ology and to Hugh Niall for his helpful discussions on the use of the protein sequencer.

Added in Proof

The partial amino acid sequence of the *C. pasteurianum* flavodoxin has been reported by Fox and Brown (1971). The placement of a lysine residue in position 46 is based on their report. In our studies, lysine and valine were observed in 1 and 2% yields, respectively, and it was not possible to decide which amino acid occupied the 46th position.

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Synthesis of Polypeptides and Oligopeptides with the Repeating Sequence L-Alanyl-L-prolylglycine*

G. P. Lorenzi,† B. B. Doyle,‡ and E. R. Blout§

ABSTRACT: The synthesis and characterization of the polytripeptide poly(Ala-Pro-Gly) and a series of oligopeptides (from the tripeptide to the octadecapeptide) with this sequence are reported. The polymer was synthesized by the active ester method, using the *N*-hydroxysuccinimide ester as the polymerizable tripeptide derivative in one preparation and the *p*-nitrophenyl ester in a second preparation. Good yields of relatively high average molecular weight polymer were obtained in both cases. The oligomers were prepared sequentially using the mixed-anhydride method, with isobutyl chloroformate as the mixed-anhydride-forming reagent. All the products were crystalline and gave only one spot on thin-layer chromatography after purification. Conformational studies on this polymer and the oligomers are reported in the accompanying paper.

One useful approach to the understanding of the physical and chemical properties of collagen is the study of model polypeptides having amino acid compositions and distributions resembling those of the natural protein. In particular, studying the conformational properties of such models in the solid state and in solution may help clarify the factors which stabilize the triple-helical structure of collagen. Thus in recent years there have been synthesized a number of polytripeptides similar to collagen in that every third residue is a glycine and at least one of the other two amino acids is a proline or hydroxyproline (Carver and Blout, 1967; Andreeva *et al.*, 1967).

To extend these studies, we have prepared the polytripeptide poly(Ala-Pro-Gly) and the first six members of the series of oligopeptides with the same sequence, having the general formula Boc-(Ala-Pro-Gly)$_n$-OMe.[1] We have also synthesized the corresponding trifluoroacetic acid salts, and some of the related Boc-protected peptide acids. The study of such oligopeptides of well-defined chemical structure and molecular weight provide a reliable complement to the study of the polydisperse fractions which were prepared from the polytripeptide. Experiments with the oligopeptides may help clarify relationships between structure and molecular weight

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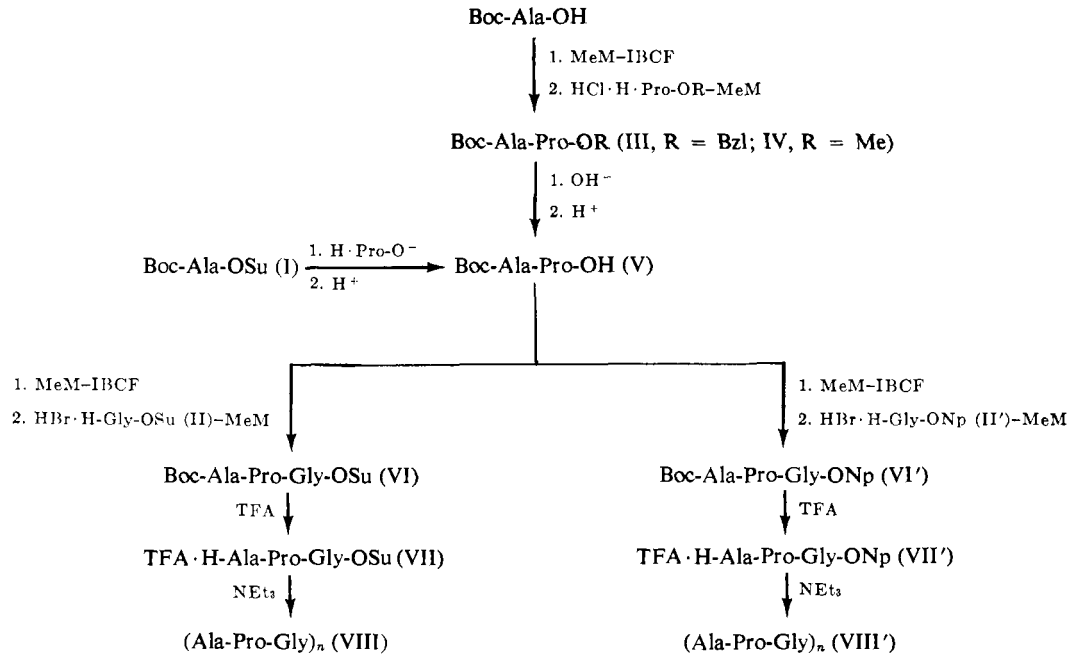
[1] The following abbreviations are used in this paper: Boc, *tert*-butyloxycarbonyl; Z, benzyloxycarbonyl; Pro, L-prolyl; Ala, L-Alanyl; Gly, glycyl; OMe, methyl ester; OBzl, benzyl ester; OSu, *N*-hydroxysuccinimide ester; ONp, *p*-nitrophenyl ester.

