oxime filtered (1.1 g., m.p. 88–89°) and recrystallized once from petroleum ether, whereupon it melted at 89.5–90°. Subsequent conversion to the amide X proceeded in the above-described manner.

2,2,6,6-d_4-N-Ethylcyclohexylamine (XXII).—The labeled amide X (200 mg.) in 20 cc. of dry ether was heated under reflux for 4 hr. with 0.5 g. of lithium aluminum hydride and 50 cc. of ether. After decomposing by the sodium sulfate technique, the ether was evaporated and the labeled cyclohexylamine (see Table III) distilled at a bath temperature of 164°.

N-Trideuterioacetylcyclohexylamine (VII).—Cyclohexylamine (50 mg.) was kept at room temperature for 2 min. in ether solution with 100 mg. of d_6-acetic anhydride. Solid potassium carbonate was added, followed by 3 cc. of water and 50 cc. of ether. The ether phase was dried and evaporated and the amide was recrystallized from petroleum ether; m.p. 105.5–106°.

Preparation of Deuterated N-Acetylcyclopentylamines (see Table II).—Cyclopentanone (3.3 g.) was dissolved in 20 g. of deuterium oxide and left at room temperature for 2 weeks with 1.5 g. of potassium carbonate, since the usual equilibration conditions with stronger base led to self-condensation. Solid potassium carbonate (4.0 g.) was added followed by 3.5 g. of hydroxylamine hydrochloride. After 5 min., the oxime was extracted with ether and the latter washed with water, dried and evaporated, leaving 3.5 g. of **2,2,5,5-d_4-cyclopentanone oxime**, m.p. 56–57°. The oxime (0.4 g.) was heated under reflux for 30 min. with excess ethereal lithium aluminum hydride and after decomposing with aqueous sodium sulfate solution, the ether phase was dried and 1 cc. of acetic anhydride was added. The ether was now removed, and the residue treated with 5 cc. of water and then mixed with solid potassium carbonate. The required **2,2,5,5-d_4-N-acetylcyclopentylamine (XVI)**[22] was extracted with ether and distilled at 0.1 mm.

1-d_1-Acetylcyclopentylamine (XIV) was prepared in an analogous fashion from unlabeled cyclopentanone oxime and substituting lithium aluminum deuteride for lithium aluminum hydride. **N-Trideuterioacetylcyclopentylamine (XV)** was obtained in the above-described manner by acetylation of cyclopentylamine with d_6-acetic anhydride in ether solution.

(22) The unlabeled amide has been described by E. K. Harvill, R. M Herbst, E. C. Schreiner and C. W. Roberts, *J. Org. Chem.*, **15**, 662 (1950).

Preparation of Deuterated N-Ethylcyclopentylamines (see Table III).—**2,2,5,5-d_4-N-Ethylcyclopentylamine (XIX)** and **N-β,β,β-d_3-ethylcyclopentylamine (XX)** were prepared by reducing the appropriate labeled amide (XV, XVI) with excess lithium aluminum hydride in ether solution (4 hr. reflux), while in the synthesis of **N-α,α-d_2-ethylcyclopentylamine (XVIII)**, lithium aluminum deuteride was employed for the reduction of N-acetylcyclopentylamine (XIII). In each instance, the amine[23] was purified by distillation (b.p. 119–120°) prior to mass spectra analysis.

Preparation of Deuterated N-Ethyl-N-acetylcyclopentylamines (Table IV).—For the preparation of the unlabeled N-ethyl-N-acetylcyclopentylamine (XXIII), the 2,2,5,5-d_4 analog XXVI, the N-α,α-d_2-ethyl-N-acetylcyclopentylamine (XXV) and the N-β,β,β-d_3-ethyl derivative XXVII, approximately 100 mg. of the appropriate amine (XVII–XX in Table III) was dissolved in 10 cc. of ether and kept at room temperature for 2 min. with 0.5 cc. of acetic anhydride. The ether was removed, 2 cc. of water was added, followed by 1.0 g. of sodium carbonate and then anhydrous magnesium sulfate. The solid mass was extracted thoroughly with ether, the solvent removed and the residual amide distilled under reduced pressure before mass spectral analysis. The trideuterioacetyl derivative XXIV was prepared in the same manner, except that d_6-acetic anhydride was employed in the last step. The unlabeled amide XXIII exhibited b.p. 241°, $n^{25}D$ 1.4721, λ_{max}^{capill} 6.06 μ and proved to be quite hygroscopic.

N-Ethyl-N-β,β,β-d_3-ethylacetamide (V).—Ethylamine was acetylated with d_6-acetic anhydride in ether solution as described above for N-ethylcyclopentylamine and the resulting N-ethyl-d_3-acetamide, in ether, was heated under reflux for 4 hr. with an excess of lithium aluminum hydride. The excess reagent was destroyed at 0° by the addition of aqueous sodium sulfate solution and the ether containing the labeled diethylamine was distilled directly from the reaction vessel into a cooled flask containing excess acetic anhydride dissolved in ether. The ether was then removed at room temperature by distillation under reduced pressure, solid sodium carbonate and water were added and the product was extracted with ether. After drying with anhydrous magnesium sulfate, the ether was removed and the amide V distilled at 24 mm. before mass spectral determination.

(23) The unlabeled amine has been reported by H. A. Shonle and J. W. Corse, U. S. Patent 2,424,063 (*Chem. Abstr.*, **41**, 7420 (1947)).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, POLYTECHNIC INSTITUTE OF BROOKLYN, BROOKLYN 1, N. Y.]

Conformational Aspects of Polypeptides. IX.[1] Synthesis of Oligomeric Peptides Derived from β-Methyl L-Aspartate

BY MURRAY GOODMAN AND FRANKLIN BOARDMAN[2]

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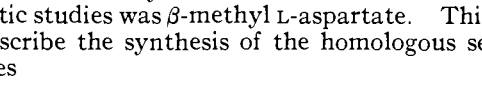
The synthesis of optically pure oligomeric peptides and polymers derived from β-methyl L-aspartate is described. The oligomers contain between 2 and 14 aspartate residues. Three general peptide synthetic methods were employed, utilizing the mixed anhydride, active ester and azide reaction sequences.

Introduction

Recent work by Karlson, Norland, Fasman and Blout,[3] and Bradbury, Downie, Elliott and Hanby[4] on the conformation of poly-β-benzyl L-aspartate has shown that a polypeptide derived from this amino acid in the L-configuration may form a left-handed helix. Past investigations of the polymers of a wide range of amino acids (*e.g.*, L-alanine, ε-benzyloxycarbonyl-L-lysine, γ-benzyl and γ-methyl L-glutamate[5,6] and L-methionine[7,8]) have shown that the L-configuration generally adopts a right-handed helix. Consequently, it was of interest to synthesize oligomers of an aspartate ester in order to determine the rotatory properties of peptides which have marginal helical stability. In order to avoid the possibility of acid solvolysis of a benzyl ester,[9] the amino acid chosen for synthetic studies was β-methyl L-aspartate. This paper will describe the synthesis of the homologous series of peptides



which may be abbreviated by the Brand–Edsall scheme as[10]

(1) This investigation was generously supported by a grant from the National Science Foundation (G8614). Previous paper in this series: M. Goodman, I. Listowsky, Y. Masuda and F. Boardman, *J. Am. Chem. Soc.*, in press.

(2) Submitted by Franklin Boardman to the faculty of the Polytechnic Institute of Brooklyn, 1962, in partial fulfillment of the requirements for the Ph.D. Degree.

(3) R. H. Karlson, K. S. Norland, G. D. Fasman and E. R. Blout, *J. Am. Chem. Soc.*, **82**, 2268 (1960).

(4) E. M. Bradbury, A. R. Downie, A. Elliott and W. E. Hanby, *Proc. Roy. Soc.* (London), **A259**, 110 (1960).

(5) E. R. Blout, in "Optical Rotatory Dispersion," by C. Djerassi, McGraw-Hill Book Co., Inc., New York, N. Y., 1960, Chapter 17.

(6) M. Goodman, E. E. Schmitt and D. A. Yphantis, *J. Am. Chem. Soc.*, **84**, 1283 (1962).

(7) S. M. Bloom, G. D. Fasman, C. DeLoze and E. R. Blout, *ibid.*, **84**, 458 (1962).

(8) G. E. Perlman and E. Katchalski, *ibid.*, **84**, 452 (1962).

(9) P. Doty, A. Wada, J. T. Yang and E. R. Blout, *J. Polymer Sci.*, **23**, 851 (1957).

(10) E. Brand and J. T. Edsall, *Ann. Rev. Biochem.*, **16**, 223 (1947).

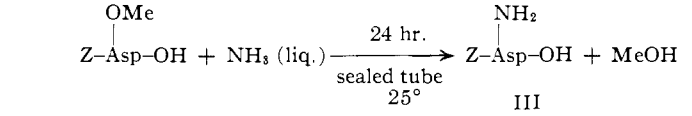
where Z is a benzyloxycarbonyl group.

It was required that all methods of synthesis produce products that can be easily separated from the reaction mixture and that are optically pure.

