mixture was filtered off, the solvent was evaporated, and the residue was recrystallized from acetone/ethanol (1:1) yielding **25** (80%): mp 223-225 °C; MS *m/e* 330 (10, M^+), 332 (12, M^+), 209 (100), 84 (31), 70 (37), 56 (17); ^1H NMR δ 2.25 (m, 2 CH_2), 3.80 (m, 4 CH_2), 7.20 (m, 4 H arom). Anal. ($C_{14}H_{20}BrClN_2$) C, H, N.

General Procedure B. Preparation of Derivatives 27-30. To a solution of 1-(3-chlorophenyl)piperazine (**12**; 0.4 g, 2 mmol) in toluene (30 mL) was added anhydrous K_2CO_3 (0.5 g). Then a solution of the appropriate acid chloride (2.5 mmol) in toluene (10 mL) was added dropwise, and the mixture was stirred for 2-3 h at room temperature. The mixture was treated with water (30 mL) and warmed up to 50 °C, and the organic layer was separated. Then the solvent was evaporated, and the residue was purified using silica gel chromatography with *n*-hexane/$CHCl_3$ (1:1). The product was dissolved with acetone (5 mL) and treated with an excess of Et_2O, saturated with HCl. Resultant salts were recrystallized from acetone/ethanol mixture.

1-(3-Chlorophenyl)-4-propionylpiperazine hydrochloride (27·HCl): yield 85%; mp 148-150 °C; MS (free base) *m/e* 252 (36, M^+), 254 (10, M^+), 166 (100), 154 (32), 56 (76). Anal. ($C_{13}H_{17}ClN_2O$·HCl) C, H, N.

4-Butanoyl-1-(3-chlorophenyl)piperazine hydrochloride (28·HCl): yield 90%; mp 123-125 °C; MS (free base) *m/e* 266 (1, M^+), 196 (20), 154 (100), 56 (12). Anal. ($C_{14}H_{19}ClN_2O$·HCl) C, H, N.

1-(3-Chlorophenyl)-4-(2-methylpropionyl)piperazine hydrochloride (29·HCl): yield 87%; mp 143-145 °C; MS (free base) *m/e* 266 (25, M^+), 268 (8, M^+), 166 (100), 154 (29), 56 (95). Anal. ($C_{14}H_{19}ClN_2O$·HCl) C, H, N.

1-(3-Chlorophenyl)-4-(phenylacetyl)piperazine hydrochloride (30·HCl·$^1/_2H_2O$): yield 85%; mp 129-131 °C; MS (free base) *m/e* 314 (51, M^+), 315 (16, M^+), 195 (19), 166 (100), 154 (39), 56 (58). Anal. ($C_{18}H_{19}ClN_2O$·HCl·$^1/_2H_2O$) C, H, N.

pK_a Measurements. Ionization constants were determined by a potentiometric titration[36] at 37 ± 0.1 °C. The pK_a values were calculated from the experimental data using the ENZFITTER program.[37]

Binding Experiments. Radioligand receptor binding studies were performed in the rat brain (hippocampus), according to the published procedure.[38] A radioligand used in the binding assays was [^{3}H]-8-OH-DPAT.

Acknowledgment. This work was supported by the State Committee for Scientific Research (KBN), Grant No. 4-1449-91-01 (1991-1994).

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Electrophilic Opioid Ligands. Oxygen Tethered α-Methylene-γ-lactone, Acrylate, Isothiocyanate, and Epoxide Derivatives of 6β-Naltrexol

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O^6-Ether derivatives of 6β-naltrexol in which the ether substituent includes various electrophilic groups have been synthesized in an effort to examine structure-activity requirements at the 6β-substituent for receptor affinity and irreversibility of binding in opioid receptor preparations. A series of tethered 6β-ethers having terminal epoxides, α-methylene-γ-lactones, and an isothiocyanate group were prepared. The stereochemistry of the α-methylene-γ-lactones was established by convergent synthesis of their reduction products from epoxides of known absolute stereochemistry. In general, the tested compounds showed comparable affinity and selectivity for the receptor subtypes. All were found with high affinity for μ-sites. The terminal epoxide ether diastereomers **8** and **9** were not bound irreversibly in the assay for total opioid receptors. The α-methylene-γ-lactone diastereomers **10** and **11**, and their O^{14}-acetyl precursors **20** and **21**, respectively, varied in their irreversible effects, but where noted these effects were μ-site selective. Methacrylate ether **7** and isothiocyanate ether **12** were bound irreversibly at both μ- and δ-sites.

A number of irreversible opioid ligands have been synthesized and their receptor binding properties have been examined.[1] Among these agents are many analogues of naltrexone (**1**), a nearly pure opioid receptor antagonist, which has a high affinity and selectivity for μ-binding sites. Incorporated into these compounds is a reactive electrophilic functionality as a C-6 substituent for covalent binding to opioid receptors. β-Chlornaltrexamine (β-CNA) (**2**), β-funaltrexamine (β-FNA) (**3**), and other compounds derived from 6β-naltrexamine have shown interesting binding properties in opioid receptor preparations.[2-4] Of

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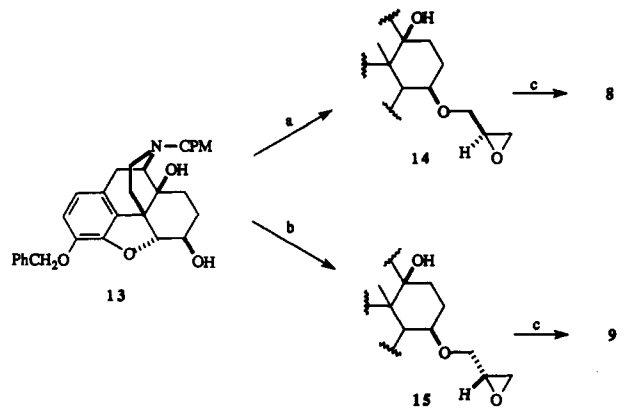
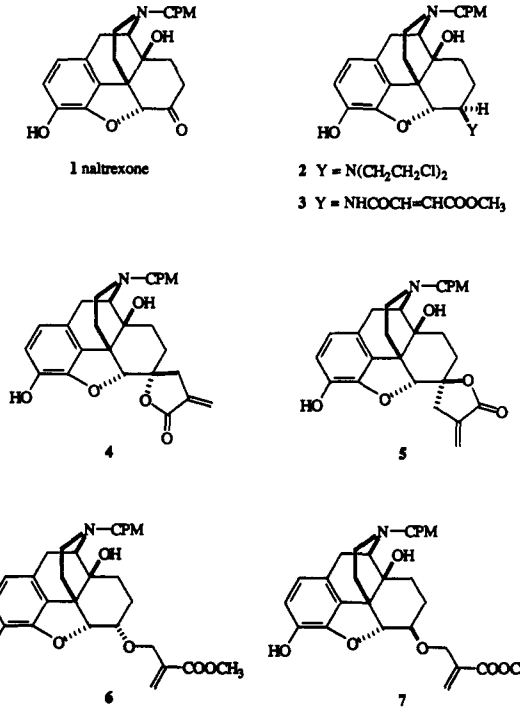
Scheme I[a]



[a] Reagents: (a) KH, THF, 2*R*-(−)-glycidyl tosylate; (b) KH, THF, 2*S*-(+)-glycidyl tosylate; (c) H_2, 5% Rh–C, EtOH.

the related electrophilic reagents, the conformationally restricted isomeric spiro-α-methylene-γ-lactones **4** and **5** containing an electrophilic α,β-unsaturated carbonyl group, **4** is bound reversibly to opioid receptors but **5** is bound irreversibly.[5,6] Of the conformationally less restricted methacrylate ether analogues of these spiro-α-methylene-γ-lactones, analogues **6** and **7**, only the 6β-ether **7** is bound irreversibly.[7] Locating the electrophilic group at the 6β-position appears to yield access to a receptor-bound nucleophile(s) for covalent binding. As previously noted, there appear to be a number of subtle factors associated with both receptor binding and apparent covalent binding at opioid receptors of agents in which the electrophile is incorporated at the C-6 substituent of the naltrexyl structure.[4,7]

Previous work has shown that O^6-substituted opioids with β-substituents are bound with high affinity at opioid receptors. Even bulky substituents like the glucuronic acid moiety in morphine-6β-glucuronide, an important metabolite of morphine, provide compounds that are highly



bound to opioid receptors, morphine-6β-glucuronide being 2–10-fold less potent than morphine in radioligand displacement assays.[8] Thus it seems possible to add a variety of 6β-substituents while retaining significant activity.

As a result of our previous success with regioselective synthesis of ethers at the oxygen of 6β-naltrexol having interesting binding properties, we extended the approach to synthesis of some related ethers. In this paper, we report the synthesis and activity in opioid radioligand displacement assays of 6β-ethers **8–12**, **20**, and **21**, molecules which have a tethered electrophilic substituent incorporated into the 6β-ether as an epoxide, an α-methylene-γ-lactone, or an isothiocyanate. Previously synthesized methacrylate ether **7**, a compound closely related to these ethers, is used for comparison in these assays. The compounds were synthesized to explore the structure–activity requirements for opioid receptor binding and irreversibility in the 6β-ether substituent.

