O^3-(2-Carbomethoxyallyl) Ethers of Opioid Ligands Derived from Oxymorphone, Naltrexone, Etorphine, Diprenorphine, Norbinaltorphimine, and Naltrindole. Unexpected O^3-Dealkylation in the Opioid Radioligand Displacement Assay

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O^3-(2-Carbomethoxyallyl) ether derivatives of some phenolic 4,5-epoxymorphinan opioid ligands have been prepared in a simple one-step procedure, and their behavior in the radioligand receptor assay was compared to their phenolic precursors. These O^3-ether ligands appeared to show significant affinity for opioid receptors, about 2-fold less than the parent phenols, and their receptor selectivities were similar. However, on close examination of the stability of a representative ether **2b** in the radioligand displacement assay, considerable O^3-dealkylation was observed. The dealkylation process occurred even after denaturation of the proteins of the membrane preparation, and it occurred in the presence of model nucleophiles imidazole and thiophenol. Thus, what apparently was unusual activity is explained by O^3-dealkylation to the parent phenol (e.g., **2a**). Saturated ether analog **2c** was not dealkylated under the conditions of the radioligand displacement assay and was a very weak opioid ligand. We conclude that the conversion of the O^3(2-carbomethoxyallyl) ether electrophilic ligands to their parent phenols accounts for their activity in the opioid radioligand displacement assay.

Electrophilic affinity probes derived from several structurally different classes of opioid agonists and antagonists have been prepared to aid in characterization of their ligand-receptor interactions.[1] Attempts to obtain site-selective alkylating agents have led to preparation of ligands in which electrophilic groups are located at various molecular sites appended to the basic opioid pharmacophore. The additional functional groups are generally restricted in size in order to obtain compounds with reasonable affinity for receptors and are located at sites that are amenable to chemical modification. Some examples of irreversible ligands for different classes of opioid receptors include ligands related to naltrexone in which the electrophile is located at the C-6 position, e.g., nitrogen mustards,[2] fumaramates,[3] and isothiocyanates[4] derived from 6α- and 6β-naltrexamine, δ-site-selective irreversible ligands including those derived from fentanyl and 3-methylfentanyl,[5] and isothiocyanates derived from naltrindole,[6] and κ-site-selective irreversible ligands derived from U-50,488H.[7]

When the known μ-receptor ligands naltrexone, morphine, and morphinone are the starting compounds, electrophilic groups are usually located in positions in the C-ring (principally C-6 or occasionally C-14[8]), sites of hetero atoms which can be derivatized readily and where substituents may be added to provide compounds with good activity. Substitution at C-8 and at the basic nitrogen atom have been less successful.[9,10]

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In these and other opioids, the phenolic oxygen atom at the 3-position in the morphinan skeleton has not been utilized, principally because ethers and esters of the earlier opioids had substantially reduced activity in vivo and/or very low potency in radioligand displacement assays. For example, codeine and oxycodone are nearly inactive in radioligand displacement assays against [^{3}H]naloxone;[11] likewise, heroin is much less potent than is morphine or O^6-acetylmorphine in radioligand displacement assays against [^{3}H]naltrexone.[12] Thus one hypothesis is that codeine and heroin are analgesic because they serve as prodrugs of morphine and of O^6-acetylmorphine and morphine, respectively.[12,13] However, some related compounds that are not expected to be prodrugs, e.g. the O^3-*tert*-butyl ethers of analgesic opioids have measurable activity in vivo but are not potent in the radioligand displacement assay.[14] Evidence exists that the expected analgetically active ether cleavage products are not observed as metabolites.[15] Even among the more potent opioids in Bentley's C-ring bridged analogs, e.g. etorphine and related compounds, compounds in which significant activity is observed in vivo among the methyl ethers,[16,17] the corresponding phenols are usually 1 or 2 orders of magnitude more potent in radioligand displacement assays,[18] suggesting these ethers could serve as prodrugs. In the antagonist series differences in vivo are even larger, up to 2000-fold.[19] However, when most of the A-ring in agonists in this series has been "carved away" by ozonolysis, the remaining compounds retain significant analgesic activity.[20] Clearly, complete conservation of structural integrity at all molecular sites in the opioids is not absolutely necessary for productive receptor binding interaction. In general, among morphinan- and 4,5-epoxymorphinan-derived agents, a free phenolic hydroxyl group is required for significant activity of opioids in radioreceptor ligand displacement assays, and those O^3-ethers that can be metabolized to the phenols are less potent in vivo than the corresponding phenols. Among O^3-ether analogs of naltrindole[21] (δ-sites) and norbinaltorphimine[22] (κ-sites), similar differences are noted in vitro, where the O^3-methyl ethers are significantly less active. However, the recent demonstration of significant opioid activity of the O^6-glucuronides of morphine and normorphine suggests assignment of activity observed in vivo to a single molecular species is probably an oversimplification.[23]

The preparation of the α,β-unsaturated carboxylic acid ester derivatives as esters, ethers, or lactones at the C-6 position of naltrexone and 6α- and 6β-naltrexol[24,25] and on the side chain of etorphine[18] suggested the possibility

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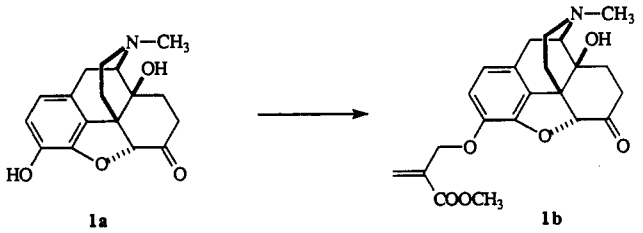
Scheme I [a]



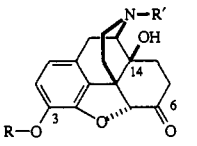
1a 1b

[a] Reagents: methyl 2-(bromomethyl)acrylate, n-$Bu_4N^+Br^-$, NaOH, H_2O, CH_2Cl_2.

