from benzene to give white needles of benzene solvate (38 mg, 2.4%): mp 169–170° dec; $[\alpha]^{25}D$ +85.3° (MeOH); ultraviolet (ethanol) 262 mμ (ε 23,000), 340 mμ (ε 5700); infrared (mineral oil mull) 2.91 (m), 2.97 (m), 3.06 (w), 5.66 (s), 6.09 (s), sh 6.19, 6.38 μ (s).

Anal. Calcd for $C_{22}H_{24}N_2O_9 \cdot C_6H_6$: C, 62.42; H, 5.62; N, 5.20. Found: C, 62.35; H, 5.58; N, 5.40.

Registry No.—IVa, 7695-46-7; IVb, 7721-33-7; Ia, 60-54-8; II, 3811-31-2; 8-hydroxy-1-tetralone, 7695-97-8; V, 7695-48-9.

Amino Acids and Peptides. IX.[1] Synthesis of a Tetrapeptide Sequence (A_{13}–A_{16}) of Glucagon

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The central tetrapeptide sequence (A_{13}–A_{16}) of the hyperglycemic hormone glucagon[2] has been formed in part by several overlapping routes within the last several years. The first preparation afforded the tripeptide N-benzyloxycarbonyl-O-benzyl-L-tyrosyl-L-leucyl-α-methyl-β-*t*-butyl-L-aspartate, which was made by coupling N-benzyloxycarbonyl-O-benzyl-L-tyrosyl-L-leucine with α-methyl-β-*t*-butyl-L-aspartate.[3] Additionally, a homolog was obtained by an azide reaction between N-benzyloxycarbonyl-O-benzyl-L-tyrosyl-L-leucine hydrazide and α-ethyl-β-*t*-butyl-L-aspartate. The second synthesis described N-*t*-butyloxycarbonyl-L-leucyl-L-asparaginyl-L-serine hydrazide, constructed by a stepwise procedure from L-serine methyl ester.[4] The third approach mentioned the simple dipeptide *p*-methoxybenzyloxycarbonyl-O-benzyl-L-tyrosyl-L-leucine methyl ester that was used as an intermediate for subsequent work.[5]

A preparation of three related tetrapeptides constituting the sequence A_{13}–A_{16} of glucagon is given here. The work began by coupling N-benzyloxycarbonyl-β-*t*-butyl-L-aspartic acid α-dicyclohexylammonium salt (I)[6-8] with L-serine methyl ester hydrochloride (II)[9] in the presence of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride[10] to yield N-benzyloxycarbonyl-β-*t*-butyl-L-aspartyl-L-serine methyl ester (III). Dipeptide III was formed in lower yield by the action of 1-cyclohexyl-3-(2-morpholinyl-4-ethyl)carbodiimide metho-*p*-toluenesulfonate.[11] Alternatively, crystalline N-benzyloxycarbonyl-β-*t*-butyl-L-aspartic acid (IV) was prepared[12] and condensed with serine salt II by use of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride, N,N′-dicyclohexylcarbodiimide,[13] and 2-ethyl-5-phenyloxazolium 3′-sulfonate.[14] Although dipeptide III was obtained in each case with the same physical constants as in the first preparation, the yields were uniformly disappointing. Later the dicyclohexylammonium salt I was treated with 2,4,5-trichlorophenol to prepare the corresponding α-2,4,5-trichlorophenyl ester (V). This particular compound is a valuable addition to the list of 2,4,5-trichlorophenyl esters[15] that have been widely employed in recent years to facilitate the construction of peptides.[16] The activated ester V on treatment with serine salt II afforded dipeptide III in good yield.

Hydrogenolysis of dipeptide III led to β-*t*-butyl-L-aspartyl-L-serine methyl ester (VI), which was combined with N-benzyloxycarbonyl-L-leucine 2,4,5-trichlorophenyl ester (VII) to form N-benzyloxycarbonyl-L-leucyl-β-*t*-butyl-L-aspartyl-L-serine methyl ester (VIII). Tripeptide VIII was obtained in higher yield by coupling N-benzyloxycarbonyl-L-leucine (IX) with amine VI in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride. Reduction of tripeptide VIII gave L-leucyl-β-*t*-butyl-L-aspartyl-L-serine methyl ester (X), which was treated with N-benzyloxycarbonyl-L-tyrosine 2,4,5-trichlorophenyl ester (XI)[15] to yield N-benzyloxycarbonyl-L-tyrosyl-L-leucyl-β-*t*-butyl-L-aspartyl-L-serine methyl ester (XII), as an amorphous solid. A 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide coupling between N-benzyloxycarbonyl-L-tyrosine (XIII) and amine X afforded the same tetrapeptide (XII).

