Notes

Amino Acids and Peptides. XVIII.[1] Synthesis of a Tetrapeptide Sequence (A_1–A_4) of Glucagon

BRIAN O. HANDFORD, THOMAS A. HYLTON, KUNG-TSUNG WANG, AND BORIS WEINSTEIN

Department of Chemistry, Stanford University, Stanford, California 94305

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The N-terminal A_1–A_4 sequence of the hyperglycemic hormone glucagon[2] has been prepared in the form of blocked derivatives by several groups in the last few years. The original scheme utilized the dipeptides N^{α}-benzyloxycarbonyl-L-histidyl-L-serine hydrazide, N^{α}-benzyloxycarbonyl-N^{im}-benzyl-L-histidyl-L-serine hydrazide, and N^{α}-benzyloxycarbonyl-L-glutaminyl glycine for the construction of larger units.[3] Later, the dipeptide N^{α}-*t*-butyloxycarbonyl-L-histidyl-O-*t*-butyl-L-serine hydrazide was employed for a related synthesis.[4] The tetrapeptide N^{α}-benzyloxycarbonyl-N^{im}-benzyl-L-histidyl-L-seryl-L-glutaminylglycine has been made by two procedures and used in a succeeding condensation reaction.[5] Finally, another variant of the tetrapeptide exists in the form of N^{α}-*t*-butyloxycarbonyl-L-histidyl-L-seryl-L-glutaminylglycine.[6]

In concluding our work on small glucagon fragments,[7-12] there are described here several new approaches to the A_1–A_4 sequence involving various protecting groups. For example, N^{α}-benzyloxycarbonyl-L-glutamine (I)[6,13-17] and glycine methyl ester hydrochloride (II)[12,18] were coupled by 2-ethyl-5-phenyloxazolium-3′-sulfonate[19,20] to yield N^{α}-benzyloxycarbonyl-L-glutaminylglycine methyl ester (III). Dipeptide III was formed in similar amount by the combination of N^{α}-benzyloxycarbonyl-L-glutamine 2,4,5-trichlorophenyl ester (IV)[21] or N^{α}-benzyloxycarbonyl-L-glutamine *p*-nitrophenyl ester (V)[22] with the glycine ester II. Removal of the N^{α}-benzyloxycarbonyl group of dipeptide III by hydrogenolysis in the presence of dilute acid gave L-glutaminylglycine methyl ester hydrochloride (VI). Treatment of L-serine (VII) with acetyl chloride[23] afforded O-acetyl-L-serine hydrochloride (VIII),[24,25] which was converted into N^{α}-benzyloxycarbonyl-O-acetyl-L-serine (IX).[26] Reaction with *p*-nitrophenol and N,N′-dicyclohexylcarbodiimide[27] produced the corresponding *p*-nitrophenyl ester (X).[28-30] Similarly, acid IX was joined with 2,4,5-trichlorophenol to yield N^{α}-benzyloxycarbonyl-O-acetyl-L-serine 2,4,5-trichlorophenyl ester (XI).

A reaction between amine VI and the activated ester X furnished N^{α}-benzyloxycarbonyl-O-acetyl-L-seryl-L-glutaminylglycine methyl ester (XII). The benzyloxycarbonyl moiety in tripeptide XII was cleaved with hydrogen bromide–acetic acid to yield O-acetyl-L-seryl-L-glutaminylglycine methyl ester hydrobromide (XIII). Alternatively, hydrogenolysis of XII formed O-acetyl-L-seryl-L-glutaminylglycine methyl ester (XIV). At this point, N^{α}-benzyloxycarbonyl-N^{im}-benzyl-L-histidine (XV)[31] was combined with 2,4,5-trichlorophenol to form N^{α}-benzyloxycarbonyl-N^{im}-benzyl-L-histidine 2,4,5-trichlorophenyl ester (XVI),[32] and neutralization of the salt XIII followed by addition of the activated ester XVI gave the desired N^{α}-benzyloxycarbonyl-N^{im}-benzyl-L-histidyl-O-acetyl-L-serine-L-glutaminylglycine methyl ester (XVII).

A second synthesis of the tetrapeptide sequence was achieved in the following manner. The trichlorophenyl ester XVI and L-serine methyl ester afforded N^{α}-benzyloxycarbonyl-N^{im}-benzyl-L-histidyl-L-serine methyl ester (XVIII),[5] which was hydrolyzed to the corresponding acid (XIX).[5] Addition of hydrazine to the ester XVIII produced N^{α}-benzyloxycarbonyl-N^{im}-benzyl-L-histidyl-L-serine hydrazide (XX). An azide coupling between XX and the amine VI furnished an amorphous N^{α}-benzyloxycarbonyl-N^{im}-benzyl-L-histidyl-L-seryl-L-glutaminylglycine methyl ester

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(XXI). Alternatively, VI and N$^{\alpha}$-benzyloxycarbonyl-L-serine (XXII) were joined by N,N′-dicyclohexylcarbodiimide[33] to yield N$^{\alpha}$-benzyloxycarbonyl-L-seryl-L-glutaminylglycine methyl ester (XXIII). Hydrogenolysis formed L-seryl-L-glutaminylglycine methyl ester (XXIV), which on reaction with the trichlorophenyl ester XVI gave crystalline tetrapeptide XXI.

