collected on the filter. A small additional crop was obtained from the mother liquors, total wt 2.7 g (57.9%), mp 84–86°.

A sample melting at 82–86° was recrystallized three times from ethyl acetate–ether–petroleum ether (1:1:5). The soft, white needles melted at 87.5–89°, $[\alpha]^{28.5}D$ −42.4° (*c* 5, dimethylformamide).

Anal. Calcd for $C_{12}H_{12}N_2O_4$ (248.23): C, 58.1; H, 4.87; N, 11.3. Found: C, 58.0; H, 4.77; N, 11.1.

A small sample was treated for 30 min with an anhydrous solution of ethylene dichloride containing 20 equiv of hydrogen bromide. The reaction mixture was then diluted with anhydrous ether. The white solid which separated was collected and crystallized from methanol–ether. Paper electrophoresis in barbital buffer at pH 8.6 and amino acid analysis both indicated isoasparagine in good yield.[12b] Its composition and reaction with sodium–ammonia–methanol are given in Table I.

Acknowledgment. We thank Miss Audrey L. Hughes, Mrs. Harriet R. Levie, and Mr. Arnold Benjamin for amino acid analyses and valuable assistance.

The Synthesis of Glycyl-L-prolylglycyl and Glycyl-L-prolyl-L-alanyl Oligopeptides and Sequential Polypeptides[1]

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Abstract: The syntheses of the sequential oligomers, H(-Gly-L-Pro-Gly-)$_n$OH and H(-Gly-L-Pro-L-Ala-)$_n$OH (n = 1, 2, and 4) are reported. Poly(glycyl-L-prolylglycine) and poly(glycyl-L-prolyl-L-alanine) were prepared by the polymerization of the *p*-nitrophenyl esters of the appropriate tripeptides. Weight-average molecular weights of up to 6000 for poly(glycyl-L-prolylglycine) and 15,000 for poly(glycyl-L-prolyl-L-alanine) were obtained.

Collagen, the most abundant structural protein, has recently been shown to have a unique triple helical structure and a gross amino acid composition strikingly different from other proteins. The triple helical structure was originally postulated by Ramachandran[3] on the basis of X-ray diffraction analysis in 1954 and subsequently modified by Rich and Crick[4] and by him.[5] Chemical analyses of several collagens reveal that approximately a third of the amino acid residues are glycine and that about one-fourth of the remaining amino acid residues are L-proline and hydroxyl-L-proline.[6] The isolation of comparatively large amounts of glycyl-L-proline[7] and the later isolation of glycyl-L-prolylhydroxy-L-proline[8] suggested a specific role for glycine and the pyrrolidine-containing amino acids. When Grassman and co-workers[9] reported data which strongly supported the idea that every third residue in collagen is a glycine, several investigators concluded that the "collagen fold" was caused by the repeating unit, glycyl-L-prolyl-X. The unusual optical rotatory behavior of poly-L-proline[10] and of the random copolymers of sarcosine and L-proline[11] suggested the study of ordered peptides containing the (glycyl-L-prolyl-X) unit. Kitaoka, Sakakibara, and Tani were first to attempt a synthesis of an ordered polymer containing the unit, L-prolyl-X-glycyl. L-Prolyl-L-leucylglycine was polymerized employing tetraethyl pyrophosphite, but the isolated polymer was not amenable to study in aqueous media.[12]

In this paper we shall describe the stepwise synthesis and physical properties of two dodecamers, glycyl-L-prolylglycylglycyl-L-prolylglycylglycyl-L-prolylglycylglycyl-L-prolylglycine and glycyl-L-prolyl-L-alanylglycyl-L-prolyl-L-alanylglycyl-L-prolyl-L-alanylglycyl-L-prolyl-L-alanine and the synthesis and certain physical properties of poly(glycyl-L-prolylglycine) and poly(glycyl-L-prolyl-L-alanine). While our work was in progress Debabov, Andreeva, and co-workers reported their studies of poly(glycyl-L-prolylhydroxyl-L-proline).[13]

The synthesis of the dodecapeptide, H(-Gly-L-Pro-Gly-)$_4$OH is summarized in Chart I.

The synthesis of glycyl-L-prolylglycine has been reported by Davis and Smith.[14] Our synthesis differed. Carbobenzyloxyglycine was converted in high yield to carbobenzyloxyglycine thiophenyl ester with

(1) (a) This is Polypeptides L. For the preceding paper in this series see E. R. Simons and E. R. Blout, *Biochim. Biophys. Acta*, **92**, 197 (1964). (b) We are pleased to acknowledge support of this work in part by the Office of the Surgeon General, Department of the Army.

(2) Research Laboratories, Polaroid Corp., Cambridge 39, Mass.

(3) G. N. Ramachandran and G. Kartha, *Nature*, **174**, 269 (1954); **176**, 593 (1955).

(4) A. Rich and F. H. C. Crick, *ibid.*, **176**, 915 (1955).

(5) (a) G. N. Ramachandran and V. Sasisekharan, *ibid.*, **190**, 1004 (1961); (b) G. N. Ramachandran in "Aspects of Protein Structure," Academic Press Inc., New York, N. Y., 1963, p 39.

(6) The amino acid contents of several collagens are summarized in a review by G. R. Tristram and R. H. Smith, *Advan. Protein Chem.*, **18**, 227 (1963).

(7) W. A. Schroeder, L. M. Kay, J. LeGette, L. Honnen, and F. C. Green, *J. Am. Chem. Soc.*, **76**, 3556 (1954).

(8) T. D. Kroner, W. Tabroff, and J. J. McGarr, *ibid.*, 77, 3356 (1955).

(9) (a) W. Grassman, K. Hannig, H. Endres, and A. Riedely, *Z. Physiol. Chem.*, 307, 87 (1956); (b) W. Grassman, K. Hannig, and M. Schleyer, *ibid.*, **322**, 71 (1960).

(10) (a) A. Berger, J. Kurtz, and E. Katchalski, *J. Am. Chem. Soc.*, **76**, 5552 (1954); (b) E. R. Blout and G. D. Fasman in "Recent Advances in Gelatin and Glue Research," G. Stainsby, Ed., Pergamon Press Ltd., London, 1958, p 122; (c) I. Z. Steinberg, W. F. Harrington, A. Berger, M. Sela, and E. Katchalski, *J. Am. Chem. Soc.*, **82**, 5263 (1960); (d) E. R. Blout, J. P. Carver, and J. Gross, *ibid.*, **85**, 644 (1963); (e) G. D. Fasman and E. R. Blout, *Biopolymers*, **1**, 3 (1963).

(11) G. D. Fasman and E. R. Blout, *ibid.*, **1**, 99 (1963).

(12) H. Kitaoka, S. Sakakibari, and H. Tani, *Bull. Chem. Soc. Japan*, **31**, 802 (1958).

(13) See N. S. Andreeva, M. I. Millionova, and Yu. N. Chirgadze in "Aspects of Protein Chemistry," Academic Press Inc., New York, N. Y., 1963, p 137.

(14) N. C. Davis and E. L. Smith, *J. Biol. Chem.*, **200**, 373 (1953).

