Cholecystokinin-Pancreozymin. 2.[1] Synthesis of a Protected Heptapeptide Hydrazide Corresponding to Sequence 17–23

Yakir S. Klausner[‡] and Miklos Bodanszky*

Department of Organic Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel, and Department of Chemistry, Case Western Reserve University, Cleveland, Ohio 44106

Received June 11, 1976

The partially protected heptapeptide hydrazide *tert*-butyloxycarbonyl-β-benzyl-L-aspartyl-L-prolyl-L-seryl-L-histidyl-L-arginyl-L-isoleucyl-L-serine hydrazide, corresponding to sequence 17–23 of cholecystokinin, was prepared in solution by stepwise chain lengthening with active esters.

The amino acid sequence of the porcine gastrointestinal hormone cholecystokinin-pancreozymin (CCK) was determined by Mutt and Jorpes.[2] The C-terminal dodecapeptide, a biologically active tryptic fragment of the 33-membered chain, was synthesized by Ondetti and his associates;[3] the N-terminal octapeptide by Bodanszky and his coworkers.[1] In position 27 of the sequence a tyrosine *O*-sulfate residue is present. Esterifiction of the phenolic hydroxyl group of tyrosine can be carried out on C-terminal sequences which do not contain a serine residue. Beyond that point, the presence of serine, unless an enzyme-catalyzed process is found, should interfere with selective esterification. It seems to be difficult to carry out an entirely stepwise synthesis[4] of CCK, because the tyrosine sulfate moiety is partially decomposed under the conditions of acidolysis generally applied for the removal of acid labile protecting groups, such as the *tert*-butyloxycarbonyl group. The alternative deblocking of α-amino groups by hydrogenolysis is impeded by the presence of methionine residues in the sequence. Thus, a fragment condensation approach was designed. The partial sequences 1–8,[1] 9–16, 17–23, 24–26, and 27–33 were selected as intermediates that can be combined in an order to be determined in exploratory experiments. Synthesis of a partially protected heptapeptide hydrazide corresponding to sequence 17–23 is reported in this paper. The scheme of the synthesis is shown in Chart I.

Stepwise chain lengthening from serine, the C-terminal residue of the heptapeptide, was uneventful until the last residue, aspartic acid, was incorporated. This was first attempted by acylation with *tert*-butyloxycarbonyl-β-benzyl-L-aspartic acid *N*-hydroxysuccinimide ester.[5] In this case, however, in addition to the desired protected heptapeptide methyl ester XI, a second product formed in which the side chain of the C-terminal serine was also acylated with *tert*-butyloxycarbonyl-β-benzyl-L-aspartic acid. Model experiments[6] revealed that the imidazole in the side chain of the histidine residue (in position 20) is responsible for extensive O-acylation. Subsequently, more favorable conditions were found and applied: the protected aspartyl residue was introduced in the form of the *p*-nitrophenyl ester[7] in the presence of 1-hydroxybenzotriazole.[8] This approach produced the expected protected heptapeptide ester XI in satisfactory yield and purity. Compound XI was then partially deblocked by hydrogenation and converted to the hydrazide XIII. In exploratory experiments,[9] XIII was treated with nitrous acid and the azide thus formed was used for the acylation of L-aspartyl-L-arginyl-L-aspartic acid methyl ester (CCK_{24-26}). The partially protected decapeptide ester could be secured, albeit in moderate yield, in homogeneous form after chromatography. Thus, the partially protected heptapeptide hydrazide XIII seems to be a suitable intermediate for the total synthesis of CCK.

‡ The Hebrew University of Jerusalem.

Experimental Section

Capillary melting points are reported uncorrected. Thin layer chromatograms (silica gel, Merck) were developed with the following solvent systems: A, 1-butanol–acetic acid–water (4:1:1); B, chloroform–methanol (8:2); C, EtOAc–pyridine–AcOH–H_2O (30:20:6:11). Spots were revealed by uv, iodine vapor, charring,[10] and by the Sakaguchi or Pauly reagents. For amino acid analysis, samples were hydrolyzed with constant boiling hydrochloric acid in evacuated, sealed ampules at 110 °C for 16 h, and analyzed by the method of Spackman, Stein, and Moore[11] on a Beckman-Spinco 120C instrument.

