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Synthesis, Opioid Receptor Binding, and Biological Activities of Naltrexone-Derived Pyrido- and Pyrimidomorphinans

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A series of pyrido- and pyrimidomorphinans (**6a**−**h** and **7a**−**g**) were synthesized from naltrexone and evaluated for binding and biological activity at the opioid receptors. The unsubstituted pyridine **6a** displayed high affinities at opioid δ, μ, and κ receptors with K_i values of 0.78, 1.5, and 8.8 nM, respectively. Compound **6a** was devoid of agonist activity in the mouse vas deferens (MVD) and guinea pig ileum (GPI) preparations but was found to display moderate to weak antagonist activity in the MVD and GPI with K_e values of 37 and 164 nM, respectively. The pyrimidomorphinans in general displayed lower binding potencies and δ receptor binding selectivities than their pyridine counterparts. Incorporation of aryl groups as putative δ address mimics on the pyrido- and pyrimidomorphinan framework gave ligands with significant differences in binding affinity and intrinsic activity. Attachment of a phenyl group at the 4′-position of **6a** or the equivalent 6′-position of **7a** led to dramatic reduction in binding potencies at all the three opioid receptors, indicating the existence of a somewhat similar steric constraint at the ligand binding sites of δ, μ, and κ receptors. In contrast, the introduction of a phenyl group at the 5′-position of **6a** did not cause any reduction in the binding affinity at the δ receptor. In comparison to the unsubstituted pyridine **6a**, the 5′-phenylpyridine **6c** showed improvements in μ/δ and κ/δ binding selectivity ratios as well as in the δ antagonist potency in the MVD. Interestingly, introduction of a chlorine atom at the *para* position of the pendant 5′-phenyl group of **6c** not only provided further improvements in δ antagonist potency in the MVD but also shifted the intrinsic activity profile of **6c** from an antagonist to that of a μ agonist in the GPI. Compound **6d** thus possesses the characteristics of a nonpeptide μ agonist/δ antagonist ligand with high affinity at the δ receptor (K_i = 2.2 nM), high antagonist potency in the MVD (K_e = 0.66 nM), and moderate agonist potency in the GPI (IC_{50} = 163 nM). Antinociceptive evaluations in mice showed that intracerebroventricular (icv) injections of **6d** produced a partial agonist effect in the 55 °C tail-flick assay and a full agonist effect in the acetic acid writhing assay (A_{50} = 7.5 nmol). No signs of overt toxicity were observed with this compound in the dose ranges tested. Moreover, repeated icv injections of an A_{90} dose did not induce any significant development of antinociceptive tolerance in the acetic acid writhing assay. The potent δ antagonist component of this mixed μ agonist/δ antagonist may be responsible for the diminished propensity to produce tolerance that this compound displays.

Introduction

The existence of at least three distinct subtypes of opioid receptors, designated μ, δ, and κ receptors, in the central nervous system and periphery is now well established.[1,2] Human μ, δ, and κ receptors have been cloned and have been shown to belong to the G protein-coupled receptor (GPCR) superfamily.[3−5] The development of potent and selective antagonist and agonist ligands for each of these opioid receptor subtypes has been the goal of medicinal chemists for many years because of their potential usefulness as pharmacological tools and as therapeutic agents.[6−8] In the search for subtype selective nonpeptide opioid ligands it has been found that a number of ligands synthetically derived from naltrexone display significant selectivity toward the δ receptors. Among these the indolomorphinan naltrindole (**1**, NTI) is presently widely used as δ selective antagonist ligand, and other ligands such as its 5′-isothiocyanate derivative (**2**, NTII), benzofuran analogue (**3**, NTB), and (*E*)-7-benzylidenenaltrexone (**4**, BNTX) have been useful in the pharmacological characterization of δ opioid receptor subtypes.[9−16] In an effort to delineate the factors contributing to the high affinity and δ selectivity of these ligands, Portoghese

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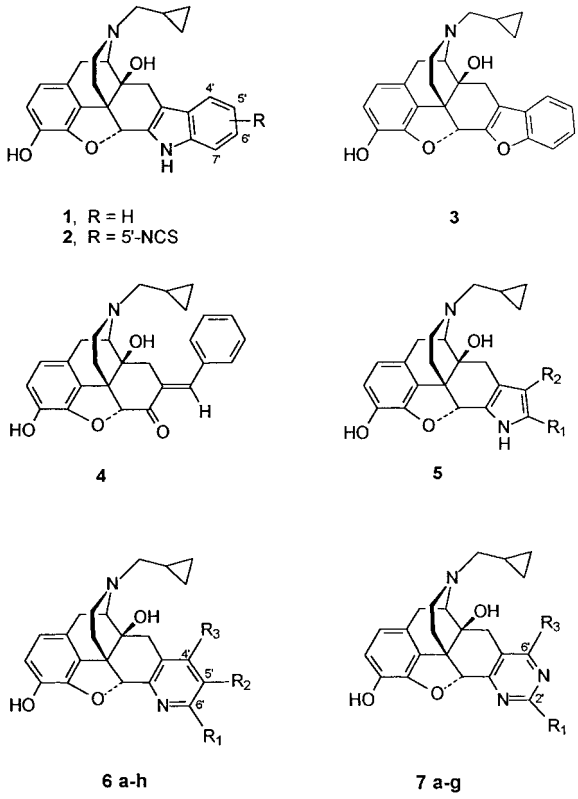
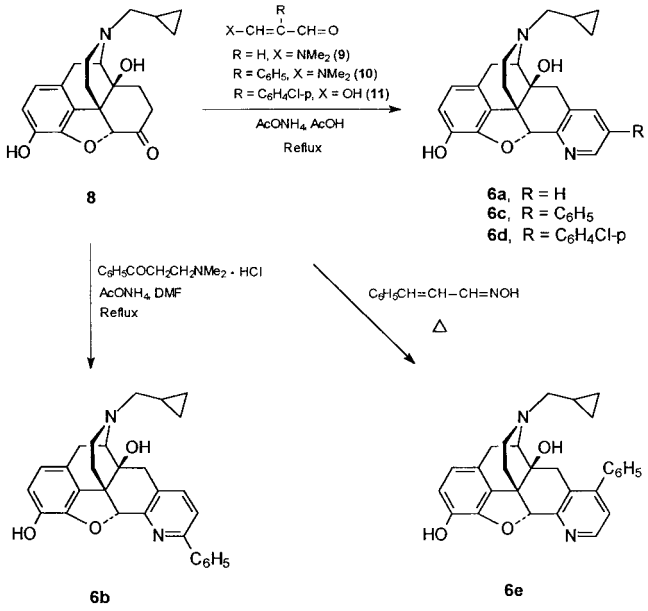
Chart 1



Scheme 1



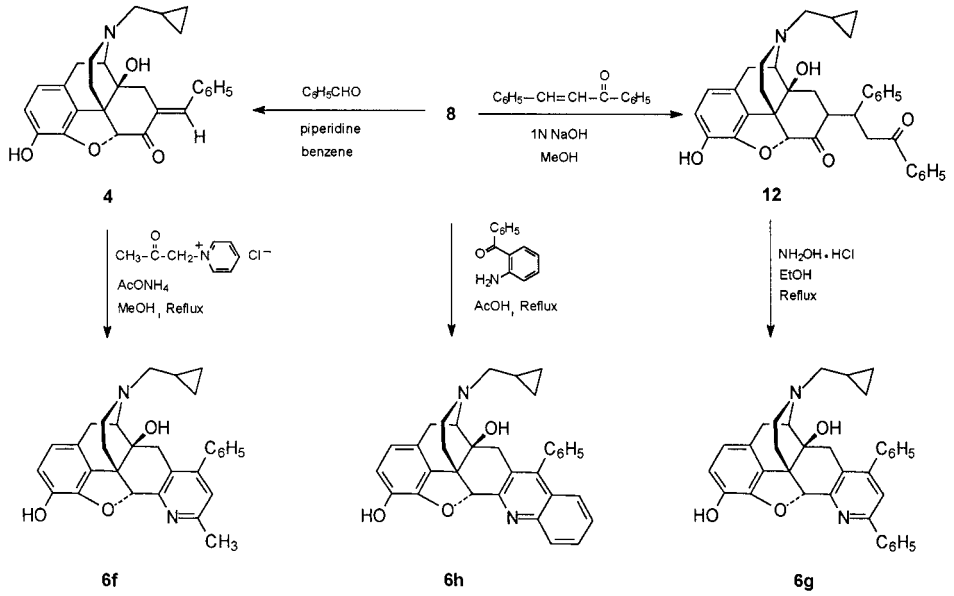
and co-workers have investigated the synthesis and evaluation of pyrrolomorphinans (**5**) carrying alkyl as well as phenyl groups that can potentially mimic the disposition of the free rotating phenyl group that is present in the δ antagonist **4**. On the basis of structure−activity relationship considerations it has been suggested that the δ selectivity displayed by some of the substituted pyrrolomorphinans can be attributed to the ability of the substituents (R_1 and R_2 in **5**) to hinder interaction at μ and κ receptors, while not affecting δ receptors.[17,18] In our research efforts directed toward the development of subtype selective nonpeptide opioid ligands,[19] we became interested in naltrexone-derived pyrido- and pyrimidomorphinans as novel molecular probes to study differences in binding and activation at the opioid δ, μ, and κ receptors. The choice of pyridine and pyrimidine heterocyclic units was based on considerations of synthetic access and the potential for substituent group variations on these templates. Since a rigid benzenoid or free rotating phenyl group, often referred to as the "address" element,[20,21] appears to be a key structural feature contributing to the δ receptor selectivity of the ligands such as **1**−**4**, we chose to focus initially on pyrido- and pyrimidomorphinan target compounds **6a**−**h** and **7a**−**g** (Chart 1) carrying phenyl substituents on the six-membered heterocyclic moiety.

