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Diaryldimethylpiperazine Ligands with μ- and δ-Opioid Receptor Affinity: Synthesis of (+)-4-[(αR)-α-(4-allyl-(2S,5S)-dimethylpiperazin-1-yl)-(3-hydroxyphenyl)methyl]-*N*-ethyl-*N*-phenylbenzamide and (−)-4-[(αR)-α-(2S,5S)-dimethylpiperazin-1-yl)-(3-hydroxyphenyl)methyl]-*N*-ethyl-*N*-phenylbenzamide

In Jong Kim,[a] Thomas Ullrich,[a,†] James W. Janetka,[a,‡] M. Scott Furness,[a,§] Arthur E. Jacobson,[a,*] Richard B. Rothman,[b] Christina M. Dersch,[b] Judith L. Flippen-Anderson,[c,‖] Clifford George[c] and Kenner C. Rice[a,*]

[a]*Laboratory of Medicinal Chemistry, Building 8, Room B1-23, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Department of Health and Human Services, Bethesda, MD 20892, USA*
[b]*Clinical Psychopharmacology Section, National Institute on Drug Abuse, Addiction Research Center, Department of Health and Human Services, Baltimore, MD 21224, USA*
[c]*Laboratory for the Structure of Matter, Naval Research Laboratory, Washington DC 20375, USA*

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Abstract—We have explored the synthesis of compounds that have good affinity for both μ- and δ-opioid receptors from the (αR,2S,5S) class of diaryldimethylpiperazines. These non-selective compounds were related to opioids that have been found to interact selectively with μ- or δ-opioid receptors as agonists or antagonists. In our initial survey, we found two compounds, (+)-4-[(αR)-α-(4-allyl-(2S,5S)-dimethylpiperazin-1-yl)-(3-hydroxyphenyl)methyl]-*N*-ethyl-*N*-phenylbenzamide **(14)** and its N–H relative, (−)-4-[(αR)-α-(2S,5S)-dimethylpiperazin-1-yl)-(3-hydroxyphenyl)methyl]-*N*-ethyl-*N*-phenylbenzamide (**15**), that interacted with δ-receptors with good affinity, and, as we hoped, with much higher affinity at μ-receptors than SNC80. The relative configuration of the benzylic position in (+)-4-[(αR)-α-(4-allyl-(2S,5S)-dimethyl-1-piperazinyl)-(3-methoxyphenyl)methyl]-benzyl alcohol **(10)** was determined by X-ray crystallographic analysis of a crystal that was an unresolved twin. The absolute stereochemistry of that benzylic stereogenic center was unequivocally derived by the X-ray crystallographic analysis from the two other centers of asymmetry in the molecule that were known. Those were established from the synthesis via a dipeptide *cyclo*-L-Ala-L-Ala in which the absolute stereochemistry was established.
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Introduction

Compounds that are very selective and have high affinity for a specific opioid receptor are useful research tools and, as therapeutic agents, avoid the side effects due to activation of the other two opioid receptors. Thus, much research has focused on a search for highly selective opioids.[1,2] Side effects due to the interaction of a ligand with a specific type of opioid receptor are, however, unavoidable. For example, selective and high affinity μ-receptor opioids not only alleviate centrally mediated pain, but also are well known to cause side effects such as respiratory depression, tolerance, and dependence. Many δ-receptor agonists, like the μ-agonists, reduce centrally mediated pain, and, as well, exhibit proconvulsant activity.[1] Lastly, κ-receptor mediated agonists have also been found to mediate analgesia, but these opioids have dysphoric effects.[3] Compounds that act as antagonists via interaction with opioid receptors have been found to have useful and

*Corresponding author. Tel.: +1-301-496-1856; fax: +1-301-402-0589e-mail: kr21f@nih.gov (K. R. Rice); e-mail: aej@helix.nih.gov (A. E. Jacobson).
†Current address: Novartis Research Institute, A-1235 Vienna, Austria.
‡Current address: AstraZeneca R & D Boston, Waltham, MA.
§Current address: Food and Drug Administration, Rockville, MD.
‖Current address: PDB, Rutgers, the State University of New Jersey, Piscataway, NJ 08854.

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interesting activities. The μ-antagonists will, of course, block the antinociceptive action of μ-agonists, but some have been found to be useful as treatment agents for the abuse of opioid agonists and alcohol.[4] The δ-antagonists have been found to attenuate or reverse μ receptor-mediated side effects such as respiratory depression, tolerance, and dependence.[5–8] Compounds with mixed receptor properties (μ and δ) have recently been found that acted as agonists.[9] We thought that it might be of interest to synthesize a different type of compound that would interact with good affinity at both μ- and δ-opioid receptors. Those that did not interact well with κ-receptors would, eventually, be further examined for their actions as μ-agonists and δ-antagonists (i.e., as analgesics with the μ-agonist side effects of tolerance and dependence blocked by the δ-antagonist action). We decided to examine this in two stages, the initial stage involved their synthesis and the evaluation of their binding affinity to opioid receptors.

Our search for this type of ligand focused on compounds with the diaryldimethylpiperazine molecular structure (Chart 1) that have been extensively reviewed.[10,11] Well known compounds in this class were [+]-4-[(α*R*)-α-[(2*S*,5*R*)-4-allyl-2,5-dimethyl-1-piperazinyl]-3-methoxybenzyl]-*N*,*N*-diethylamide (SNC 80,[12] **1**), a highly δ-selective agonist with good δ-receptor affinity,[13,14] and the much less selective and higher affinity[13] phenolic δ-agonist, the racemic 4-[(4-allyl-2,5-dimethylpiperazin-1-yl)-(3-hydroxyphenyl)methyl]-*N*,*N*-diethylbenzamide (racemic BW373U86, **2**).[15,16] This racemate and its α*R*,2*S*,5*R*-(+)-enantiomer, exhibited both δ- and μ-agonist effects. In general, in the α*R*,2*S*,5*R* diaryldimethylpiperazine series, the 3-methoxylphenyl derivatives were found to have increased δ-receptor selectivity and decreased affinity when compared with its phenolic relative.[14] Somewhat less δ-affinity was observed for α*S*,2*S*,5*R* ligands than the comparable α*R*,2*S*,5*R* ligands, but those that were examined retained δ-agonist activity.[14] We decided, initially, to try to increase the affinity of these compounds for the μ-receptor (as agonists) while retaining the δ-affinity, and convert compounds from both the benzylic α*S* and α*R* diaryldimethylpiperazine stereochemical series of δ-agonists to δ-antagonists. In order to increase μ-receptor affinity, we modified the *N*,*N*-diethylamide moiety to an *N*-ethyl,*N*-phenylbenzamide. A similar *N*-methylanilide moiety, that was also moved to the *meta*-position on the aromatic ring (i.e., **3**, Chart 1), has been shown to increase μ-agonist activity in this series).[9] The *para*-position of the *N*,*N*-diethylamide moiety had been hypothesized to be an appropriate δ-receptor address.[9,17–19] Since we eventually would want to obtain a compound with good affinity for the δ-receptor that would display δ-antagonist activity, we then considered how a δ-agonist could be converted to a δ-antagonist in the diaryldimethylpiperazine series. The normal methodology used for such conversions in the μ-series of morphinans, epoxymorphinans, and 6,7-benzomorphans (e.g., by exchange of an *N*-methyl moiety for an *N*-allyl group) was not thought to be applicable in this series since both *N*-methyl and *N*-allyl compounds are δ-agonists in the SNC80-type of diaryldimethylpiperazines. However, a change in the configuration of the 5-methyl group, from 5*R* to 5*S*, might provide a different steric interaction with the receptor, eliminating the agonist effect. Such steric influences have been known to dramatically modify μ-agonists to μ-antagonists in the phenylpiperidine series of analgesics that were also unaffected by modification of the N-substituent.[20,21] That a similar pharmacological change induced by a steric effect might be applicable to δ-ligands was indicated by the mention, in a patent, of a diaryldimethylpiperazine compound with δ-antagonist activity (as ascertained in the mouse vas deferens assay) that had an α*R*,2*S*,5*S* configuration,[22] and the experimental determination of δ-antagonist activity in a 3*R*,5*S*-diaryldimethylpiperazine compound, [*cis*-4-(α-(4-((Z)-2-butenyl)-3,5-dimethyl-1-piperazinyl)-3-hydroxybenzyl)-*N*,*N*-diethylbenzamide, **4**].[9] We, thus, decided to synthesize and determine the opioid receptor binding affinities of α*R* *N*-H and α*R* *N*-allyl (2*S*,5*S*)-2,5-*cis*-dimethylpiperazinyl)-(3-methoxyphenyl)methyl]-*N*-ethyl-*N*-phenylbenzamides.

