a literature method,[11] by hydrogenation of the corresponding Phe analogues. The synthesis of peptides was performed on a Biosearch 9500ex peptide synthesizer using Merrifield resin. A detailed synthetic protocol has been reported previously.[6] The crude peptide was extracted with 20% aqueous acetic acid, lyophilized, and purified by HPLC (21.4 mm i.d. × 25 cm, C18, 8-μm silica, Dynamax preparative HPLC, Rainin). The purified peptides, recovered by lyophilization of the HPLC fractions, were at least 95% pure and gave proton NMR, FAB-MS, and amino acid analyses consistent with the proposed structure (Table IV).

C5a Receptor Binding Assay. C5a receptor binding affinity of peptides was determined by competing for binding of radioiodinated C5a to human polymorphonuclear leukocyte (PMNL) membranes. Human PMNL membranes were isolated following cell lysis by nitrogen cavitation and Percoll density-gradient centrifugation.[12] Radiolabeled C5a was prepared by glucose oxidase-lactoperoxidase catalyzed radioiodination,[13] and the product was purified by affinity purification on a goat anti-human C5a resin.[14] Binding was performed in buffered balanced salts solution (pH 7.0) containing 0.25% gelatin, a cocktail of protease inhibitors, 50–250 pM ^{125}I-C5a, and 5-20 μg/mL PMNL membranes. Samples were incubated for 60 min at ambient temperature, and membrane-bound C5a was collected by filtration onto Millipore HVLP filters. The inhibitor concentration that displaced ^{125}I-C5a binding by 50% was determined by linear-regression analysis of the data and the apparent inhibition constant (K_i) was calculated by the method of Cheng and Prusoff.[15]

PMNL Chemokinesis. The assay procedure was based on the method of Smith et al.[16] Human peripheral blood PMNL were purified by density-gradient centrifugation on Mono-Poly Resolving Medium (Flow Laboratories, McLean, VA), subjected to brief hypotonic lysis to remove contaminating red cells, and washed three times before use. The assay was conducted in Earle's balanced salt solution buffered with 20 mM HEPES at pH 7.0 and supplemented with 0.25% gelatin to prevent adsorptive loss of C5a proteins. PMNL were suspended at 2 × 10^8 PMNL/mL in 37 °C buffer containing 0.3% SeaPlaque agarose (Marine Colloids, Rockland, ME). The cell mixture was dispensed in 1-μL droplets in a 96-well microtiter plate (Falcon Micro-Test III, Becton Dickinson, Oxnard, CA). The plates were chilled on ice for 10 min. The droplets were layered with 100 μL of buffer, and then 11 μL of buffer with or without stimulus was added. Plates were incubated for 2 h at 37 °C, and the cell-migration distance was measured from the edge of the droplet to the leading front. Chemokinetic migration was estimated by subtracting the mean of a random migration (buffer) control and expressed as a percent of the maximal response to rC5a. The highest value obtained for each peptide is shown in the tables as chemokinetic efficacy.

Acknowledgment. We thank Ms. Ruth Krause, Ms. Loan Miller, and Mr. Thomas Fey for assisting in the collection of biological data, and we thank analytical services at Abbott Laboratories for spectroscopic determinations.

Registry No. 1, 133009-93-5; 2, 133214-63-8; 3, 137232-25-8; 4, 137232-26-9; 5, 137232-27-0; 6, 137232-28-1; 7, 137232-29-2; 8, 137232-30-5; 9, 133214-67-2; 10, 133214-78-5; 11, 133253-59-5; 12, 137232-31-6; 13, 137232-32-7; 14, 133214-90-1; C5a, 80295-54-1.

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Preparation and Opioid Activity of Analogues of the Analgesic Dipeptide 2,6-Dimethyl-L-tyrosyl-*N*-(3-phenylpropyl)-D-alaninamide

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CNS Diseases Research, G. D. Searle and Company, 4901 Searle Parkway, Skokie, Illinois 60067. *Received April 24, 1991*

A number of analogues of the recently disclosed analgesic dipeptide 2,6-dimethyl-L-tyrosyl-D-alanine-phenylpropylamide (SC-39566, **2**) were prepared. These analogues contained oxymethylene, aminomethylene, ketomethylene, bismethylene, and trans double bond (including vinyl fluoride) isosteric replacements for the amide bond between the D-alanine and phenylpropylamine units in **2**. These compounds were tested in opioid binding assays and in the mouse writhing assay for analgesic activity. Though not as potent as **2**, the oxymethylene, and trans double bond isosteres showed analgesic activity. The aminomethylene analogues also showed binding activity in subnanomolar concentrations at the μ receptor. The amide bond between 2,6-dimethyl-L-tyrosine and D-alanine units seems to be critical for opioid activity.

The Tyr tyramine and Phe phenyl moieties in enkephalins were found to be the major contributors to recognition at opioid receptors.[1-3] However, the identification of several potent opioidergic peptides (dermorphin,[4] casomorphin,[5] and morphiceptin[6]) that have Phe in the third

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§ CNS Biology.
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(1) Morley, J. S. Structure–activity Relationships of Enkephalin-like Peptides. *Annu. Rev. Pharmacol. Toxicol.* **1980**, *20*, 81.
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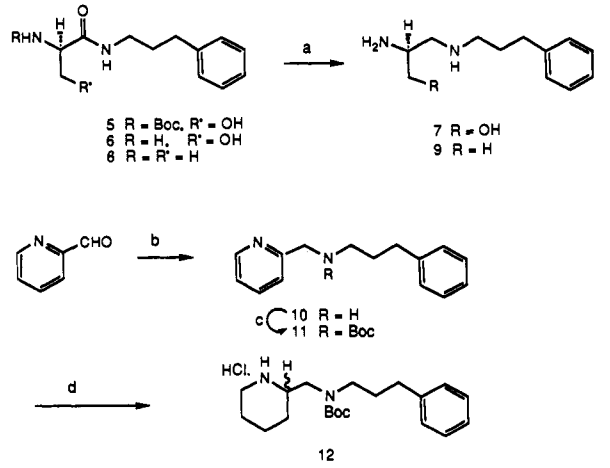
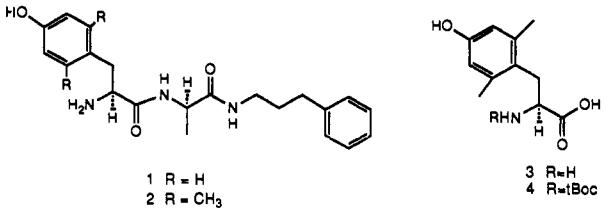
0022-2623/92/1835-0223$03.00/0

Scheme I[a]



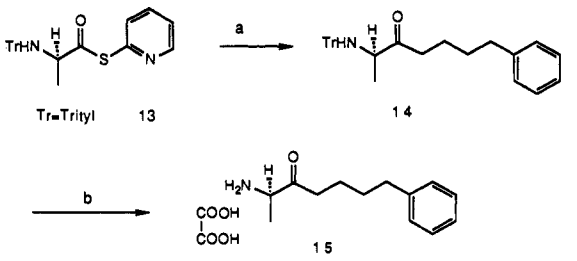
[a] Reagents: (a) B_2H_6, THF; (b) 3-phenyl-1-propylamine, H_2, 5% Pd/C; (c) $(tBoc)_2O$, Et_3N; (d) H_2, 5% Pd/C.

position suggested that although the Tyr/Phe relationship is a critical requirement for opioid activity, the exact position of Phe vs Tyr in the linear amino acid sequence can vary. That the Phe peptides can have potent opioid activity led Stewart and co-workers to elucidate the "minimum structure" of enkephalin required for opioid activity.[7-9] They found that a compound as simple as Tyr-D-Ala-$NHCH_2CH_2CH_2C_6H_5$ (1) retained potent activity in the guinea pig ileum in vitro assay and produced analgesia after icv administration in the mouse.[8,9]



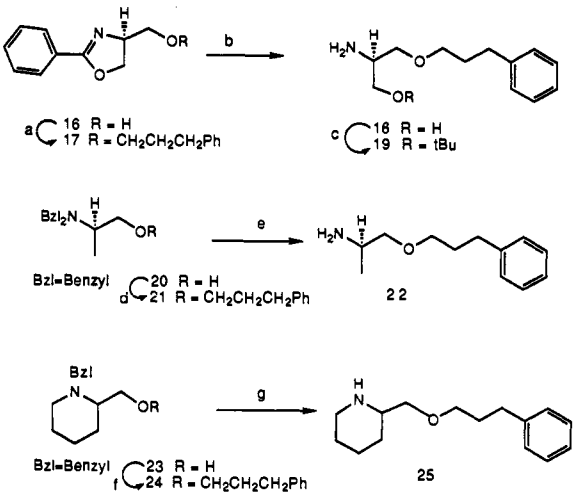
Intensive efforts[10-12] along similar lines led to 2,6-dimethyl-L-tyrosyl-D-alanine phenylpropylamide (SC-39566, 2).[12] This compound, which contains the unnatural amino acid 2,6-dimethyl-L-tyrosine (3) (DMT), was active in analgesic assays and had fewer side effects than morphine at equianalgesic doses.[12] As part of an ongoing structure–function study,[11] we investigated the functional role of the peptide backbone by separately replacing the amide bonds in 2 with groups often used as peptide bond sur-

Scheme II[a]



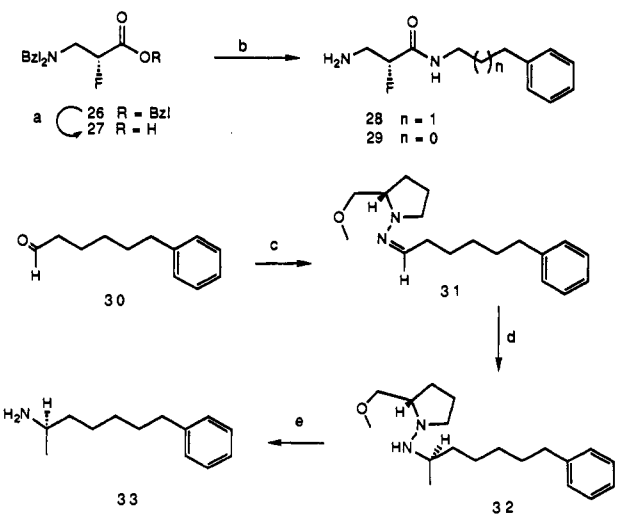
[a] Reagents: (a) $PhCH_2CH_2CH_2CH_2MgBr$, THF; (b) oxalic acid, acetic acid, H_2O.

Scheme III[a]



[a] Reagents: (a) $NaH/PhCH_2CH_2CH_2OMs$, THF; (b) 6 N, H_2SO_4; (c) isobutene/concentrated H_2SO_4; (d) $NaH/PhCH_2CH_2CH_2OMs$, THF; (e) 60 psi H_2, 20% Pd/C; (f) $NaH/PhCH_2CH_2CH_2OMs$, THF; (g) 60 psi H_2, 10% Pd/C.

Scheme IV[a]



[a] Reagents: (a) 0.5 N LiOH, THF; (b) (i) 4-methylmorpholine, isobutyl chloroformate, (ii) 3-phenyl-1-propylamine, (iii) 50 psi H_2, 20% Pd/C; (c) (S)-(−)-1-amino-2-(methoxymethyl)pyrrolidine, 60 °C; (d) $CeCl_3$, CH_3Li, THF; (e) Raney nickel, 400 psi H_2, methanol, 60 °C.

