N-Substituted 9β-Methyl-5-(3-hydroxyphenyl)morphans Are Opioid Receptor Pure Antagonists

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Received May 8, 1998

The inhibition of radioligand binding and [^{35}S]GTPγS functional assay data for *N*-methyl- and *N*-phenethyl-9β-methyl-5-(3-hydroxyphenyl)morphans (**5b** and **5c**) show that these compounds are pure antagonists at the μ, δ, and κ opioid receptors. Since **5b** and **5c** have the 5-(3-hydroxyphenyl) group locked in a conformation comparable to an equatorial group of a piperidine chair conformation, this information provides very strong evidence that opioid antagonists can interact with opioid receptors in this conformation. In addition, it suggests that the *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine class of antagonist operates via a phenyl equatorial piperidine chair conformation. Importantly, the close relationship between the 4-(3-hydroxyphenyl)piperidines and 5-(3-hydroxyphenyl)morphan antagonists shows that the latter class of compound provides a rigid platform on which to build a novel series of opioid antagonists.

The opioid receptor system has been extensively investigated, and thousands of compounds have been synthesized and evaluated in radioligand binding assays, tissue assays, and animal models.[1] Numerous structural types of opioid agonists have been discovered, and several such as methadone, meperidine, fentanyl, and pentazocine as well as others have become important drugs for the treatment of pain.[1] In contrast, there are only a few structural types that show potent, opioid pure antagonist activity.[1,2] A resurgence in heroin use in recent years coupled with the demonstrated effectiveness of opioid antagonists for the treatment of other substances of abuse has spurred new interest in the development of novel antagonists for opioid receptors.[3] The oxymorphone-related compounds such as naloxone (**1a**) and naltrexone (**1b**) (Chart 1), where the antagonist activity is dependent upon the N-substituent, have received considerable attention over the past few decades.[1] For example, pioneering studies by Portoghese and co-workers led to the development of the prototypical κ and δ opioid receptor antagonists norbinaltorphimine (**2**, nor-BNI) and naltrindole (**3**, NTI). In contrast, the N-substituted *trans*-3,4-dimethyl(3-hydroxyphenyl)piperidine (**4a**, **4b**, **4c**, **4d**) class of pure antagonist has received relatively little attention. Studies with the *N*-methyl analogue **4a** as well as many other N-substituted analogues such as **4b**, **4c** (LY255582), and **4d** showed that the pure antagonist activity was dependent on the 3-methyl substituent and its trans relative relationship to the 4-methyl substituent on the piperidine ring and, unlike the oxymorphone class, was independent of the nature of the N-substituent.[2,4−8] Interestingly, the 3,4-dimethyl cis isomer **4e** was found to be a mixed agonist−antagonist. May and co-workers[9] reported that 2,9α-dimethyl-5-(3-hydroxyphenyl)morphan (**5a**), which has the 9-methyl group in a configuration comparable to the *cis*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine (**4e**) with the 5-(3-hydroxyphenyl) group locked in an equatorial conformation relative to the piperidine ring in the morphan structure, was a weak but pure antagonist. Since the *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine (**4a**) is 40 times more potent than its cis isomer **4e**, one might predict that 2,9β-dimethyl-5-(3-hydroxyphenyl)morphan (**5b**), which has the 9-methyl group in a configuration comparable to **4a**, would also be a more potent pure antagonist than the 9α isomer **5a**. In this article, we describe the synthesis of *N*-methyl- and *N*-phenethyl-9β-methyl-5-(3-hydroxyphenyl)morphans (**5b** and **5c**, respectively) and report that these compounds, which have the 3-hydroxyphenyl group locked in an equatorial position relative to the piperidine ring in the morphan structure, are high-affinity, pure antagonists for opioid receptors.

Chemistry

The synthesis of the *N*-methyl- and *N*-phenethyl-9β-methyl-5-(3-hydroxyphenyl)morphans (**5b** and **5c**, respectively) was achieved as illustrated in Scheme 1.[10] Treatment of 1,2,6-trihydro-1,3-dimethyl-4-(3-methoxyphenyl)pyridine (**6**) with *sec*-butyllithium followed by quenching with allyl bromide provided the enamine adduct **7** which was cyclized without isolation to give 2,9-dimethyl-5-(3-methoxyphenyl)-2-azabicyclo[3.3.1]-

