($CDCl_3$) δ 1.3 [s, 12, 2-SC(CH_3)$_2$], 3.2 (s, 6, 2-NCH_3), 7.4 (s, 2, 2-CH=N).

Anal. Calcd for $C_{10}H_{20}N_2S_2$: C, 51.68; H, 8.67; N, 12.05; S, 27.59; mol wt, 232.4. Found: C, 51.76; H, 8.86; N, 11.73; S, 27.69; mol wt, 232 ($CHCl_3$).

1,1-Dimethyl 2-Substituted Aminoethanethiols (9–15). To a stirred solution containing 0.2 mol of **2, 3, 4, 5, 6, 7,** or **8** in 500 ml of ethanol at 60°, a solution containing 23.4 g (0.6 mol) of sodium borohydride in 600 ml of ethanol was added slowly at 65–70° over a 1-hr period. The stirred reaction mixture was heated at reflux for 1 hr and then allowed to cool to 30°. The reaction mixture was added to 2000 g of ice water and stirred at 0–10° for 1 hr. For **13,** the precipitate was collected by filtration, washed with water until the washings were neutral to litmus, and air dried at 25–30°. For the remainder, the viscous liquids were extracted by the addition of 1 l. of ethyl ether and filtered to remove any impurities. The separated ether layer was washed with water until the washings were neutral to litmus and dried over sodium sulfate. The ether was removed in vacuo at maximum temperature at 80–90° (1–2 mm). The data are summarized in Table II.

Hydrochloride Salts (9a–12a and 15a). To a stirred solution containing **9–12** or **15** in 300 ml of ethyl ether, hydrogen chloride gas was bubbled through the solution at 0–10° over a 0.5-hr period. The precipitate was collected by filtration, washed with 200 ml of ethyl ether, and air dried at 50°. The data are summarized in Table II.

3-[*p*-(Dimethylamino)phenyl]-5,5-dimethyl-2-thiazolidinethione (16) and 3-(*p*-Anilinophenyl)-5,5-dimethyl-2-thiazolidinethione (17). A stirred slurry containing 0.15 mol of **13** or **14,** 9.8 g (0.15 mol) of 85% potassium hydroxide, 22.8 g (0.3 mol) of carbon disulfide, and 50 ml of ethanol was heated at reflux for 24 hr. After the stirred reaction mixture was cooled to 0°, the precipitate was collected by filtration, washed successively with 25 ml of ethanol and 300 ml of water, and air dried at 25–30°. The crude **16,** mp 142–146°, and **17,** mp 165–166°, were obtained in yields of 75 and 99%, respectively. After recrystallization from ethyl acetate and toluene, respectively, **16** and **17** melted at 157–158 and 166–167°, respectively: NMR ($CDCl_3$) of **16,** δ 1.60 [s, 6, -C(CH_3)$_2$], 2.92 [s, 6, -N(CH_3)$_2$], 4.08 (s, 2, NCH_2C), 6.50–7.30 (m, 4, ArH), and **17,** δ 1.60 [s, 6, -C(CH_3)$_2$], 4.10 (s, 2, NCH_2C), 5.90 (br s, 1, -NH), 6.90–7.50 (m, 9, 2 ArH); mass spectrum *m/e* (rel intensity) of **16,** probe temperature 125°, 266 (86.8), 190 (39.1), 178 (16.7), 152 (26.8), 147 (100), 145 (31.5), 135 (22.4), 120 (21.7), 77 (22.5), and 42 (32.6), and **17,** probe temperature 200°, 314 (100), 238 (32.8), 226 (17.3), 200 (14.3) 195 (52.1), 183 (12.5), 168 (26.9), 167 (44.9), 77 (17.5), and 65 (10.1).

Anal. Calcd for $C_{13}H_{18}N_2S_2$ (**16**): C, 58.61; H, 6.81; N, 10.51; S, 24.07; mol wt, 266.4. Found: C, 58.82; H, 6.79; N, 10.46; S, 24.16; mol wt, 265 ($CHCl_3$). Calcd for $C_{17}H_{18}N_2S_2$ (**17**): C, 64.93; H, 5.77; N, 8.91; S, 20.39. Found: C, 64.80; H, 5.66; N, 8.83; S, 20.60.

Registry No.—**1,** 15581-80-3; **2,** 54410-19-4; **3,** 54410-20-7; **4,** 54410-21-8; **5,** 54410-22-9; **6,** 54410-23-0; **7,** 54410-24-1; **8,** 54410-25-2; **9,** 54410-26-3; **9a,** 54410-27-4; **10,** 54410-28-5; **10a,** 54410-29-6; **11,** 54410-30-9; **11a,** 54410-31-0; **12,** 54446-52-5; **12a,** 54410-32-1; **13,** 54410-33-2; **14,** 54410-34-3; **15,** 54410-35-4; **15a,** 39981-47-0; **16,** 54410-36-5; **17,** 54410-37-6; isobutyraldehyde, 78-84-2; sulfur chloride, 10025-67-9; *N,N*-dimethyl-*p*-phenylenediamine, 99-98-9; *N*-phenyl-*p*-phenylenediamine, 101-54-2; methylamine, 74-89-5; *m*-trifluoromethylaniline, 98-16-8; aniline, 62-53-3; *p*-chloroaniline, 106-47-8; 4-bromo-5-chloro-2-methylaniline, 30273-47-3.

Supplementary Material Available. Mass spectral fragmentation routes for **2, 9,** and **16** will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D.C. 20036. Remit check or money order for $4.00 for photocopy or $2.50 for microfiche, referring to code number JOC-75-1224.

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(8) **15a** prepared by their method kindly furnished by F. Meneghini.
(9) See paragraph at end of paper regarding supplementary material.

Preparation of Protected Peptide Intermediates for a Synthesis of the Ovine Pituitary Growth Hormone Sequence 96–135

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The preparation of seven protected peptides (tri- to dodecapeptides) which span the sequence of the "active core" region 96–135 of ovine pituitary growth hormone is described. These peptides were synthesized through modified solid-phase techniques, i.e., use of *p*-alkoxybenzyl alcohol resin and hydroxymethyl resin or successive applications of both solid-phase and conventional procedures. The purity of all peptides was carefully ascertained.

The biochemical studies of Li and others[1–6] have led to the conclusion that an intact molecule of pituitary growth hormone is not essential for the manifestation of its biological activities. Indeed, an "active core" was isolated from tryptic digest of bovine growth hormone by Sonenberg et al.[4] and its amino acid sequence delineated.[7] The compound was reported to possess 20–30% of the activity[4,8] of the intact hormone both by the tibia test[9] and the body weight gain test.[10] Moreover, the species specificity appeared to become less stringent. The bovine core peptide exhibited activity in man whereas the intact bovine growth hormone did not. Comparison of the core fragment with the amino acid sequence of bovine growth hormone[11–13] revealed its identity with the sequence region 96–133 (Chart I). In a recent communication Li et al.[14] have described a solid-phase synthesis of the corresponding region of human growth hormone (i.e., sequence 95–136) and showed that the product stimulated the growth of rat tibia. Li and Yam-

Chart I
Amino Acid Sequence in the Region of Residues 96–135 for Ovine Growth Hormone (OGH), Bovine Growth Hormone (BGH), Human Growth Hormone (HGH), and Porcine Growth Hormone (PGH)

```
          96                  100                 105
OGH: -Val-Phe-Thr-Asp-Ser-Leu-Val-Phe-Gly-Thr-Ser-Asp-    Arg-Val-
BGH: -Val-Phe-Thr-Asn-Ser-Leu-Val-Phe-Gly-Thr-Ser-Asp-    Arg-Val-
HGH: -Val-Phe-Ala-Asn-Ser-Leu-Val-Tyr-Gly-Ala-Ser-Asn-Ser-Asp-Val-

          110                 115                 120
OGH: -Tyr-Glu-Lys-Leu-Lys-Asp-Leu-Glu-Glu-Gly-Ile-Leu-Ala-Leu-Met-
BGH: -Tyr-Glu-Lys-Leu-Lys-Asp-Leu-Glu-Glu-Gly-Ile-Leu-Ala-Leu-Met-
HGH: -Tyr-Asp-Leu-Leu-Lys-Asp-Leu-Glu-Glu-Gly-Ile-Gln-Thr-Leu-Met-
PGH:                      -Asp-Leu-Glu-Glu-Gly-Ile-Gln-Ala-Leu-Met-

          125                 130                 135
OGH: -Arg-Glu-Leu-Glu-Asp-Val-Thr-Pro-Arg-Ala-Gly-
BGH: -Arg-Glu-Leu-Glu-Asp-Gly-Thr-Pro-Arg-Ala-Gly-
HGH: -Gly-Arg-Leu-Glu-Asp-Gly-Ser-Pro-Arg-Thr-Gly-
PGH: -Arg-Glu-Leu-Glu-Asp-Gly-Ser-Pro-Arg-Ala-Gly-
```

ashiro[15] have also reported the synthesis of human growth hormone-like protein by the standard solid-phase method[16–19] based on a partly incorrect amino acid sequence.[20] Conventional synthesis of protected peptide components with sequences corresponding to residues 1–27,[21] 28–52,[22] 53–67,[23] and 166–188[22] were also communicated by Li and his coworkers. Syntheses of other partial sequences of human growth hormone in attempts to locate active sites have also been reported by several authors.[24–26]

In a previous communication[27] we have described the synthesis of the COOH-terminal nonapeptide sequence H-Arg-Glu-Leu-Glu-Asp-Gly-Thr-Pro-Arg-OH of the active core of the bovine hormone. This nonapeptide possessed significant rat tibia growth promoting activity at dose levels of 5–15 μg/rat per day. However, in the rat body weight gain test, the peptide was inactive up to the doses of 50 μg/rat per day, which might be due to metabolic instability of the compound or, alternatively, might indicate that the tibia test could give misleading results with small peptides.[28] We concluded that it was desirable to synthesize and investigate a series of increasingly longer peptides. In this report, the synthesis of protected peptides needed for a total synthesis of the core peptide 96–135 of ovine growth hormone is described. These peptides were prepared through a modified solid-phase technique[29] or a combination of solid-phase[16–29] and conventional procedures.[30,31] The protected intermediates will serve to synthesize the tetracontapeptide active core by conventional fragment condensation methods.

