Formation of Oligomers on N Acylation of Tripeptides. Proton Magnetic Resonance Spectra of the Peptides[1]

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The peptides L-Phe-Gly-L-Leu, L-Leu-L-Phe-L-Ala, and L-Ala-L-Phe-Gly-L-Leu were prepared *via* controlled acylation by amino acid N-carboxyanhydrides. Acylation of the tripeptides by benzyloxycarbonyl chloride resulted in extensive formation of tripeptide oligomers, indicating that the carboxylate anion of a free peptide competes favorably with the terminal amino group as a nucleophile. The proton magnetic resonance spectra of L-Leu-L-Phe-L-Ala and its oligomers and of L-Ala-L-Phe-Gly-L-Leu are reported and briefly discussed.

Unblocked peptide fragments are potentially available, with speed and convenience, and in quantity, using the method of controlled acylation of amino acids and peptides by amino acid N-carboxyanhydrides.[3-5]

In exploring this method in our laboratory we readily prepared two free tripeptides and a tetrapeptide, and for subsequent use subjected the tripeptides to standard conditions for N acylation by benzyloxycarbonyl chloride. Extensive oligomerization of the tripeptides occurred in the acylation process. This observation, together with the high-resolution proton magnetic resonance spectra of the oligomers, is described and discussed below. The reactions reported are summarized in Figure 1.

Experimental Section[6]

General Nmr Spectra.—Proton magnetic resonance spectra were measured using Varian A-60, HA-60 and HA 100 spectrometers with occasional assistance of a Varian C-1024 time averaging computer. We are grateful to the Department of Chemistry, University of Chicago, for the use of the HA-100 instrument. Assignment of resonances was made on the basis of chemical shifts and homonuclear spin-decoupling experiments. Spectra were measured on solutions in trifluoroacetic acid, trifluoroacetic acid-*d*, dimethyl sulfoxide-d_6 and D_2O, and referred to internal tetramethylsilane, or, for aqueous solutions, to internal sodium 2,2-dimethyl-2-silapentane-5-sulfonate.

Controlled Acylation by Amino Acid N-Carboxyanhydrides (NCA).—A Waring Model 1002 Commercial Blendor with a 1-qt clover-shaped Pyrex jar was used. The tightly fitting plastic cap provided for the jar had a 2-in. central opening with a removable plug. This opening was used for addition of the solid anhydrides to the cooled and stirred reaction mixture. The outer part of the cap was bored with two holes in one corner to support and pass the leads of a single-turn cooling coil of $\frac{3}{16}$-in. stainless steel tubing. The turn of the coil was placed below the stirrer blades of the jar and the leads were brought up along one corner of the jar, carefully placed to avoid interference with the blades. Additional holes were bored in the other corners of the cap, one to support an alcohol thermometer, and one to support a combination pH electrode; the active part of each of these was placed so as to be bathed in the stirred mixture. Though the remaining hole passed a piece of Teflon tubing through which alkali was added from a syringe to maintain constant pH during the reaction.

Through the cooling coil was passed a water–glycol cooling mixture, pumped from a reservoir chilled by solid carbon dioxide. Coolant was pumped at a rate and temperature so that the *stirred* reaction mixture could be held at 0° or slightly below; with sufficient cooling for the stirred condition, the unstirred reaction mixture would freeze.

L-Phe-Gly-L-Leu (I).—A solution of 0.94 g (0.005 mol) of glycyl-L-leucine (Mann Research Laboratories) in 50 ml of trimethylammonium chloride buffer (0.42 *M* amine, 0.25 *N* hydrochloric acid) was adjusted, *at* 0°, to pH 10.4 by addition of 1.2 ml of 4 *N* sodium hydroxide. This solution was agitated at 0° in the Blendor fitted as described above, at its maximum speed, and to it was added all at once 1.05 g (0.0055 mol) of solid L-phenylalanine N-carboxyanhydride.[7] The pH of the reacting mixture was maintained at 10.4 by continuous addition of 4 *N* sodium hydroxide. Consumption of alkali ceased after several minutes; 1.20 ml of base was consumed. After 10 min the pH was adjusted to 6, using concentrated sulfuric acid. The solution was filtered and concentrated to dryness at reduced pressure. The residue was washed with a small amount of water, and the water-insoluble part, 1.7 g, was crystallized from glacial acetic acid–ether to yield 1.57 g (94%) of tripeptide, mp 233–234° dec. This product was chromatographically homogeneous in a system known to separate it from the starting dipeptide and the three amino acids involved—Eastman silica chromagram sheet, 1-butanol–acetic acid–water 7:1:2 (7:1:2 BAW). Upon hydrolysis with 4 *N* hydrochloric acid, it yielded a chromatogram with three spots corresponding to its amino acid components. An analytical sample was dried at 100° at 0.05-mm pressure.