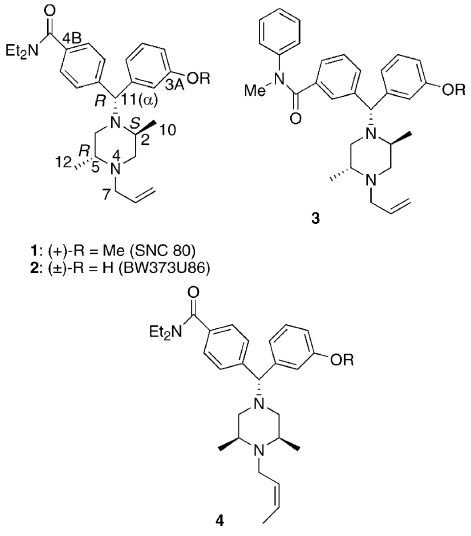
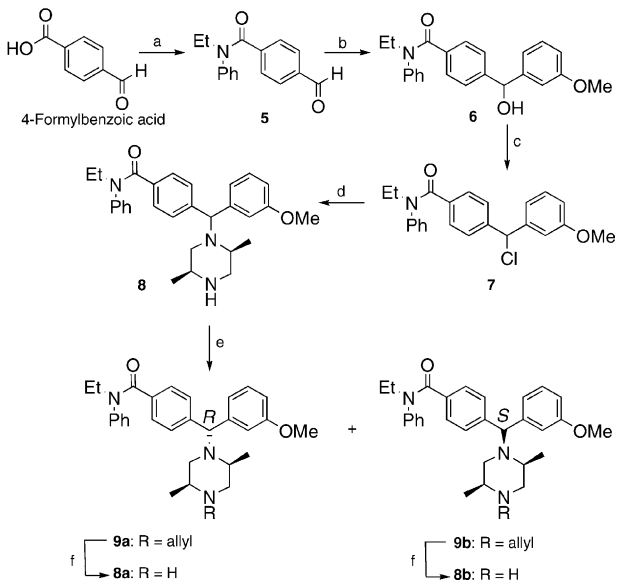


Chart 1.

Chemistry

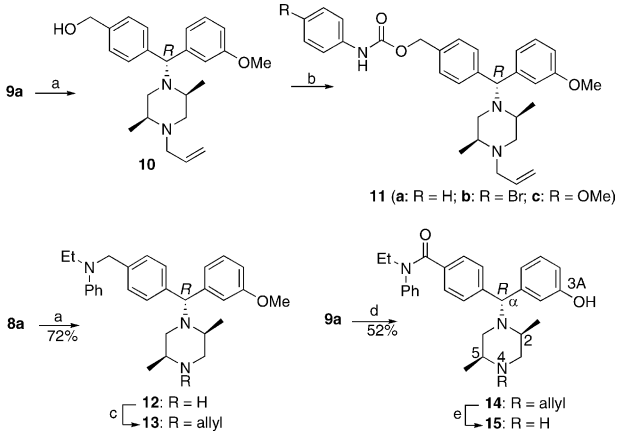
The synthesis of the intermediates leading to the designed compounds was straightforward and accomplished according to a previously reported method.[23] 4-Formyl-*N*,*N*-diethylbenzamide (**5**) was obtained from the condensation of 4-formylbenzoic acid with diethylamine in the presence of 1-(3-dimethylaminopropyl)3-ethylcarbodiimide (EDCI) in 88% yield. Reaction of **5** with (3-methoxyphenyl)magnesium bromide produced benzhydryl alcohol **6**, which was converted to the corresponding chloride **7** after treatment with concentrated hydrochloric acid (Scheme 1). Addition of (2*S*,5*S*)-dimethylpiperazine,[24] yielded an inseparable amine mixture **8**. Allylation of the mixture **8** gave a mixture **9**, from which pure **9a** and **9b** could be obtained by preparative HPLC. Pure **8a** and **8b** was obtained from pure **9a** and **9b**, respectively, by catalytic hydrogenation.



Scheme 1. Conditions: (a) *N*-ethylaniline, EDCI, 10 °C to rt, CH_2Cl_2, 88%; (b) 3-MeO–PhMgBr, 10 °C to rt, THF, 78%; (c) HCl, $CHCl_3$, rt, 97%; (d) (2*S*,5*S*)-dimethylpiperazine, $NaHCO_3$, CH_3CN, reflux, 72%; (e) allyl bromide, $NaHCO_3$, DMF, 80 °C, 95%; (f) 10% Pd/C, cat CH_3CO_2H, H_2O, reflux.

In order to determine the importance of the amide function to the pharmacological effect of the ligand, that moiety was reduced in **8a** and **9a** with lithium aluminum hydride (LAH). We were surprised to find that they reacted differently. Compound **9a** gave benzyl alcohol **10** on LAH reduction, while **8a** gave benzylamine **12**, in good yield (Scheme 2). We assumed that the difference in reactivity of **8a** and **9a** could be due to a reduction of different complexes that might be formed between **8a** or **9a** and LAH.