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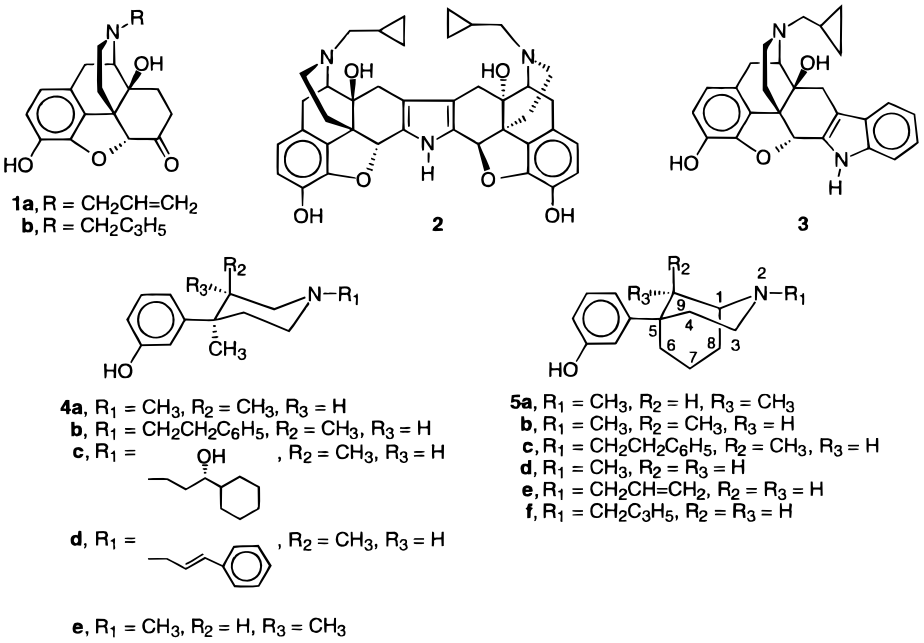
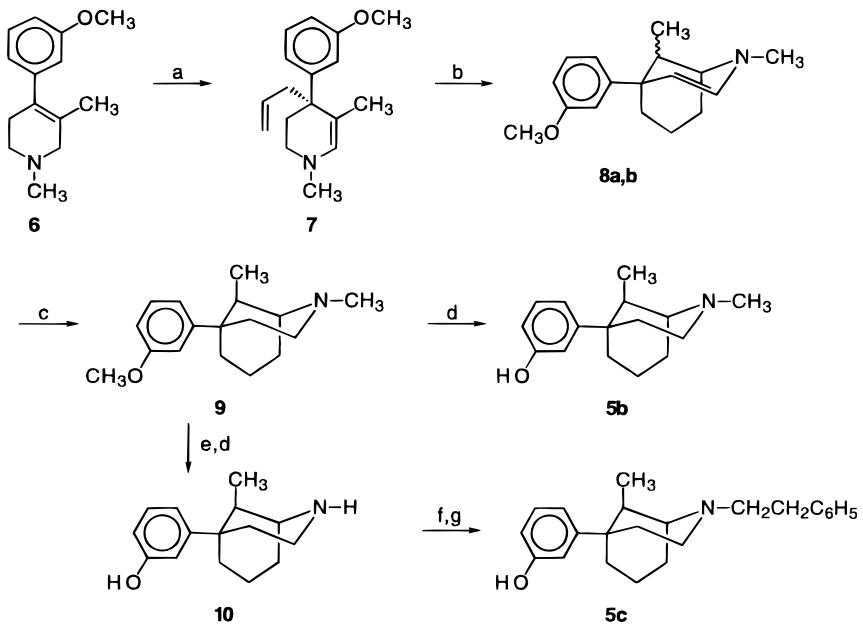
S0022-2623(98)00290-8 CCC: $15.00
Published on Web 09/16/1998

Chart 1



Scheme 1[a]



[a] (a) *s*-BuLi, allyl-Br; (b) H_3PO_4, HCO_2H; (c) $NaHB(OAc)_3$; (d) HOAc, HBr; (e) PhOCOCl, then KOH, H_2O; (f) (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate, $PhCH_2CO_2H$; (g) borane−dimethyl sulfide, THF.

non-3-ene (**8a**, **8b**) in a 3:1 9β:9α-methyl ratio, using 1:1 phosphoric and formic acid. Reduction of unpurified **8a** and **8b** using sodium borohydride triacetate followed by separation of the major isomer gave **9**. Subjection of **9** to O-demethylation using hydrobromic acid in acetic acid provided the desired phenylmorphan **5b**. Single-crystal X-ray analysis showed that **5b** had the desired 9β-methyl relative configuration (Figure 1).

The *N*-phenethyl derivative **5c** was prepared from intermediate **9**. Treatment of **9** with phenyl chloroformate followed by hydrolysis of the resulting urethane with potassium hydroxide followed by O-demethylation with hydrobromic acid in acetic acid gave **10**. Compound **10** was converted to **5c** by coupling with phenylacetic acid in the presence of (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate followed by borane reduction of the resulting amide intermediate.

Biological Results

Table 1 lists the radioligand binding data for compounds **5b** and **5c** along with data for naltrexone (**1b**). While the binding of **5b** to all three opioid receptors was weak, it is particularly interesting to note that changing the N-substituent from methyl to phenethyl (**5c**) provided a dramatic increase in binding affinity, a feature shared by the corresponding 4-(3-hydroxyphenyl)piperidine analogues **4a** and **4b** (Table 2).[11] Furthermore, the relative binding affinities displayed by **5b** and **5c**

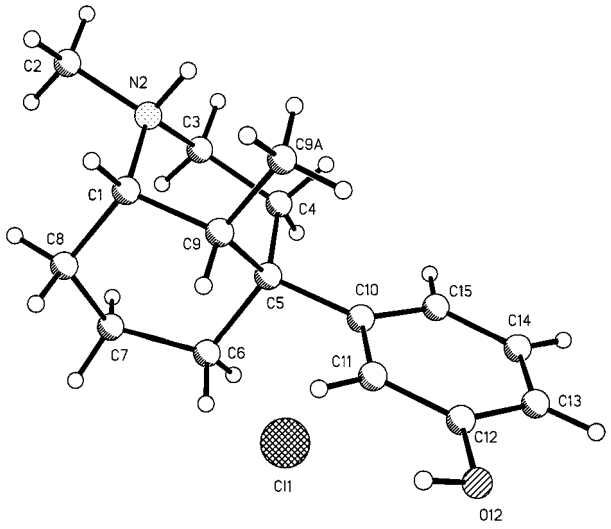


Figure 1. X-ray structure of **5b** drawn using the experimentally determined coordinates.