In a third approach, L-histidine hydrochloride monohydrate (XXV) was treated with methanol–sulfuric acid–hydrogen chloride to afford L-histidine methyl ester dihydrochloride (XXVI).[34] The older methanol–hydrogen chloride route is more troublesome.[35]

Neutralization of salt XXVI, followed by addition of *t*-butyloxycarbonyl azidoformate,[36] furnished N$^{\alpha}$-*t*-butyloxycarbonyl-L-histidine methyl ester (XXVII). N$^{\alpha}$,N^{im}-di-*t*-butyloxycarbonyl-L-histidine methyl ester (XXVIII) was also isolated from this reaction; though it slowly decomposes to the mono-*t*-butyloxycarbonyl-substituted product XXVII, it too may be used in peptide synthesis. Routine hydrolysis of the ester XXVII yielded N$^{\alpha}$-*t*-butyloxycarbonyl-L-histidine (XXIX).

The last scheme for the preparation of the tetrapeptide utilized N^{im}-benzyl-L-histidine (XXX),[37] which on blocking with *t*-butyloxycarbonyl azidoformate, gave N$^{\alpha}$-*t*-butyloxycarbonyl-N^{im}-benzyl-L-histidine (XXXI). It is noted here that this particular compound has been sought without success by others. The intermediate XXXI promises to be a valuable addition to the small list of histidine derivatives now employed in the general technique of stepwise synthesis.[38,39] Combination with 2,4,5-trichlorophenol then afforded N$^{\alpha}$-*t*-butyloxycarbonyl-N^{im}-benzyl-L-histidine 2,4,5-trichlorophenyl ester (XXXII). Addition of XXXII to the amine XXIV produced crystalline N$^{\alpha}$-*t*-butyloxycarbonyl-N^{im}-benzyl-L-histidyl-L-seryl-L-glutaminylglycine methyl ester (XXXIII), whereas an attempt to prepare XXXIII by coupling the acid XXXI and the amine XXIV with N,N′-dicyclohexylcarbodiimide furnished only an oil.

The synthesis, isolation, and characterization of the various tetrapeptides completed the efforts directed toward the preparation of seven distinct glucagon fragments. Future reports in this series will deal with the combination of these blocked units to form larger and possibly physiologically active peptides.

Experimental Section[40]

N$^{\alpha}$-Benzyloxycarbonyl-L-glutaminylglycine Methyl Ester (III).—N$^{\alpha}$-Benzyloxycarbonyl-L-glutamine (13.31 g, 0.05 mol) {mp 134–136.5°; $[\alpha]^{26.8}$D −6.9° (*c* 2.00, ethanol); $[\alpha]^{27.0}$D −3.7° (*c* 2.00, glacial acetic acid)} {lit.[5,13–17] mp 138–139.5°, 135°, 133–137°; $[\alpha]$D −7.1°, +5.8° (ethanol), and −1.7°, +7.6° (glacial acetic acid)} was dissolved in acetonitrile (250 ml) containing triethylamine (6.95 ml, 0.05 mol), and the solution was cooled to 5°. 2-Ethyl-5-phenyloxazolium-3′-sulfonate (12.63 g, 0.05 mol) was added with stirring, and the reaction mixture was allowed to warm to room temperature with complete solution occurring within 40 min. Finely powdered glycine methyl ester hydrochloride (6.27 g, 0.05 mol) was dissolved in hot N,N-dimethylformamide (250 ml), and triethylamine (6.95 ml, 0.05 mol) was added with rapid cooling (Dry Ice–isopropanol bath) to 0°. The resulting suspension was then added to the acetonitrile solution containing the "Woodward" intermediate. After 48 hr, the solution was evaporated almost to dryness and the residue dissolved in chloroform (1 l.) and washed rapidly with water (two 250-ml portions). Any delay after the first water wash may cause the chloroform phase to set to a gelatinous mass. Ethanol (200 ml) was added to the chloroform solution, and the mixture was evaporated to dryness. A solution of the residue in methanol (50 ml) deposited a hard, white, microcrystalline mass [mp 166–172°; 9.77 g (56%)]. Recrystallization form methanol–water (4:1) gave fine, white needles of N$^{\alpha}$-benzyloxycarbonyl-L-glutaminylglycine methyl ester: mp 174–175°; $[\alpha]^{28.3}$D −6.6° (*c* 1.00, N,N-dimethylformamide); R_f 0.64 (methanol); ν_{max} 3400 (NH), 2950 (CH), 1740 and 1700 (C=O), 1685 (urethan), 1655 (amide I), 1530 (amide II), 1240 and 1210 (CO), and 697 (Ph) cm^{-1}; λ_{max} 242, 247, 252, 257, 262, 264, and 268 mμ (ε 109, 132, 170, 210, 158, 172, and 113).

Anal. Calcd for $C_{16}H_{21}N_3O_6$ (351.4): C, 54.69; H, 6.02; N, 11.96. Found: C, 54.84; H, 6.02; N, 11.98.

The dipeptide was alternatively prepared in a like manner, but using acetonitrile (250 ml) in place of N,N-dimethylformamide as the solvent for glycine methyl ester hydrochloride. In this event, crude dipeptide (46%) separated in solid form from the reaction solution. Other coupling reactions included N$^{\alpha}$-benzyloxycarbonyl-L-glutamine 2,4,5-trichlorophenyl ester with glycine methyl ester hydrochloride (51%) and N$^{\alpha}$-benzyloxycarbonyl-L-glutamine *p*-nitrophenyl ester with glycine methyl ester hydrochloride (57%).

