Synthesis of Sequence Peptide Polymers Related to Collagen[1,2]

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The sequence peptide polymers poly(Pro)-Gly, poly(Hyp(H))-Gly, poly(Ala)-Pro-Gly, poly(Pro)-Hyp(H)-Gly, poly(Ser(H))-Pro-Gly, and poly(Gly)-Gly-Hyp(H)-Gly have been prepared in high optical purity from the corresponding peptide *p*-nitrophenyl esters.[3] All residues were of the L configuration.

Polymers containing proline and hydroxyproline have been prepared ranging from the homopolymers through random copolymers to polymers with repeating sequences. Much of the interest in these polymers arises from their relationship to collagen.[6]

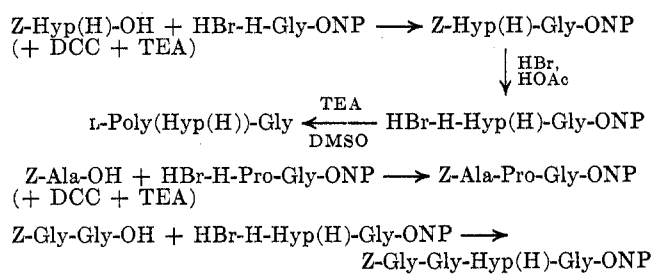
Among sequence polymers reported previously are poly(Gly)-Pro-Hyp,[7] poly(Pro)-Gly-Pro,[8] poly(Pro)-Ala-Gly,[9] poly(Gly)-Pro-Ala,[10] poly(Gly)-Pro-Gly,[10] poly(Pro)-Gly-Gly,[8a] and poly(Ala)-Pro-Gly.[11]

The present paper reports details of the synthesis of the repeating sequence polymers A–F, Table I. Some, such as poly-Pro-Hyp(H)-Gly, have collagen-related sequences, others such as poly-Pro-Gly do not. In this study we have paid special attention to the difficult questions of optical purity of intermediates and of polymers. Various physical studies on these polymers will be reported elsewhere.

Facile racemization of C-terminal residues *via* azlactone formation has been well documented,[12,13] but it is not so well known that C-terminal proline is also racemized under relatively mild conditons.[14] Evidence presented below indicates that polymerization of tripeptides by tetraethyl pyrophosphite can lead to racemization; yet this method has often been used for making collagen analogs.

In the present study we used the active ester route shown in Scheme I. The steps involve only those

SCHEME I[a]



[a] Z is benzyloxycarbonyl, HONP is *p*-nitrophenol, DCC is dicyclohexylcarbodiimide, TEA is triethylamine, DMSO is dimethyl sulfoxide.[3]

shown in previous work to be free of racemization.[5,13] We have further applied two direct criteria for evaluating optical purity. One is the rotations of intermediates (Table I); these are constant on recrystallization or on synthesis from independent batches of starting materials. The other is the rotations observed for hydrolyzed samples. According to the hydrolysis criterion all compounds in Table I are judged to be optically pure within the experimental error of 2–4%. According to the first criterion they are purer.

It is possible to compare one rotation value with a value from the literature. Poly(Pro)-Hyp(H)-Gly (Table I) had $[\alpha]^{25}D$ −400° (*c* 0.1, water). The reported value for a sample of the equivalent Gly-Pro-Hyp(H) was $[\alpha]D$ −280°, and what is described as a second form had $[\alpha]D$ −140°.[7] Poly(Pro)-Hyp(H)-Gly is rather poorly soluble in water while the reported poly(Gly)-Pro-Hyp was fairly soluble. The higher rotation and the lower solubility of polymer prepared by the *p*-nitrophenyl ester route suggests that it is of higher optical purity than polymer prepared using tetraethyl pyrophosphite.

Molecular weights (Table II) were measured by the Archibald method,[15] using both schlieren optics and the Rayleigh interference system, and also by full sedimentation equilibrium. Except for poly(Pro)-Hyp(H)-Gly in water, there is relatively little association. Number average molecular weights as measured in water with a differential vapor pressure "osmometer" are believed correct to ~10%. Dinitrophenylation of the amino end groups gave number average results which tended to be too high. The cause is not known with certainty, but, if there had been as much as 2–3% of benzyloxycarbonyl group as an impurity in the hydrobromide "monomer," then end group values would be close to those observed. Unfractionated condensation polymers such as those prepared by the *p*-nitrophenyl

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(2) With the technical assistance of E. Heimer.