The following abbreviations were used: DCC, dicyclohexylcarbodiimide; DCU, dicyclohexylurea; DMF, dimethylformamide; TFA, trifluoroacetic acid; THF, tetrahydrofuran.

Benzyloxycarbonyl-L-isoleucyl-L-serine Methyl Ester (I). To a solution of serine methyl ester hydrochloride[12] (3.2 g, 20 mmol) in DMF (25 ml), triethylamine was added, followed by benzyloxycarbonyl-L-isoleucine *p*-nitrophenyl ester[13] (8.49 g, 22 mmol). The solution was kept slightly basic by the addition of the same base. After 2 days, *unsym*-dimethylaminopropylamine (0.51 g, 5 mmol) was added.[14] One hour later, the reaction mixture was diluted with ethyl acetate (300 ml), and the organic phase was washed with 0.5 N ammonia solution (20-ml portions), twice with 1 N HCl (20 ml), water (2 × 20 ml) and saturated NaCl solution (2 × 20 ml). The solution was dried over Na_2SO_4, filtered, and concentrated in vacuo to a small volume. The precipitate that formed was filtered with the aid of several portions of ethyl acetate. The air-dried product weighed 6.0 g (82%), mp 177–178 °C. Recrystallization from ethanol raised the melting point to 178–179 °C; $[\alpha]^{25}D$ +3.7° (*c* 1, DMF); TLC R_f (B) 0.7.

Anal. Calcd for $C_{18}H_{26}N_2O_6$: C, 59.0; H, 7.1; N, 7.6. Found: C, 59.0; H, 7.1; N, 7.9.

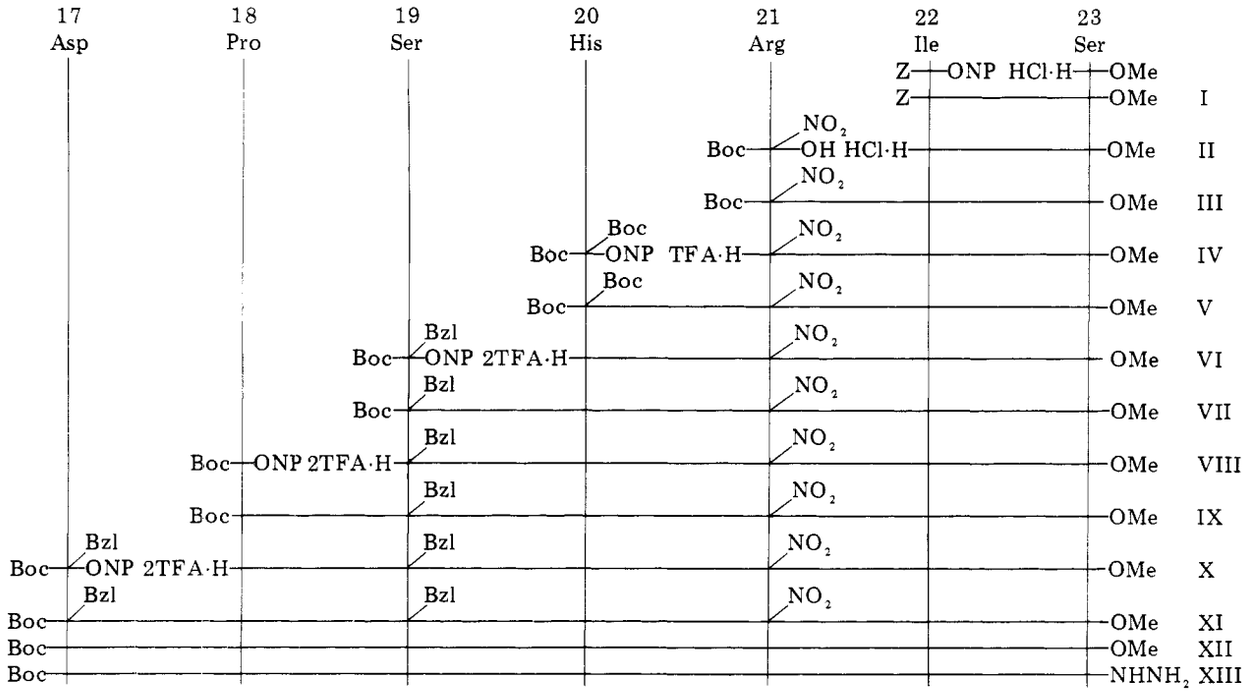
Compound I was prepared also by coupling of Z-L-Ile with Ser-OCH_3, with DCC as condensing agent. The physical properties of this preparation were the same as those of the one described above, but the yield was poor.

