Synthesis and Antireserpine Activity of Peptides of L-Dopa

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A series of dipeptides and tripeptides containing L-Dopa was prepared and examined for anti-Parkinson activity in mice. Some of the peptides were more effective in reversing reserpine-induced catatonia then L-Dopa. The peptides were relatively nontoxic and resulted in a low degree of stereotypic behavior.

Following the discovery of dopamine in the caudate nucleus of various animals,[1,2] its depletion by reserpine, and replenishment by DL-Dopa,[1] the potential use of L-Dopa in the treatment of the dopamine deficiency of Parkinson[3] has been pursued. Despite the excellent and dramatic improvement obtained with L-Dopa in Parkinson patients,[4] certain limitations to its use have appeared.[5] This has prompted the search in many laboratories for substances which could replace L-Dopa or complement its use in the treatment of this degenerative disease.

A series of di- and tripeptides containing L-Dopa was therefore synthesized and examined in mice both for their ability to reverse ptosis and catatonia brought on by prior treatment with reserpine and to produce rearing and salivation. Acute toxicities were also determined to provide some index of the relationship of toxicity to activity.

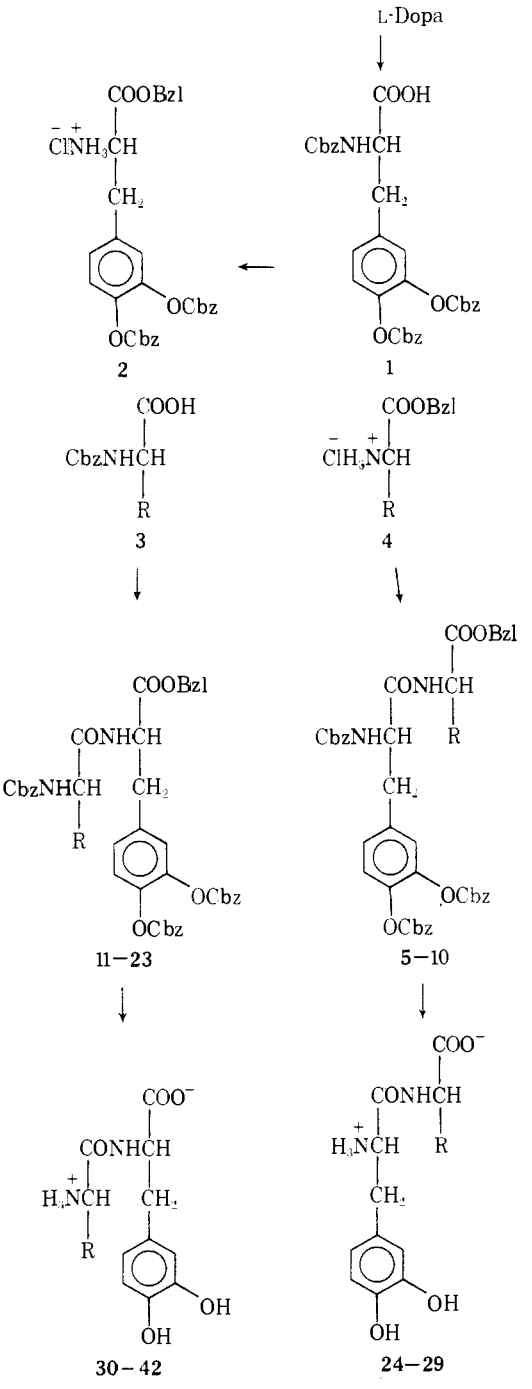
Since L-Dopa has not been found as a constituent in naturally occurring peptides or proteins, relatively little chemical work has been done with peptides containing this amino acid. Several dipeptides containing DL-Dopa at the C terminus have been synthesized by the phosphorus oxychloride and acid chloride methods.[6,7] Dipeptides with DL-Dopa at the N-terminal position were reported to have been prepared by the carbodiimide, phosphorus oxychloride, diethylcyanamide, and thiophenyl ester methods.[8] These earlier syntheses were carried out without protection of the side-chain aromatic hydroxyl groups. We found it desirable to protect the side-chain groups of L-Dopa prior to synthesis to avoid oxidation to the quinone. Moreover, our synthetic procedures were known to proceed without racemization and this was confirmed by the Manning–Moore test.[9]

