Synthesis of Naltrexone-Derived δ-Opioid Antagonists. Role of Conformation of the δ Address Moiety

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Naltrindole (1) (NTI) is a highly potent and selective δ-opioid receptor antagonist. In an effort to understand the origin of the high potency, affinity, and selectivity of NTI, we have examined the conformational role of its indolic benzene moiety through the synthesis of related naltrexone derivatives **3–8**, which contain the benzene moiety in different orientations and at different attachments in the molecule. One of these naltrexone derivatives, **5**, whose 7-indanyl benzene moiety is orthogonal to ring C of the morphinan system, is a potent δ-opioid receptor antagonist *in vitro* and *in vivo*. Computer-assisted molecular overlay studies of the minimized structures (**2–8**) revealed the importance of the position of the benzene moiety for effective interaction with δ-opioid receptors. In compounds **2**, **4**, and **5**, the aromatic ring falls in the same region of space as that of the indolic benzene moiety of NTI, and all of these ligands possessed significant activity at δ-opioid receptors. Analogues (**3** and **6–8**) which were shown to have relatively weak δ-opioid receptor antagonist potency have their aromatic groups located in a space that is different from that of the more potent analogues.

Naltrindole (1) (NTI) is a non-peptide ligand that is employed widely as a δ-selective opioid receptor antagonist.[1-3] The high δ-opioid antagonist potency and selectivity of NTI have been ascribed[3] to the indolic benzene moiety which mimics a portion of the putative "address"[4] component (Phe[4]) of enkephalin. The benzene moiety apparently confers such selectivity by simultaneously enhancing the affinity for δ sites and decreasing the affinity for κ and μ sites.[2] Very recent studies have suggested that the receptor-bound conformational requirements of the δ address component of antagonists differ from those of agonists.[5] Indeed, site-directed mutagenesis studies of the cloned δ-opioid receptor suggest there is a fundamental difference between the interaction of agonists and antagonists.[6]

In an effort to investigate the conformational role of the putative δ address mimic with regard to its ability to enhance δ antagonist potency and confer δ selectivity, we have synthesized opiates **3–8** that contain aromatic groups which possess different fixed or preferred conformations. The relationship of the aromatic group with respect to the antagonist pharmacophore and its effect on δ antagonist potency and selectivity was evaluated. Here we show that ligands whose aromatic groups share conformational space with the indolic benzene moiety of NTI possess higher δ antagonist potency than other analogues of the series.

Design Rationale

From a heuristic perspective, it has been suggested that opioid receptors contain two subsites that interact with opioid ligands.[7] The "message" subsite, which is envisaged to be a highly homologous domain in the opioid receptor family, is associated with the locus for initiation of signal transduction. The "address" subsite is considered to be unique for a specific receptor type or subtype and functions to enhance the affinity of the ligand without contributing to signal transduction.

As the high potency and δ selectivity of NTI (1) are due to the indolic benzene moiety which plays a key role in the interaction with the address subsite of the δ receptor, we have explored the optimal regio and conformational requirements of the putative address component through the pharmacologic evaluation of compounds **3–8** (Chart 1).

The (*Z*)-benzylidene **3** was synthesized in order to compare its δ antagonist potency with that of its geometric isomer, BNTX (**2**),[8] which is an established δ_1 antagonist.[9] The 7α-benzyl analogue **4** was made in order to evaluate the effect of conformational mobility of the phenyl group on δ selectivity. The spiroindan **5** and the catechol spiroketal **6** were prepared to explore how conformationally fixed orientations of the aromatic group would affect δ antagonist activity. The aromatic groups in both of these compounds are orthogonal to the C-ring of naltrexone. The epimeric anilino compounds **7** and **8** were synthesized because they represent conformationally mobile analogues of NTI.

Chemistry

Synthesis. (*Z*)-7-Benzylidenenaltrexone (**3**) was obtained from the base-catalyzed condensation of naltrexone (**9**) with benzaldehyde. This reaction was described[8] previously in connection with the synthesis of the preponderant *E*-isomer BNTX (**2**). Chromatographic analysis of the crude product indicated it to be a 98:2 mixture of *E*- and *Z*-isomers. The geometry of the double bond in these isomers was assigned on the basis of their NMR spectra. Thus, the *E*-isomer **2** exhibited a vinylic proton absorption at δ 7.59 while that for the *Z*-isomer **3** appeared at δ 7.50. This is consistent with the report[10] that the chemical shift of the β-vinyl proton in the *E*-isomers of α,β-unsaturated carbonyl compounds is downfield relative to that of the corresponding *Z*-isomers. Also, the isomeric product ratio is in harmony with the spectral data, in that it is expected that the less sterically crowded isomer **2** should be favored in the condensation reaction. In fact, **3** was slowly isomerized to **2** in solution at pH 7.4.

