factor (K') = 37.5]. After desalination on a Bio-Gel P-2 column (see above) and lyophilization, 134 mg of RPM was obtained (30 μmol, 33%): overall yield (based on the crude peptide-resin) 13.6%. The synthetic tetracontapeptide was homogeneous with regard to thin-layer chromatography and electrophoresis: HPTLC using the solvent systems 1-butanol/pyridine/acetic acid/water (20:10:3:10) and ethyl acetate/pyridine/acetic acid/water (5:5:1:3) each gave a single spot (ninhydrin and Sakaguchi detection) with R_f values of 0.25 and 0.34, respectively; electrophoreses in pyridinium acetate buffer (pH 3.45 and 6.50, 400 V, 30 min) on precoated cellulose plates each gave a single spot (ninhydrin and Sakaguchi) with R_f values of 0.42 and −0.13 relative to arginine. Amino acid analysis of an acid hydrolysate gave the following: Asp, 1.04 (1); Thr, 12.25 (13); Ser, 2.75 (3); Glu, 4.12 (4); Pro, 4.08 (4); Gly, 3.00 (3); Val, 1.97 (2); Ile, 0.94 (1); Phe, 5.18 (5); Arg, 3.90 (4). Complete enzymic digestion successively with trypsin, α-chymotrypsin, prolidase, and then with leucine aminopeptidase gave the following: Asp, 1.02 (1); Thr, 12.58 (13); Ser, 3.02 (3); Glu, 4.08 (4); Pro, 3.90 (4); Gly, 3.00 (3); Val, 1.96 (2); Ile, 0.97 (1); Phe, 5.07 (5); Arg, 3.96 (4).

Measurements of Ligand Binding. Equilibrium Dialysis. Dialysis studies were performed in cylindrical Teflon cells having a total capacity of 11.5 mL and which were divided into two equal compartments by a semipermeable membrane of 1.35-cm radius. The membranes were cut from cellulose dialysis tubings (Spectrapor 3, Spectrum Medical Industries Inc.). The permeability of the membrane to the synthetic receptor was found to be ~4% within the experimental time (48 h). The binding data were corrected for the leakage effect. Retention of the ligands to the membrane was found to be negligible. The experiments were generally started by placing 5 mL of an RMP solution in 10^{-2} M sodium phosphate buffer (pH 7.0) to one side of each cell and 5 mL of an equally buffered solution of the ^{14}C-labeled ligand to the other side of the cell. The dialysis cells prepared in this manner were shaken with a frequency of 25 oscillations per minute. After equilibration, aliquots (5 μL) were taken from each side of the cells and the concentration of the ^{14}C-labeled ligand was determined on a Packard Tri-Carb 3380 counter. Each dialysis experiment was run in duplicate. Amino acid analyses served to determine the peptide concentration. The amounts of bound and free ligands were calculated as described by Changeux et al.[77]

Gel Filtration.[78] In a typical binding experiment, a 0.8 × 60 cm column of Sephadex G-15, thoroughly equilibrated with a known solution of [Leu]-enkephalin in 10^{-2} M sodium phosphate buffer (pH 7.0), was loaded with a known solution of RMP in 1 mL of the equilibrating buffer. The elution rate was 10 mL/h, and the column effluent was monitored spectrophotometrically at 280 nm. The elution pattern displayed a first peak representing the free receptor and the ligand–RMP complex, followed by a trough (negative peak) whose area provided a measure of the amount of ligand bound to the receptor.[78]

UV Difference Spectral Measurements. UV absorptiometry at constant temperature was performed in a Cary 118 double-beam spectrophotometer, equipped with thermostatted reference and sample compartments. In a typical experiment, the peptide solutions were placed in two-celled cuvettes. On the reference side, one side of the cuvette held a solution of [Leu]-enkephalin in a 10^{-2} M sodium phosphate buffer, and the other side held an equally buffered solution of RMP. On the sample side, one side held [Leu]-enkephalin and RMP mixed at the same concentrations as in the reference cuvette, and the other held buffer. The mixture was allowed to react to equilibrium in the spectrophotometer, and the difference spectrum was recorded. Equilibrium constants were derived from the variations at distinct wavelengths of the difference spectra with varying ligand concentrations according to a method described by Rosotti and Rosotti.[42]

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Registry No. RMP, 87937-17-5; [Leu]-enkephalin, 58822-25-6; [Met]-enkephalin, 58569-55-4; des-Gly_3-[Leu]-enkephalin, 60254-81-1; $dynorphin_{1-8}$, 75790-53-3; *all*-D-[Leu]-enkephalin, 87921-92-4; [Phe_1,Leu]-enkephalin, 60254-86-6; des-NH_2-[Leu]-enkephalin, 87902-07-6; des-NH_2-[Phe_1,Leu]-enkephalin, 87902-08-7; N^{α}-Boc-(1-^{14}C)-Gly-OH, 66025-27-2; (3-*Gly*-1-^{14}C)-[Leu]-enkephalin, 87902-09-8; H-His-Phe-Arg-Trp-OH, 4289-02-5; Boc-Orn(Pht)-OH, 87902-10-1; Boc-Orn(Z)-OH, 2480-93-5; Boc-Asp(OBzl)-Pro-OH, 68939-29-7; Boc-Ser(Bzl)-Pro-OH, 87902-11-2; Boc-Asp(OBzl)-OH, 7536-58-5; Boc-Ser(Bzl)-OH, 23680-31-1; H-Pro-OMe, 2577-48-2; Boc-Asp(OBzl)-Pro-OMe, 87902-12-3; Boc-Ser(Bzl)-Pro-OMe, 76947-97-2; Boc-Glu(OBzl)-O^-·Me_4N^+, 87902-14-5; [$Orn_{1,7,24,30}$]RMP, 87937-16-4.

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Replacement of the Peptide-Backbone Amides Connecting Tyr-Gly and Gly-Gly in Leucine-enkephalin with Ketomethylene Groups: Synthesis and Biological Activity

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A peptide analogue of Leu-enkephalin was synthesized in which the amide linkages between Tyr-Gly and Gly-Gly were replaced by ketomethylene groups. The resulting analogue, **12**, had $^1/_{4000}$th and $^1/_{2400}$th the opiate receptor binding activity of Leu-enkephalin when (^3H)[D-Ala^2,D-Leu^5]enkephalin and (^3H)naloxone, respectively, were used as tritiated ligands. When tested for analgesia in mice by the tail-flick assay, **12** produced analgesia in 50% of the mice tested at a dose of 24.3 μg/mouse (icv), while the ED_{50} of Leu-enkephalin is 240 μg/mouse (icv). At a dose of 40 μg/mouse (icv) or higher, **12** caused convulsions in a dose-dependent manner. No analgesia was observed after intravenous (iv) administration of 240 μg/mouse of **12**.