Results

Synthesis. Scheme I summarizes the synthesis of the dipeptides.† Carbobenzoxylation of L-Dopa gave *N,O,O'*-tricarbobenzoxy-L-Dopa (**1**). Treatment of **1** with phosphorus pentachloride gave an intermediate *O,O'*-dicarbobenzoxy-L-Dopa *N*-carboxy anhydride which opened to *O,O'*-dicarbobenzoxy-L-Dopa benzyl ester hydrochloride (**2**) on alcoholysis with an HCl–benzyl alcohol solution. The protected N-terminal Dopa dipeptides (Table I, **5–10**) were prepared by coupling **1** with the corresponding amino acid benzyl ester salts **4** by the DCC method.[11] Coupling of **2** with the corresponding *N*-carbobenzoxyamino acids by the DCC method gave the *N*-carbobenzoxy-protected C-terminal Dopa dipeptides (Table I, **11–20**). L-Pyroglutamic acid was coupled directly with **2** to afford the partially protected dipeptide **21**. In several cases (**22**, **23**) the *N-tert*-butyloxycarbonyl-protected C-terminal Dopa dipeptides were also prepared by the DCC method. The protected dipeptides **5–21** were hydrogenated to the corresponding dipeptides (Table II, **24–40**). Compounds **22** and **23** required deprotection with trifluoroacetic acid in addition to hydrogenation to give dipeptides **41** and **42**.

The synthetic approach to the tripeptides is outlined in

Scheme I



† Abbreviations used are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature Symbols for Amino Acid Derivatives and Peptides.[10] Dopa, 3-(3,4-dihydroxyphenyl)alanine; DCC, dicyclohexylcarbodiimide; amino acid NCA, amino acid *N*-carboxy anhydride; Cbz, benzyloxycarbonyl; Bzl, benzyl; Boc, *tert*-butyloxycarbonyl; MeO, methoxy; ONP, *p*-nitrophenyl ester.

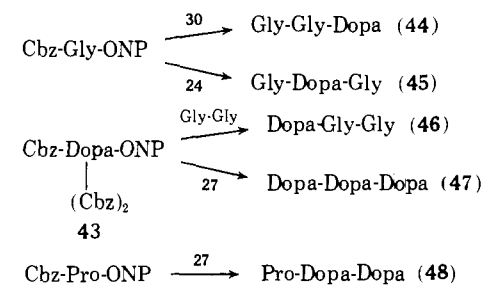
Table I. Protected Dopa Dipeptides

No.	X	Yield, %[a]	Mp, °C (solvent)	$[\alpha]^{25}D$, deg[b]	Formula	Analyses[c]
			Cbz-Dopa-X-OBzl $(Cbz)_2$			
5	-Gly-	69 (D–MC)	92–94 (MC–ET)	+6.64	$C_{42}H_{38}N_2O_{11}$	C, H, N
6	-Tyr- Cbz	53 (D–MC)	93–95 (MC–ET)	+7.34 (T)	$C_{57}H_{50}N_2O_{14}$	C, H, N
7	-3-MeO-Tyr- Cbz	60	156–160 (EA–PE)	−1.96	$C_{58}H_{52}N_2O_{15}$	C, H, N
8	-Dopa- $(Cbz)_2$	71	109–119 (EA–PE)	−7.54 (M)	$C_{65}H_{56}N_2O_{17}$	C, H, N
9	-Lys- Cbz	61	121.5–125 (EA–PE)	−1.42 (T)	$C_{54}H_{53}N_3O_{13}\cdot H_2O$	C, H, N
10	-Glu- OBzl	56	115.5–118 (EA–PE)	+2.63	$C_{52}H_{48}N_2O_{13}$	C, H, N
			X-Dopa-OBz $(Cbz)_2$			
11	Cbz-Gly-	89	Oil	−3.56	$C_{42}H_{36}N_2O_{11}$	C, H, N
12	Cbz-Ala-	79	137–139 (E)	+1.98 (T)	$C_{43}H_{40}N_2O_{11}$	C, H, N
13	Cbz-Leu-	62	107–109 (MC-PE)	−18.2 (M)	$C_{46}H_{46}N_2O_{11}$	C, H, N
14	Cbz-Val-	75	105–108 (EA–PE)	−0.66	$C_{45}H_{44}N_2O_{11}$	C, H, N[d]
15	Cbz-Pro-	65	Oil	−31.40	$C_{45}H_{42}N_2O_{11}$	C, H, N
16	Cbz-Tyr- Cbz	76	120–123 (EA–PE)	−11.54	$C_{57}H_{50}N_2O_{14}$	C, H, N
17	Cbz-3-MeO-Tyr- Cbz	63	121–124 (EA–ET)	−11.09	$C_{58}H_{52}N_2O_{15}$	C, H, N
18	Cbz-D-Dopa- $(Cbz)_2$	66	154–160 (EA–PE)	−2.98	$C_{65}H_{56}N_2O_{17}$	C, H, N
19	Cbz-Glu- OBzl	69	123.5–125 (EA–PE)	−10.70 (M)	$C_{52}H_{48}N_2O_{13}$	C, H, N
20	Cbz-Ser-	66	150–153 (EA–PE)	−21.49	$C_{43}H_{40}N_2O_{12}$	C, H, N
21	<Glu-	42 (D–MC)	133–136 (EA–PE)	−9.71	$C_{37}H_{34}N_2O_{10}$	C, H, N
22	Boc-Lys- Cbz	56	114–117 (EA–PE)	−9.55	$C_{51}H_{55}N_3O_{13}$	C, H, N
23	Boc-Glu-OBzl	72	82–85.5 (EA–PE)	+4.71	$C_{49}H_{50}N_2O_{13}$	C, H, N