Results and Discussion

The protected peptides which have been prepared to date as intermediates for a total synthesis of the ovine growth hormone fragment 96–135 are listed in Table I with some of their physicochemical characteristics. A thin layer chromatogram of these peptide intermediates is shown in Figure 1. Z-Val-Phe-Thr(Bzl)-OH (I)[32] was synthesized by a modified solid-phase technique.[27,29] Bpoc-Thr(Bzl)-OH was esterified to *p*-alkoxybenzyl alcohol resin with the aid of DCC[33,34] using 4-dimethylaminopyridine[35] as catalyst.[27] The ensuing Bpoc-Thr(Bzl)-$OCH_2C_6H_4OCH_2C_6H_4$-Resin was benzoylated[29] to block unreacted hydroxymethyl groups. Following cleavage of the Bpoc group by 0.5% TFA in CH_2Cl_2 and neutralization with 10% TEA, Bpoc-Phe-OH was coupled to the aminoacyl resin using DCC as described previously, followed by another cycle in which Z-Val-OH was coupled to the peptide resin. The protected tripeptide I was cleaved from the solid support by treatment with 50% TFA in CH_2Cl_2 (25°, 30 min) and isolated as an analytically pure, crystalline solid in 75% overall yield. Similarly prepared on the *p*-alkoxybenzyl alcohol resin were the intermediates H-Ser(Bzl)-Leu-Val-Phe-Gly-OH (II), H-Lys(Z)-Asp(OBzl)-Leu-Glu(OBzl)-OH (III), H-Gly-Ile-Leu-Ala-Leu-OH (IV), H-Arg(Tos)-Glu(OBzl)-Leu-Glu(OBzl)-Asp(OBzl)-Val-OH (V), and H-Thr(Bzl)-Pro-Arg(Tos)-Ala-Gly-OH (VI). With the exception of compound VI, which required chromatographic purification on a Sephadex LH-20 column, all of these side chain protected peptides crystallized directly after cleavage from the resin, giving pure products with overall yields of 30–90% based on the respective amino acid contents of the starting aminoacyl resins.

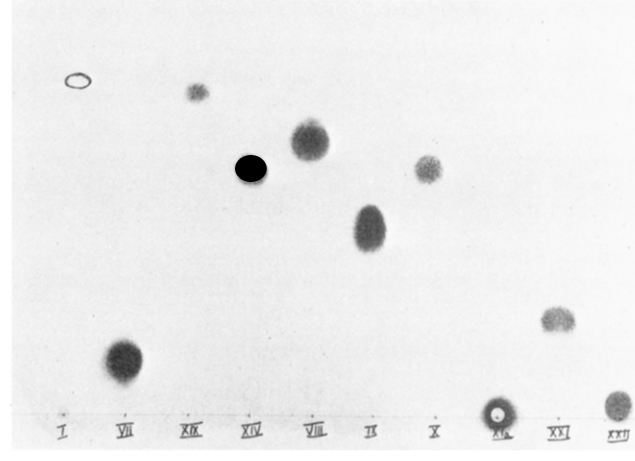


Figure 1. Thin layer chromatogram of the synthetic protected peptide intermediates. Solvent system: methanol–chloroform–acetic acid (51:6:3). Color developed by chlorine–tolidine.

Compound II, H-Ser(Bzl)-Leu-Val-Phe-Gly-OH, prepared by the solid-phase method as described above, was further treated with Boc-Asn-OSu to form the protected hexapeptide Boc-Asn-Ser(Bzl)-Leu-Val-Phe-Gly-OH (VII) as an analytically pure solid. Reactions of H-Lys(Z)-Asp(OBzl)-Leu-Glu(OBzl)-OH (III) with Boc-Leu-OSc[36] similarly gave the protected crystalline pentapeptide Boc-Leu-Lys(Z)-Asp(OBzl)-Leu-Glu(OBzl)-OH (VIII). In the same fashion the protected hexapeptide Boc-Glu(OBzl)-Gly-Ile-Leu-Ala-Leu-OH (IX) was prepared by coupling Boc-Glu(OBzl)-OSu with H-Gly-Ile-Leu-Ala-Leu-OH (IV) and the protected heptapeptide Bpoc-Met-Arg(Tos)-Glu(OBzl)-Leu-Glu(OBzl)-Asp(OBzl)-Val-OH (X) by cou-

Table I
Peptide Intermediates for the Synthesis of Ovine Growth Hormone Fragment 96–135

Compd[a]	Mp, °C	$[\alpha]^{25}$D, deg	Elemental analysis, %[b] C	H	N
^{96}Z-Val-Phe-Thr(Bzl)^{98}-OH (I)	209–211 cryst	−3.50 (*c* 0.7, MeOH)	$C_{33}H_{39}N_3O_7$ (589.69) 67.22 66.83	6.67 6.73	7.13 7.13
Boc-Asn^{99}-Ser(Bzl)-Leu-Val-Phe-Gly-OH^{104} (VII)	Amorphous	−31.92 (*c* 1, DMF)	$C_{41}H_{59}N_7O_{11}$ (825.96) 59.62 59.32	7.20 7.28	11.87 11.78
Boc-Thr^{105}(Bzl)-Ser(Bzl)-Asp(OBzl)OH (XIX)	113–116 cryst	+6.90 (*c* 1, DMSO)	$C_{37}H_{45}N_3O_{10}$ (691.75) 64.24 64.39	6.56 6.98	6.07 6.37
Boc-Arg^{108}(Tos)-Val-Tyr(Cl_2Bzl)-Glu(OBzl)-Lys^{112}(Z)-OH (XIV)	138–141 cryst	−7.49 (*c* 1, DMF)	$C_{65}H_{81}N_9O_{15}Cl_2S$ (1331.35) 58.64 58.31	6.13 6.20	9.47 9.49
Boc-Leu-Lys^{113}(Z)-Asp(OBzl)-Leu-Glu(OBzl)-OH^{117} (VIII)	155–157 cryst	−25.50 (*c* 1, DMF)	$C_{54}H_{74}N_6O_{14}$ (1031.18) 62.90 62.77	7.23 7.14	8.15 8.07
Boc-Glu^{118}(OBzl)-Gly-Ile-Leu-Ala-Leu-OH^{123} (IX)	182–184 cryst	−28.59 (*c* 1, DMF)	$C_{40}H_{64}N_6O_{11}$ (804.96) 59.68 59.56	8.01 8.24	10.44 10.42
HCl·H-Met^{124}-Arg(Tos)-Glu(OBzl)-Leu-Glu(OBzl)-Asp(OBzl)-Val-Thr(Bzl)-Pro-Arg(Tos)-Ala-Gly-NH_2^{135} (XXII)	238–241 cryst	−21.01 (*c* 1, DMF)	$C_{98}H_{133}N_{19}O_{23}S_3 \cdot HCl$ (2077.87) 56.65 56.02	6.50 6.65	12.81 12.66

[a] The numbers above the sequence refer to the position in ovine growth hormone. [b] Calculated over found. Molecular weights are given in parentheses.

pling Bpoc-Met-ONp with H-Arg(Tos)-Glu(OBzl)-Leu-Glu(OBzl)-Asp(OBzl)-Val-OH (V). All active ester couplings proceeded smoothly. Slight excess of the corresponding active esters were used.

