Articles

Enkephalin Analogs as Systemically Active Antinociceptive Agents: O- and N-Alkylated Derivatives of the Dipeptide Amide L-2,6-Dimethyltyrosyl-*N*-(3-phenylpropyl)-D-alaninamide†

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Received December 22, 1993•

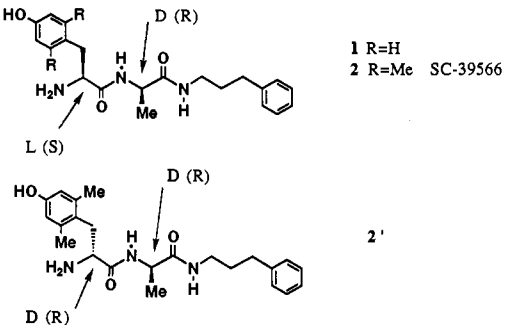
A number of O- and N-alkylated derivatives of the antinociceptive, orally active, μ-opioid-selective truncated enkephalin analog L-2,6-dimethyltyrosyl-*N*-(3-phenylpropyl)-D-alaninamide (**2**, SC-39566) were synthesized to explore the structure–activity relationships of the series. The parent molecule is quite forgiving of substitution on the tyrosyl phenolic moiety and on the alanyl nitrogen. The tyrosyl and (phenylpropyl)amide NH sites, however, appear to be critical to interactions with the receptor, for even modest changes at these sites cause great loss of binding potency.

Introduction

The palliation of severe pain has presented a therapeutic challenge to physicians from time immemorial. The search for a substance with the analgesic properties of morphine, lacking morphine's side effects, continues unabated.[1,2] The realization[3,4] of Portoghese's prediction[5] of the existence of multiple opioid receptors allowed the commencement of a rational approach to the separation of properties of the opioid analgesics, especially with the characterization of saturable, high-affinity, stereospecific opioid receptors.[6] The identification of endogenous opioid ligands[7] presented a new starting point for opioid design. In a short time, Vavrek et al.[8] characterized a dipeptide amide, L-tyrosyl-*N*-(3-phenylpropyl)-D-alaninamide (**1**), which had strong opioid properties. This work was elaborated upon in these laboratories, resulting in L-2,6-dimethyltyrosyl-*N*-(3-phenylpropyl)-D-alaninamide (SC-39566, **2**).[9-11] This compound displayed sufficiently interesting pharmacology to allow its advancement into the development process, and thus a synthetic program was undertaken to systematically alkylate **2** in order to explore the structure–activity relationships emerging from the resulting steric and electronic changes. This report describes the results of O- and N-alkylation and stereogenic carbon alkylation of **2**.

Chemistry

2,6-Dimethyl-DL-tyrosine (DL-DMT, **21**) was initially synthesized by the method of Abrash.[12] The racemic material was incorporated into the D-alanine-containing dipeptide amide, resulting in a pair of diastereomers which were separated by column chromatography. Assignments of stereochemistry at the dimethyltyrosyl center were made by comparison of the alanyl methyl NMR signal in each compound (Table 1) with those of the known diastereomer



2 (structure proof by X-ray study, to be reported separately[11]) and the corresponding D,D-diastereomer (**2'**). These assignments are supported by the data on optical rotation (see Table 5, *vide infra*); in every case the assigned L,D-diastereomer has a (much) more positive value than the corresponding D,D-diastereomer.

Later in the synthetic work, an asymmetric synthesis[13] of this unnatural amino acid was used, giving only the L-enantiomer. Scheme 1 depicts the alkylation of racemic benzyloxycarbonyl (Z)-protected DMT, using the method of Karady et al.[14] Amide bond formation was accomplished by means of a mixed anhydride reaction employing isobutyl chloroformate and *N*-methylmorpholine.[15] The reagents for mixed anhydride peptide bond formation are shown in this scheme; in subsequent schemes, they are merely referred to as "Mixed Anhyd".

The N-alkylation of the D-alanyl residue is described in Schemes 2 and 3, involving alkylation of the *N*-*tert*-butyloxycarbonyl (Boc)-protected amino acid with sodium hydride/alkyl halide (Scheme 2) or, in the case of the cyclopropylmethyl function, alkylation of the free amine (Scheme 3).

O-Alkylation of the tyrosine residue is displayed in Schemes 4 and 5, and N-alkylation in Scheme 6. Alkylation with inexpensive alkylating agents was done through the unisolated dialkylated intermediate (Scheme 4), while the intermediate DMT methyl ester (**22**) was used in Scheme 5, allowing the use of 1 equiv of alkylating agent.

† Presented in (varying) parts at the 202nd National Meeting of the American Chemical Society, New York, NY, August 1991 (MEDI 152), and at the 21st Annual Meeting of the Society for Neuroscience, New Orleans, LA, November 1991 (121.7).

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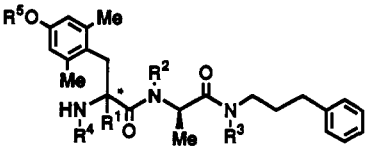
• Abstract published in *Advance ACS Abstracts*, February 15, 1994.

0022-2623/94/1837-0888$04.50/0

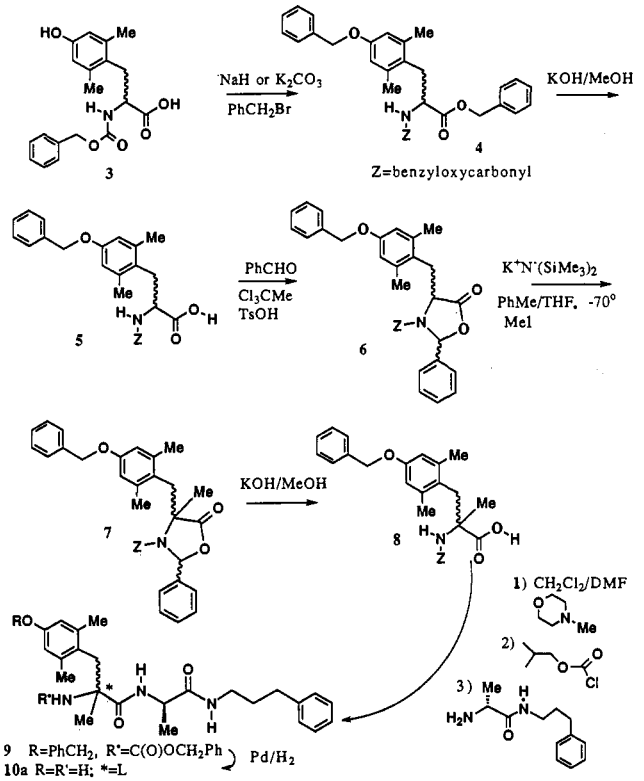
Table 1. Chemical Shifts of Selected Diastereomers



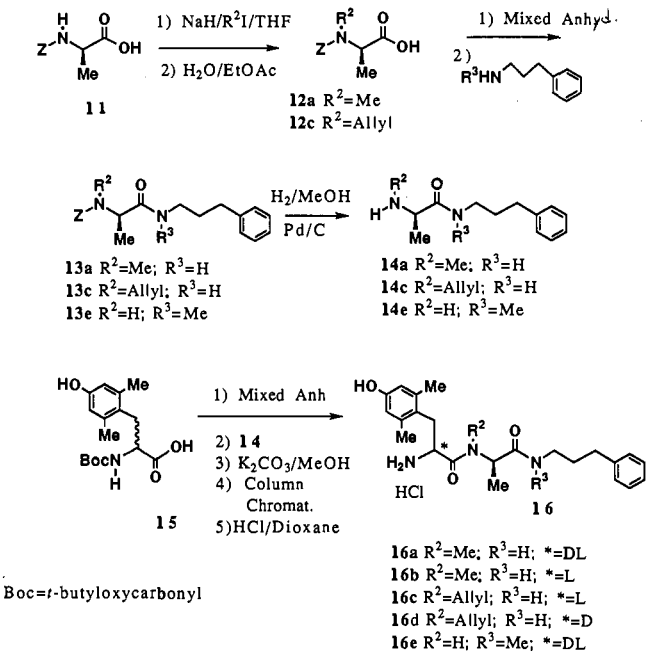
compd	R_1	R^2	R^3	R^4	R^5	*D/L	δ^a
2	H	H	H	H	H	L	0.88
2'	H	H	H	H	H	D	1.15
10a	Me	H	H	H	H	L	1.10
10b	Me	H	H	H	H	D	1.21
16c	H	allyl	H	H	H	L	1.03
16d	H	allyl	H	H	H	D	1.13
18b	H	cyclopropylmethyl	H	H	H	L	1.08
18c	H	cyclopropylmethyl	H	H	H	D	1.22
30a	H	H	H	Me	H	L	0.90
30b	H	H	H	Me	H	D	1.18
20b	H	H	H	H	Me	L	1.06
20c	H	H	H	H	Me	D	1.21
26a	H	H	H	H	$PhCH_2$	L	0.83
26b	H	H	H	H	$PhCH_2$	D	1.14
26c	H	H	H	H	*p*-F-$PhCH_2$	L	0.85
26d	H	H	H	H	*p*-F-$PhCH_2$	D	1.15
26f	H	H	H	H	*p*-NO_2-$PhCH_2$	L	0.95
26g	H	H	H	H	*p*-NO_2-$PhCH_2$	D	1.28
26j	H	H	H	H	*p*-*t*-Bu-$PhCH_2$	L	0.80
26k	H	H	H	H	*p*-*t*-Bu-$PhCH_2$	D	1.15

[a] NMR chemical shift: ppm in hertz from tetramethylsilane.

