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# Syntheses of Peptides Related to the *N*-Terminal Structure of Corticotropin. VI. The Synthesis of Ser$^1$- and Gly$^1$-Decapeptides Corresponding to the First Ten-amino-acid Sequence of Corticotropin

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*t*-Butyloxycarbonyl-seryl-(and-glycyl-)tyrosyl-seryl-methionyl-glutamyl-histidyl-phenylalanyl-arginyl-tryptophyl-glycine have been synthesized as the first step of the synthesis of adrenocorticotropically active peptides. The trifluoroacetic acid treatment of these two compounds afforded the free decapeptides, seryl-tyrosyl-seryl-methionyl-glutamyl-histidyl-phenylalanyl-arginyl-tryptophyl-glycine, which corresponded to the amino-acid sequence of positions 1 to 10 in the corticotropin molecule, and its glycine$^1$-analog. Concerning the synthesis of serine peptides, a tetrapeptide derivative, corresponding to the first four amino acid residues of corticotropin, was obtained by using *O*-benzyl-serine.

In 1961 Hofmann and Yajima reported that a synthetic tridecapeptide amide, corresponding to the first thirteen amino acid residues of corticotropin (ACTH), exhibited a very low but reproducible ACTH activity.[1] Later Li and his collaborators synthesized a pentadecapeptide which had a sequence corresponding to positions 1—10 plus positions 15—19 of the corticotropin molecule, and the product was found to be active at a low level of potency.[2] From these facts it seemed that the amino terminal decapeptide portion might have a structure functionally essential for the hormonal activity, even if the decapeptide itself did not elicit any appreciable potency.

We have recently completed the synthesis of some biologically-active peptides related to the corticotropin molecule.[3-5] Each of them was obtained by connecting the amino terminal decapeptide fragment with the carboxyl terminal moiety of the molecule. During the course of these syntheses, we prepared the free decapeptide, corresponding to the first ten amino acid residues of corticotropin, and its analog, which had a glycine in place of the amino terminal serine; we prepared, that is, seryl-tyrosyl-seryl-methionyl-glutamyl-histidyl-phenylalanyl-arginyl-tryptophyl-glycine ($\alpha^{1-10}$-ACTH) (I) and glycyl-tyrosyl-seryl-methionyl-glutamyl-histidyl-phenylalanyl-arginyl-tryptophyl-glycine ($\alpha^{1-10}$-Gly$^1$-ACTH) (II).*1 As has been stated above, peptide I may provide a functional core for the ACTH activity, while its protected derivative is a key fragment for the synthesis of adrenocorticotropically-active peptides. In this respect, peptide II may be considered to be the same as peptide I, since the hydroxyl group of the terminal serine has been recognized as being nonessential for the activity.[6] We wish to describe here the synthesis of peptides I and II in detail. Concerning the synthesis of serine peptides, the use of *O*-benzylserine will also be described.

**The Synthesis of $\alpha^{1-10}$-Gly$^1$-ACTH (II).**— Serine was acylated by using 1.1—1.2 equivalent moles of *t*-butyl azidoformate[7,8] to obtain *t*-butyloxycarbonyl-serine (III), which was first isolated in the form of a dicyclohexylamine salt[9] in a 60 per cent yield. The dicyclohexylamine salt was then treated with Dowex-50 ($H^+$-form) in order to recover compound III, which crystallized as the monohydrate. The crystalline monohydrate became sirupy in a desiccator as a result of the immediate loss of water. When a 100 per cent excess of the azide was used for the above acylation, we could not obtain the crystalline III after all; it appeared most likely that the *O*-acylation of serine had occurred to some extent as a side reaction. The *N*, *N'*-dicyclohexylcarbodiimide (DCCl)-induced coupling[10] of III with the methionine

1) K. Hofmann and H. Yajima, *J. Am. Chem. Soc.*, **83**, 2289 (1961).
2) C. H. Li, J. Ramachandran and D. Chung, ibid., **86**, 2711 (1964); **85**, 1895 (1963).
3) H. Otsuka, K. Inouye, M. Kanayama and F. Shinozaki, This Bulletin, **38**, 679 (1965).
4) H. Otsuka, K. Inouye, M. Kanayama and F. Shinozaki, ibid., **38**, 1563 (1965).
5) H. Otsuka, K. Inouye, F. Shinozaki and M. Kanayama, *J. Biochem.* **58**, 512 (1965).
*1 All amino acid residues are of the L-configuration with the exception of glycine.
6) H. E. Lebovitz and F. L. Engel, *Endocrinol.*, **73**, 573 (1963).
7) L. A. Carpino, *J. Am. Chem. Soc.*, **79**, 98 (1957); L. A. Carpino, C. A. Giza and B. A. Carpino, ibid., **81**, 955 (1959).
8) K. Inouye, M. Kanayama and H. Otsuka, *J. Chem. Soc. Japan, Pure Chem. Sect.* (*Nippon Kagaku Zasshi*), **85**, 599 (1964).
9) E. Klieger, E. Schröder and H. Gibian, *Ann.*, **640**, 157 (1961); F. Weygand and K. Hunger, *Z. Naturforsch.*, **13b**, 50 (1958).
10) J. C. Sheehan and G. P. Hess, *J. Am. Chem. Soc.*, **77**, 1067 (1955).

Table I. Amino acid compositions in the leucine aminopeptidase digests of the decapeptides I and II

| Enzyme/substrate ratio (w/w) | Molar ratio (Tyr=1.00) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Decapeptide II | | | | | | | | | |
| | 1 Gly | 2 Tyr | 3 Ser | 4 Met | 5 Glu | 6 His | 7 Phe | 8 Arg | 9 Try | 10 Gly |
| 1/50* | 1.00 | 1.00† | 0.90 | 0.88 | 0.64 | 0.59 | 0.57 | 0.51 | 0.41 | 0.42 |
| 1/20** | 1.00 | 1.00 | 0.91 | 0.96 | (0.78) | 0.87 | 0.86 | 0·80 | 0.80 | 0.77 |
| 1/10** | 1.00 | 1.00 | 0.96 | 1.00 | 0.99 | 0.92 | 0.89 | (0.99) | (0.79) | 0.85 |
| | Decapeptide I | | | | | | | | | |
| | 1 Ser | 2 Tyr | 3 Ser | 4 Met | 5 Glu | 6 His | 7 Phe | 8 Arg | 9 Try | 10 Gly |
| 1/10** | 1.00 | 1.00†† | 0.93 | 0.94 | 0.96 | 0.94 | 0.87 | 0.83 | (0.76) | 0.86 |

* In 0.05 M "Tris" buffer (pH 7.85, 0.005 M $Mg^{2+}$) - dioxane (4 : 1) at 37°C for 24 hr.