L-Glutaminylglycine Methyl Ester Hydrochloride (VI).—N$^{\alpha}$-Benzyloxycarbonyl-L-glutaminylglycine methyl ester (3.514 g, 0.01 mol) was dissolved in warm methanol (50 ml) and hydrogenolyzed in the presence of 10% palladium-on-carbon catalyst (0.100 g) and hydrochloric acid (1 *N*, 10 ml) during 2 hr. Usually, it was necessary at the beginning of the hydrogenation to keep the solution warm with an infrared lamp to prevent separation of the starting material. Removal of the catalyst and evaporation of the methanol and water furnished oily L-glutaminylglycine methyl ester hydrochloride [2.173 g, 100%; R_f 0.10 (ninhydrin positive)].

O-Acetyl-L-serine Hydrochloride (VIII).—A mixture of L-serine (1.000 g, 0.01 mol) and glacial acetic acid–acetyl chloride (1:1, 30 ml) was allowed to stand at room temperature for 20 min, then ether was added to precipitate O-acetyl-L-serine hydrochloride (1.30 g, 76%): mp 160–161° dec; $[\alpha]^{26.0}$D +18.3° (*c* 2.0, ethanol) {lit.[24,25] mp 160° dec; $[\alpha]^{27}$D −7.4° (*c* 2.2, ethanol)}.

N$^{\alpha}$-Benzyloxycarbonyl-O-acetyl-L-serine (IX).—Carbobenzyloxy chloride was added to a solution of O-acetyl-L-serine hydrochloride (4.800 g, 0.026 mol) in saturated sodium bicarbonate (150 ml) at room temperature. After 3.5 hr, the solution was washed with ether, acidified, and extracted with ethyl acetate. Washing, drying, and evaporation of the organic solution gave an oil (2.400 g, 33%) [lit.[26] oil].

N$^{\alpha}$-Benzyloxycarbonyl-O-acetyl-L-serine *p*-Nitrophenyl Ester (X).—Oily N$^{\alpha}$-benzyloxycarbonyl-O-acetyl-L-serine (7.110 g, 0.025 mol) was condensed with *p*-nitrophenol (4.173 g, 0.030 mol) using N,N′-dicyclohexylcarbodiimide (5.178 g, 0.025 mol) in ethyl acetate (50 ml) at 0°. After 3 hr, the N,N′-dicyclohexylurea was removed, and the ethyl acetate solution was washed with sodium bicarbonate (5%) and brine and dried. Evaporation gave a solid that was crystallized from ethanol (2.251 g, 23%): mp 98–99° (lit.[28–30] 94–96, 90–91, 74–76°).

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N^{α}-Benzyloxycarbonyl-O-acetyl-L-serine 2,4,5-Trichlorophenyl Ester (XI).—Oily N^{α}-benzyloxycarbonyl-O-acetyl-L-serine (3.821 g, 0.014 mol) was condensed with 2,4,5-trichlorophenol (3.160 g, 0.016 mol) using N,N′-dicyclohexylcarbodiimide (3.294 g, 0.014 mol) in ethyl acetate (50 ml) at −5°. The product was isolated as described in the previous procedure. Crystallization from benzene gave N^{α}-benzyloxycarbonyl-O-acetyl-L-serine 2,4,5-trichlorophenyl ester (2.900 g, 46%): mp 88–89°; ν_{max} 3330 (NH), 2930 (CH), 1765 (C=O), 1720 broad (C=O and urethan), 1225 (CO), and 698 (Ph) cm^{-1}; λ_{max} 267 mμ (ε 8100).

Anal. Calcd for $C_{19}H_{16}Cl_3NO_6$ (460.7); C, 50.51; H, 3.45; Cl, 23.08, N, 3.03. Found: C, 50.80; H, 3.54; Cl, 21.85; N, 3.30.

N^{α}-Benzyloxycarbonyl-O-acetyl-L-seryl-L-glutaminylglycine Methyl Ester (XII).—L-Glutaminylglycine methyl ester [freshly prepared from N^{α}-benzyloxycarbonyl-L-glutaminylglycine methyl ester (1.054 g, 0.0030 mol) and 10% palladium-on-carbon catalyst (0.100 g) in methanol (50 ml)] and N^{α}-benzyloxycarbonyl-O-acetyl-L-serine *p*-nitrophenyl ester (1.330 g, 0.0033 mol) in N,N-dimethylformamide (25 ml) was stirred for 2 days. After dilution with ether (100 ml), the gelatinous precipitate was filtered, washed with sodium bicarbonate (5%) and water, and dried. Crystallization from water gave N^{α}-benzyloxycarbonyl-O-acetyl-L-seryl-L-glutaminylglycine methyl ester (0.805 g, 52%): mp 210–212°; $[\alpha]^{25.0}D$ −6.8° (*c* 1.76, N,N-dimethylformamide); R_f 0.35; ν_{max} 3290 (OH), 2950 (CH), 1735 (C=O), 1655 broad (C=O and urethan), 1220 (CO), and 697 (Ph) cm^{-1}; λ_{max} 247, 252, 262, 264, 267, and 312 mμ (ε 282, 286, 313, 274, 285, 234, and 196).

Anal. Calcd for $C_{21}H_{28}N_4O_9$ (480.5); C, 52.49; H, 5.87; N, 11.66. Found: C, 52.68; H, 5.94; N, 11.40.

