A Marked Change of Receptor Affinity of the 2-Methyl-5-(3-hydroxyphenyl)morphans upon Attachment of an (*E*)-8-Benzylidene Moiety: Synthesis and Evaluation of a New Class of σ Receptor Ligands

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The (*E*)-8-benzylidene and (*E*)-8-(3,4-dichlorobenzylidene), 7-ketone derivatives, **5** and **6**, of the synthetic opiate 2-methyl-5-(3-hydroxyphenyl)morphan [5-(3-hydroxyphenyl)-2-methyl-2-azabicyclo[3.3.1]nonane, **1**], were synthesized from the 7-ketone derivatives **2** or **4** via the Claisen–Schmidt reaction. The corresponding enantiomers of **5** and **6** were obtained in >99% optical purity from the optical isomers of **4**, resolved with the *O,O'*-dibenzoyltartaric acids. The absolute configurations of the enantiomers of **4** were determined by conversion, via Clemmensen reduction, to the enantiomers of **1**, the configurations of which are known. The determination of the regioisomer and configurational isomer of **5**, with respect to the introduced benzylidene group, was determined from a single-crystal X-ray analysis. ^{1}H NMR data was used to confirm that **6** possessed the same configuration as **5**. Radioreceptor binding studies in rat and guinea pig brain preparations revealed that (−)-(1*S*,5*S*)-**5** displayed an 11-fold decrease in affinity for the opioid μ receptor and an increase in affinity for σ receptors of 81-fold (low nanomolar affinity) relative to the ketone precursor (+)-(1*S*,5*S*)-**4**. An analogous, albeit less dramatic, trend was seen with compound (−)-(1*S*,5*S*)-**6**. Compounds (−)-(1*S*,5*S*)-**5** and (−)-(1*S*,5*S*)-**6** are distinct from the typical σ-opiates in that they have very low affinity for either PCP sites or muscarinic receptors. The high affinity and selectivity of these novel σ receptor ligands suggests that they will be valuable for the elucidation of the functional roles of σ receptors.

The 2-methyl-5-(3-hydroxyphenyl)morphans are an interesting class of synthetic analgesics that bind to opioid receptors and were developed by May et al.[1] These compounds differ from the classical opioid compounds in that the phenolic moiety occupies an equatorial position of the piperidine ring containing the necessary basic amino group (see Figure 1). Interestingly, both optical isomers of the parent 2-methyl-5-(3-hydroxyphenyl)morphans are active analgesics. Whereas (+)-(1*S*,5*R*)-**1** was found to be an agonist with 4 times the potency of morphine, (−)-(1*R*,5*S*)-**1** was also shown to have the agonist potency of morphine with moderate antagonist properties.[2] *In vitro* binding assays reveal that both the (+) and the (−) isomer have a high affinity for the μ opioid receptor, slight affinity for the κ opioid receptor and virtually no affinity for the δ opioid receptor.[3] As part of a project to alter the selectivity of the μ opioid binding 5-(3-hydroxyphenyl)morphans for other receptors, we have found that the incorporation of a benzylidene moiety at the C-8 position significantly decreases the affinity at the μ receptor and greatly enhances the affinity for σ receptors. A diverse group of compounds are known to bind to σ receptors, including (+)-benzomorphans[4] and (+)-morphinans (unnatural opioid isomers),[5] the *cis* isomers of U50,488 and compounds developed from structure–activity relationship (SAR) studies of this compound,[6] disubstituted guanidines,[7] 3-phenylpiperidines (dopaminergics), certain neuroleptics such as phenothiazines and tricyclic antidepressants,[5] monoamine oxidase inhibitors,[8] and steroids such as progesterone.[9] The distribution of σ receptors is diverse: they are found in the brain, especially in motor areas,[10] and also in the periphery in the liver,[11] spleen,[9] vas deferens,[12] gastrointestinal tract,[13] the pineal gland,[14] the testis, ovary, adrenal gland, pituitary gland,[15] and renal tissue.[16] Therefore, it is not surprising that σ receptors have been implicated in the involvement in a wide array of physiological processes.[17] However, the study of these receptors and their heterogeneity[18] has been hampered by the lack of selective σ ligands, and so the development of selective ligands for these receptors is important. The possible role of σ receptors in neuroprotection,[19] cytotoxicity,[20] and motor disorders[21] also warrants their continued study. In an effort to discover highly selective σ ligands,

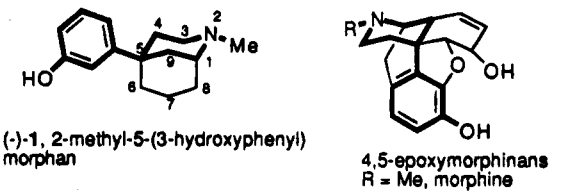

Figure 1. Orientation of phenolic moiety relative to piperidine ring in classical 4,5-epoxymorphinans (axial) as compared to the 2-methyl-5-phenylmorphans (equatorial).

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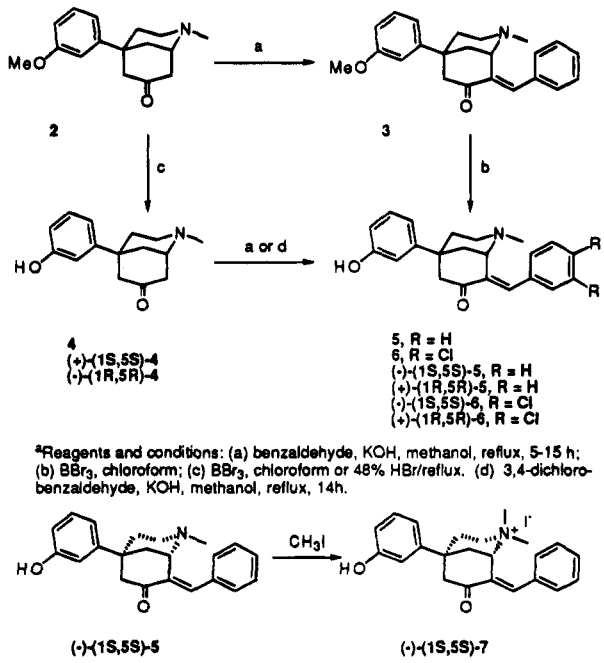
Scheme 1



[a]Reagents and conditions: (a) benzaldehyde, KOH, methanol, reflux, 5-15 h; (b) BBr_3, chloroform; (c) BBr_3, chloroform or 48% HBr/reflux. (d) 3,4-dichlorobenzaldehyde, KOH, methanol, reflux, 14h.

our modifications of the 2-methyl-5-(3-hydroxyphenyl)-morphans has introduced a new class of σ receptor binding compounds to the diverse group presently known.