** A solution of the substrate (ca. 1 μmol.) in 0.1 ml. of 0.1 N hydrochloric acid was neutralized with 0.1 N sodium hydroxide and to this were quickly added 0.5 ml. of 0.05 M "Tris" buffer (pH 7.85, 0.005 M $Mg^{2+}$) and the enzyme solution (1 mg./ml. in the same "Tris" buffer as above).*2 The mixture was then incubated at 37°C for 36 hr.

† Recovery 98%. †† Recovery 97%.

methyl ester yielded the *t*-butyloxycarbonyl-seryl-methionine methyl ester (IV) in a good yield (84%). Compound IV was treated with dry hydrogen chloride in ethyl acetate to give seryl-methionine methyl ester hydrochloride as an oil. In this case, it was found that the protective group was not split appreciably, even after 30 min. at room temperature, when anhydrous methanol was used as the solvent in place of ethyl acetate. We previously used a formyl group[11] for the protection of an amino function of the peptide containing a methionine residue.[12] The *t*-butyloxycarbonyl group,[7] which has recently become available, seems to be more advantageous than the formyl group, since the former unlike the latter,[11] usually offers no opportunity for the coupling procedures to be employed. The oily hydrochloride of the seryl-methionine methyl ester was converted into the free base (V), which then crystallized readily from ethyl acetate in a 80 per cent yield based on the IV used. The *t*-butyloxycarbonyl-glycyl-tyrosine methyl ester was easily obtained by the DCCl-mediated condensation of *t*-butyloxycarbonyl-glycine with the tyrosine methyl ester. The amorphous protected dipeptide ester, which was found to be homogeneous in thin-layer chromatography, was converted into the crystalline hydrazide (VI) in a 85 per cent yield based on the *t*-butyloxycarbonyl-glycine used. *t*-Butyloxycarbonyl-glycyl-tyrosine azide, which was derived from compound VI, was made to react with V in acetonitrile to give, in a moderate yield (62%), a crystalline-protected tetrapeptide *t*-butyloxycarbonyl - glycyl - tyrosyl - seryl - methionine methyl ester (VII). Compound VII was then converted into the corresponding hydrazide (VIII).

For the synthesis of a protected decapeptide, *t*-butyloxycarbonyl-glycyl - tyrosyl - seryl - methionyl-γ-*t*-butyl - glutamyl - histidyl - phenylalanyl - arginyl - tryptophyl-glycine (IX), compound VIII was treated with nitrous acid and the resultant azide was allowed to react with the hexapeptide, γ-*t*-butyl - glutamyl - histidyl - phenylalanyl - arginyl - tryptophyl-glycine, the synthesis of which has already been described.[13] The decapeptide (IX) was purified by reprecipitation from dimethylformamide-methanol in a yield of 63 per cent. The purified IX was found to be entirely homogeneous in thin-layer chromatography. The protective group of IX were removed by treatment with trifluoroacetic acid to liberate the decapeptide II ($\alpha^{1-10}$-Gly$^1$-ACTH). The synthetic peptide II gave a single spot ($R_f$=0.36) reactive to ninhydrin, Pauly, Ehrlich, Sakaguchi and methionine ($P_tI_6''$) reagents on paper in the *n*-butanol - acetic acid - pyridine - water (30 : 6 : 20 : 24 by volume) system.[14] Acid hydrolysis afforded a mixture of the constituent amino acids in the ratios expected by theory, with the exception of the tryptophan, which had partially decomposed with acid.[15] The intact peptide was determined spectrophotometrically to contain tyrosine and tryptophan in a molar ratio of 1 : 1.[16] The peptide II was also submitted to leucine aminopeptidase (LAP) digestion at various enzyme/substrate ratios. The results are presented in Table I, together with those obtained for peptide I. The table shows that the first half of the mol-

*2 Leucine aminopeptidase (LAP) Lot No. 5930, Worthington Biochemical Corporation, Freehold, New Jersey, U. S. A.

11) J. C. Sheehan and D-D. H. Yang, *J. Am. Chem. Soc.*, **80**, 1154 (1958).

12) K. Inouye and H. Otsuka, This Bulletin, **34**, 1 (1961).

13) K. Inouye, ibid., **38**, 1148 (1965).

14) S. G. Waley and J. Watson, *Biochem. J.*, **55**, 328 (1953).

15) D. H. Spackman, W. H. Stein and S. Moore. *Anal. Chem.*, **30**, 1191 (1958).

16) T. W. Goodwin and R. A. Morton, *Biochem. J.*, **40**, 628 (1946); G. H. Beavan and E. R. Holliday, *Advances in Protein Chemistry*, **7**, 319 (1952).

ecules could be digested completely, whereas their carboxyl terminal moieties seemed to be somewhat resistant to the action of the enzyme under the conditions used. The difficulty may have arisen from some inhibition by the accumulated products, since the hexapeptide, which was derived from γ-*t*-butyl-glutamyl-histidyl-phenylalanyl-arginyl-tryptophyl-glycine, an intermediate for the synthesis of peptides I and II, has been found to be completely digestible by the enzyme.[13] Schwyzer and Kappeler[20] described that their preparation of the decapeptide (I) was completely digested by LAP. However, the rate of the hydrolysis by LAP depends partly on the contamination of some endopeptidases, which may promote the digestion. It is, therefore, difficult to compare our results with their directly. The present data may, then, be considered to provide a proof of the optical homogeneity of our decapeptide preparations.