(3) Abbreviation conventions are those recommended by IUPAC-IUB[4] extended so as to specify side-chain substitution explicitly. *Cf.* ref 5, footnote 6. All residues in the present work were of the L configuration.

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Table I

Rotations of Intermediates and Polymers[a]

No.	Compounds	Mol wt	Concn, %	Solvent	Drude terms: $a \times 10^{-1}$	Drude terms: λ_0, nm	Molar rotation, deg: At 589	At 546	Obsd[b]	Expected[b]
1	Z-Ala-OH	223.2	2	HOAc	−10.304	167.67 ± 6	−32.7	−38.3	13.6	14.7[c]
3	Z-Pro-OH	249.3	2	EtOAc	−32.025	185.66	−102	−121	(−71)[d]	−71.4
4	HBr-H-Hyp(H)-Gly-ONP	390.2	2	Water	−91.743	171.03	−289	−341	−71.3	−70.4
5	Z-Hyp(H)-Gly-ONP	443.4	2	DMF	−67.923	220.79	−228	−272	−68.4	−70.4
6	HBr-H-Pro-Gly-ONP	374.2	1.4	DMF	−32.107	162.77	−100	−118	−72.4	−71.4
7	Z-Pro-Gly-ONP	427.4	2	DMF	−81.213	219.70	−272	−325	−72.0	−71.4
8	HBr-H-Ala-Pro-Gly-ONP	445.1	2	CH_3OH	−106.73	218.34	−357	−427	−57.1	−57.2[e]
9	Z-Ala-Pro-Gly-ONP	498.5	0.5	CH_3CN	−123.21	232.49	−421	−505	−56.8	−57.2[e]
10	HBr-H-Pro-Hyp(H)-Gly-ONP	487.1	1	CH_3OH	−143.68	203.99	−471	−560	−144	−142
11	Z-Pro-Hyp(H)-Gly-ONP	540.5	1	CH_3OH	−179.26	218.38	−599	−716	−144	−142
12	HBr-H-Ser(H)-Pro-Gly-ONP	461.1	1	CH_3OH	−105.14	217.62	−351	−419	−56.1	−55.8[f]
13	Z-Ser(H)-Pro-Gly-ONP	514.5	1	CH_3CN	−96.554	230.31	−329	−394	−52	−55.8[f]
14	HBr-H-Gly-Gly-Hyp(H)-Gly-ONP	504.1	1	CH_3OH	−112.66	216.43	−375	−448	−67.2	−70.4
15	Z-Gly-Gly-Hyp(H)-Gly-ONP	557.5	1	HOAc	−99.169	219.58	−332	−397	−70.7	−70.4
A	Poly(Hyp(H))-Gly[g]	170.2	0.6	Water	−126.5	204.91	−414	−493	−71.5	−70.4
B	Poly(Pro)-Gly[g]	154.2	0.1	Water	−132.0	212.00	−440	−525	−72.5	−71.4
C	Poly(Ala-Pro-Gly)[g]	225.2	0.5	DCA	−131.1	206.75	−435	−513	−59	−57.2
D	Poly(Pro)-Hyp(H)-Gly[g]	267.3	0.5	DCA	−248.5	210.76	−820	−980	−146	−142
			0.1	Water	−326.0	201.18	−1060	−1250		
E	Poly(Ser(H))-Pro-Gly[g]	241.2	0.5	DCA	−98.4	219.89	−330	−394	−55.5	−55.8
F	Poly(Gly)-Gly-Hyp(H)-Gly[g]	284.3	0.1	Water	−86.8	205.62	−284	−339	−71	−70.4