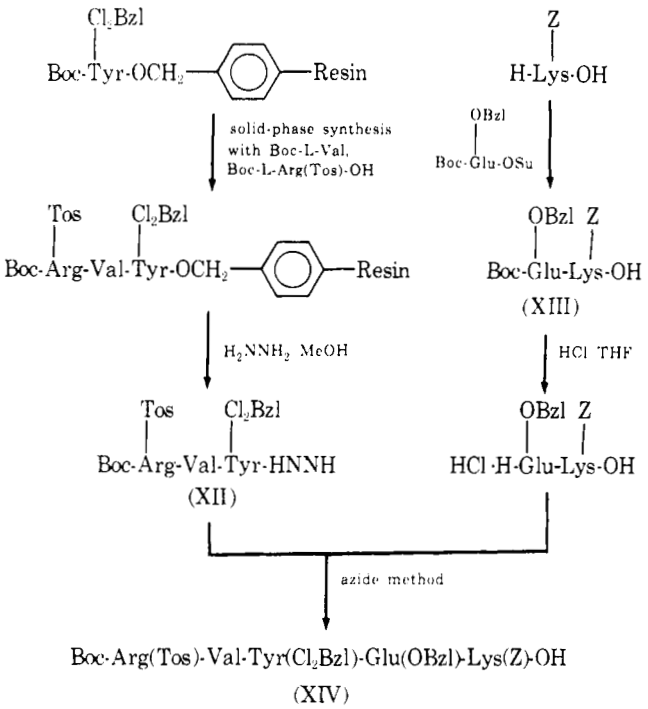
The synthesis of Boc-Thr(Bzl)-Pro-Arg(Tos)-Ala-Gly-NH_2 (XI) on hydroxymethyl resin[37] followed closely the original methodology of Merrifield,[16-19] i.e., Boc-amino acids were used in the repetitive synthetic cycles and the peptide chain was released from the resin by ammonolytic cleavage of the benzyl-ester-like anchoring bond. The protected peptapeptide amide was obtained as an amorphous solid in 60% overall yield. The amino protecting group was then removed by treatment with 3.4 *N* HCl in tetrahydrofuran to afford HCl·H-Thr(Bzl)-Pro-Arg(Tos)-Ala-Gly-NH_2 (XIa).

For the synthesis of Boc-Arg(Tos)-Val-Tyr(Cl_2Bzl)-Glu(OBzl)-Lys(Z)-OH (XIV), a combination of solid-phase and conventional procedures was employed (see Scheme I). Boc-Tyr(Cl_2Bzl)-OH[38] was esterified to the hydroxymethyl resin and the solid-phase synthesis continued by incorporation of Boc-Val-OH and Boc-Arg(Tos)-OH into the resin to form the tripeptide resin. Hydrazinolysis afforded the crystalline Boc-Arg(Tos)-Val-Tyr(Cl_2Bzl)-$HNNH_2$ (XII) in 63% overall yield. Coupling of this tripeptide with the dipeptide H-Glu(OBzl)-Lys(Z)-OH·HCl by the azide method[39] resulted in the formation of the crystalline pentapeptide XIV in 53% yield.

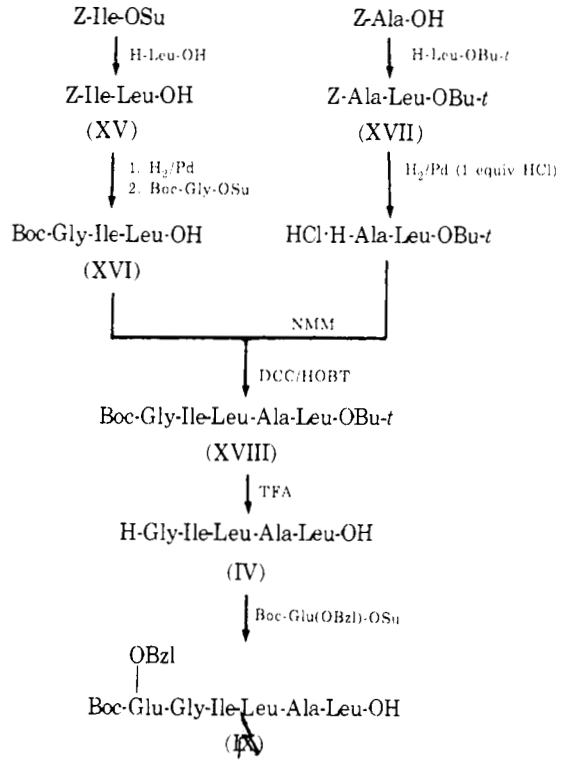
Boc-Glu(OBzl)-Gly-Ile-Leu-Ala-Leu-OH (IX) was also prepared by conventional methods as illustrated in Scheme II. Coupling of Boc-Ile-OSu with Leu gave Z-Ile-Leu-OH (XV), which on hydrogenolysis followed by coupling with Boc-Gly-OSu yielded the tripeptide Boc-Gly-Ile-Leu-OH (XVI). This tripeptide was then coupled to H-Ala-Leu-OBu-*t*·HCl derived from hydrogenolytic cleavage of the Z group from Z-Ala-Leu-OBu-*t* (XVII) using the HOBT-assisted[40] DCC reaction[41] to give the protected pentapeptide Boc-Gly-Ile-Leu-Ala-Leu-OBu-*t* (XVIII). Treatment with TFA afforded the pentapeptide H-Gly-Ile-Leu-Ala-Leu-OH (IV), which was also prepared by the above-mentioned solid-phase technique. Reaction of IV with Boc-Glu(OBzl)-OSu led smoothly to the desired product IX, a crystalline solid, in 78% yield.

Boc-Ser(Bzl)-Asp(OBzl)-OH was prepared from H-Asp(OBzl)-OH and Boc-Ser(Bzl)-OSu.[42] Deprotection of Boc group by treatment with HCl in THF gave the crystalline dipeptide salt H-Ser(Bzl)-Asp(OBzl)-OH·HCl. On reaction of this compound with Boc-Thr(Bzl)-OSu the desired protected tripeptide Boc-Thr(Bzl)-Ser(Bzl)-As-

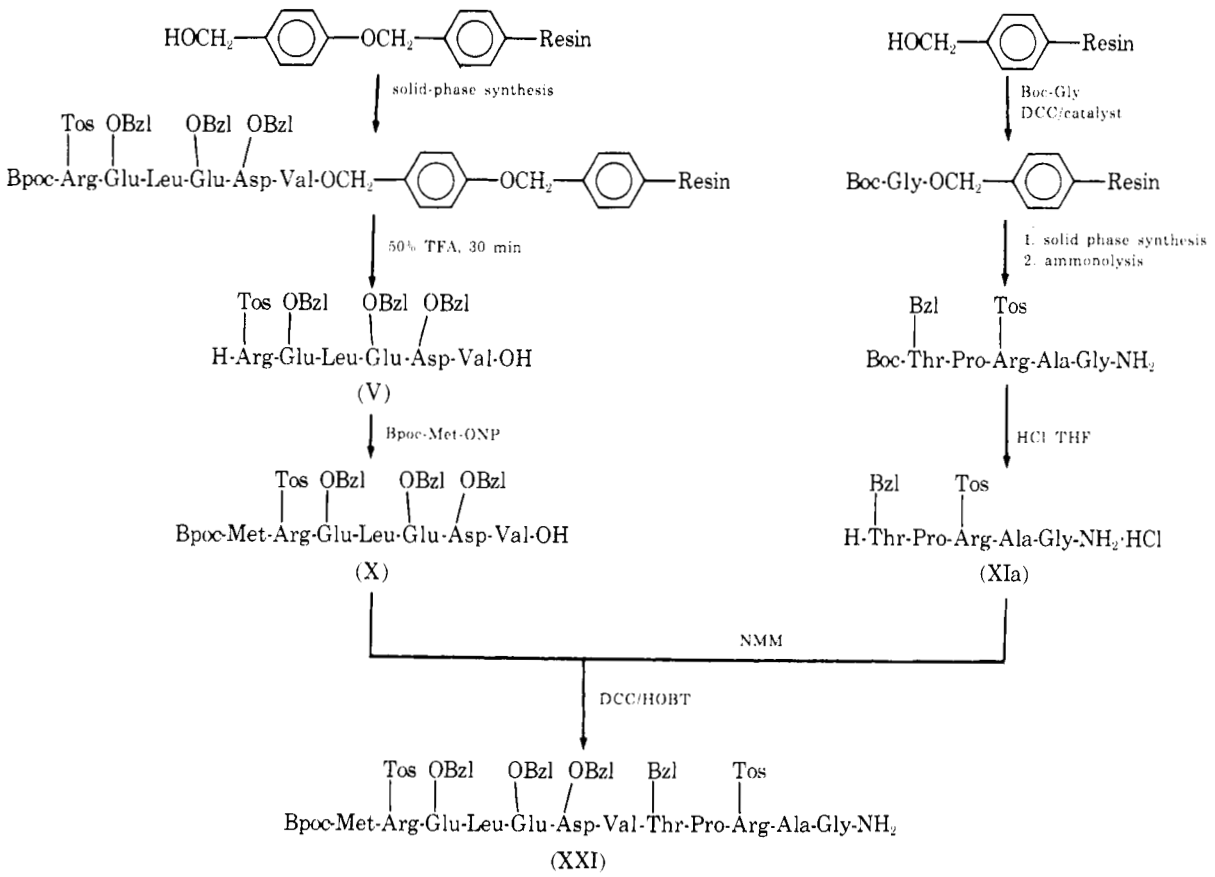
Scheme I
Synthesis of Boc-Arg(Tos)-Val-Tyr(Cl_2Bzl)-Glu(OBzl)-Lys(Z)-OH



Scheme II
Synthesis of Boc-Glu(OBzl)-Gly-Ile-Leu-Ala-Leu-OH



Scheme III
Example of Solid-Phase Fragment Approach



p(OBzl)-OH (XIX) was obtained. A similar derivative, Bpoc-Thr(Bzl)-Ser(Bzl)-Asp(OBzl)-OH, was prepared in an analogous manner from Bpoc-Thr(Bzl)-OSu and the dipeptide salt H-Ser(Bzl)-Asp(OBzl)-OH · HCl. The HCl salt of H-Thr(Bzl)-Ser(Bzl)-Asp(OBzl)-OH (XX) was obtained by treatment of either Boc-Thr(Bzl)-Ser(Bzl)-Asp(OBzl)-OH or Bpoc-Thr(Bzl)-Ser(Bzl)-Asp(OBzl)-OH with HCl in THF.