Scheme 1



Scheme 2



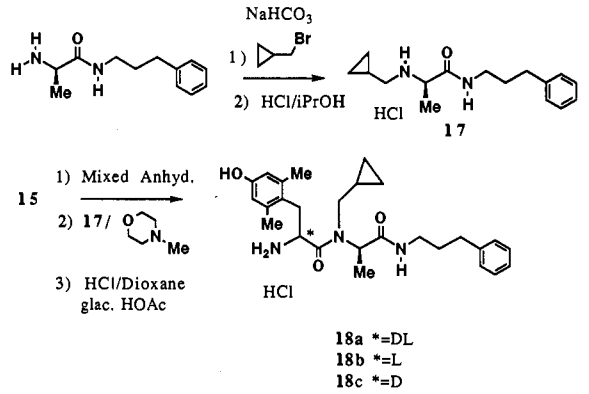
Pharmacology

Antinociceptive activities were determined in the mouse abdominal stretch (writhing) assay.[16] The opioid competitive binding assays, employing homogenized rat brain minus cerebellum, used [^{3}H]naloxone for nonselective opioid binding, [^{3}H]DAMGO (Tyr-D-Ala-Gly-MePhe-Gly-ol) for μ-opioid binding, and [^{3}H]DSLET (Tyr-D-Ser-Gly-Phe-Leu-Thr) for δ-opioid binding.[17] The concentrations of radioligands used were at or near their K_d values.

Results and Discussion

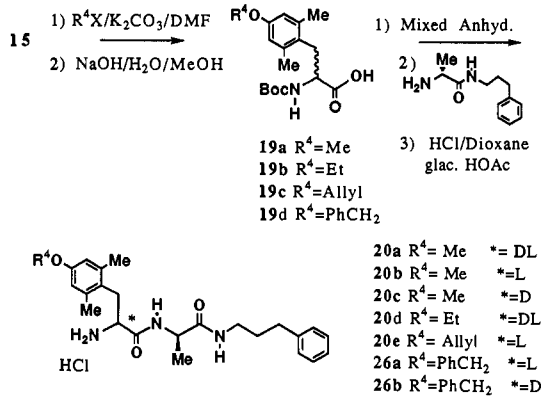
Sequential alkylation of the peptide backbone of **2** (SC-39566) resulted in compounds of widely varying activities (see Table 2). Methylation of the tyrosyl amino function gave compounds (**30a,b**) with markedly diminished affinity for the opioid receptor (as measured by displacement of tritiated naloxone, Table 2) and reduced antinociceptive activity compared to **2**. The analogous, L-DMT-containing diastereomer to **2**, **30a**, has an IC_{50} more than 3 orders of magnitude weaker than that of **2**, the unsubstituted parent. Clearly this locus on the minimal enkephalin structure is not amenable to steric modulation. Methylation on the alanyl nitrogen, on the other hand, gave compounds (**16a,b**) which largely retained their affinity for opioid sites. The pure L-DMT, D-Ala dias-

Scheme 3



18a *=DL
18b *=L
18c *=D

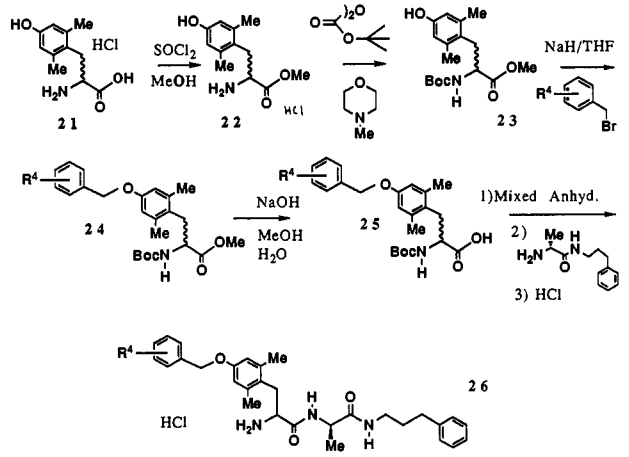
Scheme 4



19a R^4=Me
19b R^4=Et
19c R^4=Allyl
19d R^4=$PhCH_2$

20a R^4= Me *= DL
20b R^4= Me *=L
20c R^4= Me *=D
20d R^4= Et *=DL
20e R^4= Allyl *=L
26a R^4=$PhCH_2$ *=L
26b R^4=$PhCH_2$ *=D

Scheme 5



For 24 and 25:

c, d R^4=p-F
e R^4=p-CN
f, g R^4=p-NO_2
h, i R^4=p-CO_2t-Bu
j, k R^4=p-t-Bu
m R^4=o-Me

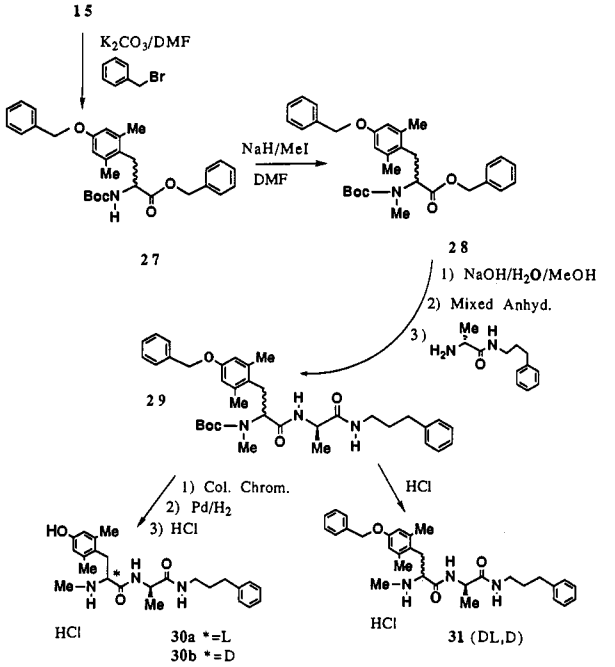
For 26:

c, d R^4=p-F
e R^4=p-CN
f, g R^4=p-NO_2
h, i R^4=p-CO_2H
j, k R^4=p-t-Bu
m R^4=o-Me

tereomer, **16b**, retains the μ- and δ-opioid affinities of the parent **2** and also retains the antinociceptive activity of the parent. Thus a modest substitution change on the alanyl nitrogen is forgiving with respect to intestinal bioavailability.

More elaborate substitution on the alanyl nitrogen, on the other hand, leads to bioavailability problems in the gut. This is shown by the analogous *N*-allyl diastereomer, **16c**, and the corresponding *N*-cyclopropylmethyl com-

Scheme 6

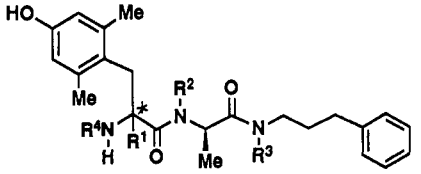


pound, **18b**. Both of these compounds bind tightly to opioid sites, and both are very potent antinociceptive agents by the subcutaneous route (Table 2). They are, however, devoid of oral antinociceptive activity at the doses tested (10 mg/kg). It will be noticed that substituents which are associated with opioid antagonists in the morphinan series (the nitrogen in the morphine skeleton corresponds to the N-terminal tyrosine nitrogen in this series, the site of R^4) are, on the other hand, associated with opioid agonists when placed at the alanyl nitrogen site (the site of R^2) of the dipeptide amides. Alkylation at the nitrogen of the (phenylpropyl)amide moiety gives a compound (**16e**) that was only characterized as a pair of diastereomers. The poor opioid binding, as shown in Table 2, suggests that this site on the parent compound is important in receptor site recognition.

Alkylation at the tyrosyl epimeric carbon gives a compound (**10a**) with diminished opioid binding (at both μ and δ sites) when compared to **2**. Antinociceptive activity remains relatively constant. This is in marked contrast with results of alkylation one atom away, on the tyrosyl nitrogen (**30a**, *vide supra*). These data emphasize the critical nature of the tyramine nitrogen, common to both peptides and rigid opioids. In the rigid opiate series, substitution at this site is heavily influential in the agonist or antagonist functional activity of the compound, and in this peptide series relatively minor substitution alterations greatly diminish opioid binding.