Chemistry

2′*R*-Epoxide **8** and 2′*S*-epoxide **9** were prepared (Scheme I) from the O^3-benzyl ether of 6β-naltrexol (**13**). Ether **13** was obtained from 6β-naltrexol[9] by selective O-benzylation

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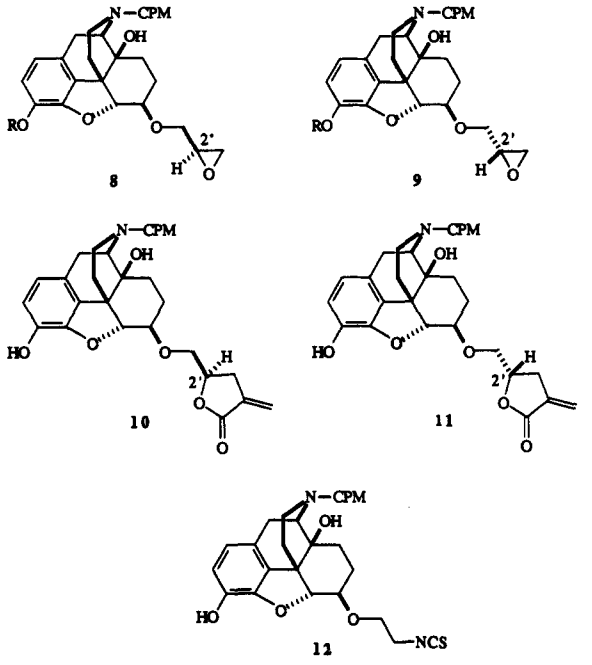

on the phenolic oxygen under mild phase transfer conditions. 2′*R*-Epoxide **8** was obtained from **13** in two steps by selective O^6-alkylation. Treatment of the dialkoxide which was generated from **13** using excess potassium hydride with 2(*R*)-(−)-glycidyl tosylate gave ether epoxide **14**. When removal of the benzyl protecting group was attempted by hydrogenolysis using palladium on carbon as the catalyst, the major products were epoxide ring-opened compounds. Milder hydrogenolysis conditions employing rhodium on carbon as the catalyst provided the desired 2′*R*-epoxide **8** in reasonable yield. Similarly, the diastereomeric O^3-benzyl 2′*S*-epoxide **15** was obtained after treatment of the dipotassium salt of **13** with 2(*S*)-(+)-glycidyl tosylate. Hydrogenolysis of **15** using rhodium on carbon as the catalyst provided 2′*S*-epoxide **9**.

The α-methylene-γ-lactones **10** and **11** were synthesized (Scheme II) from **13** via the O^6-ether aldehyde **19**. When the dialkoxide generated from **13** with excess potassium hydride was treated with bromoacetaldehyde diethyl acetal the O^6-alkylated product **16** was obtained. Hydrogenolysis with palladium on carbon as the catalyst, followed by acetylation gave the O^3,O^{14}-diacetate **18**. Acidic hydrolysis provided the free aldehyde **19**. When the aldehyde **19** was allowed to react with the allylic zinc reagent prepared from methyl 2-(bromomethyl)acrylate and activated zinc,[10] a 1:1 mixture of the two possible diastereomeric α-methylene-γ-lactones **20** and **21** was obtained. The O^3-acetate esters were cleaved in this process. These diastereomers were readily separated by flash chromatography. Removal of the O^{14}-acetate protecting group was achieved by heating **20** and **21** with methanol to give α-methylene-γ-lactones **10** and **11**, respectively.

The absolute stereochemistry at the C-2′ position of the α-methylene-γ-lactones **10**, **11**, **20**, and **21** was determined based on the known stereochemistry at the C-2′ position of the O^3-benzyl 2′*R*-epoxide **14** (Scheme III). When epoxide **14** was allowed to react with the dilithio anion of propionic acid,[5] a 1:1 mixture of two diastereomeric O^3-benzyl α-methyl-γ-lactones **22** and **23** was obtained. These lactones were readily separated by flash chromatography. Hydrogenolysis of the O^3-benzyl protecting group provided the phenolic α-methyl-γ-lactones **24** and **25**, respectively.

When α-methylene-γ-lactone **10** was catalytically hydrogenated, only a single α-methyl-γ-lactone diastereomer was isolated. The ^{1}H NMR, ^{13}C NMR, FTIR, and FAB MS spectra of this compound were identical in all respects to those obtained for α-methyl-γ-lactone **25**. Lactone **25** is the sole product isolated from hydrogenation of **10**, presumably due to steric factors requiring the addition of hydrogen to the face of the exocyclic double bond opposite the C-2′ substituent on the five-membered ring. Thus the α-methyl-γ-lactone product has substituents at C-2′ and C-4′ that have a cis relationship. Similar results have previously been reported on 2,4-disubstituted five-membered lactones.[11] On this basis, both of the chiral centers at C-2′ and C-4′ in **25** must have the *R* absolute stereochemistry because the starting epoxide has the *R* absolute stereochemistry at C-2′. Thus diastereomer **24** has the 2′*R*,4′*S* stereochemistry and **25** has the 2′*R*,4′*R* stereochemistry, as shown in Scheme III.

When α-methylene-γ-lactone **11** was hydrogenated, again only the α-methyl-γ-lactone diastereomer having a cis-2′,4′ relationship was obtained. The ^{1}H NMR, ^{13}C NMR, and FTIR spectra of the resulting product **26** were different from the spectral data obtained for either **24** or **25**, as expected. The chiral centers in **26** must have the *S* absolute stereochemistry at C-2′ and at C-4′ based on the initial 2′*S* stereochemistry and the cis relationship of substituents in **26**. We conclude that α-methylene-γ-lactones **10** and **20** possess the 2′*R* absolute stereochemistry in the chain of the C-6β ether substituent and that α-methylene-γ-lactones **11** and **21** possess the 2′*S* absolute stereochemistry.

Isothiocyanate **12** was synthesized (Scheme IV) from the diethyl acetal **16** via the diamine **31**. Acidic hydrolysis of acetal **16** provided the aldehyde **27**. Attempts to convert **27** directly to the diamine **31** by reductive amination using ammonium acetate and sodium cyanoborohydride gave primarily the dimeric secondary amine **28**. However, when the aldehyde **27** was first converted to the oxime **29**, and then reduced with lithium aluminum hydride, the diamine **30** was obtained cleanly. Hydrogenolysis of the O^3-benzyl protecting group gave the highly insoluble phenolic diamine **31** which provided the desired isothiocyanate **12**

Table I. Comparison of Opioid Receptor Binding of 6-Ethers against 0.5 nM [^{3}H]Bremazocine (total sites), against 1.0 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 1.0 nM [^{3}H]DPDPE (δ-sites) in the Guinea Pig Brain Membrane Preparation

		IC_{50} (nM)[a]			
compound	C-2′	total sites	μ-sites	κ-sites	δ-sites
epoxide **8**	*R*	11	2.9	17	12
epoxide **9**	*S*	12	3.5	17	13
α-methylene-γ-lactone **10**	*R*	19	4.2	44	9.1
α-methylene-γ-lactone **20**	*R*	15	2.2	48	7.2
α-methylene-γ-lactone **11**	*S*	18	3.4	28	19
α-methylene-γ-lactone **21**	*S*	14	2.8	25	7.7
methacrylate **7**	–	11	2.5	21	1.4
isothiocyanate **12**	–	12	2.3	34	6.2
naloxone	–	43	8.9	50	62
naltrexone **1**	–	5.7	0.73	9.5	15
6β-naltrexol	–	13	2.2	20	23
bremazocine	–	1.5	1.8	1.2	1.5