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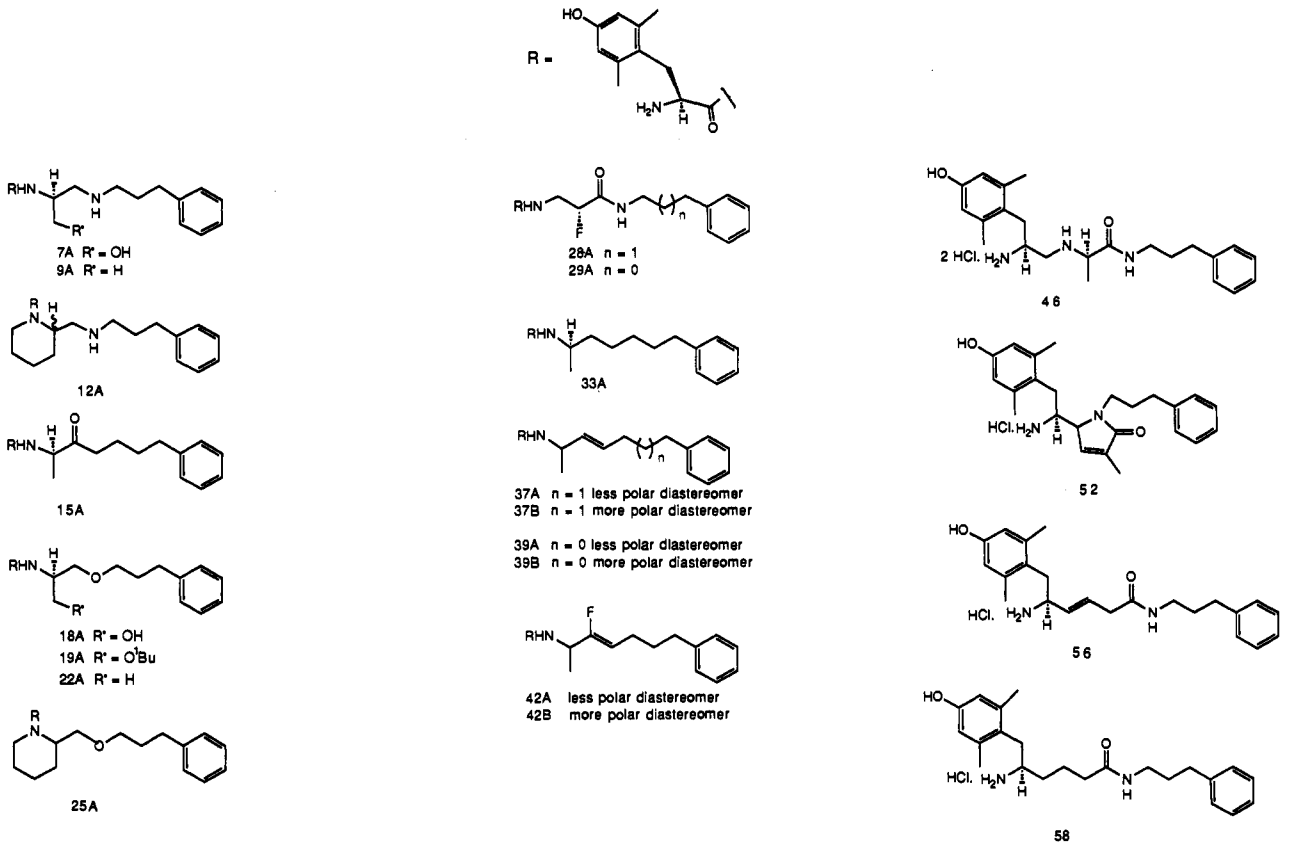
Chart I

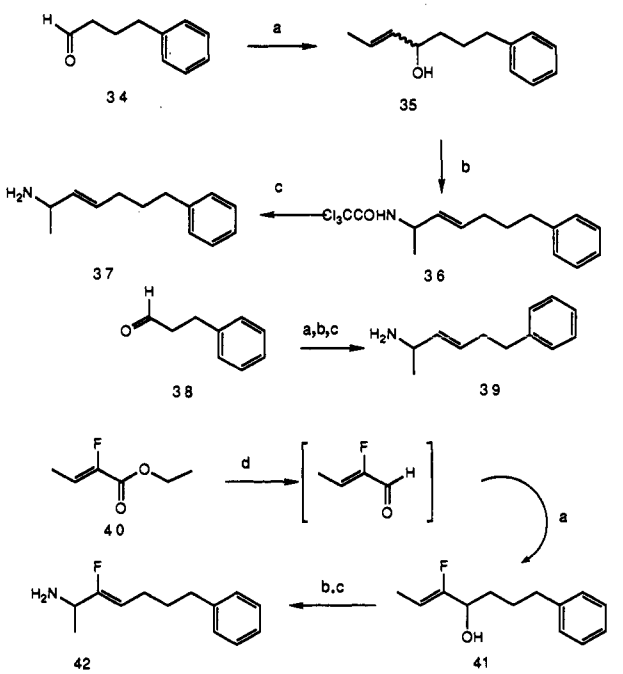


rogates.[13,14] It was anticipated that these analogues would show whether the amide bonds served mainly to orient correctly the aromatic rings or were, in fact, inherently crucial for analgesic activity.[15] The preparation of 21 analogues of compound **2** is described, and the structures are given in Chart I.

Chemistry

Numerous modifications of alanine phenylpropylamide were prepared since coupling of Boc-DMT (**4**) with a variety of amines was straightforward. In this group, the alanine nitrogen was left in place (**7A**, **9A**, **12A**), removed (**15A**), or replaced by oxygen (**18A**, **19A**, **22A**, **25A**) or carbon including a trans double bond (**33A**, **37A**, **37B**, **39A**, **39B**). Although a simple trans double bond is an adequate geometric isostere for a trans amide linkage, it is unable to mimic the electronic and hydrogen-bonding interactions that are obtained in amides and is thus unable to operate as a true bioisostere in the broadest sense. It was thought that a vinyl fluoride isostere would be somewhat more functional in this regard, since the fluoro moiety is at least a partial mimic of the amide oxygen as a hydrogen-bonding acceptor. Accordingly, fluorine-containing trans double bond compounds (**42A**, **42B**) were also prepared.[16] Also,

Scheme V[a]



[a] Reagents: (a) $CH_3CH{=}CHMgBr$, THF; (b) (i) NaH (catalytic), CCl_3CN, ether, (ii) xylene, reflux; (c) 6 N NaOH, ethanol; (d) Dibal-H.

two derivatives of β-alanine were made (**28A**, **29A**). Preparation of the required amines is shown in Schemes I–V. Amides of 2,6-dimethyl-L-tyrosine (**3**) were obtained by mixed anhydride coupling of **4** with the appropriate

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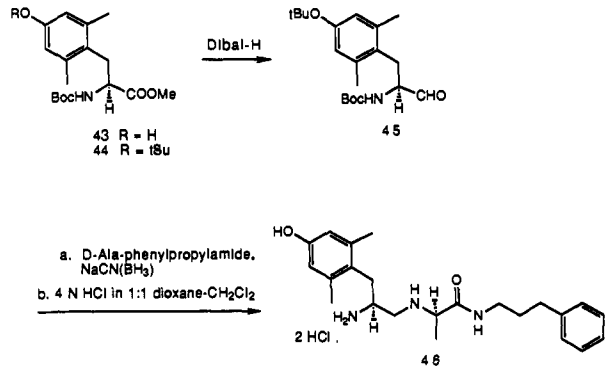
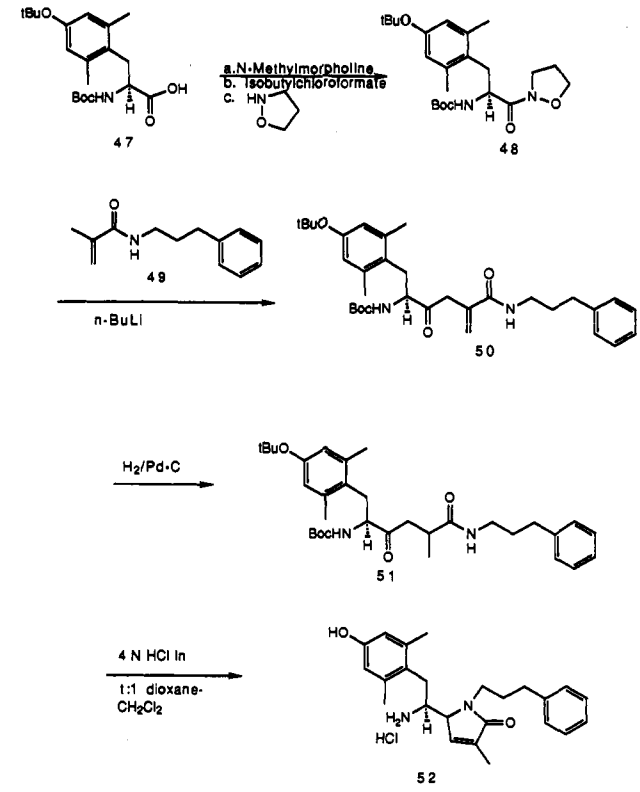
Scheme VI



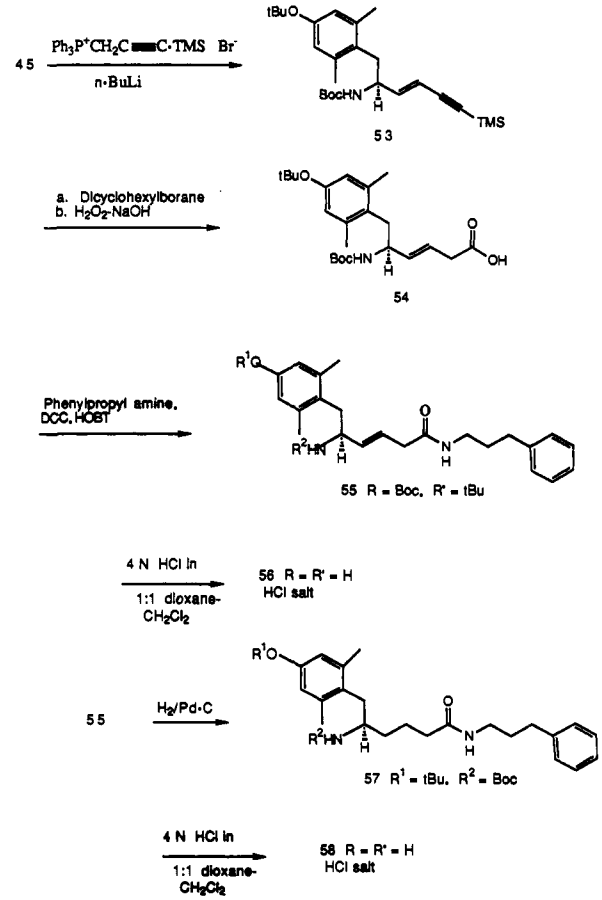
amine and deblocking with HCl in dioxane–methylene chloride. The numbering of the compounds in Chart I is coordinated with the preparation of the required amines. Racemic amine (**12**) (Scheme I) on coupling with **3** gave a inseparable mixture of diastereoisomers (**12A**) and these were tested as such. Oxalate salt (**15**) (Scheme II), prepared from D-Ala according to a known[18] method, on coupling with **3** gave a 4:1 mixture of diastereoisomers. For the preparation of **18** (Scheme III), etherification of D-Ser-derived serinol was accomplished in a stereocontrolled fashion using a precedented method.[19] The benzyl ester of the β-amino acid **27** (Scheme IV) was prepared from L-Ser using a published procedure.[20] The preparation of chiral α-methyl amine **33** (Scheme IV) used a known protocol.[22] Racemic allyl amines **37**, **39**, and **42** (Scheme V) were prepared in a stereospecific manner by the method of Overman.[23] In the sequence used for the preparation of **42**, ester **40**[24] was reduced with Dibal to give an aldehyde which was treated in situ with the Grignard reagent derived from 1-bromo-3-phenylpropane. These allyl amines on coupling with **3** gave separable mixtures of diastereoisomers.

Isosteric modification of the dimethyltyrosine-D-alanine amide junction posed a greater synthetic challenge, so only four analogues (**46**, **52**, **56**, **58**) were obtained, one of which (**52**) represented cyclization of the desired ketomethylene isostere which occurred during deblocking of **51**. The preparation of modified 2,6-dimethyl-L-tyrosines is shown in Schemes VI–VIII. Aldehyde **45** (Scheme VI) was stereochemically unstable,[25] but a freshly prepared sample of **45** underwent reductive amination with D-Ala-$CH_2CH_2CH_2C_6H_5$ to give a stereochemically homogeneous

Scheme VII



Scheme VIII



product. A combination of Weinreb's ketone synthesis[26] and Kempf's lithiation[27] technology permitted the assem-

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Table I. Pharmacological Activities[a] of Selected Analogues of SC-39566 (2)

compound	receptor binding, IC_{50}, nm					writhing mouse, sc ED_{50},[c] mg/kg
	μ	δ	κ	$IC_{50}^{\delta}/IC_{50}^{\mu}$	$IC_{50}^{\kappa}/IC_{50}^{\mu}$	
2	0.13	3.98	380	30.6	2923	0.52
9A	0.13	46	330	354	2538	4/10[b]
12A	0.045	12.1	10	269	222	3/10[b]
18A	1.67	233	5900	139	3533	2.1 (0.9–3.8)
19A	33	340	9300	10.3	282	1.7 (0.7–2.7)
22A	0.5	21	1300	42	2600	3/10[b]
25A	1.62	110	280	67.9	173	2.1 (0.9–3.8)
37B	5.4	39.3%[d]	5400		1000	2.1 (0.9–3.7)
42B	6.9	47.3%[d]	8000		1159	2.1 (1.2–3.6)

[a] The observed opioid activities of all target compounds in binding and mouse writhing test are given in the supplementary material. [b] Number of mice of those tested in which 10 mg/kg compound inhibited writhing. [c] Confidence limit in parentheses. [d] Maximum percent displacement at 100 nM.

bly of ketone **50** (Scheme VII) from the activated 2,6-dimethyl-L-tyrosine **48** and the acrylamide **49**. However removal of the *tert*-butoxycarbonyl group from **51** could not be accomplished without causing cyclization to **52**. β,γ-Unsaturated acid **54** (Scheme VIII) was prepared from **3** using a known method.[28]

Results and Discussion

The activity of these compounds was examined using two bioassays. Potency at the μ, δ, and κ opioid binding sites was determined in homogenates of rat brain. Table I shows the data for the most active compounds for which IC_{50} values could be determined. With the exception of **19A**, these compounds were very potent at the μ opioid binding site, having IC_{50} values of 7 nM or less. While these compounds demonstrated some affinity for the δ opioid binding site, they were uniformly less potent at this site and demonstrated between 10- and 350-fold selectivity for the μ opioid binding site. Similarly, all of the compounds were uniformly less potent at the κ opioid binding site. The compounds with the highest μ selectivity, **9A** and **12A**, contained an additional basic nitrogen, both nitrogens being protonated under the conditions of receptor binding measurement. Surprisingly, neither compound was active after sc administration in the writhing test. However, as both contain a basic isostere and should then exist as dications at pH 7, they may possess significantly different partitioning characteristics than the parent **2**. Thus, the absence of systemic analgesic activity may be a consequence of distribution. The retention of modest sc writhing activity by the oxymethylene isosteres **18A** and **19A**, even though they were less potent at the μ opioid receptor, is consistent with this interpretation.