Chemistry

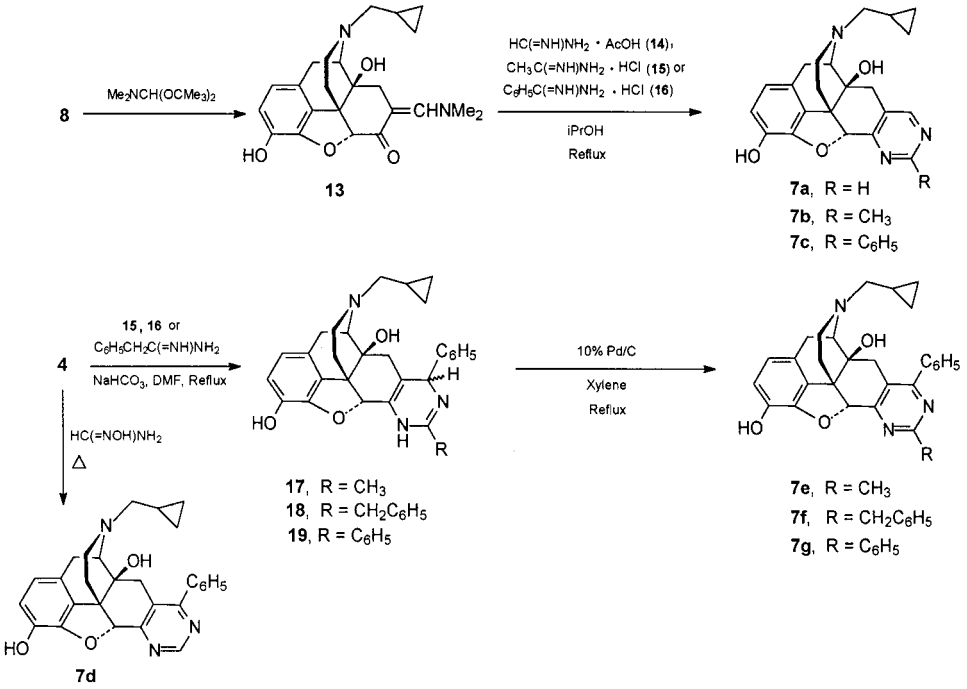
As shown in Scheme 1, the unsubstituted pyridine **6a** was synthesized from naltrexone (**8**) by condensation with 3-(dimethylamino)acrolein (**9**) and ammonium acetate in refluxing acetic acid, a method based on the reported procedure for one-step preparation of pyridines from ketones and 3-aminoacrolein.[22] The 5′-arylpyridines **6c** and **6d** were also prepared in a similar fashion using 2-phenyl-3-(dimethylamino)acrolein (**10**) or 2-(4-chlorophenyl)malondialdehyde (**11**) as the aldehyde component in the condensation reaction with **8**. The aldehydes **9** and **11** are commercially available, and **10** is readily obtainable by a literature procedure involving Vilsmeier−Haack formylation of phenylacetic acid.[23] The 6′-phenylpyridomorphinan **6b** was obtained by a pyridine annulation methodology involving the reaction of ketone **8** with dimethylaminopropiophenone hydrochloride in the presence of ammonium acetate.[24] Our initial attempts at the preparation and utilization of 3-*O*-benzyl-7-benzoylnaltrexone as a key 1,3-dicarbonyl intermediate for the synthesis of 4′-phenylpyridomorphinans **6e**−**g** proved unsatisfactory owing to the difficulties encountered in obtaining the diketone in acceptable yields. In alternative approaches that were explored, we were gratified to discover that the 4′-phenylpyridine **6e** could be obtained directly from naltrexone by a simple fusion reaction with cinnamaldoxime. The 6′-methyl-4′-phenylpyridine **6f** (Scheme 2) was synthesized by using benzylidenenaltrexone **4**[25] as the starting material in a condensation−cyclization reaction with 1-acetonylpyridinium chloride and ammonium acetate.[26] Base-catalyzed Michael addition of chalcone[27] to naltrexone (**8**) followed by cyclization of the resulting 1,5-diketone **12** with hydroxylamine hydrochoride[28] afforded the desired 4′,6′-diphenylpyridine **6g**. The 4′-phenylquinoline **6h** was readily obtained by Friedländer reaction[29] of naltrexone with 2-aminobenzophenone.

The 6′-unsubstituted pyrimidine target compounds **7a**−**c** were synthesized through the reaction of 7-(dimethylaminomethylene)naltrexone (**13**) with amidines[30] as shown in Scheme 3. The preparation of the enamino ketone **13** was best performed by reacting naltrexone (**8**) with the di-*tert*-butyl acetal of dimethylformamide since the reaction of **8** with the dimethyl acetal of dimethylformamide yields significant amounts of phenolic *O*-methylated derivative of **13**.[31] Benzylidenenaltrexone **4** was again utilized as the starting material for the synthesis of 6′-phenylpyrimidines **7d**−**g**. Condensation of **4** with acetamidine, phenylacetamidine, or

Scheme 2



Scheme 3



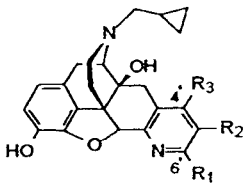
benzamidine yielded the corresponding dihydropyrimidines[32,33] **17**−**19** which were then aromatized to the pyrimidines **7e**−**g** by dehydrogenation with Pd/C in refluxing xylene.[34] The 2′-unsubstituted pyrimidine **7d**, however, could be obtained only in very low yields by this route. It occurred to us that the condensation of an α,β-usaturated ketone such as **4** with an amidoxime might directly yield aromatic pyrimidine through cyclocondensation and dehydration. Indeed, the thermal condensation of formamidoxime with **4** proved to be a facile synthetic method for the preparation of **7d**.

Biological Results and Discussion

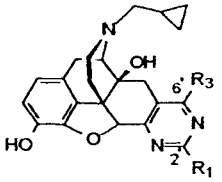
Opioid Receptor Binding and Bioassays in Smooth Muscle Preparations. The binding affinities of the target compounds for the μ and δ receptors were determined by inhibition of binding of [^{3}H]DAMGO[35] and [^{3}H]DADLE[36] to rat brain membranes. The affinities of the compounds for the κ receptors were determined by inhibition of binding of [^{3}H]U69,593[37] to guinea pig brain membranes. The δ, μ, and κ opioid receptor binding affinities along with binding selectivity ratios for the target compounds are given in Table 1. The opioid agonist and antagonist potencies of selected compounds were determined on the electrically stimulated mouse vas deferens (MVD) and guinea pig ileum (GPI) smooth muscle preparations as described previously.[19,38,39] The opioid antagonist and agonist potencies of the target compounds in the MVD and GPI are listed in Table 2.

Among the target compounds examined, the unsubstituted pyridomorphinan **6a** shows the highest binding

Table 1. Opioid Receptor Binding Affinities of Pyrido- and Pyrimidomorphinans in Homogenates of Rat or Guinea Pig Brain Membranes



6 a-h



7 a-g

				K_i (nM) ± SEM			selectivity ratio	
compd	R_1	R_2	R_3	δ[a]	μ[b]	k_1[c]	μ/δ	k_1/δ
6a	H	H	H	0.78 ± 0.06	1.5 ± 0.09	8.8 ± 0.69	1.9	11
6b	C_6H_5	H	H	1.76 ± 0.39	11.0 ± 0.65	18.4 ± 3.2	6.3	10
6c	H	C_6H_5	H	0.87 ± 0.07	13.5 ± 1.0	17.6 ± 1.6	16	20
6d	H	4-Cl−C_6H_4	H	2.2 ± 0.16	51.0 ± 8.0	20.0 ± 1.04	23	9.1
6e	H	H	C_6H_5	73.0 ± 8.0	191 ± 19	264 ± 21	2.6	3.6
6f	CH_3	H	C_6H_5	125 ± 9	154 ± 42	677 ± 63	1.2	5.4
6g	C_6H_5	H	C_6H_5	86.0 ± 9.0	652 ± 71	2116 ± 185	7.6	25
6h	CH=CH−CH=CH		C_6H_5	73.0 ± 8.5	308 ± 31	272 ± 6	4.2	3.7
7a	H		H	3.5 ± 0.24	4.15 ± 0.75	6.24 ± 0.74	1.2	1.8
7b	CH_3		H	22.7 ± 4.0	6.0 ± 0.5	25.0 ± 3.0	0.3	1.1
7c	C_6H_5		H	16.0 ± 4.0	22.0 ± 2.0	11.0 ± 1.4	1.4	0.7
7d	H		C_6H_5	230 ± 16	348 ± 67	216 ± 16	1.5	0.9
7e	CH_3		C_6H_5	325 ± 20	254 ± 50	565 ± 34	0.8	1.7
7f	$CH_2C_6H_5$		C_6H_5	100 ± 11	233 ± 44	1269 ± 243	2.3	13
7g	C_6H_5		C_6H_5	344 ± 38	1167 ± 169	2539 ± 167	3.4	7.4
8, naltrexone				39.5 ± 3.0	2.5 ± 0.21	7.0 ± 0.18	0.06	0.18
1, naltrindole				0.41 ± 0.09	99 ± 4.6	35.8 ± 4.0	241	87

[a] Displacement of [^{3}H]DADLE (1.3−2.0 nM) in rat brain membranes using 100 nM DAMGO to block binding to μ sites. [b] Displacement of [^{3}H]DAMGO (1.4−3.0 nM) in rat brain membranes. [c] Displacement of [^{3}H]U69,593 (1.2−2.2 nM) in guinea pig brain membranes.

Table 2. Opioid Antagonist and Agonist Potencies of Pyrido- and Pyrimidomorphinans in the MVD and GPI Preparations

	antagonist activity					agonist activity	
	DPDPE (δ)[a]		PL-017 (μ)[b]		K_e selectivity	MVD IC_{50} (nM)	GPI IC_{50} (nM)
compd	IC_{50} ratio	K_e (nM)[c]	IC_{50} ratio	K_e (nM)[c]	ratio μ/δ	or % max resp[d]	or % max resp[d]
6a	27.9 ± 1.2	37	7.08 ± 3.44	164	4.4	0%	0%
6b	41.1 ± 1.0	25	12.4 ± 2.8	88	3.5	0%	0%
6c	294 ± 82	3.4	24.6 ± 3.6	42	12	4.7%	0%
6d	1519 ± 797	0.66	*e*			21%	163 ± 22
6e	20.5 ± 1.2	51	6.21 ± 2.23	190	3.7	31%	14.8%
6g	21.2 ± 4.9	49	*f*			0%	0%
7a	14.4 ± 3.8	75	7.52 ± 5.1	150	2	0	0%
7b	7.6 ± 0.7	150	*f*			3	0%
7c	14.3 ± 1.5	75	34.1± 3.5	30	0.4	0%	4%
7d	4.3 ± 0.11	300	5.9 ± 3.2	204	0.7	15.4%	17%
7f	4.8 ± 1.32	260	23.8 ± 0.7	44	0.17	11.7%	14%
7g	7.63 ± 1.77	150	2.8 ± 1	560	3.7	0%	0%
1[g]	2000 ± 400	0.49	24 ± 2	43	88	16%	18%

[a] DPDPE in the MVD preparation. [b] PL-017 in the GPI preparation. [c] K_e (nM) = [antagonist]/(IC_{50} ratio − 1), where the IC_{50} ratio is the IC_{50} of the agonist in the presence of antagonist divided by the control IC_{50} in the same preparation ($n \geq 3$). [d] Partial agonist activity is expressed as the percentage inhibition of contraction at a concentration of 1 μM. [e] The agonist effects precluded the determination of antagonist effects. [f] IC_{50} ratio was not statistically different from 1. [g] Data from ref 19.

affinity at the δ receptor site with a K_i value in the subnanomolar range. The compound also displays high affinity binding at the μ and κ receptors with μ/δ and κ/δ binding selectivity ratios of 1.9 and 11, respectively. As compared to naltrexone (**8**), the pyridomorphinan **6a** binds with 50-fold higher affinity at the δ site and with nearly the same affinity as that of **8** at the μ and κ sites. The relatively low μ/δ and κ/δ binding selectivity of **6a**, as compared to that of NTI (**1**), can therefore be attributed to the inability of the pyridine ring of **6a** to interfere with and decrease the binding at the μ and κ receptors in a manner similar to that caused by the indole ring of NTI.