An alternative route to tetrapeptide XII is mentioned now. A 2-ethyl-5-phenyloxazolium 3′-sulfonate[14] coupling between N,O-dibenzyloxycarbonyl-L-tyrosine (XIV) and L-leucine methyl ester hydrochloride (XV)[9] afforded N,O-dibenzyloxycarbonyl-L-tyrosyl-L-leucine methyl ester (XVI). Dipeptide XVI on treatment with excess hydrazine formed N-benzyloxycarbonyl-L-tyrosyl-L-leucine hydrazide (XVII).[17] This particular reaction is of some interest because the protecting O-benzyloxycarbonyl group is simultaneously cleaved by hydrazine to give the corresponding mono-N-benzyloxycarbonyl derivative. An azide coupling between hydrazide XVII and 1-hydroxypiperidine[18] led to N-benzyloxycarbonyl-L-tyrosyl-L-leucine 1-hydroxypiperidine ester (XVIII). Activated ester XVIII and amine XI then gave tetrapeptide XII in moderate amount.

In an effort to improve the physical characteristics of tetrapeptide XII, N-benzyloxycarbonyl-O-benzyl-L-tyrosine *p*-nitrophenyl ester (XIX)[19] was treated with amine X to afford crystalline N-benzyloxycarbonyl-O-benzyl-L-tyrosyl-L-leucyl-β-*t*-butyl-L-aspartyl-L-serine methyl ester (XX). Treatment of N,O-dibenzyloxycarbonyl-L-tyrosine 2,4,5-trichlorophenyl ester (XXI) with amine X produced crystalline N,O-dibenzyloxycarbonyl-L-tyrosyl-L-leucyl-β-*t*-butyl-L-aspartyl-L-serine methyl ester (XXII). Alternatively, the same

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tetrapeptide XXII was formed by coupling N,O-dibenzyloxycarbonyl-L-tyrosine (XXIII) to amine X with the aid of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride.

Experimental Section[20]

N-Benzyloxycarbonyl-β-*t*-butyl-L-aspartic Acid (IV).—A mixture of N-benzyloxycarbonyl-β-*t*-butyl-L-aspartic acid α-dicyclohexylammonium salt (2.49 g, 0.00494 mole), ethyl acetate (25 ml), and 10% aqueous citric acid (25 ml) was shaken in a separating funnel for several minutes, at which point the solid had dissolved completely. After an additional 5 min of agitation, the organic phase was separated, washed with water to neutrality, dried, and evaporated to leave a colorless oil. An ether–petroleum ether solution of this material at 0° slowly deposited crystalline N-benzyloxycarbonyl-β-*t*-butyl-L-aspartic acid (1.17 g, 74%): mp 47.5–48.5°; $[\alpha]^{26}D$ −11.6° (*c* 1, pyridine), $[\alpha]^{28}D$ −2.6° (*c* 1, methanol) [lit.[12] mp 78° (!); $[\alpha]D$ −12.95° (pyridine), −3.4° (methanol)]; R_f 0.1; ν_{max} 3420 broad (OH), 2980 (CH), 1720 (C=O), 1392 and 1368 (*t*-butyl), 1240 broad (CO), and 698 (Ph) cm^{-1}; λ_{max} 247, 252, 257, 262, 264, and 267 mμ (ε 115, 157, 205, 152, 167, and 106).

Anal. Calcd for $C_{16}H_{21}NO_6$ (323.3); C, 59.43; H, 6.55; N, 4.33. Found: C, 58.99; H, 6.33; N, 4.42.