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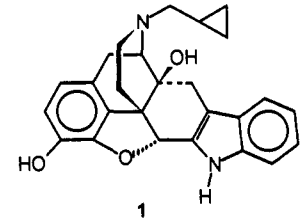
Chart 1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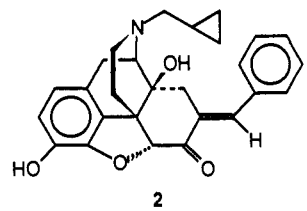
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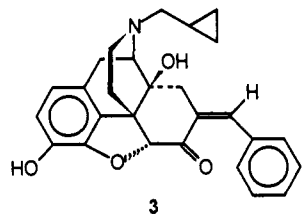
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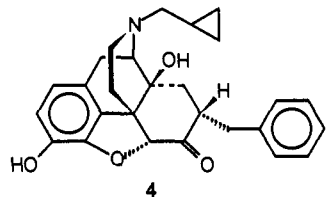
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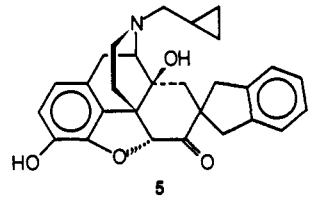
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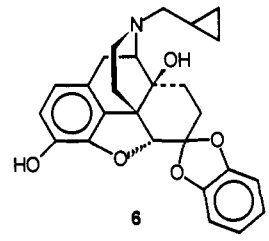
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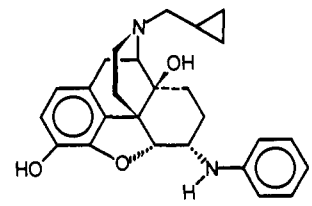
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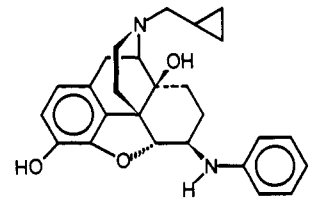
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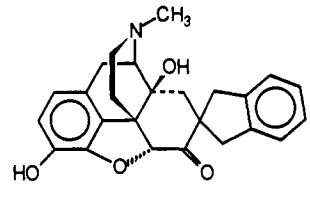
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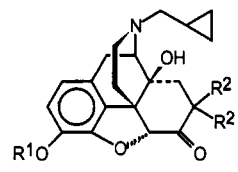
9 $R^1 = R^2 = H$
10 $R^1 = C_6H_5CH_2$, $R^2 = H$
11 $R^1 = C_6H_5CH_2$, $R^2 = (CH_2)_2C_6H_4$

Catalytic hydrogenation of 2 afforded 7α-benzylnaltrexone (4) as the only detectable diastereomer. The stereochemistry of 4 was determined from the connectivity between the benzylic and H-7 protons using COSY. The stereochemistry at C-7 was determined from the NOE difference spectra. On irradiation of H-5, NOE enhancements were observed for H-7 and H-15. Moreover, irradiation of H-7 afforded NOEs for H-5 and H-15. These results suggested H-7 as β, and therefore, the benzyl substituent has the α-configuration. The fact that the benzyl group of 4 was not epimerized upon treatment with base is consistent with the 7α-equatorial epimer because the 7β-isomer would be quite hindered as a consequence of the benzyl group occupying an axial conformation.

The spiroindan 5 was prepared from naltrexone benzyl ether (10). The lithium enolate of 10 was formed in DMSO from lithium hexamethyldisilazane. Reaction of this lithium enolate with α,α'-dibromo-*o*-xylene afforded the spiroindan 11. The proton NMR spectrum of intermediate 11 contains two doublets ($J \simeq 16$ Hz), consistent with geminal coupling of the benzylic methylene protons of the indan moiety. The ^{13}C NMR spectrum of 11 also is consistent with the assigned structure, in that it exhibits 10 pairs of methylene protons from DEPT. Also, this analysis indicated that a new quaternary carbon (δ 56.45) was formed at C-7. Catalytic hydrogenolysis of 11 afforded the desired target compound 5.

The catechol ketal 6 was synthesized from naltrexone (9) and catechol in the presence of *p*-toluenesulfonic acid using a Dean–Stark trap. The product exhibited an NMR absorption at δ 4.61 for H-5 which was upfield from that of the starting ketone 9 (δ 5.0).

The 6α- and 6β-anilino epimers 7 and 8 were synthesized by the sodium cyanoborohydride reduction of the Schiff base formed from the condensation of naltrexone (9) with aniline. The NMR spectrum of 8 showed a doublet of J = 8 Hz for H-5 which is diagnostic for the 6β-epimer. Its epimer, 7, exhibited a doublet of J = 4 Hz for H-5 which is typical for the 6α-isomer.[11]

Biological Results

Smooth Muscle Preparations. The target compounds were evaluated on the electrically stimulated guinea pig ileal longitudinal muscle[12] (GPI) and the mouse vas deferens[13] (MVD) preparations. The ligands were incubated with the preparations for 15 min prior to testing with the standard agonists. Morphine (M), ethylketazocine (EK), and [D-Ala^2,D-Leu^5]enkephalin[14] (DADLE) were employed as μ-, κ-, and δ-selective agonists, respectively. Morphine and EK were employed in the GPI, and DADLE was used in the MVD. The antagonist potencies are expressed as K_e values which were calculated from the equation K_e = [antagonist]/(IC_{50} ratio − 1), where the IC_{50} ratio represents the IC_{50} of the agonist in the presence of the antagonist divided by the control IC_{50} of the agonist.

The opioid antagonist potencies of the target compounds and the reference ligands, NTI (1) and BNTX (2), are listed in Table 1. The (*Z*)-benzylidene derivative 3 possessed about one-tenth the potency of its *E*-isomer, BNTX (2). Compounds 4 and 5 were significantly more

Table 1. Opioid Antagonist Activity of 6- or 7-Substituted Naltrexone Derivatives

