reactive guanidino carbinol or amino groups.

(10) Viomycin and capreomycin are commercially available as the sulfates. Tuberactinomycin A was isolated from a *Streptoverticillum* species (Lederle culture B0471). It is worth mentioning that hydrochloride or sulfate salts of these compounds give good ^{13}C NMR spectra whereas the carbonate salts give poorer quality spectra.

(11) G. L. Levy and G. L. Nelson, "^{13}C NMR for Organic Chemists", Wiley-Interscience, New York, N.Y., 1972, p 123.

(12) J. B. Stothers, "Carbon 13 NMR Spectroscopy", Academic Press, New York, N.Y., 1972, p 142.

(13) For comparison purposes the minimal inhibitory concentrations in μg/mL of I, II, and viomycin are given against two bacterial species in the agar dilution assay. *Mycobacterium smegmatis* ATCC 607: I, 2.5; II, 25; viomycin, 2.5. *Klebsiella pneumoniae:* I, 10; II, 100; viomycin, 25.

(14) (a) IRC-50 resin from Rohm and Haas Co., Philadelphia, Pa. (b) CM-Sephadex C-25 obtainable from Pharmacia Fine Chemicals, Inc., Piscataway, N.J. (c) Vydac column Mo. SS-2-500 A-107 from Spectra Physics, Santa Clara, Calif. (d) AG 50W-X4 from Bio-Rad Laboratories, Richmond, Calif. (e) Amberlite CG-50 is supplied by Mallinckrodt, St. Louis, Mo. (f) Cellulose CF11 from Whatman, Inc., Clifton, N.J.

Facile Synthesis of Amino Acid and Peptide Esters under Mild Conditions via Cesium Salts

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A facile procedure for the preparation of a wide variety of esters derived from protected amino acids and peptides under mild conditions is described. The carboxylic acid to be esterified is first titrated to pH 7 with cesium carbonate or cesium bicarbonate and the neutral salt obtained is then allowed to react with different alkyl halides to form the corresponding esters. The reaction is simple, easily scaled up, and proceeds without observable racemization. Many amino acid and peptide esters that might be difficult to prepare by other methods have been made by this method. The usefulness of this procedure is further demonstrated by the synthesis of methionine-enkephalin.

Many useful processes for esterification of carboxylic acids have been reported in the literature.[1-10] However, a great need still exists for a versatile and facile procedure to prepare esters under mild conditions. Such a procedure should be applicable to compounds that are sensitive to acidic, basic, or thermic conditions without depending on exotic expensive reagents. Recently, in an effort to reduce the number of side reactions encountered in solid-phase peptide synthesis,[11] a method for total esterification of Merrifield resins (chloromethylated copolystyrene–1% divinylbenzene) with the cesium salts of Boc-amino acids[12] was investigated.[13] The reaction was found to proceed rapidly and quantitatively under mild conditions. The process has since been satisfactorily utilized to prepare β-phenacyl aspartate[14] and Boc-valyl-4-(oxymethyl)phenylacetic acid.[15] Preparation of other polymer-bound benzyl esters[13,16] and polymer-bound α-methylphenacyl esters has also been described.[17] In the following, we describe the application of this principle to the synthesis of protected amino acid and protected peptide esters in solution.

Reaction of suitably protected amino acid or peptide cesium salts with alkyl halides yielded the desired esters readily under neutral conditions at room temperature. No racemization was observed during this process. The reactions were easily carried out and the yields were generally very high.

In Table I, α-carboxylic esters of amino acids and peptides prepared in this study are listed. The general procedure is as follows. The carboxylic acid to be esterified is first converted into its cesium salt by titration to neutrality with aqueous $CsHCO_3$ or Cs_2CO_3. The latter reagent is more economical. After evaporation to dryness the neutral salt is treated with an alkyl halide in DMF to form the corresponding ester. For example, the benzyl esters I–XII (Table I) were obtained within a short period of time by stirring equivalent amounts of benzyl bromide and the cesium salts of Boc-amino acids or Boc-peptides in DMF at room temperature.

The combination of amine protection by the *tert*-butyloxycarbonyl group and carboxyl protection by the benzyl ester has been the most widely used tactic in peptide synthesis.[18-20] The scope and versatility of this useful approach is now further enhanced by the facile introduction of the benzyl ester group into protected peptides, as shown in Scheme I, with a synthesis of methionine-enkephalin as an example. Thus, Boc-Phe-Met-OH was readily converted in high yield into its benzyl ester Boc-Phe-Met-OBzl (VII) by the cesium salt

Scheme I. Synthesis of H-Tyr-Gly-Gly-Phe-Met-OH (Enkephalin)