Condensation of Bpoc-Met-Arg(Tos)-Glu(OBZL(-Leu-Glu(OBzl)-Asp(OBzl)-Val-OH (X) with the HCl salt of H-Thr(Bzl)-Pro-Arg(Tos)-Ala-Gly-NH_2 (XIa) by the HOBT-assisted DCC method[40] resulted in the formation of the crystalline protected dodecapeptide Bpoc-Met-Arg(Tos)-Glu(OBzl)-Leu-Glu(OBzl)-Asp(OBzl)-Val-Thr(Bzl)-Pro-Arg(Tos)-Ala-Gly-NH_2 (XXI) in 59% yield. The α-amino protecting group was subsequently removed with 0.5 *N* HCl in tetrahydrofuran to give the crystalline hydrochloride salt HCl · H-Met-Arg(Tos)-Glu(OBzl)-Leu-Glu(OBzl)-Asp(OBzl)-Val-Thr(Bzl)-Pro-Arg(Tos)-Ala-Gly-NH_2 (XXII).

Several preparations described here represent further examples of combined use of solid phase and classical methods in peptide synthesis[29,46-49] (cf. Scheme III).

Experimental Section

Melting points are uncorrected. Amino acids analyses were performed on a Beckman Model 121 analyzer according to the procedure of Spackman et al.[43] Thin layer chromatography was carried out on precoated silica gel plates (Merck, F-254) using solvent systems described before.[27,37] Elementary analyses, optical rotation data, ir, uv, and NMR spectra were provided by the Physical Chemistry Department. Bpoc-amino acids were prepared as described in the literature[44,45] and were of L configuration. Other amino acid derivatives were purchased from Bachem Inc., Marina Del Rey, Calif., or from Chemical Dynamics Corp., South Plainfield, N.J. *p*-Alkoxybenzyl alcohol resin was prepared as reported previously.[29] Bpoc-amino acid CHA or DCHA salts were converted into free acids prior to use.[45] For hydrogenolysis, 5% Pd on $BaSO_4$ was used as catalyst.

Bpoc-Met-ONp. A suspension of Bpoc-Met-OH · DCHA (11.11 g, 19.6 mmol) in a mixture of water and ether at 0° was treated with 1 *M* citric acid until the solid dissolved. The ether layer was washed with water, dried (Na_2SO_4), and evaporated at 30° to yield Bpoc-Met-OH as an oil which was treated with 3.3 g of *p*-nitrophenol (23.5 mmol) and 4.85 g of DCC (23.5 mmol) in 200 ml of THF (0°, 2 hr; 25°, 2 hr). The insoluble by-product was filtered off and the filtrate evaporated to a syrup which was crystallized from *i*-PrOH by the addition of a small amount of petroleum ether: yield 6.8 g (68%); mp 90–92°; $[\alpha]^{25}D$ −71.1° (*c* 1, MeOH).

Anal. Calcd for $C_{27}H_{28}N_2O_6S$ (508.59): C, 63.76; H, 5.55; N, 5.51. Found: C, 63.60; H, 5.48; N, 5.44.

Bpoc-Thr(Bzl)-OSu. Bpoc-Thr(Bzl)-OH (1 g, 2.2 mmol) was stirred in 5 ml of THF with HOSu (0.27 g, 2.33 mmol) and DCC (0.49 g, 2.37 mmol). Work-up as above gave an oily material which was precipitated from ethyl acetate with petroleum ether: yield 1.19 g (97%); homogeneous on TLC; NMR spectral data agreed with expected values.

Anal. Calcd for $C_{31}H_{32}N_2O_7$ (544.59): C, 68.37; H, 5.92; N, 5.19. Found: C, 68.41; H, 6.01; N, 5.21.

Boc-Glu(OBzl)-OSu. Boc-Glu(OBzl)-OH (9.0 g, 26.7 mmol) in 200 ml of THF was treated with HOSu (3.25 g, 28.2 mmol) and DCC (5.95 g, 28.9 mmol) (0°, 1 hr; 25°, 3 hr). It was filtered and evaporated to a semisolid mass which was crystallized from *i*-PrOH: yield 9.5 g (81%); mp 103–104°; $[\alpha]^{25}D$ +23.67° (*c* 1, THF).

Anal. Calcd for $C_{21}H_{26}N_2O_8$ (434.44): C, 58.06; H, 6.03; N, 6.45. Found: C, 58.20; H, 6.15; N, 6.40.

Z-Val-Tyr(Bzl)-OCH$_3$. H-Tyr-OCH_3 · HCl (4.43 g, 13.8 mmol) in 100 ml of CH_2Cl_2 was treated with TEA (1.94 ml, 13.8 mmol), Z-Val-OH (3.48 g, 13.8 mmol), and DCC (3.27 g, 15.9 mmol) (0°, 1 hr; 25°, 3 hr). It was filtered and washed with water, dried (Na_2SO_4), and evaporated to a solid mass, crystallized from EtOH: yield 6.5 g (91%); mp 163–165°.

Anal. Calcd for $C_{30}H_{34}N_2O_6$ (518.58): C, 69.48; H, 6.61; N, 5.40. Found: C, 69.48; H, 6.58; N, 5.43.

H-Val-Tyr-OCH$_3$ · HCl. The above compound (5.45 g, 10.6 mmol) in a mixture of THF (100 ml), MeOH (80 ml), and water (20 ml) was hydrogenated at 46 psi overnight in a Parr hydrogenator in the presence of 1.76 ml of concentrated HCl and 2.5 g of catalyst. It was filtered and evaporated at 35° to give an oil which was crystallized from methanol–ether: yield 3.1 g (81%); mp 121–124°; $[\alpha]^{25}D$ +30.53° (*c* 1, MeOH).

Anal. Calcd for $C_{15}H_{22}N_2O_4 \cdot HCl \cdot CH_3OH$ (362.85): C, 52.96; H, 7.50; N, 7.72; Cl, 9.77. Found: C, 52.69; H, 7.34; N, 7.68; Cl, 9.71.

Aoc-Arg(Tos)-Val-Tyr-OCH$_3$. The HCl salt of H-Val-Tyr-OCH_3 (1.0 g, 2.76 mmol) in 30 ml of CH_2Cl_2 was stirred with 0.39 ml of TEA, 1.02 g of Aoc-Arg(Tos)-OH (2.76 mmol), and 0.66 g of DCC (3.2 mmol) (0°, 1 hr; 25°, 3 hr). The reaction mixture was filtered and diluted with 100 ml of ethyl acetate, washed (H_2O), dried (Na_2SO_4), and evaporated to an oil which on treatment with ether solidified immediately. It was reprecipitated from ethyl acetate with ether: yield 0.92 g (46.5%); homogeneous on tlc; NMR spectral data agreed with the structure; $[\alpha]^{25}D$ −1.66° (*c* 5, MeOH).

Anal. Calcd for $C_{34}H_{52}N_6O_9S$ (718.66): C, 56.81; H, 7.01; N, 11.69; S, 4.46. Found: C, 56.22; H, 6.99; N, 11.46; S, 4.51.

Z-Ile-Leu-OH (XV). Leucine (3.64 g, 27.6 mmol) was dissolved in 13 ml of Triton B (40% methanolic solution of trimethylbenzylammonium hydroxide), evaporated to dryness at 35°, twice re-evaporated each with 20-ml portions of DMF, and then treated with 10 g of Z-Leu-OSu (27.6 mmol) in 50 ml of DMF (0°, 2 hr; 25°, 24 hr). The reaction mixture was partitioned between ether and 2% citric acid (500 ml each) and the organic layer was washed several times with water, dried (Na_2SO_4), and evaporated to a solid mass. It was crystallized from ethyl acetate and petroleum ether: yield 7.86 g (75.5%); mp 139–140°; $[\alpha]^{25}D$ −28.76° (*c* 1, MeOH).