CHART I: Synthesis of the Sequential Polypeptide $(Ala\text{-}Pro\text{-}Gly)_n$.[a]



[a] MeM, *N*-methylmorpholine; IBCF, isobutyl chloroformate; TFA, trifluoroacetic acid.

and should show the effect of heterogeneity on the commonly studied physical parameters and transitions. Significant differences have been found recently between the properties of a monodisperse polytripeptide, poly(L-prolyl-L-prolylglycine) (Sakakibara *et al.*, 1968), and the results reported previously (Engel *et al.*, 1966) for fractions of this same polytripeptide that had been prepared by polycondensation. In this paper we describe in detail the synthesis and characterization of the polytripeptide and the oligopeptides indicated above.

Synthesis

The preparation of polymers with the repeating sequence -Ala-Pro-Gly- has been reported by Huggins *et al.* (1968), Heidemann and Bernhardt (1967), and Shibnev and Lazareva (1969). These groups of workers used tetraethyl pyrophosphite (Kitaoka *et al.*, 1958) to effect the polycondensation of their "monomeric" tripeptide acids; however, few other experimental details were given. While our work was in progress, we became aware of a synthesis of poly(Ala-Pro-Gly) which uses a method similar to ours and which has been reported recently by Segal and Traub (1969).

The method employing peptide active esters is known to be advantageous for synthesizing peptides, and excellent results have been obtained from the application of *N*-hydroxysuccinimide esters (Anderson *et al.*, 1964; Laufer and Blout, 1967) and *p*-nitrophenyl esters (Bodansky, 1960; Goodman and Stueben, 1959; Iselin and Schwyzer, 1960). The active ester method, using the *p*-nitrophenyl ester, has also been applied successfully to preparing polypeptides with repeating sequences (Bloom *et al.*, 1966; DeTar and Estrin, 1966; DeTar *et al.*, 1963). We chose the *N*-hydroxysuccinimide ester as one polymerizable tripeptide derivative and the *p*-nitrophenyl ester as the other. The trifluoroacetic acid derivatives VII and VII' of these two esters were synthesized as indicated in Chart I. The Boc-protected tripeptide ester VI was prepared by direct coupling of the Boc-protected dipeptide acid V with glycine *N*-hydroxysuccinimide ester using the mixed carbonic anhydride method. This product (VI) was then treated with trifluoroacetic acid to obtain the trifluoroacetic acid salt VII. The *p*-nitrophenyl ester tripeptide unit VII' was prepared in a completely analogous manner. The polycondensations of VII and VII' were carried out in a very concentrated solution in order to minimize the formation of cyclic peptides (DeTar *et al.*, 1963). Dimethyl sulfoxide was used as the solvent and an excess of triethylamine was employed to ensure that all the trifluoroacetic acid present in the starting tripeptide derivative was neutralized by this base.

The synthesis of the oligopeptides was carried out according to Chart II. The mixed-anhydride method, with isobutyl chloroformate as the-mixed-anhydride-forming reagent (Vaughan and Osato, 1952), was used in each coupling step. The trifluoroacetic acid salts XVI–XX were prepared by treatment with trifluoroacetic acid of the corresponding Boc-protected compounds. These were used as the sources of the amino component in the preparation of the Boc-protected methyl esters XI–XV; Boc-Ala-Pro-Gly-OH (IX) was used in general as the acid component.

