7′-Substituted Amino Acid Conjugates of Naltrindole. Hydrophilic Groups as Determinants of Selective Antagonism of δ_1 Opioid Receptor-Mediated Antinociception in Mice

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Received November 30, 1994®

A series of amino acid conjugates (**2**–**6**) of naltrindole (**1**) were synthesized from 7′-carboxynaltrindole (**7**) in order to obtain δ antagonists that would have minimal access to the central nervous system (CNS) upon peripheral administration. All of the ligands (**2**–**7**) were tested in smooth muscle preparations and found to be potent and selective δ opioid antagonists. Receptor binding showed **2**–**7** to be highly δ-selective, with K_i ratios (μ/δ, κ/δ) ranging from 127 to 38 000. Two of the more selective conjugates, the glycinate **2** and aspartate **3**, were evaluated by the iv and icv routes in mice, and they afforded very high iv/icv dose ratios (112 766 and 46 667, respectively) consistent with poor CNS penetration. The *in vivo* testing revealed that **2** and **3** are δ_1-selective antagonists, in contrast to naltriben and related ligands which are δ_2-selective. The fact that the binding data are not consistent with the *in vivo* data suggests that the origin of the selectivity of naltrindole congeners may be related to selective access to tissue compartments in the CNS rather than to binding affinity differences between δ opioid receptor subtypes.

It is well documented that endogenous opioid peptides mediate their pharmacologic effects via interaction with opioid receptors in both the central and peripheral nervous systems.[1] The principal target tissues in the former are the brain and the spinal cord. The major sites in the periphery are the gastrointestinal tract, lymphocytes, and a variety of other tissues. The constipating action and inhibitory modulation of the immune response by morphine are examples of such effects.

Quaternized opiates have been employed as pharmacologic tools in order to sort out peripheral from central effects.[2] However, such ligands have considerably lower affinity for opioid receptors than their tertiary amine precursors. In order to circumvent this problem, opiates with hydrophilic groups attached to the C-6 position of the morphinan system have been synthesized.[3] It was reported that such compounds, particularly opiates with zwitterionic moieties, were effective in greatly reducing access to the central nervous system (CNS) without substantially decreasing receptor activity. Similar approaches have been recently employed in the benzeneacetamide[4,5] and phenylpiperidine[6] classes of analgesics.

It would be desirable to develop an armamentarium of ligands that are selective for each of the major types (μ, δ, κ) of opioid receptors. In particular, δ-selective antagonists would be useful as probes for receptors on immune cells in view of the potent immunosuppressive[7–9] effects of naltrindole (**1**).[10,11] Here we present the first report of naltrindole-related antagonists (**2**–**6**) that have greatly reduced access to the CNS and possess high potency and pharmacologic selectivity in the antagonism of δ_1 receptor-mediated antinociception when administered by the intracerebroventricular (icv) route.

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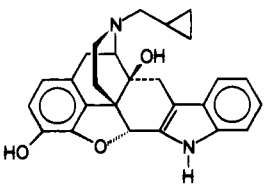

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Design Rationale and Chemistry

Structure–activity relationship studies in the naltrindole (NTI) series have revealed that the 7′-position on the indole moiety is relatively tolerant to substitution.[12] We therefore expected that the attachment of an amino acid at this position would not compromise activity. As this attachment could be accomplished through amidation of a carboxyl group, the key intermediate employed in the synthesis of target compounds **2**–**6** was 7′-carboxynaltrindole (**7**) (Scheme 1). Compound **7** was obtained via the Fischer indole synthesis[13] which involved refluxing equivalent amounts of naltrexone and 2-hydrazinobenzoic acid in glacial acetic acid (Scheme 1). The NMR spectrum of **7** possessed the characteristic downfield absorption of H-5 due to deshielding by the indole moiety. The coupling of **7** with suitably protected amino acids using the Bop reagent afforded the corresponding intermediates **8a**–**e**. Catalytic hydrogenation of these intermediates gave target compounds **2**–**6**.

Pharmacological Results

Smooth Muscle Preparations. All target compounds were tested on the electrically stimulated guinea pig ileal longitudinal muscle[14] (GPI) and mouse vas deferens[15] (MVD) preparations as described previously.[16] Antagonists were incubated with the preparations for 15 min prior to testing. Morphine (M), ethylketazocine (EK), and [D-Ala^2,D-Leu^5]enkephalin[17]

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Scheme 1

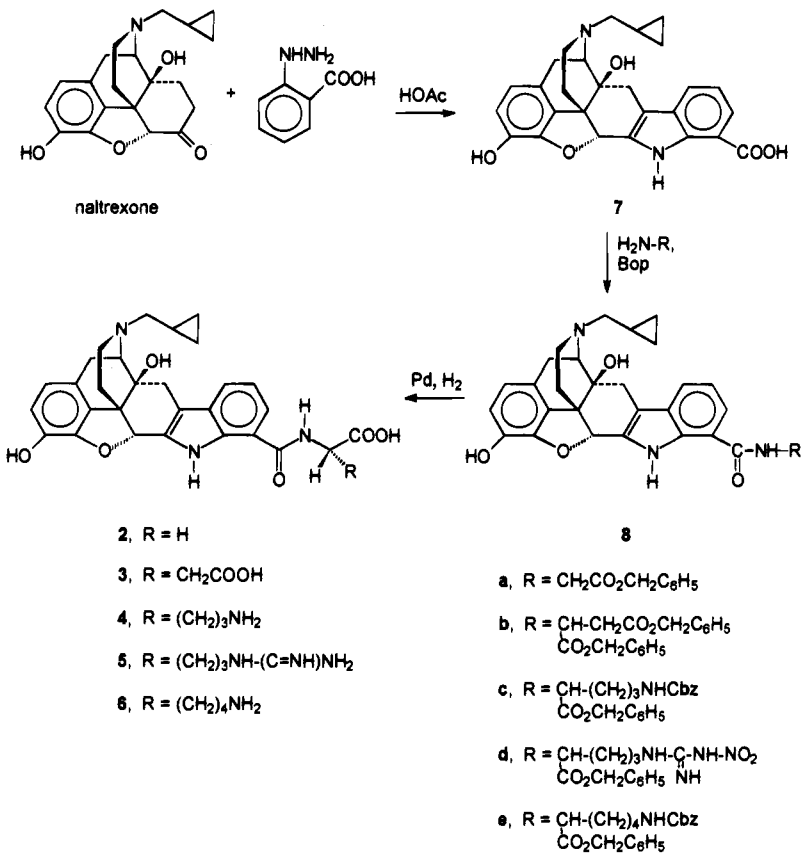

Table 1. Antagonist Potencies of 7'-Substituted Amino Acid Conjugates of Naltrindole in the MVD and GPI Preparations