Table 1. Radioligand Binding Results at the μ, δ, and κ Opioid Receptors for *N*-Methyl- and *N*-Phenethyl-9β-methyl-5-(3-hydroxyphenyl)morphans

	K_i (nM ± SD)		
compd	μ [^{3}H]DAMGO[a]	δ [^{3}H]DADLE[b]	κ [^{3}H]U69,593[c]
5b	166 ± 15	>10000	816 ± 66
5c	3.11 ± 0.21	272 ± 30	14.5 ± 0.99
1b, naltrexone	1.39 ± 0.40	94.9 ± 6.6	4.71 ± 0.7

[a] [^{3}H]DAMGO, (D-Ala^2,MePhe^4,Gly ol^5)enkephalin, tritiated ligand selective for μ opioid receptor. [b] [^{3}H]DADLE, (D-Ala^2,D-Leu^5)enkephalin, tritiated ligand selective for δ opioid receptor. [c] [^{3}H]U69,593, [^{3}H]-5α,7α,8β-(−)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl]benzeneacetamide, tritiated ligand selective for κ opioid receptor.

Table 2. Affinities of the N-Substituted 3,4-Dimethyl-(3′-hydroxyphenyl)piperidine Antagonists for the μ and κ Opioid Receptors[a]

	K_i (nM)	
compd	μ [^{3}H]Nal[b]	κ [^{3}H]EKC[c]
4a	80	833
4b	1.5	52
1b, naltrexone	0.56	3.9

[a] Data taken from ref 11. [b] [^{3}H]Naloxone (μ receptor assay). [c] [^{3}H]Ethylketocyclazocine (κ receptor assay).

for μ and κ opioid receptors are quite similar to that observed for **4a** and **4b**. These results show that the binding affinities of **5b** and **5c** are not adversely affected by the 1,5-carbon bridge present in these structures. In addition, it suggests a common binding mode for the two types of structures.

The increase in binding of [^{35}S]GTPγS stimulated by opioid agonists is an assay able to distinguish compounds of differing efficacy and intrinsic activity.[8] The antagonist properties of test compounds can be determined by measuring the inhibition of this stimulation. To assess their potency as antagonists and to verify that **5b** and **5c** retain pure antagonist activity, the compounds were analyzed for either stimulation or inhibition of agonist-stimulated GTP binding in comparison with naltrexone (Table 3). In this functional assay, neither **5b** nor **5c** stimulated GTP binding as measured up to concentrations of 10 μM, showing that both compounds were devoid of agonist activity.[12] As mentioned previously, retention of pure antagonist activity regardless of the N-substituent structure is a key feature that separates the 3,4-dimethyl-4-(3-hydroxyphenyl)piperidine class of antagonist from oxymorphone-based antagonists which display pure antagonism only for certain N-substituents such as the *N*-allyl or *N*-cyclopropylmethyl derivatives. In their ability to reverse agonist-stimulated GTP binding, compound **5c** displayed a higher potency than naltrexone. These results are striking since agonist activity in several opioid ligands is enhanced by N-substituents with two methylene groups terminated by a phenyl group (*N*-phenethyl). It is evident that the antagonist activity of **5c** is due to factors different from those of the oxymorphone-type pure antagonists.

Table 3. Inhibition by Antagonists of [^{35}S]GTPγS Binding in Guinea Pig Caudate Stimulated by DAMGO (μ), SNC80 (δ), and U69,593 (κ) Selective Opioid Agonists[a]

	K_i (nM ± SD)		
compd	μ (DAMGO)[a]	δ (SNC80)[b]	κ (U69,593)[c]
5b	21.2 ± 2.30	750 ± 85.9	105 ±10.9
5c	0.338 ± 0.028	12.6 ± 1.01	1.34 ± 0.084
1b, naltrexone	0.930 ± 0.21	19.3 ± 2.25	2.05 ± 0.21

[a] DAMGO, (D-Ala^2,MePhe^4,Gly-ol^5)enkephalin, agonist selective for μ opioid receptor. [b] SNC80, [(+)-4-[(αR)-α-(2*S*,5*R*)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide, agonist selective for δ opioid receptor. [c] U69,593, *trans*-3,4-dichloro-*N*-methyl[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide, agonist selective for κ opioid receptor.

The data in Table 3 also demonstrate that the *N*-methyl to *N*-phenethyl change, **5b** to **5c**, results in a concomitant increase in antagonist potency. Thus, as is the case for the 3,4-dimethyl-4-(3-hydroxyphenyl)-piperidines, the antagonist potency and not the agonist/antagonist behavior of the 9β-methyl-5-(3-hydroxyphenyl)morphans **5b** and **5c** is mediated by the N-substituent.

Discussion

In contrast to the naltrexone (**1b**) like antagonists, the *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidines do not rely on the N-substituent for expression of antagonist activity.[5] Instead, the effect of the N-substituent has been shown to modulate the potency of antagonism. Studies with a variety of 4-substituted 4-phenylpiperidines, each possessing both a 3-hydroxyl substituent in their 4-phenyl ring and a 3β-methyl substituent in the piperidine ring, displayed pure antagonist activity.[5,6] These studies also showed that both opioid binding affinity and antagonist activity were dependent on a trans relative relationship between the 3- and 4-methyl groups on the piperidine ring. This information combined with the observation that the 3,4-dimethyl cis isomer **4e** displayed a peculiar pattern of mixed agonist/antagonist activity led to the hypothesis that a 3-hydroxyphenyl equatorial piperidine chair conformation mediated the antagonists' properties. Further studies utilizing a variety of di- and trimethyl-substituted 4-(3-hydroxyphenyl)piperidines[7] supported this hypothesis. We have demonstrated in this study that *N*-methyl-9β-methyl-5-(3-hydroxyphenyl)morphan (**5b**) is an opioid receptor pure antagonist. In addition, we found that replacement of the *N*-methyl with an *N*-phenethyl group to give **5c** resulted in a 63-, 60-, and

