

## Synthesis of Alanine Oligopeptides with Solubility-Enhancing Blocking Groups<sup>1</sup>

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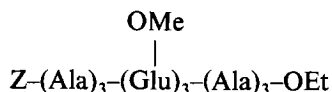
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Several series of alanine oligomers containing solubility-enhancing blocking groups were prepared. Oligomers of increased solubility were obtained through the use of 2-methoxy-[2-ethoxy-(2-ethoxy)] acetic acid (MEEA) and morpholine (Mo) as blocking groups for the N- and C-terminal ends, respectively. These two groups in combination with the standard benzyloxycarbonyl and ethyl ester blocking groups provided us with four series of alanine oligomers. These materials were prepared by the mixed anhydride method. The oligomers prepared in this manner were optically pure and were obtained in good yield.

Oligopeptides have been used as model compounds to investigate the  $\alpha$ -helix and other conformations in polypeptides and proteins. We have reported on the conformations of glutamate, aspartate, and alanine oligomers using optical rotatory dispersion (ORD), circular dichroism (CD), and ultraviolet spectroscopy (1-13). Under proper conditions  $\gamma$ -methyl-L-glutamate oligomers were found to become helical at the heptamer (1, 2, 5, 8, 11). Similarly,  $\beta$ -methyl-L-aspartate oligomers showed no helicity until the undecamer under similar conditions (6-8). Recently, the results on glutamate oligomers were confirmed using 220 MHz nuclear magnetic resonance spectroscopy (nmr) (14).

Many studies have been carried out on oligomers and polymers of L-alanine. These studies were hampered by the low solubility of these compounds in helix-supporting solvents. Gratzer and Doty studied a polymer composed of a poly-L-alanine block sandwiched between two DL-glutamic acid polymers (15). They obtained a value of 92% helicity for the alanine block in water at pH 7. The determination of the percentage of helicity was based on  $b_0$  values obtained by optical rotatory dispersion. In our laboratory we studied the conformation of alanine oligomers, but we were limited by the above-noted low solubility (12, 13). To obtain soluble oligomers we prepared cooligomers of L-alanine and  $\gamma$ -methyl-L-glutamic acid and found that



was largely helical as determined by optical rotatory dispersion and ultraviolet spectroscopy (13). Ingwall and coworkers studied a decaalanine block flanked by DL-lysine polymers and found no evidence of helicity in water (16). Recently, Gibson and Scheraga have predicted a  $\beta$ -structure for decaalanine, based on conformational energy calculations (17).

<sup>1</sup> This represents paper XXXVI in our series on Conformational Aspects of Polypeptide Structure.

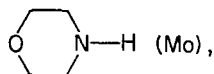
<sup>2</sup> Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the Polytechnic Institute of Brooklyn.

We believe that attempts to solubilize alanine oligomers and polymers with amino acid-derived solubilizing groups are not the only approach to the problem. One must always be concerned with the possible stereochemical influence of these groups. The nmr studies of such materials may be hindered by interference between N—H and  $\alpha$ -CH resonances in the oligomer and blocking group.

Our research has centered on finding low molecular-weight non-peptide blocking groups, which would enhance the solubility of alanine oligomers in helix-supporting solvents. In the studies to be described, 2-methoxy-[2-ethoxy-(2-ethoxy)] acetic acid,



and morpholine,



were used as blocking groups for the N-terminal and C-terminal ends, respectively. These groups are transparent in the spectroscopic regions where the oligomers absorb and are easily prepared from commercially available starting materials. These groups in combination with standard benzyloxycarbonyl (Z) and ethyl ester (OEt) blocking groups were employed in four series of alanine oligomers:

Z-(Ala) <sub>n</sub> -OEt	<i>n</i> = 2-7
MEEA-(Ala) <sub>n</sub> -OEt	<i>n</i> = 3-7
MEEA-(Ala) <sub>n</sub> -Mo	<i>n</i> = 3, 5-9
Z-(Ala) <sub>n</sub> -Mo	<i>n</i> = 2-9

The four series of compounds were prepared using the mixed anhydride method described by Anderson (18). We found that this technique provides optically pure peptides in reasonable yield.

## RESULTS AND DISCUSSION

### *Synthesis of Oligomers of L-Alanine*

The synthesis of all oligomer series was commenced by separately preparing N-terminal blocked and C-terminal blocked L-alanine. Benzyloxycarbonyl-L-alanine (I) was prepared using the Schotten-Baumann procedure described by Bergmann and Zervas [19]. The carboxylic acid group was protected by preparing the corresponding ethyl ester hydrochloride (II) according to the method of Rowlands and Young (20). The dipeptide Z-(Ala)<sub>2</sub>-OEt (III) and tripeptide Z-(Ala)<sub>3</sub>-OEt (VI) were prepared using a mixed anhydride procedure as described in the experimental section.

The extension of the oligomer chain was continued by removing either the "Z" or ethyl ester blocking group and lengthening the chain from the unprotected end. The removal of the benzyloxycarbonyl group was accomplished using a catalytic hydrogenation procedure described by Brand and Erlanger (21) and later modified by our group (9). The diblocked peptide was dissolved in either methanol or trifluoroethanol and hydrogenated in the presence of 10% Pd/C catalyst and hydrochloric acid. The resulting hydrochloride salts were used without recrystallization. L-Alanyl-L-alanine ethyl ester

hydrochloride IV as well as the analogous deblocked tripeptide were prepared using the above method.

The ethyl ester was cleaved from the oligopeptides using a basic hydrolysis procedure similar to that described by Brand et al. (21). Although we were able to saponify both di- and tripeptide ethyl esters, we were unable to cleave the ethyl ester from tetramers or higher oligomers. A similar finding was reported by Langsam (22), who was unable to displace the ethyl ester of Z-(Ala)<sub>5</sub>-OEt using a large excess of hydrazine. This circumstance presented somewhat of a limitation to our synthetic approach since we were restricted to preparing the higher oligomers using a trimer on one end.

Anderson has shown that if the mixed anhydride is formed in solvents, such as ethyl acetate or tetrahydrofuran, using an exact equivalence of all reactants, racemization-free products result. For all the oligomers prepared we used conditions identical to those described in Anderson's study. According to Anderson, the formation of the product occurs rapidly and the reaction can be worked up in a matter of minutes (18). We found that, especially for the higher oligomers, longer reaction times resulted in improved yields. In light of this we usually allowed the reaction to proceed at -20° for 2-3 hr. Then the reaction was allowed to warm slowly to room temperature. The resulting peptide was recovered using a suitable purification procedure. In general the exact procedure used depended on whether the resulting oligomers were soluble in the reaction medium or whether they precipitated from solution. The former oligomers were isolated by a suitable extraction procedure. The latter were removed from solution by filtration. Exact details of the purification procedures are presented in the experimental section.

We prepared the MEEA blocking group starting from 2-methoxy-[2-ethoxy-(2-ethoxy)] ethyl acetate. This ester was converted to the corresponding alcohol using acid-catalyzed hydrolysis in methanol. The reaction proceeded smoothly and the alcohol was obtained in better than 80% yield. We converted this alcohol to the analogous carboxylic acid by oxidation using potassium permanganate in alkaline solution. Although the yield obtained in this reaction was low (~20%) we were able to prepare a usable blocking group in this manner.

After preparing our blocking group, we commenced the synthesis of the MEEA-(Ala)<sub>n</sub>-OEt oligopeptides using many of the procedures described previously. The corresponding dimer [MEEA-(Ala)<sub>2</sub>-OEt] was a viscous oil which was difficult to purify. We, therefore, decided to begin the conformational analysis of these oligomers at the tripeptide. We also found that MEEA-(Ala)<sub>3</sub>-OH was impossible to separate from the analogous ethyl ester using recrystallization techniques. Chromatographic separations were also not particularly effective. Purification of the free acid was finally achieved using the extraction procedure described in detail in the experimental section.

