the 35 Bijvoet pairs for the 2R,3R enantiomer of (-)-5·HBr and the 2R,3S enantiomer of (-)-7·HCl, for which $|F_c(h,k,l) - F_c(h,-k,l)| > 1.1$ for (-)-5·HBr and 1.8 for (-)-7·HCl, 68 and 33 of the Fc differences, respectively, had the same sign as the corresponding F_o differences. The weighting scheme used in the later part of the refinement was $w = 1/[1 + ((|F_o| - A)/B)^2]$,[30] where $A = 10$ and $B = 9$ for (-)-5·HBr and $A = 8$ and $B = 4$ for (-)-7·HCl. The form factors used were those given by Cromer and Mann.[31] All calculations have been performed on a DEC-system-10 computer using mainly the X-ray 72 program system.[32]

The molecular conformations and atomic labeling schemes are shown in Figure 1.

Molecular Mechanics Calculations. The structural modelling was performed by use of the interactive computer graphics program MIMIC (methods for interactive modelling in chemistry).[16] Calculations were performed on a VAX 11/780 computer using Allingers MMP2 force field,[33] to which had been added parameters for the phenol[34] and amino groups.[35] Computational times ranged from 1 to 25 min/minimization.

Pharmacology. Materials and Methods. Male Sprague-Dawley rats weighing 200-300 g (ALAB, Stockholm, Sweden) were used. Reserpine and haloperidol were dissolved in a few drops of glacial acetic acid and made up to volume with 5.5% glucose solution. The other test compounds were dissolved in saline immediately before use. Injection volumes were 5 mL/kg, and injection solutions had approximately neutral pH.

Biochemistry. Brain levels of DOPA and 5-HTP were analyzed by HPLC with electrochemical detection.[21] For biochemical results and experimental details, see Tables III and IV and footnotes *a* in Tables III and IV.

Locomotor Activity. The motor activity was measured by means of photocell recordings ("M/P 40 Fc Electronic Motility Meter", Motron Products, Stockholm, Sweden) as previously described.[17] For experimental details, see footnotes *b* in Tables III and IV. Each box was equipped with a semitransparent mirror that allowed gross behavior observations of the animals during the experiments. The motor activity results are shown in Tables III and IV.

Acknowledgment. We thank Ingrid Bergh, Lucia Gaete, and Boel Göransson for skillful assistance in the pharmacological testing, Christer Sahlberg and Peter Konradsson for the capillary GC data, Bengt Andersson for the HPLC data, Berit Backlund-Höök for drawing the figures, and Tommy Liljefors and Robert E. Carter for providing access to the MIMIC program. The financial support from Astra Läkemedel AB, The Swedish Board for Technical Development, The Swedish Medical Research Council (No. 155), The Swedish Natural Science Research Council, Centrala Försöksdjursnämnden, Ingabritt och Arne Lundbergs Forskningsstiftelse, The Swedish Academy of Pharmaceutical Sciences, IF's Stiftelse and the Medical Faculty, University of Göteborg, is gratefully acknowledged.

Supplementary Material Available: Complete lists of identified low-energy conformations and steric energies and X-ray data of (-)-5·HBr and (-)-7·HCl, including positional and thermal parameters, bond lengths, and bond angles (6 pages); tables of observed and calculated structure factors (61 pages). Ordering information is given on any current masthead page.

(30) Mills, O. S.; Rollet, J. S. In *Computing Methods and the Phase Problem in X-ray Crystal Analysis*; Pergamon: London, 1961; pp 107-124.
(31) Cromer, D. T.; Mann, J. B. *Acta Crystallogr., Sect. A.* **1968**, *A24*, 321.
(32) Stewart, J. M.; Kruger, G. J.; Ammon, H. L.; Dickinson, C.; Hall, S. R. The *X*-ray System, version of June 1972; Technical Report TR-192, Computer Science Center, University of Maryland, College Park, MD, 1972.
(33) Allinger, N. L.; Yuh, Y. *Quantum Chem. Program Exchange* **1980**, *12*, 395.
(34) Dodziuk, H.; von Voitenberg, H.; Allinger, N. L. *Tetrahedron* **1982**, *38*, 2811.
(35) Profeta, S., Jr.; Allinger, N. L. *J. Am. Chem. Soc.* **1985**, *107*, 1907.
(36) Carlsson, A.; Kehr, W.; Lindqvist, M. *J. Neural Transm.* **1977**, *40*, 99.

N,N-Dialkylated Leucine Enkephalins as Potential δ Opioid Receptor Antagonists[1]

Jane A. Lovett[2] and Philip S. Portoghese*

Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, Minnesota 55455.
Received November 12, 1986

A series of N,N-dialkylated leucine enkephalins were prepared in order to study the effect of substitution on antagonist activity at the δ opioid receptor. The target peptides **1-7** were evaluated in the mouse vas deferens (MVD) and guinea pig ileum (GPI) at 1 μM. All of the compounds except [*N*,*N*-di-2-phenethyl,Leu^5]enkephalin (**7**) showed antagonist activity in the MVD against the δ receptor agonist [D-Ala^2,D-Leu^5]enkephalin. The most potent congener, [*N*,*N*-dibenzyl,Leu^5]enkephalin (**3**), was 2.5-fold more potent than [*N*,*N*-diallyl,Leu^5]enkephalin (**1**). None of the compounds at 1 μM showed any antagonist activity against agonists for other receptor types. The *N*,*N*-di-2-phenethyl (**7**) and *N*,*N*-dioctyl (**6**) analogues showed significant agonist activity at 1 μM in the MVD.

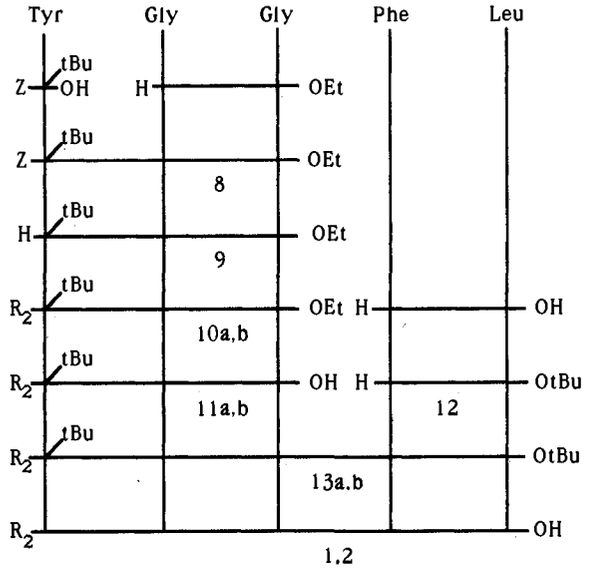
Since Martin and co-workers[3,4] reported evidence for multiple opioid receptors, a considerable amount of research has focused on the development of ligands specific for each of the receptor types. Such specific ligands, especially antagonists, can be very useful biochemical tools, both for determining differences between receptor types and for studying their roles in various biological processes.