[a] The reported Drude equation parameters summarize the optical rotations observed at 589, 578, 546, 435, and in some cases 365 nm. Temperature was 25°. The correlation uncertainty in a and λ_0 is ~1–1.5% relative.[h] The rotations at 589 and 546 nm are believed to be correct to at least 5% relative; most are good to 2% or better. DMF is dimethylformamide. DCA is dichloroacetic acid. [b] Check of optical purity. Peptide was hydrolyzed in 5 *N* HCl for 15 hr at 100°. The molar rotation is reported at 546 nm in 5 *N* HCl at 25°. Some compounds were also hydrolyzed under more vigorous conditions as a check. The expected value is that based on measurements on the amino acid plus simple derivatives, hydrolyzed as above. [c] Values reported by J. P. Greenstein and M. Winitz ("Chemistry of the Amino Acids," Wiley, New York, N. Y., p 116) were averaged by fitting to the Drude equation to give the following $[M]_{546}$ values: H-Ala-OH, 14.9; H-Hypro(H)-OH, −71.8; H-Pro-OH, −73.1°. [d] Estimated from hydrolysis at 120° for 10 hr. [e] $[M]_{546}$ summed for H-Ala-OH + H-Pro-OH = +14.3 − 71.5 = −57.2. [f] $[M]_{546}$ summed for H-Ser(H)-OH + H-Pro-OH = +15.7 − 71.5 = −55.8. [g] Based on elemental analyses for C and N, the following polymer contents (dry basis) were assigned: 92% A, 92% B, 90% C, 89% D, 95% E, 90% F. Water is not readily removed even on prolonged drying. The Drude a value, the molar rotations at 589 and 546 nm, and the observed rotation at 546 nm for hydrolyzed samples were all corrected to dry basis by the factor indicated. In this study several samples of each polymer were used and water contents varied from one to the next. [h] See ref 19b for further details.

Table II

Polymer Molecular Weight Determinations

Polymer	$\bar{V}$[a]	Wt av mol wt: Archibald[b]	FSE[c]	No. av mol wt: DVP[d]	DNP[e]	η[f]
Hyp(H)-Gly	0.667	9,800	8,300	3,700		
		12,800	9,400	5,300	8,000	0.335
			12,800	4,600	14,000	0.406
Pro-Gly	0.716	10,000	7,700	3,100	12,000	0.316
			14,200	5,300	20,000	0.432
Ala-Pro-Gly	0.723		2,500			0.275
Pro-Hyp(H)-Gly	0.701	45,000[g]	42,000		15,000	0.291
Ser(H)-Pro-Gly	0.685	11,000	10,000			0.286
Gly-Gly-Hyp(H)-Gly	0.656		17,000	5,000	30,000	0.443

[a] Partial specific volume, computed from residue values in Table III: H. K. Schachman in "Methods in Enzymology," Vol. IV, S. P. Colowick and N. O. Kaplan, Ed., Academic Press, New York, N. Y., 1957, p 70. [b] 1% solution in water, meniscus values, standard deviation of average ~15%. [c] Full sedimentation equilibrium, 0.1–1% solution in water, standard deviation of average ~8%. [d] Differential vapor pressure molecular weight using a vapor phase osmometer, in water; standard deviation of average ~15%. [e] Dinitrophenylation by "simplified" procedure; standard deviation of average ~15%. [f] Reduced viscosity in dichloroacetic acid at 30°. [g] Other preparations had mot wt 9800 and 23,000.

ester method should have a weight average molecular weight twice the number average.[16]

In spite of the marked tendency of proline peptides to form diketopiperazines,[17] it is interesting that 5–10% yields of the important dipeptide polymers poly(Pro)-Gly and poly(Hyp(H))-Gly can nevertheless be reproducibly obtained.

Proton magnetic resonance has proved especially valuable in monitoring the peptide syntheses and in evaluating the polymers. In most cases it is possible to check quantitatively for the presence of the amino acids and the protecting groups and to ascertain the presence of impurities.[18]

Experimental Section[19]

HBr-H-Hyp(H)-Gly-ONP (4).—Dry hydrogen bromide was passed through a solution of 9.0 g of Z-Hyp(H)-Gly-ONP in 50 ml of trifluoroacetic acid for 45 min. The solution was poured into 500 ml of dry ether; the slurry was stirred and filtered. The crude product was dried under vacuum, stirred with 100 ml of methanol, then filtered, and washed with ether. The yield was quantitative, mp 248° dec. Methylene chloride could be used in place of trifluoroacetic acid. This preparation consistently gave samples with bromide and *p*-nitrophenol titers within 1% (relative) of theory.