The relative configuration of the benzylic position in **10** (and, by extension, in all of the other compounds with three centers of asymmetry (e.g., **8**, **9**, **14**, and **15**) was determined by X-ray crystallographic analysis of a crystal that was an unresolved twin. Many reflections included overlap data from the other twin components.



Scheme 2. Conditions: (a) $LiAlH_4$, Et_2O, reflux; (b) arylisocyanate, C_6H_6, rt, 89–94%; (c) allyl bromide, $NaHCO_3$, DMF, 80 °C, 97%; (d) BBr_3Me_2S in CH_2Cl_2, 1,2-dichloroethane, 60 °C, 52%; (e) 10% Pd-C, cat CH_3CO_2H, H_2O, reflux, 73%.

This prevented the direct determination of absolute configuration. However, the absolute stereochemistry of the benzylic stereogenic center was unequivocally established from this X-ray crystallographic analysis of **10**·2HBr because the two other centers of asymmetry in the molecule were known. The absolute configuration of 2*S*,5*S*-dimethylpiperazine was derived by its synthesis from the dipeptide *cyclo*-L-Ala-L-Ala in which the absolute stereochemistry had been unambiguously determined. The configuration of the benzylic (C11α) center of asymmetry in **10** was, thus, assigned as *R*.

Benzyl alcohol **10** was converted to the corresponding carbamates **11** in high yield from its reaction with arylisocyanates. The benzyl amine analogue **13** was obtained from **12**, and the phenolic benzamides **14** and **15** were obtained from **9a**, as shown in Scheme 2.

Results and Discussion

We have succeeded in synthesizing (+)-4-[(α*R*)-α-(4-allyl-(2*S*,5*S*)-dimethylpiperazin-1-yl)-(3-hydroxyphenyl)methyl]-*N*-ethyl-*N*-phenylbenzamide **(14)** and the N–H enantiomer, (−)-4-[(α*R*)-α-(2*R*,5*R*)-dimethylpiperazin-1-yl)-(3-hydroxyphenyl)methyl]-*N*-ethyl-*N*-phenylbenzamide (**15**). The X-ray diffraction study of (+)-4-[(α*R*)-α-(4-allyl-(2*S*,5*S*)-dimethyl-1-piperazinyl)-(3-methoxyphenyl)methyl]-benzyl alcohol dihydrobromide (**10**·2HBr, Fig. 1), allowed determination of their absolute configuration. These compounds were found to have good (low nM) affinity to the δ-receptor, about the same as that of SNC80 (**1**, Table 1), and also have much higher affinity for the μ-opioid receptors than SNC80. They are, accordingly, less selective for those receptors, satisfying our primary initial goal. The phenolic compound **14** displayed better affinity (K_i = 55 nM) for the μ-opioid receptor, than **15**, its *N*-nor relative. Unfortu-

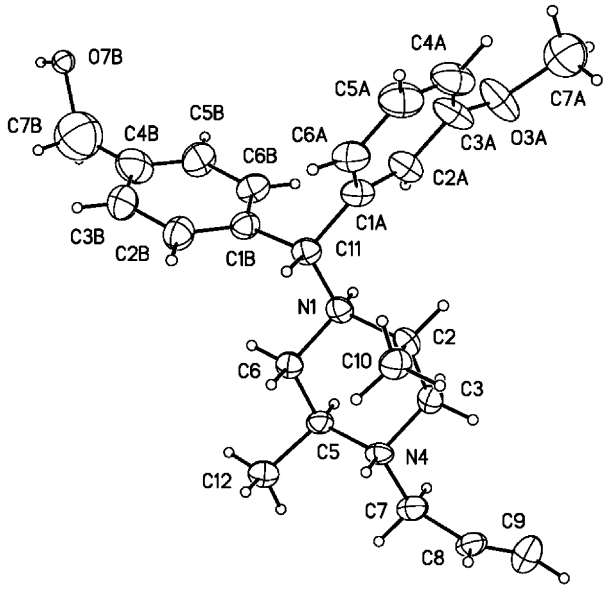
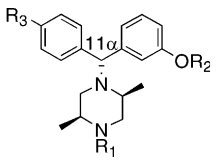


Figure 1. Displacement ellipsoid plot of (+)-4-[(α*R*)-α-(4-allyl-(2*S*,5*S*)-dimethyl-1-piperazinyl)-(3-methoxyphenyl)methyl]-benzyl alcohol dihydrobromide (**10**·2HBr) drawn at 30% probability level. The bromine ions have been omitted for clarity.

Table 1. Opioid receptor binding affinities of ligands [K_i nM ± SEM)]



8a, 8b, 9a, 9b, 12, 13, 14, 15: R_3 = *N*-ethyl-*N*-phenylamide; **10**: R_3 = hydroxymethyl; **11a**: R_3 = phenylcarbamate methyl ester; **11b**: R_3 = 4-bromophenylcarbamate methyl ester; **11c**: R_3 = 4-methoxyphenylcarbamate methyl ester

	11α	R_1	R_2	μ[a]	δ[b]	κ[c]	μ/δ	κ/δ
1[d]				2470 ± 200[d]	2.9 ± 0.35[d]	—	857	—
8a	*R*	H	Me	> 9200	35 ± 5	17,800 ± 1220	> 262	509
8b	*S*	H	Me	> 9200	99 ± 6	27,000	> 93	273
9a	*R*	Allyl	Me	> 9200	58 ± 4	2480 ± 180	> 159	43
9b	*S*	Allyl	Me	> 9200	224 ± 26	7260 ± 410	> 41	32
10	*R*	Allyl	Me	> 6540	293 ± 15	8280 ± 430	> 22	28
11a	*R*	Allyl	Me	> 6540	1310 ± 54	> 6990	> 5	> 5
11b	*R*	Allyl	Me	> 6540	> 4850	> 6990	> 1.3	> 1.4
11c	*R*	Allyl	Me	> 6540	1380 ± 84	8510 ± 370	> 5	6
12	*R*	H	Me	> 6540	1210 ± 68	> 6990	> 5	6
13	*R*	Allyl	Me	> 6540	1350 ± 33	> 6990	> 5	5
14	*R*	Allyl	H	55 ± 6	3.2 ± 0.18	36 ± 4	18	11
15	*R*	H	H	232 ± 14	2.9 ± 0.27	271 ± 19	80	93

[a]Displacement of [^{3}H]DAMGO (D-Ala^2,MePhe^{4}Gly-ol^5)enkephalin), in rat brain membranes.
[b]Displacement of [^{3}H]DADLE (D-Ala^2,D-Leu^5)enkephalin), in rat brain membranes. DAMGO (100 nM) was used to block μ receptor binding.
[c]Displacement of [^{3}H]U69,593 (*trans*-3,4-dichloro-*N*-methyl[2-(1-pyrrolidinyl)cyclohexyl]benzene acetamide), in guinea pig brain membranes.
[d]Data for SNC80 (**1**) from ref 13.

nately, **14** interacted as well with the κ- (K_i = 36 nM) as with the μ-receptor. None of the other compounds listed in Table 1 had the desired selectivity and affinity for the μ- and δ-receptors. The amide function, in this series, was found to be important for interaction with opioid receptors, and the phenolic compounds, not the methoxy ethers, had the desired selectivity and reasonable affinity. The α*S* configuration as seen in **8b** and **9b** (Table 1) did not appear to be advantageous. The two most interesting compounds, **14** and **15**, will, eventually, be further examined for their agonist and/or antagonist activity and the pharmacological data will be reported in due course.

