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Amino Acids and Peptides. XVI.[1] Synthesis of a Tetrapeptide Sequence (A_9–A_{12}) of Glucagon

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Protected peptides corresponding to the A_9–A_{12} sequence of the hyperglycemic hormone glucagon[2] have been elaborated recently by several investigators. The first synthesis gave the tripeptide N-benzyloxycarbonyl-L-asparaginyl-L-tyrosyl-L-serine methyl ester, prepared by a coupling between N-benzyloxycarbonyl-L-asparagine *p*-nitrophenyl ester and L-tyrosyl-L-serine methyl ester.[3] The second preparation yielded N-trifluoroacetyl-β-*t*-butyl-L-aspartyl-L-tyrosyl-L-serine hydrazide, formed by joining N-trifluoroacetyl-β-*t*-butyl-L-aspartic acid with either O-benzyl-L-tyrosyl-O-benzyl-L-serine benzyloxycarbonylhydrazide or L-tyrosyl-O-benzyl-L-serine benzyloxycarbonylhydrazide.[4] Later, N-benzyloxycarbonyl-β-*t*-butyl-L-aspartyl-L-tyrosyl-L-serine methyl ester was made from N-benzyloxycarbonyl-β-*t*-butyl-L-aspartic acid and L-tyrosyl-L-serine methyl ester by a mixed anhydride procedure,[5] while the tetrapeptide under discussion was incorporated into a longer fragment by stepwise addition of individual amino acids from the carboxyl end.[6]

In continuation of earlier work, there is described here the formation of a protected A_9–A_{12} peptide and other related compounds. The procedure began with N^{ϵ}-benzylidene-L-lysine[7] (I), which on treatment with benzyl chloroformate and sodium hydroxide afforded both N^{ϵ}-benzyloxycarbonyl-L-lysine[8-11] (II) and N^{α}-benzyloxycarbonyl-L-lysine[7,10-12] (III). Reaction of compound III with *t*-butyl azidoformate[13] in the presence of dicyclohexylamine[14] led to N-benzyloxycarbonyl-N^{ϵ}-*t*-butyloxycarbonyl-L-lysine dicyclohexylammonium salt[12,15-17] (IV). Alternatively, III was esterified with thionyl chloride and methanol to obtain syrupy N-benzyloxycarbonyl-L-lysine methyl ester hydrochloride[7] (V), which was converted by *t*-butyl azidoformate[13] into N-benzyloxycarbonyl-N^{ϵ}-*t*-butyloxycarbonyl-L-lysine methyl ester[16-19] (VI). Removal of the benzyloxycarbonyl group of compound VI by hydrogenation in acetic acid formed crystalline N^{ϵ}-*t*-butyloxycarbonyl-L-lysine methyl ester hydroacetate[16,17] (VII). A 2-ethyl-5-phenylisoxazolium 3′-sulfonate[20,21] coupling between the amine VII and N-benzyloxycarbonyl-L-serine (VIII) yielded N-benzyloxycarbonyl-L-seryl-N^{ϵ}-*t*-butyloxycarbonyl-L-lysine methyl ester (IX). The dipeptide IX on hydrogenation in acetic acid afforded the corresponding amine hydroacetate (X); addition of N-benzyloxycarbonyl-O-benzyl-L-tyrosine *p*-nitrophenyl ester[22] (XI) formed amorphous N-benzyloxycarbonyl-O-benzyl-L-tyrosyl-L-seryl-N^{ϵ}-*t*-butyloxycarbonyl-L-lysine methyl ester (XII). The combination of N,O-dibenzyloxycarbonyl-L-tyrosine 2,4,5-trichlorophenyl ester[23] (XIII) and the amine X produced impure N,O-dibenzyloxycarbonyl-L-tyrosyl-L-seryl-N^{ϵ}-*t*-butyloxycarbonyl-L-lysine methyl ester (XIV). A third approach to this tripeptide sequence involved an azide synthesis[24,25] between N-benzyloxycarbonyl-L-tyrosyl-L-serine hydrazide (XVI) and the amine X affording N-benzyloxycarbonyl-L-tyrosyl-L-seryl-N^{ϵ}-*t*-butyloxycarbonyl-L-lysine methyl ester (XVIII). The hydrazide XVI was prepared from N-benzyloxycarbonyl-L-tyrosyl-L-serine methyl ester[26,27] (XV) in the usual manner, and from N,O-dibenzyloxycarbonyl-L-tyrosyl-L-serine methyl ester (XVII) by warming with excess hydrazine. The latter reaction is the second example of the removal of an O-benzyloxycarbonyl blocking group by hydrazine.[23]