**The Synthesis of $\alpha^{1-10}$-ACTH (I).**—*t*-Butyloxycarbonyl-serine (III) was treated with diazomethane in order to obtain the corresponding methyl ester; the resultant oily ester was then converted into *t*-butyloxycarbonyl-serine hydrazide (X) in a yield of 88 per cent based on the III used. Iselin and Schwyzer reported the synthesis of X by another route, starting with the serine methyl ester.[17] The *t*-butyloxycarbonyl-seryl-tyrosine methyl ester (XI) was synthesized by the azide-coupling procedure from compound X and the tyrosine methyl ester in a moderate yield (66%). The melting point of our preparation agrees fairly well with the value reported for XI by Hofmann et al., but the optical rotation differs significantly from theirs.[18] The hydrazide (XII), which was derived from XI, had the same optical rotation as that given by them. Concerning the synthesis of XI, attempts were also made to achieve the DCCl-induced condensation of *t*-butyloxycarbonyl-serine (III) with the tyrosine methyl ester, but the reaction appeared so complicated that it was very difficult to isolate the pure XI from the medium. Concerning this point, it should be noted that Li et al. obtained the carbobenzoxy analog of XI in an excellent yield by the DCCl procedure.[19] *t*-Butyloxycarbonyl-seryl-tyrosine azide, which was derived from XII, was allowed to react with the seryl-methionine methyl ester (V) to yield a tetrapeptide derivative, the *t*-butyloxycarbonyl-seryl-tyrosyl-seryl-methionine methyl ester (XIII). Compound XIII failed to crystallize, but it was found to be homogeneous in thin-laryer chromatography. The optical rotation of our material agrees satisfactorily with the value described for XIII by Iselin and Schwyzer.[17] Compound XIII was then converted almost quantitatively into the crystalline hydrazide (XIV), the melting point and the optical rotation of which coincided fairly well with those reported in the literature.[17]

The protected tetrapeptide hydrazide (XIV) was transformed into the azide, and this was then coupled with γ-*t*-butyl-glutamyl-histidyl-phenylalanyl-arginyl-tryptophyl-glycine[13] to give a protected decapeptide *t*-butyloxycarbonyl-seryl-tyrosyl-seryl-methionyl-γ-*t*-butyl-glutamyl-histidyl-phenylalanyl-arginyl-tryptophyl-glycine (XV), which was found to be homogeneous in thin-layer chromatography after reprecipitation from dimethylformamide-methanol. The yield of the purified XV amounted to 80 per cent when the hydrazide XIV was used in a 50 per cent excess relative to the hexapeptide. Our preparation of XV showed a good agreement in the optical rotation with the substance as prepared by other investigators.[20,21] The free decapeptide I ($\alpha^{1-10}$-ACTH) was obtained by the trifluoroacetic acid treatment of compound XV. The peptide I was found to be homogeneous to ninhydrin, Pauly, Ehrlich, Sakaguchi and methionine ($P_tI_6''$) reagents in paper chromatography. Acid hydrolysis gave a mixture consisting of the constituent amino acids in the ratios expected by theory, with the exception of the tryptophan. The intact peptide contained tyrosine and tryptophan in a molar ratio of 1 : 1.[16]

Previously we described the use of *O*-benzyl-serine[22,23] in the synthesis of the *N*-terminal sequence of corticotropin, where the amino function of the *N*-terminal serine was protected by a carbobenzoxy group.[12] An attempt to determine if the *N*-acyl and the *O*-benzyl groups could be removed safely by reduction with sodium in liquid ammonia was made for the protected nonapeptide *N*-carbobenzoxy-*O*-benzyl-seryl-tyrosyl-*O*-benzyl-seryl-methionyl-γ-*t*-butyl-glutamyl-histidyl-phenylalanyl-$N^G$-tosyl-arginyl-tryptophan methyl ester (m. p. 220—221°C, $[\alpha]_D^{22.5}$ −10.5° (*c* 1.05, dimethylformamide)); it was proved that one of the serine residues, which might be the amino end, was transformed into glycine to an extent of 60—70 per cent upon the treatment.[24] We next synthesized the first tetrapeptide sequence of corticotropin in the form of the *t*-butyloxycarbonyl-*O*-benzyl-seryl-tyrosyl-*O*-benzyl-seryl-methionine methyl ester (XVIII) as an intermediate in the synthesis of corticotropin peptides, in order to test the role of the hydroxyl function of serine in the activity as well as to examine the behavior of the protective groups and the serine residues

17) B. Iselin and R. Schwyzer, *Helv. Chim. Acta*, **44**, 169 (1961).
18) K. Hofmann, J. Rosenthaler, R. D. Wells and H. Yajima, *J. Am. Chem. Soc.*, **86**, 4991 (1964).
19) C. H. Li, J. Meienhofer, E. Schnabel, D. Chung, T-b. Lo and J. Ramachandran, ibid., **83**, 4449 (1961).
20) R. Schwyzer and H. Kappeler, *Helv. Chim. Acta*, **44**, 1991 (1961).
21) R. Geiger, K. Sturm and W. Siedel, *Chem. Ber.*, **96**, 1080 (1963).
22) K. Okawa and H. Tani, *J. Chem. Soc. Japan, Pure Chem. Sect.* (*Nippon Kagaku Zassi*), **75**, 1199 (1954).
23) K. Okawa, This Bulletin, **29**, 486 (1956).
24) H. Otsuka and K. Inouye, unpublished.

in the treatment with sodium in liquid ammonia. We wish to describe here, however, only the synthesis of compound XVIII as an example of serine peptide. The synthetic procedure for XVIII is quite similar to that employed for compound VII. *t*-Butyloxycarbonyl-*O*-benzyl-serine (XVI), which failed to crystallize, was isolated as the dicyclohexylamine salt.[9] The coupling of compound XVI with the tyrosine methyl ester by the DDCI method involved no difficulty other than those encountered with compound XI.

We also tried to obtain *O*-benzyl-D-serine from the *N*-acetyl-*O*-benzyl-D-serine, which could be isolated from the optical resolution medium[23] after *O*-benzyl-L-serine ($[\alpha]_D^{24}$ +7.3° (*c* 2.0, N hydrochloric acid)) had been separated. The treatment of *N*-acetyl-*O*-benzyl-D-serine (m. p. 110—111°C, $[\alpha]_D^{23}$ −46.7° (*c* 2.0, acetic acid)) with 80% hydrazine hydrate at 100°C gave the desired *O*-protected D-serine in a 70 per cent yield, no fission of the *O*-benzyl group was detected. The resultant material had an optical rotation of $[\alpha]_D^{24.5}$ −7.2° (*c* 2.0, N hydrochloric acid); its *N*-*t*-butyloxycarbonyl derivative was also found to be fairly coincident with the L-antipode in optical rotation with an opposite sign.

## Experimental

The amino acids used are of the L-configuration, unless otherwise indicated.

All melting points are uncorrected.