O-Acetyl-L-seryl-L-glutaminylglycine Methyl Ester Hydrobromide (XIII).—N^{α}-Benzyloxycarbonyl-O-acetyl-L-seryl-L-glutaminylglycine methyl ester (0.200 g, 0.00042 mol) was added to a mixture of hydrogen bromide–acetic acid (30%, 1 ml) and glacial acetic acid (1 ml). After stirring for 1 hr, the solution was diluted with ether (10 ml), and the precipitated solid was collected, washed with ether, and stored *in vacuo*. Owing to its hygroscopic nature, no physical data were obtained for this product.

O-Acetyl-L-seryl-L-glutaminylglycine Methyl Ester (XIV).—A suspension of N^{α}-benzyloxycarbonyl-O-acetyl-L-seryl-L-glutaminylglycine methyl ester (0.120 g, 0.00025 mol) in methanol (20 ml) containing 10% palladium-on-carbon catalyst (0.030 g) was hydrogenated for 2 hr. The catalyst was removed by filtration and evaporation of the solvent gave O-acetyl-L-seryl-L-glutaminylglycine methyl ester as a white solid (0.087 g, 100%): R_f 0.10 (ninhydrin positive).

N^{α}-Benzyloxycarbonyl-N^{im}-benzyl-L-histidine (XV).—This compound was obtained from N^{im}-benzyl-L-histidine (6.100 g, 0.025 mol) and carbobenzoxy chloride (5 ml) in lithium hydroxide (1 *N*, 25 ml) and dioxane (15 ml) in the usual manner (7.450 g, 79%): mp 214–215° dec; $[\alpha]^{26.0}D$ +6.4° (*c* 5, glacial acetic acid) {lit.[31] mp 210–213°; $[\alpha]^{21}D$ +5.2° (*c* 5, glacial acetic acid)}.

N^{α}-Benzyloxycarbonyl-N^{im}-benzyl-L-histidine 2,4,5-Trichlorophenyl Ester (XVI).—This compound was obtained from N^{α}-benzyloxycarbonyl-N^{im}-benzyl-L-histidine (3.794 g, 0.01 mol), 2,4,5-trichlorophenol (2.369 g, 0.012 mol), and N,N′-dicyclohexylcarbodiimide (2.063 g, 0.01 mol) in N,N-dimethylformamide (25 ml) in the usual manner (4.099 g, 73%): mp 108–109° (lit.[32] mp 107–108°).

N^{α}-Benzyloxycarbonyl-N^{im}-benzyl-L-histidyl-O-acetyl-L-seryl-L-glutaminylglycine Methyl Ester (XVII).—The aforementioned O-acetyl-L-seryl-L-glutaminylglycine methyl ester hydrobromide in N,N-dimethylformamide (3 ml) was treated in turn with tri-*n*-butylamine (0.077 g, 0.00042 mol) and N^{α}-benzyloxycarbonyl-N^{im}-benzyl-L-histidine 2,4,5-trichlorophenyl ester (0.235 g, 0.0024 mol) and stirred overnight at room temperature. Addition of ether gave a solid, which was collected, washed with ammonium hydroxide (1 *N*), hydrochloric acid (1 *N*), and water, and dried. Crystallization from N,N-dimethylformamide and then ethanol gave N^{α}-benzyloxycarbonyl-N^{im}-benzyl-L-histidyl-O-acetyl-L-seryl-L-glutaminylglycine methyl ester (0.087 g, 21%): mp 197–199°.

Anal. Calcd for $C_{34}H_{41}N_7O_{10}$ (707.7): C, 57.70; H, 5.84; N, 13.86. Found: C, 57.37; H, 6.10; N, 13.90.

N^{α}-Benzyloxycarbonyl-N^{im}-benzyl-L-histidyl-L-serine Methyl Ester (XVIII).—N^{α}-Benzyloxycarbonyl-N^{im}-benzyl-L-histidine 2,4,5-trichlorophenyl ester (1.118 g, 0.002 mol) was added to a mixture of L-serine methyl ester hydrochloride (0.312 g, 0.002 mol) and triethylamine (0.30 ml, 0.002 mol) in methylene dichloride (20 ml). After 8 hr, the organic phase was washed with sodium bicarbonate (2%) and water, dried, and evaporated to dryness. Crystallization from methanol–ethyl acetate (1:3) gave white needles of N^{α}-benzyloxycarbonyl-N^{im}-benzyl-L-histidyl-L-serine methyl ester (0.801 g, 83%): mp 173.5–175° (lit.[5] mp 173–174°).

N^{α}-Benzyloxycarbonyl-N^{im}-benzyl-L-histidyl-L-serine (XIX).—This compound was obtained by treating N^{α}-benzyloxycarbonyl-N^{im}-benzyl-L-histidyl-L-serine methyl ester (0.240 g, 0.0005 mol) with sodium hydroxide (1 *N*) in dioxane (0.4 ml) in the usual manner (0.138 g, 59%): mp 181–182° (lit.[5] mp 182°).

N^{α}-Benzyloxycarbonyl-N^{im}-benzyl-L-histidyl-L-serine Hydrazide (XX).—Hydrazine (0.6 ml, 95%) was added to a solution of N^{α}-benzyloxycarbonyl-N^{im}-benzyl-L-histidyl-L-serine methyl ester (1.100 g, 0.023 mol) in ethanol (20 ml) at −10°. After 10 hr, the mixture was allowed to stand at room temperature for 48 hr. The precipitate was filtered, washed with cold ethanol, and dried. Crystallization from N,N-dimethylformamide–water (1:1) gave N^{α}-benzyloxycarbonyl-N^{im}-benzyl-L-histidyl-L-serine hydrazide (0.939 g, 85%): mp 147.5–149.0°.