[a] After recrystallization. Couplings were carried out in methylene chloride unless otherwise indicated next to yield. Solvents used for crystallization are indicated next to the melting point. Solvents abbreviated as follows: MC, methylene chloride, EA, ethyl acetate; PE, petroleum ether; M, methanol; E, ethanol; D, dimethylformamide; T, tetrahydrofuran; CH, chloroform; ET, ether; W, water; I, isopropyl alcohol. [b] Rotations were measured at concentrations of 0.9–2.1% in chloroform unless otherwise noted. [c] Analyses of the elements indicated were within ±0.4% of theory except where indicated. [d] C: calcd, 68.52; found, 67.84.

Scheme II. Coupling of the dipeptides by the *p*-nitrophenyl ester[12] method followed by hydrogenolysis gave Gly-Gly-L-Dopa (**44**), Gly-L-Dopa-Gly (**45**), L-Dopa-Gly-Gly (**46**), L-Dopa-L-Dopa-L-Dopa (**47**), and L-Pro-L-Dopa-L-Dopa (**48**). In order to determine the optical purity of the peptides prepared by these procedures, a sample of L-Dopa-L-Dopa (**27**) was hydrolyzed to Dopa and then allowed to react with L-Ala NCA.[9] The resultant Ala-Dopa diastereomers were chromatographed on an amino acid analyzer and the ratio of L-Ala-L-Dopa:L-Ala-D-Dopa determined. It was calculated that less than 0.82% of D-Dopa was generated from the entire synthesis of **27** from L-Dopa.

Scheme II



Pharmacology. Reversal of reserpine-induced catatonia is used to detect centrally acting anti-Parkinson agents.[13] Compounds that reverse reserpine-induced ptosis without affecting catatonia are assumed to be acting by a peripheral mechanism.[14] Several tripeptides (**44**, **45**, and **48**) were significantly more active than L-Dopa in reversing catatonia induced by reserpine (Tables II and III). The other peptides were in general equivalent on a mg/kg ip basis to L-Dopa. However, based on the actual content of L-Dopa in the peptides (*i.e.*, mequiv/kg) compounds **28**, **33**, **35**, and **40** were also more effective than L-Dopa. Nevertheless, no obvious structure–activity relationships were