Catalytic reduction in acetic acid of the tripeptide XVIII afforded L-tyrosyl-L-seryl-N^{ϵ}-*t*-butyloxycarbonyl-L-lysine methyl ester hydroacetate (XIX), which was coupled to N-benzyloxycarbonyl-β-*t*-butyl-L-aspartate α-2,4,5-trichlorophenyl ester[23] (XX) or the corresponding α-*p*-nitrophenyl ester[28-30] (XXI) to form the desired N-benzyloxycarbonyl-β-*t*-butyl-L-aspartyl-L-tyrosyl-L-seryl-N^{ϵ}-*t*-butyloxycarbonyl-L-lysine methyl ester (XXII). Reaction of compound XXII with hydrazine led to N-benzyloxycarbonyl-β-*t*-butyl-L-aspartyl-L-tyrosyl-L-seryl-N^{ϵ}-*t*-butyloxycarbonyl-L-

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lysine hydrazide (XXIII), an intermediate desired for future work in this series. Lastly, mention is made of N-benzyloxycarbonyl-L-tyrosyl-L-serylglycine methyl ester (XXIV), a compound prepared for the comparison of two azide coupling procedures.

Experimental Section[31]

N^{ϵ}-Benzylidene-L-lysine (I).—This compound was obtained by the addition of benzaldehyde to a chilled solution of L-lysine monohydrochloride in dilute lithium hydroxide (40.1 g, 68%), mp 205–207° (lit.[7] mp 206–208° dec).

N^{ϵ}-Benzyloxycarbonyl-L-lysine (II) and N^{α}-Benzyloxycarbonyl-L-lysine (III).—Chilled (0°) solutions of sodium hydroxide (1 *N*, 150 ml) and benzyl chloroformate (17 ml) were added in two portions within a brief period (5 min) to a cold (−1°), vigorously stirred solution of N^{ϵ}-benzylidene-L-lysine (23.4 g, 0.10 mole) in sodium hydroxide (1 *N*, 100 ml). Lower temperatures do not aid the reaction due to freezing of the sodium hydroxide solution at −2 to −3° and the conversion of the basic N^{ϵ}-benzylidene-L-lysine solution into a turgid mass below −5°. Stirring was continued for a short interval (0°, 10 min; room temperature, 10 min), after which concentrated hydrochloric acid (25 ml) was added, and the mixture was heated (50°, 5 min) and extracted with two 100-ml portions of ether. The water layer was adjusted to pH 6.2 and filtered to remove N^{ϵ}-benzyloxycarbonyl-L-lysine: mp *ca.* 235°; $[\alpha]^{25}D$ +14.0° (*c* 2.86, water containing 2 equiv of hydrochloric acid) [lit.[8-10] mp *ca.* 255, 262°; $[\alpha]D$ +14.4° (*c* 1.6, water containing 2 equiv of hydrochloric acid), $[\alpha]^{20}D$ +14.0° (*c* 0.5 *N* hydrochloric acid)]; ν_{max} 3330 (NH), 3030 (OH), 2935 (CH), 1687 (C=O), 1270 (CO), and 735 and 695 (Ph) cm^{-1}; λ_{max} 247, 252, 257, 264, and 267 mμ (ε 178, 223, 272, 237, and 172).

The remaining water solution was concentrated *in vacuo* (100 ml) and allowed to stand in the cold room to deposit slowly solid N-benzyloxycarbonyl-L-lysine, recrystallized from water (14.5 g, 52%): 230–233°; $[\alpha]^{22}D$ −10.3° (*c* 5.04, 0.2 *N* hydrochloric acid) [lit.[7,10,12] mp 235–237, 227–228°; $[\alpha]^{25}D$ −12.2° (*c* 5.6, 0.2 *N* hydrochloric acid), $[\alpha]^{20}D$ −7.3° (*c* 5, 0.5 *N* hydrochloric acid), $[\alpha]^{20}D$ −13.7° (*c* 2, 0.2 *N* hydrochloric acid)]; ν_{max} 3315 (NH), 3030 (OH), 2935 (CH), 1727 (C=O), 1242 (CO), and 740 and 698 (Ph) cm^{-1}; λ_{max} 247, 252, 258, 264, and 268 mμ (ε 143, 195, 247, 215, and 154).

N-Benzyloxycarbonyl-N^{ϵ}-*t*-butyloxycarbonyl-L-lysine Dicyclohexylammonium Salt (IV).—Treatment of N-benzyloxycarbonyl-L-lysine with *t*-butyl azidoformate gave white needles of N-benzyloxycarbonyl-N^{ϵ}-*t*-butyloxycarbonyl-L-lysine dicyclohexylammonium salt: mp 150–153°; $[\alpha]^{26}D$ +5.0° (*c* 1.00, methanol) [lit.[12,15,16] mp 156–157, 154–157, 153–155°; $[\alpha]^{20}D$ +7.82° (*c* 1, ethanol), $[\alpha]^{23}D$ +4.95° (*c* 1, dimethylformamide), $[\alpha]^{26}D$ +5.5° (*c* 4.6, methanol)]; ν_{max} 3380 broad (OH), 2925 (CH), 1690 broad (C=O), 1390 and 1360 (*t*-butyl), 1245 (CO), and 695 (Ph) cm^{-1}; λ_{max} 247, 252, 258, 262, 264, and 267 mμ (ε 153, 206, 252, 196, 216 and 154).

