

Bloor and Breen²⁷ have calculated wave functions for some of the heterocycles studied in the present research by the CNDO/2 method of Pople and Segal.²⁸ Rather good correlation was found between the ¹³C chemical shifts and the total charge densities, but not between the chemical shifts and π -electron densities in contrast to the results shown in Figure 10.

The nmr spin-spin coupling constants calculated by the extended Hückel treatment are best characterized as unsatisfactory. The geminal carbon-proton coupling constants in *all* the nitrogen heterocycles were calculated to fall between -4.9 and -6.6 Hz, although the experimental values are all positive and show considerable variation. The systematic trends evident in the data of Table I are not reflected in the calculated values. Also, the calculated vicinal carbon-proton coupling constants fall irregularly between +1.1 and +2.0 Hz.

The extended Hückel theory appears to generally predict one-bond, carbon-proton coupling constants which are considerably below the experimental values; however, trends in aliphatic systems are more-or-less faithfully reproduced.²⁹ The calculated values of the one-bond, carbon-proton coupling constants in nitrogen heterocycles were 99, 118, or 141 Hz depending on whether there were no, one, or two adjacent nitrogens.

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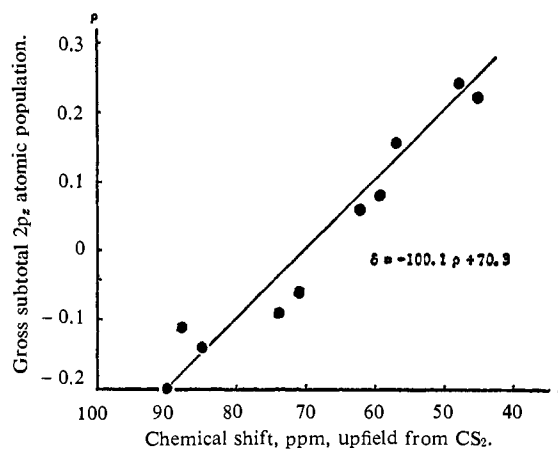


Figure 11. Correlation of calculated gross subtotal $2p_z$ atomic population with ¹³C chemical shifts for the five-membered nitrogen heterocycles.

The variation is in the right direction but the experimental trend toward higher values of J_{CH} with the accumulation of nitrogen in the molecules was not reproduced. The predicted proton-proton coupling constants are in equally poor agreement with the experimental results.

The extended Hückel theory does not seem to work well for these compounds as regards predicting of nmr spin-spin coupling constants.

Conformation of Small Peptides. II. Synthesis and Infrared Studies of Small Peptides¹

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Abstract: The synthesis of several protected peptides containing L-valine, L-alanine, L-isoleucine, L-leucine, and glycine are reported. The absorbance in the N-H stretching region of the infrared spectrum was determined as a function of concentration in deuteriochloroform. Some tetra-, penta-, and hexapeptides show intramolecular hydrogen bonds in this solvent. Evidence was found for the occurrence of the folded β conformation in the tetrapeptide BOC-L-alanyl-L-valyl-L-alanyl-L-valine methyl ester.

In recent years increased interest has arisen in the conformation or *short-range*⁶ properties of peptides. This work has been confined, in the greater part, to polymers of essentially infinite length. Since most proteins have relatively short helical segments, more information on equivalent peptides is required. It seems that caution should be exercised in attempting

to extrapolate observations of the behavior of high homopolymers to short segments of varied sequences. Goodman and coworkers⁷ studied the optical rotatory properties of oligopeptides of γ -methyl-L-glutamate and L-alanine and concluded that secondary structure first occurred in pentapeptides. They interpreted their results as the end-to-end hydrogen bonding of small helical segments at the pentapeptide with progressively greater helical behavior until this property maximized at the nonapeptide and longer chain lengths. However, as pointed out by Schellman,⁸ the conformation

(1) Part I has already appeared as a preliminary communication: J. E. Shields and S. T. McDowell, *J. Amer. Chem. Soc.*, **89**, 2499 (1967).

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(5) Case Summer Undergraduate Research Participant, 1966.

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would be expected to be dependent both on the solvent and the side-chain structure. Recently it was shown that intramolecular hydrogen bonding could be observed in a tetrapeptide with deuteriochloroform as solvent.¹

Due to the small side chains of L-alanine and glycine, it is possible that the dipeptide sequence -alanyl-glycyl- may be capable of forming a tight fold in a peptide backbone.⁹ Mizushima¹⁰ has reported observing intramolecular hydrogen bonding in acetylglycine N-methylamide in carbon tetrachloride solutions. Furthermore, poly-L-valine was reported to have a β -sheet structure.¹¹ Consequently, the structure -valylvalylalanyl-glycyl-valylvalyl- was selected as a possible sequence to give a folded peptide. Recently, Scheraga¹² reported calculations which yield the right-handed α helix as the most stable structure for poly-L-valine.

This paper reports the synthesis and infrared studies of the above hexapeptide in conjunction with several smaller peptides.

Results

Synthesis of Peptides. Cbz-L-alanine,^{13,14} Cbz-L-valine,¹⁵ BOC-L-alanine,^{13,16} BOC-glycine,¹⁶ BOC-L-valine,¹⁶ BOC-L-leucine,¹⁶ BOC-L-isoleucine,¹⁶ L-alanine methyl ester hydrochloride,¹⁷ L-valine *t*-butyl ester hydrochloride,¹⁸ L-valine methyl ester hydrochloride,¹⁹ L-leucine methyl ester hydrochloride,²⁰ and L-isoleucine methyl ester hydrochloride²¹ were prepared by standard literature procedures.

Cbz-L-alanyl glycine ethyl ester²² (I) was obtained in high yield (84%) by the coupling of Cbz-L-alanine and glycine ethyl ester hydrochloride by the dicyclohexylcarbodiimide method.²³ The dinitrophenyl active ester method used by Katsyannis and coworkers^{22b} to obtain compound I gave a yield of 72%. Treatment of compound I with hydrogen bromide in glacial acetic acid removed the Cbz protecting group and gave L-alanyl-glycine ethyl ester hydrobromide (II) which was coupled with BOC-L-valine using Woodward's Reagent K in the presence of triethylamine to give BOC-L-valyl-L-alanyl-glycine ethyl ester (III) in a yield of 34%.

Coupling of the hydrobromide II with carbobenzoxy-L-valine using the dicyclohexylcarbodiimide method²³

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gave Cbz-L-valyl-L-alanyl-glycine ethyl ester (IV) in 62% yield, mp 192-192.5°. The tripeptide IV was converted to the hydrobromide V with hydrogen bromide in glacial acetic acid and the hydrobromide V coupled with BOC-L-valine to give BOC-L-valyl-L-valyl-L-alanyl-glycine ethyl ester (VI) in 78% yield.

Attempts to prepare Cbz-L-valyl-L-valine *t*-butyl ester (VII) in reasonable yield by the coupling of Cbz-L-valine and valine *t*-butyl ester hydrochloride using Woodward's Reagent K failed. However, the dipeptide was obtained in high yield by the mixed carbonic anhydride method.²⁴ Hydrogenation of the protected peptide VII in methanol over palladium oxide and treatment of the product with hydrogen chloride gave L-valyl-L-valine *t*-butyl ester hydrochloride (VIII), mp 175-176°, which was coupled with BOC-L-valyl-L-valyl-L-alanyl-glycine (IX), obtained from the alkaline hydrolysis of the corresponding ethyl ester VI, using Woodward's Reagent K to give BOC-L-valyl-L-valyl-L-alanyl-glycyl-L-valyl-L-valine *t*-butyl ester (X) in 66% yield.