	DADLE (δ)[b]				IC$_{50}$ selectivity ratio	
compd[a]	IC$_{50}$ ratio	K_e (nM)[d]	M (μ)[c] IC$_{50}$ ratio	EK (κ)[c] IC$_{50}$ ratio	δ/μ	δ/κ
1 (NTI)[e]	459 ± 104	0.2	11.2 ± 1.8	1.3 ± 0.2	41	353
2	32 ± 8	3.2	1.5 ± 0.1	1.1 ± 0.2	21	30
3	89 ± 20	1.1	1.3 ± 0.5	2.1 ± 0.6	67	43
4	58 ± 15	1.8	3.0 ± 1	0.9 ± 0.0	20	63
5	31 ± 5	3.3	1.3 ± 0.6	1.2 ± 0.5	24	27
6	20 ± 4	5.2	1.0 ± 0.4	1.1 ± 0.0	20	20
7	25 ± 7[f]	0.4	2.0 ± 0.3	1.0 ± 0.2	122[g]	231[g]

[a] The concentration of antagonist was 100 nM unless otherwise specified. [b] [D-Ala^2,D-Leu^5]enkephalin in the MVD preparation. [c] Morphine (M) or ethylketazocine (EK) in the GPI preparation. [d] Derived from the Schild relationship (Schild, H. O. *Pharmacol. Rev.* **1957**, *9*, 242) and calculated from an average of at least three IC$_{50}$ ratio determinations by using K_e = [antagonist]/(IC$_{50}$ ratio − 1). [e] Data from ref 31. [f] The concentration of **7** in the MVD experiment was 10 nM. [g] A calculated IC$_{50}$ ratio (238) based on 100 nM **7** was employed to calculate the selectivity ratio.

(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 mediated through δ opioid receptors were 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 in the same preparation.

The conjugates **2**–**6** were potent and selective δ opioid antagonists with K_e values ranging from 1 to 5 nM (Table 1). None were more potent than NTI at δ receptors, but the δ selectivities were equivalent to or higher than that of NTI. The precursor **7** exhibited δ antagonist potency approaching that of NTI.

Binding. The opioid receptor affinities of the target compounds (Table 2) were determined on guinea pig membranes employing a modification of the method of Werling et al.[18] Binding to κ, μ, and putative "δ_1" and "δ_2" sites was evaluated by competition with [^{3}H]-U69593,[19] [^{3}H]-[D-Ala^2,MePhe^4,Gly-ol^6]enkephalin[20] ([^{3}H]-DAMGO), [^{3}H]-[D-Pen^2-D-Pen^5]enkephalin[21] ([^{3}H]DP-DPE), and [^{3}H]-[D-Ser^2-Leu^5]enkephalin-Thr^6 [17] ([^{3}H]-DSLET) in the presence of 100 nM DAMGO, respectively. All of the conjugates (**2**–**6**) and the 7'-carboxy precursor **7** possessed high affinity for δ sites. The glycinate **2** and the precursor **7** possessed 2 and 6 times greater affinity for δ_1 sites than NTI, while other members (**3**–**6**) of the series had 5–10-fold less affinity than that of NTI. All of the compounds were highly selective, with K_i ratios substantially greater than that of NTI. It is noteworthy that the glycinate conjugate **2** possessed the highest selectivity ratios, with values of μ/δ_1 and κ/δ_1 > 30 000. Interestingly, the glycine and ornithine conjugates **2** and **4** competed more effectively with [^{3}H]DSLET than with [^{3}H]DPDPE for δ sites by a factor of 8–10. The aspartate and lysine derivatives **3** and **6** exhibited a modest preference for [^{3}H]DPDPE sites. There is no obvious correlation between binding and pharmacologic antagonist potency *in vitro* or *in vivo* (see below).

***In Vivo* Studies.** Two of the more selective antagonist ligands (**2**, **3**) and NTI (**1**) were evaluated in male Swiss–Webster mice using the tail-flick assay (Table 3).[22] Mice were pretreated with the antagonist by the icv or iv route so that the peak antagonist activity coincided with the peak antinociceptive response of the

Table 2. Binding of 7′-NTI Derivatives to Guinea Pig Brain Membranes

	K_i (nM)[a]				K_i selectivity ratio		
compd	[^{3}H]DAMGO (μ)	[^{3}H]U69593 (κ)	[^{3}H]DPDPE (δ_1)	[^{3}H]DSLET (δ_2)	μ/δ_1	κ/δ_1	δ_1/δ_2
1 (NTI)[b]	3.8	332	0.03		127	11066	
2	206	162	0.005	0.00066	38166	30037	8.2
3	368	893	0.14	0.41	2574	6246	0.3
4	136	57	0.18	0.015	756	320	11.3
5	333	188	0.16	0.083	2096	1181	1.9
6	219	41	0.30	0.75	719	134	0.4
7	24	42	0.013	0.016	1846	3246	0.8

[a] The geometric mean of K_i values for three replicate determinations. [b] Data from ref 12.