Chart I

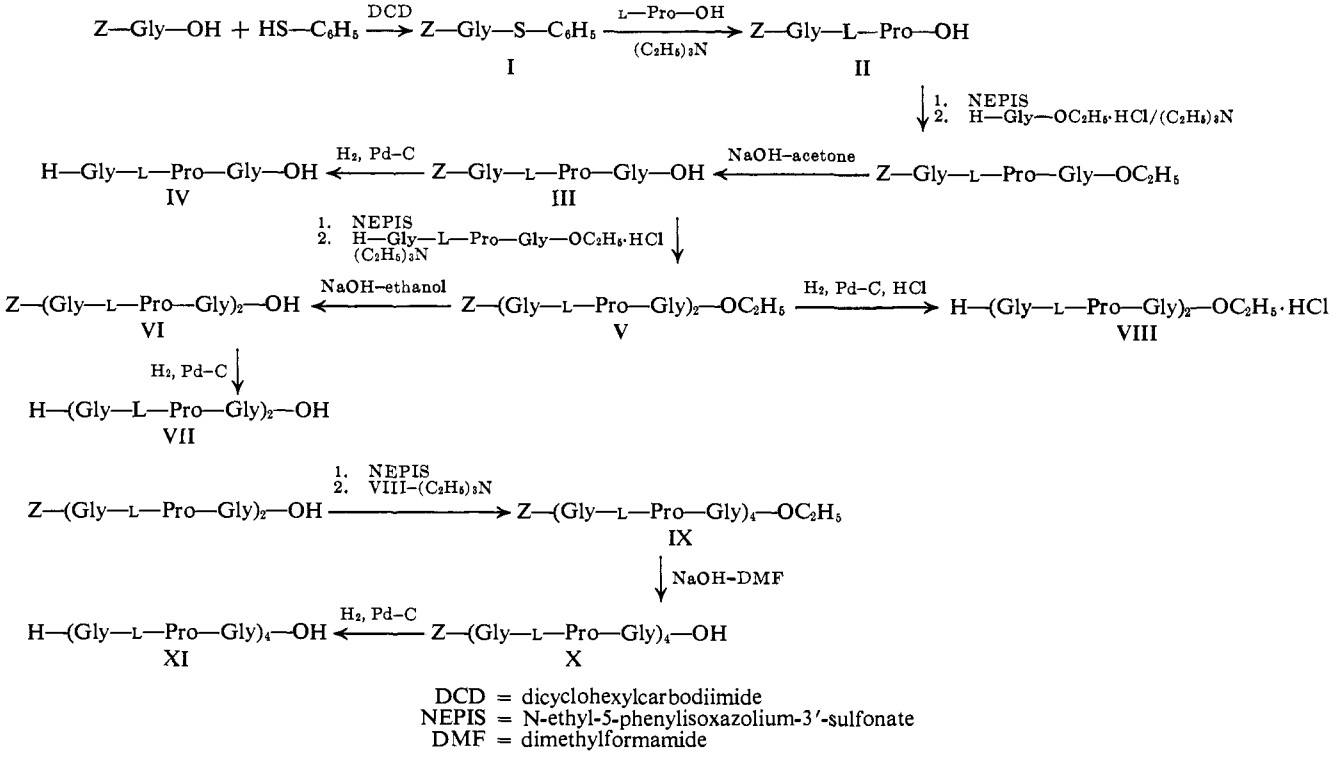
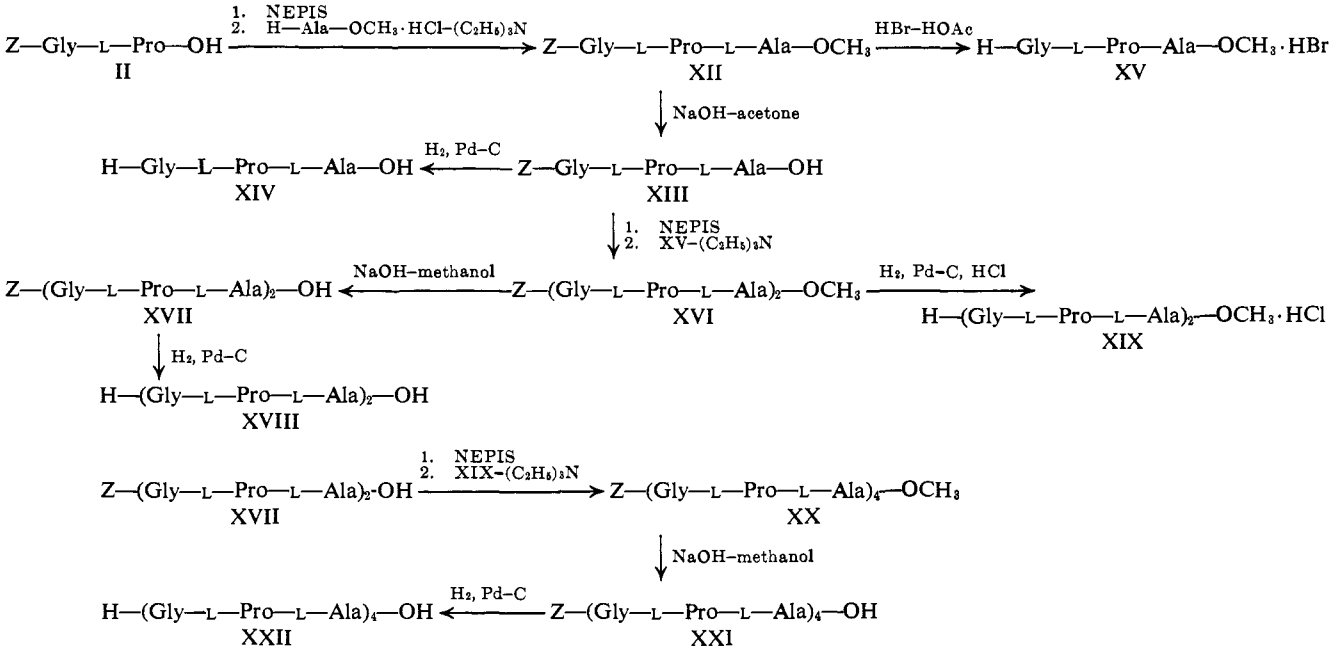


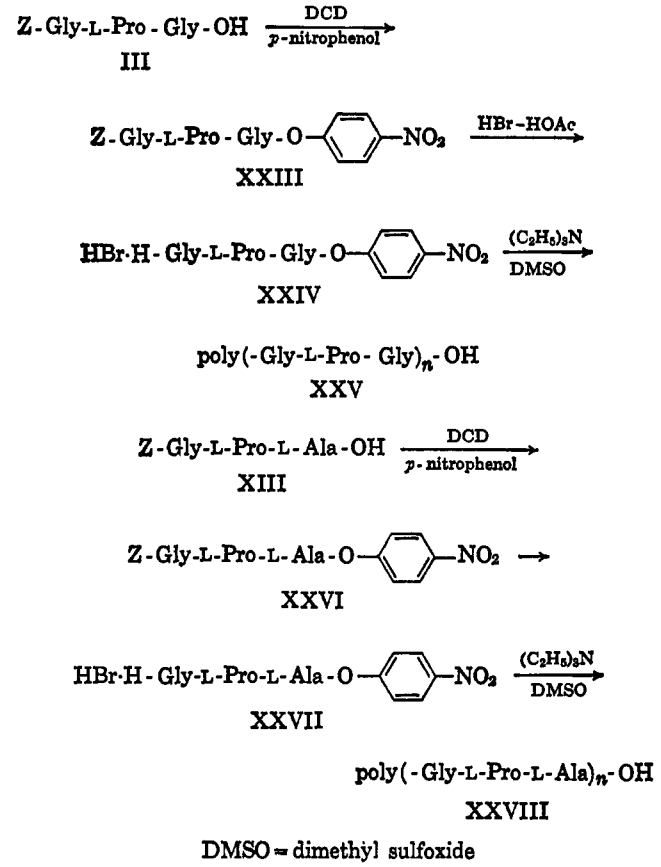
Chart II



dicyclohexylcarbodiimide rather than *via* the mixed anhydride used by Wieland.[15] The thioester was transformed to carbobenzyloxyglycyl-L-proline as described by Rydon and Smith[16] except that triethylamine rather than aqueous sodium hydroxide was used as the acid binding agent. N-Ethyl-5-phenylisoxazolium-3′-sulfonate[17] was used to condense the blocked dipeptide with ethyl glycinate. Basic hydrolysis of the syrupy product gave crystalline carbobenzyloxyglycyl-L-prolylglycine in high yield. Hydrogenolysis gave the free tripeptide.[14] The crystalline hexapeptide, glycyl-L-prolylglycylglycyl-L-prolylglycine,[18] was made by first condensing carbobenzyloxyglycyl-L-prolylglycine with glycyl-L-prolylglycine ethyl ester followed by saponification and hydrogenolysis. Recently, Reader and Smith described syntheses of the intermediates, carbobenzyloxyglycyl-L-prolylglycylglycyl-L-prolylglycine and the ethyl ester.[19] The dodecamer was made from the intermediate, carbobenzyloxyglycyl-L-prolylglycyl-