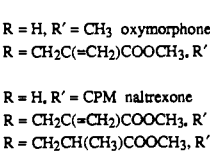
of extending the study to the previously unexplored O^3-ethers, especially since the required preparative transformation is readily achieved. The resulting electrophilic O^3-ether ligands would act as probes for the presence of nucleophilic sites in the region of the receptors involved in complexation of the A-ring 3-substituent. This binding interaction may be different for the three receptor subtypes, μ, κ, and δ. In this paper, we report the preparation of O^3-(2-carbomethoxyallyl) ethers of some standard 4,5-epoxymorphinan ligands and their behavior in opioid radioligand displacement assays for the μ-, δ-, and κ-subtypes of opioid receptors. These prepared compounds include the O^3-ethers of oxymorphone (**1a**), naltrexone (**2a**), 6β-naltrexol (**3a**) and its mono-O^6-(2-carbomethoxyallyl) ether (**4a**), etorphine (**5a**), diprenorphine (**6a**), norbinaltorphimine (**7a**), and naltrindole (**8a**). For comparison with naltrexone ether **2b**, saturated O^3-ether analog **2c** was prepared and tested as a mixture of its two diastereomers.

Chemical Synthesis. To our knowledge, the preparation of methacrylyl ethers by etherification of alcohol hydroxyl groups with methyl 2-(bromomethyl)acrylate using phase-transfer conditions[26] has not been examined previously. We find this method (Scheme I) to be generally applicable to phenolic opioid ligands. The method effectively produced good yields of ethers **1b**–**8b** when applied to each of the readily available phenols (**1a**–**3a** and **5a**–**8a**). The selectivity of this method is evident from the fact that other potentially reactive sites, including secondary and tertiary alcohols, pyrrole and indole nitrogens, and tertiary amines are inert to alkylation under the reaction conditions. It is interesting that these highly basic aqueous conditions do not cause hydrolysis of the methacrylate methyl ester group.

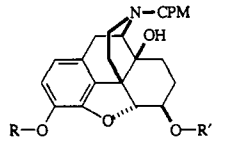
The regiochemistry of the O-alkylation for ethers **3b**, **4a**, and **4b** derived from 6β-naltrexol (**3a**) was readily determined based on analysis of the ^{1}H NMR chemical shifts of the allylic methylene protons of the methacrylate substituent.[24] The ^{1}H NMR spectrum of 6β-naltrexol-6-ether **4a** displayed signals for these protons as two doublets in the range of 4.1–4.4 ppm. The ^{1}H NMR



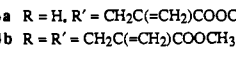
1a R = H, R′ = CH_3 oxymorphone
1b R = $CH_2C(=CH_2)COOCH_3$, R′ = CH_3



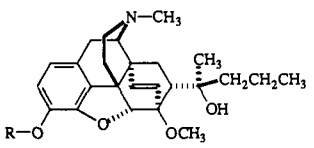
2a R = H, R′ = CPM naltrexone
2b R = $CH_2C(=CH_2)COOCH_3$, R′ = CPM
2c R = $CH_2CH(CH_3)COOCH_3$, R′ = CPM



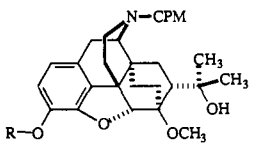
3a R = R′ = H 6β-naltrexol
3b R = $CH_2C(=CH_2)COOCH_3$, R′ = H



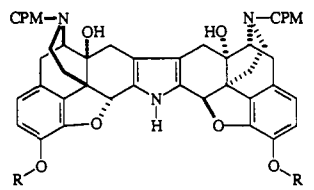
4a R = H, R′ = $CH_2C(=CH_2)COOCH_3$
4b R = R′ = $CH_2C(=CH_2)COOCH_3$



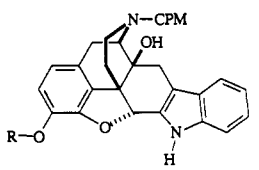
5a R = H etorphine
5b R = $CH_2C(=CH_2)COOCH_3$



6a R = H diprenorphine
6b R = $CH_2C(=CH_2)COOCH_3$



7a R = H norbinaltorphimine
7b R = $CH_2C(=CH_2)COOCH_3$



8a R = H naltrindole
8b R = $CH_2C(=CH_2)COOCH_3$

spectrum of 6β-naltrexol-3-ether **3b** displayed the analogous signals as two doublets in the range of 4.7–5.0 ppm. In the ^{1}H NMR spectrum of 6β-naltrexol-3,6-diether **4b**, two multiplets were observed in the range of 4.3–4.45 and 4.8–4.85 ppm.

Opioid Receptor Binding. To examine the possible opioid receptor binding and selectivities of the prepared compounds, their apparent IC_{50} values at all three of the generally accepted receptor types were measured in a method analogous to that developed by Goldstein and Naidu.[27] The apparent affinities of the ethers and their parent phenols for opioid receptor sites were determined in a crude membrane preparation from the guinea pig brain using [^{3}H]bremazocine (all sites), [^{3}H]DAGO (μ-sites), [^{3}H]bremazocine in the presence of unlabeled DAGO and DPDPE (κ-sites), and [^{3}H]DPDPE (δ-sites). The results of these competition binding experiments are described in Table I.

From Table I, most of the ethers seem to be reasonably potent ligands in the radioligand displacement assays, only about 2-fold less potent than the corresponding phenols, in each of the assays. Receptor subtype selectivity did not seem to be appreciably altered by this molecular modification, i.e., the relative potency at the three sites does not change greatly. Similar 2–3-fold changes appeared to occur in each of the assays. Our initial interpretation was that these parallel shifts in binding constants among the ligands indicated substantially similar modification of binding interactions must take place. However, the stability of these ethers under the conditions of these assays required verification.

Stability Studies on O^3-Ethers. When formation of naltrexone (**2a**) from ether **2b** under conditions of the radioligand displacement assay was determined by GLC,

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(26) For the etherification of phenols by phase-transfer catalysis, see for example McKillop, A.; Fiaud, J.-C.; Hug, R. P. The Use of Phase-Transfer Catalysis for the Synthesis of Phenol Ethers. *Tetrahedron* **1974**, *30*, 1379–1382.