Several theories have been advanced concerning the contribution of the peptide-backbone amide groups in enkephalins to proper binding to the analgesic receptor. In 1980, Hudson et al.[1] proposed a conformational model for the binding of Met-enkephalin to the analgesic receptor site. This model was supported by the analgesic receptor binding activity of some Met-enkephalin analogues that they had synthesized. In this model the peptide backbone was involved in receptor binding at only two points. These binding points were the terminal amine group and the carbonyl group that is part of the amide linkage connecting the two glycines.

Additional support for the Hudson et al.[1] model has been provided by studies with enkephalin analogues with trans olefin groups in place of peptide-backbone linkages.

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The lack of importance of the Tyr-Gly amide bond for receptor binding was demonstrated by Hann et al.[2] when they replaced this bond with a trans carbon–carbon double bond in Leu-enkephalin, and the resulting analogue had equivalent activity to Leu-enkephalin in the analgesic receptor binding assay. The importance of the Gly-Gly amide linkage to enkephalin activity is demonstrated by the studies of Cox et al.[3] on an enkephalin analogue, Tyr-Gly-Gly-Phe-Pro-NH_2, with a trans carbon–carbon double bond replacing the Gly-Gly amide linkage. The resulting analogue had only 0.1% of the activity of its model pentapeptide in the electrically stimulated guinea pig ileum preparation.

Recently, Almquist et al.[4] substituted a ketomethylene ($COCH_2$) linkage in place of the peptide amide linkage between Phe-Gly in the angiotensin converting enzyme (ACE) inhibitor Bz-Phe-Gly-Pro. The resulting analogue was 100 times more potent as an ACE inhibitor than the corresponding tripeptide. In addition, metabolism studies in rats[5] show that this ketomethylene tripeptide analogue is not cleaved by the peptidases of the gastrointestinal tract or in the serum after oral or intravenous administration, respectively. These results indicate that the replacement of peptide amide linkages with ketomethylenes is a viable method to stabilize biologically active peptides to peptidase cleavage and thus yield orally active peptides.

This paper describes the synthesis of a Leu-enkephalin analogue with ketomethylene groups incorporated between both Tyr-Gly and Gly-Gly. These substitutions should greatly stabilize the Leu-enkephalin molecule to peptidase degradation. Considering the Hudson et al.[1] model for binding of enkephalins to the analgesic receptor site, this new Leu-enkephalin analogue was expected to maintain binding ability similar to Leu-enkephalin.

Chemistry. Scheme I outlines the synthesis of the leucine-enkephalin bis(ketomethylene) analogue **12**. In the initial step, *N*-phthaloyl-*O*-benzyl-L-tyrosine[6] is converted to its acid chloride **1**. Reaction of **1** with the Grignard derivative of 2-(2-bromoethyl)-1,3-dioxolane[4] gave the desired ketone **2** in 63% yield. Formation of alcohol by attack of the excess Grignard derivative on the ketone group did not occur even at room temperature, but rather Grignard attack on the phthalimido carbonyl is the major side reaction.

The ketone is then ketalized with ethylene glycol to compound **3**. The acetal blocking group can then be selectively removed from **3** by using *p*-toluenesulfonic acid in acetone to yield the aldehyde **4**. Compound **4** was then condensed with another molecule of the Grignard derivative of 2-(2-bromoethyl)-1,3-dioxolane to yield **5**. Hydrazinolysis of the phthalimido group in **5** gave the amine **6**. The amino group was then protected with a (trichloroethoxy)carbonyl group, and the resulting compound was oxidized with chromic acid to the desired keto acid **7**. The (trichloroethoxy)carbonyl group was used instead of (benzyloxy)carbonyl for blocking the terminal amino group because it could be attached without first blocking the hydroxyl group, and it is more stable to the acidic Jones oxidation. The keto acid **7** was condensed with L-phenylalanyl-L-leucine methyl ester by using 1-hydroxybenzotriazole and dicyclohexycarbodiimide as coupling reagents to yield the blocked Leu-enkephalin analogue **8**. Stepwise removal of the methyl ester, benzyl ether, ketal, and (trichloroethoxy)carbonyl blocking groups yielded the desired Leu-enkephalin bis(ketomethylene) analogue **12**. The ketal must be cleaved before removing the (trichloroethoxy)carbonyl group. Opening up the ketal when the amino terminus is unblocked does not occur, perhaps because the protonated amine prevents protonation of the ketal oxygen due to repulsion of incoming protons.

Table I. Opiate Receptor Binding Studies with Selected Inhibitors

compound	IC_{50}[a] × 10^{-9}, M	
	(^{3}H)DADL	(^{3}H)naloxone
12	33 000 (3)	180 000 (3)
Leu-enkephalin	8.4 (2)	75 (2)
[D-Ala^2,D-Leu^5]enkephalin	9.0 (2)	

[a] The concentration of test compound required to inhibit the specific binding of tritiated ligand (either (^{3}H) [D-Ala^2,D-Leu^5]enkephalin or (^{3}H)naloxone) by 50%. The number of binding assays that were performed to obtain each value is given in parentheses.

Table II. Proportion of Mice Showing Analgesic Reaction in the Tail-Flick Test After the Intracerebroventricular Administration of 12 and Leu-enkephalin

compound	dose, μg/mouse	mice showing analgesic response[b]		
		2 min[c]	4 min	8 min
12	243[a]			
	60	1/2	1/3	0/3
	48	1/2	1/3	0/3
	40	1/2	1/3	0/3
	24.3	6/12		
	12.2	2/12		
saline		0/8	0/3	0/3
Leu-enkephalin	240	4/6		

[a] This dose produced severe convulsions; therefore, the tail-flick test was not administered. [b] Ratio of mice showing an analgesic response to number of mice tested. [c] Time following injection that mice were tested for analgesia.

Results and Discussion

Table I shows the results of opiate receptor binding studies with compound **12**. It is more than 1000 times less potent than Leu-enkephalin in displacing either (^{3}H)[D-Ala^2,D-Leu^5]enkephalin or (^{3}H)naloxone from the opiate receptor site. Obviously the replacement of both the Tyr-Gly and Gly-Gly amide linkages of Leu-enkephalin with ketomethylene groups has greatly reduced the ability of the resulting analogue (**12**) to bind to the opiate receptor.