Ser and its *tert*-butyl ether have been used at position 2 in linear enkephalin analogues to produce δ-selective agonists, primarily by reducing affinity for the μ opioid binding site.[31] In this regard, compounds **18A** and **19A** are notable in that they had similar substitutions. In the case of **19A**, potency at the μ opioid binding site was reduced 250-fold, while potency at the δ opioid binding site was reduced 85-fold, resulting in a compound that was less μ selective than its parent **2**. In the case of **18A**, potency at the μ opioid binding site was reduced only by 10-fold, whereas potency at the δ opioid binding site was reduced by 58-fold, resulting in a compound that was more μ selective. The fact that the selectivity of these compounds did not change in a manner consistent with that observed with linear hexapeptide analogues of enkephalin suggests that the structure–activity relationships of the compounds in this study are not comparable to those of linear enkephalin analogues.

Several of the isosteres in this study also demonstrated reasonable analgesic activity in the writhing test, having ED_{50} values of approximately 2 mg/kg sc. To determine whether this analgesia was opioid mediated, the inhibition of writhing produced by **2**, **18A**, **19A**, **25A**, **37B**, and **42B** was challenged with 1 mg/kg sc naloxone, a nonspecific opioid antagonist. In each case, the inhibition of writhing was significantly antagonized, suggesting that the analgesia was opioid mediated. The use of naloxone does not offer insight into the receptor subtypes mediating the analgesic activity of these compounds. However, given the sensitivity of the writhing test to μ, δ, and κ agonists, it appears unlikely that the analgesic activity of these isosteres is κ-mediated, given their uniformly poor potency at the κ binding site.

Aminomethylene analogues **9A** and **12A** were the most potent compounds in the opioid receptor binding assays, but were inactive in the writhing test following subcutaneous administration. Ketomethylene analogue **15A** had neither binding nor analgesic activity. It is interesting that only substances with oxygen[17] in the primary backbone (**18A**, **19A**, **25A**) were active both in vitro and in vivo. In contrast, ether analogue **22A** of the lead compound **2** was inactive in vivo, although quite potent in the binding assay. The trans double bond surrogates **37B** and **42B** were among the most active analogues of **2**. These compounds had the same relative stereochemistry and also had the same distance between the two aromatic moieties.

Analogues **46**, **56**, and **58**, in which the amide junction between the dimethyltyrosine and D-Ala was replaced with aminomethylene, trans double bond, and bismethylene, respectively, were inactive in both the opioid binding assay and in the writhing test. However, as presented earlier, changes made to the amide junction between D-Ala and the phenylpropylamine side-chain produced modest reduction of activity. These data suggest that the amide junction between dimethyltyrosine and D-Ala is more crucial than the amide junction between D-Ala and phenylpropylamine for opioid activity. It would therefore

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appear that the elements of this amide bond might be necessary for interacting with opioid receptors or to enforce particular conformations of the molecule by intramolecular hydrogen bonding. It is notable that although 2 was active following intragastric administration (ig) (0.53 mg/kg), none of the compounds in this series was active following administration of doses as high as 10 mg/kg ig. We may therefore conclude that both amide bonds of compound 2 are optimal for consistently effective binding at the μ receptor and for good subcutaneous activity in the mouse writhing test. The presence of both amide linkages, on the basis of the results presented here, also appear to be necessary for oral activity.

Experimental Section

Proton magnetic resonance (^{1}H NMR) spectra were obtained in $CDCl_3$ (unless specified otherwise) by using a GE 300-MHz spectrometer. The chemical shifts are recorded as parts per million (ppm) downfield from tetramethylsilane. During workup of reactions, after washing with water and drying over $MgSO_4$, the extracts were concentrated on a rotary evaporator at reduced pressure while the temperature was maintained below 35 °C. Room temperature refers to 23 °C. Flash chromatography was performed according to the procedure of Still[32] using Merck silica gel 60 (230–400 mesh). Microanalyses were performed at the Searle Physical Methodology Department. All nonaqueous reactions were performed under a positive pressure of argon. No attempts were made to maximize the yields of reactions.

1,1-Dimethylethyl [1(*R*)-(Hydroxymethyl)-2-oxo-2-[(3-phenylpropyl)amino]ethyl]carbamate (5). 4-Methylmorpholine (1.3 g, 125 mmol) and isobutyl chloroformate (1.7 g, 124 mmol) were added in succession to a stirred solution of *N*-Boc-D-serine (2.5 g, 122 mmol) in CH_2Cl_2 (100 mL) at 0 °C. After 0.5 h, the mixture was cooled to −50 °C and 3-phenyl-1-propylamine (1.7 g, 126 mmol) was added. The mixture was permitted to warm to room temperature over 16 h. The solution was washed with 0.5 N $KHSO_4$ and water, dried, and concentrated. The residue, on trituration with pentane, yielded 5 (3 g, 76%) as white solid: ^{1}H NMR δ 1.45 (9 H, s), 1.82 (2 H, p, J = 7.5 Hz), 2.63 (2 H, t, J = 7.5 Hz), 3.13–3.41 (3 H, m), 3.62 (1 H, m), 4.02–4.15 (2 H, m), 5.57 (1 H, J = 7.5 Hz), 6.7 (1 H, bs), 7.1–7.3 (5 H, m).

2(*R*)-Amino-3-hydroxy-*N*-(3-phenylpropyl)propanamide (6). A solution of 5 (12 g, 37.3 mmol) in 5 N HCl in dioxane was permitted to stand at room temperature for 1 h. The volatiles were removed, and the residue was extracted with dilute $NaHCO_3$ and CH_2Cl_2. The organic phase was dried and concentrated. The residue was triturated to give 6 (7.8 g, 95%) as a white solid: ^{1}H NMR δ 1.86 (2 H, p, J = 7.5 Hz), 2.06 (2 H, t, J = 7.5 Hz), 3.29 (2 H, m), 3.39 (1 H, t, J = 5 Hz), 3.67 (1 H, dd, J = 12, 5 Hz), 3.82 (1 H, dd, J = 12, 5 Hz), 7.1–7.3 (5 H, m), 7.5 (1 H, bs).

2(*S*)-Amino-3-[(3-phenylpropyl)amino]-1-propanol (7). A solution of 6 (7.6 g, 33.8 mmol) in THF (100 mL) was added dropwise over 1 h to 200 mL (200 mmol) of a 1 N solution of borane in THF. The mixture was refluxed for 9 h. It was cooled to −5 °C and 100 mL of 4 N HCl was added dropwise. The mixture was concentrated to remove most of the THF, basified with dilute NaOH to pH 12, and extracted with ethyl acetate. The organic phase was washed with brine, dried, and concentrated to give 7 (6.6 g, 92%) as an oil: ^{1}H NMR ($CDCl_3$–D_2O) δ 1.81 (2 H, p, J = 7.5 Hz), 2.55–2.72 (6 H, m), 2.96 (1 H, p, J = 5 Hz), 3.58 (2 H, d, J = 5 Hz).

General Procedure for the Mixed Anhydride Coupling of Amines to 2,6-Dimethyl-L-tyrosine (3). Example Procedure: Preparation of α(*S*)-Amino-4-hydroxy-*N*-[1(*S*)-(hydroxymethyl)-2-[(3-phenylpropyl)amino]ethyl]-2,6-dimethylbenzenepropanamide Dihydrochloride Salt (7A). To a stirred solution of *N*-Boc-2,6-dimethyl-L-tyrosine (3.6 g, 11.7 mmol) in THF (100 mL) at −23 °C was added 4-methylmorpholine (1.22 g, 12.1 mmol) and isobutyl chloroformate (1.66 g, 12.1 mmol) in succession. The mixture was stirred for 0.75 h and 7 (2.5 g, 120 mmol) was added all at once. Stirring was continued for an additional 0.5 h. The cooling bath was then removed and stirring continued for 1 h more. The mixture was concentrated and extracted with water and ethyl acetate. The organic phase was washed with water, 0.5 N $KHSO_4$, saturated $NaHCO_3$, and water, dried, and concentrated. The residue was chromatographed (93:7:1 CH_2Cl_2–CH_3OH–NH_4OH) to give the protected derivative of the title compound in 15% yield as a white solid. The solid was dissolved in a mixture of CH_2Cl_2 and 6.5 N HCl in dioxane (5 mL of each) and allowed to stand at room temperature for 10 min. The volatiles were removed, and the residue was triturated with ether containing a small amount of ethanol. The solid was filtered and dried at 74 °C (0.1 mmHg) to provide 7A as a white solid: ^{1}H NMR (DMSO-d_6) δ 1.99 (2 H, m), 2.18 (6 H, s), 2.68 (2 H, t, J = 7.5 Hz), 2.71–3.16 (8 H, m), 3.67 (1 H, dd, J = 12, 5 Hz), 4.02 (1 H, m), 5.05 (1 H, bs), 6.4 (2 H, s), 7.15–7.35 (5 H, m), 8.19 (1 H, d, J = 8 Hz), 8.4–9.1 (4 H, bs), 9.14 (1 H, bs). Anal. ($C_{23}H_{35}Cl_2N_3O_3$·0.75H_2O) C, H, N. Cl: calcd, 14.59; found, 13.94.

***N*^{1}-(3-Phenylpropyl)-1,2(*R*)-propanediamine (9).** 2(*R*)-Amino-*N*-(3-phenylpropyl)propanamide (8) (2.06 g, 10 mmol) was reduced with borane in THF (100 mmol) as described for 7 to give 9 (1.5 g, 93%) as an oil: ^{1}H NMR ($CDCl_3$–D_2O) δ 1.05 (3 H, d, J = 6.5 Hz), 1.8 (2 H, q, J = 7.5 Hz), 2.33 (1 H, dd, J = 12, 8 Hz), 2.55 (1 H, dd, J = 12, 4 Hz), 2.58–2.70 (4 H, m), 2.9 (1 H, m), 7.1–7.3 (5 H, m).

α(*S*)-Amino-4-hydroxy-2,6-dimethyl-*N*-[1(*R*)-methyl-2-[(3-phenylpropyl)amino]ethyl]benzenepropanamide Dihydrochloride Salt (9A). Diamine 9 was coupled to DMT as described for the preparation of 7A to give 2HCl salt 9A as white solid: ^{1}H NMR (DMSO-d_6) δ 0.73 (3 H, d, J = 7 Hz), 1.99 (2 H, m), 2.2 (6 H, s), 2.61 (2 H, t, J = 7 Hz), 2.63–3.05 (6 H, complex band), 3.58 (1 H, m), 4.08 (1 H, m), 6.4 (2 H, s), 7.15–7.35 (5 H, m), 8.01 (1 H, d, J = 8 Hz), 8.7–9.02 (4 H, bs), 9.15 (1 H, s). Anal. ($C_{23}H_{34}Cl_2N_3O_2$·0.5H_2O) C, H, N, Cl.

***N*-(3-Phenylpropyl)-2-pyridinemethanamine (10).** A mixture of pyridine-2-carboxaldehyde (6.885 g, 64 mmol), acetic acid (8.1 mL, 140 mmol), and 3-phenyl-1-propylamine (8.685 g, 64 mmol) in ethanol (50 mL) was shaken in the presence of 5% Pd/C in a Parr hydrogenator under a 60 psi H_2 atmosphere. The mixture was filtered and concentrated. The residue was chromatographed (0.5% NH_3 in 1:1 ethyl acetate–hexane) to give 10 (5.83 g, 37%) as an oil: ^{1}H NMR δ 1.85 (2 H, q, J = 7.5 Hz), 2.67 (2 H, t, J = 7.5 Hz), 2.71 (2 H, t, J = 7 Hz), 3.9 (2 H, s), 7.2 (7 H, m), 7.60 (1 H, td, J = 8, 2.5 Hz), 8.55 (1 H, bd, J = 8.5 Hz).