While the introduction of a phenyl group at the 6′- or 5′-position on the pyridine ring of **6a** (compounds **6b**, **6c**) did not significantly affect the binding potency, the introduction of a phenyl group at the 4′-position (**6e**) led to a dramatic (>90-fold) reduction in affinity at the δ receptor. Indeed all of the pyridines bearing a 4′-phenyl substituent (**6e**−**h**) displayed weak binding potencies at δ, μ, and κ receptors. These results suggest the presence of some common structural motif at the ligand binding site of μ, δ, and κ receptors that causes unfavorable steric interactions in the binding of C ring fused morphinans carrying a bulky substituent extending from a position adjacent to the 7-position. The binding profile of 6′- and 5′-aryl pyridines **6b**−**d** indicates that the aryl substituents, especially at the 5′-position of the pyridomorphinan framework, are better tolerated at the δ site than they are at the μ site,

thus leading to improved μ/δ biding selectivities. Comparison of the binding profiles of the pyrimidines with sterically equivalent pyridines shows parallel trends in structure−affinity relationships. The pyrimidines in general display lower δ receptor binding affinities and μ/δ and κ/δ binding selectivity ratios than their pyridine counterparts, **6a** vs **7a**, **6b** vs **7c**, **6e** vs **7d**, **6f** vs **7e**, and **6g** vs **7g**.

In the smooth muscle assays, all of the compounds tested displayed antagonist activity in the MVD. The antagonist potencies of the pyrimidines in general are lower than those of the pyridines. While **6a** is nearly one-half as potent as NTI in binding at the δ receptor, it is 75-fold weaker than NTI as an antagonist in the MVD. It has earlier been suggested that the presence of a benzenoid aromatic ring system either in a coplanar or noncoplanar orientation with the C ring of morphinan in NTI or 7-spiroindanylnaltrexone, respectively, contributes to the high δ antagonist potency of these ligands.[40] The absence of such an aromatic moiety that can interact and stabilize the antagonist state of δ receptor probably accounts for the weak δ antagonist potency of **6a**. Interestingly, while the introduction of a phenyl group at the 6′-position of the pyridomorphinan template did not significantly affect the antagonist potency (**6a**, K_e = 37 nM; **6b**, K_e = 25 nM), the introduction of a phenyl group at the 5′-position (**6c**) led to a 10-fold enhancement in δ antagonist potency in the MVD and a 3-fold increase in δ over μ antagonist selectivity. Despite these improvements, the δ antagonist potency and δ antagonist selectivity of **6c** are lower than those of NTI (**6c**, K_e = 3.4 nM, K_e μ/K_e δ = 12; **1**, K_e = 0.49 nM, K_e μ/K_e δ = 88). At the ligand binding sites, the 5′-phenyl group of **6c** can occupy the same lipophilic pocket as that occupied by the indolic benzene ring of NTI. However, the phenyl group of **6c** is likely to probe a more extended region than the benzenoid ring of NTI due to the fact that the phenyl group of **6c** is attached to the pyridine ring by a single bond whereas the indolic benzene ring of NTI is directly fused to the pyrrole unit. Moreover, while the indolic benzene moiety of NTI is rigidly held in a coplanar orientation with the C ring of morphinan, the 5′-phenyl group of **6c** is conformationally mobile and is likely to adopt an orientation that is not coplanar with the C ring of morphinan. These structural differences as well as differences in physicochemical properties between **6c** and NTI may be contributing factors for the potency and selectivity differences between these two ligands.

Interestingly, the introduction of a chlorine atom at the *para* position in the 5′-phenyl ring of **6c** conferred further enhancement in δ antagonist potency. Thus, among the compounds studied, the chlorophenyl compound **6d** is the most potent δ antagonist ligand with a K_e of 0.66 nM. Surprisingly, while all other compounds were devoid of significant agonist activity in MVD or GPI, **6d** functioned as a full agonist in the GPI with an IC_{50} of 163 nM (0.64 × morphine).[39] In the presence of 1 μM CTAP, a μ opioid selective antagonist, the dose−response curve of **6d** in the GPI was shifted rightward 5.1-fold. Testing in the presence of nor-BNI, a κ opioid selective antagonist, shifted the dose−response curve 1.6-fold rightward. At 1 μM concentration, the nonselective opioid receptor antagonist naloxone shifted the dose−response curve 5.2-fold to the right. These data show that the agonist activity of **6d** in the GPI is mediated through the opioid μ receptors. While the precise reason for the dramatic change in the intrinsic activity profile from δ and μ antagonist to a mixed δ antagonist/μ agonist brought about by the introduction of a chlorine atom on the pendant phenyl ring of **6c** remains unclear, it is worth noting that similar substitution-induced changes in intrinsic activity profiles have been reported in studies with ligands interacting with other receptor systems.[41−43]

Pharmacological Evaluations in Animals. Studies using rodents have demonstrated that δ opioid antagonists such as NTI and TIPP can prevent the development of tolerance and dependence to μ agonists such as morphine without antagonizing μ receptor mediated antinociception.[44−46] On the basis of these observations it has been suggested that the development of compounds possessing mixed μ agonist/δ antagonist properties may have considerable therapeutic potential as analgesic drugs with low propensity to produce tolerance and dependence side effects.[44,47,48] Among the compounds studied, the chlorophenylpyridine **6d** emerged as a compound of interest since it displayed the characteristics of a nonpeptide opioid ligand possessing mixed μ agonist/δ antagonist properties. Therefore this compound was evaluated for antinociceptive activity in mice. In the 55 °C tail-flick test (high-intensity stimulus) compound **6d**, administered by intracerebroventricular (icv) injections, was a partial agonist with an A_{50} value greater than 100 nmol (Figure 1). In the acetic acid writhing assay **6d** displayed full agonist activity with a calculated A_{50} value of 7.5 nmol. Morphine, a prototypic μ agonist, produced a full agonist effect following icv injection in both the 55 °C tail-flick and acetic acid writhing assays. The calculated A_{50} values for morphine were 2.94 nmol in the tail-flick and 0.004 nmol in the acetic acid writhing assays. Using a standard tolerance regimen, repeated icv injections of an A_{90} dose of morphine (×2 daily for 3 days) produced a significant rightward shift in the antinociceptive dose−response curve (12.5-fold), indicating the development of tolerance (Figure 2). Repeated icv injections of an A_{90} dose of **6d** on the other hand did not produce a significant rightward shift (<1.5-fold) in the antinociceptive dose−response curve. This indicates that compound **6d** may produce limited or no antinociceptive tolerance. The lack of development of tolerance to the antinociceptive effects of **6d** may be related to the mixed μ agonist/δ antagonist profile of this compound.

Summary and Conclusions

A series of novel pyrido- and pyrimidomorphinan ligands synthetically derived from naltrexone were prepared and evaluated for biological activities at the opioid receptors. We have found that the annulation of a pyridine or pyrimidine ring on the C ring of naltrexone markedly increases the binding affinity at the opioid δ receptors. The pyridomorphinans in general display higher affinities and greater δ receptor binding selectivities than the pyrimidines. Introduction of free rotating phenyl groups as putative δ address mimics on the pyrido- and pyrimidomorphinan template produced varying effects on the affinity as well as the activity

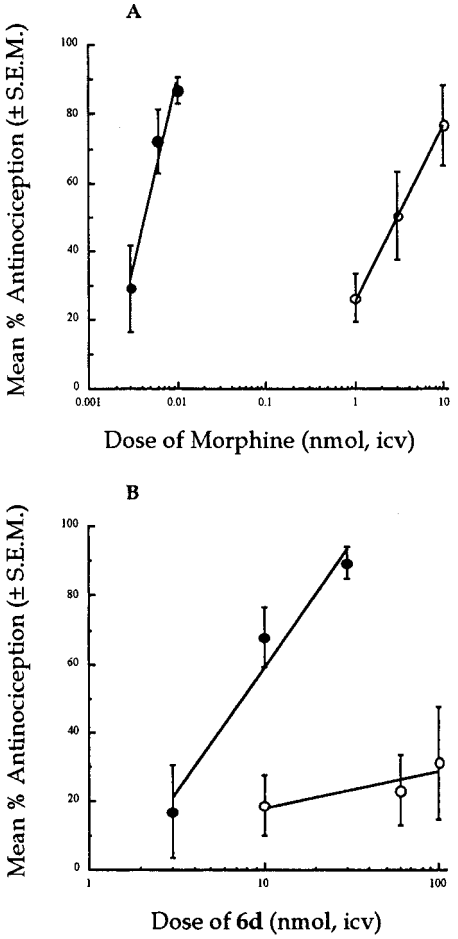


Figure 1. Antinociceptive dose−response curves for (A) icv morphine and (B) icv **6d** in the 55 °C tail-flick (open circles) and acetic acid writhing assays (closed circles).