Experimental

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded in $CDCl_3$ (unless otherwise noted) with tetramethylsilane (TMS) as the internal standard on a Varian Gemini-300 spectrometer. Chemical ionization mass spectra (MS, CI-NH_3) were recorded on a Finnigan 4600 spectrometer. Optical rotations were obtained using a Perkin-Elmer 241 MC polarimeter. Thin layer chromatography (TLC) was performed on Analtech silica gel GHLF 0.25 mm plates. Flash column chromatography was performed with Fluka silica gel 60 (220–240 mesh). Preparative HPLC were performed using the Shimadzu LC-6A system. Elemental analyses were performed by Atlantic Microlabs, Inc., Norcross, GA, USA All extracted solutions were dried over magnesium sulfate or sodium sulfate and concentrated to dryness on a rotary evaporator under reduced pressure.

***N*-Ethyl-4-formyl-*N*-phenylbenzamide (5).** 1-[3-(Dimethylamino)propyl] - 3 - ethylcarbodiimide hydrochloride (230.1 g, 1.20 mol) was added portionwise to a vigorously stirred mixture of 4-formylbenzoic acid (150 g, 1 mol) and *N*-ethylaniline (133 g, 1.1 mol) in anhydrous CH_2Cl_2 (2.5 L) at 10 °C. The mixture temperature was maintained for 30 min and then kept at room temperature for 16 h. After adding a saturated solution of sodium bicarbonate (1 L), the aqueous layer was extracted with $CHCl_3$ (3×500 mL). The combined organic extracts were washed with water, 2N-hydrochloric acid, and brine, dried ($MgSO_4$ /activated charcoal), filtered, and evaporated. The crude material was triturated with isopropyl ether to afford colorless crystals (224 g, 88%), mp 79–81 °C. ^{1}H NMR ($CDCl_3$) δ 9.91 (s, 1H), 7.64 (d, *J* = 7 Hz, 2H), 7.43 (d, *J* = 7 Hz, 2H), 7.30–7.09 (m, 3H), 7.04 (d, *J* = 7 Hz, 2H), 4.01 (q, *J* = 7 Hz, 2H), 1.26 (t, *J* = 7 Hz, 3H); MS *m*/*z* 253 (M^+). Anal. calcd for $C_{16}H_{15}NO_2 \cdot 1.0H_2O$: C, 75.86; H, 5.97; N, 5.53. Found: C, 75.66; H, 6.09; N, 5.50.

***N*-Ethyl-4-[hydroxy-(3-methoxyphenyl)methyl]-*N*-phenylbenzamide (6).** A solution of **5** (109 g, 430.3 mmol) in anhydrous THF (350 mL) was added to a vigorously stirred mixture of 3-methoxyphenylmagnesium bromide (948 mL, 474 mmol, 0.5 M solution in THF) in anhydrous THF at 10 °C. The mixture was allowed to warm to room temperature and was stirred for 30 min. After adding saturated NH_4Cl (1 L), the aqueous layer was extracted with ethyl acetate (3×300 mL). The combined organic extracts were washed with brine, dried ($MgSO_4$ /activated charcoal), filtered, and evaporated. The crude material was crystallized from isopropyl ether to afford **6** as colorless crystals (121 g, 78%), mp 106–108 °C. ^{1}H NMR ($CDCl_3$) δ 7.40–7.21 (m, 8H), 7.14 (d, *J* = 7 Hz, 2H), 6.98–6.83 (m, 3H), 5.90 (s, 1H), 4.08 (q, *J* = 7 Hz, 2H), 4.93 (s, 3H), 1.31 (t, *J* = 7 Hz, 3H); MS *m*/*z* 360 (M^+). Anal. calcd for $C_{23}H_{25}NO_3$: C, 76.43; H, 6.41; N, 3.88. Found: C, 76.39; H, 6.43; N, 3.87.

4-[Chloro-(3-methoxyphenyl)methyl]-*N*-ethyl-*N*-phenylbenzamide (7). A solution of **6** (201 g, 557 mmol) in $CHCl_3$ (800 mL) was added dropwise to 37% hydrochloric acid (2.2 L). The biphasic mixture was allowed to stir at room temperature for 16 h. The organic layer was separated, washed with water (2×200 mL), dried ($NaSO_4$ /activated charcoal), filtered, and evaporated to afford **7** as a red oil (205 g, 97%), ^{1}H NMR ($CDCl_3$) δ 7.40–7.21 (m, 8H), 7.14 (d, *J* = 7 Hz, 2H), 6.98–6.83 (m, 3H), 5.90 (s, 1H), 4.08 (q, *J* = 7 Hz, 2H), 3.93 (s, 3H), 1.31 (t, *J* = 7 Hz, 3H); MS *m*/*z* 379 (M^+). Anal. calcd for $C_{23}H_{22}ClNO_2 \cdot 0.75H_2O$: C, 70.22; H, 6.02; N, 3.56. Found: C, 70.44; H, 5.75; N, 3.55.

4-[(2*S*,5*S*)-Dimethyl-1-piperazinyl)-(3-methoxyphenyl)-methyl]-*N*-ethyl-*N*-phenylbenzamide (8). (2*S*,5*S*)-Dimethylpiperazine[24] (5.75 g, 50.39 mmol) and $NaHCO_3$ (4.23 g, 50.39 mmol) were suspended in anhydrous CH_3CN (80 mL) under argon and refluxed for 30 min.

A solution of **7** (8.6 g, 22.68 mmol) in anhydrous CH_3CN (30 mL) was added dropwise, and the mixture was refluxed for 16 h. Hydrochloric acid (2N, 300 mL) was added, and the aqueous layer was washed with ethyl acetate (3×200 mL). The aqueous phase was treated with ammonium hydroxide until pH 9, and extracted with ethyl acetate (3×300 mL). The combined organic extracts were washed with water and brine, dried ($NaSO_4$/activated charcoal), filtered, and evaporated to afford crude **8**. It was purified by column chromatography (10% MeOH in $CHCl_3$) to give **8** as a pale yellow oil (7.504 g, 72%), ^{1}H NMR ($CDCl_3$) δ 7.18–6.99 (m, 8H), 6.94–6.81 (m, 4H), 6.61 (d, $J=7$ Hz, 1H), 4.28 (s, 1H), 3.83 (q, $J=7$ Hz, 2H), 3.68 (s, 3H), 2.90–3.07 (m, 1H), 2.88–2.53 (m, 3H), 2.39–2.25 (m, 1H), 2.18–2.08 (m, 1H), 1.97–1.80 (m, 1H), 1.60 (broad s, 1H), 1.12 (t, $J=7$ Hz, 3H), 0.87–0.72 (m, 6H); MS m/z 458 $(M+1)^+$. Anal. calcd for $C_{29}H_{37}Cl_2N_3O_2{\cdot}2.25H_2O$: C, 61.09; H, 7.32; N, 7.37. Found: C, 60.94; H, 6.95; N, 7.18.