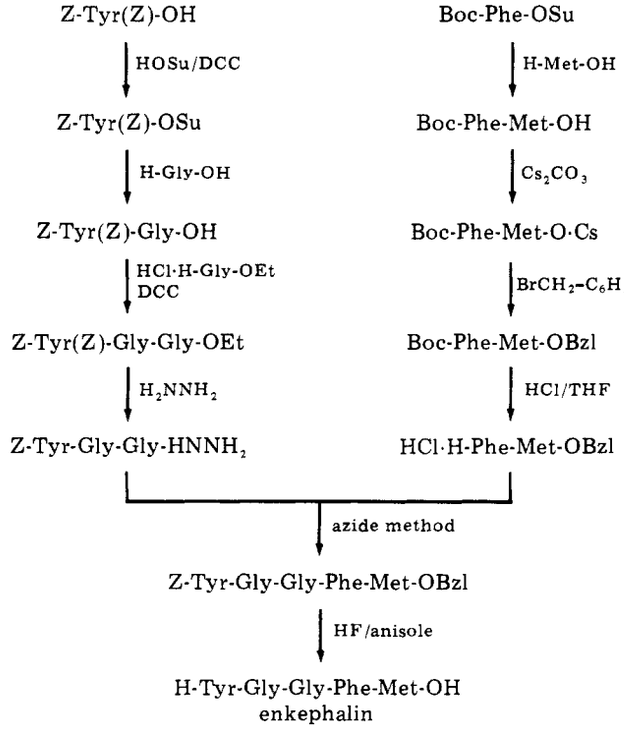

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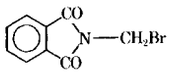
Table I. Preparation of Protected Amino Acid and Peptide Esters via Cs-Salt Method: RX + R'COO·Cs → R'COOR + CsX

RX	Registry no.	R'COOH	R'COOR	Registry no.	% yield	Mp, °C	$[\alpha]^{25}D$, deg	% C[i]	% H[i]	% N[i]
$C_6H_5CH_2Br$	100-39-0	Boc-Asn-OH	Boc-Asn-OBzl (I)	13512-57-7	90.3[a]	120–122	−17.29[f]	59.61 59.76	6.88 6.81	8.69 8.82
$C_6H_5CH_2Br$		Boc-Gln-OH	Boc-Gln-OBzl (II)	61543-21-3	86.3[a]	108–110	−22.69[f]	60.70 60.72	7.19 7.30	8.33 8.35
$C_6H_5CH_2Br$		Boc-Trp-OH	Boc-Trp-OBzl (III)	57229-67-1	86.5[b]	140–142	−2.02[g]	70.03 70.25	6.64 6.60	7.10 6.92
$C_6H_5CH_2Br$		Boc-Arg(Tos)-OH	Boc-Arg(Tos)-OBzl (IV)	61543-20-2	88.1[c]	Amorphous	+2.81[h]	57.90 57.84	6.61 6.77	10.80 10.72
$C_6H_5CH_2Br$		Boc-Phe-Gly-OH	Boc-Phe-Gly-OBzl (V)	42280-29-5	79.5[c]	132–134	−8.61[f]	66.97 67.18	6.84 6.90	6.79 6.64
$C_6H_5CH_2Br$		Boc-Phe-Phe-OH	Boc-Phe-Phe-OBzl (VI)	16879-80-4	82.0[c]	121–122	−1.59[g]	71.69 71.86	6.72 6.72	5.57 5.56
$C_6H_5CH_2Br$		Boc-Phe-Met-OH	Boc-Phe-Met-OBzl (VII)	61543-22-4	86.2[c]	99–100	−3.43[h]	64.11 64.16	7.04 6.99	5.76 5.97
$C_6H_5CH_2Br$		Boc-Pro-Pro-OH	Boc-Pro-Pro-OBzl (VIII)	29776-70-3	96.0[c]	Oil	−103.63[h]	65.65 65.31	7.51 7.49	6.96 6.78
$C_6H_5CH_2Br$		Boc-Gly-Glu(OBzl)-OH	Boc-Gly-Glu(OBzl)-OBzl (IX)	61570-89-6	75.0[a]	Oil	−17.82[h]	64.45 64.58	6.66 6.44	5.78 6.06
$C_6H_5CH_2Br$		Boc-Val-Cys(2-NO_2-Bzl)-OH	Boc-Val-Cys(2-NO_2-Bzl)-OBzl (X)	61543-23-5	67.3[c]	120–122	−65.64[g]	59.43 59.53	6.47 6.54	7.70 7.73
$C_6H_5CH_2Br$		Boc-Leu-Val-Thr(Bzl)-OH	Boc-Leu-Val-Thr(Bzl)-OBzl (XI)	61543-24-6	85.5[a]	68–72	−31.02[h]	66.75 66.71	8.07 8.08	6.86 6.81
$C_6H_5CH_2Br$		Boc-Ala-Ala-Ala-Lys(Z)-Ala-OH	Boc-Ala-Ala-Ala-Lys(Z)-Ala-OBzl (XII)	61543-25-7	72.9[c]	226–228	−29.41[f]	60.46 60.42	7.21 7.29	11.13 11.04
2-$NO_2C_6H_4CH_2Cl$	612-23-7	Boc-Glu(OBzl)-Glu(OBzl)-OH	Boc-Glu(OBzl)-Glu(OBzl)-O(2-NO_2Bzl) (XIII)	61543-26-8	89.1[c]	108–110	+3.79[g]	62.50 62.60	5.97 5.88	6.07 6.05
CH_3I	74-88-4	Boc-Ile-Leu-OH	Boc-Ile-Leu-OMe (XIV)	61543-27-9	81.0[d]	144–146	−26.00[g]	60.30 60.50	9.56 9.61	7.82 7.93
CH_3I		Z-Lys(Z)-Phe-Phe-Gly-OH	Z-Lys(Z)-Phe-Phe-Gly-OMe (XV)	61570-88-5	80.4[d]	178–180	−24.35[f]	66.22 66.06	6.33 6.30	8.98 8.94
$C_6H_5COCHMeBr$	2114-00-3	Z-Gly-OH	Z-Gly-OMPA (XVI)	61543-28-0	79.1[b]	Oil		66.85 66.88	5.61 5.71	4.10 4.08
$C_6H_5COCHMeBr$		Z-Ala-OH	Z-Ala-OMPA (XVII)	61543-29-1	81.7[d]	Oil	−21.71[g]	67.59 67.49	5.96 5.73	3.94 3.85
Ph_3CCl	76-83-5	Fmoc-Gly-OH	Fmoc-Gly-OTrt (XVIII)	61570-87-4	40.1[c]	173–175		80.13 80.15	5.42 5.27	2.60 2.54
Ph_3CCl		Z-Ile-Leu-OH	Z-Ile-Leu-OTrt (XIX)	61543-30-4	64.2[e]	152–154	−54.57[h]	75.46 75.66	7.14 7.08	4.51 4.47
Me_3CBr	507-19-7	Z-Ala-OH	Z-Ala-OBut (XX)	50300-96-4	14[c]	Oil	−10.18[g]	64.50 64.44	7.58 7.42	5.01 4.98
$MeOC_6H_4CH_2Cl$	824-94-2	Boc-Trp-OH	Boc-Trp-OPMB (XXI)	61543-31-5	68.5[c]	100–102	−4.08[g]	67.91 68.12	6.65 6.50	6.60 6.42
$MeOC_6H_4CH_2Cl$		Z-Ile-Leu-OH	Z-Ile-Leu-OPMB (XXII)	61543-32-6	73.6[c]	112–116	−19.42[h]	67.45 67.54	7.68 7.68	5.62 5.58
	5332-26-3	Boc-Ala-Ala-OH	Boc-Ala-Ala-OPIM (XXIII)	61543-33-7	81.4[b]	96–98	−19.15[g]	57.27 57.13	6.01 5.84	10.02 9.99
		Z-Ile-Leu-OH	Z-Ile-Leu-OPIM (XXIV)	61543-34-8	87.6[b]	151–153	−11.12[f]	64.79 64.75	6.56 6.49	7.82 7.71