N-Benzyloxycarbonyl-β-*t*-butyl-L-aspartate α-2,4,5-Trichlorophenyl Ester (V).—Solid N-benzyloxycarbonyl-β-*t*-butyl-L-aspartic acid α-dicyclohexylammonium salt (10.09 g, 0.0200 mole) was shaken with ethyl acetate (100 ml) and 0.5 *M* aqueous citric acid (50 ml), until only two liquid phases were seen in the container. The organic phase was separated, washed successively with water and brine, and dried. The ethyl acetate solution was treated with 2,4,5-trichlorophenol (4.13 g, 0.0220 mole),[21] followed by N,N′-dicyclohexylcarbodiimide (4.13 g, 0.0200 mole).[22] After stirring for 15 hr, the N,N′-dicyclohexylurea was removed and the solution was washed successively with saturated sodium bicarbonate solution, water, and brine and dried. Evaporation of the solvent yielded an almost colorless oil, which was redissolved in diisopropyl ether at room temperature. On standing overnight at 0°, an additional small quantity of urea was deposited; the filtered solution was diluted with petroleum ether to opalescence, warmed slightly, and left at room temperature for 15 hr and then at 0° for a additional 24 hr. The separated material was recrystallized from the same solvent system to afford shining, white needles of N-benzyloxycarbonyl-α-2,4,5-trichlorophenyl-β-*t*-butyl-L-aspartate (7.83 g, 78%): mp 73–74°; $[\alpha]^{26}D$ −21.4° (*c* 1, ethyl acetate), $[\alpha]^{26}D$ −4.8° (*c* 1, chloroform); R_f 0.89; ν_{max} 3375 (NH), 2978 (CH), 1778 and 1720 (C=O), 1395 and 1370 (*t*-butyl), 1140 (CO), and 693 (Ph) cm^{-1}; λ_{max} 258, 264, 275 (shoulder), 279, and 288 mμ (ε 365, 439, 636, 957, and 1015).

Anal. Calcd for $C_{22}H_{22}Cl_3NO_6$ (502.8); C, 52.55; H, 4.41; Cl, 21.18; N, 2.79. Found: C, 52.60; H, 4.36; Cl, 21.17; N, 3.01.

N-Benzyloxycarbonyl-β-*t*-butyl-L-aspartyl-L-serine Methyl Ester (III). A. By Use of N-Benzyloxycarbonyl-β-*t*-butyl-L-aspartic Acid α-Dicyclohexylammonium Salt and 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide Hydrochloride.—A solution of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (2.31 g, 0.0120 mole)[10] in dichloromethane (30 ml) was added to a previously prepared solution of N-benzyloxycarbonyl-β-*t*-butyl-L-aspartic acid α-dicyclohexylammonium salt [5.05 g, 0.0100 mole, mp 125.0–126.5°, $[\alpha]^{29.5}D$ +8.6° (*c* 1, 90% acetic acid); lit.[6-8] mp 126.5 and 124°, $[\alpha]D$ +5.5°, +5.8°, +7.7°)] and L-serine methyl ester hydrochloride [1.57 g, 0.0100 mole, mp 163–164°, $[\alpha]^{28}D$ +3.75° (*c* 1.33, methanol); lit.[9] mp 168°, $[\alpha]D$ +5.5° (*c* 1.8, methanol)] in dichloromethane (20 ml). After stirring at room temperature for 48 hr the solvent was evaporated and the residue was partitioned between ethyl acetate and water. The organic phase was washed successively with 1% aqueous citric acid, water, saturated sodium bicarbonate solution, and water and dried. Evaporation of the solvent left an oil, which on redissolving in dioxane–*n*-hexane deposited flat spars of N-benzyloxycarbonyl-β-*t*-butyl-L-aspartyl-L-serine methyl ester (3.37 g, 80%), mp 41–43°. Crystallization from methanol–water gave long needles of a polymorphic form: mp 57.0–58.5°; $[\alpha]^{26.0}D$ −5.4° (*c* 1, methanol); R_f 0.61; ν_{max} 3330 broad, 2977 (CH), 1720 broad (C=O), 1640 and 1525 broad (CONH), 1395 and 1368 (*t*-butyl), 1240 broad (CO), and 697 (Ph) cm^{-1}; λ_{max} 247 (shoulder), 252, 257, 264, and 267 mμ (ε 122, 160, 205, 169, and 107).

Anal. Calcd for $C_{20}H_{28}N_2O_8$ (424.5); C, 56.60; H, 6.65; N, 6.60. Found: C, 56.58; H, 6.63; H, 6.54.

The dipeptide was alternatively prepared from N-benzyloxycarbonyl-β-*t*-butyl-L-aspartic acid α-dicyclohexylammonium salt, L-serine methyl ester hydrochloride, and 1-cyclohexyl-3-(2-morpholinyl-4-ethyl)carbodiimide metho-*p*-toluenesulfonate (46%); N-benzyloxycarbonyl-β-*t*-butyl-L-aspartic acid L-serine methyl ester, and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (61%); N-benzyloxycarbonyl-β-*t*-butyl-L-aspartic acid L-serine methyl ester and N,N′-dicyclohexylcarbodiimide (61%); N-benzyloxycarbonyl-β-*t*-butyl-L-aspartic acid L-serine methyl ester and 2-ethyl-5-phenyloxazolium 3′-sulfonate (43%); and N-benzyloxycarbonyl-α-2,4,5-trichlorophenyl-β-*t*-butyl-L-aspartate and L-serine methyl ester (77%).