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(17) M. Goodman and K. C. Stueben, *J. Amer. Chem. Soc.*, **84**, 1279 (1962).

(18) A table of the nmr resonance positions will appear following these pages in the microfilm edition of this volume of the journal. Single copies may be obtained from the Business Operations Office, Books and Journals Division, American Chemical Society, 1155 Sixteenth St., N.W., Washington, D. C. 20036, by referring to code number JOC-72-4377. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche.

(19) (a) Analyses, ultracentrifuge runs, and optical rotations were performed by Mrs. L. Ross. A few analyses were performed by F. Pascher, Bonn, Germany. Infrared spectra were run on a Perkin-Elmer Infracord (137) or on the Model 21. Nmr data reported in Table III were measured on a Varian A-60. (b) Further procedures are reported by R. J. Albers, N. F. Estrin, and D. F. DeTar, *Biochem. Preparations* **13**, 34 (1971).

Anal. Calcd for $C_{13}H_{16}N_3O_6Br$: C, 40.02; H, 4.13; N, 10.77; Br, 20.48, ONP, 35.39. Found: C, 39.33; H, 4.03; N, 10.81; Br, 20.25; ONP, 35.4.

The ir spectrum (137, KBr) showed 1760 (COONP), 1655 (amide), 1620 (w), 1580 (w), 1560, 1520 cm^{-1}.

Z-Hyp(H)-Gly-ONP (5).—To a stirred mixture of 20.0 g of dicyclohexylcarbodiimide, 26.3 g of HBr-H-Gly-ONP, and 170 ml of acetonitrile at 5° was added over a period of 25 min a solution of 28.0 g of Z-Hyp(H)-OH[20] and 9.11 g of triethylamine in 50 ml of acetonitrile. After 2 hr at room temperature the reaction was complete as judged from the disappearance of carbodiimide (ir). The urea was removed by filtration and extracted twice with 25-ml portions of methylene chloride. The combined solvents were reduced to one-third of their volume and poured into 2 l. of ice–water (pH 2). The product was filtered, dissolved in methylene chloride, and dried over $MgSO_4$, and solvent removed. (In some runs the initial product was an oil; this was taken up in methylene chloride as above.) The material solidified and was crystallized from 200 ml of methanol–ether. Further recrystallization from methanol gave 24 g (55%), mp 139–141°.

Anal. Calcd for $C_{21}H_{21}N_3O_8$: C, 56.88; H, 4.77; N, 9.48; ONP, 31.15. Found: C, 57.16; H, 4.91; N, 9.55; ONP, 30.8.

The ir spectrum (137, KBr) showed 1770 (COONP), 1690 (Z), 1660 (amide), 1610 (w), 1640 (w), 1595, 1520 cm^{-1}.

Poly(Hyp(H))-Gly (A).—To a solution of 10.0 g of HBr-H-Hyp(H)-Gly-ONP in 15.0 ml of dimethyl sulfoxide at 25° was added 3.60 ml of triethylamine. After 1 week 15 ml of ether was added causing copious precipitation. The solvent was decanted; the precipitate was extracted with ethyl acetate and then dialyzed for 3 days in water. Lyophilization gave 0.30 g (7%) polymer. The major product was presumably the diketopiperazine, which was not isolated.

Anal. Calcd for $C_7H_{10}N_2O_3$: C, 49.4; H, 5.9; N, 16.5. Calcd for 92% polymer–8% water: C, 45.4; H, 6.3; N, 15.1. Found: C, 45.6; H, 6.4; N, 15.1.

The ir spectrum (21, KBr) showed 3356, 3077 (w), 2924, 1639, 1543, 1443, 1328, 1198, 1078, 1028 cm^{-1}.