Anal. Calcd for $C_{24}H_{28}N_6O_5$ (480.5): C, 59.99; H, 5.87; N, 17.49. Found: C, 59.89; H, 5.94; N, 17.41.

N^{α}-Benzyloxycarbonyl-N^{im}-benzyl-L-histidyl-L-seryl-L-glutaminylglycine Methyl Ester (XXI). A. By Use of N^{α}-Benzyloxycarbonyl-N^{im}-benzyl-L-histidyl-L-serine Hydrazide.—A solution of N^{α}-benzyloxycarbonyl-L-histidyl-L-serine hydrazide (0.192 g, 0.0004 mol) in hydrochloric acid (1 *N*, 2 ml) was cooled to 0° and treated with sodium nitrite (0.028 g, 0.0004 mol). After standing for 10 min, the solution was neutralized with potassium carbonate (5%), and the precipitated azide was collected and washed with cold water, then dissolved in N,N-dimethylformamide (1 ml), and added to a solution of L-glutaminylglycine methyl ester in N,N-dimethylformamide (3 ml), previously prepared by neutralization of the corresponding hydrochloride salt (0.087 g, 0.0004 mol). The reaction stood for 24 hr at 0°, then at room temperature for 48 hr. Afterward, the solution was evaporated, and the residue was extracted with ethyl acetate (30 ml). The organic phase was washed with water, sodium bicarbonate (5%), hydrochloric acid (5%), and brine, and dried. The addition of ether to the ethyl acetate solution formed a gum. Reprecipitation in turn from methanol–ethyl acetate and N,N-dimethylformamide–ether gave amorphous N^{α}-benzyloxycarbonyl-N^{im}-benzyl-L-histidyl-L-seryl-L-glutaminylglycine methyl ester (0.040 g, 15%): mp 247–250° dec.

B. By Use of N^{α}-Benzyloxycarbonyl-N^{im}-benzyl-L-histidine 2,4,5-Trichlorophenyl Ester.—A solution of L-seryl-L-glutaminylglycine methyl ester (0.576 g, 0.00020 mol) and N^{α}-benzyloxycarbonyl-N^{im}-benzyl-L-histidine 2,4,5-trichlorophenyl ester (0.124 g, 0.00022 mol) in N,N-dimethylformamide (3 ml) was stirred for 2 days. Sodium bicarbonate (5%, 15 ml) was added to the reaction mixture, stirring was continued for 30 min, then the solid was filtered and washed with water. Crystallization from N,N-dimethylformamide–water gave tiny, white needles of N^{α}-benzyloxycarbonyl-N^{im}-benzyl-L-histidyl-L-seryl-L-glutaminylglycine methyl ester (0.036 g, 30%): mp 246–248° dec; R_f 0.50; ν_{max} 3290 (OH), 2945 (CH), 1725 (C=O), 1660 very broad (C=O and urethan), 1160 (CO), and 692 (Ph) cm^{-1}.

Anal. Calcd for $C_{32}H_{39}H_7O_9$ (665.7): C, 57.73; H, 5.91; N, 14.73. Found: C, 57.85; H, 6.02; N, 14.70.

N^{α}-Benzyloxycarbonyl-L-Seryl-L-glutaminylglycine Methyl Ester (XXIII).—The aforementioned L-glutaminylglycine methyl ester hydrochloride (0.01 mol) and N^{α}-benzyloxycarbonyl-L-serine (2.392 g, 0.01 mol) were dissolved in N,N-dimethylformamide (50 ml). The reactants were first cooled to −40°; then triethylamine (1.39 ml, 0.01 mol) and N,N′-dicyclohexylcarbodiimide (2.166 g, 0.0105 mol) were added to the chilled solution. After 1 hr at −40°, 3 hr at −20°, and 48 hr in the cold room, the mixture was taken almost to dryness, and the residue was shaken with water. The N,N′-dicyclohexylurea was removed, the filtrate was evaporated to dryness, and the solid was crystallized from a minimum volume of hot methanol. Recrystallization from hot methanol–water (1:1) deposited colorless needles of N^{α}-benzyloxycarbonyl-L-seryl-L-glutaminylglycine methyl ester (2.622 g, 60%): mp 200.5–202.0°; $[\alpha]^{26.7}D$ −2.0° (*c* 1.0, N,N-dimethylformamide); R_f 0.60 (butanol–acetic acid–water), 0.63 (methanol); ν_{max} 3300 broad (OH), 2950 (CH), 1720 broad

(C=O), 1665 broad (C=O and urethan), 1220 (C—O) and 698 (Ph) cm^{-1}; λ_{max} 252, 257, 264, and 268 mμ (ε 223, 263, 220, and 170).

Anal. Calcd for $C_{19}H_{26}N_4O_8$ (438.4): C, 52.05; H, 5.98; N, 12.78. Found: C, 52.06; H, 5.79; N, 13.00.

If the coupling reaction was initiated at 0° and then allowed to attain room temperature, there resulted lower yields (29 and 25%) of the tripeptide. The tripeptide was alternatively prepared by use of 2-ethyl-5-phenyloxazolium-3′-sulfonate, but only an oily product was obtained in spite of many crystallization attempts.

L-Seryl-L-glutaminylglycine Methyl Ester (XXIV).—A solution of N^{α}-benzyloxycarbonyl-L-seryl-L-glutaminylglycine methyl ester (0.078 g, 0.00018 mol) in methanol (30 ml) was hydrogenolyzed in the presence of 10% palladium-on-carbon catalyst (0.021g) for 1 hr. Filtration of the catalyst and evaporation of the solvent gave oily L-seryl-L-glutaminylglycine methyl ester (0.053 g, 100%): R_f 0.25 (ninhydrin positive).