β-*t*-Butyl-L-aspartyl-L-serine Methyl Ester (VI).—N-Benzyloxycarbonyl-β-*t*-butyl-L-aspartyl-L-serine methyl ester (1.703 g, 0.00400 mole) was dissolved in methanol (30 ml) containing 10% palladium-on-carbon catalyst (0.173 g) and was hydrogenated at 0° for 1 hr. The reaction mixture was filtered and the solvent was evaporated to leave a clear, colorless oil, R_f 0.48 (ninhydrin positive). If the reaction was conducted at room temperature, some diketopiperazine formation was seen as a second spot, R_f 0.63 (ninhydrin negative).

N-Benzyloxycarbonyl-L-leucine 2,4,5-Trichlorophenyl Ester (VII).—To a solution of oily N-benzyloxycarbonyl-L-leucine (29.65 g, 0.112 mole) and 2,4,5-trichlorophenol (24.30 g, 0.123 mole)[21] in ethyl acetate (150 ml) at −10° was added N,N′-dicyclohexylcarbodiimide (24.10 g, 0.117 mole)[22] dissolved in ethyl acetate (100 ml). The cooling bath was allowed to warm to room temperature, and the reaction was stirred for an additional 17 hr. Glacial acetic acid (0.5 ml) was added, and, after 3 hr, filtration of the mixture gave N,N′-dicyclohexylurea (24.83 g, 95%). The solvent was evaporated and the oily residue was redissolved in *n*-hexane and allowed to stand at 0° overnight. The product was collected from ethanol to produce N-benzyloxycarbonyl-L-leucine 2,4,5-trichlorophenyl ester (39.78 g, 80%): mp 64–65°; $[\alpha]^{25.2}D$ −29.1° (*c* 1.44, methanol); R_f 0.92; ν_{max} 3340 (NH), 2955 (CH), 1755 and 1700 (C=O), 1170 (isopropyl), 1115 (CO), and 738 and 695 (Ph) cm^{-1}; λ_{max} 258, 264, 279, and 288 mμ (ε 444, 573, 1220, and 1280).

Anal. Calcd for $C_{20}H_{20}Cl_3NO_4$ (444.7); C, 54.01; H, 4.53; Cl, 23.92; N, 3.15. Found: C, 53.69; H, 4.65; Cl, 23.79; N, 3.38.

N-Benzyloxycarbonyl-L-leucyl-β-*t*-butyl-L-aspartyl-L-serine Methyl Ester (VIII). A. By Use of N-Benzyloxycarbonyl-L-leucine 2,4,5-Trichlorophenyl Ester.—An ice-cold, methanolic solution of β-*t*-butyl-L-aspartyl-L-serine methyl ester, freshly prepared by the hydrogenation of N-benzyloxycarbonyl-β-*t*-butyl-L-aspartyl-L-serine methyl ester (1.703 g, 0.00400 mole), was treated with N-benzyloxycarbonyl-L-leucine 2,4,5-trichlorophenyl ester (1.963 g, 0.00440 mole). The solution was evaporated and the residual oil was redissolved in ethyl acetate (10 ml) and allowed to stand at room temperature for 62 hr. The solution was partially evaporated and warmed, and *n*-hexane was added to the turbidity point. The separated material was collected and recrystallized from aqueous methanol to yield colorless needles of N-benzyloxycarbonyl-L-leucyl-β-*t*-butyl-L-aspartyl-L-serine methyl ester (1.08 g, 50%): mp 131.0–132.5°; $[\alpha]^{27.8}D$ −34.7° (*c* 1, methanol); R_f 0.71; ν_{max} 3420 broad (NH), 2950 (CH), 1710 broad (C=O), 1670 and 1520 broad (CONH), 1385 and 1365 (*t*-butyl), 1150 (CO), and 695 (Ph) cm^{-1}; ν_{max} 247, 252, 258, 262, and 264 mμ (ε 135, 169, 211, 160, 175, and 114).

(20) Melting points are uncorrected. Microanalyses were provided by Messrs. Erich H. Meier and J. Consul, Microanalytical Laboratory, Stanford University. The optical rotation and infrared (potassium bromide) and ultraviolet (methanol) measurements were obtained by Mrs. Linda D. Carroll. Thin layer chromatography employed baked silica G as the support, methanol–chloroform (1:9) as the solvent, and iodine for detection purposes. Evaporations were performed under reduced pressure in a rotary evaporator at minimum temperature, while high-boiling solvents were removed at reduced pressure (0.2–0.5 mm). Acetonitrile and dimethylformamide were spectroscopic quality and petroleum ether had a bp 30–60°. Magnesium sulfate was generally used for drying purposes.