[a] Reaction time 6 h. [b] Reaction time 1 h. [c] Reaction time 17 h. [d] Reaction time 30 min. [e] Reaction time 64 h. [f] c 1, DMF. [g] c 1, EtOAc. [h] c 1, $CHCl_3$. [i] Anal. (calcd over found).

method which was subsequently condensed with the tripeptide Z-Tyr-Gly-Gly-$HNNH_2$ via the azide method.[21] The protected pentapeptide Z-Tyr-Gly-Gly-Phe-Met-OBzl was obtained as a crystalline pure compound. It is interesting that there was no indication for thioether alkylation at the methionine residue during the synthesis of VII. Apparently, carboxyl group benzylation proceeded much faster than thioether alkylation. The absence of S-alkylation was similarly observed during the synthesis of Boc-Val-Cys(2-NO_2-Bzl)-OBzl (X).

To ascertain the absence of gross racemization during esterification, the Manning and Moore test[22] was applied to a sample of crude Boc-Phe-Phe-OBzl (VI). A small amount of D isomer (0.30% D-Phe) was found. However, this value coincided within experimental variation with the D-Phe content (0.27%) of the hydrolyzed starting material Boc-Phe-Phe-OH. It is therefore concluded that racemization during the preparation of this ester, if any, was below the limit of detection by this test. Since phenylalanine is more racemization prone than most other amino acids the generalization that esterification of α-amino acids via the cesium salt proceeds generally free of observable racemization appears justified.

Similar to benzyl esters, methyl esters of protected peptides can be prepared efficiently from their cesium salts and methyl iodide. The reaction was found to proceed rapidly, requiring only a few minutes for completion (see compounds XIV, XV). Presumably, ethyl iodide could be used in a similar fashion for the preparation of peptide ethyl esters. The photolyzable 2-nitrobenzyl ester[23,24] and α-methylphenacyl esters[25] were also obtained by the cesium salt method using the readily available 2-nitrobenzyl chloride and α-bromopropiophenone, respectively, as alkylating agents. Reaction of amino acids or peptide cesium salts with triphenylmethyl chloride provided trityl esters[26-28] in moderate yields (see compounds XVIII and XIX). Z-Ala-OBut (XX) was prepared from the cesium salt of Boc-Ala-OH with 2-bromo-2-methylpropane, although the yield was low, probably owing to the instability and low reactivity of this alkylating agent. As illustrated by the synthesis of XXI and XXII, *p*-methoxybenzyl esters[29] were satisfactorily obtained from cesium salts and *p*-methoxybenzyl chloride.[30] This ester may become more important as a temporary blocking group for the carboxylic function since the selective removal of the Boc group by *p*-toluenesulfonic acid in the presence of *p*-methoxybenzyl ester has recently been described.[31] The cesium ester method was equally suitable for the preparation of phthalimidomethyl esters[32] as exemplified by the synthesis of XXIII and XXIV. The phthalimidomethyl ester is an attractive protection for carboxyl group in peptide synthesis since it can be removed selectively in the presence of many other widely used blocking groups under very mild conditions.[18-20]

In conclusion, the cesium salt method provides facile preparation of a wide variety of esters of protected amino acids and peptides under mild, neutral conditions at room temperature. The procedure is simple, easily scaled up, and proceeds without observable racemization. Its great versatility suggests that the procedure might be useful beyond amino acid and peptide chemistry.