(+)-4-[(αR)-α-(4-Allyl-(2*S*,5*S*)-dimethyl-1-piperazinyl)-(3-methoxyphenyl)methyl]-*N*-ethyl-*N*-phenylbenzamide (9a) and (+)-4-[(αS)-α-(4-Allyl-(2*S*,5*S*)-dimethyl-1-piperazinyl)-(3-methoxy- phenyl)methyl]-*N*-ethyl-*N*-phenylbenzamide (9b). A suspension of **8** (750 mg, 1.64 mmol), allyl bromide (199 mg, 1.64 mmol) and $NaHCO_3$ (276 mg, 3.28 mmol) in anhydrous DMF (10 mL) was stirred under argon for 2 h at 80 °C. After the solvent was removed, the residue was diluted with ethyl acetate (50 mL). The organic layer was washed with water (3×5 mL) and brine, dried (sodium sulfate/activated charcoal), filtered and evaporated to afford **9** as a yellow oil, a mixture of two diastereomers (777 mg, 95%). Separation of the diastereomeric mixture was accomplished by preparative HPLC (Alltech econosphere column (silica, 10 μm, 22×250 mm), UV detection at 254 nm). The mixture was dissolved in 40% ethyl acetate in hexanes (~50 mg/mL), filtered through a 0.45 μm filter, and ~50 mg (1 mL/injection) was separated at a time. The mobile phase contained 40% ethyl acetate in hexanes with a flow rate of 5 mL/min. The retention time of **9a** and **9b** was about 28 and 30 min, respectively, and ~70% separation was achieved in each injection. **9a**: dihydrochloride salt from diethyl ether, mp 192–193 °C; $[\alpha]_{20}^{D}$ +5.9° (*c* 0.44, MeOH); ^{1}H NMR (free base) ($CDCl_3$) δ 7.30–7.08 (m, 8H), 6.97 (d, $J=8$ Hz, 2H), 6.91–6.82 (m, 2H), 6.71 (d, $J=8$ Hz, 1H), 5.93–5.77 (m, 1H), 5.22–5.08 (m, 2H), 4.42 (s, 1H), 3.97 (q, $J=8$ Hz, 2H), 3.75 (s, 3H), 3.44–3.31 (m, 1H), 2.98–2.84 (m, 1H), 2.83–2.71 (m, 1H), 2.57 (dd, $J=15$ Hz, $J=4$ Hz, 1H), 2.44–2.28 (m, 1H), 2.22–2.15 (m, 1H), 1.21 (t, $J=8$ Hz, 3H), 1.00 (d, $J=8$ Hz, 3H), 0.92 (d, $J=8$ Hz, 3H). Anal. calcd for $C_{32}H_{41}Cl_2N_3O_2{\cdot}1.75H_2O$: C, 63.83; H, 7.45; N, 6.98. Found: C, 63.86; H, 7.26; N, 6.79. **9b**: dihydrochloride salt from diethyl ether, mp 159–160 °C, $[\alpha]_{20}^{D}$ +12.2° (*c* 0.36, MeOH); ^{1}H NMR (free base) ($CDCl_3$) δ 7.28–7.05 (m, 8H), 7.05–6.88 (m, 4H), 6.91–6.82 (m, 2H), 6.71 (d, $J=8$ Hz, 1H), 5.93–5.77 (m, 1H), 5.22–5.08 (m, 2H), 4.40 (s, 1H), 3.97 (q, $J=8$ Hz, 2H), 3.77 (s, 3H), 3.44–3.31 (m, 1H), 2.84–2.66 (m, 2H), 2.62–2.48 (m, 1H), 2.47–2.29 (m, 2H), 2.29–2.16 (m, 1H), 1.21 (t, $J=8$ Hz, 3H), 0.97 (d, $J=8$ Hz, 3H), 0.95 (d, $J=8$ Hz, 3H). Anal. calcd for $C_{32}H_{41}Cl_2N_3O_2{\cdot}1.75H_2O$: C, 63.83; H, 7.45; N, 6.98. Found: C, 63.91; H, 7.16; N, 6.85.

General procedure for deallylation of 9a and 9b. The allyl-substituted amine (~0.4 mmol) was suspended in glacial acetic acid (50 mg) and water (3.0 mL), and 10% palladium on charcoal (15 mg) was added. The mixture was stirred at reflux temperature for 16 h, cooled to room temperature, filtered through Celite, treated with ammonium hydroxide until pH 12 and extracted with $CHCl_3$ (3×5 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and evaporated to give the crude material. Chromatographic purification of the crude material with 10% MeOH in $CHCl_3$ afforded **8a** or **8b** as a colorless oil.

4-[(αR)-α-(2*S*,5*S*)-dimethyl-1-piperazinyl)-(3-methoxyphenyl)methyl]-*N*-ethyl-*N*-phenylbenzamide (8a). Deallylation of **9a** (200 mg, 0.402 mmol) gave **8a** (130 mg, 73%); dihydrochloride salt from methanol, mp 174–175 °C, $[\alpha]_{20}^{D}$ −7.9 (*c* 0.47, MeOH); ^{1}H NMR (free base) ($CDCl_3$) δ 7.28-7.08 (m, 8H), 6.99 (d, $J=8$ Hz, 2H), 6.97- 6.88 (m, 2H), 6.69 (d, $J=8$ Hz, 1H), 4.37 (s, 1H), 3.97 (q, $J=8$ Hz, 2H), 3.76 (s, 3H), 3.13-3.03 (m, 1H), 2.95-2.83 (m, 1H), 2.82–2.76 (m, 1H), 2.75–2.63 (m, 1H), 2.22 (dd, $J=15$ Hz, $J=4$ Hz, 1H), 2.00–1.87 (m, 1H), 1.57 (broad s, 1H), 1.22 (t, $J=8$ Hz, 3H), 0.96 (d, $J=8$ Hz, 3H), 0.94 (d, $J=8$ Hz, 3H). Anal. calcd for $C_{29}H_{37}Cl_2N_3O_2{\cdot}2.0H_2O$: C, 59.76; H, 7.40; N, 7.21. Found: C, 60.03; H, 7.06; N, 6.81.