Assignments of stereochemistry at the tyrosyl site are made by study of the NMR signal due to the D-alanyl methyl group (Table 1). The parent (**2**) was crystallized as the free base, characterized by X-ray analysis,[11] and compared to its diastereomer D-2,6-dimethyltyrosyl-*N*-(3-phenylpropyl)-D-alaninamide (**2'**). The alanyl methyl signal of **2** was upfield of the corresponding signal of **2'**. Comparison of the optical rotations of **2** and **2'** show that the $[\alpha]_D$ for **2** is +536°, compared with −356° for **2'**. In every case in this study in which both diastereomers (L,D and D,D) were isolated, the compound with the higher field alanyl methyl group was the compound with the more

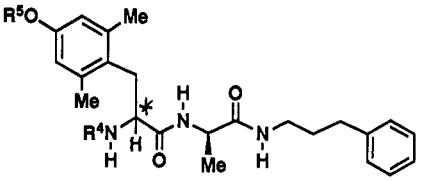
Table 2. Pharmacological Profile of Products of Alkylation on the Amide Backbone



compd	R^1	R^2	R^3	R^4	D/L	nalo[a]	μ[b]	δ[c]	writhing assay[d] sc[e]	writhing assay[d] ig[f]
2	H	H	H	H	L	0.1	0.2	8.6	0.5 (0.26–1.0)	2 (0.7–3.0)
2'	H	H	H	H	D	50			0.6	2.4
10a	Me	H	H	H	L	150	2.0	64	3.0 (1.1–8.1)	3.7 (1.0–35.1)
10b	Me	H	H	H	D	640			I	I
16a	H	Me	H	H	DL	0.6			1.7 (0.9–2.4)	1.3 (0.5–2.7)
16b	H	Me	H	H	L		0.1	4.4	0.07 (0.03–0.1)	3.1 (1.7–5.4)
16c	H	allyl	H	H	L	1.3			0.7 (0.2–1.7)	I
16d	H	allyl	H	H	D	280			I	I
16e	H	H	Me	H	DL	300			I	I
18a	H	cyclopropylmethyl	H	H	DL	10			2.4 (1.5–3.8)	I
18b	H	cyclopropylmethyl	H	H	L		0.4	9.4	0.5 (0.2–1.0)	I
18c	H	cyclopropylmethyl	H	H	D		23.7	409	I	I
30a	H	H	H	Me	L	140			I	I
30b	H	H	H	Me	D	1800			I	I

[a] Displacement of tritiated naloxone from rat brain membranes [IC_{50} (nM)]. [b] Displacement of tritiated DAMGO from rat brain membranes [IC_{50} (nM)]. [c] Displacement of tritiated DSLET from rat brain membranes [IC_{50} (nM)]. [d] Mouse writhing assay: ED_{50} (mg/kg). 95% confidence limits in parentheses. "I" indicates no significant inhibition at 10 mg/kg, the highest dose tested. [e] Subcutaneous administration. [f] Intragastric administration.

Table 3. Pharmacological Profile of Products of Alkylation on the Tyrosyl Phenolic Oxygen



compd	R^4	R^5	D/L	nal[a]	μ[b]	δ[c]	writhing assay sc[d]	writhing assay ig[e]
20a	H	Me	DL	290			4.3 (1.5–60.5)	2.3 (0.7–5.0)
20b	H	Me	L	50	57	>1000	0.7 (0.3–1.4)	I
20c	H	Me	D	420			3.5 (1.8–7.3)	2.7 (0.9–6.3)
20d	H	Et	DL	130			I	I
20e	H	allyl	L			600	12.4 (9.3–14.8)	14.9 (7.4–21.3)
26a	H	$PhCH_2$	L	360	4.0	55	1.8 (0.8–3.2)	2.4 (0.9–4.9)
26b	H	$PhCH_2$	D	22			2.3 (0.7–5.0)	12.9 (6.0–41.7)
26c	H	*p*-F-$PhCH_2$	L	18	17.5	385	2.7 (1.6–4.7)	I
26d	H	*p*-F-$PhCH_2$	D	150			I	I
26e	H	*p*-CN-$PhCH_2$	L	17	28.6	>1000	I	I
26f	H	*p*-NO_2-$PhCH_2$	L	2.9	3.5	127	2.3 (0.7–5.0)	6.8 (3.7–11.6)
26g	H	*p*-NO_2-$PhCH_2$	D	95			I	I
26h	H	*p*-COOH-$PhCH_2$	L		12.3	882	I	I
26i	H	*p*-COOH-$PhCH_2$	D	83			I	I
26j	H	*p*-*t*-Bu-$PhCH_2$	L	0.5			I	I
26k	H	*p*-*t*-Bu-$PhCH_2$	D	160			I	I
26m	H	*o*-Me-$PhCH_2$	L				I	I
31	Me	$PhCH_2$	L	53			~10	I

[a] Displacement of tritiated naloxone from rat brain membranes [IC_{50} (nM)]. [b] Displacement of tritiated DAMGO from rat brain membranes [IC_{50} (nM)]. [c] Displacement of tritiated DSLET from rat brain membranes [IC_{50} (nM)]. [d] Mouse writhing assay: ED_{50} (mg/kg). 95% confidence limits in parentheses. "I" indicates no significant inhibition at 10 mg/kg, the highest dose tested. [e] Subcutaneous administration. [f] Intragastric administration.

positive $[\alpha]_D$ (Table 5). This diastereomer was assigned the L,D configuration, and the diastereomer with the lower field alanyl methyl signal (and the negative $[\alpha]_D$) was assigned the D,D configuration. It will be noticed that in every case except that of **26a** and **26b**, the L,D-diastereomer thus assigned is more biologically active than the D,D-diastereomer (opioid displacement, Tables 2 and 3).

Substitution at the phenolic oxygen generally gave compounds with poorer binding to the opioid receptor, but with some retention of antinociceptive activity (Table 3). The *O*-methyl L,D-diastereomer, **20b**, for example, binds with 2 orders of magnitude less potency than **2** but has similar antinociceptive activity. It is not known to what extent this disparity reflects metabolism of **20b** to **2**, or greater bioavailability of **20b** compared to **2**. Certain substituted *O*-benzyl compounds, however, do possess intrinsic binding properties, as demonstrated by the *p*-nitrobenzyl ether **26f**. μ binding is 1 order of magnitude removed from the parent but still has a nanomolar value. The *p*-*tert*-butylbenzyl ether **26j** has about the same binding potency as **2** but lacks antinociceptive activity.

Table 4. Opioid Subtype Binding for Selected Compounds

compd	μ^a	δ^b	κ^c	writhing mouse[d]
20b	32.0 ± 0.5	2094.0 ± 957	19763.0 ± 3533	0.8
16b	0.2 ± 0.01	8.0 ± 2.6	316.0 ± 11	0.07
10a	1.1 ± 0.005	124.0 ± 44	1375.0 ± 180	2.6
18b	0.5 ± 0.005	10.0 ± 1.5	1000.0 ± 40	0.5
26a	0.92 ± 0.46	133.3 ± 1.1	2520.0 ± 53.3	1.9
26b	44.7 ± 5.5	1799.0 ± 140	17470.0 ± 280	2.3
26c	8.73 ± 1.5	367.3 ± 13.2	10800.0 ± 1130	2.7
2	0.13 ± 0.03	9.57 ± 2.46	221.8 ± 9.1	0.5

[a] Displacement of tritiated DAMGO from rat brain membranes [IC_{50} (nM) ± SEM]. [b] Displacement of tritiated DSLET from rat brain membranes [IC_{50} (nM) ± SEM]. [c] Displacement of tritiated U-69593 from rat brain membranes [IC_{50} (nM) ± SEM]. [d] ED_{50} (mg/kg, sc).

Alkylation at both the oxygen and nitrogen sites of the tyrosine moiety (**31**) gave, as expected, a relatively inactive compound.

Comparison of the SAR in this series with the SAR of alkylated enkephalin analogs in other synthetic series must be done with great care, since Lovett and Portoghese showed that "The effects of the *N*-alkyl substitution and peptide chain modification [of *N,N*-dialkylleucine enkephalin analogues] are not additive".[18] Pert et al. demonstrated that the tyrosyl-alkylated compound *N*-allyl-[D-Ala]^2-Met-enkephalin was a weak agonist/antagonist compared to the parent [D-Ala]^2-Met-enkephalin[19] (ID_{50} with NaCl present was 500 nM for the parent and 50 000 nM for the *N*-allyl derivative). The tyrosyl *N*-allyl pentapeptide amide allyl-Tyr-D-Ala-Phe-Gly-Phe-NH_2 bound to the μ-opioid receptor with less than one-tenth the potency of the parent[20] and to the δ receptor with about one-fifth of the potency.[20] A related tetrapeptide, allyl-Tyr-D-Ala-Phe-Gly-NH_2,[20] was a much poorer ligand with respect to its parent, displaying one-thirtieth of the parent's μ potency.

