Synthesis of Sequential Polypeptides Containing L-β-3,4-Dihydroxyphenyl-α-Alanine (DOPA) and L-Glutamic Acid

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Synopsis

Sequential polypeptides with the repeating units L-glutamyl-L-DOPA, L-DOPA-L-glutamyl-L-DOPA, L-glutamyl-L-glutamyl-L-DOPA, L-DOPA-L-DOPA-L-glutamyl-L-DOPA, and L-glutamyl-L-glutamyl-L-glutamyl-L-DOPA have been synthesized by solution polymerization of the *p*-nitrophenyl esters of the corresponding di-, tri-, and tetrapeptides. The O,O′-dimethyl and γ-methyl groups were used to protect side chains of L-DOPA and L-glutamic acid. The monomers for the polytripeptides and polytetrapeptides were prepared by stepwise elongation, using the dicyclohexylcarbodiimide coupling method. Moderately high molecular weight sequential polypeptides were obtained. The protected groups of the side chain were removed simultaneously by use of boron tribromide in chloroform. Trimethylphosphate-soluble sequential polypeptides containing L-DOPA were obtained.

INTRODUCTION

The amino acid, L-β-3,4-dihydroxyphenyl-α-alanine (DOPA) has been used in the treatment of the dopamine deficiency of Parkinson patients, resulting in excellent and dramatic improvement.[1,2] However, DOPA has not been found as a constituent in naturally occurring peptides or proteins, and little chemical work has been done about the nature of peptides and polypeptides containing this amino acid. In the previous papers of this series,[3–8] we have reported the synthesis and conformational studies of poly(L-DOPA) and a series of random copolymers of L-DOPA with L-glutamic acid (Glu). To study further poly(L-DOPA), we widened the experiments on the polypeptide to include the sequential polypeptides. Since chemically related sequential polypeptides containing L-tyrosine (Tyr) and L-Glu have been synthesized, and since the conformational properties of some of these polypeptides have also been studied,[9–15] polydi-, tri-, and tetrapeptides containing L-DOPA are important aromatic polypeptides in elucidating the optical properties. Furthermore, since we have reported enzymelike hydrolytic activities of poly(L-Tyr, L-Glu) and poly(L-Tyr-L-Glu),[9,16] it may be possible to use the sequential polypeptides containing L-DOPA as functional polymers.

Five kinds of sequential polypeptides were synthesized by the polycondensation of the *p*-nitrophenyl esters of the corresponding di-, tri-, and

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Glu(OMe) → Nps-Glu(OMe) DCHA
(I)

DOPA(diMe) → Z-DOPA(diMe) → Z-DOPA(diMe)-ONp →
(II) (III)

HBr·DOPA(diMe)-ONp
(IV)

I + IV → Nps-Glu(OMe)-DOPA(diMe)-ONp →
(V)

HCl·Glu(OMe)-DOPA(diMe)-ONp → Poly[Glu(OMe)-DOPA(diMe)]
(VI) (VII)

→ Poly(Glu-DOPA)
(VIII)

Scheme I. Preparation of sequential polydipeptide. Abbreviations used: OMe, γ-methyl ester; Nps, *o*-nitrophenylsulfenyl; DCHA, dicyclohexylamine salt; diMe, *O,O'*-dimethyl; Z, carbobenzoxy; ONp, *p*-nitrophenyl ester.

tetrapeptides. Boron tribromide was used successfully for the cleavage of ether and ester protecting groups. The synthetic route to the sequential polypeptides is outlined in Schemes I–III.

EXPERIMENTAL

L-DOPA and *o*-nitrophenylsulfenyl chloride were purchased from the Tokyo Chemical Industry Co., Ltd. *p*-Nitrophenol and boron tribromide were purchased from the Wako Pure Chemical Industries Ltd. γ-Methyl-L-glutamate, carbobenzoxy chloride, dicyclohexylcarbodiimide [(cH × N═)$_2$C] and 25% hydrogen bromide were purchased from the Protein Research Foundation. N,N-Dimethylformamide (HCONMe$_2$; dried over potassium hydroxide) and dichloroacetic acid (Cl_2AcOH), both from Wako, were distilled at 40°C/10 mm Hg and at 102°C/20 mm Hg, respectively, and stored in brown bottles. Dioxane and triethylamine