1,1-Dimethylethyl (3-Phenylpropyl)(2-pyridinylmethyl)-carbamate (11). A mixture of 10 (5.8 g, 26 mmol), triethylamine (3.6 mL, 26 mmol), and di-*tert*-butyl dicarbonate (5.7 g, 26 mmol) in CH_2Cl_2 (80 mL) was stirred at room temperature for 3 h. The mixture was concentrated and the residue extracted with ether. The organic phase was washed with water, dried, and concentrated. The residue was chromatographed (40% ethyl acetate in hexane) to give 11 (6.6 g, 79%) as yellow oil: ^{1}H NMR δ 1.3–1.5 (9 H, 2 bs), 1.75–1.95 (2 H, bm), 2.5–2.65 (2 H, bm), 3.2–3.4 (2 H, bm), 4.5–4.6 (2 H, bm), 7.1–7.3 (7 H, m), 7.64 (dt, J = 8, 2 Hz), 8.52 (1 H, bd, J = 6 Hz).

1,1-Dimethylethyl (3-Phenylpropyl)(2-piperidinylmethyl)carbamate (12). Hydrogenation of 11 (6.527 g, 20 mmol) in ethanol (100 mL) and concentrated HCl (1.65 mL) with 5% Pt/C in a 60 psi H_2 atmosphere gave the hydrochloride of 12 as white solid: ^{1}H NMR (DMSO-d_6) δ 1.4 (9 H, bs), 1.6–1.9 (5 H, bm), 2.5–2.6 (2 H, distorted t, J = 7 Hz), 2.7–2.8 (1 H, m), 3.2–3.6 (5 H, m), 7.2–7.4 (5 H, m).

1-[2(*S*)-Amino-3-(4-hydroxy-2,6-dimethylphenyl)-1-oxopropyl]-2-[[(3-phenylpropyl)amino]methyl]piperidine Dihydrochloride Salt (12A). Hydrochloride 12 was coupled to DMT as described in the preparation of 7A to afford 2HCl salt 12A in 20% yield as a white solid: ^{1}H NMR (DMSO-d_6) δ 0.7–1.65 (6 H, m), 1.85–2.35 (8 H, m), 2.55–3.1 (10 H, m), 3.8, 4.25, 4.45, 4.75, and 4.79 (0.5 H, bm), 6.42 and 6.44 (1 H, s), 7.15–7.35 (5 H, m), 8.6 (4 H, bm), 9.2 (1 H, m). Anal. ($C_{26}H_{39}Cl_2N_3O_2$·0.75H_2O) C, H, N, Cl.

7-Phenyl-2(*R*)-[(triphenylmethyl)amino]-3-heptanone (14). To Mg (1.25 g, 544 mmol) stirred in THF (10 mL) at room temperature was added a solution of 1-bromo-4-phenylbutane (9.48 g, 445 mmol) in THF (60 mL) over 1 h. The mixture was

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further stirred for 2 h at 45 °C, cooled to 0 °C, and transferred via a cannula to a solution of S-2-pyridinyl 2(R)-(triphenylmethyl)aminopropanethioate (13)[16] (6 g, 142 mmol) in THF (60 mL). After 3 h, the mixture was added to saturated NH_4Cl solution and extracted with ether. The organic phase was washed with 1 N NaOH and brine, dried, and concentrated. The residue was chromatographed (3% ethyl acetate in hexane) to give 14 (3 g, 47%) as a white solid: ^1H NMR δ 1.21 (3 H, d, J = 7 Hz), 1–1.5 (8 H (including d at 1.21), m), 2.05 (1 H, m), 2.48 (2 H, t, J = 7.5 Hz), 3.22 (1 H, d, J = 8 Hz), 3.39 (1 H, p, J = 7.5 Hz), 7.0–7.5 (20 H, m).

2-Amino-7-phenyl-3-heptanone Oxalate Salt (15). A mixture of 14 (2.4 g), oxalic acid (0.48 g), acetic acid (27 mL), and water (13 mL) was stirred at room temperature for 5.5 h. The volatiles were removed in vacuo, and the residue was triturated with water (100 mL) and filtered. The filtrate was concentrated in vacuo. The residue was shaken with ether and vacuum filtered, to yield 15 (1.42 g, 90%) as a white solid: ^1H NMR (DMSO-d_6) δ 1.36 (3 H, d, J = 7.5 Hz), 1.4–1.6 (4 H, m), 2.47–2.67 (4 H, m), 4.07 (1 H, q, J = 7.5 Hz), 7.08–7.3 (5 H, m), 8.0–8.5 (4 H, bs).

2,6-Dimethyl-N-[(phenylmethoxy)carbonyl]-L-tyrosine (z-DMT). To a mixture of 3[11] (40 g, 163 mmol) in water (600 mL) and 12.5% aqueous NaOH (65 mL) was added while stirring benzyl chloroformate (32 g, 187 mmol) all at once. Then 12.5% aqueous NaOH (135 mL) was added dropwise over 1.75 h. The mixture was stirred for 16 h more and the aqueous phase was filtered through Celite. The clear filtrate was cooled in an ice bath and acidified to pH = 1 with concentrated HCl. The mixture was extracted with ethyl acetate. The organic phase was dried and concentrated. The residue was dried in vacuo at 55 °C to give the title compound as white solid (43.8 g, 70%).

α-Amino-4-hydroxy-2,6-dimethyl-N-[1(R)-methyl-2-oxo-6-phenylhexyl]benzenepropanamide Hydrochloride Salt (15A). Oxalate salt 15 was coupled to z-DMT as described for the preparation of 7A and deprocted by hydrogenation over 5% Pd/C under 20 psi of H_2 pressure, in presence of 1 equiv of hydrochloric acid to give 15A (69%) as a white solid: ^1H NMR (DMSO-d_6) δ 0.8 and 1.08 (3 H (3:1), d, J = 7.5 Hz), 1.3–1.55 (4 H, m), 2.14 and 2.16 (6 H (3:1), s), 2.45–2.6 (5 H, m), 2.85–3.1 (2 H, m), 3.7–3.85 (1 H, m), 4.17 (1 H, p, J = 7.5 Hz), 6.4 and 6.42 (2 H (1:3), s), 7.1–7.3 (5 H, m), 8.15 (1 H, d, J = 7.5 Hz), 8.5–8.6 (3 H, bs), 9.1 (1 H, s). Anal. ($C_{24}H_{33}ClN_2O_3 \cdot 0.5H_2O$) C, H, N. Cl: calcd, 8.02; found, 8.44.

4,5-Dihydro-2-phenyl-4(S)-[(3-phenylpropoxy)methyl]-oxazole (17). A 60% suspension of NaH in mineral oil (0.787 g, 19.68 mmol) was washed twice with hexane and suspended in THF (40 mL) at 0 °C. A solution of 4,5-dihydro-2-phenyloxazole (16)[19] (3 g, 16.9 mmol) in THF (20 mL) was added dropwise to the stirred suspension of NaH. The mixture was stirred at room temperature for 1 h and at 50 °C for 2 h. The mixture was cooled to 0 °C and 3-phenyl-1-propyl methanesulfonate (3.8 g, 17.76 mmol) was added. The mixture was heated at 40 °C for 16 h and refluxed for 2 h. After cooling to 0 °C, water and ether were added. The organic phase was washed with water, dried, and concentrated. The residue was chromatographed (40% ethyl acetate in hexane) to give 17 (4.8 g, 96%) as a pale yellow oil: ^1H NMR δ 1.89 (2 H, m), 2.66 (2 H, t, J = 7.5 Hz), 3.4–3.58 (3 H, m), 3.73 (1 H, m), 4.32 (1 H, m), 4.42–4.56 (2 H, m), 7.1–7.5 (8 H, m), 7.95 (2 H, m).

2(R)-Amino-3-(3-phenylpropoxy)-1-propanol (18). A mixture of 17 (4.8 g) and 6 N H_2SO_4 (10 mL) was refluxed for 16 h. The mixture was cooled and basified with 50% NaOH and extracted with ether. The extract was dried and concentrated to yield 18 (1.95 g, 58%) as an oil: ^1H NMR δ 1.88 (2 H, m), 2.48–2.65 (3 H, bs), 2.68 (2 H, t, J = 7.5 Hz), 3.08 (1 H, bt, J = 5 Hz), 3.34 (1 H, dd, J = 9, 6 Hz), 3.38–3.53 (8 H, m), 3.62 (1 H, dd, J = 12, 4 Hz), 7.1–7.3 (5 H, m).

α(S)-Amino-4-hydroxy-N-[1(R)-(hydroxymethyl)-2-(3-phenylpropoxy)ethyl]-2,6-dimethylbenzenepropanamide Hydrochloride Salt (18A). Compound 18 was coupled to DMT as described in the preparation of 7A to give 18A (68%) as a white solid: ^1H NMR ($CDCl_3$–CD_3OD) δ 1.82 (2 H, p, J = 7.5 Hz), 2.26 (6 H, s), 2.62 (2 H, t, J = 7.5 Hz), 3.1–3.19 (2 H, m), 3.27 (2 H, dd, J = 12, 5 Hz), 3.25–3.52 (5 H, m), 3.94 (1 H, m), 6.5–6.55 (2 H, distorted s), 7.1–7.3 (5 H, m). Anal. ($C_{23}H_{33}ClN_2O_4 \cdot 0.25H_2O$) C, H, N, Cl.

1-(1,1-Dimethylethoxy)-3-(3-phenylpropoxy)-2(S)-propanamine (19). A mixture of 18 (1.05 g, 5 mmol), concentrated H_2SO_4 (0.543 g, 5.33 mmol), and isobutene (100 mL) in CH_2Cl_2 (150 mL) was shaken in a Parr shaker for 16 h. The mixture was carefully basified with saturated $NaHCO_3$ and extracted with ether. The extract was washed with water, dried, and concentrated. The residue was chromatographed (8:11:1, ethyl acetate–hexane–triethylamine) to give 19 (0.65 g, 48%) as an oil: ^1H NMR δ 1.2 (9 H, s), 1.9 (2 H, m), 2.68 (2 H, t, J = 7 Hz), 3.09 (1 H, q, J = 7 Hz), 3.19–3.52 (6 H, m), 7.1–7.3 (5 H, m).

α(S)-Amino-N-[1(S)-[(1,1-dimethylethoxy)methyl]-2-(3-phenylpropoxy)ethyl]-4-hydroxy-2,6-dimethylbenzenepropanamide Hydrochloride Salt (19A). Compound 19 was coupled to z-DMT as described for the preparation of 7A in 92% yield; deprotection was effected by hydrogenolysis in ethanol with 10% Pd/C under 5 psi of H_2 pressure; the crude product was treated with ethanolic HCl to give 19A (92%) as a white solid: ^1H NMR (DMSO-d_6) δ 1.08 (9 H, s), 1.77 (2 H, p, J = 7.5 Hz), 2.2 (6 H, bs), 2.56 (2 H, t, J = 7.5 Hz), 2.91 (1 H, m), 3.07–3.5 (8 H, m), 3.92 (1 H, m), 6.2 (1 H, m), 6.5 (2 H, s), 6.68 (1 H, bs), 7.1–7.3 (5 H, m), 8.45–8.6 (3 H, bs). Anal. ($C_{27}H_{41}ClN_2O_4$) C, H, N, Cl.

N-[1(R)-Methyl-2-(3-phenylpropoxy)ethyl]-N-(phenylmethyl)benzenemethanamine (21). 2(R)-[Bis(phenylmethyl)amino]-1-propanol (20) was alkylated as described for the preparation of 17. The crude product was chromatographed (10% ethyl acetate in hexane containing 5% triethylamine) to give 21 as an oil (24%): ^1H NMR δ 1.07 and 1.08 (3 H, 2 d, J = 7 and 7.5, respectively), 1.8–1.96 (2 H, m), 2.63–2.74 (2 H, m), 2.95–3.1 (1 H, m), 3.3–3.79 (8 H, m), 7.1–7.4 (15 H, m).

1-(3-Phenylpropoxy)-2(R)-propanamine (22). Compound 21 was hydrogenated in ethanol in the presence of 20% Pd/C as catalyst under a 60 psi H_2 atmosphere to give 22 (25%) as an oil after chromatography (50% ethyl acetate in hexane containing 10% triethylamine): ^1H NMR δ 1.03 (3 H, d, J = 6 Hz), 1.3 (2 H, bs), 1.9 (2 H, m), 2.7 (2 H, t, J = 7.5 Hz), 3.05–3.51 (5 H, m), 7.1–7.3 (5 H, m).