Methods

Chemical Synthesis. The racemic compound **2** was prepared by following a reported literature procedure.[22] The benzylidene moiety of racemic **3** was incorporated using Claisen–Schmidt conditions (85%). Under the reaction conditions used, one predominant isomer was formed [(*E*)-8] out of four possible isomers. Compound **3** was demethylated using BBr_3, forming racemic **5.** Compound **6** was prepared by a Claisen–Schmidt reaction of 3,4-dichlorobenzaldehyde directly with the phenolic **4** prepared by demethylation of **2** with either BBr_3 or 48% HBr at reflux. The optical isomers of **5** and **6** were prepared from the resolved isomers of **4**, (+)-(1*S*,5*S*)-**4** and (−)-(1*R*,5*R*)-**4** using Claisen–Schmidt conditions and in all cases the ^{1}H NMRs were superimposable with those of the racemic compounds. The quaternary methylammonium iodide salt of (−)-(1*S*,5*S*)-**5**, compound (−)-(1*S*,5*S*)-**7**, was prepared by reaction with methyl iodide in refluxing acetone.

Resolution, Determination of Optical Purity, and Absolute Configuration. Racemic **4** was converted to its dibenzoyl tartrate salts and these were resolved through fractional crystallization from DMF/methanol. The optical purity of each isomer was shown to be >99% by HPLC on a Daicel Chiralcel OD column (1.0 mL/min, hexanes/2-propanol/0.1% $HNEt_2$) observing at 254 nm. The approximate retention times for (+)-(1*S*,5*S*)-**4** and (−)-(1*R*,5*R*)-**4** were 9.2 and 7.1 min, respectively. The absolute configurations of (+)-(1*S*,5*S*)-**4** and (−)-(1*R*,5*R*)-**4** were revealed by converting them via Clemmensen reduction into the parent compounds (−)-(1*R*,5*S*)-**1** and (+)-(1*S*,5*R*)-**1**, respectively. The absolute configuration of (−)-(1*R*,5*S*)-**1** has been previously determined.[23]

X-ray Crystallography of 5. The six-membered heterocyclic ring of **5** has a normal chair conformation with the methyl group equatorial to the ring. The six-membered ring fused to the heterocyclic ring has a half-chair conformation. The aromatic rings themselves are almost parallel to one another (angle between the two ring planes is 32.8°). The distance between the center of the benzylidene phenyl ring and N2 is 4.81 Å, while the distance between the center of the phenolic ring and N2 is 5.71 Å. The Br ion forms hydrogen bonds to two symmetrically related molecules of **5** (N—H···Br, where N—H = 0.91 Å, H···Br = 2.40, N···Br = 3.22 Å, and N—H···Br = 150.0°, and O—H···Br where O—H = 0.82 Å, H···Br = 2.44, O···Br = 3.23 Å, and O—H···Br = 162.6°).

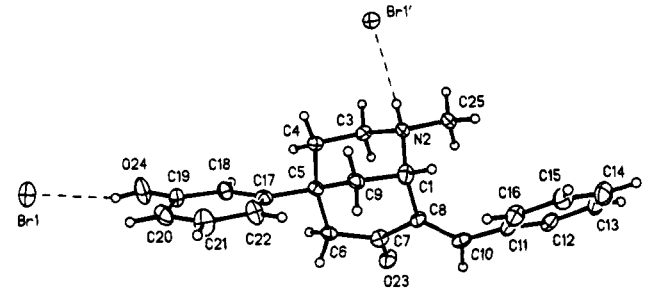


Figure 2. Results of the X-ray study on **5**. The figure is drawn using the experimentally determined coordinates with the thermal ellipsoids at the 20% probability level. Br1', a symmetry mate of Br1, has been included to illustrate the second hydrogen bond.

Results and Discussion

The reactions producing the benzylidene compounds were straightforward and consistently gave predominantly the (*E*)-8 isomers. This isomer configuration was determined by single-crystal X-ray diffraction analysis of the hydrobromide salt of racemic **5** (ORTEP is shown in Figure 2). Presumably enolate production is favored at the C-8 position as opposed to the C-6 position, and steric factors influence the geometry of the double bond as water is eliminated.[24] The configuration of **6** was determined to be the same as that of racemic **5** based on the similarities of their ^{1}H NMR spectra. The 1-H protons displayed marked downfield shifts relative to the 1-H of **4** due to the benzylidene groups at C-8. The methyls on N-2 were shielded by the introduced aromatic ring. The 6-H protons appeared at almost identical chemical shifts: 2.96 (dd, 1H, J = 3.1, 18.0 Hz, 6-H_{eq}) and 2.60 (dd, 1H, J = 1.5, 18.0 Hz, 6-H_{ax}) for **6** and 2.95 (dd, 1H, J = 3.2, 17.8 Hz, 6-H_{eq}) and 2.61 (dd, 1H, J = 1.3, 17.9 Hz, 6-H_{ax}) for **5**. The 6-H protons could be assigned as axial or equatorial based on the following: (1) axial protons usually resonate at a higher field than equatorial protons by about 0.5 ppm and (2) long-range w coupling in chair 6-membered rings is usually between 1,3-diequatorial protons.[25] Finally, the benzylidene protons appeared within 0.2 ppm of each other at approximately 7.6–7.8 ppm.

Binding assays for opioid receptors (μ, δ, and κ_1), the σ receptor, the PCP site, and muscarinic receptors were performed. Classical σ-opiates typically bind to PCP sites,[26] and it was for this reason that these binding affinities were determined. None of the compounds showed any appreciable affinity for PCP sites with most having K_i's >100 μM (see Table 1). This difference between the binding properties of these 2-methyl-5-(3-hydroxyphenyl)morphan derivatives and the more common σ-opiates such as (+)-SKF 10,047 might be a reflection of their phenyl-equatorial configuration. In an analogous fashion, this difference in configuration

Table 1. Inhibition of Radioligand Binding to Guinea Pig Brain σ Receptors and Rat Brain PCP Sites and Muscarinic Receptors

compd	K_i (nM ± SD)		
	[^{3}H]-(+)-pentazocine (σ)[39]	[^{3}H]TCP (PCP)[39]	[^{3}H]NMS (muscarinic)[40]
2	5.2 (±0.2) × 10^3	>10^5	23.3 (±1.0) × 10^3
3	137.7 ± 9.6	>5 × 10^4	11.6 (±0.9) × 10^3
4	1.4 (±0.1) × 10^3	>10^5	46.4 (±16.2) × 10^3
(+)-(1*S*,5*S*)-**4**	762.3 ± 81.6	>10^5	39.1 (±2.8) × 10^3
(−)-(1*R*,5*R*)-**4**	31.8 (±1.8) × 10^3	>10^5	25.3 (±3.2) × 10^3
5	23.3 ± 4.0	>10^5	14.4 (±0.6) × 10^3
(−)-(1*S*,5*S*)-**5**	9.4 ± 1.3	>10^5	7.4 (±0.3) × 10^3
(+)-(1*R*,5*R*)-**5**	1.3 (±0.1) × 10^3	~10^5	26.0 (±12.4) × 10^3
6	65.3 ± 5.0	>10^5	no inhibition
(−)-(1*S*,5*S*)-**6**	32.0 ± 2.9	>10^5	50.3 (±14.3) × 10^3
(+)-(1*R*,5*R*)-**6**	3.2 (±0.2) × 10^3	>10^5	18.6 (±2.3) × 10^3
(−)-(1*S*,5*S*)-**7**	5.4 (±1.7) × 10^3	no inhibition	14.7 (±6.3) × 10^3