Cbz-L-valyl-L-alanine methyl ester (XI) was synthesized by the mixed carbonic anhydride method²⁴ in yields of 80%, mp 165-167°. Hydrogenation of the dipeptide XI over palladium oxide and coupling of the product with BOC-L-alanine by the mixed carbonic anhydride method²⁴ gave BOC-L-alanyl-L-valyl-L-alanine methyl ester (XII) in 83% yield. Removal of the N-terminal protecting group in peptide XII with 1 *N* HCl in glacial acetic acid and coupling of the product with BOC-L-valine gave BOC-L-valyl-L-alanyl-L-valyl-L-alanine methyl ester (XIII) in a yield of 61%. BOC-L-alanyl-L-valine methyl ester (XIV), BOC-L-valyl-L-alanyl-L-valine methyl ester (XV), and BOC-L-alanyl-L-valyl-L-alanyl-L-valine methyl ester (XVI) were prepared in a similar manner.

BOC-L-alanyl-L-alanine methyl ester (XVII) was obtained in yields of 65-82% by the coupling of BOC-L-alanine and L-alanine methyl ester hydrochloride by the mixed carbonic anhydride method.²⁴ Treatment of the dipeptide XVII with trifluoroacetic acid gave the trifluoroacetate of L-alanyl-L-alanine methyl ester which on coupling with BOC-L-alanine by the mixed carbonic anhydride method²⁴ gave BOC-L-alanyl-L-alanyl-L-alanine methyl ester (XVIII). Similar treatment of the tripeptide XVIII gave BOC-L-alanyl-L-alanyl-L-alanyl-L-alanine methyl ester (XIX).

Cbz-L-valyl-L-valine methyl ester (XX), mp 116-119°, was obtained in yields of 69-74% by coupling carbobenzoxy-L-valine with L-valine methyl ester hydrochloride by the mixed carbonic anhydride method.²⁴ Treatment of the dipeptide XX with hydrogen bromide in acetic acid followed by coupling with Cbz-L-valine gave Cbz-L-valyl-L-valyl-L-valine methyl ester (XXI), mp 218°. The Cbz group of the tripeptide XXI was removed by hydrogenation over palladium oxide in methanol and the product coupled with BOC-L-valine to give BOC-L-valyl-L-valyl-L-valyl-L-valine methyl ester (XXII), mp 247-248° dec, in 76% yield. Treatment of the tetrapeptide XXII with trifluoroacetic acid and coupling of the product with BOC-L-valine gave BOC-L-valyl-L-valyl-L-valyl-L-valyl-L-valine methyl ester (XXIII), mp >300°, in 65% yield.

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BOC-L-leucyl-L-leucine methyl ester (XXIV) was prepared in 89% yield by coupling BOC-L-leucine and L-leucine methyl ester hydrochloride by the mixed carbonic anhydride method. Treatment of peptide XXIV with trifluoroacetic acid followed by coupling with BOC-L-leucine by the mixed carbonic anhydride method gave BOC-L-leucyl-L-leucyl-L-leucine methyl ester (XXV) in 68% yield. Similar treatment of the tripeptide XXV gave BOC-L-leucyl-L-leucyl-L-leucyl-L-leucine methyl ester (XXVI) in 57% yield.

BOC-L-isoleucyl-L-isoleucine methyl ester (XXVII), BOC-L-isoleucyl-L-isoleucyl-L-isoleucine methyl ester (XXVIII), and BOC-L-isoleucyl-L-isoleucyl-L-isoleucyl-L-isoleucine methyl ester (XXIX) were prepared in a manner similar to that used for the corresponding L-leucine derivatives.

Physical Measurements. The molecular weight of the tetrapeptide VI was measured in chloroform at three concentrations using a vapor pressure osmometer. The molecular weights found are listed in Table I.

Table I. Molecular Weight Determinations of the Tetrapeptide VI in Chloroform

Concn, M	Apparent mol wt
0.0670	804
0.0392	689
0.0196	594
Monomer mol wt	472.5

A detailed study of the infrared absorption of many peptides in deuteriochloroform solutions was made. The dilution technique of Mizushima¹⁰ was employed as a method of observing N-H stretch absorption due to intramolecular hydrogen bonding. The technique employs the fact that intermolecular hydrogen bonding is concentration dependent whereas intramolecular hydrogen bonding is concentration independent. In this work the authors have used the ratio of the absorbance of hydrogen-bonded N-H (A_H) to that of free N-H (A_F) as a measure of hydrogen bonding in a molecule. In a theoretical case this ratio should decrease as concentration is decreased until it ultimately reaches zero in a case where no intramolecular hydrogen bonding occurs. It has been found that a value of 0.1-0.2 is indicative of no intramolecular hydrogen bonding as illustrated by the data on di- and tripeptides which are unable to form these bonds (Table II). On the other

Table II. Infrared Results on Di- and Tripeptides

Peptide	Concn, M	Frequency		A_H/A_F
		of free N-H, cm^{-1}	of H-bonded N-H, cm^{-1}	
I	1.622×10^{-1}	3430	3340	0.37
	1.622×10^{-2}	3430	3340	0.093
	1.622×10^{-3}	3430	3340	0.046
XI	1.37×10^{-2}	3430	3330	0.34
	1.37×10^{-3}	3430	3330	0.11
III	5.35×10^{-3}	3430	3330	0.45
	5.35×10^{-4}	3430	3330	0.19
	5.35×10^{-5}	3430	3330	0.12
XVIII	1.41×10^{-2}	3430	3332	0.77
	1.41×10^{-3}	3430	3332	0.30
	1.41×10^{-4}	3430	3332	0.24

hand, values of A_H/A_F above 0.4 at the lowest concentrations indicate stable intramolecular hydrogen bonding.

Normally tenfold dilutions were made until the ratio A_H/A_F appeared fairly constant, and in order to obtain reasonable spectra the path length of the solution was increased a corresponding amount.

Table II shows the ratio of the absorbances of hydrogen-bonded to free N-H stretch at various concentrations of di- and tripeptides and in all cases A_H/A_F reaches a value less than 0.2 except for the tripeptide XVIII which gave a value of 0.24. Table III gives

Table III. Infrared Results of Tetra- and Higher Peptides

Peptide	Concn, M	Frequency		A_H/A_F
		of free N-H, cm^{-1}	of H-bonded N-H, cm^{-1}	
VI	1.45×10^{-2}	3427	3300	3.36
	1.45×10^{-3}	3432	3329	1.08
	1.45×10^{-4}	3432	3335	0.83
	1.45×10^{-5}	3434	3335	0.74
XIX	6×10^{-5}	3427	3342	0.63
	3×10^{-5}	3428	3345	0.69
XXVI	1.33×10^{-2}	3430	3345	0.73
	0.795×10^{-3}	3430	3340	0.67
	0.795×10^{-4}	3432	3340	0.64
XVI	1.36×10^{-2}	3431	3334	0.79
	1.36×10^{-3}	3431	3336	0.65
	1.36×10^{-4}	3433	3340	0.48
	0.34×10^{-4}	3435	3338	0.48
XXII	9.23×10^{-3}	3428	3315	1.24
	9.23×10^{-4}	3430	3335	0.32
	9.23×10^{-5}	3432	3335	0.1
XXIX	1.09×10^{-3}	3440	3345	0.28
	1.09×10^{-4}	3435	3345	0.15
XIII	5.23×10^{-3}	3427	3325	0.54
	5.23×10^{-4}	3427	3325	0.17
XXIII	2.55×10^{-3}	3425	3290	3.64
	2.55×10^{-4}	3425	3310	1.10
	5.0×10^{-5}	3430	3330	0.61
X	2.55×10^{-5}	3430	3330	0.59
	3×10^{-4}	3431	3330	1.56
	3×10^{-5}	3430	3331	1.82

corresponding data for tetra-, penta-, and hexapeptides. Taking a minimum limiting value of A_H/A_F of 0.4 as indicating intramolecular hydrogen bonding, it can be seen that several tetrapeptides and the penta- and hexapeptides show intramolecular hydrogen bonding.