The synthesis of the four series of L-alanine oligomers was quite similar. A typical synthesis is shown in Scheme 1 for the MEEA-(Ala)<sub>n</sub>-Mo series. A summary of the melting points and optical rotations of the oligomers prepared is presented in Table 1.

After completing the synthesis of the desired oligopeptides we began to characterize some of their physical properties. As discussed earlier, one of our primary goals was the improved solubility of these oligomers in helix-supporting solvents. Trifluoroethanol (TFE) has been shown to be a helix-supporting solvent for oligopeptides of  $\gamma$ -ethyl-L-glutamate (14) and  $\beta$ -methyl-L-aspartate (8). Our investigations indicated that all peptides in both alanine series were soluble enough in TFE to be examined using circular dichroism. Results of this conformational analysis will be presented in a later paper.

TABLE I  
SUMMARY OF PHYSICAL PROPERTIES OF NEW L-ALANINE OLIGOMERS

Compound	mp	$[\alpha]_D^{25}$	Calculated			Found		
			C	H	N	C	H	N
Z-(Ala) <sub>4</sub> -OEt	251–253°	–95.1°	56.89	6.94	12.06	56.94	6.76	11.51
Z-(Ala) <sub>6</sub> -OEt	>275°d	–119.3°	55.43	6.98	13.85	54.91	6.74	13.46
Z-(Ala) <sub>7</sub> -OEt	>275°d	–125.4°	54.94	6.99	14.47	54.66	6.94	14.24
Z-(Ala) <sub>2</sub> -Mo	135–136°	–35.4°	59.49	6.93	11.56	59.25	7.05	11.46
Z-(Ala) <sub>3</sub> -Mo	198–200°	–62.5°	58.05	6.96	12.90	57.84	6.81	13.02
Z-(Ala) <sub>4</sub> -Mo	270–271°	–78.0°	57.02	6.98	13.85	56.84	6.86	13.67
Z-(Ala) <sub>5</sub> -Mo	>275°d	–92.0°	56.24	6.99	14.57	56.13	7.02	14.48
Z-(Ala) <sub>6</sub> -Mo	>275°d	–102.5°	55.63	7.00	15.14	55.38	7.02	14.84
Z-(Ala) <sub>7</sub> -Mo	>275°d	–108.0°	55.14	7.01	15.59	55.05	7.12	15.38
Z-(Ala) <sub>8</sub> -Mo	>275°d	–117.1°	54.74	7.02	15.96	54.92	7.13	15.81
Z-(Ala) <sub>9</sub> -Mo	>275°d	–121.6°						
MEEA-(Ala) <sub>3</sub> -OEt	138.5–140°	–70.7°	51.54	7.93	10.02	51.38	8.08	10.04
MEEA-(Ala) <sub>4</sub> -OEt	240–241°	–89.2°	51.42	7.81	11.42	51.31	7.65	11.59
MEEA-(Ala) <sub>5</sub> -OEt	>250°d	–98.5°	51.33	7.72	12.47	51.22	7.56	12.36
MEEA-(Ala) <sub>6</sub> -OEt	>250°d	–108.2°	51.26	7.65	13.28	50.99	7.69	13.29
MEEA-(Ala) <sub>7</sub> -OEt	>250°d	–119.0°	51.20	7.59	13.93	50.44	7.35	13.49
MEEA-(Ala) <sub>3</sub> -Mo	110–111°	–49.9°	52.16	7.88	12.17	52.02	7.89	12.09
MEEA-(Ala) <sub>5</sub> -Mo	250°d	–87.5°	51.82	7.69	13.94	51.68	7.81	13.76
MEEA-(Ala) <sub>6</sub> -Mo	>275°d	–94.5°	51.70	7.63	14.55	51.54	7.80	14.44
MEEA-(Ala) <sub>7</sub> -Mo	>275°d	–102.3°	51.60	7.58	15.04	51.41	7.75	14.90
MEEA-(Ala) <sub>8</sub> -Mo	>275°d	–104.4°	51.52	7.54	15.45			
MEEA-(Ala) <sub>9</sub> -Mo	>275°d	–110.2°	51.45	7.50	15.79			

#### Molar Rotation Studies on Alanine Oligomers

We previously indicated that optical purity is a necessary (3) prerequisite for the formation of stable secondary structures by polypeptides and oligopeptides. In order for our conformational analysis to be meaningful, therefore, it was of paramount importance that all oligomers be optically pure. We demonstrated that in a helix-breaking solvent a plot of molar rotation ( $[\phi]_M$ ) versus the number of residues ( $n$ ) in an oligopeptide should be linear (3, 13). Deviations from linearity may then be attributed either to impurities or racemization. Applying this method we measured the optical activity of our oligopeptides at the sodium D-line (589 nm) in trifluoroacetic acid (TFA). Figures 1 and 2 show that the molar rotation values for all oligomer series fall on straight lines within experimental error. The slopes of all lines are nearly equal indicating the same contribution to the optical activity for internal residues in each series. We conclude that little if any racemization occurred during the synthetic procedures.

## EXPERIMENTAL

### Materials

L-Alanine was purchased from the Ajinomoto Chemical Co., Inc., Japan. *N*-Methyl morpholine, morpholine, and benzyl-chloroformate were purchased from the Aldrich Chemical Co. Isobutyl chloroformate was purchased from either the Frinton Chemical Co. or Fisher Scientific Co. The above reagents were obtained in the purest form avail-