	DADLE[a] (δ)		M[b] (μ)		EK[b] (κ)		K_e ratio	
compd[c]	IC_{50} ratio[d]	K_e[e]	IC_{50} ratio[d]	K_e[e]	IC_{50} ratio[d]	K_e[e]	μ/δ	κ/δ
1 (NTI)	152 ± 34[f] (5)	0.13	7.8 ± 1.6[g] (3)	29	5.4 ± 0.8[g] (3)	45	223	346
2 (BNTX)[h]	49 ± 5 (4)	2.9	13 ± 0.9 (3)	8.3	2.7 ± 1 (3)	100	3	44
3	5.2 ± 0.8 (3)	24	0.7 (1)	*i*	9 (1)	53	*i*	2
4	67 ± 13 (3)	1.5	5.8 ± 1.6 (3)	21	1.3 ± 0.1 (3)	*i*	14	*i*
5	130 ± 30 (3)	0.78	25 ± 4 (3)	4.2	1.9 ± 0.5 (6)	105	5	135
6	0.9 ± 0.9 (3)	*i*	3.5 ± 0.8 (3)	40	1.3 ± 0.2 (3)	*i*	*i*	*i*
7	12 ± 3 (3)	8.8	33 ± 8 (3)	3.2	1.9 ± 0.3 (3)	100	0.4	12
8	2.0 ± 0.4 (3)	100	78 ± 16 (3)	1.3	18 ± 4 (3)	6.0	0.01	0.06

[a] [D-Ala²,D-Leu⁵]enkephalin in the MVD preparation. [b] Morphine (M) and ethylketazocine (EK) in the GPI. [c] Unless otherwise stated, 100 nM concentration was employed. [d] IC_{50} ratio is the IC_{50} in the presence of antagonist divided by the control IC_{50} value. [e] K_e (nM) = [antagonist]/(IC_{50} ratio − 1). [f] Concentration of NTI was 20 nM.[2] [g] Concentration was 200 nM.[2] [h] Reference 9. [i] Due to an IC_{50} ratio that is not statistically different from 1, the K_e values and selectivity ratios could not be calculated.

potent in antagonizing the δ-selective agonist DADLE as compared to the other target compounds. However, neither of these more potent ligands was as effective as NTI (1) in antagonizing DADLE. In this regard, NTI was 6-fold more potent as a δ antagonist than **5**. The spiroketal **6** was virtually devoid of antagonist activity at all three receptor types. The anilino compounds also showed significant δ antagonist potency differences in that the α-epimer **7** was at least 10 times more potent than the β-epimer **8**. It is noteworthy that **7** and **8** are both μ-selective.

The most δ-selective of the target compounds **3–8** was 7-benzylnaltrexone (**4**). The spiroindan **5** had somewhat lower selectivity even though it was twice as potent as **4**. BNTX (**2**) ranked third in regard to δ selectivity. BNTX (**2**) was more selective than its isomer, **3**, due to its 8-fold greater δ antagonist potency.

The agonist activity of the target compounds (1 μM) was evaluated on the GPI and MVD preparations. None of the compounds possessed full agonist activity. The greatest partial agonist effect was observed with **7**, which exhibited 34% and 21% maximal effects in the GPI and MVD, respectively. Its epimer, **8**, was of similar magnitude, and the remainder of the compounds possessed lower agonist responses. Two of the compounds (**4** and **5**) enhanced the electrically stimulated twitch, but these were of marginal significance relative to that of BNTX (**2**) which afforded an enhancement of 35% in the MVD.

Binding. Opioid receptor binding experiments using guinea pig brain were carried out for target compounds **3–8** using a modification of the procedure of Werling et al.[15] Binding to μ sites was evaluated by competition of the target compounds with [³H][D-Ala²,MePhe⁴,Gly-ol⁶]-enkephalin[16] ([³H]DAMGO), to δ_1 sites with [³H][D-Pen²,D-Pen⁵]enkephalin[17] ([³H]DPDPE), to δ_2 sites with [³H][D-Ser²,Leu⁵]enkephalin-Thr⁶[14] ([³H]DSLET) in the presence of 1 μM DAMGO, and to κ sites with [³H]-(5α,7α,8β)-(−)-*N*-methyl-*N*-(1-pyrrolidinyl)-1-oxaspiro-[4,5]dec-8-ylbenzeneacetamide ([³H]U69593).[18]

The K_i values of the target compounds **3–8** are presented in Table 2. Binding data for all of these ligands, except spiroindan **5**, were obtained using [³H]DPDPE and [³H]-DSLET to label δ_1 and δ_2 sites, respectively. The binding of **5** could not be determined using these radioligands due to the very flat competition curves which made estimation of K_i values very difficult. However, when [³H]NTI was employed, a reliable K_i value was obtained for **5**. On this basis, the affinity of **5** was determined to be one-sixth that of NTI, which is identical to the antagonist potency difference observed in the MVD. Among the target compounds, spiroindan **5** appears to have the highest affinity for δ receptors.

Table 2. Opioid Receptor Binding of 6- and 7-Substituted Naltrexone Derivatives

	K_i, nM[a]				
compd	[³H]-NTI (δ)	[³H]-DPDPE (δ_1)	[³H]-DSLET (δ_2)	[³H]-DAMGO (μ)	[³H]-U69593 (κ)
1 (NTI)	0.0072			14.4	14.0
2 (BNTX)[b]		0.10	10	13.3	59
3		0.83	1.8	1.9	66
4		0.86	0.12	1.4	25
5	0.047			1.4	12.5
6		121	15	5.2	25
7		0.60	1.5	0.19	2.2
8		4.5	7.8	1.4	2.2

[a] Geometric means of three replicate determinations. [b] Binding data from ref 9.

With respect to selectivity, the spiroketal **6** and the anilino epimers **7** and **8** were the only compounds that had greater affinity for μ receptors. The remaining target compounds **3–5** were δ-selective, with **5** having the greatest selectivity.

In regard to affinity differences between δ_1 and δ_2 receptors, the target compounds tended to favor δ_1 sites, except for the 7-benzyl derivative **4** which bound with greater affinity to δ_2 sites. In any case, the affinity differences between the δ subtypes were not greater than 8-fold.