II + VI → Z-DOPA(diMe)-Glu(OMe)-DOPA(diMe)-ONp →
(IX)

HBr·DOPA(diMe)-Glu(OMe)-DOPA(diMe)-ONp →
(X)

Poly[DOPA(diMe)-Glu(OMe)-DOPA(diMe)] → Poly(DOPA-Glu-DOPA)
(XI) (XII)

I + VI → Nps-Glu(OMe)-Glu(OMe)-DOPA(diMe)-ONp
(XIII)

HCl-Glu(OMe)-Glu(OMe)-DOPA(diMe)-ONp →
(XIV)

Poly[Glu(OMe)-Glu(OMe)-DOPA(diMe)]
(XV)

→ Poly(Glu-Glu-DOPA)
(XVI)

Scheme II. Preparation of sequential polytripeptides.

II + X → Z-DOPA(diMe)-DOPA(diMe)-Glu(OMe)-DOPA(diMe)-ONp
(XVII)
→ HBr·DOPA(diMe)-DOPA(diMe)-Glu(OMe)-DOPA(diMe)-ONp →
(XVIII)
Poly[DOPA(diMe)-DOPA(diMe)-Glu(OMe)-DOPA(diMe)] →
(XIX)
Poly(DOPA-DOPA-Glu-DOPA)
(XX)
I + XIV → Nps-Glu(OMe)-Glu(OMe)-Glu(OMe)-DOPA(diMe)-ONp →
(XXI)
HCl·Glu(OMe)-Glu(OMe)-Glu(OMe)-DOPA(diMe)-ONp →
(XXII)
Poly[Glu(OMe)-Glu(OMe)-Glu(OMe)-DOPA(diMe)] →
(XXIII)
Poly(Glu-Glu-Glu-DOPA)
(XXIV)

Scheme III. Preparation of sequential polytetrapeptides.

(Et_3N) from Wako were refluxed over sodium and then fractionally distilled at atmospheric pressure. The 2.8*N* solution of hydrogen chloride in dioxane was prepared by purifying the gas with sulfuric acid and then bubbling it into dioxane. All other solvents and reagents were reagent grade and were used without further purification.

Melting points were determined using a Yamato capillary melting point apparatus MP-1 and are uncorrected. Optical rotations were measured with a Jasco DIP-4 polarimeter at 589 nm. The ir spectra were measured with a Jasco IR DS-301 spectrophotometer as KBr pellets or films on sodium chloride plate. Elemental analyses were carried out with a Yanagimoto CHN recorder MT-2.

The intrinsic viscosities were measured in Cl_2AcOH at 25°C using an Ubbelohde viscometer and are listed in Table I. The molecular weights were estimated from empirical equations $[\eta] = 3.2 \times 10^{-2} M_r^{0.66}$ for poly(O-carbobenzoxy-L-Tyr)[17] and $[\eta] = 2.78 \times 10^{-5} M_r^{0.87}$ for poly(γ-benzyl-L-glutamate)[18], both measured in Cl_2AcOH at 25°C.

Synthesis

N-α-o-Nitrophenylsulfenyl-γ-Methyl-L-Glutamate Dicyclohexylamine Salt (*I*)

A modification of the procedure of Ledger and Stewart was used.[19] γ-Methyl-L-glutamate (32.2 g) was suspended in 250 ml of water and 250 ml of dioxane. Sodium bicarbonate (18.4 g) and magnesium oxide (9.6 g) were added, and the mixture was stirred to a smooth paste. *o*-Nitrophenylsulfenyl chloride (41.8 g) was added in portions over 15 min, and stirring continued for 1 hr. The mixture was acidified to Congo red with

TABLE I
Molecular Weights of the Protected Sequential Polypeptides

Polypeptide	$[\eta]^{25}_{Cl_2AcOH}$	Molecular Weight (DP)[a]	
[Glu(OMe)-DOPA(diMe)]$_n$	0.28	28,100 (160)[b]	39,300 (220)[c]
[DOPA(diMe)-Glu(OMe)-DOPA(diMe)]$_n$	0.19	15,300 (80)	
[Glu(OMe)-Glu(OMe)-DOPA(diMe)]$_n$	0.24		33,100 (200)
[DOPA(diMe)-DOPA(diMe)-Glu(OMe)-DOPA(diMe)]$_n$	0.20	17,200 (90)	
[Glu(OMe)-Glu(OMe)-Glu(OMe)-DOPA-(diMe)]$_n$	0.42		62,900 (400)

[a] Degree of polymerization.
[b] Calculated from $[\eta] = 3.2 \times 10^{-2} M_r^{0.66}$.
[c] Calculated from $[\eta] = 2.78 \times 10^{-5} M_r^{0.87}$.