Anal. Calcd for $C_{31}H_{51}N_3O_6$ (561.7); C, 66.28; H, 9.15; N, 7.48. Found: C, 66.13; H, 9.11; N, 7.43.

N-Benzyloxycarbonyl-L-lysine Methyl Ester Hydrochloride (V).—Esterification of N-benzyloxycarbonyl-L-lysine with a mixture of thionyl chloride and methanol produced a syrup, which resisted crystallization by a variety of techniques.

N-Benzyloxycarbonyl-N^{ϵ}-*t*-butyloxycarbonyl-L-lysine Methyl Ester (VI).—The product from the foregoing preparation was reacted with *t*-butyl azidoformate to yield colorless needles of N-benzyloxycarbonyl-N^{ϵ}-*t*-butyloxycarbonyl-L-lysine methyl ester: mp 62–63°; $[\alpha]^{28}D$ −10.0° (*c* 1.0, acetone); R_f 0.79 [lit.[16,18,19] mp 62–63, 57°; $[\alpha]^{26}D$ −9.3° (*c* 2.05, acetone), $[\alpha]^{26}D$ −10.6° (*c* 1.90, acetone)]; ν_{max} 3350 (NH), 2950 (CH), 1720 very broad (C=O), 1375 and 1350 (*t*-butyl), 1170 (CO), and 697 (Ph) cm^{-1}; λ_{max} 247, 252, 257, 261, 264, and 267 mμ (ε 141, 204, 230, 183, 193, and 135).

N^{ϵ}-*t*-Butyloxycarbonyl-L-lysine Methyl Ester Hydroacetate (VII).—Hydrogenolysis of N-benzyloxycarbonyl-N^{ϵ}-*t*-butyloxycarbonyl-L-lysine methyl ester in methanol with 1 equivalent of acetic acid formed needles of N^{ϵ}-*t*-butyloxycarbonyl-L-lysine methyl ester hydroacetate: mp 78–79°; $[\alpha]^{27}D$ +15.3° (*c* 1.0, methanol) [lit.[16] mp 78–79°; $[\alpha]^{26}D$ +17.0° (*c* 2.1, methanol)]; R_f 0.24; ν_{max} 3380 (NH), 2970 (CH), 1720 very broad (C=O), 1390 and 1365 (*t*-butyl), and 1180 (CO) cm^{-1}; no selective absorption occurred in the ultraviolet region.

Anal. Calcd for $C_{14}H_{28}N_2O_6$ (320.4): C, 52.48; H, 8.81; N, 8.74. Found: C, 52.53; H, 8.79; N, 8.98.

N-Benzyloxycarbonyl-L-seryl-N^{ϵ}-*t*-butyloxycarbonyl-L-lysine Methyl Ester (IX).—Triethylamine (1.39 ml, 0.010 mole) was added to a stirred suspension of N-benzyloxycarbonyl-L-serine (2.39 g, 0.010 mole) and 2-ethyl-5-phenylisoxazolium 3′-sulfonate (2.53 g, 0.010 mole) in acetonitrile (50 ml) at 0°. After 18 hr at 0°, there was added to the almost clear mixture a solution of N^{ϵ}-*t*-butoxycarbonyl-L-lysine methyl ester hydroacetate (3.20 g, 0.010 mole) and triethylamine (1.39 ml, 0.010 mole) in acetonitrile (50 ml) at 0°. The reaction was stirred at 0° for 24 hr, after which evaporation of the solvent yielded an oil that was distributed between ethyl acetate and water. The organic phase was washed with 0.5 *M* aqueous citric acid, water, saturated sodium bicarbonate solution, and brine, dried and taken to dryness. The residue was dissolved in ether and on standing at room temperature furnished white needles of N-benzyloxycarbonyl-L-seryl-N^{ϵ}-*t*-butyloxycarbonyl-L-lysine methyl ester (2.35 g, 49%): mp 106.5–107.5°; $[\alpha]^{26}D$ −11.2° (*c* 1.0, methanol); R_f 0.64; ν_{max} 3330 broad (OH), 2940 (CH), 1753, 1720, and 1710 (C=O), 1685 (urethane), 1645 (amide I), 1535 (amide II), 1392 and 1367 (*t*-butyl), 1250 broad (CO), and 697 (Ph) cm^{-1}; λ_{max} 247, 252, 257, 261, 264, and 267 mμ (ε 117, 158, 206, 154, 168, and 108).

Anal. Calcd for $C_{23}H_{35}N_3O_8$ (481.5): C, 57.37; H, 7.33; N, 8.73. Found: C, 57.36; H, 7.34; N, 8.96.

If the initial reaction mixture was allowed to warm to room temperature, a clear solution of the "Woodward" intermediate resulted after *ca.* 30 min. From this point onward, when the solution was maintained at 0° and the experiment executed in a manner identical with the above description, thin layer chromatography showed the final oil to be composed of two components (R_f 0.46 and 0.66), and the yield of pure dipeptide was low (17%).