4-[(αS)-α-(2*S*,5*S*)-Dimethyl-1-piperazinyl)-(3-methoxyphenyl)methyl]-*N*-ethyl-*N*-phenylbenzamide (8b). Deallylation of **9b** (210 mg, 0.422 mmol) gave **8b** (140 mg, 73%); dihydrochloride salt from methanol, mp 177–178 °C, $[\alpha]_{20}^{D}$ −10.0° (*c* 0.38, MeOH); ^{1}H NMR (free base) ($CDCl_3$) δ 7.28–7.08 (m, 8H), 7.05–6.87 (m, 4H), 6.69 (d, $J=8$ Hz, 1H), 4.35 (s, 1H), 3.97 (q, $J=8$ Hz, 2H), 3.78 (s, 3H), 3.13–3.03 (m, 1H), 2.88–2.78 (m, 1H), 2.77–2.62 (m, 2H), 2.21 (dd, $J=15$ Hz, $J=4$ Hz, 1H), 2.05–1.83 (m, 1H), 1.52 (broad s, 1H), 1.20 (t, $J=8$ Hz, 3H), 0.95 (d, $J=8$ Hz, 3H), 0.87 (d, $J=8$ Hz, 3H). Anal. calcd for $C_{29}H_{37}Cl_2N_3O_2{\cdot}1.25H_2O$: C, 63.08; H, 7.19; N, 7.61. Found: C, 63.00; H, 7.11; N, 7.55.

(+)-4-[(αR)-α-(4-Allyl-(2*S*,5*S*)-dimethyl-1-piperazinyl)-(3-methoxyphenyl)methyl]-benzyl alcohol (10). 1 M-$LiAlH_4$ in THF (0.957 mL, 0.957 mmol was added dropwise to a solution of **9a** (396.3 mg, 0.798 mmol) in Et_2O (10 mL) at 0 °C. After refluxing 2 h, the reaction mixture was cooled to 0 °C and quenched by addition of saturated NH_4Cl solution. The heterogeneous mixture was filtered through a pad of Celite and washed with EtOAc. The combined filtrate was washed with water and brine, dried over Na_2SO_4, filtered and concentrated to dryness. Chromatography of the crude with 40% ethyl acetate in hexanes gave **10** as a colorless oil (303.6 mg, quantitative), $[\alpha]_{20}^{D}$ +23.3° (*c* 0.45, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.44 (br-d, $J=8$ Hz, 2H), 7.24 (br-d, $J=8$ Hz, 2H), 7.15 (t, $J=8$ Hz, 1H), 7.01–6.97 (m, 2H), 6.69 (br-dd, $J=8$ and 2 Hz, 2H), 5.92–5.78 (m, 1H), 5.19–5.10 (m, 2H), 4.60 (br–s, 2H), 4.51 (br-s, 1H), 3.75

(s, 3H), 3.41 (br-dd, $J=14$ and 5 Hz, 1H), 3.03–3.00 (m, 1H), 2.80 (dd, $J=14$ and 8 Hz, 1H), 2.60 (dd, $J=11$ and 3 Hz, 1H), 2.47–2.38 (m, 3H), 2.28–2.21 (m, 1H), 1.03 (d, $J=6$ Hz, 3H), 0.95 (d, $J=6$ Hz, 3H); MS m/z 381 $(M+1)^+$; HRMS (FAB) m/z calcd for $C_{12}H_{24}N_2O_2$, 381.2543; found, 381.2541. The dihydrobromide salt was prepared and crystallized slowly from ethanol in a diethyl ether atmosphere (mp 159–160 °C) for X-ray crystallographic analysis (Fig. 1).

General procedure for syntheses of carbamates 11. A mixture of benzyl alcohol **10** (~0.12 mmol), arylisocyanate (~0.125 mmol) and a catalytic amount of dibutyl dilaurate (3 μL) in benzene (1.5 mL) was stirred at room temperature overnight. The reaction mixture was diluted with ethyl acetate (20 mL), washed with water and brine, dried over Na_2SO_4, filtered, and concentrated to dryness. Column chromatography of the crude product with 20% ethyl acetate in hexanes afforded carbamates **11** as a colorless film (89–94%).

Carbamate 11a. Reaction of benzyl alcohol **10** (44. 6 mg, 0.117 mmol) and phenylisocyanate (13 μL, 0.12 mmol) gave carbamate **11a** (52.3 mg, 89%), $[\alpha]_{20}^{D}$ +23.3° (*c* 0.49, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.47 (br-d, $J=8$ Hz, 2H), 7.38–7.26 (m, 6H), 7.18–6.96 (m, 3H), 6.71–6.64 (m, 2H), 5.93–5.79 (m, 1H), 5.20–5.15 (m, 2H), 5.13 (br-s, 2H), 4.53 (br-s, 1H), 3.76 (s, 3H), 3.42 (dd, $J=14$ and 5 Hz, 1H), 3.03–3.01 (m, 1H), 2.83 (dd, $J=14$ and 8 Hz, 1H), 2.63 (dd, $J=11$ and 3 Hz, 1H), 2.50–2.39 (m, 3H), 2.31–2.23 (m, 1H), 1.04 (d, $J=6$ Hz, 3H), 0.97 (d, $J=6$ Hz, 3H); MS m/z 500 $(M+1)^+$. Dihydrochloride salt from methanol, mp 168 °C (dec). Anal. calcd for $C_{31}H_{39}Cl_2N_3O_3{\cdot}0.5H_2O$: C, 64.02; H, 6.93; N, 7.23. Found: C, 64.10; H, 6.97; N, 7.23.

Carbamate 11b. Reaction of benzyl alcohol **10** (46.7 mg, 0.123 mmol) and *p*-bromophenylisocyanate (24.8 mg, 0.125 mmol) gave carbamate **11b** (66.8 mg, 94%), $[\alpha]_{20}^{D}$ +22.0° (*c* 0.45, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.47 (br-d, $J=8$ Hz, 2H), 7.41–7.38 (m, 2H), 7.29–7.24 (m, 3H), 7.16 (t, $J=8$ Hz, 1H), 7.00–6.96 (m, 2H), 6.72–6.68 (m, 1H), 6.62 (br–s, 1H), 5.92–5.79 (m, 1H), 5.20–5.12 (m, 4H), 4.53 (s, 1H), 3.76 (s, 3H), 3.42 (dd, $J=14$ and 5 Hz, 1H), 3.03–3.00 (m, 1H), 2.82 (dd, $J=14$ and 8 Hz, 1H), 2.2 (dd, $J=11$ and 3 Hz, 1H), 2.49–2.38 (m, 3H), 2.32–2.23 (m, 1H), 1.03 (d, $J=6$ Hz, 3H), 0.97 (d, $J=6$ Hz, 3H); MS m/z 578 (M^+). Dihydrochloride salt from methanol, mp 165 °C (dec). Anal. calcd for $C_{31}H_{38}BrCl_2N_3O_3$: C, 57.15; H, 5.88; N, 6.45. Found: C, 56.83; H, 6.08; N, 6.14.