The discovery of the enkephalins[5] introduced a new class of ligands for the opioid receptor and another receptor type, the δ receptor.[6] Because the classical alkaloid opiate antagonists, such as naloxone and naltrexone, interact preferentially with the μ receptor,[7,8] attention was turned

(1) Presented, in part, at the 192nd National Meeting of the American Chemical Society, Anaheim, CA, September 7-11, 1986; MEDI 73.
(2) Present address: Department of Chemistry, California State University Sacramento, Sacramento, CA 95819.
(3) Martin, W. R.; Eades, C. G.; Thompson, J. A.; Huppler, R. E.; Gilbert, P. E. *J. Pharmacol. Exp. Ther.* **1976**, *197*, 517-532.
(4) Gilbert, P. E.; Martin, W. R. *J. Pharmacol. Exp. Ther.* **1976**, *198*, 66-82.
(5) Hughes, J.; Smith, T. W.; Kosterlitz, H. W.; Fothergill, L. A.; Morgan, B. A.; Morris, H. R. *Nature (London)* **1975**, *258*, 577-579.
(6) Lord, J. A. H.; Waterfield, A. A.; Hughes, J.; Kosterlitz, H. W. *Nature (London)* **1977**, *267*, 495-499.
(7) Kosterlitz, H. W.; Paterson, S. J. *Proc. R. Soc. London, B* **1980**, *210*, 113-122.
(8) Wood, P. L.; Charleson, S. E.; Lane, D.; Hudgin, R. L. *Neuropharmacology* **1981**, *20*, 1215-1220.

0022-2623/87/1830-1144$01.50/0

Scheme I



a R = allyl
b R = cyclopropylmethyl

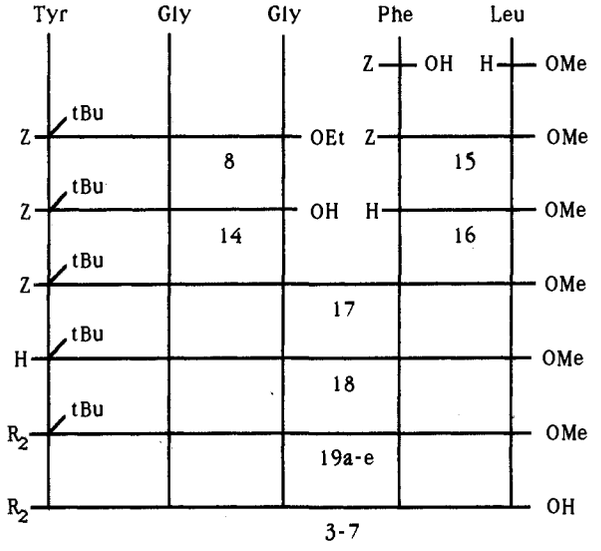
to enkephalin analogues as possible δ receptor antagonists. Since *N*-alkyl groups such as allyl and cyclopropylmethyl confer opioid antagonist properties to these alkaloids, this modification was examined as a way of preparing δ receptor antagonists from the enkephalins. However, introduction of a single such alkyl group at the amine terminus of the enkephalins yielded, at best, weak mixed agonist/antagonists.[9-12] The first highly selective δ receptor antagonist was successfully prepared by Shaw and co-workers[13] by introducing two allyl groups at the amine terminus to give the methyl ester of *N*,*N*-diallyl leucine enkephalin (1). They have prepared additional analogues of 1 with various peptide chain modifications in order to improve potency and stability against enzymatic degradation and to study the differences in structure–activity relationships for agonism vs. antagonism.[13,14]

We have synthesized a series of N,N-dialkylated leucine enkephalins (1–7) to study the effect of substitution on δ receptor antagonism, with the goal of using this information to design irreversible δ antagonists.[15]

R_2Tyr-Gly-Gly-Phe-Leu

1, R = allyl
2, R = cyclopropylmethyl
3, R = benzyl
4, R = *n*-propyl
5, R = *n*-pentyl
6, R = *n*-octyl
7, R = phenethyl

Scheme II



a R = benzyl
b R = n-propyl
c R = n-pentyl
d R = n-octyl
e R = phenethyl

Chemistry

The compounds were prepared in solution by one of two routes, as depicted in Schemes I and II. The initial compounds, where R = allyl (1) and cyclopropylmethyl (2), were prepared according to Scheme I. *N*,*N*-Diallyl leucine enkephalin, 1,[13,14] was prepared in order to evaluate reaction conditions for the preparation of other N,N-dialkylated analogues and as a standard for the biological testing. The *N*,*N*-bis(cyclopropylmethyl) derivative, **2**, was chosen for study because, for the alkaloid antagonists, replacement of the *N*-allyl of naloxone with a cyclopropylmethyl group to give naltrexone results in an increase in receptor binding affinity and antagonist potency.[8,16] The synthesis of these two leucine enkephalin analogues involved a 3 + 2 strategy. Couplings were performed by the mixed-anhydride procedure. Benzyloxycarbonyl groups were used as temporary amine protecting groups and were removed by catalytic transfer hydrogenation.[17] Carboxylic acids were temporarily protected as the alkyl esters, which were removed by alkaline hydrolysis. Both the phenol of tyrosine and the C-terminal carboxylic acid were protected by *tert*-butyl groups, which were removed in the last step by 50% trifluoroacetic acid in CH_2Cl_2 containing anisole as a scavenger. Alkylation was performed on the tripeptide **9**, with excess alkyl bromide and *N*,*N*-diisopropylethylamine in refluxing toluene, according to the procedure of Laguzza and Ganem.[18] After hydrolysis of the esters, the tripeptides **11a** and **11b** were each coupled to **12** to give **13a** and **13b**, respectively, which were deprotected to give the target leucine enkephalin analogues 1 and **2**. Yields for these and other reactions in the preparation of 1 and **2** were generally greater than 80%.

When the bis(cyclopropylmethyl) derivative **2** did not prove to be any more potent as a δ receptor antagonist than *N*,*N*-diallyl leucine enkephalin, 1 (see below), several ad-

(9) Hahn, E. F.; Fishman, J.; Shiwaku, Y.; Foldes, F. F.; Nagashima, H.; Duncalf, D. *Res. Commun. Chem. Pathol. Pharmacol.* **1977**, *18*, 1–9.
(10) Morgan, B. A.; Bower, J. D.; Guest, K. P.; Handa, B. K.; Metcalf, G.; Smith, C. F. C. *5th American Peptide Symposium*; Goodman, M., Meienhofer, J., Eds.; Wiley: New York, 1977; pp 111–113.
(11) Pert, C. B.; Bowie, D. L.; Pert, A.; Morell, J. L.; Gross, E. *Nature (London)* **1977**, *269*, 73–75.
(12) Summers, M. C.; Hayes, R. J. *J. Biol. Chem.* **1981**, *256*, 4951–4956.
(13) Shaw, J. S.; Miller, L.; Turnbull, M. J.; Gormley, J. J.; Morley, J. S. *Life Sci.* **1982**, *31*, 1259–1262.
(14) Belton, P.; Cotton, R.; Giles, M. B.; Gormley, J. J.; Miller, L.; Shaw, J. S.; Timms, D.; Wilkinson, A. *Life Sci.* **1983**, *33* (Suppl 1), 443–446.
(15) Lovett, J. A.; Portoghese, P. S. *J. Med. Chem.*, submitted.
(16) Magnan, J.; Paterson, S. J.; Tavani, A.; Kosterlitz, H. W. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1982**, *319*, 197–205.
(17) Anwer, M. K.; Spatola, A. F. *Synthesis* **1980**, 929–932.
(18) Laguzza, B. C.; Ganem, B. *Tetrahedron Lett.* **1981**, *22*, 1483–1486.