Experimental Section

Melting points are uncorrected. Thin layer chromatography was carried out on precoated silica gel plates (Merck, F-254) with the solvent systems given previously.[33] Elemental analyses and physicochemical measurements (IR, UV, $[\alpha]^{25}_D$, NMR) were performed by the Hoffmann-La Roche Physical Chemistry Department. All the new compounds described in these experiments have been examined by UV, IR, and NMR spectrophotometry. Each compound gave spectra agreeing with its structure.

Amino acid derivatives were either purchased from Bachem Inc., Marina Del Rey, Calif., or prepared in our laboratories. All the optically active amino acids were of the L configuration. Benzyl bromide, *o*-nitrobenzyl chloride, 2-bromo-2-methylpropane, α-bromopropiophenone, methyl iodide, and *p*-methoxybenzyl alcohol were obtained from Aldrich Chemical Co. and triphenylmethyl chloride and *N*-bromomethylphthalimide were the products of Eastman Organic Chemicals. Cesium carbonate was bought from Atomergic Chemical Co., Long Island, N.Y. *p*-Methoxybenzyl chloride was prepared from *p*-methoxybenzyl alcohol according to the literature procedure.[30] Other chemicals and solvents used were reagent grade products available from commercial sources.

Since the procedures utilized to make the esters listed in Table I are very similar from one to the other, the synthesis of only a few representative compounds will be described in the following examples.

A. General Examples of Esterification. Boc-Asn-OBzl (I). Boc-Asn-OH (11.0 g, 47.5 mmol) was dissolved in 200 mL of MeOH and 20 mL of water was added. The solution was titrated to pH 7.0 (pH paper) with a 20% aqueous solution of Cs_2CO_3 (~55 mL). The mixture was evaporated to dryness and the residue reevaporated twice from 120 mL of DMF (45 °C). The white solid cesium salt obtained was stirred with 8.9 g (52 mmol) of benzyl bromide in DMF (120 mL) for 6 h. On evaporation to dryness and treatment with a large volume of water (500 mL) the product solidified. It was taken into ethyl acetate, washed with water, dried over Na_2SO_4, evaporated to a solid mass, and crystallized from ethyl acetate with petroleum ether: yield 13.8 g (90.3%); mp 120–122 °C; $[\alpha]^{25}_D$ −17.29° (*c* 1, DMF).

Anal. Calcd for $C_{16}H_{22}N_2O_5$ (322.36): C, 59.61; H, 6.88; N, 8.69. Found: C, 59.76; H, 6.81; N, 8.82.

Boc-Glu(OBzl)-Glu(OBzl)-O(2-NO_2-Bzl) (XIII). H-Glu(OBzl)-OH (4.74 g, 20 mmol) was finely ground in a mortar and pestle and stirred with 8.7 g of Boc-Glu(OBzl)-OSu[34] (20 mmol) and NMM (4.8 mL) in DMF (150 mL) for 36 h. The clear solution obtained was evaporated to a syrup and treated with a large volume of water. The oily product was taken up in ethyl acetate, washed with 5% HOAc followed by water (three times), dried over Na_2SO_4, and evaporated to dryness. The colorless, clear oil (12.2 g) failed to crystallize. TLC indicated that the product, Boc-Glu(OBzl)-Glu(OBzl)-OH, is homogeneous, $[\alpha]^{25}_D$ −7.59° (*c* 1, DMF).

Anal. Calcd for $C_{29}H_{36}N_2O_9$ (556.60): C, 62.57; H, 6.52; N, 5.03. Found: C, 62.37; H, 6.34; N, 5.01.

The oily Boc-Glu(OBzl)-Glu(OBzl)-OH (6.0 g, 10.8 mmol) was dissolved in 100 mL of EtOH when 10 mL of H_2O was added. The solution was then titrated to pH 7 with 20% Cs_2CO_3 and evaporated to dryness. The residue was reevaporated twice from DMF (100 mL) and the cesium salt obtained was stirred with 1.88 g (11 mmol) of *o*-nitrobenzyl chloride in 50 mL of DMF for 17 h. Evaporation of the solvent and treatment of the residue with water gave a crude solid product. It was collected by suction, dissolved in ethyl acetate, washed with water, dried over Na_2SO_4, and evaporated to a solid mass. Crystallized from ethyl acetate and petroleum ether: yield 6.66 g (89.1%); mp 108–110 °C $[\alpha]^{25}_D$ +3.79° (*c* 1, EtOAc).

Anal. Calcd for $C_{36}H_{41}N_3O_{11}$ (691.73): C, 62.51; H, 5.97; N, 6.07. Found: C, 62.60; H, 5.88; N, 6.05.

Z-Lys(Z)-Phe-Phe-Gly-OCH_3 (XV). Z-Lys(Z)-Phe-Phe-Gly-OH[35] (1.0 g, 1.3 mmol) was dissolved in 50 mL of THF and 5 mL of water was added. The solution was neutralized to pH 7 with 20% Cs_2CO_3 and evaporated to dryness as described above to give the cesium salt. It was then stirred with 0.22 g (1.55 mmol) of CH_3I in DMF (20 mL) for 30 min. Upon removal of the solvent by evaporation and treatment with water, a solid product was obtained. It was collected and washed with water on the funnel and dried to a white powder. Crystallized from THF and water: yield 0.82 g (80.4%); mp 178–180 °C; $[\alpha]^{25}_D$ −24.35° (*c* 1, DMF).