***t*-Butyloxycarbonyl-serine Dicyclohexylamine Salt.**—To a solution of 10.51 g. (0.1 mol.) of serine and 22.0 g. (0.24 mol.) of sodium bicarbonate in 200 ml. of water and 200 ml. of dioxane, 17.20 g. (0.12 mol.) of *t*-butyl azidoformate[7,8] was added drop by drop at a bath temperature of 45°C; the mixture was then stirred for 20 hr. at the above temperautre. The reaction mixture was chilled in an ice-bath, neutralized by the addition of 57.5 ml. of ice-cold 4 N hydrochloric acid (pH 4.6—4.8), and concentrated to about 80 ml. at 40°C in vacuo. The concentrated solution was then acidified to below pH 2 with ice-cold 4 N hydrochloric acid in the presence of 200 ml. of ethyl acetate at 0°C. The aqueous phase was extracted three times with 100 ml.-portions of cold ethyl acetate. The organic extracts were then pooled, dried over anhydrous sodium sulfate, and evaporated at 35—40°C in vacuo, thus affording the product as a sirup (13.83 g., 67.5%). The product was dissolved in 100 ml. of ether, and to this was added an ethereal solution of 12.20 g. (0.0675 mol.) of dicyclohexylamine. After the mixture had been stored in a refrigerator overnight, the crystals which separated were filtered off, washed with ether and dried; 23.56 g. (61.0%), m. p. 143—148°C. A 21.8 g.-sample was recrystallized from 220 ml. of hot ethyl acetate to give 19.6 g. of needles; m. p. 142—144°C, $[\alpha]_D^{25.5}$ +13.3±0.5° (*c* 3.044, methanol).

Found: C, 62.17; H, 9.80; N, 7.36. Calcd. for $C_8H_{15}O_5N \cdot C_{12}H_{23}N$: C, 62.15; H, 9.91; N, 7.25%.

***t*-Butyloxycarbonyl-serine Monohydrate (III).**—A solution of 17.40 g. (0.045 mol.) of the dicyclohexylamine salt which was obtained above in 200 ml. of 50% ethanol was shaken in the presence of 45 ml. (wet volume) of Dowex-50WX8 ($H^+$-form) at room temperature for 30 min. After the resin had been removed by filtration, the filtrate was concentrated to a small volume at 40—45°C in vacuo. The crystalline monohydrate, which separated soon when the mixture was chilled in an ice-bath, was collected and air-dried; 9.81 g. (97.7%), m. p. 45—47°C (sintering at 42°C), $[\alpha]_D^{26}$ −3.0±1° (*c* 2.330, acetic acid), $[\alpha]_D^{25}$ −7.6±0.5° (*c* 2.590, water).

Found: C, 43.25; H, 7.88; N, 6.52; $H_2O$, 7.19. Calcd. for $C_8H_{15}NO_5 \cdot H_2O$: C, 43.04; H, 7.68; N, 6.28; $H_2O$, 8.07%.

***t*-Butyloxycarbonyl-serine Hydrazide (X).**—To a solution of 5.58 g. (0.025 mol.) of III in 20 ml. of dioxane there was added, at 0°C, an ethereal solution of diazomethane until the yellow color persisted. After 10 more min. the solvent was removed in vacuo at 30°C. The resultant sirup was dissolved in 25 ml. of anhydrous ethanol; to this was added 3.0 ml. (0.06 mol.) of hydrazine hydrate, and the mixture was kept at room temperature for 24 hr. After the solvent had been removed in vacuo, the sirupy residue was treated with ethyl acetate to afford a crystalline solid. Recrystallization from hot ethyl acetate gave fine needles; 4.82 g. (88.0%), m. p. 109—110°C, $[\alpha]_D^{25.5}$ −9.0±0.5° (*c* 4.127, methanol). Lit.[17] m. p. 112—114°C, $[\alpha]_D^{27}$ −9.4±1° (*c* 4.1, methanol).

Found: C, 43.80; H, 7.91; N, 19.19;. Calcd. for $C_8H_{17}N_3O_4$: C, 43.83; H, 7.82; N, 19.17%.

***t*-Butyloxycarbonyl-seryl-methionine Methyl Ester (IV).**—A mixture of 5.99 g. (0.03 mol.) of methionine methyl ester hydrochloride, 6 ml. of water and 30 ml. of methylene chloride was chilled in an ice-bath and shaken with 15 ml. of ice-cold 50% potassium carbonate. The aqueous phase was then extracted again with cold methylene chloride. The organic extracts were combined, dried over anhydrous sodium sulfate, and evaporated at a bath temperature of 20°C in vacuo, affording the free base as an oil, which was then redissolved in methylene chloride. To this solution there were added 6.13 g. (0.0275 mol.) of compound III and a methylene chloride solution of 5.68 g. (0.0275 mol.) of *N*, *N'*-dicyclohexylcarbodiimide (DCCl); the reaction mixture was then kept in a refrigerator overnight. The *N*, *N'*-dicyclohexylurea (DCU) which formed was removed (5.81 g., 94.1%), and the filtrate was evaporated in vacuo. The residue was dissovled in ethyl acetate, and the solution was washed with ice-cold N hydrochloric acid, M sodium bicarbonate and saturated sodium chloride, dried over anhydrous sodium sulfate, and evaporated in vacuo. The resultant oily residue was dissolved in a small volume of acetonitrile, and the slight amount of the urea which separated upon chilling was removed. The evaporation of the solvent afforded an oily residue which crystallized during storage in a refrigerator. The crystals were washed with ether - petroleum ether and dried; 8.78 g. (91.1%), m. p. 64—66°C. Recrystallization from ethyl acetate - petroleum ether gave a yield of 8.21 g. (83.8%); m. p. 66—67.5°C, $[\alpha]_D^{25.5}$ −29.5±1° (*c* 2.220, methanol).

Found: C, 48.06; H, 7.61; N, 8.20; S, 9.34. Calcd. for $C_{14}H_{26}N_2O_6S$: C, 47.98; H, 7.48; N, 7.99; S,

9.15%.

**Seryl-methionine Methyl Ester (V).**—To a solution of 1.402 g. (4 mmol.) of IV in 2ml. of ethyl acetate there was added 2.5 ml. of 20% hydrogen chloride in ethyl acetate, the mixture was then allowed to stand at room temperature for 60 min. The solvent was evaporated in vacuo and the resultant oily ester hydrochloride was dissolved in methylene chloride. The solution was then treated with 50% potassium carbonate at 0°C, and the organic phase was dried over anhydrous sodium sulfate and evaporated at a bath temperature of 20°C in vacuo to give a sirupy residue, which crystallized upon the addition of ethyl acetate; 0.825 g. (82.5%), m. p. 84—85°C, $[\alpha]_D^{24.5}$ $-16.3\pm1°$ (*c* 1.791, methanol). Lit.[19] m. p. 86—88°C, $[\alpha]_D^{24}$ $-21°$ (*c* 1, N hydrochloric acid).