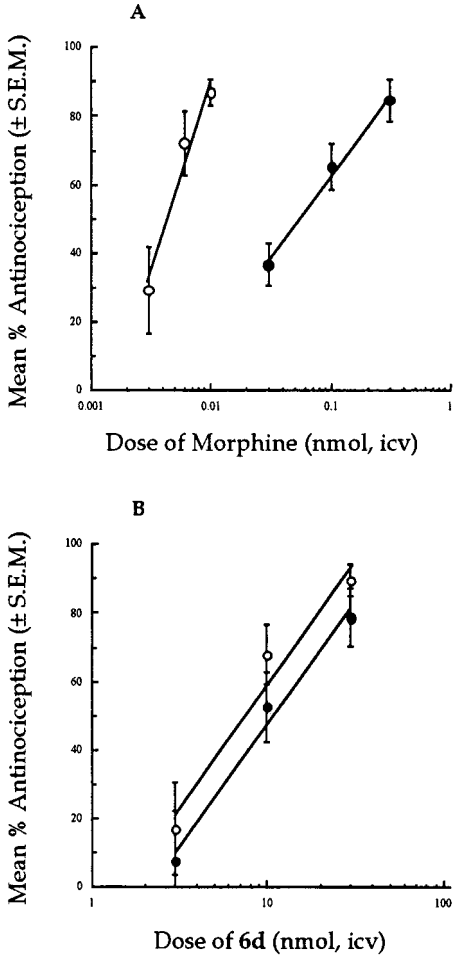


Figure 2. Antinociceptive dose−response curves for (A) icv morphine and (B) icv **6d** in the acetic acid writhing assay in naive control mice (open circles) and mice injected repeatedly (closed circles) with morphine or **6d**. Approximate A_{90} doses of morphine (0.01 nmol) or **6d** (30 nmol) were given twice daily for 3 days.

profiles depending upon the position of attachment of the phenyl group. On the pyridomorphinan template, while the introduction of a phenyl group at the 6′-position does not significantly affect the binding profile, the placement of phenyl group at the 4′-position leads to a profound decrease in binding affinities due to unfavorable steric interactions at the binding sites at δ, μ, and κ receptors. In contrast, the introduction of a phenyl group at the 5′-position improves the δ receptor binding selectivity as well as the δ antagonist potency. Surprisingly, the placement of a 4-chlorophenyl group at the 5′-position yielded a ligand (**6d**) possessing high binding potency at the δ receptor, high δ antagonist potency in bioassays in the MVD, and moderate μ agonist potency in the GPI. In the antinociceptive studies, **6d** displayed partial agonist activity in the tail-flick assay and a full agonist activity in the acetic acid writhing assay. Of significant interest is the finding that this compound, possessing mixed μ agonist/δ antagonist properties, did not induce tolerance to its antinociceptive effects and did not display any overt signs of toxicity in mice in the dose ranges tested. The results of the present study suggest that molecular manipulations such as substituent variations at the 5′-position of the pyridomorphinan template may provide novel ligands with improved δ antagonist or mixed μ agonist/δ antagonist activity profiles at the opioid receptors.

Experimental Section

General Methods. Melting points were determined in open capillary tubes with a Mel-Temp melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Nicolet 300NB spectrometer operating at 300.635 MHz. Chemical shifts are expressed in parts per million downfield from tetramethylsilane. Spectral assignments were supported by proton decoupling. Mass spectra were recorded on a Varian MAT 311A double-focusing mass spectrometer in the fast atom bombardment (FAB) mode. Elemental analyses were performed by Atlantic Microlab, Inc. (Atlanta, GA) or the Spectroscopic and Analytical Laboratory of Southern Research Institute. Analytical results indicated by elemental symbols were within ±0.4% of the theoretical values. Thin-layer chromatography (TLC) was performed on Analtech silica gel GF 0.25 mm plates. Reverse phase TLC was performed using 0.2 mm Whatman PLKC18F silica gel 60 Å plates. Flash column chromatography was performed with E. Merck silica gel 60 (230−400 mesh). All organic extracts were dried over anhydrous Na_2SO_4 and concentrated to dryness on a rotary evaporator under reduced pressure. Naltrexone hydrochloride was obtained from Mallinckrodt. Cinnamaldehyde oxime was purchased from Lancaster, and 1-acetonylpyridinium chloride was obtained from TCI America. 2-(4-Chlorophenyl)malondialdehyde was purchased from Acros Organics. All other reagents were obtained from Aldrich Chemical Co., or Fluka.

Chemistry. General Procedures. 17-(Cyclopropylmethyl)-6,7-didehydro-3,14-dihydroxy-4,5α-epoxypyrido-[2′,3′:6,7]morphinan (6a). To a solution of naltrexone (**8**)

(1.02 g, 3.0 mmol) and ammonium acetate (0.92 g, 12.0 mmol) in glacial acetic acid (20 mL) was added 3-(dimethylamino)-acrolein (0.6 mL, 6.0 mmol), and the mixture was stirred under reflux at 135−140 °C (oil bath) for 6 h. After cooling, the reaction mixture was concentrated under reduced pressure, the residue was suspended in water (20 mL), and the pH of the mixture was adjusted to 7 with concentrated aqueous NH_4-OH. The resulting suspension was extracted with $CHCl_3$ (3 × 80 mL) and washed with water (160 mL). The extract was dried, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography over a column of silica using $CHCl_3$−MeOH (98:2) as the eluent followed by recrystallization from EtOAc to obtain **6a** (0.58 g, 51%): mp 207−209 °C dec; TLC R_f 0.6 ($CHCl_3$−MeOH, 9:1); ^{1}H NMR (DMSO-d_6) δ 0.12−0.17 and 0.46−0.52 (2m, 4H, cyclopropyl CH_2CH_2), 0.83−0.94 (m, 1H, cyclopropyl C*H*), 1.57 (app d, 1H, J = 12.5 Hz, C-15 H), 2.18 (app td, 1H, J = 12.0, 2.5 Hz, C-15 H), 2.29 (app dd, 1H, J = 12.8, 4.8 Hz, C-16 H), 2.39 (app d, 2H, J = 6.4 Hz, NCH_2-cyclopropyl), 2.55−2.73 (m, 4H, C-8 H_2, C-10 H and C-16 H), 3.08 (app d, 1H, J = 18.5 Hz, C-10 H), 3.22 (app d, 1H, J = 6.2 Hz, C-9 H), 4.77 (bs, 1H, C-14 OH), 5.30 (s, 1H, C-5 H), 6.50 (app s, 2H, C-1 H and C-2 H), 7.25 (dd, 1H, J = 7.7, 4.6 Hz, C-5′ H), 7.47 (dd, 1H, J = 7.7, 1.3 Hz, C-4′ H), 8.49 (dd, 1H, J = 4.6, 1.5 Hz, C-6′ H), 9.04 (bs, 1H, C-3 OH); MS m/z 377 $(MH)^+$. Anal. ($C_{23}H_{24}N_2O_3$·0.5H_2O) C, H, N.

17-(Cyclopropylmethyl)-6,7-didehydro-3,14-dihydroxy-4,5α-epoxy-6′-phenylpyrido[2′,3′:6,7]morphinan (6b). A solution of naltrexone (**8**) (1.02 g, 3.0 mmol) in DMF (15 mL) was heated to reflux under argon. To the solution was added in portions a suspension of 3-(dimethylaminopropiophenone) hydrochloride (0.77 g, 3.6 mmol) and ammonium acetate (1.2 g, 15.6 mmol) in DMF (10 mL) over a period of 45 min. The refluxing was continued for an additional 4 h. The mixture was allowed to cool to room temperature and stir overnight. The mixture was diluted with water (20 mL), the pH was adjusted to 8 with 1 N aqueous NH_4OH, and the mixture was extracted with CH_2Cl_2. The extract was washed with saturated aqueous NaCl and dried, and the solvent was removed under reduced pressure. The residue was purified by chromatography over a column of silica, using $CHCl_3$−MeOH−NH_4OH (98:1.5:0.5) as the eluent, followed by recrystallization from CH_2Cl_2 to yield **6b** (0.44 g, 33%): mp 156−158° C dec; TLC R_f 0.43 ($CHCl_3$−MeOH−NH_4OH, 95:5:0.5); ^{1}H NMR (DMSO-d_6) δ 0.13−0.18 and 0.47−0.54 (2m, 4H, cyclopropyl CH_2CH_2), 0.85−0.97 (m, 1H, cyclopropyl C*H*), 1.60 (app d, 1H, J = 12.3 Hz, C-15 H), 2.20 (app td, 1H, J = 12, 3.0 Hz, C-15 H), 2.33 (app dd, 1H, J = 12.5, 4.6 Hz, C-16 H), 2.40 (app d, 2H, J = 6.4 Hz, NCH_2-cyclopropyl), 2.58−2.72 (m, 4H, C-8 H_2, C-10 H and C-16 H), 3.10 (d, 1H, J = 18.5 Hz, C-10 H), 3.25 (app d, 1H, J = 6.2 Hz, C-9 H), 4.80 (bs, 1H, C-14 OH), 5.40 (s, 1H, C-5 H), 6.52 (app s, 2H, C-1 H, C-2 H), 7.41−7.54 (m, 3H, C-3″ H, C-4″ and C-5″ H), 7.58 (d, 1H, J = 8.1 Hz, C-4′ H), 7.83 (d, 1H, J = 8.1 Hz, C-5′ H), 8.09 (dd, 2H, J = 6.8, 1.5 Hz, C-2″ H and C-6″ H), 9.05 (br s, 1H, C-3 OH); MS m/z 453 $(MH)^+$. Anal. ($C_{29}H_{28}N_2O_3$·0.75H_2O) C, H, N.

17-(Cyclopropylmethyl)-6,7-didehydro-3,14-dihydroxy-4,5α-epoxy-5′-phenylpyrido[2′,3′:6,7]morphinan (6c). A stirred mixture of naltrexone (**8**) (1.0 g, 2.9 mmol), 3-(dimethylamino)-2-phenylacrolein (1.03 g, 5.9 mmol), and ammonium acetate (0.9 g, 11.2 mmol) in AcOH (15 mL) was heated to reflux in an oil bath at 130−135 °C under an argon atmosphere for 26 h. The solvent was removed under reduced pressure, and the residue was partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$. The layers were separated, the aqueous layer was extracted with CH_2Cl_2, the combined extracts were washed with saturated aqueous NaCl and dried, and the solvent was removed. The crude product thus obtained was chromatographed on a column of silica using $CHCl_3$−MeOH−NH_4OH (99:0.5:0.5) as the eluent. Fractions containing the desired product were combined, the solvent was removed, and the residue was purified by preparative reverse phase TLC using CH_3CN as the solvent to obtain **6c** (0.10 g, 8%): mp >158° C dec; TLC R_f 0.44 ($CHCl_3$−MeOH−NH_4OH, 95:5:0.5); ^{1}H NMR (DMSO-d_6) δ 0.12−0.18 and 0.47−0.51 (2m, 4H, cyclopropyl CH_2CH_2), 0.79−0.90 (m, 1H, cyclopropyl C*H*), 1.60 (app d, 1H, J = 12.1 Hz, C-15 H), 2.15−2.25 (m, 1H, C-15 H), 2.33 (app dd, 1H, J = 12.7, 4.6 Hz, C-16 H), 2.40 (app d, 2H, J = 6.4 Hz, NCH_2-cyclopropyl), 2.60−2.75 (m, 4H, C-8 H_2, C-10 H and C-16 H), 3.10 (d, 1H, J = 18.5 Hz, C-10 H), 3.26 (app d, 1H, J = 6.4 Hz, C-9 H), 4.82 (bs, 1H, C-14 OH), 5.36 (s, 1H, C-5 H), 6.52 (app s, 2H, C-1 H and C-2 H), 7.41−7.51 (m, 3H, C-3″ H, C-4″ and C-5″ H), 7.69 (dd, 2H, J = 7.0, 1.5 Hz, C-2″ H and C-6″ H), 7.74 (d, 1H, J = 2.0 Hz, C-4′ H), 8.79 (d, 1H, J = 2.2 Hz, C-6′ H), 9.04 (br s, 1H, C-3 OH); MS m/z 453 $(MH)^+$. Anal. ($C_{29}H_{28}N_2O_3$·0.25H_2O) C, H, N.