These results appear to be in conflict with the model proposed by Hudson et al.[1] for binding of enkephalins to the analgesic receptor site. Compound **12** has the basic structure of Leu-enkephalin, including its terminal amine and the Gly-Gly carbonyl group, but binds very poorly to the opiate receptor site. Perhaps the inclusion of two ketomethylene linkages adjacent to one another in the Leu-enkephalin molecule prevents the resulting analogue (**12**) from attaining the conformation necessary for binding to the opiate receptor site.

Even though **12** binds poorly to the opiate receptor site it still has analgesic activity when given intracerebroventricularly (icv) to mice (Table II). Compound **12** was

(2) Hann, M. M.; Sammes, P. G.; Kennewell, P. D.; Taylor, J. B. *J. Chem. Soc., Trans. Perkin 1* **1982**, 307–314.
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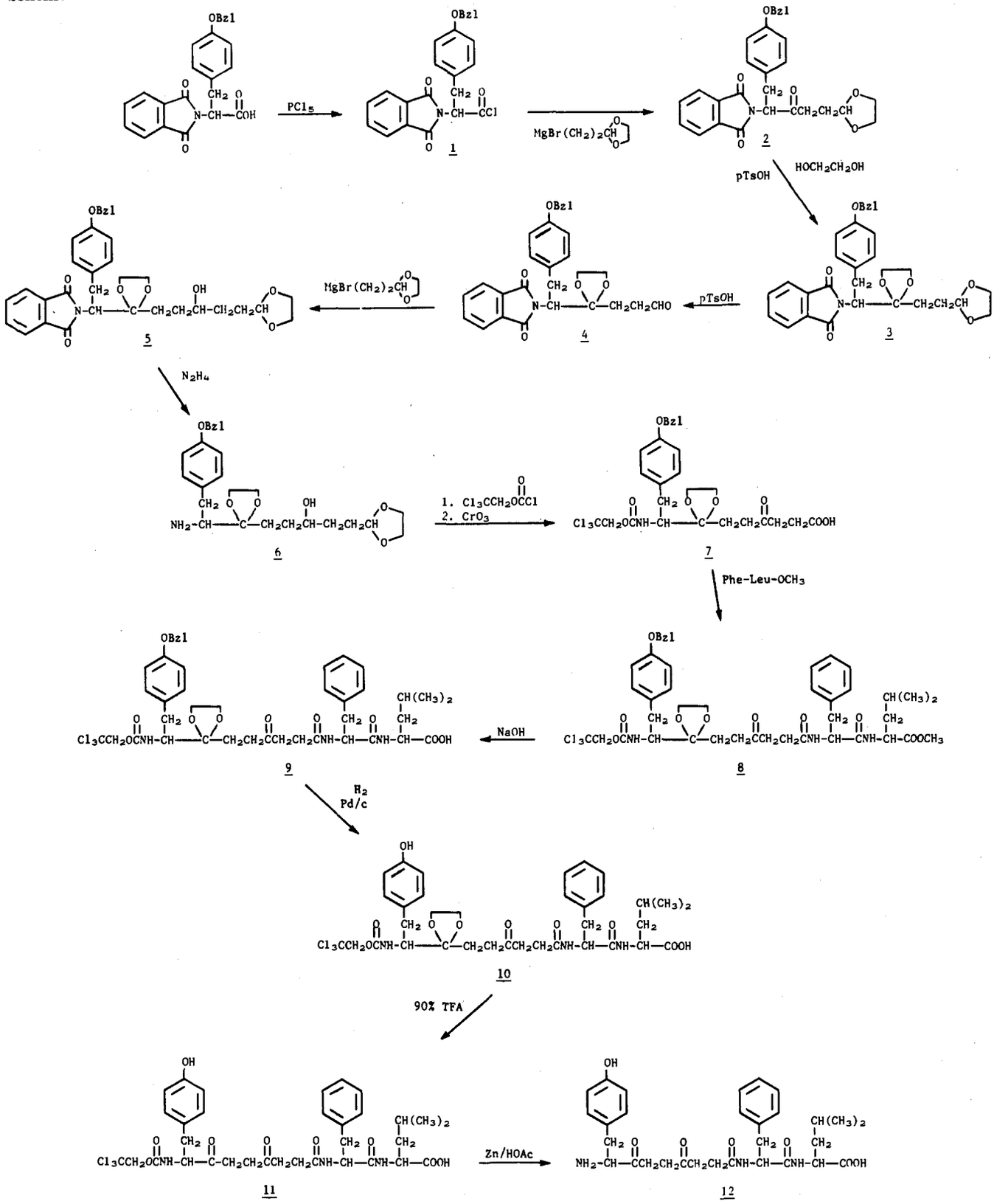
Scheme I



tested in the tail-flick test in mice and produced an analgesic response in 6 out of 12 mice at a dose of 24.3 μg/mouse (icv). Leu-enkephalin showed an analgesic response in four out of six mice at 240 μg/mouse (icv), which was the dose reported by Buscher et al.[7] to be the ED_{50} for Leu-enkephalin in this assay. Compound **12** at doses of 40 μg/mouse (icv) and higher produced convulsions in a dose-dependent manner. One animal out of eight convulsed at the dose level of 40 μg, two out of eight at 48 μg, three out of eight at 60 μg, and six out of six at 243 μg. Because of the convulsions produced by **12** at doses of 40 μg/mouse (icv) and higher, an ED_{50} value for the analgesic effect of **12** was not determined.

In a preliminary test to determine duration of analgesic effect, three groups of eight animals each were given 60,

(7) Buscher, H. H.; Hill, R. C.; Romer, D.; Cardinaux, F.; Closse, A.; Hauser, D.; Pless, J. *Nature (London)* **1976**, *261*, 423–425.

48, and 40 μg/mouse (icv), respectively, and tested for analgesia at 2, 4, and 8 min after treatment. At these lower doses the convulsions were short term enough to allow us to pick mice for analgesic testing that were not in a convulsive state during the time they were tested. Table II shows that no significant prolongation of analgesic effect was seen. Because of the convulsions and compound limitation, these studies were not repeated.

In another preliminary study, nine mice were injected with 240 μg/mouse intravenously and given the tail-flick test 5, 10, and 15 min after treatment. No appreciable analgesic effect or convulsion was seen in any of these animals. Compound limitation prevented further studies at higher doses.

These results demonstrate that even though compound **12** has less than $^1/_{10000}$th the opiate receptor binding ability of Leu-enkephalin, it still has approximately 10 times the analgesic activity of Leu-enkephalin. The low analgesic activity of Leu-enkephalin at 2 min following icv injection in mice is due to its rapid degradation by various peptidases in the brain. Compound **12**, on the other hand, is greatly stabilized to such peptidase degradation by its ketomethylene linkages. Assuming the analgesic activity seen with compound **12** is due to its binding with the enkephalin receptor, one explanation for this surprising in vivo activity is that **12** is approximately 10 000 times more stable to peptidase degradation than Leu-enkephalin.