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

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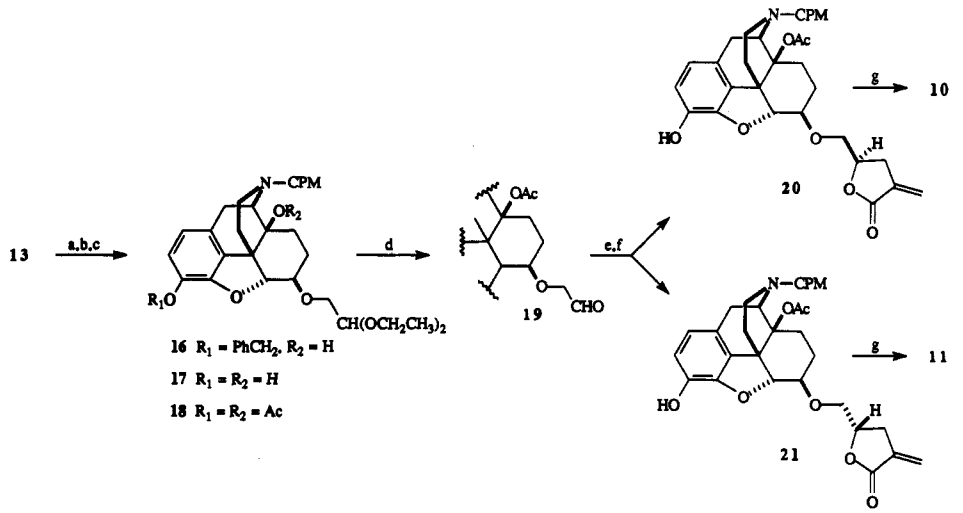
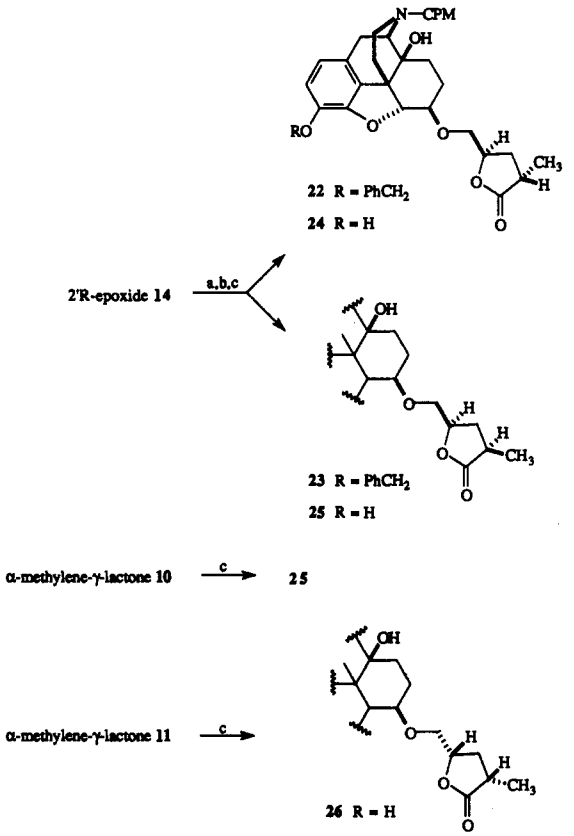
Scheme II[a]



[a] Reagents: (a) KH, THF, $BrCH_2CH(OCH_2CH_3)_2$; (b) H_2, 10% Pd–C, EtOH; (c) Ac_2O; (d) aqueous oxalic acid; (e) $BrZnCH_2C(CH_2)CO-OCH_3$, THF; (f) separate; (g) MeOH, 50 °C.

Scheme III[a]



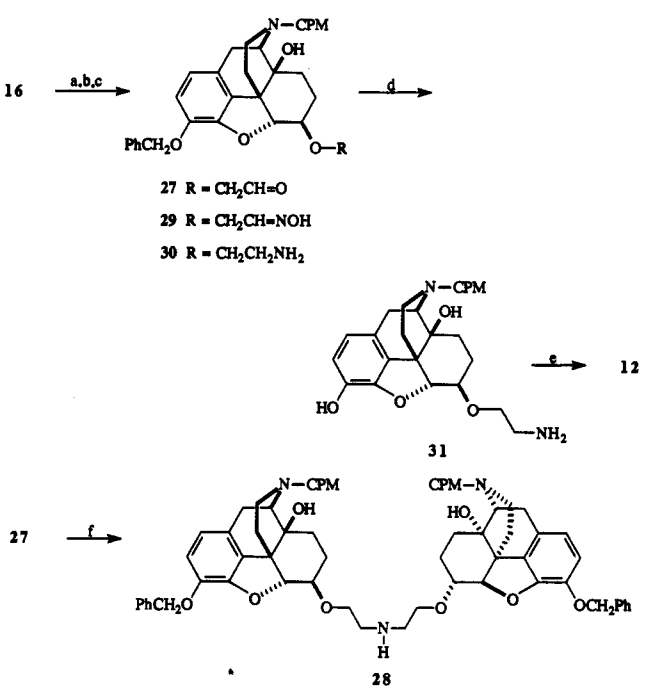
[a] Reagents: (a) CH_3CH_2COOH, LDA, THF; (b) separate; (c) H_2, 10% Pd–C, EtOH.

after treatment with thiophosgene.

Opioid Receptor Binding

The affinity of epoxides **8** and **9**, α-methylene-γ-lactones **10**, **11**, **20**, and **21**, methacrylate ether **7**, and isothiocyanate **12** at the three opioid receptor binding sites (μ, κ, and δ) was determined in a crude membrane preparation from the guinea pig brain (Table I). The displacement assays were run with [^{3}H]bremazocine (all sites), [^{3}H]DAGO (μ-sites), [^{3}H]bremazocine in the presence of unlabeled DAGO and unlabeled DPDPE (κ-sites), and [^{3}H]DPDPE (δ-sites). All tested compounds exhibited similar activity (IC_{50} = 11 to 19 nM) in the assay for total opioid receptors labeled

Scheme IV[a]



[a] Reagents: (a) aqueous oxalic acid; (b) $NH_2OH{\cdot}HCl$, $NaHCO_3$, MeOH, H_2O; (c) $LiAlH_4$, THF; (d) H_2, 10% Pd–C, AcOH, H_2O; (e) $Cl_2C{=}S$, $NaHCO_3$, CH_2Cl_2; (f) NH_4OAc, $NaBH_3CN$, MeOH.

by [^{3}H]bremazocine. When the displacement assay was run with [^{3}H]DAGO, good activity was displayed with all compounds (IC_{50} = 2.2 to 4.2 nM), however these affinity values were 3–6 times lower than that obtained for naltrexone (**1**). These results suggest that the binding affinity imparted by the naltrexyl skeleton of these molecules largely determines the high affinity for μ sites observed with these agents. It is noteworthy that the O^{14}-acetyl-α-methylene-γ-lactone **20** exhibits nearly twice the affinity for μ-sites as the corresponding O^{14}-hydroxy α-methylene-γ-lactone **10**. In the binding assay for determining κ-site affinity all compounds displayed lower affinity (IC_{50}s = 17–49 nM) than at other sites. The epoxides **8** and **9** exhibited the highest affinity (IC_{50} = 17 nM for each) for κ-sites of all the tested agents. When the displacement assay was run using [^{3}H]DPDPE, the tested compounds exhibited variable affinity (IC_{50} = 1.4–19 nM). With the

Table II. Irreversibility of Total Opioid Receptor Binding and Protection by 1 μM Naloxone

compound	C-2′	conc (nM)	percent recovery of 0.5 nM [^{3}H]bremazocine binding[a] unwashed	washed	protected
naloxone	–	1000	0	100	–
epoxide 8	R	50	16	91	95
epoxide 9	S	50	10	79	88
α-methylene-γ-lactone 10	R	100	13	56	66
α-methylene-γ-lactone 20	R	50	27	68	84
α-methylene-γ-lactone 11	S	100	17	51	56
α-methylene-γ-lactone 21	S	50	20	54	61
methacrylate 7[7]	–	20	35	60	90
isothiocyanate 12	–	50	19	55	63

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

exception of α-methylene-γ-lactone 11, all compounds had higher affinity for δ-sites than did naltrexone (1).

These data suggest that the presence of a substituent of extended chain length at C-6 provides analogues of naltrexone (1) with enhanced δ-site affinity. The methacrylate ether 7 (IC_{50} = 1.4 nM) displays the highest affinity for δ-sites of the compounds tested. This high δ-binding may be associated with the presence of extended planarity (over five atoms) of the acrylate group in the 6β-ether side chain. The extended planarity of this π-system is similar to the planarity of the π-system of the indole ring fused to the 4,5α-epoxymorphinan 6- and 7-positions in naltrindole, a potent δ-ligand. With the exception of methacrylate ether 7, all compounds clearly displayed μ-site selectivity.

Even the very bulky tethered α-methylene-γ-lactone group does not decrease binding to a great extent. It seems that the region of the binding site taken up by the tethered C-6 ether substituent may be quite large, thereby allowing a large hydrophobic O^6-substituent to be present without significant distortion of the drug–receptor interaction. The large degree of structural variation on the 6- and 7-positions of the other opioid ligands including the highly potent C-ring bridged analogs[12] supports this view.

Irreversibility Studies

To determine whether any of the ligands were bound irreversibly in opioid receptor preparations, concentrations that were ca. 70–90% inhibitory against 0.5 nM [^{3}H]bremazocine in the binding assay were incubated for 1 h at 25 °C with guinea pig brain membranes. The membranes were then washed thoroughly as described in the Experimental Section and assayed with [^{3}H]bremazocine (Table II). The α-methylene-γ-lactones 10, 11, and 21, the methacrylate ether 7, and the isothiocyanate 12 appeared to be bound irreversibly as washing restored less than 70% of the binding of [^{3}H]bremazocine. As with other naltrexone-derived epoxides,[5,6] epoxides 8 and 9 did not appear to be bound irreversibly. We conclude that, under the conditions of the radiodisplacement assay, relatively more reactive electrophiles are needed for irreversible activity, and/or these epoxide groups are not properly aligned for reaction with a nucleophile on the receptor binding site.