HBr-H-Pro-Gly-ONP (6).—Dry hydrogen bromide was passed through a stirred solution of 356 g of Z-Pro-Gly-ONP in 3 l. of dry methylene chloride (distilled from P_2O_5). The product was separated by filtration, washed with dry ether, with two 400-ml portions of acetonitrile, and with ether, and then dried under reduced pressure at 50° to give 291 g (94%) of hydrobromide, mp 197–198° dec.

Anal. Calcd for $C_{13}H_{16}N_3O_5Br$: C, 41.73; H, 4.31; N, 11.23; Br, 21.35; ONP, 36.9. Found: C, 41.34; H, 3.99; N, 11.13, Br, 20.97; ONP, 36.4.

The ir spectrum (137, KBr) 1760 (COONP), 1660 (amide), 1610 (w), 1590 (w), 1555, 1525 cm^{-1}.

Z-Pro-Gly-ONP (7).—To a stirred mixture of 148.5 g of dicyclohexylcarbodiimide, 190.0 g of HBr-H-Gly-ONP, and 2 l. of acetonitrile at 5° was added over a period of 20 min a solution of 200.0 g of Z-Pro-OH[21,22] and 67.3 g of triethylamine in 600 ml of acetonitrile. After 1 hr at room temperature, the mixture was filtered. The filtrate was reduced to half-volume and poured into 4 l. of ice–water adjusted to pH 2 (HCl). The oily precipitate solidified on stirring and was collected by filtration. The filter cake was extracted with two 200-ml portions of dimethylformamide and these were poured in ice–water at pH 2 (HCl). The combined precipitates were crystallized from methanol–water and then from ethyl acetate giving 211.0 g (70%) of product, mp 139–141°.

Anal. Calcd for $C_{21}H_{21}N_3O_7$: C, 59.01; H, 4.95; N, 9.83; ONP, 32.3. Found: C, 59.48; H, 5.26; N, 9.91; ONP, 31.3.

The ir spectrum (137, KBr) showed 1770 (COONP), 1690 (Z), 1660 (amide), 1610 (w), 1640 (w), 1595, 1520 cm^{-1}.

Poly(Pro)-Gly (B). i.—To a solution of 10.0 g of HBr-H-Pro-Gly-ONP in 10.0 ml of dimethyl sulfoxide at 25° was added 3.7 ml of triethylamine. The solution gelled in 5 min; an additional 5.0 ml of dimethyl sulfoxide was added. After 3 days, 120 ml of ethyl acetate was added causing precipitation of triethylamine hydrobromide and polymer. The precipitate was dissolved in methanol and reprecipitated with ether giving 2.1 g of white powdery solid. This was dialyzed with water for 2 days. Lyophilization gave 1.00 g of polymer.

ii.—Polymerization on the same scale was carried out in 14.0 ml of dimethyl sulfoxide for 3 days. The solvent was removed at room temperature under high vacuum, and the residue was dissolved in 25 ml of water and dialyzed against water. After 2 days neither *p*-nitrophenol nor triethylamine hydrobromide could be detected. The solvent was removed by lyophilization, giving 0.200 g of polymer.

Anal. Calcd for $C_7H_{10}N_2O_2$: C, 54.5; H, 6.5; N, 18.2. Calcd for 96% polymer–4% water: C, 52.4; H, 6.7; N, 17.4. Found: C, 52.4; H, 7.2; N, 17.1.

The ir spectrum (21, KBr) showed 3390, 3077, 3040, 2882, 1639, 1527, 1439, 1323, 1235, 1187, 1157, 1020 cm^{-1}.

HBr-H-Ala-Pro-Gly-ONP (8).—Hydrogen bromide was passed through a suspension of 10.0 g of Z-Ala-Pro-Gly-ONP in 60 ml of acetic acid. The product was precipitated by pouring the solution into 500 ml of dry ether: yield 8.4 g (94%). This material was hydroscopic but upon stirring in warm ethyl acetate was converted to 8.2 g of nonhydroscopic material, mp 190° dec.

Anal. Calcd for $C_{16}H_{21}N_4O_6Br$: C, 43.16; H, 4.75; N, 12.58; Br, 17.95; ONP, 31.01. Found: C, 40.69; H, 4.73; N, 12.6; Br, 18.3; ONP, 29.1.

