inequivocally resolved by vibrational analysis.[11] One may conclude, however, from data on molecules of constant length dissolved in solvents differing in polarity[27] (*e.g.*, retinal, Figure 6) that electronic bond equalization contributes appreciably to length-dependent shifts in ν(C=C).

The fact that the downshift of ν(C=C) levels off earlier in the sequence than does ν_{max} is the corollary of general principles. Two nondegenerate, close-in-energy eigenstates of an unperturbed (*e.g.*, vibrational) Hamiltonian would be repelled by the presence of an additional perturbation connecting these two states. (This is how the perturbing Kuhn potential repels the S_1 state from the S_0 electronic state of the linear trough.) Carrying the analogy to an idealized case of molecular vibration, a mode dominated by C=C stretching (*e.g.*, the ethylenic mode) would tend to coalesce with a mode dominated by C–C stretching when these two types of bonds tend to equalize. The off-diagonal terms in the vibrational Hamiltonian admix the internal coordinates and thus prevent the approach due to bond equalization of the ethylenic toward the C–C stretching modes which would be expected from progressive bond equalization. Thus the Raman spectra of long-chain polyenes indicate a higher degree of apparent bond alternation than that inferred from ν_{max} of the absorption spectra.

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Solid-Phase Synthesis of Selectively Protected Peptides for Use as Building Units in the Solid-Phase Synthesis of Large Molecules

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Abstract: Self-catalyzed transesterification with 2-dimethylaminoethanol at room temperature was found to be effective for the removal of a variety of protected peptides from the Merrifield resin after solid-phase synthesis. The 2-dimethylaminoethyl function was removed from the resultant protected peptide esters by treatment with water at room temperature, to yield the corresponding protected peptide acids. The series of reactions comprising transesterification and ester hydrolysis was found to proceed with 0.3 ± 0.1% racemization of the C-terminal amino acid. The transesterification was found to be very slow when the peptide contained a C-terminal proline residue and in this case the reaction was expedited by the addition of thallium 2-dimethylaminoethoxide. *tert*-Butyloxycarbonylglycyl-L-alanine, prepared by the above methods, was utilized as a building unit in a further solid-phase synthesis using a variety of coupling procedures. The extent of racemization obtained in the coupling reaction was measured in each case. The highest yield and lowest degree of racemization was obtained using *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline as the coupling agent in dioxane solution. The procedures described herein were developed to provide a route for the solid-phase synthesis of selectively protected peptides which are required as building units for the solid-phase synthesis of large molecules.

The unequivocal chemical synthesis of proteins has long been a major goal of peptide chemistry and recent achievements in this direction are well known.[2-5] However, both solid-phase and solution methods of synthesis suffer from a number of disadvantages[6-14] when applied to large molecules, and it is evident that considerable modification of present techniques will be required before the synthesis of homogeneous proteins of defined structure can be readily accomplished.

It is possible that the major problems of the solid-phase method will be alleviated by the adoption of a fragment condensation strategy whereby the peptide chain will be assembled from a series of preformed protected peptide building units. The advantages of

(1) (a) This investigation was supported by grants from The Banting Research Foundation, The National Research Council of Canada, the Medical Research Council of Canada, and the University of Alberta Medical Research Fund. (b) Presented in part by M. A. Barton and R. U. Lemieux at the 14th Annual Meeting of the Canadian Federation of Biological Societies, Toronto, June 15–18, 1971, Abstract No. 35. (c) Abbreviations used are: Boc, *tert*-butyloxycarbonyl; Cbz, benzyloxycarbonyl; DMAE, 2-dimethylaminoethanol, DMF, *N*,*N*-dimethylformamide; EEDQ, *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline; DCC, *N*,*N*′-dicyclohexylcarbodiimide.

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this approach derive from the fact that purification of the protected peptides will be possible before these become incorporated into the larger peptide chain. Thus a more homogeneous final product can be expected and failure sequences will be separable on the basis of molecular size. Studies of the solid-phase fragment condensation procedure have been initiated in a number of laboratories[15-18] using direct activation of the free terminal carboxylic acid function of selectively protected peptides prepared by solution methods. However, it is clear that the approach will not become generally useful unless these protected peptides can be prepared by rapid methods. There is, therefore, a need for the development of solid-phase procedures which will allow the preparation of selectively protected peptides containing a free terminal carboxylic acid group, for subsequent use as building units in the solid-phase synthesis of larger molecules. Some advances have been made in this direction by the use of modified methods of attachment of the peptide to the polymer support,[19-22] but as yet none of these procedures has achieved general practical application.