Results and Discussion

Structure Proof of Starting Material.—The basic starting material for the oligomers is β-methyl L-aspartate hydrochloride (I). This compound was easily synthesized from L-aspartic acid by Fischer esterification under dilute conditions.[11] In view of the conformational studies that were to be carried out on the oligomers it was necessary to prove that the β-methyl ester hydrochloride was not contaminated with the isomeric α-methyl ester hydrochloride. Berger and Katchalski had been faced with an analogous problem in their synthesis of polymers of β-benzyl L-aspartate.[12] The proof of structure for that compound was performed by conversion of the amino acid ester to its N-benzyloxycarbonyl derivative, followed by ammonolysis of the ester group. The product, benzyloxycarbonyl-L-asparagine (III), is a highly crystalline compound whose structure has been proved.[12-14] It undergoes a 20° melting point depression when mixed with its isomer benzyloxycarbonylisoasparagine. When pure, both asparagine derivatives have the same melting point, 165°.[12]

The sequence of reactions utilized by Berger and Katchalski was applied to β-methyl L-aspartate to form benzyloxycarbonyl asparagine (compound III).



The amide III was produced in 90% yield and showed no melting point depression when mixed with authentic material prepared by the carbobenzoxylation of L-asparagine according to the directions of Rudinger and Zaoral.[13] It was therefore concluded that the β-methyl L-aspartate hydrochloride synthesized was reasonably pure.

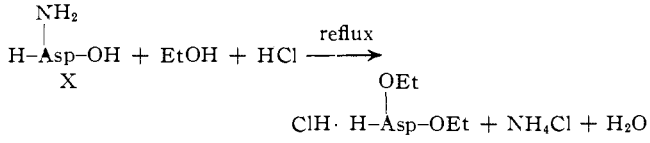
Polymers of β-Methyl L-Aspartate.—Polymers of β-methyl aspartate were prepared by basic initiation of the appropriate α-amino acid N-carboxyanhydride, according to the directions of Coleman for poly-β-methyl DL-aspartate.[15] The amino acid ester II was treated with phosgene in dioxane for 2 hr. at 60° to yield the N-carboxyanhydride of β-methyl L-aspartate (IVa).

The anhydride IVa was crystallized with difficulty from ethyl acetate–hexane. It was decided to use the crystalline anhydride without extensive recrystallization and add excess initiator to neutralize the hydrogen chloride (assumed to be the major impurity). Since the amount of hydrogen chloride was not exactly determined, the relationship between the anhydride to initiator (A/I) ratio and the degree of polymerization is not ascertainable.

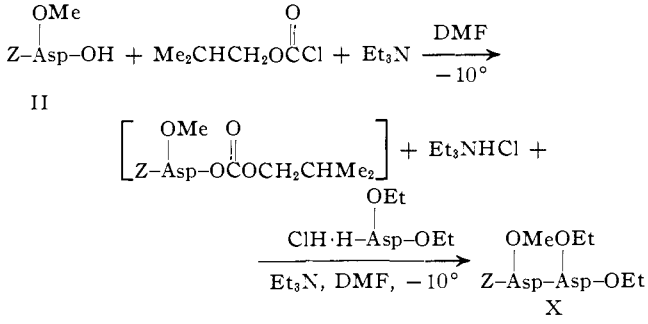
A high molecular weight polymer was prepared by initiation with sodium methoxide (A/I = 970) following Blout's directions for the production of high molecular weight poly-γ-benzyl L-glutamate.[16] A lower molecular weight species was prepared (A/I = 48) by the use of diethylamine as initiator in tetrahydrofuran solution. Attempts to obtain higher or lower molecular weight polymers by changing the A/I in this medium failed, paralleling the results of Bradbury, *et al.*,[4] in their preparations of poly-β-benzyl L-aspartate using diethylamine. In order to produce polymers having a degree of polymerization near twenty, the solvent was changed to *t*-butyl alcohol, thus ensuring that lower molecular weight polymers were not left in solution by the precipitation techniques used in the production of the higher polymers. Two low molecular weight polymers were obtained, A/I of 5.30 and 1.32, respectively. These polymers were washed with ether to remove any α-*t*-butyl-β-methyl-L-aspartate that might have been formed as well as any unreacted N-carboxyanhydride. The lower of the two polymers was an oily, white solid and the higher polymer was a white solid.

Synthesis of Oligomers.—Three general coupling methods were employed to prepare the oligomeric peptides. These include the mixed carboxylic–carbonic anhydride reaction for producing dipeptide and tetra- through hexapeptides as well as intermediates,[17-19] the active ester procedure used in producing the tripeptide and intermediates,[20,21] and the azide coupling reaction used in producing the octamer, undecamer and tetradecamer.[22-25]

The reactions used to produce the di- and tripeptides were modeled after the scheme adopted by Goodman, Schmitt and Yphantis[6] for the synthesis of oligomeric peptides derived from γ-methyl L-glutamate. Diethyl L-aspartate hydrochloride (IX) was prepared by Fischer esterification of L-asparagine and served as the C-terminal residue on all of the oligomers.[26]



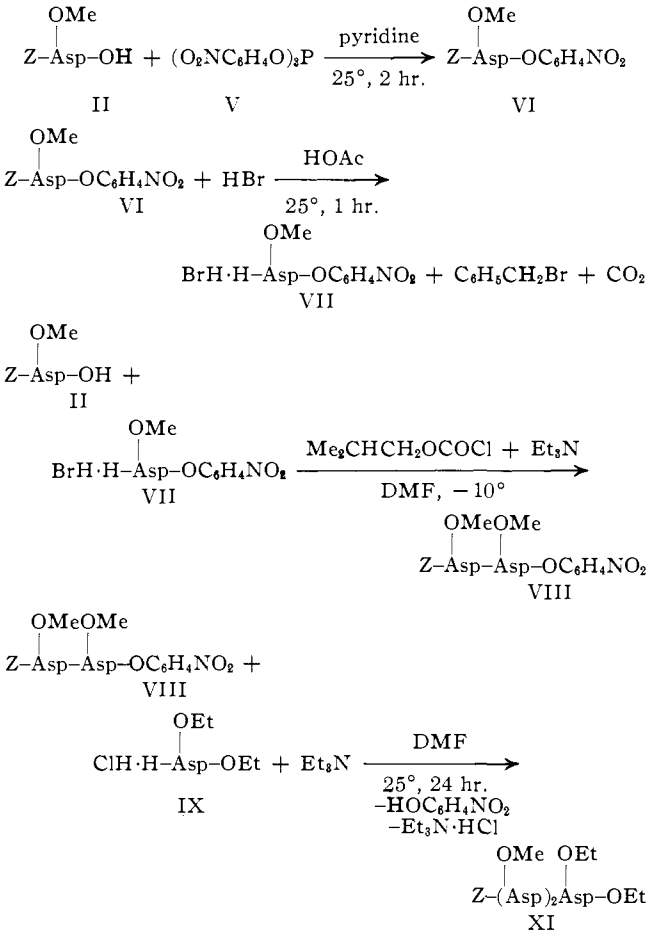
A dipeptide could now be produced by the mixed anhydride procedure utilizing benzyloxycarbonyl-β-methyl-L-aspartate (II) and diethyl L-aspartate hydrochloride (IX).



The tripeptide XI was synthesized by a combination of the mixed anhydride and active ester procedures. Benzyloxycarbonyl-β-methyl-L-aspartate was converted to its α-*p*-nitrophenyl ester by the technique of Schwyzer.[23] Removal of the benzyloxycarbonyl group af-

(11) N. de Groot and N. Lichtenstein, *Bull. Research Council Israel*, **8A**, 116 (1959).
(12) A. Berger and E. Katchalski, *J. Am. Chem. Soc.*, **73**, 4084 (1951).
(13) J. Rudinger and M. Zaoral, *Coll. Czech. Chem. Commun.*, **24**, 1993 (1959).
(14) M. Bergmann and L. Zervas, *Ber.*, **65**, 1192 (1932).
(15) D. Coleman, *J. Chem. Soc.*, 2294 (1951).
(16) E. R. Blout and R. H. Karlson, *J. Am. Chem. Soc.*, **78**, 941 (1956).
(17) J. R. Vaughan and R. L. Osato, *ibid.*, **74**, 676 (1952).
(18) R. A. Boissonas, *Helv. Chim. Acta*, **34**, 874 (1951).
(19) T. Wieland and H. Bernhard, *Ann.*, **572**, 190 (1959).
(20) M. Bodanszky and V. duVigneaud, *J. Am. Chem. Soc.*, **81**, 5688 (1959).
(21) B. Iselin, W. Rittel, P. Sieber and R. Schwyzer, *Helv. Chim. Acta*, **40**, 373 (1957).
(22) T. Curtius, *Ber.*, **35**, 3226 (1902).
(23) R. Schwyzer, *Angew. Chem.*, **71**, 742 (1959).
(24) E. Klieger and H. Gibian, *Ann.*, **649**, 183 (1961).
(25) J. Rudinger and J. Honzl, *Coll. Czech. Chem. Commun.*, **26**, 2333 (1961).
(26) E. Fischer and E. Koenigs, *Ber.*, **43**, 661 (1910).