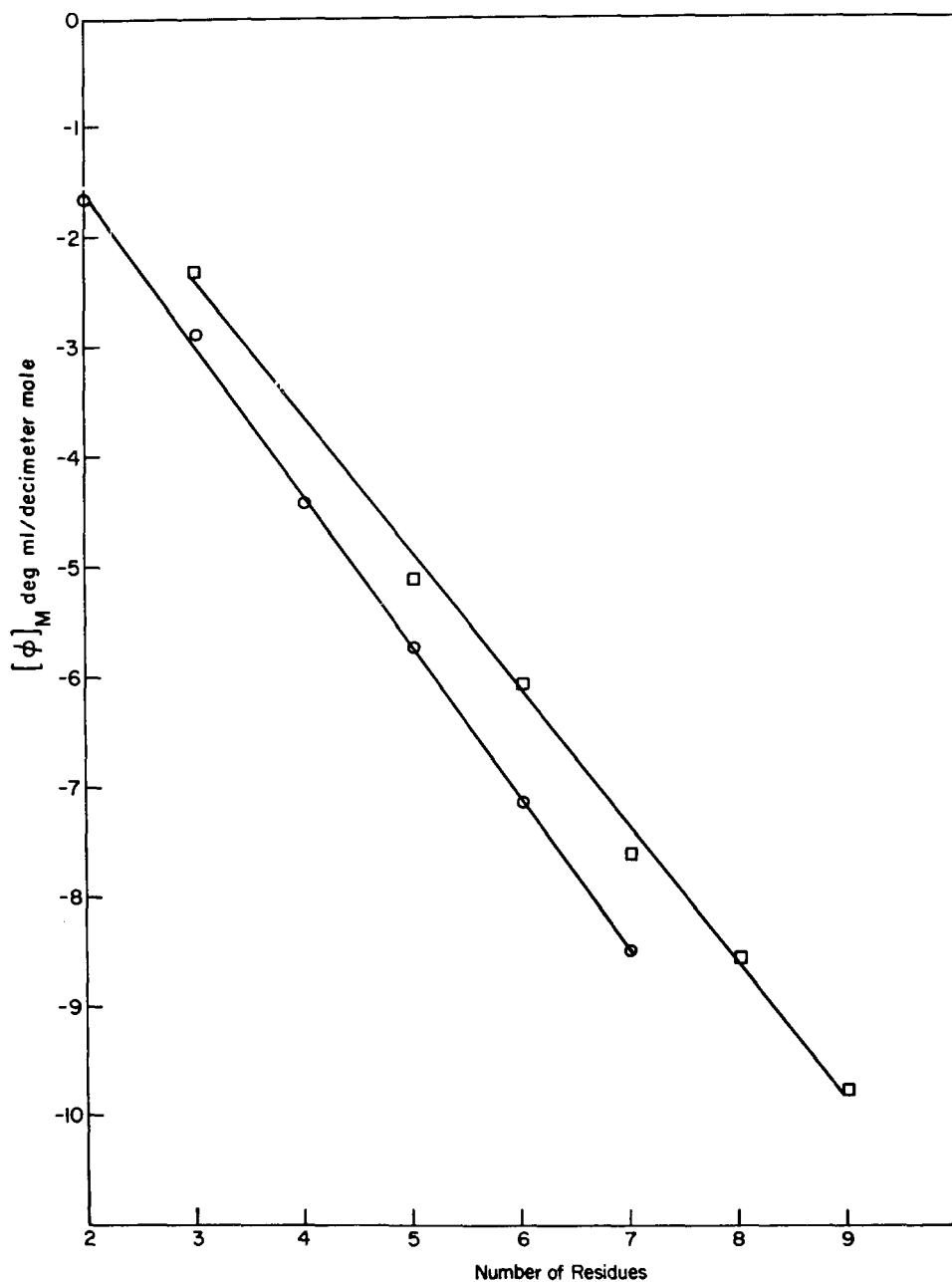


FIG. 1. Molar rotations in trifluoroacetic acid;  $Z-(Ala)_n-OEt$  ○,  $MEEA-(Ala)_n-Mo$  □.

able. They were used without further purification. All solvents used were reagent grade chemicals. Methanol, ethyl acetate, chloroform, carbon tetrachloride, and ethyl ether were obtained from the Mallinkrodt Chemical Co. Dimethylformamide and tetrahydrofuran were purchased from Matheson, Coleman, and Bell. Both these solvents

contained less than 0.05% water and were stored over No. 5A molecular sieves prior to use in the coupling reactions. Trifluoroacetic acid (Aldrich) and trifluoroethanol (Halocarbon Corp.) were obtained in the highest purity available and used without further purification. Sulfuric acid, hydrochloric acid, sodium bicarbonate, and anhydrous magnesium sulfate were purchased from the Allied Chemical Co. 2[2-(2-Methoxy ethoxy)] ethyl acetate was obtained from Matheson, Coleman, and Bell.

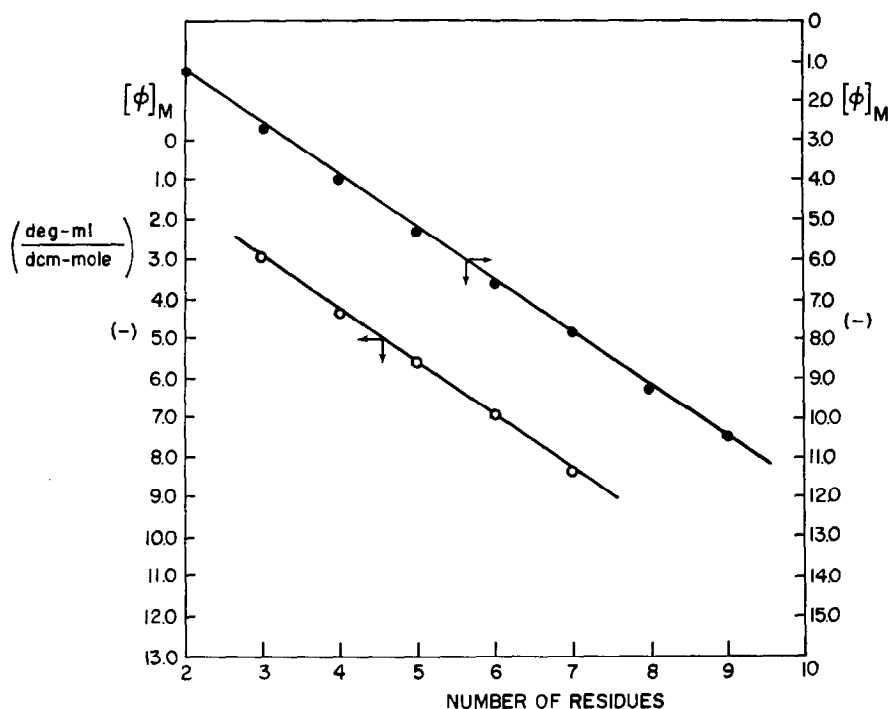


FIG. 2. Molar rotations in trifluoroacetic acid; MEEA-(Ala)<sub>n</sub>-OEt ○, Z-(Ala)<sub>n</sub>-Mo ●.

### Preparation of Compounds

**Benzyloxycarbonyl-L-alanine (I).** Benzyloxycarbonyl-L-alanine was prepared using the method described by Bergmann and Zervas (19) on a 0.8-molar scale. After recrystallization from chloroform-petroleum ether the product was obtained in 75% yield, mp 87°,  $[\alpha]_D^{25} -13.7^\circ$  (c2, HOAc) [lit. (19) mp 87°,  $[\alpha]_D^{25} -13.9^\circ$  (c2, HOAc)].

**L-Alanine ethyl ester hydrochloride(II).** The ethyl ester hydrochloride of L-alanine was prepared using the method of Rowlands and Young on a 0.2 molar scale. The desired product was obtained in ~100% yield, mp 76° [lit. (23) mp 76°].

**Benzyloxycarbonyl-L-alanyl-L-alanine ethyl ester (III).** This material was prepared from benzyloxycarbonyl-L-alanine and L-alanine ethyl ester hydrochloride, using the mixed anhydride method described by Anderson (18). The crude dimer weighed 31 g. It was recrystallized from ethyl acetate-hexane to give 28.5 g (89% yield) of a white crystalline product, mp 116–116.5° [lit. (24) mp 116°];  $[\alpha]_D^{25} -51.1^\circ$  (c 0.5, trifluoroacetic acid) [lit. (12)  $[\alpha]_D^{25} = -50.78^\circ$  (c 0.25, trifluoroacetic acid)].

**L-Alanyl-L-alanine ethyl ester hydrochloride (IV).** Cleavage of the benzyloxycarbonyl blocking group from the diblocked dimer was accomplished using catalytic hydrogenolysis in methanol according to the method of Brand and Erlanger (21). The product

thus obtained was an oily, hygroscopic solid. This material (98% yield) was used without further purification.