Anal. Calcd for $C_{43}H_{49}N_5O_9$ (779.90): C, 66.22; H, 6.33; N, 8.98. Found: C, 66.06; H, 6.30; N, 8.94.

Z-Ala-OMPA (XVII). Z-Ala-OH (1.12 g, 5 mmol) was converted into its cesium salt as described above and stirred with 1.17 g (5.5 mmol) of α-bromopropiophenone for 30 min in DMF (25 mL). Workup as usual gave an oil (1.45 g, 81.7%) which failed to crystallize, $[\alpha]^{25}_D$ −21.71° (*c* 1, EtOAc).

Anal. Calcd for $C_{20}H_{21}NO_5$ (355.38): C, 67.59; H, 5.96; N, 3.94. Found: C, 67.49; H, 5.73; N, 3.85.

Z-Ile-Leu-OTrt (XIX). Z-Ile-Leu-OH[34] (1.0 g, 2.64 mmol) was converted into its cesium salt as described above and stirred with 1.0 g (3.6 mmol) of triphenylmethyl chloride for 64 h in DMF (15 mL). Workup as usual gave a solid material which was crystallized from ethyl acetate and petroleum ether: yield 1.05 g (64.2%); mp 152–154 °C; $[\alpha]^{25}_D$ −54.57° (*c* 1, $CHCl_3$).

Anal. Calcd for $C_{39}H_{44}N_2O_5$ (620.77): C, 75.46; H, 7.14; N, 4.51. Found: C, 75.66; H, 7.08; N, 4.47.

Z-Ile-Leu-OPMB (XXII). Z-Ile-Leu-OH (1.0 g, 2.64 mmol) was converted into its cesium salt and reacted with 0.47 g (3.0 mmol, freshly prepared) of *p*-methoxybenzyl chloride for 17 h in DMF (10 mL). Workup as usual gave a crude solid which was crystallized from THF and petroleum ether: yield 0.97 g (73.6%); mp 112–116 °C; $[\alpha]^{25}_D$ −19.42° (*c* 1, $CHCl_3$).

Anal. Calcd for $C_{28}H_{38}N_2O_6$ (498.62): C, 67,45; H, 7.68; N, 5.62. Found: C, 67.54; H, 7.68; N, 5.58.

Z-Ala-OBut (XX). Z-Ala-OH (2.33 g, 10 mmol) and solid Cs_2CO_3 (3.25 g, 10 mmol) were ground together in a mortar and pestle and the resulting fine powder was stirred in DMF (30 mL) with 2.85 g (20 mmol) of 2-bromo-2-methylpropane for 17 h. The solvent was then removed by evaporation and the residue was treated with water.[36] The product was taken into ethyl acetate and washed with water, 15% $NaHCO_3$, and water followed by drying (Na_2SO_4). Evaporation of the solvent left a clear, colorless oil (0.39 g, 14%): homogeneous on TLC; $[\alpha]^{25}_D$ −10.18° (*c* 1, EtOAc).

Anal. Calcd for $C_{15}H_{21}NO_4$ (279.33): C, 64.50; H, 7.58; N, 5.01. Found: C, 64.44; H, 7.42; N, 4.98.

Boc-Ala-Ala-OPIM (XXIII). Boc-Ala-Ala-OH (1.3 g, 5 mmol) was converted into its cesium salt in the usual manner (20% Cs_2CO_3 and aqueous MeOH; DMF) and stirred with 1.32 g (5.5 mmol) of *N*-bromomethylphthalimide for 1 h in DMF (15 mL). Removal of the solvent and treatment with water gave a solid which was worked up as usual and crystallized from ethyl acetate and petroleum ether: yield 1.70 g (81.4%); mp 96–98 °C $[\alpha]^{25}_D$ −19.15° (*c* 1, EtOAc).

Anal. Calcd for $C_{20}H_{25}N_3O_7$ (419.42): C, 57.27; H, 6.01; N, 10.02. Found: C, 57.13; H, 5.84; N, 9.99.

H-Cys(2-NO_2-Bzl)-OH. Cysteine hydrochloride monohydrate (17.56 g, 100 mmol) was dissolved in 200 mL of 1 N NaOH that had been purged with argon gas for 15 min. To this mixture, 17.4 g (102 mmol) of *o*-nitrobenzyl chloride in 100 mL of peroxide-free dioxane[37] was added dropwise under argon during 90 min. The solution was stirred for an additional 60 min and the pH adjusted to 6.2. A heavy suspension of crystalline product formed. The solid was collected and washed with water, *i*-PrOH, and ether: yield 22.0 g (85.6%); mp 198–200 °C.

Anal. Calcd for $C_{10}H_{12}N_2O_4S$ (256.28): C, 46.87; H, 4.72; N, 10.93; S, 12.51. Found: C, 47.02; H, 4.74; N, 10.94; S, 12.62.