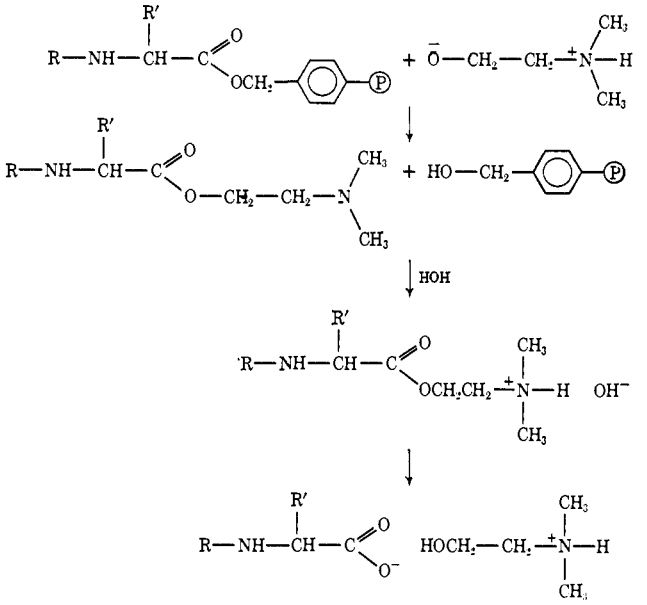
In the present paper we describe a route by which protected peptides containing a free terminal carboxylic acid group can be obtained following synthesis on the standard solid-phase support.[23] These peptides can be utilized as building units in fragment condensation solid-phase synthesis.

Discussion

The most commonly used method of solid-phase synthesis[23] employs a benzyl ester bond for the linkage of the peptide to the polymer support, and this bond is known to be susceptible to transesterification by methanol or ethanol in the presence of base catalysis.[24-27] In order to extend this procedure to provide a practical method for the preparation of protected peptide acids we have studied the use of 2-dimethylaminoethanol (DMAE) as a transesterification agent. This alcohol was chosen because previous work[28] has shown that DMAE esters possess unusual base lability.

We have found that, after solid-phase synthesis, peptides can be released from the solid support by treatment with DMAE at room temperature. The resultant protected peptide DMAE esters are obtained by evaporation of the solution, and the DMAE ester function can subsequently be removed by treatment with aqueous DMF at room temperature (Scheme I).

Scheme I



Detailed studies of the reaction were undertaken as follows.

Influence of Solvent on the Rate of Transesterification with DMAE. The effect of solvent on the course of the transesterification was investigated as shown in Table I, using Boc-glycine linked to the chloromethylated polystyrene resin. It was found that a mixture of DMAE with DMF (1:1 v/v) gave almost quantitative transesterification after 70 hr at room temperature, whereas a less complete reaction was observed with other solvent combinations. The addition of methylene chloride inhibited the transesterification, and it was subsequently observed that methylene chloride readily reacts with DMAE to yield a quaternary ammonium salt. We have also observed that DMAE and DMAE esters will react slowly with chloro groups on the polymer when the chloromethylated resin is used. However, this latter reaction apparently has no deleterious effect when a large molar excess of DMAE is incorporated in the reaction solvent.

Table I. Effect of Solvent in the Transesterification of Boc-glycyl-polymer with DMAE[a,b]

Solvent	% removal of amino acid from polymer
DMAE	68
DMAE–methylene chloride (1:1)	23
DMAE–dioxane (1:1)	65
DMAE–DMF (1:1)	95

[a] Samples of Boc-glycyl-polymer (50 mg; 3.62 μmol) suspended in the solvent (2 ml), suspensions rocked at room temperature for 70 hr, filtered, washed with ethanol (3 × 2 ml), dried (40°, *in vacuo*), weighed, and hydrolyzed 24 hr in dioxane (5 ml) plus 12 *N* hydrochloric acid (5 ml) at reflux temperature. Glycine estimated by amino acid analysis. [b] Boc-glycyl-polymer previously washed with tetraethylammonium chloride to remove noncovalently bound Boc-glycine.

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The rate of transesterification in DMAE–DMF (1:1 v/v) was studied more extensively using a polymer-linked pentapeptide Boc-*O*-Bzl-L-Ser-L-Gln-NO_2-L-Arg-N^{im}-Bzl-L-His-Gly-*O*-polymer (Figure 1). The reaction rate was comparable with that obtained under similar conditions with Boc-Gly-*O*-polymer. The length of the peptide chain therefore did not appear to influence the rate of transesterification.

Influence of the C-Terminal Amino Acid on the Rate of Transesterification. Preparative experiments were performed using Boc-*O*-Bzl-L-Ser-L-Gln-NO_2-L-Arg-N^{im} Bzl-L-His-Gly-*O*-polymer ,Cbz-L-Ala-L-Phe-*O*-polymer, and Boc-Gly-L-Ala-*O*-polymer. The DMAE esters were obtained as oils which were characterized by thin layer chromatography and nmr spectroscopy. The characterization of these peptides was completed after the conversion to the crystalline carboxylic acids. Nmr spectra of the esters and acids confirmed that Boc and Cbz groups remained intact during the transesterification. The DMAE esters were isolated in yields of 85–95% after 3 days of reaction at room temperature. Thus, the nature of the C-terminal amino acid appeared to exert little effect on the rate of transesterification, in the cases of glycine, alanine, and phenylalanine. However a marked decrease in the rate of reaction was observed when proline was investigated as a C-terminal residue. It is likely that low reaction rates will also apply in the case of severely hindered amino acids such as valine. In cases of low reactivity it is advantageous to catalyze the reaction by increasing the concentration of 2-dimethylaminoethoxide ion. This was investigated in the case of proline using a catalytic amount of thallium 2-dimethylaminoethoxide[29] included in the reaction mixture. In the presence of the catalyst a quantitative transesterification of Boc-prolyl-polymer was obtained in a 22-hr reaction at room temperature. Catalysis of the reaction using sodium 2-dimethylaminoethoxide was slightly less effective (83% transesterification after 21 hr at room temperature).