**Benzyloxycarbonyl-L-alanyl-L-alanine (V).** Benzyloxycarbonyl-L-alanyl-L-alanine ethyl ester (6.44 g, 0.02 mole) was dissolved in 150 ml of methanol and the solution heated to 37° with stirring. Sodium hydroxide (2 N, 11 ml, 0.022 mole) was added to the reaction medium and the resultant mixture was stirred at 37° for 2 hr. At this time the solution was cooled in an ice bath and acidified to pH 2 (hydron paper) with concentrated hydrochloric acid. The solvent methanol was then removed under reduced

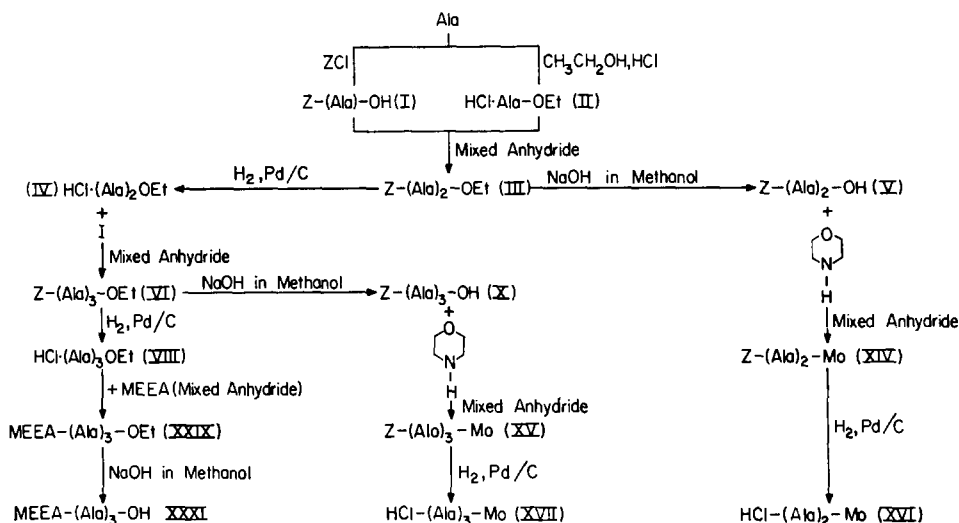


FIG. 3. Preparation of MEEA-(Ala)<sub>n</sub>-Mo (n = 3, 5-9).

pressure at low temperature (~35°). The resulting solid was recrystallized from hot chloroform to give 4.1 g (70%) of the white crystalline product, mp 149–151° [lit. (24) mp 153°],  $[\alpha]_D^{25} -49.7^\circ$  (c 0.47, trifluoroacetic acid).

**Benzyloxycarbonyl-di-(L-alanyl)-L-alanine ethyl ester (VI).** Benzyloxycarbonyl-di-(L-alanyl)-L-alanine ethyl ester was prepared using the same procedure used for the analogous dimer. The reaction was run on a 0.0446 molar scale. After recrystallization from ethyl acetate-hexane 14 g (80%) of white crystalline trimer were obtained, mp 188–190° [lit. (25) mp 192°],  $[\alpha]_D^{25} -73.0^\circ$  (c 0.5, trifluoroacetic acid) [lit. (12)  $[\alpha]_D^{25} -72.01^\circ$  (c 0.5, trifluoroacetic acid)].

**Benzyloxycarbonyl-tri-(L-alanyl)-L-alanine ethyl ester (VII).** Benzyloxycarbonyl-L-alanyl-L-alanine (0.88 g, 0.003 mole) was reacted with L-alanyl-L-alanine ethyl ester hydrochloride using the mixed anhydride procedure. After two hr the solution had warmed to room temperature and the mixture was filtered through a sintered-glass funnel. The resulting solid was thoroughly washed with 1 N hydrochloric acid, saturated sodium bicarbonate, and distilled water. It was further purified by slurring with methanol and ether. The product obtained after drying was recrystallized from hot ethanol to give 0.86 g (62%) of a white powdery solid, mp 251–253°d,  $[\alpha]_D^{25} -95^\circ$  (c 0.50, trifluoroacetic acid).

*Anal.* Calcd for C<sub>22</sub>H<sub>32</sub>N<sub>4</sub>O<sub>7</sub>: C, 56.89; H, 6.94; N, 12.06. Found: C, 56.94; H, 6.76; N, 11.51.

*Di-(L-alanyl)-L-alanine ethyl ester hydrochloride (VIII).* Benzyloxycarbonyl-di-(L-alanyl)-L-alanine ethyl ester (7.0 g, 0.0178 mole) was hydrogenated in 200 ml of methanol using the procedure employed for the analogous dimer. In order to obtain complete solution it was necessary to first dissolve the trimer in hot methanol and then add the cooled solution to the hydrogenation flask. The product, a hygroscopic solid, was obtained in 85% yield (4.5 g), mp 144–146°d. This material was used without further purification.

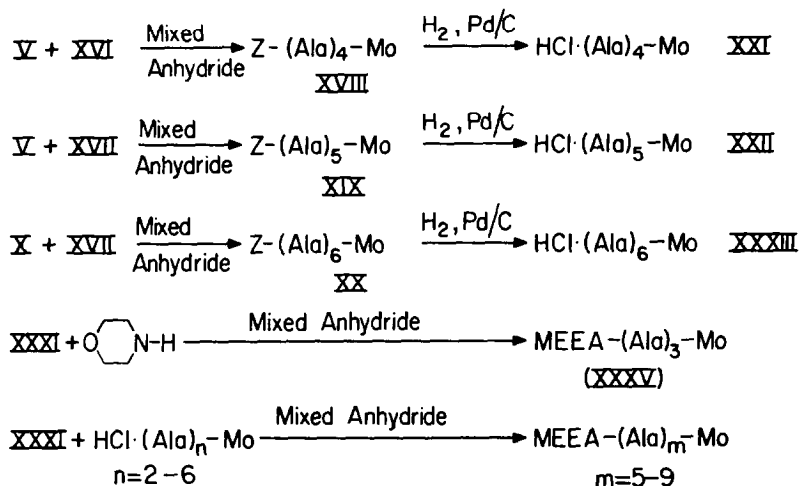


FIG. 4. Synthetic scheme for the preparation of 2-methoxy-[2-ethoxy-(2-ethoxy)] acetyl L-alanine oligopeptide morpholine amide derivatives.

*Tri-L-alanyl-L-alanine ethyl ester hydrochloride (IX).* Benzyloxycarbonyl-tri-(L-alanyl)-L-alanine ethyl ester (0.5 g, 0.0011 mole) was hydrogenated in 50 ml of trifluoroethanol using a procedure identical to that described for the analogous dimer. The resulting white crystalline material (0.36 g, 91% yield) was used without further purification, mp 240°d.

*Benzyloxycarbonyl-di-(L-alanyl)-L-alanine (X).* Benzyloxycarbonyl-di-(L-alanyl)-L-alanine ethyl ester (7.86 g, 0.02 mole) was saponified using a procedure similar to that described for the analogous dimer. The crude product was recrystallized from hot ethyl acetate to give 5.1 g (70%) of a white crystalline solid, mp 224–226° [lit. (26) mp 225–226°],  $[\alpha]_D^{25} -74.6^\circ$  (c 0.45, trifluoroacetic acid). It should be noted that approximately 4 liters of ethyl acetate were necessary to recrystallize the product.

*Benzyloxycarbonyl-tetra-(L-alanyl)-L-alanine ethyl ester (XI).* This compound was prepared from benzyloxycarbonyl-(L-alanyl)-L-alanine and di-(L-alanyl)-L-alanine ethyl ester hydrochloride, using the same procedure employed in the synthesis of benzyloxycarbonyl-tri-(L-alanyl)-L-alanine ethyl ester. The reaction was run on a 0.01-mole scale. Recrystallization from hot trifluoroethanol–ethanol gave a white crystalline material in 75% yield, mp 250°;  $[\alpha]_D^{25} -107.1^\circ$  (c 0.5, trifluoroacetic acid) [lit. (12) mp 250°;  $[\alpha]_D^{25} -104.9^\circ$  (c 0.5, trifluoroacetic acid)].

*Anal.* Calcd for  $\text{C}_{25}\text{H}_{37}\text{N}_5\text{O}_8$ : C, 56.06; H, 6.96; N, 13.08. Found: C, 55.83; H, 6.88; N, 13.01.