***tert*-Butyloxycarbonylnitro-L-arginyl-L-isoleucyl-L-serine Methyl Ester (III). A.** The protected dipeptide I (3.66 g, 10 mmol) was hydrogenated for 3 h in a mixture of methanol (120 ml) and 1 N aqueous HCl (10 ml) in the presence of a 10% Pd on charcoal catalyst (750 mg). The reaction mixture was filtered from the catalyst and evaporated in vacuo to a small volume, and the dipeptide hydrochloride (II) was precipitated with dry ether (50 ml). The product was collected on a filter and dried in vacuo over P_2O_5 and KOH. Compound II [2.5 g, 93%, mp 197–198 °C, TLC R_f (A) 0.48] was dissolved in DMF (20 ml), and *tert*-butyloxycarbonylnitro-L-arginine (2.97 g, 9.3 mmol) and 1-hydroxybenzotriazole[15] (1.62 g, 12 mmol) were added with stirring. After complete dissolution, the mixture was cooled in ice, and triethylamine (1.3 ml, 9.3 mmol) was added, followed by DCC (1.92 g, 9.3 mmol). Stirring in the cold was continued for 30 min, then overnight at room temperature. The DCU was filtered off, the solvent removed in vacuo, and the residue dissolved in ethyl acetate (250 ml). The solution was successively washed with 3% citric acid (2 × 15 ml), saturated bicarbonate solution (3 × 1 ml), and water (4 × 15 ml). The solvent was evaporated without prior drying. The crystals were collected and washed on a filter with ethyl acetate. The air-dried product weighed 3.53 g (66%): mp 174–175 °C dec; $[\alpha]D$ −9° (*c* 1, DMF); TLC R_f (A) 0.72, R_f (B) 0.6. Amino acid analysis: Arg + Orn 0.9, Ile 1.0, Ser 0.9. Recrystallization from methanol–ether did not raise the melting point.

Anal. Calcd for $C_{21}H_{39}N_7O_9$: C, 47.3; H, 7.3; N, 18.4. Found: C, 47.1; H, 7.1; N, 18.3.

B.[16] Compound II (6.65 g, 24.8 mmol) was dissolved in DMF (50

Chart I. Scheme of the Synthesis of Boc-Asp-Pro-Ser-His-Arg-Ile-Ser-$NHNH_2$ (XIII)



ml), and triethylamine (3.5 ml, 25 mmol) and *tert*-butyloxycarbonylnitro-L-arginine *N*-hydroxysuccinimide ester[3] (14.85 g, 34.5 mmol) were added. After 5 h at room temperature, the mixture was diluted with EtOAc to 1 l.; the solution was washed with a 2% solution of citric acid (4 × 200 ml), water (200 ml), 1 M $NaHCO_3$ (4 × 200 ml), and water (3 × 200 ml), dried over Na_2SO_4, and concentrated to about 200 ml. Next day, the crystals were filtered and washed with hot EtOAc, hot tetrahydrofuran, and once more with hot EtOAc. They weighed 9.1 g (69%), mp 180–182 °C dec. A less pure second crop (2.0 g) melted (after sintering at 174 °C) at 178–179 °C dec. Recrystallization of a sample of the first crop from DMF–EtOAc raised the melting point to 183–185 °C dec, $[\alpha]^{23}D$ −9° (*c* 2, DMF).

Anal. Found: C, 47.3; H, 7.6; N, 18.4.

