components of the interactants. This hydrophobic bonding is characterized by an enthalpy change of approximately 0 and a large positive entropy change. A model for hydrophobic bonding as the partial reversal of the solution process for hydrophobic molecules is presented.

Studies on Polypeptides. XL. Synthetic Routes to Peptides Containing β-(Pyrazolyl-1)- and β-(Pyrazolyl-3)-alanine

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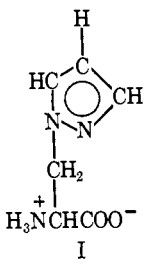
Abstract: Certain derivatives of β-(pyrazolyl-1)- and of β-(pyrazolyl-3)-alanine which are of interest for peptide synthesis are described. These include N^{α}-benzyloxycarbonyl-β-(pyrazolyl-1)-alanine and its *p*-nitrophenyl ester, N^{α}-*t*-butoxycarbonyl-β-(pyrazolyl-1)-alanine, N^{α}-benzyloxycarbonyl-β-(pyrazolyl-3)-alanine and its hydrazide, N^{α}-*t*-butoxycarbonyl-β-(pyrazolyl-3)-alanine and its hydrazide, and N^{α}-N^{pyr}-dibenzyloxycarbonyl-β-(pyrazolyl-3)-alanine and its *p*-nitrophenyl and N-hydroxysuccinimide esters. N^{α}-Benzyloxycarbonyl-β-(pyrazolyl)-3)- and N^{α}-*t*-butoxycarbonyl-β-(pyrazolyl-3)-alanine are shown to form optically active anhydro compounds when treated with N,N′-dicyclohexylcarbodiimide in anhydrous solvents. These anhydro compounds react with nucleophiles to form the respective carboxylic acid derivatives. Anhydro compound formation and ring opening proceed without racemization. The preparation of β-(pyrazolyl-3)-alanylaspartic acid, β-(pyrazolyl-3)-alanylmethionylaspartic acid *d*-sulfoxide, β-(pyrazolyl-3)-alanylphenylalanylarginyltryptophylglycine, and β-(pyrazolyl-1)-alanylphenylalanylarginyltryptophylglycine is described. The similarity between histidine and β-(pyrazolyl-3)-alanine as concerns reactions which are of interest from the point of view of peptide synthesis is discussed.

The replacement, in physiologically active peptides, of histidine by the isosteric pyrazolylalanines provides a useful tool for assessing the importance for biological activity of the characteristic acid–base properties of the imidazole portion of histidine. The rationale for this approach has been presented.[4] To date we have replaced histidine by β-(pyrazolyl-3)-alanine (II) in S-peptide$_{1-12}$ amide,[4] S-peptide$_{1-14}$[5] of ribonuclease A, [5-glutamine]-β-corticotropin$_{1-20}$ amide,[6] and [5-valine]-angiotensin II[7] and have evaluated the effects of this substitution on biological activity.



I

This article describes the preparation of a number of derivatives of β-(pyrazolyl-1)- (I) and particularly of β-(pyrazoly-3)-alanine (II) which are useful for introducing these amino acids into peptides. Also, applications of some of these derivatives to the synthesis of selected peptides are presented. The methyl ester dihydrochlorides and amides of I and II have been described previously.[4]

Preparative Aspects

Exposure of β-(pyrazolyl-1)-alanine (I) to benzyl chloroformate in aqueous sodium hydroxide gives the crystalline N^{α}-benzyloxycarbonyl derivative. This compound forms a crystalline *p*-nitrophenyl ester on treatment with *p*-nitrophenol and N,N′-dicyclohexylcarbodiimide.[8] β-(Pyrazolyl-1)-alanine (I) affords a crystalline N^{α}-*t*-butoxycarbonyl derivative with *t*-butyl azidoformate[9] and magnesium oxide in dioxane–water.

The chemistry of β-(pyrazolyl-3)-alanine (II) (Scheme I) is more complex than that of β-(pyrazolyl-1)-alanine since this unnatural amino acid contains a readily substitutable hydrogen on the pyrazole ring. When β-(pyrazolyl-3)-alanine is treated with 1 equiv of benzyl chloroformate in aqueous sodium hydroxide, the crystalline N^{α}-benzyloxycarbonyl derivative III is formed.

Methyl N^{α}-benzyloxycarbonyl-β-(pyrazolyl-3)-alaninate (V) is obtained as an oil from methyl β-(pyrazolyl-3)-alaninate (IV) and benzyl chloroformate in chloroform containing triethylamine.

(1) The authors wish to express their appreciation to the U. S. Public Health Service for generous support of this investigation.

(2) Except for glycine the amino acid residues in the various peptides and peptide derivatives are of the L variety.

(3) The following abbreviations are used: BOC = *t*-butoxycarbonyl; Z = benzyloxycarbonyl; ONP = *p*-nitrophenylate; ONHS = N-hydroxysuccinimido; Met→O = methionine *d*-sulfoxide; Pyr(3)Ala = β-(pyrazolyl-3)-alanine; Pyr(1)Ala = β-(pyrazolyl-1)-alanine; DCC = N,N′-dicyclohexylcarbodiimide; DMF = dimethylformamide; CMC = carboxymethylcellulose; TEA = triethylamine; AP-M = aminopeptidase M [G. Pfleiderer, P. G. Celliers, M. Stanulovic, E. D. Wachsmuth, H. Determann, and G. Braunitzer, *Biochem. Z.*, **340**, 552 (1964)].

(4) K. Hofmann and H. Bohn, *J. Amer. Chem. Soc.*, **88**, 5914 (1966).

(5) F. M. Finn and K. Hofmann, *ibid.*, **89**, 5298 (1967).