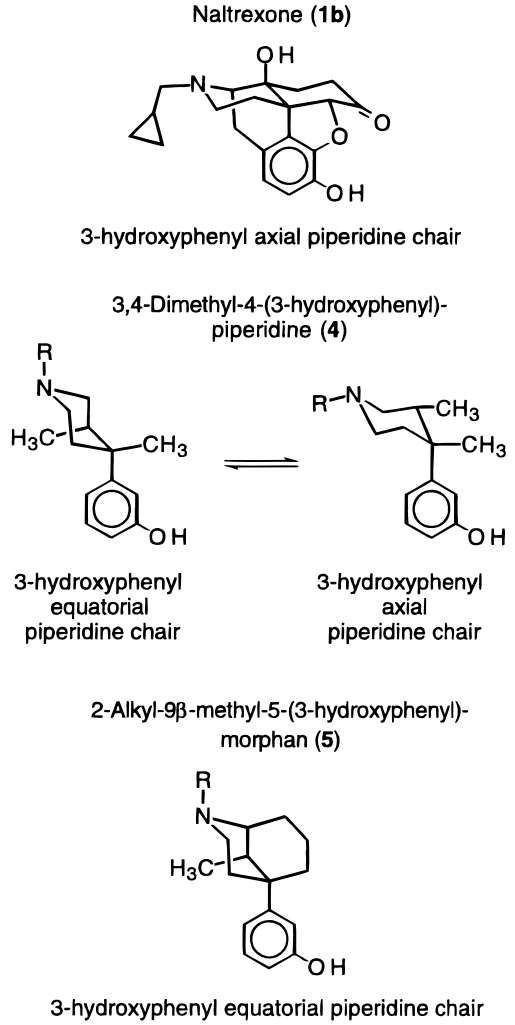


Figure 2. Conformational representation of naltrexone (**1b**), N-substituted 3,4-dimethyl-4-(3-hydroxyphenyl)piperidine, and 2-alkyl-9β-5-(3-hydroxyphenyl)morphan.

70-fold increase in antagonist potency at the μ, δ, and κ opioid systems. These results are particularly interesting since changing an *N*-methyl to an *N*-phenethyl substituent in all opioid systems which have the 3-hydroxyphenyl group in an axial relationship relative to the piperidine ring results in an increase in opioid agonist activity.[1] This information strongly suggests that **5b** and **5c** are acting as conformationally rigid analogues of the *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine class of opioid antagonists where the 3-hydroxyphenyl group is in an equatorial position relative to the piperidine ring.

In opioid alkaloids such as naloxone (**1a**) and naltrexone (**1b**), the 3-hydroxyphenyl ring is fixed in an axial orientation relative to the piperidine ring by the rigid framework of the structure (Figure 2). The 3-hydroxyphenyl ring in the 3,4-dimethyl-4-(3-hydroxyphenyl)piperidine analogues **4** can be in either axial or equatorial positions (Figure 2). ^{1}H and ^{13}C NMR studies[13,14] as well as molecular modeling studies[11] suggest a preference for the 3-hydroxyphenyl equatorial conformation. 5-(3-Hydroxyphenyl)morphans such as **5a**, **5b**, and **5c** are sterically constrained 4-(3-hydroxyphenyl)piperidines with the 3-hydroxyphenyl ring fixed in the equatorial position (Figure 2). The pure antagonist activity of the morphans **5b** and **5c** provides convincing evidence that opioid ligands of the phenylpiperidine class express potent opioid antagonist activity with their 3-hydroxyphenyl group in an equatorial position. Ong and co-workers[15] reported that changing the *N*-methyl group of the opioid agonist *N*-methyl-5-(3-hydroxyphenyl)morphan (**5d**) to an *N*-allyl- or *N*-cyclopropyl group (**5e** and **5f**) did not convert **5d** to an opioid antagonist. Thus, an equatorial 3-hydroxyphenyl group even when combined with an *N*-allyl- or *N*-cyclopropylmethyl substituent is not sufficient to express opioid antagonism. Since **5b** and **5d** differ only by the presence of a 9β-methyl group in **5b**, it is apparent that this 9β-methyl substituent is essential for pure opioid antagonist activity. Since both the *N*-methyl and *N*-phenethyl analogues (**5b** and **5c**, respectively) are pure opioid antagonists, the antagonist properties are not dependent on the N-substituent structure which is the same behavior seen in the *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine class of antagonists.

A comparison of the radioligand and [^{35}S]GTPγS binding properties of the N-substituted 9β-methyl-5-(3-hydroxyphenyl)morphans **5b** and **5c** to those of the N-substituted 3,4-dimethyl-4-(3-hydroxyphenyl)piperidines **4a** and **4b** strongly suggests that these two types of compounds are interacting with opioid receptors in a similar mode. The pure antagonist activity of **5b**, which is increased when the *N*-methyl group is replaced by a phenethyl group to give **5c**, properties unique to the 3,4-dimethyl-4-(3-hydroxyphenyl)piperidine class of antagonist, strongly supports the original hypothesis that this class of opioid antagonist expresses pure antagonist activity with the 4-(3-hydroxyphenyl) group in an equatorial conformation.[16]

The difference in SARs required for the N-substituents of the oxymorphone class of antagonists, which possess an axial 3-hydroxyphenyl group, and the 5-(3-hydroxyphenyl)morphan class, which has an equatorial 3-hydroxyphenyl group, can be accounted for by an extension of the model that Portoghese used to explain the N-substituent requirement differences between the benzomorphan and morphan classes of opioid agonists.[17,18] In a comparison of the opioid agonists of the phenylmorphan series (phenyl equatorial) to those of the benzomorphan series (phenyl axial), Portoghese proposed that differences in behavior between the two systems possessing the same N-substituents could be explained by assuming a common binding site for the phenyl rings in these compounds with the piperidine nitrogen ends of the molecules sharing a common ionic site of interaction but with their N-substituents situated in different receptor environments.[17,18] Viewed from this point, the very different behaviors associated with the N-substituents of the 3-hydroxyphenyl axial antagonists compared with the 3-hydroxyphenyl equatorial antagonists are easily understood. The N-substituent structure in the phenyl axial series is the trigger for antagonism, whereas the N-substituent structure in the phenyl equatorial series only governs potency. It is the axial 3-methyl substituent (phenylpiperidines) or the 9β-methyl group (phenylmorphans) which triggers antagonist behavior.