An investigation of the use of sodium alkoxide in a similar transesterification with benzyl alcohol showed that this procedure also provides a useful route for the solid-phase synthesis of the benzyl esters of protected peptides. These intermediates are of synthetic value because the benzyl ester function can be selectively removed by catalytic hydrogenation. Benzyloxycarbonyl-L-alanyl-L-phenylalanine benzyl ester was obtained in 95% yield after recrystallization, and no racemization was observed, within the limits of the nmr spectroscopy technique.[30]

Hydrolysis of DMAE Esters. Esters of DMAE have previously been shown to hydrolyze in sodium bicarbonate solution.[28] However, we have observed that in many cases the hydrolysis of the protected peptide DMAE esters occurs at useful rates in aqueous DMF in the absence of added alkali. The rate of hydrolysis was found to be markedly dependent on the relative proportions of DMF and water in the reaction mixture, the rate being greatest in solutions containing a large percentage of water. For example Boc-Gly DMAE ester was completely converted to Boc-glycine after 2 hr at room temperature when DMF–water (1:5 v/v) was employed as the reaction solvent. When the same reaction was performed using a 1:1 mixture of DMF–water as the solvent, the time for complete reaction was increased to 12 hr; the progress of the reaction was followed in each case by thin layer chromatography.

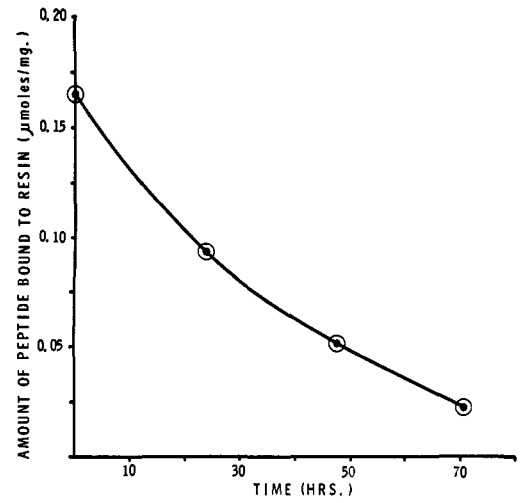

Figure 1. Transesterification of samples of Boc-*O*-Bzl-L-Ser-L-Gln-NO_2-L-Arg-N^{im}-Bzl-L-His-Gly-*O*-polymer (25 mg) in DMAE–DMF (1.0 ml; 1:1 v/v) at room temperature. The amount of peptide remaining bound to the resin at the end of the reaction time was measured by amino acid analysis of a hydrolyzed sample.

The use of a large proportion of water in the reaction mixture was precluded when the water solubility of the peptide was low, but we found that in these circumstances the addition of a trace amount of imidazole (0.1 equiv) caused a useful increase in the reaction rate. Thus the dipeptide benzyloxycarbonyl-L-alanyl-L-phenylalanine 2-dimethylaminoethyl ester was incompletely hydrolyzed to benzyloxycarbonyl-L-alanyl-L-phenylalanine after 5 days at room temperature in DMF–water (1:1 v/v), but when this reaction was repeated with the addition of imidazole (0.1 equiv), complete hydrolysis was obtained in 24 hr at room temperature. Benzyloxycarbonyl-L-alanyl-L-phenylalanine was isolated in a yield of 84% after recrystallization from aqueous acetic acid and this product was characterized by nmr spectroscopy and comparison of the melting point with the previously reported value.[31] Under the same conditions (DMF–water, 1:1; 0.1 equiv of imidazole) Boc-Gly-L-Ala DMAE ester was completely converted to Boc-Gly-L-Ala-OH after a 24-hr reaction at room temperature and the product was isolated in a yield of 69% after dry column chromatography. Similarly the pentapeptide acid Boc-*O*-Bzl-L-Ser-L-Gln-NO_2-L-Arg-N^{im}-Bzl-L-His-Gly-OH was obtained from the corresponding ester after a 20-hr reaction at room temperature; the product was isolated in 79% yield following recrystallization from ethanol.

Thus we conclude that the hydrolysis of peptide DMAE esters can generally be accomplished in aqueous DMF at room temperature provided the reaction mixture contains more than 50% of water. When the proportions of DMF and water are approximately equal, it can be advantageous to incorporate a trace of

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imidazole in the reaction mixture. The hydrolysis of DMAE esters of peptides of extremely low water solubility will probably be best accomplished by the sodium bicarbonate procedure.[28]