Experimental Section

Melting points were determined on a Thomas-Hoover Uni-melt and are uncorrected. Optical rotations were measured on a Perkin-Elmer 141 automatic polarimeter. Mass spectra were taken with either an LKB 9000 GC-MS spectrometer or a Reibermag Model R-10-10-C using desorption chemical ionization. ^{1}H NMR spectra were taken with a Varian EM 390 spectrometer. ^{13}C NMR spectra were taken on a Varian XL-100 FT (25.17 MHz) spectrometer or on a Nicolet 300 FT (75.46 MHz) spectrometer. Evaporations were performed at 40 °C under house vacuum (35 mmHg) on a Büchi rotovapor unless otherwise stated. Elemental analyses were conducted by Eric Meier, Stanford University, Palo Alto, CA, or by Galbraith Laboratories, Inc., Knoxville, TN. Amino acid analysis was performed by Peninsula Laboratories, Inc., San Carlos, CA. Thin-layer chromatography was carried out on Uniplates from Analtech coated with 250 μm of silica gel GF. Analytical high-performance LC was carried out on a Waters ALC-201 HPLC with UV visualization at 210 nm with a Schoeffel GM770 UV spectrometer. The Waters Radialpak B column in a RCM 100 unit was used for organic phase high-performance LC, and the Waters reverse-phase μBondapak C_{18} (P/N 27324) column was used for aqueous phase analytical high-performance LC. Preparative high-performance LC was performed with the Waters Prep LC/System 500 with silica gel and reverse-phase cartridges or 2.5 cm diameter stainless-steel columns from Waters packed with either normal phase or reverse phase packing material purchased from Waters. Some of the noncrystalline compounds could not be totally freed of solvent even on heating under reduced pressure. The elemental analyses of these recorded with solvent present. The existence of solvents of crystallization was confirmed by ^{1}H NMR whenever possible.

***N*-Phthaloyl-*O*-benzyl-L-tyrosyl Chloride (1).** A mixture of *N*-phthaloyl-*O*-benzyl-L-tyrosine[6] (1.00 g, 2.49 mmol), phosphorous pentachloride (0.570 g, 2.74 mmol), and dry benzene (100 mL) was heated with stirring under N_2 at 55 °C for 1 h. The mixture was evaporated (azeotroping twice with toluene (2 × 50 mL) to a white powder, which was crystallized from toluene–petroleum ether (bp 35–60 °C) to white solid 1: yield 0.976 g (93.0%); mp 134–137 °C; $[\alpha]_D^{20}$ −168° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 3.50 (d, 1, *J* = 10 Hz, PhCH_2CH), 3.52 (d, 1, *J* = 7 Hz, PhCH_2CH), 4.92 (s, 2, OCH_2Ph), 5.27 (dd, 1, *J* = 7 and 10 Hz, CH), 6.77 (d, 2, *J* = 10 Hz, CH_2*Ph*O), 7.04 (d, 2, *J* = 10 Hz, CH_2*Ph*O), 7.32 (s, 5, OCH_2*Ph*), 7.75 (m, 4, phthalimide); MS, *m/e* 419 (M^+); IR 1800 (m, COCl), 1770, 1700 (m, phthalimide) cm^{-1}. Anal. ($C_{24}H_{18}NO_4Cl$) C, H, N, Cl.

2-[5-[4-(Benzyloxy)phenyl]-3-oxo-4(*S*)-phthalimidopentyl]-1,3-dioxolane (2). In an argon atmosphere, a solution of 2-(2-bromoethyl)-1,3-dioxolane (from Tridom Chemical Co.; 13.6 g, 75.0 mmol) in dry THF (125 mL) was added over 1 h to a stirred mixture of magnesium turnings (2.01 g, 82.5 mmol) in dry THF (25 mL) at 35–40 °C. The mixture was then stirred for an additional 45 min at 40–45 °C and transferred with filtering to a dry addition funnel containing dry THF (100 mL). In an argon atmosphere, 160 mL of this solution was added over 45 min to a stirred solution of 1 (15.2 g, 36.2 mmol) in dry THF (500 mL) with external cooling to maintain a reaction temperature of 0–5 °C. After the addition, the mixture was stirred at ambient temperature for 1 h and then poured into 10% NH_4Cl solution (1 L). The mixture was extracted with EtOAc (1 L), and the EtOAc layer was washed successively with 0.1 N NaOH (2 × 500 mL) and saturated NaCl solution (1 L). The EtOAc solution was dried (Drierite) and evaporated to a yellow solid. This solid was crystallized from EtOAc–petroleum ether (bp 35–60 °C) to a pale yellow solid 2: yield 9.07 g; mp 109–111 °C. The mother liquor was evaporated to a yellow solid which was eluted through a silica gel (90–200 mesh, 200 g) column with EtOAc–petroleum ether (bp 35–60 °C), 1:1. After 400 mL of effluent was discarded, the next 400 mL of effluent was collected and evaporated to a light yellow solid, which was crystallized from EtOAc–petroleum ether (bp 35–60 °C) to more white solid 2: yield 2.02 g (total yield 63.1%); mp 111–115 °C; TLC R_f 0.43 [isopropyl acetate–petroleum ether (bp 35–60 °C), 3:2]; $[\alpha]^{25}_D$ −150° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.00 (m, 2, CH_2CH_2CH), 2.59 (t, 2, *J* = 7.5 Hz, $COCH_2CH_2$), 3.47 (m, 2, PhCH_2CH), 3.85 (m, 4, acetal CH_2's), 4.92 (s, 2, OCH_2Ph), 4.95 (m, 2, CH and acetal CH), 6.70 (d, 2 *J* = 9 Hz, CH_2*Ph*O), 7.03 (d, 2, *J* = 9 Hz, CH_2*Ph*O), 7.32 (s, 5, OCH_2*Ph*), 7.68 (m, 4, phthalimide); MS, *m/e* 485 (M^+); IR 1720 (s, ketone) cm^{-1}. Anal. ($C_{29}H_{27}NO_6$) C, H, N.