(15) T. Wieland, W. Schafer, and E. Bokelman, *Ann.*, **573**, 99 (1951).
(16) H. N. Rydon and P. W. G. Smith, *J. Chem. Soc.*, 3642 (1956).
(17) R. B. Woodward, R. A. Olafson, and H. Mayer, *J. Am. Chem. Soc.*, **83**, 1010 (1961).
(18) V. G. Debabov and V. A. Shibinev, *Izv. Akad. Nauk SSSR, Otd. Khim. Nauk*, 1031 (1962).
(19) J. A. Reader and P. W. G. Smith, *J. Chem. Soc.*, 3479 (1965).

Chart III



DMSO = dimethyl sulfoxide

glycyl-L-prolylglycine ethyl ester. On hydrogenolysis with Pd–C in the presence of hydrochloric acid a crystalline hydrochloride was obtained which could be condensed with carbobenzyloxyglycyl-L-prolylglycylglycyl-L-prolylglycine using N-ethyl-5-phenylisoxazolium-3′-sulfonate. The blocked dodecamer, carbobenzyloxyglycyl-L-prolylglycylglycyl-L-prolylglycylglycyl-L-prolylglycylglycyl-L-prolylglycine ethyl ester on saponification and hydrogenolysis gave crystalline glycyl-L-prolylglycylglycyl-L-prolylglycylglycyl-L-prolylglycylglycyl-L-prolylglycine. The synthesis of the dodecapeptide, H(-Gly-L-Pro-Ala-)$_4$OH, is summarized in Chart II.

The steps outlined in Chart II are very similar to those involved in the synthesis of H(-Gly-L-Pro-Gly-)$_4$OH, and require no further comment.

The polymers were synthesized by the polymerization of the tripeptide *p*-nitrophenyl esters employing the conditions of DeTar.[20] This particular application of *p*-nitrophenyl esters had been suggested by Bodansky.[21] Carbobenzyloxyglycyl-L-prolylglycine and carbobenzyloxyglycyl-L-prolyl-L-alanine were converted to their *p*-nitrophenyl esters with dicyclohexylcarbodiimide and the carbobenzyloxy blocking groups removed with dry hydrogen bromide in acetic acid. The glycyl-L-prolylglycine *p*-nitrophenyl ester hydrobromide[22] and glycyl-L-prolyl-L-alanine *p*-nitrophenyl ester hydrobromide were neutralized and polymerized in dimethyl sulfoxide. The molecular weights of the polymers were determined[23] by the Archibald approach to equilibrium method (see Chart III). The poly(glycyl-L-prolylglycine) was estimated to have a weight-average molecular weight of 6000 and poly(glycyl-L-prolyl-L-alanine) of 15,000.

Previous workers[17] found little or no racemization when N-ethyl-5-phenylisoxazolium-3′-sulfonate was used to form peptide bonds. However, the importance of optical purity in relation to structural determinations of the peptides and polypeptides reported here containing L-alanyl residues necessitates determinations of the extent of racemization, if any. The optical purity of the alanyl residues in XIV, XXIII, and XXVIII is being determined by enzymic methods. Preliminary results indicate that little or no racemization has occurred with the procedures used.

Experimental Section

General. N-Ethyl-5-phenylisoxazolium-3′-sulfonate (Woodward's reagent K) was purchased from Pilot Chemical Co., Watertown, Mass., and is referred to as NEPIS for brevity. All analytical samples were dried at 100° under high vacuum for 12 hr. The recorded melting points are uncorrected. On examination employing thin layer chromatography using butanol–acetic acid–pyridine–water (30:6:24:20), the analytical samples moved as single spots. The plates were prepared with silica gel H (E. Merck). The Gly-L-Pro-Gly unblocked oligomers in formic–acetic acid buffer moved as single spots using high-voltage paper electrophoresis.

Carbobenzyloxyglycine Thiophenyl Ester (I). Thiophenol (16.5 g, 0.15 mole) was dissolved in 300 ml of dry dichloromethane. The reaction vessel was externally cooled and magnetically stirred. Carbobenzyloxyglycine (30.5 g, 0.15 mole) and 31.4 g (0.02 mole) of dicyclohexylcarbodiimide were then added. The temperature was kept at 0° for 2 hr and then at room temperature overnight. The precipitated dicyclohexylurea was removed. The filtrate was concentrated to a small volume *in vacuo* and was again filtered to remove an additional small amount of dicyclohexylurea. The dichloromethane was removed *in vacuo* and the resultant oil was taken up in 25 ml of ethyl acetate. Hexane was added and the solution was allowed to stand at 5° overnight. The product crystallized and was washed with 2% sodium bicarbonate solution followed by water, mp 71–73°, 34.5 g (76%) (lit.[15] mp 72°).

Carbobenzyloxyglycyl-L-proline (II). Carbobenzyloxyglycine thiophenyl ester (30.1 g, 0.1 mole), L-proline (11.5 g, 0.1 mole), and triethylamine (27.6 ml, 0.2 mole) were dissolved in 150 ml of methanol and refluxed overnight. The solvent was removed under reduced pressure. Water (100 ml) was added and the oil and aqueous layer so obtained were extracted with ether. The aqueous layer was separated from the ether layer and carefully acidified to pH 2 with 3 *N* hydrochloric acid. The resulting oil crystallized on scratching, weight 19.9 g (66.0%), mp 155–157° (lit.[16] mp 155°).

Carbobenzyloxyglycyl-L-prolylglycine (III). NEPIS (15.1 g, 0.06 mole) was suspended in 120 ml of dry acetonitrile and magnetically stirred. Carbobenzyloxyglycyl-L-proline (18.4 g, 0.06 mole) in 120 ml of acetonitrile containing 8.64 ml (0.06 mole) of triethylamine was added maintaining the reaction vessel below room temperature. After about 2 hr a clear solution was obtained. A suspension of 8.34 g (0.06 mole) of ethyl glycinate hydrochloride in 120 ml of acetonitrile containing 8.64 ml (0.06 mole) of triethylamine was added to the clear solution and stirring was continued overnight. After filtration, the solvent was removed under reduced pressure and 300 ml of water added. The oily residue was extracted into chloroform (four 200-ml portions). The chloroform layer was washed with 2% sodium bicarbonate solution and brine, and dried over anhydrous magnesium sulfate. Removal of the solvent under reduced pressure gave carbobenzyloxyglycyl-L-prolylglycine ethyl ester as a thick oil weighing 23.2 g (99.1%). The product was dissolved in 114 ml of ethanol and cooled, and 62.6 ml of 1 *N* sodium hydroxide solution was added. The mixture was

(20) D. F. DeTar, W. Honsberg, U. Honsberg, A. Wieland, M. Gouge, H. Bach, A. Tahara, W. S. Brinigar, and F. F. Rogers, Jr., *J. Am. Chem. Soc.*, **85**, 2873 (1963).