Table 3. Antagonist Profiles of Compounds **2** and **3** after Intracerebroventricular (icv) and Intravenous (iv) Administration in Mice

	pretreatment[b]			
agonist[a]	compd	dose (route of injection)	control ED_{50} (95% CL)[c]	ED_{50} ratio (95% CL)[d]
morphine (μ)	**2**	20 pmol/kg (icv)	9.7 (8.6 – 10.9) μmol/kg, sc	0.9 (0.8 – 1.1)
	3	20 pmol/kg (icv)		1.0 (0.7 – 1.5)
U50488H (κ)	**2**	20 pnmol/kg (icv)	27.3 (8.5 – 52.8) μmol/kg, sc	0.8 (0.7 – 0.9)
	3	20 pmol/kg (icv)		0.7 (0.4 – 1.2)
DSLET (δ_2)	**2**	20 pmol/kg (icv)	0.7 (0.3 – 1.0) nmol, icv	1.1 (0.8 – 1.6)
	3	20 pmol/kg (icv)		0.5 (0.3 – 1.1)
DPDPE (δ_1)	**2**	20 pmol/kg (icv)	8.6 (6.5 – 11.1) nmol, icv	5.3 (3.6 – 7.7)*
	3	20 pmol/kg (icv)		2.6 (2.0 – 3.6)*
	2	0.9 μmol/kg (iv)		2.7 (1.9 – 4.0)*
	3	0.8 μmol/kg (iv)		2.3 (1.7 – 3.1)*
	1	0.4 nmol/kg (icv)		2.6 (1.9 – 3.9)*
	1	12.5 μmol/kg (iv)		6.7 (4.6 – 10.0)*

[a] Peak antinociceptive activity times of agonists were morphine (30 min), U50488H (20 min), DSLET (10 min), and DPDPE (20 min). [b] Antagonists were administered so that their peak activities coincided with the peak agonist activities. Pretreatment times were **2** (30 min icv, 90 min iv), **3** (20 min icv, 30 min iv), and 1 (30 min icv, 40 min iv). [c] Antinociceptive activities were determined by tail-flick assay in mice. [d] ED_{50} with antagonist/control ED_{50}.

Table 4. Dose Required To Produce an Equivalent Antagonism of DPDPE-Induced Antinociception[a]

compd	icv	iv	ratio (iv/icv)
1	0.3 nmol/kg	2.19 μmol/kg	7300
2	4.7 pmol/kg	0.53 μmol/kg	112766
3	12.5 pmol/kg	0.62 μmol/kg	49600

[a] Dose required to double the ED_{50} of DPDPE (antagonist dose/ED_{50} ratio – 1).

selective agonists. The nonpeptide μ and κ agonists, morphine and *trans*-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide[23] (U50488), were administered sc; the peptide agonists (DPDPE and DSLET) were injected icv.

When administered either iv or icv, the glycine conjugate **2** effectively antagonized the antinociceptive effect of the δ_1 agonist, DPDPE. No significant antagonism of DSLET (δ_2), morphine (μ), or U50488 (κ) was observed. Similarly, the aspartate conjugate **3** selectively antagonized DPDPE.

The doses required to produce equivalent antagonism icv and iv are listed in Table 4. These doses were determined by calculating the dose of antagonist required to double the ED_{50} dose of DPDPE. The iv/icv dose ratios of **2** and **3** were 15 and 7 times greater than that of NTI.

Discussion

Earlier structure–activity studies on the δ opioid receptor antagonist naltrindole (**1**; NTI) have shown that the attachment of a substituent to the 7′-position of its indole moiety has minimal affect on antagonist potency, selectivity, and binding. In the present study we have taken advantage of this finding in the design of δ-selective antagonists that are less accessible to the CNS after peripheral administration, as such compounds may be useful tools to factor the central from the peripheral effects mediated by δ opioid receptors. Our approach involved the attachment of an amino acid to a 7′-carbonyl function to afford NTI derivatives **2**–**6** that are more polar than their parent compound, **1**.

The conjugates **2**–**6** and their precursor, **7**, were found to be potent δ opioid receptor antagonists in the mouse vas deferens preparation, and they displayed little, if any, antagonism toward μ- and κ-selective agonists in the guinea pig ileum. In fact, the δ/μ and δ/κ IC_{50} selectivity ratios of these ligands were equal to or greater than that of NTI.

Binding studies revealed that the K_i values of these antagonists were in the sub-nanomolar range for δ receptors. It is noteworthy that some of the conjugates (**2**, **4**) appeared to have greater affinity for δ_2 relative to δ_1 putative binding sites, while others (**3**, **6**) exhibited a modest preference for δ_1 over δ_2 sites. The significance of these results in uncertain in view of the fact that the classification of δ subtypes[24,25] has been based primarly on *in vivo* pharmacologic antagonism of putative δ_1 (DPDPE) and δ_2 (DSLET and deltorphin II) agonists by the selective δ antagonists, naltriben (NTB) (δ_2),[25] naltridole-5′-isothiocyanate (5′-NTII) (δ_2),[26] and [D-Ala^2,-Leu^5]enkephalin-Cys^6 (δ_1).[27] The only reported[28,29] binding studies using [^{3}H]DPDPE and [^{3}H]DSLET have not revealed any clear selectivity differences in competition studies with the δ_2 antagonists (NTB and NTII), and only 7-benzylidinenaltrexone (BNTX) was reported[30] to display δ_1-selective binding. The fact that these binding selectivity ratios do not correlate with the *in vivo* δ_1/δ_2 selectivity ratios of **2** and **3** (see below) suggests the limitation of binding data to predict pharmacologic potency of antagonists in these studies.