Extent of Racemization during Transesterification with DMAE and Hydrolysis of DMAE Esters. The extent of racemization of the carboxyl terminal amino acid was measured in the case of the dipeptide L-Leu-L-Ala. Transesterification of Boc-L-Leu-L-Ala-*O*-polymer was performed at room temperature in DMAE–DMF solution (1:1 v/v). The product was hydrolyzed in aqueous DMF and the amino-protecting group was removed by mild acidolysis. Quantitation of the dipeptide diastereoisomers was performed by ion-exchange chromatography using a Beckman 120C amino acid analyzer.[32] The overall extent of racemization for the combined series of reactions was found to be 0.3 ± 0.1%; *i.e.*, significantly less racemization occurred in the combined transesterification and DMAE ester hydrolysis procedure than was found on saponification of the methyl ester of the same dipeptide.[33]

Protected Peptides as Building Units in Solid-Phase Synthesis. In order to investigate the use of the protected peptides, obtained by the above procedure, as building units in further solid-phase synthesis, a study was made of the coupling of Boc-Gly-L-Ala-OH with H-L-Leu-polymer. These components were chosen because it is possible to separate and quantitate the two diastereoisomeric tripeptides, Gly-L-Ala-L-Leu and Gly-D-Ala-L-Leu, by ion-exchange chromatography using an amino acid analyzer.[34,35]

Thus we determined both yield of coupling and extent of racemization using a variety of coupling procedures as shown in Table II.

In DMF solution the highest coupling yield (83%) was obtained using EEDQ and the extent of racemization under these conditions was 8.5%. When the EEDQ coupling was repeated using dioxane as the solvent, the yield was 91% and the extent of racemizaton was reduced to 1.3%. A reduction of the molar ratio of EEDQ from 10 to 1.5 equiv gave identical results in dioxane solution (89% coupling; 1.2% racemization). The use of a large excess of coupling agent was therefore of no practical advantage.

An investigation of the use of the protected dipeptide acylphosphonium salt, derived from hexamethylphosphoramide, was attempted using hexamethylphosphoramide as solvent in the coupling reaction. This procedure has been shown to be effective in the solution synthesis of a variety of peptides.[36] However, we found that successful swelling of the polymer could not be achieved in this solvent alone. When a mixture of hexamethylphosphoramide and DMF (5:3 v/v) was employed as the solvent, no coupling occurred in the presence of 1.5 equiv of protected dipeptide carboxylate salt and 1.5 equiv of tosic anhydride in 15 equiv of hexamethylphosphoramide. When the reaction was repeated with the same ratio of reactants but with DMF as the solvent in the coupling step, a yield of 47% was obtained, but extensive racemization (41%) also occurred under these conditions.

Table II. Coupling of Boc-Gly-L-Ala-OH[a] with H-L-Leu-polymer[b,c]

Coupling agent	Solvent	Coupling,[d] %	Racemization,[e,f] %
EEDQ[g] (10 equiv)	DMF	83	8.5
CDI[h] (10 equiv)	DMF	0	
DCC[i] (10 equiv)	DMF	69	18.3
EEDQ (10 equiv)	Dioxane	91	1.3
EEDQ (1.5 equiv)	Dioxane	89	1.2
Tosic anhydride (1.5 equiv) plus HMPA[j] (15 equiv)	HMPA–DMF (5:3)	0	
Tosic anhydride (1.5 equiv) plus HMPA (15 equiv)	DMF	47	41

[a] 1.5 equiv, 0.127 mmol. [b] 1.0 equiv, 0.085 mmol. [c] Reactions performed for 24 hr at room temperature in 0.5 ml of solvent. [d] The yield was determined from amino acid ratios after washing of the peptide-polymer with 6 × 1 ml each of DMF, methylene chloride, methanol, acetic acid, and methanol, followed by hydrolysis in 12 *N* hydrochloric acid (5 ml) plus dioxane (5 ml) for 24 hr at reflux temperature. [e] The peptide-polymer (0.076 mmol) was treated with trifluoroacetic acid (5 ml) plus hydrogen bromide gas for 15 min at room temperature; quantitation of the diastereoisomers was accomplished using a Beckman amino acid analyzer (Model 120 C) as described by F. H. C. Stewart, *Aust. J. Chem.*, **23**, 1073 (1970). [f] Racemization = [D,L/(D,L + L,L)] × 100%. [g] *N*-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline. [h] 1,1′-Carbonyldiimidazole. [i] Dicyclohexylcarbodiimide. [j] Hexamethylphosphoramide.

Of the four methods studied we find that EEDQ is the most satisfactory reagent for the coupling of protected peptides in solid-phase reactions. A similar result was obtained by Yajima and Kawatani[18] in a comparison of yields obtained in coupling a protected pentapeptide onto a polymer-linked peptide using EEDQ, DCC, DCC plus pentachlorophenol and *N*-ethyl-5-phenylisoxazolium-3′-sulfonate as coupling agents. We also find that coupling with EEDQ causes relatively little racemization when dioxane is employed as the solvent. The figure of 1.2% represents the overall extent of racemization obtained in the combined series of reactions consisting of transesterification, hydrolysis of the DMAE ester, and EEDQ coupling of the peptide acid. The extent of racemization in the first two steps was shown to be approximately 0.3% and thus the EEDQ coupling procedure apparently results in 0.9% racemization when employed in dioxane solution.