Found: C, 43.29; H, 7.29; N, 11.06; S, 12.66. Calcd. for $C_9H_{18}N_2O_4S$: C, 43.18; H, 7.25; N, 11.19; S, 12.81%.

***t*-Butyloxycarbonyl-glycyl-tyrosine Hydrazide (VI).**—To a solution of 2.32 g. of tyrosine methyl ester hydrochloride in 2 ml. of water, 10 ml. of ice-cold 50% potassium carbonate was added at 0°C. The crystalline precipitates which separated were filtered off, washed with cold water, and dried to give the free base of the tyrosine methyl ester; 1.81 g. (93.0%) the free base of the tyrosine methyl ester; 1.81 g. (93.0%), m. p. 134—135°C, $[\alpha]_D^{23.5}$ $+26.9\pm0.8°$ (*c* 2.411, methanol). Lit.[25] m. p. 135°C.

*t*-Butyloxycarbonyl-glycine (4.85 g., 0.0276 mol.) and 5.40 g. (0.0276 mol.) of the tyrosine methyl ester were dissolved in acetonitrile, and to this solution was added 5.72 g. (0.0276 mol.) of DCCl; the mixture was then kept in a refrigerator overnight. After the DCU which separated had been filtered off, the filtrate was evaporated in vacuo. The residue was dissolved in ethyl acetate, and the solution was washed successively with ice-cold N hydrochloric acid, water, M sodium bicarbonate and water, dried over anhydrous sodium sulfate, and evaporated in vacuo. The resultant amorphous dipeptide, which gave a single spot in thin-layer chromatography ($R_f$=0.50, Silica-gel G in ethyl acetate), was dissolved in 85 ml. of methanol, and 3.35 ml. of hydrazine hydrate was added. The mixture was then allowed to stand at room temperature for 48 hr. The crystalline precipitates which separated were collected, washed with a mixture of methanol and ether, and then ether, and dried in vacuo to give compound VI; 8.21 g. (84.5%), m. p. 187—188°C. A sample was recrystallized from methanol for analyses; m. p. 189.5—190°C, $[\alpha]_D^{26.5}$ $+0.5\pm1°$ (*c* 2.002, dimethylformamide).

Found: C, 54.56; H, 6.97; N, 15.93. Calcd. for $C_{16}H_{24}N_4O_5$: C, 54.53; H, 6.87; N, 15.93%.

***t*-Butyloxycarbonyl-seryl-tyrosine Methyl Ester (XI).**—Compound X (2.19 g., 0.01 mol.) was dissolved in 20 ml. of ice-cold N hydrochloric acid, and to this was added 6 ml. of ice-cold 2 M sodium nitrite at an ice-salt-bath temperature. After 4 min. the separated azide was taken into ethyl acetate. The organic solution was then washed with ice-cold M potassium carbonate and sodium chloride, and dried over anhydrous sodium sulfate. To this ethyl actate solution 1.95 g. (0.01 mol.) of the tyrosine methyl ester (free base) was added; the mixture was then concentrated to a small volume at a bath temperature of 5—10°C in vacuo, and about 25 ml. of tetrahydrofuran was introduced. The mixture was then left to stand at 0—4°C for 68 hr. The solvent was removed in vacuo, and the residue was redissolved in ethyl acetate. The solution was washed in an ordinary manner with ice-cold hydrochloric acid and sodium bicarbonate and dried. The evaporation of the solvent gave a foamy residue, which crystallized from ether; 2.50 g. (65.5%), m. p. 115—118°C, $[\alpha]_D^{25}$ $-5.0\pm2°$ (*c* 1.036, methanol). Lit.[18] m. p. 117—119°C, $[\alpha]_D^{28}$ $-13.3°$ (*c* 0.97, methanol).

Found: C, 55.94; H, 6.92; N, 7.56. Calcd. for $C_{18}H_{26}N_2O_7$: C, 56.53; H, 6.85; N, 7.33%.

***t*-Butyloxycarbonyl-seryl-tyrosine Hydrazide (XII).**—To a solution of 1.99 g. of compound XI in 20 ml. of anhydrous ethanol 1.3 ml. of hydrazine hydrate was added; the mixture was then allowed to stand at room temperature overnight. The crystalline precipitates which separated were collected, washed with ethanol and ether, and dried; 1.76 g. (88.5%), m. p. 188—190°C (decomp.). A sample was recrystallized from water for analyses; m. p. 193—195°C decomp., $[\alpha]_D^{24}$ $-19.8\pm1°$ (*c* 2.035, 50% acetic acid). Lit.[18] m. p. 199—200°C (decomp.), $[\alpha]_D^{28}$ $-18.6°$ (*c* 1.20, 50% acetic acid).

Found: C, 51.50; H, 7.15; N, 14.97. Calcd. for $C_{17}H_{26}N_4O_6\cdot{}^1/_2H_2O$: C, 52.16; H, 6.95; N, 14.31%.

***t*-Butyloxycarbonyl-glycyl-tyrosyl-seryl-methionine Methyl Ester (VII).**—To a solution of 1.76 g. (5.0 mmol.) of compound VI in 12.5 ml. of ice-cold N hydrochloric acid 3.0 ml. of ice-cold 2M sodium nitrite was added at an ice-salt bath temperature. After 4 min. 10 ml. of saturated sodium chloride was introduced, and the separated azide was placed in 20-ml. portions of ether. The organic extracts were combined, washed with ice-cold M sodium bicarbonate, dried over anhydrous sodium sulfate, and evaporated in vacuo to give a foamy residue, which was then redissolved in cold acetonitirle. To this solution 1.25 g. (5.0 mmol.) of compound V was added and the mixture was left to stand at 0—4°C for two days. The crystalline tetrapeptide VII which separated was filtered off, washed with cold ethyl acetate and ether, and dried; 1.40 g. (61.5%), m. p. 183—185°C (softening at 181°C). A suspension of this slightly-colored material in acetonitrile was boiled for a few minutes and then refrigerated. The treatment gave the colorless crystals with a recovery of 96.7 per cent; m. p. 183—185°C, $[\alpha]_D^{24}$ $-16.5\pm2°$ (*c* 1.15, methanol). A single spot was visible in thin-layer chromatography; $R_f$=0.48 (Silica-gel G, methanol-ethyl acetate (15:85)).

Found: C, 52.44; H, 7.01; N, 9.98; S, 5.70. Calcd. for $C_{25}H_{38}N_4O_9S$: C, 52.62; H, 6.71; N, 9.82; S, 5.62%.