Experimental Section

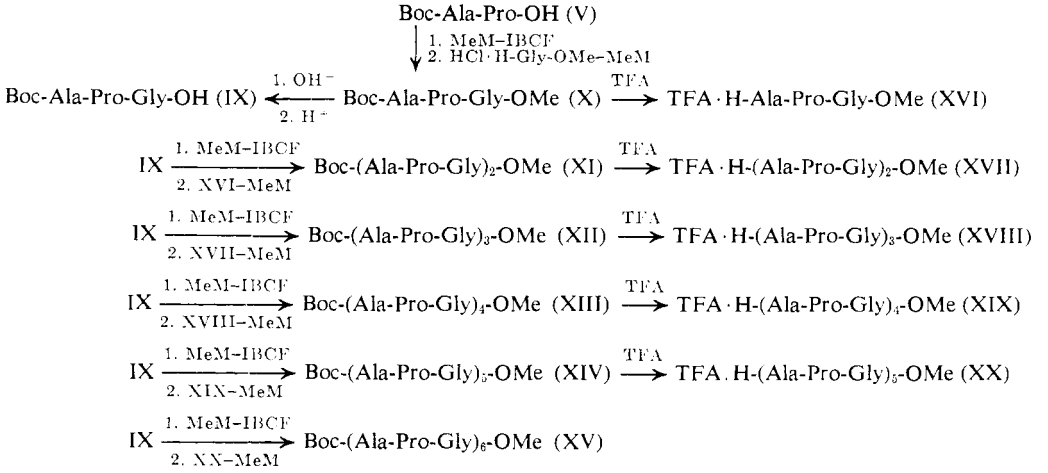
A. Reagents and Solvents. All amino acids and their derivatives except the *N*-hydroxysuccinimide esters and the hydrobromide of glycine *p*-nitrophenyl ester were commercial products, and were used in general as recieved. HCl·H-Pro-OMe was received as a syrup in methanol and was freed of this solvent by drying *in vacuo*. HCl·H-Pro-OBzl was purified by recrystallization from methanol–ether, mp 149–151° (lit. (Neuman and Smith, 1951) mp 148–148.5°).

N-Methylmorpholine was distilled and the fraction boiling at 115.5° was used.

The triethylamine was purified following the procedure of Kovacs *et al.* (1966).

The trifluoroacetic acid was purified by distillation.

CHART II: Synthesis of Oligopeptides with the Sequence Ala-Pro-Gly.[a]



[a] MeM = *N*-methylmorpholine; IBCF = isobutyl chloroformate; TFA, trifluoroacetic acid.

The chloroform used as the solvent for the mixed-anhydride-coupling reactions was purified by distillation immediately prior to use.

The dimethoxyethane to be used for the same purpose was purified by distillation over lithium aluminum hydride.

The other solvents were of reagent grade and were used without further purification.

B. Analysis and Characterization of the Intermediate and Final Products. The microanalyses were performed by the Spang Microanalytical Laboratory, Ann Arbor, Mich., the Scandinavian Microanalytical Laboratory, Herlev, Denmark, and the Werby Laboratories, Inc., Boston, Mass. The elemental analysis data of the Boc-protected oligopeptides XIII–XV, which were crystallized from water-containing solutions, gave persistently low values for carbon and nitrogen content and high values for hydrogen even after drying at 85° and at 0.05 mm for 48 hr. A good agreement was found between the experimental and calculated values of the carbon-to-nitrogen ratio, so it seems reasonable to assume that some water remained in the samples even after intensive drying (Stewart, 1966). In the case of the polymer anomalous elemental analyses were also obtained, indicating retention of water and possibly other solvents.

Thin-layer chromatography was performed on silica gel H (Merck, AG), using in general the solvent system $CHCl_3$–CH_3OH (9:1, v/v). Compounds were located with a ninhydrin spray, with the chlorine–*o*-tolidine method, or with iodine.

The melting points were taken on a Kofler apparatus and are uncorrected.

The infrared spectra were obtained using a Perkin-Elmer 521 spectrophotometer with the samples in KBr pellets.