(21) M. Bodansky, private communication to E. R. Blout and S. M. Bloom.

(22) Reader and Smith recently reported the synthesis of this compound[19] by substantially the same route as reported in this paper. We have included our experimental details since slight changes in the quantity of hydrogen bromide associated with the tripeptide *p*-nitrophenyl ester hydrobromide affects the degree of polymerization (*vide infra*).

(23) P. J. Oriel and E. R. Blout, *J. Am. Chem. Soc.*, **88**, 2041 (1966).

magnetically stirred for 6 hr at room temperature, filtered, cooled, and acidified with 3 *N* hydrochloric acid. After concentrating under reduced pressure, the oil which separated was taken up in chloroform, washed with water, and dried with anhydrous magnesium sulfate. The solvent was removed under reduced pressure and residual material was dissolved in a minimal volume of ethanol–ethyl acetate (1:1). The crystalline product separated on standing in the refrigerator overnight, 13.0 g (60.4%), mp 137–139°, $[\alpha]^{25}_{546}$ −87.2° (*c* 1.2, ethanol) (lit.[14] mp 144–145°, $[\alpha]^{21}D$ −80.9° (*c* 1.0, H_2O)).

Glycyl-L-prolylglycine (IV). The procedure of Davis and Smith[14] was employed. The crystalline tripeptide was obtained by hydrogenolysis of III, $[\alpha]^{25}_{546}$ −115° (*c* 0.14, H_2O), $[\alpha]^{24}D$ −101° (*c* 0.14, water), (lit.[14] $[\alpha]^{21}D$ −108°).

Carbobenzyloxyglycyl-L-prolylglycylglycyl-L-prolylglycine Ethyl Ester (V). To a cold suspension of carbobenzyloxyglycyl-L-prolylglycine (7.2 g, 0.02 mole) in dry acetonitrile (46 ml), anhydrous triethylamine (2.96 ml, 0.021 mole) was added. After magnetically stirring a few minutes, a homogeneous solution was obtained to which 46 ml of dry acetonitrile and NEPIS (5.09 g, 0.02 mole) were added in the cold. The solution became clear after about 4 hr. To a chilled suspension of finely powdered glycyl-L-prolylglycine ethyl ester hydrochloride[15] (5.91 g, 0.02 mole) in 90 ml of dry acetonitrile was added 2.96 ml (0.021 mole) of anhydrous triethylamine. After stirring for 10 min, the free peptide ester was transferred to the reaction vessel. After stirring for 6 hr, the product precipitated, 8.2 g, mp 130–140°. Crystallization of this material from ethanol–ethyl acetate (1:19) afforded 5.8 g of V, mp 157–158°. Excess acetonitrile from the original filtrate was evaporated off under reduced pressure. The residue was diluted with 150 ml of cold water and continuously extracted with 200 ml of ethyl acetate. On cooling crystals separated from the ethyl acetate, 1.8 g, mp 159–160°. The total yield obtained was 7.6 g (62.8%). For analysis the compound was again crystallized from ethanol–ethyl acetate, mp 161–163°, $[\alpha]^{24}_{546}$ −107.5° (*c* 0.18, ethanol) (lit.[19] mp 163–164°, $[\alpha]^{29}D$ −88° (*c* 0.62, ethanol)).

Anal. Calcd for $C_{28}H_{38}O_9N_6$: C, 56.67; H, 6.23; N, 13.67. Found: C, 56.59; H, 6.60; N, 13.91.

Carbobenzyloxyglycyl-L-prolylglycylglycyl-L-prolylglycine (VI). Carbobenzyloxyglycyl-L-prolylglycylglycyl-L-prolylglycine ethyl ester (2 g, 59.5 mmoles) was dissolved in 40 ml of warm ethanol. After cooling to room temperature, 6.6 ml of cold 1 *N* NaOH solution was added. The mixture was stirred for 5 hr and acidified carefully to pH 2 with 1 *N* HCl. Five milliliters of water was added and the clear solution was concentrated under reduced pressure at 45°. On standing at 3° for 48 hr, 1.75 g (89.5%) of the free acid was obtained, mp 212–214°. A small portion was crystallized from ethyl acetate–ethanol, mp 216–217°, $[\alpha]^{24}_{546}$ −125° (*c* 0.21, water) (lit.[19] mp 218–219°, $[\alpha]^{29}D$ −80° (*c* 0.42 ethanol)).

Anal. Calcd for $C_{26}H_{36}O_{10}N_6H_2O$: C, 52.69; H, 6.12; N, 14.18. Found: C, 53.43; H, 6.11; N, 14.00.

Glycyl-L-prolylglycylglycyl-L-prolylglycine (VII). Carbobenzyloxyglycyl-L-prolylglycylglycyl-L-prolylglycine monohydrate (0.59 g, 1.81 mmoles) was dissolved in a warm mixture of 100 ml of ethanol and 25 ml of water, and cooled to room temperature. A suspension of 0.3 g of 10% Pd–C in 30 ml of absolute ethanol was added and the hydrogenolysis was allowed to proceed with magnetic stirring for 6 hr. An additional 90 ml of water was then added and the hydrogenolysis was continued for another 10 hr. The reaction mixture was filtered and the filtrate was concentrated to a small volume at 40° under reduced pressure and again filtered. Anhydrous ethanol (20 ml) was added and the mixture was concentrated under reduced pressure at room temperature. On standing at 3° precipitation occurred. The solid was collected and dried, 0.36 g (76.7%). A small portion was crystallized for analysis by dissolving the compound in a minimal amount of water and adding three times the volume of ethanol. On standing at 3° for 10 hr, the compound crystallized, $[\alpha]^{24}_{546}$ −110.8° (*c* 0.16, ethanol) (lit.[18] $[\alpha]^{32}D$ −76° (*c* 0.5, acetic acid)).

Anal. Calcd for $C_{18}H_{28}N_6O_8$: C, 47.36; H, 6.18; N, 18.41. Found: C, 47.09; H, 6.61; N, 18.25.

Glycyl-L-prolylglycylglycyl-L-prolylglycine Ethyl Ester Hydrochloride (VIII). Carbobenzyloxyglycyl-L-prolylglycylglycyl-L-prolylglycyl ethyl ester (2 g, 59.5 mmoles) was dissolved in 90 ml of warm ethanol and cooled, and 0.5 ml of 10 *N* HCl was added. After addition of 0.9 g of 10% Pd–C suspended in 15 ml of ethanol, the solution was stirred magnetically for 10 hr during hydrogenolysis. A solid precipitated, 30 ml of water was added, and the hydrogenolysis was continued for another 10 hr. The reaction mixture was filtered and the filtrate was evaporated under reduced pressure at 40° to a small volume. Thirty milliliters of absolute ethanol was added and the solution was evaporated again. The process was repeated giving an amorphous material which was dissolved in 20 ml of warm absolute ethanol and filtered. On cooling a powder separated, was filtered, air dried, and stored in a vacuum desiccator over sodium hydroxide pellets, 1.41 g (84.4%), mp 220–222°, $[\alpha]^{24}_{546}$ −130.8° (*c* 0.13, water).

Anal. Calcd for $C_{20}H_{33}N_6O_7Cl$: C, 47.57; H, 6.54; Cl, 6.94. Found: C, 47.04; H, 6.48; Cl, 6.57.