2-[2-[4-(Benzyloxy)phenyl]-1(*S*)-phthalimidoethyl]-2-[2-(1,3-dioxolan-2-yl)ethyl]-1,3-dioxolane (3). A mixture of 2 (14.2 g, 29.2 mmol), dry toluene (250 mL), ethylene glycol (100 mL), and *p*-toluenesulfonic acid (5.55 g, 29.2 mmol) was stirred and slowly distilled. Each time 50 mL of distillate had collected an additional 50 mL of fresh toluene was added to the reaction mixture. After 2 h and 4 h, more ethylene glycol (2 × 50 mL) was also added to the reaction mixture. After 6 h of this procedure, the reaction was allowed to cool, and the ethylene glycol was separated from the toluene in a separatory funnel. The toluene volume was adjusted to 250 mL and washed successively with 0.1 N NaOH (2 × 200 mL) and saturated sodium chloride solution (200 mL). The ethylene glycol was extracted with EtOAc (200 mL) and washed successively with the above 0.1 N NaOH and saturated sodium chloride solutions. The toluene and EtOAc extracts were combined, dried (Drierite), and evaporated to a dark amber foam. This foam was crystallized from EtOAc to give racemic product (0.678 g; mp 126–127 °C), which was discarded. The mother liquor was purified by preparative high-performance LC on a silica gel cartridge using petroleum ether (bp 35–60 °C)–EtOAc–ethyl propionate, 68:29:2.5, as the eluting solvent. The first 1750 mL of effluent was discarded, and the next 1650 mL was evaporated to an amber foam. This foam was crystallized from EtOAc–petroleum ether (bp 35–60– °C) to give more racemic product (0.742 g), which was discarded. The mother liquor that contained optically active product was evaporated to a gummy amber foam, 3: yield 9.16 g (64.5%); TLC R_f 0.34 [isopropyl acetate–petroleum ether (bp 35–60 °C), 3:2]; $[\alpha]^{23}_D$ −91.3° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.77 (m, 2, CH_2), 2.07 (m, 2, CH_2), 3.10 (dd, 1, *J* = 3.5 and 11 Hz, 1 H of PhCH_2CH), 3.95 (m, 9, acetal CH_2's, ketal CH_2's, and 1 H of PhCH_2CH), 4.52 (dd, 1, *J* = 3.5 and 11 Hz, CH), 4.87 (s, 2, OCH_2Ph), 4.89 (t, 1, *J* = 4.5 Hz, acetal CH), 6.73 (d, 2, *J* = 9 Hz, CH_2*Ph*O), 7.06 (d, 2, *J* = 9 Hz, CH_2*Ph*O), 7.30 (s, 5, OCH_2*Ph*), 7.62 (m, 4, phthalimide); MS, *m/e* 528 (M − H). Anal. ($C_{31}H_{31}NO_7$) C, H, N.

2-[2-[4-(Benzyloxy)phenyl]-1(*S*)-phthalimidoethyl]-2-(3-oxopropyl)-1,3-dioxolane (4). A mixture of 3 (9.16 g, 17.3 mmol), acetone (500 mL), and *p*-toluenesulfonic acid (2.47 g, 13.0 mmol) was stirred at ambient temperature for 16 h. The mixture was evaporated at 25 °C in vacuo to one-half the original volume, poured into EtOAc (750 mL), and washed with saturated $NaHCO_3$ solution (400 mL). The aqueous phase was reextracted with EtOAc (250 mL), and the organic extracts were combined, washed

with saturated NaCl solution (400 mL), dried (Drierite), and evaporated to an amber foam. This foam was purified by preparative high-performance LC on a silica gel cartridge using petroleum ether (bp 35–60 °C)–EtOAc, 2:1, as eluant. The first 1900 mL of effluent was discarded, and the next 1050 mL was evaporated to light yellow gum **4**: yield 5.41 g. The next 1250 mL was evaporated to give 2.01 g of material, which was reacted with acetone (150 mL) and *p*-toluenesulfonic acid (541 mg, 2.84 mmol) for 16 h. After the same workup and purification procedure as above, more **4** was obtained: yield 1.05 g (total yield from **3**, 76.9%); TLC R_f 0.24 [petroleum ether (bp 35–60 °C)–EtOAc, 2:1]; $[\alpha]^{24}_D$ −97.4° (*c* 1.0, $CHCl_3$); ^1H NMR δ 2.39 (s, 4, CH_2's), 3.10 (dd, 1, J = 3.5 and 11 Hz, 1 H of PhCH_2CH), 3.95 (m, 5, ketal CH_2's and 1 H of PhCH_2CH), 4.57 (dd, 1, J = 3.5 and 11 Hz, CH), 4.89 (s, 2, OCH_2Ph), 6.73 (d, 2, J = 9 Hz, CH_2PhO), 7.06 (d, 2, J = 9 Hz, CH_2PhO), 7.30 (s, 5, OCH_2Ph), 7.65 (m, 4, phthalimide), 9.64 (s, 1, CHO); MS calcd for $C_{29}H_{27}NO_6$, 485.1838; found, M^+ 485.1853; IR 1720 (s, aldehyde) cm^{-1}. Anal. ($C_{29}H_{27}NO_6$) C, H, N.

2-[2-[4-(Benzyloxy)phenyl]-1(*S*)-phthalimidoethyl]-2-[5-(1,3-dioxolan-2-yl)-3-hydroxypentyl]-1,3-dioxolane (5). In an argon atmosphere, a solution of 2-(2-bromoethyl)-1,3-dioxolane (1.81 g, 10.0 mmol) in dry THF (15 mL) was added over 20 min to a stirred mixture of magnesium turnings (270 mg, 11.0 mmol) in dry THF (2 mL) at 35–40 °C. The mixture was then stirred for an additional 45 min at 40–45 °C. This amber solution was allowed to cool, and dry THF (20 mL) was added. In an argon atmosphere, 0.8 mL of this solution was added over 30 min via syringe to a stirred solution of **4** (100 mg, 0.206 mmol) in dry THF (5 mL) at 20 °C. After the addition, the mixture was stirred at ambient temperature for 15 min and then poured into 10% NH_4Cl solution (20 mL). The mixture was extracted with EtOAc (25 mL) and the EtOAc extract was washed successively with saturated $NaHCO_3$ solution (25 mL) and saturated NaCl solution (25 mL). The organic layer was dried (Drierite) and evaporated to a clear film. This crude material was purified by preparative silica gel TLC ($CHCl_3$–CH_3OH, 19:1) to give the product (R_f 0.21) as a colorless gum: yield 76.9 mg (63.3%); $[\alpha]^{23}_D$ −89.7° (*c* 1.0 $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.8 (m, 9, alkyl CH_2's and CHO*H*), 3.10 (dd, 1, J = 3.5 and 11 Hz, 1 H of PhCH_2CH), 3.85 (m, 10, acetal CH_2's, ketal CH_2's, C*H*OH, and 1 H of PhCH_2CH), 4.57 (dd, 1, J = 3.5 and 11 Hz, NCH), 4.89 (t, 1, J = 4.5 Hz, acetal CH), 4.92 (s, 2, OCH_2Ph), 6.73 (d, 2, J = 9 Hz, CH_2PhO), 7.06 (d, 2, J = 9 Hz, CH_2PhO), 7.30 (s, 5, OCH_2Ph), 7.65 (m, 4, phthalimide); MS, *m/e* 486 (M − $C_5H_9O_2$), 356 (M − $C_{11}H_{19}O_5$), 231 (M − $C_{23}H_{18}NO_3$); IR 3400 (m, hydroxyl) cm^{-1}. Anal. ($C_{34}H_{37}NO_8$), C, H, N.