Boc-Val-Cys(2-NO_2-Bzl)-OBzl (X). Boc-Val-OSu (3.15 g, 10 mmol) and 2.56 g (10 mmol) of H-Cys(2-NO_2-Bzl)-OH were allowed to react in DMF (75 mL) for 36 h in the presence of 1.5 mL of Et_3N. Evaporation of the solvent and trituration of the residue gave a gum which was taken up in ethyl acetate, washed with water, dried over Na_2SO_4, and evaporated to a slightly yellowish oil (4.2 g) that failed to crystallize. It was thus converted into the cesium salt as described above and treated with 1.62 g of benzyl bromide in a manner similar to that for the preparation of VII. The mixture was left stirring overnight and worked up as usual to give a solid which was crystallized from ethyl acetate and petroleum ether: yield 3.67 g (67.3%); mp 120–122 °C; $[\alpha]^{25}_D$ −65.64° (*c* 1, EtOAc).

Anal. Calcd for $C_{27}H_{35}N_3O_7S$ (545.64): C, 59.43; H, 6.47; N, 7.70. Found: C, 59.53; H, 6.54; N, 7.73.

Boc-Gly-Glu(OBzl)-OBzl (IX). H-Glu(OBzl)-OH (1.19 g, 5 mmol), finely ground, was stirred with 1.43 g (5.25 mmol) of Boc-Gly-OSu and 0.7 mL (5 mmol) of Et_3N in DMF (35 mL) for 36 h. The clear solution obtained was evaporated to a syrup and treated with acidified water. The oily product was taken up in ethyl acetate, washed with 5% AcOH followed by water, dried over Na_2SO_4, and evaporated to dryness. The colorless, clear oil failed to crystallize. TLC indicated that the product Boc-Gly-Glu(OBzl)-OH was homogeneous, $[\alpha]^{25}_D$ +0.55° (*c* 1.8, MeOH).

Anal. Calcd for $C_{19}H_{26}N_2O_7$ (394.43): C, 57.86; H, 6.64; N, 7.10. Found: C, 57.98; H, 6.74; N, 6.98.

The oily Boc-Gly-Glu(OBzl)-OH was dissolved in 35 mL of EtOH when 5 mL of water was added. The solution ws then titrated to pH 7 with 20% Cs_2CO_3 and evaporated to dryness. The residue was reevaporated twice from DMF (25 mL) and the cesium salt obtained was stirred with 0.86 g (5 mmol) of benzyl bromide for 6 h. The solvent was then removed by evaporation and the residue treated with water. The product was taken into ethyl acetate, washed with water, and dried over Na_2SO_4. Evaporation of the solvent left a clear, colorless oil, which failed to crystallize. TLC indicated that the product Boc-Gly-Glu(OBzl)-OBzl (1.82 g, 75%) was homogeneous, $[\alpha]^{25}_D$ −17.82° (*c* 2.1, MeOH).

Anal. Calcd for $C_{26}H_{32}N_2O_7$ (484.56): C, 64.45; H, 6.66; N, 5.78. Found: C, 64.58; H, 6.44; N, 6.06.

B. Synthesis of Methionine-Enkephalin. Z-Tyr(Z)-OSu. Z-Tyr(Z)-OH[38] (5 g, 11.1 mmol) in THF was cooled in an ice bath and treated with 1.54 g (13.3 mmol) of HOSu and 2.52 g (12.2 mmol) of DCC. The mixture was gently stirred at 0 °C for 1 h and at 25 °C for 2.5 h. The insoluble by-product was then filtered off and the filtrate evaporated at 30 °C to leave a solid mass, recrystallized from *i*-PrOH: yield 5.9 g (97.2%); mp 153–154 °C; $[\alpha]^{25}_D$ +8.77° (*c* 1, $CHCl_3$).

Anal. Calcd for $C_{29}H_{26}N_2O_9$ (546.54): C, 63.73; H, 4.79; N, 5.12. Found: C, 63.94; H, 5.07; N, 5.17.

Z-Tyr(Z)-Gly-OH. Glycine (0.75 g, 10 mmol) was dissolved in 4.9 mL of 40% Triton B in MeOH (10.5 mmol), evaporated at 35 °C to a white solid residue, reevaporated twice from DMF (50 mL), and mixed with 6.01 g (11 mmol) of Z-Tyr(Z)-OSu in DMF. The reaction mixture was stirred for 17 h during which time some additional Et_3N was added occasionally to maintain the pH at about 8.0 (moist pH paper). Acetic acid was added to about pH 3 and the solvents removed under reduced pressure. The product was extracted into ethyl acetate, washed with 5% HOAc and water, dried over Na_2SO_4, and evaporated to a white solid, crystallized from ethyl acetate with petroleum ether: yield 4.8 g (94.7%); mp 174–176 °C; $[\alpha]^{25}_D$ −20.08° (*c* 1, DMF).

Anal. Calcd for $C_{27}H_{26}N_2O_8$ (506.52): C, 64.03; H, 5.17; N, 5.53. Found: C, 63.99; H, 5.13; N, 5.45.