*Benzyloxycarbonyl-penta-(L-alanyl)-L-alanine ethyl ester (XII).* This compound was prepared from benzyloxycarbonyl-di-(L-alanyl)-L-alanine and di-(L-alanyl)-L-alanine



ethyl ester hydrochloride on a 0.01-mole scale, using the same procedure employed in the preparation of benzyloxycarbonyl-tri-(L-alanyl)-L-alanine ethyl ester. Recrystallization from hot trifluoroethanol gave a white crystalline material in 60% yield, mp  $> 275^{\circ}\text{C}$ ;  $[\alpha]_{\text{D}}^{25} -119.3^{\circ}$  (*c* 0.25, trifluoroacetic acid).

*Anal.* Calcd for  $\text{C}_{28}\text{H}_{42}\text{N}_6\text{O}_9$ : C, 55.43; H, 6.98; N, 13.85. Found: C, 54.91; H, 6.74; N, 13.46.

*Benzyloxycarbonyl-hexa-(L-alanyl)-L-alanine ethyl ester (XIII).* This compound was prepared from benzyloxycarbonyl-di-(L-alanyl)-L-alanine and tri-(L-alanyl)-L-alanine ethyl ester hydrochloride on a 0.002-mole scale, using the same technique employed in the synthesis of benzyloxycarbonyl-tri-(L-alanyl)-L-alanine ethyl ester. Recrystallization from hot trifluoroethanol-ethanol gave a white crystalline material in 62% yield, mp  $275^{\circ}\text{C}$ ;  $[\alpha]_{\text{D}}^{25} -125.4^{\circ}$  (*c* 0.25, trifluoroacetic acid).

*Anal.* Calcd for  $\text{C}_{31}\text{H}_{47}\text{N}_7\text{O}_{10}$ : C, 54.94; H, 6.99; N, 14.47. Found: C, 54.66; H, 6.94; N, 14.24.

*Benzyloxycarbonyl-L-alanyl-L-alanine morpholine amide (XIV).* Benzyloxycarbonyl-L-alanyl-L-alanine (2.94 g, 0.01 mole) was dissolved in 100 ml of dry tetrahydrofuran in a 250-ml three-necked round-bottomed flask. The solution was cooled to  $-20^{\circ}$  and *N*-methyl morpholine (1.01 g, 0.01 mole) was added. Isobutyl chloroformate was then added and a white precipitate immediately formed. This mixture was stirred at  $-20^{\circ}$  for 5 min at which time morpholine (0.96 g, 0.011 mole) was added to the reaction flask. The reaction proceeded at  $-20^{\circ}$  for 2 hrs and then the flask was allowed to slowly warm to room temperature. The solution was filtered and the tetrahydrofuran removed under reduced pressure. The resulting solid was dissolved in 400 ml of chloroform and extracted with 25 ml of 1 *N* hydrochloric acid, 50 ml of saturated sodium bicarbonate, and 25 ml of distilled water. The chloroform layer was dried over magnesium sulfate and the solvent removed under reduced pressure. The resulting white solid (2.7 g, 75%) was recrystallized from ethyl acetate, mp  $135-136^{\circ}$ ,  $[\alpha]_{\text{D}}^{25} -35.4^{\circ}$  (*c* 0.47, trifluoroacetic acid).

*Anal.* Calcd for  $\text{C}_{18}\text{H}_{25}\text{N}_3\text{O}_5$ : C, 59.49; H, 6.93; N, 11.56. Found: C, 59.25; H, 7.05; N, 11.46.

*Benzyloxycarbonyl-di-(L-alanyl)-L-alanine morpholine amide (XV).* Benzyloxycarbonyl-di-(L-alanyl)-L-alanine (2.0 g, 0.0055 mole) was dissolved in 400 ml of dry tetrahydrofuran in a 500-ml three-necked round-bottomed flask. The solution was cooled to  $-20^{\circ}$  in a Dry Ice-carbon tetrachloride bath and *N*-methyl morpholine (0.553 g, 0.0055 mole) was added. Isobutyl chloroformate (0.751 g, 0.0055 mole) was then added and a white precipitate immediately formed. This mixture was stirred for 5 min at  $-20^{\circ}$  at which time morpholine (0.494 g, 0.0057 mole) was added. The solution was stirred for 2 hr at  $-20^{\circ}$  and allowed to warm slowly to room temperature. After the white precipitate was removed by filtration, the tetrahydrofuran was removed under reduced pressure. The resulting solid was dissolved in 400 ml of chloroform and extracted with 50 ml of 1 *N* hydrochloric acid, 100 ml of saturated sodium bicarbonate, and 100 ml of distilled water. The chloroform layer was dried over magnesium sulfate and the solvent removed under reduced pressure. The resulting white powder (2.1 g, 87%) was recrystallized from hot ethyl acetate, mp  $198-200^{\circ}$ ,  $[\alpha]_{\text{D}}^{25} -59.84^{\circ}$  (*c* 0.48, trifluoroacetic acid).

*Anal.* Calcd for  $\text{C}_{21}\text{H}_{30}\text{N}_4\text{O}_6$ : C, 58.05; H, 6.96; N, 12.90. Found: C, 57.84; H, 6.81; N, 13.02.

*L-Alanyl-L-alanine morpholine amide hydrochloride (XVI).* Benzyloxycarbonyl-L-alanyl-L-alanine morpholine amide (2.7 g, 0.0075 mole) was hydrogenated in 100 ml of methanol using a 10% Pd/C catalyst (0.1 g) in the presence of concentrated hydrochloric acid (0.83 ml, 0.009 mole). The hydrogen uptake subsided after approximately 20 min

but the reaction was allowed to proceed for 2 hr to ensure completion. At that time the solution was filtered and the methanol removed under reduced pressure. Subsequent addition and evaporation of added ethanol resulted in the solidification of the hydrochloride salt. After slurrying this solid in ethyl acetate and filtering, 1.75 g (88%) of a white crystalline product was obtained, mp 230–232°d. The material was used without further purification.

*Di-(L-Alanyl)-L-alanine morpholine amide hydrochloride (XVII).* Benzyloxycarbonyl-di-(L-alanyl)-L-alanine morpholine amide (1 g, 0.0023 mole) was hydrogenated in 100 ml of methanol using a similar procedure to that described for the analogous dimer. The 10% Pd/C catalyst was activated by washing with glacial acetic acid just prior to the reaction. After the remaining water had been eliminated via azeotropic distillation with ethanol, a white crystalline product was recovered (0.73 g, 94%), mp 135°d. This salt was used without further purification.

*Benzyloxycarbonyl-tri-(L-alanyl)-L-alanine morpholine amide (XVIII).* Benzyloxycarbonyl-L-alanyl-L-alanine (0.882 g, 0.003 mole) was dissolved in 100 ml of dry tetrahydrofuran in a 250-ml three-necked round-bottomed flask. The solution was cooled with stirring to –20° in a dry ice–carbon tetrachloride bath and *N*-methyl morpholine (0.303 g, 0.003 mole) was added. Isobutyl chloroformate (0.411 g, 0.003 mole) was then added and a white precipitate immediately formed. The mixture was stirred at –20° for 5 min, at which time a solution of L-alanyl-L-alanine morpholine amide hydrochloride (0.795 g, 0.003 mole) and *N*-methyl morpholine (0.303 g, 0.003 mole) in 40 ml of dry dimethylformamide was added to the reaction flask. The resulting suspension was noted to have thickened considerably. The reaction was allowed to proceed at –20° for 2 hr and then slowly warmed to room temperature. The reaction solution was filtered through a sintered-glass funnel and the remaining solid was washed several times with hot chloroform. The chloroform washings were combined with the reaction solution and diluted with 400 ml of chloroform. This organic layer was extracted with 150 ml of 1 *N* hydrochloric acid, 100 ml of saturated sodium bicarbonate, and 100 ml of distilled water. The organic layer was then dried over magnesium sulfate and evaporated under reduced pressure. The resulting solid (600 mg, 40%) was recrystallized from hot ethanol, mp 270–271°,  $[\alpha]_D^{25} = -78.0^\circ$  (*c* 0.46, trifluoroacetic acid).