α(S)-Amino-4-hydroxy-2,6-dimethyl-N-[1(R)-methyl-2-(3-phenylpropoxy)ethyl]benzenepropanamide Hydrochloride Salt (22A). Compound 22 was coupled to DMT as described for the preparation of 7A to give 22A (80%) as a white solid: ^1H NMR (DMSO-d_6) δ 0.77 (3 H, d, J = 7 Hz), 1.77 (2 H, p, J = 7.5 Hz), 2.19 (6 H, s), 2.60 (2 H, t, J = 7.5 Hz), 2.87–3.4 (6 H, m), 3.62–3.73 (1 H, m), 3.83 (1 H, m), 6.4 (2 H, s), 7.1–7.3 (5 H, m), 7.68 (1 H, d, J = 7.5 Hz), 8.48 (3 H, bs), 9.05 (1 H, bs). Anal. ($C_{23}H_{33}ClN_2O_3 \cdot 0.25H_2O$) C, H, N, Cl.

1-(Phenylmethyl)-2-[(3-phenylpropoxy)methyl]piperidine (24). 1-(Phenylmethyl)-2-piperidinemethanol (23) was alkylated as described for the preparation of 18. The crude product was chromatographed (1:4:0.5, ethyl acetate–hexane–aqueous NH_3) to give 23 (41%) and 24 (38%) as oil: ^1H NMR δ 1.22–1.57 (4 H, m), 1.61–1.8 (2 H, m), 1.9 (2 H, m), 2.01 (1 H, ddd, J = 13, 10, 3 Hz), 2.5 (1 H, m), 2.69 (2 H, t, J = 7.5 Hz), 2.75 (1 H, dt, J = 12, 3 Hz), 3.32 (1 H, d, J = 13 Hz), 3.37–3.5 (3 H, m), 4.11 (1 H, d, J = 13 Hz), 7.1–7.4 (10 H, m).

2-[(3-Phenylpropoxy)methyl]piperidine (25). Compound 24 (3 g) was hydrogenated in ethanol (50 mL) and acetic acid (0.62 g) in the presence of 10% Pd/C, at 50 °C, under a 60 psi H_2 atmosphere. The solvent was evaporated and the residue treated with excess aqueous $NaHCO_3$ and extracted with ether. The organic phase was dried and concentrated to yield 25 (1.18 g, 55%) as an oil: ^1H NMR δ 1.11 (1 H, m), 1.11 (1 H, m), 1.23–1.67 (5 H, m), 1.78 (1 H, m), 1.89 (2 H, m), 2.58–2.78 (4 H, m), 3.05 (1 H, m), 3.22 (2 H, m), 3.32–3.59 (3 H, m), 7.15–7.4 (5 H, m).

1-[2(S)-Amino-3-(4-hydroxy-2,6-dimethylphenyl)-1-oxopropyl]-2-[(3-phenylpropoxy)methyl]piperidine Hydrochloride Salt (25A). Compound 25 was coupled to DMT as described for the preparation of 7A to give 25A as a mixture of diastereoisomers: ^1H NMR ($CDCl_3$–CD_3OD) δ 1.08–1.96 (8 H, m), 2.63 and 2.65 (2 H, 2 t, J = 7.5 Hz), 2.87–3.58 (8 H, m), 4.5 (1 H, m), 6.56–6.57 (2 H, 3 s), 7.1–7.42 (5 H, m). Anal. ($C_{26}H_{37}ClN_2O_3 \cdot 0.25H_2O$) C, H, N, Cl.

3-[Bis(phenylmethyl)amino]-2(R)-fluoropropanoic Acid (27). To a solution of phenylmethyl 3-[bis(phenylmethyl)-amino]-2(R)-fluoropropanoate (26)[20] (6.03 g, 16 mmol) in THF (100 mL) at 0 °C was added a 0.5 N solution of LiOH (35 mL,

17.5 mmol). The mixture was stirred at room temperature for 16 h and concentrated to remove THF. The residue was extracted with ethyl acetate. The aqueous phase was acidified with acetic acid and extracted with ethyl acetate. The organic phase was washed with water and brine, dried, and concentrated to give **27** (4.35 g, 93%) as a thick gum: ^{1}H NMR δ 3.15 (2 H, dd, J_{HF} = 17.5, J_{HH} = 6 Hz), 3.84 (2 H, d, J = 13 Hz), 3.93 (2 H, d, J = 13 Hz), 4.83 (1 H, dt, J_{HF} = 47.5, J_{HH} = 6 Hz), 7.3–7.4 (10 H, m).

α(*S*)-Amino-*N*-[2-fluoro-3-oxo-3-[(3-phenylpropyl)amino]propyl]-4-hydroxy-2,6-dimethylbenzenepropanamide Hydrochloride Salt (28A). Compound **27** was coupled to 3-phenyl-1-propylamine by the mixed anhydride coupling method and the crude product on hydrogenolysis in ethyl acetate with 20% Pd/C under 50 psi of H_2 pressure gave 3-amino-2(*S*)-fluoro-*N*-(3-phenylpropyl)propanamide (**28**) as a syrup. Crude amine **28** was coupled to DMT as described in the preparation of **7A** to give **28A** in 51% overall yield from **27**, as a colorless solid: ^{1}H NMR (DMSO-d_6) δ 1.73 (2 H, m), 2.56 (2 H, J = 7.5), 2.83–3.21 (5 H, m), 3.51–3.82 (2 H, m), 4.72 (1 H, ddd, J_{HF} = 51 Hz, J = 8.2 Hz), 6.4 (2 H, s), 7.2–7.4 (5 H, m), 8.3–8.4 (2 H, m), 8.5 (3 H, bs), 9.1 (1 H, bs). Anal. ($C_{22}H_{26}FN_3O_3$) C, H, N, F.

α(*S*)-Amino-*N*-[2-fluoro-3-oxo-3-[(2-phenylethyl)amino]propyl]-4-hydroxy-2,6-dimethylbenzenepropanamide (29A). Substitution of phenethylamine for 3-phenyl-1-propylamine in the sequence described above for the preparation of **28** provided 3-amino-2(*S*)-fluoro-*N*-(2-phenylethyl)propanamide (**29**) as a white solid. Crude amine **29** was coupled to DMT as described in the preparation of **7A** and the product was purified by chromatography to give the corresponding free base (**29A**) in 15% overall yield from **27** as a white solid: ^{1}H NMR ($CDCl_3$–D_2O) δ 2.3 (6 H, s), 2.69 (1 H, dd, J = 14, 10 Hz), 2.84 (2 H, t, J = 7 Hz), 3.16 (1 H, dd, J = 14, 5 Hz), 3.5–3.9 (6 H, m), 4.89 (1 H, ddd, J_{HF} = 50 Hz, J_{HH} = 7 and 4 Hz), 6.54 (2 H, s), 7.15–7.35 (5 H, m). Anal. ($C_{23}H_{31}ClFN_3O_3 \cdot 0.25H_2O$) C, H, N, Cl, F.

Benzenehexanal (30). Sodium hydride (1.7 g, 60% suspension in mineral oil, 43 mmol) was washed with hexane and suspended in THF (70 mL) at 0 °C. The mixture was stirred and methyl diethylphosphonoacetate (7.45 mL, 40.6 mmol) was added dropwise with stirring. After 0.5 h, **34** (6 g) was added. After 15 min, saturated NH_4Cl was added. The mixture was extracted with ether and the organic phase was washed with water, dried, and concentrated. The residue was chromatographed (20% ethyl acetate in hexane) to give methyl 6-phenyl-2(*E*)-hexenoate (6.5 g, 74%) as an oil: ^{1}H NMR δ 1.79 (2 H, p, J = 7.5 Hz), 2.24 (2 H, m), 2.64 (2 H, t, J = 7.5 Hz), 3.72 (3 H, s), 5.83 (1 H, dd, J = 16, 5 Hz), 6.98 (1 H, dt, J = 16, 7.5 Hz), 7.12–7.32 (5 H, m).

The above oil was hydrogenated in methanol in the presence of Pd/C under 5 psi of H_2 pressure to give methyl benzenehexanoate in 98% yield: ^{1}H NMR δ 1.36 (2 H, m), 1.64 (4 H, m), 2.31 (2 H, t, J = 7.5 Hz), 2.61 (2 H, t, J = 7.5 Hz), 3.67 (3 H, s), 7.13–7.31 (5 H, m).

To a solution of 3.15 g (15.3 mmol) of the above crude product in CH_2Cl_2 (200 mL) at −78 °C was added with stirring a 1 M solution of Dibal-H (15.5 mL, 15.5 mmol) in toluene. After 15 min, 1.5 mL of methanol was added followed by 100 mL of a saturated solution of Rochelle's salt. The mixture was warmed to room temperature and extracted with ether. The organic phase was washed with 1 N HCl and brine, dried, and concentrated to give **30** (2.75 g, 94%) as an oil: ^{1}H NMR δ 1.38 (2 H, m), 1.58–1.72 (4 H, m), 2.42 (2 H, td, J = 7.5, 2 Hz), 2.62 (2 H, t, J = 7.5 Hz), 7.1–7.3 (5 H, m), 9.77 (1 H, t, J = 2 Hz).

2-(Methoxymethyl)-*N*-(6-phenylhexylidene)-1-pyrrolidinamine (31). To **30** (2.5 g, 14.2 mmol) at room temperature was added dropwise with stirring *S*-(−)-1-amino-2-(methoxymethyl)pyrrolidine (1.85 g, 14.2 mmol). The mixture was heated to 60 °C and allowed to cool back to room temperature, where it was stirred for 16 h. The mixture was taken up in ether, washed with water, dried, and concentrated. The residue was distilled at 0.4 mmHg bulb-to-bulb (bath temperature = 170 °C) to give **31** (3.8 g, 93%): ^{1}H NMR δ 1.3–2.0 (10 H, m), 2.21 (2 H, m), 2.61 (2 H, t, J = 7.5 Hz), 2.70 (1 H, m), 3.31–3.44 (6 H, includes a singlet at 3.38, m), 3.56 (1 H, m), 6.63 (1 H, t, J = 6 Hz), 7.1–7.3 (5 H, m).

2-(Methoxymethyl)-*N*-[1(*R*)-methyl-6-phenylhexyl]-1-pyrrolidinamine (32). To a suspension of $CeCl_3$ (prepared from 7.21 g of $CeCl_3 \cdot 7H_2O$ as described in ref 21) in THF (125 mL) at −78 °C was added with stirring a 1 M ether solution of CH_3Li (13.8 mL, 19.32 mmol). The dark brown mixture was stirred for 1 h, and **32** (2.52 g, 8.75 mmol) in 2 mL of THF was added. The mixture was stirred for 3 h and allowed to warm to room temperature. Methanol (10 mL) was added and the mixture was extracted with water and ether. The organic phase was washed with water and brine, dried, and concentrated. The residue was chromatographed (20% ethyl acetate in hexane) to give **31** (1.01 g, 40%) and **32** (1.14 g, 40%) as an oil: ^{1}H NMR δ 1.01 (3 H, d, J = 6 Hz), 1.53–1.98 (10 H, m), 2.07–2.27 (2 H, m), 2.5–2.6 (3 H, m), 2.80 (1 H, m), 3.3–3.43 (2 H, m), 3.35 (3 H, s), 3.53 (1 H, dd, J = 9, 4 Hz), 7.1–7.32 (5 H, m).

α(*R*)-Methylbenzenehexanamine (33).[22] The above oil was hydrogenated in methanol in the presence of Raney nickel under a 400 psi H_2 atmosphere at 60 °C to give, after chromatography (10% triethylamine in ether), **33** (0.56 g, 78%) as an oil: ^{1}H NMR δ 1.05 (3 H, d, J = 7 Hz), 1.12 (2 H, m), 1.32 (6 H, m), 1.51–1.71 (2 H, m), 2.60 (2 H, t, J = 7.5 Hz), 2.85 (1 H, m), 7.1–7.32 (5 H, m).

α(*S*)-Amino-4-hydroxy-2,6-dimethyl-*N*-[1(*R*)-methyl-6-phenylhexyl]benzenepropanamide (33A). Compound **33** was coupled to DMT as described in the preparation of **7A** to give **33A** as a white solid in 79% yield: ^{1}H NMR (DMSO-d_6) δ 0.69 (3 H, d, J = 7 Hz), 1.23 (6 H, m), 1.52 (2 H, m), 2.18 (6 H, s), 2.54 (2 H, t, J = 7.5 Hz), 2.88 (1 H, dd, J = 13, 4 Hz), 2.97 (1 H, t, J = 13 Hz), 3.57–3.71 (2 H, m), 6.4 (2 H, s), 7.1–7.3 (5 H, m), 7.57 (1 H, d, J = 8 Hz), 8.4 (3 H, bs), 9.1 (1 H, s). Anal. ($C_{24}H_{35}ClN_2O_2 \cdot 0.5H_2O$) C, H, N, Cl.