5′-(4-Chlorophenyl)-17-(cyclopropylmethyl)-6,7-didehydro-3,14-dihydroxy-4,5α-epoxypyrido[2′,3′:6,7]morphinan (6d). A stirred mixture of naltrexone hydrochloride (**8**·HCl) (4.72 g, 12.5 mmol), 2-(4-chlorophenyl)malondialdehyde (2.5 g, 13.7 mmol), and ammonium acetate (1.93 g, 25 mmol) in AcOH (75 mL) was heated to reflux in an oil bath at 130−135 °C under an argon atmosphere until TLC analysis of the reaction mixture using EtOAc:cyclohexane:Et_3N (1:1:0.02) as the solvent system indicated complete disappearance of naltrexone (approximately 20 h). The reaction mixture was cooled to room temperature, and the solvent was removed under reduced pressure. The residue was treated with water, and the pH of the mixture was adjusted to 8 with saturated aqueous $NaHCO_3$. The solid that separated was collected by filtration and dried. The crude product was chromatographed over a column of silica, using $CHCl_3$−MeOH (98:2) as the eluent, and then recrystallized from EtOAc/cyclohexane to give **6d** (2.12 g, 35%): mp >175 °C dec; TLC R_f 0.45 ($CHCl_3$−MeOH, 97:3); ^{1}H NMR ($CDCl_3$) δ 0.15−0.19 and 0.56−0.61 (2m, 4H, cyclopropyl CH_2CH_2), 0.83−0.93 (m, 1H, cyclopropyl C*H*), 1.81−1.88 (m, 1H, C-15 H), 2.33−2.53 (m, 4H, C-15 H, C-16 H and NCH_2-cyclopropyl), 2.61−2.84 (m, 4H, C-8 H_2, C-10 H and C-16 H), 3.17 (app d, 1H, J = 18.6 Hz, C-10 H), 3.31 (app d, 1H, J = 6.3 Hz, C-9 H), 4.5−5.5 (broad hump, 2H, C-3 OH and C-14 OH), 5.59 (s, 1H, C-5 H), 6.59 and 6.68 (AB−System, 2H, J = 8.1 Hz, C-1 H and C-2 H), 7.38−7.44 (m, 4H, C-2″ H, C-3″ H, C-5″ and C-6″ H), 7.48 (d, 1H, J = 2.2 Hz, C-4′ H), 8.69 (d, 1H, J = 1.9 Hz, C-6′ H); MS m/z 487 $(MH)^+$. Anal. ($C_{29}H_{27}ClN_2O_3$) C, H, N, Cl.

17-(Cyclopropylmethyl)-6,7-didehydro-3,14-dihydroxy-4,5α-epoxy-4′-phenylpyrido[2′,3′:6,7]morphinan (6e). A mixture of naltrexone (**8**) (2.0 g, 5.9 mmol) and cinnamaldehyde oxime (4.8 g, 32.6 mmol) was heated in an oil bath at 140 °C under an argon atmosphere for 8 h and cooled to room temperature. The mixture was partitioned between CH_2Cl_2 and 1 N aqueous HCl. The organic layer was extracted twice with 1 N aqueous HCl, and the combined acid extracts were cooled in ice/water bath and basified to pH 10 with concentrated aqueous NH_4OH. The resulting mixture was extracted three times with CH_2Cl_2. The combined organic extracts were washed with saturated aqueous NaCl and dried. The solvent was removed, and the residue was chromatographed over a column of silica using $CHCl_3$−MeOH−NH_4OH (99:1.0:0.2) as the eluent. The product was recrystallized from $CHCl_3$ to give **6e** (0.624 g, 24%): mp >156° C dec; TLC R_f 0.40 ($CHCl_3$−MeOH−NH_4OH, 95:5:0.5); ^{1}H NMR (DMSO-d_6) δ 0.07−0.10 and 0.42−0.46 (2m, 4H, cyclopropyl CH_2CH_2), 0.78−0.90 (m, 1H, cyclopropyl C*H*), 1.58 (app d, 1H, J = 11.7 Hz, C-15 H), 2.17−2.38 (m, 4H, C-15 H, C-16 H and NCH_2-cyclopropyl), 2.44 (app s, 2H, C-8 H_2), 2.58 (dd, 1H, J = 18.8, 6.7 Hz, C-10 H), 2.62−2.66 (m, 1H, C-16 H), 3.03 (app d, 1H, J = 18.8 Hz, C-10 H), 3.09 (app d, 1H, J = 6.2 Hz, C-9 H), 4.7 (bs, 1H, C-14 OH), 5.37 (s, 1H, C-5 H), 6.52 and 6.55 (AB−System, 2H, J = 8.2 Hz, C-1 H and C-2 H), 7.19 (d, 1H, J = 5.0 Hz, C-5′ H), 7.21−7.24 (m, 2H, C-2″ and C-6″ H), 7.40−7.47 (m, 3H, C-3″ H, C-4″ H and C-5″ H), 8.55 (d, 1H, J = 5.0 Hz, C-6′ H), 9.06 (br s, 1H, C-3 OH); MS m/z 453 $(MH)^+$. Anal. ($C_{29}H_{28}N_2O_3$·0.25H_2O) C, H, N.

17-(Cyclopropylmethyl)-6,7-didehydro-3,14-dihydroxy-4,5α-epoxy-6′-methyl-4′-phenylpyrido[2′,3′:6,7]morphinan (6f). Under an argon atmosphere, a mixture of 7-benzylidenenaltrexone (**4**) (0.45 g, 1.05 mmol) and 1-acetonylpyri-

dinium chloride (0.18 g, 1.05 mmol) and ammonium acetate (0.73 g, 9.45 mmol) in MeOH (7 mL) was heated under refux for 48 h. The solvent was removed, and the residue was dissolved in CH_2Cl_2. The solution was clarified by filtration. Removal of CH_2Cl_2 and chromatographic purification of the residue on a column of silica using $CHCl_3$−MeOH (99:1 to 97:3 gradient) gave 0.275 g (56%) of the product. A solution of this compound in EtOH was treated with 1.0 M solution of hydrogen chloride in ether to obtain the dihydrochloride of **6f**: mp >240 °C dec; TLC R_f 0.38 ($CHCl_3$−MeOH−NH_4OH, 95:5:0.5); ^{1}H NMR of the free base ($CDCl_3$) δ 0.09−0.13 and 0.49−0.55 (2m, 4H, cyclopropyl CH_2CH_2), 0.78−0.89 (m, 1H, cyclopropyl C*H*), 1.78−1.85 (m, 1H, C-15 H), 2.26−2.51 (m, 5H, C-8 H, C-15 H, C-16 H and NCH_2-cyclopropyl), 2.55 (s, 3H, C-6′ CH_3), 2.51−2.66 (m, 3H, C-8 H, C-10 H, C-16 H), 2.68−2.80 (m, 1H, C-16 H), 3.09 (app d, 1H, J = 18.5 Hz, C-10 H), 3.18 (app d, 1H, J = 6.6 Hz, C-9 H), 4.8−5.2 (bs, 2H, C-3 OH and C-14 OH), 5.57 (s, 1H, C-5 H), 6.56 and 6.69 (AB−System, 2H, J = 8.3 Hz, C-1 H and C-2 H), 6.96 (s, 1H, C-5′ H), 7.17−7.20 (m, 2H, C-2″ and C-6″ H), 7.35−7.42 (m, 3H, C-3″ H, C-4″ H and C-5″ H); MS *m*/*z* 467 $(MH)^+$. Anal. ($C_{30}H_{30}N_2O_3$·2HCl·H_2O) C, H, N, Cl.

17-(Cyclopropylmethyl)-6,7-didehydro-3,14-dihydroxy-4′,6′-diphenyl-4,5α-epoxy-pyrido[2′,3′:6,7]morphinan (6g). To a mixture of naltrexone hydrochloride (**8**·HCl) (0.60 g, 1.59 mmol) and *trans*-chalcone (0.33 g, 1.59 mmol) in MeOH (24 mL) was added, after bubbling argon through the mixture for a few minutes, 1 N aqueous NaOH (12.0 mL, 12.0 mmol) and the resulting solution was left standing in the refrigerator for 1 week. The reaction mixture was partitioned between saturated aqueous NH_4Cl and CH_2Cl_2. The aqueous layer was extracted twice with CH_2Cl_2, and the combined organic extracts were washed with saturated aqueous NaCl and dried. Removal of the solvent and recrystallization of the residue from MeOH yielded 0.426 g (49%) of the 1,5-diketone **12**: mp >240 °C dec; TLC R_f 0.53 ($CHCl_3$−MeOH, 9.0:1.0); ^{1}H NMR ($CDCl_3$) δ 0.09−0.14 and 0.50−0.55 (2m, 4H, cyclopropyl CH_2CH_2), 0.76−0.87 (m, 1H, cyclopropyl C*H*), 1.46−1.54 (m, 2H, C-8 H and C-15 H), 1.93 (dd, 1H, J = 12.9, 4.1 Hz), 2.11(app td, 1H, J = 11.9, 4.5 Hz, C-16 H), 2.29−2.45 (m, 3H, C-16 H and NCH_2-cyclopropyl), 2.54 (dd, 1H, J = 18.9, 6.3 Hz, C-10 H), 2.65 (app dd, 1H, J = 11.9, 4.5 Hz, C-16 H), 3.02 (app d, 1H, J = 18.5 Hz, C-10 H), 3.13 (app d, 1H, J = 5.9 Hz, C-9 H), 3.20 (dd, 1H, J = 16.5, 8.4 Hz, C_6H_5COC*H*), 3.26 (dd, 1H, J = 16.5, 5.6 Hz, C_6H_5COC*H*), 3.35 (ddd, 1H, J = 13.4, 6.2, 4.2 Hz, C-7 H), 3.86−3.93 (m, 1H, C_6H_5C*H*), 4.67 (s, 1H, C-5 H), 5.2−5.8 (br hump, 2H, C-3 OH and C-14 OH), 6.59 and 6.73 (AB−System, 2H, J = 8.1 Hz, C-1 H and C-2 H), 7.09−7.20 (m, 5H, C_6H_5CH), 7.35−7.55 (m, 3H, *m* and *p*-H of C_6H_5CO), 7.83−7.86 (m, 2H, *o*-H of C_6H_5CO); MS *m*/*z* 550 $(MH)^+$. Anal. ($C_{35}H_{35}NO_5$) C, H, N.