The results described above indicate that the DMAE transesterification procedure provides a useful route for the synthesis of a variety of protected peptides; however, it is evident that a nontransesterifiable protecting group will be required if the method is to be used for the provision of peptides containing protected carboxylic acid side chains. It is possible that *tert*-butyl ester side chain protection could be utilized in combination with the *p*-diphenylisopropoxycarbonyl amino protecting group as suggested by others.[19,22] Work is also in progress in this laboratory to develop a more acid-stable transesterification-resistant protect-

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ing group. Since the extent of racemization is small, it is also conceivable that problems of carboxylic acid side chain protection can be eliminated by employing a synthetic strategy in which aspartic or glutamic acids are utilized as single units while other parts of the molecule are built up from peptide blocks synthesized by the methods described herein.

The methods described in this paper have subsequently been employed in the peptide fragment solid-phase synthesis of the 1–20 amino-terminal peptide of human encephalitogenic basic protein.[37]

Experimental Section

DMAE obtained from Eastman Organic Chemicals was dried by distillation with two volumes of dry benzene. The fraction boiling at 135° (760 mm) was collected and kept over Linde molecular sieve (Type 4A) and was filtered through a column of alumina (B. D. H., Brockman activity II) immediately before use. Chloromethylated copolystyrene, 2% divinylbenzene, containing 1.8–2.2 mequiv of chlorine per g, was obtained from Schwartz Bioresearch Inc. The hydroxymethylated polymer was prepared by the method of Mizoguchi.[38] *tert*-Butyloxycarbonyl amino acids were obtained from Cyclo Chemical Corp. or were synthesized by the method of Schnabel.[39] Thallous ethoxide was obtained from Aldrich Chemical Co. DL-Leu-DL-Ala was obtained from Sigma Chemical Co. DMF was purified and tested for the absence of dimethylamine as described by Stewart and Young.[40] Amino acid analyses were performed on a Beckman amino acid analyzer, Model 120C. Acid hydrolysis of peptide resin samples was performed in dioxane–12 *N* hydrochloric acid (1:1) at reflux temperature, for 24 hr. Hydrolysis of peptide samples was performed in 6 *N* hydrochloric acid *in vacuo* at 110° for 24 hr. Nuclear magnetic resonance spectra were obtained on Varian A-60 or Varian HA-100 instruments.

Thin layer chromatography was performed on silica gel G (Brinkman Instruments, (Canada) Ltd.). The following solvent systems were used: tlc-1, 1-butanol–acetic acid–water (6:3:3 v/v); tlc-2, methyl ethyl ketone–acetic acid–water (5:35:25 v/v); tlc-3, DMF–chloroform, (2:3 v/v); tlc-4, 1-butanol–DMF–water (6:3:3 v/v); tlc-5, pyridine–chloroform (1:5 v/v); tlc-6, pyridine–chloroform 1:1 v/v); tlc-7, DMF–chloroform (1:1 v/v).

The plates were sprayed with the chlorine peptide spray.[41] Synthesis of polymer-linked peptides was performed by the method of Marshall and Merrifield.[23] The progress of these syntheses was monitored at each step by amino acid analysis of hydrolysates of aliquots of the peptide resin. The chloromethylated resin was used in all syntheses except that of Boc-Gly-L-Ala-*O*-polymer in which the hydroxymethylated polymer was employed. Acetylation of the residual hydroxyl groups was performed by standard methods[42] after attachment of the first amino acid.

Thallous 2-dimethylaminoethoxide solution was prepared by mixing thallous ethoxide (0.75 g; 3 mmol), DMAE (21 ml), DMF (42 ml), and benzene (8 ml). Distillation was performed at 50° (20 mm) until the volume of the solution was reduced by approximately 10 ml. The volume of the thallous alkoxide solution was measured and the titer of thallium salt calculated, for further use as transesterification catalyst. Precautions were taken to ensure that skin contact and contamination of work areas with thallium derivatives was scrupulously avoided.[29]

Boc-glycyl-L-alanine 2-Dimethylaminoethyl Ester. The protected dipeptide resin (22.7 g; 15.7 mmol) was washed with DMF (3 × 70 ml) and the washings were discarded. The resin was stirred for 24 hr at room temperature in DMF–DMAE (180 ml; 1:1 v/v). The solvent was removed by filtration and the resin was resuspended in DMF–DMAE (180 ml; 1:1 v/v). The reaction procedure was repeated a further three times with the same batch of resin. The filtrates were combined and evaporated *in vacuo* at 20° to give the product (4.69 g; 94%; oil). Thin layer chromatography showed one major spot having the following R_f values: (tlc-1) 0.58, (tlc-2) 0.49, (tlc-3) 0.45, (tlc-4) 0.63, (tlc-5) 0.60. Nmr (A60, $CDCl_3$) τ 8.61 (d, CH_3CH–, alanine), 8.55 (s, $(CH_3)_3C$, Boc group; 12 H with 8.61 peak), 7.72 (s, 6 H, $(CH_3)_2N$, DMAE group), 7.41 (t, 2 H, $-OCH_2-CH_2N$, DMAE group), 6.19 (d, 2 H, NCH_2CO–, glycine), 5.76 (t, 2 H, $-OCH_2CH_2N$, DMAE group), 5.38 (m, 1 H, $-CHCH_3$, alanine).