0.5*N* sulfuric acid and extracted three times with ethyl acetate. The organic layer was washed with water and dried over sodium sulfate. Dicyclohexylamine (40 ml) was added to the filtered solution, whereupon the product precipitated. It was recrystallized from methanol. Yield, 60.0 g (60.5%); mp, 192°C; $[\alpha]_D^{25} = -30.4°$ (c = 1.0, methanol).

ANAL.: *Calcd. for* $C_{24}H_{37}N_3O_6S$: C, 58.16; H, 7.53; N, 8.48. *Found:* C, 58.33; H, 7.56; N, 8.37.

N-α-Carbobenzoxy-O,O′-Dimethyl-L-DOPA (*II*)

O,O′-Dimethyl-L-DOPA hydrochloride was prepared from the procedure of our previous paper.[7] To a solution of O,O′-dimethyl-L-DOPA hydrochloride (22.7 g) in 40 ml of water, 173 ml of 1*N* sodium hydroxide was added at 0 to −5°C. The solution was stirred vigorously under nitrogen while carbobenzoxy chloride (17.8 g) in 40 ml of ether and 104 ml of 1*N* sodium hydroxide were added in five equal portions over a period of 1 hr. After stirring an additional 1 hr at 0°C and 1 hr at 25°C, the solution was acidified to Congo red with 1*N* hydrochloric acid. After cooling for 30 min, the product was filtered and recrystallized from ethanol and *n*-hexane. Yield, 26.7 g (85.6%); mp, 117°C; $[\alpha]_D^{25} = 13.4°$ (c = 1.0, ethanol).

ANAL.: *Calcd. for* $C_{19}H_{21}NO_6$: C, 63.50; H, 5.89; N, 3.90. *Found:* C, 63.48; H, 6.00; N, 3.89.

N-α-Carbobenzoxy-O,O′-Dimethyl-L-DOPA p-Nitrophenyl Ester (*III*)

Compound II (12.4 g) was dissolved in 450 ml of ethyl acetate, the solution cooled to 0°C, and (cH × N=)$_2$C (7.9 g) added. After 10 min, *p*-nitrophenol (5.8 g) was added to the cold solution, and the reaction mixture was stirred at 0°C for 2 hr and allowed to stand overnight at 5°C. A few drops of acetic acid were added, and the insoluble dicyclohexylurea was filtered off. The filtrate was washed successively three times with a 1% sodium bicarbonate solution, water, 0.5*N* hydrochloric acid, and water and then dried over sodium sulfate. After filtration, the solvent was evaporated under reduced pressure. Ethyl acetate was added to the residue. The additional dicyclohexylurea which precipitated was filtered off, and on

addition of *n*-hexane, compound III crystallized. This was recrystallized from ethyl acetate and *n*-hexane. Yield, 13.5 g (81.4%); mp, 138°C; $[\alpha]_D^{25} = -10.0°$ (c = 1.0, ethyl acetate).

ANAL.: *Calcd. for* $C_{25}H_{24}N_2O_8$: C, 62.49; H, 5.03; N, 5.83. *Found:* C, 62.60; H, 4.95; N, 5.80.

O,O'-Dimethyl-L-DOPA p-*Nitrophenyl Ester Hydrobromide (IV)*

Compound III (13.0 g) was dissolved in 260 ml of dry dioxane, and 88 ml of 3.1*N* hydrogen bromide in glacial acetic acid was added, after which the solution was allowed to stand at room temperature for 2 hr. The solvent was evaporated under reduced pressure. The residue was repeatedly triturated with dry ether until a white powder was obtained. The product was recrystallized from methanol and ether. Yield, 11.2 g (96.9%); mp, 219°C; $[\alpha]_D^{25} = 20.5°$ (c = 1.0, methanol).

ANAL.: *Calcd. for* $C_{17}H_{19}N_2O_6Br$: C, 47.79; H, 4.48; N, 6.56. *Found:* C, 47.71; H, 4.45; N, 6.46.