Z-Tyr(Z)-Gly-Gly-OEt. Glycine ethyl ester HCl (1.27 g, 9.1 mmol) was allowed to react with 4.6 g (9.1 mmol) of Z-Tyr(Z)-Gly-OH, 2.07 g (10 mmol) of DCC, and 1.02 mL (9.1 mmol) of NMM in a mixture of DMF and CH_2Cl_2 at 0 °C for 1 h and then at 25 °C for 24 h. After removal of the by-product and the solvent, the syrup obtained was dissolved in ethyl acetate, washed with water, dried (Na_2SO_4), and evaporated to dryness. The product was crystallized from ethyl acetate and petroleum ether: yield 5.1 g (94.8%); mp 89–91 °C.

Anal. Calcd for $C_{31}H_{33}N_3O_9$ (591.62); C, 62.93; H, 5.62; N, 7.10. Found: C, 62.97; H, 5.52; N, 7.21.

Z-Tyr-Gly-Gly-HNNH$_2$. Z-Tyr(Z)-Gly-Gly-OEt (4.4 g, 7.44 mmol) was dissolved in EtOH (50 mL) and treated with 5 mL of H_2NNH_2. The product precipitated as a granular solid during overnight standing, recrystallized from EtOH: yield 2.5 g (75.8%); mp 198–201 °C; $[\alpha]^{25}_D$ −21.86 (*c* 1, DMF).

Anal. Calcd for $C_{21}H_{25}N_5O_6$ (443.46): C, 56.89; H, 5.68; N, 15.79. Found: C, 56.60; H, 5.80; N, 15.62.

Boc-Phe-Met-OH. Methionine (3.5 g, 24.45 mmol) was dissolved in 11.57 mL (25.4 mmol) of 40% Triton B and evaporated to an oil. It was reevaporated twice with DMF (45 mL) and the salt obtained was stirred with 9.35 g (26.9 mmol) of Boc-Phe-OSu in DMF (0 °C, 1 h; 25 °C, 17 h). The mixture was adjusted to pH 3 and worked up as usual to give an oil which was crystallized from ethyl acetate and petroleum ether: yield 7.5 g (80.7%); mp 137–139 °C; $[\alpha]^{25}_D$ +23.01° (*c* 1, $CHCl_3$).

Anal. Calcd for $C_{19}H_{28}N_2O_5S$ (396.50): C, 57.56; H, 7.12; N, 7.06; S, 8.09. Found: C, 57.82; H, 7.25; N, 7.14; S, 8.26.

Boc-Phe-Met-OBzl (VII). Boc-Phe-Met-OH (6.0 g, 15.12 mmol) was converted into its cesium salt as described above except that all the operations were performed under an argon atmosphere. The white solid salt was dissolved in DMF (50 mL, purged with argon for 15 min) and treated with 2.72 (15.8 mmol) of benzyl bromide added dropwise during a 30-min period of time. The reaction mixture was worked up as usual, after 17 h of gentle stirring, to give a solid which was crystallized from ethyl acetate with petroleum ether: yield 6.4 g (86.2%); mp 99–100 °C; $[\alpha]^{25}_D$ −3.43° (*c* 1, $CHCl_3$).

Anal. Calcd for $C_{26}H_{34}N_2O_5S$ (486.63): C, 64.11, H, 7.04; N, 5.76; S, 6.59. Found: C, 64.16; H, 6.99; N, 5.97; S, 6.68.

HCl·H-Phe-Met-OBzl. Boc-Phe-Met-OBzl (4.38 g, 9.02 mmol) was treated with freshly prepared peroxide-free[37] 4.0 N HCl in THF for 90 min. Evaporation of the excess HCl and solvent gave a white solid which was crystallized from EtOH and ether: yield 2.66 g (69.7%); mp 140–142 °C; $[\alpha]^{25}_D$ −8.57° (*c* 1, 0.1 N HCl).

Anal. Calcd for $C_{21}H_{27}N_2O_3S$ Cl (422.97): C, 59.63; H, 6.43; N, 6.62; S, 7.58; Cl, 8.38. Found: C, 59.50; H, 6.44; N, 6.57; S, 7.49; Cl, 8.45.

Z-Tyr-Gly-Gly-Phe-Met-OBzl. Z-Tyr-Gly-Gly-HNNH$_2$ (2.0 g, 4.51 mmol) was dissolved in 45 ml of DMF, cooled to −20 °C and treated with 5.65 mL of 4.0 N HCl in THF followed by 9.1 mL of 10% isoamyl nitrite in DMF. After 30 min, the mixture was cooled down to −30 °C when 3.79 mL of Et_3N and HCl·H-Phe-Met-OBzl (1.9 g, 4.51 mmol) were added. The mixture was stirred gently at 4 °C for 32 h during which time some more Et_3N was added in order to maintain the pH slightly basic. Evaporation of the solvent and trituration of the residue gave a yellowish powder which was taken up in ethyl acetate, washed with H_2O, dried over Na_2SO_4, and evaporated to a smaller volume when crystallization began. Recrystallized from ethyl acetate: yield 2.40 g (66.9%); mp 182–185 °C; $[\alpha]^{25}_D$ −26.82° (*c* 1, DMF).

Anal. Calcd for $C_{42}H_{47}N_5O_9S$ (797.93): C, 63.22; H, 5.94; N, 8.78;

S, 4.02. Found: C, 63.16; H, 5.83; N, 8.83, S, 4.13.