Carbobenzyloxyglycyl-L-prolylglycylglycyl-L-prolylglycylglycyl-L-prolylglycylglycyl-L-prolylglycine Ethyl Ester (IX). Carbobenzyloxyglycyl-L-prolylglycylglycyl-L-prolylglycine (1.57 g, 2.73 mmoles) was suspended in 20 ml of dry acetonitrile and 0.38 ml (2.74 mmoles) of anhydrous triethylamine was added. After stirring for 15 min, another 20 ml of dry acetonitrile and 0.692 g (2.73 mmoles) of NEPIS were added. The suspension was magnetically stirred for 4 hr, an almost homogeneous solution being obtained. The solution was filtered and added to a cold suspension of finely powdered glycyl-L-prolylglycylglycyl-L-prolylglycine ethyl ester hydrochloride (1.38 g, 2.74 mmoles) in 20 ml of dry acetonitrile containing 0.38 ml (2.74 mmoles) of anhydrous triethylamine. An additional 15 ml of dry acetonitrile was used for complete transfer of the above suspension. On stirring for 2 hr, the reaction mixture became progressively turbid and powdery material started to separate. The stirring was continued for 36 hr at room temperature. The precipitated material was filtered and washed with 5 ml of cold ethanol, 1.94 g, mp 245–255° dec. The compound was dissolved in a warm mixture of 75 ml of ethanol and 21 ml of water, filtered, and concentrated under reduced pressure at 45° to a small volume. On standing in the cold for 15 hr, a crystalline solid, 1.18 g (42.4%), was obtained, mp 274–276° dec, $[\alpha]^{24}_{546}$ −126.8° (*c* 0.18, 1:1 DMF–water).

Anal. Calcd for $C_{46}H_{64}O_{13}N_2$: C, 53.91; H, 6.25; N, 16.41. Found: C, 53.81; H, 6.68; N, 15.81.

Carbobenzyloxyglycyl-L-prolylglycylglycyl-L-prolylglycylglycyl-L-prolylglycylglycyl-L-prolylglycine (X). The ester IX (125 mg, 1.41 mmoles) was dissolved in a warm mixture of 15 ml of water and 3 ml of spectro quality dimethylformamide and the solution was cooled to 15°. Sodium hydroxide (1 *N*, 0.15 ml) was added and the reaction mixture was magnetically stirred for 6 hr at room temperature. The solution was acidified in the cold with 1 *N* HCl to pH 2, filtered, and refrigerated for 15 hr during which time some crystalline material separated. The compound was filtered and air dried, 58 mg, mp 259–260°. The filtrate was evaporated under reduced pressure to dryness and dissolved in 1 ml of water by warming; 3 ml of dry ethanol was added. On standing in the cold for 12 hr, an additional 44 mg of the acid was obtained, mp 254–255°. A mixture melting point of the two fractions melted from 258 to 259°. The yield was 84%. A small amount was crystallized from a mixture of ethanol and water, mp 262–263° dec, $[\alpha]^{24}_{546}$ −121.3° (*c* 0.18, 50% ethanol).

Anal. Calcd for $C_{44}H_{60}N_{12}O_{15}$: C, 53.01; H, 6.02; N, 16.86. Calcd for $C_{44}H_{60}N_{12}O_{15}\cdot 2.5H_2O$; C, 50.46; H, 6.61; N, 16.04. Found: C, 50.72; H, 6.25; N, 16.14.

Glycyl-L-prolylglycylglycyl-L-prolylglycylglycyl-L-prolylglycylglycyl-L-prolylglycine (XI). Carbobenzyloxyglycyl-L-prolylglycylglycyl-L-prolylglycylglycyl-L-prolylglycylglycyl-L-prolylglycine (0.343 g, 0.330 mmole) was dissolved in 35 ml of 1:1 methanol–water by warming. The solution was magnetically stirred and hydrogenated at atmospheric pressure with a stream of hydrogen employing 0.4 g of 10% Pd–C in 15 ml of 1:1 methanol–water. After 6 hr 20 ml of water was added and the hydrogenolysis was continued for 24 hr. After filtering twice through Celite, the filtrate was concentrated to near dryness under reduced pressure at 45°. On addition of ethanol a white solid precipitated which was filtered and dried, 140 mg. The solid was taken up in water and filtered, and the solution was lyophilized. Seventy-five milligrams of a white solid was obtained which was dried *in vacuo* for analysis, $[\alpha]^{25}_{546}$ −142.6° (*c* 0.10, water).

Anal. Calcd for $C_{36}H_{54}N_{12}O_{13}\cdot 2H_2O$: C, 48.11; H, 6.46; N, 18.71; O, 26.62. Found: C, 48.19; H, 6.78; N, 18.63; O, 26.58.

Carbobenzyloxyglycyl-L-prolyl-L-alanine Methyl Ester (XII). N-Ethyl-5-phenylisoxazolium-3′-sulfonate (2.52 g, 0.01 mole) was suspended in 20 ml of dry acetonitrile, and a solution of 3.06 g (0.01 mole) of carbobenzyloxyglycyl-L-proline and 1.44 ml (0.01 mole) of triethylamine in 20 ml of dry acetonitrile was added. The mixture was stirred magnetically at 15° for 30 min and then stirred at room temperature until a clear solution resulted. A suspension of 1.39 g (0.01 mole) of L-alanine methyl ester hydro-

chloride, 1.44 ml of triethylamine, and 20 ml of acetonitrile was added to the above solution and the mixture was stirred magnetically for 72 hr. A clear solution resulted. The acetonitrile was removed *in vacuo*, 50 ml of water was added, and the product was extracted into ethyl acetate by continuous liquid–liquid extraction for 12 hr. Upon concentration of the ethyl acetate extract *in vacuo*, white crystals were obtained, 2.5 g (64%), mp 155–158°. On crystallization from ethyl acetate–hexane, the compound melted from 159 to 160°, $[\alpha]^{25}_{546}$ −128° (*c* 0.16, methanol).

Anal. Calcd for $C_{19}H_{25}O_6N_3$: C, 58.30; H, 6.44; N, 10.74. Found: C, 58.58; H, 6.59; N, 10.66.

Carbobenzyloxyglycyl-L-prolyl-L-alanine (XIII). Carbobenzyloxyglycyl-L-prolyl-L-alanine methyl ester (1.0 g, 2.56 mmoles) was dissolved in 5 ml of reagent methanol and 2.7 ml of 1 *N* sodium hydroxide added dropwise at room temperature. After stirring for 6 hr, the solution was cooled and acidified to pH 1.8 with 4 *N* hydrochloric acid. The methanol was evaporated in a rotary evaporator and the turbid solution was stored at 3°. The crystalline acid XIII (0.55 g, mp 140–142°) was obtained. After extraction of the mother liquor with six 10-ml portions of ethyl acetate and washing with saturated brine, an additional 0.3 g, mp 141–142°, was obtained raising the total yield to 91%, $[\alpha]^{25}_{546}$ −120° (*c* 0.12, methanol).

Anal. Calcd for $C_{18}H_{23}O_6N_3$: C, 57.3; H, 6.1; N, 11.14. Found: C, 57.24; H, 6.23; N, 11.10.

Glycyl-L-prolyl-L-alanine (XIV). Carbobenzyloxyglycyl-L-prolyl-L-alanine (1 g, 2.65 mmoles) was dissolved in a mixture of 22 ml of absolute methanol and 1 ml of water and magnetically stirred in an atmosphere of hydrogen in presence of 0.5 g of 10% Pd–C. After 6 hr, 20 ml of distilled water was added, and stirring was continued for 48 hr. Following filtration through Celite, the alcohol–water mixture was evaporated under reduced pressure at 40°, the glycyl-L-prolyl-L-alanine crystallizing on concentration. The product was filtered after standing in the cold, 0.62 g (96.1%). The compound was crystallized from ethanol–water (7:3), $[\alpha]^{25}_{546}$ −177° (*c* 0.08, water) (lit.[18] $[\alpha]^{22}_{D}$ −132° (*c* 0.5, acetic acid)).