forded the active ester hydrobromide by the technique of Ben-Ishai and Berger.[27] Coupling of the active ester hydrobromide with benzyloxycarbonyl-β-methyl-L-aspartate by the mixed anhydride method gave a dipeptide active ester. The tripeptide was formed by displacement of the *p*-nitrophenoxy group by the amino group of diethyl L-aspartate, following the general procedure for the synthesis of a tripeptide developed by Goodman and Stueben.[28]

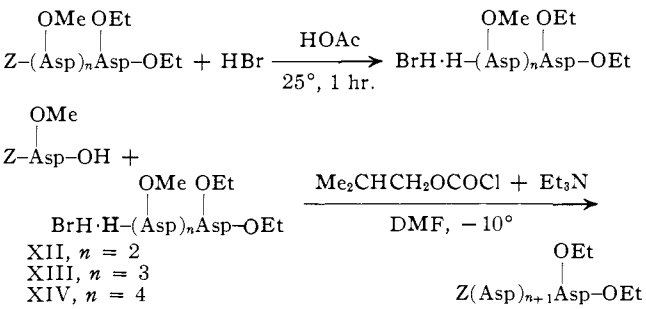


A pentapeptide was synthesized by removing the blocking group on the N-terminal end of the tripeptide XI and coupling the peptide with dipeptide active ester VIII. However, the product was contaminated heavily with dipeptide active ester and other means were sought to produce higher oligomers.

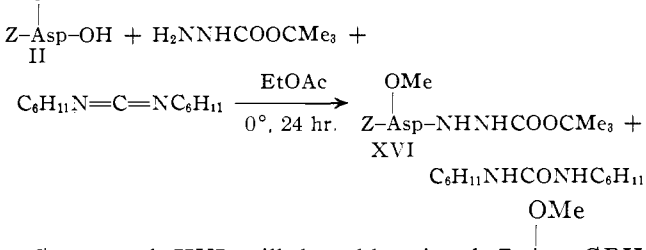
Before abandoning the use of dipeptide active ester, it was necessary to determine whether the poor yield was due to incomplete decarbobenzoxylation of the tripeptide or incomplete dipeptide active ester coupling. The benzyloxycarbonyl group was removed by catalytic hydrogenation. The extent of reaction was determined by titrating the carbon dioxide produced, using the method of Patchornik and Shalitin.[29]

The reactions utilized have been run at 25° or lower. The need for such mild conditions is dictated by the tendency of aspartic derivatives to undergo rearrangement. Bernhard, Berger, *et al.*, have isolated the intermediate benzyloxycarbonyl-L-aminosuccinimide in the hydrolysis of a peptide containing benzyloxycarbonyl-β-benzyl-L-aspartate.[30] They have proposed a mechanism to account not only for imide formation but also for the resulting products benzyloxycarbonyl-α-(and β-)benzyl-L-aspartate. Poly-L-aspartic acid has also been shown to rearrange to poly-L-aminosuccinimide by Kovacs.[31] Such rearrangements involving the exchange of groups on the α- and β-carboxyl groups would destroy the validity of the conformational studies for which the peptides were being synthesized. Reversal of imide formation may lead to either α- or β-esters depending on the particular carbonyl group attacked by the alcoholate ion. Similarly, hydrolysis of only one of the acyl imide linkages will result in either of the two possible amides.

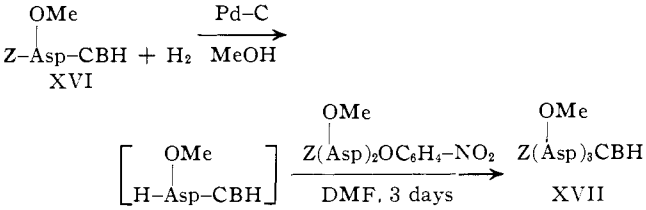
To avoid any possible isomerizations[30-31] and rearrangements of the sort encountered with aspartate esters, only mild reactions were used.



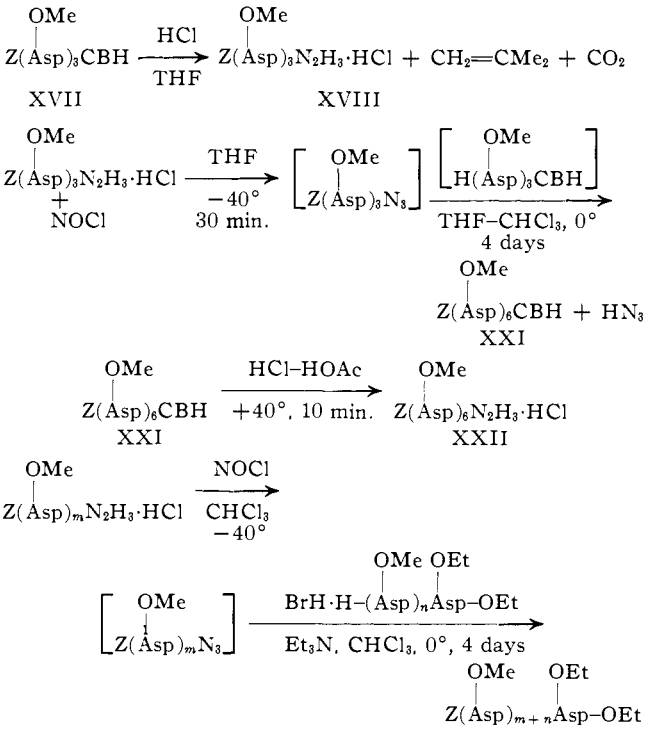
Since the yields for these reactions did not exceed 60%, the synthesis of higher oligomers could not be carried out by these procedures. Only the azide method in peptide synthesis has been shown to join blocks of peptides and retain complete optical purity in the product.[22-25] In order to utilize the azide coupling reaction, a scheme of synthesis suggested by Schwyzer[23] and utilized by Gibian and Klieger[24] on glutamic peptides was employed. The main feature of this reaction is the use of the blocking group *t*-butoxycarbonyl hydrazide, $NH_2NHCOOCMe_3$, on the C-terminal end of the peptide chain. At the proper time, this blocking group can be removed by acid solvolysis to yield a hydrazide, which upon treatment with nitrosyl chloride can be converted to a reactive acyl azide. The required intermediate for these reactions, *t*-butoxycarbonyl hydrazide, can be prepared by the method of Carpino.[32] The *t*-butoxycarbonyl hydrazide is allowed to react with compound II



Compound XVI will be abbreviated Z–Asp(OMe)–CBH where CBH stands for carbo-*t*-butoxy hydrazide (or *t*-butoxycarbonyl hydrazide). The entire azide synthetic scheme is



(27) D. Ben-Ishai and A. Berger, *J. Org. Chem.*, **17**, 1564 (1952).
(28) M. Goodman and K. C. Stueben, *J. Am. Chem. Soc.*, **81**, 3980 (1959).
(29) A. Patchornik and Y. Shalitin, *Anal. Chem.*, **33**, 1887 (1961).
(30) S. A. Bernhard, A. Berger, H. Carter, E. Katchalski, M. Sela and Y. Shalitin, *J. Am. Chem. Soc.*, **84**, 2421 (1962).
(31) J. Kovacs in "Polyamino Acids, Polypeptides, and Proteins," edited by M. A. Stahmann, University of Wisconsin Press, Madison, Wis., 1962, Chapter 4.
(32) L. A. Carpino, *J. Am. Chem. Soc.*, **79**, 98 (1957).



XX, $m = 3$, $n = 4$, octapeptide
XXIII, $m = 6$, $n = 4$, undecapeptide
XXIV, $m = 6$, $n = 7$, tetradecapeptide

Removal of the CBH blocking group proceeds in yields of 80–90%. The azide coupling steps, however, proceed in yields of only 30–50%. Of the various methods of converting hydrazides to acyl azides, the method of Rudinger and Honzl (employing the reagent nitrosyl chloride) gave the highest yields of pure final product.[25]

In utilizing this series of reactions it is important to obtain the tripeptide-CBH XVII in a high state of purity. The failure of this compound to crystallize can be taken as a warning that the azide coupling step will not take place. As the precursor XVI is an oil, purification of the starting material, benzyloxycarbonyl-β-methyl-L-aspartate, is essential. This can be accomplished by conversion of that acid to its piperazonium salt by the method of Prigot and Pollard.[33] The salt can be recrystallized with greater ease than the parent acid, and the acid liberated from the salt by the action of hydrochloric acid is now easily recrystallized.

The best solvents for the azide coupling reaction are ethyl acetate, tetrahydrofuran and chloroform. When dimethylformamide or dimethylacetamide is employed, no crystalline product is obtained. The number of aspartyl residues that can be joined by this method is limited by the requirement of solubility of the reactants in the medium employed. As the tetradecapeptide appeared to be only partially soluble in chloroform, the best solvent found for the oligomers, the synthesis of higher peptides by this method appears unlikely.