Table 2. Inhibition of Radioligand Binding to Rat Brain μ and δ Receptors and Guinea Pig Brain κ_1 Receptors

compd	K_i (nM ± SD)			
	[^{3}H]DAMGO (μ)[41]	[^{3}H]DADLE (δ)[42]	[^{3}H]U69,593 (κ_1)[43]	σ/μ (K_i/K_i)
4[a]	5.5 ± 0.5	320 ± 26	1013 ± 86	2.5 × 10^2
(+)-(1*S*,5*S*)-**4**	35.5 ± 5.2	504.9 ± 113.3	2536 ± 370	21.5
(−)-(1*R*,5*R*)-**4**	8.7 ± 0.9	121.5 ± 28.7	491 ± 47	3.7 × 10^3
5[a]	33.9 ± 3.5	>500	576 ± 31	0.687
(−)-(1*S*,5*S*)-**5**	392 ± 48	1989 ± 283	889 ± 97	0.024
(+)-(1*R*,5*R*)-**5**	37.6 ± 2.2	734.7 ± 108.9	1091 ± 102	35
6	6.1 ± 1.6	240.4 ± 44.9	>500	11
(−)-(1*S*,5*S*)-**6**	45.1 ± 4.3	>500	>500	0.710
(+)-(1*R*,5*R*)-**6**	4.5 ± 1.3	271 ± 35	>500	7.2 × 10^2
(−)-(1*S*,5*S*)-**7**	>700	>1000	>5000	<7.7

[a] *O*-Methyl derivatives had K_i values (μ, δ, and κ_1) > 500 nM.

is thought to be responsible for the inability of common "antagonist" *N*-alkyl substituents from the 4,5-epoxymorphinan, morphinan, and benzomorphans to impart any antagonist properties to the 5-phenylmorphans through opioid receptors.[27] Opioid receptor binding assays revealed that the parent ketone **4** had the most affinity for μ receptors (5.5 nM) and slight affinity for the δ receptor (320 nM) (see Table 2). The affinity of **4** for the other receptors was relatively weak (>1 μM). The resolution of **4** into its optical isomers and their conversion to (+)-(1*S*,5*R*)- and (−)-(1*R*,5*S*)-**1** of known absolute configuration revealed that it was (−)-(1*R*,5*R*)-**4** that had most of the affinity for the μ opioid receptor (8.7 nM) whereas (+)-(1*S*,5*S*)-**4** showed a slight affinity for the σ receptor at 762 nM. However, upon the attachment of the (*E*)-8-benzylidene group to racemic **4** giving racemic **5**, the affinity for μ receptors decreased by 6-fold whereas the affinity for the σ site increased by 62-fold, resulting in a large change in σ/μ (K_i/K_i) from 2.5 × 10^2 for **4** to 0.687 for **5**. The attachment of the (*E*)-8-benzylidene group to the (+)-(1*S*,5*S*)-**4** isomer producing (−)-(1*S*,5*S*)-**5** decreased the affinity at μ receptors by 11-fold while increasing the affinity for σ receptors by 81-fold, thereby dramatically decreasing the σ/μ (K_i/K_i) from 21.5 for (+)-(1*S*,5*S*)-**4** to 0.024 for (−)-(1*S*,5*S*)-**5**. Thus, compound (−)-(1*S*,5*S*)-**5** had >40-fold selectivity for σ receptors (9.4 nM) over μ receptors. It appears that the σ receptor is about 7 times more sensitive to the (*E*)-8-benzylidene substitution than is the μ opioid receptor. Compound (−)-(1*S*,5*S*)-**5** has an affinity for the σ receptor that is about 140-fold greater than its enantiomer (+)-(1*R*,5*R*)-**5**, indicating an enantioselective interaction. This is quite different from the enantioselectivity of the σ receptor for the benzomorphan isomer pairs, which is typically about 20-fold.[4] On the other hand, the enantioselectivity of the μ receptor for the isomers of **5** is reversed and differs by only 10-fold. The high affinity of the *cis* isomers of U50,488, and compounds developed from SAR studies of this compound,[28] for the σ receptor prompted our incorporation of a 3,4-dichlorobenzylidene moiety under analogous conditions, producing (±)-**6**, (−)-(1*S*,5*S*)-**6**, and (+)-(1*R*,5*R*)-**6**. Interestingly, the addition of the (*E*)-8-(3,4-dichlorobenzylidene) group to the parent ketone (+)-(1*S*,5*S*)-**4** yielding compound (−)-(1*S*,5*S*)-**6** had virtually no effect on the affinities for the opioid receptors, but did enhance the affinity for σ receptors by 24-fold, resulting in a σ/μ (K_i/K_i) of 0.710 for (−)-(1*S*,5*S*)-**6** as opposed to a σ/μ (K_i/K_i) of 21.5 for (+)-(1*S*,5*S*)-**4**. Therefore, the (*E*)-8-(3,4-dichlorobenzylidene) substitution resulted in a decrease in binding affinity for the σ receptor of about 3-fold for racemic **6** as well as its optical isomers relative to **5** and its optical isomers, respectively, and was unsuccessful in decreasing the affinity for the opioid receptors. The superior selectivity of the (*E*)-8-benzylidene derivative (−)-(1*S*,5*S*)-**5** for σ receptors relative to the (*E*)-8-(3,4-dichlorobenzylidene) derivative (−)-(1*S*,5*S*)-**6** is evident.

Other studies have shown that σ receptor activation decreases the maximal stimulation of phosphoinositide turnover produced by cholinergic agonists, possibly by internalization of cholinergic receptors.[29] Involvement of direct antagonist activity at the muscarinic receptor must be ruled out.[30] Therefore, σ ligands required for these studies must not bind to cholinergic receptors, and it was for this reason that the binding affinities at muscarinic receptors were determined for these (*E*)-8-benzylidene-substituted 2-methyl-5-(3-hydroxyphenyl)-morphans. These assays revealed that none of the compounds tested had any appreciable affinity for muscarinic receptors: the most potent σ ligands, (−)-(1*S*,5*S*)-**5** and (−)-(1*S*,5*S*)-**6** with [^{3}H]-(+)-pentazocine displacement binding affinities of 9.4 and 32.0 nM, respectively, had displacement binding affinities for [^{3}H]-*N*-methylscopolamine at muscarinic receptors of 7.4 × 10^3 and 50.3 × 10^3 nM, respectively. Thus, these

compounds are potential ligands for studying the relationship between σ and cholinergic receptors.