A mixture of the above diketone (0.20 g, 0.37 mmol) and NH_2OH·HCl (0.064 g, 0.92 mmol) in EtOH (2 mL) under argon was refluxed for 24 h. After cooling, the mixture was partitioned between CH_2Cl_2 and water. The aqueous layer was extracted twice with CH_2Cl_2. The combined organic extracts were washed with saturated aqueous NaCl and dried, and the solvent was removed. The residue was purified by chromatography over a column of silica using EtOAc−hexanes−EtOH−Et_3N (20:79:1:0.1) as the eluent to obtain 0.146 g (75%) of the product as the free base. This material was converted to the hydrochloride salt by treating its solution in Et_2O with 1 M HCl in Et_2O, and it was recrystallized from EtOH to obtain **6g**: mp >240 °C dec; TLC R_f 0.52 ($CHCl_3$−MeOH−NH_4OH, 95:5:0.5); ^{1}H NMR (DMSO-d_6) δ 0.35−0.72 (m, 4H, cyclopropyl CH_2CH_2), 1.01−1.13 (m, 1H, cyclopropyl C*H*), 1.81−1.88 (m, 1H, C-15 H), 2.55−2.93 (m, 6H, C-8 H_2, C-16 H and NCH_2-cyclopropyl), 3.08−3.19 (m, 2H, C-10 H and C-16 H), 3.34−3.45 (m, 1H, C-10 H), 3.95−4.05 (m, 1H, C-9 H), 5.65 (s, 1H, C-5H), 6.39 (s, 1H, C-14 OH), 6.64 and 6.69 (AB−System, 2H, J = 8.1 Hz, C-1 H and C-2 H), 7.38−7.55 (m, 8H, Ar−H), 7.77 (s, 1H, C-5′ H), 8.16 (app d, 2H, J = 7.0 Hz, *o*-Ar−H), 8.9 (br s, 1H, N−H^+), 9.40 (s, 1H, C-3 OH); MS *m*/*z* 529 $(MH)^+$. Anal. ($C_{35}H_{32}N_2O_3$·HCl·1.25H_2O) C, H, N.

17-(Cyclopropylmethyl)-6,7-didehydro-3,14-dihydroxy-4,5α-epoxy-4′-phenyl-quinolino[2′,3′:6,7]morphinan (6h). A mixture of naltrexone hydrochloride (**8**·HCl)(0.378 g, 1.0 mmol) and 2-aminobenzophenone (0.217 g, 1.1 mmol) in AcOH (5 mL) was refluxed by heating in an oil bath at 130 °C for 16 h. The reaction mixture was concentrated under reduced pressure, and the residue was partitioned between aqueous $NaHCO_3$ and $CHCl_3$. The aqueous layer was extracted twice with $CHCl_3$, and the combined $CHCl_3$ extracts were washed with water and dried. The solvent was removed under reduced pressure, and the residue was purified by chromatography over a column of silica using $CHCl_3$−MeOH (98:2) as the eluent to obtain **6h** (0.22 g, 44%): mp >154 °C dec; TLC R_f 0.32 ($CHCl_3$−MeOH, 97:3); ^{1}H NMR (DMSO-d_6) δ 0.07−0.12 and 0.42−0.48 (m, 4H, cyclopropyl CH_2CH_2), 0.81−0.90 (m, 1H, cyclopropyl C*H*), 1.64 (app d, 1H, C-15 H), 2.16−2.26 (m, 1H, C-15 H), 2.30−2.42 (m, 5H, C-8 H_2, C-16 H and NCH_2-cyclopropyl), 2.45−2.54 (m, 1H, C-10 H), 2.65−2.73 (m, 1H, C-16 H), 3.03 (app d, 1H, J = 18.7 Hz, C-10 H), 3.07 (app d, 1H, C-9 H), 4.78 (s, 1H, C-14 OH), 5.56 (s, 1H, C-5 H), 6.50 and 6.54 (AB−System, 2H, J = 8.0 Hz, C-1 H and C-2 H), 7.13−7.18 (m, 1H, C-6′ H), 7.18−7.23 (m, 2H, C-3″ H and C-5″ H), 7.45−7.53 (m, 3H, C-5′ H, C-2″ H and C-6″ H), 7.55−7.61 (m, 1H, C-4″ H), 7.71−7.77 (m, 1H, C-7′ H), 8.08 (app d, 1H, J = 8.0 Hz, C-8′ H), 9.12 (s, 1H, C-3 OH); MS *m*/*z* 503 $(MH)^+$. Anal. ($C_{33}H_{30}N_2O_3$·0.25H_2O) C, H, N.

7-(Dimethylaminomethylene)naltrexone (13). A mixture of naltrexone (**8**) (2.0 g, 5.8 mmol) and *N*,*N*-dimethylformamide di-*tert*-butyl acetal (5.0 mL, 20.8 mmol) was stirred at room temperature for 5 h under an atmosphere of argon. The reaction mixture was concentrated under reduced pressure, and the residue was chromatographed over a column of silica using $CHCl_3$−MeOH−Et_3N (98.5:0.5:1.0) as the eluent to give 1.32 g (57%) of **13**: mp 162−164 °C dec; TLC R_f 0.53 ($CHCl_3$−MeOH, 9.0:1.0); ^{1}H NMR ($CDCl_3$) δ 0.05−0.2 and 0.40−0.6 (2m, 4H, cyclopropyl CH_2CH_2), 0.79−0.95 (m, 1H, cyclopropyl C*H*), 2.05−2.31 (m, 3H, C-8 H, C-15 H and C-16 H), 2.32−2.45 (m, 3H, C-8 H and NCH_2-cyclopropyl), 2.50−2.70 (m, 3H, C-8 H, C-10 H and C-16 H), 3.0 [s, 6H, $N(CH_3)_2$], 3.09 (app d, 1H, J = 18.2 Hz, C-10 H), 3.12 (app d, 1H, J = 6.38 Hz, C-9 H), 4.28 (s, 1H, C-5 H), 4.45−4.80 (br hump, 1H, C-14 OH), 6.45−6.58 (m, 2H, C-1 H and C-2 H), 7.36 (s, 1H, =CH), 9.06 (br s, 1H, C-3 OH); MS *m*/*z* 397 $(MH)^+$. This material was used in subsequent reactions without further purification.

17-(Cyclopropylmethyl)-6,7-didehydro-3,14-dihydroxy-4,5α-epoxypyrimido[4′,5′:6,7]morphinan (7a). A mixture of **13** (0.263 g, 0.66 mmol), and formamidine acetate (1.1 g, 10.5 mmol) in *i*-PrOH (20 mL) under argon was heated to reflux for 5 h. The reaction mixture was cooled, and the solvent was removed under reduced pressure. The residue was partitioned between CH_2Cl_2, and saturated aqueous $NaHCO_3$. The aqueous layer was extracted twice with CH_2Cl_2 and the combined organic extracts were washed with saturated aqueous NaCl and dried and the solvent was removed. The residue was chromatographed on a column of silica using $CHCl_3$−MeOH−NH_4OH (99:0.5:0.5) as the eluent. The product obtained was crystallized from CH_2Cl_2 to give **7a** as a colorless crystalline solid (0.115 g, 46%): mp 146−148 °C (start dec at 136 °C); TLC R_f 0.31 ($CHCl_3$−MeOH−NH_4OH, 95:5:0.5); ^{1}H NMR (DMSO-d_6) δ 0.11−0.18 and 0.46−0.53 (2m, 4H, cyclopropyl CH_2CH_2), 0.83−0.94 (m, 1H, cyclopropyl C*H*), 1.57 (app d, 1H, J = 12.5 Hz, C-15 H), 2.17 (app td, 1H, J = 12.1, 2.5 Hz, C-15 H), 2.31 (app dd, 1H, J = 12.6, 4.7 Hz, C-16 H), 2.39 (app d, 2H, J = 6.4 Hz, NCH_2-cyclopropyl), 2.62 (app dd, 1H, J = 12.5, 6.8 Hz, C-10 H), 2.45−2.74 (m, 3H, C-8 H_2, and C-16 H), 3.09 (app d, 1H, J = 18.7 Hz, C-10 H), 3.27 (app d, 1H, J = 6.2 Hz, C-9 H), 4.86 (s, 1H, C-14 OH), 5.26 (s, 1H, C-5 H), 6.53 (app s, 2H, C-1 H and C-2 H), 8.58 (s, 1H, C-6′ H), 9.11 (s, 1H, C-2′ H), 9.12 (s, 1H, C-3 OH); MS *m*/*z* 378 $(MH)^+$. Anal. ($C_{22}H_{23}N_3O_3$·H_2O) C, H, N.

17-(Cyclopropylmethyl)-6,7-didehydro-3,14-dihydroxy-4,5α-epoxy-2′-methylpyrimido[4′,5′:6,7]morphinan (7b). Compound **13** (0.50 g, 1.26 mmol) was reacted with acetami-

dine hydrochloride (0.477 g, 5.04 mmol) in *i*-PrOH (50 mL) according to the procedure described for the synthesis of **7a** to provide the title compound **7b** (0.39 g, 79%) as a colorless crystalline solid: mp 234−238 °C; TLC R_f 0.29 ($CHCl_3$−MeOH−NH_4OH, 95:5:0.5); ^{1}H NMR (DMSO-d_6) δ 0.11−0.17 and 0.46−0.52 (2m, 4H, cyclopropyl CH_2CH_2), 0.82−0.94 (m, 1H, cyclopropyl C*H*), 1.56 (app d, 1H, J = 12.1 Hz, C-15 H), 2.17 (app td, 1H, J = 12.7, 2.6 Hz, C-15 H), 2.28 (app dd, 1H, J = 12.7, 4.4 Hz, C-16 H), 2.38 (app d, 2H, J = 6.6 Hz, NCH_2-cyclopropyl), 2.45−2.50 (m, 1H, C-8 H), 2.61 (s, 3H, C-2′ CH_3), 2.57−2.70 (m, overlapped with CH_3, 3H, C-8 H, C-10 H and C-16 H), 3.08 (app d, 1H, J = 18.7 Hz, C-10 H), 3.25 (app d, 1H, J = 6.2 Hz, C-9 H), 2.45−2.74 (m, 3H, C-8 H_2, and C-16 H), 3.09 (app d, 1H, J = 18.7 Hz, C-10 H), 3.25 (app d, 1H, J = 6.2 Hz, C-9 H), 4.82 (br s, 1H, C-14 OH), 5.20 (s, 1H, C-5 H), 6.53 (app s, 2H, C-1 H and C-2 H), 8.45 (s, 1H, C-6′ H), 9.12 (br s, 1H, C-3 OH); MS m/z 392 $(MH)^+$. Anal. ($C_{23}H_{25}N_3O_3$·0.2H_2O) C, H, N.