Boc-Gly-L-Ala. Boc-Gly-L-Ala DMAE ester (316 mg; 1 mmol) was kept at room temperature in a mixture of DMF (10 ml) and water (10 ml) in the presence of a trace amount of imidazole (6.8 mg; 0.1 mmol). The reaction was monitored by thin layer chromatography (tlc-3) and was found to be complete after 24 hr. The reaction mixture was evaporated *in vacuo* at 20° and was re-evaporated with ethyl acetate (3 × 20 ml) followed by 0.1 *N* hydrochloric acid (2 × 10 ml). The residue was chromatographed on a dry column of silica gel (12.5 × 1 cm) in 1-butanol–acetic acid–water (6:3:3) (fraction size, 0.5 ml). Fractions were monitored by thin layer chromatography (tlc-2). Fractions 1–12 were combined and evaporated to yield the *product* (169 mg; 69%; crystals): mp 124–129°; nmr (A60, $CDCl_3$) τ 8.66 (d, CH_3CH, alanine), 8.60 (s, $(CH_3)_3C$, Boc group, 12 H with 8.66 peak), 6.26 (d, 2 H, $-NCH_2CO$–, glycine), 5.59 (m, 1 H, $-CHCH_3$, alanine); thin layer chromatography, single spot, R_f 0.78 (tlc-2).

Anal. Calcd for $C_{10}H_{18}N_2O_5$: C, 48.77; H, 7.37; N, 11.38. Found: C, 48.45; H, 7.27; N, 11.41.

Benzyloxycarbonyl-L-Alanyl-L-phenylalanine 2-Dimethylaminoethyl Ester. Benzyloxycarbonyl-L-alanyl-L-phenylalanyl resin (500 mg; 0.126 mmol of peptide) was stirred 3 days at room temperature in a DMF–DMAE mixture (20 ml; 1:1 v/v). The resin was removed by filtration and was washed with DMF (3 × 10 ml). The filtrate and washings were evaporated *in vacuo* at room temperature to yield the product (52 mg; 93%; oil); nmr (A-60, $CDCl_3$) τ 8.68 (d, 3 H, CH_3CH–, alanine), 7.72 (s, 6 H, $(CH_3)_2N$–, DMAE group), 7.46 (t, 2 H, $-CH_2CH_2N$, DMAE group), 6.90 (m, $C_6H_5CH_2CH$–, phenylalanine), 5.79 (t, $-OCH_2CH_2$–, DMAE group), 5.76 (q, $-CHCH_3$, alanine, 3 H with 5.79 peak), 5.21 (m, 1 H, $-CHCH_2C_6H_5$, phenylalanine), 4.90 (s, 2 H, $C_6H_5CH_2O$, Cbz group), 4.51 (d, broad, 1 H, $-NHCH$–), 3.35 (d, broad, 1 H, $-NHCH$–), 2.79 and 2.66 (m superimposed on s, 10 H, two C_6H_5, Cbz group and phenylalanine); tlc-6, R_f 0.73 (slight trace remained at origin).

Benzyloxycarbonyl-L-alanyl-L-phenylalanine. Benzyloxycarbonyl-L-alanyl-L-phenylalanine 2-dimethylaminoethyl ester (74 mg; 0.168 mmol) was kept at room temperature in a mixture of DMF (2 ml) and water (2 ml) in the presence of a trace amount of imidazole (0.0168 mmol). The reaction was monitored by thin layer chromatography (tlc-6) and was found to be complete after 24 hr. The reaction mixture was evaporated *in vacuo* at 20° and was redissolved in ethyl acetate (15 ml). The solution was extracted with 0.01 *N* hydrochloric acid (2 × 10 ml) and the aqueous phase was washed with ethyl acetate (2 × 10 ml). The combined ethyl acetate extracts were dried ($MgSO_4$) and evaporated *in vacuo*. The residue was recrystallized from aqueous acetic acid to give benzyloxycarbonyl-L-alanyl-L-phenylalanine (50 mg; 84%): mp, 125° (softened at 115°). Recrystallization from ethyl acetate–hexane gave material having mp 124–126° [lit.[31] mp 122°]: nmr (DMF-d_7) τ 9.06 (d, 3 H, CH_3CH–, alanine), 7.40 (m, $C_6H_5CH_2-CH$–, phenylalanine), 5.73 (q, 1 H $-CHCH_3$, alanine), 6.14 (m, 1 H, $-CHCH_2C_6H_5$, phenylalanine), 5.31 (s, 2 H, $C_6H_5CH_2O$–, Cbz group), 3.12 and 3.02 (two s, 10 H, $2C_6H_5$, phenylalanine and Cbz group).