Carbamate 11c. Reaction of benzyl alcohol **10** (47.9 mg, 0.126 mmol) and *p*-methoxyphenylisocyanate (16.6 μL, 0.128 mmol) gave carbamate **11c** (60.1 mg, 91%), $[\alpha]_{20}^{D}$ +21.7° (*c* 0.30, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.46 (br–d, $J=9$ Hz, 2H), 7.29–7.25 (m, 3H), 7.16 (t, $J=8$ Hz, 1H), 7.00–6.96 (m, 2H), 6.86–6.82 (m, 2H), 6.71–6.68 (m, 1H), 6.51 (br-s, 1H), 5.92–5.79 (m, 1H), 5.20–5.11 (m, 4H), 4.53 (s, 1H), 3.77 (s, 3H), 3.76 (s, 3H), 3.42 (br-dd, $J=14$ and 5 Hz, 1H), 3.03–3.01 (m, 1H), 2.82 (dd, $J=14$ and 8 Hz, 1H), 2.62 (dd, $J=11$ and 3 Hz, 1H), 2.49–2.39 (m, 3H), 2.30–2.23 (m, 1H), 1.03 (d, $J=6$ Hz, 3 Hz), 0.97 (d, $J=6$ Hz, 3H); MS m/z 530 $(M+1)^+$. Dihydrochloride salt from methanol, mp 160–161 °C (dec). Anal. calcd for $C_{32}H_{41}Cl_2N_3O_4{\cdot}1.0H_2O$: C, 61.73; H, 7.29; N, 6.75. Found: C, 61.96; H, 6.89; N, 6.44.

(+)-4-[(αR)-α-(2S,5S)-dimethyl-1-piperazinyl)-(3-methoxyphenyl)methyl]-*N*-ethyl-*N*-phenylbenzylamine (12). To a mixture of **8a** (88 mg, 0.193 mmol) in THF (2 mL) was added dropwise 1.0 M-$LiAlH_4$ in THF (0.23 mL, 0.23 mmol) at 0 °C, and the mixture was refluxed for 2 h. The reaction was quenched by addition of saturated. NH_4Cl solution, filtered through a pad of Celite and washed with EtOAc. The combined filtrate was washed with water and brine, dried over Na_2SO_4, filtered, and concentrated to dryness. Column chromatography of the crude with 10% MeOH in $CHCl_3$ provided **8** (61.0 mg, 72%) as a colorless oil, $[\alpha]_{20}^{D}$ +2.2° (*c* 0.55, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.36 (br-d, $J=8$ Hz, 2H), 7.19–7.10 (m, 5H), 7.03–6.99 (m, 2H), 6.72–6.62 (m, 4H), 4.43 (br-s, 3H), 3.77 (s, 3H), 3.42 (q, $J=7$ Hz, 2H), 3.15 (dd, $J=12$ and 3 Hz, 1H), 3.00–2.86 (m, 2H), 2.73 (dd, $J=12$ and 2 Hz, 1H), 2.43 (dd, $J=12$ and 3 Hz, 1H), 2.03 (t, $J=11$ Hz, 1H), 1.17 (t, $J=7$ Hz, 3H), 0.99 (d, $J=6$ Hz, 3H), 0.98 (d, $J=6$ Hz, 3H); MS m/z 444 $(M+1)^+$. Trihydrochloride salt from diethyl ether, mp 173 °C (dec). Anal. calcd for $C_{29}H_{40}Cl_3N_3O{\cdot}2.25H_2O$: C, 58.68; H, 7.56; N, 7.08. Found: C, 58.76; H, 7.37; N, 6.72.

(+)-4-[(αR)-α-(4-Allyl-(2S,5S)-dimethylpiperazin-1-yl)-(3-methoxyphenyl)methyl]-*N*-ethyl-*N*-phenylbenzylamine (13). Allylation was performed according to the procedure described in conversion of **8** to **9**. Reaction of **12** (32.3 mg, 0.073 mmol) and allyl bromide (6.6 μL, 0.077 mmol) gave **13** (34.1 mg, 97%) as a colorless oil, $[\alpha]_{20}^{D}$ +24.2° (*c* 0.99, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.37 (br–d, $J=8$ Hz, 2H), 7.19–7.10 (m, 5H), 7.00–6.97 (m, 2H), 6.72–6.20 (m, 4H), 5.92–5.78 (m, 1H), 5.19–5.10 (m, 2H), 3.76 (m, 3H), 3.46–3.37 (m, 3H), 3.00–2.98 (m, 1H), 2.79 (br-dd, $J=14$ and 8 Hz, 1H), 2.59 (dd, $J=11$ and 3 Hz, 1H), 2.46–2.36 (m, 3H), 2.27–2.20 (m, 1H), 1.17 (t, $J=7$ Hz, 3H), 1.02 (d, $J=6$ Hz, 3H), 0.96 (d, $J=6$ Hz, 3H); MS m/z 484 $(M+1)^+$. Trihydrochloride salt from diethyl ether, mp 187 °C (dec). Anal. calcd for $C_{32}H_{44}Cl_3N_3O{\cdot}1.50H_2O$: C, 61.98; H, 7.64; N, 6.78. Found: C, 61.98; H, 7.73; N, 6.52.

(+)-4-[(αR)-α-(4-Allyl-(2S,5S)-dimethylpiperazin-1-yl)-(3-hydroxyphenyl)methyl]-*N*-ethyl-*N*-phenylbenzamide (14). To a solution of **9a** (179 mg, 0.3596 mmol) in 1,2-dichloroethane (4.5 mL) was added dropwise 1 M-$BBr_3{\cdot}Me_2S$ in CH_2Cl_2 (1.26 mL, 1.26 mmol) at 0 °C and the mixture was heated at 60 °C for 3.5 h. The reaction mixture was poured into ice-cold ammonia solution and extracted with 10% MeOH in ethyl acetate (2×). The combined organic layer was washed with water and brine, dried over Na_2SO_4, filtered and concentrated to dryness. Column chromatography of the crude with 40% ethyl acetate in hexanes provided **14** (91.2 mg, 52%) as a colorless oil, $[\alpha]_{20}^{D}$ +16.8° (*c* 0.41, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.23–6.56 (m, 8H), 5.88–5.75 (m, 1H), 5.18–5.09 (m, 2H), 4.46 (s, 1H), 3.96 (q, $J=7$ Hz, 2H), 3.33 (br-dd, $J=14$ and 5 Hz, 1H), 2.90–2.77 (m, 2H),

2.55 (dd, $J=11$ and 4 Hz, 1H), 2.43–2.34 (m, 2H), 2.20–2.11 (m, 2H), 1.20 (t, $J=7$ Hz, 3H), 0.99 (d, $J=6$ Hz, 3H), 0.93 (d, $J=6$ Hz, 3H); MS m/z 484 $(M+1)^+$. Dihydrochloride salt from methanol, mp 173–174 °C. Anal. calcd for $C_{31}H_{39}Cl_2N_3O_2\cdot 0.5H_2O$: C, 65.83; H, 7.13; N, 7.43. Found: C, 65.95; H, 7.08; N, 7.44.