Discussion

The use of the concentration dependence of the ratio of hydrogen-bonded N-H absorption to nonhydrogen-bonded absorption (A_H/A_F) appears to serve as a useful tool in illustrating the presence of intramolecular hydrogen bonding within a molecule.¹⁰ At high concentrations the value of A_H/A_F will in almost all cases be high due to the presence of intermolecular hydrogen bonding, but as intermolecular interactions are decreased and finally reduced to negligible amount by dilution, the ratio will reflect the situation on the molecular scale.

As expected, neither di- nor tripeptides show any stable intramolecular hydrogen bonding, as indicated by the low value of A_H/A_F reached at low concentrations

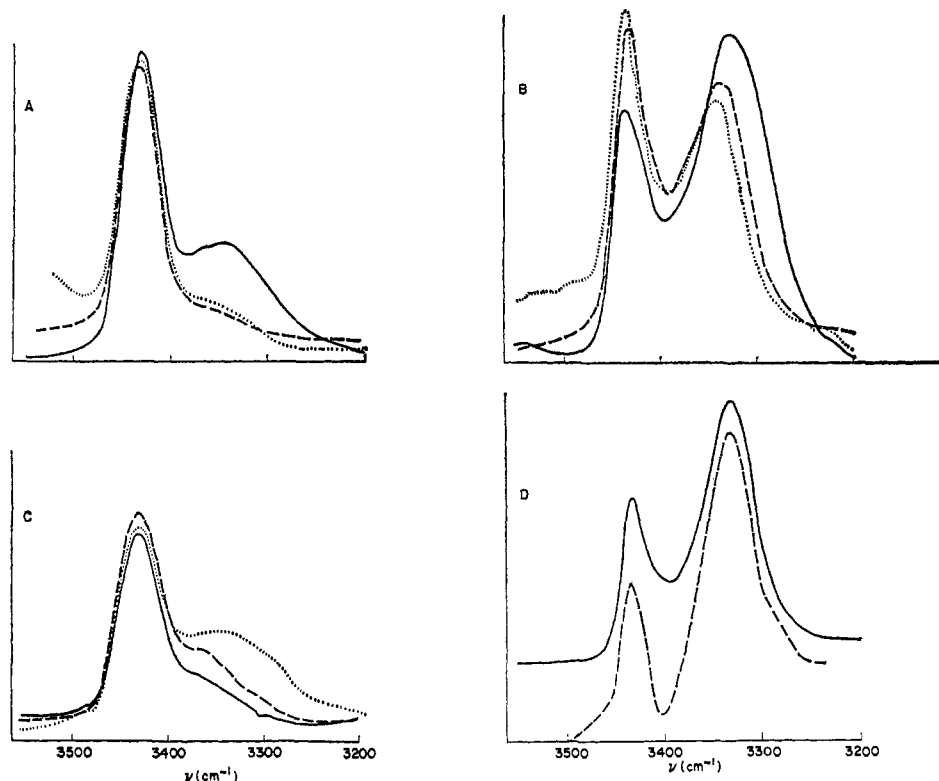


Figure 1. (A) Cbz-Ala-Gly-OEt: —, 1.622×10^{-1} ; ---, 1.622×10^{-2} ; ···, 1.622×10^{-3} . (B) *t*-BOC-Val-Val-Ala-Gly-OEt: —, 1.45×10^{-2} ; ---, 1.45×10^{-3} ; ···, 1.45×10^{-4} . (C) *t*-BOC-Val-Ala-Gly-OEt: ···, 5.35×10^{-3} ; ---, 5.35×10^{-4} ; —, 5.35×10^{-5} . (D) *t*-BOC-Val-Val-Ala-Gly-Val-Val-OBu: —, 3×10^{-4} ; ---, 3×10^{-5} . Concentrations in moles/liter.

(Table II). Typical spectra of di- and tripeptides having no intramolecular structure are shown in Figure 1A and 1C. The hydrogen-bonded N-H absorption at 3340 and 3330 cm^{-1} , respectively, is seen to fall very drastically on dilution.

As reported earlier,¹ stable secondary structure has been indicated for the tetrapeptide VI, which gave a limiting value of *ca.* 0.7 for $A_{\text{H}}/A_{\text{F}}$ (Table III, Figure 1B). The fact that the N-H hydrogen-bonded absorption observed at these low concentrations could not be attributed to intermolecular interactions was substantiated by molecular weight determinations in chloroform which indicated that at concentrations as high as $1.96 \times 10^{-2} M$ only a small amount of association occurred (Table I). It seems reasonable to assume that associated species comprise a negligible fraction of the total peptide at the concentrations of 10^{-4} to $10^{-5} M$ which we used in our study.

The tetra-L-alanine (XIX) and tetra-L-leucine (XXVI) derivatives and the peptide BOC-L-alanyl-L-valyl-L-alanyl-L-valine methyl ester all show intramolecular hydrogen bonding as indicated by values of 0.69, 0.64, and 0.48, respectively, for $A_{\text{H}}/A_{\text{F}}$ at concentrations in the region of $5 \times 10^{-5} M$. However, it is not possible to generalize and assume that all tetrapeptides show intramolecular hydrogen bonding since the tetra-L-valine (XXII) (Figure 2B) and tetra-L-isoleucine (XXIX) derivatives and BOC-L-valyl-L-alanyl-L-valyl-L-alanine give ratios of 0.1, 0.15, and 0.17 for $A_{\text{H}}/A_{\text{F}}$, respectively, at a concentration of *ca.* $10^{-4} M$. Thus there is a structural dependence on whether a tetrapeptide can form stable intramolecular hydrogen bonds. It appears that multiple substitution on the β carbons of the amino

acids hinders the ability of the peptide to fold or form any type of helix.

Although one usually speaks of helices⁶ when referring to conformation of small peptides, the folded β form^{1,25,26} is strongly indicated by the observation that peptide XVI forms intramolecular hydrogen bonds while XIII does not. The two peptides do not exhibit drastic differences when molecular models of each in helical forms are examined. Only in the folded β conformation does a difference appear. When protected tetrapeptides are put into this conformation, the side chains of residues 1 and 3 are axial to the plane of the two intramolecular hydrogen bonds, and side chains 2 and 4 are equatorial. It would appear that peptide XIII, with valine residues at 1 and 3, cannot assume the folded β form, while XVI, with valine at 2 and 4, can. This is consistent with the observations of intramolecular hydrogen bonding in peptide VI, which has valines in positions 1 and 2, and its absence in the tetravaline derivative XXII. Thus, bulky substituents or multiple substitution at the β carbons of residues in positions 1 and 3 result in disruption of folded β conformations in tetrapeptides.

Intramolecular hydrogen bonding was observed in

(25) R. Schwyzer, *Ciba Found. Symp. Amino Acids Peptides Antimetab. Activity*, 1958, 171 (1958).