Table II. Dipeptides of L-Dopa

No.	Compound	Yield, %	Mp, °C	$[\alpha]^{25}D$, deg[a]	Formula	Analyses[f]	Reserpine reversal: Ptosis[i] ip	Ptosis[i] po	Catatonia[j] ip	Catatonia[j] po	Stereotypic behavior: Rearing,[j] ip	Salivation,[j] ip	Acute toxicity,[k] ip
	L-Dopa				$C_9H_{11}N_1O_4$		220 ± 90	330 ± 170	300 ± 55	520 ± 175	750 ± 60	800 ± 40	1140 ± 66
24	Dopa-Gly	84	160–163[b] (W–I)	−53.97	$C_{11}H_{14}N_2O_5$	C, H, N	96 ± 34	124 ± 34	300 ± 157	300 ± 157	670 ± 33	600 ± 22	1650 ± 660
25	Dopa-Tyr	65	178–180 (W–I)	+21.13 (M)	$C_{18}H_{20}N_2O_6 \cdot 2H_2O$	C, H, N	210 ± 157	>800	710 ± 209	>800	>800	>800	>800
26	Dopa-3-MeO-Tyr	24	164–167 (W–I)	+17.41	$C_{19}H_{22}N_2O_7 \cdot 1.5H_2O$	C, H, N	165 ± 56		>800		>40	>40	
27	Dopa-Dopa	65	174–176 (E)	+23.09	$C_{18}H_{20}N_2O_7$	C, H, N	108 ± 14	180 ± 50	285 ± 51	550 ± 82	665 ± 45	540 ± 44	>800
28	Dopa-Lys	37	150 dec (E–ET)	+16.91	$C_{15}H_{23}N_3O_5 \cdot 2H_2O$[c]	C, H, N[g]	148 ± 86	295 ± 154	240 ± 162	395 ± 41	>800	<800	>800
29	Dopa-Glu	81	157–159 (W–I)	+22.47	$C_{14}H_{18}N_2O_7 \cdot 0.5H_2O$	C, H, N[h]	440 ± 42	380 ± 131	>800	>800	>800	>800	>800
30	Gly-Dopa	36	171–173[d] (W–I)	+46.07	$C_{11}H_{14}N_2O_5$	C, H, N	185 ± 70	225 ± 68	285 ± 63	225 ± 91	705 ± 63	540 ± 40	>800
31	Ala-Dopa	73	225–226 dec (W–I)	+24.91	$C_{12}H_{16}N_2O_5$	C, H, N	205 ± 82	183 ± 53	450 ± 171	375 ± 105	>800	>800	>800
32	Leu-Dopa	55	Amorphous (I)	+39.99 (M)	$C_{15}H_{22}N_2O_5 \cdot 1.5H_2O$	C, H, N	50 ± 42	140 ± 60	275 ± 168	600 ± 60	>400	>400	>400
33	Val-Dopa	62	156 dec (W–E)	+26.93	$C_{14}H_{20}N_2O_5 \cdot 1.5H_2O$	C, H, N	180 ± 35	335 ± 91	210 ± 69	460 ± 113	>800	>800	>800
34	Pro-Dopa	60	170 dec (W–I)	−27.95	$C_{14}H_{18}N_2O_5 \cdot H_2O$	C, H, N	270 ± 92	295 ± 150	500 ± 360	420 ± 189	>800	>800	>800
35	Tyr-Dopa	78	169–171 (M)	+21.66	$C_{18}H_{20}N_2O_6 \cdot 2CH_4OH$	C, H, N	160 ± 70	500 ± 60	245 ± 120	>800	>400	>400	>400
36	3-MeO-Tyr-Dopa	58	164–166 (W–I)	+21.33	$C_{19}H_{22}N_2O_7 \cdot H_2O$	C, H, N[e]	285 ± 149	540 ± 247	770 ± 416	>800			
37	D-Dopa-Dopa	33	171–175 (W–I)	+15.81	$C_{18}H_{20}N_2O_7 \cdot H_2O$	C, H, N	390 ± 183	>800	390 ± 183	>800	>800	>800	>800
38	Glu-Dopa	32	163–165 (W–E)	+17.89	$C_{14}H_{18}N_2O_7 \cdot 0.5H_2O$	C, H, N	215 ± 123	255 ± 108	400 ± 153	275 ± 118			
39	Ser-Dopa	84	149 dec (W–I)	+23.29	$C_{12}H_{16}N_2O_6 \cdot 0.5H_2O$	C, H, N	195 ± 100	92 ± 32	>800	440 ± 146	>800	730 ± 31	>800
40	<Glu-Dopa	85	120 dec	0.49	$C_{14}H_{16}N_2O_6 \cdot 1.5H_2O$	C, H, N	295 ± 150	>800	195 ± 97	>800	>800	>800	>800
41	Lys-Dopa	96	Amorphous	+5.86	$C_{15}H_{23}N_3O_5 \cdot H_2O$[c]	C, H, N	340 ± 95	430 ± 185	550 ± 306	>800	>800	>800	
42	γ-Glu-Dopa	52	Amorphous	+22.08	$C_{14}H_{18}N_2O_7 \cdot H_2O$	C, H, N	520 ± 396	>800	470 ± 184	>800	>800	>800	>800

[a] All rotations were measured in 1.0 *M* HCl unless otherwise noted at concentrations of 0.8–2.0%. [b] Resolidifies and melts again at 258–263° dec. [c] Obtained as the monoacetate salt. [d] Resolidifies and melts again at 259–265° dec. [e] Obtained as the ditrifluoroacetate salt. [f] Analyses of the elements indicated were within ±0.4% of theory except where indicated. [g] C: calcd, 48.45; found, 48.94. [h] C: calcd, 50.18; found, 50.67. H: calcd, 5.72; found, 6.14. [i] C: calcd, 55.88; found, 56.37. [j] ED_{50} ± S.E. (mg/kg). [k] LD_{50} ± S.E. (mg/kg).

Table III. Tripeptides of L-Dopa

No.	Compound	Mp, °C	$[\alpha]^{25}D$, deg[a]	Formula[b]	Reserpine reversal: Ptosis[c] ip	Ptosis[c] po	Catatonia[c] ip	Catatonia[c] po	Stereotypic behavior: Rearing,[c] ip	Salivation,[c] ip	Acute toxicity,[d] ip
44	Gly-Gly-Dopa	170–172 (W–I)	+17.31	$C_{13}H_{17}N_3O_6 \cdot 0.5H_2O$	68 ± 39	492 ± 91	110 ± 37	440 ± 168	930 ± 102	730 ± 42	
45	Gly-Dopa-Gly	217–220 dec (W–I)	+22.10	$C_{13}H_{17}N_3O_6 \cdot 0.5H_2O$	112 ± 39	570 ± 142	112 ± 39	>800	>800	650 ± 87	
46	Dopa-Gly-Gly	186–188 (W–I)	+55.60	$C_{13}H_{17}N_3O_6 \cdot 1.5H_2O$	185 ± 60		285 ± 156		>800	660 ± 10	
47	Dopa-Dopa-Dopa	Amorphous	+13.76	$C_{27}H_{33}N_3O_{10} \cdot 4H_2O$	96 ± 41	118 ± 51	360 ± 83	560 ± 257	750 ± 22	700 ± 14	>800
48	Pro-Dopa-Dopa	260–261 dec (W–I)	+104.0 (D)	$C_{23}H_{27}N_3O_8 \cdot 0.5H_2O$	94 ± 51	>100	94 ± 51	>100	770 ± 71	490 ± 43	>800