This assumed alignment for the two antagonist classes also provides an explanation for other differences

noted between the two classes of antagonists. For example, it is interesting to note that the N-substituted 5-(3-hydroxyphenyl)morphans **5b** and **5c** were more potent as inhibitors of agonist-stimulated [^{35}S]GTPγS binding than as inhibitors in the binding assay (compare data from Tables 1 and 3). In contrast, the radioligand binding and GTP binding data for naltrexone, particularly at the μ and κ opioid receptors, are very similar. In this regard, we recently reported that a series of N-substituted (3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidines such as **4c** and **4d** were also more potent as inhibitors of agonist-stimulated [^{35}S]GTPγS binding than as inhibitors in the radioligand binding assay.[8] While these differences could at least in part be linked to changes in tissue and radioligands, this does not account for the differences seen between the opioid ligands **5b**, **5c**, and **4c** with those of naltrexone (**1b**). This difference could be due to the fact that the N-substituents of the two classes of antagonists are interacting with different parts of the opioid receptor.

In summary, we have demonstrated that 9β-methyl-5-(3-hydroxyphenyl)morphans are a new structural type of pure opioid antagonist. The data also strongly support the proposed 4-(3-hydroxyphenyl) equatorial piperidine chair mode of interaction for the *trans*-3,4-dimethyl-(3-hydroxyphenyl)piperidine class of opioid antagonist.[5,16] This new antagonist class provides a more suitable structure on which to attach receptor subtype-selective recognition elements.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary tube apparatus and are not corrected. Elemental analyses were obtained by Atlantic Microlabs, Inc., and are within ±0.4% of the calculated values. ^{1}H NMR spectra were determined on a Bruker WM-250 spectrometer using tetramethylsilane as an internal standard. Silica gel 60 (230−400 mesh) was used for all column chromatography. All reactions were followed by thin-layer chromatography using Whatman silica gel 60 TLC plates and were visualized by UV or by charring using 5% phosphomolybdic acid in ethanol. All solvents were reagent grade. Tetrahydrofuran and diethyl ether were dried over sodium benzophenone ketyl and distilled prior to use.

The [^{3}H]DAMGO, DAMGO, and [^{3}H][D-Ala^2,D-Leu^5]enkephalin were obtained via the Research Technology Branch, NIDA, and were prepared by Multiple Peptide Systems (San Diego, CA). The [^{3}H]U69,593 and [^{35}S]GTPγS (s.a. = 1250 Ci/mmol) were obtained from DuPont New England Nuclear (Boston, MA). U69,593 was obtained from Research Biochemicals International (Natick, MA). Levallorphan was a generous gift from Kenner Rice, Ph.D., NIDDK, NIH (Bethesda, MD). GTPγS and GDP were obtained from Sigma Chemical Co. (St. Louis, MO). The sources of other reagents are published.[19]

1,2,3,4-Tetrahydro-4-allyl-1,5-dimethyl-4-(3-methoxyphenyl)pyridine (7). To a solution of 500 mg (2.3 mmol) of 1,2,6-trihydro-1,3-dimethyl-4-(3-methoxyphenyl)pyridine (**6**) in 15 mL of THF at −42 °C was added *s*-BuLi in cyclohexane (1.3 M, 2.9 mmol). After 1 h, allyl bromide (2.3 mmol) was added, and the color of the solution changed from dark red to yellow. After been stirred for 1 h at −42 °C, the mixture was allowed to warmed to 0 °C and then quenched with water (10 mL). Diethyl ether (10 mL) was added, and the aqueous layer was extracted with ether (2×). The combined ether layers were washed with water (10 mL), saturated $NaHCO_3$, and brine and dried over Na_2SO_4. Evaporation of solvent afforded 590 mg (~100%) of crude **7**. The crude product was used directly in the next step without further purification: ^{1}H NMR ($CDCl_3$) δ 7.26 (m, 1 H), 7.01 (m, 2 H), 6.74 (m, 1 H), 5.89 (s, 1 H), 5.82 (m, 1 H), 5.13 (m, 2 H), 3.80 (s, 3 H), 2.68−2.40 (m, 3 H), 2.55 (s, 3 H), 2.22 (m, 1 H), 1.66 (m, 2 H), 1.52 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 159.2, 151.1, 136.7, 135.8, 128.7, 119.8, 117.4, 114.3, 110.1, 107.7, 55.1, 46.1, 43.1, 43.0, 41.7, 36.4, 17.3.

2,9-Dimethyl-5-(3-methoxyphenyl)-2-azabicyclo[3.3.1]-non-3-ene (8a, 8b). A solution of 300 mg (1.17 mmol) of **7** in 6 mL of 85% H_3PO_4/HCO_2H (1:1) was stirred at room temperature for 72 h. The resulting dark-brown mixture was diluted with water (6 mL) and cooled in an ice bath while NaOH (25% w/w) was added until pH 8. The aqueous solution was extracted with $CHCl_3$ (3×). The combined organic layers were washed with aqueous $NaHCO_3$ and brine and dried over Na_2SO_4. Evaporation of the solvent gave 270 mg (90%) of crude products **8a** and **8b** in a ratio of 3:1. The crude products were used directly in the next step without further purification: ^{1}H NMR ($CDCl_3$) of the mixture δ 7.24−6.70 (m, 4 H), 6.16 (d, 1 H, J = 9.2 Hz), 4.34 (d, 1 H, J = 7.0 Hz), 4.13 (d, 1 H, J = 9.1 Hz), 3.80 (s, 3 H), 2.80 (s, 3 H), 3.10−1.40 (m, 8 H), 0.74 (d, 3 H, J = 8.6 Hz), 0.57 (d, 3 H, J = 8.1 Hz).