N-α-o-Nitrophenylsulfenyl-γ-Methyl-L-Glutamyl-O,O'-Dimethyl-L-DOPA p-*Nitrophenyl Ester (V)*

To a solution of oily α-*o*-nitrophenylsulfenyl-γ-methyl-L-glutamate (isolated from 4.6 g of compound I using dilute sulfuric acid[20]) in 30 ml of dry dioxane, (cH × N═)$_2$C (2.1 g) was added at 5°C. After 10 min, O,O'-dimethyl-L-DOPA *p*-nitrophenyl ester (isolated from 4.0 g of compound IV using 1.3 ml of Et_3N) in 50 ml of dry chloroform was added to the dioxane solution, and the mixture was stirred for 2 hr at 5°C and for 15 hr at room temperature.[19] The product was worked up in a manner similar to compound III. Yield, 5.4 g (90.0%); mp, 136–138°C; $[\alpha]_D^{25} = -32.4°$ (c = 1.0, ethyl acetate).

ANAL.: *Calcd. for* $C_{29}H_{30}N_4O_{11}S$: C, 54.20; H, 4.71; N, 8.72. *Found:* C, 54.38; H, 4.68; N, 8.66.

γ-Methyl-L-Glutamyl-O,O'-Dimethyl-L-DOPA p-*Nitrophenyl Ester Hydrochloride (VI)*

Compound V (4.1 g) was dissolved in 20 ml of dry dioxane. Then 5.5 ml of 2.8*N* hydrogen chloride in dry dioxane was added to the solution. After 10 min, dry ether was added to the solution, and the mixture was kept for 6 hr in a refrigerator. The precipitate was washed with ether and dried. The product was recrystallized from ethanol and ether. Yield, 3.3 g (98.2%); mp, 95–97°C; $[\alpha]_D^{25} = 9.0°$ (c = 1.0, methanol).

ANAL.: *Calcd. for* $C_{23}H_{28}N_3O_9Cl$: C, 52.52; H, 5.37; N, 7.99. *Found:* C, 52.58; H, 5.26; N, 8.13.

Poly(γ-Methyl-L-Glutamyl-O,O'-Dimethyl-L-DOPA) (VII)

Compound VI (3.3 g) was dissolved in 3.0 ml of $HCONMe_2$. Under cooling, 1.04 ml of Et_3N was added to the solution with stirring. An appreciable increase in solution viscosity was observed after 1 hr, and the

mixture solidified after 2 hr. More solvent (2 ml) was added to the solid after 1 day. The thick semisolid was kept at room temperature for 8 days and poured into 400 ml of ethanol, yielding a pale yellow precipitate. The precipitated polymer was centrifuged, washed with methanol and ether, and dried. Yield, 1.9 g (88.0%). The molecular weight of the polypeptide is shown in Table I.

ANAL.: *Calcd. for* $(C_{17}H_{22}N_2O_6)_n$: C, 58.28; H, 6.33; N, 8.00. *Found:* C, 58.40; H, 6.30; N, 8.04.

Poly(L-Glutamyl-L-DOPA) (VIII)

Compound VII (1000 mg) was dissolved in 200 ml of chloroform, and boron tribromide (5.4 g) in 50 ml of chloroform was added dropwise with stirring. Stirring was continued for 8 hr at 50°C. The reaction mixture was evaporated under reduced pressure, triturated with ether, and dried. The residue was washed thoroughly with water, centrifuged, and dried. Yield, 828 mg (94.1%). The ester band of compound VII at 1732 cm^{-1} shifted to 1723 cm^{-1} (free carboxyl group), and the ether band at 1030 cm^{-1} disappeared.

ANAL.: *Calcd. for* $(C_{14}H_{16}N_2O_6)_n$: C, 54.54; H, 5.23; N, 9.09. *Found:* C, 54.37; H, 5.29; N, 8.80.

N-α-Carbobenzoxy-O,O'-Dimethyl-L-DOPA-γ-Methyl-L-Glutamyl-O,O'-Dimethyl-L-DOPA p-*Nitrophenyl Ester (IX)*

Compound II (2.9 g) in dioxane and γ-methyl-L-glutamyl-O,O'-dimethyl-L-DOPA *p*-nitrophenyl ester (isolated from 4.3 g of compound VI using 1.1 ml of Et_3N) were coupled by the $(cH \times N{=})_2C$ procedure described for the synthesis of compound V, except that chloroform was used to purify the product. After recrystallization from chloroform and *n*-hexane, compound IX was obtained. Yield, 5.9 g (86.8%); mp, 180°C; $[\alpha]_D^{18} = -16.2°$ (c = 1.0, $HCONMe_2$) and $-6.5°$ (c = 1.0, chloroform).