Anal. Calcd for $C_{17}H_{25}O_4N_3$: C, 60.88; H, 7.51; N, 12.53. Found: C, 60.48; H, 7.70; N, 12.38.

This experiment was repeated in the same apparatus at ten times the scale (0.005 mol) with no loss in yield.

L-Ala-L-Phe-Gly-L-Leu (II).—A procedure analogous to that described above was used. L-Phe-Gly-L-Leu (I) (12.0 g, 0.036 mol), prepared as above, and L-alanine N-carboxyanhydride[8] (4.6 g, 0.04 mol) were the reactants. The crude product obtained from the reaction mixture, after acidification, evaporation to dryness, and washing with a small amount of water, contained four components; in order of decreasing R_f in 7:1:2 BAW they were initial tripeptide, tetrapeptide, unknown (possibly alanylanine), and alanine. The major component, tetrapeptide, crystallized first from acetic acid–ether; 7.3 g (50% was obtained.[9] Separation of tri- and tetrapeptide could also be achieved making use of the fact that the tripeptide is insoluble but the tetrapeptide soluble in 1-butanol or 1-pentanol.

A chromatographically pure analytical sample, mp 191–192° dec, was obtained from acetic acid–ether, but although dried at 100° and 0.05 mm for 24 hr, it appeared to retain 1 mol of water.

Anal. Calcd for $C_{20}H_{30}O_5N_4 \cdot H_2O$: C, 56.59; H, 7.60; N, 13.20. Found: C, 56.97; H, 7.30; N, 13.08.

L-Leu-L-Phe-L-Ala (V).—A solution of 1.78 g (0.02 mol) of

(1) Work supported by research grants from the National Science Foundation (GB 4514) and the U. S. Public Health Service, National Institute of General Medical Sciences (GM 14069).

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(3) R. G. Denkewalter, *et al.*, *J. Amer. Chem. Soc.*, **88**, 3163 (1966).

(4) R. Hirschmann, *et al.*, *J. Org. Chem.*, **32**, 3415 (1967).

(5) D. F. Veber, K. Pfister, and R. Hirschmann, *J. Med. Chem.*, **10**, 986 (1967).

(6) Elemental analyses performed by Micro-Tech Laboratories, Skokie, Ill. Melting points are corrected.

(7) M. Sela and A. Berger, *J. Amer. Chem. Soc.*, **77**, 1893 (1955).

(8) J. L. Bailey, *J. Chem. Soc.*, 3461 (1950); A. Elliott, *Proc. Roy. Soc.* (London), **A211**, 104 (1954).

(9) Reaction on this scale overloaded our apparatus; mixing and cooling were not optimal. On an 0.005-mol scale the yield was 60%.

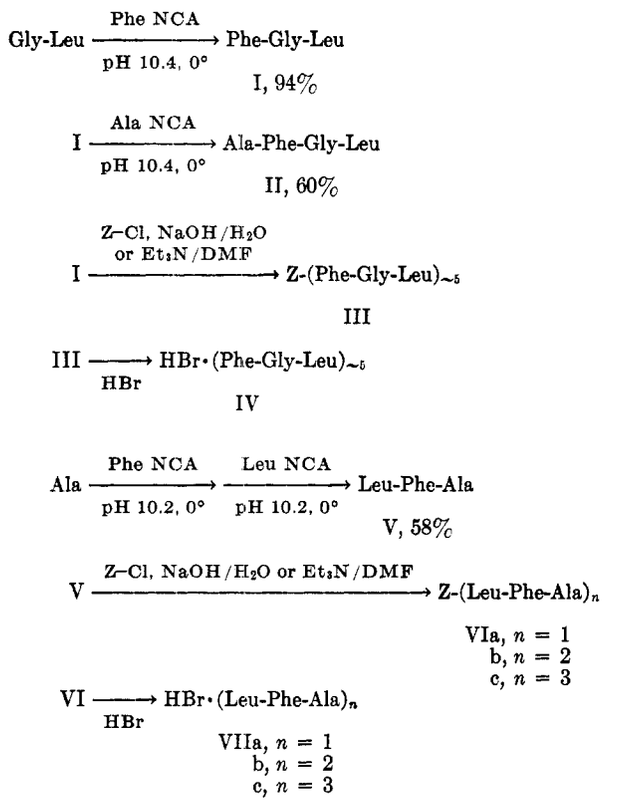


Figure 1.—Peptide couplings and oligomerizations discussed in this report. Abbreviations: NCA, N-carboxyanhydride; Z benzyloxycarbonyl; DMF, dimethylformamide. All dissymmetric amino acid residues are L series. Yields shown are of isolated, homogeneous products.