Anal. Calcd for $C_{10}H_{17}O_4N_3 \cdot 0.5H_2O$: C, 47.62; H, 7.14; N, 16.66. Found: C, 47.15; H, 7.26; N, 16.41.

Glycyl-L-prolyl-L-alanine Methyl Ester Hydrobromide (XV). Carbobenzyloxyglycyl-L-prolyl-L-alanine methyl ester (3.64 g, 0.93 mmole) was dissolved in 22 ml of glacial acetic acid at room temperature and 29 ml of 4 *N* hydrogen bromide in glacial acetic acid was added. After the reaction had proceeded for 4 hr, the hydrogen bromide and acetic acid were removed under reduced pressure at 50°. The gummy residue was twice dissolved in dry methanol and the solvent was removed. The residue was dissolved in 10 ml of dry methanol and precipitated by the addition of dry ethyl ether, mp 172–174°, 2.54 g (81.7%). The compound was stored in a desiccator.

Carbobenzyloxyglycyl-L-prolyl-L-alanylglycyl-L-prolyl-L-alanine Methyl Ester (XVI). Carbobenzyloxyglycyl-L-prolyl-L-alanine (2.83 g, 7.5 mmoles) was suspended in 18 ml of dry acetonitrile and 0.48 ml (7.5 mmoles) of anhydrous triethylamine added. After stirring for a few minutes, a clear solution was obtained, and 18 ml of dry acetonitrile containing NEPIS (1.9 g, 7.5 mmoles) was added in the cold. The reaction mixture became clear upon stirring for 4 hr. After cooling, a suspension of finely powdered glycyl-L-prolyl-L-alanine methyl ester hydrobromide (2.54 g, 7.5 mmoles) in 18 ml of dry acetonitrile containing 0.48 ml (7.5 mmoles) of anhydrous triethylamine was added. After 15 min, the solution became clear. The stirring was continued for 4 hr, during which time the reaction mixture became viscous and solid material separated. On standing for 15 hr, the product crystallized, 2.81 g (58%), mp 202–204°. The compound was crystallized from methanol–ethyl acetate, mp 208–210°, $[\alpha]^{25}_{546}$ −167° (*c* 0.11, methanol).

Anal. Calcd for $C_{29}H_{40}O_9N_6$: C, 56.5; H, 6.5; N, 13.64. Found: C, 56.22; H, 6.81; N, 13.76.

Carbobenzyloxyglycyl-L-prolyl-L-alanylglycyl-L-prolyl-L-alanine (XVII). The hexapeptide ester XVI (1.4 g, 2.27 mmoles) was dissolved in 65 ml of warm methanol. On cooling, 2.58 ml of 1 *N* sodium hydroxide was added carefully and the reaction stirred for 5.5 hr. The solution was diluted with water and adjusted to pH 6.7 with 1 *N* hydrochloric acid. Excess methanol was removed under reduced pressure at room temperature and the residual material extracted with two 10-ml portions of chloroform. On washing the chloroform layer with brine, drying with anhydrous magnesium sulfate, and evaporation of the solvent, 0.34 g of unchanged ester, mp 206–208°, was obtained. The aqueous layer on acidification to pH 2 with 0.5 *N* hydrochloric acid and cooling yielded 0.6 g of acid XVII, mp 219–220° dec. The yield was 57% on the basis of ester consumed, $[\alpha]^{25}_{546}$ −164° (*c* 0.10, methanol).

Anal. Calcd for $C_{28}H_{38}O_9N_6$: C, 55.81; H, 6.31; N, 13.95. Found: C, 55.88; H, 6.50; N, 13.87.

Glycyl-L-prolyl-L-alanylglycyl-L-prolyl-L-alanine Methyl Ester Hydrochloride (XIX). The hexapeptide ester XVI (1.2 g, 1.99 mmoles) was dissolved in 65 ml of methanol and magnetically stirred with 0.5 g of 10% Pd–C in a hydrogen atmosphere until carbon dioxide evolution ceased. The reaction mixture was filtered through Celite and evaporated at room temperature under reduced pressure to dryness. The residue was dissolved in a minimal quantity of methanol and 30 ml of dry ether to which a small quantity of a cold, saturated solution of hydrogen chloride gas in dry ether was added. On standing in the cold an oil separated which was dissolved in dry methanol and concentrated under reduced pressure at 40°. The product crystallized during evaporation, 0.71 g (68%), mp 215–216° dec. It was used for the subsequent condensation step without further purification.

Glycyl-L-prolyl-L-alanylglycyl-L-prolyl-L-alanine (XVIII). Carbobenzyloxyglycyl-L-prolyl-L-alanylglycyl-L-prolyl-L-alanine (0.3 g, 0.487 mmole) was dissolved in 10 ml of warm methanol and cooled, and a suspension of 0.15 g of 10% Pd–C in 10 ml of ethanol was added. The reaction was stirred under hydrogen for 6 hr, 15 ml of water was added, and the stirring was continued for another 24 hr. The reaction mixture was filtered through Celite and the filtrate was evaporated under reduced pressure. Another 10 ml of absolute ethanol was added and the filtrate was again concentrated under reduced pressure. The product crystallized, 0.18 g (73.1%). It was recrystallized by dissolving in a minimum amount of water (4 ml) and adding 30 ml of absolute ethanol, 0.13 g (56.5%), $[\alpha]^{25}_{546}$ −198° (*c* 0.10, water).

Anal. Calcd for $C_{20}H_{32}O_7N_6$: C, 51.28; H, 6.84; N, 17.95. Found: C, 50.96; H, 7.26; N, 18.02.

Carbobenzyloxyglycyl-L-prolyl-L-alanylglycyl-L-prolyl-L-alanylglycyl-L-prolyl-L-alanylglycyl-L-prolyl-L-alanine Methyl Ester (XX). Carbobenzyloxyglycyl-L-prolyl-L-alanylglycyl-L-prolyl-L-alanine (0.4 g, 0.67 mmole) was dissolved in 4 ml of dry acetonitrile, and 0.093 ml (0.67 mmole) of anhydrous triethylamine was added. A clear solution was obtained after a few minutes to which 0.169 g (0.67 mmole) of NEPIS diluted with 4 ml of dry acetonitrile was added. After 3 hr, a solution was obtained, cooled, and a suspension of 0.347 g (0.67 mmole) of glycyl-L-prolyl-L-alanylglycyl-L-prolyl-L-alanine methyl ester hydrochloride in 4 ml of dry acetonitrile containing 0.093 ml of anhydrous triethylamine added. On stirring for 15 hr, the dodecapeptide methyl ester separated as a solid from the reaction mixture, 0.25 g (35%), mp 260–265° dec. The compound was crystallized from a mixture of methanol–water, mp 263–264° dec, $[\alpha]^{25}_{546}$ −211° (*c* 0.15, 50% MeOH).

Anal. Calcd for $C_{49}H_{70}N_{12}O_{15}$: C, 55.16; H, 6.57; N, 15.76. Found: C, 55.42; H, 6.85; N, 15.61.

Carbobenzyloxyglycyl-L-prolyl-L-alanylglycyl-L-prolyl-L-alanylglycyl-L-prolyl-L-alanylglycyl-L-prolyl-L-alanine (XXI). Carbobenzyloxyglycyl-L-prolyl-L-alanylglycyl-L-prolyl-L-alanylglycyl-L-prolyl-L-alanylglycyl-L-prolyl-L-alanine methyl ester (0.33 g, 0.31 mmole) was dissolved with warming in 36 ml of 1:1 methanol–water, the solution was magnetically stirred, and 0.41 ml of 1 *N* sodium hydroxide was added. After 5.5 hr, 5 ml of water was added and the pH was adjusted to 6.6 with 0.5 *N* hydrochloric acid. The solution was evaporated under reduced pressure at room temperature to a small volume and filtered through Celite. On acidification to pH 2 and storage in the refrigerator, XXI crystallized, 200 mg, mp 252–254° dec. The compound was crystallized from water, mp 252–254° dec, $[\alpha]^{25}_{546}$ −242° (*c* 0.05, 1:1 methanol–water).