(27) Goldstein, A.; Naidu, A. Multiple Opioid Receptors: Ligand Selectivity Profiles and Binding Site Signatures. *Mol. Pharmacol.* **1989**, *36*, 265–272.

Table I. Opioid Receptor Binding of the 3-Ethers against 0.5 nM [^{3}H]Bremazocine (total receptors), against 1 nM [^{3}H]DAGO (μ-sites), against 0.5 nM [^{3}H]Bremazocine in the Presence of 100 nM Unlabeled DAGO and 100 nM Unlabeled DPDPE (κ-sites), and against 1 nM [^{3}H]DPDPE (δ-sites) in the Guinea Pig Brain Membrane Preparation

	IC_{50} (nM)[a]			
	total receptors	μ-sites	κ-sites	δ-sites
oxymorphone 3-ether **1b**	110	2.4	880	21
oxymorphone (**1a**)	90	1.4	630	45
naltrexone 3-ether **2b**	10.3	1.5	19	17
naltrexone (**2a**)	5.7	0.73	9.5	15
saturated naltrexone 3-ether **2c**	>3000	840	>3000	>1000
6β-naltrexol 3-ether **3b**	27	2.8	47	34
6β-naltrexol (**3a**)	13	2.2	20	23
6β-naltrexol 3,6-diether **4b**	14	2.8	42	3.2
6β-naltrexol 6-ether **4a**	11.1	2.5	21	1.4
etorphine 3-ether **5b**	2.4	0.75	4.9	1.2
etorphine (**5a**)	1.7	0.48	3.4	1.3
diprenorphine 3-ether **6b**	2.2	1.0	3.6	1.3
diprenorphine (**6a**)	1.3	0.71	1.8	0.68
norbinaltorphimine 3,3′-diether **7b**	22	47	8.3	50
norbinaltorphimine (**7a**)	11	18	6.0	22
naltrindole 3-ether **8b**	80	96	175	0.80
naltrindole (**8a**)	47	37	100	0.32
naloxone	43	8.9	50	62
bremazocine	1.5	1.8	1.2	1.5

[a] Values are averages of duplicate determinations, ±10 to 15%.

Table II. Conversion of Ether **2b** to Phenol **2a** under Conditions of the Opioid Radioligand Displacement Assay and in the Presence of Model Nucleophiles

	% conversion[a] to **2a**
Opioid Displacement Assay	
naltrexone 3-ether **2b** (100 nM)	71
2b buffer only	0
2b heat denaturation (100 °C for 30 min)	80
2b acid precipitation, resuspended pellet	78
2b acid precipitation, supernatant	0
saturated naltrexone 3-ether **2c** (100 nM)	0
Model Nucleophiles	
2b (100 nM) + imidazole (1 μM)	15
imidazole (1 mM)	75
2b (100 nM) + thiophenol (1 μM)	22
thiophenol (1 mM)	77

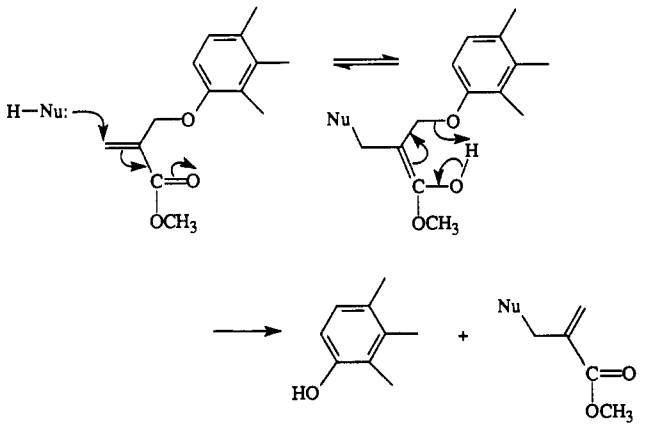
[a] Averages of duplicate determinations, ±10% or less.

electron-capture detection,[28] about 70% of **2b** was converted to the phenol (Table II). In control experiments, we noted the dealkylation did not occur in buffer alone; however, denaturation of the membrane preparation by heat or by acid precipitation followed by resuspension showed an increase in the amount of dealkylation. Additionally, use of model nucleophiles imidazole and thiophenol also resulted in dealkylation of **2b**.

To determine whether the α,β-unsaturated ester moiety was required for this dealkylation process to occur, the saturated ether **2c** was subjected to the radioreceptor assay conditions and both displacement and conversion to **2a** were determined. The compound was only weakly active in the radioligand displacement assay (Table I) and no phenol was formed.

Consistent with these observations a chemical mechanism for the O-dealkylation process is suggested in Scheme II. Conjugate addition by nucleophiles present in the membrane preparation is suggested, followed by

Scheme II



loss of the phenol. Allylic displacement of the phenol by an S_N2' process is also a possibility. The phenol appears to be required as the leaving group since analogous ethers of C-6 alcohols of the same type are stable under the conditions of the radioligand displacement assay.

Conclusion. We have synthesized and examined the binding of a handful of O^3-methacrylate ethers derived from various classes of opioids. The demonstrated ease of preparation of these phenolic derivatives suggests comparable ease of preparation of such derivatives from opioids and other molecules possessing a phenolic hydroxyl group. However, the instability of **2b** in the binding assay suggests similar instability of the other phenolic derivatives examined which would prevent their use as potential electrophilic ligands for reaction with nucleophilic sites at opioid receptors. The potency of the O^3-(2-carbomethoxyallyl) ether ligands in the radioligand displacement assay appears to be associated with formation of the parent phenol under the conditions of the assay. Whether this type of functional group can be used to develop possible drug delivery systems of phenols like this or other pharmacophores remains to be examined.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 1610 FTIR. NMR spectra were recorded on a Varian VXR-300 spectrometer. Chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane as an internal standard. Electron impact (EI) mass spectra were obtained on a VG-7070 mass spectrometer and FAB mass spectra on a VG-70 SEQ mass spectrometer, both by direct insertion probe. Optical rotations were measured on a JASCO-DIP-4 digital polarimeter. Analytical thin-layer chromatography (TLC) was performed on Analtech silica gel HLF glass plates. Flash chromatography[29] was performed using Merck silica gel 60 (230–400 mesh). Dichloromethane was stored over 3-Å molecular sieves prior to use. All reactions were run under an argon atmosphere. Microanalyses were performed by Desert Analytics, Tucson, AZ. Where indicated by the symbols of the elements, analyses were within ±0.4% of theoretical values.