2-[1(*S*)-Amino-2-[4-(benzyloxy)phenyl]ethyl]-2-[5-(1,3-dioxolan-2-yl)-3-hydroxypentyl]-1,3-dioxolane (6). A solution of **5** (3.72 g, 6.33 mmol) and hydrazine (1.62 g, 50.6 mmol) in absolute ethanol (100 mL) was refluxed under N_2 for 15 h. The mixture was evaporated to a semisolid residue. This residue was dissolved in CH_2Cl_2 (300 mL) and 0.3 N NaOH (300 mL). After the solution was shaken, the CH_2Cl_2 layer was collected, and the aqueous phase was reextracted with CH_2Cl_2 (300 mL). The CH_2Cl_2 extracts were combined, washed with water (300 mL), dried (Drierite), and evaporated to a pale yellow oil: yield 2.88 g (99.3%); TLC R_f 0.16, ninhydrin absorbing ($CHCl_3$–CH_3OH, 97:3); $[\alpha]^{20}_D$ −11.9° (*c* 0.31, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.7 (m, 11, alkyl CH_2's, CHO*H* and NH_2), 2.28 (dd, 1, J = 3 and 14 Hz, 1 H of PhCH_2CH), 3.0 (m, 2, H_2NCH and 1 H of PhCH_2CH), 3.55 (m, 1, C*H*OH), 3.88 (m, 4, acetal CH_2's), 4.00 (s, 4, ketal CH_2's), 4.87 (t, 1, J = 4.5 Hz, acetal CH), 5.02 (s, 2, OCH_2Ph), 6.87 (d, 2, J = 9 Hz, CH_2PhO), 7.12 (d, 2, J = 9 Hz, CH_2PhO), 7.35 (m, 5, OCH_2Ph); MS calcd for $C_{26}H_{34}NO_6$, 456.2386; found, M^+ 456.2422. Anal. ($C_{26}H_{35}NO_6$) H, N; C: calcd, 68.25; found, 66.65.

2-[2-[4-(Benzyloxy)phenyl]-1(*S*)-[[(2,2,2-trichloroethoxy)carbonyl]amino]ethyl]-2-(5-carboxy-3-oxopentyl)-1,3-dioxolane (7). To a stirred solution of **6** (2.51 g, 5.49 mmol) and *N,N*-diisopropylethylamine (942 mg, 7.29 mmol) in CH_2Cl_2 (50 mL) under N_2 at 0 °C was added 2,2,2-trichloroethyl chloroformate (1.45 g, 6.86 mmol) dropwise. Stirring was continued at 0 °C for 15 min, and the mixture was then reduced in volume by evaporation at 20 °C in vacuo to a volume of approximately 5 mL. Acetone (100 mL) was added, and the mixture was reduced in volume as before. Acetone (500 mL) was added, the mixture was cooled in an ice bath with stirring, and an ice-cold solution of chromium trioxide (6.04 g, 60.4 mmol) in 35% sulfuric acid (230 mL) was added dropwise. The mixture was stirred for 30 min at 0 °C following the addition and was then poured into a separatory funnel containing $CHCl_3$ (1 L) and ice (500 g) and shaken. The $CHCl_3$ layer was collected, and the aqueous phase was reextracted with $CHCl_3$ (1 L). The $CHCl_3$ extracts were combined and washed successively with H_2O (2 × 1 L) and saturated NaCl solution (1 L). The $CHCl_3$ layer was dried (Na_2SO_4) and evaporated to an amber gummy foam. This crude material was purified on a silica gel (90–200 mesh, 300 g) column using $CHCl_3$–CH_3OH–acetic acid, 95:5:0.1, as eluant. Fractions corresponding to product were combined, evaporated, and evaporated successively from toluene (2 × 10 mL) and Et_2O (2 × 10 mL) to give a white solid foam **7**: yield 1.95 g (58.9%); TLC R_f 0.57 ($CHCl_3$–CH_3OH–acetic acid, 90:10:0.5); $[\alpha]^{28}_D$ −18.0° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.5 (m, 9, alkyl CH_2's and 1 H of PhCH_2CH), 3.97 (s, 4, ketal CH_2's), 4.10 (m, 1, 1 H of PhCH_2CH), 4.55 (s, 2, Cl_3CCH_2O), 4.95 (s, 2, OCH_2Ph), 5.13 (d, 1, J = 10 Hz, NH), 6.81 (d, 2, J = 9 Hz, CH_2PhO), 7.08 (d, 2, J = 9 Hz, CH_2PhO), 7.37 (s, 5, OCH_2Ph), 7.8 (s, 1, COOH); MS, *m/e* 472 (M − $C_6H_9O_3$), 453 (M − $C_2H_2Cl_3$ − OH), 201 (M − $C_{18}H_{17}Cl_3NO_3$); IR 1750 and 1720 (s, ketone and carbamate), 1610 (m, carboxylate) cm^{-1}; ^{13}C NMR ($CDCl_3$) δ (peak height, mm) 27.7 (59), 28.2 (58), 34.5 (39), 36.6 and 37.7 (81, 77, H_2CCOCH_2), 56.9 (40), 65.5 and 65.7 (81, 90, ketal CH_2's), 69.9 (120, OCH_2Ph), 74.1 (OCH_2CCl_3), 95.8 (8, CCl_3), 110.9 (35), 114.7 (195), 127.3 (210), 127.7 (111), 128.4 (173), 129.9 (187), 130.2 (23), (137.0 (72), 154.4 [23, OC(O)NH], 157.3 (39), 177.5 [38, C(O)OH], 208.0 [27, CC(O)C]. Anal. ($C_{27}H_{30}Cl_3NO_8$·0.08$CHCl_3$) C, H, N, Cl.