2,9β-Dimethyl-5-(3-methoxyphenyl)-2-azabicyclo[3.3.1]-nonane (9). A solution of 270 mg (1.05 mmol) of **8a** and **8b** mixture and acetic acid (1.05 mmol, 0.061 mL) in 5 mL of dichloroethane was treated with $NaBH(OAc)_3$ under N_2 atmosphere. The reaction was stirred at room temperature for 2 h. The reaction was quenched by adding 10% NaOH to pH ~ 10. The mixture was extracted with ether (3×) and washed with water and brine. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. Isolation of the major isomer by chromatography (1% Et_3N/EtOAc) gave 135 mg (50%) of **9** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 7.26 (m, 1 H), 6.94 (m, 2 H), 6.70 (m, 1 H), 3.80 (s, 3 H), 3.05−2.90 (m, 2 H), 2.71 (m, 1 H), 2.43 (s, 3 H), 2.42−2.30 (m, 2 H), 2.28−2.15 (m, 1 H), 2.00−1.35 (m, 6 H), 0.86 (d, 3 H, J = 8.25 Hz); ^{13}C NMR ($CDCl_3$) 159.2, 152.0, 128.9, 118.0, 112.3, 109.6, 59.7, 55.1, 51.1, 43.1, 42.5, 40.0, 38.3, 29.1, 25.6, 23.4, 14.8. Anal. ($C_{17}H_{25}NO$) C, H, N.

2,9β-Dimethyl-5-(3-hydroxyphenyl)-2-azabicyclo[3.3.1]-nonane (5b). Compound **9** was treated with 4 mL of glacial acetic acid and 4 mL of 48% aqueous hydrobromic acid at reflux temperature for 20 h. The reaction was cooled to room temperature and diluted with 10 mL of water. The pH was adjusted to 10 by using 50% NaOH with ice cooling. The product was extracted into a mixture of 3:1 1-butanol/toluene, dried over Na_2SO_4, and concentrated under reduced pressure. Separation by chromatography [50% (80% $CHCl_3$, 18% MeOH, 2% NH_4OH) in chloroform] provided 199 mg (84%) of **5b** as a white solid: ^{1}H NMR ($CDCl_3$) δ 7.15 (m, 1 H), 6.87−6.75 (m, 2 H), 6.61 (m, 1 H), 3.10−2.90 (m, 2 H), 2.77 (m, 1 H), 2.44 (s, 3 H), 2.50−2.30 (m, 2 H), 2.25−2.10 (m, 1 H), 2.00−1.60 (m, 5 H), 1.60−1.40 (m, 1 H), 0.80 (d, 3 H, J = 8.3 Hz); ^{13}C NMR ($CDCl_3$) δ 155.9.

The hydrochloride salt was prepared and crystallized from ether/methanol using 1 N HCl in ethyl ether: 152.0, 129.1, 117.5, 113.0, 112.4, 59.7, 51.0, 43.0, 42.0, 40.2, 38.0, 29.0, 25.6, 23.2, 14.6. The structure of this compound was determined by single-crystal X-ray analysis. Anal. ($C_{16}H_{24}ClNO$) C, H, N.

5-(3-Hydroxyphenyl)-9β-methyl-2-azabicyclo[3.3.1]-nonane (10). A solution of 200 mg (1.28 mmol) of phenyl chloroformate was added dropwise to 300 mg (1.16 mmol) of **9** in 10 mL of dichloromethane at room temperature under a nitrogen atmosphere. The reaction was heated to reflux for 6 h. Since the reaction was not complete by TLC, the solvent was then changed to dichloroethane and the reflux was continued for another 12 h. The mixture was cooled to room temperature and concentrated under reduced pressure. The resulting oil was treated with 10 mL of 1 N NaOH and stirred with slight warming for 15 min. The product carbamate was then extracted with ether, and the ether layer was washed with 1 N HCl and water. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was then treated with 5 mL of ethanol and 1.5 mL of 50% aqueous KOH at reflux for 70 h. The mixture was cooled and concentrated under reduced pressure. The resulting concen-

trate was extracted with ether (2×), and the ether layers were concentrated in vacuo. The resulting oil was dissolved into 10 mL of 1 N HCl and washed with ether. The aqueous layer was then made strongly basic (pH > 12) with 50% NaOH with ice cooling. The desired amine was extracted into ether (2×), and the ether extracts were washed, dried over Na_2SO_4, and concentrated under reduced pressure to give 207 mg (70%) of a light-yellow oil. This was treated with 4 mL of glacial acetic acid and 4 mL of 48% aqueous hydrobromic acid at reflux temperature for 20 h. The reaction was cooled to room temperature and diluted with 10 mL of water. The pH was adjusted to 10 by using 50% NaOH with ice cooling. The product was extracted into a mixture of 3:1 1-butanol/toluene, dried over Na_2SO_4, and concentrated under reduced pressure to yield 100 mg (51%) of **10** as a semisolid. The crude product **10** was used directly in the next step without further purification: ^{1}H NMR (CD_3OD) δ 7.15 (m, 1 H), 6.79−6.75 (m, 2 H), 6.65 (m, 1 H), 3.70−3.30 (m, 3 H), 2.70 (m, 1H), 2.45−1.70 (m, 8 H), 0.87 (d, 3 H, J = 8.3 Hz).