A Cary 15 spectrophotometer was used for the ultraviolet measurements, with water as the solvent. Concentrations of the higher oligopeptides were evaluated on the basis of nitrogen analysis of the samples used. The absorption data reported in this paper give the mean residue extinction coefficient (ϵ_{res}).

All molecular weights were determined by the Archibald approach to equilibrium method (Archibald, 1947; Schachman, 1957) on a Beckman Model E ultracentrifuge.

C. Preparation of the Polytripeptide (Ala-Pro-Gly)$_n$. Boc-Ala-OSu (I). It was prepared and purified following the procedure of Anderson *et al.* (1964), mp 166–167° [lit. (Anderson *et al.* (1964) have obtained two different forms of Boc-Ala-OSu) mp 143–144 and 167°].

HBr·H-Gly-OSu (II). Z-Gly-OSu was prepared and purified following the procedure of Anderson *et al.* (1964) mp 113–114.5° (lit. (Anderson *et al.*, 1964) mp 113–114°).

Anhydrous hydrogen bromide was bubbled for 2 hr into a stirred ice-cold solution of 5.96 g (19.1 mmoles) of Z-Gly-OSu in a mixture of 160 ml of glacial acetic acid and 50 ml of methylene chloride. Then the solvents were removed by evaporation and the residual product was purified by washing with methylene chloride. The hydrobromide II, mp 166–168° (dec), was obtained in a nearly quantitative yield.

HBr·H-Gly-ONp (II′). It was prepared from Z-Gly-ONp according to the literature (Goodman and Stueben, 1959).

Boc-Ala-Pro-OBzl (III). A solution of 4.96 g (26.2 mmoles) of Boc-Ala-OH in 46.5 ml of chloroform was stirred and chilled to −15° in an ice–salt bath. *N*-Methylmorpholine (2.93 ml, 26.2 mmoles) was added, followed by isobutyl chloroformate (3.60 ml, 27.6 mmoles), giving a precipitate. After 15 min, HCl·H-Pro-OBzl (6.35 g, 26.2 mmoles) and *N*-methylmorpholine (2.95 ml) were added; gas evolution ensued. The reaction mixture was kept stirring for 1 hr between −10 and 4° and for 23 hr at room temperature; then water (50 ml) was added. After shaking, the water layer was removed, and the organic layer was washed successively with 5% $NaHCO_3$ solution (50 ml) and saturated sodium chloride solution (two 50-ml portions). After drying the chloroform layer over anhydrous sodium sulfate and removing the solvent under reduced pressure, a yellow oil was obtained. The oil was chromatographically pure, but could not be crystallized: yield 9.44 g (96%).

Boc-Ala-Pro-OMe (IV). The procedure adopted for the synthesis and purification of III was followed, except that the L-proline methyl ester hydrochloride was added as a concentrated solution in chloroform. Again an oil was obtained (63.5% yield) which could not be crystallized, but which was apparently pure by thin-layer chromatography.

Boc-Ala-Pro-OH (V). To a solution of III (9.45 g, 25.1 mmoles) in 50 ml of methanol, 25.1 ml of 1 N NaOH was added. The solution was stirred for 11 hr; then most of the methanol was evaporated under reduced pressure at 40°. Water (50 ml) was added, and the resulting solution was acidified to pH 2 with citric acid. The product, which crystallized

immediately, was redissolved in dimethoxyethane, and this solution was then dried over anhydrous magnesium sulfate and concentrated under reduced pressure. On addition of hexane, crystals were obtained: mp 156–158°, yield 5.84 g (81%).

The hydrolysis of the methyl ester IV to give the same compound was carried out in an analogous manner.

Compound V was also prepared by reacting I with proline in an aqueous medium containing sodium bicarbonate (Anderson *et al.*, 1964). After recrystallization from ethyl acetate–hexane, the product so obtained showed a melting point of 155–156° (yield 74%). *Anal.* Calcd for $C_{13}H_{22}N_2O_5$: C, 54.54; H, 7.78; N, 9.81. Found: C, 54.53; H, 7.75; N, 9.78.