(21) Commercial 2,4,5-trichlorophenol (Dow Chemical Co.) was thrice crystallized from *n*-heptane and twice sublimed *in vacuo*, mp 67–68°.

(22) D. F. Elliott and D. W. Russel, *Biochem. J.*, **66**, 49P (1957).

Anal. Calcd for $C_{26}H_{39}N_3O_9$ (537.6); C, 58.09; H, 7.31; N, 7.82. Found: C, 57.89; H, 7.21; N, 7.70.

The tripeptide was alternatively prepared from N-benzyloxycarbonyl-L-leucine, β-*t*-butyl-L-aspartyl-L-serine methyl ester, and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (74%).

N,O-Dibenzyloxycarbonyl-L-tyrosyl-L-leucine Methyl Ester (XVI).—To a stirred solution of N,O-dibenzyloxycarbonyl-L-tyrosine (13.32 g, 0.0300 mole) in acetonitrile (60 ml) held at 0° was added 2-ethyl-5-phenyloxazolium-3′-sulfonate (7.59 g, 0.0300 mole)[14] and triethylamine (4.17 ml, 0.0300 mole). After 10 min the ice–water bath was removed, and complete solution occurred as the reaction mixture warmed to room temperature. L-Leucine methyl ester hydrochloride (5.45 g, 0.0300 mole, mp 151–152°) was dissolved in hot acetonitrile (60 ml), and, after cooling and adding triethylamine (4.17 ml, 0.0300 mole), this solution was added to the solution of the "Woodward" intermediate, and the coupling was left at room temperature for 24 hr. The solvent was evaporated and the residue was dissolved in ethyl acetate and washed successively with 0.5 *M* aqueous citric acid, saturated sodium bicarbonate solution (some tendency to form emulsions), and brine. On concentration, the solution deposited white needles, which on recrystallization from ethyl acetate–diisopropyl ether afforded N,O-dibenzyloxycarbonyl-L-tyrosyl-L-leucine methyl ester (9.54 g, 55%): mp 163.5–164.5°; $[\alpha]^{26}D$ −17.2° (*c* 1, methanol); R_f 0.82; ν_{max} 3300 (NH), 2950 (CH), 1745 broad (C=O), 1530 broad (CONH), 1250 broad (CO), and 740 and 697 (Ph) cm^{-1}; λ_{max} 252, 257, 262, 264, and 267 mμ (ε 520, 688, 664, 677, and 483).

Anal. Calcd for $C_{32}H_{36}N_2O_8$ (576.7); C, 66.65; H, 6.29; N, 4.86. Found: C, 66.52; H, 6.27; N, 4.83.

N-Benzyloxycarbonyl-L-tyrosyl-L-leucine Hydrazide (XVII).—A solution of N,O-dibenzyloxycarbonyl-L-tyrosyl-L-leucine methyl ester (5.77 g, 0.0100 mole) and 95% hydrazine (1.59 ml, 0.0500 mole) in methanol (50 ml) was heated under reflux for 1 hr. On standing, the solution deposited fluffy, white needles (mp 212–223°), which were shown to consist of two components [R_f 0.66 (major) and 0.73, methanol]. As the component of higher R_f was more soluble in hot methanol than that of lower R_f the latter material was purified by the repeated suspension of the mixture in small amounts of methanol at reflux for 5–10 min. Crystallization of the product from methanol furnished white needles of N-benzyloxycarbonyl-L-tyrosyl-L-leucine hydrazide (1.46 g, 33%): mp 225–226° (lit.[17] mp 226°); $[\alpha]^{26}D$ −17.0° (*c* 1, dimethylformamide); R_f 0.66 (methanol); ν_{max} 3400 (NH), 2950 (CH), 1670 broad (CONH), 1240 broad (CO), and 695 (Ph) cm^{-1}; λ_{max} 225, 252 (shoulder), 258 (shoulder), 264 (shoulder), 268 (shoulder), 277, and 284 (shoulder) mμ (ε 10,760, 461, 680, 956, 1183, 1622, and 1381).

Anal. Calcd for $C_{23}H_{30}N_4O_5$ (442.5); C, 62.43; H, 6.83; N, 12.66. Found: C, 62.34; H, 6.88; N, 12.72.