5-[2-[2-[4-(Benzyloxy)phenyl]-1(*S*)-[[(2,2,2-trichloroethoxy)carbonyl]amino]ethyl]-1,3-dioxolan-2-yl]-3-oxopentanoyl-L-phenylalanyl-L-leucine Methyl Ester (8). To an ice-cold, stirred solution of **7** (2.04 g, 3.38 mmol), L-phenylalanyl-L-leucine methyl ester hydrochloride (2.56 g, 7.78 mmol), 1-hydroxybenzotriazole (555 mg, 3.72 mmol), and triethylamine (786 mg, 7.77 mmol) in CH_2Cl_2 (27 mL) was added a solution of dicyclohexylcarbodiimide (DCC; 766 mg, 3.71 mmol) in CH_2Cl_2 (6 mL) dropwise. The mixture was stirred for 64 h at ambient temperature and then cooled to 0 °C, and acetic acid (0.5 mL) was added to convert excess DCC to dicyclohexylurea (DCU). The suspension was filtered, and the filtrate was adjusted to 200 mL with CH_2Cl_2. This solution was washed successively with ice cold 2 N HCl (200 mL), 0.3 N NaOH (200 mL), and H_2O (2 × 200 mL). The CH_2Cl_2 layer was dried (Na_2SO_4) and evaporated to a straw-colored solid. This solid was dissolved in EtOAc (20 mL), cooled, and filtered (remove DCU). The filtrate was crystallized by addition of petroleum ether (bp 35–60 °C) to give white solid **8**: yield 892 mg; mp 136–138 °C. The mother liquor was purified by preparative high-performance LC on a silica gel cartridge using EtOAc–petroleum ether (bp 35–60 °C)–2-propanol, 1:1:0.02, as eluant. The first 2.45 L of effluent was discarded, and the next 1.7 L was evaporated and crystallized as above to give more white **8**: yield 763 mg (total yield from **7**, 55.9%); mp 135–137 °C; TLC R_f 0.27 [EtOAc–petroleum ether (bp 35–60 °C)–2-propanol, 1:1:0.04]; $[\alpha]^{23}_D$ −33.1° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.86 (m, 6, leucine CH_3's), 3.65 (s, 3, ester CH_3), 3.97 (s, 4, ketal CH_2's), 4.55 (s, 2, Cl_3CCH_2O), 4.97 (s, 2, OCH_2O), 5.16 (d, 2, J = 10 Hz, carbamate NH), 6.26 and 6.46 (2 d, 2, J = 8 Hz each, amide NH's), 6.81 (d, 2, J = 9 Hz, CH_2PhO), 7.08 (d, 2, J = 9 Hz, CH_2PhO), 7.23 (s, 5, phenylalanine phenyl), 7.36 (s, 5, OCH_2Ph); MS, *m/e* 727 (M − $C_2H_2Cl_3$ − OH). Anal. ($C_{43}H_{52}Cl_3N_3O_{10}$·0.5$H_2O$) C, H, N, Cl.

5-[2-[2-[4-(Benzyloxy)phenyl]-1(*S*)-[[(2,2,2-trichloroethoxy)carbonyl]amino]ethyl]-1,3-dioxolan-2-yl]-3-oxopentanoyl-L-phenylalanyl-L-leucine (9). To a stirred solution of **8** (673 mg, 0.768 mmol) in THF (6.1 mL) was added 1 N NaOH (1.15 mL). The resulting emulsion was stirred vigorously 1.5 h. The mixture was diluted with H_2O (15 mL) and acidified to pH 2 with dilute HCl, and the THF was evaporated under reduced pressure. The resulting gum was extracted with CH_2Cl_2 (3 × 75 mL), and the organic phases were combined and then washed with saturated NaCl solution. The CH_2Cl_2 layer was dried (Na_2SO_4) and evaporated to an off-white foam, which was crystallized from $CHCl_3$–ether to white solid **9**: yield 330 mg; mp 126–129 °C. The mother liquor was purified by preparative high-performance LC on a reverse-phase column (2.5 × 30 cm, C_{18}/Porasil B) using

CH_3OH–0.05% TFA in H_2O, 3:1, as eluant. The first 680 mL of effluent were discarded, and the next 180 mL was evaporated to a white foam, which was crystallized from $CHCl_3$–ether to more white solid **9**: yield 84.8 mg (total yield from **8**, 62.6%); mp 126–129 °C; TLC R_f 0.52 ($CHCl_3$–CH_3OH–acetic acid, 9:1:0.1); $[\alpha]^{21}_D$ −41.0° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.86 (m, 6, leucine CH_3's), 3.95 (s, 4, ketal CH_2's), 4.55 (s, 2, Cl_3CCH_2O), 4.97 (s, 2, OCH_2Ph), 6.78 (d, 2, *J* = 9 Hz, CH_2PhO), 7.05 (d, 2, *J* = 9 Hz, CH_2PhO), 7.15 (s, 5, phenylalanine phenyl), 7.32 (s, 5, OCH_2Ph), 9.30 (1, COOH); MS, *m/e* 472 (M − $C_{21}H_{29}N_2O_5$). Anal. ($C_{42}H_{50}Cl_3N_3O_{10}$) C, H, N, Cl.

5-[2-[2-(4-Hydroxyphenyl)-1(*S*)-[[(2,2,2-trichloroethoxy)carbonyl]amino]ethyl]-1,3-dioxolan-2-yl]-3-oxopentanoyl-L-phenylalanyl-L-leucine (10). A mixture of **9** (521 mg, 0.603 mmol) and 10% Pd/C (521 mg) in glacial acetic acid (25 mL) were stirred at room temperature under 1 atmosphere of hydrogen for 6.5 h. The mixture was then filtered through Celite and evaporated in vacuo at 30 °C (azeotroping with toluene) to an off-white foam. This foam was crystallized from $CHCl_3$ to white solid **10**: yield 237 mg (50.9%); mp 140 °C; TLC R_f 0.40 ($CHCl_3$–CH_3OH–acetic acid, 9:1:0.1); $[\alpha]^{20}_D$ −21.1° (*c* 1.0, EtOH); ^1H NMR (acetone-d_6) δ 0.91 (d, 6, *J* = 4.5 Hz, leucine CH_3's), 4.00 (s, 4, ketal CH_2's), 4.60 (s, 2, Cl_3CCH_2O), 6.67 (d, 2, *J* = 9 Hz, CH_2PhO), 7.06 (d, 2, *J* = 9 Hz, CH_2PhO), 7.22 (s, 5, phenylalanine phenyl). Anal. ($C_{35}H_{44}Cl_3N_3O_{10}$), C, H, N, Cl.