It was necessary to prove that the methods of synthesis employed did not lead to any detectable racemization. The pentapeptide, β-methyl L-aspartate hydrochloride, L-aspartic acid and a low molecular weight polypeptide were hydrolyzed in hydrochloric acid at 120° and the respective specific rotations recorded. All gave readings close to +16°. Since $[\alpha]^{25}D$ for L-aspartic acid, without previous heating, is +25°, some reaction must have taken place during hydrolysis. The nature of this reaction is now under study in our laboratories. Karlson, *et al.*, reported that the hydrolysis of poly-β-benzyl L-aspartate proceeded with complete retention of configuration, but attempts to repeat their work led to a value of +18° rather and the expected +25°.[3]

In order to determine the validity of the above measurements, a sample of L-aspartic acid was mixed with a known amount of DL-aspartic acid and subjected to the same conditions. The rotation of this mixture proved to be lower than 16° by exactly the proportion of DL-acid added. Consequently, the measurements are valid on empirical grounds. It should be noted that the observed rotations of the pentapeptide were large enough to allow the detection of one D-residue.

Racemization of L-aspartic acid on treatment with hydrochloric acid has been noted previously by Michael and Wing.[34,35] However, these authors conducted their work at 180° while a temperature of 120° was employed in the present work. At lower temperatures solution of the peptides does not take place.

Conformation of Peptides.—The molar rotation of each peptide in dichloroacetic acid gives values which differ from each other by a constant amount from the dipeptide through tetradecapeptide. Since this solvent is expected to destroy secondary structure, the constancy of the difference in rotations for the homologous oligomeric series confirms the previous conclusion that a high degree of optical purity was achieved during synthesis. A similar deduction can be made from the rotation values of peptides in dimethylformamide. However, the peptides higher than the octamer are insoluble in dimethylformamide. In chloroform, secondary structure appears at the octamer and larger peptides as can be seen by the optical rotation data.

On the basis of these data, it can be assumed that dichloroacetic acid and dimethylformamide are both solvents which support random coil structure for the oligomers.[36] Chloroform, however, supports secondary structure, which may be either in the form of an α-helix or intermolecular association. The conformations of each peptide will be discussed in the accompanying paper.[37]

Conclusions.—The methods described for the preparation of the oligomeric peptides and polymers derived from β-methyl L-aspartate result in products of high chemical and optical purity. The optical rotations obtained for the peptides indicate that secondary structure is possible in chloroform solution for peptides larger than an octamer. Difficulties in obtaining high yields using some methods indicate that the methyl ester side chains are close enough to the main peptide chain to hinder the approach of the large amine peptide molecules. Specifically, the inability of a tripeptide with a free amino group to displace *p*-nitrophenoxyl groups from a dipeptide active ester must be due to the influence of the side chains, since the analogous reaction in the γ-methyl L-glutamate series takes place in high yield.[6]

Extensive use has been made of the azide coupling reaction and the CBH blocking group for the synthesis of large peptides. It is felt by the authors that this procedure is the best one for the synthesis of such peptides despite the relatively low yields obtained.

Experimental[38]

Materials.—β-Methyl L-aspartate hydrochloride, diethyl L-aspartate hydrochloride and β-benzyl L-aspartate were purchased

(33) M. Prigot and C. B. Pollard, *J. Am. Chem. Soc.*, **70**, 2758 (1948).

(34) A. Michael and J. Wing, *Ber.*, **17**, 2984 (1884).

(35) A. Michael and J. Wing, *Am. Chem. J.*, **7**, 278 (1885–1886).

(36) M. Goodman, I. Listowsky and E. E. Schmitt, *J. Am. Chem. Soc.*, **84**, 1296 (1962).

(37) M. Goodman, F. Boardman and I. Listowsky, *ibid.*, **85**, 2491 (1963).

TABLE I

SUMMARY OF PHYSICAL DATA OF PREPARED OLIGOMERIC PEPTIDES DERIVED FROM β-METHYL L-ASPARTATE

Formula	Peptide	M.p., °C.	Molar rotation × 10^{-3} in Dichloroacetic acid[a]	Yield, %	Calculated % C	Calculated % H	Calculated % N	Found % C	Found % H	Found % N
X	Di-	80–81	+0.80	50	55.74	6.23	6.19	55.59	6.36	6.27
XI	Tri-	127–128	− .05	57	53.70	6.07	7.22	53.85	6.24	7.26
XII	Tetra-	143–144	− .90	62	52.39	5.96	7.88	52.13	5.95	7.94
XIII	Penta-	161–163	−1.65	51	51.49	5.88	8.34	51.78	5.76	8.12
XIV	Hexa-	175–178	−2.55	61	50.82	5.83	8.67	50.84	5.89	8.44
XX	Octa-	207 dec.	−4.15	30	49.82	5.75	9.13	49.62	5.83	8.89
XXIII	Undeca-	224 dec.	−6.85	31	49.08	5.68	9.54	49.38	5.93	9.29
XXIV	Tetradeca-	233 dec.	−9.15	25	48.60	5.64	9.80	48.48	5.65	10.50

[a] The molar rotation of each aspartyl residue is −0.80 to −0.85°. The optical rotation data for the oligomers in a variety of solvents are treated more fully in the next paper.[37]

from the Cyclo Chemical Corp., Los Angeles, Calif. L-Aspartic acid, L-asparagine monohydrate and carbobenzoxy chloride (benzyloxycarbonyl chloride) were purchased from Mann Research Laboratories, New York, N. Y. Triethylamine, diethylamine and *t*-butyl alcohol [Matheson, Coleman and Bell (M. C. and B.)] were distilled from barium oxide at atmospheric pressure. Dimethylformamide (M. C. and B.) was fractionally distilled at 0.5 mm. pressure. The central fraction was utilized in these experiments. Tetrahydrofuran and dioxane (both M. C. and B.) were distilled from sodium ribbon at atmospheric pressure, after reflux for 8 hr. The remaining organic compounds were employed without further purification: chloroform (A.C.S. reagent grade, Brothers Chemical Corp., Orange, N. J.), ethyl acetate (anhydrous grade, M. C. and B.), isobutyl chloroformate (Eastman, White Label), anhydrous ether (Mallinckrodt) and *p*-nitrophenol (Fisher, reagent grade). Dichloroacetic acid (Fisher, laboratory grade) was used without purification if colorless; otherwise fractional vacuum distillation at 0.5 mm. was necessary. Glacial acetic acid was distilled from boron triacetate. Nitrosyl chloride, phosgene, hydrogen bromide, hydrogen chloride, prepurified nitrogen, and prepurified hydrogen were obtained in cylinders from the Matheson Company, East Rutherford, N. J.

Preparation of Compounds. **β-Methyl L-aspartate hydrochloride** (I) was prepared by the method of de Groot and Lichtenstein[11] from L-aspartic acid and methanol. Recrystallization of the crude product from methanol–ether gave white needles in 70% yield, m.p. 204° dec., $[\alpha]^{25}D$ +12.4° (*c* 1, 1:3 ethanol–water). For the same compound, Schwarz, *et al.*,[39] report m.p. 191–193° dec., $[\alpha]^{25}D$ +21.4° (*c* 1, 1:3 ethanol–water). A commercial sample of this compound supplied by the Cyclo Chemical Corp. possessed m.p. 200° dec. and $[\alpha]^{25}D$ +15.3° under the same conditions. Recrystallization of this sample lowered $[\alpha]$ to +12.9°. It was concluded that the literature value for the specific rotation was in error.

Anal. Calcd. for $C_5H_{12}NO_4Cl$: C, 32.71; H, 5.49; N, 7.63. Found: C, 32.51; H, 5.62; N, 7.39.

Benzyloxycarbonyl-β-methyl-L-aspartate (II) was prepared by modification of the method of Schwarz, *et al.*[39] To 100 ml. of water at 0° were added β-methyl L-aspartate hydrochloride (I, 19 g., 0.10 mole) and sodium carbonate (12 g., 0.11 mole). When the evolution of carbon dioxide had ceased, carbobenzoxy chloride (17.5 ml., 0.12 mole) and sodium carbonate solution (7 g., 0.06 mole; dissolved in 50 ml. of water) were added dropwise simultaneously to the vigorously stirred reaction mixture. When the addition of reagents was complete, stirring was continued and the reaction mixture was allowed to warm to room temperature. After 3 hr. the stirring was stopped and the reaction mixture was extracted with three 100-ml. portions of ether. The aqueous layer was acidified to pH 1 and then extracted with four 100-ml. portions of ethyl acetate. The combined ethyl acetate extracts were dried over magnesium sulfate and evaporated *in vacuo* to yield an oil which solidified after storage for 2 days. The oil was best purified by converting it to the piperazonium salt by the method of Prigot and Pollard.[33] After one recrystallization of the salt from boiling acetone, the pure salt, m.p. 128°, was obtained. The salt was suspended in a mixture of 200 ml. of ether and 200 ml. of 2 *N* hydrochloric acid. The ether layer was dried over magnesium sulfate and evaporated to half its volume. Upon addition of petroleum ether to the cloud point, crystallization commenced. Filtration of the crystals and drying *in vacuo* afforded 16 g. (60%) of product, m.p. 97–98° (lit.[37] m.p. 98°), $[\alpha]^{25}D$ −18.5° (*c* 2.5, pyridine); lit.[37] $[\alpha]^{25}D$ −17.4° (*c* 2.5, pyridine).