Anal. Calcd for $C_{48}H_{68}N_{12}O_{15} \cdot H_2O$: C, 53.83; H, 6.58; N, 15.69. Found: C, 54.16; H, 6.05; N, 15.62.

Glycyl-L-prolyl-L-alanylglycyl-L-prolyl-L-alanylglycyl-L-prolyl-L-alanylglycyl-L-prolyl-L-alanine (XXII). Compound XXI (100 mg, 0.098 mmole) was dissolved in warm 1:1 ethanol–water (10 ml) 100 mg of Pd black was added, and the mixture was hydrogenated. After 5 hr another 20 ml of water was added and the hydrogenolysis was continued for 24 hr. The reaction mixture was filtered through Celite and concentrated under reduced pressure at 40° to 5 ml. An additional 5 ml of water was added and the solution again filtered. On lyophilization, 69 mg (79%) of a white solid was obtained, $[\alpha]^{25}_{546}$ −284° (*c* 0.1, H_2O).

Anal. Calcd for $C_{40}H_{62}N_{12}O_{13} \cdot 4H_2O$: C, 48.48; H, 6.71. Found: C, 48.45; H, 6.84.

Carbobenzyloxyglycyl-L-prolylglycine *p*-Nitrophenyl Ester (XXIII). Carbobenzyloxyglycyl-L-prolylglycine (12.0 g, 33.0

mmoles), 4.6 g (33.0 mmoles) of *p*-nitrophenol, and 6.8 g (33.0 mmoles) of dicyclohexylcarbodiimide were dissolved in 400 ml of dichloromethane and the solution was stirred magnetically for 1 day at room temperature. The dicyclohexylurea was filtered off and the solvent was removed. Scratching and trituration with isopropyl alcohol induced crystallization. The product was filtered off and dried, 15.0 g (93% yield), mp 144–146°. For analysis the compound was recrystallized twice from isopropyl alcohol, mp 146.5–148°, and dried at 80° under high vacuum (lit.[19] mp 151–153°).

Anal. Calcd for $C_{23}H_{24}N_4O_8$: C, 57.02; H, 4.99; N, 11.57. Found: C, 57.76; H, 5.31; N, 11.51.

Glycyl-L-prolylglycine *p*-Nitrophenyl Ester Hydrobromide (XXIV). Carbobenzyloxyglycyl-L-prolylglycine *p*-nitrophenyl ester (15.0 g, 31.0 mmoles) was dissolved in 600 ml of glacial acetic acid. Anhydrous hydrogen bromide was bubbled into the magnetically stirred acetic acid solution previously cooled to incipient crystallization. After 45 min the hydrogen bromide gas flow was stopped and replaced by a nitrogen stream until excess hydrogen bromide gas was eliminated. The solvent was removed under reduced pressure until a few milliliters of acetic acid remained. Hexane was added to precipitate the product. The crude hydrobromide was washed with hexane and dried, 19.5 g, mp 160–169° dec. On crystallization from methanol–isopropyl alcohol, a melting point of 196–197° dec was obtained twice. In later runs a melting point of 179–181° was obtained (lit.[19] mp 175–176°). After drying at 80° under high vacuum the purified compound, 7.0 g (52%), was submitted for analysis.

Anal. Calcd for $C_{15}H_{19}N_4O_6Br$: C, 41.77; H, 4.44; Br, 18.53. Found: C, 41.46; H, 4.96; Br, 18.60.

Poly(glycyl-L-prolylglycine) (XXV). Glycyl-L-prolylglycine *p*-nitrophenyl ester hydrobromide (0.863 g, 2 mmoles), was dissolved in 1.4 ml of dimethyl sulfoxide and to this 0.28 ml (2 mmoles) of triethylamine was added at once with efficient stirring of the reaction mixture. The stirring was continued at room temperature overnight. The product was precipitated by adding dichloromethane to the reaction mixture. The polymer was collected by centrifugation and washed several times with dichloromethane until free from *p*-nitrophenol, yielding 0.145 g (31%). Ten milligrams of the above crude polymer was dissolved in 1 ml of water and dialyzed *vs* water (about 10 ml) for 24 hr, changing water every 8 hr. On lyophilization, 1 ml of solution yielded 3.3 mg of the pure polymer. The hydrobromide, mp 196–197°, gave polymer with a higher average molecular weight than the hydrobromide, mp 179–181°. During the polymerization it was noted that the effective pH was slightly lower with the higher melting hydrobromide. Apparently slight changes in acidity (or a purer tripeptide preparation) have a large effect on the degree of polymerization. This observation is being studied; $[\eta]^{25}$ (80% HCOOH) 0.05, $[\alpha]^{25}D$ −120° (*c* 0.1, 10% formic acid), $[\alpha]^{25}D$ −198° (*c* 0.1, methanol).

Anal. Calcd for $(C_9H_{13}N_3O_3)_n$: C, 51.18; H, 6.16; N, 19.90. Found: C, 51.04; H, 6.61; N, 19.67.

Carbobenzyloxyglycyl-L-prolyl-L-alanine *p*-Nitrophenyl Ester (XXVI). Carbobenzyloxyglycyl-L-prolyl-L-alanine (10.7 g, 28.4 mmoles), 3.94 g (28.4 mmoles) of *p*-nitrophenol, and 5.84 g (28.4 mmoles) of dicyclohexylcarbodiimide were dissolved in 350 ml of dichloromethane and the solution was magnetically stirred overnight. Dicyclohexylurea crystallized out as the reaction proceeded and was filtered off. The dichloromethane was removed at reduced pressure and the syrup was triturated with a small amount of dichloromethane to remove more urea. On addition of hexane to the cloud point and storage at 3°, the product was obtained as colorless needles, mp 139–142°, 9.0 g (63%). Repeated crystallization from dichloromethane–hexane raised the melting point to 153–155°.

Anal. Calcd for $C_{24}H_{26}O_8N_4$: C, 57.84; H, 5.22; N, 11.24. Found: C, 57.6; H, 5.3; N, 11.5.

Glycyl-L-prolyl-L-alanine *p*-Nitrophenyl Ester Hydrobromide (XXVII). Carbobenzyloxyglycyl-L-prolyl-L-alanine *p*-nitrophenyl ester (8.4 g, 16.9 mmoles) was dissolved in 85 ml of glacial acetic acid, and 42.5 ml of 4 *N* hydrogen bromide in acetic acid was added. The mixture was kept at room temperature for 4 hr before removal of the solvent under reduced pressure at 55°. The syrup obtained was dissolved in a minimal amount of dry methanol and dry ethyl ether was added to the cloud point. On standing the product crystallized out, mp 176–178°, 6.0 g (80%). The compound was recrystallized from methanol–ether for analysis, mp 177–178°.

Anal. Calcd for $C_{16}H_{21}O_6N_4Br$: C, 43.14; H, 4.71; Br, 17.97. Found: C, 43.0; H, 4.7; Br, 17.80.