ANAL.: *Calcd. for* $C_{42}H_{46}N_4O_{14}$: C, 60.72; H, 5.58; N, 6.74. *Found:* C, 60.74; H, 5.60; N, 6.75.

O,O'-Dimethyl-L-DOPA-γ-Methyl-L-Glutamyl-O,O'-Dimethyl-L-DOPA p-*Nitrophenyl Ester Hydrobromide (X)*

Compound IX (5.8 g) was treated with 23 ml of 3.1*N* hydrogen bromide by the same procedure described for the synthesis of compound IV. Yield, 5.0 g (91.9%); mp, 103–113°C (broad); $[\alpha]_D^{18} = 3.9°$ (c = 1.0, ethanol).

ANAL.: *Calcd. for* $C_{34}H_{41}N_4O_{12}Br$: C, 52.51; H, 5.31; N, 7.21. *Found:* C, 52.61; H, 5.32; N, 7.03.

Poly(O,O'-Dimethyl-L-DOPA-γ-Methyl-L-Glutamyl-O,O'-Dimethyl-L-DOPA) (XI)

Compound X (2.5 g) in 3 ml of $HCONMe_2$ was polymerized as described previously for the synthesis of compound VII using 0.54 ml of Et_3N. Yield, 1.5 g (83.8%). The molecular weight is shown in Table I.

ANAL.: *Calcd. for* $(C_{28}H_{35}N_3O_9)_n$: C, 60.31; H, 6.33; N, 7.54. *Found:* C, 60.19; H, 6.31; N, 7.53.

Poly(L-DOPA-L-Glutamyl-L-DOPA) (XII)

Compound XI (800 mg) was treated with boron tribromide (3.0 g) as described previously for the synthesis of compound VIII. Yield, 612 mg (87.4%).

ANAL.: *Calcd. for* $(C_{23}H_{25}N_3O_9)_n$: C, 56.67; H, 5.17; N, 8.62. *Found:* C, 56.30; H, 5.23; N, 8.49.

N-α-o-Nitrophenylsulfenyl-γ-Methyl-L-Glutamyl-γ-Methyl-L-Glutamyl-O,O′-Dimethyl-L-DOPA p-*Nitrophenyl Ester (XIII)*

N-α-o-Nitrophenylsulfenyl-γ-methyl-L-glutamate (isolated from 4.5 g of compound I) and γ-methyl-L-glutamyl-O,O′-dimethyl-L-DOPA *p*-nitrophenyl ester (isolated from 4.8 g of compound VI) were coupled as described previously using $(c\mathrm{H} \times \mathrm{N}{=})_2\mathrm{C}$ (2.1 g) for the synthesis of compound IX. Yield, 6.0 g (83.7%); mp, 125–127°C; $[\alpha]_D^{25} = -55.3°$ (c = 1.0, chloroform).

ANAL.: *Calcd. for* $C_{35}H_{39}N_5O_{14}S$: C, 53.50; H, 5.00; N, 8.91. *Found:* C, 53.53; H, 5.02; N, 8.92.

γ-Methyl-L-Glutamyl-γ-Methyl-L-Glutamyl-O,O′-Dimethyl-L-DOPA p-*Nitrophenyl Ester Hydrochloride (XIV)*

Compound XIII (6.0 g) was treated with 6.6 ml of 2.8*N* hydrogen chloride as described previously. Yield, 4.9 g (95.9%); mp, 95–100°C; $[\alpha]_D^{20} = -13.4°$ (c = 1.0, ethanol).

ANAL.: *Calcd. for* $C_{29}H_{37}N_4O_{12}Cl$: C, 52.06; H, 5.57; N, 8.37. *Found:* C, 52.09; H, 5.55; N, 8.26.

Poly(γ-Methyl-L-Glutamyl-γ-Methyl-L-Glutamyl-O,O′-Dimethyl-L-DOPA) (XV)

Compound XIV (2.4 g) in 2 ml of $HCONMe_2$ was polymerized as described previously using Et_3N (0.60 ml). Yield, 1.7 g (96.0%). The molecular weight is shown in Table I.