Anal. Calcd. for $C_{12}H_{15}NO_6$: C, 55.51; H, 5.38; N, 4.98. Found: C, 55.73; H, 5.44; N, 5.04.

Benzyloxycarbonyl-L-asparagine (III).—Benzyloxycarbonyl-β-methyl-L-asparate (II, 1.04 g., 0.00370 mole) was dissolved in 5 ml. of liquid ammonia and kept in a sealed tube for 1 day at room temperature. The tube was opened and the ammonia evaporated cautiously. The remaining gray powder was triturated with 10 ml. of 1 *N* hydrochloric acid for 5 min. and the residue was removed by filtration. Recrystallization of the residue from boiling water gave 0.84 g. (90%) of product, m.p. 163° (lit.[12] 165°). The melting point was not depressed when the product was mixed with material prepared according to the method of Zaoral and Rudinger.[13]

β-Methyl L-Aspartate-N-carboxyanhydride (IVa) was prepared according to the method of Coleman from β-methyl L-aspartate hydrochloride (I) and phosgene in dioxane solution.[15] The product was a colorless oil which crystallized slowly from ethyl acetate–hexane in 40–50% yields, m.p. 80° dec. (lit. for DL-compound, m.p. 84° dec.), $[\alpha]^{25}D$ −72.8° (*c* 3, chloroform).

Poly-β-methyl L-Aspartate (IVb).—Four polymers were produced by basic initiation of β-methyl L-aspartate-N-carboxyanhydride (IVa).

(1) A/I = 970: To 5 ml. of dimethylformamide were added anhydride IVa (0.519 g., 0.0030 mole) and a stock sodium methoxide solution. (This stock solution was prepared by diluting 0.010 ml. of 0.300 *N* sodium methoxide solution in 1:3 methanol–benzene with 10 ml. of dimethylformamide. After 5 min. a gel appeared in the reaction flask. After storage overnight at room temperature, ether (200 ml.) was added and the mixture stirred for 1 hr. at room temperature. The yield of dry polymer isolated by filtration was 0.345 g. (90%), m.p. 230° dec.

(2) A/I = 48: A lower molecular weight polymer was prepared by the use of tetrahydrofuran as solvent and 0.060 ml. of diethylamine as initiator. The same weight of IVa as in the preceding section was employed. The yield of dry polymer was 0.33 g. (85%), m.p. 210–225° dec.

(3) A/I = 5.30: This polymer was produced by preparing a reaction mixture consisting of anhydride IVa (0.445 g., 0.00257 mole) and diethylamine (0.50 ml., 0.000486 mole) in 10 ml. of *t*-butyl alcohol; 10 min. after the anhydride had dissolved, a faint turbidity appeared in the reaction flask. After standing overnight, a white granular precipitate appeared. The solvent was removed by lyophylization. The yield of dry polymer was 0.33 g. (85%), m.p. 210–225° dec.

(4) A/I = 1.32: This polymer was prepared according to the directions for A/I = 5.30 with the exception that 0.200 ml. of diethylamine was employed as initiator. Upon lyophylization, the polymer had the form of an oily white solid. The yield of polymer was 0.33 g. (85%), m.p. 215–230° dec.

Tris-(*p*-nitrophenyl) Phosphite (V).—A modification of the method of Strecker and Grossman was employed.[40] *p*-Nitrophenol (41.7 g., 0.300 mole) was dissolved in 125 ml. of 1,2-dichloroethane by warming the mixture to reflux. After cooling to room temperature, phosphorus trichloride (8.6 ml., 0.100 mole) was added dropwise. The solution was then refluxed overnight in the hood. Upon cooling to 0°, the phosphite V precipitated as a gray powder which was filtered and washed with ether. Recrystallization from boiling toluene (to which Norit-A was added) gave slightly gray needles in 33% yield, m.p. 167–170° dec (lit. 170–171° dec.).

Benzyloxycarbonyl-α-*p*-nitrophenyl-β-methyl-L-aspartate (VI). Schwyzer's method for the synthesis of *p*-nitrophenyl esters was employed.[21] To 16 ml. of pyridine were added with stirring benzyloxycarbonyl-β-methyl-L-aspartate (II, 10 g., 0.0357 mole) and tris-(*p*-nitrophenyl) phosphite (V, 9.6 g., 0.0216 mole). The reaction was allowed to proceed at room temperature for 3 hr., by which time complete solution had taken place. The yellow reaction mixture was diluted with 200 ml. of ethyl acetate and

(38) All melting points are corrected. Analyses were carried out by Schwarzkopf Laboratories, Woodside, Long Island, N. Y.

(39) H. Schwarz, F. M. Bumpus and I. H. Page, *J. Am. Chem. Soc.*, **79**, 5697 (1957).

(40) W. Strecker and C. Grossman, *Ber.*, **58**, 1042 (1925).

extracted three times with 40-ml. portions of 1 *N* hydrochloric acid, 30% potassium chloride and 10% sodium bicarbonate–30% potassium chloride. Upon drying of the ethyl acetate layer with magnesium sulfate and evaporation *in vacuo*, a yellow oil was obtained which crystallized after an hour. Recrystallization from boiling absolute ethanol furnished 10 g. (70%) of product, m.p. 105–106°, $[\alpha]^{25}D$ −43.7° (*c* 2, dimethylformamide).

Anal. Calcd. for $C_{14}H_{18}N_2O_8$: C, 56.62; H, 4.51; N, 6.96. Found: C, 56.78; H, 4.67; N, 6.99.

α-*p*-Nitrophenyl-β-methyl-L-aspartate Hydrobromide (VII).—To 5 ml. of a saturated solution of hydrogen bromide in glacial acetic acid was added benzyloxycarbonyl-α-*p*-nitrophenyl-β-methyl-L-aspartate (VI, 3.8 g., 0.0095 mole). After standing for 1 hr. at room temperature complete solution occurred and the evolution of carbon dioxide ceased. Absolute ether was added to the cloud point in order to crystallize the hydrobromide (VII). After standing overnight at room temperature the hydrobromide was filtered and recrystallized from methanol–ether to yield 2.0 g. (60%), of long white needles, m.p. 118° dec., $[\alpha]^{25}D$ +13.8° (*c* 0.85, ethanol).

Anal. Calcd. for $C_{10}H_{13}N_2O_6Br$: C, 37.87; H, 3.75; N, 8.02. Found: C, 38.15; H, 4.02; N, 8.20.

Benzyloxycarbonyl-β-methyl-L-aspartyl-α-*p*-nitrophenyl-β-methyl-L-aspartate (VIII) (Dipeptide Active Ester).—To a solution of benzyloxycarbonyl-β-methyl-L-aspartate (II, 0.834 g., 0.00297 mole) in dimethylformamide at −10° were added consecutively isobutyl chloroformate (0.040 ml., 0.00297 mole) and triethylamine (0.300 ml., 0.00297 mole) with stirring. Twenty minutes later, a solution of α-*p*-nitrophenyl-β-methyl-L-aspartate hydrobromide (VII, 1.03 g., 0.00297 mole) in 5 ml. of dimethylformamide cooled to −10° was added to the reaction mixture, followed by the slow addition of triethylamine (0.300 ml., 0.00297 mole). Stirring was continued for 4 hr. while the reaction mixture was allowed to come to room temperature. The reaction mixture was diluted with 150 ml. of ethyl acetate and extracted three times with 40-ml. portions of 1 *N* hydrochloric acid, 30% potassium chloride and 10% sodium bicarbonate–30% potassium chloride. After drying of the ethyl acetate layer over magnesium sulfate and evaporation of the solvent under reduced pressure, a yellowish solid was obtained. Recrystallization from boiling absolute ethanol furnished 0.980 g. (74%) of dipeptide active ester VIII, m.p. 170–171°, $[\alpha]^{25}D$ −44.6° (*c* 2, dimethylformamide). When ten times the quantities of reactants were used, the yield dropped to 40%.

Anal. Calcd. for $C_{18}H_{25}N_3O_{11}$: C, 54.24; H, 4.74; N, 7.90. Found: C, 54.12; H, 4.86; N, 8.12.

Diethyl L-aspartate hydrochloride (IX) was prepared by a modification of the method of Fischer and Koenigs.[26] Anhydrous L-asparagine was prepared by heating commercial L-asparagine monohydrate to 100° at 0.5 mm. pressure overnight. The anhydrous product (25 g., 0.189 mole) was then suspended in 125 ml. of absolute ethanol to which acetyl chloride (45 ml., 0.635 mole) had been added previously. The reaction mixture was refluxed overnight and then evaporated *in vacuo* to a volume of 25 ml. After removing the precipitated ammonium chloride by filtration, ether was added to the cloud point. The mixture stood for a day at room temperature before the product IX was removed by filtration. After recrystallization from ethanol–ether, 25 g. (59%) of diethyl L-aspartate hydrochloride was obtained, m.p. 105–106° dec. (lit.[41] 109–110° dec., $[\alpha]^{25}D$ +7.9° (*c* 1, water); lit.[39] $[\alpha]^{24}D$ +8.1° (*c* 1, water)).