7-Phenyl-2(*E*)-hepten-4-ol (35). A solution of 1-bromo-1-propene (7.2 mL, 84 mmol) in THF (50 mL) was added dropwise during 1 h to Mg (2.2 g, 96 mmol) suspended in THF (25 mL) at 45 °C. (The reaction was initiated by adding a small crystal of I_2.) After 45 min the mixture was diluted with ether (200 mL) and cooled to −78 °C. To this was added benzenebutanal (**34**) (10 g, 69 mmol). After 0.5 h the mixture was warmed to room temperature and saturated NH_4Cl was added. The mixture was extracted with ether. The organic phase was washed with water and brine, dried, and concentrated to give crude **35** (13 g, 100%) as a mixture of isomers which was used in the next reaction without further purification: ^{1}H NMR δ 1.4–1.78 (8 H, m), 2.58–2.70 (2 H, m), 4.05 and 4.49 (1 H (3:7), m), 5.35–5.72 (2 H, m), 7.1–7.3 (5 H, m).

Trichloro-*N*-(1-methyl-6-phenyl-2(*E*)-hexenyl)acetamide (36). To a suspension of NaH (1 g, 42 mmol) in ether (300 mL) at 0 °C was added dropwise **35** (8 g, 42 mmol). The cooling bath was removed and stirring continued for 1 h. The mixture was cooled to 0 °C and trichloroacetonitrile (4.22 mL, 42 mmol) was added. After 10 min the cooling bath was removed and stirring continued for 1 h. Methanol (1.7 mL) was added and the mixture concentrated in vacuo. The residue was shaken with a mixture of ether (200 mL) and hexane (100 mL) and filtered. The filtrate was concentrated. The residue was dissolved in xylene (300 mL) and refluxed for 14 h. The mixture was cooled to room temperature and filtered through silica gel (100 g). The filtrate was concentrated and the residue chromatographed (7% ethyl acetate in hexane) to give **36** (9.5 g, 67%) as a colorless oil: ^{1}H NMR δ 1.32 (3 H, d, J = 7 Hz), 1.71 (2 H, p, J = 7.5 Hz), 2.08 (2 H, J = 7.5 Hz), 2.62 (2 H, t, J = 7.5 Hz), 4.48 (1 H, m), 5.45 (1 H, dd, J = 16 and 6 Hz), 5.69 (1 H, J = 16 and 7.5 Hz), 6.5 (1 H, bs), 7.1–7.3 (5 H, m).

7-Phenyl-3(*E*)-hepten-2-amine (37).[23] A mixture of **36** (9.5 g, 27 mmol), 6 N NaOH (135 mL), and 95% ethanol (145 mL) was stirred under an N_2 atmosphere for 40 h. The mixture was extracted with ether. The organic phase was dried over K_2CO_3 and concentrated. The residue was distilled bulb-to-bulb at 0.5 mm (bath temperature = 90 °C) to give **37** (5.3 g, 99%) as a colorless oil: ^{1}H NMR δ 1.13 (3 H, d, J = 7 Hz), 1.70 (2 H, J = 7.5 Hz), 2.04 (2 H, q, J = 7.5 Hz), 2.61 (2 H, t, J = 7.5 Hz), 3.45 (1 H, p, J = 7), 5.45 (1 H, dd, J = 15, 7 Hz), 5.02 (1 H, dt, J = 15, 7 Hz), 7.1–7.3 (5 H, m).

α(*S*)-Amino-4-hydroxy-2,6-dimethyl-*N*-[1-methyl-6-phenyl-2(*E*)-hexenyl]benzenepropanamide Hydrochloride Salts (37A and 37B). Allylamine **37** was coupled to 4 as described in the preparation of **7A** to give a mixture of diastereomeric products which were separated by chromatography and deprotected to afford **37A** (less polar) and **37B** (more polar), each in

42% yield, as a white solids. **37A**: ^{1}H NMR (DMSO-d_6) δ 1.06 (3 H, d, J = 7 Hz), 1.57 (2 H, m), 1.96 (2 H, q, J = 6 Hz), 2.14 (6 H, s), 2.57 (2 H, t, J = 7.5 Hz), 2.88 (1 H, dd, J = 13, 4 Hz), 3.02 (1 H, dd, J = 13, 12 Hz), 3.69 (1 H, m), 4.28 (1 H, m), 4.96–5.13 (2 H, m), 6.42 (2 H, s), 7.04–7.34 (5 H, m), 7.92 (1 H, d, J = 8 Hz), 8.44 (3 H, bs), 9.04 (1 H, bs). Anal. ($C_{24}H_{33}ClN_2O_2$·0.25H_2O) C, H, N, Cl. **37B**: ^{1}H NMR (DMSO-d_6) δ 0.76 (3 H, d, J = 7 Hz), 1.58 (2 H, p, J = 7.5 Hz), 1.93 (2 H, q, J = 7 Hz), 1.99 (6 H, s), 2.52 (2 H, t, J = 7.5 Hz), 2.81–3.03 (2 H, m), 3.64 (1 H, m), 4.19 (1 H, q, J = 6 Hz), 5.27 (1 H, dd, J = 16, 6 Hz), 5.50 (1 H, dt, J = 16, 7 Hz), 6.4 (2 H, s), 7.1–7.3 (5 H, m), 7.78 (1 H, d, J = 8 Hz), 8.51 3 H, bs), 9.06 (1 H, bs). Anal. ($C_{24}H_{33}ClN_2O_2$) C, H, N, Cl.

α(*S*)-Amino-4-hydroxy-2,6-dimethyl-*N*-[1-methyl-5-phenyl-2(*E*)-pentenyl]benzenepropanamide Hydrochloride Salts (39A and 39B). Substitution of hydrocinnamaldehyde (**38**) for **34** in the sequence of reactions described above for the preparation of **39A** and **39B** provided HCl salts **39A** (less polar) and **39B** (more polar), in 38% and 40% yield, respectively, as white solids. **39A**: ^{1}H NMR (DMSO-d_6) δ 1.02 (3 H, d, J = 7 Hz), 2.1–2.22 (8 H, including singlet at 2.15, m), 2.53 (2 H, m), 2.9 (1 H, dd, J = 13, 4 Hz), 3.0 (1 H, dd, J = 13, 12 Hz), 3.7 (1 H, dd, J = 12, 4 Hz), 4.27 (1 H, m), 5.0 (1 H, dt, J = 16, 7 Hz), 5.10 (1 H, dd, J = 10, 5 Hz), 6.42 (2 H, s), 7.1–7.3 (5 H, m), 7.98 (1 H, d, J = 8 Hz), 8.5 (3 H, bs), 9.1 (1 H, s). Anal. ($C_{23}H_{31}ClN_2O_2$) C, H, N, Cl. **39B**: ^{1}H NMR (DMSO-d_6) δ 0.77 (3 H, d, J = 7 Hz), 1.96–2.32 (8 H, including s at 2.18, m), 2.60 (2 H, t, J = 7.5 Hz), 2.84–3.06 (2 H, m), 3.66 (1 H, m), 4.2 (1 H, m), 5.33 (1 H, dd, J = 16, 5 Hz), 5.56 (1 H, dt, J = 16, 5 Hz), 6.42 (2 H, s), 7.14–7.34 (5 H, m), 7.82 (1 H, d, J = 7.5 Hz), 8.42 (3 H, bs), 9.14 (1 H, s). Anal. ($C_{23}H_{31}ClN_2O_2$) C, H, N, Cl.

3-Fluoro-7-phenyl-3(*Z*)-hepten-2-amine (42). To a stirred solution of **40**[24] (5.59 g, 42.3 mmol) in THF (350 mL) at −78 °C was added a 1 N toluene solution of Dibal-H (42 mL, 42 mmol) dropwise over 15 min. The mixture was stirred for 2 h to yield solution A. In a separate flask Mg (2.67 g, 116 mmol) was stirred in THF (5 mL) and treated with a solution of 1-bromo-3-phenylpropane (15.5 mL, 102 mmol) in THF (20 mL) over 45 min. (A small amount of the bromide solution was added to the Mg at first and stirred to initiate the reaction; the rest of the bromide was then added slowly to maintain a gentle reflux.) The mixture was further stirred for 1 h, cooled to −78 °C, and cannulated into solution A. After 1 h, saturated NH_4Cl was added followed by 200 mL of a saturated solution of Rochelle's salt. The mixture was allowed to warm to room temperature. The aqueous phase was extracted with ether, and the combined organic phase was washed with water and brine, dried, and concentrated. The residue was distilled at 130 °C (0.1 mmHg) to give 3-fluoro-7-phenyl-2(*Z*)-hexen-4-ol (**41**) (3 g, 34%) as an oil: ^{1}H NMR δ 1.55–1.83 (8 H, m), 2.59–2.7 (2 H, m), 4.46 (1 H, dq, J = 20, 7 Hz), 5.19 (1 H, dq, J = 28, 7.5 Hz), 7.1–7.3 (5 H, m). **41** (4.29 g, 20.6 mmol) was treated with NaH (60 mg, 2.5 mmol) and trichloroacetonitrile (2 mL, 20 mmol) and the product refluxed in xylene as described in the procedure for the preparation of **36** to give trichloro-*N*-(2-fluoro-1-methyl-6-phenyl-2-hexenyl)acetamide as an oil in 83% yield after chromatography (5% ethyl acetate in hexane): ^{1}H NMR δ 1.42 (3 H, d, J = 7 Hz), 1.71 (2 H, p, J = 7.5), 2.13 (2 H, qd, J = 7.5), 2.61 (2 H, t, J = 7.5 Hz), 4.59 (1 H, m), 4.88 (1 H, dt, J_{HF} = 38 Hz, J_{HH} = 7.5 Hz), 7.1–7.3 (5 H, m). Hydrolysis of the above amide as described for **37** gave **42** in 84% yield as an yellowish oil: ^{1}H NMR δ 1.24 (3 H, d, J = 7.5 Hz), 1.69 (2 H, p, J = 7.5 Hz), 2.12 (2 H, q, J = 7.5 Hz), 2.62 (2 H, t, J = 7.5 Hz), 3.48 (1 H, m), 4.69 (1 H, dt, J_{HF} = 38 Hz, J_{HH} = 7.5 Hz), 7.1–7.3 (5 H, m).

α(*S*)-Amino-*N*-[2-fluoro-1-methyl-7-phenyl-2(*Z*)-heptenyl]-4-hydroxy-2,6-dimethylbenzenepropanamide Hydrochloride Salts (42A and 42B). Allylamine **42** was coupled to Boc-DMT as described in the preparation of **7A**, giving a mixture of diastereomeric products which were separated by chromatography and deprotected to give **42A** (less polar) and **42B** (more polar) in 19% and 22% yield, respectively, as white solids. **42A**: ^{1}H NMR (DMSO-d_6) δ 1.13 (3 H, d, J = 7 Hz), 1.54 (2 H, m), 1.77–2.03 (2 H, m), 2.15 (6 H, s), 2.53 (2 H, t, J = 7.5 Hz), 2.92 (1 H, dd, J = 13, 4 Hz), 3.01 (1 H, dd, J = 13, 12 Hz), 3.76 (1 H, dd, J = 12, 4 Hz), 4.2 (1 H, dt, J_{HF} = 38 Hz, J_{HH} = 7.5 Hz), 4.39 (1 H, m), 6.4 (2 H, s), 7.0 (5 H, m), 8.37 (1 H, d, J = 8 Hz), 8.65 (3 H, bs), 9.15 (1 H, s). Anal. ($C_{24}H_{32}ClFN_2O_2$) C, H, N, Cl, F. **42B**: ^{1}H NMR (DMSO-d_6) δ 0.84 (3 H, d, J = 7 Hz), 1.58 (2 H, m), 1.96 (2 H, m), 2.54 (2 H, t, J = 7.5 Hz), 2.91–3.09 (2 H, m), 3.73 (2 H, m), 4.31 (2 H, m), 4.9 (1 H, dt, J_{HF} = 38 Hz, J_{HH} = 7.5 Hz), 6.42 (2 H, s), 7.1–7.3 (5 H, m), 8.14 (1 H, d, J = 7.5 Hz), 8.65 (3 H, bs), 9.15 (1 H, bs). Anal. ($C_{24}H_{32}ClFN_2O_2$) C, H, N, Cl, F.