L-Histidine Methyl Ester Dihydrochloride (XXVI).—This compound was obtained by refluxing for 1 hr a solution of L-histidine hydrochloride (37.5 g, 0.196 mol) in methanol (350 ml) containing sulfuric acid (10 ml), then saturating with hydrogen chloride gas for 2 hr. After standing in the cold room overnight, the product was filtered and dried *in vacuo* (41.0 g, 87%): mp 205–206° dec (lit.[34] mp 200–201°); $[\alpha]^{24.2}D$ +11.0° (*c* 1.17, water).

N^{α}-*t*-Butyloxycarbonyl-L-histidine Methyl Ester (XXVII).—A suspension of L-histidine methyl ester dihydrochloride g, 0.100 mol) in chloroform (100 ml) at 0° was treated with ammonia gas for 10 min. After filtration of the precipitated ammonium chloride, the chloroform was evaporated, and the oily residue was dissolved in pyridine (25 ml) and treated with *t*-butyloxycarbonyl azidoformate (15.730 g, 0.11 mol). After standing for 72 hr at room temperature, the solvent was removed, and the oily residue was dissolved in ethyl acetate, washed with water to remove traces of histidine, and exhaustively extracted with citric acid (0.5 *M*). The combined acid phases were covered with an equal volume of fresh ethyl acetate, and solid sodium hydrogen carbonate was added portionwise with vigorous stirring until the aqueous solution was basic (pH 8.5). The organic layer was washed with brine, dried, and on concentration furnished colorless needles of N^{α}-*t*-butyloxycarbonyl-L-histidine methyl ester: mp 125.5–126.0°; $[\alpha]^{27.0}D$ −11.7° (*c* 1.0, methanol); $[\alpha]^{27.3}D$ −13.0° (*c* 2.0, pyridine) {lit.[6] mp 124–125°; $[\alpha]^{20}D$ −13.6° (*c* 2.0, pyridine)}; R_f 0.27; ν_{max} 3462 (NH), 2972 (CH), 1750, and 1705 (C=O), 1685 (urethan), 1390 and 1365 (*t*-butyl), and 1160 broad (CO) cm^{-1}; λ_{max} 270 very broad mμ (ε 107).

Anal. Calcd for $C_{12}H_{19}N_3O_4$ (269.3): C, 53.52; H, 7.11; N, 15.60. Found: C, 53.39; H, 6.95; N, 15.63.

N^{α},N^{im}-Di-*t*-butyloxycarbonyl-L-histidine Methyl Ester (XXVIII).—The original ethyl acetate solution from the aforementioned preparation was washed twice more with citric acid, saturated hydrogen carbonate solution, and brine, dried, and taken to dryness. The residual oil was dissolved as rapidly as possible in the minimum quantity of warm diisopropyl ether. The tepid solution was seeded with a sample of N-*t*-butyloxycarbonyl-L-histidine methyl ester and allowed to stand for several hours at room temperature, whereupon the deposited crystals were discarded. The decanted liquor was kept at 0° and slowly deposited clusters of colorless needles, recrystallization of which under the same conditions gave N^{α},N^{im}-di-*t*-butyloxycarbonyl-L-histidine methyl ester: mp *ca.* 90°; R_f 0.73; ν_{max} 3380 (NH), 2975 (CH), 1750 and 1710 (C=O), 1685 (urethan), 1387 and 1330 (*t*-butyl), and 1160 broad (CO) cm^{-1}; λ_{max} 236 mμ (ε 2910).

Anal. Calcd for $C_{17}H_{27}N_3O_6$ (369.4): C, 55.27; H, 7.37; N, 11.37. Found: C, 55.41; H, 7.38; N, 11.27.

This compound decomposes slowly at room temperature and more rapidly in solution.

N^{α}-*t*-Butyloxycarbonyl-L-histidine (XXIX).—Sodium hydroxide (1 *N*, 10 ml) was added with stirring to a solution of N^{α}-*t*-butyloxycarbonyl-L-histidine methyl ester (2.693 g, 0.01 mol) in dioxane (10 ml). After 30 min the solution was evaporated to a small volume, diluted with water, washed with ethyl acetate, and stirred with a strong cation-exchange resin (Bio Rad AG50W-X2, 200–400 mesh, exchange capacity 0.7 mequiv/ml of resin bed, analytical grade, 14.3 ml, 0.01 equiv) for 5 min. The resin was removed, and the water solution was evaporated to yield an oil, which was dissolved in the minimum volume of cold methanol, and acetonitrile added to opalescence at room temperature. A few drops of methanol restored a clear solution, which deposited glistening parallelepipeds of N^{α}-*t*-butyloxycarbonyl-L-histidine (1.818 g, 71%): mp 191.0–191.5°; $[\alpha]^{27.6}D$ −10.6° (*c* 1.0, N,N-dimethylformamide); ν_{max} 3400 (OH), 2975 (CH), 1705 (C=O), 1390 and 1367 (*t*-butyl), and 1168 (C—O); λ_{max} 214 mμ (ε 5850).

Anal. Calcd for $C_{11}H_{17}N_3O_4$ (255.3): C, 51.76; H, 6.71; N, 16.46. Found: C, 51.75; H, 6.92; N, 16.38.

This compound becomes pale yellow and finally pale pink at room temperature.