To determine whether the irreversible effects of these agents are μ-site selective we repeated the incubations with concentrations of ligands that were ca. 70–90% inhibitory against 1.0 nM [^{3}H]DAGO, a μ-selective ligand, in the binding assay. After washing, the membranes were assayed with [^{3}H]DAGO (Table III). All compounds tested are

Table III. Irreversibility of μ Opioid Receptor Binding and Protection by 1 μM Naloxone

compound	C-2′	conc (nM)	percent recovery of 1.0 nM [^{3}H]DAGO binding[a] unwashed	washed	protected
naloxone	–	1000	0	100	–
α-methylene-γ-lactone 10	R	10	14	61	88
α-methylene-γ-lactone 20	R	10	10	49	94
α-methylene-γ-lactone 11	S	10	25	62	77
α-methylene-γ-lactone 21	S	10	21	55	71
methacrylate 7	–	10	21	59	95
isothiocyanate 12	–	8	12	39	65

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

Table IV. Irreversibility of δ Opioid Receptor Binding and Protection by 1 μM Naloxone

compound	C-2′	conc (nM)	percent recovery of 1.0 nM [^{3}H]DPDPE binding[a] unwashed	washed	protected
naloxone	–	1000	0	100	–
α-methylene-γ-lactone 10	R	25	26	86	104
α-methylene-γ-lactone 20	R	22	19	71	86
α-methylene-γ-lactone 11	S	70	21	79	80
α-methylene-γ-lactone 21	S	17	23	66	71
methacrylate 7	–	4	5	42	97
isothiocyanate 12	–	20	20	50	59

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

bound irreversibly at μ-sites. Naloxone protected against these irreversible effects for 2′R-α-methylene-γ-lactones 10 and 20, and the methacrylate ether 7. The lack of complete protection by naloxone in the cases of 2′S-α-methylene-γ-lactones 11 and 21 and the isothiocyanate 12 is not yet understood. Several factors might contribute to the lack of protection, including rate of dissociation from the receptor, as well as nonspecific partitioning of ligand into lipid with slow redistribution after washing, or nonspecific covalent interactions. Such observations have been noted previously.[2,12–14]

Because compounds 10 and 20 both have the R absolute configuration at the C-2′ position, their irreversible effects at a particular binding site are expected to be similar. This is the case since both compounds display μ-selective irreversible binding which is subject to naloxone protection. Likewise, compounds 11 and 21 with the S absolute configuration at the C-2′ position display comparable irreversible effects at the μ-site, but with decreased naloxone protection. It is possible that the rate of the covalent binding reaction competes with the rate of ligand–receptor complex dissociation, being more rapid at μ-sites with α-methylene-γ-lactones 11 and 21 than with 10 and 20.

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(13) Bidlack, J. M.; Frey, D. K.; Sayed-Mozaffari, A.; Archer, S. 14β-(Bromoacetamido)-morphine Irreversibly Labels μ Opioid Receptors in Rat Brain Membranes. *Biochemistry* **1989**, *28*, 4333–4339.

(14) Simon, E. J.; Hiller, J. M.; Groth, J.; Edelman, I. Further Properties of Stereospecific Opiate Binding Sites in Rat Brain: On the Nature of the Sodium Effect. *J. Pharmacol. Exp. Ther.* **1979**, *192*, 531–537.

Perhaps 11 and 21 are more effectively aligned with a receptor nucleophile than are the diastereomeric lactones 10 and 20.

To determine whether irreversible effects occur at δ-sites we repeated the incubations with concentrations of ligands that were ca. 70–90% inhibitory against 1.0 nM [^{3}H]-DPDPE in the binding assay. After washing, the membranes were assayed with [^{3}H]DPDPE (Table IV). Of the compounds tested only the methacrylate ether 7 and the isothiocyanate 12 appeared to be bound irreversibly at δ-sites. Naloxone protected against the irreversible effects of methacrylate ether 7 at δ-sites as was observed at μ-sites. The results suggest a comparable mode of irreversible binding by this agent at both receptor types. The lack of protection of 12 at μ- and/or δ-sites may be a result of the high degree of reactivity of this electrophilic group toward receptor nucleophiles relative to the reactivity of the conjugate addition ligands having α,β-unsaturated ester or lactone groups. Clearly, the irreversible effects observed with α-methylene-γ-lactones 10, 11, 20, and 21 are μ-site selective since washing restored more than two-thirds of the [^{3}H]DPDPE binding with all four compounds. It is possible that the nucleophilic group associated with δ-sites is in a different location or orientation than that at μ-sites. For some receptor alkylating ligands, the degree of affinity for a particular binding site may be accentuated by rapid irreversible binding taking place even in the presence of competing radioligand. This may explain the high affinity and high degree of irreversibility of methacrylate ether 7 at δ-sites.

Our data suggests that some differences exist between μ- and δ-sites. With regard to δ-sites the bulkiness of the α-methylene-γ-lactone group makes access of the receptor nucleophile for conjugate addition to this electrophile more difficult than for other ligands. The smaller methacrylate and isothiocyanate groups have greater conformational freedom so they can realign more readily for reaction with a nucleophile than can the α-methylene-γ-lactones. Apparently at μ-sites there is greater conformational freedom for the O^6-substituent. The data suggest that the μ-site can accommodate the α-methylene-γ-lactones readily in a conformation that allows for orientation with receptor nucleophile(s).

Conclusion

Clearly, affinity is retained in compounds with a wide variety of 6β-ether substituents. Compounds have been prepared with similar affinity for μ-opioid receptors as naltrexone, and with higher affinity for δ-sites. All of the α-methylene-γ-lactones 10, 11, 20, and 21 appear to be bound irreversibly at opioid receptors, and this effect seems to be μ-site selective. The methacrylate ether 7 and the isothiocyanate 12 appear to be bound irreversibly at both μ- and δ-sites. The results with these agents provide information concerning the nature of regions of opioid binding sites involved in complexation of the 6β-substituents. Some understanding of structural limits in the 6β-substituent toward irreversible binding at μ- and δ-sites was obtained. This, combined with previously reported binding data on substituted naltrexone-derived spirolactones,[5,6] 6α- and 6β-naltrexol ethers,[7] and 6α- and 6β-naltrexamine-derived irreversible ligands[2-4] provides a basis for additional investigation into structure–activity requirements of 6-substituted 4,5α-epoxymorphinan opioid binding.

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 Kodak (100 μm) plastic-backed plates. Flash chromatography[15] was performed using Merck silica gel 60 (230–400 mesh). Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl immediately prior to use. All reactions were run in flame-dried flasks under an argon atmosphere. Microanalyses were performed by Galbraith Laboratories, Knoxville, TN or by Desert Analytics, Tucson, AZ. Where indicated by the symbols of the elements, analyses were within ±0.4% of theoretical values.

3-(Benzyloxy)-4,5α-epoxy-6β,14-dihydroxy-17-(cyclopropylmethyl)morphinan (13). To a solution of 6β-naltrexol[9] (1.0 g, 2.9 mmol), benzyl bromide (0.9 mL, 7.6 mmol), and tetra-*n*-butylammonium hydrogen sulfate (200 mg, 0.6 mmol) in CH_2Cl_2 (25 mL) was added a solution of NaOH (200 mg, 5 mmol) in water (25 mL). The mixture was stirred vigorously for 5 h and then extracted with ether (3 × 30 mL). The combined extracts were dried ($MgSO_4$), and volatiles were evaporated. The residue was purified by flash chromatography (125 g silica gel) eluting with ether (600 mL) followed by 4% triethylamine–ether (300 mL) to give ether 13 (1.07 g, 85% yield) as a viscous oil: $[\alpha]_D$ = −103° (*c* = 1.00, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 0.05–0.15 (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), 1.25–1.4 (m, 1 H, C-8 H), 1.45–1.7 (m, 3 H, C-7 H, C-8 H, and C-15 H), 1.85–2.0 (m, 1 H, C-7 H), 2.05–2.3 (m, 2 H, C-15 H and C-16 H), 2.34 (d, *J* = 6.4 Hz, 2 H, NCH_2-cyclopropyl), 2.57 (dd, *J* = 5.7 and 18.2 Hz, 1 H, C-10α H), 2.6–2.7 (m, 1 H, C-16 H), 2.8 (br s, 1 H, movable, OH), 3.01 (d, *J* = 18.2 Hz, 1 H, C-10β H), 3.07 (d, *J* = 5.7 Hz, 1 H, C-9 H), 3.4–3.5 (m, 1 H, C-6 H), 4.45 (d, *J* = 5.7 Hz, 1 H, C-5 H), 5.16 (d, *J* = 13.7 Hz, 1 H, benzylic CH), 5.25 (d, *J* = 13.7 Hz, 1 H, benzylic CH), 6.55 (d, *J* = 8.2 Hz, 1 H, C-1 H), 6.75 (d, *J* = 8.2 Hz, 1 H, C-2 H), 7.25–7.45 (m, 5 H, phenyl); ^{13}C NMR ($CDCl_3$) δ 3.88, 3.98, 9.47, 22.71, 25.22, 29.20, 31.32, 43.70, 46.93, 59.23, 62.08, 70.03, 71.69, 72.00, 95.51, 116.67, 118.50, 125.85, 127.36 (2 carbons), 127.67, 128.24 (2 carbons), 132.12, 137.33, 141.99, 144.40; FTIR (KBr) 3600–3200 (OH), 1633, 1605, 1497 cm^{-1}; EIMS (M^+) 433.