Benzyloxycarbonyl-β-methyl-L-asparty-Diethyl L-Aspartate (X) (Dipeptide).—In 20 ml. of dimethylformamide at −10° were dissolved with stirring benzyloxycarbonyl-β-methyl-L-aspartate (II, 1.16 g., 0.0041 mole), isobutyl chloroformate (0.53 ml., 0.0041 mole) and triethylamine (0.0055 ml., 0.0041 mole). After 20 min., a solution of diethyl L-aspartate hydrochloride (IX, 0.81 g., 0.0036 mole) in 5 ml. of dimethylformamide cooled to −10° was added followed by triethylamine (0.55 ml., 0.0041 mole) which was added in five portions. Stirring was continued for another 2 hr. while the temperature of the reaction mixture was allowed to rise to 25°. The mixture was diluted with 250 ml. of ethyl acetate and extracted with 1 *N* hydrochloric acid, 30% potassium chloride and 10% sodium bicarbonate–30% potassium chloride solutions. The ethyl acetate layer was dried over magnesium sulfate and the solvent was removed by distillation to yield an oily solid. Recrystallization from chloroform–hexane furnished 0.65 g. (50%) of dipeptide X, m.p. 80–81°, $[\alpha]^{25}D$ −25.7° (*c* 2.80, dimethylformamide).

Anal. Calcd. for $C_{21}H_{28}N_2O_9$: C, 55.74; H, 6.23; N, 6.19. Found: C, 55.59; H, 6.36; N, 6.27.

Benzyloxycarbonyldi-(β-methyl-L-asparty)-Diethyl L-Aspartate (XI) (Tripeptide).—In 30 ml. of dimethylformamide were dissolved benzyloxycarbonyl-β-methyl-L-aspartyl-α-*p*-nitrophenyl-β-methyl-L-aspartate (VIII, 4.9 g., 0.0093 mole) and diethyl L-aspartate hydrochloride (IX, 4.2 g., 0.186 mole). To the vigorously stirred reaction mixture was now added triethylamine (2.6 ml., 0.186 mole) in five portions over a period of 30 min. The reaction mixture was stirred overnight at room temperature and then diluted with 300 ml. of ethyl acetate. After extraction with 1 *N* hydrochloric acid, 30% potassium chloride and 10% sodium bicarbonate–30% potassium chloride, the ethyl acetate layer was dried over magnesium sulfate and the solvent was removed by distillation. The pale yellow solid obtained was crystallized from ethyl acetate–hexane to yield 3.0 g. (57%) of tripeptide, m.p. 127–128°, $[\alpha]^{25}D$ −34.5° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{36}H_{35}N_3O_{12}$: C, 53.70; H, 6.07; N, 7.22. Found: C, 53.85; H, 6.24; N, 7.26.

An attempt to prepare the tripeptide using one equivalent of each reagent resulted in a 20% recovery of VIII, but no peptide.

Benzyloxycarbonyltri-(β-methyl-L-asparty)–Diethyl L-Aspartate (XII) (Tetrapeptide).—To 1.1 ml. of a saturated solution of hydrogen bromide in glacial acetic acid was added tripeptide XI (0.893 g., 0.00143 mole). After standing 1 hr. at room temperature, complete solution had occurred and the evolution of carbon dioxide ceased. The product (tripeptide hydrobromide) was precipitated with ether and the supernatant liquid decanted. The residue was taken up in chloroform and reprecipitated with ether; the supernatant liquid was again decanted. The method of purification by solution, precipitation and decantation was repeated three times. As a result, no odor of hydrogen bromide could be detected in the hydrobromide. After drying the hydrobromide for 2 hr. at 0.05 mm. and 25° in a tared flask, 0.755 g. (92%) was obtained. No attempt was made to filter the oily hydrobromide.

In 10 ml. of dimethylformamide at −10° were dissolved benzyloxycarbonyl-β-methyl-L-aspartate (II, 0.401 g., 0.00143 mole), isobutyl chloroformate (0.189 ml., 0.00143 mole) and triethylamine (0.200 ml., 0.00143 mole). The last reagent was added in four portions to the vigorously stirred cold reaction mixture. Twenty minutes later, a solution of the tripeptide hydrobromide (0.755 g., 0.00133 mole) in 10 ml. of dimethylformamide at −10° was added to the reaction mixture, followed by triethylamine (0.186 ml., 0.00133 mole) in five portions. Vigorous stirring was maintained throughout the additions. Two hours later, the reaction mixture was allowed to come to room temperature, diluted with 200 ml. of ethyl acetate and worked up in the same way as the tripeptide XI. The crude white product was recrystallized from chloroform–ether to give 0.589 g. (62%), m.p. 143–144°, $[\alpha]^{25}D$ −13.1° (*c* 1, dichloroacetic acid).

Anal. Calcd. for $C_{31}H_{42}N_4O_{15}$: C, 52.39; H, 5.96; N, 7.88. Found: C, 52.13; H, 5.95; N, 7.94.

Benzyloxycarbonyltetra-(β-methyl-L-asparty)–Diethyl L-Aspartate (XIII) (Pentapeptide). Method A.—The pentapeptide was prepared in the same manner as the tetrapeptide. The decarbobenzoxylation of the tetrapeptide (XII, 1.25 g., 0.00176 mole) afforded a quantitative yield of the oily tetrapeptide hydrobromide. This hydrobromide, after neutralization with triethylamine, was treated with a mixed anhydride made from benzyloxycarbonyl-β-methyl-L-aspartate (II, 0.542 g., 0.00193 mole), isobutyl chloroformate (0.371 ml., 0.00193 mole) and triethylamine (0.393 ml., 0.00193 mole). The pentapeptide was obtained pure as recrystallization from chloroform–ether did not change its melting point, 161–163°. The yield of recrystallized material was 0.750 g. (51%), $[\alpha]^{25}D$ −19.1° (*c* 0.75, dichloroacetic acid).

Anal. Calcd. for $C_{38}H_{49}N_5O_{18}$: C, 51.49; H, 5.88; N, 8.34. Found: C, 51.78; H, 5.76; N, 8.12.

Method B.—Hydrogenolysis of tripeptide XI was carried out by the quantitative method of Patchornik and Shalitin.[29] To a magnetically stirred mixture of 15 ml. of dimethylformamide and 15 ml. of ethanol was added tripeptide (1.00 g., 0.00172 mole). Nitrogen was passed through for 10 min. and then 0.1 g. of 10% palladium-on-charcoal was added. Nitrogen was passed through for another 10 min.

The nitrogen and hydrogen from the reaction mixture were passed directly into a solution of benzylamine (3.6 ml., 0.036 mole) in 20 ml. of ethanol containing 3 drops of thymol blue indicator. The carbon dioxide was bubbled into the solution, causing the thymol blue to impart a yellow color to the originally blue solution. Before the nitrogen stream was stopped, the benzylamine solution was titrated with 0.1772 *N* sodium methoxide in 1:3 methanol–benzene to a blue end point; 0.500 ml. of titrant was required.

Hydrogen was now passed into the system and both solutions were stirred magnetically. After 10 min., the benzylamine solution turned yellow, indicating that decarbobenzoxylation and concomitant carbon dioxide evolution were taking place. Titrant (7.28 ml., 75% of theory) was added to restore the blue color. After 2 hr., 9.04 ml. (93%) of titrant had been added to the benzylamine solution. Since further passage of hydrogen did not result in a change of color from blue to yellow, the hydrogen stream was stopped and the system flushed with nitrogen for 15 min. The palladium catalyst was removed by filtration and the filtrate evaporated *in vacuo*. The oily unblocked tripeptide was dissolved in 15 ml. of dimethylformamide containing dipeptide

(41) R. E. Neumann and E. L. Smith, *J. Biol. Chem.*, **193**, 97 (1951).

active ester VIII (0.913 g., 0.00172 mole) and the reaction mixture stirred overnight at room temperature. After the usual dilution with ethyl acetate and aqueous extractions, the crude yield of pentapeptide plus dipeptide active ester was obtained. Purification was achieved by three recrystallizations from chloroform–ether.

The final yield of pure pentapeptide was 0.430 g. (29%), m.p. 155–156°. Attempts to increase both the yield and purity of the product by increasing the reaction time of the active ester coupling failed. No attempt was made to make the ratio of tripeptide to dipeptide active ester greater than one, as any increase in yield would be offset by the use of excessive amounts of the more precious reactant, the tripeptide.