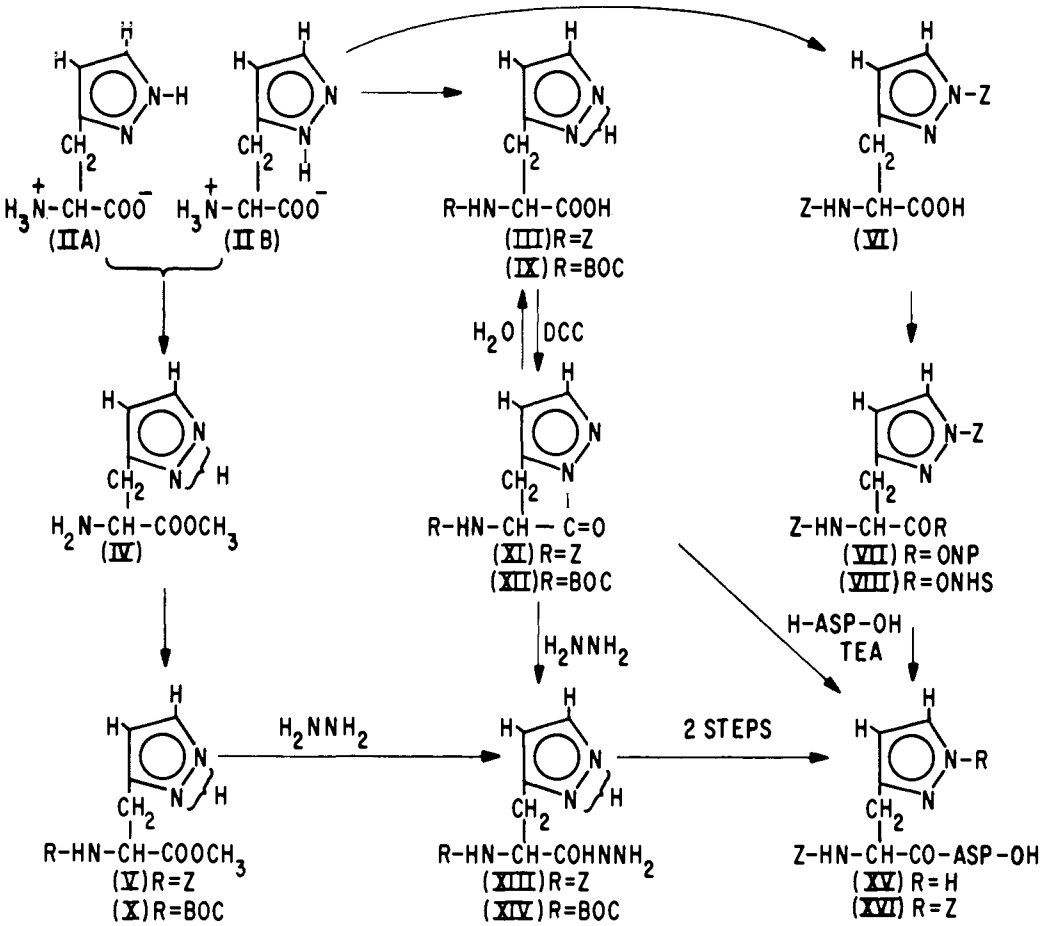
(6) K. Hofmann, H. Bohn, and R. Andreatta, *ibid.*, **89**, 7126 (1967).

(7) K. Hofmann, R. Andreatta, J. P. Buckley, W. E. Hageman, and A. P. Shapiro, *ibid.*, **90**, 1654 (1968).

(8) (a) D. F. Elliott and D. W. Russell, *Biochem. J.*, **66**, 49P (1957); (b) M. Rothe and F. W. Kunitz, *Ann. Chem.*, **608**, 88 (1957).

(9) L. A. Carpino, C. A. Giza, and B. A. Carpino, *J. Amer. Chem. Soc.*, **81**, 955 (1959).

Scheme I



Exposure of II to an excess of benzyl chloroformate in aqueous sodium carbonate affords the N^{α},N^{pyr}-dibenzyloxycarbonyl derivative which thus far has resisted crystallization.

Since β-(pyrazolyl-3)-alanine can exist in the form of the two tautomeric structures IIA and IIB, the N^{pyr}-benzyloxycarbonyl group may be located on either the 1 or the 2 position of the ring. In analogy to histidine where the 1 position on the imidazole ring is more hindered than the 3 position, we have assigned structure VI to the dibenzyloxycarbonyl derivative. This assignment conforms to that proposed for the N^{im}-benzyloxycarbonyl group in N^{α},N^{im}-dibenzyloxycarbonylhistidine by Patchornik, *et al.*[10] N^{α},N^{pyr}-Dibenzyloxycarbonyl-β-(pyrazolyl-3)-alanine appears to possess more stability than N^{α},N^{im}-dibenzyloxycarbonylhistidine. Samples have been stored at room temperature for more than 2 years without showing signs of decomposition.

The crystalline *p*-nitrophenyl ester of N^{α},N^{pyr}-dibenzyloxycarbonyl-β-(pyrazolyl-3)-alanine (VII) is readily prepared by the customary procedure.[8] The N-hydroxysuccinimide ester VIII of VI is amorphous. On exposure to *t*-butyl azidoformate and magnesium oxide in aqueous dioxane, β-(pyrazolyl-3)-alanine forms the crystalline N^{α}-*t*-butoxycarbonyl derivative IX. Even in the presence of a large excess of acylating component, formation of a di-*t*-butoxycarbonyl derivative is not observed. Methyl N^{α}-*t*-butoxycarbonyl-β-(pyrazolyl-3)-alaninate (X) is obtained in crystalline form from methyl β-(pyrazolyl-3)-alaninate (IV)[4] and *t*-butyl azidoformate in pyridine solution containing TEA.

With N,N′-dicyclohexylcarbodiimide under anhydrous conditions both N^{α}-benzyloxycarbonyl-β-(pyrazolyl-3)-alanine (III) and N^{α}-*t*-butoxycarbonyl-β-(pyrazolyl-3)-alanine (IX) form the crystalline, optically active anhydro compounds XI and XII.

An analogous optically active anhydro compound in the histidine series was obtained by Sheehan, *et al.*,[11] when they exposed N^{α}-*p*-nitrobenzyloxycarbonyl-L-histidine to the action of N,N′-diisopropylcarbodiimide in dioxane. This anhydro compound reacts with benzylamine to give the benzylamide of N^{α}-*p*-nitrobenzyloxycarbonylhistidine.

Refluxing with water for several hours converts the anhydro compound XI into N^{α}-benzyloxycarbonyl-β-(pyrazolyl-3)-alanine (III). The melting point and optical rotation of this material agree with those of the benzyloxycarbonyl derivative prepared directly from β-(pyrazolyl-3)-alanine. The amorphous hydrazides of N^{α}-benzyloxycarbonyl- (XIII) and of N^{α}-*t*-butoxycarbonyl-β-(pyrazolyl-3)-alanine (XIV) were prepared by the reaction of hydrazine with either the respective methyl esters or the anhydro compounds XI and XII. Apparently cyclization and ring opening proceed without racemization.

Having explored some aspects of the chemistry of I and II we turned to the preparation of peptides

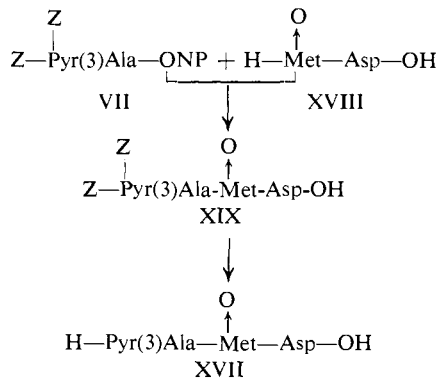
(10) A. Patchornik, A. Berger, and E. Katchalski, *J. Amer. Chem. Soc.*, 79, 6416 (1957).