*Anal.* Calcd for  $C_{24}H_{35}N_5O_7$ : C, 57.02; H, 6.98; N, 13.85. Found: C, 56.84; H, 6.86; N, 13.67.

*Benzyloxycarbonyl-tetra-(L-alanyl)-L-alanine morpholine amide (XIX).* Benzyloxycarbonyl-L-alanyl-L-alanine (0.735 g, 0.0025 mole) was dissolved in 100 ml of dry tetrahydrofuran in a 250-ml three-necked round-bottomed flask. The solution was cooled to –20° and *N*-methyl morpholine (0.256 g, 0.0025 mole) was added followed by isobutyl chloroformate (0.346 g, 0.0025 mole). A white precipitate was observed to form immediately. After this mixture was stirred at –20° for 5 min, a solution of di-(L-alanyl)-L-alanine morpholine amide hydrochloride (0.85 g, 0.0025 mole) and *N*-methyl morpholine (0.256 g, 0.0025 mole) in 25 ml of dimethylformamide was added to the reaction flask. The reaction medium thickened noticeably. The reaction proceeded at –20° for 3 hr and then was allowed to warm to room temperature. The resulting suspension was filtered through a sintered-glass funnel to give a thick white solid. This solid was carefully washed on the filter funnel with 1 *N* hydrochloric acid (three times), saturated sodium bicarbonate (three times), and distilled water (until the washes were neutral). The remaining solid was washed several times with ether until a dry powder resulted. This powder was slurried several times with boiling ethanol to remove any impurities. In this manner 0.95 g (65%) of a white powdery product were obtained, mp > 275°d,  $[\alpha]_D^{25} -92^\circ$  (*c* 0.45, trifluoroacetic acid).

*Anal.* Calcd for  $C_{27}H_{40}N_6O_8$ : C, 56.24; H, 6.99; N, 14.57. Found: C, 56.13; H, 7.02; N, 14.48.

*Benzylloxycarbonyl-penta-(L-alanyl)-L-alanine morpholine amide (XX).* Benzylloxycarbonyl-di-(L-alanyl)-L-alanine (1.1 g, 0.003 mole) was allowed to react with di-(L-alanyl)-L-alanine morpholine amide hydrochloride using the mixed anhydride procedure employed to prepare the analogous pentamer. The product was worked up in an identical manner and the final purification was effected by slurrying the solid in hot ethanol. The product was a white powdery solid (1.58 g, 82%), mp > 275°d,  $[\alpha]_D^{25} -102.5^\circ$  (c 0.45, trifluoroacetic acid).

*Anal.* Calcd for  $C_{30}H_{45}N_7O_9$ : C, 55.63; H, 7.00; N, 15.14. Found: C, 55.38; H, 7.02; N, 14.82.

*Tri-(L-alanyl)-L-alanine morpholine amide hydrochloride (XXI).* Benzylloxycarbonyl-tri-(L-alanyl)-L-alanine morpholine amide (0.505 g, 0.001 mole) was hydrogenated in 100 ml of methanol using a procedure similar to those described for the analogous dimer and trimer. In order to obtain complete solution it was necessary to dissolve the reactant in hot methanol, and then add the cooled solution to the hydrogenation flask. The white crystalline solid so obtained (0.39 g, 97%), mp 245°d, was used without further purification.

*Tetra-(L-alanyl)-L-alanine morpholine amide hydrochloride (XXII).* Benzylloxycarbonyl-tetra-(L-alanyl)-L-alanine morpholine amide (0.900 g, 0.0016 mole) was hydrogenated in 75 ml of trifluoroethanol using a procedure identical to that described for the analogous dimer. The uptake of hydrogen subsided in approximately 15 min. The white crystalline solid thus obtained (0.67 g, 90%), mp > 250°d, was used without further purification.

*Penta-(L-alanyl)-L-alanine morpholine amide hydrochloride (XXIII).* Benzylloxycarbonyl-penta-(L-alanyl)-L-alanine morpholine amide (1.00 g, 0.0015 mole) was hydrogenated in 100 ml of trifluoroethanol using a procedure similar to that described for the corresponding dimer. In order to completely dissolve the hexamer it was necessary to first heat it in trifluoroethanol and then add the cooled solution to the hydrogenation flask. The product recovered after the cleavage was a white crystalline solid (0.800 g, 95%), mp > 250°d. The material was used without further purification.

*Benzylloxycarbonyl-hexa-(L-alanyl)-L-alanine morpholine amide (XXIV).* Benzylloxycarbonyl-di-(L-alanyl)-L-alanine (0.147 g, 0.0005 mole) was reacted with tetra-(L-alanyl)-L-alanine morpholine amide hydrochloride using a mixed anhydride procedure. The technique was identical to that employed to prepare the analogous pentamer except that the hydrochloride salt was added in a dimethylsulfoxide solution. The ratio of dimethylsulfoxide to tetrahydrofuran was held at 1:9. In this manner a white powdery solid (0.130 g, 35%) was obtained, mp > 275°d,  $[\alpha]_D^{25} -108.1^\circ$  (c 0.45, trifluoroacetic acid).

*Anal.* Calcd for  $C_{33}H_{50}N_8O_{10}$ : C, 55.14; H, 7.01; N, 15.59. Found: C, 55.05; H, 7.02; N, 15.38.

*Benzylloxycarbonyl-hepta-(L-alanyl)-L-alanine morpholine amide (XXV).* Benzylloxycarbonyl-di-(L-alanyl)-L-alanine (0.183 g, 0.0005 mole) was coupled with tetra-(L-alanyl)-L-alanine morpholine amide hydrochloride using a mixed anhydride coupling procedure. The method was similar to that used to prepare the analogous pentamer except that the hydrochloride salt was added in a dimethylsulfoxide solution. The ratio of the dimethylsulfoxide to tetrahydrofuran was held at 1:9. In order to purify the crude product obtained after the usual washings the octamer was dissolved in hexafluoroisopropanol and crystallized by adding distilled water. The final product was a

white powdery solid (0.150 g, 38 %), mp  $> 275^{\circ}\text{C}$ ,  $[\alpha]_{\text{D}}^{25} -117.1^{\circ}$  ( $c$  0.44, trifluoroacetic acid).

*Anal.* Calcd for  $\text{C}_{36}\text{H}_{55}\text{N}_9\text{O}_{11}$ : C, 54.74; H, 7.02; N, 15.96. Found: C, 54.92; H, 7.13; N, 15.81.

*Benzyloxycarbonyl-octa-(L-alanyl)-L-alanine morpholine amide (XXVI).* Benzyloxycarbonyl-di-(L-alanyl)-L-alanine (0.183 g, 0.0005 mole) was coupled with penta-(L-alanyl)-L-alanine morpholine amide hydrochloride using a mixed anhydride reaction. The procedure used was identical to that described for the analogous octamer. Final purification of the crude nonamer was effected as follows: The nonamer was first washed with hot trifluoroethanol. The remaining solid was dissolved in trifluoroacetic acid and filtered through a sintered-glass funnel into ether where it precipitated out. The recovered purified product was a white powdery material (0.170 g, 40 %), mp  $> 275^{\circ}\text{C}$   $[\alpha]_{\text{D}}^{25} -121.6^{\circ}$  ( $c$  0.20, trifluoroacetic acid).