The structural diversity of the many σ ligands has probably hampered many attempts to define the pharmacophore. Largent et al.[5] defines the pharmacophore as containing a 3- or 4-phenylpiperidine as well as lipophilic *N*-alkyl substituents. They also indicate the lack of enantiospecificity required by the receptor, although good enantioselectivity with many enantiomeric pairs is noted when conditions which selectively determine interaction with σ receptor subtypes are used.[4,16b,18b,h] Other studies have shown that a 1-phenyl-2-aminopropane is the simplest pharmacophore required for σ receptor binding, provided that the amine is not primary. Furthermore, lipophilic phenylalkyl substituents on the amine nitrogen can greatly enhance the binding affinity.[31] Various σ receptor models have been presented.[32] Considering that all of these models hold that the basic nitrogen usually contains a lipophilic substituent, it is possible that the (*E*)-8-benzylidene moiety of (−)-(1*S*,5*S*)-**5** is acting as an equivalent of this lipophilic moiety, even though it is not directly connected to the nitrogen. The two most recent σ_1 receptor models[32e,f] are quite similar in their proposals. Gilligan et al.[32e] propose that optimal σ_1 ligands will contain a distal hydrophobic group and a proximal hydrophobic group with distances of 4−8 and 2−4 Å from the basic nitrogen center, respectively. The model also includes a hydrogen bonding center 2−4 Å from the basic nitrogen. The second σ_1 model, proposed by Glennon et al.,[32f] equates a primary hydrophobic site B with the distal hydrophobic group proposed by Gilligan et al.,[32e] and a secondary binding site A (tolerates bulk) comparable to the proximal hydrophobic group, with distances from the basic nitrogen ranging from 6−10 and 2.5−3.9 Å, respectively. The X-ray crystallographic data for compound **5** reveals that the distance between N2 and the centroid of the benzylidene phenyl ring is 4.81 Å, while that from N2 to the centroid of the phenolic ring is 5.71 Å. Unlike the compounds used to define these models, **5** is a rigid bicylic structure, and the distances between the phenyl groups and the amine nitrogen cannot vary significantly. The σ_1 receptor models[32e,f] were derived from the binding data for flexible molecules, and this limits the certainty with which the spatial relationships between the binding sites can be determined. It seems likely that the benzylidene and the phenol of (−)-(1*S*,5*S*)-**5** constitute the two necessary hydrophobic groups, and considering these distances, the benzylidene moiety might interact with the secondary binding site A of the Glennon et al. model, which is tolerant of bulk, while the phenolic group interacts with primary hydrophobic site B.

Su et al. point out that the various σ receptor models may need further refining to explain how several of their σ ligands still maintained relatively high affinity for the σ receptor even after quaternization of the amine nitrogen.[32d] However, Glennon et al. contend that quaternary amines do not readily bind to σ_1 receptors.[32f] Others support the contention that the basic nitrogen is in its unprotonated form upon receptor binding and that in fact it is possible to have compounds without basic nitrogens, such as progesterone, that bind to the sigma receptor.[32b] In fact, the model reported by Su et al. indicates that the carbonyl of progesterone is acting in place of the amine lone pair.[32d] It was of interest to us to determine if the nitrogen lone pair was in fact necessary for the binding of the (*E*)-8-benzylidene or if perhaps it was the ketone carbonyl that might be important for σ receptor affinity. Quaternization of the basic amine group of the potent σ ligand (−)-(1*S*,5*R*)-**5** producing (−)-(1*S*,5*S*)-**7** decreased σ receptor binding capability almost 600-fold with a K_i for displacement of [^{3}H]-(+)-pentazocine of 5.4 μM. This highlights the importance of the nonquaternary amine for σ receptor affinity for this class of ligands.

Conclusion

By the incorporation of an (*E*)-8-benzylidene group onto the (+)-2-methyl-5-(3-hydroxyphenyl)morphan, opioid binding affinities were decreased and the binding affinities for σ receptors greatly enhanced. These compounds represent a new class of potent σ ligands with characteristics quite distinct from other σ-opiates such as the benzomorphans: (1) the nitrogen does not contain a lipophilic group as is required for the benzomorphans, (2) the enantioselectivity of the σ receptors for this new class of compounds is much higher than for the benzomorphans, and (3) these compounds have very low affinity for the PCP site, unlike the benzomorphans which can bind at this site. The compounds display no significant affinity for muscarinic receptors (>7 μM) and therefore have potential as ligands for the study of the relationship between σ receptors and cholinergic receptors. Thus, these compounds constitute a new class of selective ligands for the σ receptor with high binding affinity.

Experimental Section

General Instrumentation and Methods. ^{1}H NMR spectra were recorded for the free bases of all compounds in $CDCl_3$ (unless otherwise noted) on a Varian Gemini-300 spectrometer, and the data are reported in the following format: chemical shift (all relative to Me_4Si), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet, ap = apparent), integration, coupling constants, and exchangeability after D_2O addition. Electron impact (EI) mass spectra were recorded on a VG-Micromass 7070F spectrometer, and chemical ionization (CI) mass spectra were recorded on a Finnigan 1015. IR spectra were recorded on a Bio-Rad FTS-45 spectrophotometer. Polarimetric measurements were taken using a Perkin-Elmer 241MC polarimeter. High-pressure liquid chromatography (HPLC) was performed on a Shimadzu LC-6A equipped with a SPD-SAV detector, a CR-601 plotter, and a Daicel Chiralcel OD column. Thin layer chromatography (TLC) was performed on Analtech silica gel GHLF 0.25-mm plates. Preparative TLC was performed on Analtech silica gel GF 2.00-mm plates. Elemental microanalyses were performed by Atlantic Microlab, Inc. Melting points were recorded on a Thomas-Hoover capillary apparatus and are corrected. The yields reported are not optimized.

5-(3-Hydroxyphenyl)-2-methyl-2-azabicyclo[3.3.1]nonan-7-one (4). Method A. A solution of **2**[22] (499 mg, 1.92 mmol) in 10 mL of ethanol-free, amylene-stabilized chloroform was added over a period of 2 min to a well-stirred solution of 0.95 mL (10.0 mmol) of BBr_3 in 25.0 mL of chloroform maintained at room temperature. Stirring was continued for 15 min. The reaction mixture was poured into a mixture of 4 mL of concentrated NH_4OH and 15 g of ice while stirring vigorously. The two-phase mixture was stirred at 0 °C for 30 min. The organic phase was saturated with NaCl and was extracted with chloroform (3 × 25 mL). The chloroform layers were combined, dried (Na_2SO_4), and evaporated to give an off-white foam. The foam was dissolved in 2-propanol (3 mL), and 48% HBr was added to pH 3. Compound **4**·HBr was filtered and rinsed with 2-propanol (4 mL) and with petroleum ether (bp 30−60 °C, 5 mL), yielding 514 mg (82%) of a white crystalline solid, mp 233−235 °C. An analytical sample was prepared

by recrystallization from ethanol: mp 234–236 °C; ^{1}H NMR δ 7.20 (t, 1H, J = 7.9 Hz), 6.86 (d, 1H, J = 7.8 Hz), 6.79 (s, 1H), 6.68 (dd, 1H, J = 2.0, 7.9 Hz), 3.50 (m, 1H), 2.96 (qd, 1H, J = 2.2, 17.2 Hz), 2.76 (m, 2H), 2.32–2.52 (m, 3H), 2.42 (s, 3H), 2.19 (m, 2H), 1.84–2.06 (m, 2H); IR (KBr) 3432.7, 2940.1, 2809.9, 1700.4, 1685.6, 1616.3, 1585.8, 1482.8, 1448.1, 1376.6, 1361.9, 1335.9, 1284.4, 1225.7, 1180.0, 1137.4, 1083.7, 925.1, 736.9, 699.2 cm^{-1}; MS (EI) m/z 245 (M^+), 188 ($M^+ - C_3H_7N$). Anal. ($C_{15}H_{19}NO_2$·HBr) C, H, N.