(26) Although one can construct several variations of the folded β structure (Figure 1 of ref 1) with Dreiding models, we refer in all cases to that in which the side chain of residue 3 is axial to the plane of the α -carbon atoms. This can be written in the conventional notation [J. T. Edsall, P. J. Flory, J. C. Kendrew, A. M. Liquori, G. Némethy, G. N. Ramachandran, and H. A. Scheraga, *J. Biol. Chem.*, **241**, 1004 (1966)] as $\phi_1 = 30^\circ$, $\psi_1 = 330^\circ$, $\phi_2 = 150^\circ$, $\psi_2 = 270^\circ$, $\phi_3 = 330^\circ$, $\psi_3 = 150^\circ$, $\phi_4 = 330^\circ$, and $\psi_4 = 30^\circ$ in a rough approximation from Table III of Edsall, *et al.*

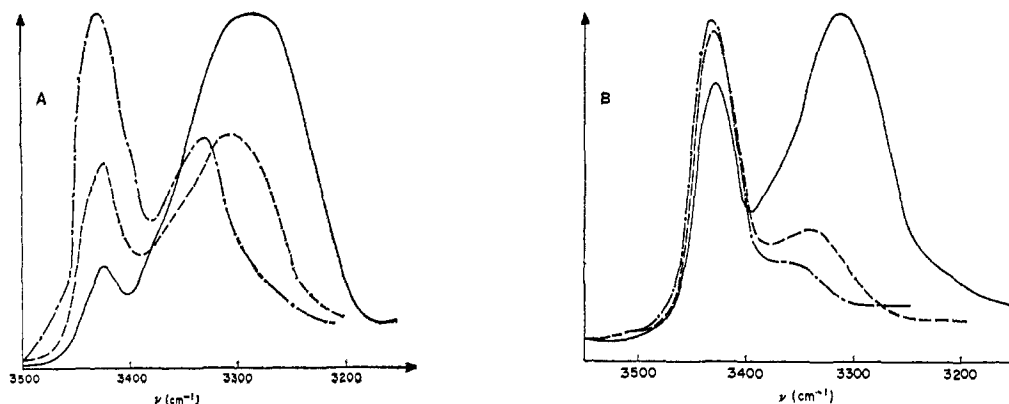


Figure 2. (A) *t*-BOC-Val₅OMe: —, 2.55×10^{-3} ; ---, 2.55×10^{-4} ; -·-, 5.1×10^{-5} . (B) *t*-BOC-Val₄OMe: —, 9.23×10^{-3} ; ---, 9.23×10^{-4} ; -·-, 9.23×10^{-5} . Concentrations in moles/liter.

the pentapeptide XXIII (Figure 2A), a value of 0.59 being obtained for A_H/A_F at a concentration of 2.55×10^{-5} M. A folded or an α -helical structure for the pentapeptide XXIII would permit formation of two intramolecular hydrogen bonds, whereas a 3_{10} helix would permit three of these bonds. If this compound does not exist as an equilibrium mixture of the helical and the open-chain forms, the results could best be interpreted as resulting from a structure containing two hydrogen bonds.

The hexapeptide X derived from the tetrapeptide VI undoubtedly exists in a stable conformation in deuteriochloroform as exhibited by the high value of A_H/A_F (ca. 1.7) (Figure 1D). In this case the folded β -sheet and α -helix structures require formation of three intramolecular hydrogen bonds and the 3_{10} helix four such bonds. It appears that the hexapeptide X contains at least three hydrogen bonds in order to account for the value of 1.7 for A_H/A_F .

Using the concentration-independent hydrogen-bonded N-H absorption band at 3300–3350 cm^{-1} as evidence of intramolecular hydrogen bonding, and by inference as evidence of some form of stable conformation, we have investigated a series of peptide derivatives in deuteriochloroform solutions. These ranged in size from dipeptides to a pentapeptide and a hexapeptide. None of the dipeptides or tripeptides showed hydrogen bonding at low concentrations, and so may be presumed to lack stable conformations which involve hydrogen bonding. Tetrapeptides form stable intramolecular hydrogen bonds except when side-chain steric effects prevent this. In one case, it appears that the alternatives are the folded β form or a non-hydrogen-bonded conformation. Other tetrapeptides behave in ways consistent with this suggestion. Finally, the two cases of higher peptides examined both show intramolecular hydrogen bonding.

Experimental Section

Infrared measurements were made using a Beckman Model IR7 infrared spectrophotometer. "Pyrosil" cells, Type S-22-350, were used throughout the work. Deuteriochloroform was purified by washing in rapid succession with water, concentrated sulfuric acid, 1 N aqueous sodium hydroxide, and water (six times), drying over anhydrous calcium sulfate, and distilling in the dark. The solvent was stored under nitrogen in the dark. Deuteriochloroform solutions of the peptides were left in cells fitted with drying tubes filled with phosphorus pentoxide until no water could be detected in the infrared when air was used as reference (usually overnight).

The final spectra were obtained using deuteriochloroform, which had been treated in a similar manner, as reference.

Molecular weight measurements were made in spectroquality chloroform using a Mechrolab vapor pressure osmometer, Model 301A. The instrument was calibrated using biphenyl.

L-Valine, L-alanine, L-leucine, L-isoleucine, glycine, and glycine ethyl ester hydrochloride were obtained from Mann Research Laboratories, Inc. Woodward's Reagent K was obtained from Pilot Chemicals.

Tetrahydrofuran was purified by distillation from calcium hydride. Acetonitrile was purified by distillation from phosphorus pentoxide. Dichloromethane was purified by washing with saturated aqueous sodium hydrogen carbonate and drying (CaCl_2) followed by distillation. Organic extracts were dried over anhydrous magnesium sulfate. All melting points were determined in capillaries and are uncorrected.

Cbz-L-alanylglycine Ethyl Ester (I). Cbz-L-alanine (9.36 g, 42 mmol) and glycine ethyl ester hydrochloride (7.1 g, 50 mmol) were suspended in dichloromethane (250 ml) and cooled to -5° . The mixture was treated with dicyclohexylcarbodiimide (10.5 g, 50 mmol) in dichloromethane (30 ml) and triethylamine (5.05 g, 50 mmol) and stirred magnetically for 1 hr at -5° and 48 hr at 4° . Filtration and evaporation of the solvent gave a solid residue which was dissolved in ethyl acetate, washed successively with 5% aqueous citric acid (100 ml), water (100 ml), aqueous potassium hydrogen carbonate (100 ml), and water, and dried. The solvent was evaporated under vacuum and the residue was crystallized from ethyl acetate-hexane, yielding 10.94 g (84.5%), mp $100\text{--}103^\circ$ (lit.^{2b} mp $98\text{--}99^\circ$), $[\alpha]^{25}_D -28^\circ$ (c 2.0, methanol).

Anal. Calcd for $\text{C}_{13}\text{H}_{20}\text{N}_2\text{O}_5$ (308.3): C, 58.43; H, 6.54; N, 9.08. Found: C, 58.46; H, 6.52; N, 9.31.

BOC-L-valyl-L-alanylglycine Ethyl Ester (III). BOC-L-valine (2.18 g, 10 mmol) and Woodward's Reagent K (2.53 g, 10 mmol) were suspended in nitromethane (75 ml), and triethylamine (1.01 g, 10 mmol) was added. The solution became homogeneous after 25 min when L-alanylglycine ethyl ester hydrobromide (from treatment of compound I with 20% hydrogen bromide in glacial acetic acid) (2.54 g, 10 mmol) in nitromethane (10 ml) and triethylamine (1.01 g, 10 mmol) were added and the reaction mixture was stirred overnight.

The solution was evaporated to dryness and the residue taken up in an ethyl acetate-water mixture. The organic layer was washed successively with saturated aqueous potassium chloride, 5% aqueous citric acid, water, 2 N aqueous potassium bicarbonate, and water. The solution was dried with anhydrous magnesium sulfate, filtered, and evaporated under vacuum. The residue was crystallized from ethanol, yielding 1.28 g (34%), mp $147\text{--}150^\circ$, $[\alpha]^{25}_D -33^\circ$ (c 2.0, methanol).

Anal. Calcd for $\text{C}_{17}\text{H}_{21}\text{N}_3\text{O}_6$ (373.4): C, 54.67; H, 8.37; N, 11.26. Found: C, 54.75; H, 8.71; N, 11.26.