5-(3-Hydroxyphenyl)-9β-methyl-2-(2′-phenylethyl)-2-azabicyclo[3.3.1]nonane (5c). To a solution of 100 mg (0.43 mmol) of **10**, 190 mg (0.43 mmol) of BOP reagent, and 0.19 mL (1.38 mmol) of triethylamine in 15 mL of THF was added phenylacetic acid (70.25 mg, 0.52 mmol). The mixture was stirred at room temperature for 1 h. The reaction was diluted with 45 mL of water and ether (45 mL). The aqueous layer was extracted with ether (2×). The combined ether layers were washed with $NaHCO_3$ and brine and dried over Na_2SO_4. Evaporation of solvent provided the crude product as a colorless oil. The crude amide was dissolved in THF (8 mL). The solution was cooled to 0 °C, and borane−methyl sulfide complex (0.4 mL, 0.8 mmol) was added dropwise. After vigorous reaction ceased, the resulting mixture was slowly heated to reflux and maintained at that temperature for 4 h. The reaction mixture was cooled to 0 °C, 6 mL of methanol was added, and the mixture was stirred for 1 h. Anhydrous hydrogen chloride in ether (1 mL) was added to attain a pH < 2, and the resulting mixture was gently refluxed for 1 h. After the mixture was cooled to room temperature, methanol was added, and the solvents were removed on a rotovap. The residue obtained was made basic (pH > 12) by adding 25% NaOH and extracted with ether (3×). The combined ether layers were dried over Na_2SO_4 and concentrated under reduced pressure. Separation by chromatography (1% Et_3N/50% EtOAc/hexanes) gave 38 mg (71%) of amine **5c** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 7.30−7.14 (m, 6 H), 6.85 (m, 2 H), 6.63 (m, 1 H), 4.71 (br s, 1 H), 3.05 (m, 2 H), 2.88 (m, 1 H), 2.79 (s, 4 H), 2.43−2.15 (m, 3 H), 1.94−1.65 (m, 5 H), 1.65−1.45 (m, 1 H), 0.83 (d, 3 H, J = 8.2 Hz); ^{13}C NMR ($CDCl_3$) δ 155.7, 152.5, 140.9, 129.1, 128.8, 128.3, 125.9.

The hydrochloride salt was prepared and crystallized from ether/methanol using 1 N HCl in ethyl ether: 117.7, 113.0, 112.4, 57.4, 57.2, 49.5, 42.4, 40.0, 38.7, 34.1, 29.1, 26.2, 23.4, 14.7. Anal. ($C_{23}H_{30}ClNO$) C, H, N.

Opioid Binding Assays. μ Binding sites were labeled using [^{3}H][D-Ala^2-MePhe^4,Gly-ol^5]enkephalin ([^{3}H]DAMGO) (2.0 nM, s.a. = 45.5 Ci/mmol), and δ binding sites were labeled using [^{3}H][D-Ala^2,D-Leu^5]enkephalin (2.0 nM, s.a. = 47.5 Ci/mmol) using rat brain membranes prepared as described.[20] κ-1 binding sites were labeled using [^{3}H]U69,593 (2.0 nM, s.a. = 45.5 Ci/mmol) and guinea pig membranes pretreated with BIT and FIT to deplete the μ and δ binding sites.[19]

[^{3}H]DAMGO binding proceeded as follows: 12- × 75-mm polystyrene test tubes were prefilled with 100 μL of the test drug which was diluted in binding buffer (BB: 10 mM Tris-HCl, pH 7.4, containing 1 mg/mL BSA), followed by 50 μL of BB and 100 μL of [^{3}H]DAMGO in a protease inhibitor cocktail (10 mM Tris-HCl, pH 7.4), which contained bacitracin (1 mg/mL), bestatin (100 μg/mL), leupeptin (40 μg/mL), and chymostatin (20 μg/mL). Incubations were initiated by the addition of 750 μL of the prepared membrane preparation containing 0.2 mg/mL protein and proceeded for 4−6 h at 25 °C. The ligand was displaced by 10 concentrations of test drug, in triplicate, 2×. Nonspecific binding was determined using 20 μM levallorphan. Under these conditions, the K_d of [^{3}H]-DAMGO binding was 4.35 nM. Brandel cell harvesters were used to filter the samples over Whatman GF/B filters, which were presoaked in wash buffer (ice-cold 10 mM Tris-HCl, pH 7.4).

[^{3}H][D-Ala^2,D-Leu^5]enkephalin binding proceeded as follows: 12- × 75-mm polystyrene test tubes were prefilled with 100 μL of the test drug which was diluted in BB, followed by 100 μL of a salt solution containing choline chloride (1 M, final concentration of 100 mM), $MnCl_2$ (30 mM, final concentration of 3.0 mM), and, to block μ sites, DAMGO (1000 nM, final concentration of 100 nM), followed by 50 μL of [^{3}H][D-Ala^2,D-Leu^5]enkephalin in the protease inhibitor cocktail. Incubations were initiated by the addition of 750 μL of the prepared membrane preparation containing 0.41 mg/mL protein and proceeded for 4−6 h at 25 °C. The ligand was displaced by 10 concentrations of test drug, in triplicate, 2×. Nonspecific binding was determined using 20 μM levallorphan. Under these conditions the K_d of [^{3}H][D-Ala^2,D-Leu^5]enkephalin binding was 2.95 nM. Brandel cell harvesters were used to filter the samples over Whatman GF/B filters, which were presoaked in wash buffer (ice-cold 10 mM Tris-HCl, pH 7.4).