L-Seryl-N^{ϵ}-butyloxycarbonyl-L-lysine Methyl Ester Hydroacetate (X).—A solution of N-benzyloxycarbonyl-L-seryl-N^{ϵ}-*t*-butyloxycarbonyl-L-lysine methyl ester (0.963 g, 0.002 mole) in methanol (10 ml) was hydrogenolyzed during 2 hr in the presence of 10% palladium on carbon (0.045 g) and acetic acid (0.13 ml, 0.0022 mole). The reaction mixture was filtered and the solvent evaporated to leave a clear oil, R_f 0.10.

N-Benzyloxycarbonyl-O-benzyl-L-tyrosyl-L-seryl-N^{ϵ}-*t*-butyloxycarbonyl-L-lysine Methyl Ester (XII).—The aforementioned product was swirled with a solution of N-benzyloxycarbonyl-O-benzyl-L-tyrosine *p*-nitrophenyl ester (1.053 g, 0.002 mole) in chloroform (20 ml). After 48 hr, the solution was evaporated to dryness and the residue dissolved in ethyl acetate and washed with 0.5 *M* aqueous citric acid, saturated sodium bicarbonate solution, and brine. The dried solution was concentrated to a small volume and ether added to the opalescent point. On standing at room temperature, a gel deposited, which was collected, washed with ether, and dried. Reprecipitation from ethyl acetate–ether and drying furnished N-benzyloxycarbonyl-O-benzyl-L-tyrosyl-L-seryl-N^{ϵ}-*t*-butyloxycarbonyl-L-lysine methyl ester as an amorphous white powder (0.861 g, 59%): mp 118–120°; $[\alpha]^{26}D$ −8.4° (*c* 1.0, methanol; R_f 0.70; ν_{max} 3350 very broad (OH), 2930 (CH), 1690 very broad (C=O) and urethane), 1520 (amide II), 1392 and 1367 (*t*-butyl), 1245 (CO), and 695 (Ph) cm^{-1}; λ_{max} 227, 252, 258, 264, 267, 276, and 283 mμ (ε 16,000, 698, 970, 1210, 1350, 1600, and 1360).

Anal. Calcd for $C_{39}H_{50}N_4O_{10}$ (734.9): C, 63.75; H, 6.86; N, 7.62. Found: C, 63.75; H, 7.00; N, 7.74.

N,O-Dibenzyloxycarbonyl-L-tyrosyl-L-seryl-N^{ϵ}-*t*-butyloxycarbonyl-L-lysine Methyl Ester (XIV).—To an ethyl acetate (5 ml) solution of L-seryl-N^{ϵ}-*t*-butyloxycarbonyl-L-lysine methyl ester hydroacetate, freshly prepared by the hydrogenation of N-benzyloxycarbonyl-L-seryl-N^{ϵ}-*t*-butyloxycarbonyl-L-lysine-methyl ester (0.159 g, 0.00033 mole), was added N,O-dibenzyl-

(31) Melting points are uncorrected. Microanalyses were provided by Messrs. Erich H. Meier and J. Consul, Microanalytical Laboratory, Stanford University. The optical rotation, infrared (potassium bromide), and ultraviolet (95% ethanol) measurements were obtained by Mrs. L. D. Carroll. Thin layer chromatography employed silica gel G (freshly activated) as the support, methanol–chloroform (1:9) for development, and iodine for visualization. Evaporations were performed under reduced pressure (water pump) in a rotatory evaporator at minimum temperature, while high-boiling solvents were removed at vacuum pressure (0.2–0.5 mm). Magnesium sulfate was used for drying purposes. Acetonitrile and dimethylformamide were spectroscopic quality; other solvents were reagent grade and light petroleum had bp 30–60°.

oxycarbonyl-L-tyrosine 2,4,5-trichlorophenyl ester (0.207 g, 0.00033 mole) in ethyl acetate (2 ml), followed by triethylamine (0.00033 mole). After 48 hr at room temperature, the reaction mixture was diluted with ethyl acetate (15 ml) and washed with 0.5 *M* aqueous citric acid, saturated sodium bicarbonate solution, and brine. The dried solution was evaporated to a small bulk and diisopropyl ether added to the opalescence point. On standing at room temperature, a gel separated, which was washed with diisopropyl ether and dried to give N,O-dibenzyloxycarbonyl-L-tyrosyl-L-seryl-N$^{\epsilon}$-*t*-butyloxycarbonyl-L-lysine methyl ester as an amorphous powder (0.172 g): mp 116–118°; R_f 0.75. No further work was done to characterize this product.