4,5α-Epoxy-6-oxo-14-hydroxy-3-[(2-carbomethoxyallyl)-oxy]-17-methylmorphinan (1b). To a solution of oxymorphone (**1a**) (100 mg, 0.33 mmol), methyl 2-(bromomethyl)acrylate (176 mg, 0.99 mmol), and tetra-*n*-butylammonium bromide (21 mg, 0.07 mmol) in CH_2Cl_2 (2 mL) was added 0.8 M aqueous NaOH solution (2 mL, 1.6 mmol) and stirred vigorously for 2 h. The mixture was extracted with ether (3 × 15 mL), and then the volatiles were evaporated. The residue was purified by flash chromatography (21 g of silica gel) eluting with 2% triethylamine–

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ethyl acetate (200 mL) to give the ether **1b** (130 mg, 100% yield) as white crystals: mp 131–135 °C; $[\alpha]_D$ = −152° (c = 1.00, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 1.5–1.6 (m, 1 H, C-15 H), 1.62 (dt, J = 3.3 and 14.3 Hz, 1 H, C-8 H), 1.85–1.95 (m, 1 H, C-8 H), 2.1–2.2 (m, 1 H, C-16 H), 2.28 (dt, J = 14.3 and 3.0 Hz, 1 H, C-7 H), 2.35–2.5 (m, 2 H, C-15 H and C-16 H), 2.40 (s, 3 H, NCH_3), 2.55 (dd, J = 6.5 and 18.5 Hz, 1 H, C-10α H), 2.87 (d, J = 5.9 Hz, 1 H, C-9 H), 3.02 (dt, J = 5.0 and 14.4 Hz, 1 H, C-7 H), 3.15 (d, J = 18.5 Hz, 1 H, C-10β H), 3.79 (s, 3 H, CO_2CH_3), 4.67 (s, 1 H, C-5 H), 4.92 (s, 2 H, allylic CH_2), 6.04 (d, J = 1.5 Hz, 1 H, E-vinylic CH), 6.37 (d, J = 1.3 Hz, 1 H, Z-vinylic CH), 6.61 (d, J = 8.2 Hz, 1 H, C-1 H), 6.72 (d, J = 8.1 Hz, 1 H, C-2 H); ^{13}C NMR ($CDCl_3$) δ 21.91, 30.44, 31.33, 36.05, 42.63, 45.09, 50.07, 51.81, 64.37, 67.94, 70.18, 90.24, 117.33, 119.31, 125.66, 126.89, 129.48, 135.69, 141.02, 145.13, 165.76, 208.02; FTIR (KBr) 3364, 1727, 1714, 1636, 1614, 1499 cm^{-1}; HREIMS calcd for $C_{22}H_{25}NO_6$ 399.1682, obsd 399.1653. Anal. ($C_{22}H_{25}NO_6$) C, H, N.

4,5α-Epoxy-6-oxo-14-hydroxy-3-[(2-carbomethoxyallyl)-oxy]-17-(cyclopropylmethyl)morphinan (2b). Ether **2b** was prepared from naltrexone (**2a**) as described above for the synthesis of **1b** from oxymorphone (**1a**). After flash chromatography eluting with CH_2Cl_2 followed by ether, the ether **2b** was obtained (100% yield) as a clear viscous oil: $[\alpha]_D$ = −157.5° (c = 1.00, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 0.1–0.2 (m, 2 H, cyclopropyl CH_2), 0.5–0.6 (m, 2 H, cyclopropyl CH_2), 0.8–0.95 (m, 1 H, cyclopropyl CH), 1.5–1.6 (m, 1 H, C-15 H), 1.63 (dt, J = 3.2 and 13.8 Hz, 1 H, C-8 H), 1.85–1.95 (m, 1 H, C-8 H), 2.13 (dt, J = 3.6 and 12.1 Hz, 1 H, C-16 H), 2.29 (dt, J = 14.3 and 3.0 Hz, 1 H, C-7 H), 2.35–2.45 (m, 1 H, C-15 H), 2.40 (d, J = 6.7 Hz, 2 H, NCH_2 cyclopropyl), 2.58 (dd, J = 6.0 and 18.6 Hz, 1 H, C-10α H), 2.70 (dd, J = 4.6 and 11.9 Hz, 1 H, C-16 H), 3.03 (dt, J = 5.0 and 14.4 Hz, 1 H, C-7 H), 3.05 (d, J = 18.9 Hz, 1 H, C-10β H), 3.18 (d, J = 5.9 Hz, 1 H, C-9 H), 3.79 (s, 3 H, CO_2CH_3), 4.68 (s, 1 H, C-5 H), 4.92 (s, 2 H, allylic CH_2), 6.04 (s, 1 H, E-vinylic CH), 6.37 (s, 1 H, Z-vinylic CH), 6.59 (d, J = 8.2 Hz, 1 H, C-1 H), 6.71 (d, J = 8.2 Hz, 1 H, C-2 H); ^{13}C NMR ($CDCl_3$) δ 3.80, 3.97, 9.39, 22.61, 30.65, 31.43, 36.12, 43.47, 50.67, 51.81, 59.07, 61.87, 67.96, 69.98, 90.29, 117.34, 119.26, 125.67, 126.89, 129.65, 135.71, 141.01, 145.16, 165.77, 208.05; FTIR (neat) 3500–3200 (OH), 1726, 1716, 1634, 1608, 1496 cm^{-1}; HREIMS calcd for $C_{25}H_{29}NO_6$ 439.1995, obsd 439.1992. Anal. ($C_{25}H_{29}NO_6$) C, H, N.