Cbz-L-valyl-L-alanylglycine Ethyl Ester (IV). A stirred mixture of Cbz-L-valine (5.52 g, 22 mol) and L-alanylglycine ethyl ester hydrobromide (21.9 mmol) in acetonitrile (75 ml) was cooled to -5° and treated with dicyclohexylcarbodiimide (5.15 g, 25 mmol) and triethylamine (2.22 g, 22 mmol) in acetonitrile (50 ml). Stirring was continued at 4° for several days. The reaction mixture was filtered, the solvent removed, and the residue extracted thoroughly with chloroform. The chloroform extract was washed successively

with 1 *N* hydrochloric acid (two 100-ml portions) and water (100 ml) and dried. The solvent was evaporated under vacuum and the residue dissolved in dimethylformamide and allowed to stand for 2 hr. The solution was filtered and the product precipitated with water, isolated by filtration, and dried. Recrystallization from ethanol-ethyl acetate mixtures yielded 5.54 g (62%), mp 192–192.5°, $[\alpha]^{25}_D - 5^\circ$ (*c* 2.0, dimethylformamide).

Anal. Calcd for $C_{20}H_{23}N_3O_6$ (407.5): C, 58.95; H, 7.17; N, 10.31. Found: C, 59.31; H, 7.17; N, 10.23.

L-Valyl-L-alanylglycine Ethyl Ester Hydrobromide (V). Cbz-L-valyl-L-alanylglycine ethyl ester (1.92 g, 4.7 mmol) was treated with 20% hydrogen bromide in acetic acid for 40 min at room temperature. The hydrobromide V, yielding 1.29 g (77%), was precipitated by addition of ether and was recrystallized from ethanol-ether, mp 181.5–182°, $[\alpha]^{25}_D - 33^\circ$ (*c* 2.0 water).

Anal. Calcd for $C_{19}H_{24}BrN_3O_4$ (354): C, 40.69; H, 6.83; N, 11.62; Br, 22.56. Found: C, 40.5; H, 6.8; N, 11.75; Br, 22.7.

BOC-L-valyl-L-valyl-L-alanylglycine Ethyl Ester (VI). L-Valyl-L-alanylglycine ethyl ester hydrobromide (3.54 g, 10 mmol) and BOC-L-valine (2.39 g, 11 mol) were suspended in dichloromethane (200 ml), treated with triethylamine (1.01 g, 10 mmol), and cooled in an ice-ethanol bath. Dicyclohexylcarbodiimide (2.57 g, 12.5 mmol) in dichloromethane (20 ml) was added and the reaction mixture stirred overnight at 0°.

The residue, after filtration and evaporation of the filtrate to dryness, was dissolved in chloroform, washed successively with 5% aqueous citric acid (two 100-ml portions), water, 2 *N* aqueous potassium hydrogen carbonate, and water, and dried. Evaporation of the solvent gave a white solid which was precipitated from hot dimethylformamide by water, air dried, and recrystallized from ethyl acetate to give 3.88 g (78%), mp 204.5–206.5°, resolidified and melted at 212–213°, $[\alpha]^{25}_D - 77^\circ$ (*c* 2.0, methanol).

Anal. Calcd for $C_{22}H_{30}N_4O_7$ (472.5): C, 55.91; H, 8.53; N, 11.86. Found: C, 55.94; H, 8.51; N, 11.75.

Cbz-L-valyl-L-valine *t*-Butyl Ester (VII). a. *Via Woodward's Reagent K.* Woodward's Reagent K (7.60 g, 30 mmol) was added to a stirred solution of Cbz-L-valine (7.54 g, 30 mmol) and triethylamine (3.03 g, 30 mmol) in acetonitrile (150 ml) at 0°. After *ca.* 3.5 hr, most of the Woodward's Reagent K had dissolved and L-valine *t*-butyl ester hydrochloride (6.29 g, 30 mmol) in acetonitrile (30 ml) was added followed by triethylamine (3.03 g, 30 mmol). The mixture was stirred overnight at 0°.

Filtration and evaporation of the filtrate to dryness gave a residue which was dissolved in ethyl acetate, washed with 5% aqueous citric acid, saturated aqueous potassium chloride, 2 *N* aqueous potassium hydrogen carbonate, and saturated aqueous potassium chloride, and dried. The ethyl acetate solution was cooled to 0° and some insoluble material removed by filtration. Evaporation of the filtrate to dryness and crystallization of the residue from pentane gave a total yield of 4.36 g (36%) of product, the first crop melting at 72–75°.

b. *Via Mixed Carbonic Anhydride.* Triethylamine (5.56 g, 55 mmol) and isobutyl chloroformate (6.55 ml, 50 mmol) were added to a magnetically stirred cooled (–10°) solution of Cbz-L-valine (13.8 g, 55 mol) in purified tetrahydrofuran (300 ml). The solution was stirred below –5° for 1 hr when L-valine *t*-butyl ester hydrochloride (10.5 g, 50 mmol) and triethylamine (5.05 g, 50 mmol) were added. Stirring was continued overnight at 0°.

Filtration and evaporation of the solvent gave a colorless oil which was dissolved in ethyl acetate, washed successively with 5% aqueous citric acid (two 100-ml portions), water (two 100-ml portions), 2 *N* aqueous potassium hydrogen carbonate (two 100-ml portions), water (two 100-ml portions), and saturated aqueous potassium chloride (100 ml), and dried. The oil obtained on evaporation of the solvent was crystallized from pentane, yielding 14.7 g (72%) of product, mp 67–70°, $[\alpha]^{25}_D - 40^\circ$ (*c* 2.0, methanol).

Anal. Calcd for $C_{22}H_{34}N_2O_5$ (406.52): C, 65.01; H, 8.43; N, 6.89. Found: C, 64.97; H, 8.50; N, 7.03.

L-Valyl-L-valine *t*-Butyl Ester Hydrochloride (VIII). Cbz-L-valyl-L-valine *t*-butyl ester (8.13 g, 20 mmol) and palladium oxide (1.0 g) in methanol (100 ml) were treated with hydrogen until the catalyst coagulated. Filtration and evaporation of the solvent gave a colorless oil which, on treatment with methanol containing hydrogen chloride (20 mmol) and evaporation of the methanol, gave 5.9 g (96%), mp 167–169°, raised to 175–176° from ethanol-ether. Recrystallization is rendered difficult by the hydroscopic nature of the product and a sample from another run, mp 166–169°, was submitted for analysis, $[\alpha]^{25}_D - 25.1^\circ$ (*c* 2.0, water).

Anal. Calcd for $C_{14}H_{20}N_2O_3Cl$ (308.89): C, 54.44; H, 9.46; N, 9.07; Cl, 11.48. Found: C, 54.27; H, 9.58; N, 9.31; Cl, 11.40.

BOC-L-valyl-L-valyl-L-alanylglycine (IX). BOC-L-valyl-L-valyl-L-alanylglycine ethyl ester (6.4 g, 13.6 mmol) in methanol (50 ml) was treated with 1 *N* aqueous sodium hydroxide (14.5 ml, 14.5 mmol) at room temperature for 30 min. The reaction mixture was diluted with water (600 ml), filtered, and extracted with ether and the ether discarded. The aqueous solution was acidified to pH 3 with solid citric acid and extracted with ethyl acetate (three 150-ml portions); the combined extracts were washed with 5% aqueous citric acid and dried. Evaporation of the solvent gave a gel which, on dissolving in dimethylformamide and evaporation to dryness, gave a white solid which, on dissolving in dimethylformamide and reprecipitation by water, gave 3.7 g (57%), which decomposed slowly above 163°. Repeated precipitation from dimethylformamide by water gave the product which decomposed slowly above 219° and was pure by thin layer chromatography on silica gel, $[\alpha]^{25}_D - 10^\circ$ (*c* 1.0, dimethylformamide).