L-alanine in 100 ml of trimethylammonium chloride buffer (0.42 *M* amine, 0.25 *M* hydrochloric acid) was adjusted at 0° to pH 10.2 (2.6 ml of 4 *N* sodium hydroxide) and agitated in the Blendor at 0°. To this solution was added, all at once, 4.05 g (0.021 mol) of L-phenylalanine N-carboxyanhydride and the pH was held at 10.10–10.25 by continuous addition of 4 *N* sodium hydroxide (8.1 ml). After 10 min, 18 *N* sulfuric acid (3.3 ml) was added to bring the pH to 3.0, and 5 min later 7.2 ml of 4 *N* sodium hydroxide was used to readjust the pH to 10.15 *at* 0°.

To the chilled and agitated reaction mixture was now added all at once 3.46 g (0.021 mol) of L-leucine N-carboxyanhydride[10] and pH was maintained at 10.10–10.25 by continuous addition of 4 *N* sodium hydroxide (7.1 ml). At the completion of reaction, pH was adjusted first to 3.0 and then to 6.

The reaction mixture was filtered to remove a small amount of insoluble (polymeric?) material. On thin layer chromatography the soluble material was resolved into L-Leu-L-Phe-L-Ala (V), the predominant product, and L-Phe-L-Ala; it appeared to contain very little else. Concentration under reduced pressure to a volume of 10–15 ml caused precipitation of crystalline tripeptide, most of the dipeptide remaining in the mother liquors. The crystalline product was washed with alcohol and recrystallized from glacial acetic acid–ether to yield 4.0 g (58%) of tripeptide. This product was chromatographically single and free of dipeptide and amino acids.

An analytical sample was crystallized from water–dioxane and ethanol–dioxane and dried at 0.05 mm and 100° for 24 hr. It decomposed above 220° without melting. Thin layer chromatography of a hydrolysate (4 *N* hydrochloric acid) showed the constituent three amino acids.

Anal. Calcd for $C_{18}H_{21}O_4N_3 \cdot 0.5H_2O$: C, 60.31; H, 7.87; N, 11.72. Found: C, 60.22, 60.14; H, 7.79, 7.83; N, 11.40, 11.42.

Benzyloxycarbonylation of L-Phe-Gly-L-Leu. A. In Water.—To a vigorously stirred solution of 0.84 g (0.0025 mol) of L-Phe-Gly-L-Leu (I) in 1.25 ml of 2 *N* sodium hydroxide were added, in alternating portions of one-half, 0.51 g (0.003 mol) of benzyloxycarbonyl chloride and 1.5 ml of 2 *N* sodium hydroxide. The addition required about 30 min and was conducted at 0°. After 30 min of further stirring, the reaction mixture was acidified to pH 4 using 4 *N* hydrochloric acid; then the precipitated product was collected by filtration and washed with alcohol and petroleum ether (bp 30–60°); it weighed 0.83 g After reprecipitation from acetic acid solution by addition of ether, the product had mp 285–288° dec.

B. In Dimethylformamide.—To a stirred solution of 0.84 g (0.0025 mol) of L-Phe-Gly-L-Leu (I) in 30 ml of dimethylformamide cooled in an ice bath were added, first, 0.73 ml (0.0052 mol) of triethylamine and, second, 0.47 g (0.0028 mol) of benzyloxycarbonyl chloride, drop by drop. After 1 hr of stirring at ice bath temperature, 1.25 ml of 2 *N* hydrochloric acid was added, the mixture was filtered free of precipitated triethylammonium chloride, and the filtrate was concentrated to dryness at reduced pressure. The dried residue was triturated with petroleum ether and then with water, to yield 0.8 g of solid, mp 276–280° dec. This was purified by precipitation from dimethylformamide solution on addition of ether, with almost quantitative recovery, mp 285–288° dec.

Anal. Calcd for pentamer III, $C_{93}H_{123}O_{18}N_{15}$: C, 64.23; H, 7.13; N, 12.08. Found: C, 63.36; H, 7.03; N, 12.10.

Benzyloxycarbonylation of L-Leu-L-Phe-L-Ala. A. In Water.—To a vigorously stirred solution of 0.87 g (0.0025 mol) of L-Leu-L-Phe-L-Ala in 2.5 ml of *N* sodium hydroxide at 0° were added, in alternating portions of about one-half, 0.51 g (0.003 mol) of benzyloxycarbonyl chloride and 1.5 ml of 2 *N* sodium hydroxide. Addition required about 30 min and stirring was continued 30 min at 0° and 30 min more without cooling.

The reaction mixture, which contained a precipitate, was acidified to pH 4, using 4 *N* hydrochloric acid, and vigorously shaken with 30 ml of ethyl acetate. An insoluble product was collected by filtration and purified by repeated precipitation induced by adding ether to a dimethylformamide solution. This substance, mp 310–313° dec, was obtained in 13% yield (0.12 g). It was assigned the structure of N-benzyloxycarbonyl nonapeptide (trimer VIc).