4,5α-Epoxy-6-oxo-14-hydroxy-3-(2-carbomethoxypropoxy)-17-(cyclopropylmethyl)morphinan (2c). To a solution of allylic ether **2b** (50 mg, 0.11 mmol) in EtOH (20 mL) was added 10% Pd on carbon (10 mg), and it was treated with hydrogen gas at 1 atm pressure for 2 h. The mixture was diluted with EtOH (50 mL) and then filtered through a one-half-in. pad of Celite under suction. The EtOH was evaporated under vacuum, and then the residue was treated with saturated aqueous $NaHCO_3$ solution (10 mL). The mixture was extracted with CH_2Cl_2 (3 × 15 mL). The combined extracts were dried (Na_2SO_4), and the solvent was evaporated. The residue was purified by flash chromatography (6 g of silica gel) eluting with 4% triethylamine–ether (50 mL) to give ether **2c** (40 mg, 80% yield) as a 1:1 mixture of two diastereomers as a viscous oil: ^1H NMR ($CDCl_3$) δ 0.1–0.2 (m, 2 H, cyclopropyl CH_2), 0.5–0.6 (m, 2 H, cyclopropyl CH_2), 0.8–0.95 (m, 1 H, cyclopropyl CH), 1.29 (d, J = 7.2 Hz, 3 H, CH_3), 1.5–1.7 (m, 2 H, C-8 H and C-15 H), 1.8–1.95 (m, 1 H, C-8 H), 2.05–2.2 (m, 1 H, C-16 H), 2.25–2.35 (m, 1 H, C-7 H), 2.35–2.5 (m, 3 H, C-15 H and NCH_2 cyclopropyl), 2.5–2.65 (m, 1 H, C-10α H), 2.65–2.75 (m, 1 H, C-16 H), 2.9–3.1 (m, 3 H, C-7 H, C-10β H, and C*H*$COOCH_3$), 3.18 (d, J = 5.5 Hz, 1 H, C-9 H), 3.71 and 3.72 (two s, 3 H, CO_2CH_3), 4.15–4.3 [m, 1 H, $OCH_2CH(CH_3)CO_2CH_3$], 4.35–4.45 [m, 1 H, $OCH_2CH(CH_3)CO_2CH_3$], 4.66 (s, 1 H, C-5 H), 5.2 (br s, 1 H, movable, OH), 6.58 (d, J = 8.2 Hz, 1 H, C-1 H), 6.65–6.75 (m, 1 H, C-2 H). FAB MS (M + 1)$^+$ calcd for $C_{25}H_{32}NO_6$ 442.2230, obsd 442.2202. Anal. ($C_{25}H_{31}NO_6 \cdot 0.25H_2O$) C, H, N.

4,5α-Epoxy-6β,14-dihydroxy-3-[(2-carbomethoxyallyl)-oxy]-17-(cyclopropylmethyl)morphinan (3b) and 4,5α-Epoxy-14-hydroxy-3,6β-bis[(2-carbomethoxyallyl)oxy]-17-(cyclopropylmethyl)morphinan (4b). Ethers **3b** and **4b** were prepared from 6β-naltrexol (**3a**)[30] as described above for the synthesis of **1b** from oxymorphone (**1a**). After flash chromatography eluting with ether the 3,6-diether **4b** was obtained (6% yield) as a clear viscous oil, $[\alpha]_D$ = −50° (c = 0.50, CH_2Cl_2), followed after further elution with 4% triethylamine–ether by the 3-ether **3b** (73% yield) as a clear viscous oil, $[\alpha]_D$ = −90.5° (c = 1.00, CH_2Cl_2).

3b: ^1H NMR ($CDCl_3$) δ 0.1–0.2 (m, 2 H, cyclopropyl CH_2), 0.5–0.6 (m, 2 H, cyclopropyl CH_2), 0.8–0.9 (m, 1 H, cyclopropyl CH), 1.3–1.4 (m, 1 H, C-8 H), 1.4–1.5 (m, 1 H, C-15 H), 1.55–1.7 (m, 2 H, C-7 H and C-8 H), 1.9–2.0 (m, 1 H, C-7 H), 2.11 (dt, J = 3.3 and 11.9 Hz, 1 H, C-16 H), 2.24 (dt, J = 4.7 and 12.3 Hz, 1 H, C-15 H), 2.36 (d, J = 6.6 Hz, 2 H, NCH_2-cyclopropyl), 2.55–2.7 (m, 2 H, C-10α H and C-16 H), 3.02 (d, J = 18.6 Hz, 1 H, C-10β H), 3.10 (d, J = 5.7 Hz, 1 H, C-9 H), 3.4–3.6 (m, 2 H, 1 H movable, OH and C-6 H), 3.81 (s, 3 H, CO_2CH_3), 4.47 (d, J = 5.8 Hz, 1 H, C-5 H), 4.77 (d, J = 14.3 Hz, 1 H, allylic CH), 4.93 (d, J = 14.2 Hz, 1 H, allylic CH), 5.2 (br s, 1 H, OH), 5.95 (s, 1 H, E-vinylic CH), 6.34 (s, 1 H, Z-vinylic CH), 6.57 (d, J = 8.2 Hz, 1 H, C-1 H), 6.73 (d, J = 8.2 Hz, 1 H, C-2 H); ^{13}C NMR ($CDCl_3$) δ 3.81, 3.92, 9.37, 22.67, 25.25, 29.22, 31.16, 43.64, 46.90, 51.97, 59.15, 62.00, 68.18, 69.96, 71.91, 95.63, 116.97, 118.52, 126.15, 126.57, 132.18, 136.03, 141.35, 144.42, 166.03; FTIR (neat) 3700–3100 (OH), 1721, 1634, 1607, 1496 cm^{-1}; FAB MS (M + 1)$^+$ calcd for $C_{25}H_{32}NO_6$ 442.2230, obsd 442.2202. Anal. ($C_{25}H_{31}NO_6$) C, H, N.