(11) J. C. Sheehan, K. Hasspacher, and Y. L. Yeh, *ibid.*, 81, 6086 (1959).

containing these amino acids. Coupling *via* the Rudinger procedure[12] of N^{α}-benzyloxycarbonyl-β-(pyrazolyl-3)-alanine azide with triethylammonium aspartate affords crystalline N^{α}-benzyloxycarbonyl-β-(pyrazolyl-3)-alanylaspartic acid (XV). This compound is also obtained in poor yield when the anhydro compound XI reacts with aspartic acid in aqueous DMF containing triethylamine. Amorphous N^{α},N^{pyr}-dibenzyloxycarbonyl-β-(pyrazolyl-3)-alanylaspartic acid (XVI) ensues when *p*-nitrophenyl N^{α},N^{pyr}-dibenzyloxycarbonyl-β-(pyrazolyl-3)-alaninate (VII) reacts with triethylammonium aspartate in aqueous DMF. Both protected dipeptides are readily deblocked by hydrogenolysis to give the crystalline β-(pyrazolyl-3)-alanylaspartic acid.

The *d*-sulfoxide of β-(pyrazolyl-3)-alanylmethionylaspartic acid (XVII), an intermediate for the synthesis of 12-β-(pyrazolyl-3)-alanine S-peptide$_{1-14}$,[5] was prepared (Scheme II) in crystalline form from *p*-nitrophenyl N^{α},N^{pyr}-dibenzyloxycarbonyl-β-(pyrazolyl-3)-alaninate (VII) and triethylammonium methionylaspartate *d*-sulfoxide (XVIII) followed by deblocking of the ensuing protected intermediate XIX by reduction with sodium in liquid ammonia. The observation that sodium in liquid ammonia reduction does not reduce the sulfoxide substantiates previous findings of Iselin.[13]

Scheme II



The pentapeptide β-(pyrazolyl-3)-alanylphenylalanylaryginyltryptophylglycine, a key intermediate for the synthesis of [5-glutamine,6-β-(pyrazolyl-3)-alanine]-β-corticotropin$_{1-20}$amide,[6] was prepared by coupling *p*-nitrophenyl N^{α},N^{pyr}-dibenzyloxycarbonyl-β-(pyrazolyl-3)-alaninate (VII) with phenylalanylarginyltryptophylglycine[14] and deblocking the protected intermediate by hydrogenolysis. N-Hydroxysuccinimido-N^{α},N^{pyr}-dibenzyloxycarbonyl-β-(pyrazolyl-3)-alaninate (VIII) has also been employed to acylate phenylalanylarginyltryptophylglycine.

For the synthesis of β-(pyrazolyl-1)-alanylphenylalanylarginyltryptophylglycine, phenylalanylarginyltryptophylglycine was acylated with *p*-nitrophenyl N^{α}-benzyloxycarbonyl-β-(pyrazolyl-1)-alaninate, and the protected intermediate was decarbobenzoxylated by hydrogenolysis. Both pentapeptides are completely digestible by aminopeptidase M (AP-M).

(12) J. Honzl and J. Rudinger, *Collect. Czech. Chem. Commun.*, **26**, 2333 (1961).
(13) B. Iselin, *Helv. Chim. Acta*, **44**, 61 (1961).
(14) K. Hofmann and S. Lande, *J. Amer. Chem. Soc.*, **83**, 2286 (1961).

Discussion

Despite marked differences in the acid–base characteristics of their ring portions, histidine and β-(pyrazolyl-3)-alanine exhibit rather similar bevavior[15] in those situations which are of importance to peptide chemistry.

Like histidine, β-(pyrazolyl-3)-alanine forms a mono- and a dibenzyloxycarbonyl derivative. Exposure to carbodiimides in anhydrous solvents converts the N^{α}-monobenzyloxycarbonyl derivatives into optically active anhydro compounds. The anhydro compound of N^{α}-benzyloxycarbonyl-β-(pyrazolyl-3)-alanine regenerates the starting material on refluxing with water and forms the hydrazide when treated with hydrazine. With triethylammonium aspartate in aqueous DMF N^{α}-benzyloxycarbonyl-β-(pyrazolyl-3)-alanylaspartic acid is obtained in poor yield. The anhydro compound of N^{α}-*p*-nitrobenzyloxycarbonylhistidine reacts with benzylamine to give the benzylamide.

The methyl esters of the N^{α}-benzyloxycarbonyl derivatives of histidine and of β-(pyrazolyl-3)-alanine form normal hydrazides when exposed to hydrazine and these, *via* the corresponding azides, can be coupled with amino acids and peptides.

Ring-acylated derivatives of β-(pyrazolyl-3)-alanine appear to exhibit considerably more stability than do corresponding derivatives in the histidine series. The dibenzyloxycarbonyl derivatives of histidine and β-(pyrazolyl-3)-alanine can be converted into "active" esters. Our experience suggests the crystalline *p*-nitrophenyl ester of N^{α},N^{pyr}-dibenzyloxycarbonyl-β-(pyrazolyl-3)-alanine (VII) as the most useful compound for the synthesis of peptides containing β-(pyrazolyl-3)-alanine. This compound can be readily prepared and its reaction with peptides proceeds cleanly and in good yields. The nonreactivity with azides of the pyrrole nitrogen in β-(pyrazolyl-3)-alanine and the inertness of the pyrazole ring toward nitrite open the way to the synthesis of peptides containing this amino acid by the azide method without ring protection.

Experimental Section[16]

N^{α}-Benzyloxycarbonyl-β-(pyrazolyl-1)-alanine. Benzyl chloroformate (1 ml) and 4 *N* sodium hydroxide (1.94 ml) were added to an ice-cold solution of β-(pyrazolyl-1)-alanine (1.0 g)[4] in 4 *N* sodium hydroxide (1.62 ml) in three equal portions over a period of 20 min. The mixture was stirred for 30 min at ice-bath temperature and was then extracted with ether. The aqueous phase was acidified to congo red with 6 *N* hydrochloride acid and extracted with ethyl acetate. The ethyl acetate extract was washed in the usual manner, dried over sodium sulfate, and evaporated. The solid

(15) For a recent review of histidine chemistry, see E. Schröder and K. Lübke, "The Peptides," Vol. 1, Academic Press, New York, N.Y., 1965, p 177.
(16) Melting points are uncorrected. Rotations were determined with a Zeiss precision polarimeter. Measurements were carried out with a mercury lamp at 546 and 578 mμ and extrapolated to the 589-mμ sodium line. Elemental analyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y. The amino acid composition of acid and enzymic hydrolysates was determined with a Beckman-Spinco Model 120 amino acid analyzer according to the method of S. Moore, D. H. Spackman, and W. H. Stein, *Anal. Chem.*, 30, 1185 (1958). Paper chromatograms were performed on Whatman No. 1 filter paper by the descending technique with the following solvent systems: R_f^{I}, 1-butanol–acetic acid–water, 4:1:5 (upper phase); R_f^{3}, 1-butanol–pyridine–acetic acid–water, 45:30:9:36. Thin layer chromatograms were performed with the following solvent systems: R_f^{I}, 1-butanol–acetic acid–water, 60:20:20; R_f^{III}, 1-butanol–pyridine–acetic acid–water 30:20:6:24. AP-M digests were performed as described in K. Hofmann, F. M. Finn, M. Limetti, J. Montibeller, and G. Zanetti, *J. Amer. Chem. Soc.*, **88**, 3633 (1966).

residue was recrystallized from ethyl acetate as needles, 1.7 g (91%), mp 170–171°; $[\alpha]^{25}D$ −53.6° (*c* 1.0, DMF).