N-Benzyloxycarbonyl-L-tyrosyl-L-serine Methyl Ester (XV).—Dicyclohexylcarbodiimide (1.236 g, 0.0061 mole) was added to a solution of N-benzyloxycarbonyl-L-tyrosine[32] [1.576 g, 0.005 mole; mp 101–101°; $[\alpha]^{26}D$ +43.3° (*c* 2.01, chloroform)], L-serine methyl ester hydrochloride (0.776 g, 0.005 mole), and triethylamine (0.70 ml, 0.005 mole) in acetonitrile–dioxane (1:1, 50 ml) held at 0°. The usual work-up procedure led to N-benzyloxycarbonyl-L-tyrosyl-L-serine methyl ester (1.638 g, 79%): mp 151–152°; $[\alpha]^{25.5}D$ −3.3° (*c* 0.98, methanol) [lit.[26,27] mp 152°, 156–158°; $[\alpha]^{23}D$ −3.1° (*c* 2.2, methanol), $[\alpha]^{22}D$ −2.8° (*c* 2, methanol); ν_{max} 3340 very broad (OH), 1695 very broad (C=O and urethane), 1515 (amide II), 1240 very broad (OH), and 697 (Ph) cm^{-1}; λ_{max} 226, 252, 258, 264, 267, 277, and 284 mμ (ε 7760, 350, 535, 782, 986, 1380, and 1130).

Anal. Calcd for $C_{21}H_{24}N_2O_7$ (416.4): C, 60.57; H, 5.81; N, 6.73. Found: C, 60.55; H, 5.79; N, 6.88.

N,O-Dibenzyloxycarbonyl-L-tyrosyl-L-serine Methyl Ester (XVII).—A hot solution of L-serine methyl ester hydrochloride (4.655 g, 0.030 mole) in dimethylformamide (20 ml) was added to a solution of triethylamine (4.17 ml, 0.030 mole) in acetonitrile (100 ml) held at −20° (acetone–Dry Ice bath). While the temperature was maintained, N,O-dibenzyloxycarbonyl-L-tyrosine (13.49 g, 0.030 mole) was added, with the aid of a powder funnel and a little acetonitrile, followed by dicyclohexylcarbodiimide (6.49 g, 0.0315 mole). After 2 hr at 0°, the mixture was allowed to warm to room temperature and stirred overnight. After filtration from the precipitated dicyclohexylurea, the solvent was removed and the oily residue was redissolved in dichloromethane and washed with 0.5 *M* aqueous citric acid, saturated sodium bicarbonate solution, and brine. Evaporation of the dried organic phase furnished a white solid, which was crystallized from methanol to yield needles of N,O-dibenzyloxycarbonyl-L-tyrosyl-L-serine methyl ester (13.41 g, 81%): mp 176–177°; $[\alpha]^{22}D$ −4.2° (*c* 0.962, dimethylformamide); R_f 0.66; ν_{max} 3400 very broad (OH), 2950 (CH), 1750 (C=O), 1680 very broad (C=O and urethane), 1530 (amide II), 1265 (CO), and 696 (Ph) cm^{-1}; λ_{max} 252, 257, 262, 264, and 268 mμ (ε 525, 642, 613, 619, and 452).

Anal. Calcd for $C_{29}H_{30}N_2O_8$ (534.6): C, 65.16; H, 5.66; N, 5.24. Found: C, 65.17; H, 5.59; N, 5.55.

N-Benzyloxycarbonyl-L-tyrosyl-L-serine Hydrazide (XVI). A. From N-Benzyloxycarbonyl-L-tyrosyl-L-serine Methyl Ester.—A solution of N-benzyloxycarbonyl-L-tyrosyl-L-serine methyl ester (0.833 g, 0.002 mole) and 95% hydrazine (0.334 ml, 0.010 mole) in methanol (10 ml) on standing at room temperature for 48 hr formed white needles of N-benzyloxycarbonyl-L-tyrosyl-L-serine hydrazide (0.762 g, 91%): mp 226–227°; $[\alpha]^{21.5}D$ −4.9° (*c* 1.0, dimethylformamide), $[\alpha]^{26}D$ −20.9° (*c* 1.05, 1 *N* hydrochloric acid); ν_{max} 3400 very broad (OH), 3395 (NH), 2950 (CH), 1660 very broad (C=O) and urethane), 1515 (amide II), 1240 broad (CO), and 697 (Ph) cm^{-1}; λ_{max} 225, 252, 258, 264, 267, 277, and 283 mμ (ε 9860, 391, 576, 845, 1070, 1460, and 1190).

Anal. Calcd for $C_{20}H_{24}N_4O_6$ (416.4): C, 57.69; H, 5.81; N, 13.45. Found: C, 57.34; H, 6.00; N, 13.70.

B. From N,O-Dibenzyloxycarbonyl-L-tyrosyl-L-serine Methyl Ester.—N,O-Dibenzyloxycarbonyl-L-tyrosyl-L-serine methyl ester (5.506 g, 0.010 mole) was treated with hydrazine (3.34 ml, 0.100 mole) in methanol (70 ml) under reflux for 30 min and the reaction mixture left to crystallize at room temperature. Recrystallization of the product from methanol furnished N-benzyloxycarbonyl-L-tyrosyl-L-serine hydrazide (2.750 g, 66%), identical in all respects with the material from **A**.