The glycinate **2** and aspartate **3** conjugates were evaluated for their effectiveness as antinociceptive

agents in mice by peripheral (iv) and central (icv) routes of administration. Both **2** and **3** were δ_1-selective in that they antagonized the δ_1 agonist, DPDPE, but not the δ_2 agonist, DSLET. It is not entirely clear why the binding selectivity of **2** and **3** differs from their pharmacologic selectivity, but one possibility is that accessibility to the δ_1 subtype *in vivo* might be favored by the presence of hydrophilic groups in these compounds. Thus, it is conceivable that the greater water solubility of the conjugates might facilitate access to tissue compartments in the CNS that contain δ_1 sites or prevent access to compartments that contain δ_2 sites. An alternate possibility is that identical δ receptors are located in different tissue compartments whose accessibility is governed by the polarity of the antagonist. Significantly, the *in vivo* δ_2-selective antagonists, naltriben[25] and benzylnaltrindole,[31] are considerably more lipophilic than **2** or **3**.

The iv/icv dose ratios of **2** and **3** to produce equivalent antagonism of DPDPE-induced antinociception were very high (>49 000) and, as expected, greater than that determined for NTI (Table 4). This ratio was 15- and 7-fold greater than that of NTI for **2** and **3**, respectively, which indicates that NTI penetrates the CNS more readily than the conjugates. It was surprising that the aspartate **3** has a lower iv/icv dose ratio than the glycinate **2** in view of the greater polarity of the aspartate relative to the glycinate residue. This is counter to current dogma which would predict that greater polarity would lead to lower CNS penetration.

In conclusion, our approach to design δ antagonists that penetrate the CNS less readily than NTI has been successful, as amino acid conjugates **2** and **3** are highly potent δ opioid antagonists when administered centrally and many orders of magnitude less potent by the peripheral route. It is proposed that the *in vivo* δ_1 selectivity of **2** and **3** may be a function of the greater hydrophilic character imparted to the ligands by the amino acid residue. If, for example, this promotes greater accessibility of the ligand to the δ_1 receptor subtype in neural tissues, it may suggest a new approach to the design of *in vivo* pharmacologically selective antagonist ligands for δ opioid receptor subtypes localized in the CNS. If the origin of the pharmacologic selectivity of NTI congeners for central δ opioid receptor subtypes is in part related to selective access to tissue compartments rather than to binding selectivity, this would explain the lack of correlation between *in vivo* pharmacologic selectivity and binding selectivity. For this reason, the subtype selectivity of **2** and **3** for antagonism of antinociception may not be the same for δ receptors at peripheral sites.

Experimental Section

Melting points were determined in open capillary tubes with a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ, and are within ±0.4 % of the theoretical values. IR spectra were obtained on a Perkin-Elmer 281 infrared spectrometer, and peak positions are expressed in cm^{-1}. NMR spectra were recorded at ambient temperature on GE-300 MHz and Bruker AC-200 MHz instruments, and chemical shifts are reported as δ values (ppm) relative to TMS. Mass spectra were obtained on a VG 7070E-HF instrument. All TLC data were determined with E. Merck Art. 5554 DC-Alufolien Kieselgel 60 F_{254}. Column chromatography was carried out on E. Merck silica gel 60 (230–400 mesh). The eluents used during column chromatography and reverse phase preparative HPLC, $CHCl_3$–MeOH–NH_4OH and MeOH–H_2O–CH_3CN, are denoted by CMA and MWA, respectively.

Dimethylformamide was distilled over calcium hydride. All other solvents and reagents were used without any further purifications unless specified. Naltrexone hydrochloride salt was supplied by Mallinckrodt.

7'-Carboxy-17-(cyclopropylmethyl)-6,7-didehydro-3,14-dihydroxy-4,5α-epoxy-6,7:2',3'-indolomorphinan Hydrochloride (7). Naltrexone hydrochloride (2.00 g, 5.3 mmol) and 2-hydrazinobenzoic acid (1.2 equiv, 1.1 g) were dissolved in glacial acetic acid (75 mL). The reaction mixture was refluxed for 6 h. The solvent was evaporated under reduced pressure and the residue dissolved in a minimum amount of MeOH. The crude product was purified on column chromatography (silica gel) with CMA (95:5:0.5). Upon evaporation of solvents under reduced pressure, the product was isolated as a solid which was recrystallized from methanol/ether to afford **7** (56%, 1.2 g): mp > 230 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.95 (d, 1H, COOH), 9.03 (s, 1H, PhOH), 7.75 (d, 1H, J = 7.50 Hz, Ph), 7.61 (d, 1H, J = 7.50 Hz, Ph), 7.04 (t, 1H, J = 7.20 Hz, Ph), 6.45 (m, 2H, Ph), 5.63 (s, 1H, H_5), 3.15 (m, 3H, H_9, H_{10}), 2.90–2.60 (m, 8H, H_8, H_{18}, H_{16}, H_{15}), 1.62 (m, 1H, H_{15}), 0.58 (m, 1H, H_{19}), 0.51 (m, 2H, H_{20}, H_{21}), 0.42 (m, 2H, H_{20}, H_{21}); ^{13}C NMR (50 MHz, DMSO-d_6) δ 168.35, 143.11, 140.12, 131.04, 130.23, 127.70, 124.70, 123.53, 123.14, 118.57, 118.22, 117.28, 109.80, 83.39, 72.23, 61.40, 57.92, 46.78, 44.30, 29.97, 28.74, 23.10, 7.82, 4.41, 3.12; IR (KBr pellet) 3200, 1677 cm^{-1}; HRMS (FAB) 459 (M + H^+), calcd 459.1919, obsvd 459.1911. Anal. ($C_{27}H_{26}O_5N_2 \cdot H_2O \cdot HCl$) C, H, N.