*2-Methoxy-[2-ethoxy-(2-ethoxy)]ethanol (XXVII).* 2-Methoxy-[2-ethoxy-(2-ethoxy)]ethyl acetate (200 g, 0.97 mole) was dissolved in 250 ml of anhydrous methanol in an open one-necked 1-liter round-bottomed flask. *p*-Toluenesulfonic acid (1 g) was added to this solution and the resulting mixture was heated with stirring at  $65-70^{\circ}$  overnight. During this time most of the methanol and the methyl acetate which formed distilled off. The next morning the rest of the solvent methanol was removed at atmospheric pressure. The remaining solution was cooled and then distilled under high vacuum. In this manner 128 g (81 %) of 2-methoxy-[2-ethoxy-(2-ethoxy)]ethanol, bp  $92-95^{\circ}$ , 0.1 Torr, were obtained. The product showed no absorption in the infrared carbonyl region,  $1600-1800\text{ cm}^{-1}$ .

*2-Methoxy-[2-ethoxy-(2-ethoxy)] acetic acid (XXVIII).* 2-Methoxy-[2-ethoxy-(2-ethoxy)]ethanol (164 g, 1.0 mole) was dissolved in 250 ml of deionized water containing sodium hydroxide (40 g, 1.0 mole). The mixture was stirred vigorously, and a solution of potassium permanganate (310 g, 2 moles) in 1500 ml of deionized water was added. The addition of the oxidizing agent was followed by an immediate temperature rise accompanied by the formation of a dark-brown precipitate. The reaction was allowed to proceed overnight to insure completion. The next morning the suspension was strongly acidified with concentrated sulfuric acid. Sodium bisulfite powder was then added until all of the brown precipitate (manganese dioxide) had dissolved. It was necessary to add more sulfuric acid to keep the reaction medium acidic during this latter procedure. The resulting solution was extracted with 1 liter of ether for 3 days using a continuous liquid-liquid extractor. The ethereal layer was dried over magnesium sulfate and the solvent was removed under reduced pressure. The remaining liquid was fractionated under very high vacuum. The desired product was found to be the highest boiling fraction and was a water-white viscous liquid, bp  $135-138^{\circ}$ ,  $10^{-4}$  Torr. Examination of the product (38 g, 21 %) using nmr showed all the expected resonances in the correct ratio. The recovered acid had a neutralization eq. of 182 (theoretical 178).

*2-Methoxy-[2-ethoxy-(2-ethoxy)]acetyl-di-(L-alanyl)-L-alanine ethyl ester (XXIX).* 2-Methoxy-[2-ethoxy-(2-ethoxy)] acetic acid (2.58 g, 0.0145 mole) was coupled with di-(L-alanyl)-L-alanine ethyl ester hydrochloride using a mixed anhydride reaction. The procedure used was similar to that employed in the preparation of benzyloxycarbonyl-L-alanyl-L-alanine ethyl ester. After the reaction solution had been allowed to warm to room temperature it was diluted with 500 ml of chloroform. The chloroform layer was extracted with three 50-ml portions of 1 *N* hydrochloric acid, saturated sodium bicarbonate, and distilled water. These washings were then extracted several times with fresh chloroform. The combined chloroform solutions were dried over magnesium sulfate and the solvent was removed under reduced pressure. The recovered product

was a white soft waxy material. It was purified by recrystallization from chloroform-ether. The product so obtained (4.5 g, 75%) had a melting point of 138.5–140°,  $[\alpha]_D^{25}$  –70.7° (c 0.55, trifluoroacetic acid).

*Anal.* Calcd for  $C_{18}H_{33}N_3O_8$ : C, 51.54; H, 7.93; N, 10.02. Found: C, 51.38; H, 8.08; N, 10.04.

*2-Methoxy-[2-ethoxy-(2-ethoxy)]acetyl-tri-(L-alanyl)-L-alanine ethyl ester (XXX).* 2-Methoxy-[2-ethoxy-(2-ethoxy)] acetic acid (1.2 g, 0.0068 mole) was coupled with tri-(L-alanyl)-L-alanine ethyl ester hydrochloride using a mixed anhydride reaction. The procedure used was identical to that described for the corresponding trimer. The crude product recovered from the chloroform washings was recrystallized from absolute ethanol. The final product (1.5 g, 45%) was a white powdery solid, mp 240–241°,  $[\alpha]_D^{25}$  –89.2° (c 0.49, trifluoroacetic acid).

*Anal.* Calcd for  $C_{21}H_{38}N_4O_9$ : C, 51.42; H, 7.81; N, 11.42. Found: C, 51.39; H, 7.65; N, 11.59.

*2-Methoxy-[2-ethoxy-(2-ethoxy)]acetyl di-(L-alanyl)-L-alanine (XXXI).* 2-Methoxy-[2-ethoxy-(2-ethoxy)]acetyl-di-(L-alanyl)-L-alanine ethyl ester (1.7 g, 0.0041 mole) was dissolved in 40 ml of methanol and warmed to 37°. Sodium hydroxide (2 N, 2.3 ml, 0.0046 mole) was added to the above solution and the resulting mixture was stirred for 2 hr at 37°. At that time the solution was cooled to 0° and acidified to pH 2 with concentrated hydrochloric acid. After the solvent had been completely removed under high vacuum the remaining solid was dissolved in hot chloroform and separated from sodium chloride by filtration. The chloroform was again removed under reduced pressure to yield a crude white solid. Thin-layer chromatography (silica plates–ethanol eluent) indicated that the recovered solid was a mixture of unreacted ester and the free acid. These two compounds were separated by dissolving them in a small amount of distilled water and extracting the solution four or five times with an equal quantity of chloroform. The unreacted ester went completely into the chloroform solution. The desired free acid remained in the aqueous layer. The product was recovered from the water solution by freeze drying (0.95 g, 60%). Thin-layer chromatography showed that the final material was one broad spot, mp 114–116°,  $[\alpha]_D^{25}$  –79.8° (c 0.55, water). Repetition of this experiment using different hydrolysis times resulted in the same product with the same specific rotation. The latter result indicated that little if any racemization occurred during the hydrolysis.

*2-Methoxy-[2-ethoxy-(2-ethoxy)]acetyl-tetra-(L-alanyl)-L-alanine ethylester (XXXII).* 2-Methoxy-[2-ethoxy-(2-ethoxy)]acetyl-di-(L-alanyl)-L-alanine (0.100 g, 0.00026 mole) was coupled with L-alanyl-L-alanine ethyl ester hydrochloride using a mixed anhydride procedure. The reaction conditions and workup were similar to those used in the preparation of benzyloxycarbonyl-tetra-(L-alanyl)-L-alanine. In this procedure the final purification was attained by recrystallizing the crude product from absolute ethanol. In this manner 0.060 g (42% yield) of pure product were attained, mp > 250°d,  $[\alpha]_D^{25}$  –98.5° (c 0.2, trifluoroacetic acid).

*Anal.* Calcd for  $C_{24}H_{43}N_5O_{10}$ : C, 51.33; H, 7.72; N, 12.47. Found: C, 51.22; H, 7.56; N, 12.36.

*2-Methoxy-[2-ethoxy-(2-ethoxy)]acetyl-penta-(L-alanyl)-L-alanine ethyl ester (XXXIII).* 2-Methoxy-[2-ethoxy-(2-ethoxy)]acetyl-di-(L-alanyl)-L-alanine (0.100 g, 0.00026 mole) was coupled with di(L-alanyl)-L-alanine ethyl ester hydrochloride using a mixed anhydride procedure. The reaction conditions and workup were similar to those used in the preparation of benzyloxycarbonyl-tetra-(L-alanyl)-L-alanine ethyl ester. The crude product was recrystallized from trifluoroethanol–water. In this manner 0.100 g (61%) of product was obtained, mp > 250°d,  $[\alpha]_D^{25}$  –108.2° (c 0.45, trifluoroacetic acid).