Method B. A solution of **2**·HBr[33] (10.02 g, 29.45 mmol) and 48% aqueous HBr (90 mL) was heated to reflux for 1.5 h. The solution was cooled with a dry ice–acetone bath, and then 70 mL of concentrated NH_4OH was slowly and carefully added with vigorous stirring, bringing the pH to 9. The resultant aqueous solution was extracted with chloroform (4 × 250 mL) and the chloroform layers were combined, dried (Na_2SO_4), and evaporated, yielding a yellow oil. This was triturated with 50 mL of boiling 2-propanol and made acidic by adding 5 mL of 48% HBr. The first crop of slightly off-white crystals weighed 8.81 g (92%) and had mp 234–236 °C. The filtrate was evaporated, and the residue was dissolved in 35 mL of water and made basic to pH 9 by the addition of 1 M NH_4OH. This was extracted with chloroform (3 × 50 mL). The extracts were combined, dried (Na_2SO_4), and evaporated. The foam was dissolved in 7 mL of hot 2-propanol and made acidic with 48% HBr. The off-white crystals were filtered and dried, yield 0.22 g (2%, total yield 94%), mp 225–228 °C. Both crops were converted to the free base with 0.30 M NH_4OH (100 mL) and extracted with chloroform (3 × 100 mL). The chloroform extracts were dried (Na_2SO_4) and evaporated. The resulting foam was mixed with petroleum ether (50 mL) and after standing, crystallization ensued. The off-white free base **4** was filtered and dried, 6.52 g (90%), mp 165–166 °C; the free base is a white crystalline solid with a mp 165–166 °C. Anal. ($C_{15}H_{19}NO_2$) C, H, N.

Optical Resolution of (±)-5-(3-Hydroxyphenyl)-2-methyl-2-azabicyclo[3.3.1]nonan-7-one (4). A hot solution of 98% (−)-*O,O'*-dibenzoyl-L-tartaric acid (3.66 g, 10.2 mmol) in 20 mL of MeOH was added to a hot solution of **4** (4.91 g, 20.0 mmol) in 50 mL of methanol. Crystals began to form within 5 min. The isolated crystals were recrystallized four times by dissolving them in warm DMF (15–10 mL) and adding hot methanol (100–70 mL) to a constant mp of 210–211 °C dec, yield 3.22 g (72%); $[\alpha]^{22}_D$ (salt in DMF, c 0.94) = −28.2°. Anal. ($C_{15}H_{19}NO_2$·0.5$C_{18}H_{14}O_8$·1.25H_2O) C, H, N. The solvent volume was decreased with each recrystallization in the ranges specified. The salt was free-based with 50 mL 0.5 M NH_4OH and extracted with chloroform (5 × 30 mL). Drying (K_2CO_3) of the chloroform extracts, evaporation, and trituration with ether/petroleum ether (1:1) yielded white needles of (+)-(1*S*,5*S*)-**4**, 1.72 g (70%); mp 177–179 °C; $[\alpha]^{22}_D$ (free base in MeOH, c 1.12) = +8.8°. Anal. ($C_{15}H_{19}NO_2$) C, H, N. Optical purity was shown to be >99% by HPLC (1.0 mL/min, hexanes/2-propanol/0.1% $HNEt_2$), observing at 254 nm. The approximate retention time was 9.2 min.

All of the filtrates from the above resolution were evaporated to dryness on a rotary evaporator equipped with a high-vacuum line, and the residue was treated with 100 mL of 0.5 M NH_4OH. This aqueous solution was extracted with chloroform (4 × 100 mL). The chloroform layers were dried (K_2CO_3), and evaporation gave a slightly yellow oil that was dissolved in 50 mL of hot methanol. A hot solution of 2.30 g (6.42 mmol) of 99% (+)-*O,O'*-dibenzoyl-D-tartaric acid in hot methanol (15 mL) was added, and the salt crystallized within several minutes. The isolated crystals were recrystallized three times by dissolving them in warm DMF (15–10 mL) and adding hot methanol (80–70 mL) to a constant mp of 210–211 °C dec, yield 3.40 g (76%); $[\alpha]^{22}_D$ (salt in DMF, c 1.04) = +28.5°. Anal. ($C_{15}H_{19}NO_2$·0.5$C_{18}H_{14}O_8$·1.25H_2O) C, H, N. The solvent volume was decreased with each recrystallization in the ranges specified. The salt was free-based with 50 mL 0.5 M NH_4OH and extracted with chloroform (5 × 30 mL). Drying (K_2CO_3) of the chloroform extracts, evaporation, and trituration with ether/petroleum ether (1:1) yielded white needles of 1.77 g (72%) of (−)-(1*R*,5*R*)-**4**: mp 177–179 °C; $[\alpha]^{22}_D$ (free base in MeOH, c 1.27) = −9.0°. Anal. ($C_{15}H_{19}NO_2$) C, H, N. Optical purity was shown to be >99% by HPLC (1.0 mL/min, hexanes/2-propanol/0.1% $HNEt_2$), observing at 254 nm. The approximate retention time was 7.1 min.

(+)-(1*S*,5*R*)-5-(3-Hydroxyphenyl)-2-methyl-2-azabicyclo[3.3.1]nonane [(+)-(1*S*,5*R*)-1]. Compound (−)-(1*R*,5*R*)-**4** (>99% ee) (918.7 mg, 3.745 mmol) was placed in a solution of HCl(g) saturated ether (103 mL) at 0 °C. Activated Zn dust[34] (14 g, 214 mmol) was added in small portions over a 30-min period, and HCl gas was bubbled through the reaction solution until all of the Zn^0 was gone. The reaction solution was poured into a mixture of water (500 mL) and chloroform (250 mL). The pH of the aqueous layer was carefully adjusted to 9 with solid Na_2CO_3, and the insoluble material was removed by filtration through a Celite pad. The filtrate was placed in a separatory funnel, and the chloroform layer was separated. The aqueous layer was extracted with chloroform (6 × 100 mL) and all chloroform layers were combined, dried (K_2CO_3), and evaporated. The resulting oil was dissolved in methanol, and the insoluble material was filtered. The methanol solution was acidified with 48% HBr, and the solution was evaporated to dryness. To the resulting yellow oil was added 5 mL of acetone. White needles were filtered and air-dried, yield 660.3 mg (52%), mp 228–229 °C. An analytical sample was prepared by diffusion of acetone into a methanol solution of (+)-(1*S*,5*R*)-**1** in a closed chamber, yield 480.3 mg of colorless needles; mp 228–229 °C; $[\alpha]^{21}_D$ (salt in H_2O, c 0.69) = +5.5°. A sample was free-based with chloroform/0.5 M NH_4OH, and the resulting oil was triturated with 0.5 M NH_4OH until it crystallized: $[\alpha]^{22}_D$ (free base in MeOH, c 1.12) = +15.2° (lit.[2] $[\alpha]^{20}_D$ = +12.4°). A proton NMR of (+)-(1*S*,5*R*)-**1** was identical to that of an authentic sample of racemic **1**.[35]

