloric acid. Addition of dioxan, followed by evaporation under reduced pressure, gave the *N*-protected dipeptide, which was separated from salt by dissolving in acetone. Evaporation of the acetone yielded *N*-protected dipeptide.

N-(o-*Nitrophenylthio*)*glycyl*-(*O-benzyl*)*tyrosine* (7) was obtained in 97% yield, m.p. 111 °C (Found: C, 59·8; H, 4·55; N, 8·75. $C_{24}H_{23}N_3O_6S$ requires C, 59·9; H, 4·8; N, 8·75%), $[\alpha]_D^{25}$ +16 ± 0·5° (*c* 0·5 EtOAc).

N-o-*Nitrophenylthio*-(*O-benzyl*)*tyrosylglycine* (8) was obtained in quantitative yield, m.p. 137 °C (Found: C, 59·7; H, 4·65; N, 8·75. $C_{24}H_{23}N_3O_6S$ requires C, 59·85; H, 4·8; N, 8·75%), $[\alpha]_D^{25}$ +71·6 ± 0·5° (*c* 0·5 dimethylformamide).

Removal [8] *of* o-*Nitrophenylthio-group*.—The Nps-derivative was dissolved in the minimum volume of acetone, to which was added ether saturated with hydrogen chloride. The precipitated hydrochloride was collected and washed with ether and acetone until no trace of yellow colour remained in the white crystalline solid.

Deprotection of dipeptide (5) yielded the *hydrochloride* of *glycyl*-(*O-benzyl*)*tyrosine ethyl ester* (95% yield) as white needles, m.p. 158 °C (Found: C, 60·8; H, 6·45; N, 7·1. $C_{20}H_{25}ClN_2O_4$ requires C, 61·2; H, 6·4; N, 7·15%), $[\alpha]_D^{25}$ +18·4 ± 0·5° (*c* 0·5 water).

Deprotection of dipeptide (6) yielded white needles (quantitative yield) of the *hydrochloride* of (*O-benzyl*)*tyrosylglycine methyl ester*, m.p. 172 °C (subl.) (Found: C, 60·25; H, 6·5; N, 7·0. $C_{19}H_{23}ClN_2O_4$ requires C, 60·25; H, 6·05; N, 7·3%), $[\alpha]_D^{25}$ +44 ± 0·5° (*c* 0·5 water).

Synthesis of Tetrapeptides. General Procedure.—The Nps-dipeptide (0·015 mol), the dipeptide ester hydrochloride (0·015 mol), 2,4,5-trichlorophenol (0·015 mol), and DCCI (0·015 mol) were mixed in cold (−40 °C) ethyl acetate (50 cm^3) containing triethylamine (0·015 mol). After 8 h the triethylammonium chloride and didyclohexylurea were removed by filtration. Evaporation of the filtrate under reduced pressure gave the protected tetrapeptides which were further purified by washing with ethanol and ether, followed by recrystallisation.

Coupling of dipeptide derivative (8) to the dipeptide ester (9) gave N-o-*nitrophenylthio*-(*O-benzyl*)*tyrosylglycyl-glycyl*-(*O-benzyl*)*tyrosine ethyl ester* (11) as yellow granules (85% yield) (from ether–petroleum), m.p. 115—120 °C (Found: C, 64·85; H, 5·65; N, 8·3. $C_{44}H_{45}N_5O_9S$ requires C, 64·5; H, 5·55; N, 8·6%), $[\alpha]_D^{25}$ +36 ± 0·5° (*c* 0·5 dimethylformamide).

Coupling of dipeptide derivative (8) to the dipeptide ester (10) gave N-(o-*nitrophenylthio*)-(*O-benzyl*)*tyrosylglycyl*-(*O-benzyl*)*tyrosylglycine methyl ester* (12) as fine yellow needles (91% yield) [from ethyl acetate–acetone (1 : 3) and petroleum], m.p. 191 °C (Found: C, 64·15; H, 5·35; N, 8·6. $C_{43}H_{43}N_5O_9S$ requires C, 64·1; H, 5·35; N, 8·75%), $[\alpha]_D^{25}$ +41 ± 0·5° (*c* 0·5 dimethylformamide).

Coupling of dipeptide derivative (7) to the dipeptide ester (9) gave N-o-(*nitrophenylthio*)*glycyl*-(*O-benzyl*)*tyrosyl-glycyl*-(*O-benzyl*)*tyrosine ethyl ester* (13) as yellow needles (92% yield) [from ethyl acetate–acetone (1 : 3) and petroleum], m.p. 175 °C (Found: C, 64·8; H, 5·6; N, 8·5. $C_{44}H_{45}N_5O_9S$ requires C, 64·5; H, 5·55; N, 8·6%), $[\alpha]_D^{25}$ −4·8 ± 0·5° (*c* 0·5 dimethylformamide).

Coupling of dipeptide derivative (7) with dipeptide ester (10) gave N-o-*nitrophenylthioglycyl*-(*O-benzyl*)*tyrosyl*-(*O-benzyl*)*tyrosylglycine methyl ester* (14) as fine yellow needles

* For details of Supplementary Publications, see Notice to Authors No. 7 in *J.C.S. Perkin I*, 1973, Index issue.

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(93% yield) [from ethyl acetate–acetone (1 : 3) and petroleum], m.p. 183—184 °C (Found: C, 64·35; H, 5·2; N, 8·6. $C_{43}H_{43}N_5O_9S$ requires C, 64·1; H, 5·35; N, 8·75%), $[\alpha]_D^{25}$ −15 ± 0·5° (*c* 0·5 dimethylformamide).