Anal. Calcd for $C_{62}H_{63}O_{12}N_9$: C, 64.96; H, 7.30; N, 11.00. Found: C, 65.07; H, 7.38; N, 10.73.

The ethyl acetate phase was dried over magnesium sulfate and concentrated to dryness at reduced pressure. The residue was taken up in methanol, and after the solution had been filtered, a small amount of water was added to induce crystallization of a second product. This product was recrystallized from methanol–water to yield 0.15 g (15%) of what proved to be the benzyloxycarbonyl hexapeptide (dimer VIb), mp 228–231° dec.

Anal. Calcd for $C_{44}H_{58}O_9N_6$: C, 64.84; H, 7.17; N, 10.31. Found: C, 64.55; H, 7.16; N, 10.08.

From the methanolic mother liquors of the hexapeptide an oil was thrown down by further addition of water. This was triturated with water until it solidified, and then recrystallized several times from ethyl acetate–petroleum ether to afford 0.40 g (33%) of benzyloxycarbonyl tripeptide, VIa, mp 142–144°.

Anal. Calcd for $C_{26}H_{33}O_6N_3$: C, 64.58; H, 6.88; N, 8.69. Found: C, 64.84; H, 6.99; N, 8.75.

Repetition of the above experiment resulted in isolated yields of monomer, dimer and trimer of 21, 25, and 21% respectively. From an experiment using 2 equiv of benzyloxycarbonyl chloride and an appropriate amount of base were isolated 17, 13, and 23% respectively, of the three peptides.

B. In Dimethylformamide.—To a stirred solution of 0.87 g (0.0025 mol) of L-Leu-L-Phe-L-Ala (V) in 30 ml of dimethylformamide cooled in an ice bath were added, first, 0.73 ml (0.0052 mol) of triethylamine and, second, 0.47 g (0.0028 mol) of benzyloxycarbonyl chloride. After 30 min 1.25 ml of 2 *N* hydrochloric acid was added. The reaction mixture was filtered to remove precipitated triethylammonium chloride. The filtrate was evaporated to dryness under reduced pressure and the resultant was triturated with petroleum ether and then water to yield 0.6 g of a solid. This was extracted with ethyl acetate. The ethyl acetate insoluble material, 0.4 g, was trimer VIc, mp 310–312° dec.

The ethyl acetate solution, on evaporation, yielded 0.2 g of dimer, VIb, mp 226–230° dec. No benzyloxycarbonyl tripeptide was obtained.

Hydrogen Bromide Cleavage of Z-L-Leu-L-Phe-L-Ala (VIa) and Its Dimer (VIb).—The benzyloxycarbonyl peptides, 0.15 g,

(10) D. Coleman, *J. Chem. Soc.*, 3222 (1950); J. L. Bailey, *ibid.*, 3461 (1950); A. C. Farthing and R. J. W. Reynolds, *Nature*, **165**, 647 (1950).

were dissolved in 5 ml of 30% hydrogen bromide in acetic acid, and the solutions were allowed to remain at room temperature for 1 hr. Anhydrous ether was then added to precipitate apparently crystalline solids, which were collected on a glass frit funnel and washed copiously with ether.

The cleavage product from the tripeptide derivative was recrystallized from absolute ethanol–ether to give 0.15 g of tripeptide hydrobromide VIIa, mp 218–220° dec.

Anal. Calcd for $C_{18}H_{28}O_4N_3Br$: C, 50.23; H, 6.56; N, 9.76; Br, 18.57. Found: C, 50.83; H, 6.73; N, 9.76; Br, 18.12.

The cleavage product from the hexapeptide derivative was purified by precipitation from dimethylformamide by the addition of ether to yield 0.15 g of hydrobromide, mp 290–300° dec.

Anal. Calcd for $C_{36}H_{53}O_7N_6Br$: Br, 10.49. Found: Br, 9.58.

Hydrogen Bromide Cleavage of Z-(L-Leu-L-Phe-L-Ala)$_3$ (VIc) and Z-(L-Phe-Gly-L-Leu)$_n$ (III).—The benzyloxycarbonyl peptides, 0.11 g, were dissolved in 3 ml of trifluoroacetic acid and to these solutions were added 0.5-ml portions of 30% hydrogen bromide in acetic acid. After 1 hr at room temperature dry ether was added to precipitate solid products, which were collected on a glass frit funnel and washed copiously with ether.

The cleavage product of VIc was purified by precipitation from trifluoroacetic acid solution on addition of dry ether; 0.1 g of product VIIc was obtained.