N^{α}-N^{im}-Bis(*tert*-butyloxycarbonyl)-L-histidylnitro-L-arginyl-L-isoleucyl-L-serine Methyl Ester (V). The protected tripeptide III (3.21 g, 6 mmol) was dissolved in TFA (15 ml). After 15 min, the acid was evaporated in vacuo, and dry ether (70 ml) was added. The tripeptide trifluoroacetate IV was collected on a filter, washed with ether, and dried in vacuo over P_2O_5 and KOH [mp 104–105 °C, TLC R_f (A) 0.45]. It was dissolved in DMF (15 ml), and diisopropylethylamine[17] (1.95 ml, 12 mmol) was added, followed by N^{α}-N^{im}-bis(*tert*-butyloxycarbonyl)-L-histidine *p*-nitrophenyl ester[18] (3.43 g, 7.2 mmol). After 2 days, petroleum ether (50 ml) was added. The mixture was vigorously stirred, and the petroleum ether was decanted. This procedure was repeated once more, then ether (100 ml) was added. The product solidified within a few minutes. It was collected and thoroughly washed with ether and ethyl acetate. The material was treated with a few milliliters of THF and precipitated with ethyl acetate. The air-dried product weighed 4.16 g (88%), mp 133–134 °C. Reprecipitation of a sample from THF–ethyl acetate did not raise the melting point: $[\alpha]^{24}D$ −10° (*c* 1, DMF); TLC R_f (A) 0.78, R_f (B) 0.62. Amino acid analysis: His 1.0, Arg + Orn 0.9, Ile 1.0, Ser 0.9.

Anal. Calcd for $C_{32}H_{54}O_{12}N_{10}\cdot H_2O$: C, 48.7; H, 7.2; N, 17.8. Found: C, 49.0; H, 7.1; N, 17.6.

***tert*-Butyloxycarbonyl-*O*-benzyl-L-seryl-L-histidylnitro-L-arginyl-L-isoleucyl-L-serine Methyl Ester (VII).** The protected tetrapeptide V (4.74 g, 6 mmol) was dissolved in TFA (15 ml). After 15 min, the acid was removed in vacuo and the product was precipitated with dry ether (60 ml). It was filtered, washed with ether, and dried in vacuo: 5.2 g (97%); mp 88–89 °C; TLC R_f (A) 0.17. The tetrapeptide ditrifluoroacetate VI was added to a solution of *tert*-butyloxycarbonyl-*O*-benzyl-L-serine *p*-nitrophenyl ester[18] (7.5 mmol) in DMF (15 ml) containing diisopropylethylamine (1.95 ml, 12 mmol). The solution was kept slightly basic, and 2 days later the DMF was removed in vacuo. Dry ether (60 ml) was added and, after a few hours in the cold, the supernatant solution was decanted. This treatment was repeated twice. The residue was dissolved in a few milliliters of DMF and poured into ethyl acetate (400 ml) in a separatory funnel. The organic phase was washed with 0.5 N ammonia solution and then with several portions of water until neutral. The ethyl acetate was removed in vacuo, and the crystalline residue was filtered and washed with ethyl acetate. The air-dried product weighed 3.35 g (66%): mp 128–129 °C; $[\alpha]D$ −15° (*c* 1, DMF); TLC R_f (A) 0.54, R_f (B) 0.37. Amino acid analysis: Ser 2.0, Arg + Orn 0.9, His 1.0, Ile 1.1. For analysis, a sample was recrystallized from ethyl acetate, without change in the melting point.

Anal. Calcd for $C_{37}H_{57}N_{11}O_{12}$ (847.9): C, 52.4; H, 6.8; N, 18.2. Found: C, 52.2; H, 7.0; N, 18.0.