N-Benzyloxycarbonyl-L-tyrosyl-L-leucine 1-Hydroxypiperidine Ester (XVIII).—N-Benzyloxycarbonyl-L-tyrosyl-L-leucine hydrazide (1.33 g, 0.00300 mole) was dissolved in dimethylformamide (9 ml) with slight warming, and the solution was cooled to −40°. Hydrochloric acid in tetrahydrofuran (2.808 *N*, 4.27 ml, 0.0120 mole of hydrochloric acid) was added slowly, followed by *n*-butyl nitrite (0.41 ml, 0.00360 mole). After an additional 30 min at −40°, triethylamine (1.68 ml, 0.0120 mole) and then 1-hydroxypiperidine (0.606 g, 0.00600 mole)[18] were added and the reaction mixture was kept at 0° for 24 hr. The solvents were evaporated and the residue was distributed between ethyl acetate and water. The organic phase was washed successively with 0.5 *M* aqueous citric acid, water, saturated sodium bicarbonate solution, and brine and dried. After removal of most of the ethyl acetate, petroleum ether was added to the opalescence point, followed by ethyl acetate dropwise until a clear solution was obtained again at room temperature. This solution on standing at 0° rapidly deposited a pale yellow oil, which solidified after 3 months. The material was crystallized from ethyl acetate–diisopropyl ether to give colorless needles of N-benzyloxycarbonyl-L-tyrosyl-L-leucine 1-hydroxypiperidine ester (0.770 g, 50%): mp 150–151°; R_f 0.58; $[\alpha]^{25.8}D$ +1.6° (*c* 1, chloroform); ν_{max} 3300 (NH), 2950 (CH), 1751 (C=O), 1730–1650 broad (C=O and CONH), 1512 (CONH), 1262 and 1230 (CO), and 695 (Ph) cm^{-1}; λ_{max} 226, 253 (shoulder), 258 (shoulder), 264 (shoulder), 277, and 283 (shoulder) mμ (ε 9970, 428, 644, 932, 1625, and 1381).

Anal. Calcd for $C_{28}H_{37}N_3O_6$ (511.6); C, 65.73; H, 7.29; N, 8.21. Found: C, 65.64; H, 7.14; N, 8.19.

L-Leucyl-β-*t*-butyl-L-aspartyl-L-serine Methyl Ester (X).—N-Benzyloxycarbonyl-L-leucyl-β-*t*-butyl-L-aspartyl-L-serine methyl ester (0.541 g, 0.00100 mole) was dissolved in methanol (25 ml) containing 10% palladium-on-carbon catalyst (0.067 g) and hydrogenated for 2 hr at atmospheric pressure. The reaction mixture was filtered and the solvent was evaporated to leave a colorless oil (0.388 g, 96%), R_f 0.40 (ninhydrin positive).

N-Benzyloxycarbonyl-L-tyrosyl-L-leucyl-β-*t*-butyl-L-aspartyl-L-serine Methyl Ester (XII). A. By Use of N-Benzyloxycarbonyl-L-tyrosine and 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide Hydrochloride.—A solution of L-leucyl-β-*t*-butyl-L-aspartyl-L-serine methyl ester, freshly prepared by the hydrogenation of N-benzyloxycarbonyl-L-leucyl-β-*t*-butyl-L-aspartyl-L-serine methyl ester (0.544 g, 0.00100 mole), and N-benzyloxycarbonyl-L-tyrosine (0.299 g, 0.00100 mole) in dichloromethane (15 ml) was treated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.215 g, 0.00110 mole)[10] and stirred at room temperature for 2 days. The solvent was evaporated and the residue was partitioned between ethyl acetate and water. The organic phase was washed successively with 1% aqueous citric acid, water, saturated sodium bicarbonate solution and water, then dried, and evaporated to leave a white gel. The material was precipitated from methanol–water to give the tetrapeptide (0.252 g): mp 155–157° (softening at 150°), R_f 0.50 with a minor impurity at R_f 0.65. The analytical sample was obtained from acetone–petroleum ether as a white powder (0.143 g, 21%): mp 159–160°; R_f 0.50; $[\alpha]^{27.3}D$ −25.9° (*c* 1, dimethylformamide); ν_{max} 3300 broad (NH), 2950 (CH), 1720–1640 very broad (C=O and CONH), 1520 (CONH), 1390 and 1365 (*t*-butyl), 1240 broad (CO), and 735 and 690 (Ph) cm^{-1}; λ_{max} 226, 253 (shoulder), 258 (shoulder), 268 (shoulder), 277, and 283 (shoulder) mμ (ε 9830, 440, 650, 1170, 1625, and 1340).