4,7-Dioxo-9-(4-hydroxyphenyl)-8(*S*)-[[(2,2,2-trichloroethoxy)carbonyl]amino]nonanoyl-L-phenylalanyl-L-leucine (11). A solution of **10** (234 mg, 0.303 mmol) in 9:1 trifluoroacetic acid–water (2.3 mL) was stirred at room temperature for 6 h. The mixture was evaporated at 30 °C (azeotroping with water) to give a light amber solid foam, which was crystallized from $CHCl_3$ to give colorless solid **11**: yield 166 mg (75.1%); mp ~80 °C; TLC R_f 0.50 ($CHCl_3$–CH_3OH–acetic acid, (9:1:0.1); $[\alpha]^{20}_D$ −23.7° (*c* 1.0, EtOH); ^1H NMR (acetone-d_6) δ 0.94 (d, 6, *J* = 4.5 Hz, leucine CH_3's), 4.72 (s, 2, Cl_3CCH_2O), 6.72 (d, 2, *J* = 9 Hz, CH_2PhO), 7.09 (d, 2, *J* = 9 Hz, CH_2PhO), 7.24 (s, 5, phenylalanine phenyl). Anal. ($C_{33}H_{40}Cl_3N_3O_9 \cdot 0.6CHCl_3$) C, H, N, Cl.

8(*S*)-Amino-4,7-dioxo-9-(4-hydroxyphenyl)nonanoyl-L-phenylalanyl-L-leucine Trifluoroacetate (12). A mixture of **11** (134 mg, 0.184 mmol) and activated zinc (100 mg) was stirred at room temperature in glacial acetic acid (1 mL) for 2.5 h. The mixture was filtered through Celite, and the filtrate was evaporated to a tan foam. This foam was purified by preparative high-performance LC on a reverse-phase column (2.5 × 30 cm, C_{18}/Porasil B) using 0.05% TFA in H_2O–CH_3OH, 11:9, as eluant. The first 200 mL of effluent was discarded, and the next 400 mL was evaporated at 30 °C to give a colorless glass. This glass was dissolved in 0.05% TFA in H_2O (20 mL) and lyophilized to fluffy white **12**: yield 59.3 mg (52.6%); TLC R_f 0.30 ($CHCl_3$–CH_3OH–acetic acid, 17:3:0.1), 0.72 (butanol–acetic acid–H_2O, 10:3:1); $[\alpha]^{22}_D$ −13.5° (*c* 0.17, H_2O); ^1H NMR (D_2O, 300 MHz) (DHO peak at δ 4.8) δ 0.86 (m, 6, leucine CH_3's), 1.58 [m, 3, $CH_2CH(CH_3)_2$], 2.42 (m, 2), 2.85 (m, 6), 3.08 (m, 2), 3.35 (m, 2), 4.27 (m, 1), 6.88 (d, 2, *J* = 9 Hz, CH_2PhOH), 7.17 (d, 2, *J* = 9 Hz, CH_2PhOH), 7.28 (m, 5, phenylalanine phenyl); ^{13}C NMR (D_2O) δ (peak height, mm) 20.6, 22.2 (78, 89, leucine CH_3's), 24.4 [105, leucine $CH(CH_3)_2$], 29.1 (42), 33.5 (56), 34.7 (44), 35.6 (36), 37.0 (82), 39.5 (84), 51.7 (24), 54.9 (35), 60.1 (49), 116.1, 116.2, 125.5, 127.0, 127.1, 128.8, 129.0, 129.2, 129.5, 130.8, 136.4, (43, 48, 44, 24, 36, 51, 23, 52, 22, 41, 49, aromatic C's), 155.3 (39, tyrosine C-OH), 163.2 (m, CF_3CO_2), 173.2, 174.8, 176.1 (42, 35, 30, amide and carboxyl CO's), 206.6, 212.0 (41, 38, ketone CO's). Anal. ($C_{30}H_{39}N_3O_7 \cdot CF_3COOH \cdot 1.5H_2O$) C, H, N. Amino acid analysis: Phe, 1.01; Leu, 0.99.

Opiate Receptor Binding Assay. Binding assays were performed essentially as described by Pasternak et al.[8] Male Sprague–Dawley rats (175–200 g) were decapitated, and the brains were rapidly removed. Whole brains minus cerebella were suspended in 40 vol of ice-cold 50 mM Tris-HCl, pH 7.7 (at 25 °C). The tissue was homogenized in a Brinkman polytron and centrifuged at 4 °C for 15 min at 40000*g*. The pellets were suspended to a concentration of 10 mg of original tissue/mL in Tris buffer and incubated at 37 °C for 1 h. The tissue was then centrifuged at 4 °C and resuspended in fresh Tris buffer (containing 50 μg/mL of bacitracin or prevent proteolytic breakdown of the leucine enkephalin) at 10 mg of tissue/mL. Receptor-binding incubations contained 1.8 mL of tissue suspension, either (^3H)naloxone (0.8 nM) or (^3H)-D-Ala-D-Leu-enkephalin (1.0 nM), and unlabeled drugs in a total volume of 2.0 mL. The tubes were incubated at 25 °C for 40 min and then placed on ice for an additional 10 min. The samples were then filtered rapidly through Whatman GF/B filters, followed by two 4-mL buffer washes. Radioactivity bound to filters was determined by liquid scintillation spectrometry.

Tail-Flick Test of Analgesia. A standard tail-flick test[9] was used for bioassay of **12** and Leu-enkephalin in mice. Male mice of the Swiss-Webster strain weighing 19 to 22 g wre given two predrug tests, administered 30 min apart. The mean of the two reaction times of each animal was considered to be its base-line score. The test substances were dissolved in sterile saline, and for intracerebroventricular injections, a volume of 4 μL/mouse was administered. The accuracy of injections was confirmed macroscopically with the aid of India ink.[10] Since Büscher et al.[7] reported that intracerebroventricularly administered Leu-enkephalin was maximally active 2 min after injection, the two peptides were evaluated 2 min after treatment. (Our preliminary study to determine the duration of the analgesic effect of intracerebroventricularly administered **12** indicated that the greatest percentage of mice showed analgesic response at 2 min after injection rather than 4 or 8 min later.) In accordance with the criterion stated by Büscher et al.,[7] an animal was considered to have shown analgesic response if its reaction time was more than 75% of its base-line score. The mean base-line reaction time for the mice tested was 2.76 ± 0.40 s.

Registry No. 1, 88036-14-0; **2**, 88036-15-1; **3**, 88036-16-2; **4**, 88036-17-3; **5**, 88036-18-4; **6**, 88036-19-5; **7**, 88036-20-8; **8**, 88036-21-9; **9**, 88036-22-0; **10**, 88036-23-1; 11, 88036-24-2; **12**, 88036-25-3; *N*-phthaloyl-*O*-benzyl-L-tyrosine, 2130-97-4; 2-(2-bromoethyl)-1,3-dioxolane, 18742-02-4; ethylene glycol, 107-21-1; 2,2,2-trichloroethyl chloroformate, 17341-93-4; L-phenylalanyl-L-leucine methyl ester hydrochloride, 38155-45-2.

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