[a] All rotations were taken in 1.0 *M* HCl at concentrations of 1.0–2.0% unless otherwise noted. [b] Analyses for C, H, and N were within ±0.4% of theory. [c] ED_{50} ± S.E. (mg/kg). [d] LD_{50} ± S.E. (mg/kg).

observed since dipeptides with neutral, acidic, or basic amino acids at either the N-terminal (**24–29**) or C-terminal (**30–42**) position were about equally active. Several dipeptides (**25, 26, 29, 36,** and **39**) were less active than L-Dopa in reversing reserpine-induced catatonia but were effective in reversing reserpine-induced ptosis. It was concluded that these compounds were acting mainly by a peripheral mechanism.

The peptides of L-Dopa were also examined for induction of stereotypic behavior (rearing) and salivation. Rearing behavior in mice may be a possible indication of the involuntary motor activity observed in patients receiving anti-Parkinson drugs and salivation may be primarily due to peripheral effects of the drug.[14] In general, the peptides caused a lower degree of stereotypic behavior in mice than L-Dopa (Tables II and III). Acute toxicity studies in mice also revealed that the peptides were relatively nontoxic.

Experimental Section

Biological Measurements. Male CF-15 mice (Carworth Farms, New City, N. Y.) weighing 17–25 g were used in all pharmacological test procedures. Reversal of reserpine-induced catatonia and ptosis was determined by the administration of test compounds orally or intraperitoneally to animals pretreated 4 hr earlier with reserpine (5 mg/kg ip). The mice were tested for catatonia by placing the animals on size No. 1 corks in uncomfortable positions and observing them for 5 min. Mice that remained on the corks during the period were considered catatonic. Mice with complete closure of both eyelids were considered to exhibit ptosis. The animals were tested for reversal of catatonia and ptosis 15–20 min after administration of the test compound. A total of 15–30 test animals were used for each peptide; in the case of L-Dopa a total of 90–140 mice were used for calculations.

Incidence of rearing and salivation was determined 60–75 min after the intraperitoneal administration of test compounds to nonpretreated mice. The mice were placed in 3 × 3 in. observation cages and closely observed for 15 min for rearing behavior. At the end of the observation period the mice were visually checked for evidence of salivation. In addition, the mice received intraperitoneal doses of the test compounds and were observed 24 hr for mortality to determine acute toxicities. The ED_{50} and LD_{50} values for each procedure were calculated by the method of Miller and Tainter.[15]

Materials and Methods. L-Dopa was obtained from Hoffmann-La Roche Inc. and found to contain <0.20% of the D isomer by the Manning-Moore method[9] using L-alanine *N*-carboxy anhydride. D-Dopa was purchased from Cyclo Chemical Co. Carbobenzoxy chloride (CbzCl) (technical grade) and dicyclohexylcarbodiimide (reagent grade) were obtained from Aldrich Chemical Co. Phosphorus pentachloride (reagent grade) was obtained from Mallinckrodt. Benzyl alcohol (BzOH) (reagent grade) was purchased from Fisher Scientific Co. DMF (reagent grade, dried over Linde type 4A molecular sieves) and trifluoroacetic acid (TFA) were supplied by Matheson Coleman and Bell. Hydrogen chloride and hydrogen (prepurified) were purchased from Matheson Gas Products. Palladium (5%) on $BaSO_4$ was obtained from Englehard Industries. Carbobenzoxyamino acids and other amino acid derivatives were synthesized or purchased from Fox Chemical Co. and examined for purity by thin-layer chromatography prior to usage. All amino acid derivatives used were of the L configuration unless otherwise stated. All other reagents and solvents were reagent grade and used without further purification. Amino acid analyses were performed on the Jeol 5AH Analyzer. Melting points were determined on a Reichert hot-stage apparatus and are uncorrected. All new compounds were characterized by infrared and nmr spectra. C, H, and N microanalyses were determined to within ±0.4% of the theoretical values. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter. Thin-layer chromatography was performed on all peptides using silica gel G in three separate systems and developed with ninhydrin and Cl_2-tolidine [BuOH-AcOH-EtOAc-H_2O (1:1:1:1); BuOH-AcOH-H_2O (4:1:1); BuOH-AcOH-pyridine-H_2O (15:3:10:12)].