Anal. Calcd for $C_{35}H_{48}N_4O_{11}$ (700.8); C, 59.58; H, 6.90; N, 7.99. Found: C, 59.67; H, 6.84; N, 7.85.

The tetrapeptide was alternatively prepared from N-benzyloxycarbonyl-L-tyrosyl-L-leucine 1-piperidyl ester and β-*t*-butyl-L-aspartyl-L-serine methyl ester (20%); and N-benzyloxycarbonyl-L-tyrosine 2,4,5-trichlorophenyl ester and L-leucyl-β-*t*-butyl-L-aspartyl-L-serine methyl ester (impure product).

N-Benzyloxycarbonyl-O-benzyl-L-tyrosyl-L-leucine-β-*t*-butyl-L-aspartyl-L-serine Methyl Ester (XX).—A freshly prepared solution of L-leucyl-β-*t*-butyl-L-aspartyl-L-serine methyl ester, obtained by the hydrogenation of N-benzyloxycarbonyl-L-leucyl-β-*t*-butyl-L-aspartyl-L-serine methyl ester (2.154 g, 0.00400 mole), and N-benzyloxycarbonyl-O-benzyl-L-tyrosine *p*-nitrophenyl ester (2.107 g, 0.00400 mole)[19] in dimethylformamide (9 ml) was allowed to stand for 7.5 days. The solvent was removed and the residual oil was precipitated as a gel from ethyl acetate–ether. The material was collected and crystallized from aqueous methanol to afford a white powder of N-benzyloxycarbonyl-O-benzyl-L-tyrosyl-L-leucyl-β-*t*-butyl-L-aspartyl-L-serine methyl ester (2.391 g, 76%): mp 174–176°; R_f 0.83; $[\alpha]^{27.8}D$ −23.0° (*c* 1, dimethylformamide); ν_{max} 3245 (NH), 2950 (CH), 1725–1640 very broad (C=O and CONH), 1520 (CONH), 1390 and 1368 (*t*-butyl), 1240 broad (CO), and 735 and 695 (Ph) cm^{-1}; λ_{max} 227, 252, 258, 264, 267, 276, and 283 mμ (ε 2890, 134, 183, 226, 252, 296, and 253).

Anal. Calcd for $C_{42}H_{54}N_4O_{11}$ (790.9); C, 63.77; H, 6.88; N, 7.08. Found: C, 63.72; H, 6.98; N, 7.18.

N,O-Dibenzyloxycarbonyl-L-tyrosyl-L-leucyl-β-*t*-butyl-L-aspartyl-L-serine Methyl Ester (XXII). A. By Use of N,O-Dibenzyloxycarbonyl-L-tyrosine 2,4,5-Trichlorophenyl Ester.—A solution of L-leucyl-β-*t*-butyl-L-aspartyl-L-serine methyl ester in ethyl acetate (15 ml), freshly prepared by the hydrogenation of N-benzyloxycarbonyl-L-leucyl-β-*t*-butyl-L-aspartyl-L-serine methyl ester (0.538 g, 0.00100 mole), was added to N,O-dibenzyloxycarbonyl-L-tyrosine 2,4,5-trichlorophenyl ester [0.651 g, 0.00100 mole, mp 142–143°, $[\alpha]^{21.8}D$ −32.4° (*c* 1.11 dimethylformamide); lit.[15] mp 143–144°, $[\alpha]^{21}D$ −36°] dissolved in ethyl acetate (5 ml). The reaction was warmed, and, on standing at room temperature for 20 hr, a large amount of solid separated from the solution. After 60 hr the crystals were collected and crystallized from both ethyl acetate–*n*-hexane and aqueous methanol, to yield N,O-dibenzyloxycarbonyl-L-tyrosyl-L-leucyl-β-*t*-butyl-L-aspartyl-L-serine methyl ester (0.522 g, 63%): mp 163.5–165.0°; $[\alpha]^{27.3}D$ −21.0° (*c* 1, dimethylformamide); R_f 0.73; ν_{max} 3290 (NH), 2950 (CH), 1760–1640 very broad (C=O and CONH), 1520 (CONH), 1390 and 1368 (*t*-butyl), 1240 broad (CO), and 740

and 695 (Ph) cm^{-1}; λ_{max} 252, 257, 262, 264, 267, and 280 (shoulder) mμ (ε 577, 742, 738, 765, 644, and 300).