ANAL.: *Calcd. for* $(C_{23}H_{31}N_3O_9)_n$: C, 55.98; H, 6.33; N, 8.51. *Found:* C, 56.10; H, 6.16; N, 8.50.

Poly(L-Glutamyl-L-Glutamyl-L-DOPA) (XVI)

Compound XV (900 mg) was treated with boron tribromide (3.1 g) as described previously. Yield, 721 mg (90.4%).

ANAL.: *Calcd. for* $(C_{19}H_{23}N_3O_9)_n$: C, 52.17; H, 5.30; N, 9.61. *Found:* C, 51.92; H, 5.26; N, 9.48.

N-α-Carbobenzoxy-O,O′-Dimethyl-L-DOPA-O,O′-Dimethyl-L-DOPA-γ-Methyl-L-Glutamyl-O,O′-Dimethyl-L-DOPA p-*Nitrophenyl Ester (XVII)*

Compound II (1.1 g) and O,O′-dimethyl-L-DOPA-γ-methyl-L-glutamyl-O,O′-dimethyl-L-DOPA *p*-nitrophenyl ester (isolated from 2.4 g of

compound X) were coupled as described previously using ($cH \times N{=})_2C$ (0.64 g). Yield, 2.6 g (81.0%); mp, 170–173°C; $[\alpha]_D^{23} = -14.1°$ (c = 1.0, chloroform).

ANAL.: *Calcd. for* $C_{53}H_{59}N_5O_{17}$: C, 61.32; H, 5.73; N, 6.75. *Found:* C, 61.40; H, 5.67; N, 6.86.

O,O′-Dimethyl-L-DOPA-O,O′-Dimethyl-L-DOPA-γ-Methyl-L-Glutamyl-O,O′-Dimethyl-L-DOPA p-*Nitrophenyl Ester Hydrobromide (XVIII)*

Compound XVII (2.6 g) was treated with 8.1 ml of 3.1*N* hydrogen bromide as described previously. Yield, 1.8 g (72.9%); mp, 122–132°C (broad); $[\alpha]_D^{23} = -6.6°$ (c = 1.0, methanol).

ANAL.: *Calcd. for* $C_{45}H_{59}N_5O_{15}Br$: C, 54.88; H, 5.53; N, 7.11. *Found:* C, 54.94; H, 5.67; N, 7.00.

Poly(O,O′-Dimethyl-L-DOPA-O,O′-Dimethyl-L-DOPA-γ-Methyl-L-Glutamyl-O,O′-Dimethyl-L-DOPA) (XIX)

Compound XVIII (1.8 g) was polymerized as described previously using Et_3N (0.31 ml). Yield, 1.2 g (82.1%). The molecular weight is shown in Table I.

ANAL.: *Calcd. for* $(C_{39}H_{48}N_4O_{12})n$: C, 61.25; H, 6.33; N, 7.33. *Found:* C, 61.07; H, 6.44; N, 7.34.

Poly(L-DOPA-L-DOPA-L-Glutamyl-L-DOPA) (XX)

Compound XIX (600 mg) was treated with boron tribromide (1.7 g) as described previously. Yield, 447 mg (85.5%).

ANAL.: *Calcd. for* $(C_{32}H_{34}N_4O_{12})_n$: C, 57.65; H, 5.14; N, 8.40. *Found:* C, 57.62; H, 5.07; N, 8.20.

N-α-o-Nitrophenylsulfenyl-γ-Methyl-L-Glutamyl-γ-Methyl-L-Glutamyl-γ-Methyl-L-Glutamyl-O,O′-Dimethyl-L-DOPA p-*Nitrophenyl Ester (XXI)*

N-α-*o*-Nitrophenylsulfenyl-γ-methyl-L-glutamate (isolated from 1.8 g of compound I) and γ-methyl-L-glutamyl-γ-methyl-L-glutamyl-O,O′-dimethyl-L-DOPA *p*-nitrophenyl ester (isolated from 2.4 g of compound XIV) were coupled as described previously using ($cH \times N{=})_2C$ (0.82 g). Yield, 3.2 g (96.1%); mp, 162–164 °C; $[\alpha]_D^{23} = -41.7°$ (c = 1.0, chloroform).