7'-[[(Glycine benzyl ester)-*N*-yl]carbonyl]-17-(cyclopropylmethyl)-6,7-didehydro-3,14-dihydroxy-4,5α-epoxy-6,7:2',3'-indolomorphinan (8a). Carboxylic acid **7** (365 mg, 0.7 mmol) was dissolved in a mixture of DMF/CH_2Cl_2 (40 mL) (1:1), in the presence of Et_3N (5.0 equiv 483 μL), [(benzotriazol-1-yl)oxy]tris(dimethylamino)phosphonium hexafluorophosphate (Bop reagent; Aldrich) (1.5 equiv 460 mg), and benzyl glycinate *p*-toluenesulfonate salt (1.0 equiv, 257 mg). The reaction mixture was extracted with ethyl acetate, and the organic layer was washed with saturated $NaHCO_3$, 10% citric acid, and brine. The organic layer was dried over $MgSO_4$ and the solvent removed *in vacuo*. The crude product was eluted on a silica gel column CMA (98:2:0.5) and purified further on preparative TLC (silica gel, 1 mm) with CMA (99:1:0.5). The desired product **8a** was isolated as an oil (150 mg, 36%) which was crystallized from chloroform/hexanes: mp 118–121 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.16 (s, 1H, PhOH), 7.52 (d, 1H, J = 7.50 Hz, Ph), 7.37 (m, 4H, Ph), 6.94 (d, 1H, J = 7.20 Hz, Ph), 6.89 (m, 1H), 6.64 (d, 1H, J = 8.40 Hz, H_2), 6.55 (d, 1H, J = 8.50 Hz, H_1), 5.64 (s, 1H, H_5), 5.24 (s, 2H, COOBn), 4.29 (d, 2H, J = 4.80 Hz, CH_2NH), 3.37 (d, 1H, J = 6.00 Hz, H_9), 3.10 (d, 1H, J = 18.30 Hz, H_{10}), 2.83–2.59 (m, 6H, H_{16}, H_{18}, H_{10}, NH), 2.45–2.29 (m, 3H, H_{15}, H_8), 1.80 (d, 1H, J = 11.10 Hz, H_{15}), 0.87 (m, 1H, H_{19}), 0.55 (m, 2H, H_{21}, H_{20}), 0.16 (m, 2H, H_{20}, H_{21}); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.43, 168.78, 163.45, 143.99, 140.22, 136.65, 135.89, 131.37, 130.92, 129.28, 129.25, 129.14, 129.11, 129.04, 128.57, 125.28, 123.65, 121.08, 119.48, 118.62, 118.12, 115.41, 111.35, 85.55, 73.49, 67.91, 62.89, 60.09, 48.66, 44.34, 42.18, 37.43, 37.19, 32.06, 29.32, 23.83, 10.09, 4.78, 4.42; HRMS (FAB) 606 (M + H^+), calcd 606.2604, obsvd 606.2625. Anal. ($C_{36}H_{35}O_6N_3$) C, H, N.

7'-[[(Dibenzyl L-aspartate)-*N*-yl]carbonyl]-17-(cyclopropylmethyl)-6,7-didehydro-3,14-dihydroxy-4,5α-epoxy-6,7:2',3'-indolomorphinan (8b). Carboxylic acid **7** (426 mg, 0.9 mmol) was dissolved in a mixture of DMF/CH_2CL_2 (40 mL) (1:1), in the presence of Et_3N (6.0 equiv, 720 μL), Bop reagent (1.5 equiv, 571 mg), and dibenzyl L-aspartate *p*-toluenesulfonate salt (1.2 equiv, 502 mg). The workup and purification were carried out as described for compound **8a**. The desired product **8b** was isolated in 30% yield (187 mg, 0.2 mmol) and crystallized from chloroform/hexanes: mp 115–118 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.06 (s, 1H, OH), 7.59 (d, 1H, J = 7.50 Hz, Ph), 7.32 (m, 7H, Ph), 7.13 (d, 1H, J = 7.50 Hz, Ph), 6.85 (d, 1H, J = 7.20 Hz, Ph), 6.76 (d, 1H, J = 8.40 Hz, H_2), 6.65 (d, 1H, J = 8.40 Hz, H_1), 6.13 (m, 1H, NH), 5.77 (s, 1H, H_5), 5.21 (m, 2H, CH_2Ph), 5.13 (m, 2H, CH_2Ph),

5.09 (m, 1H, CHN), 3.38 (d, 2H, J = 4.80 Hz, CH_2COOBn), 3.26 (d, 1H, J = 5.10 Hz, H_9), 3.21 (d, 1H, J = 19.50 Hz, H_{10}), 2.89–2.71 (m, 3H), 2.57–2.33 (m, 5H), 1.84 (d, 1H, J = 9.90 Hz, H_{15}), 0.87 (m, 1H, H_{19}), 0.55 (m, 2H, H_{20}, H_{21}), 0.17 (m, 2H, H_{20}, H_{21}); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.66, 172.26, 168.02, 143.81, 139.59, 136.34, 135.94, 135.58, 131.30, 129.30, 129.25, 129.11, 128.09, 128.02, 123.47, 121.48, 119.70, 118.09, 118.04, 117.85, 115.28, 115.25, 111.89, 86.31, 73.55, 68.61, 67.85, 62.84, 60.11, 49.83, 48.83, 44.37, 37.33, 32.44, 29.33, 23.83, 10.05, 4.78, 4.41; HRMS (FAB) (M + H^+), calcd 754.3128, obsvd 754.3138.