***N,O,O'*-Tricarbobenzoxy-L-Dopa (1).** L-Dopa (140 g, 0.71 mol) was added to a solution of 711 ml of 1 *M* NaOH in 1480 ml of H_2O and the solution stirred vigorously under nitrogen at −10° while CbzCl (404 g, 2.33 mol) in 2 l. of Et_2O and 2370 ml of 1 *M* NaOH were added simultaneously over a period of 75 min. After stirring an additional 1 hr at 0° and 2 hr at 25°, the crystalline sodium salt was collected by filtration, washed with Et_2O and H_2O, and then partitioned between 2 l. each of Et_2O and 1 *M* citric acid. The Et_2O layer was washed with H_2O, dried over Na_2SO_4, and evaporated, and the product was crystallized from CH_2Cl_2–petroleum ether: yield 330.2 g (78%); mp 82–84°; $[\alpha]^{25}D$ −0.67° (*c* 5.5, MeOH). *Anal.* ($C_{33}H_{29}NO_{10}$) C, H, N.

***N,O,O'*-Tricarbobenzoxy-D-Dopa (1a).** The D isomer of 1 was prepared from D-Dopa by the method outlined above. The product was obtained as white prisms: mp 80–85°; $[\alpha]^{25}D$ 1.35° (*c* 3.3, MeOH). *Anal.* ($C_{33}H_{29}NO_{10}$) C, H, N.

***O,O'*-Dicarbobenzoxy-L-Dopa Benzyl Ester Hydrochloride (2).** A solution of 1 (230 g, 0.385 mol) in 5.3 l. of Et_2O was allowed to react with PCl_5 (97.4 g, 0.47 mol) to give a clear solution. Evaporation gave a colorless syrup which was immediately allowed to react with 690 ml of 1 *M* HCl in BzOH at 50° for 30 min. Following the evolution of CO_2 15 vol of Et_2O was added. The solid product obtained was recrystallized from MeOH-Et_2O to give 144.4 g (63%) of white prisms: mp 114–117°; $[\alpha]^{25}D$ −10.36° (*c* 1, MeOH). *Anal.* ($C_{32}H_{30}ClNO_8$) C, H, N.

***O,O'*-Dicarbobenzoxy-D-Dopa Benzyl Ester Hydrochloride (2a).** *N,O,O'*-Tricarbobenzoxy-D-Dopa (1a) was treated by the method outlined above for the L isomer. The product crystallized from MeOH-Et_2O as white prisms: mp 107–113°; $[\alpha]^{25}D$ +10.35° (*c* 1, MeOH). *Anal.* ($C_{32}H_{30}ClNO_8 \cdot 2H_2O$) C, H, N.

***N,O*-Dicarbobenzoxy-3-methoxy-L-tyrosine (3a).** This compound was prepared from 3-methoxy-L-tyrosine (5.0 g, 23.7 mmol) by the procedure outlined for the synthesis of 1 using 2 equiv of CbzCl. The reaction proceeded at −10° for 1.5 hr and 25° for 1 hr, was transferred to a separatory funnel, and washed with Et_2O, and the aqueous layer was acidified with saturated citric acid and extracted with Et_2O. The extract was washed with H_2O, dried over $MgSO_4$, filtered, evaporated to dryness, and triturated with petroleum ether to give 9.4 g (82.9%) of white prisms: mp 126–129°; $[\alpha]^{25}D$ +39.55° (*c* 2.1, $CHCl_3$). *Anal.* ($C_{26}H_{25}NO_8$) C, H, N.

***O*-Carbobenzoxy-3-methoxy-L-tyrosine Benzyl Ester Hydrochloride (4a).** A solution of **3a** (6.6 g, 13.8 mmol) in $CHCl_3$ (200 ml) was treated with PCl_5 (2.88 g, 13.8 mmol) with stirring over a 1-hr period. The mixture was filtered and the filtrate evaporated several times from $CHCl_3$ to give a colorless oil which analyzed correctly for *O*-carbobenzoxy-3-methoxy-L-tyrosine *N*-carboxy anhydride. *Anal.* ($C_{19}H_{17}NO_7$) C, H, N. The oil was treated with a freshly prepared solution of 0.7 *M* HCl in BzOH (150 ml) at 50° for 35 min and then crystallized by addition of Et_2O (450 ml)–petroleum ether (900 ml). Recrystallization from EtOH-Et_2O–petroleum ether gave 2.7 g (40% overall) of white crystals: mp 76–80°; $[\alpha]^{25}D$ −12.93° (*c* 2, MeOH). *Anal.* ($C_{25}H_{26}ClNO_6 \cdot H_2O$) H, N; C: calcd, 61.29; found, 61.76.