The ir spectrum (137, KBr) showed 1760 (COONP), 1690 (amide), 1600 (w), 1680 (w), 1510 cm^{-1}.

Z-Ala-Pro-Gly-ONP (9).—To a stirred mixture (5°) of 61.9 g of dicyclohexylcarbodiimide, 110.0 g of HBr-H-Pro-Gly-ONP and 2.2 l. of acetonitrile was added during 1 hr a solution of 72.1 g of Z-Ala-OH[23] and 41.0 ml of triethylamine in 1.1 l. of acetonitrile. After 4 hr at 5°, the mixture was filtered and the filter cake extracted with 250 ml of dimethylformamide. The combined solvents were reduced to one-half volume under reduced pressure and poured into 4 l. of ice–water (pH 2). The precipitate was collected, dissolved in 1 l. of warm methanol, and allowed to crystallize. The prouct was dried (107 g, 73%) and recrystallized from 2.5 l. of methanol to give 91.0 g (62%) of product, mp 155–156°.

Anal. Calcd for $C_{24}H_{26}N_4O_8$: C, 57.82; H, 5.26; N, 11.24; ONP, 27.7. Found: C, 57.5; H, 5.13; N, 11.2; ONP, 27.6.

The ir spectrum (137, KBr) showed 1740 (COONP), 1680 (Z), 1640 (amide), 1610 (w), 1580 (w), 1540, 1510 cm^{-1}.

Poly(Ala)-Pro-Gly (C).—To 6.22 g of HBr-H-Ala-Pro-Gly-ONP in 10.0 ml of dimethyl sulfoxide was added 1.81 ml of triethylamine. After 4 hr 5.0 ml more of solvent was added to promote stirring. This was continued for 4 days. The solvent was removed under vacuum at room temperature, and the residue was transferred to a dialysis bag with water. Some precipitation occurred; after 2 days the water was removed by lyophilization leaving on drying 1.30 g (41%) of polymer.

Anal. Calcd for $C_{10}H_{15}N_3O_3$: C, 53.2; H, 6.7; N, 18.7. Calcd for 89% polymer–11% water: C, 47.4; H, 7.1; N, 16.6. Found: C, 47.7; H, 7.1; N, 16.3.

The ir spectrum (21, KBr) showed 3390, 3077 (sh), 3000 (sh), 1645, 1546, 1379, 1342, 1238, 1198, 1110, 1076 cm^{-1}.

HBr-H-Pro-Hyp(H)-Gly-ONP (10).—Dry hydrogen bromide was passed through a solution of 7.0 g of Z-Pro-Hyp(H)-Gly-ONP in 40 ml of trifluoroacetic acid. This solution was poured into 500 ml of dry ether and stirred in the cold until the precipitate was filterable. The solid was washed with 10% acetonitrile in ether, dried under vacuum, and then stirred with 200 ml of warm ethyl acetate to give 5.4 g (85%) of product, mp 170° dec.

Anal. Calcd for $C_{18}H_{23}N_4O_7Br$: C, 44.36; H, 4.76; N, 11.50; Br, 16.40; ONP, 28.34. Found: C, 43.49; H, 4.63; N, 11.3; Br, 15.6; ONP, 26.2.

The ir spectrum (21, KBr) showed 1779 (COONP), 1684 (amide), 1647 (amide), 1621 (w), 1597 (w), 1567 (w), 1538 cm^{-1}.

Z-Pro-Hyp(H)-Gly-ONP (11).—To a mixture of 20.0 g of HBr-H-Hyp(H)-Gly-ONP, 10.8 g of dicyclohexylcarbodiimide, and 130 ml acetonitrile was added a solution of 14.6 g of Z-Pro-OH and 7.1 ml of triethylamine in 120 ml of acetonitrile over a period of 1 hr. After 4 hr the urea was removed and washed with acetonitrile. The solutions were combined and evaporated to dryness. The residue was slurried in 200 ml of ethyl acetate leving triethylamine hydrobromide undissolved. The product was precipitated by adding 400 ml of ether. After thorough drying, the product was slurried in water to remove traces of triethylamine hydrochloride which otherwise causes the material to become gummy: yield 11.0 g (46%), mp 90–92°.