7′-[[[Benzyl N^{δ}-(carbobenzyloxy)-L-ornithinate]-N^2-yl]carbonyl]-17-(cyclopropylmethyl)-6,7-didehydro-3,14-dihydroxy-4,5α-epoxy-6,7:2′,3′-indolomorphinan (8c). Carboxylic acid **7** (426 mg, 0.8 mmol) was dissolved in a mixture of DMF/CH_2Cl_2 (1:1) (30 mL) at room temperature, in the presence of Bop reagent (1.5 equiv, 536 mg), Et_3N (5.0 equiv, 564 μL), and benzyl N^{δ}-Cbz-L-ornithinate trifluoroacetate salt[32] (380 mg, 0.8 mmol). The reaction mixture was stirred overnight. The workup and purification were carried out as described for compound **8a**. The desired product **8c** was isolated as an oil in 56% yield (362 mg): ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.09 (br s, 1H, PhOH), 7.51 (d, 1H, J = 8.70 Hz, Ph), 7.35 (m, 10H), 7.10 (d, 1H, J = 8.30 Hz, Ph), 6.88 (m, 1H, Ph), 6.61 (d, 1H, J = 8.40 Hz, H_2), 6.54 (d, 1H, J = 8.40 Hz, H_1), 5.57 (s, 1H, H_5), 5.20 (m, 2H, COOBn), 5.06 (m, 2H, Cbz), 4.90 (m, 1H, CHCOOBn), 3.34 (d, 1H, J = 6.30 Hz, H_9), 3.13 (m, 3H, H_{10} -CH_2NHCbz), 2.81–2.57 (m, 3H, H_{10}, H_{16}), 2.45–2.32 (m, 4H, H_8, H_{18}), 1.76 (m, 6H, CH_2's H_{15}), 0.90 (m, 1H, H_{19}), 0.57 (m, 2H, H_{20}, H_{21}), 0.17 (m, 2H, H_{20}, H_{21}); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.56, 167.91, 156.87, 143.24, 139.55, 136.18, 139.55, 136.68, 136.18, 135.32, 130.67, 130.40, 128.64, 128.45, 128.30, 128.15, 127.98, 124.67, 123.29, 120.58, 118.84, 118.04, 117.50, 115.12, 110.99, 84.73, 72.81, 67.28, 66.60, 62.32, 59.47, 52.41, 48.06, 43.70, 40.48, 31.51, 29.52, 28.70, 26.21, 22.46, 9.47, 3.81; HRMS (FAB) 797 (M + H^+), calcd 797.3550, obsvd 797.3547; IR (neat) 3300, 1719, 1703, 1613 cm^{-1}. Anal. ($C_{47}H_{48}O_8N_4$) C, H, N.

7′-[[(Benzyl N^{ω}-nitro-L-argininate)-N^2-yl]carbonyl]-17-(cyclopropylmethyl)-6,7-didehydro-3,14-dihydroxy-4,5α-6,7:2′,3′-indolomorphinan Hydrochloride (8d). Carboxylic acid **7** (350 mg, 0.7 mmol) was dissolved in CH_2Cl_2/DMF (1:1) (50 mL), with Bop reagent (1.5 equiv, 440 mg), Et_3N (5.0 equiv, 463 mL), and benzyl N^{α}-amino-N^{ω}-nitro-L-arginate trifluoroacetate salt[32] (1.1 equiv, 309 mg). The workup and purification steps were similar to those described for compound **8a**. The desired product **8d** was isolated as a solid in 9% yield (33 mg): mp >240 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.01 (br s, 1H, PhOH), 8.80 (br s, 1H, OH), 7.63 (m, 1H, Ph), 7.47 (m, 6H, Ph), 7.05 (m, 1H, $H_{5'}$), 6.68 (d, 1H, J = 7.50 Hz, H_2), 6.55 (d, 1H, J = 7.50 Hz, H_1), 5.67 (s, 1H, H_5), 5.18 (br m, 2H, COOBn), 5.04 (m, 1H, CHCOOBn), 3.34 (d, 1H, J = 6.30 Hz, H_9), 3.13 (m, 3H, H_{10} -CH_2NH), 2.88–2.60 (m, 5H), 2.45–2.32 (m, 4H), 1.81 (m, 6H, -CH_2's H_{15}), 0.89 (m, 1H, H_{19}), 0.58 (m, 2H, H_{21}, H_{20}), 0.17 (m, 2H, H_{20}, H_{21}); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.14, 167.04, 143.78, 140.22, 131.16, 129.07, 128.95, 128.80, 128.75, 125.10, 123.69, 121.59, 119.38, 118.83, 117.67, 111.05, 84.97, 73.58, 67.75, 62.71, 59.88, 44.26, 41.08, 32.00, 29.09, 23.52, 9.74, 4.39, 3.89; IR (KBr) 3500, 1735, 1637 cm^{-1}; HRMS (FAB) 750 (M + H^+), calcd 750.3251, obsvd 750.3242.

7′-[[[Benzyl N^{ϵ}-(carbobenzyloxy)-L-lysinate]-N^2-yl]carbonyl]-17-(cyclopropylmethyl)-6,7-didehydro-3,14-dihydroxy-4,5α-epoxy-6,7:2′,3′-indolomorphinan (8e). Carboxylic acid **7** (385 mg, 0.93 mmol) was dissolved in a mixture of DMF/CH_2Cl_2 (40 mL) (1:1), in the presence of Et_3N (5.0 equiv, 509 μL), Bop reagent (1.5 equiv, 484 mg), and benzyl N^{ϵ}-Cbz-L-lysinate trifluoroacetate salt[32] (1.0 equiv, 353 mg). The reaction mixture was stirred overnight at room temperature. The workup and purification procedure were similar to those described for compound **8a**. The desired product **8e** was isolated as an oil in 25% yield (170 mg): ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.12 (s, PhOH), 7.54 (d, 1H, J = 8.70 Hz, Ph), 7.43 (d, 1H, J = 7.20 Hz, Ph), 7.33 (m, 7H, Ph), 7.00 (m, 2H, Ph), 6.60 (d, 2H, J = 8.00 Hz, H_2), 6.53 (d, 2H, J = 8.00 Hz, H_1), 5.56 (s, 1H, H_5), 5.23 (m, 2H, COOBn), 5.04 (s, 2H, Cbz), 4.92 (m, 1H, CHCOOBn), 3.32 (d, 1H, J = 6.20 Hz, H_9), 3.09 (m, 3H, H_{10}, CH_2NHCbz), 2.90–2.60 (br m, 3H, H_{10}, H_{16}), 2.50–2.30 (br m, 5H, H_{16}, H_8, H_{10}, H_{18}), 1.85 (br m, 3H, H_8 CH_2), 1.40 (br m, 6H, H_{15} CH_2's), 0.90 (m, 1H, H_{19}), 0.58 (m, 2H, H_{20}, H_{21}), 0.17 (m, 2H, H_{20}, H_{21}); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.67, 167.83, 156.88, 143.24, 139.52, 136.62, 136.38, 135.57, 130.64, 130.54, 128.65, 128.45, 128.35, 127.99, 124.69, 123.32, 120.42, 118.80, 118.13, 117.40, 115.32, 110.95, 84.75, 72.75, 67.24, 66.62, 62.33, 59.48, 52.42, 43.71, 40.41, 31.89, 31.45, 29.25, 28.73, 23.14, 22.45, 9.46, 4.10, 3.81; HRMS (FAB) 811 (M + H^+), calcd 811.3706, obsvd 811.3716; IR (neat) 3200, 1743, 1721, 1637 cm^{-1}.