Anal. Calcd for $C_{43}H_{54}N_4O_{13}$ (834.9); C, 61.83; H, 6.52; N, 6.71. Found: C, 61.66; H, 6.68; N, 6.67.

The tetrapeptide was alternatively prepared from N,O-dibenzyloxycarbonyl-L-tyrosine, L-leucyl-β-*t*-butyl-L-aspartyl-L-serine methyl ester, and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (68%).

Registry No.—Glucagon, 35-25-6; IV, 5545-52-8; V, 7635-36-1; III, 7646-47-1; VI, 7646-48-2; VII, 7646-49-3; VIII, 7646-50-6; XVI, 7641-11-4; XVII, 7646-51-7; XVIII, 7646-52-8; X, 7646-53-9; XII, 7688-12-2; XX, 7646-54-0; XXII, 7688-13-3.

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Cyclization of ω-Haloamides to Lactams

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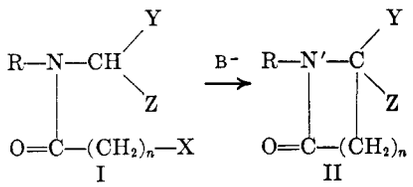
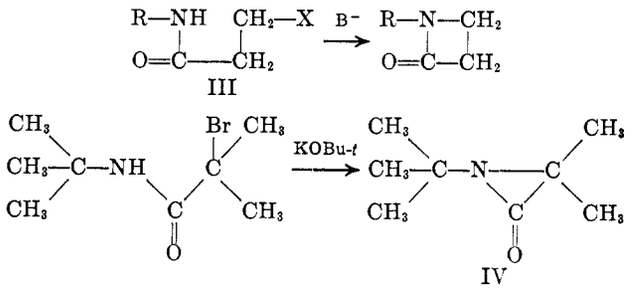
β-Lactams (n = 1) and γ-lactams (n = 2) of type II have been prepared in high yield by the cyclization of the intermediate I under the influence of a base[2-6] when Y and Z groups are electron-withdrawing groups, such as esters or nitriles.

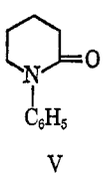


An interesting aspect of the cyclization of ω-haloacylaminomalonic esters (I, Y = Z = CO_2R) under the conditions used by Sheehan and Bose[2] is that it can yield only four- and five-membered lactams (n = 1 or 2). For reasons that are not clear neither the six- nor the seven-membered lactams (n = 3 or 4) can be prepared by this method.[3,6]

Another cyclization method that has been used for the synthesis of several β-lactams is that developed by Knunyants.[7] This involves the treatment of a β-haloamide of type III with a base, *e.g.*, sodium and liquid ammonia. That this method can be extended to three-membered heterocycles has been shown by Baumgarten[8] and Sheehan[9] in their syntheses of α-lactams (IV). Since no information can be found about the usefulness of this method for the synthesis of lactams of larger ring size we undertook a study on the cyclization of ω-haloamides (see Table I for pertinent data). We were particularly interested in finding out whether this cyclization had the same limitations as the cyclization of ω-haloacylaminomalonates.



In our study, sodium in liquid ammonia was used as the base for the cyclization of ω-haloamides. From Table II it will be seen that β-lactams could be prepared by this method in yields from 50 to 90%. The cyclization reaction was found to be equally successful for the synthesis of γ-lactams (48–79%). This method of lactam formation was also extended to the six-membered system[10] (V), but all attempts to cyclize ε-bromocaproic acid anilide under the Knunyants' conditions to get a seven-membered lactam failed and the starting amide was recovered.



V

We have also investigated the possibility of employing the readily available base, dimsyl anion, which is more convenient to work with than sodium in liquid ammonia. Corey[11] and others[12-14] have extensively used dimsyl anion ($CH_2^-SOCH_3$) as a base in various reactions. We have tested sodium hydride–dimethyl sulfoxide (DMSO) as well as potassium *t*-butoxide–DMSO as cyclization reagents for lactam synthesis from suitable haloamides. It was noticed that dimsyl anion could be used successfully to obtain four-, five-, and six-membered lactams. However, this base failed to product the seven-membered heterocyclic ring.

A comparative study on the use of various bases to effect the cyclization of β-bromopropionanilide was undertaken. Sodium in liquid ammonia, sodium hydride in DMSO, and potassium *t*-butoxide in DMSO were the bases examined. The best yield (95%) of 1-phenyl-2-azetidinone was obtained with sodium hydride in DMSO. Sodium in liquid ammonia and potassium *t*-butoxide in DMSO gave 68 and 70% of cyclized product, respectively. Since other haloamides

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