17-(Cyclopropylmethyl)-6,7-didehydro-3,14-dihydroxy-4,5α-epoxy-2′-phenylpyrimido[4′,5′:6,7]morphinan (7c). Compound **13** (0.40 g, 1.0 mmol) was reacted with benzamidine hydrochloride (0.632 g, 4.0 mmol) in *i*-PrOH (30 mL) according to the procedure described for the synthesis of **7a**. The product obtained after column chromatographic purification was crystallized from EtOH to yield the title compound **7c** (0.37 g, 82%) as a colorless crystalline solid: mp 169−173 °C (softens at 150 °C); TLC R_f 0.59 ($CHCl_3$−MeOH−NH_4OH, 95:5:0.5); ^{1}H NMR (DMSO-d_6) δ 0.12−0.18 and 0.47−0.53 (2m, 4H, cyclopropyl CH_2CH_2), 0.83−0.96 (m, 1H, cyclopropyl C*H*), 1.60 (app d, 1H, J = 12.7 Hz, C-15 H), 2.20 (app td, 1H, J = 12.1, 2.4 Hz, C-15 H), 2.34 (app dd, overlapped with NCH_2-cyclopropyl, 1H, C-16 H), 2.40 (app d, 2H, J = 6.4 Hz, NCH_2-cyclopropyl), 2.57 (d, 1H, J = 17.0 Hz, C-8 H), 2.64 (d, overlapped with C-10 H and C-16 H, 1H, J = 17.0 Hz, C-8 H), 2.61−2.75 (m, 2H, C-10 H and C-16 H), 3.11 (app d, 1H, J = 18.7 Hz, C-10 H), 3.29 (app d, 1H, J = 6.2 Hz, C-9 H), 4.88 (br s, 1H, C-14 OH), 5.36 (s, 1H, C-5 H), 6.54 (app s, 2H, C-1 H and C-2 H), 7.52−7.56 (m, 3H, C-3″ H, C-4″ H and C-5″ H), 8.37−8.40 (m, 2H, C-2″ H and C-6″ H), 8.68 (s, 1H, C-6′ H), 9.14 (s, 1H, C-3 OH); MS m/z 454 $(MH)^+$. Anal. ($C_{28}H_{27}N_3O_3$) C, H, N.

17-(Cyclopropylmethyl)-6,7-didehydro-3,14-dihydroxy-4,5α-epoxy-6′-phenylpyrimido[4′,5′:6,7]morphinan (7d). A solid mixture of 7-benzylidenenaltrexone (**4**) (0.5 g, 1.16 mmol) and formamidoxime (1.2 g, 20 mmol), under a nitrogen atmosphere, was heated in an oil bath. The mixture became a homogeneous melt at 100 °C, and an exothermic reaction set in at 120 °C. The mixture was held at that temperature for 30 min and then allowed to cool to room temperature. Chromatographic purification of the residue on a column of silica ($CHCl_3$−MeOH, 98:2 to 95:5 gradient) followed by crystallization from $CHCl_3$ afforded 0.148 g (33%) of **7d**: mp 151−153° C dec (softens at 137 °C); TLC R_f 0.37 ($CHCl_3$−MeOH−NH_4OH, 95:5:0.5); ^{1}H NMR ($CDCl_3$) δ 0.10−0.15 and 0.51−0.56 (2m, 4H, cyclopropyl CH_2CH_2), 0.78−0.92 (m, 1H, cyclopropyl C*H*), 1.81−1.84 (m, 1H, C-15 H), 2.29−2.39 (m, 3H, C-15 H, C-16 H and C-17 H), 2.45 (dd, 1H, J = 12.5, 6.2 Hz, C-17 H), 2.58 (d, 1H, J = 16.3 Hz, C-8 H), 2.67 (dd, 1H, J = 19.0, 6.8 Hz, C-10 H), 2.71−2.74 (m, 1H, C-16 H), 2.77 (d, 1H, J = 16.3 Hz, C-8 H), 3.15 (app d, 1H, J = 18.7 Hz, C-10 H), 3.22 (app d, 1H, J = 6.4 Hz, C-9 H), 4.9−5.2 (br hump, 2H, C-3 OH and C-14 OH), 5.49 (s, 1H, C-5 H), 6.63 and 6.72 (AB−System, 2H, J = 8.1 Hz, C-1 H and C-2 H), 7.45 (app s, 5H, C_6H_5), 9.2 (s, 1H, C-2′ H); MS m/z 454 $(MH)^+$. Anal. ($C_{28}H_{27}N_3O_3$·0.25H_2O) C, H, N.

17-(Cyclopropylmethyl)-6,7-didehydro-3,14-dihydroxy-4,5α-epoxy-2′-methyl-6′-phenylpyrimido[4′,5′:6,7]morphinan (7e). A mixture of 7-benzylidenenaltrexone (**4**) (0.59 g, 1.37 mmol), acetamidine hydrochloride (0.39 g, 4.12 mmol), and $NaHCO_3$ (0.346 g) in DMF (5 mL) was heated under an argon atmosphere at 70 °C for 5 h and then at reflux for 3 h. The mixture was concentrated under reduced pressure, and the residue was partitioned between CH_2Cl_2 and water. The aqueous layer was extracted once with CH_2Cl_2, and the combined organic extracts were dried. The solvent was removed, and the residue was chromatographed over a column of silica using $CHCl_3$−MeOH−Et_3N (95:5:0.2 to 87:12:4 gradient) as the eluent to obtain 0.49 g (76%) of the dihydropyrimidine **17** as a light brown solid: TLC R_f 0.34 ($CHCl_3$−MeOH−NH_4OH, 83:15:2); ^{1}H NMR (MeOH-d_4) δ 0.11−0.15 and 0.48−0.54 (m, 4H, cyclopropyl CH_2CH_2), 0.82−0.93 (m, 1H, cyclopropyl C*H*), 1.56 (d, 1H, J = 18.0 Hz, C-8 H), 1.63−1.71 (m, 2H, C-8 H and C-15 H), 1.93 (s, 3H, C-2′ CH_3), 2.23−2.44 (m, 5H, C-10 H, C-15 H, C-16 H and NCH_2-cyclopropyl), 2.68−2.76 (m, 1H, C-16 H), 2.98 (app d, 1H, J = 18.7 Hz, C-10 H), 3.12 (app d, 1H, J = 6.6 Hz, C-9 H), 4.84 and 4.86 (2s, 2H, C-5 H and C-6′ H), 6.42 and 6.61 (AB−System, 2H, J = 8.1 Hz, C-1 H and C-2 H), 6.87−6.91 (m, 2H, C-2″ H and C-6″ H), 7.09−7.16 (m, 3H, C-3″ H, C-4″ H and C-5″ H); MS m/z 470 $(MH)^+$. To a suspension of the above dihydropyrimidine **17** (0.424 g, 0.9 mmol) in xylenes (20 mL) was added 0.2 g of 10% palladium on carbon under an argon atmosphere. The mixture was heated under reflux for 8 h. An additional 0.2 g of the catalyst was added and the mixture was refluxed further for 8 h, cooled, and filtered through a pad of Celite. The filtrate was concentrated under reduced pressure and the residue was purified by chromatography over a column of silica using $CHCl_3$−MeOH (99:1) as the eluent to afford **7e** (0.193 g, 46%): mp 160−162 °C dec (softens at 144 °C); TLC R_f 0.37 ($CHCl_3$−MeOH−NH_4OH 95:5:0.5); ^{1}H NMR ($CDCl_3$) δ 0.09−0.14 and 0.50−0.56 (2m, 4H, cyclopropyl CH_2CH_2), 0.76−0.88 (m, 1H, cyclopropyl C*H*), 1.79−1.85 (m, 1H, C-15 H), 2.28−2.38 (m, 3H, C-15 H, C-16 H and NC*H*-cyclopropyl), 2.45 (dd, 1H, J = 13, 6.6 Hz, NC*H*-cyclopropyl), 2.51 (d, 1H, J = 16.5 Hz, C-8 H), 2.65 (dd, 1H, J = 19.0, 7.0 Hz, C-10 H), 2.69 (d, 1H, J = 16.5 Hz, C-8 H), 2.69−2.75 (m, 1H, C-16 H), 2.78 (s, 3H, C-2′ CH_3), 3.13 (app d, J = 18.5 Hz, C-9 H), 3.20 (app d, 1H, J = 6.6 Hz, C-10 H), 4.90−5.15 (br hump, 2H, C-3 OH and C-14 OH), 5.45 (s, 1H, C-5 H), 6.61 and 6.71 (AB−System, 2H, J = 8.0 Hz, C-1 H and C-2 H), 7.42 (s, 5H, C_6H_5); MS m/z 468 $(MH)^+$. Anal. ($C_{29}H_{29}N_3O_3$·H_2O) C, H, N.

2′-Benzyl-17-(cyclopropylmethyl)-6,7-didehydro-3,14-dihydroxy-4,5α-epoxy-6′-phenylpyrimido[4′,5′:6,7]morphinan (7f). A mixture of benzylidenenaltrexone (**4**) (0.43 g, 1.0 mmol), phenylacetamidine (0.403 g, 3.0 mmol), and $NaHCO_3$ (0.252 g, 3.0 mmol) in DMF (5 mL) was heated at 100 °C for 18 h. Workup of the reaction mixture and chromatographic purification over silica using $CHCl_3$−MeOH−Et_3N (95:5:0.2) as the eluent gave 0.43 g (79%) of the dihydropyrimidine **18**, which was dehydrogenated with palladium on carbon and purified as described for **7e** to yield the title compound **7f** (0.18 g, 42%): mp 131−135 °C dec; TLC R_f 0.52 ($CHCl_3$−MeOH−NH_4OH, 95:5:0.5); ^{1}H NMR ($CDCl_3$) δ 0.09−0.13 and 0.49−0.55 (2m, 4H, cyclopropyl CH_2CH_2), 0.75−0.86 (m, 1H, cyclopropyl C*H*), 1.77−1.81 (m, 1H, C-15 H), 2.27−2.34 (m, 3H, C-15 H, C-16 H and NC*H*-cyclopropyl), 2.43 (dd, 1H, J = 12.6, 6.3 Hz, NC*H*-cyclopropyl), 2.52 (d, 1H, J = 16.1 Hz, C-8 H), 2.59−2.76 (m, 3H, C-8 H, C-10 H and C-16 H), 3.12 (app d, 1H, J = 18.9 Hz, C-10 H), 3.18 (app d, 1H, J = 6.3 Hz, C-9 H), 4.33 (s, 2H, C$H_2C_6H_5$), 5.20−5.40 (bs, 2H, C-3 OH and C-14 OH), 5.44 (s, 1H, C-5 H), 6.61 and 6.71 (AB−System, 2H, J = 8.75 Hz, C-1 H and C-2 H), 7.21−7.31(m, 10H, Aryl-H); MS m/z 544 $(MH)^+$. Anal. ($C_{35}H_{33}N_3O_3$·0.5H_2O) C, H, N.