Anal. Calcd for $C_{14}H_{15}O_4N_3$: C, 58.1; H, 5.2; N, 14.5. Found: C, 58.1; H, 5.2; N, 14.6.

***p*-Nitrophenyl N$^{\alpha}$-Benzyloxycarbonyl-β-(pyrazolyl-1)-alaninate.** DCC (310 mg) was added to an ice-cold solution of N$^{\alpha}$-benzyloxycarbonyl-β-(pyrazolyl-1)-alanine (435 mg) and *p*-nitrophenol (250 mg) in 15 ml of a 2:1 mixture of dioxane–ethyl acetate, and the solution was stirred at ice-bath temperature for 2 hr. After standing for 16 hr at room temperature the suspension was filtered; the filtrate was evaporated to dryness *in vacuo* and the residue dissolved in ethyl acetate (20 ml). The solution was kept at 0° for a few hours, then the precipitate was removed by filtration, and the solution was evaporated to dryness. The solid residue was recrystallized twice from ethanol, 486 mg (79%), mp 128–129°; $[\alpha]^{26}D$ −44.6° (*c* 1.0, DMF).

Anal. Calcd for $C_{20}H_{18}O_6N_4$: C, 58.5; H, 4.4; N, 13.7. Found: C, 58.6; H, 4.5; N, 13.4.

N$^{\alpha}$-*t*-Butoxycarbonyl-β-(pyrazolyl-1)-alanine. A solution of *t*-butyl azidoformate (900 mg) in dioxane (4.5 ml) was added to a suspension of magnesium oxide (240 mg) in water (4 ml) containing β-(pyrazolyl-1)-alanine (465 mg), and the mixture was stirred at 45° for 20 hr. Water (45 ml) was then added, and the mixture was extracted with three 25-ml portions of ethyl acetate. The aqueous phase was filtered, cooled at 0°, acidified at pH 4 with 10% citric acid, and extracted with three 50-ml portions of ethyl acetate. The organic phases were washed with saturated sodium chloride, dried over sodium sulfate, and evaporated to dryness *in vacuo*. The solid was washed with ether and recrystallized from ethyl acetate, 418 mg (55%), mp 145° dec; $[\alpha]^{25}D$ −32.5° (*c* 1.0, MeOH).

Anal. Calcd for $C_{11}H_{17}O_4N_3$: C, 51.8; H, 6.7; N, 16.5. Found: C, 51.9; H, 6.7; N, 16.3.

N$^{\alpha}$-Benzyloxycarbonyl-β-(pyrazolyl-3)-alanine (III). a. By the Standard Procedure. Benzyl chloroformate (2 ml) and 4 *N* sodium hydroxide (3.87 ml) were added to an ice-cold solution of β-(pyrazolyl-3)-alanine (2.0 g)[4] in 2 *N* sodium hydroxide (6.48 ml) over a period of 20 min. The mixture was stirred at 0° for 1 hr and was then extracted with ether. The aqueous phase was cooled to 0°, acidified to congo red with 6 *N* hydrochloric acid, and extracted with ethyl acetate. The ethyl acetate extracts were washed with water, dried over sodium sulfate, and evaporated to dryness. The solid residue was recrystallized from water as needles, 2.28 g (61%), mp 138–139°; $[\alpha]^{25}D$ −29.6° (*c* 1.22, DMF); R_f^I 0.75.

Anal. Calcd for $C_{14}H_{15}O_4N_3$: C, 58.1; H, 5.2; N, 14.5. Found: C, 58.3; H, 5.4; N, 14.8.

The dicyclohexylammonium salt was prepared by adding dicyclohexylamine (0.1 ml) to III (100 mg) in ethyl acetate (3 ml). The gelatinous precipitate was collected, washed with ice-cold ethyl acetate and ether, and dried; yield, 163 mg, mp 182–183° dec; $[\alpha]^{25}D$ +20.7° (*c* 2.69, EtOH).

Anal. Calcd for $C_{26}H_{33}O_4N_4$: C, 66.4; H, 8.1; N, 11.9; O, 13.6. Found: C, 66.4; H, 8.4; N, 11.9; O, 13.5.

b. By Hydrolysis of the Anhydro Compound XI. Water (10 ml) was added to anhydro-N$^{\alpha}$-benzyloxycarbonyl-β-(pyrazolyl-3)-alanine (136 mg), and the mixture was refluxed until all of the starting material had dissolved (~4 hr). Upon cooling of the solution, the desired product crystallized. The mixture was placed in a refrigerator for 1 hr, and the solid was collected and washed with a small amount of ice–water; yield (needles from water), 121 mg (83%), mp 138–139°; mixture melting point with authentic material gave no depression; $[\alpha]^{27}D$ −28.5° (*c* 2.05, DMF).

Anhydro-N$^{\alpha}$-benzyloxycarbonyl-β-(pyrazolyl-3)-alanine (XI). DCC (1.07 g) was added with stirring to an ice-cold solution of N$^{\alpha}$-benzyloxycarbonyl-β-(pyrazolyl-3)-alanine (1.50 g) in ethyl acetate (50 ml). The mixture was stirred at 0° for 2 hr and at room temperature for 1 hr, and the N,N′-dicyclohexylurea was removed by filtration. The filtrate was evaporated to dryness and the oily residue solidified when kept in a refrigerator for 12 hr. The solid was washed with ethyl acetate and ether and recrystallized from ethyl acetate, 972 mg (69%), mp 160–162°; $[\alpha]^{25}D$ −39.5° (*c* 1.8, DMF).

Anal. Calcd for $C_{14}H_{13}O_3N_3$: C, 62.0; H, 4.8; N, 15.5. Found: C, 62.2; H, 4.8; N, 15.8.

N$^{\alpha}$-*t*-Butoxycarbonyl-β-(pyrazolyl-3)-alanine (IX). A solution of *t*-butyl azidoformate (9 ml) in dioxane (25 ml) was added to a suspension of magnesium oxide (2.2 g) in water (25 ml) containing β-(pyrazolyl-3)-alanine (2.0 g), and the mixture was stirred for 3 days at 30–35°. Water (50 ml) was added; the suspension was filtered, and the filtrate was extracted with three 50-ml portions of ethyl acetate. The aqueous phase was cooled at 0°, acidified to pH 3 with ice-cold 10% citric acid, and extracted with ethyl acetate. The ethyl acetate extracts were washed with saturated sodium chloride and dried over sodium sulfate, and the solvent was removed *in vacuo*. The solid was recrystallized from ethyl acetate, 2.72 g (82%), mp 163–164°; $[\alpha]^{24}D$ −22.6° (*c* 6.2, DMF); R_f^I 0.70.

Anal. Calcd for $C_{11}H_{17}O_4N_3$: C, 51.8; H, 6.7; N, 16.5. Found: C, 51.9; H, 6.8; N, 16.2.