Table I. Agonist and Antagonist Activities of *N,N*-Dialkyl Leucine Enkephalins in the Mouse Vas Deferens (MVD) and Guinea Pig Ileum (GPI)

	MVD[a]			GPI[a]		
		antagonism: IC_{50} ratio[c]			antagonism: IC_{50} ratio[c]	
compd	agonism:[b] % inhibn	DADLE	morphine	agonism:[b] % inhibn	morphine	EK
1	10.0 ± 4.1	2.7 ± 0.6[d]	0.8 ± 0.1	−6.7, 0[e]	0.8, 1.1[e]	0.6, 1.1[e]
2	27.3 ± 1.7	1.9 ± 0.2	1.4 ± 0.5	2.2, 2.5[e]	0.7, 1.0[e]	0.6, 1.2[e]
3	11.4 ± 5.9	6.6 ± 1.4[f]	0.6 ± 0.2	1.9, 2.9	1.0, 1.0	0.8, 1.2
4	11.9 ± 7.2	1.8 ± 0.3	0.9 ± 0.2	−2.6, 0	0.5, 1.2	0.4, 1.5
5	20.4 ± 7.9	3.3 ± 1.0[g]	0.8 ± 0.2	15.9 ± 12.4	1.3 ± 0.6	0.6 ± 0.2
6	39.2 ± 8.7	1.4 ± 0.2	0.7 ± 0.2	8.1, 26.2	0.6, 0.8	0.7 ± 0.1
7	60.5 ± 6.2	0.9 ± 0.1[h]	1.1 ± 0.4[h]	14.7 ± 4.4	0.6 ± 0.1	0.8 ± 0.1

[a] Compounds were tested at 1 μM. Results are mean ± standard error of the mean (SEM, $n = 3$) or individual values ($n = 2$). [b] Percent inhibition of the twitch after a 10-min incubation (unless otherwise indicated) of the tissue with the test compound. [c] The IC_{50} of the agonist in the presence of the test compound divided by that of the agonist in the absence of the test compound in the same tissue. [d] The K_e value, calculated according to the formula K_e = (antagonist dose)/(IC_{50} ratio − 1), is 740 ± 220 nM. [e] 30-min incubation. [f] K_e = 210 ± 50 nM. [g] K_e = 1.4 ± 1.1 μM. [h] Because of the agonism of 7 (IC_{50} = 1.5 ± 0.4 μM), the concentration was 0.2 μM.

ditional derivatives were prepared. In order to minimize the number of steps from a common intermediate to each new analogue, the synthesis was modified and the alkylation of the amine terminus postponed to the pentapeptide stage (Scheme II). The methyl rather than the *tert*-butyl ester was used to protect the C-terminus, since this allowed selective deprotection of the carboxylic acid and coupling to give C-terminal extended peptides. Other protecting groups and the coupling method were the same as described above; the common intermediate **18** was obtained in good yield.

The yields for the alkylation depended on the identity of the alkyl group. Alkylation of pentapeptide **18** with benzyl bromide gave the corresponding *N,N*-dibenzyl pentapeptide **19a** in good yield when the reaction was conducted at room temperature in acetonitrile.[19,20] However, attempts to prepare analogues by alkylation of **18** with other alkyl bromides, namely *n*-propyl, *n*-pentyl, *n*-octyl, and 2-phenethyl bromides, yielded mixtures of the N-monoalkylated and N,N-dialkylated pentapeptides. The use of the alkyl tosylates was examined as a way to improve the yields of the desired N,N-dialkylated pentapeptides. But even under optimized reaction conditions (refluxing toluene, 4 h)[21] with this better leaving group, the yields of the N,N-dialkylated pentapeptides **19b–e** were less than 50% and N-monoalkylated peptides were still obtained. An alternative approach, reductive amination of the peptide with excess aldehyde and sodium cyanoborohydride, proved unsatisfactory because it yielded a mixture of products. The pentapeptides **19a–e** were deprotected to give the desired leucine enkephalin analogues **3–7**.

Pharmacology

The target compounds were tested at 1 μM on the electrically stimulated longitudinal muscle of the guinea pig ileum (GPI)[22] and in the mouse vas deferens (MVD)[23] preparations. Antagonist activity was measured against morphine (a μ agonist[24]) and ethylketazocine (EK, a κ agonist[24]) in the GPI, and against morphine and [D-Ala^2,D-Leu^5]enkephalin (DADLE, a δ agonist[24]) in the MVD; the potency is expressed as the ratio of the IC_{50} of the antagonist-treated preparation divided by that of the control in the same tissue.

All of the compounds, except the *N,N*-diphenethyl derivative (**7**), showed antagonist activity in the MVD against DADLE (Table I). *N,N*-Diallyl leucine enkephalin, **1**, was an antagonist only at the δ receptor and caused a 2.7-fold shift in the DADLE dose–response curve. These findings are consistent with previously reported results,[14] although the observed K_e value[25] (740 nM) is higher than that previously reported (200 nM). The *N,N*-bis(cyclopropylmethyl) derivative **2** was slightly less active as a δ antagonist than **1** and showed weak agonist activity on the MVD. The *N,N*-dibenzyl peptide **3** was the most potent δ antagonist of the compounds tested, causing a 6.7-fold shift of the DADLE dose–response curve (K_e = 210 nM). For the analogues **4–6** containing saturated alkyl chains, antagonist activity was greatest for chains of middle length, with the *N,N*-dipentyl derivative **5** showing a 3.3-fold shift in the DADLE dose–response curve. Agonist activity in the MVD increased with increasing chain length for these analogues, so that the *N,N*-dioctyl derivative **6** showed significant agonist activity. The *N,N*-diphenethyl compound **7** also exhibited significant agonist activity, causing a 60% inhibition of the muscle twitch at 1 μM; the IC_{50} for this compound was 1.5 μM. At its IC_{20} (0.2 μM) it was inactive as an antagonist toward DADLE. All of the compounds were inactive at 1 μM as antagonists against morphine in the MVD. They were also inactive as antagonists in the GPI at 1 μM against both morphine and EK, and their agonist activity in this tissue was minimal.

Discussion

These results demonstrate that, for *N,N*-dialkyl leucine enkephalins, the constitution as well as the number of substituents on the amine terminus is important in determining the type of activity observed (agonist vs. antagonist) and the compound's potency. A π-electron system, although contained in two of the more potent δ receptor antagonists (**1** and **3**), was not required for δ receptor antagonism nor was it sufficient to guarantee antagonist activity. The poor antagonist activity but significant agonist activity of the *N,N*-dioctyl derivative **6** indicates that the maximum length of the alkyl chain that is tolerated by the receptor is different for agonists and antagonists.

These analogues, like several *N,N*-diallyl leucine enkephalin analogues,[14] were not as potent antagonists at the

(19) Reaction of **12** with benzyl bromide in refluxing toluene did not yield any of the desired product.
(20) These reaction conditions also give good yields when the alkyl halide is allyl bromide.
(21) Longer reaction times with the alkyl tosylates resulted in decomposition and decreased yields.
(22) Rang, H. P. *Br. J. Pharmacol.* **1965**, *22*, 356–365.
(23) Henderson, G.; Hughes, J.; Kosterlitz, H. W. *Br. J. Pharmacol.* **1972**, *46*, 764–766.
(24) Iwamoto, E. T.; Martin, W. R. *Med. Res. Rev.* **1981**, *1*, 411–440.
(25) Kosterlitz, H. W.; Watt, A. J. *Br. J. Pharmacol.* **1968**, *33*, 266–276.