Boc-Ala-Pro-Gly-OSu (VI). A solution of 0.509 g (1.77 mmoles) of V in 20 ml of chloroform was chilled to −15° with stirring. Then 0.20 ml (1.78 mmoles) of *N*-methylmorpholine and 0.25 ml (1.92 mmoles) of isobutyl chloroformate were pipetted in. Thirty minutes later 0.450 g (1.78 mmoles) of II was added. Finally, a solution of 0.20 ml of *N*-methylmorpholine in 4 ml of chloroform was added dropwise. Then the cooling bath was removed, and the reaction was allowed to proceed at room temperature for 18 hr. After washing with water (three 25-ml portions), the chloroform solution was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was recrystallized twice from ethyl acetate–hexane, giving 0.39 g of product, mp 128.5–130.5° (yield 50%). For the elemental analysis a fraction was recrystallized once more: mp 129.5–131°. *Anal.* Calcd for $C_{19}H_{28}N_4O_8$: C, 51.81; H, 6.41; N, 12.72. Found: C, 52.17; H, 6.26; N, 12.75.

Boc-Ala-Pro-Gly-ONp (VI′). A procedure analogous to that used for VI was followed to synthesize this product from V and II′, and to purify it: mp 191–192.5°, yield 75%. *Anal.* Calcd for $C_{21}H_{28}N_4O_8$: C, 54.30; H, 6.08; N, 12.06. Found: C, 53.87; H, 6.06; N, 11.93.

TRIFLUOROACETIC ACID·H-Ala-Pro-Gly-OSu (VII) AND TRIFLUOROACETIC ACID·H-Ala-Pro-Gly-ONp (VII′). These salts were obtained from the corresponding Boc-protected active esters VI and VI′ by reaction with trifluoroacetic acid. Conditions similar to those described in section D for the preparation of the trifluoroacetates XVI–XX were adopted. The final products gave only one spot by thin-layer chromatography, but their weight was slightly higher than that expected, suggesting trifluoroacetic acid was present in excess.

$(Ala\text{-}Pro\text{-}Gly)_n$ (VIII AND VIII′). The entire product of the reaction with trifluoroacetic acid of 4.16 mmoles of VI, was dissolved in 3.0 ml of dimethyl sulfoxide with stirring, and 1.15 ml (8.26 mmoles) of triethylamine were added; 1.15 ml more of this base was added 21 hr later. The polymerizing mixture became a milk-like, viscous suspension after the second addition of triethylamine, and this was kept stirring at room temperature for 4 days and then at 50° for 1 day. Diethyl ether was then added, and the product was separated by centrifugation. The polymer (VIII) was washed several times with diethyl ether, then with methylene chloride, and finally dried *in vacuo* over P_2O_5: weight, 0.766 g (yield, 81%).

By starting from the *p*-nitrophenyl ester VII′ and using comparable conditions, a 60% yield of polymer VIII′ was obtained. *Anal.* Calcd for $(C_{10}H_{15}N_3O_3)_n$: C, 53.32; H, 6.71; N, 18.65; C/N, 2.85. Found: C, 50.87; H, 6.70; N, 18.15; C/N, 2.80. Amino acid analysis gave a ratio of 1.00:0.95:1.00 for Ala:Pro:Gly.

Both products had the same infrared spectrum, confirming their identity, and indicating no substantial amount of impurity was present. The unfractionated polymers were insoluble in most organic solvents, partially soluble in water, soluble in hexafluoroisopropyl alcohol, 2 M LiBr aqueous solution, trifluoroethanol (upon heating), and formic acid (50% aqueous solution).

Both polymer samples (VIII and VIII′) were fractionated by dialysis and gave water-soluble and -insoluble fractions having different average molecular weights. VIII was fractionated using water alone as the solvent. The fractionation of VIII′ was carried out using a LiBr aqueous solution as follows: 0.86 g of the polymer was dissolved in 200 ml of 2 M LiBr solution, and this was dialyzed against 1000 ml of 2 M LiBr solution. Two changes of the outside solvent were made, allowing 24 hr for equilibration after each change. Then to remove the LiBr from the polymer solutions, they were dialyzed against 1000 ml of distilled water, which was changed every 24 hr for 6 days, until conductivity measurements on the water outside the bag and ultraviolet absorption measurements on the polymer solution inside the bag indicated that LiBr was no longer present. About 0.5 g of the polymer precipitated out of solution in the dialysis bag on dilution of the LiBr, while the rest remained in aqueous solution. The water-insoluble fraction had a weight-average molecular weight of approximately 14,000 based on an Archibald ultracentrifuge run done in 60% formic acid; the water-soluble fraction, run in water using the same method, gave a molecular weight value of 5500.