4b: ^1H NMR ($CDCl_3$) δ 0.1–0.2 (m, 2 H, cyclopropyl CH_2), 0.5–0.6 (m, 2 H, cyclopropyl CH_2), 0.75–0.9 (m, 1 H, cyclopropyl CH), 1.33 (dt, J = 2.6 and 13.6 Hz, 1 H, C-8 H), 1.4–1.5 (m, 1 H, C-15 H), 1.55–1.65 (m, 1 H, C-8 H), 1.7–1.8 (m, 1 H, C-7 H), 1.95 (dq, J = 2.8 and 12.9 Hz, 1 H, C-7 H), 2.08 (dt, J = 3.4 and 12.0 Hz, 1 H, C-16 H), 2.24 (dt, J = 5.0 and 12.4 Hz, 1 H, C-15 H), 2.36 (d, J = 6.4 Hz, 2 H, NCH_2-cyclopropyl), 2.5–2.65 (m, 2 H, C-10α H and C-16 H), 3.02 (d, J = 18.5 Hz, 1 H, C-10β H), 3.08 (d, J = 5.7 Hz, 1 H, C-9 H), 3.2–3.35 (m, 1 H, C-6 H), 3.73 (s, 3 H, CO_2CH_3), 3.78 (s, 3 H, CO_2CH_3), 4.3–4.45 (m, 1 H, allylic CH_2), 4.58 (d, J = 6.4 Hz, 1 H, C-5 H), 4.8–4.85 (m, 2 H, allylic CH_2), 5.0 (br s, 1 H, OH), 6–6.05 (m, 2 H, E-vinylic CH), 6.28 (s, 1 H, Z-vinylic CH), 6.38 (s, 1 H, Z-vinylic CH), 6.58 (d, J = 8.1 Hz, 1 H, C-1 H), 6.74 (d, J = 8.2 Hz, 1 H, C-2 H); ^{13}C NMR ($CDCl_3$) δ 3.79, 3.93, 9.44, 22.64, 23.76, 29.57, 30.59, 43.79, 47.58, 51.65, 59.09, 62.14, 67.30, 67.88, 69.91, 80.21, 94.98, 116.62, 118.42, 125.91 (2 carbons), 126.30, 132.36, 135.97, 137.13, 141.94, 143.98, 165.69, 166.14; FTIR (neat) 3385 (OH), 1723, 1634, 1608, 1496 cm^{-1}; FAB MS (M + 1)$^+$ calcd for $C_{30}H_{38}NO_8$ 540.2594, obsd 540.2555. Anal. ($C_{30}H_{37}NO_8$) C, H, N.

3-*O*-(2-Carbomethoxyallyl)-6,7,8,14-tetrahydro-7α-(2-hydroxy-2-pentyl)-6,14-*endo*-ethenooripavine (5b). Ether **5b** was prepared from etorphine (**5a**)[20] as described above for the synthesis of **1b** from oxymorphone (**1a**). After flash chromatography eluting with CH_2Cl_2 followed by ether, the ether **5b** was obtained (100% yield) as a clear viscous oil which crystalized on standing: mp 96–99 °C; $[\alpha]_D$ = −51.5° (c = 0.50, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 0.80 (dd, J = 8.2 and 12.7 Hz, 1 H, C-8 α H), 0.91 (t, J = 7.1 Hz, 3 H, C-19 propyl CH_3), 0.96 (s, 3 H, C-19 CH_3), 1.15–1.55 (m, 4 H, C-19 propyl CH_2CH_2), 1.75–2.0 (m, 3 H, C-7β H and C-15 CH_2), 2.3–2.45 (m, 2 H, C-10α H and C-16 H), 2.37 (s, 3 H, NCH_3), 2.45–2.55 (m, 1 H, C-16 H), 2.82 (dd, J = 9.2 and 12.7 Hz, 1 H, C-8β H), 3.12 (d, J = 6.3 Hz, 1 H, C-9 H), 3.21 (d, J = 18.6 Hz, 1 H, C-10β H), 3.75 (s, 3 H, C-6 OCH_3), 3.77 (s, 3 H, CO_2CH_3), 4.54 (s, 1 H, C-5 H), 4.7–4.85 (m, 2 H, allylic CH_2), 4.89 (s, 1 H, OH), 5.43 (d, J = 8.9 Hz, 1 H, C-17 H), 5.9–6.0 (m, 2 H, C-18 H and E-vinylic CH), 6.34 (s, 1 H, Z-vinylic CH), 6.49 (d, J = 8.2 Hz, 1 H, C-1 H), 6.64 (d, J = 8.0 Hz, 1 H, C-2 H); ^{13}C NMR ($CDCl_3$) δ 14.79, 15.88, 22.35, 24.05, 30.61, 33.55, 42.80, 43.23, 43.53, 45.45, 46.77, 47.07, 51.84, 55.08, 59.87, 68.00, 74.79, 84.04, 98.76, 116.27, 119.23, 124.90, 125.99, 129.16, 134.58, 135.11, 136.02, 139.96, 148.43, 165.71; FTIR (neat) 3495 (OH), 1722, 1632, 1600, 1494 cm^{-1}; FAB MS (M + 1)$^+$ calcd for $C_{30}H_{40}NO_6$ 510.2852, obsd 510.2803. Anal. ($C_{30}H_{39}NO_6$) C, H, N.

3-*O*-(2-Carbomethoxyallyl)-21-cyclopropyl-6,7,8,14-tetrahydro-7α-(2-hydroxy-2-propyl)-6,14-*endo*-ethanonororipavine (6b). Ether **6b** was prepared from diprenorphine (**6a**)[20] as described above for the synthesis of **1b** from oxymorphone (**1a**). After flash chromatography eluting with 4% triethylamine–15% CH_2Cl_2–hexanes the ether **6b** was obtained (74% yield) as a clear viscous oil: $[\alpha]_D$ = −84° (c = 1.00, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 0.1–0.2 (m, 2 H, cyclopropyl CH_2), 0.45–0.55 (m, 2 H,