[^{3}H]U69,593 binding proceeded as follows: 12- × 75-mm polystyrene test tubes were prefilled with 100 μL of the test drug which was diluted in BB, followed by 50 μL of BB, followed by 100 μL of [^{3}H]U69,593 in the standard protease inhibitor cocktail with the addition of captopril (1 mg/mL in 0.1 N acetic acid containing 10 mM 2-mercaptoethanol to give a final concentration of 1 μg/mL). Incubations were initiated by the addition of 750 μL of the prepared membrane preparation containing 0.4 mg/mL protein and proceeded for 4−6 h at 25 °C. The ligand was displaced by 10 concentrations of test drug, in triplicate, 2×. Nonspecific binding was determined using 1 μM U69,593. Under these conditions the K_d of [^{3}H]U69,593 binding was 3.75 nM. Brandel cell harvesters were used to filter the samples over Whatman GF/B filters, which were presoaked in wash buffer (ice-cold 10 mM Tris-HCl, pH 7.4) containing 1% PEI.

For all three assays, the filtration step proceeded as follows: 4 mL of the wash buffer was added to the tubes, was rapidly filtered, and was followed by two additional wash cycles. The tritium retained on the filters was counted, after an overnight extraction into ICN Cytoscint cocktail, in a Taurus beta counter at 44% efficiency.

[^{35}S]GTPγS Binding Assay. Ten frozen guinea pig brains (Harlan Bioproducts for Science, Inc., Indianapolis, IN) were thawed, and the caudate putamen were dissected and homogenized in buffer A (3 mL/caudate) (buffer A: 10 mM Tris-HCl, pH 7.4 at 4 °C, containing 4 μg/mL leupeptin, 2 μg/mL chymostatin, 10 μg/mL bestatin, and 100 μg/mL bacitracin) using a polytron (Brinkman) at setting 6 until a uniform suspension was achieved. The homogenate was centrifuged at 30000*g* for 10 min at 4 °C and the supernatant discarded. The membrane pellets were washed by resuspension and centrifugation twice more with fresh buffer A, aliquotted into microfuge tubes, and centrifuged in a Tomy refrigerated microfuge (model MTX 150) at maximum speed for 10 min. The supernatants were discarded, and the pellets were stored at −80 °C until assayed.

For the [^{35}S]GTPγS binding assay, all drug dilutions were made up in buffer B (50 mM Tris-HCl, pH 7.7/0.1% BSA). Briefly, 12- × 75-mm polystyrene test tubes received the following additions: (a) 50 μL of buffer B with or without an agonist, (b) 50 μL of buffer B with or without 60 μM GTPγS for nonspecific binding, (c) 50 μL of buffer B with or without an antagonist, (d) 50 μL of salt solution which contained in buffer B 0.3 nM [^{35}S]GTPγS, 600 mM NaCl, 600 μM GDP, 6 mM dithiothreitol, 30 mM $MgCl_2$, and 6 mM EDTA, and (e) 100 μL of membranes in buffer B to give a final concentration of 10 μg/tube. The final concentrations of the reagents were 100 mM NaCl, 5 mM $MgCl_2$, 1 mM EDTA, 1 mM dithiothreitol, 100 μM GDP, 0.1% BSA, 0.05−0.1 nM [^{35}S]GTPγS, 500 nM or 10 μM agonists, and varying concentrations (at least 10 different concentrations) of antagonists. The reaction was

initiated by the addition of membranes and terminated after 4 h by addition of 3 mL of ice-cold (4 °C) purified water (Milli-Q UV-Plus, Millipore) followed by rapid vacuum filtration through Whatman GF/B filters presoaked in purified water. The filters were then washed once with 5 mL of ice-cold water. Bound radioactivity was counted by liquid scintillation spectroscopy using a Taurus (Micromedic) liquid scintillation counter at 98% efficiency after an overnight extraction in 5 mL of Cytoscint scintillation fluid. Nonspecific binding was determined in the presence of 10 μM GTPγS. Assays were performed in triplicate, and each experiment was performed at least 3 times.

Data Analysis. The data of the two separate experiments (opioid binding assays) or three experiments ([^{35}S]GTPγS assay) were pooled and fit, using the nonlinear least-squares curve-fitting language MLAB-PC (Civilized Software, Bethesda, MD), to the two-parameter logistic equation[21] for the best-fit estimates of the IC_{50} and slope factor. The K_i values were then determined using the equation: $IC_{50}/(1 + ([L]/K_d))$.

Single-Crystal X-ray Analysis of 5b. Crystals of **5b** were grown from ethyl ether/methanol. Data were collected on a computer-controlled automatic diffractometer, Siemens P4, with a graphite monochromator on the incident beam. Data were corrected for Lorentz and polarization effects, and a face-indexed absorption correction was applied. The structure was solved by direct methods with the aid of the program SHELXS[22] and refined by full-matrix least-squares on F_2 values using the program SHELXL.[22] The parameters refined included the coordinates and anisotropic thermal parameters for all non-hydrogen atoms. Hydrogen atoms on carbons 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. H angles were idealized and U_{iso}(H) values set at fixed ratios of U_{iso} values of bonded atoms. Coordinates were refined for H atoms bonded to nitrogen and oxygen. Additional experimental and structural analysis including an ORTEP figure and tables of atomic coordinates, bond lengths, and band angles are available as Supporting Information. Atomic coordinates are also available from the Cambridge Crystallographic Data Centre (Cambridge University Chemical Laboratory, Cambridge CB2 1EW, U.K.).

Acknowledgment. This research was supported by the National Institute on Drug Abuse, Grant DA09045.

Supporting Information Available: Crystal data, structural refinement analysis, atomic coordinates, bond lengths, bond angles, anisotropic displacement parameters, hydrogen coordinates, and isotropic displacement parameters of **5b** (6 pages). Ordering information is given on any current masthead page.

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JM980290I