17-(Cyclopropylmethyl)-6,7-didehydro-3,14-dihydroxy-2′,6′-diphenyl-4,5α-epoxy-pyrimido[4′,5′:6,7]morphinan (7g). A mixture of compound **4** (0.40 g, 0.93 mmol), benzamidine hydrochloride (0.44 g, 2.8 mmol), and $NaHCO_3$ (0.235 g, 2.8 mmol) in DMF (5 mL) was heated at reflux for 4 h. Workup of the reaction mixture and column chromatographic purification over silica using EtOAc−EtOH−Et_3N (97:3:0.3) as the eluent gave 0.45 g (91%) of the dihydropyrimidine **19**. A small portion of the free base was dissolved in Et_2O, and treated with 1 M HCl in Et_2O to obtain **19**·2HCl·H_2O: mp >240 °C dec; TLC R_f 0.11 (EtOAc−EtOH−Et_3N, 94.5:5.0:0.5); ^{1}H NMR (DMSO-d_6) δ 0.31−0.72 (m, 4H, cyclopropyl CH_2CH_2), 0.99−1.09 (m, 1H, cyclopropyl C*H*), 1.65 (d, 1H, J = 18 Hz, C-8 H), 1.79−1.88 (m, 1H, C-15 H), 2.22 (d, 1H, J = 18 Hz, C-8 H), 2.49−2.75 (m, 3H, C-15 H, C-16 H and NC*H*-cyclopropyl), 2.87−2.97 (m, 2H, C-10 H and NC*H*-cyclopropyl), 3.07−3.15

(m, 1H, C-16 H), 3.23−3.34 (m, 1H, C-10 H), 3.98−4.05 (m, 1H, C-9 H), 5.33 (s, 2H, C-5 H and C-6′ H) 6.55 and 6.81 (AB−System, 2H, J = 8.1 Hz, C-1 H and C-2 H), 6.95−6.98 (m, 3H, N−H and *o*-Ar−H), 7.20−7.32 (m, 3H, *m*- and *p*-Ar−H), 7.60−7.65 (m, 2H, *m*-Ar−H), 7.74−7.81 (m, 3H, *o*- and *p*-Ar−H), 9.0 (br, s, C-14 OH), 9.6 (br s, 1H, C-3 OH), 11.4 (br, s, NH^+), 12.1 (br s, 1H, NH^+); MS *m*/*z* 532 $(MH)^+$. Anal. ($C_{34}H_{33}N_3O_3$·2HCl·H_2O) C, H, N, Cl

Dehydrogenation of 386 mg (0.77 mmol) of the free base of **19** with 10% palladium on carbon in refluxing xylenes followed by chromatographic purification over silica using $CHCl_3$ to $CHCl_3$−MeOH (99.5:0.5) gradient as the eluent gave 0.277 g (72%) of the title compound. The free base was converted to the hydrochloride in the usual way and crystallized from EtOH to obtain the hydrochloride of **7g** as colorless crystals: mp >240 °C dec; TLC R_f 0.56 ($CHCl_3$−MeOH 9.0:1.0); ^{1}H NMR of the free base ($CDCl_3$) δ 0.10−0.15 and 0.51−0.56 (m, 2H, cyclopropyl CH_2CH_2), 0.78−0.88 (m, 1H, cyclopropyl C*H*), 1.83−1.89 (m, 1H, C-15 H), 2.30−2.49 (m, 4H, C-15 H, C-16 H and NCH_2-cyclopropyl), 2.60 (d, 1H, J = 16 Hz, C-8 H), 2.62−2.75 (m, 2H, C-10 H and C-16 H), 2.77 (d, 1H, J = 16 Hz, C-8 H), 3.15 (app d, 1H, J = 18.8 Hz, C10), 3.23 (app d, 1H, J = 6.4 Hz, C-9 H), 4.8−5.3 (br hump, 2H, C-3 OH and C-14 OH), 5.57 (s, 1H, C-5 H), 6.63 and 6.72 (AB−System, 2H, J = 8.2 Hz, C-1 H and C-2 H), 7.43−7.48 (m, 6H, *m*- and *p*-Ar−H), 7.53−7.56 (m, 2H, *o*-Ar−H), 8.48−8.51 (m, 2H, *o*-Ar−H); MS *m*/*z* 550 $(MH)^+$. Anal. ($C_{34}H_{31}N_3O_3$·HCl·0.25H_2O) C, H, N, Cl.

Biological Assays. Radioligand Binding Assays for μ, δ, and κ Receptors. Mu binding sites were labeled using [^{3}H]-DAMGO (1−3 nM) and rat brain membranes as previously described[35] with several modifications. Rat membranes were prepared each day using a partially thawed frozen rat brain which 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 30000*g* 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 (50 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).[35] The nonspecific binding was determined using 20 μM of levallorphan. Delta binding sites were labeled using [^{3}H]DADLE (2 nM) and rat brain membranes as previously described,[36] with several modifications. Rat membranes were prepared each day using a partially thawed frozen rat brain which 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 30000*g* 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 (50 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 $MnCl_2$, 100 nM DAMGO to block binding to μ sites, and PIC. Nonspecific binding was determined using 20 μM levallorphan. Kappa binding sites were labeled using [^{3}H]U69,593 (2 nM) as previously described,[37] with several modifications. Guinea pig brain membranes were prepared each day using partially thawed guinea pig brain which was homogenized with a polytron in 10 mL/brain of ice-cold 10 mM Tris-HCl, pH 7.0. The membranes were then centrifuged twice at 30000*g* 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 (75 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 ^{3}H ligand was displaced by 8−10 concentrations of test drug, two times. 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 $K_i = IC_{50}/(1 + [L]/K_d)$.

GPI and MVD Bioassays.[38,39] Electrically induced smooth muscle contractions of mouse vas deferens and strips of guinea pig ileum longitudinal muscle myenteric plexus were used. Tissues came from male ICR mice weighing 25−40 g and male Hartley guinea pigs weighing 250−500 g. The tissues were tied to gold chain with suture silk, suspended in 20 mL baths containing 37 °C oxygenated (95% O_2, 5% CO_2) Krebs bicarbonate solution (magnesium free for the MVD), and allowed to equilibrate for 15 min. The tissues were then stretched to optimal length previously determined to be 1 g tension (0.5 g for MVD) and allowed to equilibrate for 15 min. The tissues were stimulated transmurally between platinum wire electrodes at 0.1 Hz, 0.4 ms pulses (2-ms pulses for MVD), and supramaximal voltage. An initial dose−response curve of DPDPE or PL-017 was constructed at the start of each assay to establish tissue effects, allowing each tissue to be used as its own control. Tissues not producing typical results were not used. Experimental compounds were added to the baths in 14−60 μL volumes. Succeeding doses of agonist were added cumulatively to the bath at 3 min intervals to produce a concentration−response curve. The tissues were then washed extensively with fresh buffer until the original contraction height was reestablished. Agonist effects of the compounds at 1 μM were measured as percent inhibition of contraction height 10 min after addition to the bath. Antagonist effects to DPDPE and PL-017 were assayed after incubation of the tissues with 1 μM concentration of the compound in the bath for 30 min. The tissues were then washed with fresh buffer for 30 min, and the agonist dose−response curve was repeated. Rightward shifts in the dose−response curves were calculated by dividing the antagonized dose−response curve IC_{50} value by the unantagonized IC_{50} value. IC_{50} values represent the mean of two to four tissues. IC_{50} estimates and their associated standard errors were determined by using a computerized nonlinear least-squares method.[49]

Antinociceptive Studies. Male ICR mice (Harlan) were used for all evaluations. Mice were housed in a temperature and humidity controlled vivarium on a 12:12 h light:dark cycle with unlimited access to food and water prior to the formal procedures. Graded doses of morphine or **6d** were injected intracerebroventricularly (icv) under light ether anesthesia. Morphine sulfate was dissolved in distilled water and injected in a volume of 5 μL. Compound **6d** was dissolved in 100% DMSO and injected in a volume of 5 μL. Antinociceptive assays were performed at various times after injection.

Tail-Flick Assay. Naive mice were baselined in the 55 °C tail-flick test as previously described.[50] Doses of morphine or **6d** were injected icv, and antinociception was assessed at 10, 20, 30, 45 and 60 min postinjection. Percent antinociception was calculated using the formula: %MPE (maximal possible effect) = 100 × (test − control)/(cutoff − control) where control is the predrug observation, test is the postdrug observation, and cutoff is the maximal stimulus allowed (15 s for tail-flick). Antinociceptive A_{50} values and 95% confidence intervals were determined using linear regression software (FlashCalc). In this assay morphine was a full agonist (A_{50} = 2.94 nmol) and **6d** was a partial agonist (A_{50} > 100 nmol) (see Figure 1).

Acetic Acid Writhing Assay. Acetic acid (0.6%) was injected by the intraperitoneal (ip) route in a volume of 10 mL/kg body weight. Immediately after injection, each mouse was placed in a clear Plexiglas container and observed for 15 min. The number of abdominal writhes was recorded during this time period. Injections of morphine or **6d** preceded the injection of acetic acid by 10 min in an effort to match peak drug effects with the nociceptive stimulus. Percent antinociception was calculated using the formula: %MPE (maximal possible effect) = 100 − ((# writhes individual mouse/mean # writhes control group) × 100). The control group was a separate group of mice that received 5 μL icv injections of 100% DMSO (vehicle) followed by ip injections of 0.6% acetic acid. In this assay, both morphine and **6d** displayed full agonist activity with A_{50} (95%

confidence interval) values of 0.004 nmol (0.003−0.006 nmol) and 7.5 nmol (5.3−10.5 nmol), respectively (Figure 1).

Tolerance Regimen. Mice were injected twice daily (8 a.m. and 8 p.m.) with an approximate A_{90} dose of morphine (0.01 nmol) or **6d** (30 nmol) for 3 days. Antinociceptive dose−response curves in the acetic acid writhing assay were generated on the morning of the fourth day using the procedures outlined above. Repeated icv injections of an A_{90} dose of morphine (twice daily for 3 days) produced a significant rightward shift (12.5-fold) in the morphine dose−response curve. The calculated A_{50} value (and 95% confidence intervals) for the morphine multiple-injection group was 0.05 nmol (0.03−0.08 nmol). Repeated icv injections of an A_{90} dose of **6d** (twice daily for 3 days) did not produce a significant shift in the **6d** dose−response curve. The calculated A_{50} value (and 95% confidence intervals) for the **6d** multiple-injection group was 10.9 nmol (7.2−16.5 nmol) (Figure 2).

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