***tert*-Butyloxycarbonyl-L-prolyl-*O*-benzyl-L-seryl-L-histidylnitro-L-arginyl-L-isoleucyl-L-serine Methyl Ester Dihydrate (IX).** The protected pentapeptide VII (2.97 g, 3.5 mmol) was dissolved in TFA (10 ml). After 15 min, the TFA was removed in vacuo, and the product (VIII) was precipitated with dry ether (60 ml) and dried in vacuo: 3.43 g (quantitative); mp 109–110 °C; TLC R_f (A) 0.30. The entire amount was added to a solution of *tert*-butyloxycarbonyl-L-proline *p*-nitrophenyl ester[19] (4 mmol) in DMF (10 ml) containing triethylamine (1 ml, 7 mmol). After 2 days, the DMF was removed in vacuo, and ether (50 ml) was added. The mixture was cooled and the ether was decanted. This procedure was repeated twice. The residue was dissolved in methanol, and the solution was poured into ethyl acetate (350 ml). The organic phase was washed with 0.5 N ammonia and then with water. Evaporation of the ethyl acetate (without prior drying) and precipitation with ether yielded 2.54 g (75%), mp 202–203 °C dec, with softening at 200 °C. Recrystallization from methanol gave mp 204–205 °C dec; $[\alpha]^{24}D$ −29° (*c* 1, DMF); TLC R_f (A) 0.48, R_f (B) 0.31. Amino acid analysis: Pro 1.0, Ser 1.8, His 1.1, Arg + Orn 1.0, Ile 1.0.

Anal. Calcd for $C_{42}H_{64}N_{12}O_{13}\cdot 2H_2O$ (981.1): C, 51.4; H, 7.0; N, 17.1. Found: C, 51.3; H, 6.8; N, 17.0.

***tert*-Butyloxycarbonyl-β-benzyl-L-aspartyl-L-prolyl-*O*-benzyl-L-seryl-L-histidylnitro-L-arginyl-L-isoleucyl-L-serine Methyl Ester (XI). A.** The protected hexapeptide IX (8.5 g, 8.7 mmol) was dissolved in TFA (30 ml). After 15 min, the acid was removed in vacuo, and the product was precipitated with dry ether (100 ml). The yield after drying in vacuo was 9.3 g, mp 97–98 °C, TLC R_f (A) 0.19.

The hexapeptide ditrifluoroacetate (X) was dissolved in DMF (10 ml). Triethylamine (2.2 ml, 16 mmol) was added, followed by *tert*-butyloxycarbonyl-β-benzyl-L-aspartic acid *N*-hydroxysuccinimide ester[5] (4.63 g, 11 mmol) and 1-hydroxybenzotriazole (1.08 g, 8 mmol). The reaction mixture was kept slightly basic. Next day, the solvent was removed in vacuo, and the residue was dissolved in ethyl acetate (600 ml). The solution was washed five times with saturated $NaHCO_3$

solution and with water until neutral and concentrated in vacuo. The product was precipitated by the addition of ether (100 ml), collected on a filter, and washed with ether. The air-dried material weighed 8.15 g. The crude product showed two major spots on TLC, R_f (A) 0.65 and 0.74, R_f (B) 0.45 and 0.71. For the separation of the components and isolation of XI in pure form, cf. the following paper.

B. The hexapeptide ditrifluoroacetate X (100 mg, 0.1 mmol) was dissolved in DMF (0.6 ml) and treated with diisopropylethylamine (0.017 ml, 0.11 mmol), *tert*-butyloxycarbonyl-β-benzyl-L-aspartic acid *p*-nitrophenyl ester (Bachem, 42 mg, 0.1 mmol), and 1-hydroxybenzotriazole (13 mg, 0.1 mmol). Next day, ether (40 ml) was added and the mixture was kept overnight in the refrigerator. The resulting solid was disintegrated, filtered, washed with ether (25 ml), and dried over P_2O_5 in vacuo. The product weighed 115 mg (98%), mp 109–110 °C, $[\alpha]^{24}D$ −35° (*c* 1, DMF). On TLC, in addition to the main product [R_f (A) 0.65, R_f (B) 0.45], only a trace of the *O*-acyl derivative [R_f (A) 0.74, R_f (B) 0.71] could be detected. Amino acid analysis: Asp 1.0, Ser 1.7, Pro 1.0, Ile 1.0, His 1.1, Arg + Orn 1.0.

Anal. Calcd for $C_{53}H_{75}N_{13}O_{16}\cdot H_2O$: C, 54.4; H, 6.6; N, 15.6. Found: C, 54.5; H, 6.7; N, 15.6.