(−) - 4 - [(αR) - α - (2S,5S) - dimethylpiperazin - 1 - yl) - (3-hydroxyphenyl) - methyl] - N - ethyl - N - phenylbenzamide (15). A mixture of **14** (52.0 mg, 0.1075 mmol), glacial acetic acid (three drops) and water (2.0 mL) and 10% palladium on charcoal (10 mg) was refluxed for 14 h. The reaction was filtered through Celite, washed with 10% MeOH in ethyl acetate and treated with ammonium hydroxide until pH 12. The organic extracts were dried over Na_2SO_4, filtered and evaporated to give the crude material. Chromatographic purification of the crude material with 10% MeOH in $CHCl_3$ afforded **15** as a colorless oil, $[\alpha]_{20}^{D}$ −2.8° (c 0.88, MeOH); ^{1}H NMR (CD_3OD) δ 7.32–6.60 (m, 8H), 4.54 (s, 1H), 3.94 (q, $J=7$ Hz, 2H), 3.32–3.27 (m, 3H), 3.12 (br-d, $J=11$ Hz, 1H), 2.56–2.34 (m, 2H), 1.20–1.15 (m, 6H), 1.08 (d, $J=7$ Hz, 3H); MS m/z 444 $(M+1)^+$. Dihydrochloride salt from methanol, mp 176–177 °C. Anal. calcd for $C_{28}H_{35}Cl_2N_3O_2\cdot 1.5H_2O$: C, 61.87; H, 7.05; N, 7.73. Found: C, 61.77; H, 6.96; N, 7.50.

Single-crystal X-ray diffraction analysis of (+)-4-[(αR)-α-(4-allyl-(2S,5S)-dimethyl-1-piperazinyl)-(3-methoxyphenyl)methyl]-benzyl alcohol dihydrobromide (10·2HBr). C_{24} H_{34} N_2^{2+} O_2 $2(Br^-)$ F.W. = 542.35, monoclinic space group $P2_1$, $a=7.444(1)$, $b=9.046(1)$, $c=21.471(1)$ Å, $\beta=98.17(1)°$. $V=1431.1(3)$ Å^3, $Z=2$, $\rho_{calc}=1.259$ mg mm^{-3}, λ (Cu K_α) = 1.54178 Å, $\mu=3.736$ mm^{-1}, $F(000)=556$, $T=293$ K.

A clear colorless 0.27×0.12×0.02 mm crystal was used for data collection with a Bruker SMART 6K CCD detector on a Platform goniometer. The Rigaku rotating Cu anode source was equipped with incident beam Gobel mirrors. Lattice parameters were determined using SAINT[25] from 4214 reflections within $5.31<\theta<61.37°$. Data were collected to $2\theta=133.9°$. A set of 7113 reflections was collected in the ω scan mode. There were 3882 unique reflections. Corrections were applied for Lorentz, polarization, and absorption effects. The structure was solved with SHELXTL[26] and refined with the aid of the SHELX97[26] system of programs. The full-matrix least-squares refinement on F^2 used 3 restraints and varied 230 parameters including atom coordinates and anisotropic thermal parameters. H atoms were included using a riding model [coordinate shifts of C applied to attached H atoms, C-H distances set to 0.96–0.93 Å, H angles idealized, U_{iso}(H) were set to 1.2° to 1.5 U_{eq}(C). Final residuals were $R1=0.099$ for the 3132 observed data with $F_o>4\sigma(F_o)$ and 0.109 for all data. Final difference Fourier excursions of 1.82 and −0.74 eÅ^{-3}. A definitive assignment was made for the third center of asymmetry in Fig. 1 assuming the 2S,5S absolute configuration of the methyl groups on the piperazine molecule. This absolute configuration of the (2S,5S)-2,5-dimethylpiperazine was assured by its synthesis from a peptide of established configuration. Crystallographic data (excluding structure factors) have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no CCDC 21160. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-1223-336033 or email: deposit@ccdc.cam.ac.uk).

Opioid binding assays

Opioid binding assays proceeded according to published procedures.[27,28] Mu receptors were labeled with [^{3}H]DAMGO (D-Ala^2,MePhe^{4}Gly-ol^5)enkephalin, 2 nM). Rat membranes for mu and delta receptor binding assays were prepared each day using a partially thawed frozen rat brain that was homogenized with a polytron in 10 mL/brain of ice-cold 10 mM Tris–HCl, pH 7.0. Membranes were then centrifuged twice at 30,000g for 10 min and resuspended with ice-cold buffer following each centrifugation. After the second centrifugation, the membranes were resuspended in 50 mM Tris–HCl, pH 7.4 (60 mL/brain), at 25 °C. Incubations proceeded for 2 h at 25 °C in 50 mM Tris–HCl, pH 7.4, along with a protease inhibitor cocktail (PIC). The nonspecific binding was determined using 20 μM of levallorphan. Delta binding sites were labeled using [^{3}H]DADLE (D-Ala^2, D-Leu^5)enkephalin, 2 nM) and rat brain membranes. Rat membranes were prepared each day as described above. After the second centrifugation, the membranes were resuspended in 50 mM Tris–HCl, pH 7.4 (60 mL/brain), at 25° C. Incubations proceeded for 2 h at 25 °C in 50 mM Tris–HCl, pH 7.4, containing 100 mM choline chloride, 3 mM MnCl2, 100 nM DAMGO to block binding to mu sites, and PIC. Nonspecific binding was determined using 20 μM levallorphan. Kappa binding sites were labeled using [^{3}H]U69,593 (*trans*-3,4-dichloro-*N*-methyl[2-(1-pyrrolidinyl)cyclohexyl]benzene acetamide, 2 nM). Guinea pig brain membranes were prepared each day using partially thawed guinea pig brain that was homogenized with a polytron in 15 mL/brain of ice-cold 10 mM Tris–HCl, pH 7.0. The membranes were then centrifuged twice at 30,000g for 10 min and resuspended with ice-cold buffer following each centrifugation. After the second centrifugation, the membranes were resuspended in 50 mM Tris–HCl, pH 7.4 (85 mL/brain), at 25° C. Incubations proceeded for 2 h at 25 °C in 50 mM Tris–HCl, pH 7.4, containing 1 μg/mL of captopril and PIC. Nonspecific binding was determined using 1 μM U69,593. Each radioligand was displaced by 8-10 concentrations of test drug. Compounds were prepared as 1 mM solution with 10 mM Tris buffer (pH 7.4) containing 10% DMSO before drug dilution. All drug dilutions were done in 10 mM Tris–HCl, pH 7.4, containing 1 mg/mL bovine serum albumin. All washes were done with ice-cold 10 mM Tris–HCl, pH 7.4. The IC_{50} and slope factor (N) were obtained by using the program MLAB-PC (Civilized Software, Bethesda, MD). K_i values were calculated according to the equation $K_i=IC_{50}/(1+[L]/K_d)$.

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