Method C.—Treatment of tripeptide XII (0.452 g., 0.000778 mole) with 0.60 ml. of a saturated solution of hydrogen bromide in acetic acid afforded the tripeptide hydrobromide (0.411 g., 100%). The hydrobromide was dissolved in 5 ml. of tetrahydrofuran and neutralized with triethylamine (0.109 ml., 0.000778 mole). This mixture was shaken for a few minutes and then cooled in an ice-bath to await further use.

In 5 ml. of tetrahydrofuran was suspended dipeptidehydrazide XIX (0.330 g., 0.000778 mole). The suspension was stirred vigorously and cooled in a methanol–Dry Ice bath for 5 min. At this time, nitrosyl chloride (2 ml. of a saturated solution of nitrosyl chloride in tetrahydrofuran, 0.00778 mole) was added and the Dry Ice bath was removed. After solution of the hydrazide, the stirring was stopped and the reaction mixture was chilled once more in Dry Ice for 5 min. The mixture was diluted with 10 ml. of ethyl acetate (which had been cooled to 0°) and extracted twice with 10-ml. portions of 10% sodium bicarbonate–30% potassium chloride solution (also chilled) in a cold separatory funnel. The ethyl acetate layer was dried over magnesium sulfate and filtered directly into the cooled solution of unblocked tripeptide previously prepared.

The new reaction mixture was stored at −10° for 3 days. At that time, it was diluted with 100 ml. of ethyl acetate and extracted with aqueous acid and base. Upon evaporation of the ethyl acetate, 0.260 g. (40%) of pentapeptide was obtained, m.p. 160–163°, $[\alpha]^{25}D$ −19.3° (*c* 0.75, dichloroacetic acid). The azide coupling gave pentapeptide which was identical with that prepared by the mixed anhydride coupling in method A.

Benzyloxycarbonylpenta-(β-methyl-L-aspartyl)–Diethyl L-Aspartate (XIV) (Hexapeptide).—The hexapeptide was prepared in the same manner as the tetrapeptide. Treatment of pentapeptide XIII (0.504 g., 0.000601 mole) with hydrogen bromide and acetic acid resulted in pentapeptide hydrobromide (0.458 g., 97%). After neutralization with triethylamine (0.084 ml., 0.000601 mole) the hydrobromide was allowed to react with the mixed anhydride formed from benzyloxycarbonyl-β-methyl-L-aspartate (II, 0.169 g., 0.000601 mole), isobutyl chloroformate (0.079 ml., 0.000601 mole) and triethylamine (0.084 ml., 0.000601 mole). The hexapeptide obtained after the usual dilution with ethyl acetate and aqueous extractions amounted to 0.348 g. (61%), m.p. 175–178°. Recrystallization from chloroform–ether did not raise the melting point; $[\alpha]^{25}D$ −26.6° (*c* 0.7, dichloroacetic acid).

Anal. Calcd. for $C_{41}H_{56}N_6O_{21}$: C, 50.82; H, 5.83; N, 8.67. Found: C, 50.84; H, 5.89; N, 8.44.

Benzyloxycarbonyl-β-methyl-L-aspartyl-*t*-butyloxycarbonylhydrazide (XVI).—To 100 ml. of ethyl acetate at 0° were added with stirring benzyloxycarbonyl-β-methyl-L-aspartate (II, 31.3 g., 0.0758 mole) and *t*-butyloxycarbonyl hydrazide[32] (10.0 g., 0.0758 mole). When complete solution had occurred, N,N′-dicyclohexylcarbodiimide (15.6 g., 0.0758 mole) was added and the stirring continued. A precipitate of N,N′-dicyclohexylurea appeared after 5 min. After 1 hr. the temperature of the reaction mixture was allowed to rise to 25° and the stirring was continued overnight. After removal of the urea by filtration, the filtrate was diluted with 100 ml. of ethyl acetate and extracted in the usual manner. The ethyl acetate layer was dried over magnesium sulfate and then evaporated to yield 28.4 g. (90%) of product in the form of an oil. Upon drying at 25° and 0.05 mm., the product hardened to a gum which could not be crystallized. It was used in the next step without further purification.

Benzyloxycarbonyltri-(β-methyl-L-aspartyl)–*t*-Butyloxycarbonyl Hydrazide (XVII) (Tripeptide-CBH).—To a solution of 30 ml. of ethyl alcohol and 50 ml. of ethyl acetate was added benzyloxycarbonyl-β-methyl-L-aspartyl-*t*-butyloxycarbonyl hydrazide (XVI, 3.63 g., 0.0875 mole). Nitrogen was passed through the system and then 0.3 g. of 10% palladium-on-charcoal was added. Nitrogen was passed through for another 5 min. and then replaced by hydrogen for 4 hr. During this time the reaction mixture was stirred magnetically. Hydrogen was removed by flushing with nitrogen for 5 min. The catalyst was removed by filtration. The filtrate was evaporated *in vacuo* in a tared flask. The product, an oil, weighed 2.58 g. (90%). This oil was dissolved in 10 ml. of dimethylformamide containing benzyloxycarbonyl-β-methyl-L-aspartyl-α-*p*-nitrophenyl-β-methyl-L-aspartate (VIII, 4.65 g., 0.0875 mole). The solution was stirred for 3 days at room temperature before dilution with 100 ml. of ethyl acetate and the usual extractions with aqueous acid and base. Evaporation of the ethyl acetate afforded 3.00 g. (50%) of an oil which crystallized after trituration with ether. After recrystallization from dioxane–hexane, the pure product obtained amounted to 2.70 g. (40%), m.p. 96–97°, $[\alpha]^{25}D$ −50.3° (*c* 0.16, dimethylformamide).

Anal. Calcd. for $C_{28}H_{39}N_5O_{13}$: C, 51.45; H, 6.01; N, 10.72. Found: C, 51.61; H, 6.09; N, 10.50.

Benzyloxycarbonyltri-(β-methyl-L-aspartyl) Hydrazide Hydrochloride (Tripeptide-hydrazide) (XVIII).—Tripeptide-CBH (XVII, 0.982 g., 0.00142 mole) was suspended in 5 ml. of a saturated solution of anhydrous hydrogen chloride in tetrahydrofuran. The peptide dissolved with evolution of a gas. After 1 hr. at room temperature, a gel appeared in the reaction flask. Crystallization of the gel was effected by trituration with 10 ml. of absolute ether. The crude hydrazide obtained was recrystallized by dissolving in hot tetrahydrofuran and adding ether after the solution had cooled to room temperature. The yield of pure hydrazide was 0.845 g. (84%), m.p. 157–160° dec., $[\alpha]^{26}D$ −26.0° (*c* 0.2, dimethylformamide).

Anal. Calcd. for $C_{23}H_{33}ClN_5O_{11}$: C, 46.74; H, 5.63; N, 11.85. Found: C, 46.56; H, 5.59; N, 11.70.

Benzyloxycarbonyldi-(β-methyl-L-aspartyl) Hydrazide (XIX) (Dipeptide-Hydrazide).—To 50 ml. of tetrahydrofuran at 25° were added with stirring dipeptide active ester VIII (0.531 g., 0.001 mole) and hydrazine hydrate (0.050 ml., 0.001 mole). The hydrazine hydrate dissolved 10 min. after addition. Twenty minutes later, the solvent was evaporated *in vacuo* to yield a yellow solid that was crystallized by trituration with ether. The ether suspension was cooled for 30 min. in an ice-bath and stirred. The resultant off-white precipitate was separated by filtration and dried overnight *in vacuo*. The final yield amounted to 0.30 g. (71%), m.p. 145–147° dec., $[\alpha]^{25}D$ −25.7° (*c* 0.66, dimethylformamide).

Benzyloxycarbonylhepta-(β-methyl-L-aspartyl)–Diethyl L-Asparate (XX) (Octapeptide).—The octapeptide was prepared according to the directions for the preparation of pentapeptide XIII by method C (the azide reaction). Pentapeptide XIII (0.870 g., 0.00104 mole) was treated with hydrogen bromide in acetic acid to yield the pentapeptide hydrobromide (0.72 g., 88%). After neutralization with an equivalent of triethylamine (0.128 ml.) in 5 ml. of tetrahydrofuran, the mixture was shaken and cooled in an ice-bath to await further use.

The tripeptide azide was prepared by suspending the tripeptidehydrazide XVIII (0.514 g., 0.000916 mole) in 5 ml. of tetrahydrofuran which was cooled to Dry Ice–methanol bath temperature. To the suspension was added 0.300 ml. of tetrahydrofuran containing 0.00916 mole of nitrosyl chloride. The azide solution was diluted with ethyl acetate, extracted with sodium bicarbonate solution, dried over magnesium sulfate, and added to the solution of the unblocked pentapeptide. The reaction mixture was stored at −10° for 4 days, at which time most of the octapeptide precipitated from solution. It was redissolved in 200 ml. of hot chloroform and after cooling extracted twice with 40-ml. portions of 1 *N* hydrochloric acid. The chloroform was evaporated at 0.05 mm. to yield a yellowish semisolid. This solid was purified by repeated washing with 50% aqueous ethanol until the color had almost disappeared and then dried at 0.05 mm. and 25° for 6 hr. Recrystallization of the crude product from chloroform–ether afforded 0.340 g. (30%), m.p. 207° dec., $[\alpha]^{25}D$ −35.1° (*c* 0.5, dichloroacetic acid).