Anal. Calcd for $C_{20}H_{30}N_2O_5$ (378.47): C, 63.47; H, 7.99; N, 7.40. Found: C, 63.48; H, 7.90; N, 7.37.

H-Ile-Leu-OH · HCl. The above compound (5.7 g, 15.1 mmol) was hydrogenated at 50 psi in a mixture of MeOH (100 ml), H_2O (33 ml), and 1 *N* HCl (16.6 ml) overnight in the presence of 1.5 g of catalyst. It was filtered and evaporated to an oil, and crystallized from EtOH and ether: 3.79 g (89%); mp 222–224°.

Anal. Calcd for $C_{12}H_{24}N_2O_3 \cdot HCl$ (280.79): C, 51.33; H, 8.95; N, 9.99. Found: C, 51.07; H, 8.82; N, 9.89.

Boc-Gly-Ile-Leu-OH (XVI). Boc-Gly-OSu (3.6 g, 13.2 mmol)[36] in DMF (50 ml) was stirred with 3.79 g of H-Ile-Leu-OH · HCl (13.2 mmol) and 1.91 ml of TEA (0°, 2 hr; 25°, 17 hr). A few drops of TEA were added occasionally during this time in order to maintain the reaction slightly basic. The mixture was diluted with 650 ml of ethyl acetate, washed with 460 ml of 0.1 *N* HCl followed by water (three times), dried (Na_2SO_4), and evaporated to a small volume. Upon addition of petroleum ether, crystallization began. The crude product was recrystallized from ethyl acetate and petroleum ether: yield 4.82 g (91%); mp 144–146°; $[\alpha]^{25}D$ −30.92° (*c* 1, MeOH).

Anal. Calcd for $C_{19}H_{35}N_3O_6$ (401.49): C, 56.84; H, 8.79; N, 10.47. Found: C, 57.02; H, 9.03; N, 10.28.

Z-Ala-Leu-OBu-*t* (XVII). H-Leu-OBu-*t* · HCl (5 g, 22.4 mmol) in 100 ml of CH_2Cl_2 was stirred with 5 g of Z-Ala-OH (22.4 mmol), 3.16 ml of TEA, and 5.36 g of DCC (26 mmol) (0°, 1 hr; 25°, 2 hr). It was filtered, washed (H_2O), dried (Na_2SO_4), and evaporated to give an oil, which was crystallized from ether and petroleum ether: yield 6.9 g (78%); mp 97–98°.

Anal. Calcd for $C_{21}H_{32}N_2O_5$ (392.49): C, 64.26; H, 8.22; N, 7.14. Found: C, 64.54; H, 8.06; N, 7.17.

Boc-Gly-Ile-Leu-Ala-Leu-OBu-*t* (XVIII). Z-Ala-Leu-OBu-*t* (4.9 g, 12.5 mmol) was hydrogenated in a mixture of MeOH (75 ml), H_2O (25 ml), and 1 *N* HCl (12.5 ml) overnight at 50 psi in the presence of 2 g of catalyst and worked up as usual to give 3.53 g of amorphous H-Ala-Leu-OBu-*t* · HCl. This was treated with Boc-Gly-Ile-Leu-OH (4.82 g, 12 mmol), 1.5 ml of NMM, HOBT (3.24 g, 22 mmol), and DCC (2.9 g, 14 mmol) in 100 ml of DMF (−10°, 4 hr; 25°, 72 hr). It was filtered and evaporated to a syrup which on treatment with ethyl acetate solidified immediately. It was taken up in CH_2Cl_2, washed (H_2O), dried (Na_2SO_4), evaporated to an oil, and crystallized from ethyl acetate: yield 5.45 g (72%); mp 227–228°; $[\alpha]^{25}D$ −64.94° (*c* 1, MeOH).

Anal. Calcd for $C_{32}H_{59}N_5O_8$ (641.85): C, 59.88; H, 9.27; N. 10.91. Found: C, 59.75; H, 9.46; N, 11.01.

H-Ser(Bzl)-Asp(OBzl)-OH · HCl. H-Asp(OBzl)-OH (15 g, 67.2 mmol) in 500 ml of DMF was stirred with 9.45 ml of TEA (67.2 mmol) and Boc-Ser(Bzl)-OSu (26.3 g, 67.2 mmol) in an analogous manner as in the preparation of XVI and worked up as usual to give an oil which resisted attempts at crystallization. It was thus treated with 400 ml of freshly prepared 3.1 *N* HCl in THF for 30 min to remove the Boc group. Evaporation at 35° gave an oil which was crystallized from *i*-PrOH and ether: yield 19.0 g (65%); mp 160–162°; $[\alpha]^{25}D$ +12.73° (*c* 1, MeOH).

Anal. Calcd for $C_{21}H_{24}N_2O_6 \cdot HCl$ (436.89): C, 57.73; H, 5.77; N, 6.41; Cl, 8.11. Found: C, 57.56; H, 5.60; N, 6.38; Cl, 8.26.

Boc-Thr(Bzl)-Ser(Bzl)-Asp(OBzl)-OH (XIX). Part of the above compound (13.5 g, 30.9 mmol) was stirred in 250 ml of DMF with 8.7 ml of TEA (61.8 mmol) and Boc-Thr(Bzl)-OSu (12.6 g, 30.9 mmol) (0°, 2 hr; 25°, 24 hr) and treated in an analogous manner as in the preparation of XVI. The product started to crystallize

when the ethyl acetate solution was concentrated to a small volume. It was recrystallized from ethyl acetate: yield 11.1 g (52%); mp 113–116°; $[\alpha]^{25}D$ +6.90° (*c* 1, DMSO); NMR spectral data agreed with expected values.

Anal. Calcd for $C_{37}H_{45}N_3O_{10}$ (691.75): C, 64.24; H, 6.56; N, 6.07. Found: C, 64.39; H, 6.68; N, 6.37.

H-Thr(Bzl)-Ser(Bzl)-Asp(OBzl)-OH · HCl (XX). H-Ser(Bzl)-Asp(OBzl)-OH (0.1 g, 0.23 mmol) in 3 ml of DMF was treated with Bpoc-Thr(Bzl)-OSu (0.125 g, 0.23 mmol) and 65 mg of TEA (0° 90 min, 25° 24 h). The rction mixture was poured into a small volume of water and acidified to pH 3 with 1 *M* citric acid. The product was extracted into ethyl acetate and washed with H_2O, dried (Na_2SO_4), and evaporated to dryness. The oily residue was dissolved in 7 ml of freshly prepared 0.3 *N* HCl in THF and evaporated to dryness after 10 min standing. The compound was crystallized from THF and ether: yield 0.075 g (52%); mp 160–162°; $[\alpha]^{25}D$ +9.85° (*c* 0.5, MeOH).

Anal. Calcd for $C_{32}H_{37}N_3O_8 \cdot HCl$ (628.11): C, 61.19; H, 6.10; N, 6.69; Cl, 5.64. Found: C, 61.37; H, 6.20; N, 6.80; Cl, 5.63.

The same compound was also prepared from Boc-Thr(Bzl)-Ser(Bzl)-Asp(OBzl)-OH (XIX) by a 30-min treatment with 3 *N* HCl in THF and similar work-up: yield 71%; mp 160–162°.

Boc-Glu(OBzl)-Lys(Z)-OH (XIII). Boc-Glu(OBzl)-OSu (12.98 g, 30 mmol) and H-Lys(Z)-OH (8.42 g, 29.8 mmol) in DMF (260 ml) were allowed to react in the presence of 4.2 ml of TEA (30 mmol) in a manner similar to that for the preparation of XVI and worked up. The oily product crystallized on addition of ether. It was recrystallized from ether: yield 14.9 g (83%); mp 115–118°; $[\alpha]^{25}D$ −2.77° (*c* 1, MeOH).

Anal. Calcd for $C_{31}H_{41}N_3O_9$ (599.66): C, 62.09; H, 6.89; N, 7.01 Found: C, 62.06; H, 7.16; N, 7.02.

Treatment of this compound with 100 ml of 4.7 *N* HCl in THF for 60 min and work-up as usual gave an amorphous H-Glu(OBzl)-Lys(Z)-OH · HCl (13.4 g), homogeneous on TLC. Without further purification, part of the product was used as described below.

Boc-Arg(Tos)-Val-Tyr(Cl_2Bzl)-HNNH$_2$ (XII). Boc-Tyr-(Cl_2Bzl)-O$CH_2C_6H_4$-Resin[37] (13.9 g, 8.64 mmol) was deprotected (50% TFA, 30 min) and neutralized (10% TEA, 10 min) and the standard solid-phase synthesis[17,18] conducted by sequential incorporation of Boc-Val-OH (4.7 g, 21.6 mmol) and Boc-Arg(Tos)-OH (7.6 g, 21.6 mmol) into the resin to give Boc-Arg(Tos)-Val-Tyr(Cl_2Bzl)-O$CH_2C_6H_4$-Resin (18.9 g). It was hyrazinolyzed in 700 ml of MeOH containing 70 ml H_2NNH_2 for 72 hr (25°) and worked up to produce an oil which was crystallized from *i*-PrOH: yield 4.66 g (62.5%); mp 189–191°; $[\alpha]^{25}D$ −6.19° (*c* 0.5, DMSO).