***In Vivo* Studies.** As the spiroindan derivative **5** was the most potent δ antagonist among the target compounds **3–8**, it was tested using the tail-flick procedure in mice.[19] Administration of **5** (2 nmol icv) and standard agonists was timed so that the peak effect (20 min) coincided with the center of the observation period. The ED_{50} values of the standard agonists, DPDPE (δ_1), DSLET (δ_2), morphine (μ), and *trans*-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide[20] (U50488) (κ) were then determined and expressed as ED_{50} ratios (treated/control). The results of these studies (Table 3) indicated that DPDPE was antagonized to the greatest degree, with weaker antagonism of DSLET and morphine. Since DSLET does possess a small μ agonist component, the ED_{50} ratio represents a maximum value for δ_2-mediated antagonism. The κ agonist, U50488, was not significantly antagonized. No significant agonist activity for **5** was observed at doses as high as 32 nmol/mouse.

Discussion

The conformationally restricted spiro compounds **5** and **6** both have their aromatic groups fixed orthogonally with

Table 3. Antagonism by 5 of the Antinociceptive Effect of Opioid Agonists in Mice

agonist	selectivity	ED_{50}, nmol/mouse or μmol/kg control	ED_{50}, nmol/mouse or μmol/kg treated[a]	ED_{50} ratio[b]
DPDPE[c]	δ_1	6.4 (4.7–8.2)	46.3 (33.9–62.4)	11.1 (7.6–17.2)
DSLET[c]	δ_2	0.7 (0.4–0.9)	2.8 (2.0–3.8)	6.1 (3.9–9.3)
morphine[d]	μ	6.9 (6.1–8.2)	21.1 (18.2–24.5)	8.2 (6.6–10.0)
U50488[d]	κ	8.6 (6.7–11.0)	12.2 (9.7–16.5)	3.0 (2.1–4.5)

[a] Treated icv with 2 nmol of 5 (peak time 20 min). [b] Treated ED_{50} divided by control ED_{50}. [c] Administered icv; ED_{50} values expressed as nmol/mouse. [d] Administered icv; ED_{50} values expressed as μmol/kg.

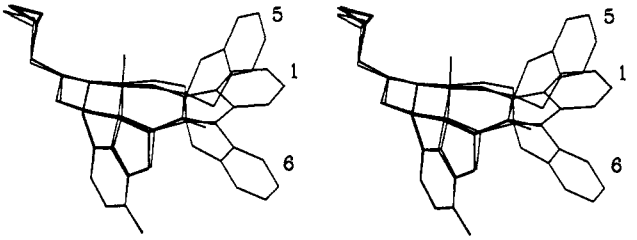

Figure 1. Superposition of naltrindole (1) and spiro compounds 5 and 6.

respect to ring C of the morphinan structure. It is noteworthy that only in 5 does the aromatic group share some of the conformational space occupied by the coplanar indolic benzene moiety of NTI (1) (Figure 1). Significantly, 5 displayed potent δ-opioid antagonist activity whereas 6 was ineffective as an antagonist at all three receptor types. This suggests that there may be a subsite of the δ-opioid receptor which recognizes the aromatic moiety of NTI (1) and 5. The absence of δ-antagonist activity and low affinity of 6 can be ascribed to hindrance to binding due to the location of its aromatic group. Although 5 is a potent δ antagonist, it possesses one-sixth the potency and affinity of NTI (1). This suggests that the coplanar indolic benzene moiety of 1 is more capable of stabilizing the antagonist state of the δ receptor than the orthogonally oriented aromatic moiety of the indanyl group.

The fact that the indan benzene moiety of 5 and the phenyl group of BNTX (2) are in somewhat similar conformations is consistent with their highest δ antagonist potency relative to those of other members of the series. These results are in harmony with the feeble δ antagonist potency of the (*Z*)-benzylidene isomer 3, as the orientation of its phenyl group is directed away from the conformational space occupied by 2 and 5. Similarly, the comparable antagonist potency of 2 and 7-benzylnaltrexone (4) is consistent with the overlapping minimum energy conformations of the phenyl group in these compounds.

The most dramatic example of the role of conformation in δ antagonist potency is seen with the 6-anilino epimers 7 and 8. Although these epimers are closely structurally related to NTI (1), they have weak or no δ antagonist activity and are μ-selective. It may be significant that the anilino phenyl group of 7 and 8 does not share conformational space with the aromatic residues of the more potent ligands. Superposition of compounds 1–8 in their energetically preferred conformations illustrates (Figure 2) that the aromatic groups of the more potent δ antagonists (1, 2, 4, and 5) share conformational space that differs from those that are weakly active (3 and 6–8).

Interestingly, none of the δ-opioid antagonists synthesized in the present study possessed δ selectivity approaching that of NTI (1). Part of the reason for this is related to the considerably higher antagonist potency and affinity of NTI at δ sites. The most potent member (5) of the present series possesses a K_e value which is 6-fold greater than that of NTI. This, coupled with the (2–3)-fold smaller K_e values for μ and κ receptor interactions with 5, contributes to its lower selectivity. Thus, it appears that the conformation of the benzene moiety in 5 is a critical determinant of δ selectivity both by direct interaction with δ receptors and through its propensity to sterically interfere with binding to other opioid receptor types. In regard to the latter, the *in vitro* antagonist data suggest that the orthogonally oriented benzene moiety of 5 is not as effective as the coplanar benzene moiety in NTI.