***tert*-Butyloxycarbonyl-L-aspartyl-L-prolyl-L-seryl-L-histidyl-L-arginyl-L-isoleucyl-L-serine Hydrazide (XIII).** A sample (1.12 g) of the protected heptapeptide ester XI was dissolved in a mixture of 95% ethanol (125 ml), H_2O (20 ml), and AcOH (2.5 ml) and hydrogenated in the presence of a 10% Pd on charcoal catalyst (0.3 g) for 3 days. The catalyst was removed by filtration and the solvent by evaporation in vacuo, and the residue was treated with dry ether (50 ml). The solid product was collected on a filter, washed with dry ether, and dried in vacuo over P_2O_5 and KOH for 24 h. The product (XII) weighed 0.80 g: mp 77–78 °C; $[\alpha]^{24}D$ −57° (*c* 1, MeOH); TLC R_f (A) 0.33. No significant uv absorption was found at 270 nm. Elemental analysis suggests the retention of both acetic acid and water.

Anal. Calcd for $C_{39}H_{64}N_{12}O_{14}\cdot 2.5CH_3COOH\cdot 2H_2O$: C, 47.7; H, 7.2; N, 15.5. Found: C 47.5; H, 7.1; N, 15.6.

A sample (0.88 g) of the partially protected heptapeptide methyl ester XII was dissolved in CH_3OH (18 ml) and treated with hydrazine hydrate (2 ml). After 12 h, the solvent was removed in vacuo, and the residue dried in vacuo over P_2O_5 and concentrated H_2SO_4 for 24 h. The product was dissolved in H_2O and freeze-dried. This was repeated four times. The hydrazide XIII (0.88 g) melted at 99–100 °C; $[\alpha]^{24}D$ −53° (*c* 1, CH_3OH); TLC R_f (A) 0.18, R_f (C) 0.33. Amino acid analysis: Asp 1.0, Ser 1.8, Pro 0.95, Ile 1.0, His 1.0, Arg 1.0.

Anal. Calcd for $C_{38}H_{64}N_{14}O_{13}\cdot 6H_2O$: C, 44.2; H, 7.6; N, 19.5. Found: C, 43.9; H, 7.5; N, 19.3.

Loss of weight on drying at room temperature for 24 h and then at 60 °C for 16 h was 8.3%. Calcd (for $6H_2O$): 10.7. After this drying, the melting point changed to 124–133 °C dec, with sintering at 118 °C.

Acknowledgment. This study was supported by a grant from the U.S. Public Health Service (NIH AM-12473).

Registry No.—I, 60526-22-9; II, 60562-29-0; III, 60526-23-0; IV, 60562-30-3; V, 60526-24-1; VI, 60526-26-3; VII, 60526-27-4; VIII, 60526-29-6; IX, 60526-30-9; X, 60526-32-1; XI, 60512-76-7; XII, 60526-33-2; XIII, 60526-34-3; serine methyl ester hydrochloride, 5680-80-8; benzyloxycarbonyl-L-isoleucine nitrophenyl ester, 2130-99-6; z-L-Ile, 3160-59-6; Ser-OCH_3, 2788-84-3; *tert*-butyloxycarbonylnitro-L-arginine, 2188-18-3; *tert*-butyloxycarbonylnitro-L-argine *N*-hydroxysuccinimide ester, 60526-35-4; N^{α}-N^{im}-bis(*tert*-butyloxycarbonyl)-L-histidine *p*-nitrophenyl ester, 20866-47-1; *tert*-butyloxycarbonyl-*O*-benzyl-L-serine *p*-nitrophenyl ester, 16948-39-3; *tert*-butyloxycarbonyl-L-proline *p*-nitrophenyl ester, 28310-65-8; *tert*-butyloxycarbonyl-β-benzyl-L-aspartic acid *N*-hydroxysuccinimide ester, 13798-75-9; *tert*-butyloxycarbonyl-β-benzyl-L-aspartic acid *p*-nitrophenyl ester, 26048-69-1.