***t*-Butyloxycarbonyl-glycyl-tyrosyl-seryl-methionine Hydrazide Hemihydrate (VIII).**—Hydrazine hydrate (0.6 mol.) was added to a solution of 1.20 g. of compound VII in 6 ml. of dimethylformamide; the mixture was allowed to stand at room temperature for 40 hr., after which time the hydrazide VIII was precipitated by the addition of 45 ml. of ethyl acetate in the form of a gelatinous mass. This amorphous solid was crystallized from hot water; 1.14 g. (93.9%), m. p. 202—204°C (decomp.), $[\alpha]_D^{23}$ $-17.8\pm1°$ (*c*

25) J. Ramachandran and C. H. Li, *J. Org. Chem.*, **28**, 173 (1963).

1.940, 50% acetic acid).

Found: C, 49.59; H, 6.95; N, 14.39; S, 5.49. Calcd. for $C_{24}H_{38}N_6O_8S\cdot{}^1/_2H_2O$: C, 49.73; H, 6.78; N, 14.50; S, 5.53%.

***t*-Butyloxycarbonyl-seryl-tyrosyl-seryl-methionine Methyl Ester (XIII).**—Compound XII (2.87 g., 7.5 mmol.) was dissolved in 19 ml. of ice-cold N hydrochloric acid containing 2 g. of sodium chloride, and to this was added 4.5 ml. of ice-cold 2M sodium nitrite at an ice-salt bath temperature. After 4 min. the azide was extracted three times with ethyl acetate. The organic extracts were combined, washed with ice-cold M sodium bicarbonate and saturated sodium chloride, and dried over anhydrous sodium sulfate. To this solution 1.33 g. (5.3 mmol.) of V was added, and the resultant suspension was concentrated to about 20—25 ml. at a bath temperature of 5—10°C in vacuo. During the concentration a greater part of the solid disappeared. The mixture was then kept at 0—4°C for 2 days. A little water was added to dissolve the gelatinous precipitates, which had separated, and the resultant clear solution was washed with ice-cold N hydrochloric acid, M sodium bicarbonate and water, dried over anhydrous sodium sulfate, and evaporated in vacuo to give a amorphous solid mass, which was then reprecipitated from hot ethyl acetate; 1.42 g. (44.8%), m. p. 125—130°C. A sample was reprecipitated from ethyl acetate for analyses; m. p. 129—134°C, $[\alpha]_D^{23}$ $-29.3\pm1°$ (*c* 2.053, methanol). Lit.[17] m. p. 115—125°C, $[\alpha]_D^{27}$ $-29.8\pm1°$ (*c* 2.1, methanol). A single spot was detected in thin-layer chromatography; $R_f=0.57$, methanol-ethyl acetate (15 : 85).

Found: C, 52.15; H, 6.88; N, 19.55; S, 5.38. Calcd. for $C_{26}H_{40}N_4O_{10}S$: C, 51.99; H, 6.71; N, 19.33; S, 5.34%.

***t*-Butyloxycarbonyl-seryl-tyrosyl-seryl-methionine Hydrazide (XIV).**—Compound XIII (0.78 g.) was dissolved in 5 ml. of dimethylformamide, and to this was added 0.3 ml. of hydrazine hydrate. The mixture was kept at 0—4°C for 40 hr. The tetrapeptide hydrazide XIV was then precipitated by the addition of ethyl acetate. The precipitates crystallized upon treatment with hot water; 0.76 g. (96.9%), m. p. 187—189°C (decomp.) (sintering at 175°C). A sample was recrystallized from water for analyses without any change in the melting point; $[\alpha]_D^{23.5}$ $-35.8°$ (*c* 1.966, 50% methanol). Lit.[17] m. p. 191—193°C, $[\alpha]_D^{25}$ $-36.7\pm1°$ (*c* 2.0, water-methanol 1 : 1).

Found: C, 49.09; H, 6.79; N, 13.99; S, 5.42. Calcd. for $C_{25}H_{40}N_6O_9S$: C, 49.99; H, 6.79; N, 13.99; S, 5.34%.

***t*-Butyloxycarbonyl-glycyl-tyrosyl-seryl-methionyl-γ-*t*-butyl-glutamyl-histidyl-phenylalanyl-arginyl-tryptophyl-glycine Dihydrate (IX).**—To a solution of compound VIII (0.348 g.., 0.6 mmol.) in 3.5 ml. of dimethyl-formamide 1.8 ml. of ice-cold N hydrochloric acid and 0.66 ml. of M sodium nitrite were added at an ice-salt-bath temperature. After 4 min. saturated sodium chloride (20 ml.) was introduced, and the separated azide was taken into ethyl acetate. The organic extracts were combined, washed with ice-cold M sodium bicarbonate and water, and dried over anhydrous sodium sulfate. To this was added a solution of 0.496 g. (0.5 mmol.) of γ-*t*-butyl-glutamyl-histidyl-phenylalanyl-arginyl-tryptophyl-glycine[13] and 0.21 ml. of triethylamine in 15 ml. of dimethylformamide, and the mixture was concentrated at 5—10°C in vacuo until it became clear. The reaction mixture was then allowed to stand at 0—4°C. The freshly prepared azide (derived from 0.232 g. (0.4 mmol.) of VIII as described above) was added after 20 hr. of reaction. After 40 hr. acetic acid (0.5 ml.) was added, and the mixture was poured into 200 ml. of cold ethyl acetate to separate the crude protected decapeptide, which were collected by filtration (0.607 g.). The crude product was twice reprecipitated from dimethylformamide-methanol (1 : 2); 0.461 g. (63.1%), m. p. 213—216°C (decomp.), $[\alpha]_D^{24}$ $-10.8\pm2°$ (*c* 1.077, dimethylformamide). A single spot was detected in thin-layer chromatography; $R_f=0.60$ (Silica-gel G, dimethylformamide - ethyl acetate - acetic acid (15 : 10 : 2).

Found: C, 55.04; H, 6.65; N, 15.30; S, 2.04. Calcd. for $C_{67}H_{92}N_{16}O_{17}S\cdot2H_2O$: C, 55.05; H, 6.62; N, 15.33; S, 2.19%.