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(21) A. Berger, J. Kurtz, and E. Katchalski, *J. Amer. Chem. Soc.*, **76**, 5552 (1954).

(22) R. Roeske, F. H. C. Stewart, R. J. Stedman, and V. du Vigneaud, *ibid.*, **78**, 5883 (1956).

(23) A. A. Patchett and B. Witkop, *ibid.*, **79**, 185 (1957).

Anal. Calcd for $C_{26}H_{28}N_4O_9$: C, 57.77; H, 5.22; N, 10.37; ONP, 25.55. Found: C, 57.26; H, 5.36; N, 10.4; ONP, 24.7.

The ir spectrum (21, KBr) showed 1751 (COONP), 1681 (br, Z, amide), 1647 (amide), 1618 (w), 1594 (w), 1546 (w), 1524 cm^{-1}.

Poly(Pro)-Hyp(H)-Gly (D).—To a solution of 2.00 g of HBr-H-Pro-Hyp(H)-Gly-ONP in 4.8 ml of dimethyl sulfoxide was added 0.57 ml of triethylamine. During 4 hr 6.0 ml more of solvent was added as gelling caused the solution to become unstirrable. After 2 days the solvent was removed by lyophilization. The residue was extracted with ether, then dialyzed against water for 3 days. (After 2 days no bromide ion could be detected.) The polymer was separated by lyophilization and dried at 80° under high vacuum: yield 0.65 g (59%).

Anal. Calcd for $C_{12}H_{17}N_3O_4$: C, 53.9; H, 6.4; N, 15.7. Calcd for 95% polymer–5% water: C, 51.2; H, 6.6; N, 14.9. Found: C, 51.6; H, 6.7; N, 14.8.

The ir curve (21, KBr) showed 3413, 2923, 1633, 1546, 1445, 1401, 1333, 1195, 1159, 1081, 1026 cm^{-1}.

HBr-H-Ser(H)-Pro-Gly-ONP (12).—This preparation was similar to that of **10**; the yield was quantitative, mp 202° dec.

Anal. Calcd for $C_{16}H_{21}N_4O_7Br$: C, 41.66; H, 4.59; N, 12.15; Br, 1732; ONP, 29.9. Found: C, 40.46; H, 3.97; N, 12.12; Br, 17.01; ONP, 29.5.

The ir spectrum (137, KBr) showed 1770 (COONP), 1680 (Z), 1640 (amide), 1610 (w), 1580, 1515 cm^{-1}.

Z-Ser(H)-Pro-Gly-ONP (13).—To a cold (5°) stirred mixture of 28.1 g of dicyclohexylcarbodiimide, 50.0 g of HBr-H-Pro-Gly-ONP and 1 l. of acetonitrile was added during 3.5 hr a solution of 35.0 g of Z-Ser(H)-OH and 17.0 ml of triethylamine in 500 ml of acetonitrile. After 1.5 hr the solids were separated and the filter cake was extracted twice with 200 ml of warm acetonitrile. The solvents were combined and taken to dryness; the residue was dissolved in 400 ml of warm ethyl acetate from which the product separated upon cooling and stirring for several hours. This material was washed with water (pH 2) and recrystallized from ethyl acetate giving 33.6 g (49%) of product, mp 142–144°.

Anal. Calcd for $C_{24}H_{26}N_4O_9$: C, 56.03; H, 5.09; N, 10.89; ONP, 26.8. Found: C, 55.69; H, 5.32; N, 10.85; ONP, 26.7.

The ir spectrum (137, KBr) showed 1770 (COONP), 1770 (Z), 1660 (amide), 1640 (amide), 1620 (w), 1530 cm^{-1}.

Poly(Ser(H))-Pro-Gly (E).—To a stirred solution of 6.00 g of HBr-H-Ser(H)-Pro-Gly-ONP in 10.0 ml of dimethyl sulfoxide was added 1.79 ml of triethylamine. After 3 days the solvent was removed by lyophilization. The residue was washed successively with 250-ml portions of ether, methanol, chloroform, and ether employing a preparative centrifuge (10,000 rpm). The polymer was dried under vacuum at 80° for 3 days: yield 1.90 g (61%).