Boc-*O*-Bzl-L-Ser-L-Gln-NO_2-L-Arg-N^{im}-Bzl-L-His-Gly DMAE Ester. The protected pentapeptide resin (7.9 g; 1.30 mmol) was rocked for 24 hr at room temperature in DMF–DMAE (40 ml; 1:1 v/v). The solvent was removed by filtration and, after washing with DMF (30 ml), the resin was resuspended in DMF–DMAE (40 ml; 1:1 v/v). The reaction procedure was repeated a further three times with the same batch of resin. The filtrates and washings were combined and evaporated *in vacuo* at 20° to give a yellow oil which on trituration with ether gave the product (1.08 g; 85%; pale cream powder): thin layer chromatography, single spot, R_f 0.30 (tlc-7); nmr (HA-100, DMF-d_7) τ 8.63 (s, 9 H, $(CH_3)_3C$, Boc group), 7.68 (s, 6 H, $(CH_3)_2N$, DMAE group), 5.82 (t, 2 H, $-OCH_2-CH_2N$, DMAE group), 5.50 (s, 2 H, $C_6H_5CH_2O$, benzylserine group), 4.84 (s, 2 H, $C_6H_5CH_2N$, benzylhistidine group), 3.03 (s, 1 H, imidazole $-H_4$), 2.72 (m, 10 H, $C_6H_5CH_2$–, benzylserine and benzylhistidine groups), 2.32 (s, 1 H, imidazole-H_2).

Boc-*O*-Bzl-L-Ser-L-Gln-NO_2-L-Arg-N^{im}-Bzl-L-His-Gly-OH. The protected pentapeptide DMAE ester (500 mg, 0.5 mmol) was kept at room temperature in a mixture of DMF (5 ml) and water (5 ml) in the presence of a trace amount of imidazole (0.05 mmol). The reaction was monitored by thin layer chromatography (tlc-7) and was found to be complete after 20 hr. The reaction mixture was

(37) Manuscript in preparation.
(38) See J. M. Stewart and J. D. Young, "Solid Phase Peptide Synthesis," W. H. Freeman, San Francisco, Calif., 1969, p 27.
(39) E. Schnabel, *Justus Liebigs Ann. Chem.*, 702, 188 (1967).
(40) Reference 38, p 31.
(41) R. H. Mazur, B. W. Ellis, and F. S. Cammarata, *J. Biol. Chem.*, 237, 1619 (1962).
(42) Reference 38, p 33.

evaporated *in vacuo* at 20° and was reevaporated with water (10 ml). The residue was recrystallized from ethanol to give the *product* (364 mg; 79%): mp 198–200°; nmr (HA-100; DMF-d_7) τ 8.79 (s, 9 H, $(CH_3)_3C$, Boc group), 5.47 (s, 2 H, $C_6H_5CH_2O$–, benzylserine group), 4.80 (s, 2 H, $C_6H_5CH_2N$–, benzylhistidine group), 2.98 (s, 1 H, imidazole-H_4) 2.70 (m, 10 H, $C_6H_5CH_2$–, benzylserine and benzylhistidine groups) 2.24 (s, 1 H, imidazole-H_2). Thin layer chromatography, single spot, R_f 0.14 (tlc-7). $[\alpha]^{25}D$ −4.67° (*c* 1.9, DMF).

Anal. Calcd for $C_{41}H_{56}O_{12}N_{12}$: C, 54.18; H, 6.21; N, 18.49. Found: C, 53.99; H, 5.97; N, 18.37.

Transesterification of *tert*-Butoxycarbonyl-L-prolyl Resin. (i) With Thallium Alkoxide Catalysis. *tert*-Butoxycarbonyl-L-prolyl resin (1.5 g; 0.675 mmol of proline) was rocked for 2 days at room temperature in DMF (8 ml). The washings were discarded and the resin was suspended in a DMF–DMAE mixture (9 ml; 2:1 v/v) containing thallous 2-dimethylaminoethoxide (0.068 mmol; 0.1 equiv), and the reaction mixture was rocked for 22 hr at room temperature. The solution was separated from the resin by filtration and passed through a column (10 × 2 cm) of Amberlite IRC-50 (column poured in DMF) in order to remove the thallium salt. The resin was washed with DMF (3 × 10 ml) and the washings were passed through the ion-exchange resin column as above.

All filtrates and washings were combined and evaporated *in vacuo* at 30° to yield the product (186 mg; 96%; oil): thin layer chromatography showed a single spot; R_f values (tlc-1) 0.63 and (tlc-5) 0.40; nmr (A60, DMF-d_7) τ 9.00 (s, 9 H, $(CH_3)_3C$–, Boc group), 8.41 (m, 4 H, $-CH_2CH_2$–, proline), 8.16 (s, 6 H, $(CH_3)_2N$–, DMAE group), 7.85 (t, 2 H, $-CH_2CH_2N$, DMAE group), 7.00 (m, 2 H, $-CH_2N$–, proline), 6.17 (m, 3 H, $-OCH_2CH_2$– and $-CH$–, DMAE group and proline). Amino acid analyses of the prolyl resin indicated 99% removal of proline from the resin.