Anal. Calcd. for $C_{51}H_{70}N_8O_{27}$: C, 49.82; H, 5.75; N, 9.13. Found: C, 49.62; H, 5.83; N, 8.89.

Benzyloxycarbonylhexa-(β-methyl-L-aspartyl)–*t*-Butyloxycarbonyl Hydrazide (XXI) (Hexapeptide-CBH).—Hydrogenolysis of tripeptide-CBH XVII (0.654 g., 0.001 mole) was effected in a mixture of 10 ml. of methanol and 30 ml. of ethyl acetate using 10% palladium-on-charcoal as described in the preparation of tripeptide-CBH (XVIII). A crude yield of 0.496 g. (96%) was obtained. This product was coupled with tripeptide hydrazide XVIII (0.564 g., 0.000956 mole) which had been previously converted to the corresponding azide, employing nitrosyl chloride (0.310 ml. of a tetrahydrofuran solution, 0.00956 mole) as described in the preparation of the pentapeptide *via* azide coupling.

As in the preparation of the octapeptide XX, the product precipitated from solution during storage at −10°. This precipitate was separated by filtration, washed with chloroform at room temperature and dissolved in hot dioxane. After filtration of the dioxane solution, the solvent was lyophilized. The residue was mixed with ether and filtered to yield 0.210 g. (26%), m.p. 172° dec.

The mother liquor from the reaction gave additional product. The solution was evaporated under reduced pressure to yield a yellowish solid, which was dissolved in dioxane, filtered and lyophilized to yield 0.122 g. (15%) of product, m.p. 172° dec. The combined yield amounted to 0.332 g. (41%).

Anal. Calcd. for $C_{43}H_{60}N_8O_{22}$: C, 49.62; H, 5.81; N, 10.77. Found: C, 49.49; H, 5.81; N, 10.89.

Benzyloxycarbonylhexa-(β-methyl-L-aspartyl) Hydrazide Hydrochloride (XXII) (Hexapeptide Hydrazide).—In 5 ml. of a saturated solution of hydrogen chloride in glacial acetic acid was suspended hexapeptide-CBH (XXI, 0.340 g., 0.000292 mole). The expected solution and gas evolution did not take place until the mixture was heated to 60°. Ten minutes later 3 ml. of tetrahydrofuran was added, followed by the dropwise addition of ether, until crystallization occurred. The product was separated by filtration and washed with ether. After drying, 0.236 g. (82%) of product was obtained, m.p. 165° dec.

Benzyloxycarbonyldeca-(β-methyl-L-aspartyl)-Diethyl L-Aspartate (XXIII) (Undecapeptide).—The undecapeptide was prepared according to the directions for the preparation of the pentapeptide by method C (azide coupling). Pentapeptide XIII (0.200 g., 0.000219 mole) was treated with hydrogen bromide in acetic acid to yield the pentapeptide hydrobromide (0.172 g., 100%). This compound was neutralized with triethylamine (0.031 ml., 0.000219 mole) in 5 ml. of tetrahydrofuran and cooled to 0° to await further use.

A hexapeptide azide was prepared by treatment of hexapeptide hydrazide XXII (0.214 g., 0.000219 mole) with nitrosyl chloride (0.76 ml. of a tetrahydrofuran solution, 0.00219 mole) in 5 ml. of tetrahydrofuran. Since warming the reaction mixture did not produce solution of the hydrazide, cold chloroform (10 ml.) was added until solution did take place. The reaction mixture was then chilled in a Dry Ice–acetone bath and extracted (without further dilution) with aqueous sodium bicarbonate–potassium chloride. The chloroform layer was dried over magnesium sulfate and added to the solution of the neutralized pentapeptide hydrobromide. The reaction mixture was stored at −10° for 4 days. The chloroform solution was extracted with 1 *N* hydrochloric acid, dried over magnesium sulfate and evaporated *in vacuo*. The gelatinous residue was redissolved in chloroform and precipitated with ether. After filtration and drying, the yield of undecapeptide amounted to 0.110 g. (31%), m.p. 224° dec., $[\alpha]^{25}D$ −42.9° (*c* 0.5, dichloroacetic acid).

Anal. Calcd. for $C_{66}H_{91}N_{11}O_{36}$: C, 49.08; H, 5.68; N, 9.54. Found: C, 49.38; H, 5.93; N, 9.29.

Benzyloxycarbonyltrideca-(β-methyl-L-aspartyl)-Diethyl-L-Aspartate (XXIV) (Tetradecapeptide).—The tetradecapeptide was prepared according to the directions for the preparation of pentapeptide, method C (azide coupling). Octapeptide XX (0.326 g., 0.000266 mole) was converted to its hydrobromide by treatment with 0.4 ml. of a saturated solution of hydrogen bromide in acetic acid. After purification of the salt by the usual method, it was dissolved in 5 ml. of hot chloroform. The solution was cooled to room temperature and triethylamine (0.037 ml., 0.000266 mole) was added. The mixture was shaken and cooled in an ice-bath to await further use.

A hexapeptide azide was prepared by treatment of hexapeptide hydrazide XXII (0.260 g., 0.000266 mole) suspended in 15 ml. of chloroform with nitrosyl chloride (1.12 ml. of a saturated solution in tetrahydrofuran, 0.000266 mole) in a Dry Ice–methanol bath. Upon warming to room temperature, solution occurred, indicating the conversion of hydrazide to azide. The chloroform solution was rechilled, extracted twice with 10-ml. portions of chilled sodium bicarbonate–potassium chloride solution, and, after drying with magnesium sulfate, the chloroform layer was added to the previously prepared solution of octapeptide. This new reaction mixture was kept at −10° for 4 days.

The chloroform solution was diluted with 15 ml. of chloroform and extracted with 1 *N* hydrochloric acid. An emulsion formed at this point, but separation of the layers was achieved by diluting the chloroform layer with ethyl acetate, and the aqueous layer with 30% sodium chloride solution. The bottom layer, consisting of chloroform, ethyl acetate and product, was evaporated *in vacuo* to yield 1.2 g. of an off-white solid which was mostly inorganic salt. Washing the solid with water removed the salt and gave crude product which was then washed with ethanol, ether, and dried for 3 hr. *in vacuo* at room temperature. The final yield of product amounted to 0.129 g. (25%), m.p. 233° dec., $[\alpha]^{25}D$ −46.0° (*c* 0.3, dichloroacetic acid).

Anal. Calcd. for $C_{81}H_{112}N_{14}O_{45}$: C, 48.60; H, 5.64; N, 9.80. Found: C, 48.48; H, 5.65; N, 10.50.

Hydrolysis of Peptides as a Check on Optical Purity.—Samples of pentapeptide, poly-β-methyl L-aspartate (A/I = 5.30), β-methyl L-aspartate hydrochloride, β-benzyl L-aspartate, and L-aspartic acid were each treated as follows:

In a micro-Carius tube were placed 0.03 to 0.06 g. of sample and 2.50 ml. of 6 *N* hydrochloric acid. The tube was sealed and heated at 120° for 18 hr. and then cooled to room temperature. The tube was then opened and the solution was filtered through a cotton plug into a polarimeter tube. The resultant optical rotations are shown in Table II.

TABLE II

SPECIFIC ROTATIONS OF DERIVATIVES OF β-METHYL L-ASPARTATE AFTER HYDROLYSIS WITH 6 *N* HYDROCHLORIC ACID

Rotations reported at 589 mμ, polarimeter tube length of 2 dm. at 25.0°

Compound	Wt., g.	Conditions	Rotations—Observed	Rotations—Specific[a]
ClH·H-Asp(OMe)-OH	0.0449	120°, 18 hr.	+0.423°	+16.3°
ClH·H-Asp(OMe)-OH	.0406	120°, 36 hr.	+ .190	+ 8.10
ClH·H-Asp(OMe)-OH	.0538	25°, 10 min.	+ .450	+23.1
H-Asp(OBz)-OH	.0466	120°, 18 hr.	+ .380	+17.6
H-Asp(OH)-OH (all L)	.0391	120°, 18 hr.	+ .510	+16.4
H-Asp(OH)-OH (L + DL)	.0283 (L) .0131 (DL)	120°, 18 hr.	+ .370	+11.4[b]
Pentapeptide[c]	.0377	120°, 18 hr.	+ .380	+15.9
Polymer (A/I = 1.26)	.0342	120°, 18 hr.	+ .470	+16.6

[a] The values for specific rotation have been corrected for the loss of weight suffered by the compounds upon conversion to aspartic acid. The values are the specific rotation of aspartic acid and not the parent compound. [b] The calculated specific rotation for this mixture is 70% of 16.3 or 11.4°. [c] As the pentapeptide samples produced by synthetic methods A and C (see Experimental section) are identical, all methods of synthesis employed for the oligomers have been tested by the one pentapeptide sample.

It was necessary to heat the pentapeptide and polymer for 36 hr. for complete hydrolysis.