***O*-Carbobenzoxy-L-tyrosine Benzyl Ester Hydrochloride (4b).** A suspension of *N,O*-dicarbobenzoxy-L-tyrosine[16] (9.0 g, 0.022 mol) in 500 ml of Et_2O was allowed to react with 5.0 g of PCl_5 for 60 min. The solvent was evaporated and the residue evaporated several times from Et_2O. The residue was treated with 100 ml of 1 *M* HCl in BzOH and stirred at 50° for 1 hr. The product was precipitated by addition of 10 vol of Et_2O and recrystallized from MeOH-Et_2O to give 5.1 g (57.8%) of white crystals: mp 196–197°; $[\alpha]^{25}D$ −12.23° (*c* 1, MeOH). *Anal.* ($C_{24}H_{24}ClNO_5$) Cl, H, N; C: calcd, 65.23; found, 64.39.

General Procedure for the Synthesis of Protected Dopa Dipeptides 5–23. For the synthesis of protected dipeptides containing Dopa at the N-terminal position, the appropriate amino acid benzyl ester hydrochloride and 1 were dissolved in CH_2Cl_2 (in several cases addition of DMF was required to bring about complete solution), cooled to 0°, and treated with 1 equiv of Et_3N. DCC (1 equiv) was added and the reaction mixture stirred at 0° for 1 hr and then at 25° for 16 hr. The insoluble by-product was filtered off and the filtrate evaporated to dryness, taken up in $CHCl_3$, and washed with 5% $NaHCO_3$ and 0.05 *M* HCl. The $CHCl_3$ layer was dried over $MgSO_4$, filtered, evaporated to dryness, and crystallized from appropriate solvents as indicated in Table I.

For the synthesis of protected dipeptides containing Dopa at the C-terminal position, the appropriate N-protected amino acid was allowed to react with **2** by a procedure similar to that described above. The solvents used for crystallization of the products are also indicated in Table I.

Dipeptides of L-Dopa 24–42 (Table II). The protected dipeptide (**5–23**) was dissolved in MeOH (50 ml) (in several cases addition of THF was required to bring about complete solution) and

glacial AcOH (5 ml), followed by addition of 2-5 g of 5% Pd-$BaSO_4$. Hydrogenation was carried out in a Parr apparatus at 35-50 psi for 16 hr. The catalyst was removed by filtration through Celite. The filtrate was evaporated to dryness, reevaporated from water, and crystallized.

N,O,O'-**Tricarbobenzoxy-L-Dopa** *p*-**Nitrophenyl Ester** (**43**). A solution of 1 (81.3 g, 0.136 mol) in 700 ml of CH_2Cl_2 was allowed to react with *p*-nitrophenol (22.35 g, 0.16 mol) and DCC (33.2 g, 0.162 mol) at 0° for 21 hr. Filtration followed by evaporation of the solvents gave an oil which solidified immediately upon treatment with EtOH. Recrystallization from EtOH gave 81 g (82%) of the desired compound: mp 115-117°; $[\alpha]^{25}D$ −16.80° (*c* 1, DMF). *Anal.* ($C_{39}H_{32}N_2O_{12}$) C, H, N.

Gly-Gly-L-Dopa (**44**). A mixture of Gly-L-Dopa (**30**, 3.0 g, 11.8 mmol) and Cbz-Gly-ONP (3.98 g, 12.0 mmol) in 50 ml of DMF was cooled to 0°. Et_3N (1.195 g, 11.8 mmol) was added and the solution stirred at 0° for 2 hr and at 25° for 90 hr under N_2. Glacial AcOH (1.5 ml) was added and the solution evaporated to remove DMF. It was triturated with Et_2O, dissolved in 5% $NaHCO_3$, and extracted with Et_2O. The aqueous solution was acidified with 1 *M* HCl, extracted into EtOAc, dried over $MgSO_4$, and evaporated to dryness. The resultant oil was dissolved in 130 ml of 10% AcOH in MeOH and hydrogenated in the presence of 1.2 g of 5% Pd-$BaSO_4$. The reaction mixture was treated with H_2O, filtered, and evaporated several times from H_2O. The product was crystallized from H_2O-*i*-PrOH to give 1.04 g (27.4% overall).

Gly-L-Dopa-Gly (**45**). A mixture of L-Dopa-Gly (**24**, 3.0 g, 11.8 mmol) and Cbz-Gly-ONP (3.98 g, 12.0 mmol) was reacted, worked up, and hydrogenated as described for 44 to give 475 mg (17.4% overall) of the desired compound after crystallization from H_2O-*i*-PrOH.

L-Dopa-Gly-Gly (**46**). A mixture of Gly-Gly·HCl (1.24 g, 6.66 mmol) and **43** (5.05 g, 7.0 mmol) in 50 ml of DMF was cooled to 0° and Et_3N (1.35 g, 13.3 mmol) added to the reaction and hydrogenation carried out as described for 44 to give 776 mg (36.2% overall) of white crystals.