Anal. Calcd for $C_{54}H_{78}O_{10}N_9Br$: N, 11.53; Br, 7.31. Found: N, 11.33; Br, 7.73.

The cleavage product (IV) of III was also purified by precipitation from trifluoroacetic acid–ether; 0.1 g was obtained.

Anal. Calcd for pentamer, $C_{85}H_{113}N_{15}O_{16}Br$: C, 60.55; H, 7.06; N, 12.46; Br, 4.74. Found: C, 60.40; H, 7.00; N, 12.09; Br, 4.42.

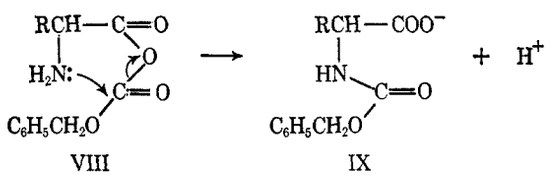
Discussion

Synthesis.—In preparing peptides I, II and V (Figure 1) *via* the N-carboxyanhydride method, we followed the procedure described by the Merck group,[3-5] with the exception that, rather than the original borate buffer, we used a trimethylamine–trimethylammonium system, 0.4 *M* in total amine concentration. We chose trimethylamine because its pK_A at 0°, 10.7,[11] is closer to the required pH for minimization of side reactions (10.2–10.4),[3,4] and therefore at a given concentration it could be expected to provide greater buffer capacity than borate (pK_A at 0°, 9.5[12]). It was nonetheless necessary to control pH by addition of concentrated alkali during acylation.

Oligomerization.—When the tripeptide Phe-Gly-Leu was subjected to Schotten–Baumann conditions for N acylation by benzyloxycarbonyl chloride,[13,14] there was obtained, almost quantitatively, a peptide of solubility characteristics different from those expected for the benzyloxycarbonyl tripeptide. The integral of the proton magnetic resonance spectrum of this product (trifluoroacetic acid solution) indicated a ratio of one benzyloxycarbonyl methylene (5.1 ppm) to four or five phenylalanyl methylenes. Treatment with hydrogen bromide in trifluoroacetic acid afforded in high yield a peptide hydrobromide with bromine content consistent with the hydrobromide of tripeptide pentamer. A similar oligomeric product was obtained, also in excellent yield, when the acylation was carried out in dimethylformamide solution, using triethylamine as base.

With the tripeptide Leu-Phe-Ala (V), treatment with benzyloxycarbonyl chloride in aqueous base resulted in a mixture of three discrete compounds: the N-benzyloxycarbonyl derivatives of the tripeptide (VIa), of its dimer (VIb) and of its trimer (VIc). The yield of VIa was not increased by use of 2 equiv of acylating agent. When acylation was carried out in dimethylformamide solution (triethylamine as base) only the trimeric (VIc) and dimeric (VIb) derivatives were isolated.

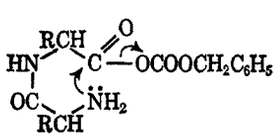
Reaction of an acyl halide with an amino acid anion, or with the anion of an unblocked peptide, can occur at either of the two nucleophilic centers, amino and carboxylate. Benzyloxycarbonylation of amino acids in aqueous base consistently affords the N-benzyloxycarbonyl derivatives in good yield,[13] and would do probably so regardless of the initial site of reaction. Reaction at the carboxylate group leads to the mixed anhydride VIII, which can readily undergo intramolecular acyl transfer to the stable N-acyl derivative IX. An indication that some C acylation occurs,



VIII IX

however, is given by the observation that reaction of glycine in bicarbonate buffer affords benzyloxycarbonylglycylglycine as a contaminant (10%) of the desired benzyloxycarbonylglycine.[15]

Carboxyl acylation of a dipeptide anion would be expected to result in formation of a diketopiperazine, *via* the six-membered intermediate state shown as X,



X

by analogy with the facile cyclization of even unactivated dipeptide esters. In our laboratory we have, in other work, successfully benzyloxycarbonylated glycylglycine and glycyltyrosine under Schotten–Baumann conditions, indicating that in these peptides containing N-terminal glycine at least, acylation occurs predominantly on nitrogen.

In case of a tripeptide, a mixed anhydride formed by acylation at the C terminus cannot readily undergo intramolecular reaction with the terminal amino group; with one exception,[16] attempts at cyclization of tripeptides have failed.[17] Carboxylate acylation should therefore be unproductive (hydrolysis of the anhydride) or should lead to oligomerization, that is, peptide bond formation by intermolecular reaction of amino groups

(11) Measured pH at half-neutralization of 0.47 *M* solution at 0°.

(12) "Handbook of Chemistry and Physics," 46th ed, Chemical Rubber Co., Cleveland, Ohio, 1965, p D-80.