There have been a number of reports that strongly implicate δ-opioid receptor subtypes in antinociception.[21-24] In this connection, BNTX (2) has been reported[9] to be a selective δ_1-opioid antagonist, while naltriben (NTB) and naltrindole-5′-isothiocyanate (5′-NTII) are antagonists of δ_2 receptors.[21,22] The *in vivo* putative δ_1 agonists are DPDPE and DADLE, while [D-Ala^2,Glu^4]deltorphin and DSLET have pharmacologic selectivity for δ_2 receptors. It has been noted that the mouse vas deferens preparation is not a predictive model for discrimination between δ subtypes inasmuch as it may contain a δ subtype that differs from that in the CNS.[9,25,26] Moreover, with the exception of BNTX, the aforementioned δ-selective antagonists have not exhibited selective binding that has a clear correlation with their *in vivo* antagonism of δ_1- and δ_2-selective agonists.[27,28] In view of the reported low correlation between *in vitro* antagonist activity and binding, it was not surprising that compounds 3–8 of the present series exhibited a rank order for δ-opioid antagonist potency in the MVD that only partially corresponded with that of the binding data.

Because the spiroindan 5 exhibited potent and selective δ-opioid antagonist and binding data, it was evaluated *in vivo* (Table 3). Although 5 (2 nmol/mouse) was capable of antagonizing DPDPE-induced antinociception most effectively, it was not highly selective in this regard. The δ_2 agonist, DSLET, and the μ agonist, morphine, both were antagonized less potently than the δ_1 agonist, DPDPE. The κ-opioid agonism that was induced by U50488 was not significantly affected by pretreatment with 5. Thus, it appears that 5 is somewhat selective for δ_1 receptors. It is noteworthy that 5 was substantially less potent *in vivo* than NTI (1), which is active icv at a dose of 10 pmol/mouse.[21] As was discussed in connection with the *in vitro* data, the lower potency and selectivity may in part be related to the orientation of the benzene moiety of the indan substituent. Of significance is the report that the *N*-methyl analogue of 5, SIOM (12), is both an agonist and an antagonist *in vivo* at δ_1 receptors.[5] The possibility of multiple recognition sites on a single receptor system was discussed as an explanation for these results. The close structural relationship between 5 and 12 and the fact that there was not effective competition between 5 and δ-selective agonist radioligands in binding, suggest that different binding sites may be involved. These different sites may have different conformational requirements. The recent report[6] that a δ receptor whose conserved Asp 95 in helix 2 was replaced by Asn exhibited greatly decreased affinity for δ agonists, but not antagonists, is consistent with the idea of different sites.

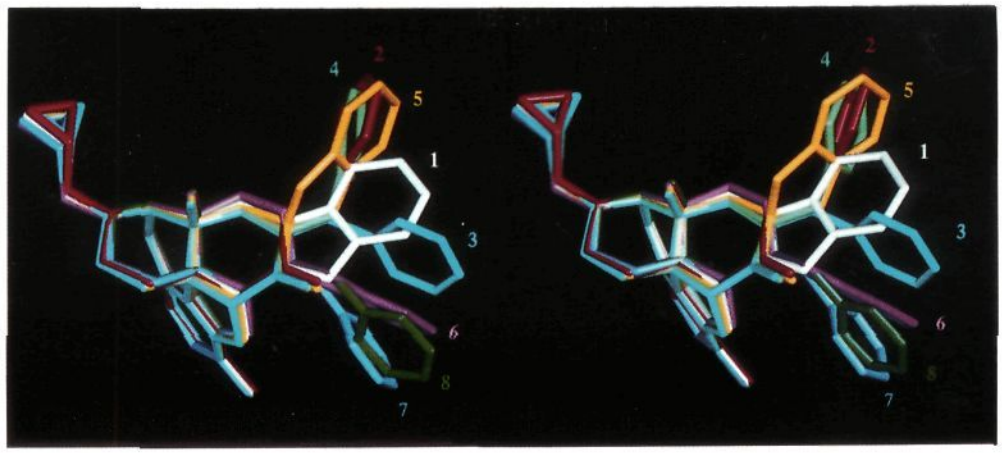

Figure 2. Superposition of compounds 1–8 in their energetically preferred conformations. Note that the more potent δ antagonists (1, 2, 4, and 5) and weakly active ligands (3, 6–8) share different conformational space.

Experimental Section

Melting points were determined in open capillary tubes on a Thomas-Hoover apparatus and are uncorrected. Elemental analyses were performed by MHW Laboratories (Phoenix, AZ). 1D and 2D NMR spectra were recorded at ambient temperature on IBM Bruker AC-300 and Varian 500 MHz spectrometers using Me_4Si as an internal standard. Infrared spectra were recorded on a Varian FT-IR spectrometer. Mass spectra were obtained on a Finnigan 4000 or a VG707EHF spectrometer. All TLC data were determined with Analtech silica gel (CHLF 21521). Spinning thin-layer chromatography was performed on Analtech 457G silica gel using a chromatotron (Harrison Research, Palo Alto, CA). Vacuum flash chromatography was performed on 150-Å pore size 35–75-μm particle size flash column silica gel (Analtech). All solvents were dried prior to use in reactions.