***t*-Butyloxycarbonyl-seryl-tyrosyl-seryl-methionyl-γ-*t*-butyl-glutamyl-histidyl-phenylalanyl-arginyl-tryptophyl-glycine Dihydrate (XV).**—The azide, which was derived from 0.685 g. (1.14mmol.) of compound XIV, and 0.750 g. (0.76 mmol.) of γ-*t*-butyl-glutamyl-histidyl-phenylalanyl-arginyl-tryptophyl glycine[13] were made to react in an ethyl acetate-dimethylformamide solution at 0—4°C for 44 hr. in almost the same manner as in the case of the synthesis of IX. After the addition of 0.75 ml. of acetic acid, the reaction mixture was introduced into cold ethyl acetate; the resultant precipitates of crude XV were filtered off to give 1.041 g. The crude product was reprecipitated from dimethylformamide-methanol to afford 0.910 g. (80.2%) of the pure material, which was found to be homogeneous in thin-layer chromatography, $R_f=0.47$ (Silica-gel G, dimethylformamide - ethyl acetate - acetic acid (15 : 10 : 2)).; m. p. 202°C (decomp.), $[\alpha]_D^{24}$ $-13.2\pm2°$ (*c* 1.045, dimethylformamide). Lit. m. p. 203°C decomp., $[\alpha]_D$ $-12.4\pm0.8°$ (*c* 1.328, dimethylformamide);[20] m. p. 201—204°C (decomp.), $[\alpha]_D^{22}$ $-13.0\pm1°$ (*c* 1, dimethylformamide).[21]

Found: C, 55.24; H, 7.25; N, 14.99; S, 2.56. Calcd. for $C_{68}H_{94}N_{16}O_{18}S\cdot2H_2O$: C, 54.75; H, 6.62; N, 15.02; S, 2.15%.

**Seryl-tyrosyl-seryl-methionyl-glutamyl-histidyl-phenylalanyl-arginyl-tryptophyl-glycine (I).**—The protected decapeptide XV (50 mg.) was dissolved in 1 ml. of anhydrous trifluoroacetic acid, and the solution was ketp at room temperature for 60 min. The solvent was removed by evaporation at a bath temperature of 35—40°C in vacuo to give a sirupy residue, which was dried over sodium hydroxide in vacuo. The residue was then dissolved in water, and to this was added 2 ml. (wet volume) of Amberlite CG-4B (acetate form), and the mixture was stirred at room temperature for 40 min. After the resin had been removed by filtration, the filtrate was lyophilized to give quantitatively the decapeptide I as a colorless powder with a silky luster; it gave a single spot ($R_f=0.52$) reactive to ninhydrin, Pauly, Ehrlich, Sakaguchi and methionine ($PtI_6''$) reagents on paper in the solvent system of *n*-butanol - acetic acid - pyridine - water (30 : 6 : 20 : 24);[14] $\lambda_{max}^{N/10\ HCl}=280$ mμ (ε 6490), $\lambda_{shoulder}^{N/10\ HCl}=288.5$ mμ (ε 4870), $\lambda_{max}^{N/10\ NaOH}$ 281.5 mμ (ε 6810),

288.5 mμ (ε 6600), $[\alpha]_D^{24}$ −30.3±2° (*c* 0.551, N acetic acid). Amino acid ratios in acid hydrolysate:[15] Ser 1.83, Tyr 1.06, Met 1.03, Glu 1.00, His 0.99, Phe 1.02, Arg 0.94, Gly 0.92, Try 0.55,*3 $NH_3$ 0.51.

**Glycyl-tyrosyl-seryl-methionyl-glutamyl-histidyl-phenylalanyl-arginyl-tryptophyl-glycine (II).**—The protected decapeptide IX (60 mg.) was treated with trifluoroacetic acid in exactly the same manner as has been described above for I, thus giving the free decapeptide II as a colorless fluffy powder, which gave a single spot ($R_f$=0.37) reactive to ninhydrin, Pauly, Ehrlich, Sakaguchi and methionine ($PtI_6''$) reagents in paper chromatography in *n*-butanol-acetic acid-pyridine-water (30 : 6 : 20 : 24);[14] $\lambda_{max}^{N/10\ HCl}$ =280 mμ (ε 6320), $\lambda_{shoulder}^{N/10\ HCl}$=288.5 mμ (ε 4820), $\lambda_{max}^{N/10\ NaOH}$=281.5 mμ (ε 7220), 288.5 mμ (ε 6950), $[\alpha]_D^{13.5}$ −32.2±2° (*c* 0.631, N acetic acid). Amino acid ratios in acid hydrolysate:[15] Gly 1.80, Tyr 1.04, Ser 0.87, Met 0.98, Glu 1.00, His 0.95, Phe 0.92, Arg >0.83, Try 0.57,*3 $NH_3$ 0.37.

***N-t*-Butyloxycarbonyl-*O*-benzyl-serine Dicyclohexylamine Salt (XVI).**—*O*-Benzyl-serine[22,23] (4.88 g., 0.025 mol.) was acylated with 5.37 g. (0.0375 mol.) of *t*-butyl azidoformate and sodium bicarbonate in a water-dioxane (1 : 1) solution in almost the same manner as was used for compound III. The sirupy product (7.1 g.) was converted into the dicyclohexylamine salt as usual; 9.25 g. (77.6%), m. p. 135—136°C. Recrystallization from ethanol-ether gave a sample for analyses; m. p. 135.5—136°C, $[\alpha]_D^{24.5}$ +24.3±1° (*c* 2.494, methanol).

Found: C, 68.23; H, 9.34; N, 5.91. Calcd. for $C_{15}H_{21}NO_5 \cdot C_{12}H_{23}N$: C, 68.03; H, 9.03; N, 5.88%.

***N-t*-Butyloxycarbonyl-*O*-benzyl-seryl-tyrosine Hydrazide (XVII).**—A solution of 3.58 g. (7.5 mmol.) of compound XVI in 40 ml. of 50% ethanol was shaken in the presence of 10 ml. (wet volume) of Dowex-50W X8 ($H^+$ form) at room temperature for 30 min. After the resin had been removed by filtration, the filtrate was evaporated at 40—45°C in vacuo. The sirupy residue was dissolved in ether, and the solution was dried over sodium sulfate and evaporated in vacuo to give *N-t*-butyloxycarbonyl-*O*-benzyl-serine as a clear oil. To this were added a methylene chloride solution of 1.47 g. (7.5 mmol.) of the tyrosine methyl ester and then a methylene chloride solution of 1.55 g. (7.5 mmol.) of DCCI. The mixture was kept in a refrigerator overnight. Subsequent treatment was carried out in almost the same manner as for compound IV. The resulting dipeptide ester (3.60 g.) failed to crystallized, but it was found to be homogeneous in thin-layer chromatography; ($R_f$=0.53 (Silica-gel G, ethyl acetate)).