Poly(glycyl-L-prolyl-L-alanine) (XXVIII). Glycyl-L-prolyl-L-alanine *p*-nitrophenyl ester hydrobromide (1.78 g, 4 mmoles) was dissolved in 3 ml of dimethyl sulfoxide and to this 0.56 ml (4 mmoles) of triethylamine was added at once while stirring the reaction mixture efficiently. The reaction was allowed to proceed at room temperature overnight. The polymer was precipitated by the addition of 300 ml absolute ethanol, collection by centrifugation, and washing twice with ethanol to remove *p*-nitrophenol and triethylamine hydrobromide, 0.280 g (33%). The polymer was dissolved in 28 ml of water and dialyzed *vs.* water (280 ml) for 24 hr, changing water every 6 hr. On lyophilization, 0.222 g of polymer was obtained, $[\eta]^{25}$ (0.2 *M* NaCl) 0.16; $[\alpha]^{25}_{546}$ −206° (*c* 0.08, H_2O).

Anal. Calcd for $(C_{10}H_{15}N_3O_3)_n$: C, 53.33; H, 6.66; N, 18.66. Found: C, 53.10; H, 6.97; N, 18.46.

Discussion

The ordered oligomers and polymers of glycyl-L-prolylglycine and glycyl-L-prolyl-L-alanine were synthesized as models for investigations of the conformational requirements of the "collagen fold." In the following paper[23] some physical–chemical measurements of these compounds are described, while in this paper we report only the measurements used to characterize the compounds. Although the difficulties in extracting structural information from rotation measurements at a single wavelength are well known, nonetheless, it appears possible to draw certain conclusions from such measurements on the homologous oligopeptides described above.

For example, the specific rotations at 589 mμ for the oligomers of glycyl-L-prolylglycine (Table I) provide data for the calculation of the residue rotation, $[R_{Pro}]D$, of an L-prolyl group in a random peptide chain.

Table I

	$[\alpha]D$ (H_2O), deg	$[R_{Pro}]D$ (H_2O), deg
H-Gly-L-Pro-Gly-OH	−101	−248
H(-Gly-L-Pro-Gly-)$_2$OH	−104	−236
H(-Gly-L-Pro-Gly-)$_4$OH	−117	−270
H(-Gly-L-Pro-Gly-)$_n$OH	−120[a]	−260
H(-Gly-L-Pro-Gly-)$_n$OH	−198[b]	

[a] Determined in 10% formic acid. [b] Determined in methanol.

The average residue rotation so obtained is −253 ± 17° for the L-prolyl group and is a value close to that estimated by other means.[24] Two further conclusions are suggested by the limited measurements reported in Table I. Firstly, the relatively small differences in $[\alpha]D$ observed with the different oligomers indicate that they do not have structure in solution. Secondly, since the $[\alpha]D$ of poly(-Gly-L-Pro-Gly-) in 10% formic acid is essentially identical with that of the dodecamer in water, the polymer in formic acid most probably is also structureless. Finally, it may be inferred from the much increased negative value of the rotation of the polymer in methanol solution (compared to the value in 10% formic acid) that in alcohol solution the polymer is nonrandom.

The $[\alpha]D$ values of the oligomers of glycyl-L-prolyl-L-alanine and poly(glycyl-L-prolyl-L-alanine) are recorded in Table II.

(24) W. F. Harrington and P. H. von Hippel, *Advan. Protein Chem.*, 16, 1 (1961).

Table II

	$[\alpha]_D$ (H_2O), deg
H-Gly-L-Pro-L-Ala-OH·0.52H_2O	−140
H(-Gly-L-Pro-L-Ala-)$_2$OH	−200
H(-Gly-L-Pro-L-Ala-)$_4$OH·4H_2O	−234
H(-Gly-L-Pro-L-Ala-)$_n$OH	−206

The specific rotations of the Gly-L-Pro-L-Ala oligomers are considerably more negative than those of the analogous Gly-L-Pro-Gly peptides reflecting the substantial rotatory contribution of the L-alanyl residue. If the prolyl residue contribution is taken as that observed with the Gly-L-Pro-Gly peptides then from the above data, in theory, one should be able to obtain the alanyl residue contribution. Such calculations have been made and indicate either that the alanyl residue contribution is greater than that generally assumed for a random polypeptide chain ($\sim -110°$)[25] *or* that there are residue–residue interactions which substantially affect the rotations.

(25) P. Urnes and P. Doty, *Advan. Protein Chem.*, 16, 401 (1961).

On the Structure of Gly-Pro-Gly and Gly-Pro-Ala Oligopeptides and Sequential Polypeptides[1-3]

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Abstract: Physical–chemical studies of the chain regularity and interaction of Gly-L-Pro-X oligopeptides and sequential polypeptides are described. It is shown that association of single chains of Gly-L-Pro-Gly polymer stabilizes the formation of partly helical structures, whereas Gly-L-Pro-L-Ala polymers do not show regular structure in solution.

Conformational studies of single-chain synthetic homopolypeptides and amino acid copolymers have helped to elucidate the influences of hydrogen bonding, side-chain steric hindrance, electrostatic interactions, and other short-range interactions on the formation of ordered polypeptide chain structures in solution. Many of these same forces serve to stabilize protein structures, but additional stabilization of structure in proteins appears to come from disulfide bonds and specific short-range interactions which frequently occur on widely separated parts of the peptide chains or on altogether different chains. Since the studies on polypeptides heretofore have been for the most part confined to homopolyamino acids or random copolyamino acids, it is of considerable interest to examine polypeptide models in which the amino acid contents and sequences more closely approximate those found in proteins.

In this communication, we describe conformational studies of ordered sequences of oligomers and polymers of (glycyl-L-prolyl-X)$_n$, where X is glycine or alanine. These sequences were selected to serve as models of collagen since collagen contains approximately 30–35% glycine, 20–25% of imino acids (L-proline and L-hydroxyproline), and approximately 10% L-alanine.[5] Collagen proteins apparently have large portions of the sequence Gly-L-Pro-X,[6] although X is in many cases an amino acid other than glycine or alanine or an imino acid. It will be shown in this communication that the oligomers and polymers investigated appear to have no structural regularity as single chains, but upon association with other chains form structures resembling collagen in many respects. In addition, the choice of the third amino acid in the trimer sequence has a marked influence on the ability of the polymer to associate to regular structures.

Experimental Section

Materials. The syntheses of the oligomers and polymers used in this study are described in the accompanying paper.[2] All optically active amino acids used in the syntheses were the L isomers. All oligomers and the Gly-Pro-Ala polymer were water soluble. Solutions of these materials were made by dissolving the lyophilized material directly in distilled water. For the Gly-Pro-Gly polymer which is acid soluble, a high concentration of acid was used for initial solvation with subsequent dilution with distilled water. Concentrations were calculated from the weight of material used and were checked for internal consistency with ultraviolet spectra using the 190–195-mμ maxima. The dichloroacetic acid (DCA) used was freshly distilled. Other solvents used were reagent or spectral grade.

Optical Rotatory Dispersion. Optical rotatory dispersion measurements were made with a Cary 60 recording spectropolarimeter. In most measurements dispersions were taken using Opticell cuvettes with optical path lengths from 0.1 mm to 5 cm. The temperature of the cell compartment was 25–28°. Temperature variation and kinetic studies utilized jacketed Opticell cuvettes. The temperature of the solution was measured by means of a calibrated thermistor probe. Refractive index corrections were not made because of the paucity of refractive index dispersion data for mixed solvents at short wavelengths.

(1) This is Polypeptides LI. For the previous paper in this series see ref 2.

(2) S. M. Bloom, S. K. Dasgupta, R. P. Patel, and E. R. Blout, *J. Am. Chem. Soc.*, 88, 2035 (1966).

(3) This work has been supported in part by the Office of the Surgeon General, Department of the Army.

(4) Postdoctoral Research Fellow of the Public Health Service.

(5) K. A. Piez and J. Gross, *Biochem. Biophys. Acta*, 34, 24 (1959).

(6) W. F. Harrington and P. H. von Hippel, *Advan. Protein Chem.*, 16, 1 (1961).