7′-[(Glycine-N-yl)-carbonyl]-17-(cyclopropylmethyl)-6,7-didehydro-3,14-dihydroxy-4,5α-epoxy-6,7:2′,3′-indolomorphinan Hydrochloride (2). Intermediate **8a** (100 mg, 0.16 mmol) was dissolved in methanol (10 mL) in the presence of a catalytic amount of 10% Pd-on-carbon and 2 N HCl (1.5 equiv, 12 μL). The hydrogenation reaction was run at atmospheric pressure for 1 h. The mixture was filtered over Celite, the Celite was washed several times with MeOH, and the mixture was concentrated under reduced pressure. Slow addition of EtOAc to the concentrated methanolic solution of **8a** led to the precipitation of a solid which was purified by HPLC using a C_{18} reverse phase column (silica gel) with MWA (4:3:3) plus a few drops of NH_4OH, and the desired hydrochloride salt **2** (t_R = 50 min) was isolated in 75% yield (68 mg): mp >240 °C; ^{1}H NMR (300 MHz, methanol-d_4) δ 10.76 (br s, 1H), 7.61 (m, 2H, $H_{6'}$, $H_{4'}$), 7.06 (t, 1H, J = 7.35 Hz, $H_{5'}$), 6.64 (m, 2H, H_1, H_2), 5.75 (s, 1H, H_5), 4.15 (d, 2H, J = 6.20 Hz, CH_2N), 3.47–3.27 (m, 2H, H_9, H_{10}), 3.18–2.86 (m, 4H, H_{18}, H_{10}, H_8), 2.77–2.67 (m, 2H, H_8, H_{15}), 1.94 (br d, 1H, J = 13.50 Hz, H_{15}), 1.14 (m, 1H, H_{19}), 0.78 (m, 2H, H_{20}, H_{21}), 0.52 (m, 2H, H_{20}, H_{21}); ^{13}C NMR (75 MHz, methanol-d_4) δ 171.57, 169.58, 141.39, 130.79, 128.54, 123.23, 123.11, 123.00, 121.78, 121.75, 119.89, 118.89, 118.76, 109.01, 84.08, 72.80, 62.82, 58.01, 51.78, 41.32, 41.21, 29.43, 28.99, 24.22, 6.04, 5.44, 2.48; HRMS (FAB) 516 (M + H^+), calcd 516.2134, obsvd 516.2134. Anal. ($C_{29}H_{30}O_6N_3$·HCl) C, H, N.

7′-[(L-Aspartate-N-yl)carbonyl]-17-(cyclopropylmethyl)-6,7-didehydro-3,14-dihydroxy-4,5α-epoxy-6,7:2′,3′-indolomorphinan Hydrochloride (3). Intermediate **8b** (180 mg, 0.2 mmol) was dissolved in methanol (15 mL), in the presence of a catalytic amount of 10% Pb-on-carbon and 6 N HCl (2.0 equiv, 0.5 μL). The hydrogenation and purification procedures were as described for compound **2**. The crude product was purified by reverse phase HPLC (MWA, 3:6:1), and the diacid (t_R = 9.50 min) was isolated in 90% yield (130 mg, 0.2 mmol): mp > 210 °C dec; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.04 (s, 1H, PhOH), 9.04 (br s, 1H, OH), 8.38 (d, 1H, J = 7.20 Hz, NH), 7.58 (d, 1H, J = 7.50 Hz, Ph), 7.53 (d, 1H, J = 7.50 Hz, Ph), 7.00 (t, 1H, J = 7.50 Hz, Ph), 6.48 (s, 2H, H_2, H_1), 5.63 (s, 1H, H_5), 4.06 (m, 1H, CHCOOH), 3.45 (m, 1H, H_9), 3.29 (d, 2H, J = 3.60 Hz, CH_2COOH), 3.23–3.11 (m, 3H), 3.04–2.61 (m, 5H), 1.93 (d, 1H, J = 11.10 Hz, H_{15}), 1.18 (m, 1H, H_{19}), 0.72 (m, 2H, H_{20}, H_{21}), 0.50 (m, 2H, H_{20}, H_{21}); ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.63, 173.68, 167.07, 144.16, 141.09, 136.21, 132.16, 128.70, 123.19, 121.89, 119.62, 119.12, 118.30, 118.01, 110.03, 104.55, 104.13, 84.54, 73.28, 64.05, 62.47, 59.09, 50.01, 47.93, 45.50, 29.80, 24.02, 5.42, 4.23, 1.21; HRMS (FAB) 574 (M + H^+), calcd 574.2189, obsvd 574.2191. Anal. ($C_{31}H_{32}O_8N_3$·HCl) C, H, N.