δ receptor as naloxone (K_e = 24 nM against [Leu^5]enkephalin in the MVD[26]). But unlike naloxone, they were selective for the δ receptor. While this work was in progress, Cotton et al.[26] reported the activity of *N,N*-diallyl[Aib^2,Aib^3,Leu^5]enkephalin,[27] which was a δ selective antagonist with a potency (K_e = 30 nM against [Leu^5]-enkephalin) similar to that of naloxone.

These results indicate that the nature of the peptide chain also influences the antagonist potency of different *N,N*-dialkyl leucine enkephalin analogues. The effects of the *N*-alkyl substitution and peptide chain modification are not additive. For the Gly-containing peptides, the *N,N*-dibenzyl derivative was a more potent δ receptor antagonist than the *N,N*-diallyl analogue, while the reverse was found for the Aib-containing peptides.[15]

In conclusion, substitution of different *N*-alkyl groups yielded *N,N*-dibenzyl leucine enkephalin (**3**), which is a more potent δ receptor antagonist than *N,N*-diallyl leucine enkephalin (**1**).

Experimental Section

^{1}H NMR spectra were recorded on either a JEOL FX-90 FT in $CDCl_3$ or a Nicolet NT 300-MHz spectrophotometer in Me_2SO-d_6; all compounds gave the expected spectra. Chemical-ionization mass spectra, using NH_3 as the reagent gas, were recorded on a Finnigan 4000 spectrophotometer. Optical rotations were recorded on either a Perkin-Elmer 141 or a Rudolph Autopol III polarimeter, using a 10-cm, 1-mL polarimeter cell. Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by MHW Laboratories, Phoenix, AZ.

Analytical silica gel TLC was performed on either glass-backed plates (silica gel GF, 0.25-mm thickness, Analtech, Newark, DE) or plastic-backed plates (silica gel 60 F-254, 0.20-mm thickness, E. Merck, obtained from American Scientific Products). Octadecyl (C_{18}) reverse-phase (RP) TLC plates ($KC_{18}F$, 200-μm thickness) were obtained from Whatman, Clifton, NJ. Silica gel chromatography was performed with either Davisil silica gel, grade 633, 200–425 mesh, or Merck silica gel, grade 60, 230–400 mesh, 60A, both from Aldrich Chemical Co., Milwaukee, WI, and reverse-phase chromatography was performed with C_8, 40-μm reverse-phase resin from Analytichem International, Harbor City, CA. Unless otherwise noted, all column chromatography was performed with a medium-pressure liquid chromatography system consisting of Michel-Miller columns (Ace Glass, Inc., Vineland, NJ) and either a Lab Pump or Lab Pump, Jr. (Fluid Metering, Inc., Oyster Bar, NY). High-performance liquid chromatography (HPLC) was performed on an Altex 110A system equipped with a programmable controller, UV detector (280 nm), and a recorder/integrator (Hewlet Packard 3390A). The column was an Altex Lichrosorb C_8, 5-μm reverse-phase column (4.6 × 250 mm) fitted with a 3.0 × 50 mm C_8, 5-μm precolumn, and the solvent system was a mixture of 0.01 M NH_4OAc buffer, pH 4.0, and MeOH (both HPLC grade) used at a flow rate of 1 mL/min; t_m = 3.35 min.

All solvents and chemicals were reagent grade, unless otherwise specified. THF was freshly distilled from sodium and benzophenone under N_2; dioxane and toluene were distilled in a like manner and stored until use under N_2 over alumina (activity 1) and 4A molecular sieves, respectively. Acetonitrile was distilled from CaH_2 and stored under N_2 over 3A sieves. DMF (Gold Seal) was obtained from Aldrich. Amino acids were obtained from Sigma Chemical Co., St. Louis, MO; all are the L configuration. Isobutylene gas was obtained from Matheson, East Rutherford, NJ. Other reagents were obtained from Aldrich.

Alkyl tosylates were generally prepared by Tipson's procedure[28] and except for 2-phenethyl tosylate, which was recrystallized from Et_2O/30–60 °C petroleum ether, were vacuum distilled.

Mixed Carbonic Anhydride Coupling (Method A). Peptide bonds were formed by the mixed carbonic anhydride coupling procedure, using isobutyl chloroformate and *N*-methylmorpholine under "Anderson conditions"[29] in either THF or, when the amine component was an HCl salt, a THF/DMF mixture; ref 30 describes an illustrative procedure. When the peptide contained a *tert*-butyl protecting group, cold 10% citric acid was used to wash the organic extract rather than 1 N HCl.

Catalytic Transfer Hydrogenation (Method B). The *N*-benzyloxycarbonyl protecting group was removed with ammonium formate (4.0 equiv) and 10% palladium on carbon (0.25–0.50 wt/wt peptide) in MeOH (5 mL/mmol of peptide) for 30–40 min essentially according to the procedure of Anwer and Spatola.[17] After filtration and evaporation of the reaction mixture, the residue was dissolved in EtOAc and washed twice with saturated NaCl, 5% $NaHCO_3$, and again twice with saturated NaCl. The extract was then dried (Na_2SO_4) and evaporated to yield the amine.

Alkaline Hydrolysis of Esters (Method C). Amino acid or peptide esters were hydrolyzed with a mixture of 0.25 N NaOH (1.1–1.2 equiv) and THF (3/7, v/v), starting at 0–5 °C and then at 25 °C for 1.5–4 h. After neutralization with 1 N HCl, concentration in vacuo, and dilution with H_2O, the reaction mixture was extracted with Et_2O. The aqueous layer was then cooled, acidified to ca. pH 3 with cold 10% citric acid, and extracted with EtOAc. The extract was washed with H_2O until neutral and then with saturated NaCl, dried (Na_2SO_4), and evaporated to give the desired acid.

Deprotection with Trifluoroacetic Acid (Method D). Cold trifluoroacetic acid (1.5 mL/mmol of peptide) was added to a cold solution of the protected peptide and anisole in CH_2Cl_2 (1.5 mL/mmol of peptide) and the reaction mixture stirred under N_2 at 0–5 °C for 5–10 min and then at 25 °C for 2.5–3.5 h. After evaporation, the peptide was purified by column chromatography.

***N*-(Benzyloxycarbonyl)-*O*-*tert*-butyltyrosylglycylglycine Ethyl Ester (8).** *N*-(Benzyloxycarbonyl)-*O*-*tert*-butyltyrosine ethyl ester, which was prepared from *N*-(benzyloxycarbonyl)-tyrosine ethyl ester[31] with condensed isobutylene and concentrated H_2SO_4 in CH_2Cl_2[32] and purified by silica gel column chromatography (Waters 500A preparative LC system, hexane/20% EtOAc), was hydrolyzed (method C) to give *N*-(benzyloxycarbonyl)-*O*-*tert*-butyltyrosine[32] (89.7%). *N*-(Benzyloxycarbonyl)-*O*-*tert*-butyltyrosine (7.07 g, 19.0 mmol) was coupled (method A) to glycylglycine ethyl ester hydrochloride (3.74 g, 19.0 mmol) to give tripeptide **8** (9.02 g, 92.3%) as a glass. A sample was crystallized from Et_2O/hexane: mp 103.5–105 °C; $[\alpha]^{27}_D$ −18.5° (c 1.0, DMF); R_f (CH_2Cl_2/4% MeOH) 0.27. Anal. ($C_{27}H_{35}N_3O_7$) C, H, N.