ANAL.: *Calcd. for* $C_{41}H_{48}N_6O_{17}S$: C, 53.01; H, 5.21; N, 9.05. *Found:* C, 53.12; H, 5.11; N, 8.88.

γ-Methyl-L-Glutamyl-γ-Methyl-L-Glutamyl-γ-Methyl-L-Glutamyl-O,O′-Dimethyl-L-DOPA p-*Nitrophenyl Ester Hydrochloride* (*XXII*)

Compound XXI (3.2 g) was treated with 3.0 ml of 2.8*N* hydrogen chloride as described previously. Yield, 1.8 g (64.3%); mp, 164–166°C; $[\alpha]_D^{24} = -21.8°$ (c = 1.0, methanol).

ANAL.: *Calcd. for* $C_{35}H_{46}N_5O_{15}Cl$: C, 51.76; H, 5.71; N, 8.62. *Found:* C, 51.89; H, 5.77; N, 8.78.

Poly(*γ-Methyl-L-Glutamyl-γ-Methyl-L-Glutamyl-γ-Methyl-L-Glutamyl-O,O′-Dimethyl-L-DOPA*) (*XXIII*)

Compound XXII (1.8 g) in 4 ml of $HCONMe_2$ was polymerized as described previously using Et_3N (0.37 ml). Yield, 1.4 g (100%). The molecular weight is shown in Table I.

ANAL.: *Calcd. for* $(C_{29}H_{40}N_4O_{12})_n$: C, 54.71; H, 6.33; N, 8.80. *Found:* C, 54.56; H, 6.35; N, 8.79.

Poly(*L-Glutamyl-L-Glutamyl-L-Glutamyl-L-DOPA*) (*XXIV*)

Compound XXIII (800 mg) was treated with boron tribromide (2.0 g) as described previously. Yield, 660 mg (92.7%).

ANAL.: *Calcd. for* $(C_{24}H_{30}N_4O_{12})_n$: C, 50.88; H, 5.33; N, 9.89. *Found:* C, 50.82; H, 5.26; N, 9.71.

RESULTS AND DISCUSSION

Since 1963 *p*-nitrophenyl active esters have been successfully used to produce a number of high-molecular-weight sequential polypeptides.[21,22] Although L-DOPA is rapidly oxidized into a deep red-colored quinone-type derivative and although care must be observed in handling it, all reactions (Schemes I–III) leading to the five monomer-active ester hydrohalides proceeded smoothly and in good yields. The intermediates were characterized by elemental analysis, melting point determination, ir spectra, and optical rotation. Purification by successive extraction with dilute acid, water, dilute base, and water was sufficient for most intermediate compounds. In the case of peptide hydrohalides, recrystallization from alcohol and ether yielded compounds with satisfactory purity.

Harwood and Cassidy[23] and Fuller et al.[24] have successfully used the O,O′-diacetyl group to synthesize high-molecular-weight poly(DL- or L-DOPA). However, since the O-acetyl protecting group was known to cleave easily and cause a partial racemization by alkaline treatment (even mild), we chose our O,O′-dimethyl group to protect the O,O′-dihydroxyl group in the side chain. In addition, γ-methyl ester was much better than γ-benzyl ester, since the latter was found to cleave partially by the treatment of hydrogen bromide in acetic acid.

When we polymerized the di-, tri-, and tetrapeptides, we obtained the corresponding polypeptides in yields of 82–100%. The yields of glutamyl

polypeptides (VII, XV, and XXIII; 88–100%) were higher than those of DOPA polypeptides (XI and XIX; 82–84%). Likewise, the molecular weights of glutamyl polypeptides (DP 160–400) were much higher than those of DOPA polypeptides (DP 80–90). In any case, the intrinsic viscosities and molecular weights of the five sequential polypeptides were satisfactory (Table I).

Boron tribromide has been used for the cleavage of ethers (methyl and benzyl),[25–27] esters (methyl, benzyl, and *t*-butyl)[25] and amine protecting groups (carbobenzoxy and *t*-butoxycarbonyl).[25,28,29] The peptide bond, however, was completely unchanged, and the products were not racemized by the reagent.[25] In our previous paper we have reported the synthesis of random sequence poly(L-DOPA, L-Glu) via poly(O,O′-dimethyl-L-DOPA, γ-benzyl-L-glutamate).[8] In the same manner the protected sequential polypeptides were easily converted to the deprotected polypeptides.

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