Anhydro N$^{\alpha}$-*t*-Butoxycarbonyl-β-(pyrazolyl-3)-alanine (XII). DCC (1.62 g) was added with stirring to an ice-cold solution of N$^{\alpha}$-*t*-butoxycarbonyl-β-(pyrazolyl-3)-alanine (2.0 g) in 300 ml of a 1:1 mixture of dioxane and ethyl acetate and stirring was continued at 0° for 2 hr. The suspension was kept in a refrigerator for 20 hr; the N,N′-dicyclohexylurea was removed by filtration, and the filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in ethyl acetate; the solution was chilled, and undissolved N,N′-dicyclohexylurea was removed by filtration. The filtrate was evaporated to dryness, and the residue was washed with ether and dried. Recrystallization from ethyl acetate gave rosettes of needles, 1.43 g (77%), mp 187–189°; $[\alpha]^{23}D$ −41.0° (*c* 6.0, DMF); $[\alpha]^{25}D$ −12.5° (*c* 2.14, MeOH), rotation unchanged after standing for 3.5 hr at room temperature in methanol.

Anal. Calcd for $C_{11}H_{15}O_3N_3$: C, 55.7; H, 6.4; N, 17.7. Found: C, 55.6; H, 6.6; N, 17.2.

Methyl N$^{\alpha}$-*t*-Butoxycarbonyl-β-(pyrazolyl-3)-alaninate (X). TEA (3.01 ml) was added to an ice-cold solution of methyl β-(pyrazolyl-3)-alaninate dihydrochloride (2.29 g)[4] in pyridine (25 ml). This was followed by *t*-butyl azidoformate (4 ml), and the solution was stirred at 37° for 48 hr. The solvent was removed *in vacuo*, the residue dissolved in ice-cold ethyl acetate (100 ml), and the solution washed with three 100-ml portions of ice-cold 10% citric acid, three 100-ml portions of ice-cold 1 *N* sodium bicarbonate, and saturated sodium chloride. The oil which was obtained on evaporation of the ethyl acetate was evaporated twice with petroleum ether and was then kept under petroleum ether in a refrigerator until crystallization occurred. The compound was recrystallized from a mixture of ether and petroleum ether; colorless plates, 1.82 g (71%), mp 105–106°; $[\alpha]^{26}D$ −27.5° (*c* 1.72, DMF).

Anal. Calcd for $C_{12}H_{19}O_4N_3$: C, 53.5; H, 7.1; N, 15.6. Found: C, 53.7; H, 7.3; N, 15.8.

N$^{\alpha}$,N^{pyr}-Dibenzyloxycarbonyl-β-(pyrazolyl-3)-alanine (VI). β-(Pyrazolyl-3)-alanine (2.96 g) was dissolved in 0.5 *N* sodium hydroxide (39 ml), and sodium carbonate monohydrate (5.95 g) was added. After cooling to 0°, benzyl chloroformate (6.8 ml) was added in five portions over a period of 1 hr with vigorous stirring. The mixture was stirred for an additional 30 min at room temperature; water (50 ml) was added to dissolve the oily precipitate, and the solution was extracted with ether. The aqueous phase was cooled at 0° and acidified to congo red with ice-cold 6 *N* hydrochloric acid, and the precipitate was extracted into ethyl acetate. The ethyl acetate extracts were washed with saturated sodium chloride and dried over sodium sulfate, and the solvent was removed. The resulting gelatinous residue was washed with cold ether containing 15% petroleum ether (bp 30–60°) and dried, 7.80 g (96%), mp 104–106°; $[\alpha]^{24}D$ −31.1° (*c* 3.2, DMF).

Anal. Calcd for $C_{22}H_{21}O_6N_3$: C, 62.4; H, 5.0; N, 9.9; O, 22.7. Found: C, 62.6; H, 5.2; N, 10.1; O, 23.2.

***p*-Nitrophenyl N$^{\alpha}$,N^{pyr}-Dibenzyloxycarbonyl-β-(pyrazolyl-3)-alaninate (VII).** DCC (4.95 g) was added to an ice-cold solution of N$^{\alpha}$,N^{pyr}-dibenzyloxycarbonyl-β-(pyrazolyl-3)-alanine (10.16 g) and *p*-nitrophenol (4.0 g) in ethyl acetate (200 ml). After stirring for 1 hr at 0° and 1 hr at room temperature, a few drops of glacial acetic acid were added, and the mixture was chilled. The N,N′-dicyclohexylurea was removed by filtration, and the solvent was evaporated. The residue was washed with ice-cold ethanol and ether and dried; yield, 10.35 g as silky needles from ethanol (79%), mp 156–158°; $[\alpha]^{26}D$ −18.0° (*c* 1.80, DMF).

Anal. Calcd for $C_{28}H_{24}O_8N_4$: C, 61.8; H, 4.4; N, 10.3. Found: C, 61.8; H, 4.4; N, 10.2.

N-Hydroxysuccinimido-N$^{\alpha}$,N^{pyr}-dibenzyloxycarbonyl-β-(pyrazolyl-3)-alaninate (VIII). DCC (413 mg) was added with stirring to an ice-cold solution of N$^{\alpha}$,N^{pyr}-dibenzyloxycarbonyl-β-(pyrazolyl-3)-alanine (847 mg) and N-hydroxysuccinimide (253 mg) in ethyl acetate (20 ml). The mixture was stirred for 1 hr at 0° and 1 hr at room temperature, and the N,N′-dicyclohexylurea was removed by filtration. Evaporation of the solvent gave an amorphous solid which was washed with ether and dried, 979 mg (94%); $[\alpha]^{24}D$ −43.8° (*c* 4.2, DMF).

Anal. Calcd for $C_{26}H_{24}O_8N_4$: C, 60.0; H, 4.6; N, 10.8. Found: C, 60.4; H, 4.8; N, 11.0.

N^{α}-Benzyloxycarbonyl-β-(pyrazolyl-3)-alanine Hydrazide (XIII). a. From the Methyl Ester with Hydrazine. TEA (2.17 ml) was added to an ice-cold suspension of methyl β-(pyrazolyl-3)-alaninate dihydrochloride monohydrate[4] (1.61 g) in chloroform (13 ml) to give a clear solution. Benzyl chloroformate (0.68 ml) was added with stirring and stirring was contined for 2 min when TEA (0.87 ml) was added followed by an additional 0.68 ml of benzyl chloroformate. Stirring was continued for 30 min at room temperature when the solution was evaporated to dryness *in vacuo*. The residue was dissolved in ice-cold ethyl acetate (150 ml) and the solution was extracted with three 20-ml portions of ice-cold 10% citric acid followed by three 20-ml portions of 1 *N* sodium bicarbonate. The ethyl acetate layer was dried over sodium sulfate and evaporated to dryness *in vacuo*. The ensuing oil was dissolved in methanol (5 ml) and hydrazine hydrate (0.62 ml) was added. After standing at room temperature for 24 hr the solvent was removed, and the residue was dried over concentrated sulfuric acid. The material was dissolved in ethyl acetate (500 ml); the solution was washed with three 20-ml portions of 1 *N* sodium bicarbonate and dried over sodium sulfate, and the solvent was removed. The amorphous hydrazide was dissolved in dioxane–water, and the solution was lyophilized, 1.21 g (60%); $[\alpha]^{23}D$ −10.6° (*c* 2.42, 80% DMF).