H-Tyr-Gly-Gly-Phe-Met-OH (Methionine-Enkephalin). The protected pentapeptide Z-Tyr-Gly-Gly-Phe-Met-OBzl (2.4 g, 3.0 mmol) was treated with HF[39] (35 mL) at 0 °C for 45 min in the presence of 13.3 mL of anisole and 6.6 mL of diethyl sulfide. After removal of the acid, the residue was taken up in 5% HOAc, washed a few times with peroxide-free ether, and lyophilized to give 2.01 g of crude product. It showed two minor impurities on TLC which were eliminated after passing through a Sephadex G-10 column (5 × 100 cm) using 0.2 M HOAc as eluent: yield 0.99 g; $[\alpha]^{25}_D$ +23.80° (*c* 1, 5% HOAc).

Anal. Calcd for $C_{27}H_{35}N_5O_7S \cdot H_2O$ (591.68): C, 54.80; H, 6.30; N, 11.83. Found: C, 54.90; H, 6.14; N, 11.69.

Amino Acid Anal.[40] Gly, 2.00; Met, 0.98; Tyr, 1.00; Phe, 1.00.

Racemization Test for Boc-Phe-Phe-OBzl Prepared by the Cesium Salt Method. Samples of Boc-Phe-Phe-OH and *unpurified* crude Boc-Phe-Phe-OBzl obtained from Boc-Phe-Phe-OH by the cesium salt method were hydrolyzed separately in 6 N HCl at 135 °C for 4 h. They were then evaporated to dryness and separately made up to a concentration of 6 μmol/mL (Phe) with pH 10.2 borate buffer. Derivatization and analyses of these samples were carried out according to Manning and Moore[22] with the results: Boc-Phe-Phe-OH contained 0.27% D-phenylalanine after hydrolysis whereas Boc-Phe-Phe-OBzl (crude) contained 0.30% D-phenylalanine after hydrolysis. Thus, within the experimental variation, they contained equal amounts of D-phenylalanine.

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Registry No.—Boc-Asn-OH Cs salt, 61543-35-9; Boc-Gln-OH Cs salt, 61543-36-0; Boc-Trp-OH Cs salt, 61543-37-1; Boc-Arg(Tos)-OH Cs salt, 61543-38-2; Boc-Phe-Gly-OH Cs salt, 61543-39-3; Boc-Phe-Phe-OH Cs salt, 61543-40-6; Boc-Phe-Met-OH Cs salt, 61543-41-7; Boc-Pro-Pro-OH Cs salt, 61543-42-8; Boc-Gly-Glu(OBzl)-OH Cs salt, 61543-43-9; Boc-Val-Cys(2NO_2-Bzl)-OH Cs salt, 61543-44-0; Boc-Leu-Val-Thr(Bzl)-OH Cs salt, 61543-45-1; Boc-Ala-Ala-Ala-Lys(Z)-Ala-OH Cs salt, 61570-86-3; Boc-Glu(OBzl)-Glu(OBzl)-OH Cs salt, 61543-46-2; Boc-Ile-Leu-OH, 61543-47-3; Z-Lys(Z)-Phe-Phe-Gly-OH, 61604-89-5; Z-Gly-OH, 61543-48-4; Z-Ala-OH, 61543-49-5; Fmoc-Gy-OH, 61543-50-8; Z-Ile-Leu-OH, 61543-51-9; Boc-Ala-Ala-OH, 61543-52-0; H-Glu(OBzl)-OH, 1676-73-9; Boc-Glu(OBzl)-OSu, 32886-40-1; Boc-Glu(OBzl)-Glu(OBzl)-OH, 32886-41-2; H-Cys(2-NO_2-Bzl)-OH, 61543-53-1; Cys HCl, 52-89-1; Boc-Val-OSu, 3392-12-9; Boc-Gy-OSu, 3392-07-2; Boc-Gly-Glu(OBzl)-OH, 56357-41-6; Z-Tyr(Z)-OSu, 6461-23-0; Z-Tyr(Z)-OH, 29713-96-0; HOSu, 6066-82-6; Z-Tyr(Z)-Gly-OH, 14297-20-2; Gly, 56-40-6; Z-Tyr(Z)-Gly-Gly-OEt, 61543-54-2; Gly-OEt HCl, 623-33-6; Z-Tyr-Gly-Gly-$HNNH_2$, 61543-55-3; Boc-Phe-Met-OH, 61543-56-4; Met, 63-68-3; Boc-Phe-OSu, 3674-06-4; HCl·H-Phe-Met-OBzl, 61543-57-5; Z-Tyr-Gly-Gly-Phe-Met-OBzl, 61543-58-6; H-Tyr-Gly-Gly-Phe-Met-OH, 58569-55-4.

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(12) Abbreviations used: Boc, *tert*-butyloxycarbonyl; But, *tert*-butyl; Bzl, benzyl; Bpoc, 2-(biphenylyl)-2-propyloxycarbonyl; Fmoc, 9-fluorenylmethyloxycarbonyl; MPA, α-methylphenacyl; 2-NO_2-Bzl, 2-nitrobenzyl; PIM, phthalimidomethyl; Trt, trityl; Z, benzyloxycarbonyl; NMM, *N*-methylmorpholin; TLC, thin layer chromatography; THF, tetrahydrofuran; HOSu, *N*-hydroxysuccinimide; DCC, dicyclohexylcarbodiimide; HOBT, *N*-hydroxybenzotriazole; PMB, *p*-methoxybenzyl; DMF, dimethylformamide.
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