Computer-assisted molecular overlay studies were conducted for compounds using Biosym software[29] (Insight II version 2.0.0 and Discover version 2.7) operating in an IRIX 3.3.2 environment on a 35/TG personal iris workstation. All chemical structures were minimized using a quasi-Newton–Raphson algorithm (VA09A).[30] Each minimized structure was subjected to high-temperature molecular dynamics (700 K) for 1 ps. The resulting structure was then reminimized using VA09A, and the minimum energy conformation was compared to the initial minimized structure. Discover calculations used a consistent valence *forcefield (cvff)*[31] *supplied with the Biosym package.*

7-Benzylidenenaltrexones 2 and 3. To a stirred solution of **9**·HCl (754 mg, 2 mmol) in MeOH (16 mL) at 0 °C was added NaOH (1 N, 16 mL). The reaction mixture was cooled to −5 °C and stirred under argon for 15 min. Benzaldehyde (2 mL, 14.8 mmol) was added to the stirred solution, and the mixture was kept in the freezer for 12 h. It was then acidified to pH 4.5 with 1 N HCl (20–25 mL). The acidified solution was stirred for 20 min at room temperature and then evaporated *in vacuo*. The resulting residue was dissolved in H_2O (40 mL) and extracted with EtOAc (3 × 40 mL). The combined organic phase was dried (anhydrous Na_2SO_4) and evaporated to give an oil which was chromatographed on a chromatotron (ethyl acetate/hexane/NH_3 80:20:1) to give a solid (600 mg, 70%) consisting of a mixture of **2** and **3** in a 98:2 ratio. The crude mixture (300 mg) of **2** and **3** was subjected to flash chromatography using silica gel 60 (230–400 mesh, $CHCl_3$/MeOH/NH_4OH 95:5:0.5), and the major product was isolated as a gummy residue which was crystallized from ethanol to give the pure *E*-isomer **2**. The free base was dissolved in ethyl acetate cooled to −5 °C, ether saturated with HCl was added dropwise to the solution, and the precipitate was collected and crystallized (ethanol) to give **2**·HCl (280 mg, 65%): mp 225 °C dec; R_f 0.72 ($CHCl_3$/MeOH/NH_4OH 9.8:0.2:3 drops); IR (KBr, cm^{-1}) 1680 (C=O), 1614 (C=C); ^1H NMR (DMSO-d_6) δ 7.59 (s, 1H, vinylic), 7.38–7.46 (m, 5H, ArH), 6.70 (dd, 2H, H-1 and H-2), 4.78 (s, 1H, H-5), 0.85 (m, 1H, cyclopropane), 0.49 (d, 1H, cyclopropane), 0.14 (d, 1H, cyclopropane); FAB-MS *m/z* 429 (M^+). Anal. ($C_{27}H_{27}NO_4$·HCl) C, H, N.

The crude solid (300 mg) which contained **2** and **3** in a 98:2 ratio was subjected to HPLC (silica gel) ($CHCl_3$/hexane/MeOH/NH_4OH 98:12:2:1, v/v) in order to separate the minor isomer. The two bands were separated with a retention time (RT) of 10.98 min (98%, **2**, *E*-isomer) and 9.54 min (2%, **3**, *Z*-isomer). The minor band with a retention time of 9.54 min was further purified by reinjection and elution ($CHCl_3$/hexane/MeOH/NH_4OH 98:12:2:1, v/v) to give pure **3**, 5 mg (0.6%, based on naltrexone): R_f 0.74 ($CHCl_3$/MeOH/NH_4OH 98:2:1); IR (Nujol, cm^{-1}) 1690 (C=O), 1620 (C=C); ^1H NMR (DMSO-d_6) δ 7.50 (s, 1H, vinylic proton), 7.26–7.50 (m, 5H, ArH), 6.68 (dd, 2H, H-1 and H-2), 5.11 (s, 1H, H-5), 0.85 (m, 1H, cyclopropane), 0.49 (d, 1H, cyclopropane), 0.14 (d, 1H, cyclopropane); FAB-MS *m/z* 429 (M^+). No analysis was obtained because of insufficient quantity. The purity of **3** was evaluated further from TLC in two different solvent systems: R_f 0.45 (hexane/EtOAc/NH_4OH 70:30:0.1) and R_f 0.71 ($CHCl_3$/MeOH/NH_4OH 97:3:0.1).

17-(Cyclopropylmethyl)-7α-benzyl-4,5α-epoxy-3,14-dihydroxymorphinan-6-one (4). A mixture of **2** (80 mg, 0.18 mmol) and 10% Pd/C (50 mg) in EtOH (20 mL) was hydrogenated at an initial pressure of 35 psi for 4 h. The mixture was filtered over Celite, and the filtrate was evaporated to dryness. The resulting solid was purified by flash chromatography (hexane/EtOAc/NH_4OH 70:30:1) to give **4** (30 mg, 38%): mp 210 °C; R_f 0.70 ($CHCl_3$/MeOH/NH_4OH 9.8:0.2:3 drops); IR (KBr, cm^{-1}) 1730 (C=O); ^1H NMR (DMSO-d_6) δ 7.82–7.12 (m, 5H, ArH), 6.63 (d, 1H, H-1, *J* ≃ 8.5 Hz), 6.59 (d, 1H, H-2, *J* ≃ 8.5 Hz), 4.81 (s, 1H, H-5), 3.30 (m, 1H, H-7), 3.02 (dd, 2H, CH_2, J_{gem} = 18 Hz, $J_{1'-7\beta} \simeq 5.7$ Hz), 1.7 (dd, 1H, H-8α, $J_{8\alpha-8\beta} \simeq 18$ Hz, $J_{8\alpha-8\beta} \simeq 6$ Hz), 1.4 (dd, 1H, H-8β, $J_{8\beta-8\alpha} \simeq 18$ Hz, $J_{8\beta-7\beta} \simeq 2.4$ Hz), 0.79 (m, 1H, cyclopropane), 0.49 (d, 1H, cyclopropane), 0.10 (d, 1H, cyclopropane); FAB-MS *m/z* 431 (M^+). The free base was converted to its HCl salt. Anal. ($C_{27}H_{29}O_4N$·HCl) C, H, N.