D. Preparation of the Oligopeptides with the Sequence Ala-Pro-Gly (X–XXII). Boc-Ala-Pro-Gly-OMe (X). A solution of 7.55 g (26.3 mmoles) of V in 65 ml of dimethoxyethane was chilled to about −15° with stirring; then 2.94 ml (26.3 mmoles) of *N*-methylmorpholine and 3.60 ml (27.6 mmoles) of isobutyl chloroformate were added giving an immediate precipitate. Fifteen minutes later, 3.30 g (26.3 mmoles) of HCl·H-Gly-OMe and 2.95 ml of *N*-methylmorpholine were added. The reaction mixture was stirred between −15 and 10° for 1 hr and at room temperature for 22 hr. Then water was added, and the dimethoxyethane was removed by evaporation almost completely. The residual solution was made up to 50 ml with water, and extracted with ethyl acetate (four 50-ml portions). The combined ethyl acetate extracts were washed with 5% aqueous sodium bicarbonate (two 50-ml portions) and with saturated sodium chloride solution (50 ml) and dried over anhydrous sodium sulfate. Removal of the solvent under reduced pressure yielded an oil which crystallized immediately upon addition of hexane. After drying, the weight of the crystals, mp 139–140°, was 7.20 g (yield 77%). For the elemental analysis, the compound was recrystallized from hot ethyl acetate–hexane: mp 141–142°; ultraviolet spectrum: λ_{max} 185 mμ; ϵ_{res} at λ_{max} 5520. *Anal.* Calcd for $C_{16}H_{27}N_3O_6$: C, 53.77; H, 7.61; N, 11.76. Found: C, 53.65; H, 7.68; N, 11.55.

Boc-Ala-Pro-Gly-OH (IX). Compound X (8.61 g, 24.1 mmoles) was dissolved in 80 ml of methanol and 24.1 ml of 1 N aqueous NaOH was added. The solution was stirred for 23 hr; then 24.1 ml of 1 N aqueous hydrogen chloride was added dropwise, under vigorous stirring. The solvents were almost completely removed by evaporation under reduced pressure and anhydrous sodium sulfate was added to the semisolid residue to remove the residual water. The product was extracted with ethyl acetate. Concentration of the ethyl acetate solution and addition of hexane caused the crystallization of the product. The crystals were collected, washed with a mixture of ethyl acetate and hexane (1:3, v/v), and dried. Their weight was 8.27 g (99% yield), mp 134–136°. *Anal.* Calcd for

TABLE I

Synthesis of	Starting Solution: IX (mmoles)	Solvent	ml	Total Duration of the Reaction (hr)
XI	9.73	DME[a]	80	24.5
XII	9.23	$CHCl_3$	80	51
XIII	3.93	$CHCl_3$	50	37
XIV	1.66	$CHCl_3$	50	43
XV	0.43	$CHCl_3$	25	92

[a] DME, dimethoxyethane.

$C_{15}H_{25}N_3O_6$: C, 52.40; H, 7.36; N, 12.20. Found: C, 52.47; H, 7.34; N, 12.24.

TRIFLUOROACETIC ACID·H-Ala-Pro-Gly-OMe (XVI) AND TRIFLUOROACETATES OF THE HIGHER OLIGOPEPTIDE METHYL ESTERS (XVII–XX). The following general procedure was adopted. The Boc-protected peptide ester was dissolved or suspended (from XII on, the higher Boc-protected peptides proved to be insoluble) in chloroform (6–10 g of peptide in 100 ml of chloroform), and trifluoroacetic acid was added (about 20-fold excess). The reaction mixture, a solution, was kept under stirring at room temperature for 4–5 hr. Then the solvent and the excess trifluoroacetic acid were removed by evaporation under reduced pressure at 40° as completely as possible. The residual oil was triturated with anhydrous ether giving a powdery product. This was collected by filtration, washed with plenty of ether, and dried overnight *in vacuo* over P_2O_5. In most cases, even if the thin-layer chromatography did not show the presence of any unreacted material, the final weight was slightly higher than the expected one, suggesting that the removal of the excess trifluoroacetic acid had not been completed. Therefore, a suitable excess of *N*-methylmorpholine was used in such cases in the coupling reactions in order to ensure the complete liberation of the peptide ester from its salt.