7′-[(L-Ornithine-N^2-yl)carbonyl]-17-(cyclopropylmethyl)-6,7-didehydro-3,14-dihydroxy-4,5α-epoxy-6,7:2′,3′-indolomorphinan Hydrochloride (4). Intermediate **8c** (233 mg, 0.3 mmol) was dissolved in MeOH (20 mL) with 1 N HCl (3 drops, 1.0 equiv) and a catalytic amount of 10% Pd-on-carbon. The hydrogenation and purification were similar to those described for **2**. The product was isolated in 85% yield (155 mg): mp >240 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.92 (s, PhOH), 8.70 (d, 1H, J = 7.20 Hz, Ph), 7.67 (br s, 1H, Ph), 7.61 (d, 1H, J = 7.20 Hz, Ph), 7.50 (d, 1H, J = 7.50 Hz, Ph), 6.98 (t, 1H, J = 7.50 Hz, Ph), 6.45 (s, 2H, NH), 5.60 (s, 1H, H_5), 4.36 (m, 1H, CHCOOH), 3.20–3.00 (m, 4H, H_9, H_{10} CH_2NH_2), 2.90–2.40 (m, 8H, H_8, H_{10}, H_{18}, H_{16}, H_{15}), 1.90–1.70 (m, 5H, H_{15} CH_2's), 0.85 (m, 1H, H_{19}), 0.46 (m, 2H, H_{20}, H_{21}), 0.12 (m, 2H, H_{21}, H_{20}); ^{13}C NMR (75 MHz, methanol-d_4) δ 171.16, 167.70, 144.13, 140.82, 136.30, 132.16, 132.05, 128.69, 125.39, 123.18,

121.87, 119.37, 118.96, 117.99, 117.91, 111.17, 84.78, 80.25, 73.32, 62.61, 59.69, 50.46, 48.35, 44.41, 42.36, 32.26, 29.74, 28.79, 23.71, 22.57, 10.32, 4.99, 4.52; HRMS (FAB) 573 (M^+), calcd 573.2713, obsvd 573.2711. Anal. ($C_{32}H_{37}O_6N_4$·HCl) C, N; H: calcd, 6.08; found, 6.59.

7'-[(L-Arginine-N^2-yl)carbonyl]-17-(cyclopropylmethyl)-6,7-didehydro-3,14-dihydroxy-4,5α-epoxy-6,7:2',3'-indolomorphinan Hydrochloride (5). Intermediate **8d** (33 mg, 0.04 mmol) was dissolved in MeOH (5 mL), with a catalytic amount of 10% Pd-on-carbon and HCl (3.0 equiv, 100 μL). The hydrogenation and purification steps were as described for compound **2**. The desired compound was isolated in 90% yield (20 mg): mp > 250 °C dec; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.05 (br s, 1H, PhOH), 7.38 (m, 3H, Ph), 6.70 (d, 1H, J = 7.50 Hz, H_2), 6.52 (d, 1H, J = 7.50 Hz, H_1), 5.63 (s, 1H, H_5), 4.33 (m, 1H, CH), 3.35–3.12 (m, 4H, H_9, H_{10} CH_2NH), 2.80–2.62 (m, 5H, H_{10}, H_{18}, H_{16}), 2.45–2.32 (m, 3H, H_8, H_{15}), 1.81–1.60 (m, 5H, H_{15} CH_2's), 0.85 (m, 1H, H_{19}), 0.46 (m, 2H, H_{20}, H_{21}), 0.12 (m, 2H, H_{20}, H_{21}); IR (KBr) 3200, 1735, 1644 cm^{-1}; HRMS (FAB) 617 (M^+), calcd 617.3087, obsvd 617.3087. Anal. ($C_{33}H_{39}O_6N_6$·HCl) C, H, N.

7'-[(L-Lysine-N^2-yl)carbonyl]-17-(cyclopropylmethyl)-6,7-didehydro-3,14-dihydroxy-4,5α-epoxy-6,7:2',3'-indolomorphinan Hydrochloride (6). Intermediate **8e** (170 mg, 0.2 mmol) was dissolved in MeOH (20 mL) in the presence of a catalytic amount of 10% Pd-on-carbon and 1 N HCl (3.0 equiv, 600 mL). The hydrogenation and purification were as described for compound **2**. The desired hydrochloride salt was isolated in 65% yield (82 mg): mp >270 °C dec; ^{1}H NMR (300 MHz, methanol-d_4) δ 7.64 (d, 1H, J = 7.50 Hz, Ph), 7.57 (d, 1H, J = 7.50 Hz, Ph), 7.05 (t, 1H, J = 7.50 Hz, Ph), 6.61 (br s, 2H, Ph), 5.73 (s, 1H, H_5), 4.16 (d, 1H, J = 4.80 Hz, CHCOOH), 3.32–3.15 (m, 4H, H_9, H_{10}, CH_2), 3.04–2.97 (m, 3H, H_{10}, H_{18}, H_{16}), 2.87–2.67 (m, 4H, H_{18}, H_{16}. H_8), 1.96 (m, 4H, H_{15} CH_2), 1.71 (m, 2H, CH_2), 1.51 (m, 2H, CH_2), 0.90 (m, 1H, H_{19}), 0.85 (m, 2H, H_{21}, H_{20}), 0.51 (m, 2H, H_{20}, H_{21}); ^{13}C NMR (75 MHz, methanol-d_4) δ 174.95, 168.23, 144.20, 141.66, 136.41, 131.92, 130.29, 128.70, 123.45, 122.77, 122.55, 120.04, 119.09, 118.88, 117.39, 109.49, 84.03, 73.21, 62.08, 60.87, 57.85, 53.39, 47.07, 39.44, 31.01, 29.82, 29.33, 27.62, 24.70, 24.02, 6.86, 6.31, 3.65; HRMS (FAB) 587 (M^+), calcd 587.2869, obsvd 587.2857; IR (KBr) 3300, 1728, 1637 cm^{-1}. Anal. ($C_{33}H_{39}O_6N_4$·HCl) C, H, N.

Acknowledgment. This research was supported by the National Institute on Drug Abuse. We thank Veronika Phillips, Michael Powers, Joan Naeseth, Mary Lunzer, and Idalia Sanchez for their capable technical assistance.

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JM940808S