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cyclopropyl CH_2), 0.7–0.85 (m, 2 H, cyclopropyl CH and C-17 H), 1.0–1.15 (m, 2 H, C-8α H and C-17 H), 1.20 (s, 3 H, C-19-CH_3), 1.38 (s, 3 H, C-19-CH_3), 1.6–1.7 (m, 1 H, C-15 H), 1.75–1.85 (m, 2 H, C-18 CH_2), 1.92 (t, J = 10.0 Hz, 1 H, C-7β H), 2.04 (dt, J = 5.4 and 12.7 Hz, 1 H, C-15 H), 2.15–2.45 (m, 4 H, C-10α H, C-16 H, and NCH_2-cyclopropyl), 2.63 (dd, J = 4.8 and 11.7 Hz, 1 H, C-16 H), 2.8–2.95 (m, 1 H, C-8β H), 2.95–3.05 (m, 2 H, C-9 H and C-10β H), 3.53 (s, 3 H, C-6-OCH_3), 3.78 (s, 3 H, CO_2CH_3), 4.41 (s, 1 H, C-5 H), 4.8–4.95 (m, 2 H, allylic CH_2), 5.08 (s, 1 H, OH), 6.02 (d, J = 1.4 Hz, 1 H, *E*-vinylic CH), 6.37 (s, 1 H, *Z*-vinylic CH), 6.53 (d, J = 8.3 Hz, 1 H, C-1 H), 6.74 (d, J = 8.0 Hz, 1 H, C-2 H); ^{13}C NMR ($CDCl_3$) δ 3.34, 4.20, 9.39, 17.48, 22.61, 24.85, 29.75, 29.87, 32.25, 35.52, 35.80, 43.60, 46.79, 47.79, 51.79, 52.54, 57.99, 59.76, 68.04, 74.22, 80.29, 96.92, 116.64, 119.02, 126.00, 129.59, 132.86, 136.04, 139.75, 147.22, 165.66; FTIR (neat) 3484 (OH), 1722, 1631, 1599, 1494 cm^{-1}; HREIMS calcd for $C_{31}H_{41}NO_6$ 523.2934, obsd 523.2939. Anal. ($C_{31}H_{41}NO_6$) C, H, N.

3,3′-Bis[(2-carbomethoxyallyl)oxy]-17,17′-bis(cyclopropylmethyl)-6,6′,7,7′-tetradehydro-4,5:4′,5′-diepoxy-6,6′-imino[7,7′-bimorphinan]-14,14′-diol (7b). To a solution of norbinaltorphimine (**7a**)[31] (40 mg, 0.06 mmol), methyl 2-(bromomethyl)acrylate (43 mg, 0.24 mmol), and tetra-*n*-butylammonium bromide (4 mg, 0.012 mmol) in CH_2Cl_2 (1 mL) was added 0.35 M aqueous NaOH solution (1 mL, 0.35 mmol) and stirred vigorously for 2 h. The mixture was treated with half-saturated aqueous $NaHCO_3$ solution (20 mL) and extracted with CH_2Cl_2 (3 × 6 mL). The combined extracts were dried (Na_2SO_4), and the solvent was evaporated. The residue was purified by flash chromatography (21 g of silica gel) eluting with 2% triethylamine–ethyl acetate (200 mL) to give the diether **7b** (50 mg, 97%) as white crystals: mp 121–124 °C; $[\alpha]_D$ = −162° (c = 0.50, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 0.1–0.2 (m, 4 H, cyclopropyl CH_2), 0.5–0.6 (m, 4 H, cyclopropyl CH_2), 0.8–0.95 (m, 2 H, cyclopropyl CH), 1.69 (d, J = 10.3 Hz, 2 H, C-15 H), 2.15–2.45 (m, 12 H, NCH_2-cyclopropyl, C-8 CH_2, C-15 H, and C-16 H), 2.6–2.75 (m, 4 H, C-10α H and C-16 H), 3.06 (d, J = 18.5 Hz, 2 H, C-10β H), 3.18 (d, J = 6.2 Hz, 2 H, C-9 H), 3.80 (s, 6 H, CO_2CH_3), 4.72 (d, J = 14.4 Hz, 2 H, allylic CH), 4.85 (d, J = 14.5 Hz, 2 H, allylic CH), 5.47 (s, 2 H, C-5 H), 5.93 (s, 2 H, *E*-vinylic CH), 6.34 (s, 2 H, *Z*-vinylic CH), 6.51 (d, J = 8.3 Hz, 2 H, C-1 H), 6.64 (d, J = 8.2 Hz, 2 H, C-2 H), 8.21 (s, movable, 1 H, NH); ^{13}C NMR ($CDCl_3$) δ 3.73, 3.99, 9.35, 23.01, 28.77, 31.46, 43.54, 47.66, 51.85, 59.24, 62.15, 67.53, 72.49, 85.04, 115.60, 115.87, 118.02, 124.60, 126.21, 126.98, 130.97, 135.55, 141.43, 144.79, 165.87; FTIR (KBr) 3600–3100 (OH), 1719, 1636, 1609, 1498 cm^{-1}; FAB MS $(M + 1)^+$ calcd for $C_{50}H_{56}N_3O_{10}$ 858.3959, obsd 858.3977. Anal. ($C_{50}H_{55}N_3O_{10}$) C, H, N.