3-(Benzyloxy)-4,5α-epoxy-14-hydroxy-6β-[(2*R*)-2,3-epoxypropoxy]-17-(cyclopropylmethyl)morphinan (14). A 35% dispersion of KH in mineral oil (2.3 g, 20 mmol) was rinsed free of oil with hexanes (3 × 25 mL), and then THF (20 mL) was added. A solution of O^3-benzyl-6β-naltrexol (13) (1.45 g, 3.35 mmol) in THF (50 mL) was added. After stirring for 6 h, the mixture was filtered through a flame-dried double-ended scintered glass filtration device (Kontes) under positive argon pressure to remove unreacted KH. A solution of 2*R*-(−)-glycidyl tosylate (870 mg, 3.8 mmol) in THF (4 mL) was added, and the solution was stirred for 19 h. The solution was treated with 10% aqueous $NaHCO_3$ solution (20 mL) and extracted with CH_2Cl_2 (3 × 50 mL). The combined extracts were dried (Na_2SO_4), and the volatiles were evaporated. The residue was purified by flash chromatography (210 g silica gel) eluting with 2% methanol–CH_2Cl_2 (1 L) to give benzyl epoxide 14 (1.37 g, 83% yield) as a viscous oil: $[\alpha]_D$ = −120° (*c* = 1.00, CH_2Cl_2); ^{1}H 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), 1.25–1.5 (m, 2 H, C-8 H and C-15 H), 1.55–1.65 (m, 1 H, C-8 H), 1.7–1.85 (m, 1 H, C-7 H), 1.9–2.15 (m, 2 H, C-7 H and C-16 H), 2.15–2.3 (m, 1 H, C-15 H), 2.35 (d, *J* = 6.4 Hz, 2 H, NCH_2-cyclopropyl), 2.5–2.7 (m, 3 H, C-10α H, C-16 H, and C-3′ H), 2.75 (t, *J* = 4.5 Hz, 1 H, C-3′ H), 3.01 (d, *J* = 18.5 Hz, 1 H, C-10β H), 3.08 (d, *J* = 5.6 Hz, 1 H, C-9 H), 3.1–3.2 (m, 1 H, C-2′ H), 3.2–3.3 (m, 1 H, C-6 H), 3.47 (dd, *J* = 5.9 and 11.4 Hz, 1 H, C-1′ H), 3.95 (dd, *J* = 2.6 and 11.4 Hz, 1 H, C-1′ H), 4.56 (d, *J* = 6.2 Hz, 1 H, C-5 H), 5.0 (br s, 1 H, movable OH), 5.18 (s, 2 H, benzylic CH_2), 6.55 (d, *J* = 8.0 Hz, 1 H, C-1 H), 6.75 (d, *J*

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= 8.0 Hz, 1 H, C-2 H), 7.2–7.5 (m, 5 H, phenyl); ^{13}C NMR ($CDCl_3$) δ 3.70, 3.86, 9.35, 22.51, 23.96, 29.49, 30.49, 43.66, 44.38, 47.42, 50.98, 58.94, 61.99, 69.73, 70.77, 71.69, 81.17, 94.88, 116.91, 118.30, 125.63, 127.12 (2 carbons), 127.48, 128.10 (2 carbons), 132.14, 137.22, 142.20, 143.98; FTIR (film) 3378 (OH), 1633, 1605, 1497 cm^{-1}; FAB MS (M + 1)$^+$ 490.

3-(Benzyloxy)-4,5α-epoxy-14-hydroxy-6β-[(2*S*)-2,3-epoxypropoxy]-17-(cyclopropylmethyl)morphinan (15). Benzyl epoxide 15 was prepared from *O*^{3}-benzyl-6β-naltrexol (13) and 2*S*-(+)-glycidyl tosylate as described above for the synthesis of benzyl epoxide 14 from 13 and 2*R*-(−)-glycidyl tosylate. Benzyl epoxide 15 was obtained (75% yield) as a viscous oil: $[\alpha]_D$ = −117° (*c* = 1.00, CH_2Cl_2); ^{1}H 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* = 2.8 and 14.4 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), 1.7–1.85 (m, 1 H, C-7 H), 1.94 (dq, *J* = 2.0 and 12 Hz, 1 H, C-7 Hz), 2.07 (dt, *J* = 3.4 and 11.4 Hz, 1 H, C-16 H), 2.15–2.3 (m, 1 H, C-15 H), 2.34 (d, *J* = 6.4 Hz, 2 H, NCH_2-cyclopropyl), 2.5–2.7 (m, 3 H, C-10α H, C-16 H, and C-3′ H), 2.72 (t, *J* = 4.0 Hz, 1 H, C-3′ H), 3.00 (d, *J* = 18.5 Hz, 1 H, C-10β H), 3.07 (d, *J* = 5.5 Hz, 1 H, C-9 H), 3.1–3.2 (m, 1 H, C-2′ H), 3.25–3.35 (m, 1 H, C-6 H), 3.70 (dd, *J* = 5.4 and 12.4 Hz, 1 H, C-1′ H), 3.78 (dd, *J* = 3.5 and 12.4 Hz, 1 H, C-1′ H), 4.56 (d, *J* = 6.2 Hz, 1 H, C-5 H), 4.9 (br s, 1 H, movable, OH), 5.19 (s, 2 H, benzylic CH_2), 6.55 (d, *J* = 8.2 Hz, 1 H, C-1 H), 6.75 (d, *J* = 8.2 Hz, 1 H, C-2 H), 7.2–7.5 (m, 5 H, phenyl); ^{13}C NMR ($CDCl_3$) δ 3.70, 3.86, 9.34, 22.51, 23.70, 29.44, 30.48, 43.67, 44.31, 47.41, 50.66, 58.93, 61.98, 69.55, 69.73, 71.69, 80.66, 94.78, 116.88, 118.32, 125.62, 127.13 (2 carbons), 127.48, 128.10 (2 carbons), 132.14, 137.20, 142.21, 143.97; FTIR (film) 3378 (OH), 1633, 1605, 1497 cm^{-1}; FAB MS (M + 1)$^+$ 490.

4,5α-Epoxy-3,14-dihydroxy-6β-[(2*R*)-2,3-epoxypropoxy]-17-(cyclopropylmethyl)morphinan (8). To a solution of benzyl epoxide 14 (500 mg, 1 mmol) in ethanol (50 mL) under argon was added 5% Rh on carbon (150 mg). The mixture was treated with hydrogen gas at atmospheric pressure while stirring for 90 min and then filtered through a pad of Celite under suction. The solids were rinsed with ethanol (2 × 10 mL), and the combined filtrates were evaporated. The residue was purified by flash chromatography (200 g silica gel) eluting with ethyl acetate (2 L) to give unreacted benzyl epoxide 14 (70 mg, 14%) followed by phenol epoxide 8 (200 mg, 50% yield) as a white powder (mp 173–174 °C): $[\alpha]_D$ = −109.5° (*c* = 0.96, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 0.05–0.2 (m, 2 H, cyclopropyl CH_2), 0.45–0.6 (m, 2 H, cyclopropyl CH_2), 0.75–0.9 (m, 1 H, cyclopropyl CH), 1.34 (dt, *J* = 3.0 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), 1.7–1.85 (m, 1 H, C-7 H), 1.94 (dq, *J* = 2.4 and 13.0 Hz, 1 H, C-7 H), 2.05–2.3 (m, 2 H, C-15 H and C-16 H), 2.36 (d, *J* = 6.5 Hz, 2 H, NCH_2-cyclopropyl), 2.5–2.7 (m, 3 H, C-10α H, C-16 H, and C-3′ H), 2.82 (t, *J* = 4.6 Hz, 1 H, C-3′ H), 3.01 (d, *J* = 18.6 Hz, 1 H, C-10β H), 3.09 (d, *J* = 5.5 Hz, 1 H, C-9 H), 3.1–3.2 (m, 1 H, C-2′ H), 3.2–3.35 (m, 1 H, C-6 H), 3.57 (dd, *J* = 5.9 and 12.1 Hz, 1 H, C-1′ H), 3.93 (dd, *J* = 2.8 and 11.9 Hz, 1 H, C-1′ H), 4.55 (d, *J* = 6.4 Hz, 1 H, C-5 H), 5.5 (br s, 2 H, movable, OH), 6.55 (d, *J* = 8.1 Hz, 1 H, C-1 H), 6.72 (d, *J* = 8.1 Hz, 1 H, C-2 H); ^{13}C NMR ($CDCl_3$) δ 3.83, 3.99, 9.48, 22.61, 23.93, 29.55, 30.44, 43.83, 44.48, 47.85, 51.42, 59.12, 62.15, 70.13, 71.17, 80.80, 95.14, 116.98, 118.78, 124.32, 131.59, 139.49, 142.18; FTIR (KBr) 3600–3000 (OH), 1622, 1502 cm^{-1}; HREIMS calcd for $C_{23}H_{29}NO_5$ 399.2046, obsd 399.2044. Anal. ($C_{23}H_{29}NO_5$·0.5H_2O) C, H, N.