Anal. Calcd for $C_{20}H_{26}N_4O_7$ (444.5): C, 54.04; H, 8.16; N, 12.60. Found: C, 53.84; H, 8.23; N, 12.53.

BOC-L-valyl-L-valyl-L-alanylglycyl-L-valyl-L-valyl *t*-Butyl Ester (X). A solution containing the tetrapeptide VIII (0.222 g, 0.5 mmol) and triethylamine (0.05 g, 0.5 mmol) in 15 ml of a 1:2 mixture of acetonitrile and dimethylformamide at 0° was added dropwise over a period of 2 hr to a suspension of Woodward's Reagent K (0.127 g, 0.5 mmol) in acetonitrile (2 ml) at 0°. The solution became homogeneous after 1 hr and stirring was continued for a further hour; then L-valyl-L-valine *t*-butyl ester hydrochloride (0.154 g, 0.5 mmol) and triethylamine (0.05 g, 0.5 mmol) were added; stirring was continued overnight at 0°. The reaction mixture was diluted with water (10 ml) and the product isolated by filtration, washed with water, aqueous potassium hydrogen carbonate, and water, and dried under vacuum to give 0.23 g (66%), mp 230°, raised to 234–235° from ethanol, $[\alpha]^{25}_D - 43^\circ$ (*c* 0.466, methanol).

Anal. Calcd for $C_{34}H_{52}N_8O_8$ (698.88): C, 58.41; H, 8.94; N, 12.02. Found: C, 58.35; H, 8.99; N, 12.08.

Cbz-L-valyl-L-alanine methyl ester (XI) was prepared in 80% yield, mp 165.5–167° (from aqueous ethanol), from Cbz-L-valine and L-alanine methyl ester hydrochloride in a manner similar to that used for the preparation of the peptide XI, $[\alpha]^{25}_D - 49.2^\circ$ (*c* 2.0, methanol).

Anal. Calcd for $C_{17}H_{24}N_2O_5$ (336.39): C, 60.70; H, 7.19; N, 8.33. Found: C, 60.79; H, 7.19; N, 8.42.

BOC-L-alanyl-L-valyl-L-alanine Methyl Ester (XII). Cbz-L-valyl-L-alanine methyl ester (11.02 g, 32.8 mmol) in methanol (250 ml) was hydrogenated over palladium oxide (1.4 g) as catalyst. The mixture was filtered and the filtrate treated with methanolic hydrochloric acid (32.8 mmol) and evaporated to dryness. The white solid residue was dissolved in tetrahydrofuran and cooled to –20°. The cooled solution was added to a solution of BOC-L-alanine (6.3 g, 33.3 mmol) in tetrahydrofuran (250 ml) which had been allowed to react with isobutyl chloroformate (4.29 ml, 32.8 mmol) and triethylamine (3.36 g, 33.3 mmol) for 45 min at –25°. This was followed by addition of triethylamine (3.34 g, 32.8 mmol) and stirring at –20° for 1 hr and then at 4° overnight.

The reaction mixture was filtered and the filtrate evaporated to dryness. The residue was dissolved in ethyl acetate and washed successively with 5% aqueous citric acid, water, 2 *N* aqueous potassium hydrogen carbonate, and water, and dried. Evaporation of the solvent gave a white powder which was recrystallized from chloroform-ethyl acetate to give 10.19 g (83%), mp 179–180°, $[\alpha]^{25}_D - 14.2^\circ$ (*c* 0.31, methanol).

Anal. Calcd for $C_{17}H_{24}N_2O_5$ (373.44): C, 54.68; H, 8.37; N, 11.25. Found: C, 54.88; H, 8.54; N, 11.16.

BOC-L-valyl-L-alanyl-L-valyl-L-alanine Methyl Ester (XIII). BOC-L-valine (2.17 g, 10 mmol) was dissolved in tetrahydrofuran (100 ml) and cooled to –30° when triethylamine (1.01 g, 10 mmol) and isobutyl chloroformate (1.20 ml, 9.2 mmol) was added. The reaction mixture was stirred at –30° for 40 min when L-alanyl-L-valyl-L-alanine methyl ester hydrochloride (from treatment of peptide IX with 1 *N* HCl in glacial acetic acid) and triethylamine (0.93 ml, 9.2 mmol) were added. The mixture was stirred for 2 hr at –25° and then at 4° overnight. A similar work-up to that used for the tripeptide XII gave 2.65 g (61%) of product, mp 249–250° (from aqueous ethanol), $[\alpha]^{25}_D - 21.2^\circ$ (*c* 0.144, methanol).

Anal. Calcd for $C_{22}H_{30}N_4O_7$ (472.57): C, 55.92; H, 8.53; N, 11.86. Found: C, 56.19; H, 8.59; N, 11.83.

BOC-L-alanyl-L-valine Methyl Ester (XIV). BOC-L-alanine (7.78 g, 41.1 mmol) was dissolved in tetrahydrofuran (250 ml) and cooled to –40°, when triethylamine (4.15 g, 41.1 mmol) and isobutyl chloroformate (5.25 ml, 40 mmol) were added. The mixture was stirred at –25° for 45 min when L-valine methyl ester hydro-

chloride (6.70 g, 40 mmol) and triethylamine (4.04 g, 40 mmol) were added. The mixture was stirred at -20° for 1 hr and then at 4° overnight.

The reaction mixture was filtered and the filtrate evaporated to dryness. The residue was dissolved in ethyl acetate, washed successively with 5% aqueous citric acid, water, 2 *M* aqueous potassium hydrogen carbonate, and water, and dried. Evaporation of the solvent gave 11.2 g of product which on recrystallization from ethyl acetate-hexane gave 9.7 g (80%), mp $87-89^{\circ}$ (hygroscopic), $[\alpha]^{25D} -22.5^{\circ}$ (*c* 0.35, methanol).

Anal. Calcd for $C_{14}H_{26}N_2O_5$ (302.36): C, 55.61; H, 8.67; N, 9.26. Found: C, 55.76; H, 8.69; N, 9.25.

BOC-L-valyl-L-alanyl-L-valine methyl ester (XV) was prepared in 67% yield, mp $159.5-161^{\circ}$ (aqueous ethanol), $[\alpha]^{25D} -13.9^{\circ}$ (*c* 0.223, methanol), by coupling BOC-L-valine and L-alanyl-L-valine methyl ester hydrochloride (from treatment of peptide XIV with 1 *N* HCl in glacial acetic acid) in a manner similar to that used for the dipeptide XIV.

Anal. Calcd for $C_{15}H_{33}N_3O_6$ (401.49): C, 56.84; H, 8.78; N, 10.47. Found: C, 56.82; H, 8.80; N, 10.62.

BOC-L-alanyl-L-valyl-L-alanyl-L-valine methyl ester (XVI) was prepared in 69% yield, mp $228.5-229.5^{\circ}$ dec (from ethanol), $[\alpha]^{25D} -15.9^{\circ}$ (*c* 0.211, methanol), by coupling of BOC-L-alanine and L-valyl-L-alanyl-L-valine methyl ester hydrochloride (from treatment of peptide XV with 1 *N* HCl in glacial acetic acid) in a manner similar to that used for peptide XIV.

Anal. Calcd for $C_{22}H_{40}N_4O_7$ (472.57): C, 55.92; H, 8.53; N, 11.86. Found: C, 55.95; H, 8.65; N, 11.89.

BOC-L-alanyl-L-alanine Methyl Ester (XVII). Isobutyl chloroformate (5.95 ml, 50 mmol) was added to a stirred solution containing BOC-L-alanine (9.45 g, 50 mmol) and triethylamine (5.05 g, 50 mmol) in tetrahydrofuran (600 ml) at -10° . After 20 min, a solution containing L-alanine methyl ester hydrochloride (6.30 g, 45 mmol) and triethylamine (4.55 g, 45 mmol) in dimethylformamide (25 ml) at -10° was added and the reaction mixture stirred overnight at 0° .