L-Dopa-L-Dopa-L-Dopa (**47**). A mixture of L-Dopa-L-Dopa (**27**, 4.17 g, 0.011 mol) and **43** (8.8 g, 0.012 mol) in 50 ml of DMF was chilled in ice, Et_3N (1.71 ml, 0.0122 mol) added, and the reaction proceeded as described for **44**. Evaporation of the solvent gave a white powder after trituration with Et_2O. It was dissolved in EtOAc, washed with H_2O, dried over Na_2SO_4, and evaporated to a glassy solid. Hydrogenation in 150 ml of MeOH-AcOH-H_2O (10:1:1) containing 2.7 g of 5% Pd-$BaSO_4$, followed by removal of catalyst and evaporation of solvents, gave a heavy syrup which was precipitated from MeOH (30 ml)-THF (700 ml) to give 1.8 g (30% overall) of a white amorphous solid. Final purification was achieved by preparative electrophoresis of 40 mg using 0.4 *M* pyridine acetate (pH 5) to give 24.5 mg of analytically pure material.

L-Pro-L-Dopa-L-Dopa (**48**). A mixture of L-Dopa-L-Dopa (**27**, 1.8 g, 4.8 mmol) and Cbz-Pro-ONP (1.8 g, 4.9 mmol) in 25 ml of DMF was cooled to 0° and Et_3N (0.68 ml, 4.68 mmol) added. The reaction proceeded as described for **44**. Evaporation of the solvent gave 2.1 g of white powder after precipitation from MeOH-Et_2O. A portion (1.2 g) was partitioned between 50 ml each of CH_2Cl_2 and H_2O and the aqueous layer lyophilized to give 0.8 g of white powder. Hydrogenation proceeded in 30 ml of MeOH-AcOH-H_2O (10:1:10) containing 0.35 g of 5% Pd-$BaSO_4$. The catalyst was removed by filtration and the filtrate was evaporated to an oily residue which was taken up in a small volume of H_2O and treated with *i*-PrOH to give 0.32 g (25%) of crystalline product.

L-Ala-D-Dopa (**49**). Condensation of Cbz-L-Ala and *O,O'*-dicarbobenzoxy-D-Dopa benzyl ester hydrochloride (**2a**) proceeded by the DCC method. The protected dipeptide crystallized from EtOAc-Et_2O: mp 140-142°; $[\alpha]^{25}D$ −4.75° (*c* 2.1, $CHCl_3$). *Anal.* ($C_{43}H_{40}N_2O_{11}$) C, H, N. Hydrogenation by the standard procedure gave product which crystallized from MeOH-*i*-PrOH: mp 175-178°; $[\alpha]^{25}D$ −21.55° (*c* 1.1, 1 *M* HCl). *Anal.* ($C_{12}H_{16}N_2O_5 \cdot H_2O$) C, H, N.

Determination of the Optical Purity of L-Dopa. A sample of L-Dopa (98.5 mg, 0.50 mmol) was dissolved in 9.0 ml of borate buffer (pH 10.2, 0.45 *M*) and allowed to react at −10° under argon with L-Ala NCA (57.5 mg, 0.50 mmol) with rapid stirring for 2 min. The solution was acidified with 1 ml of concentrated HCl and the reaction mixture diluted to 25.0 ml and adjusted to pH 2.0. A dilute solution was prepared from 1.0 ml of this solution by addition of 0.01 *M* HCl to 50.0 ml. Resolution of the reaction mixture was achieved on a 30 × 300 mm jacketed column at 60° using Joelco custom resin AR-15 to a column height of 25 cm. Citrate buffer (pH 4.00, 0.20 *M*) was passed at a rate of 0.8 ml/min. Under these conditions the major peaks emerged at 32 min (Ala), 59 min (Dopa), 131 min (L-Ala-D-Dopa), and 156 min (L-Ala-L-Dopa). The ratio of L-Dopa-L-Dopa to L-Ala-D-Dopa was determined from the dilute and concentrated solutions, respectively. L-Ala-L-Dopa (**31**) and L-Ala-D-Dopa (**49**) were independently synthesized to determine retention times and ninhydrin color value for the calculations. Under these conditions the per cent D-Dopa in our starting material was determined to be 0.14%.

Optical Purity of L-Dopa-L-Dopa. A sample of L-Dopa-L-Dopa (**27**, 94.1 mg, 0.50 mequiv) was hydrolyzed with 4 ml of 6 *M* HCl in a sealed tube at 110° for 24 hr. After removal of excess HCl the residue was allowed to react with L-Ala NCA and the mixture of diasteriomers chromatographed as outlined above. The content of L-Ala-D-Dopa in this sample was found to be 2.10%. As a control, L-Dopa was also subjected to the same hydrolytic conditions and then allowed to react with L-Ala NCA. There was 1.28% of D-Dopa found in this sample. Thus, it can be concluded that the synthetic L-Dopa-L-Dopa (**27**) probably[17] contained less than 0.82% of D-Dopa.

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