(−)-(1*R*,5*S*)-5-(3-Hydroxyphenyl)-2-methyl-2-azabicyclo[3.3.1]nonane [(−)-(1*R*,5*S*)-1]. The procedure is identical to that for the preparation of (+)-(1*S*,5*R*)-**1**, starting with 906.4 mg of (+)-(1*S*,5*S*)-**4** (>99% ee). The yield of (−)-(1*R*,5*S*)-**1** was 766.1 mg (66%) and the mp 226 °C. Analytical sample yield was 559.6 mg and had mp 228–229 °C (lit.[23] mp 232–233 °C); $[\alpha]^{20}_D$ (salt in H_2O, c 0.54) = −5.5° (lit.[23] $[\alpha]^{20}_D$ = −4.2°). A proton NMR of (−)-(1*R*,5*S*)-**1** was identical to that of an authentic sample of racemic **1**.[35]

(*E*)-8-Benzylidene-5-(3-methoxyphenyl)-2-methyl-2-azabicyclo[3.3.1]nonan-7-one Perchlorate (3). A solution of **2**·HBr (2.00 g, 5.88 mmol), 87% potassium hydroxide (1.97 g, 30.5 mmol), and benzaldehyde (5.0 mL, 50 mmol) in methanol (125 mL) was heated at reflux for 5.25 h. The reaction solution was evaporated to a low volume, the residue was suspended in saturated brine (80 mL), and this was extracted with chloroform (3 × 100 mL). The extracts were dried (K_2CO_3) and were evaporated. The excess benzaldehyde was removed on a rotary evaporator equipped with a high-vacuum line. The resulting oil was dissolved in 2-propanol (6 mL) and made acidic with concentrated perchloric acid. The crystalline solid was filtered and rinsed with cold 2-propanol (10 mL) and cold methanol (5 mL) followed by excess petroleum ether (bp 30–60 °C): yield 2.24 g (85%); mp 222–224 °C. An analytical sample was prepared by recrystallization from methanol/H_2O (1:1), giving off-white needles: mp 230–231 °C; ^{1}H NMR δ 7.79 (s, 1H), 7.39 (m, 5H), 7.30 (t, 1H, J = 8.0 Hz), 6.97 (br d, 1H, J = 7.8 Hz), 6.92 (t, 1H, J = 2.1 Hz), 6.80 (dd, 1H, J = 2.3, 7.9 Hz), 4.48 (t, 1H, J = 3.4 Hz), 3.82 (s, 3H), 2.97 (dd, 1H, J = 3.0, 17.9 Hz), 2.67 (ddd, 1H, J = 1.9, 5.2, 13.3 Hz), 2.63 (dd, 1H, J = 1.5, 17.8 Hz), 2.52 (m, 1H), 2.43 (m, 1H), 2.30 (td, 1H, J = 2.9, 12.9 Hz), 2.08 (m, 1H), 2.02 (s, 3H), 1.93 (m, 1H); IR (KBr) 3430.6, 2961.4, 2928.2, 2852.4, 2793.3, 1682.5, 1602.3, 1490.9, 1447.4, 1433.2, 1371.8, 1288.9, 1261.2, 1231.3, 1197.8, 1169.4, 1140.0, 1095.9, 1050.8, 1025.4, 870.1, 803.7, 772.0, 700.0 cm^{-1}; MS (CI-NH_3) m/z 348 (MH^+). Anal. ($C_{23}H_{25}NO_2$·$HClO_4$) C, H, N.

(*E*)-8-Benzylidene-5-(3-hydroxyphenyl)-2-methyl-2-azabicyclo[3.3.1]nonan-7-one Hydrobromide (5). Compound **3** (2.24 g, 5.00 mmol) was partitioned between 1 M NH_4OH (30 mL), saturated brine (20 mL), and chloroform (100 mL). The chloroform layer was separated, and the aqueous layer was extracted with chloroform (3 × 50 mL). The chloroform extracts were dried (Na_2SO_4) and evaporated. A solution of the free base in ethanol-free, amylene-stabilized chloroform (50.0 mL) was cooled for 5 min with a dry ice/acetone bath. To this a solution of BBr_3 (2.40 mL, 25.4

mmol) in ethanol-free, amylene-stabilized chloroform (15 mL) was added slowly over 5 min under an argon atmosphere. The cooling bath was removed, and the mixture was allowed to warm to room temperature. This was poured into a stirring mixture of concentrated NH_4OH (14 mL) and ice (80 g). This mixture was stirred on an ice bath for 30 min. The chloroform layer was separated, and the aqueous layer was extracted with chloroform (50 mL). The chloroform layers were combined, dried (Na_2SO_4), and evaporated. The residue was dissolved in 2-propanol (5.0 mL) and made acidic with 48% HBr (0.6 mL). The filtered crystalline solid was washed with 2-propanol (6 mL) and then with petroleum ether: yield 1.43 g (69%); mp 204–206 °C. An analytical sample was prepared by recrystallizing a small portion from ethanol: mp 205–207 °C; ^{1}H NMR δ 7.79 (s, 1H), 7.38 (m, 5H), 7.24 (t, 1H, J = 8.0 Hz), 6.94 (d, 1H, J = 8.0 Hz), 6.85 (t, 1H, J = 2.1 Hz), 6.71 (dd, 1H, J = 2.0, 8.1 Hz), 5.27 (s, 1H, ex w/D_2O), 4.48 (t, 1H, J = 3.2 Hz), 2.95 (dd, 1H, J = 3.2, 17.8 Hz, 6-H_{eq}), 2.67 (m, 1H), 2.61 (dd, 1H, J = 1.3, 17.9 Hz, 6-H_{ax}), 2.50 (m, 1H), 2.43 (m, 1H), 2.29 (td, 1H, J = 2.8, 12.8 Hz), 2.05 (m, 1H), 2.02 (s, 3H), 1.91 (qd, 1H, J = 2.3, 13.0 Hz); IR (KBr) 3429.2, 2935.0, 2856.8, 2792.6, 1680.9, 1611.8, 1595.1, 1492.6, 1446.8, 1369.8, 1304.9, 1262.4, 1233.3, 1182.9, 1142.4, 872.5, 803.0, 774.0, 699.5 cm^{-1}; MS (CI-NH_3) m/z 334 (MH^+). Anal. ($C_{22}H_{23}NO_2$·HBr·0.25H_2O) C, H, N. It is also possible to obtain another crystalline form of the HBr salt from the same procedure that has mp 247–248 °C and Anal. ($C_{22}H_{23}NO_2$·HBr) C, H, N.

(−)-(1*S*,5*S*)-(*E*)-8-Benzylidene-5-(3-hydroxyphenyl)-2-methyl-2-azabicyclo[3.3.1]nonan-7-one Perchlorate [(−)-(1*S*,5*S*)-5]. A solution of (+)-(1*S*,5*S*)-**4** (351.1 mg, 1.431 mmol), 87% potassium hydroxide (0.45 g, 7.0 mmol), and benzaldehyde (1.75 mL, 17.3 mmol) in methanol (35 mL) was heated at reflux for 15 h. The reaction solution was evaporated, and the residue was taken up in half-saturated brine (40 mL) and extracted with methylene chloride (3 × 40 mL). The extracts were dried (Na_2SO_4) and evaporated on a rotary evaporator equipped with a high-vacuum line to remove the excess benzaldehyde. The resulting oil was dissolved in 2-propanol (12 mL) and was made acidic with perchloric acid. The resulting crystalline solid was filtered and washed with 2-propanol (10 mL) and petroleum ether (10 mL). This was recrystallized by dissolving it in warm DMF (1.5 mL), filtering the insolubles, and adding hot 2-propanol (35 mL): yield 339 mg (55%); mp 264–265 °C; ^{1}H NMR was superimposable with that of racemic **5**; $[\alpha]^{22}_D$ (salt in DMF, c 1.03) = −236.4°; $[\alpha]^{22}_D$ (free base in MeOH, c 0.64) = −159.0°. Anal. ($C_{22}H_{23}NO_2$·$HClO_4$) C, H, N.