In a different study using analgesia as an endpoint, methylation on the Tyr and Phe sites of a set of Met-enkephalin derivatives resulted in compounds either equivalent to the parent [*Me*-Tyr-D-Met(O)-Gly-Me-Phe-Met(O)-ol compared to Tyr-D-Met(O)-Gly-Me-Phe-Met-(O)-ol][21] or less potent than the parent [Tyr-D-Ala-Gly-*Me*-Phe-Met(O)-ol compared to Tyr-D-Ala-Gly-Phe-Met(O)-ol].

A study of two Met-enkephalin analogs methylated on the tyrosyl asymmetric center (corresponding to **10a** and **10b** in this paper) showed that both diastereomeric compounds [α-Me-Tyr^1-D-Ala^2-Met-enk-NH_2 and α-Me-D-Tyr^1-D-Ala^2-Met-enk-NH_2] had ED_{50}'s at the mouse vas deferens greater than 1000 nM, compared to a 3.7 nM ED_{50} for the parent D-Ala^2-Met-enk-NH_2.[22]

A selected number of compounds were assessed in a κ-opioid assay (Table 4). At the same time, they were reassessed in the μ and δ assays, explaining the slight (within experimental error) differences for these values in Tables 2–4. The compounds are all relatively weakly active at the κ site defined by U-69593, and it is unlikely that any antinociceptive action is mediated through a κ pathway, although no selective antagonists were used in the mouse to confirm this.

In summary, the parent molecule **2**, a minimal enkephalin analog, is quite forgiving of substitution on the tyrosyl phenolic moiety and on the alanyl nitrogen. The tyrosyl and (phenylpropyl)amide NH sites, however, are critical to interactions with the receptor, and even modest changes at these sites cause great loss of binding potency.

Experimental Section

Proton (^{1}H) NMR spectra were recorded at 400 MHz on a Varian VXR-400 using DMSO-d_6 as solvent. Optical rotations were measured in MeOH in a 10-cm cell at 25 °C and at a concentration of 0.1% (see Table 5). Readings were taken at 589 nm on a Perkin-Elmer PE241 polarimeter. Chemical shifts are reported in ppm (δ) using tetramethylsilane as the internal standard. Spectroscopy was performed under the direction of Mr. A. Damascus. Elemental analyses were determined by the Searle Microanalytical Section under the direction of Mr. E. Zielinski. Microanalyses were performed for the stated elements and were within 0.4% of the theoretical values for the stated formula. High-resolution mass spectra are EI except where indicated otherwise and were obtained on a Finnigan MAT-8430, under the direction of Dr. J. Hribar. Column chromatography was performed at low pressure on Porasil or Merck silica. Melting points were taken on a Fisher-Johns hot stage apparatus and are uncorrected. Reverse-phase HPLC was run on a Zorbax RX C-8 column, with eluents of acetonitrile–triethylamine phosphate buffer (pH 2.5). Mixed anhydride yields shown in Table 5 are not optimized and are measured after purification of the N-protected dipeptide amide product.

***N*-Carbobenzoxy-2,6-dimethyl-DL-tyrosine (3).** 2,6-Dimethyltyrosine hydrochloride[13] (20.0 g) was dissolved in water (1 L). The pH was adjusted to 8.5 (10% NaOH in H_2O), and benzyl chloroformate (14.3 g) was added in one portion. The pH was maintained between 7 and 8 with aqueous NaOH for 2 h. The reaction mixture was then acidified (concentrated HCl) and extracted with ethyl acetate (EtOAc). The aqueous layer was saturated with NaCl and then extracted three times with EtOAc. The organic fractions were combined, dried (Na_2SO_4), filtered, and stripped to an oil. This oil was triturated with Et_2O/hexane, giving a solid. The solid was ground and dried overnight at 42 °C and 110 Torr. Mp: 148–151 °C. NMR: δ 4.97 (s, benzyl methylene); δ 7.25–7.36 (Z-phenyl); δ 7.62 (d, J = 9 Hz, exchangeable, 1 H (amino proton)). HRMS: m/z M^+ 343.1374, $C_{19}H_{21}NO_5$ requires 343.1420. Anal. ($C_{19}H_{21}NO_5$) C, H, N.

***O*-Benzyl-*N*-carbobenzoxy-2,6-dimethyl-DL-tyrosine Benzyl Ester (4).** **3** (15 g, 43.7 mmol) was dissolved in dimethylformamide (DMF, 200 mL) and treated with benzyl bromide (29.9 g, 20.0 mL, 174.9 mmol) and K_2CO_3 (18.1 g, 131.2 mmol) at room temperature for 16 h. The reaction mixture was diluted to 1.7 L with H_2O and then extracted twice with CH_2Cl_2. The organic fractions were combined, dried ($MgSO_4$), filtered, and stripped to an oil. This oil was subjected to column chromatography on silica gel, using a EtOAc–hexane eluent. The major product was *N*-Z-2,6-dimethyl-DL-tyrosine benzyl ester. This material (11.1 g, 25.6 mmol) was used directly: Sodium hydride dispersion in mineral oil (28.2 mmol) was washed with petroleum ether and suspended in DMF (200 mL). All of the *N*-Z-2,6-dimethyl-DL-tyrosine benzyl ester was added thereto. After 10 min of stirring, benzyl bromide (3.20 mL, 4.60 g, 26.9 mmol) was added all at once. The mixture was stirred 16 h and then worked up as describe above, giving 13 g (56%) of dibenzylated product (4). HRMS: m/z M^+ 523.2365, $C_{33}H_{33}NO_5$ requires 523.2359.

***O*-Benzyl-*N*-carbobenzoxy-2,6-dimethyl-DL-tyrosine (5).** To 4 (6.0 g, 11.5 mmol) suspended in MeOH (75 mL) was added a cooled solution of NaOH (3.67 g, 91.8 mmol) in water (10 mL). The mixture was stoppered and stirred for 3 h. A solution of $KHSO_4$ (12.5 g, 91.8 mmol) in 100 mL of water was added to the reaction mixture, and this mixture was reduced in volume and extracted thrice with CH_2Cl_2. The organic fractions were combined, dried ($MgSO_4$), filtered, and stripped to give the product (**5**, 4.6 g, 92%). HRMS: m/z M^+ 433.1887, $C_{26}H_{27}NO_5$ requires 433.1889.

Benzyl 4-[[2,6-Dimethyl-4-(phenylmethoxy)phenyl]-methyl]-5-oxo-2-phenyl-3-oxazolidinecarboxylate (6). **5** (12 g, 27.7 mmol), 1,1,1-trichloroethane (350 mL), benzaldehyde (5.62 mL, 5.86 g, 55.4 mmol), and *p*-toluenesulfonic acid monohydrate (5.27 g, 27.7 mmol) were placed in a 500-mL round-bottom single-neck flask fitted with a Soxhlet extractor whose thimble was filled with 5A molecular sieves (8–12-mesh beads).[14] The flask