3-*O*-Benzyl-17-(cyclopropylmethyl)-4,5α-epoxy-14-hydroxymorphinan-6-one (10). A mixture of naltrexone (**9**) (0.97 g, 2.84 mmol), benzyl bromide (0.68 mL, 0.97 g, 5.69 mmol), and K_2CO_3 (3.0 g) in acetone (50 mL) was refluxed for 3 h under anhydrous conditions. The reaction mixture was then cooled to 25 °C and filtered through fritted glass. The inorganic solids were washed with (3 × 30 mL) acetone. The combined washings and filtrate were concentrated under vacuum to afford a residue which was chromatographed on a 1- × 5-in. vacuum flash column (eluted with $CHCl_3$) to give **10** (1.05 g, 85.8%): mp 135–136 °C; ^1H NMR ($CDCl_3$) δ 7.47–7.26 (m, 5H, ArH), 6.65 (dd, 2H, C-1 and C-2), 5.25 (dd, 2H, -O-CH_2-Ph), 4.70 (s, 1H, C-5), 0.90 (m, 1H, cyclopropane), 0.55 (d, 2H, cyclopropane), 0.20 (d, 1H, cyclopropane); DEPT CH_2 (3.80, 3.96, 22.66, 30.71, 31.46, 36.20, 59.18, 72.09), CH (9.41, 62.01, 90.40, 118.02, 119.34, 127.02, 127.73, 128.33), quat (50.72, 70.12, 125.62, 129.82, 137.49, 141.76, 145.52, 208.2); MS (EI) *m/z* 341.5 (M^+).

3-*O*-Benzyl-17-(cyclopropylmethyl)-4,5α-epoxy-14-hydroxymorphinan-6-one-7-spiro-2′-indan (11). To a solution of hexamethyldisilazane (0.63 mL, 2.9 mmol) in dry DMSO (5 mL) was added a 1.6 M solution of *n*-butyllithium (1.6 mL, 2.56

mmol) in hexane under nitrogen. After the reaction mixture was stirred for 15 min at 25 °C, a solution of 3-benzylnaltrexone (10) (0.70 g, 1.62 mmol) in DMSO (4 mL) was added and the mixture stirred for 15 min. A solution of α,α'-dibromo-o-xylene (1.2 g, 4.6 mmol) in DMSO (2 mL) was then added. The mixture was kept at 25 °C for 1 h and then poured into H_2O (50 mL). After addition of brine (10 mL) and extraction with EtOAc (4 × 30 mL), the combined extracts were washed with brine (10 mL) and dried (anhydrous Na_2SO_4) and the solvent was removed under vacuum. The residue was chromatographed using spinning thin-layer chromatography (2-mm plate, elution with 5% MeOH/$CHCl_3$). The solvent was evaporated under vacuum to give 250 mg (36.3%) of a solid which was further purified by vacuum flash chromatography (1- × 5-in. column, elution with hexane/EtOAc 8:1) to give pure 11 (160 mg, 23%). The HCl salt was precipitated by adding ethereal HCl to an ether solution of 11. The salt was recrystallized from EtOH/ether to afford pure 11·HCl: mp 271–272 °C dec; ^{1}H NMR ($CDCl_3$) δ 7.46–7.09 (m, 9H, ArH), 6.67 (dd, 2H, C-1 and C-2), 5.27 (dd, 2H-O-CH_2-Ph), 5.01 (s, 1H, C-5), 3.82 (d, 1H, indan CH_2, J = 16.8 Hz), 3.51 (d, 1H, indan CH_2, J = 15.6 Hz), 3.22 (d, 1H, indan CH_2, J = 15.6 Hz), 2.39 (d, 1H, indan CH_2, J = 15.6 Hz), 0.85 (m, 1H, cyclopropane), 0.55 (d, 2H, cyclopropane), 0.12 (d, 1H, cyclopropane); DEPT CH_2 (3.86, 3.93, 22.65, 30.35, 41.74, 41.91, 42.49, 43.63, 56.45, 72.46), CH (9.41, 62.29, 89.21, 118.99, 119.39, 123.98, 124.38, 126.37, 126.62, 127.73, 127.85, 128.33), quat (51.39, 56.45, 69.82, 125.69, 130.01, 137.63, 140.40, 141.82, 141.87, 145.58, 208.2); IR (KBr, cm^{-1}) 1721.2 (6-C=O); MS (EI) m/z 533 (M^+). Anal. ($C_{35}H_{36}NO_4Cl\cdot{}^1/_2H_2O$) C, H, N.