(13) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," John Wiley & Sons, Inc., New York, N. Y., 1961, pp 887–901.

(14) M. Rothe, H. Brunig and G. Eppert, *J. Prakt. Chem.*, [4] **8**, 323 (1959).

(15) T. Ito, reported at the 2nd Symposium on Peptide Synthesis, Osaka, Japan, 1963. The bicarbonate buffer method of Greenstein and Winitz, p 895 of ref 13, was used.

(16) M. Roth and K. D. Steffen, *Angew. Chem.*, **77**, 347 (1965); *Angew. Chem. Intern. Ed. Engl.*, **4**, 356 (1965).

(17) E. Schroeder and K. Lubke, "The Peptides," Vol. I, E. Gross, Translator, Academic Press, New York, N. Y., 1965, p 271 ff.

with the mixed anhydride, in the usual manner for carbonic–carboxylic anhydrides.[18]

The tripeptide glycyclglycylglycine can be N acylated with benzyloxycarbonyl chloride in aqueous alkali; a 65% yield of N-acyltripeptide has reported.[14] We have repeated this acylation without detecting higher polyglycine derivatives chromatographically.[19] Thus it appears that N acylation is favored for glycylglycylglycine, but, considering the extent of oligomerization described above, tripeptides L-Phe-Gly-L-Leu and L-Leu-L-Phe-L-Ala must undergo predominant carboxyl acylation.

The evidence just cited is admittedly scanty, since N acylation of free peptides is not a common piece of synthetic strategy, but it does appear that the intrinsic reactivities toward an acyl halide of amino and carboxylate groups in peptides are quite similar. The difference between the course of reaction of triglycine, on the one hand, and that of the two tripeptides discussed here, on the other, is very likely the result of steric inhibition by the side chain of the N-terminal residue.

Nmr Spectra.—Chemical shift data are given in Table I for the aliphatic protons of Z-Leu-Phe-Ala

TABLE I

ALIPHATIC PROTON RESONANCES OF L-LEU-L-PHE-L-ALA DERIVATIVES AT 31°[a]

	Proton	VIa (DMSO)[b]	VIIa (TFA)[b]
Leu	α	3.98	4.44
	α, int.[c]	~4.2	~4.7
	δ[e]	0.81	1.04
Phe	α	4.58	5.02
	β[d]	2.95	3.19
Ala	α	4.24	4.64
	α, int.[c]	~4.2	~4.7
	β	1.29	1.52[f]
	β, int.[c]	1.20	1.41

[a] Peptide concentration 30–50 mg/ml. Chemical shifts are in parts per million and refer to internal tetramethylsilane [b] Abbreviations: DMSO, dimethyl sulfoxide-d_6; TFA, trifluoroacetic acid. [c] Internal residues in oligomers. [d] See Discussion. [e] Overlapping 6-Hz doublets, $\Delta\nu$ = 0.02–0.06 ppm. [f] In oligomers 1.56.

(VIa) in dimethyl sulfoxide, and for the corresponding unblocked peptide (VIIa) in trifluoroacetic acid. Except for differences between internal and terminal residues, noted in the table, the chemical-shift values for protons of the dimeric and trimeric analogs (VIb and c and VIIb and c) do not differ significantly from those given for VIa and VIIa. In the spectrum of the carbobenzyloxy trimer (VIc), however, the resonance lines are unusually broad. In VIc the alanyl methyl lines appear only as a broad unresolved peak, while in other cases they are two (internal and C terminal) cleanly resolved 7-Hz doublets, spaced about 0.1 ppm between centers. This increase in line width for VIc suggests that in VIc (in dimethyl sulfoxide) there may be some rigid tertiary structure. (The 0.4–0.5-ppm upfield shift of a α-proton resonances that is apparently associated with helix formation in amino acid homopolymers[20] is not observed in comparing VIb and VIc.)

(18) T. Wieland and D. Stimming, *Ann.*, **579**, 97 (1953); J. R. Vaughan and R. L. Osato, *J. Amer. Chem. Soc.*, **74**, 676 (1952).

(19) Studies by Miss Anita Go.

(20) See, for example, E. N. Bradbury, C. Crane-Robinson, H. Goldman, and H. W. E. Rattle, *Nature*, **217**, 812 (1968).