References and Notes

(1) Preceding paper: M. Bodanszky, N. Chaturvedi, D. Hudson, and M. Itoh, *J. Org. Chem.*, **37**, 2303 (1972).
(2) V. Mutt and J. E. Jorpes, *Eur. J. Biochem.*, **6**, 156 (1968); *Biochem. J.*, **125**, 570 (1971).
(3) M. A. Ondetti, J. Pluscec, E. F. Sabo, J. T. Sheehan, and N. J. Williams, *J. Am. Chem. Soc.*, **92**, 95 (1970).
(4) M. Bodanszky, *Ann. N.Y. Acad. Sci.*, **88**, 655 (1960).
(5) D. A. Laufer and E. R. Blout, *J. Am. Chem. Soc.*, **89**, 1246 (1967).
(6) M. Bodanszky, M. L. Fink, Y. S. Klausner, S. Natarajan, K. Tatemoto, A. E. Yiotakis, and A. Bodanszky, *J. Org. Chem.*, following paper in this issue.
(7) M. Bodanszky, *Nature (London)*, 685 (1955).
(8) W. Konig and R. Geiger, *Chem. Ber.*, **106**, 3626 (1973).
(9) S. Natarajan and M. Bodanszky, unpublished.
(10) T. Ziminski and E. Borowski, *J. Chromatogr.*, **23**, 480 (1966).
(11) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.*, **30**, 1190 (1958).
(12) J. I. Harris and J. S. Fruton, *J. Biol. Chem.*, **191**, 143 (1951).
(13) M. Bodanszky and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 5688 (1959).
(14) M Löw and L. Kisfaludy, *Acta Chim. Acad. Sci. Hung.*, **44**, 61 (1965).
(15) W. Konig and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).
(16) In version B, compound III was prepared by K. Tatemoto.
(17) M. Bodanszky and A. Bodanszky, *Chem. Commun.*, 591 (1967).
(18) E. Schnabel, H. Herzog, P. Hoffmann, E. Klauke, and I. Ugi, *Justus Liebigs Ann. Chem.*, 175 (1968); cf. also D. Yamashiro, J. Blake, and C. H. Li, *J. Am. Chem. Soc.*, **94**, 2855 (1972).
(19) Prepared according to the general procedure described in *Biochem. Prep.*, **9**, 110 (1962). The active ester, an oil, was used without purification.

Side Reactions in Peptide Synthesis. 4. Extensive O-Acylation by Active Esters in Histidine Containing Peptides[1]

Miklos Bodanszky,* Mary Lynn Fink, Yakir S. Klausner,‡ Sesha Natarajan, Kazuhiko Tatemoto, Athanasios E. Yiotakis, and Agnes Bodanszky

Department of Chemistry, Case Western Reserve University, Cleveland, Ohio 44106, and Department of Organic Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel

Received June 11, 1976

Acylation of the partially protected hexapeptide Pro-Ser(Bzl)-His-Arg(NO_2)-Ile-Ser-OCH_3 with the *N*-hydroxysuccinimide ester of *tert*-butyloxycarbonyl-β-benzyl-L-aspartic acid in the presence of 1-hydroxybenzotriazole produced, in addition to the desired heptapeptide derivative, a by-product, often in significant amounts. Examination of this material revealed that acylation of the free amino group of the partially protected hexapeptide was accompanied by acylation of the unprotected hydroxyl group of the C-terminal serine residue. Model experiments demonstrated that the extensive O-acylation was due to the presence f a histidine residue in the amino component. The imidazole in the side chain of this amino acid acts as catalyst in the alcoholysis of the active esters used for chain lengthening. Conditions that can reduce the extent of O-acylation were explored. The implications of this side reaction on the problems of minimal vs. global protection and of the application of excess acylating agents ("the principle of excess") are also discussed.

In the course of our continued effort[2] toward the synthesis of the gastrointestinal hormone cholecystokinin,[3] a protected heptapeptide corresponding to the partial sequence 17–23 of the 33-membered chain was prepared. Building of the protected derivative *tert*-butyloxycarbonyl-β-benzyl-L-aspartyl-L-prolyl-*O*-benzyl-L-seryl-L-histidylnitro-L-ar-

‡ The Hebrew University of Jerusalem.