SYNTHESIS OF Boc-(Ala-Pro-Gly)$_2$-OMe (XI) AND OF THE HIGHER OLIGOTRIPEPTIDES XII–XV. The synthesis was carried out using the following general procedure. A solution of IX in either dimethoxyethane or chloroform was chilled to about −15° with stirring and then 1 equiv of *N*-methylmorpholine and a 5% excess of isobutyl chloroformate were added. Fifteen minutes later the required trifluoroacetate (1 equiv) was added followed by *N*-methylmorpholine. The reaction mixture was stirred between −15° and 0° for 1 hr, then the cooling bath was removed, and the reaction mixture was maintained at room temperature for a variable time. Table I gives the data referring to the specific cases. The reaction mixture was worked up as described in the following section.

E. Isolation and Purification of the Oligotripeptides XI–XV

Boc-(Ala-Pro-Gly)$_2$-OMe (XI). The solvent was removed under reduced pressure from the reaction mixture and replaced by 170 ml of *tert*-butyl acetate. The resulting suspension was filtered, and 1 ml of water was added to the filtrate; upon stirring, crystallization began. The suspension was kept stirring overnight; then the crystals were collected, air-dried, and dissolved in water. The aqueous solution was shaken with the acidic, cation-exchange resin Dowex 50W-X12. Then the water was removed, and the product was chromatographed on silica gel H using a mixture of chloroform and methanol as the eluent. Two fractions were collected, weight 1.67 and 2.93 g (yield 81%). The first fraction, mp 125–147°, was crystallized twice from ethyl acetate to give the analytically pure product as long, very thin needles: mp 147–149°; ultraviolet spectrum: λ_{max} 189 mμ; ϵ_{res} at λ_{max} 5760. *Anal.* Calcd for $C_{26}H_{42}N_6O_9$: C, 53.59; H, 7.27; N, 14.43. Found: C, 53.61; H, 7.32; N, 14.29. From XI, using conditions similar to those adopted in the synthesis of Boc-Ala-Pro-Gly-OH, Boc-(Ala-Pro-Gly)$_2$-OH (XXI) was prepared, mp 165–166°. *Anal.* Calcd for $C_{25}H_{40}N_6O_9$: C, 52.80; H, 7.09; N, 14.78. Found: C, 52.29; H, 6.95; N, 14.37.

Boc-(Ala-Pro-Gly)$_3$-OMe (XII). The reaction mixture, a solution, was washed with 100 ml of water. The water layer was extracted with chloroform (three 100-ml portions). The chloroform was removed from the combined organic layers and replaced with water. The resulting aqueous solution was shaken successively with the acid resin Dowex 50W-X12 and the basic resin Dowex 1-X1. Then the water was removed, and the product was chromatographed twice through silica gel H using methanol as the eluent. By concentrating the methanolic eluate, and adding an excess of ethyl acetate to it, a crop of crystals was obtained: mp 214.5–216°, weight 4.59 g (yield 62%). Crystallization from hot methanol–ethyl acetate afforded the chromatographically pure product: mp 218–219°; ultraviolet spectrum: λ_{max} 190.5 mμ; ϵ_{res} at λ_{max} 5780. *Anal.* Calcd for $C_{36}H_{57}N_9O_{12}$: C, 53.51; H, 7.11; N, 15.60. Found: C, 53.17; H, 7.27; N, 15.34. The product was also prepared starting from XXI and XVI using synthesis conditions similar to those indicated above.