Anal. Calcd for $C_{39}H_{52}N_8O_8SCl_2$ (863.86): C, 54.23; H, 6.07; N, 12.97; S, 3.71; Cl, 8.21. Found: C, 53.87; H, 6.50; N, 12.92; S, 3.70; Cl, 8.00.

Boc-Arg(Tos)-Val-Tyr(Cl_2Bzl)-Glu(OBzl)-Lys(Z)-OH (XIV). Boc-Arg(Tos)-Val-Tyr(Cl_2Bzl)-HNNH$_2$ (5.63 g, 6.5 mmol) was dissolved in 65 ml of DMF, cooled to −20°, and treated with 10.6 ml of 3.68 *N* HCl in THF followed by 12.5 ml of 10% isoamyl nitrite in DMF. After stirring for 30 min at −20° the temperature was lowered to −30°, when 6.27 ml of TEA was added followed by 3.75 g of the HCl salt of H-Glu(OBzl)-Lys(Z)-OH (7.0 mmol). The mixture was stirred at −20° for 30 min and then at 0° for 48 hr. Small amounts of TEA were added periodically in order to maintain the reaction medium slightly basic. The insoluble by-products were filtered off and the filtrate evaporated to 40° to an oil. The material was taken up in 150 ml of ethyl acetate, washed (3% acetic acid and then water), and evaporated several times with benzene to give a solid mass, recrystallized from *i*-PrOH: yield 4.74 g (53%); mp 138–141°; $[\alpha]^{25}D$ −7.49° (*c* 1, DMF); NMR spectral data agreed with the expected values.

Anal. Calcd for $C_{65}H_{81}N_9O_{15}Cl_2S$ (1331.35): C, 58.64; H, 6.13; N, 9.47; Cl, 5.32. Found: C, 58.31; H, 6.20; N, 9.49; Cl, 5.42.

Amino Acid Anal. Glu, 1.00; Val, 0.95; Tyr, 0.89; Lys, 1.07; Arg, 1.10.

H-Gly-Ile-Leu-Ala-Leu-OH (IV). Boc-Gly-Ile-Leu-Ala-Leu-OBu-*t* (XVIII) (5.45 g, 8.5 mmol) was stirred in 140 ml of TFA for 150 min. The product was precipitated with ether and dried over NaOH in vacuo to give 4.24 g of white powder. The solid was triturated in hot MeOH: yield, 4.1 g (89%); mp > 300°; homogeneous on TLC; NMR spectral data agreed with expected values.

Anal. Calcd for $C_{23}H_{43}N_5O_6 \cdot \frac{1}{2}CF_3COOH$ (542.62): C, 53.29; H, 8.08; N, 12.90; F, 5.27. Found: C, 53.19; H, 8.10; N, 12.94; F, 5.12.

The same peptide was also prepared by the solid-phase method. *p*-Alkoxybenzyl alcohol resin[29] (10 g) was stirred in 100 ml of CH_2Cl_2 with pyridine (2.18 ml), Bpoc-Leu-OH (9.75 g, 26.5 mmol), and DCC (5.56 g, 26.9 mmol) for 2 hr. The protected aminoacyl resin thus obtained (10.25 g, 0.41% N, 0.29 mmol/g) was treated with benzoyl chloride,[29] giving rise to 10.3 g of Bpoc-Leu-OCH_2-$C_6H_4OCH_2C_6H_4$-Resin. Solid-phase synthesis was then conducted with Bpoc-Ala-OH (3.04 g), Bpoc-Leu-OH (3.42 g), Bpoc-Ile-OH (3.42 g), and Boc-Gly-OH (1.62 g) using DCC (2.01 g) as coupling agent in each cycle.[50,51] The protected pentapeptide resin was then stirred with 300 ml of 50% TFA in CH_2Cl_2 for 30 min. The liberated pentapeptide was separated from the resin by filtration and concentrated to a syrup, again evaporated several times with fresh CH_2Cl_2, and treated with ether. The solid obtained was washed with CH_3CN, H_2O, and DMF: yield 0.44 g (30.2%); mp > 320°; homogeneous on TLC; NMR spectrum agreed with the structure.

Anal. Calcd for $C_{23}H_{43}N_5O_6 \cdot H_2O$ (503.63): C, 54.95; H, 9.01; N, 13.98. Found: C, 54.80; H, 8.89; N, 13.75.

Amino Acid Anal. Gly, 0.95; Ala, 1.01; Ile, 0.99; Leu, 2.03.

Boc-Glu(OBzl)-Gly-Ile-Leu-Ala-Leu-OH (IX). H-Gly-Ile-Leu-Ala-Leu-OH (3.9 g, 7.18 mmol) suspended in 130 ml of DMF was allowed to react with Boc-Glu(OBzl)-OSu (4.7 g, 10.7 mmol) in the presence of 2.5 ml of TEA (17.8 mmol) for 24 hr. The reaction mixture was then diluted with 1500 ml of ethyl acetate and shaken with 1200 ml of 3% citric acid. The product in the organic layer was washed with water, during which time crystallization began. The suspension was mixed with 20 ml of DMF to dissolve the solid and evaporated to a syrup which on evaporation with fresh DMF gave a solid mass. It was crystallized from DMF (80 ml) with ether (240 ml): yield 4.55 g (78.7%); mp 182–184°; $[\alpha]^{25}D$ −28.59° (*c* 1, DMF); NMR spectral data agreed with the structure.

Anal. Calcd for $C_{40}H_{64}N_6O_{11}$ (804.96): C, 59.68; H, 8.01; N, 10.44. Found: C, 59.56; H, 8.24; N, 10.42.

Amino Acid Anal. Glu, 1.03; Gly, 1.00; Ala, 0.99; Ile, 0.98; Leu, 2.06.

Z-Val-Phe-Thr(Bzl)-OH (I). *p*-Alkoxybenzyl alcohol resin (10 g, 18.5 mmol) was stirred in 100 ml of CH_2Cl_2 with 3.3 g of 4-dimethylaminopyridine (26.9 mmol), Bpoc-Thr(Bzl)-OH (11.86 g, 26.5 mmol), and DCC (5.56 g, 26.9 mmol) for 2 hr. The esterified resin was treated with benzoyl chloride (3.75 ml) and pyridine (3.2 ml)[29] to give Bpoc-Thr(Bzl)-O$CH_2C_6H_4OCH_2C_6H_4$-Resin (11.35 g, 0.55% N, 0.39 mmol/g). Ten grams of this material (3.9 mmol) was used for the solid-phase synthesis with Bpoc-Phe-OH (4.24 g, 11.9 mmol) and Z-Val-OH (2.96 g, 11.9 mmol) successively coupled to the amino group on the resin under the conditions described previously.[50,51] The tripeptide resin was then stirred in 300 ml of 50% TFA in CH_2Cl_2 for 30 min and worked up as usual to give a gel which on treatment with benzene began to crystallize (1.9 g, mp 211–213°). It was recrystallized from ethyl acetate with petroleum ether: yield 1.75 g (75.6%); mp 209–211°; $[\alpha]^{25}D$ −3.50° (*c* 0.6, MeOH); NMR spectral data agreed with the expected values.

Anal. Calcd for $C_{33}H_{39}N_3O_7$ (589.69): C, 67.22; H, 6.67; N, 7.13. Found: C, 66.63; H, 6.73; N, 7.13.

Amino Acid Anal. Thr, 1.04; Val, 0.96; Phe, 1.00.

H-Ser(Bzl)-Leu-Val-Phe-Gly-OH (II). Bpoc-Gly-OCH_2C_6-$H_4OCH_2C_6H_4$-Resin (10 g, 4.0 mmol) was used for solid-phase synthesis in which 12 mmol each of Bpoc-Phe-OH (4.84 g), Bpoc-Val-OH (4.26 g), Bpoc-Leu-OH (4.44 g), and Bpoc-Ser(Bzl)-OH · CHA (6.4 g) were successively incorporated into the growing peptide chain on the resin[50,51] to produce the pentapeptide resin (13.8 g). Cleavage with 50% TFA in CH_2Cl_2 (30 min) was followed by the usual work-up. A white solid (1.75 g) obtained was crystallized from a small volume of MeOH: yield 0.81 g (33%); mp 247° dec; $[\alpha]^{25}D$ −29.20° (*c* 0.7, HOAc); NMR spectral data agreed with the structure.

Anal. Calcd for $C_{32}H_{45}N_5O_7$ (611.74): C, 62.83; H, 7.41; N, 11.45. Found: C, 62.76; H, 7.58; N, 11.34.

Amino Acid Anal. Ser, 0.99; Gly, 1.03; Leu, 1.01; Val, 0.91; Phe, 1.07.