***N*-[(1,1-Dimethylethoxy)carbonyl]-*O*-(1,1-dimethylethyl)-2,6-dimethyl-L-tyrosine Methyl Ester (44).**[28] To a stirred solution of *N*-[(1,1-dimethylethoxy)carbonyl]-2,6-dimethyl-L-tyrosine methyl ester (**43**) (10 g, 31 mmol) in CH_2Cl_2 (700 mL) at 0 °C was added BF_3·OEt_2 (3.8 mL, 30.9 mmol) dropwise. Isobutene was bubbled slowly until the volume increased to ca. 750 mL. The mixture was stirred for 16 h and allowed to warm to 18 °C. The mixture was extracted with ether and water. The organic phase was washed with brine, dried, and concentrated. The residue was chromatographed (10% ethyl acetate in hexane) to give **44** (7.3 g, 62%) as a gum: ^{1}H NMR δ 1.25–1.4 (18 H, m), 2.3 (6 H, s), 3.0 (2 H, m), 3.6 (3 H, bs), 4.5 (1 H, m), 5.05 (1 H, m), 6.65 (2 H, s).

1,1-Dimethylethyl [2-[4-(1,1-Dimethylethoxy)-2,6-dimethylphenyl]-1(*S*)-formylethyl]carbamate (45). To a stirred solution of **44** (3.16 g, 8.34 mmol) in toluene (175 mL) at −78 °C was added dropwise, using a syringe pump, 17 mL of a 1 M toluene solution of Dibal-H (17 mmol) over 1 h. The cooling bath was removed and CH_3OH (3 mL) was added. The mixture was poured into 350 mL of saturated Rochelle's salt and stirred for 1.5 h. The mixture was extracted with ether. The ether extract was washed with water and brine, dried, and concentrated to give crude **45** (3.15 g): partial ^{1}H NMR δ 2.96 (1 H, dd, J = 14.8 Hz), 3.17 (1 H, dd, J = 14.8 Hz), 4.34 (1 H, q, J = 8 Hz).

2(*R*)-[[2(*S*)-Amino-3-(4-hydroxy-2,6-dimethylphenyl)-propyl]amino]-*N*-(3-phenylpropyl)propanamide Dihydrochloride Salt (46). To a stirred mixture of the above obtained crude **45**, D-Ala-3-phenyl-1-propylamide[11] (2 g, 9.7 mmol), NaOAc (1.6 g), and 4-Å molecular sieves (1 g) in CH_3OH (40 mL) at room temperature was added $NaBH_3CN$ (1.2 g, 14 mmol). The mixture was stirred for 3 h and 1 N HCl was added dropwise to pH = 3.5. The mixture was basified with saturated $NaHCO_3$ and most of the methanol was removed in vacuo. The mixture was extracted with ethyl acetate. The organic phase was washed with water and brine, dried, and concentrated. The residue was chromatographed (8:1:1 ethyl acetate–hexane–triethylamine) to yield the corresponding alcohol (1 g, 35%) followed by 1 g of a gum. The latter was allowed to stand in solution with 10 mL of 4 N HCl in 1:1 CH_2Cl_2–dioxane for 10 min. The volatiles were removed, and the residue was chromatographed (15% ethanol in CH_2Cl_2 saturated with NH_3) to give the free diamine (389 mg, 12%) of **46**. Dihydrochloride salt **46** was obtained as white solid: ^{1}H NMR (DMSO-d_6) δ 1.37 (3 H, d, J = 7 Hz), 1.71 (2 H, p, J = 7.5 Hz), 2.59 (t, J = 7.5 Hz), 2.67–3.10 (4 H, m), 3.24–3.43 (2 H, m), 6.45 (2 H, s), 7.15–7.35 (5 H, m), 8.62 (1 H, t, J = 5 Hz), 8.64–8.9 (3 H, bm), 9.2 (1 H, bs). Anal. ($C_{23}H_{35}Cl_2N_3O_2$) C, H, N, Cl.

***N*-[(1,1-Dimethylethoxy)carbonyl]-*O*-(1,1-dimethylethyl)-2,6-dimethyl-L-tyrosine (47).** To a stirred solution of **44** (5 g, 13 mmol) in THF (60 mL) at 0 °C was added a 0.5 N solution of 26 mL of LiOH (13 mmol). After 15 min the mixture was concentrated and the residue acidified with 0.5 N $KHSO_4$ to pH = 5. The mixture was extracted with ethyl acetate. The organic phase was washed with water, dried, and concentrated to give **47** (4.8 g, 99%) as white solid: ^{1}H NMR δ 1.13 (6 H, bs), 1.3–1.4 (12 H, distorted s), 2.30–2.40 (6 H, distorted s), 3.05 (1 H, dd, J = 14, 11 Hz), 3.16 (1 H, dd, J = 14, 5 Hz), 4.53 (1 H, m), 4.98 (0.3 H, bd, J = 7.5 Hz), 6.18 (2 H, s), 7.22 (0.7 H, dd, J = 7.5 Hz).

1,1-Dimethyl [2-[4-(1,1-Dimethylethoxy)-2,6-dimethylphenyl]-1(*S*)-[2-isoxazolidinylcarbonyl]ethyl]carbamate (48). Isoxazolidine hydrochloride[29] was coupled to **4** using the general mixed anhydride coupling procedure described for the preparation of **7A** to give, after chromatography (40% ethyl acetate in hexane), **48** (84%) as a gum: ^{1}H NMR δ 1.3–1.4 (12 H, bm), 2.0–2.25 (2 H, m), 2.33 (6 H, s), 2.90–3.15 (3 H, m), 3.47–3.82 (3 H, m), 5.08 (1 H, m), 5.34 (1 H, m), 6.14 (2 H, s).

2-Methyl-*N*-(3-phenylpropyl)-2-propenamide (49). To a solution of methacryloyl chloride (10 mL, 102 mmol) in CH_2Cl_2 (100 mL) at 0 °C was added dropwise while stirring a mixture of Et_3N (14.5 mL, 104 mmol) and 3-phenyl-1-propylamine (14.75

mL, 104 mmol). After 0.5 h the mixture was washed with water, 1 N $KHSO_4$, and saturated $NaHCO_3$, dried, and concentrated. The residue was distilled. The fraction with bp = 150–160 °C was chromatographed (30% ethyl acetate in hexane) to give **49** (14 g, 67%) as an oil: ^1H NMR δ 1.80–1.84 (5 H, m), 2.65 (2 H, t, J = 7.5 Hz), 3.32 (2 H, q, J = 7 Hz), 5.26 (1 H, m), 5.60 (1 H, m), 6.02 (1 H, bs), 7.12–7.31 (5 H, m).

1,1-Dimethyl [1(*S*)-[[4-(1,1-Dimethylethoxy)-2,6-dimethylphenyl]methyl]-4-methylene-2,5-dioxo-5-[(3-phenylpropyl)amino]pentyl]carbamate (50).[27] To a stirred solution of **49** (9.35 g, 46 mmol) in THF (350 mL) at −78 °C was added dropwise a 2.5 M hexane solution of *n*-BuLi (37.5 mL, 94 mmol). After 3 min the temperature was raised to 0 °C and stirring continued for 0.5 h. The orange-yellow mixture was cooled to −78 °C and a solution of **48** (8.8 g, 21 mmol) in THF (20 mL) was added. After 2.5 h, the mixture was added to saturated NH_4Cl and extracted with ethyl acetate. The organic phase was washed with water, dried, and concentrated. The residue was chromatographed (1:1 ethyl acetate–hexane) to give, in the order of elution, tainted **50** (3.3 g, contaminated with a less polar impurity) and pure **50** (1.8 g) as white solids. **50**: ^1H NMR δ 0.96–1.27 (18 H, complex m), 1.96–2.10 (2 H, m), 2.3 (6 H, s), 2.55–2.75 (2 H, m), 2.97–3.09 (2 H, m), 3.23 (1 H, m), 3.50–3.65 (1 H, m), 4.08–4.26 (2 H, m), 5.35 (1 H, bs), 6.04 (1 H, bt, J = 5 Hz), 6.65 (2 H, s), 7.1–7.3 (5 H, m).

1,1-Dimethylethyl [1(*S*)-[[4-(1,1-Dimethylethoxy)-2,6-dimethylphenyl]methyl]-4-methyl-2,5-dioxo-5-[(3-phenylpropyl)amino]pentyl]carbamate (51). Hydrogenation of the above two fractions, described in the previous experimental, in THF with Pd/C under 5 psi of H_2 pressure followed by chromatography (3% ethanol in CH_2Cl_2) gave **51** (2.34 g, 20% overall yield from **48**) as a white solid: ^1H NMR δ 1.0–1.29 (22 H, m with a doublet at 1.25, J = 7.5 Hz), 1.91–2.11 (2 H, m), 2.28–2.32 (6 H, bs), 2.58–2.85 (2 H, m), 3.0 (1 H, dd, J = 15, 6 Hz), 3.04–3.24 (2 H, m), 3.44–3.58 (1 H, m), 4.03–4.26 (1 H, m), 6.65 (2 H, s), 7.1–7.3 (6 H, m).

5-[1-Amino-2-(4-hydroxy-2,6-dimethylphenyl)ethyl]-1,5-dihydro-3-methyl-1-(3-phenylpropyl)-2*H*-pyrrol-2-one Hydrochloride Salt (52). Compound **51** (100 mg) was deprotected using 4 N HCl in 1:1 CH_2Cl_2–dioxane or 1:1 trifluoroacetic acid–CH_2Cl_2. The product was extracted with saturated $NaHCO_3$ and CH_2Cl_2. The organic phase was dried and concentrated. The residue was chromatographed (CH_2Cl_2–CH_3OH–NH_4OH, 14:1:0.1) to give **52** (60 mg, 87%) as a white solid (Treatment of **51** with trimethylsilyl bromine in CH_2Cl_2 followed by methanolic HCl also gave the HCl salt of **52**: ^1H NMR δ 1.71–1.86 (2 H, m), 2.58 (2 H, t, J = 7.5 Hz), 2.72 (2 H, apparent d, J = 7 Hz), 3.03 (2 H, ddd, J = 14, 8.5, 7.5), 3.28 (1 H, td, J = 7, 2.5), 3.84 (1 H, ddd, J = 14, 8.5, 7.5), 3.97 (1 H, m), 6.5 (2 H, s), 6.72 (1 H, bs), 7.08–7.3 (6 H, m); IR ($CHCl_3$) 1660, 1630 cm^{-1}. Anal. ($C_{24}H_{30}N_2O_2$) C, H, N.

1,1-Dimethylethyl [1(*S*)-[[4-(1,1-Dimethylethoxy)-2,6-dimethylphenyl]methyl]-5-(trimethylsilyl)-2(*E*)-penten-4-ynyl]carbamate (53). To a stirred suspension of triphenyl(3-trimethylsilyl-prop-2-ynyl)phosphonium bromide (6 g, 13.24 mmol) in THF (300 mL) at −78 °C was added a 2.5 M hexane solution of *n*-BuLi (5.3 mL, 13.25 mmol). After 0.5 h, a solution of **45** (4.26 g, 12.27 mmol) in THF (30 mL) was added. After 0.5 h, the mixture was allowed to warm to 0 °C over 1 h and added to water and ether. The organic phase was washed with brine, dried, and concentrated. The residue was chromatographed (10% ethyl acetate in hexane) to give **53** (2.95 g, 67%) as a colorless gum. ^1H NMR δ 0.16 (9 H, s), 1.12–1.26 (18 H, complex m), 2.12 (6 H, s), 2.73 (1 H, dd, J = 14, 7.5 Hz), 2.85 (1 H, dd, J = 14, 7.5 Hz), 4.43–4.55 (2 H, m), 5.54 (1 H, d, J = 16 Hz), 6.08 (1 H, dd, J = 14, 5 Hz), 6.66 (2 H, s).

5(*S*)-[[(1,1-Dimethylethoxy)carbonyl]amino]-6-[4-(1,1-dimethylethoxy)-2,6-dimethylphenyl]-3(*E*)-hexenoic Acid (54). To cyclohexene (0.4 mL, 3.9 mmol) in THF (10 mL) at −10 °C was added with stirring a 1 M THF solution or borane–THF complex (2 mL, 2 mmol). After 1 h, **53** (0.9 g, 2 mmol) in THF (2 mL) was added dropwise. After 0.5 h, CH_3OH (1 mL) and 3 N NaOH (1 mL) were added followed by a 30% solution of H_2O_2 (1 mL). After 0.5 h 2 N NaOH (1 mL) was added and the mixture extracted with ether and water. The aqueous phase was acidified with 10% citric acid to pH 4 and extracted with ether. The ether extract dried and concentrated to give **54** (0.5 g, 61%) as colorless solid: ^1H NMR δ 1.2–1.4 (18 H, bs), 2.3 (6 H, s), 2.73 (1 H, dd, J = 18, 7.5 Hz), 2.89 (1 H, dd, J = 18, 7.5), 4.37 (1 H, m), 5.5–5.6 (2 H, m), 6.65 (2 H, s).