(+)-(1*R*,5*R*)-(*E*)-8-Benzylidene-5-(3-hydroxyphenyl)-2-methyl-2-azabicyclo[3.3.1]nonan-7-one Perchlorate [(+)-(1*R*,5*R*)-5]. A procedure analogous to that for (−)-(1*S*,5*S*)-**5** was carried out with the following quantities of materials: (−)-(1*R*,5*R*)-**4** (353.4 mg, 1.440 mmol), 87% potassium hydroxide (0.48 g, 7.4 mmol), benzaldehyde (1.75 mL, 17.3 mmol), and methanol (35 mL). The recrystallized yield was 374 mg (59%): mp 264–265 °C; ^{1}H NMR was superimposable with that of racemic **5**; $[\alpha]^{22}_D$ (salt in DMF, c 1.02 excluding water of crystallization) = +233.7°; $[\alpha]^{22}_D$ (free base in MeOH, c 0.61) = +154.5°. Anal. ($C_{22}H_{23}NO_2$·$HClO_4$·$^1/_3H_2O$) C, H, N.

(*E*)-8-(3,4-Dichlorobenzylidene)-5-(3-hydroxyphenyl)-2-methyl-2-azabicyclo[3.3.1]nonan-7-one Hydrobromide (6). A solution of **4** (245.9 mg, 1.002 mmol), 87% potassium hydroxide (0.30 g, 4.6 mmol), and 3,4-dichlorobenzaldehyde (0.45 g, 2.57 mmol) in methanol (20 mL) was heated at reflux under argon for 14 h. The reaction solution was evaporated to dryness, and the residue was taken up in water (20 mL) and saturated brine (5 mL). The pH was adjusted to 9, and this was extracted with chloroform (3 × 20 mL). The extracts were dried (Na_2SO_4) and evaporated. The residue was dissolved in a minimum amount of chloroform, and this solution was loaded onto a preparative TLC plate. The plate was eluted with hexanes/ethyl acetate (1:1), and the appropriate band (R_f = 0.48 with chloroform/methanol/NH_4OH, 95:5:0.1) was removed and extracted with the ethyl acetate. This gave an light yellow foam that weighed 260.5 mg (65%). An analytical sample was prepared by a second purification by preparative TLC as above, and the resulting light yellow foam was dissolved in 2-propanol (8 mL) and made acidic with 48% HBr. The light yellow crystalline salt weighed 239.4 mg and had mp 245–247 °C dec: ^{1}H NMR δ 7.63 (s, 1H), 7.51 (d, 1H, J = 1.8 Hz), 7.48 (d, 1H, J = 8.4 Hz), 7.23 (m, 2H), 6.92 (d, 1H, J = 7.4 Hz), 6.84 (t, 1H, J = 2.1 Hz), 6.71 (dd, 1H, J = 1.9, 8.0 Hz), 4.30 (t, 1H, J = 3.1 Hz), 2.96 (dd, 1H, J = 3.1, 18.0 Hz, 6-H_{eq}), 2.69 (ddd, 1H, J = 1.6, 5.2, 13.5 Hz), 2.60 (dd, 1H, J = 1.5, 18.0 Hz, 6-H_{ax}), 2.48 (m, 2H), 2.22 (td, 1H, J = 2.7, 6.5 Hz), 2.09 (s, 3H), 2.04 (m, 1H), 1.88 (qd, 1H, J = 1.5, 13.1 Hz); MS (CI-NH_3) m/z 402 (MH^+). Anal. ($C_{22}H_{21}Cl_2NO_2$·HBr) C, H, N.

(−)-(1*S*,5*S*)-(*E*)-8-(3,4-Dichlorobenzylidene)-5-(3-hydroxyphenyl)-2-methyl-2-azabicyclo[3.3.1]nonan-7-one Hydrobromide [(−)-(1*S*,5*S*)-6]. A procedure analogous to that for **6** was carried out with (+)-(1*S*,5*S*)-**4**, yield 214.3 mg (57%). An analytical sample was prepared as an HBr salt analogous to the procedure used for **6**: yield 196.6 mg; mp 250–251 °C dec; ^{1}H NMR was identical to that for racemic **6**; $[\alpha]^{22}_D$ (salt in DMF, c 0.95) = −234.0°; $[\alpha]^{22}_D$ (free base in MeOH, c 0.87) = −78.8°. Anal. ($C_{22}H_{21}Cl_2NO_2$·HBr) C, H, N.

(+)-(1*R*,5*R*)-(*E*)-8-(3,4-Dichlorobenzylidene)-5-(3-hydroxyphenyl)-2-methyl-2-azabicyclo[3.3.1]nonan-7-one Hydrobromide [(+)-(1*R*,5*R*)-6]. A procedure analogous to that for **6** was carried out with (−)-(1*R*,5*R*)-**4**, yield 209.4 mg (60%). An analytical sample was prepared as an HBr salt analogous to the procedure used for **6**: yield 198.1 mg; mp 250–251 °C dec; ^{1}H NMR was identical to that for racemic **6**; $[\alpha]^{22}_D$ (salt in DMF, c 1.02) = +229.9°; $[\alpha]^{22}_D$ (free base in MeOH, c 0.79) = +79.8°. Anal. ($C_{22}H_{21}Cl_2NO_2$·HBr) C, H, N.

(−)-(1*S*,5*S*)-(*E*)-8-Benzylidene-5-(3-hydroxyphenyl)-2,2-dimethyl-2-azoniabicyclo[3.3.1]nonan-7-one Iodide [(−)-(1*S*,5*S*)-7]. Compound (−)-(1*S*,5*S*)-**5** (172 mg, 0.516 mmol) was dissolved in hot acetone (8 mL). The solution was cooled to room temperature, methyl iodide (0.212 mL, 3.40 mmol) was added, and this solution was heated to reflux for 1 h. A solid began to crystallize from the reaction solution after 5 min. The reaction mixture was cooled on an ice–water bath, and the solid was filtered, rinsed with cold acetone (4 mL), and dried, yield 223 mg (91%). An analytical sample was prepared by recrystallization from water: yield 170 mg; mp 206–208 °C; ^{1}H NMR (DMSO-d_6) δ 9.45 (s, 1H, ex w/D_2O), 7.87 (s, 1H), 7.56 (m, 5H), 7.20 (t, 1H, J = 7.9 Hz), 6.93 (d, 1H, J = 8.6 Hz), 6.84 (t, 1H, J = 1.8 Hz), 6.70 (dd, 1H, J = 1.8, 7.9 Hz), 5.17 (br s, 1H), 3.52 (br d, 1H, J = 14.0 Hz), 3.28 (s, 3H), 3.06–2.75 (m, 5H), 2.70 (s, 3H), 2.28 (m, 2H); MS (EI) m/z 347 (M^+-HI); MS (CI-NH_3) m/z 348 (MH^+ − HI); $[\alpha]^{20}_D$ (DMSO, c 0.99) = −257.0°. Anal. ($C_{23}H_{26}INO_2$) C, H, N.