Boc-Asn-Ser(Bzl)-Leu-Val-Phe-Gly-OH (VII). Boc-Asn-OSu (0.83 g, 2.5 mmol) was treated with H-Ser(Bzl)-Leu-Val-Phe-Gly-OH (1.02 g, 1.66 mmol) in 85 ml of DMF which contained 0.35 ml of TEA under the conditions analogous to that for preparation of XVI for 24 hr. The reaction was evaporated to about ⅓ of the volume and mixed with 100 ml of 5% citric acid and the solid formed was washed with water and ethyl acetate to give a white powder. It was dissolved in 100 ml DMF, evaporated to dryness, and precipitated from a small volume of DMF with ethyl acetate: amorphous solid; yield 1.21 g (88%); $[\alpha]^{25}D$ −31.92° (*c* 1, DMF); homogeneous on TLC; NMR data agreed with expected values.

Anal. Calcd for $C_{41}H_{59}N_7O_{11}$ (825.96): C, 59.62; H, 7.20; N, 11.87. Found: C, 59.32; H, 7.28; N, 11.78.

Amino Acid Anal. Asp, 1.00; Ser, 0.92; Gly, 0.93; Leu, 1.06; Val, 1.03; Phe, 1.00.

H-Lys(Z)-Asp(OBzl)-Leu-Glu(OBzl)-OH (III). Solid-phase synthesis was conducted[51] with Bpoc-Glu(OBzl)-$OCH_2C_6H_4$-$OCH_2C_6H_4$-Resin (10 g, 3.15 mmol) using 9.45 mmol each of Bpoc-Leu-OH (3.49 g), Bpoc-Asp(OBzl)-OH (4.36 g), and Bpoc-Lys(Z)-OH (4.9 g) sequentially incorporated into the growing peptide chain to give tetrapeptide resin (13.9 g). Treatment with 50% TFA in CH_2Cl_2 (300 ml) for 30 min and work-up yielded a solid mass (2.83 g, mp 183–186°) which on recrystallization from EtOH afforded soft needles: yield 1.89 g (69.3%): mp 187–188°; $[\alpha]^{25}D$ −12.20° (*c* 0.8, DMF); NMR spectrum agreed with the structure.

Anal. Calcd for $C_{43}H_{55}N_5O_{11} \cdot C_2H_5OH$ (863.98): C, 62.56; H, 7.08; N, 8.12. Found: C, 62.50; H, 6.72; N, 8.36.

Amino Acid Anal. Asp, 0.97; Glu, 1.01; Leu, 1.04; Lys, 0.96.

Boc-Leu-Lys(Z)-Asp(OBzl)-Leu-Glu(OBzl)-OH (VIII). Boc-Leu-OSu (0.99 g, 3.02 mmol) in 100 ml of DMF was stirred with H-Lys(Z)-Asp(OBzl)-Leu-Glu(OBzl)-OH (1.74 g, 2.01 mmol) and 0.5 ml of TEA for 24 hr. The solvent was evaporated off and the residue was taken up in a mixture of ethyl acetate and 5% citric acid. The organic layer was washed (H_2O), dried, and concentrated to a small volume when crystallization began (2.0 g, mp 152–155°). It was recrystallized from ethyl acetate: yield 1.69 g (82%); mp 155–157°; $[\alpha]^{25}D$ −25.50° (*c* 1, DMF); NMR spectrum agreed with the structure.

Anal. Calcd for $C_{54}H_{74}N_6O_{14}$ (1031.18): C, 62.90; H, 7.23; N, 8.15. Found: C, 62.77; H, 7.14; N, 8.07.

Amino Acid Anal. Asp, 0.97; Glu, 1.00; Leu, 1.92; Lys, 1.05.

H-Arg(Tos)-Glu(OBzl)-Leu-Glu(OBzl)-Asp(OBzl)-Val-OH (V). Solid-phase synthesis was carried out[51] with Bpoc-Val-$OCH_2C_6H_4OCH_2C_6H_4$-Resin (9.08 g, 3.54 mmol) by successive incorporation of 11.1 mmol each of Bpoc-Asp(OBzl)-OH (4.9 g), Bpoc-Glu(OBzl)-OH · CHA (6.1 g), Bpoc-Leu-OH (3.9 g), Bpoc-Glu(OBzl)-OH · CHA (6.1 g), and Bpoc-Arg(Tos)-OH (6.0 g) into the resin. The protected hexapeptide resin (12.7 g) was treated with 300 ml of 50% TFA in CH_2Cl_2 for 30 min. Work-up as usual produced an oil which became a white powder when treated with ethyl acetate. It was crystallized from DMF and ethyl acetate: yield 3.8 g (90.5%); mp 207–209°; $[\alpha]^{25}D$ −11.84° (*c* 0.5, HOAc); NMR spectral data agreed with the expected values.

Anal. Calcd for $C_{59}H_{77}N_9O_{15}S \cdot H_2O$ (1202.39): C, 58.89; H, 6.63; N, 10.51. Found: C, 58.59; H, 6.55; N, 10.42.

Amino Acid Anal. Asp, 0.95; Glu, 2.00; Leu, 1.08; Val, 0.91; Arg, 1.06.

Bpoc-Met-Arg(Tos)-Glu(OBzl)-Leu-Glu(OBzl)-Asp-(OBzl)-Val-OH (X). Bpoc-Met-ONp (0.84 g, 1.65 mmol) in DMF (80 ml) that had been deaerated by purging with argon gas for 45 min was allowed to react with H-Arg(Tos)-Glu(OBzl)-Leu-Glu(OBzl)-Asp(OBzl)-Val-OH (1.32 g, 1.1 mmol) and TEA (0.25 ml) (0°, 1 hr; 25°, 24 hr) under argon. A few more drops of TEA were added occasionally to maintain the reaction slightly basic. The reaction mixture was neutralized, evaporated to dryness at 35°, and triturated with ethyl acetate. The solid powder (1.3 g, mp 196–199°) was dissolved in DMF and evaporated to near dryness, when *i*-PrOH was added. A crystalline product appeared when stored in the refrigerator overnight: yield 1.05 g (62%); mp 196–199°; $[\alpha]^{25}D$ −18.85° (*c* 1, MEOH); NMR spectral data agreed with the expected values.

Anal. Calcd for $C_{80}H_{100}N_{10}O_{18}S_2$ (1553.86): C, 61.84; H, 6.49; N, 9.01. Found: C, 61.30; H, 6.51; N, 9.01.

Amino Acid Anal. Asp, 1.00; Glu, 2.06; Val, 0.92; Met, 0.89; Leu, 1.01; Arg, 0.97.

H-Thr(Bzl)-Pro-Arg(Tos)-Ala-Gly-OH (VI). Bpoc-Gly-$OCH_2C_6H_4OCH_2C_6H_4$-Resin (10 g, 4.0 mmol) was used for solid-phase synthesis in which 12 mmol each of Bpoc-Ala-OH (3.93 g), Bpoc-Arg(Tos)-OH (6.8 g), Bpoc-Pro-OH (4.25 g), and Bpoc-Thr(Bzl)-OH (5.37 g) were successively incorporated into the growing peptide chain on the resin under the conditions detailed before[50,51] to produce 14.1 g of the protected pentapeptide resin. The peptide was released from the solid support by a 30-min treatment with 300 ml of 50% TFA in CH_2Cl_2. Evaporation of the solvent left a syrup which on treatment with ether solidified immediately. The product was taken up in *i*-PrOH and precipitated with ether, yielding 3.15 g of an amorphous solid. Reprecipitation from hot CH_3CN gave 1.45 g of material melting at 150–151°. TLC revealed the presence of two minor impurities. Chromatography on a Sephadex LH-20 column (2.5 × 85 cm) using *i*-PrOH–H_2O (7:3) as an eluent eliminated the contaminants: yield 0.94 g (30%); NMR spectrum agreed with the structure.

Anal. Calcd for $C_{34}H_{48}N_8O_9S \cdot 2H_2O$ (780.92): C, 52.21; H, 6.71; N, 14.33. Found: C, 51.99; H, 6.54; N, 13.95.

Amino Acid Anal. Thr, 0.93; Pro, 1.02; Gly, 0.96; Ala, 0.96; Arg, 1.11.

H-Thr(Bzl)-Pro-Arg(Tos)-Ala-Gly-NH_2 · HCl (XIa). Boc-Gly-$OCH_2C_6H_4$-Resin[37] (9.23 g, 5.55 mmol) was used in a synthesis following the standard Merrifield technique[52] with 13.8 mmol each of Boc-Ala-OH (2.61 g), Boc-Arg(Tos)-OH (4.9 g), Boc-Pro-OH (2.98 g), and Boc-Thr(Bzl)-OH (4.27 g) sequentially coupled to the resin to afford 12.3 g of pentapeptide resin. Ammonolysis in 500 ml of MeOH saturated with dry NH_3 gas for 4 days (25°) and work-up as usual[50] gave a syrup which on treatment with ether solidified immediately. The product was taken up in ethyl acetate, washed with H_2O, 5% NH_3, 5% citric acid, and H_2O, dried (Na_2SO_4), and concentrated to a clear oil. It was dissolved in *i*-PrOH and precipitated as a colorless, amorphous solid (XI) by slow addition of ether: yield 2.8 g (60.3%); $[\alpha]^{25}D$ −36.95° (*c* 1, MeOH); NMR spectrum agreed with the structure. TLC indicated that the product was contaminated with a minor fast-moving component. Without further purification at this stage, the product was converted into the HCl salt as described below.