Anal. Calcd for $C_{14}H_{17}O_3N_5$: C, 55.4; H, 5.7; N, 23.1. Found: C, 55.4; H, 5.7; N, 23.4.

b. From the Anhydro Compound XI with Hydrazine. Hydrazine hydrate (0.25 ml) was added to a methanol solution (10 ml) containing the anhydro compound XI (300 mg), and the solution was kept at room temperature for 24 hr. The solvent was removed; the residue was dried over concentrated sulfuric acid and dissolved in ethyl acetate (100 ml), and this solution was extracted with three 10-ml portions of 1 *N* sodium bicarbonate. The ethyl acetate extract was dried over sodium sulfate; the solvent was evaporated and the residue lyophilized from water, 328 mg (98%); $[\alpha]^{23}D$ −12.0° (*c* 2.32, 80% aqueous DMF).

Anal. Calcd for $C_{14}H_{17}O_3N_5$: C, 55.4; H, 5.7; N, 23.1. Found: C, 55.5; H, 5.9; N, 23.0.

N^{α}-*t*-Butoxycarbonyl-β-(pyrazolyl-3)-alanine Hydrazide (XIV). a. From the Methyl Ester with Hydrazine. Hydrazine hydrate (1.35 ml) was added to a solution of methyl N^{α}-*t*-butoxycarbonyl-β-(pyrazolyl-3)-alaninate (X) (1.8 g) in methanol (10 ml), and the solution was kept at room temperature for 24 hr. The solvent was evaporated; the residue was kept *in vacuo* over concentrated sulfuric acid for 12 hr and was then lyophilized from water to give an amorphous powder, 1.8 g (100%); $[\alpha]^{25}D$ −4.2° (*c* 9.5, DMF); $[\alpha]^{24}D$ +1.8° (*c* 3.4, EtOH).

Anal. Calcd for $C_{11}H_{19}O_3N_5$: C, 49.1; H, 7.1; N, 26.0. Found: C, 48.8; H, 7.2; N, 25.9.

b. From the Anhydro Compound XII with Hydrazine. Anhydro-N^{α}-*t*-butoxycarbonyl-β-(pyrazolyl-3)-alanine (XII) (300 mg) was added to an ice-cold solution of hydrazine hydrate (0.25 ml) in methanol (10 ml), and the solution was kept at 0° for 2 hr and at 4° for 24 hr. The solvent was evaporated *in vacuo;* the residue was dried for 12 hr over concentrated sulfuric acid and was then dissolved in ice-cold ethyl acetate (100 ml). The ethyl acetate solution was washed with three 10-ml portions of ice-cold 1 *N* sodium bicarbonate, dried over sodium sulfate, and evaporated to dryness *in vacuo*. The solid residue was lyophilized from water, 297 mg (87%); $[\alpha]^{25}D$ −4.2° (*c* 3.19, DMF); $[\alpha]^{23}D$ +2.0° (*c* 4.35, EtOH).

Anal. Calcd for $C_{11}H_{19}O_3N_5$: C, 49.1; H, 7.1; N, 26.0. Found: C, 49.2; H, 7.3; N, 26.0.

N^{α}-Benzyloxycarbonyl-β-(pyrazolyl-3)-alanylaspartic Acid Monohydrate (XV). a. From the Hydrazide XIII. *t*-Butyl nitrite (0.14 ml) was added to a stirred solution cooled at −25° of N^{α}-benzyloxycarbonyl-β-(pyrazolyl-3)-alanine hydrazide (XIII) (364 mg) in DMF (6 ml) containing 5.83 *N* hydrogen chloride in dioxane (0.62 ml). The mixture was stirred for 20 min at −25°, then cooled at −60°, and TEA (0.5 ml) was added. After 10 min a solution of aspartic acid (160 mg) in DMF–water 3:2 (5 ml) containing TEA (0.34 ml) was added, and the mixture was stirred at −10° for 1 hr, at 0° for 4 hr, and at 4° for 28 hr. The solution was evaporated to dryness *in vacuo;* the residue was dissolved in water (200 ml) and the solution applied to an AG1-X2 column (1.2 × 33 cm). The column was eluted with 150 ml of 10% acetic acid and 300 ml of 20% acetic acid. The 10% acetic acid eluates contained unreacted aspartic acid; the protected dipeptide was located in the 20% acetic acid eluates by the chlorine reaction. Fractions containing this material were pooled and evaporated to dryness, and the residue was recrystallized from water as fine needles, 264 mg (52%), mp 158–160°; $[\alpha]^{23}D$ −8.7° (*c* 3.04, 50% EtOH); R_f^{I} 0.56; amino acid ratios in acid hydrolysate: $Pyr(3)Ala_{0.98}\ Asp_{1.02}$.

Anal. Calcd for $C_{18}H_{20}O_7N_4 \cdot H_2O$: C, 51.2; H, 5.3; N, 13.3. Found: C, 51.6; H, 5.4; N, 13.2.

b. From the Anhydro Compound XI. A solution of aspartic acid (80 mg) in water (2 ml) and TEA (0.2 ml) was added to an ice-cold solution of anhydro N^{α}-benzyloxycarbonyl-β-(pyrazolyl-3)-alanine (136 mg) in DMF (3 ml). The mixture was stirred at 0° for 1 hr, and another portion of DMF (1 ml) was added to get a clear solution. The reaction mixture was allowed to stand at room temperature for 20 hr, then the bulk of the solvent was removed *in vacuo*, and water (30 ml) was added to the residue. A very small amount of insoluble material was removed by filtration, and the solution was applied to a column of AG1-X2 (1 × 12 cm) which was eluted successively with water (150 ml) and aqueous acetic acid as follows: 5% (200 ml), 10% (200 ml), and 15% (200 ml). The 5% acetic acid eluates contained unreacted aspartic acid; the 10% acetic acid eluates contained N^{α}-benzyloxycarbonyl-β-(pyrazolyl-3)-alanine (61 mg), mp 138–139°, identical with authentic sample. The 15% acetic acid eluates which contained the desired material were evaporated, and the residue was recrystallized from water, 80 mg (38%), mp 157–159° (mixture melting point with authentic material showed no depression); $[\alpha]^{26}D$ −8.3° (*c* 2.10, 50% EtOH).