Single-Crystal X-ray Analysis of 5. Crystals of **5** were grown by evaporation from methanol/acetonitrile. Data were collected on a computer-controlled automatic diffractometer, Siemens R3m/V, and corrected for Lorentz and polarization effects. Semiempirical absorption corrections from Ψ-scans were applied (min and max transmissions 0.718 and 0.788). The structure was solved by direct methods with the aid of program SHELXTL[36] and refined by full-matrix least-squares on F^2 values using program SHELXLS-93.[37] The parameters refined include the coordinates and anisotropic thermal parameters for all non-hydrogen atoms. Hydrogen atoms were included using a riding model in which the coordinate shifts of their covalently bonded atoms were applied to the attached hydrogens with C–H = 0.96 Å, N–H = 0.91 Å, and O–H = 0.82 Å. H angles were idealized and U_{iso}(H) set at fixed ratios of U_{iso} values of bonded atoms. The absolute configuration was confirmed by the least-squares refinement based on the anomalous scattering of the Br ion.[38] Additional experimental and structural analysis details are given in Table 3, and tables of crystal coordinates, bond distances, and bond angles are available as supplementary material.

Radioligand Binding Assays. [^{3}H]*N*-(1-(2-Thienyl)-cyclohexyl)[3,4-^{3}H]piperidine ([^{3}H]TCP) Binding Assay. The method used was as previously described.[39]

[^{3}H]-(+)-Pentazocine Binding Assay. The method used was as previously described.[18h,39]

[^{3}H]*N*-Methylscopolamine Binding Assay. Male Sprague–Dawley rats (Taconic Farms, Germantown, NY) were decapitated, and the cortex was rapidly dissected, pooled, and homogenized in 10 volumes of ice-cold 0.32 M sucrose in a glass–Teflon homogenizer. The homogenate was centrifuged at 1000g for 10 min. After discarding the pellet, the super-

Table 3. Crystal and Refinement Data for **5**[a]

formula	$C_{22}H_{24}NO_2^+Br^-$
formula weight	414.33
crystal color, habit	colorless, prism
crystal dimensions, mm	0.47 × 0.20 × 0.18
crystal system	orthorhombic
space group	$Pna2_1$
a, Å	13.216(7)
b, Å	19.279(9)
c, Å	7.717(5)
V, Å^3	1966(2)
Z	4
ϱ(calc), g cm^{-3}	1.40
μ, absorption coef, mm^{-1}	2.11
temp, °C	22
diffractometer	Siemens R3m/V
cell determination	
reflections, 2θ range	29, 22 – 32
λ, wavelength, Å	Mo Kα, 0.71073
2θ max (deg) scan mode	45, $\theta/2\theta$
total reflections measured	1439
unique data	1385
observed data ($I > 2\sigma I$)	923
R_{int}	0.012
refinement on F^2 using all data	
parameters refined	239
R,[b] R_w,[c]	0.045, 0.081
R,[b] R_w,[c] S[d] (for all data)	0.092, 0.098, 1.05
data:parameter ratio	6:1
final Δ_{max}/σ	0.04
Fourier excursions, e Å^{-3}	0.27, −0.28

[a] Tables of crystal coordinates, bond distances, and bond angles are available as supplementary material and from the Cambridge Structural Database. [b] $\Sigma|\Delta|/\Sigma|F_o|$. [c] $[\Sigma(w\Delta^2)^2/\Sigma(wF_o^2)^2]^{1/2}$. [d] $[\Sigma(w\Delta^2)^2/(N_o - N_p)]^{1/2}$.

natant was decanted and homogenized with a Brinkman polytron (setting 6, 20 s) and kept on ice until needed.

Binding to homogenates was determined as described using a modification of the literature procedure.[40] In a 1-mL incubation volume, 50 μL of the tissue preparation, 50 μL of [^{3}H]NMS (80.4 Ci/mmol: New England Nuclear, Boston, MA) for a final concentration of 0.8 nM, 850 μL of binding buffer (10 mM Tris HCl, pH 7.4; 5 mM $MgCl_2$; 100 mM NaCl), and 50 μL of buffer, test compound, or 10 μM atropine (for determination of nonspecific binding). After a 120-min incubation at 25 °C, the reaction was terminated by rapid filtration using a Brandel cell harvester (Brandel Instrument Co., Gaithersburg, MD) through no. 32 Schleicher and Schuell glass fiber filters. The filters were washed with four 3-mL aliquots of ice-cold assay buffer and placed in counting vials with 4 mL of CytoScint ES (ICN Biomedicals, Irvine CA) scintillation cocktail and allowed to stand overnight before counting in a Packard Tri-Carb 2200CA liquid scintillation counter (Packard Instrument Co., Downers Grove, IL).

The data was analyzed using GraphPAD software (ISI Software, Philadelphia, PA). Each concentration was done in triplicate, and the resulting values are the mean of at least two experiments.

[^{3}H]DAMGO, [^{3}H]DADLE, and [^{3}H]U69,593 Binding Assays. μ binding sites were labeled using [^{3}H]DAMGO (1–3 nM) and rat brain membranes as previously described.[41] Briefly, incubations proceeded for 4 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 levallorphan. δ binding sites were labeled using [^{3}H]DADLE (1.7–2.5 nM) and rat brain membranes as previously described.[42] Briefly, incubations proceeded for 3–4 h at 25 °C in 10 mM Tris-HCl, pH 7.4, containing 100 mM choline chloride, 3 mM $MnCl_2$, 100 nM DAMGO to block binding to μ sites and PIC. Nonspecific binding was determined using 20 μM levallorphan. κ_1 binding sites were labeled using [^{3}H]U69,593 (1.2–2.3 nM) and guinea pig brain membranes depleted of μ and δ binding sites by pretreatment with irreversible ligands BIT and FIT as previously described,[43] except that the incubation temperature was at 25 °C. Briefly, incubations proceeded for 4–6 h at 25 °C in 50 mM Tris-HCl, pH 7.4, containing PIC and 1 μg/mL of captopril. Nonspecific binding was determined using 1 μM U69,593.

Each [^{3}H] ligand was displaced by 8–10 concentrations of test drug, two times. All drug dilution was done in 10 mM Tris-HCl, pH 7.4, containing 1 mg/mL bovine serum albumin. Compounds **6**, (+)-(1*R*,5*R*)-**6**, and (−)-(1*S*,5*S*)-**6** were prepared as 1 mM solutions with 10 mM Tris buffer (pH 7.4) containing 10% DMSO, 8% Emulphor EL-620 before drug dilution. The IC_{50} and slope factor (N) were obtained by using the program MLAB.

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Supplementary Material Available: Tables of crystallographic data for (±)-**5** including bond lengths, bond angles, and atomic coordinates (4 pages). Ordering information is given on any current masthead page.

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