17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan-6-one-7-spiro-2′-indan (5). A solution of the 11 (0.160 g, 0.300 mmol) in absolute EtOH (50 mL) and concentrated HCl (1.5 mL) was hydrogenated at 25 °C and 50 psi for 48 h over 35 mg of 10% Pd/C (Fluka Chemicals). The catalyst was removed by filtering through Celite which was then washed with EtOH (3 × 25 mL). After removal of solvent under vacuum, the residue was dissolved in $CHCl_3$ (20 mL) and EtOH (1 mL) and stirred with saturated aqueous $NaHCO_3$ (20 mL) for 10 min. The mixture was extracted with $CHCl_3$ (3 × 35 mL) and washed with brine (10 mL), and the solvent was evaporated under vacuum to give 100 mg (75%) of crude 5. This material was further purified by spinning thin-layer chromatography (2-mm silica gel, hexane/EtOAc 3:1). Fractions containing 5 were pooled, and the solvent was removed under vacuum. The residue was dissolved in EtOH/ether, and ethereal HCl was added to precipitate the HCl salt. Recrystallization (MeOH/ether) gave 34.9 mg (24%) of 5·HCl: mp >285 °C; IR (KBr, cm^{-1}) 1718.1 (C=O); ^{1}H NMR ($CDCl_3$) δ 0.133 (m, 2H, H-19β, H-20β), 0.525 (m, 2H, H-19a, H-20α), 0.842 (m, 1H, H-18), 1.52 (dd, 1H, H-15e), 1.80 (d, 1H, H-8), 2.05 (d, 1H, H-8), 2.57 (dd, 1H, H-10e), 2.69 (dd, 1H, H-16e), 3.02 (d, 1H, H-10a), 3.11 (d, 1H, H-9), 3.18 (d, 1H, indan CH_2), 3.48 (d, 1H, indan CH_2), 3.82 (d, 1H, indan CH_2), 4.95 (s, 1H, H-5), 6.57 (d, 1H, H-1), 6.71 (d, 1H, H-2), 7.11 (m, 4H, indan CH), 5.66 (b s, 1H, phenol OH), 1.89 (b s, 1H, 14-OH); MS m/z 444 (M^+). Anal. ($C_{28}H_{30}NO_4Cl\cdot{}^1/_5H_2O$) C, H, N.

17-(Cyclopropylmethyl)-6,7-didehydro-4,5α-epoxy-3,14-dihydroxy-6-(1′-spirobenzodioxalanyl)morphinan (6). To a solution of naltrexone (9) (341 mg, 1 mmol) in toluene (20 mL) were added catechol (110 mg, 1 mmol) and p-toluenesulfonic acid (200 mg). The resulting mixture was refluxed in a Dean–Stark trap (3–4 h). After removal of excess solvent *in vacuo*, the gummy residue was dissolved in $CHCl_3$ (20 mL) and washed with $NaHCO_3$ (30%, 3 × 20 mL) and then with brine. The organic phase was dried (anhydrous Na_2SO_4) and the solvent removed under vacuum. The residue was purified on preparative thin-layer chromatography ($CHCl_3$/MeOH/NH_4OH 97.5:2.5:0.05). The faster moving material was isolated as an amorphous solid (151 mg, 35%): R_f 0.85 ($CHCl_3$/MeOH/NH_4OH 8:2:0.05); mp 210 °C dec; ^{1}H NMR (DMSO-d_6) δ 8.80 (b s, 1H, OH), 8.23 (s, 1H, OH), 6.78–6.50 (m, 4H, ArH), 6.50 (dd, 2H, H-1 and H-2), 4.45 (s, 1H, H-5), 0.90 (m, 1H, cyclopropane), 0.55 (d, 2H, cyclopropane), 0.20 (d, 1H, cyclopropane); FAB-MS m/z 433.3 (M^+). Anal. ($C_{28}H_{27}O_5N$) C, H, N.

17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxy-6-anilinomorphinans 7 and 8. To a suspension of 9·HCl (377 mg, 1 mmol) in toluene (10 mL) were added aniline (111.5 mg, 1.2 mmol) and a catalytic amount of p-toluenesulfonic acid monohydrate. The reaction mixture was refluxed for 2–3 h in a Dean–Stark trap until no more H_2O separation was observed. The toluene was evaporated *in vacuo*, the residue was dissolved in methanolic buffer (KOAc 19.63 mL, HOAc 0.08 mL, MeOH 20 mL), and $NaCNBH_3$ (620 mg, 10 mmol) was added along with molecular sieves (4 Å, grade 514). After the mixture was stirred for 12 h, the MeOH was removed *in vacuo* and the crude product was dissolved in EtOAc (20 mL) and washed with dilute $NaHCO_3$ (20%, 20 mL). The organic layer was collected, dried with Na_2SO_4, and evaporated to afford an oil (280 mg) which was purified further using preparative thin-layer chromatography ($CHCl_3$/MeOH/NH_4OH 80:20:0.1). The α-anilino derivative 7 was isolated as a solid (110 mg, 26%): mp 230 °C dec; R_f 0.49 ($CHCl_3$/MeOH/NH_4OH 8:2:0.1); ^{1}H NMR (DMSO-d_6) δ 8.93 (b s, 1H, OH), 7.07 (t, 2H, ArH), 6.58 (m, 5H, H-1, H-2, and ArH), 5.02 (d, 1H, NH), 4.58 (d, 1H, H-5, $J_{5\beta\text{-}6\beta}$ = 4 Hz), 3.90 (m, 1H, H-6), 0.94 (m, 1H, cyclopropane), 0.47 (d, 1H, cyclopropane), 0.12 (d, 1H, cyclopropane); FAB-MS: m/z 418.23 (M^+). Anal. ($C_{26}H_{30}N_2O_3$) C, H, N. The β-epimer 8 was obtained as crystals (165 mg, 39%): mp 260 °C dec; R_f 0.53 ($CHCl_3$/MeOH/NH_4OH 8:2:0.1); ^{1}H NMR (DMSO-d_6) δ 9.09 (b s, 1H, OH), 6.34–6.59 (m, 5H, H-1, H-2, and ArH), 6.97 (t, 2H, ArH), 5.8 (d, 1H, J = 8.5 Hz, NH), 4.42 (d, 1H, H-5, $J_{5\beta\text{-}6\alpha}$ = 8 Hz), 3.36 (m, 1H, C-6), 0.94 (m, 1H, cyclopropane), 0.74 (d, 1H, cyclopropane), 0.42 (d, 1H, cyclopropane); FAB-MS m/z 418.23 (M^+). Anal. ($C_{26}H_{30}N_2O_3$) C, H, N.

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