N-Benzyloxycarbonyl-L-tyrosyl-L-seryl-N$^{\epsilon}$-*t*-butyloxycarbonyl-L-lysine Methyl Ester (XVIII).—N-Benzyloxycarbonyl-L-tyrosyl-L-serine hydrazide (1.249 g, 0.003 mole) was dissolved in dimethylformamide (9 ml) by warming and the solution cooled to −40° (acetone–Dry Ice bath). Hydrochloric acid (1.86 *N*) in tetrahydrofuran (6.45 ml, 0.012 mol of hydrochloric acid) was then added, followed by *n*-butyl nitrite (0.41 ml, 0.0036 mole). After 30 min at −40°, triethylamine (1.67 ml, 0.012 mole) was added, and then a solution of N$^{\epsilon}$-*t*-butyloxycarbonyl-L-lysine methyl ester hydroacetate (1.057 g, 0.0033 mole) and triethylamine (0.46 ml, 0.0033 mole) in dimethylformamide (9 ml) at −20°. The final reaction mixture was left at 0° in the cold room for 48 hr.

Removal of the solvent left a residue, which was distributed between ethyl acetate and water. The organic phase was washed with 0.5 *M* aqueous citric acid, saturated sodium bicarbonate solution, and brine, and the dried solution evaporated, and the residue redissolved in tetrahydrofuran. The addition of light petroleum gave a gel, which was collected and washed with light petroleum. Reprecipitation of this product followed by washing and drying yielded N-benzyloxycarbonyl-L-tyrosyl-L-seryl-N$^{\epsilon}$-*t*-butyloxycarbonyl-L-lysine methyl ester (1.358 g, 70%): mp 116–118°; $[\alpha]^{25}D$ −8.3° (*c* 0.96, methanol), $[\alpha]^{27}D$ −6.2° (*c* 0.96, dimethylformamide); R_f 0.77; ν_{max} 3320 very broad (OH), 2950 broad (CH), 1680 very broad (C=O and urethane), 1515 (amide II), 1392 and 1365 (*t*-butyl), 1250 broad (CO) and 698 (Ph) cm^{-1}; λ_{max} 226, 253, 258, 264, 268, 277, and 283 mμ (ε 9670, 428, 668, 960, 1230, 1650, and 1410).

Anal. Calcd for $C_{32}H_{44}N_4O_{10}$ (644.7): C, 59.62; H, 6.88; N, 8.69. Found: C, 59.72; H, 6.98; N, 8.60.

L-Tyrosyl-L-seryl-N$^{\epsilon}$-*t*-butyloxycarbonyl-L-lysine Methyl Ester Hydroacetate (XIX).—N-Benzyloxycarbonyl-L-tyrosyl-L-seryl-N$^{\epsilon}$-*t*-butyloxycarbonyl-L-lysine methyl ester (1.289 g, 0.002 mole) was hydrogenated in methanol (20 ml) in the presence of 10% palladium-on-carbon catalyst (0.050 g) and acetic acid (0.12 ml, 0.002 mole). After removal of the catalyst, the solvent was evaporated to leave an oil, which was obtained as a gel from hot ethyl acetate–ether. Washing with ether and drying furnished L-tyrosyl-L-seryl-N$^{\epsilon}$-*t*-butyloxycarbonyl-L-lysine methyl ester hydroacetate as a white powder (0.927 g, 81%): mp 103–105°; $[\alpha]^{24.5}D$ −5.1° (*c* 0.98, methanol); ν_{max} 3320 very broad (OH), 2950 broad (CH), 1670 very broad (C=O and urethane), 1510 (amide II), 1395 and 1363 (*t*-butyl), and 1250 broad (CO) cm^{-1}.

Anal. Calcd for $C_{26}H_{42}N_4O_{10}$ (570.6): C, 54.73; H, 7.42; N, 9.82. Found: C, 54.80; H, 7.31; N, 9.97.