Anal. Calcd for $C_{39}H_{59}N_9O_{10}S \cdot H_2O$ (862.00): C, 54.38; H, 6.88; N, 14.66. Found: C, 54.72; H, 6.95; N, 14.65.

The above compound (XI, 4.43 g, 5.13 mmol) was treated with 3.4 *N* HCl in THF (130 ml) for 45 min. Evaporation of the solvent gave an oil which was reevaporated three times with fresh THF, leaving a glassy solid (4.03 g). The product was dissolved in hot *i*-PrOH and an amorphous solid accumulated on standing. Reprecipitation from the same solvent gave 2.2 g (55%) of the desired material: homogeneous on TLC; NMR spectrum agreed with the structure; $[\alpha]^{25}D$ −25.57° (*c* 1, MeOH).

Anal. Calcd for $C_{34}H_{49}N_9O_8S \cdot HCl \cdot H_2O$ (798.35): C, 51.15; H, 6.67; N, 15.78. Found: C, 51.28; H, 6.68; N, 15.56.

Bpoc-Met-Arg(Tos)-Glu(OBzl)-Leu-Glu(OBzl)-Asp-(OBzl)-Val-Thr(Bzl)-Pro-Arg(Tos)-Ala-Gly-NH_2 (XXI). Compound XIa (0.47 g, 0.6 mmol) was dissolved in 20 ml of DMF that had been flushed with argon gas for 45 min. The solution was cooled (0°) and treated with 0.07 ml of NMM (0.6 mmol), 0.93 g of compound X (0.6 mmol), 0.161 g of HOBT (1.19 mmol), and 0.15 g of DCC (0.72 mmol). A few more drops of NMN were added to render the reaction mixture slightly basic. The reaction mixture was stirred at 0° for 1 hr and then at 25° for 42 hr. A few drops of HOAc were added to neutralize the mixture. It was filtered and evaporated (38°) to leave a yellow oil which on trituration with MeOH gave a colorless solid (1.06 g, mp 198–201°). The product was crystallized from DMF and MeOH: yield 0.80 g (58.7%); mp 195–199°; $[\alpha]^{25}D$ −23.79° (*c* 1, DMF); NMR spectrum agreed with the structure.

Anal. Calcd for $C_{114}H_{147}N_{19}O_{25}S_3$ (2279.68): C, 60.06; H, 6.56; N, 11.57. Found: C, 59.33; H, 6.61; N, 11.51.

Amino Acid Anal. Asp, 1.02; Thr, 0.89; Glu, 2.00; Pro, 1.01; Gly, 0.99; Ala, 0.95; Val, 1.03; Met, 0.92; Leu, 0.98; Arg, 2.11.

H-Met-Arg(Tos)-Glu(OBzl)-Leu-Glu(OBzl)-Asp(OBzl)-Val-Thr(Bzl)-Pro-Arg-(Tos)-Ala-Gly-NH_2 · HCl (XXII). Compound XXI (0.75 g, 0.33 mmol) was dissolved in 6 ml of argon-treated DMF and mixed with 100 ml of freshly prepared 0.5 *N* HCl in THF (argon treated). A white, crystalline solid started to appear immediately. After standing at 25° for 10 min, the mixture was diluted with more THF (220 ml) and left at 4° overnight. The solid was collected and recrystallized from DMF and THF: yield 0.46 g (67%); mp 238–241°; $[\alpha]^{25}D$ −21.01° (*c* 1, DMF); NMR spectrum agreed with the structure.

Anal. Calcd for $C_{98}H_{133}N_{19}O_{23}S_3 \cdot HCl$ (2077.87): C, 56.65; H, 6.50; N, 12.81. Found: C, 56.02; H, 6.65; N, 12.66.

Amino Acid Anal. Asp, 1.06; Thr, 1.04; Glu, 2.10; Pro, 0.98; Gly, 0.99; Ala, 0.99; Val, 1.00; Met, 0.96; Leu, 0.92; Arg, 2.00; NH_3, 1.22.

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Registry No.—I, 54244-38-1; II, 54244-39-2; III, 54244-40-5; IV, 54244-41-6; V, 54244-42-7; VI, 54244-43-8; VII, 54244-44-9; VIII,

54244-45-0; IX, 54244-46-1; X, 54244-47-2; XI, 54325-73-4; XIa, 54325-74-5; XII, 54244-48-3; XIII, 54244-49-4; XIV, 54244-50-7; XV, 38972-95-1; XVI, 54244-51-8; XVII, 27167-65-3; XVIII, 54244-52-9; XIX, 54244-53-0; XX, 54244-54-1; XXI, 54325-75-6; XXII, 54325-76-7; Bpoc-Met-OH · DCHA, 18635-05-7; *p*-nitrophenol, 100-02-7; Bpoc-Met-ONp, 54244-55-2; Bpoc-Thr(Bzl)-OSu, 54244-56-3; Bpoc-Thr(Bzl)-OH, 47733-62-0; HOSu, 6066-82-6; Boc-Glu(OBzl)-OSu, 32886-40-1; Boc-Glu(OBzl)-OH, 13574-13-5; Z-Val-Tyr(Bzl)-OCH_3, 54325-77-8; H-Tyr-OCH_3 · HCl, 3417-91-2; Z-Val-OH, 1149-26-4; H-Val-Tyr-OCH_3 · HCl, 54244-57-4; Aoc-Arg(Tos)-Val-Tyr-OCH_3, 54325-78-9; H-Val-Tyr-OCH_3, 54244-58-5; Aoc-Arg(Tos)-OH, 54244-59-6; leucine, 61-90-5; Z-Leu-OSu, 3397-35-1; H-Ile-Leu-OH · HCl, 54244-60-9; Boc-Gly-OSu, 3392-07-2; H-Leu-OBu-*t* · HCl, 2748-02-9; Z-Ala-OH, 1142-20-7; H-Ala-Leu-OBu-*t* · HCl, 54244-61-0; Boc-Gly-Ile-Leu-OH, 54244-51-8; H-Asp(OBzl)-OH, 3479-47-8; Boc-Ser(Bzl)-OSu, 13650-73-2; Boc-Thr(Bzl)-OSu, 32886-43-4; H-Ser(Bzl)-Asp(OBzl)-OH · HCl, 54244-62-1; H-Ser(Bzl)-Asp(OBzl)-OH, 54244-63-2; H-Lys(Z)-OH, 1155-64-2; Boc-Tyr(Cl_2Bzl)-OCH_2-Ph, 54244-64-3; Boc-Val-OH, 13734-41-3; Boc-Arg(Tos)-OH, 13836-37-8; H-Glu(OBzl)-Lys(Z)-OH · HCl, 54244-65-4; Bpoc-Leu-OH, 18634-99-6; Bpoc-Ala-OH, 23631-89-2; Bpoc-Ile-OH, 47553-71-9; Boc-Gly-OH, 4530-20-5; Bpoc-Phe-OH, 40099-50-1; Bpoc-Gly-OCH_2-C_6H_4-$OCH_2C_6H_5$, 54244-66-5; Bpoc-Ser(Bzl)-OH · CHA, 25692-83-5; Boc-Asn-OSu, 42002-18-6; Bpoc-Glu(OBzl)-$OCH_2C_6H_4OCH_2C_6H_5$, 54244-67-6; Bpoc-Asp(OBzl)-OH, 25692-81-3; Bpoc-Lys(Z)-OH, 25692-93-7; Bpoc-Val-$OCH_2C_6H_4OCH_2C_6H_5$, 54244-68-7; Bpoc-Glu(OBzl)-OH · CHA, 25692-85-7; Bpoc-Arg(Tos)-OH, 47820-38-2; Bpoc-Pro-OH, 25775-07-9; Boc-Gly-OCH_2-Ph, 54244-69-8; Boc-Ala-OH, 15761-38-3; Boc-Pro-OH, 2812-46-6; Boc-Leu-OSu, 3392-09-4.

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(32) Abbreviations used: Aoc, *tert*-amyloxycarbonyl; Boc, *tert*-butyloxycarbonyl; Bpoc, 2-(*p*-biphenylyl)-2-propyloxycarbonyl; Bu-*t*, *tert*-butyl; Bzl, benzyl; Tos, *p*-toluenesulfonyl; Cl_2Bzl, 2,6-dichlorobenzyl; Z, benzyloxycarbonyl; TFA, trifluoroacetic acid; TEA, triethylamine; DCC, dicyclohexylcarbodiimide; HOBT, 1-hydroxybenzotriazole; DMF, dimethylformamide; THF, tetrahydrofuran; CHA, cyclohexylamine; DCHA, dicyclohexylamine; NMM, *N*-methylmorpholine; DMSO, dimethyl sulfoxide.
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