Anal. Calcd for $C_{10}H_{15}N_3O_4$: C, 49.8; H, 6.3; N, 17.4. Calcd for 95% polymer–5% water: C, 47.3; H, 6.5; N, 16.6. Found: C, 47.3; H, 6.3; N, 16.5.

The ir spectrum (21, KBr) showed 3356, 3076, 2941, 1639, 1529, 1443, 1330, 1235, 1053 cm^{-1}.

HBr-H-Gly-Hyp(H)-Gly-ONP (14).—Hydrogen bromide was passed through a solution of 4.4 g of Z-Gly-Gly-Hyp(H)-Gly-ONP and 25 ml of trifluoroacetic acid for 1 hr. The solvents were evaporated at 25° and 300 ml isopropyl alcohol added. The precipitate was stirred in the cold overnight, collected, washed with additional isopropyl alcohol, then with ether, and dried at 50° under vacuum: yield 4.0 g, mp 212° dec.

Anal. Calcd for $C_{17}H_{22}N_5O_8Br$: C, 40.48; H, 4.37; N, 13.89; Br, 15.87; ONP, 27.38. Found: C, 40.17; H, 4.55; N, 13.84; Br, 15.57; ONP, 26.6.

The ir spectrum (21, KBr) showed 1767 (COONP), 1681 (amide), 1653 (amide), 1631 (amide), 1600, 1563, 1527, 1492 (w) cm^{-1}.

Z-Gly-Gly-Hyp(H)-Gly-ONP (15).—To a stirred mixture of 5.28 g of dicyclohexylcarbodiimide, 10.0 g of HBr-H-Hyp(H)-Gly-ONP, and 100 ml of acetonitrile at room temperature was added during 2 hr a solution of 8.52 g of Z-Gly-Gly-OH and 2.86 g of triethylamine in 200 ml of acetonitrile. The mixture was stirred for 18 hr and filtered. The filter cake was slurried in 100 ml of warm dimethylformamide which was filtered into 1.5 l. of ice–water (pH 2) and the mixture was stirred overnight. The precipitate was collected, dried, and recrystallized from methanol giving 5.0 g (35%) of product, mp 184–187°.

Anal. Calcd for $C_{25}H_{27}N_5O_{10}$: C, 53.86; H, 4.88; N, 12.56; ONP, 24.77. Found: C, 53.34; H, 5.04; N, 12.68; ONP, 25.0.

The ir spectrum (21, KBr) showed 1773 (COONP), 1695 (Z), 1661 (amide), 1621 (amide), 1595 (w), 1565, 1548, 1524 cm^{-1}.

Poly(Gly)-Gly-Hyp(H)-Gly (F).—To a stirred solution of 3.50 g of HBr-H-Gly-Gly-Hyp(H)-Gly-ONP and 7.0 ml dimethyl sulfoxide was added 0.96 ml of triethylamine. The solution was stirred for 3 days and then lyophilized. The residue was washed into a dialysis bag with water and dialyzed for 1 week against water. The water was removed by lyophilization, and the powders were dried at 80° for 2 days under vacuum to yield 0.90 g of polymer (46%).

Anal. Calcd for $C_{11}H_{16}N_4O_5$: C, 46.47; H, 5.67; N, 19.71; O, 28.14. Found: C, 46.4; H, 5.8; N, 18.63.

The ir spectrum (21, KBr) showed 3344, 2933 (sh), 1650, 1538, 1471, 1408, 1333, 1235, 1198 (w), 1160 (w), 1079, 1027 cm^{-1}.

Registry No.—**1,** 1142-20-7; **3,** 1148-11-4; **4,** 35016-72-9; **5,** 35006-40-7; **6,** 35016-73-0; **7,** 6464-84-2; **8,** 36358-38-0; **9,** 35006-34-9; **10,** 36358-40-4; **11,** 36358-41-5; **12,** 35761-25-2; **13,** 36358-43-7; **14,** 36358-44-8; **15,** 35006-39-4; A, 28186-03-0; B, 27252-06-8; C, 26523-49-9; D, 25734-60-5; E, 26523-51-3; F, 28186-05-2.