(ii) **With Sodium Alkoxide Catalysis.** The *tert*-butoxycarbonyl-L-prolyl resin (200 mg; 0.090 mmol of proline) was stirred for 2 days in DMF (3 ml), filtered, and washed with DMF (2 × 3 ml). The washings were discarded and the resin was suspended in DMF–DMAE (3 ml; 2:1 v/v) containing sodium 2-dimethylaminoethoxide (0.03 mmol; 0.33 equiv) and the reaction mixture was stirred for 21 hr at room temperature. The solution was separated from the resin by filtration and passed through a column (10 × 1 cm) of Amberlite IRC-50 (column poured in DMF) in order to remove the sodium salt. The resin was washed with DMF (3 × 3 ml) and the washings were passed through the ion-exchange resin as above. The column was then washed with DMF (10 ml). All filtrates and washings were combined and evaporated *in vacuo* at 30° to yield the product (21.4 mg; 84%; oil). Thin layer chromatography showed one spot: tlc-3, R_f 0.63; tlc-8, R_f 0.40. Amino acid analysis of the prolyl resin indicated 83% removal of proline from the resin. The nmr spectrum (A60) was identical with that of the protected proline ester obtained by transesterification with thallium alkoxide catalysis.

Cbz-L-alanyl-L-phenylalanine Benzyl Ester. Cbz-L-alanyl-L-phenylalanyl resin (2.60 g; 1 mmol of peptide) was suspended in dry benzyl alcohol (30 ml). A solution containing sodium benzoxide [0.1 mmol) in benzyl alcohol (0.29 ml) was added and the reaction mixture was stirred for 30 min at room temperature. The mixture was filtered and the resin was washed with ether (3 × 40 ml). The filtrate and washings were combined, washed with water (50 ml), dried ($MgSO_4$), and evaporated *in vacuo* (35°; 0.1 mm Hg) to give a solid residue (259 mg) which was recrystallized from chloroform–petroleum ether to yield benzyloxycarbonyl-L-alanyl-L-phenylalanine benzyl ester (240 mg; 52%), mp 119–120°. (*Anal.* Calcd for $C_{27}H_{28}N_2O_5$: C, 70.42; H, 6.13; N, 6.08. Found: C, 70.24; H, 6.12; N, 6.09.) The resin sample from the above transesterification reaction was retreated with benzyl alcohol (30 ml) plus sodium benzoxide (0.1 mmol). The reaction mixture was stirred for 4 hr at room temperature. The isolation procedure was repeated as described above to yield a further amount of the *product* (200 mg; 43%), mp 118–119°; *i.e.*, the total recovery of protected peptide ester was 95%; nmr (A 60, $CDCl_3$) τ 8.77 (d, 3 H, CH_3CH–, alanine), 6.98 (m, 2 H, $C_6H_5CH_2CH$–, phenylalanine), 5.76 (m, 1 H, $C_6H_5CH_2CH$–, phenylalanine), 4.99 and 4.96 (two s, 4 H, two $C_6H_5CH_2O$–, benzyl ester and Cbz group), 2.95 and 2.76 (m and s superimposed, 15 H, three C_6H_5–, benzyl ester, Cbz group, and phenylalanine).

Extent of Racemization during Transesterification of Boc-L-Leu-L-Ala-*O*-polymer with DMAE and Hydrolysis of the DMAE Ester. The protected dipeptide resin samples (100 mg) were rocked for 24 to 48 hr in a mixture of DMAE–DMF (1:1, 2 ml). The samples were then filtered, washed with DMF, and the filtrates evaporated *in vacuo* at 30°. The crude dipeptide ester samples were dissolved in DMF (0.5 ml), water was added (2 ml), and the mixture was stirred for 24 to 68 hr at room temperature. The reaction mixtures were then evaporated *in vacuo* and reevaporated with 1 *N* HCl in water. Quantitation of diastereoisomers was performed by the method of Manning and Moore[32] using a Beckman amino acid analyzer Model 120C (59 × 0.9 cm column packed with Beckman UR-30 resin; pH 4.30; flow rate 50 ml/hr; 57°). Peak positions of the diastereoisomers were determined using DL-Leu-DL-Ala (107 and 126 ml). The extent of racemization (per cent D–L isomer) was 0.3 ± 0.1%. The time of transesterification and hydrolysis had no observable effect on the extent of racemization.

Microwave Structural Study of Benzvalene (Tricyclo[3.1.0.0^{2,6}]hex-3-ene)

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Abstract: The microwave spectra of six isotopic species of benzvalene (tricyclo[3.1.0.0^{2,6}]hex-3-ene) have been observed and analyzed. These data and those reported previously have permitted the determination of a complete "substitution" molecular structure. The internuclear distances are found to be (in Å): C_1–C_6 = 1.452 ± 0.001, C_1–C_2 = 1.529 ± 0.003, C_2–C_3 = 1.503 ± 0.006, C_3–C_4 = 1.339 ± 0.001, C_1–H = 1.078 ± 0.001, C_2–H = 1.082 ± 0.002, C_3–H = 1.078 ± 0.001; and the dihedral angle of the four-membered ring is 106.0 ± 0.3°. Isotopic labeling studies have shown an interesting internal molecular rearrangement.

In a previous communication[1] we reported the microwave spectrum and dipole moment of the normal isotopic species of benzvalene (tricyclo[3.1.0.0^{2,6}]-hex-3-ene). These results plus those reported here for six monosubstituted isotopic species of benzvalene are sufficient for a complete structural determination. This structural study continues a series of investigations of polycyclic hydrocarbons in our laboratory,

(1) R. D. Suenram and M. D. Harmony, *J. Amer. Chem. Soc.*, 94, 5915 (1972).