TABLE II

ALIPHATIC PROTON RESONANCES OF L-ALA-L-PHE-GLY-L-LEU AT 31°[a]

	Proton	D_2O, pH 2	D_2O, pH 6–8	D_2O, pH 12	DMSO[b]	TFA[b]
Ala	α	4.03	4.01	3.45	3.75	4.80
	β[c]	1.43	1.38	1.16	1.23[h]	1.71
Phe	α	4.60	4.58	4.61	4.40	5.00
	β[g]	3.11	3.10	3.13	3.00	3.22
Gly	α[g]	3.85[d]	3.82[e]	3.90[d]	3.72[e]	4.21[d]
Leu	α	4.47	4.28	4.25	4.20	4.49
	δ[f]	0.89	0.88	0.93	0.87	1.03

[a] Peptide concentration 30–50 mg/ml; chemical shifts (in parts per million) refer to internal sodium 2,2-dimethyl-2-silapentane-5-sulfonate in water and to tetramethylsilane in organic solvents. [b] Abbreviations: DMSO, dimethyl sulfoxide-d_6; TFA, trifluoroacetic acid. [c] Doublet, J = 7 Hz. [d] Singlet. [e] AB pattern, $\Delta\nu$ = 0.16 ppm, J_{AB} = 17 Hz. [f] Overlapping 6-Hz doublets, $\Delta\nu$ = 0.04 ppm. [g] See Discussion. [h] Concentration dependent: 1.23 at 12.5 mg/ml, 1.32 at 150 mg/ml.

Table II reports chemical-shift values for protons of Ala-Phe-Gly-Leu in water under acidic, neutral and basic conditions, as well as in dimethyl sulfoxide and trifluoroacetic acid. In the aqueous solutions, the chemical shifts of the terminal alanyl and leucyl residues change with state of ionization in the manner and to the extent already observed in dipeptides.[21] The internal glycyl residue has apparently identical α protons in aqueous acid, aqueous base and trifluoroacetic acid, but in neutral aqueous solution and in dimethyl sulfoxide these protons differ in chemical shift (0.16 ppm). Nonequivalence of glycine α protons is frequently observed in dipeptides, and, as in the present case, depends on the state of ionization of the peptide, being most common in the dipolar form.[22–24] Whereas in zwitterionic dipeptides the chemical shift difference might possibly result from the stereochemical nonidentity of the two protons, not averaged by free rotation, and the presence of a strong electric field gradient,[24] this explanation is less likely for tetrapeptide II. In II the glycyl residue is internal, removed from the charged terminii of the dipolar form, and the electric field gradient will be weaker; yet the chemical-shift difference in II is not smaller than that in the dipeptides. It is more reasonable to ascribe the observed α-proton nonequivalence to conformational preferences that are more pronounced in the dipolar form than in the charged forms.

For both II and VIa the phenylalanyl β protons are sufficiently nonequivalent in dimethyl sulfoxide (0.2 ppm) and in water (0.08 ppm) to permit analysis of their spin–spin splitting patterns. The geminal coupling constant is 14 Hz, but the significant observation is that in each case the α proton is coupled to the higher field β proton by about 10 Hz, and to the lower field β proton by about 4 Hz. These α–β couplings indicate that the favored α–β rotamer for this residue is one with a *trans* arrangement of vicinal protons. One such conformation, XI, has been established for phenylalanyl side chains in the crystal structures of L-threonyl-L-

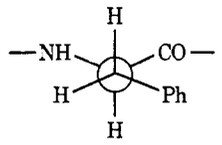
(21) M. Sheinblatt, *J. Amer. Chem. Soc.*, **88**, 2845 (1966).

(22) O. Jardetzky and A. Nakamura, *Proc. Natl. Acad. Sci. U. S.*, **58**, 2212 (1967).

(23) J. W. Westley and B. Weinstein, *Chem. Commun.*, 1232 (1967).

(24) V. J. Morlino and R. B. Martin, *J. Amer. Chem. Soc.*, **89**, 3107 (1967).

phenylalanyl nitrobenzyl ester and glycyclphenylalanyl-glycine.[25]



XI

(25) A. V. Lakshminarayanan, V. Sasisekharan, and G. M. Ramachandran in "Conformation of Biopolymers," Vol. 1, G. N. Ramachandran, Ed., Academic Press, New York, N. Y., p 61.

In the higher analogs of VI, which contain more than one phenylalanyl residue, overlapping of the β-proton patterns precludes analysis of the β-proton spectra. For trifluoroacetic acid solutions of all of the peptides, the phenylalanyl β protons appear as a doublet in the pmr spectra.

Registry No.—I, 19459-22-4; II, 19459-23-5; III, 19471-37-5; IV, 19459-24-6; V, 6514-26-7; VIa, 7625-14-1; VIb, 19459-27-9; VIc, 19459-28-0; VIIa, 19459-29-1; VIIb, 19459-30-4; VIIc, 19459-31-5.