*Anal.* Calcd for  $C_{27}H_{48}N_6O_{11}$ : C, 51.26; H, 7.65; N, 13.28. Found: C, 50.99; H, 7.69; N, 13.29.

*2-Methoxy-[2-ethoxy-(2-ethoxy)]acetyl-hexa-(L-alanyl)-L-alanine ethyl ester (XXXIV).* 2-Methoxy-[2-ethoxy-(2-ethoxy)]acetyl-di-(L-alanyl)-L-alanine (0.161 g, 0.00041 mole) was coupled with tri-(L-alanyl)-L-alanine ethyl ester hydrochloride using a mixed anhydride procedure. The reaction conditions and workup were similar to those used in the preparation of benzyloxycarbonyl-tetra-(L-alanyl)-L-alanine ethyl ester. The crude product was purified by slurrying with hot chloroform and hot ethanol. In this manner 0.090 g (31%) of product was obtained, mp  $> 250^{\circ}\text{d}$ ,  $[\alpha]_D^{25} -119.0^{\circ}$  (c 0.5, trifluoroacetic acid).

*Anal.* Calcd for  $C_{30}H_{53}N_7O_{12}$ : C, 51.20; H, 7.59; N, 13.93. Found: C, 50.44; H, 7.35; N, 13.49.

*2-Methoxy-[2-ethoxy-(2-ethoxy)]acetyl-di-(L-alanyl)-L-alanine morpholine amide (XXXV).* This compound was prepared from 2-methoxy-[2-ethoxy-(2-ethoxy)]acetyl-di-(L-alanyl)-L-alanine and morpholine on a 0.002-mole scale, using the same method as employed in the synthesis of benzyloxycarbonyl-(L-alanyl)-L-alanine morpholine amide. The product was extremely soluble in water and organic solvents and no suitable recrystallization solvent could be found. Therefore, this material was purified by preparative thin-layer chromatography on a silica-gel plate using ethanol as a solvent. A white sticky solid material was obtained in 30% yield, mp  $110-111^{\circ}$ ;  $[\alpha]_D^{25} -49.9^{\circ}$  (0.44, trifluoroacetic acid).

*Anal.* Calcd for  $C_{20}H_{36}N_4O_8$ : C, 52.16; H, 7.88; N, 12.17. Found: C, 52.02; H, 7.89; N, 12.09.

*2-Methoxy-[2-ethoxy-(2-ethoxy)]acetyl-tetra-(L-alanyl)-L-alanine morpholine amide (XXXVI).* This compound was prepared from 2-methoxy-[2-ethoxy-(2-ethoxy)]acetyl-di-(L-alanyl)-L-alanine and L-alanyl-L-alanine morpholine amide hydrochloride on a 0.001-mole scale, using the same method employed in the preparation of benzyloxycarbonyl-tri-(L-alanyl)-L-alanine ethyl ester. Recrystallization from hot trifluoroethanol-ethanol gave the product in 55% yield, mp  $250^{\circ}\text{d}$ ;  $[\alpha]_D^{25} -87.5^{\circ}$  (c 0.4, trifluoroacetic acid).

*Anal.* Calcd for  $C_{26}H_{46}N_6O_{10}$ : C, 51.82; H, 7.69; N, 13.94. Found: C, 51.68; H, 7.81; N, 13.76.

*2-Methoxy-[2-ethoxy-(2-ethoxy)]acetyl-penta-(L-alanyl)-L-alanine morpholine amide (XXXVII).* This compound was prepared from 2-methoxy-[2-ethoxy-(2-ethoxy)]acetyl-di-(L-alanyl)-L-alanine and di-(L-alanyl)-L-alanine morpholine amide hydrochloride on a 0.001-mole scale, using the same method employed in the synthesis of benzyloxy-carbonyl-tri-(L-alanyl)-L-alanine ethyl ester. Recrystallization from trifluoroethanol-ethanol gave the product in 52% yield, mp  $> 275^{\circ}\text{d}$ ;  $[\alpha]_D^{25} -94.5^{\circ}$  (c 0.21, trifluoroacetic acid).

*Anal.* Calcd for  $C_{29}H_{51}N_7O_{11}$ : C, 51.70; H, 7.63; N, 14.55. Found: C, 51.54; H, 7.80; N, 14.44.

*2-Methoxy-[2-ethoxy-(2-ethoxy)]acetyl-hexa-(L-alanyl)-L-alanine morpholine amide (XXXVIII).* This compound was prepared from 2-methoxy-[2-ethoxy-(2-ethoxy)]acetyl-di-(L-alanyl)-L-alanine and tri-(L-alanyl)-L-alanine morpholine amide hydrochloride on a 0.001-mole scale, using the same method used to prepare benzyloxycarbonyl-tri-(L-alanyl)-L-alanine ethyl ester. Recrystallization from hot trifluoroethanol-ethanol gave the product in 43% yield, mp  $> 275^{\circ}\text{d}$ ;  $[\alpha]_D^{25} -102.3^{\circ}$  (c 0.23, trifluoroacetic acid).

*Anal.* Calcd for  $C_{32}H_{56}N_8O_{12}$ : C, 51.60; H, 7.58; N, 15.04. Found: C, 51.41; H, 7.75; N, 14.90.

*2-Methoxy-[2-ethoxy-(2-ethoxy)]-acetyl-hepta-(L-alanyl)-L-alanine morpholine amide (XXXIX).* This compound was prepared from 2-methoxy-[2-ethoxy-(2-ethoxy)]-acetyl-di(L-alanyl)-L-alanine and tetra-(L-alanyl)-L-alanine morpholine amide hydrochloride on a 0.001-mole scale, using a similar procedure to that employed in the synthesis of benzyloxycarbonyl-tri-(L-alanyl)-L-alanine ethyl ester. In this case, dimethylsulfoxide was used to dissolve the amine hydrochloride. Recrystallization from trifluoroethanol-ethanol gave the product in 37% yield, mp > 275°d;  $[\alpha]_D^{25}$  -104.4° (c 0.25, trifluoroacetic acid).

*2-Methoxy-[2-ethoxy-(2-ethoxy)]-acetyl-octa-(L-alanyl)-L-alanine Morpholine (XL).* This compound was prepared from 2-methoxy-[2-ethoxy-(2-ethoxy)]-acetyl-di-(L-alanyl)-L-alanine and penta-(L-alanyl)-L-alanine morpholine amide hydrochloride on a 0.001-mole scale, using a similar procedure to that employed in the synthesis of benzyloxycarbonyl-tri-(L-alanyl)-L-alanine ethyl ester. In this case, dimethylsulfoxide was used to dissolve the amine hydrochloride. Recrystallization from hot trifluoroethanol-ethanol gave the product in 32% yield, mp > 275°d;  $[\alpha]_D^{25}$  -110.2° (c 0.22, trifluoroacetic acid).

#### *Note on Elemental Analyses*

It should be noted that the elemental analyses for three oligopeptides are not reported in each alanine oligomer series. For these compounds, carbon, hydrogen, and nitrogen analyses were all found to be significantly lower than the calculated values. Other investigators in our group have had similar experiences with the higher oligopeptides and polypeptides. It is extremely difficult to combust these compounds completely. We believe that the molar rotation and CD investigations reported in the succeeding papers give evidence for the high purity of our oligomers.

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