Table 5. Physical Properties and Synthetic and Microanalytical Data

compd	scheme	$[\alpha]_D$[a]	synth[b]	yield[c]	analyses	formula	mp (°C)	HPLC time[d]	HPLC area[e]
10a	1	+90.1	A	40	C,H,N	$C_{24}H_{33}N_3O_3$	219–220	11.77	96.8
10b	1	−23.2	A	40	C,H,N	$C_{24}H_{33}N_3O_3 \cdot 0.25H_2O$	132–133	12.11	82.8
16a	2	+36.3	B, C, D	86	C,H,N,Cl	$C_{24}H_{33}N_3O_3 \cdot HCl \cdot 0.25H_2O$			
16b	2	+31.3	B, C, D	45	C,H,N,Cl	$C_{24}H_{33}N_3O_3 \cdot HCl \cdot 0.75H_2O$			
16c	2	+117.9	B, C, D	9	C,H,N,Cl	$C_{26}H_{35}N_3O_3 \cdot HCl \cdot 0.5H_2O$	143–152	16.05	96.4
16d	2	−48.6	B, C, D	14	C,H,N,Cl	$C_{26}H_{35}N_3O_3 \cdot HCl \cdot 0.5H_2O$	146–155	16.22	95.4
16e	2	+21.2	B, D	41	C,H,N,Cl	$C_{24}H_{33}N_3O_3 \cdot HCl \cdot 0.5H_2O$	146–148	14.1, 15.1	51, 42
18a	3	+13.6	A	10	C,H,N,Cl	$C_{27}H_{37}N_3O_3 \cdot HCl \cdot 0.5H_2O$	152–154	16.8, 17.1	37, 59
18b	3	+106.0	A	11	C,H,N,Cl	$C_{27}H_{37}N_3O_3 \cdot 1.125HCl \cdot H_2O$	164–175	16.73	91.8
18c	3	−50.0	A	14	C,H,N,Cl	$C_{27}H_{37}N_3O_3 \cdot 1.125HCl \cdot 0.25H_2O$	158–162 dec	17.21	79.2
20a	4	+29.7	A	89	C,H,N,Cl	$C_{24}H_{33}N_3O_3 \cdot HCl \cdot 0.5H_2O$	130–133	29.5, 31.3	50, 49
20b	4	+76.2	A	*f*	C,H,N	$C_{24}H_{33}N_3O_3 \cdot 0.25H_2O$	130–132	30.25	100
20c	4	−37.8	A	*f*	C,H,N	$C_{24}H_{33}N_3O_3 \cdot 0.25H_2O$			
20d	4	+11.8	A	55	C,H,N,Cl	$C_{25}H_{35}N_3O_3 \cdot 1.25HCl \cdot 0.75AcOH \cdot 0.125H_2O$			
20e	4	+130.7	A	64[g]	C,H,N,Cl	$C_{26}H_{35}N3O_3 \cdot HCl \cdot 0.5H_2O$	129–133	19.06	91.1
26a	5	+115.7	A	36	C,H,N,Cl	$C_{30}H_{37}N_3O_3 \cdot HCl \cdot 0.75H_2O$	176–177	19.37	96.2
26b	5	−69.7	A	38	C,H,N,Cl	$C_{30}H_{37}N_3O_3 \cdot 1.125HCl \cdot 0.5H_2O$	200.5–202	19.00	92.3
26c	5	+94.0	A, E	30	C,H,N,Cl,F	$C_{30}H_{36}N_3O_3F \cdot HCl \cdot 0.75H_2O$	125–127	21.55	91.4
26d	5	−64.2	A, E	28	C,H,N,Cl,F	$C_{30}H_{36}N_3O_3F \cdot 1.125HCl \cdot 0.75H_2O$	133–136	21.12	89.3
26e	5	+90.2	A, E	32	C,H,N,Cl	$C_{31}H_{36}N_4O_3 \cdot HCl \cdot 0.25H_2O$			
26f	5	+72.7	A, E	21	C,H,N,Cl	$C_{30}H_{36}N_4O_5 \cdot 1.25HCl \cdot 1.5H_2O$			
26g	5	−27.9	A, E	25	C,H,N,Cl	$C_{30}H_{36}N_4O_5 \cdot HCl \cdot 1.25H_2O$	118–124	20.95	89.8
26h	5		A, E	20	C,H,N,Cl	$C_{31}H_{37}N_3O_5 \cdot 1.125HCl \cdot 0.75H_2O$			
26i	5	−64.5	A, E	31	C,H,N,Cl	$C_{31}H_{37}N_3O_5 \cdot HCl \cdot 1.375H_2O$	155–157	17.58	83.5
26j	5	+102.4	A, E	35	C,H,N,Cl	$C_{34}H_{45}N_3O_3 \cdot HCl \cdot 0.5H_2O$	128–130	25.01	90.3
26k	5	−50.0	A, E	33	C,N,Cl;H[h]	$C_{34}H_{45}N_3O_3 \cdot HCl \cdot 0.5H_2O$			
26m	5	+108.4	A, E	17	C,H,N,Cl	$C_{31}H_{39}N_3O_3 \cdot HCl \cdot 0.75H_2O$	128–131	20.02	98.1
30a	6	+112	A	6	C,H,N,Cl	$C_{24}H_{33}N_3O_3 \cdot 1.125HCl \cdot H_2O$	193–240 dec	11.74	90.6
30b	6	−51.0	A	3	C,H,N,Cl	$C_{24}H_{33}N_3O_3 \cdot HCl \cdot 1.25H_2O$			
31	6	+95.5	A	21	C,H,N,Cl	$C_{31}H_{39}N_3O_3 \cdot HCl$		19.62	98.1
2		+536.0						10.78	93.1
2′		−356.9						11.15	93.3

[a] 589 nM. [b] Synthetic method A, B, C, D, and/or E (see the Experimental Section). [c] Percent yield in final mixed anhydride condensation, after chromatography (when appropriate). [d] Crest time in minutes, reverse-phase HPLC using a Zorbax RX C-8 column (see the Experimental Section). [e] Percent area under curve at given crest time. [f] Isolated by chromatographic separation of diastereomers of **20a**. [g] Yield of phenolic alkylation step (allyl bromide/K_2CO_3/KI/refluxing acetone) performed on *N*-Boc-2. [h] H: calcd, 8.04; found, 7.55.

was immersed in an oil bath (bath temperature 120 °C), and the mixture was refluxed for 16 h. The reaction was then cooled, and the mixture was washed with saturated aqueous $NaHCO_3$. The aqueous wash was back-washed twice with CH_2Cl_2. All the organic fractions were combined, dried ($MgSO_4$), filtered, stripped, and subjected to column chromatography on silica with eluents of EtOAc–hexane. The title compound (6) was isolated as a solid (9.8 g, 68%) which was washed with ethanol and then ether. NMR: aromatic proton δ 4.21 (m), benzhydryl proton under benzyl peaks δ 4.99. Integration shows three benzyl(idene) groups.

Benzyl 4-[[2,6-Dimethyl-4-(phenylmethoxy)phenyl]-methyl]-4-methyl-5-oxo-2-phenyl-3-oxazolidinecarboxylate (7). A 500-mL round-bottom flask was charged with dry THF (150 mL) and was cooled to −60 °C. A solution of potassium hexamethyldisilazane in toluene (0.653 M, 21.0 mL, Callery Chemical Co., Callery, PA) was added all at once (N_2 atmosphere). The solution was cooled back to −70 °C, and a solution of 6 (5.5 g, 10.6 mmol) in THF (100 mL) was added dropwise rapidly, keeping the reaction temperature at or below −62 °C. The mixture was stirred in the cold bath for 30 min, and the cold bath was then removed. Stirring at room temperature for another 30 min elevated the reaction temperature to −12 °C. The reaction mixture was then reimmersed in the cold bath, and the temperature returned to −70 °C. Methyl iodide (1.05 mL, 2.40 g, 16.9 mmol) was added all at once, and after another 10 min the cold bath was removed. The reaction mixture was permitted to warm to room temperature, and 3 h after removal of the cold bath the mixture was partitioned between a mixture of H_2O (200 mL), 0.5 N $KHSO_4$ (50 mL), saturated brine (200 mL), and ether (200 mL). The aqueous phase was washed with ether, and the organic fractions were combined, dried ($MgSO_4$), filtered, and stripped to an oil. The oil was applied to silica gel column chromatography, using EtOAc–hexane as eluent, giving 7 (3.1 g, 55%). NMR: a methyl group at δ 2.71, 3 protons.

α,2,6-Trimethyl-4-(phenylmethoxy)-α-[[(phenylmethoxy)carbonyl]amino]benzenepropanoic Acid (8). 7 (2.42 g) was dissolved in 5 mL of 1:1 CH_2Cl_2–methanol, and the mixture was added to methanolic NaOH (1 N, 100 mL). After 3 h of stirring, the mixture was concentrated to 75 mL, diluted to 500 mL with water, and extracted twice with ether to remove nonacidic contaminants. The aqueous phase was made acidic with 0.5 N $KHSO_4$ and then extracted four times with CH_2Cl_2. The CH_2Cl_2 fractions were combined, dried ($MgSO_4$), filtered, and stripped to give the title compound (1.75 g, 74%). NMR: a methyl group at δ 2.75.

Method A. α,2,6-Trimethyl-*N*-[(phenylmethoxy)carbonyl]-*O*-(phenylmethyl)tyrosyl-*N*-(3-phenylpropyl)alaninamide (9). 8 (1.75 g, 3.91 mmol) and 5A molecular sieves (2 g, 8–12 mesh) in 30 mL of CH_2Cl_2 were cooled to −15 °C (N_2 atmosphere), and *N*-methylmorpholine (NMM, 0.42 g, 4.11 mmol) was added. The reaction mixture was allowed to warm to 5 °C and then cooled to −60 °C. Isobutyl chloroformate (0.53 mL, 4.03 mmol) was added. The flask was then immersed in an ice bath (0 °C), and the reaction was run at 0 °C for 30 min. The flask was then reimmersed in a dry ice–acetone bath and cooled to −70 °C. NMM (0.42 g, 4.11 mmol) was then added, followed by the dropwise addition of *N*-(3-phenylpropyl)-D-alaninamide hydrochloride[9] (0.85 g, 4.11 mmol) in CH_2Cl_2 (10 mL), keeping the reaction temperature at or below −55 °C. After the addition was complete, the reaction mixture was allowed to warm to room temperature, and stirring was continued another 1.5 h. The reaction mixture was then filtered. The filtrate was washed with 0.5 M $KHSO_4$. The resulting aqueous layer was then washed with fresh CH_2Cl_2. The organic fractions were combined, dried ($MgSO_4$), filtered, and stripped to a hard foam (2.32 g). The product was subjected to column chromatography on silica gel, using ethanol–methyl *tert*-butyl ether–ammonium hydroxide eluent. The two diastereomers were thus separated, giving a faster-emerging and a slower-emerging isomer. The more rapidly emerging isomer (1.0 g) contained the D-tyrosyl moiety (**9b**), and the more slowly emerging isomer (1.0 g) contained the L-tyrosyl moiety (**9a**).