***O*-*tert*-Butyltyrosylglycylglycine Ethyl Ester (9).** The tripeptide **8** (103 mg, 0.200 mmol) was deprotected according to method B to give **9** (66 mg, 86.5%): mp 112.5–115 °C; $[\alpha]^{25}_D$ −26.1° (c 0.63, DMF); R_f (CH_2Cl_2/10% MeOH) 0.48, (RP, MeOH/25% H_2O) 0.54. Anal. ($C_{19}H_{29}N_3O_5$) C, H, N.

***N,N*-Diallyl-*O*-*tert*-butyltyrosylglycylglycine Ethyl Ester (10a).** Compound **9** (500 mg, 1.32 mmol) was dissolved with heating in dry toluene (2.75 mL) under N_2, the solution was cooled to 0 °C, and *N,N*-diisopropylethylamine (445 mg, 3.44 mmol) followed by allyl bromide (1.40 g, 11.6 mmol) was added dropwise. The reaction mixture was warmed to 25 °C and then refluxed under N_2 for 3.25 h. The reaction mixture was cooled and the amine salt that precipitated upon cooling was filtered and washed well with toluene (~10 mL). After evaporation of the solvent in vacuo, the residue was applied to a silica gel column and eluted with CH_2Cl_2/2.5% MeOH to give pure **10a** as an oil (525 mg, 86.7%): $[\alpha]^{26}_D$ −21.0° (c 1.0, DMF); R_f (CH_2Cl_2/5% MeOH) 0.59; CI-MS, *m/e* 460 (M^+ + 1), 462 (M^+ + 3). Anal. ($C_{25}H_{37}N_3O_5$)

(26) Cotton, R.; Giles, M. G.; Miller, L.; Shaw, J. S.; Timms, D. *Eur. J. Pharmacol.* **1984**, *97*, 331–332.

(27) The structure of *N,N*-diallyl-Tyr-Aib-Aib-Phe-Leu, ICI 174864, was incorrectly reported in ref 26.

(28) Tipson, R. S. *J. Org. Chem.* **1944**, *9*, 235–241.

(29) Anderson, G. W.; Zimmerman, J. E.; Callahan, F. M. *J. Am. Chem. Soc.* **1967**, *89*, 5012–5017.

(30) Meienhofer, J. In *The Peptides: Analysis, Synthesis, Biology: Volume 1; Major Methods of Peptide Bond Formation*; Gross, E., Meienhofer, J., Eds.; Academic: New York, 1979; Chapter 6, pp 263–314.

(31) Bergmann, M.; Zervas, L. *Ber. Dtsch. Chem. Ges.* **1932**, *65*, 1192–1201.

(32) Schroeder, E. *Liebigs Ann. Chem.* **1963**, *670*, 127–136.

C, H, N.

***N,N*-Bis(cyclopropylmethyl)-*O*-*tert*-butyltyrosylglycylglycine Ethyl Ester (10b).** Compound **9** (500 mg, 1.32 mmol), *N,N*-diisopropylethylamine (445 mg, 3.44 mmol), (bromomethyl)cyclopropane (1.56 g, 11.6 mmol), and dry toluene (2.75 mL) were combined as described for **10a** and refluxed under N_2 for 2.25 h. Isolation and purification as described above gave **10b** as an oil (576 mg, 89.7%): $[\alpha]^{28.5}_D$ +0.49° (c 1.1, DMF); R_f (CH_2Cl_2/5% MeOH) 0.57; CI-MS, *m/e* 488 (M^+ + 1). Anal. ($C_{27}H_{41}N_3O_5$) C, H, N.

***N,N*-Diallyl-*O*-*tert*-butyltyrosylglycylglycine (11a).** Ester **10a** (320 mg, 0.70 mmol) was hydrolyzed (method C) and the reaction mixture then diluted with H_2O (7 mL) and most of the THF removed in vacuo at 25 °C. The aqueous layer was then extracted with Et_2O (2 × 10 mL), neutralized to pH 6.0, saturated with NaCl, and extracted exhaustively with $CHCl_3$ (5 × 20 mL). The combined $CHCl_3$ extracts were dried (Na_2SO_4), filtered through a fine sintered glass funnel, and evaporated to give **11a** as a glass (240 mg, 80.0%), which resisted crystallization: $[\alpha]^{29}_D$ −18.0° (c 1.0, DMF); R_f (RP, MeOH/30% H_2O) 0.55; CI-MS, *m/e* 432 (M^+ + 1). Anal. Calcd for $C_{23}H_{33}N_3O_5$: C, 64.02; H, 7.71; N, 9.74. Found: C, 61.35; H, 7.37; N, 9.07 (95.7% organic).

***N,N*-Bis(cyclopropylmethyl)-*O*-*tert*-butyltyrosylglycylglycine (11b).** Ethyl ester **10b** (425 mg, 0.87 mmol) was hydrolyzed (method C) and isolated as described for **11a** to give **11b** as a glass (395 mg, 98.6%), which was crystallized from CH_2Cl_2/Et_2O (yield 262 mg, 85.9%): mp 97–99 °C; $[\alpha]^{31}_D$ +1.0° (c 1.0, DMF); R_f (RP, MeOH/30% H_2O) 0.54. Anal. ($C_{25}H_{37}N_3O_5$) C, H, N.

Phenylalanylleucine *tert*-Butyl Ester (12). A cooled solution of phenylalanylleucine (1.00 g, 3.59 mmol) in dry dioxane (7.0 mL) in a thick-walled pressure reaction bottle fitted with a crown cap and gasket was treated with concentrated H_2SO_4 (0.70 mL, 13 mmol) and condensed isobutylene (~7 mL) according to the procedure described by Roeske.[33] After shaking at 25 °C for 5.25 h, the reaction mixture was poured into a cold (0 °C) mixture of 1 N NaOH (40 mL) and Et_2O (70 mL). The layers were separated, the aqueous layer was extracted further with Et_2O (3 × 25 mL), and the combined organic layers were washed with saturated NaCl (4 × 25 mL), dried (Na_2SO_4), and evaporated to give **12** as an oil (1.014 g, 84.4%); $[\alpha]^{28}_D$ −35.2° (c 1.0, DMF); R_f (CH_2Cl_2/2% MeOH/2% NH_4OH) 0.43, (RP, MeOH/25% H_2O) 0.38; CI-MS, *m/e* 335 (M^+ + 1). Anal. ($C_{19}H_{30}N_2O_3$) C, H, N.

***N,N*-Diallyl-*O*-*tert*-butyltyrosylglycylglycylphenylalanylleucine *tert*-Butyl Ester (13a).** Tripeptide **11a** (30.0 mg, 0.0695 mmol) was reacted with dipeptide **12** (23.2 mg, 0.0694 mmol) according to method A. After 1 h at −15 to −20 °C and an additional 1 h at 25 °C, the reaction mixture was evaporated in vacuo. The residue was dissolved in EtOAc (20 mL) and the organic solution washed with saturated $NaHCO_3$ (5 mL), H_2O (5 mL), and saturated NaCl (5 mL), dried (Na_2SO_4), and evaporated to give **13a** as a glass (48 mg, 92.3%): $[\alpha]^{28}_D$ −23.4° (c 0.50, DMF); R_f (CH_2Cl_2/5% MeOH) 0.43, (EtOAc/5% EtOH) 0.68. Anal. ($C_{42}H_{61}N_5O_7$) C, H, N.