Heterocyclic Amino Sugar Derivatives. I. Derivatives of 2-Amino-2-deoxy-D-allopyranose[1]

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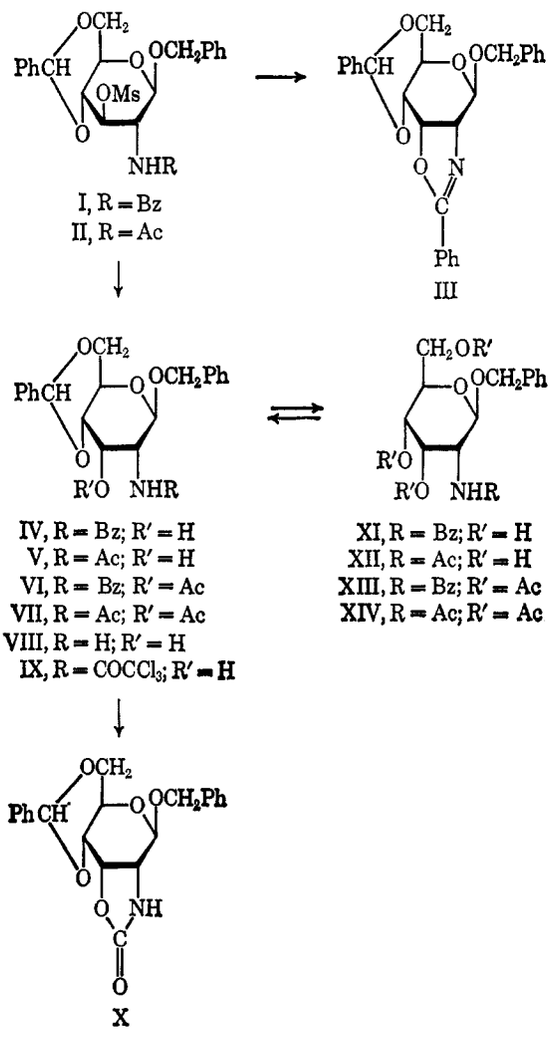
A facile synthetic route from D-glucosamine to benzyl 2-amino-4,6-*O*-benzylidene-2-deoxy-β-D-allopyranoside (VIII) was developed. A number of D-allosamine derivatives were prepared for characterization. Reaction of benzyl 2-amino-4,6-*O*-benzylidene-2-deoxy-β-D-allopyranoside (VIII) with phosgene, diphenylcarbonate, *N*,*N*′-carbonyldiimidazole, or hexachloroacetone gave benzyl 4,6-*O*-benzylidene-β-D-allopyranosido[2,3:4′,5′]-2′-oxazolidone (X) in excellent yield. A new method developed in this investigation is the utilization of hexachloroacetone to prepare a *N*-trichloroacetamido compound which is subsequently cyclized to give 2-oxazolidone X.

2-Amino-2-deoxy-D-allose (D-allosamine), an amino sugar not as yet found in nature, and some of its derivatives have been previously synthesized.[3-7] In view of the possible use of this amino sugar for the synthesis of antibiotics and nucleosides,[3] two derivatives which should be useful intermediates, benzyl 2-amino-4,6-*O*-benzylidene-2-deoxy-β-D-allopyranoside (VIII), and the cyclic carbamate benzyl 4,6-*O*-benzylidene-β-D-allopyranosido[2,3:4′,5′]-2′-oxazolidone (X) have been synthesized (Scheme I). Compound VIII would provide for a variety of anomerically pure, *N*-substituted derivatives, in analogy to corresponding derivatives of D-glucosamine prepared by Gross and Jeanloz,[8] and compound X would provide for an excellent acid-stable, alkali-labile protective group for positions 2 and 3.

It was found that sulfonate can be eliminated with the 2-methoxyethanol–sodium acetate reagent[4] from benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methylsulfonyl-β-D-glucopyranoside (II) to give benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-allopyranoside (V) in excellent yield without any laborious purification process. This approach seems to be superior to other known methods in large-scale preparations. However, we could not convert by this method the α anomer of II into the α anomer of V. Similarly, Rhoads and Gross[9] observed that eliminations of the sulfonate from benzyl 2-benzyloxycarbonylamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methylsulfonyl-D-glucopyranosides proceeded only with the β anomer.

SCHEME I



(1) A preliminary communication was presented at the 155th National Meeting of the American Chemical Society, San Francisco, Calif., March 1968, by K. Miyai and P. H. Gross, Abstracts C-017. Taken from the doctoral thesis of K. Miyai, University of the Pacific, 1968. This work was partially supported by Grant No. GP-4587 of the U. S. National Science Foundation.

(2) To whom inquiries should be directed.

(3) E. J. McEvoy, M. J. Weiss, and B. R. Baker, *J. Amer. Chem. Soc.*, **82**, 205 (1960).

(4) R. W. Jeanloz, *ibid.*, **79**, 2591 (1957).

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(8) P. H. Gross and R. W. Jeanloz, *ibid.*, **32**, 2759 (1967).

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