3-[(2-Carbomethyoxyallyl)oxy]-17-(cyclopropylmethyl)-6,7-dehydro-4,5α-epoxy-3,14-dihydroxy-6,7-[2′,3′-indolo]morphinan (8b). Ether 8b was prepared from naltrindole (**8a**)[21] as described above for the synthesis of **1b** from oxymorphone (**1a**). After flash chromatography eluting with ether, the ether **8b** was obtained (77% yield) as a white solid foam: $[\alpha]_D$ = −167.5° (c = 0.50, CH_2Cl_2), HCl salt (recrystallized from 2-propanol–ether) (dec above 170 °C); ^1H NMR (free amine 8b) ($CDCl_3$) δ 0.1–0.2 (m, 2 H, cyclopropyl CH_2), 0.5–0.6 (m, 2 H, cyclopropyl CH_2), 0.8–0.95 (m, 1 H, cyclopropyl CH), 1.7–1.8 (m, 1 H, C-15 H), 2.2–2.5 (m, 4 H, NCH_2-cyclopropyl, C-15 H, and C-16 H), 2.62 (d, J = 15.6 Hz, 1 H, C-8 H), 2.65–2.75 (m, 1 H, C-16 H), 2.81 (dd, J = 6.5 and 18.7 Hz, 1 H, C-10α H), 2.90 (d, J = 15.6 Hz, 1 H, C-8 H), 3.12 (d, J = 18.6 Hz, 1 H, C-10β H), 3.38 (d, J = 6.3 Hz, 1 H, C-9 H), 3.73 (s, 3 H, CO_2CH_3), 4.66 (d, J = 14.3 Hz, 1 H, allylic CH_2), 4.76 (d, J = 14.2 Hz, 1 H, allylic CH_2), 5.0 (br s, movable, 1 H, OH), 5.64 (s, 1 H, C-5 H), 5.77 (s, 1 H, *E*-vinylic CH), 6.17 (s, 1 H, *Z*-vinylic CH), 6.54 (d, J = 8.2 Hz, 1 H, C-1 H), 6.60 (d, J = 8.2 Hz, 1 H, C-2 H), 6.98 (t, J = 7.3 Hz, 1 H, C-5′ H), 7.08 (t, J = 7.5 Hz, 1 H, C-6′ H), 7.20 (d, J = 7.9 Hz, 1 H, C-7′ H), 7.38 (d, J = 7.4 Hz, 1 H, C-4′ H), 8.58 (s, 1 H, NH); ^{13}C NMR ($CDCl_3$) δ 3.75, 4.02, 9.39, 23.08, 28.78, 31.33, 43.52, 47.73, 51.76, 59.34, 62.10, 67.60, 72.29, 85.01, 111.03, 111.20, 116.12, 118.49, 118.65, 118.88, 122.38, 126.46, 126.54 (2 carbons), 128.79, 131.29, 135.63, 136.98, 141.33, 144.53, 165.84; FTIR (KBr) 3374 (OH), 1718, 1636, 1609, 1498 cm^{-1}; FAB MS $(M + 1)^+$ calcd for $C_{31}H_{33}N_2O_5$ 513.2386, obsd 513.2340. Anal. ($C_{31}H_{32}N_2O_5$·HCl) C, H, N.

Opioid Receptor Binding. Guinea pig brain homogenate was prepared as described by Lin and Simon.[32] The radioligand used was [^3H]bremazocine (New England Nuclear) (37.0 Ci/mmol) at a concentration of 0.5 nM for determination of total opioid binding sites. For μ-binding sites, 1.0 nM [^3H]DAGO [D-Ala^2-NMePhe^4-Gly-ol^5-enkephalin] (Amersham, 60.0 Ci/mmol) was used. For δ-binding sites, 1.0 nM [^3H]DPDPE [D-Pen^2-D-Pen^5-enkephalin] (Amersham, 50 Ci/mmol) was used. For κ-binding sites, 0.5 nM bremazocine was used in the presence of 100 nM unlabeled DAGO and 100 nM unlabeled DPDPE to block μ- and δ-sites, respectively. Nonspecific binding was determined with naloxone (10 μM). Nine concentrations of each ligand to be tested were examined in competition experiments with radioligand. The samples were incubated in 50 mM Tris-HCl buffer (pH 7.4) at 25 °C for 1 h, then rapidly filtered through Whatman GF/B filters, rinsed three times with cold buffer (2 mL each), and after standing overnight in Aquasol II scintillation fluid (10 mL) were counted in a scintillation counter. IC_{50} values were determined using log-probit analysis.

Assay for Detection of Naltrexone (2a). Membrane homogenate (8 mL) at a concentration in Tris buffer used in the binding assays described above was incubated with naltrexone 3-methacrylate ether **2b** (100 nM) at 25 °C for 1 h. Naloxone (62.5 nM), which served as the internal standard, was added, and the mixture was vortexed and then centrifuged at 20000*g* for 15 min at 4 °C. The supernatant was transferred to a clean tube and then treated with $NaHCO_3$ (150 mg). The mixture was extracted with CH_2Cl_2 (3 × 5 mL). The combined extracts were evaporated under a stream of nitrogen gas at 40–50 °C. The residue was derivatized with pentafluorobenzyl bromide under phase-transfer catalysis conditions as described by Meffin and Smith[26] with minor modifications. The final dilution was with 40 μL of hexane instead of 100 μL. A 5-μL aliquot was injected onto a 30-m DB-17 capillary column on a HP 4890A gas chromatograph, injector temperature (250 °C), EC detector temperature (300 °C), initial oven temperature (280 °C) for 5 min and then ramped at 5 °C/min to a final oven temperature (300 °C). The O^3-pentafluorobenzyl ether derivative of naloxone had a retention time of about 14 min and the O^3-pentafluorobenzyl ether derivative of naltrexone had a retention time of about 18.5 min. A standard curve was prepared for samples of membrane homogenate (8 mL) containing naltrexone at concentrations of 5 nM to 125 nM. After these samples were incubated at 25 °C for 1 h, naloxone (62.5 μM) was added, and then the samples were vortexed, centrifuged, extracted, and derivatized as described above.

Heat Denaturation. The tubes containing membrane homogenate (8 mL) were heated in boiling water (100 °C) for 30 min. After cooling, the membrane mixture was incubated with naltrexone 3-ether **2b** (100 nM), as described above.

Acid Precipitation. The tubes containing membrane homogenate (8 mL) were treated with aqueous 3 M trichloroacetic acid solution (1 mL). After 30 min at ambient temperature, the samples were centrifuged at 20000*g* for 15 min at 4 °C. The supernatant was transferred to a clean tube and then neutralized with $NaHCO_3$ (~250 mg). The pellet in the original tube was resuspended in Tris buffer (8 mL) by vortexing. Both tubes were incubated with naltrexone 3-ether (100 nM) as described above.

Incubations with Imidazole or Thiophenol. Naltrexone 3-ether (100 nM) in Tris buffer (8 mL) was incubated with either imidazole or thiophenol at a concentration of 1 μM or 1 mM at 25 °C for 1 h. The samples were then extracted with CH_2Cl_2 and derivatized as described above.

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