***N,N*-Bis(cyclopropylmethyl)-*O*-*tert*-butyltyrosylglycylglycylphenylalanylleucine *tert*-Butyl Ester (13b).** Pentapeptide **13b** (67 mg, 86.2%) was obtained as a glass from tripeptide **11b** (46.0 mg, 0.100 mmol) and dipeptide **12** (33.4 mg, 0.100 mmol) by the procedure described above for compound **13a**: $[\alpha]^{26}_D$ −11.2° (c 0.50, DMF); R_f (CH_2Cl_2/5% MeOH) 0.30. Anal. ($C_{44}H_{65}N_5O_7$) C, H, N.

***N,N*-Diallyltyrosylglycylglycylphenylalanylleucine ([*N,N*-Diallyl,Leu^5]enkephalin, 1).** Protected peptide **13a** (48.6 mg, 0.065 mmol) was treated with 50% trifluoroacetic acid in CH_2Cl_2 (2.0 mL total volume) containing anisole (70 μL, 0.64 mmol) for 3.25 h at 25 °C, as described in method D. Purification by silica gel column chromatography (CH_2Cl_2/20% MeOH) and washing with Et_2O and hexane gave 1 trifluoroacetate (44 mg, 86.1%): mp 150–152 °C; $[\alpha]^{28}_D$ −22.0° (c 0.48, MeOH); R_f (CH_2Cl_2/10% MeOH) 0.26; HPLC (MeOH/30% buffer) k' = 1.59 (t_r = 6.6 min), (MeOH/50–30% buffer gradient over 20 min) k' = 8.59 (t_r = 24.4 min). Anal. ($C_{34}H_{45}N_5O_7 \cdot CF_3CO_2H \cdot 2H_2O$) C, H, N.

***N,N*-Bis(cyclopropylmethyl)tyrosylglycylphenylalanylleucine ([*N,N*-Bis(cyclopropylmethyl),Leu^5]enkephalin, 2).** Protected pentapeptide **13b** (62 mg, 0.080 mmol) was deprotected in 50% trifluoroacetic acid in CH_2Cl_2 (2.5 mL total volume) containing anisole (85 μL, 0.78 mmol) for 3.25 h at 25 °C and purified as described for **1** above to give **2** trifluoroacetate (57.5 mg, 88.3%): mp 136.5–138.5 °C; $[\alpha]^{27}_D$ +18.2° (c 0.52, MeOH); R_f (CH_2Cl_2/10% MeOH) 0.38; HPLC (MeOH/30% buffer) k' = 1.59 (t_r = 6.6 min), (MeOH/50–30% buffer gradient over 20 min) k' = 7.24 (t_r = 21.0 min). Anal. ($C_{36}H_{49}N_5O_7 \cdot CF_3CO_2H \cdot 2H_2O$) C, H, N.

***N*-(Benzyloxycarbonyl)-*O*-*tert*-butyltyrosylglycylglycylphenylalanylleucine Methyl Ester (17).** Tripeptide **8** (8.73 g, 17.0 mmol) was hydrolyzed (method C) to give the acid **14** (6.66 g, 80.7%) as a solid; a sample was recrystallized from EtOAc/hexane: mp 118–121 °C (lit.[34] mp 109–111 °C); $[\alpha]^{25}_D$ −20.4° (c 1.0, DMF) (lit.[34] $[\alpha]^{25}_D$ −17.1° (c 1, DMF)); R_f (RP, MeOH/H_2O, 2/1) 0.59. The C-terminal dipeptide **16** was prepared by coupling (method A) *N*-(benzyloxycarbonyl)phenylalanine and leucine methyl ester hydrochloride to give **15**[35–37] (97.7%), which was crystallized from EtOAc/hexane (95.1%). Deprotection (method B)[38] gave **16** as the HCl salt[39] (4.20 g, 90.8%), which was recrystallized from EtOAc/Et_2O (3.88 g, 78.7%). Tripeptide **14** (5.08 g, 10.5 mmol) was then coupled to dipeptide **16** (3.44 g, 10.5 mmol) by method A to give pentapeptide **17** (7.35 g, 92.4%), which was crystallized from EtOAc/hexane (6.74 g, 84.8%): mp 178–180.5 °C; $[\alpha]^{25}_D$ −17.8° (c 1.0, MeOH); R_f (CH_2Cl_2/5% MeOH) 0.32, (EtOAc) 0.43. Anal. ($C_{41}H_{53}N_5O_9$) C, H, N.

***O*-*tert*-Butyltyrosylglycylglycylphenylalanylleucine Methyl Ester (18).** Pentapeptide **17** (6.50 g, 8.55 mmol) was deprotected according to method B to give **18** (5.12 g, 95.6%) as a glass, which was crystallized from EtOAc/hexane (4.91 g, 91.9%): mp 76–80.5 °C; $[\alpha]^{25}_D$ −3.2° (c 1.0, MeOH); R_f (CH_2Cl_2/5% MeOH) 0.17. Anal. ($C_{33}H_{47}N_5O_7$) C, H, N.

***N,N*-Dibenzyl-*O*-*tert*-butyltyrosylglycylglycylphenylalanylleucine Methyl Ester (19a).** *N,N*-Diisopropylethylamine (186 mg, 1.44 mmol) followed by benzyl bromide (0.863 g, 5.04 mmol) was added to a cold (5 °C) solution of **18** (325 mg, 0.519 mmol) in dry AcCN (1.0 mL), and the reaction mixture was stirred at 25 °C under N_2 overnight. The reaction mixture was diluted with EtOAc (100 mL) and the extract washed with H_2O (50 mL) and saturated NaCl, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography (CH_2Cl_2/0–5% MeOH gradient) to give 376 mg (89.8%) of **19a**. Crystallization from EtOAc/petroleum ether gave 330 mg (78.8%) of **19a**: mp 167.5–169.5 °C; $[\alpha]^{25}_D$ −14.9° (c 1.0, MeOH); R_f (CH_2Cl_2/10% MeOH) 0.60, (EtOAc) 0.40. Anal. ($C_{47}H_{59}N_5O_7$) C, H, N.

***N,N*-Dipentyl-*O*-*tert*-butyltyrosylglycylglycylphenylalanylleucine Methyl Ester (19c).** *N,N*-Diisopropylethylamine (186 mg, 1.44 mmol) followed by pentyl tosylate (0.660 g, 2.72 mmol) was added to a cold (5 °C) solution of **18** (325 mg, 0.519 mmol) in dry toluene (1.25 mL). The reaction mixture was refluxed for 4 h; additional toluene (0.2 mL) was added to the reaction mixture after 3 h to maintain the original volume. After cooling, the reaction mixture was diluted with EtOAc (100 mL) and the extract washed with H_2O (25 mL) and saturated NaCl, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography (CH_2Cl_2/0–10% MeOH gradient) to give 146 mg (36.8%) of **19c**, which was triturated with hexane: mp 144.5–149.5 °C; $[\alpha]^{25}_D$ −10.4° (c 1.0, MeOH); R_f (CH_2Cl_2/10% MeOH) 0.46, (EtOAc) 0.47. Anal. ($C_{43}H_{67}N_5O_7$) C, H, N.