N-Benzyloxycarbonyl-β-*t*-butyl-L-aspartyl-L-tyrosyl-L-seryl-N$^{\epsilon}$-*t*-butyloxycarbonyl-L-lysine Methyl Ester (XXII). A. By Use of L-Tyrosyl-L-seryl-N$^{\epsilon}$-*t*-butyloxycarbonyl-L-lysine Methyl Ester Hydroacetate and N-Benzyloxycarbonyl-β-*t*-butyl-L-aspartate α-2,4,5-Trichlorophenyl Ester.—A dimethylformamide (15 ml) solution of L-tyrosyl-L-seryl-N$^{\epsilon}$-*t*-butyloxycarbonyl-L-lysine methyl ester hydroacetate, freshly prepared by the hydrogenation of N-benzyloxycarbonyl-L-tyrosyl-L-seryl-N$^{\epsilon}$-*t*-butyloxycarbonyl-L-lysine methyl ester (1.928 g, 0.003 mole), was treated with N-benzyloxycarbonyl-β-*t*-butyl-L-aspartate α-2,4,5-trichlorophenyl ester[23] (1.508 g, 0.003 mole). After 48 hr, the solvent was evaporated and the residue redissolved in ethyl acetate and washed with 0.5 *M* aqueous citric acid, saturated sodium bicarbonate solution, and brine. On concentrating to a small volume and keeping at 0° for 24 hr, the dried ethyl acetate solution deposited a gel, which was collected with the aid of ether. Rewashing with ether and drying afforded N-benzyloxycarbonyl-β-*t*-butyl-L-aspartyl-L-tyrosyl-L-seryl-N$^{\epsilon}$-*t*-butyloxycarbonyl-L-lysine methyl ester as an amorphous, white powder (1.740 g, 71%): mp 94–96°; $[\alpha]^{27}D$ −26.1° (*c* 1.0, methanol); R_f 0.43; ν_{max} 3345 very broad (OH), 2975 (CH), 1700 very broad (C=O and urethane), 1510 (amide II), 1390 and 1365 (*t*-butyl), 1248 broad (CO) and 697 (Ph) cm^{-1}; λ_{max} 226, 252, 258, 264, 267, 277, and 283 mμ (ε 9560, 400, 615, 885, 1110, 1585, and 1345).

Anal. Calcd for $C_{40}H_{57}N_5O_{13}$ (815.9): C, 58.88; H, 7.04; N, 8.58. Found: C, 58.93; H, 7.16; N, 8.73.

B. The tetrapeptide was alternatively prepared from N-benzyloxycarbonyl-β-*t*-butyl-L-aspartate *p*-nitrophenyl ester and L-tyrosyl-L-seryl-N$^{\epsilon}$-*t*-butyloxycarbonyl-L-lysine methyl ester hydroacetate (68%).

N-Benzyloxycarbonyl-β-*t*-butyl-L-aspartyl-L-tyrosyl-L-seryl-N$^{\epsilon}$-*t*-butyloxycarbonyl-L-lysine Hydrazide (XXIII).—A solution of N-benzyloxycarbonyl-β-*t*-butyl-L-aspartyl-L-tyrosyl-L-seryl-N$^{\epsilon}$-*t*-butyloxycarbonyl-L-lysine methyl ester (0.816 g, 0.001 mole) and 95% hydrazine (0.17 ml, 0.005 mole) in methanol (15 ml) on standing at room temperature for 20 hr produced microcrystalline white needles, which were collected, washed with methanol, and dried to yield N-benzyloxycarbonyl-β-*t*-butyl-L-aspartyl-L-

(32) M. Bergmann and L. Zervas, *Ber.*, **65**, 1192 (1932).

tyrosyl-L-seryl-N$^{\epsilon}$-*t*-butyloxycarbonyl-L-lysine hydrazide (0.585 g, 72%): mp 186–188°; R_f 0.16.

Anal. Calcd for $C_{39}H_{57}N_7O_{12}$ (815.9): C, 57.41; H, 7.04; N, 12.02. Found: C, 57.17; H, 7.34; N, 12.17.

A number of attempts to secure pure tetrapeptide hydrazide from slightly impure tetrapeptide ester were unsuccessful; the tetrapeptide hydrazide was invariably obtained as a gel.

N-Benzyloxycarbonyl-L-tyrosyl-L-serylglycine Methyl Ester (XXIV).—A solution of sodium nitrite (0.210 g, 0.00305 mole) in water (3 ml) was added to a solution of N-benzyloxycarbonyl-L-tyrosyl-L-serine hydrazide (1.249 g, 0.003 mole) in acetic acid (18 ml), 2 *N* hydrochloric acid (12 ml), and water (10 ml) maintained at 0°. The resulting azide was precipitated with sodium chloride and extracted into three 10-ml portions of cold ethyl acetate. The combined extracts were washed free of acid with saturated sodium bicarbonate solution and dried (the drying agent was not removed until the coupling reaction was complete). A solution of glycine methyl ester, previously prepared by dissolving glycine methyl ester hydrochloride (0.414 g, 0.0033 mole) in dimethylformamide (30 ml) with warming, rapidly cooling the solution to 0°, and immediately adding triethylamine (0.46 ml, 0.0033 mole), was poured into the ethyl acetate solution of the azide. After 48 hr at 0°, the mixture was filtered, the solvents evaporated, and the residue dissolved in the minimum amount of hot 50% aqueous methanol. On cooling, the solution deposited almost colorless needles of N-benzyloxycarbonyl-L-tyrosyl-L-serylglycine methyl ester (1.005 g, 71%): mp 204–206°; $[\alpha]^{27}D$ −7.0° (*c* 1.0, dimethylformamide); R_f 0.19; ν_{max} 3400 very broad (OH), 2950 (CH), 1745 (methyl C=O), 1689 (urethane C=O), 1642 (amide I), 1512 (amide II), 1230 broad (OH), and 698 (Ph) cm^{-1}; λ_{max} 226, 257, 264, 268, 274, 277, and 284 mμ (ϵ 9760, 688, 1000, 1330, 1650, 1790, and 1380).