Deprotection of the Tetrapeptide at the N- *and* C-*Terminal Groups*.—Removal of the ester groups from the tetrapeptide derivatives (11)—(14) was carried out using the modified procedure of Wunsch [4] as described for the dipeptides above. Each of the Nps-tetrapeptides was purified at this stage and gave satisfactory elemental and spectral analyses. These Nps-derivatives were in turn deprotected at the *N*-terminal position using hydrogen chloride in acetone–ether solutions,[9] as described above for the dipeptides, to give the following hydrochlorides of the *O*-benzyl-protected tetrapeptides.

The *hydrochloride* of (O-*benzyl*)*tyrosylglycylglycyl*-(O-*benzyl*)*tyrosine* (15) was obtained in 90% yield, m.p. 158 °C (Found: C, 64·2; H, 5·55; N, 8·55. $C_{36}H_{39}ClN_4O_7$ requires C, 64·0; H, 5·8; N, 8·3%), $[\alpha]_D^{24}$ 24 ± 0·5° (*c* 1 dimethylformamide).

The *hydrochloride* of (O-*benzyl*)*tyrosylglycyl*-(O-*benzyl*)-*tyrosylglycine* (16) was obtained in 95% yield, m.p. 218 °C (fused) (Found: C, 63·6; H, 5·1; N, 8·35%), $[\alpha]_D^{24}$ +7·9 ± 0·5° (*c* 1 dimethylformamide).

The *hydrochloride* of *glycyl*-(O-*benzyl*)*tyrosylglycyl*-(O-*benzyl*)*tyrosine* (17) was obtained in 97% yield, m.p. 207 °C (fused) (Found: C, 64·65; H, 6·3; N, 8·3%), $[\alpha]_D^{24}$ −8·3 ± 0·5° (*c* 1 dimethylformamide).

The *hydrochloride* of *glycyl*-(O-*benzyl*)*tyrosyl*-(O-*benzyl*)-*tyrosylglycine* (18) was obtained in 70% yield, m.p. 267 °C (fused) (Found: C, 64·3; H, 4·85; N, 8·45%), $[\alpha]_D^{24}$ −22·8 ± 0·5° (*c* 1 dimethylformamide).

Removal of the O-*Benzyl Groups*.—Each *O*-benzyl-protected tetrapeptide (15)—(18) (0·5 g) in turn was placed in a two-necked flask [connected to a drying tube (P_2O_5) and delivery tube] containing dry glacial acetic acid, and a dry stream of hydrogen bromide was passed into the acid suspension for 3—5 min. After 15 min, dry ether (200 cm³) was added to precipitate the peptide. The precipitate was collected, washed with ether, and then re-dissolved in acetone (15 cm³). The peptide was re-precipitated from the acetone with ether. This process was repeated several times to give the tetrapeptide salts as amorphous hygroscopic solids. The hydrobromide salts of the tetrapeptides did not give reproducible elemental analyses, probably owing to their hygroscopic character. Conversion into the hydrochloride salts followed by gel filtration on Sephadex LH-20 using methanol as eluant gave the tetrapeptides as amorphous solids.

Tyrosylglycylglycyltyrosine hydrochloride (1) was obtained from tetrapeptide (15) (98% yield as the hydrobromide), m.p. 146 °C (Found: C, 52·55; H, 5·3; N, 10·35. $C_{22}H_{27}ClN_4O_7,0{\cdot}5H_2O$ requires C, 52·5; H, 5·35; N, 10·85%), $[\alpha]_D^{20}$ +26·4 ± 0·5° (*c* 0·5 dimethylformamide).

Tyrosylglycyltyrosylglycine hydrochloride (2) was obtained from tetrapeptide (16) (95% yield as the hydrobromide), m.p. 154 °C (Found: C, 52·55; H, 5·65; N, 10·35. $C_{22}H_{27}ClN_4O_7,0{\cdot}5H_2O$ requires C, 52·5; H, 5·35; N, 10·85%), $[\alpha]_D^{20}$ +19·8 ± 0·5° (*c* 0·5 dimethylformamide).

Glycyltyrosylglycyltyrosine hydrochloride (3) was obtained from tetrapeptide (17) (95% yield as the hydrobromide), m.p. 170 °C (Found: C, 51·4; H, 5·0; N, 10·35. $C_{22}H_{27}ClN_4O_7,H_2O$ requires C, 51·5; H, 5·25; N, 10·9%), $[\alpha]_D^{20}$ +2·6 ± 0·5° (*c* 0·5 dimethylformamide).[20]

Glycyltyrosyltyrosylglycine hydrochloride (4) was obtained from tetrapeptide (18) (95% yield as the hydrobromide), m.p. 163 °C (Found: C, 52·55; H, 5·35; N, 10·35. $C_{22}H_{27}ClN_4O_7,0{\cdot}5H_2O$ requires C, 52·4; H, 5·35; N, 10·85%), $[\alpha]_D^{20}$ −14·4 ± 0·5° (*c* 0·5 dimethylformamide).

All tetrapeptides gave satisfactory amino-acid analyses and were chromatographically homogeneous on t.l.c. plates in the system ethyl acetate–acetic acid–ethanol–petroleum (3 : 2 : 3 : 10).

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