Also obtained from the column was *N*-pentyl-*O*-*tert*-butyltyrosylglycylglycylphenylalanylleucine methyl ester (140 mg, 38.7%) as a glass: $[\alpha]^{25}_D$ −14.9° (c 1.0, MeOH); R_f (CH_2Cl_2/10%

(33) Roeske, R. *J. Org. Chem.* **1963**, *28*, 1251–1253.

(34) Bower, J. D.; Guest, K. P.; Morgan, B. A. *J. Chem. Soc., Perkin Trans. 1* **1976**, 2488–2492.

(35) Pravola, Z.; Poduska, K.; Blaha, K. *Coll. Czech. Chem. Commun.* **1964**, *29*, 2626–2632.

(36) Woodward, R. B.; Olofson, R. A.; Mayer, H. *Tetrahedron Suppl.* **1966**, *8*, 321–346.

(37) Yamada, S.; Takaeuchi, Y. *Tetrahedron Lett.* **1971**, 3595–3598.

(38) Ethereal HCl was added to the concentrated extract prior to complete evaporation of the solvent.

(39) Elliott, R. L.; Marks, N.; Berg, M. J.; Portoghese, P. S. *J. Med. Chem.* **1985**, *28*, 1208–1216.

MeOH) 0.36, (EtOAc) 0.20. Anal. ($C_{38}H_{57}N_5O_7$) C, H, N.

***N*,*N*-Dipropyl-*O*-*tert*-butyltyrosylglycylglycylphenylalanylleucine Methyl Ester (19b).** Compound **18** (325 mg, 0.519 mmol), *N*,*N*-diisopropylethylamine (186 mg, 1.44 mmol), and propyl tosylate (1.12 g, 5.20 mmol) in dry toluene (1.00 mL) were combined as described for **19c** and refluxed under N_2 for 6.5 h; no additional toluene was added during the course of the reaction. After cooling and evaporation of the solvent, the residue was applied directly to a silica gel column and eluted with a CH_2Cl_2/0–100% EtOAc gradient to give 160 mg (43.3%) of **19b**, which was crystallized from EtOAc/petroleum ether: mp 71.5–74 °C; $[\alpha]_D$ −8.0° (c 1.1, MeOH); R_f (CH_2Cl_2/10% MeOH) 0.47, (EtOAc) 0.36. Anal. ($C_{39}H_{59}N_5O_7$) C, H, N.

***N*,*N*-Dioctyl-*O*-*tert*-butyltyrosylglycylglycylphenylalanylleucine Methyl Ester (19d).** Compound **18** (325 mg, 0.519 mmol), *N*,*N*-diisopropylethylamine (186 mg, 1.44 mmol), and octyl tosylate[40] (0.765 g, 2.69 mmol) in dry toluene (1.25 mL) were combined as described for **19c** and refluxed under N_2 for 4 h. Isolation and column chromatography as described above yielded 169 mg (32.8%) of **19d** as a glass: $[\alpha]^{25}_D$ −11.9° (c 1.0, MeOH); R_f (CH_2Cl_2/10% MeOH) 0.61, (EtOAc) 0.50. Anal. ($C_{49}H_{79}N_5O_7$) C, H, N.

Also obtained from the column was *N*-octyl-*O*-*tert*-butyltyrosylglycylglycylphenylalanylleucine methyl ester (110 mg, 28.7%) as a glass: $[\alpha]^{25}_D$ −13.4° (c 0.94, MeOH); R_f (CH_2Cl_2/10% MeOH) 0.51, (EtOAc) 0.21. Anal. ($C_{41}H_{63}N_5O_7 \cdot {}^1/_2H_2O$) C, H, N.

***N*,*N*-Di-2-phenethyl-*O*-*tert*-butyltyrosylglycylglycylphenylalanylleucine Methyl Ester (19e).** Compound **18** (325 mg, 0.519 mmol), *N*,*N*-diisopropylethylamine (186 mg, 1.44 mmol), and 2-phenethyl tosylate (0.725 g, 2.62 mmol) in dry toluene (1.25 mL) were combined as described for **19c** and refluxed under N_2 for 4 h. Isolation and column chromatography as described above yielded 108 mg (24.9%) of **19e**, which was crystallized from EtOAc/petroleum ether: mp 86–88 °C; $[\alpha]^{25}_D$ −1.1° (c 0.56, MeOH); R_f (CH_2Cl_2/10% MeOH) 0.58, (EtOAc) 0.40. Anal. ($C_{49}H_{63}N_5O_7$) C, H, N.

Also obtained from the column was *N*-2-phenethyl-*O*-*tert*-butyltyrosylglycylglycylphenylalanylleucine methyl ester (195 mg, 51.3%) as a glass: $[\alpha]^{25}_D$ −18.9° (c 1.0, MeOH); R_f (CH_2Cl_2/10% MeOH) 0.49, (EtOAc) 0.20. Anal. ($C_{41}H_{55}N_5O_7 \cdot {}^1/_2H_2O$) C, H, N.

***N*,*N*-Dibenzyltyrosylglycylglycylphenylalanylleucine ([*N*,*N*-Dibenzyl,Leu⁵]enkephalin, 3).** The methyl ester **19a** (100 mg, 0.124 mmol) was hydrolyzed at 25 °C in 0.25 N NaOH (0.60 mL, 0.15 mmol) and THF (1.25 mL) for 5.75 h according to method C. After dilution with H_2O (30–40 mL), the basic solution was extracted with Et_2O (3 × 10 mL). The Et_2O extracts were back-extracted with 0.25 N NaOH (2 × 2 mL) and the combined aqueous layers neutralized to pH 6 with 1 N HCl and extracted exhaustively with $CHCl_3$ (5 × 20 mL). The $CHCl_3$ extracts were dried (Na_2SO_4) and evaporated to give the free acid [*N*,*N*-dibenzyl,*O*-*tert*-butyl,Leu⁵]enkephalin (72 mg, 73.2%): R_f (CH_2Cl_2/10% MeOH) 0.28. This compound was then deprotected with 50% trifluoroacetic acid and 10% anisole in CH_2Cl_2 (1.5 mL total volume) at 25 °C for 2.75 h, as described in method D. Purification by reverse-phase column chromatography (H_2O/50–100% MeOH gradient) gave 60 mg (89.7%) of **3** as a solid: $[\alpha]^{25}_D$ −23.2° (c 0.50, MeOH); HPLC (MeOH/30% buffer) k' = 2.22 (t_r = 10.7 min), (MeOH/40–20% buffer over 10 min) k' = 3.64 (t_r = 16.9 min). Anal. ($C_{42}H_{49}N_5O_7 \cdot {}^1/_2H_2O$) C, H, N.