1,1-Dimethylethyl [1(*S*)-[[4-(1,1-dimethylethoxy)-2,6-dimethylphenyl]methyl]-5-oxo-5-[(3-phenylpropyl)amino]-2-(*E*)-pentenyl]carbamate (55). To a stirred mixture of **54** (0.67 g, 1.65 mmol), 3-phenyl-1-propylamine (0.235 mL, 1.65 mmol), and 1-hydroxybenzotriazole hydrate (0.25 g, 1.85 mmol) in DMF (7 mL) at −23 °C was added DCC (0.34 g, 1.65 mmol). The mixture was allowed to warm to room temperature over 2.5 h. The mixture was extracted with ethyl acetate and the extract washed with water, 0.5 N $KHSO_4$, and saturated $NaHCO_3$, dried, and concentrated. The residue was chromatographed (60% ethyl acetate in hexane) to give **55** (0.58 g, 89%) as a white solid: ^1H NMR δ 1.3 (3 H, s), 1.4 (3 H, s), 1.82 (2 H, p, J = 7.5 Hz), 2.62 (2 H, t, J = 7.5 Hz), 2.73 (1 H, dd, J = 14, 8 Hz), 2.83–2.95 (3 H, m), 3.72 (2 H, m), 4.21 (1 H, m), 5.39–5.53 (2 H, m), 6.65 (2 H, s), 7.12–7.3 (5 H, m).

5(*S*)-Amino-6-(4-hydroxy-2,6-dimethylphenyl)-*N*-(3-phenylpropyl)-3(*E*)-hexenamide Hydrochloride Salt (56). Compound **55** was deprotected as in the general procedure to give **56** as a white solid: ^1H NMR (DMSO-d_6) δ 1.67 (2 H, p, J = 7.5 Hz), 2.1.8 (6 H, s), 2.55 (2 H, t, J = 7.5 Hz), 2.7–3.07 (6 H, m), 3.68 (1 H, m), 5.42–5.59 (2 H, m), 6.4 (2 H, s), 7.1–7.3 (5 H, m), 7.82 (1 H, J = 5), 8.4 (3 H, bs). Anal. ($C_{23}H_{31}N_2O_2Cl$·0.5H_2O) C, H, N, Cl.

δ(*R*)-Amino-4-hydroxy-2,6-dimethyl-*N*-(3-phenylpropyl)benzenehexanamide Hydrochloride Salt (58). Compound **55** (310 mg) was hydrogenated in ethanol with 5% Pd/C under a 5 psi H_2 atmosphere to give **57** (300 mg) as a white solid, which was deprotected as in the preparation of **7A**, giving **58** as a white solid: ^1H NMR (DMSO-d_6) δ 1.27–1.72 (6 H, m), 2.02 (2 H, t, J = 6 Hz), 2.55 (2 H, t, J = 7.5 Hz), 2.74 (1 H, dd, J = 14, 10 Hz), 2.86 (1 H, dd, J = 14, 5 Hz), 3.0 (2 H, q, J = 6 Hz), 3.14 (1 H, bs), 6.43 (2 H, s), 7.1–7.3 (5 H, m), 7.86 (1 H, t, J = 6 Hz), 8.02 (3 H, bs), 9.1 (1 H, bs). Anal. ($C_{23}H_{33}N_2O_2Cl$·0.25H_2O) C, H, N, Cl.

Pharmacological Methods. Writhing Assay. Male Charles River albino mice (CD-1/HAM/1LR) weighing between 20 and 30 g were used. Twenty-five minutes after subcutaneous or intragastric administration of the test compound (0.1 mL/10 g of body weight), 0.025% (w/v) phenylbenzoquinone was injected intraperitoneally (0.1 mL/g of body weight). Five minutes later each mouse was placed in a large glass beaker and the number of writhes that occurred in the subsequent 10 min was counted. A writhe consisted of a dorsoflexion of the back, extension of the hind limbs and strong contraction of the abdominal musculature. The test compound was considered to have produced analgesia in a mouse if the number of writhes elicited by phenylbenzoquinone was equal to or less than one-half the median number of writhes recorded for the saline-treated group that day. The results were expressed as the number of mice (out of 10 possible) in which the test compound produced analgesia. The test compound was rated active if the number of writhes in the drug-treatment group was significantly less than the number of writhes in the saline-treatment group as determined by a one-way analysis of variance. If the initial test dose of 10 mg/kg inhibited writhing in greater than six out of 10 mice, the effect of additional doses was evaluated and an ED_{50} value and 95% confidence limits were calculated using a maximum likelihood function. For those compounds that dose-dependently inhibited writhing, an additional experiment was conducted to determine whether the antinociception was opioid mediated. An ED_{70} dose of test compound was administered to each of two groups of 10 mice. Fifteen minutes later, one group was injected sc with 1 mg/kg naloxone hydrochloride and the other group was injected sc with 0.1 mL/10 g of body weight saline. Fifteen minutes later, each mouse was injected with phenylbenzoquinone and the writhing test was conducted as described above. The mean number of writhes in the naloxone-treated group was compared to that in the saline-treated group using a one-way analysis of variance.

Binding Assays. Binding at μ and δ receptors was measured in a twice-washed P2 membrane fraction obtained from whole rat brain (minus cerebellum) using a 50 mM Tris-HCl buffer (pH 7.4 at 37 °C). Assay tubes contained 0.8 mL of membrane hom-

ogenate (0.5 mg of protein), 0.1 mL of ^{3}H-labeled ligand (1.0 nM DTLET or 1.0 nM DSLET for δ, 2.0 nM DAMGO for μ), and 0.1 mL of the test compound in replicates of three. After incubation for 60 min at 37 °C, the reactions were terminated by rapid filtration on Whatman GF/B glass-fiber filters and subsequent 10 mL wash of ice-cold buffer. Filters were prepared for liquid scintillation counting. Specific binding was calculated as the difference in radioactivity bound in the absence and presence of 10 μL of levorphanol. IC_{50} values, the concentration of test compound that inhibited ^{3}H-labeled ligand binding by 50%, were obtained by regression analysis of a log-logit transformation of binding data as described by Limbird.[30]

Affinity for the κ opioid receptor was assessed using a crude membrane homogenate prepared from guinea pig brain in KRH buffer (millimolar: HEPES, 25; NaCl, 118; KCl, 4.8; $CaCl_2$, 2.5; and $MgCl_2$, 1.2; pH adjusted to 7.4) according to the method described by Takemori et al.[33] Test tubes containing approximately 1 mg of protein, 4 nM of [^{3}H]-U-69,593 (New England Nuclear), and enough KRH buffer to bring the final volume to 0.5 mL were incubated at 37 °C for 60 min before the contents of the tubes were filtered through Whatman GF/C filters which were presoaked in 0.3% polyethylenimine for 1 h. The filters were washed three times with 4 mL of 5 mM Tris-HCl, pH 7.4. Radioactivity on the filters was determined by liquid scintillation spectrometry. Nonspecific binding was defined as the amount of binding that was not inhibited by 1 μM of (-)ethylketocyclazocine.

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Registry No. **3**, 123715-02-6; **3** (R = Cbz), 137764-59-1; **4**, 99953-00-1; **5**, 92137-66-1; **6**, 137649-87-7; **7**, 137649-88-8; **7A**·2HCl, 137650-32-9; **7A** (free base), 137650-54-5; **8**, 99952-51-9; **9**, 137649-89-9; **9A**·2HCl, 137650-33-0; **9A** (free base), 137650-55-6; **10**, 137649-90-2; **11**, 137649-91-3; **12**·HCl, 137649-92-4; (*R*)-**12A**·2HCl, 137650-34-1; (*S*)-**12A**·2HCl, 137650-35-2; (*R*)-**12A** (free base), 137650-56-7; (*S*)-**12A** (free base), 137650-57-8; **13**, 109608-86-8; **14**, 137649-93-5; **15**·oxalate, 137649-95-7; **15A**·HCl, 137650-36-3; **15A** (free base), 137650-58-9; **16**, 137649-96-8; **17**, 137649-97-9; **18**, 137649-98-0; **18A**·HCl, 137650-37-4; **18A** (free base), 137650-59-0; **19**, 137649-99-1; **19A**·HCl, 137650-38-5; **19A** (free base), 137650-60-3; **20**, 60479-64-3; **21**, 137650-00-1; **22**, 137650-01-2; **22A**·HCl, 137650-39-6; **22A** (free base), 137650-61-4; **23**, 137650-02-3; **24**, 137650-03-4; **25**, 137650-04-5; (*R*)-**25A**·HCl, 137650-40-9; (*S*)-**25A**·HCl, 137650-41-0; (*R*)-**25A** (free base), 137650-62-5; (*S*)-**25A** (free base), 137650-63-6; **26**, 82770-45-4; **27**, 137650-05-6; **28A**·HCl, 137650-42-1; **28A** (free base), 137650-64-7; **29A**·HCl, 137650-43-2; **29A** (free base), 137650-65-8; **30**, 16387-61-4; **31**, 137650-06-7; **32**, 137650-07-8; **33**, 137650-08-9; **33A**, 137650-44-3; **34**, 18328-11-5; (*E*)-**35**, 137650-09-0; (*Z*)-**35**, 137650-31-8; **36**, 137650-10-3; **37** (R = H); (*R*)-**37**·HCl (R = DMT), 137650-45-4; (*S*)-**37**·HCl (R = DMT), 137650-46-5; (*R*)-**37** (R = DMT, free base), 137650-66-9; (*S*)-**37** (R = DMT, free base), 137650-67-0; **38**, 104-53-0; (*R*)-**39**·HCl (R = DMT), 137650-47-6; (*S*)-**39**·HCl (R = DMT), 137650-48-7; (*R*)-**39** (R = DMT, free base), 137650-68-1; (*S*)-**39** (R = DMT, free base), 137650-69-2; **40**, 58089-70-6; **41**, 137650-13-6; **42** (R = H), 137650-14-7; **42** (R = Cl_3CCO), 137650-12-5; (*R*)-**42**·HCl (R = DMT), 137650-49-8; (*S*)-**42**·HCl (R = DMT), 137650-50-1; (*R*)-**42** (R = DMT, free base), 137650-70-5; (*S*)-**42** (R = DMT, free base), 137650-71-6; **43**, 137650-15-8; **44**, 137650-16-9; **45**, 137650-17-0; **46**·2HCl, 137650-18-1; **46** (free base), 137650-51-2; **47**, 137650-19-2; **48**, 137650-20-5; **49**, 137650-21-6; **50**, 137650-22-7; **51**, 137650-23-8; **52**·HCl, 137650-24-9; **52** (free base), 137650-52-3; **53**, 137650-25-0; **54**, 137650-26-1; **55**, 137650-27-2; **45**·HCl, 137650-28-3; **56** (free base), 137650-53-4; **57**, 137650-29-4; **58**·HCl, 137650-30-7; **58** (free base), 137668-03-2; (*E*)-$Ph(CH_2)_3CH{=}CHCOOMe$, 55283-02-8; $Ph(CH_2)_5COOMe$, 5581-76-0; $Ph(CH_2)_3NH_2$, 2038-57-5; Boc-D-Ser, 6368-20-3; $Ph(CH_2)_4Br$, 13633-25-5; $Ph(CH_2)_3OMs$, 69804-99-5; $PhCH_2CH_2NH_2$, 64-04-0; $(EtO)_2P(O)Ch_2COOMe$, 1067-74-9; $CH_3CH{=}CHBr$, 590-14-7; $Br(CH_2)_3Ph$, 637-59-2; D-Ala-$NH(CH_2)_3Ph$, 99952-51-9; $H_2C{=}C(CH_3)COCl$, 920-46-7; $Ph_3P^+CH_2C{\equiv}CSiMe_3{\cdot}Br^-$, 42134-49-6; 2-pyridinecarboxaldehyde, 1121-60-4; (*S*)-(-)-1-amino-2-(methoxymethyl)pyrrolidine, 59983-39-0; isoxazolidine hydrochloride, 39657-45-9.

Supplementary Material Available: Observed opioid activities of all target compounds in the binding and mouse writhing tests (3 pages). Ordering information is given on any current masthead page.

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β-Proline Analogues as Agonists at the Strychnine-Sensitive Glycine Receptor

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3-Carboxy-3,4-dehydropyrrolidine was found to bind with affinity equal to that of glycine in a [^{3}H]strychnine binding assay. Simple substitution of the 1-, 2-, 4-, or 5-position resulted in marked loss of affinity. A decline in affinity was also found upon enlargement, contraction, or saturation of the 5-membered ring. However, β-proline and azetidine-3-carboxylic acid retained significant binding affinity. Despite its good affinity in [^{3}H]strychnine binding, 3-carboxy-3,4-dehydropyrrolidine showed only weak agonist activity in intracellular recordings of cultured murine spinal cord neurons. This apparent lack of correlation between binding and functional results is discussed in light of the current models of the strychnine-sensitive glycine receptor.

Introduction

The strychnine-sensitive glycine receptor is a member of the ligand-gated ion channel family of receptors.[1] Within this family, it is most closely related to the GABA, receptor.[2] Like the $GABA_A$ receptor, the glycine receptor

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