The reaction mixture was filtered and the solvent evaporated. The residue was dissolved in ethyl acetate, washed successively with 5% aqueous citric acid (two 100-ml portions), water (100 ml), 2 *N* aqueous potassium hydrogen carbonate (two 100-ml portions), and water (100 ml), and dried. Removal of the solvent and crystallization of the residue from ethyl acetate-hexane gave 5.41 g, mp $110-111^{\circ}$. Mother liquors gave 4.62 g, mp $105-108^{\circ}$, yield 81.5%, $[\alpha]^{25D} -63.8^{\circ}$ (*c* 0.87, methanol).

Anal. Calcd for $C_{12}H_{22}N_2O_5$ (274.32): C, 52.54; H, 8.08; N, 10.21. Found: C, 52.60; H, 8.09; N, 10.17.

BOC-L-alanyl-L-alanyl-L-alanine Methyl Ester (XVIII). Isobutyl chloroformate (1.47 ml, 11.1 mmol) was added to a stirred solution containing BOC-L-alanine (2.27 g, 12 mmol) and triethylamine (1.21 g, 12 mmol) in tetrahydrofuran (140 ml) at -1° . After 20 min at -1° , a cooled solution containing L-alanyl-L-alanine methyl ester trifluoroacetate (from treatment of the dipeptide XI with trifluoroacetic acid) (2.28 g, 7.92 mmol) and triethylamine (1.12 g, 11.1 mmol) in dimethylformamide (20 ml) was added and stirring continued overnight at 3° . The product, 1.62 g (60%), was isolated in a manner similar to that used for the dipeptide XVII. Recrystallization of the product from ethyl acetate gave 0.99 g (37%), mp $192-193^{\circ}$, $[\alpha]^{25D} -81.2^{\circ}$ (*c* 2.0, methanol).

Anal. Calcd for $C_{15}H_{27}N_3O_6$ (345.40): C, 52.17; H, 7.88; N, 12.16. Found: C, 52.10; H, 7.90; N, 12.30.

BOC-L-alanyl-L-alanyl-L-alanyl-L-alanine methyl ester (XIX) was prepared in 22% yield, mp 246° dec (from ethyl acetate), from BOC-L-alanine and the tripeptide XVIII by a method similar to that used for the tripeptide XVIII; $[\alpha]^{25D} -39.5^{\circ}$ (*c* 1.0, dimethylformamide).

Anal. Calcd for $C_{18}H_{32}N_4O_7$ (416.48): C, 51.92; H, 7.75; N, 13.45. Found: C, 51.90; H, 7.90; N, 13.46.

Cbz-L-valyl-L-valine methyl ester (XX) was prepared in 69% yield, mp $116-119^{\circ}$ (from hexane-ethyl acetate), from Cbz-L-valine and L-valine methyl ester hydrochloride in a manner similar to that used for the dipeptide VIII (method b), $[\alpha]^{25D} -24.3^{\circ}$ (*c* 0.3, methanol).

Anal. Calcd for $C_{15}H_{25}N_2O_5$ (364.44): C, 62.62; H, 7.74; N, 7.69. Found: C, 62.56; H, 7.85; N, 7.74.

Cbz-L-valyl-L-valyl-L-valine Methyl Ester (XXI). Cbz-L-valine (5.02 g, 20 mmol) was dissolved in purified tetrahydrofuran (100 ml), cooled to -10° , and treated with triethylamine (2.02 g, 20 mmol) and isobutyl chloroformate (2.8 ml, 20 mmol). After 1 hr, a solution containing L-valyl-L-valine methyl ester hydrobromide (from treatment of Cbz-L-valyl-L-valine methyl ester with hydrogen bromide in acetic acid) (20 mmol) in tetrahydrofuran (50 ml) at

-10° was added followed by triethylamine (2.02 g, 20 mmol). The reaction mixture was stirred overnight at 0° .

Filtration of the reaction mixture gave a white solid which was washed thoroughly with distilled water and dried to give 0.52 g of product, mp $215-216^{\circ}$. The filtrate was evaporated to dryness, and the residue was taken up in chloroform, washed successively with 5% aqueous citric acid, water, 2 *M* aqueous sodium hydrogen carbonate, and water, and dried. Removal of the solvent gave 7.25 g of product, mp $196-202^{\circ}$, raised to 218° from ethanol. Total yield was 84%, $[\alpha]^{25D} -20.3^{\circ}$ (*c* 0.41, methanol).

Anal. Calcd for $C_{24}H_{37}N_3O_6$ (463.57): C, 62.18; H, 8.04; N, 9.06. Found: C, 62.16; H, 8.02; N, 9.47.

BOC-L-valyl-L-valyl-L-valyl-L-valine Methyl Ester (XXII). Triethylamine (0.5 g, 5 mmol) and isobutyl chloroformate (0.655 ml, 5 mmol) were added to a solution of BOC-L-valine (1.09 g, 5 mmol) in tetrahydrofuran (100 ml) at -10° . After 1 hr, L-valyl-L-valyl-L-valine methyl ester (from hydrogenation of the tripeptide XXI over palladium oxide) (5 mmol) in tetrahydrofuran (100 ml) at -10° was added and the mixture stirred overnight at 0° .

The precipitate was removed by filtration, washed thoroughly with distilled water, and dried to give 1.4 g of product, mp $244-246^{\circ}$ dec.

The filtrate was evaporated to dryness and the residue taken up in chloroform. The chloroform solution was washed successively with 5% aqueous citric acid, water, 2 *M* aqueous sodium hydrogen carbonate, and water, and dried. Removal of the solvent and crystallization of the residue from chloroform-hexane gave a further 0.59 g of product, mp $247-248^{\circ}$ dec. Total yield was 76.5%, $[\alpha]^{25D} -72.7^{\circ}$ (*c* 0.0244, methanol).

Anal. Calcd for $C_{28}H_{48}N_4O_7$ (528.69): C, 59.07; H, 9.15; N, 10.59. Found: C, 59.10; H, 9.14; N, 10.63.

BOC-L-valyl-L-valyl-L-valyl-L-valyl-L-valine methyl ester (XXIII) was prepared in 65% yield, mp $>300^{\circ}$, by coupling of BOC-L-valine and L-valyl-L-valyl-L-valyl-L-valine methyl ester trifluoroacetate (from treatment of the tetrapeptide XXII with trifluoroacetic acid) by the mixed carbonic anhydride method using dimethylformamide as solvent for the trifluoroacetate. The product was isolated in a manner similar to that used for the tetrapeptide XXII; $[\alpha]^{25D} -13^{\circ}$ (*c* 0.195, trifluoroethanol).

Anal. Calcd for $C_{31}H_{57}N_5O_8$ (627.82): C, 59.28; H, 9.15; N, 11.15. Found: C, 59.27; H, 9.10; N, 11.06.

BOC-L-leucyl-L-leucine methyl ester (XXIV) was prepared in 89% yield, mp $141-142^{\circ}$ (from ethyl acetate-hexane), $[\alpha]^{25D} -25.7^{\circ}$ (*c* 0.3, methanol), from BOC-L-leucine and L-leucine methyl ester hydrochloride by a method similar to that used for the dipeptide XIV.

Anal. Calcd for $C_{18}H_{34}N_2O_5$ (358.48): C, 60.32; H, 9.56; N, 7.81. Found: C, 60.47; H, 9.73; N, 7.84.