α,2,6-Trimethyl-D-tyrosyl-*N*-(3-phenylpropyl)-D-alaninamide (10b). The more rapidly emerging title isomer **9b** (1.0 g) was hydrogenolyzed in methanol with a palladium black catalyst (0.31 g) at room temperature for 17 h at 60 psi of hydrogen. The mixture was filtered to remove the catalyst, refiltered to remove fine particles, and stripped. The residue was dissolved in ethanol–water–methanol, filtered, reduced in volume with a N_2 stream, and lyophilized to give the title compound (0.45 g), the *D,D*-isomer, mp 132–133 °C. NMR: α-methyl group at δ 2.11 and 2.25 for two rotamers; alanyl methyl at δ 1.21 (d, J = 7 Hz). $[\alpha]_D$: −23.2°. Anal. ($C_{24}H_{33}N_3O_3 \cdot {}^1/_4H_2O$, MW 416.050) C, H, N.

α,2,6-Trimethyl-L-tyrosyl-*N*-(3-phenylpropyl)-D-alaninamide (10a). The more slowly emerging isomer **9a** was treated as described for **9b** to give **10a**, the L,D-isomer, mp 219–220 °C. NMR: δ 1.10 (d, J = 7 Hz). $[\alpha]_D$: +90.1°. Anal. ($C_{24}H_{33}N_3O_3$, MW 411.54) C, H, N.

Method C. *N*-Methyl-*N*-carbobenzoxy-D-alanine (12a). *N*-Carbobenzoxy-D-alanine (11, Sigma Chemical Co., St. Louis, MO, 22.3 g, 100 mmol) was dissolved in THF (300 mL). Methyl iodide (114 g, 800 mmol) was added, and the mixture was cooled to −5 °C. Sodium hydride (300 mmol, 50% suspension in mineral oil) was added over a 1-h period. The temperature was maintained at 10 °C for another hour. Another 300 mL of THF was added, and the mixture was stirred at room temperature for 68 h. EtOAc (500 mL) was added to the reaction mixture, followed by H_2O (10 mL). This mixture was concentrated and then partitioned between water and Et_2O. The aqueous layer was washed twice with ether; the organic fractions were combined and rinsed with saturated aqueous $NaHCO_3$. The aqueous fractions were combined. The organic layers were then discarded, and the aqueous fraction was acidified with citric acid solution to pH 4. The acidified aqueous fraction was extracted with EtOAc. The organic fraction was washed twice with 5% $Na_2S_2O_3$ solution and once with water. The organic fraction was dried ($MgSO_4$), filtered, and stripped to an oil (22.2 g). Crystallization was effected with EtOAc–Skelly B mixtures. NMR ($CDCl_3$): N-Me δ 2.89 (s); alanylmethyl δ 1.43. $[\alpha]_D$ (ethanol): +24.5°. For the preparation of **12c**, allyl iodide replaced methyl iodide.

Method D. N^{α}-Methyl-*N*-(3-phenylpropyl)-D-alaninamide (14a). **12a** (7.12 g, 30.0 mmol) was treated with *N*-methylmorpholine (3.04 g, 30.0 mmol), isobutyl chloroformate (4.10 g, 30.0 mmol), and 3-phenylpropylamine (4.06 g, 30.0 mmol), as described in the preparation of (**9**), giving 9.87 g of a light yellow oil (**13a**). This oil was subjected to hydrogenation (60 psi of H_2) in methanol at 25 °C with a 10% Pd/C catalyst to give the deprotected N^{α}-methyl-*N*-(3-phenylpropyl)-D-alaninamide (**14a**) as an oil (9.58 g) after filtration and concentration. For the preparation of **14c**, **12c** replaced **12a**. For the preparation of **14e**, Z-D-alanine replaced **12a**, and *N*-methyl-3-phenylpropylamine[23] replaced 3-phenylpropylamine.

Method B. 2,6-Dimethyl-DL-tyrosyl-N^{α}-methyl-*N*-(3-phenylpropyl)-D-alaninamide Hydrochloride (16a). **14a** (2.88 g, 13.05 mmol) replaced *N*-(3-phenylpropyl)-D-alaninamide in the mixed anhydride syntheses described in the procedure for **9** (method A). It was reacted with the intermediate formed by the reaction of Boc-2,6-dimethyltyrosine[12,13] (**15**, 4.00 g, 13.05 mmol) with isobutyl chloroformate (3.58 g, 26.1 mmol; *notice that 2 mol of isobutyl chloroformate are used—the second mole acts as a protecting group for the free phenol*) in the presence of NMM (2.64 g, 26.1 mmol) in CH_2Cl_2. The final amide synthesis from the mixed anhydride was run overnight at 25 °C. After the workup described in method A, the material was used as is, without chromatography.

O-(Isobutoxycarbonyl)-Boc-2,6-dimethyl-DL-tyrosyl-N^{α}-methyl-*N*-(3-phenylpropyl)-D-alanylphenylpropylamide (6.71 g) was dissolved in methanol (100 mL) and stirred with K_2CO_3 (1.98 g) for 4 h. The mixture was concentrated and partitioned between CH_2Cl_2 and 0.5 N $KHSO_4$. The organic layer was extracted twice with 0.5 N $KHSO_4$. Each aqueous wash was back-washed with fresh CH_2Cl_2. The organic fractions were combined, washed thrice with saturated $NaHCO_3$ (back-wash with CH_2Cl_2), washed with saturated brine, dried (Na_2SO_4 followed by $CaSO_4$), filtered, and stripped to an oil (5.50 g). The oil was subjected to column chromatography on Porasil silica, using EtOAc–CH_2Cl_2 eluents, thus separating the mixture of disastereomers (3.47 g). This oil was triturated with hexane–Et_2O mixtures to give 3.18 g of a foam. This foam was treated with HOAc and HCl/dioxane as described above to give **16a**. Anal. ($C_{24}H_{33}N_3O_3 \cdot HCl \cdot 0.25H_2O$, MW 452.51) C, H, N.

2,6-Dimethyl-DL-tyrosyl-*N*-methyl-*N*-(3-phenylpropyl)-D-alaninamide Hydrochloride (16e). **15** (3.54 g, 11.5 mmol) and **14e** (2.50 g) were reacted by method A, and the subsequent column chromatography did not separate the diastereomers. A portion of this mixture (0.50 g) was treated with dioxane (20 mL) and 6.8 N HCl/dioxane (2.88 mL) for 20 h at 25 °C. The mixture was stripped to a foam, triturated thrice with Et_2O, and dried in an abderhalden apparatus at 78 °C at 0.01 Torr for 2 h. The product was the desired hydrochloride hemihydrate **16e**, mp 146–148 °C. $[\alpha]_D$: +21.2°. NMR: *N*-methyl δ 2.79 (t), collapses to δ 2.82 (d) at 60°; alanyl methyl δ 1.12 (m), 0.81 (m); these collapse to doublets at 60 °C. These multiplets are probably caused by restricted rotations due to interactions of the alanyl and (3-phenylpropyl)-amide methyl functions. Anal. ($C_{24}H_{33}N_3O_3 \cdot HCl \cdot {}^1/_2H_2O$, MW 457.02) C, H, N, Cl.

2(*R*)-[(Cyclopropylmethyl)amino]-*N*-(3-phenylpropyl)-propanamide (17). A mixture of *N*-(3-phenylpropyl)-D-alaninamide (0.825 g, 4.00 mmol), $NaHCO_3$ (1.00 g, 1.20 mmol), (bromomethyl)cyclopropane (0.64 g, 4.74 mmol), and 10 mL of EtOH (2B) was heated at reflux for 8 h with stirring. The reaction mixture was partitioned between EtOAc and water. The organic layer was separated and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the resultant oil was purified by column chromatography on a Porasil column eluting with 3/96.9/0.2 MeOH/$CHCl_3$/NH_4OH. NMR ($CDCl_3$): D-Ala methyl δ 1.28 (d, J = 9 Hz); cyclopropylmethyl δ 0–0.25 (complex, 2H), δ 0.30–0.65 (complex, 2H); δ 0.65–1.00 (complex, 1H); δ 2.39 (dd, J_1 = 3.5 Hz, J_2 = 8 Hz). HRMS chemical ionization: m/z MH^+ 261.1942, $[C_{16}H_{25}N_2O]^+$ requires 261.197.