N^{α},N^{pyr}-Dibenzyloxycarbonyl-β-(pyrazolyl-3)-alanylaspartic Acid (XVI). Aspartic acid (666 mg) in 50% aqueous DMF (20 ml) containing TEA (1.4 ml) was added to an ice-cold solution of *p*-nitrophenyl N^{α},N^{pyr}-dibenzyloxycarbonyl-β-(pyrazolyl-3)-alaninate (2.72 g) in DMF (40 ml), and the mixture was stirred for 30 min at 0°. Additional ice-cold DMF (20 ml) was added to give a clear solution which was kept at room temperature for 24 hr. The solvent was evaporated; the residue was dissolved in 50% aqueous EtOH (150 ml), and the solution was applied to a column of AG1-X2 (3 × 19 cm). The column was eluted successively with 50% EtOH (400 ml) and acetic acid in 50% EtOH as follows: 1% (500 ml), 5% (500 ml), 10% (500 ml), and 15% (500 ml). The 15% acetic acid eluates containing the desired product were pooled, and the solvent was evaporated. Precipitation from ethyl acetate with ether gave an amorphous solid, 2.36 g (87%), mp 144–145°; $[\alpha]^{27}D$ −22.8° (*c* 1.98, DMF); R_f^{I} 0.73; R_f^{III} 0.60; single chlorine-positive spot; amino acid ratios in acid hydrolysate: $Pyr(3)\text{-}Ala_{0.99}Asp_{1.01}$.

Anal. Calcd for $C_{26}H_{26}O_9N_4$: C, 58.0; H, 4.9; N, 10.4; O, 26.8. Found: C, 58.0; H, 4.9; N, 10.3; O, 27.0.

β-(Pyrazolyl-3)-alanylaspartic Acid. a. From N^{α}-Benzyloxycarbonyl-β-(pyrazolyl-3)-alanylaspartic Acid (XV). The benzyloxycarbonyl derivative XV (81 mg) was hydrogenated in 50% aqueous MeOH (10 ml) over palladium. The catalyst was removed by filtration, and the solvent was evaporated. The residue was crystallized from water–EtOH as needles, 41 mg (80%), mp 201–203° dec; $[\alpha]^{24}D$ +13.0° (*c* 2.8, water); R_f^{1} 0.26; R_f^{3} 1.1 × His; R_f^{I} 0.33; R_f^{III} 0.36; single chlorine- and ninhydrin-positive spot; amino acid ratios in AP-M digest: $Pyr(3)Ala_{1.00}Asp_{1.00}$.

Anal. Calcd for $C_{10}H_{14}O_5N_4$: C, 44.4; H, 5.2; N, 20.7. Found: C, 44.7; H, 5.1; N, 20.5.

b. From N^{α},N^{pyr}-Dibenzyloxycarbonyl-β-(pyrazolyl-3)-alanylaspartic Acid (XVI). The dibenzyloxycarbonyl derivative XVI (2.0 g) was hydrogenated in 50% aqueous MeOH (50 ml) over palladium in the usual manner. The catalyst was removed by filtration; the filtrate was evaporated, and the residue was crystallized from water–EtOH as needles, 992 mg (99%), mp 202–203° dec; $[\alpha]^{27}D$ +14.8° (*c* 1.97, water); R_f^{1} 0.26; R_f^{3} 1.14 × His; R_f^{I} 0.33; R_f^{III} 0.36; single chlorine- and ninhydrin-positive spot.

N^{α},N^{pyr}-Dibenzyloxycarbonyl-β-(pyrazolyl-3)-alanylmethionylaspartic Acid *d*-Sulfoxide Methanol Solvate (XIX). To an ice-cold solution of *p*-nitrophenyl N^{α},N^{pyr}-dibenzyloxycarbonyl-β-(pyrazolyl-3)-alaninate (1.36 g) in DMF (25 ml) an ice-cold solution of methionylaspartic acid *d*-sulfoxide monohydrate (XVIII)[16] (746 mg) and TEA (0.7 ml) in 50% aqueous DMF (15 ml) was added while stirring. After a few minutes a white material started to precipitate. The reaction mixture was stirred for 1 hr at 0° and was then allowed to reach room temperature. DMF (25 ml) was added to dissolve the precipitate, and the clear solution was allowed to stand overnight at room temperature. The solvents were evaporated *in vacuo;* the oily residue was triturated with ethyl acetate (30 ml), ether (30 ml) was added, and the mixture was kept in a freezer for 1 hr. The colorless solid was collected, washed with several portions of ether, and dried. This material was dissolved in glacial acetic acid (10 ml) and precipitated by addition of water (125 ml). The mixture was kept in a refrigerator for 1 hr; the gelatinous material was collected and washed with two 20-ml portions of ice–water. The compound was dissolved in hot MeOH,

and the precipitate which formed on cooling was collected and dried, 1.31 g (73%), mp 155–157°; $[\alpha]^{25}D$ +19.0° (*c* 1.21, MeOH); single chlorine-positive spot with R_f^{I} 0.93; R_f^{I} 0.66; R_f^{III} 0.59.

Anal. Calcd for $C_{31}H_{37}O_{11}N_5S \cdot CH_3OH$: C, 53.6; H, 5.5; N, 9.8; S, 4.5. Found: C, 53.3; H, 5.4; N, 10.0; S, 4.4.

β-(Pyrazolyl-3)-alanylmethionylaspartic Acid *d*-Sulfoxide (XVII). N^{α},N^{pyr}-Dibenzyloxycarbonyl-β-(pyrazolyl-3)-alanylmethionylaspartic acid *d*-sulfoxide methanol solvate (3.03 g) was dissolved in sodium-dried liquid ammonia (approximately 150 ml), and sodium was added in small pieces with stirring until a permanent blue color remained. After 5 min Dowex-50 (ammonium cycle) (15 g) was added, and the ammonia was allowed to evaporate at room temperature. A slow stream of nitrogen was passed over the residue to remove the last traces of ammonia. The product was extracted with several portions of water containing a few drops of 10% ammonia (total volume approximately 250 ml), and the extracts were combined, filtered, and evaporated to dryness. The ensuing oily residue was dissolved in water (50 ml) and the solution applied to a column of AG1-X2 (3 × 20 cm). The resin was washed with water (approximately 250 ml), and the product was eluted with 0.25 *N* acetic acid (approximately 350 ml). Chlorine-positive fractions were pooled, and the solvent was evaporated. The peptide was crystallized by cooling and scratching. Recrystallization from water–EtOH gave 1.38 g (78%) of the desired product, mp above 236°, slow dec; $[\alpha]^{25}D$ +44.9° (*c* 3.12, water); single chlorine- and ninhydrin-positive spot with R_f^{I} 0.29; R_f^{I} 0.22; R_f^{III} 0.25; amino acid ratios in AP-M digest: $Pyr(3)Ala_{0.96}$-$Met{\rightarrow}O_{1.00}Asp_{1.04}$ (96%).

Anal. Calcd for $C_{17}H_{23}O_7N_5S$: C, 43.2; H, 5.6; N, 16.8; O, 26.8; S, 7.7. Found: C, 43.2; H, 5.8; N, 16.2; O, 27.2; S, 7.8.