N^{im}-Benzyl-L-histidine (XXX).—Benzyl chloride was added to a sodium–liquid ammonia solution of L-histidine hydrochloride monohydrate (41.92 g, 0.20 mol) according to the original literature procedure (22.055 g, 45%): mp 200–201° dec; $[\alpha]^{26.7}D$ +19.1° (*c* 1.0, 2 *N* hydrochloric acid) {lit.[36] mp 248–249°; $[\alpha]^{34}D$ +20.5° (*c* 2, water containing 1 equiv of hydrochloric acid)}; R_f 0.16 (butanol–acetic acid–water), 0.17 (methanol). Other preparations had mp 243–246 dec.

N^{α}-*t*-Butyloxycarbonyl-N^{im}-benzyl-L-histidine (XXXI).—*t*-Butyloxycarbonyl azidoformate (5.36 ml, 0.0375 mol) in methanol (25 ml) was added to a solution of N^{im}-benzyl-L-histidine (6.133 g, 0.025 mol) in lithium hydroxide (1 *N*, 25 ml). The reaction mixture was stirred at *ca.* 40° for 72 hr, then taken to dryness, and the residue was triturated with cold water (50 ml) and chloroform (20 ml). The suspension was filtered; the aqueous filtrate was washed again with chloroform, neutralized at 0° with dilute sulfuric acid (1 *N*, 25.0 ml), and evaporated to dryness. The solid was thoroughly extracted with cold acetone, and the combined organic phases were filtered and concentrated to a small volume. On standing at room temperature there were deposited colorless needles of N^{α}-*t*-butyloxycarbonyl-N^{im}-benzyl-L-histidine (3.510 g, 41%): mp 189–190°; $[\alpha]^{28.3}D$ +23.2° (*c* 1.0, methanol); R_f 0.38 (butanol–acetic acid–water); neut equiv 352; ν_{max} 3400 (NH), 3030 (OH), 2970 (CH), 1695 very broad (C=O and urethan), 1387 and 1362 (*t*-butyl), 1245 (CO), 1170 (OH), and 710 (Ph) cm^{-1}; λ_{max} 246, 252, 257, 261, 264, and 267 mμ (ε 150, 164, 202, 171, 162, and 123).

Anal. Calcd for $C_{18}H_{23}N_3O_4$ (345.4): C, 62.59; H, 6.71; N, 12.17. Found: C, 62.46; H, 6.78; N, 12.09.

N^{α}-*t*-Butyloxycarbonyl-N^{im}-benzyl-L-histidine 2,4,5-Trichlorophenyl Ester (XXXII).—To a solution of N^{α}-*t*-butyloxycarbonyl-N^{im}-benzyl-L-histidine (0.691 g, 0.002 mol) in methylene chloride (20 ml) at −20° was added 2,4,5-trichlorophenol (0.395 g, 0.002 mole) and N,N′-dicyclohexylcarbodiimide (0.412 g, 0.002 mol). After stirring for 4 hr at −20° and 48 hr at 0°, the reaction mixture was taken almost to dryness, and the residue was suspended in chilled ethyl acetate. The N,N′-dicyclohexylurea was removed, and the organic solution was washed with cold, saturated sodium bicarbonate solution and brine and dried. Rapid concentration of the ethyl acetate solution at 10°, then addition of chilled diisopropyl ether to the opalescence point, followed by sufficient ethyl acetate to give a clear solution at 10°, and prolonged storage in a cold room furnished the product as white needles. Recrystallization using the above conditions led to N^{α}-*t*-butyloxycarbonyl-N^{im}-benzyl-L-histidine 2,4,5-trichlorophenyl ester (0.615 g, 59%): mp 123.5–124.5°; $[\alpha]^{25.7}D$ −9.9° (*c* 1.0, chloroform); R_f 0.76; ν_{max} 3420 (NH), 2975 (CH), 1777 (C=O), 1710 (urethan), 1395 and 1368 (*t*-butyl), 1120 (CO), and 697 (Ph); λ_{max} 289 and 300 (sh) mμ (ε 208 and 179).

Anal. Calcd for $C_{24}H_{24}Cl_3N_3O_4$ (524.8); C, 54.92; H, 4.61; Cl, 20.27; N, 8.01. Found: C, 43.72; H, 4.70; Cl, 20.09, N, 7.94.

N^{α}-*t*-Butyloxycarbonyl-N^{im}-benzyl-L-histidyl-L-seryl-L-glutaminylglycine Methyl Ester (XXXIII).—N^{α}-Benzyloxycarbonyl-L-seryl-L-glutaminylglycine methyl ester (0.438 g, 0.001 mol) in N,N-dimethylformamide (10 ml) was hydrogenated in the presence of 10% palladium-on-charcoal catalyst (0.025 g) and acetic acid (0.06 ml, 0.001 mol). On removal of the catalyst, the solution was cooled to −20° and N^{α}-*t*-butyloxycarbonyl-N^{im}-benzyl-L-histidine 2,4,5-trichlorophenyl ester (0.525 g, 0.001 mol) was added with stirring. After 1 hr, the reaction was maintained at 0° for 5 days. The solvent was evaporated to leave an oil which was dissolved in a small quantity of methanol. The addition of ethyl acetate precipitated a white solid, which was collected and washed immediately with ether. Reprecipitation in the same fashion gave N^{α}-*t*-butyloxycarbonyl-N^{im}-benzyl-L-histidyl-L-seryl-L-glutaminylglycine methyl ester as a white powder (0.321 g, 51%): mp 188–190° dec; $[\alpha]^{26.3}D$

$-14.1°$ (c 1.0, methanol); R_f 0.60 (methanol); ν_{max} 3300 broad (OH), 2900 (CH), 1740 (C=O), 1670 very broad (C=O and urethan), 1393 and 1669 (*t*-butyl), 1165 (CO), and 695 (Ph) cm^{-1}; λ_{max} 247, 252, 258, 261, 264, and 267 mμ (ε 162, 160, 180, 143, 145, and 100).