A 5.85 g.-sample of the dipeptide ester obtained as described above was dissolved in 40 ml. of anhydrous ethanol, and to this was added 1.45 ml. of hydrazine hydrate. The mixture was allowed to stand at room temperature for 40 hr. and then refrigerated. The crystalline product which separated was filtered off, washed with cold ethanol-ether and ether, and dried; 3.62 g., m. p. 178—181°C (decomp.) (sintering at 165°C). The concentration of the mother liquor afforded an additional 1.00 g. of the crystals with a m.p. of 168—170°C (decomp.). The total yield amounted to 4.62 g. (81.7%). Recrystallization from anhydrous ethanol gave, in a recovery of 93 per cent, crystals with a m. p. of 179—181°C decomp. (sintering at 168°C), $[\alpha]_D^{23.5}$ −12.2±1° (*c* 2.037, dimethylformamide).

Found: C, 61.02; H, 6.90; N, 11.88. Calcd. for $C_{24}H_{32}N_4O_6$: C, 61.00; H, 6.83; N, 11.86%.

***N-t*-Butyloxycarbonyl-*O*-benzyl-seryl-methionine Methyl Ester (XVIII).**—*N-t*-Butyloxy-carbonyl-*O*-benzyl-serine, which was derived from 4.76 g. (0.01 mol.) of the dicyclohexylamine salt (XVI) with the Dowex-50 treatment described above, and the methionine methyl ester (prepared from 2.40 g. (0.012 mol.) of the hydrochloride) were coupled in methylene chloride by the DCCI procedure, in almost the same manner as was used in the case of the synthesis of Compound IV, to give the protected dipeptide XVIII, which crystallized from ethyl acetate-petroleum ether; 4.11 g. (93.4%), m. p. 40—43°C. Recrystallization from the same solvent gave fine needles; m. p. 51—52°C, $[\alpha]_D^{25}$ −13.4±1° (*c* 2.169, methanol).

Found: C, 57.35; H, 7.51; N, 6.60; S, 7.52. Calcd. for $C_{21}H_{32}O_6N_2S$: C, 57.25; H, 7.32; N, 6.36; S, 7.28%.

***N-t*-Butyloxycarbonyl-*O*-benzyl-seryl-tyrosyl-*O*-benzyl-seryl-methionine Methyl Ester (XIX).**—Compound XVIII (2.20 g., 5.0 mmol.) was treated with hydrogen chloride in ethyl acetate to obtain *O*-benzyl-seryl-methionine methyl ester hydrochloride, which was then converted into the free base by treatment with 50% aqueous potassium carbonate in ether. Compound XVII (2.36 g., 5.0 mmol.) was dissolved in 8 ml. of dimethylformamide and chilled in an ice-salt bath. To this solution 20 ml. of ice-cold N hydrochloric acid and 2.75 ml. of ice-cold 2 M sodium nitrite were added. After 3 min. the azide which separated was taken into ether. The etheral solution was washed successively with ice-cold M potassium carbonate, water and saturated sodium chloride, and dried over sodium sulfate. This solution was then added to the oily dipeptide ester obtained above, and the reaction mixture was allowed to stand at 0—4°C for three days. The separated crystalline precipitates of the tetrapeptide XIX were filtered off, washed with cold ether, and dried; 1.41 g., m. p. 123.5—126°C. The mother liquor was washed with acid and bicarbonate as usual, and concentrated to a small volume to yield an additional quantity of XIX; 0.99 g., m. p. 124—127°C. The total yield amounted to 2.40 g. (61.3%). These crystals were recrystallized from ethyl acetate-petroleum ether in a recovery of 96.2 per cent; m. p. 127—129°C, $[\alpha]_D^{25.5}$ −11.1±0.7° (*c* 2.982, methanol).

Found: C, 61.67; H, 6.85; N, 7.26; S, 4.16. Calcd. for $C_{40}H_{52}N_4O_{10}S$: C, 61.52; H, 6.71; N, 7.17; S, 4.11%.

***O*-Benzyl-D-serine.**—*N*-Acetyl-*O*-benzyl-D-serine[23] (9.50 g., m. p. 110—111°C, $[\alpha]_D^{23}$ −46.7±1° (*c* 2.660, acetic acid); lit.[22] m. p. 103°C) was dissolved in 95 ml. of 80% hydrazine hydrate, and the mixture was heated under reflux at 100°C for 6 hr. Most of the hydrazine was removed by evaporation at 50—60°C in vacuo; the residue was dried over sulfuric acid in vacuo. The resultant crystalline solid was washed with cold water, ethanol and ether to give *O*-benzyl-D-serine; 5.69 g. (72.8%). A sample was recrystallized from water for analyses; $[\alpha]_D^{24.5}$ −7.2±0.3° (*c* 2.046, N hydrochloric acid).

*3 Tryptophan had been partially destroyed with acid.

Found: C, 61.55; H, 6.72; N, 7.18. Calcd. for $C_{10}H_{13}NO_3$: C, 61.45; H, 6.91; N, 6.81%.

***N-t*-Butyloxycarbonyl-*O*-benzyl-D-serine Dicyclohexylamine Salt.**—*O*-Benzyl-D-serine (0.98 g., 5.0 mmol.) and 1.375 g. (1.25 mmol.) of sodium carbonate were dissolved in 5 ml. of water, and to this was added 1.80 g. (7.5 mmol.) of *t*-butyl *p*-nitrophenyl carbonate[26] which had been dissolved in 7 ml. of *t*-butanol. The mixture was heated under reflux at 100°C for 30 min. and then evaporated to dryness in vacuo. The residue was extracted with cold water. The aqueous extract was neutralized to pH 5 by 4 N hydrochloric acid and washed with ether to remove the *p*-nitrophenol. The aqueous solution was then acidified to below pH 2 at 0°C and extracted with ether. The ether extract was dried over anhydrous sodium sulfate, and to this was added 1 ml. of dicyclohexylamine to afford the crystalline salt of *N-t*-butyloxycarbonyl-*O*-benzyl-D-serine; 1.22 g. (71.3%), m. p. 130—131°C, $[\alpha]_D^{24}$ $-23.6\pm1°$ (*c* 2.28, methanol).

Found: C, 68.54; H, 9.48; N, 5.96. Calcd. for $C_{15}H_{21}NO_5 \cdot C_{12}H_{23}N$: C, 68.03; H, 9.30; N, 5.88%.

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