4,5α-Epoxy-3,14-dihydroxy-6β-[(2*S*)-2,3-epoxypropoxy]-17-(cyclopropylmethyl)morphinan (9). Phenol epoxide 9 was prepared from benzyl epoxide 15 as described above for the synthesis of 8 from 14. Phenol epoxide 9 was obtained (86% yield) as a white powder (mp 173–175 °C): $[\alpha]_D$ = −118° (*c* = 0.96, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 0.05–0.2 (m, 2 H, cyclopropyl CH_2), 0.45–0.6 (m, 2 H, cyclopropyl CH_2), 0.75–0.9 (m, 1 H, cyclopropyl CH), 1.31 (dt, *J* = 3.0 and 13.5 Hz, 1 H, C-8 H), 1.4–1.55 (m, 1 H, C-15 H), 1.55–1.65 (m, 1 H, C-8 H), 1.65–1.8 (m, 1 H, C-7 H), 1.91 (dq, *J* = 2.0 and 13.0 Hz, 1 H, C-7 H), 2.05–2.3 (m, 2 H, C-15 H and C-16 H), 2.36 (d, *J* = 6.4 Hz, 2 H, NCH_2-cyclopropyl), 2.5–2.7 (m, 3 H, C-10α H, C-16 H, and C-3′ H), 2.84 (t, *J* = 4.5 Hz, 1 H, C-3′ H), 2.99 (d, *J* = 18.5 Hz, 1 H, C-10β H), 3.08 (d, *J* = 5.9 Hz, 1 H, C-9 H), 3.2–3.35 (m, 2 H, C-6 H and C-2′ H), 3.39 (dd, *J* = 7.2 and 12.2 Hz, 1 H, C-1′ H), 3.98 (dd, *J* = 2.2 and 12.2 Hz, 1 H, C-1′ H), 4.56 (d, *J* = 6.6 Hz, 1 H, C-5 H), 5.3 (br s, 2 H, movable, OH), 6.56 (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 3.86, 4.01, 9.50, 22.66, 24.02, 29.67, 30.40, 43.87, 44.24, 47.85, 52.24, 59.15, 62.20, 70.14, 71.14, 81.50, 95.17, 117.43, 118.83, 124.49, 131.62, 139.64, 142.58; FTIR (KBr) 3600–3000 (OH), 1621, 1504 cm^{-1}; HREIMS calcd for $C_{23}H_{29}NO_5$ 399.2046, obsd 399.2045. Anal. ($C_{23}H_{29}NO_5$) C, H, N.

3-(Benzyloxy)-4,5α-epoxy-14-hydroxy-6β-(2,2-diethoxyethoxy)-17-(cyclopropylmethyl)morphinan (16). A 35% dispersion of KH in mineral oil (3.8 g, 33 mmol) was rinsed free of oil with hexanes (3 × 25 mL), and then THF (50 mL) was added. A solution of ether 13 (2.4 g, 5.5 mmol) in THF (20 mL) was added. The mixture was stirred for 4 h, and then bromoacetaldehyde diethyl acetal (1.0 mL, 6.6 mmol) was added over 10 min. The mixture was stirred for 15 h and then heated at 50 °C for 2 h. After cooling to ambient temperature, a saturated aqueous NH_4Cl solution (30 mL) was added carefully followed by water (100 mL). The mixture was extracted with CH_2Cl_2 (3 × 75 mL). The combined extracts were washed with water (2 × 50 mL), and the volatiles were evaporated. The residue was purified by flash chromatography (165 g silica gel) eluting with 2% methanol–CH_2Cl_2 (2 L) to give ether 16 (2.05 g, 67% yield) as a viscous oil: $[\alpha]_D$ = −99.5° (*c* = 1.00, CH_2Cl_2), followed by unreacted ether 13 (300 mg, 12%); ^{1}H NMR ($CDCl_3$) δ 0.05–0.15 (m, 2 H, cyclopropyl CH_2), 0.45–0.55 (m, 2 H, cyclopropyl CH_2), 0.75–0.9 (m, 1 H, cyclopropyl CH), 1.16 (t, *J* = 7.0 Hz, 3 H, acetal CH_3), 1.18 (t, *J* = 7.0 Hz, 3 H, acetal CH_3), 1.33 (dt, *J* = 2.8 and 13.5 Hz, 1 H, C-8 H), 1.4–1.5 (m, 1 H, C-15 H), 1.5–1.65 (m, 1 H, C-8 H), 1.7–1.8 (m, 1 H, C-7 H), 1.85–2.0 (m, 1 H, C-7 H), 2.07 (dt, *J* = 3.4 and 11.9 Hz, 1 H, C-16 H), 2.23 (dt, *J* = 4.8 and 12.3 Hz, 1 H, C-15 H), 2.35 (d, *J* = 6.5 Hz, 2 H, NCH_2-cyclopropyl), 2.5–2.65 (m, 2 H, C-10α H and C-16 H), 3.00 (d, *J* = 18.4 Hz, 1 H, C-10β H), 3.07 (d, *J* = 5.7 Hz, 1 H, C-9 H), 3.2–3.3 (m, 1 H, C-6 H), 3.45–3.75 (m, 6 H, C-6 OCH_2 and acetal OCH_2), 4.57 (d, *J* = 6.4 Hz, 1 H, C-5 H), 4.6 (br s, 1 H, movable, OH), 4.66 [t, *J* = 5.2 Hz, 1 H, $(EtO)_2CH$], 5.18 (s, 2 H, benzylic CH), 6.55 (d, *J* = 8.2 Hz, 1 H, C-1 H), 6.75 (d, *J* = 8.2 Hz, 1 H, C-2 H), 7.2–7.5 (m, 5 H, phenyl); ^{13}C NMR ($CDCl_3$) δ 3.79, 3.91, 9.44, 15.34 (2 carbons), 22.64, 23.87, 29.60, 30.60, 43.78, 47.49, 59.09, 61.69, 62.17 (2 carbons), 69.90, 70.48, 71.84, 81.15, 94.83, 101.04, 117.05, 118.26, 125.70, 127.36 (2 carbons), 127.57, 128.17 (2 carbons), 132.35, 137.27, 142.31, 144.19; FTIR (film) 3381 (OH), 1633, 1605, 1497 cm^{-1}; FAB MS (M + 1)$^+$ 550.

4,5α-Epoxy-3,14-dihydroxy-6β-(2,2-diethoxyethoxy)-17-(cyclopropylmethyl)morphinan (17). To a solution of ether 16 (2.0 g, 3.6 mmol) in ethanol (100 mL) under argon was added 10% Pd on carbon (300 mg) and treated with hydrogen gas at atmospheric pressure for 2 h. The mixture was filtered through a 0.5-in. pad of Celite under suction, and the solids were rinsed with ethanol (20 mL). The volatiles were evaporated to give phenol 17 (1.6 g, 96% yield) as white crystals (dec above 205 °C): $[\alpha]_D$ = −115° (*c* = 1.00, 40% ethanol–CH_2Cl_2); ^{1}H 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), 1.21 (t, *J* = 7.0 Hz, 6 H, acetal CH_3), 1.25–1.4 (m, 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), 1.7–1.85 (m, 1 H, C-7 H), 1.85–2.0 (m, 1 H, C-7 H), 2.05–2.3 (m, 2 H, C-15 H and C-16 H), 2.37 (d, *J* = 6.4 Hz, 2 H, NCH_2-cyclopropyl), 2.5–2.7 (m, 2 H, C-10α H and C-16 H), 3.01 (d, *J* = 18.5 Hz, 1 H, C-10β H), 3.10 (d, *J* = 5.3 Hz, 1 H, C-9 H), 3.2–3.3 (m, 1 H, C-6 H), 3.5–3.8 (m, 6 H, C-6 OCH_2 and acetal OCH_2), 4.56 (d, *J* = 6.4 Hz, 1 H, C-5 H), 4.65 [t, *J* = 5.2 Hz, 1 H, $(EtO)_2CH$], 6.55 (d, *J* = 8.1 Hz, 1 H, C-1 H), 6.71 (d, *J* = 8.1 Hz, 1 H, C-2 H); ^{13}C NMR ($CDCl_3$) δ 3.80, 3.94, 9.38, 15.30 (2 carbons), 22.61, 23.62, 29.55, 30.41, 43.84, 47.76, 59.08, 61.98, 62.11, 62.20, 69.95, 70.10, 80.98, 95.10, 101.06, 116.70, 118.65, 124.19, 131.54, 139.37, 142.01; FTIR (KBr) 3600–2800 (OH), 1638, 1624, 1503 cm^{-1}; FAB MS (M + 1)$^+$ 460.