N^{α},N^{pyr}-Dibenzyloxycarbonyl-β-(pyrazolyl-3)-alanylphenylalanylarginyltryptophylglycine Monohydrate. a. From the Tetrapeptide with *p*-Nitrophenyl N^{α},N^{pyr}-Dibenzyloxycarbonyl-β-(pyrazolyl-3)-alaninate. To an ice-cold solution of phenylalanylarginyltryptophylglycine acetate monohydrtae (2.25 g)[14] in DMF (100 ml) *p*-nitrophenyl N^{α},N^{pyr}-dibenzyloxycarbonyl-β-(pyrazolyl-3)-alaninate (VII) (2.5 g) and TEA (0.75 ml) were added. The reaction mixture was stirred for 1 hr at 0° and 24 hr at room temperature. Ethyl acetate (500 ml) was added; the precipitate was collected, washed with ethyl acetate, and dried. The material was dissolved in 90% acetic acid (60 ml) and the desired product precipitated by addition of water (200 ml). The gelatinous mass was filtered, washed with water, and dried *in vacuo* over KOH pellets, 3.40 g (94%); R_f^{I} 0.66; single chlorine- and Ehrlich-positive spot; $[\alpha]^{24}D$ −12.1° (*c* 1.34, 90% acetic acid).

Anal. Calcd for $C_{50}H_{55}O_{10}N_{11} \cdot H_2O$: C, 60.8; H, 5.8; N, 15.6; O, 17.8. Found: C, 61.0; H, 6.0; N, 15.4; O, 17.8.

b. From the Tetrapeptide with N-Hydroxysuccinimido-N^{α},N^{pyr}-dibenzyloxycarbonyl-β-(pyrazolyl-3)-alaninate. Phenylalanylarginyltryptophylglycine acetate monohydrate[14] (450 mg) was treated in DMF (20 ml) containing 0.15 ml of TEA with N-hydroxysuccinimido-N^{α},N^{pyr}-dibenzyloxycarbonyl-β-(pyrazolyl-3)-alaninate (VIII) (611 mg) in the manner described under a above; 536 mg (74%); $[\alpha]^{22}D$ −11.8° (*c* 1.65, 90% acetic acid); R_f^{I} 0.66; amino acid ratios in acid hydrolysate: $Pyr(3)Ala_{1.09}Phe_{0.99}Arg_{0.96}$-$Gly_{1.01}$.

Anal. Calcd for $C_{50}H_{55}O_{10}N_{11} \cdot H_2O$: C, 60.8; H, 5.8; N, 15.6. Found: C, 60.4; H, 5.8; N, 15.2.

β-(Pyrazolyl-3)-alanylphenylalanylarginyltryptophylglycine Dihydrate. A suspension of N^{α},N^{pyr}-dibenzyloxycarbonyl-β-(pyrazolyl-3)-alanylphenylalanylarginyltryptophylglycine hydrate (3.3 g) in glacial acetic acid–50% aqueous MeOH (1:1) (100 ml) was hydrogenated over palladium. The catalyst was removed by filtration; the solvents were evaporated, and the ensuing pink oil was dissolved in water (400 ml). This solution was added to a CMC column (4 × 28 cm) which was eluted successively with water (1000 ml) and the following pH 6.8 ammonium acetate buffers: 0.01 *M* (1000 ml), 0.02 *M* (1000 ml), and 0.025 *M* (3000 ml). Individual fractions, 50 ml each, were collected at a flow rate of 10 ml/min. The 0.025 *M* eluates exhibiting a single Ehrlich-positive spot on thin layer chromatography (R_f^{I} 0.29; R_f^{III} 0.51) were pooled, the bulk of the water was removed *in vacuo*, and the residue was lyophilized to constant weight from small volumes of water, 2.25 g (90%); $[\alpha]^{24}D$ −7.2° (*c* 1.90, 50% acetic acid); R_f^{I} 0.46; R_f^{I} 0.29; single chlorine-, ninhydrin-, Ehrlich-, and Sakaguchi-positive spot; amino acid ratios in AP-M digest: $Pyr(3)Ala_{0.89}$-$Phe_{1.03}Arg_{0.99}Trp_{1.08}Gly_{1.01}$.

Anal. Calcd for $C_{34}H_{43}O_6N_{11} \cdot 2H_2O$: C, 55.3; H, 6.4; N, 20.9; O, 17.3. Found: C, 55.0; H, 6.7; N, 20.6; O, 17.1.

N^{α}-Benzyloxycarbonyl-β-(pyrazolyl-1)-alanylphenylalanylarginyltryptophylglycine Monohydrate. To a stirred solution of phenylalanylarginyltryptophylglycine acetate monohydrate[14] (490 mg) in DMF (4 ml) and TEA (0.21 ml) was added at room temperature *p*-nitrophenyl N^{α}-benzyloxycarbonyl-β-(pyrazolyl-1)-alaninate (350 mg) dissolved in DMF (1 ml). A heavy precipitate began to form after 30 min, and this was redissolved by addition of 20 ml of DMF. The solution was kept at room temperature for 48 hr when the product was precipitated by addition of ethyl acetate (30 ml). The precipitate was collected, washed with ethyl acetate and 2% acetic acid, and dried. The protected peptide was purified by precipitation from 90% acetic acid (10 ml) with water (100 ml). The precipitate was collected and dried, 368 mg (56%); $[\alpha]^{28}D$ −18.5° (*c* 1.0, 90% acetic acid); R_f^{I} 0.90; R_f^{I} 0.70.

Anal. Calcd for $C_{42}H_{49}O_8N_{11} \cdot H_2O$: C, 59.1; H, 6.0; N, 18.0; O, 16.9. Found: C, 58.5; H, 6.1; N, 18.0; O, 16.9.

β-(Pyrazolyl-1)-alanylphenylalanylarginyltryptophylglycine Diacetate Monohydrate. The above protected peptide (275 mg) was hydrogenated over palladium in 90% acetic acid (25 ml). The catalyst was removed by filtration; the solvent was evaporated, and the residue was lyophilized from water.

The crude peptide was dissolved in water (400 ml) (addition of dioxane facilitated solubilization), and this solution was added to a column of CMC (3 × 30 cm). The column was then eluted with water (200 ml), 0.01 *M* ammonium acetate (500 ml), 0.02 *M* ammonium acetate (500 ml), and finally 0.025 *M* ammonium acetate (1000 ml). Absorption at 280 mμ served to locate the peptide in the 0.025 *M* eluates. The desired fractions were pooled, evaporated to dryness, and lyophilized to constant weight from dilute acetic acid, 147 mg (54%); $[\alpha]^{27}D$ +18.2° (*c* 0.66, 90% acetic acid); single Ehrlich-, ninhydrin-, Sakaguchi-, and chlorine-positive spot with R_f^{I} 0.71; R_f^{I} 0.48; amino acid ratios in acid hydrolysate: $Pyr(1)Ala_{1.03}Phe_{1.00}Arg_{0.97}Gly_{0.99}$; amino acid ratios in AP-M digest: $Pyr(1)Ala_{1.10}Phe_{1.00}Arg_{0.91}Trp_{0.28}Gly_{1.00}$.

Anal. Calcd for $C_{34}H_{43}O_6N_{11} \cdot 2CH_3COOH \cdot H_2O$: C, 54.3; H, 6.4; N, 18.3; O, 21.0. Found: C, 54.0; H, 6.3; N, 18.1; O, 20.9.

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