***N*,*N*-Dipropyltyrosylglycylglycylphenylalanylleucine ([*N*,*N*-Dipropyl,Leu⁵]enkephalin, 4).** The methyl ester **19b** (87.5 mg, 0.123 mmol) was hydrolyzed at 25 °C for 7.5 h and the free acid [*N*,*N*-dipropyl,*O*-*tert*-butyl,Leu⁵]enkephalin (67.5 mg, 78.7%) isolated as described above under compound **3**: R_f (CH_2Cl_2/10% MeOH) 0.22. The acid was treated with 50% trifluoroacetic acid and 10% anisole in CH_2Cl_2 (1.5 mL total volume) at 25 °C for 2.5 h and purified first by silica gel column chromatography (CH_2Cl_2/0–20% MeOH gradient) and then by C_8 reverse-phase column chromatography (MeOH/50% H_2O) to give 42.5 mg (68.4%) of **4** as a solid: $[\alpha]^{25}_D$ +20.1° (c 0.50, MeOH); R_f (CH_2Cl_2/20% MeOH) 0.25, (RP, MeOH/30% H_2O) 0.38; HPLC (MeOH/40% buffer) k' = 1.24 (t_r = 7.9 min), (MeOH/50–30% buffer over 10 min) k' = 2.97 (t_r = 15.5 min). Anal. ($C_{34}H_{49}N_5O_7 \cdot H_2O$) C, H, N.

***N*,*N*-Dipentyltyrosylglycylglycylphenylalanylleucine ([*N*,*N*-Dipentyl,Leu⁵]enkephalin, 5).** The methyl ester **19c** (95 mg, 0.124 mmol) was hydrolyzed at 25 °C for 6 h and the acid (43.5 mg, 46.7%) isolated as described under compound **3**: R_f (CH_2Cl_2/10% MeOH) 0.25. Treatment with 50% trifluoroacetic acid and 10% anisole in CH_2Cl_2 (1.1 mL total volume) at 25 °C for 3 h and purification by C_8 reverse-phase column chromatography (H_2O/50–100% MeOH gradient) gave 22.5 mg (56.0%) of **5** as a solid: $[\alpha]^{25}_D$ +5.3° (c 0.50, MeOH); R_f (RP, MeOH/15% H_2O) 0.68; HPLC (MeOH/30% buffer) k' = 1.90 (t_r = 9.6 min), (MeOH/40–20% buffer over 10 min) k' = 3.44 (t_r = 15.2 min). Anal. Calcd for $C_{38}H_{57}N_5O_7$: C, 65.59; H, 8.26; N, 10.06. Found: 62.04; H, 7.77; N, 9.41 (94.1% organic).

***N*,*N*-Dioctyltyrosylglycylglycylphenylalanylleucine ([*N*,*N*-Dioctyl,Leu⁵]enkephalin, 6).** The methyl ester **19d** (104 mg, 0.127 mmol) was hydrolyzed at 25 °C for 5.75 h and the acid (68.5 mg, 67.2%) isolated as described above under compound **3**: R_f (CH_2Cl_2/10% MeOH) 0.32. Treatment with 50% trifluoroacetic acid and 10% anisole in CH_2Cl_2 (1.65 mL total volume) at 25 °C for 3 h and purification by C_8 reverse-phase column chromatography (H_2O/50–100% MeOH gradient) gave 38.5 mg (60.2%) of **6** as a solid: $[\alpha]^{25}_D$ +8.3° (c 0.50, MeOH); HPLC (MeOH/20% buffer) k' = 3.58 (t_r = 14.9 min), (MeOH/30–10% buffer over 10 min) k' = 4.49 (t_r = 18.5 min). Anal. ($C_{44}H_{69}N_5O_7$) C, H, N.

***N*,*N*-Di-2-phenethyltyrosylglycylglycylphenylalanylleucine ([*N*,*N*-Diphenethyl,Leu⁵]enkephalin, 7).** The methyl ester **19e** (53 mg, 0.064 mmol) was hydrolyzed at 25 °C for 5.5 h as described above. The reaction mixture was neutralized with 1 N HCl to pH 6, the THF evaporated, and the crude product purified by C_8 reverse-phase column chromatography (H_2O/50–100% MeOH gradient) to give 43 mg (82.5%) of the acid: R_f (CH_2Cl_2/10% MeOH) 0.20. Deprotection with 50% trifluoroacetic acid and 10% anisole in CH_2Cl_2 (1.1 mL total volume) at 25 °C for 3 h as described above and purification by C_8 reverse-phase column chromatography (H_2O/50–100% MeOH gradient) gave 35.5 mg (88.5%) of **7** as a solid: $[\alpha]^{25}_D$ +3.1° (c 0.50, MeOH); HPLC (MeOH/30% buffer) k' = 2.56 (t_r = 12.2 min), (MeOH/40–20% buffer over 10 min) k' = 3.85 (t_r = 17.3 min). Anal. ($C_{44}H_{53}N_5O_7 \cdot {}^1/_2H_2O$) C, H, N.

Acknowledgment. This research was supported by individual service award grant DA-05263 (J.A.L.) and research grant DA-01533 from the National Institute on Drug Abuse. We thank Victoria Elliott and Michael Powers for their capable assistance in the biological testing and Prof. A. E. Takemori and Dr. D. L. Larson for their assistance and advice.

Registry No. 1, 85835-41-2; 1·TFA, 107941-88-8; 2, 107941-75-3; 2·TFA, 107959-99-9; 3, 107941-76-4; 4, 107941-77-5; 5, 107941-78-6; 6, 107941-79-7; 7, 107941-80-0; 8, 107941-81-1; 9, 107941-82-2; 10a, 107941-83-3; 10b, 107941-89-9; 11a, 88282-65-9; 11b, 107941-90-2; 12, 28635-78-1; 13a, 107941-84-4; 13b, 107960-00-9; 14, 62565-7-7; 15, 3850-45-1; 16·HCl, 38155-45-2; 17, 107941-85-5; 18, 107941-86-6; 19a, 107941-87-7; 19a (hydrolyzed), 107941-98-0; 19b, 107941-91-3; 19b (hydrolyzed), 107941-99-1; 19c, 107941-92-4; 19c (hydrolyzed), 107942-00-7; 19d, 107941-93-5; 19d (hydrolyzed), 107942-01-8; 19e, 107941-94-6; 19e (hydrolyzed), 107942-02-9; *N*-(benzyloxycarbonyl)-*O*-*tert*-butyltyrosine ethyl ester, 66328-78-7; *N*-(benzyloxycarbonyl)-*O*-*tert*-butyltyrosine, 5545-54-0; glycylglycine ethyl ester hydrochloride, 2087-41-4; allyl bromide, 106-95-6; (bromomethyl)cyclopropane, 7051-34-5; phenylalanylleucine, 3303-55-7; isobutylene, 115-11-7; *N*-(benzyloxycarbonyl)phenylalanine, 1161-13-3; leucine methyl ester hydrochloride, 7517-19-3; benzyl bromide, 100-39-0; pentyl tosylate, 4450-76-4; *N*-pentyl-*O*-*tert*-butyltyrosylglycylglycylphenylalanylleucine methyl ester, 107941-95-7; propyl tosylate, 599-91-7; octyl tosylate, 3386-35-4; *N*-octyl-*O*-*tert*-butyltyrosylglycylglycylphenylalanylleucine methyl ester, 107941-96-8; 2-phenethyl tosylate, 4455-09-8; *N*-2-phenethyl-*O*-*tert*-butyltyrosylglycylglycylphenylalanyl leucine methyl ester, 107941-97-9.

(40) Bp 188–192 °C (1.13 mmHg).