3,14-Diacetoxy-4,5α-epoxy-6β-(2,2-diethoxyethoxy)-17-(cyclopropylmethyl)morphinan (18). A solution of phenol 17 (1.6 g, 0.35 mmol) in acetic anhydride (10 mL) was heated at 90 °C for 45 min, and then volatiles were evaporated. The residue was dissolved in CH_2Cl_2 (50 mL) and washed with 5% aqueous $NaHCO_3$ solution (30 mL) followed by water (30 mL). The organic layer was dried (Na_2SO_4), and volatiles were evaporated to give diacetyl acetal 18 (1.78 g, 94%) as a viscous oil: $[\alpha]_D$ = −153° (*c*

= 1.00, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 0.0–0.15 (m, 2 H, cyclopropyl CH_2), 0.4–0.55 (m, 2 H, cyclopropyl CH_2), 0.75–0.9 (m, 1 H, cyclopropyl CH), 1.22 (t, J = 7.0 Hz, 6 H, acetal CH_3), 1.25–1.45 (m, 2 H, C-8 H and C-15 H), 1.6–1.8 (m, 2 H, C-7 CH_2), 2.11 (s, 3 H, C-14 acetoxy CH_3), 2.0–2.15 (m, 1 H, C-16 H), 2.28 (s, 3 H, C-3 acetoxy CH_3), 2.2–2.45 (m, 3 H, C-15 H and NCH_2-cyclopropyl), 2.45–2.6 (m, 2 H, C-10α H and C-8 H), 2.6–2.7 (m, 1 H, C-16 H), 3.07 (d, J = 18.5 Hz, 1 H, C-10β H), 3.2–3.3 (m, 1 H, C-6 H), 3.5–3.8 (m, 6 H, C-6 OCH_2 and acetal CH_2), 4.39 (d, J = 4.9 Hz, 1 H, C-9 H), 4.6–4.7 [m, 2 H, C-5 H and $(EtO)_2CH$], 6.55 (d, J = 8.1 Hz, 1 H, C-1 H), 6.71 (d, J = 8.1 Hz, 1 H, C-2 H); ^{13}C NMR ($CDCl_3$) δ 3.63, 3.82, 9.60, 15.36 (2 carbons), 20.56, 22.22, 23.35, 23.72, 25.13, 29.78, 43.83, 48.05, 55.51, 59.27, 61.50, 61.94, 70.41, 80.60, 82.39, 95.70, 100.72, 118.57, 122.17, 131.25, 131.82, 133.24, 146.56, 168.27, 169.74; FTIR (film) 1768, 1732, 1493 cm^{-1}; FAB MS $(M + 1)^+$ 544.

3,14-Diacetoxy-4,5α-epoxy-6β-(2-oxoethoxy)-17-(cyclopropylmethyl)morphinan (19). A solution of diacetyl acetal 18 (480 mg, 0.88 mmol) in 10% aqueous oxalic acid solution (30 mL) was heated at 50 °C for 45 min. The solution was cooled in an ice bath and then poured over a mixture of $NaHCO_3$ (6 g) and water (25 mL). The mixture was extracted with CH_2Cl_2 (3 × 35 mL). The combined extracts were dried (Na_2SO_4), and the solvent was evaporated. The residue was dissolved in ethyl acetate (100 mL) and then filtered through silica gel (20 g) under suction. The solids were rinsed with ethyl acetate (100 mL) and the combined filtrates were evaporated to give diacetoxy aldehyde 19 (350 mg, 84% yield) as a viscous oil: $[\alpha]_D$ = −102° (c = 0.50, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 0.0–0.15 (m, 2 H, cyclopropyl CH_2), 0.4–0.55 (m, 2 H, cyclopropyl CH_2), 0.7–0.85 (m, 1 H, cyclopropyl CH), 1.25–1.45 (m, 2 H, C-8 H and C-15 H), 1.65–1.85 (m, 2 H, C-7 CH_2), 2.0–2.15 (m, 1 H, C-16), 2.12 (s, 3 H, C-14 acetoxy CH_3), 2.29 (s, 3 H, C-3 acetoxy CH_3), 2.2–2.45 (m, 3 H, C-15 H and NCH_2-cyclopropyl), 2.45–2.7 (m, 3 H, C-8 H, C-10α H, and C-16 H), 3.08 (d, J = 18.7 Hz, 1 H, C-10β H), 3.3–3.4 (m, 1 H, C-6 H), 4.30 (s, 2 H, C-6 OCH_2), 4.39 (d, J = 5.0 Hz, 1 H, C-9 H), 4.66 (d, J = 6.3 Hz, 1 H, C-5 H), 6.68 (d, J = 8.2 Hz, 1 H, C-1 H), 6.80 (d, J = 8.2 Hz, 1 H, C-2 H), 9.73 (s, 1 H, CHO); ^{13}C NMR ($CDCl_3$) δ 3.64, 3.82, 9.58, 20.59, 22.21, 23.35, 23.92, 25.20, 29.72, 43.76, 48.17, 55.47, 59.27, 76.13, 81.39, 82.20, 95.74, 118.92, 122.21, 131.35, 131.61, 133.29, 146.32, 168.44, 169.71, 200.71; FTIR (KBr) 1766, 1732, 1494 cm^{-1}; EIMS (M^+) 469.

14-Acetoxy-4,5α-epoxy-3-hydroxy-6β-[[(2*R*)-2-hydroxy-4-carboxy-4-*n*-pentenyl]oxy]-17-(cyclopropylmethyl)morphinan γ-Lactone (20) and 14-Acetoxy-4,5α-epoxy-3-hydroxy-6β-[[(2*S*)-2-hydroxy-4-carboxy-4-*n*-pentenyl]oxy]-17-(cyclopropylmethyl)morphinan γ-Lactone (21). To a solution of diacetoxy aldehyde 19 (550 mg, 1.17 mmol) in THF (15 mL) was added freshly activated zinc dust (225 mg, 3 equiv). The mixture was stirred at 40–50 °C while a solution of methyl 2-(bromomethyl)acrylate (170 μL, 1.4 mmol) in THF (8 mL) was added over 30 min. The mixture was stirred at 40–50 °C for an additional 90 min and then cooled in an ice bath. The mixture was treated with saturated aqueous NH_4Cl solution (20 mL) and then treated with saturated $NaHCO_3$ solution (50 mL). The mixture was extracted with CH_2Cl_2 (3 × 50 mL). The combined extracts were dried (Na_2SO_4), and the volatiles were evaporated. The residue was purified by flash chromatography (165 g silica gel) eluting with 1% methanol–CH_2Cl_2 (1.5 L) to give α-methylene-γ-lactone **20** (200 mg, 35% yield) as a white powder (mp 173–175 °C): $[\alpha]_D$ = −198.5° (c = 0.85, CH_2Cl_2), followed by α-methylene-γ-lactone 21 (200 mg, 35% yield) as a white powder (mp 173–175 °C), $[\alpha]_D$ = −140.5° (c = 1.00, CH_2Cl_2).

20: ^1H NMR ($CDCl_3$) δ 0.0–0.15 (m, 2 H, cyclopropyl CH_2), 0.4–0.55 (m, 2 H, cyclopropyl CH_2), 0.7–0.8 (m, 1 H, cyclopropyl CH), 1.2–1.3 (m, 1 H, C-8 H), 1.35–1.45 (m, 1 H, C-15 H), 1.5–1.75 (m, 2 H, C-7 CH_2), 2.0–2.15 (m, 1 H, C-16 H), 2.10 (s, 3 H, C-14 acetoxy CH_3), 2.2–2.35 (m, 3 H, C-15 H and NCH_2-cyclopropyl), 2.4–2.7 (m, 3 H, C-8 H, C-10α H, and C-16 H), 2.9–3.1 (m, 3 H, C-10β H and C-3′ CH_2), 3.15–3.25 (m, 1 H, C-6 H), 3.62 (dd, J = 2.1 and 12.8 Hz, 1 H, C-1′ H), 3.78 (d, J = 12.8 Hz, 1 H, C-1′ H), 4.31 (d, J = 5.3 Hz, 1 H, C-9 H), 4.34 (d, J = 6.6 Hz, 1 H, C-5 H), 4.6–4.7 (m, 1 H, C-2′ H), 5.7–5.75 (m, 1 H, lactone vinyl CH), 6.3–6.4 (m, 1 H, lactone vinyl CH), 6.54 (d, J = 8.2 Hz, 1 H, C-1 H), 6.73 (d, J = 8.2 Hz, 1 H, C-2 H); ^{13}C ($CDCl_3$) δ 3.54, 3.73, 9.54, 22.16, 22.91, 23.82, 25.03, 28.89, 29.63, 43.91, 47.95, 55.49, 59.16, 70.71, 76.86, 82.74, 83.64, 93.20, 117.34, 118.60, 121.84, 124.10, 130.38, 134.46, 140.49, 141.87, 169.72, 171.91; FTIR (KBr) 3700–3000 (OH), 1741, 1618, 1508 cm^{-1}; FAB MS $(M + 1)^+$ 496. Anal. ($C_{28}H_{33}NO_7 \cdot 0.5H_2O$) C, H, N.