A sample of the above product was dissolved in MeOH and treated with sufficient HCl gas dissolved in 2-propanol to render acid. Anhydrous Et_2O was added to the point of turbidity and the mixture cooled to 0 °C. The resultant solid was filtered and dried at reduced pressure under an inert atmosphere. NMR: shift of D-alanyl methyl δ 1.43 (d, 3H, J = 9 Hz); cyclopropyl methyl δ 0.20–0.70 (4H, complex); δ 0.80–1.30 (1H, complex); 2.4–2.9 (2H, hidden).

2,6-Dimethyl-DL-tyrosyl-N^{α}-(cyclopropylmethyl)-*N*-(3-phenylpropyl)-D-alaninamide Monohydrochloride (18a). **17** (9.60 mmol) replaced *N*-(3-phenylpropyl)-D-alaninamide and Boc-2,6-dimethyl-DL-tyrosine (**15**, 2.97 g, 9.60 mmol) replaced compound **8** in the mixed anhydride synthesis described in the preparation of **9**. It was reacted using isobutyl chloroformate (1.31 g, 9.60 mmol) and NMM (0.98 g, 9.60 mmol). DMF replaced CH_2Cl_2 as the solvent. The reaction mixture was worked up as described above, and the resultant foam (4.4 g) was purified by column chromatography on Merck silica eluting with 3% methanol–CH_2Cl_2. The purified material (0.51 g, 0.924 mmol) was treated with glacial acetic acid (5 mL) and 6.2 N HCl/dioxane (1.36 mL) as described for **16e** to give the desired **18a**, mp 152–154 °C. $[\alpha]_D$: +13.6°. NMR ($CDCl_3$): cyclopropyl δ 0–1.0 (complex, 5H); δ 2.25 (2H partially hidden by 2,6-dimethyl groups (tyrosine)); D-Ala methyl shifts δ 1.23 and 1.41 (3H). Anal. ($C_{27}H_{37}N_3O_3 \cdot HCl \cdot {}^1/_2H_2O$, MW 497.08) C, H, N, Cl.

***N*-Boc-*O*,2,6-trimethyl-D,L-tyrosine (19a).** **15** (3.0 g, 9.70 mmol) was stirred with methyl iodide (6.88 g, 48.5 mmol) and potassium carbonate (5.36 g, 38.8 mmol) in DMF (50 mL) for 17 h in a 100-mL flask protected from moisture. The reaction mixture was then partitioned between water and Et_2O. The aqueous phase was washed twice with Et_2O, and the organic fractions were combined, dried ($MgSO_4$), filtered, and stripped to a white solid. NMR: methoxy singlets at δ 3.51 and 3.66. HRMS: m/z M^+ 337.1898, $C_{18}H_{27}NO_5$ requires M^+ 337.189. This material was dissolved in MeOH (70 mL) and cooled in an ice bath. A solution of NaOH (3.1 g, 77.6 mmol) in water was added. The mixture was stirred for 3 h and worked up as described for compound **5**, using 10.6 g of $KHSO_4$. The product **19a** (2.6 g) displayed a single NMR methoxy singlet at δ 3.66. The analogous *N*-Boc-*O*-benzyl-2,6-dimethyl-D,L-tyrosine (**19d**), when prepared as above from **15** with benzyl bromide replacing methyl iodide, displayed a HRMS m/z M^+ 399.2037, $C_{23}H_{29}NO_5$ requires 399.2046.

***O*,2,6-Trimethyl-DL-tyrosyl-*N*-(3-phenylpropyl)-D-ala-**

ninamide Monohydrochloride (20a). A mixed anhydride procedure as described for **9** was carried out on **19a** (2.52 g, 7.79 mmol). After the preparation of the mixed anhydride intermediate, NMM (0.86 mL, 7.79 mmol) was added, followed by the dropwise addition of *N*-(3-phenylpropyl)-D-alaninamide hydrochloride (1.89 g, 7.79 mmol) in CH_2Cl_2 (10 mL). The reaction and workup were as described in **9**, resulting in a hard foam (4.51 g). This material was purified by column chromatography on Woelm silica. The eluent was CH_2Cl_2–EtOH (2B)–NH_4OH (concentrated) (98:2:0.1).

The resulting material (4.45 g) was dissolved in glacial acetic acid (50 mL) and treated with 6.8 N HCl in dioxane (12 mL). After 1.5 h of reaction at room temperature, the mixture was stripped to a syrup. The syrup was dissolved in MeOH, filtered, stripped, and triturated repeatedly with Et_2O. The resulting solid was dried in a vacuum desiccator to give the product (**20a**) as the hydrochloride hemihydrate, mp 130–133 °C. $[\alpha]_D$: +29.7°. Anal. ($C_{24}H_{33}N_3O_3 \cdot HCl \cdot {}^1/_2H_2O$, MW 457.02) C, H, N, Cl.

2,6-Dimethyl-DL-tyrosine Methyl Ester Hydrochloride (22). Thionyl chloride (38.74 g, 325 mmol) was added dropwise to MeOH (250 mL) at −70 °C (N_2 atmosphere), keeping the reaction temperature at or below −60 °C. After the addition was complete, the mixture was warmed to 0 °C. 2,6-Dimethyl-DL-tyrosine hydrochloride[12,13] (**21**, 40 g, 162 mmol) was added, and the mixture was stirred under N_2 at room temperature overnight. The reaction mixture was then filtered to remove traces of solid and stripped to an oil, which was triturated with Et_2O and allowed to stand. The oil solidified overnight and was dried in a vacuum oven at 30 °C, giving the methyl ester hydrochloride (**22**). NMR: methoxy δ 3.45 (s); 3,5-diH on aromatic ring δ 3.45 (s); 3,5-diH on aromatic ring δ 6.44 (s). HRMS: *m/z* M^+ 223.1194, $C_{12}H_{17}NO_3$ requires 223.1208.

***N*-Boc-2,6-dimethyl-DL-tyrosine Methyl Ester (23).** **22** (40 g, 154 mmol) was suspended in $CHCl_3$ (800 mL). NMM (15.5 g, 16.94 mL, 154 mmol) was added, and the mixture was stirred under nitrogen for 40 min. Di-*tert*-butyl dicarbonate (33.61 g, 35.42 mL, 154 mmol) was added, and the mixture was stirred overnight. The mixture was washed twice with water, dried ($MgSO_4$), filtered, and stripped. The residue was triturated and filtered with hexane, giving the title compound **23**. NMR: methoxy δ 3.50 (s), Boc-methyls δ 1.35 (s). Anal. ($C_{17}H_{25}NO_5$, MW 323.39) C, H, N.

Method E. *O*-[(4-Cyanophenyl)methyl]-*N*-Boc-2,6-dimethyl-DL-tyrosine (25e). A 50% dispersion of NaH in oil (27.3 mmol) was weighed into a 1-L round-bottom flask containing a magnetic stirrer. The dispersion was washed with hexane to remove the mineral oil, and the flask was immediately charged with THF (200 mL). *N*-Boc-2,6-dimethyl-DL-tyrosine methyl ester (**23**, 8 g, 24.8 mmol) was added, and a drying tube was inserted. The mixture was stirred for 2 h. α-Bromo-*p*-toluonitrile (5.24 g, 26.8 mmol) was then added, and the mixture was stirred at room temperature overnight. A thin-layer chromatogram (2:1 hexane–EtOAc) was then run to confirm completeness of reaction. In some runs starting material was still present, NaH dispersion (${}^1/_2$ molar amount) and alkylating agent (${}^1/_2$ molar amount) were added, and the reaction was run for another 24 h at room temperature. The mixture was then poured into water (1.2 L) and rapidly extracted thrice with CH_2Cl_2. The organic fractions were combined, dried ($MgSO_4$), and stripped to give a product (*O*-(*p*-cyanobenzyl)-Boc-2,6-dimethyl-DL-tyrosine methyl ester, **24e**), which was directly hydrolyzed with NaOH as described above to give **25e**. NMR: benzyl methylene δ 5.10 (s); tyrosyl aromatic protons δ 6.60 (s); benzyl aromatic protons centered at δ 7.65. Anal. ($C_{23}H_{28}NO_5$, MW 399.49) C, H, N. **24**- and **25c,d,f-m** were synthesized in a similar manner and used in the preparation of the corresponding dipeptide amides (**26**).

***O*-[(2- and 4-Substituted phenyl)methyl]-*N*-Boc-2,6-dimethyltyrosine (26c-m).** The appropriate compound **25** was treated as described in method A. For **26h,i**, the final Boc deprotection also served to release the *tert*-butyl ester on the benzyl group. See Table 5 for the physical properties of the final products.