Boc-(Ala-Pro-Gly)$_4$-OMe (XIII). The reaction mixture, a solution, was added to 30 ml of water, and, after shaking, the two phases were separated. Upon standing, crystallization of the product occurred in each of them. The two fractions were collected separately by filtration; the material crystallized from the organic phase (after washing with chloroform) weighed 1.07 g, mp 229–231° dec; the material crystallized from the aqueous phase (after washing with a mixture of water and dimethoxyethane (1:1, v/v)) weighed 1.73 g, mp 233–235° dec. The total yield was 69%. The two fractions were combined, and the product was crystallized successively from water, and from water–dimethoxyethane to give the pure product, mp 235–236° dec (in both recrystallizations a mixture of methanol and water was used as the initial solvent, but most of the methanol was subsequently removed by evaporation under reduced pressure at 40–45°): ultraviolet spectrum: λ_{max} 191 mμ; ϵ_{res} at λ_{max} 5900. *Anal.* Calcd for $C_{46}H_{72}N_{12}O_{15}$: C, 53.47; H, 7.02; N, 16.27; C/N, 3.28. Found: C, 52.85; H, 7.09; N, 15.97; C/N, 3.30. XII was also prepared starting from XXI and using XVII as the amino component source.

Boc-(Ala-Pro-Gly)$_5$-OMe (XIV). The chloroform was removed from the reaction mixture, a solution, by evaporation and the residue was washed with a mixture of dimethoxyethane and water (1:1, v/v). Basic impurities were removed by shaking an ice-cold solution of the product in 100 ml of a mixture of trifluoroethanol and water (about 1:3, v/v) with the cation-exchange resin Dowex 50W-X12. After filtering off the resin, the solution was concentrated; upon standing at room temperature, the product crystallized, weight after drying 1.45 g (yield 69%): mp >250° dec; ultraviolet spectrum: λ_{max} 191 mμ; ϵ_{res} at λ_{max} 6180. For the elemental analysis the product was recrystallized from trifluoroethanol–water. *Anal.* Calcd for $C_{56}H_{87}N_{15}O_{18}$: C, 53.44; H, 6.97; N, 16.69; C/N, 3.20. Found: C, 52.44; H, 7.16; N, 16.47; C/N, 3.18.

Boc-(Ala-Pro-Gly)$_6$-OMe (XV). The chloroform was evaporated from the reaction mixture, a suspension, and the residue was first washed with water, and then dissolved in a mixture of trifluoroethanol and water. The solution was cooled to 0° and shaken with the cation-exchange resin Dowex 50W-X12; then, after filtering off the resin, the solution was concentrated by evaporation at 40° under diminished pressure until the product started to precipitate. After standing at room temperature overnight, the product was collected by filtration and dried *in vacuo* over P_2O_5; weight 0.12 g; thin-layer chromatography showed the presence of a trace of a low R_F (developing system: *n*-BuOH–AcOH–H_2O, 1:1:1, v/v) impurity; ultraviolet spectrum: λ_{max} 191 mμ; ϵ_{res} at λ_{max} 5920. *Anal.* Calcd for $C_{66}H_{102}N_{18}O_{21}$: C, 53.43; H, 6.93; N, 16.99; C/N 3.14. Found: C, 51.46; H, 7.28; N, 16.35; C/N, 3.14. By adding dimethoxyethane to the stirred mother liquors, more product precipitated and was collected by centrifugation. The weight after drying of this second, less pure fraction, was 0.27 g (total yield 61%).

Discussion

This work is a further example of the practicality of the active ester method in preparing sequential polypeptides. Both tripeptide active esters used gave good yields of poly(Ala-Pro-Gly) with relatively high average molecular weights. Approximately 58% of the polymer obtained using the *p*-nitrophenyl ester method consisted of a water-insoluble fraction having molecular weight ($\overline{M}_w$) 14,000. Since the determination has been carried out in a relatively strong acid medium—60% formic acid aqueous solution—which should not favor association, the value found should reflect the true average molecular size of the individual macromolecules.

The oligopeptides have been synthesized using conventional synthetic methods of peptide chemistry. All of them were crystalline compounds, and after purification showed only one spot by thin-layer chromatography.

Both the polymers and the various oligomers can be assumed to be optically pure, since all reactions which involved peptides as reagents were carried out on peptides having glycine or proline as the C-terminal amino acid.

The polymer and the oligopeptides with the sequence Ala-Pro-Gly have been synthesized in order to study the structures they may adopt both in the solid state and in solution and to see if indeed they have collagen-like properties. Conformational studies have been carried out and are reported in the accompanying paper (Doyle *et al.*, 1971). Such studies emphasize the value of having a series of well-characterized oligomers, as well as the polymer, for interpreting the data obtained.

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