Anal. Calcd for $C_{29}H_{41}N_7O_9 \cdot H_2O$ (649.7): C, 53.61; H, 6.66; N, 15.09. Found: C, 53.88; H, 6.46; N, 15.62.

The tetrapeptide was alternatively prepared by use of N,N'-dicyclohexylcarbodiimide, but only an oily product was obtained in spite of many crystallization attempts.

Registry No.—III, 17115-09-2; XI, 17791-44-5; XII, 17791-45-6; XVII, 17791-46-7; XX, 17791-47-8; XXI, 17791-48-9; XXIII, 17791-49-0; XXVII, 2488-14-4; XXVIII, 17791-51-4; XXIX, 17791-52-5; XXXI, 13734-45-7; XXXII, 17791-54-7; XXXIII, 17818-04-1.

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Angular Methylation of 4-Methyl-$\Delta^{4(10)}$-1-octalone[1]

P. S. WHARTON AND C. E. SUNDIN,

Department of Chemistry, University of Wisconsin, Madison, Wisconsin 53706

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This Note reports the direct angular methylationof 4-methyl-$\Delta^{4(10)}$-1-octalone (**1** → **2**), a synthetic step previously suggested[2] for $\Delta^{4(10)}$-1-octalones in general but unrealized[3] in one attempt to methylate $\Delta^{4(10)}$-1-octalone itself. In conjunction with a synthesis of $\Delta^{4(10)}$-1-octalones *via* 1,4 cycloaddition to 1-vinylcyclohexenes[4] the present work forms the basis of a useful synthetic approach to germacrane and elemane sesquiterpenes.[5]

4-Methyl-$\Delta^{4(10)}$-1-octalone (**1**) is rapidly and completely isomerized by ethanolic sodium hydroxide to its corresponding conjugated double-bond isomer (**7**).[6] The dire consequences of this isomerization were anticipated, but their establishment proved to be useful.[7] Addition of methyl iodide to the enolate mixture formed by stirring equimolar amounts of **7** and sodium hydride in dimethoxyethane for 36 hr to ensure partial, if not complete, equilibration of enolate ions gave a multicomponent distillate in 85% yield from which the predominant component (47% by glpc) was isolated and shown to be 4,9-dimethyl-$\Delta^{5(10)}$-1-octalone, a product of angular methylation but with undesirable double-bond isomerization. Thus, of the five interconvertible enolates in this system, the most stable is the $\Delta^{1(9),5(10)}$-hexalin, in accord with the relative stabilities of the unsubstituted hexalins and other related systems.[8]

Methylation of **7** under conditions favoring kinetic control of enolate formation[9] yielded no trace of **2**. Treatment of **7** in dimethoxyethane at room temperature with an excess of both sodium hydride and methyl iodide gave in high yield 2,2,4-trimethyl-Δ^9-1-octalone (**9**) *via* the glpc detectable intermediates, *cis*- and *trans*-2,4-dimethyl-Δ^9-1-octalones (**8**, see the lower half of Scheme I). This result is consistent with exemplary data indicating that α' protons of α,β-unsaturated ketones can be more acidic than γ protons in the kinetic, if not thermodynamic, sense.[10]

In relation to the angular methylation of **1**, the foregoing results emphasized the need for a procedure involving kinetic control of enolate formation from the β,γ-unsaturated ketone. Treatment of **1** in dimethoxyethane at room temperature with 1 equiv of sodium hydride and excess methyl iodide afforded a multicomponent distillate in 83% yield, from which was obtained, by repetitive preparative glpc, a sample of the desired ketone **2**. Determination of the efficacy of this chosen[11] procedure was effected by analyzing the reaction of **1** with an excess of both sodium hydride and methyl iodide. Analysis and separation were simplified by treating each aliquot removed from the reaction vessel with base to ensure complete isomerization of β,γ-unsaturated ketones **5** and **6** to α,β-unsaturated ketones **8** and **9**. Results of the glpc analysis are shown in Table I and Figure 1. A seven-component mixture developed and converged to a single final product which was isolated and character-

TABLE I

"KINETIC" METHYLATION OF 1[a]

Time min	%						
	4	*trans*[b] **3**	**2**	*cis*[b] **3**	*trans*[b] **8**	**7**	*cis*[b] **8**
0						100	
18			3.3			96.7	
33			18.4			81.0	0.6
42		0.8	41.1	1.7	1.2	48.2	6.8
49		2.1	57.0	5.7	2.1	24.5	8.4
60	1.1	7.5	66.6	12.7	0.4	0.7	10.6
82	10.0	27.7	18.4	41.4	0.6	0.3	1.4
159	55.5	18.5		26.0			
258	76.3	10.7		13.0			
645	88.9	9.9		1.2			
1530	99.4	0.3		0.3			
2055	99.6	0.1		0.3			

[a] Compounds are listed in order of increasing glpc retention time. Other detectable peaks never amounted to more than 0.8%. [b] Arbitrary isomer assignments based on a rationalization of steric interference with adsorption.

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