***O*-Benzyl-*N*-Boc-2,6-dimethyl-DL-tyrosine Benzyl Ester (27).** **15** (5.0 g, 16.2 mmol) was dissolved in DMF (100 mL), and K_2CO_3 (6.69 g, 48.5 mmol) was added. The mixture was stirred under a drying tube in a 250-mL round-bottom single-necked flask. Benzyl bromide (11.1 g, 64.6 mmol) was added and the mixture stirred 24 h. Then another portion of benzyl bromide (5.46 g, 31.9 mmol) and of K_2CO_3 (3.4 g, 24.6 mmol) was added, and stirring was continued another 24 h. The mixture was then partitioned between water and Et_2O. The aqueous phase was washed with Et_2O. The organic fractions were combined, dried (M_gSO_4), filtered, and stripped to an oil. The oil was shaken with petroleum ether. This mixture was seeded, and product crystallized rapidly. NMR: benzyl protons (4 protons) δ 4.99 (s). Total aromatic integration 12 protons. Anal. ($C_{30}H_{35}NO_5$, MW 489.61) C, H, N. HRMS: *m/z* M^+ 489.250, $C_{30}H_{35}NO_5$ requires 489.252.

***O*-Benzyl-*N*-Boc-*N*,2,6-trimethyl-DL-tyrosine Benzyl Ester (28).** **27** (1.73 g, 3.53 mmol) was treated with sodium hydride (7.06 mmol, rinsed with petroleum ether) in DMF (22 mL) in a 100-mL pear-shaped flask, protected with a drying tube. After 15 min, CH_3I (2.51 g, 17.7 mmol) was added. After 2.5 h, the reaction mixture was diluted to 150 mL with 0.5 N $KHSO_4$, and the mixture was extracted thrice with Et_2O. The organic fractions were combined, dried ($MgSO_4$), filtered, and stripped to an oil (2.27 g). The oil was subjected to column chromatography on Woelm silica, with EtOAc–CH_2Cl_2 eluents, giving **28**. NMR: CO_2-Me δ 3.65 (s); benzyl protons δ 5.03 (2 protons), δ 7.35 (5.7 protons); N-Me δ 2.55 (s); Boc-methyl δ 1.30 (asym d).

***O*-Benzyl-*N*-Boc-*N*,2,6-trimethyl-DL-tyrosyl-*N*-(3-phenylpropyl)-D-alaninamide (29).** Ester **28** (0.85 g, 2.06 mmol) was hydrolyzed with NaOH (0.66 g, 16.5 mmol) as described above. NMR: benzyl protons δ 5.01, δ 7.35; N-Me δ 2.59 (s); Boc-methyls δ 1.27 (asym d). This resulting free acid, *O*-benzyl-*N*-Boc-*N*,2,6-trimethyltyrosine (0.70 g, 1.69 mmol), was treated with NMM (0.18 g, 1.78 mmol), isobutyl chloroformate (0.24 g, 1.75 mmol), and *N*-(3-phenylpropyl)-D-alaninamide (free base, 0.367 g, 1.78 mmol) as in method A. The resulting oil was subjected to column chromatography on Merck silica, using EtOAc–CH_2Cl_2 eluents. The resulting material was further purified on a 4-mm Chromatotron plate (centrifugal thick-layer chromatography), using hexane–EtOAc eluents. The resulting mixture of diastereomers (0.45 g) was subjected to hydrogenation in tetrahydrofuran (30 mL) in the presence of palladium black (0.045 g) under 60 psi of hydrogen at 25 °C for 22 h. Then another portion of palladium black (0.045 g) was added, and the same conditions were reapplied for 65 h. The resulting mixture was filtered, stripped to a solid, and subjected to column chromatography on Woelm silica with eluents of EtOH–CH_2Cl_2. The first emerging compound was unchanged *O*-benzyl-*N*-Boc-*N*,2,6-trimethyl-DL-tyrosyl-*N*-(3-phenylpropyl)-D-alanylphenylpropylamide (**29**). This was saved for deblocking. The next compound (*vide infra*) was the expected O-deprotected product: Boc-*N*,2,6-trimethyl-DL-tyrosyl-*N*-(3-phenylpropyl)-D-alaninamide (**30**).

***N*,2,6-Trimethyl-DL-tyrosyl-*N*-(3-phenylpropyl)-D-alaninamide (30b).** The mixture of diastereomers **30** was subjected to another column chromatography on Woelm silica, using a gradient elution of EtOH–CH_2Cl_2, 2.5:97.5–5.5:94.5. The first emerging compound (**30b**) and the second emerging compound (**30a**) were separately deblocked as described above. See Table 5 for analytical data.

***O*-Benzyl-*N*,2,6-trimethyl-DL-tyrosyl-*N*-(3-phenylpropyl)-D-alaninamide (31).** **29** was treated with methanol and 6.8 N HCl in dioxane for 24 h. The mixture was evaporated in a stream of nitrogen and dissolved in aqueous MeOH. The solution was filtered through Whatman 50 filter paper, reduced in volume in a nitrogen stream, and lyophilized, mp 186–187 °C. Anal. ($C_{31}H_{39}N_3O_3 \cdot HCl \cdot {}^3/_4H_2O$, MW 551.64) C, H, N.

Pharmacology: Writhing Assay. Charles River male albino mice, weighing 20–30 g, were used. Thirty minutes after intragastric administration to 10 mice of 10 mg/kg of body weight of compound, 0.1 mg/10 g of body weight of a 0.025% w/v solution of phenylbenzoquinone (PBQ) was injected intraperitoneally into each mouse. Ten mice which were given saline in place of a test compound were used as a control group. Five minutes later, each mouse was individually placed into a glass beaker for observation, and the number of writhes occurring during the following 10-min period was counted.

A test compound was considered to have produced analgesia in a mouse if, in accordance with the conditions set forth above, after the administration of 10 mg/kg of body weight of compound

to the mouse, the number of writhes elicited by a mouse injected with PBQ was equal to, or less than, half the median number of writhes recorded for the saline-treated control group of mice that day, as described by Taber.[16]

Opiate Binding Assay. Male Charles River Sprague–Dawley albino rats weighing 150–300 g were stunned and decapitated. Their forebrains (minus the cerebellum and associated hindbrain) were quickly removed and rinsed in ice-cold 50 mM Tris buffer, pH 7.4, and homogenized in 20 volumes of buffer with a Polytron (Brinkman) at setting 6 for 30 s. The membranes were washed by centrifugation for 20 min at 30000*g*, followed by resuspension to twice the original volume. The homogenate was incubated at 25 °C for 1 h, followed by centrifugation as above.

The resulting homogenate was then assayed for protein content according to the method described by Itzhaki et al.[24] The final pellet was resuspended to a protein concentration of 10 mg of protein/mL (assuming 6% of wet weight is protein), and 4-mL aliquots were rapidly frozen in liquid N_2.

The binding of compounds to the rat brain opiate receptor membrane preparation containing either δ- or μ-opioid receptors was measured using a modification of the method of C. B. Pert et al.[25]

The opiate binding assays were conducted in triplicate at 37 °C in 50 mM Tris/HCl buffer at pH 7.4 in a final volume of 1 mL, using varying concentrations of compound. Each of three tubes contained 0.8 mL of homogenate containing approximately 1 mg/mL of protein. [^{3}H]DAMGO (2.0 nM) and [^{3}H]DSLET (1.0 nM) were used to label the μ- and δ-opiate rat brain receptors, respectively.

The "percent displacement" of radiolabeled ligand ([^{3}H]-DAMGO for the μ receptors and [^{3}H]DSLET for the δ receptors) bound to the μ- or δ-opioid receptors by a compound was determined at different concentrations of the compound (10 μM, 1 μM, 100 nM, and/or 1 nM). Because the radiolabeled ligand and the compound compete with each other for the opiate receptor binding sites, the greater the percent of displacement of the bound radiolabeled ligand, the better the compound is in terms of its ability to bind to the opiate receptors and, thus, the more potent the compound is. "Specific binding" of a compound of the present invention to the μ- or the δ-opiate rat brain receptors was defined as the difference between total binding and that in the presence of 10 μM of levorphanol.

For those compounds which bound particularly well to the opiate receptors, the mean IC_{50} value (that concentration of a particular compound which is required to have 50% of the bound radiolabeled ligand displaced from the opiate receptors) was calculated (nM). IC_{50} values were determined from log–logit plots of concentration vs percent displacement. Comparison of IC_{50} values in this assay system provides a measure of the receptor specificity of the tested compounds.

Finally, for those compound for which a mean IC_{50} value was calculated for both the μ- and δ-opioid receptors, the ratio of the mean IC_{50} values for the μ- and δ-opioid receptors was determined. The ratio indicates how specific a particular compound is for the δ-opioid receptors. Thus, if the ratio of the mean IC_{50} values is 1.0, the compound is approximately equally potent for both the μ- and the δ-opioid receptors. The greater the number is above 1.0, the more specific the compound is for the δ-opioid receptors.

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