Anal. Calcd for $C_{23}H_{27}N_3O_8$ (473.5): C, 58.35; H, 5.75; N, 8.87. Found: C, 58.52; H, 5.69; N, 9.03.

The tripeptide was alternatively prepared by use of *t*-butyl nitrite, N-benzyloxycarbonyl-L-tyrosyl-L-serine hydrazide and glycine methyl ester hydrochloride (74%).

Registry No.—II, 1155-64-2; III, 2212-75-1; IV, 2212-76-2; VI, 2389-49-3; VII, 3252-80-0; IX, 3236-14-4; XII, 15376-59-7; XIV, 15523-36-1; XV, 15364-45-1; XVI, 2480-91-3; XVII, 15364-47-3; XVIII, 15364-48-4; XIX, 15523-37-2; XXII, 15364-49-5; XXIII, 15364-50-8; XXIV, 15364-51-9.

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Derivatives of Morphine. V.[1] The Structure of Anhydrometathebainol

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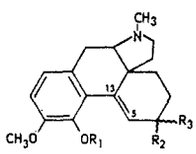
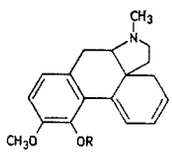
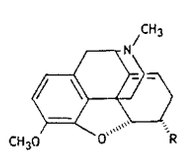
During their investigation of metathebainone (I), Small and Meitzner[2] described the preparation of "anhydrometathebainol" by dehydration of metathebainol (II) with ethanolic KOH at 160°. The authors were not able to assign a structure to the product, although they considered formula III, resulting from straightforward dehydration of II. However, they discarded III on the grounds that the dihydro derivative, obtained by catalytic hydrogenation,[3] seemed to be different from dihydrodesoxymetacodeine, the product of Wolff–Kishner reduction of I. The latter was assumed to be V and should hence be identical with dihydro-III. On this basis they concluded that "the formation of anhydrometathebainol has involved a deeper-seated structural change then mere dehydration."



I, R_1 = H; R_2R_3 = O
II, R_1 = R_2 = H; R_3 = OH
IV, R_1 = Ac; R_2 = OAc; R_3 = H
V, R_1 = R_2 = R_3 = H
VI, R_1 = Ac; R_2 = R_3 = H



III, R = H
VII, R = Ac



VIII, R = OH
IX, R = H

We were interested in the possibility of such a rearrangement and have reinvestigated this work in order to establish the structures of anhydrometathebainol and of the related dihydro derivatives. Metathebainol (II) was prepared either by catalytic hydrogenation[2] or, more conveniently, by borohydride reduction of metathebainone (I). In our hands the former procedure was erratic and gave mixtures, possibly containing products of hydrogenolysis which were not further investigated. Borohydride reduction resulted in a mixture of epimers which could be used directly for the subsequent step. The same crystalline diacetate (IV) was obtained from both preparations.

Dehydration of metathebainol by the method of Small and Meitzner[2] or by a slightly modified procedure gave crude anhydrometathebainol which could not be purified by crystallization. The melting points of this and of the other free phenols in this series were low and indefinite due to variable solvation and were therefore not suitable for characterization. Acetylation of anhydrometathebainol furnished the acetate, which could be purified readily and was identical with an authentic specimen.[2] The nmr spectrum showed a complex multiplet (3 H) in the olefinic region (δ 5.5–6.3), which was resolved into three distinct multiplets (one proton each), centered at δ 6.1, 5.92, and 5.67, on a 100-Mc instrument. The long-wavelength band in the uv region (see Experimental Section) showed a bathochromic shift (~18 mμ) with respect to metathebainol diacetate (IV) and to dihydroanhydrometathebainol acetate (see below). The strong negative circular dichroism ($\Delta\epsilon_{286-288}$ −11.3) and optical rotatory dispersion (complex negative effect, first extremum at 313 mμ, [Φ] −19,700°) are compatible with the presence of an inherently dissymmetric chromophore such as the nonplanar phenylbutadiene system. The above evidence is consistent with structure III for anhydrometathebainol which is further corroborated by the study of the dihydro derivative.

Catalytic hydrogenation of anhydrometathebainol acetate resulted in the uptake of 1 mol of hydrogen. The dihydro derivative was found to be identical with the product obtained on acetylation of dihydrodeoxymetacodeine (V), prepared[2] by Wolff–Kishner reduction of I. The structure of this was confirmed by spectroscopic data (see Experimental Section), which are consistent with VI. Of particular interest in the nmr

(1) Part IV: U. Weiss and S. J. Daum, *J. Med. Chem.*, **8**, 123 (1965).
(2) L. F. Small and E. Meitzner, *J. Amer. Chem. Soc.*, **55**, 4602 (1933).
(3) Throughout the metathebainone series, $\Delta^{8(13)}$ is relatively inert to catalytic hydrogenation.