BOC-L-leucyl-L-leucyl-L-leucine methyl ester (XXV) was prepared in 68% yield, mp $156-159^{\circ}$ (from ethyl acetate-hexane), $[\alpha]^{25D} -21.7^{\circ}$ (*c* 0.4, methanol), from BOC-L-leucine and L-leucyl-L-leucyl methyl ester trifluoroacetate (from treatment of the dipeptide XXIV with trifluoroacetic acid) by a method similar to that used for the dipeptide XIV.

Anal. Calcd for $C_{24}H_{45}N_3O_6$ (471.64): C, 61.15; H, 9.62; N, 8.91. Found: C, 61.30; H, 9.61; N, 8.84.

BOC-L-leucyl-L-leucyl-L-leucyl-L-leucine methyl ester (XXVI) was prepared in 57% yield, mp $207-208^{\circ}$ (from aqueous ethanol), $[\alpha]^{25D} -16.4^{\circ}$ (*c* 0.25, trifluoroethanol), from BOC-L-leucine and L-leucyl-L-leucyl-L-leucine methyl ester trifluoroacetate (from treatment of tripeptide XXV with trifluoroacetic acid) by a method similar to that used for the dipeptide XIV.

Anal. Calcd for $C_{30}H_{56}N_4O_7$ (584.8): C, 61.62; H, 9.65; N, 9.58. Found: C, 61.45; H, 9.65; N, 9.58.

BOC-L-isoleucyl-L-isoleucine methyl ester (XXVII) was prepared in 67% yield, mp $158-159^{\circ}$ (from ethyl acetate-hexane), $[\alpha]^{25D} -23.7^{\circ}$ (*c* 0.314, methanol), from BOC-L-isoleucine and L-isoleucine methyl ester hydrochloride in a manner similar to that used for the corresponding L-leucine compound.

Anal. Calcd for $C_{18}H_{34}N_2O_5$ (358.48): C, 60.32; H, 9.56; N, 7.81. Found: C, 60.25; H, 9.48; N, 7.77.

BOC-L-isoleucyl-L-isoleucyl-L-isoleucine methyl ester (XXVIII) was prepared in 50% yield, mp $190-191^{\circ}$ (from ethyl acetate-hexane), $[\alpha]^{25D} -29.5^{\circ}$ (*c* 0.262, methanol), from BOC-L-isoleucine and the trifluoroacetate of peptide XXVII by the method used for the preparation of the corresponding L-leucine compound XXV.

Anal. Calcd for $C_{24}H_{45}N_3O_6$ (471.64): C, 61.15; H, 9.62; N, 8.91. Found: C, 61.20; H, 9.70; N, 8.98.

BOC-L-isoleucyl-L-isoleucyl-L-isoleucine Methyl Ester (XXIX). Triethylamine (0.712 g, 7.5 mmol) and isobutyl chloro-

formate (0.983 ml, 7.5 mmol) were added to a solution of BOC-L-isoleucine (1.73 g, 7.5 mmol) in tetrahydrofuran (75 ml) at -40° . The mixture was stirred at -20° for 1 hr. A solution of L-leucyl-L-leucyl-L-leucyl methyl ester trifluoroacetate (from treatment of 3.54 g of tripeptide XXVIII with trifluoroacetic acid) in dimethylformamide (15 ml) was added, followed by triethylamine (0.712 g, 7.5 mmol) and the mixture stirred at -20° for 30 min and at 4° overnight. The usual work-up gave 1.1 g (25%) of impure product. Elution from a column of silica gel by chloroform and recrystallization from ethyl acetate gave pure product, mp 240° dec, $[\alpha]_D^{25} -43.5^{\circ}$ (c 0.2, trifluoroethanol).

Anal. Calcd for $C_{80}H_{56}N_4O_7$ (584.8): C, 61.62; H, 9.65; N, 9.58. Found: C, 61.62; H, 9.36; N, 9.32.

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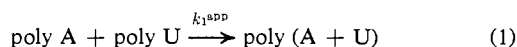
Polynucleotides. IX.¹ Temperature Dependence of Kinetics of Complex Formation in Equimolar Mixtures of Polyriboadenylate and Polyribouridylate²

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Abstract: The kinetics of formation of poly (A + U) in equimolar mixtures of poly A and poly U in 0.01 M Na⁺, as a function of temperature, have been followed spectrophotometrically at specific (isochromic) wavelengths near 280 m μ . It was observed that the second-order rate constant, k_1 , for the formation of poly (A + U) decreases with increasing temperature, approaching zero within 0.2° of the temperature corresponding to the inflection point of the absorbance-temperature profile. This behavior is similar to that previously reported by Ross and Sturtevant (1960) on the basis of measurements at 259 m μ , where the validity of quantitative interpretation is in doubt. Some observations on the transient formation of poly (A + U + U) are also reported. The kinetics of formation of both complexes are discussed in relation to hypothetical schemes of elementary processes for helix formation. The model proposed by Saunders and Ross (1960) and by Flory (1961), and amended by Kallenbach, Crothers, and Mortimer (1963) is found to be inadequate. In the Appendix, Crothers, Davidson, and Kallenbach present a more complex model that anticipates the observed temperature dependence of the kinetics and allows the conclusion that more than a single base pair is needed to establish a stable nucleus for helix growth.

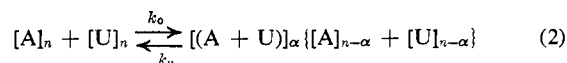
Ross and Sturtevant^{4,5} have reported that in equimolar⁶ mixtures (0.50X_U)⁷ of poly A and poly U the apparent second-order rate constant, k_1^{app} , for the formation of poly (A + U)



first increases with increasing temperature and then decreases linearly, approaching zero in the vicinity of the dissociation temperature (T_m). Such unusual tem-

perature dependence has been explained by Ross and Sturtevant⁵ in terms of a general model for helix formation^{8,9} that involves a sequence of elementary processes.

The formation of poly (A + U) may be conveniently described according to the following specific illustration of that model. The sequence is initiated by a bimolecular nucleation step



where $[A]_n$ and $[U]_n$ are concentrations of poly A and poly U residues, respectively, here assumed to be of equal chain length n . k_o is the second-order rate constant for nucleation, which for the sake of simplicity is treated here as a concerted event. k_u is the corresponding first-order rate constant for the opposing (strand separation) reaction. In this model, nucleation involves the synergic reaction of an unknown number of neighboring residues, α .

Nucleation is followed by a multistep, first-order, "zipping-up" process or helical growth, which may be described by eq 3 where k_f and k_b are the first-order forward and opposing rate constants, respectively. While eq 3 implies that the formation of one (A + U) base

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(3) U. S. Public Health Service Predoctoral Trainee, 1963-1967.

(4) P. D. Ross and J. M. Sturtevant, *Proc. Natl. Acad. Sci. U. S.*, **46**, 1360 (1960).

(5) P. D. Ross and J. M. Sturtevant, *J. Am. Chem. Soc.*, **84**, 4503 (1962).

(6) Concentration refers to nucleotide residue concentrations.

(7) The following abbreviations have been used: poly A = polyriboadenylate; poly U = polyribouridylate; poly (A + U) = the two-stranded helix containing one strand of poly A and one of poly U; poly (A + U + U) = the three-stranded helix containing one strand of poly A and two of poly U; T_m = the temperature at the midpoint of the appropriate absorbance change; $T_{m_{3 \rightarrow 2}}$ = T_m of the dissociation of the three-stranded to the two-stranded helix, whereas $T_{m_{2 \rightarrow 1}}$ signifies dissociation of the latter to the homopolymers; T_i = the temperature of the inflection point of an absorbance-temperature profile; X_U = the mole fraction of U residues in mixtures of poly A and poly U.

(8) M. Saunders and P. D. Ross, *Biochem. Biophys. Res. Commun.*, **3**, 314 (1960).

(9) P. J. Flory, *J. Polymer Sci.*, **49**, 105 (1961).