21: ^1H NMR ($CDCl_3$) δ 0.0–0.15 (m, 2 H, cyclopropyl CH_2), 0.4–0.55 (m, 2 H, cyclopropyl CH_2), 0.7–0.8 (m, 1 H, cyclopropyl CH), 1.2–1.45 (m, 2 H, C-8 H and C-15 H), 1.5–1.75 (m, 2 H, C-7 CH_2), 2.0–2.2 (m, 1 H, C-16 H), 2.15 (s, 3 H, C-14 acetoxy CH_3), 2.2–2.7 (m, 6 H, C-8 H, C-10α H, C-15 H, C-16 H, NCH_2-cyclopropyl), 2.8–3.1 (m, 2 H, C-3′ CH_2), 3.03 (d, J = 19.3 Hz, 1 H, C-10β H), 3.2–3.3 (m, 1 H, C-6 H), 3.7–3.85 (m, 2 H, C-1′ CH_2), 4.35 (d, J = 4.9 Hz, 1 H, C-9 H), 4.53 (d, J = 6.3 Hz, 1 H, C-5 H), 4.6–4.7 (m, 1 H, C-2′ H), 5.6–5.7 (m, 1 H, lactone vinyl CH), 6.2–6.3 (m, 1 H, lactone vinyl CH), 6.56 (d, J = 8.1 Hz, 1 H, C-1 H), 6.71 (d, J = 8.1 Hz, 1 H, C-2 H); ^{13}C ($CDCl_3$) δ 3.72, 3.90, 9.69, 22.36, 23.09, 23.74, 25.23, 29.63, 29.94, 44.08, 48.32, 55.63, 59.36, 71.39, 75.84, 81.50, 82.73, 94.80, 117.16, 118.93, 122.10, 125.40, 130.83, 134.09, 139.56, 141.92, 169.78, 170.34; FTIR (KBr) 3600–3000 (OH), 1726, 1624, 1507 cm^{-1}; FAB MS $(M + 1)^+$ 496. Anal. ($C_{28}H_{33}NO_7 \cdot 0.5H_2O$) C, H, N.

4,5α-Epoxy-3,14-dihydroxy-6β-[[(2*R*)-2-hydroxy-4-carboxy-4-*n*-pentenyl]oxy]-17-(cyclopropylmethyl)morphinan γ-Lactone (10). A solution of α-methylene-γ-lactone **20** (200 mg, 0.4 mmol) in methanol (10 mL) was stirred at 50 °C for 24 h. Water (25 mL) was added, and the solution was extracted with CH_2Cl_2 (3 × 20 mL). The combined extracts were dried (Na_2SO_4), and the volatiles were evaporated. The residue was purified by flash chromatography (20 g silica gel) eluting with 3% methanol–CH_2Cl_2 (500 mL) to give α-methylene-γ-lactone **10** (175 mg, 96% yield) as a white powder (mp 84–86 °C): $[\alpha]_D$ = −77° (c = 0.80, 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, cyclopropyl CH_2), 0.75–0.9 (m, 1 H, cyclopropyl CH), 1.28 (dt, J = 2.8 and 13.4 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), 1.65–1.75 (m, 1 H, C-7 H), 1.85 (dq, J = 2.4 and 12.9 Hz, 1 H, C-7 H), 2.05–2.25 (m, 2 H, C-15 H and C-16 H), 2.35 (d, J = 6.4 Hz, 2 H, NCH_2-cyclopropyl), 2.5–2.65 (m, 2 H, C-10α H and C-16 H), 2.9–3.1 (m, 4 H, C-9 H, C-10β H, and C-3′ CH_2), 3.1–3.2 (m, 1 H, C-6 H), 3.61 (dd, J = 2.1 and 12.2 Hz, 1 H, C-1′ H), 3.78 (d, J = 12.2 Hz, 1 H, C-1′ H), 4.33 (d, J = 6.6 Hz, 1 H, C-5 H), 4.6–4.7 (m, 1 H, 2′ H), 5.70 (t, J = 2.5 Hz, 1 H, lactone vinyl CH), 6.32 (t, J = 2.5 Hz, 1 H, lactone vinyl CH), 6.53 (d, J = 8.1 Hz, 1 H, C-1 H), 6.72 (d, J = 8.1 Hz, 1 H, C-2 H); ^{13}C ($CDCl_3$) δ 3.84, 3.94, 9.48, 22.60, 24.08, 29.00, 29.53, 30.36, 43.83, 47.65, 59.11, 62.24, 70.12, 70.66, 76.99, 84.46, 93.66, 117.35, 118.63, 121.94, 123.38, 131.27, 134.62, 140.25, 142.21, 172.16; FTIR (KBr) 3700–3000 (OH), 1760, 1743, 1618, 1508 cm^{-1}; HREIMS calcd for $C_{26}H_{31}NO_6$ 453.2151, obsd 453.2150. Anal. ($C_{26}H_{31}NO_6 \cdot 0.5H_2O$) C, H, N.

4,5α-Epoxy-3,14-dihydroxy-6β-[[(2*S*)-2-hydroxy-4-carboxy-4-*n*-pentenyl]oxy]-17-(cyclopropylmethyl)morphinan γ-Lactone (11). α-Methylene-γ-lactone 11 was prepared from α-methylene-γ-lactone 21 as described above for the synthesis of 10 from **20**. α-Methylene-γ-lactone 11 was obtained (96% yield) as a white powder (mp 176–181 °C): $[\alpha]_D$ = −100° (c = 0.96, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 0.05–0.2 (m, 2 H, cyclopropyl CH_2), 0.45–0.6 (m, 2 H, cyclopropyl CH_2), 0.75–0.9 (m, 1 H, cyclopropyl CH), 1.32 (dt, J = 3.0 and 13.4 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), 1.65–1.75 (m, 1 H, C-7 H), 1.89 (dq, J = 2.3 and 12.7 Hz, 1 H, C-7 H), 2.0–2.3 (m, 2 H, C-15 H and C-16 H), 2.36 (d, J = 6.6 Hz, 2 H, NCH_2-cyclopropyl), 2.5–2.7 (m, 2 H, C-10α H and C-16 H), 2.8–3.1 (m, 4 H, C-9 H, C-10β H, and C-3′ CH_2), 3.15–3.3 (m, 1 H, C-6 H), 3.72 (dd, J = 4.2 and 10.8 Hz, 1 H, C-1′ H), 3.79 (dd, J = 4.4 and 10.8 Hz, 1 H, C-1′ H), 4.52 (d, J = 6.4 Hz, 1 H, C-5 H), 4.6–4.7 (m, 1 H, C-2′ H), 5.64 (t, J = 2.6 Hz, 1 H, lactone vinyl CH), 6.24 (t, J = 2.9 Hz, 1 H, lactone vinyl CH), 6.56 (d, J = 8.2 Hz, 1 H, C-1 H), 6.71 (d, J = 8.2 Hz, 1 H, C-2 H); ^{13}C ($CDCl_3$) δ 3.87, 4.00, 9.48, 22.64, 23.84, 29.54, 29.74, 30.54, 43.84, 47.85, 59.15, 62.19, 70.11, 71.13, 75.78, 81.86, 95.14, 116.97, 118.84, 121.99, 124.46, 131.55, 134.13, 139.35, 142.05, 170.32; FTIR (KBr) 3700–3000 (OH), 1763, 1618, 1501 cm^{-1}; HREIMS calcd for $C_{26}H_{31}NO_6$ 453.2151, obsd 453.2115. Anal. ($C_{26}H_{31}NO_6 \cdot 0.25H_2O$) C, H, N.

3-(Benzyloxy)-4,5α-epoxy-14-hydroxy-6β-[(2*R*,4*S*)-2-hydroxy-4-carboxy-*n*-pentoxy]-17-(cyclopropylmethyl)-morphinan γ-Lactone (22) and 3-(Benzyloxy)-4,5α-epoxy-14-hydroxy-6β-[(2*R*,4*R*)-2-hydroxy-4-carboxy-*n*-pentoxy]-

17-(cyclopropylmethyl)morphinan γ-Lactone (23). To a solution of diisopropylamine (0.39 mL, 2.8 mmol) in THF (2 mL), cooled in an ice bath, was added a 2.5 M solution of *n*-BuLi in hexanes (1.1 mL, 2.8 mmol) over 3 min. The solution was stirred at 0 °C for 15 min, and then propionic acid (100 μL, 1.4 mmol) was added in one portion. After stirring at 0 °C for 3 min, the cooling bath was removed and the mixture was heated at 30 °C for 30 min. A solution of 2′*R*-epoxide **14** (170 mg, 0.35 mmol) in THF (4 mL) was added, and the mixture was heated at 75 °C for 20 h. The THF was evaporated, and the residue was treated with half-saturated aqueous $NaHCO_3$ solution (30 mL). The mixture was extracted with ether (3 × 50 mL). The combined extracts were dried ($MgSO_4$), and the solvent was evaporated. The residue was dissolved in 5% aqueous H_2SO_4 solution (10 mL) and heated at 70 °C for 22 h. After cooling, the solution was poured over $NaHCO_3$ (4 g). The mixture was diluted with water (40 mL) and extracted with ether (3 × 20 mL). The combined extracts were dried ($MgSO_4$), and the solvent was evaporated. The residue was purified by flash chromatography (21 g silica gel) eluting with 4% triethylamine–ether (200 mL) to give (2′*R*,4′*S*)-α-methyl-γ-lactone **22** (40 mg, 21% yield) as a viscous oil followed by (2′*R*,4′*R*)-α-methyl-γ-lactone **23** (30 mg, 16% yield) as a viscous oil.

22: ^{1}H NMR ($CDCl_3$) δ 0.05–0.2 (m, 2 H, cyclopropyl CH_2), 0.45–0.6 (m, 2 H, cyclopropyl CH_2), 0.75–0.9 (m, 1 H, cyclopropyl CH), 1.22 (d, *J* = 7.2 Hz, 3 H, C-4′ CH_3), 1.25–1.4 (m, 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), 1.8–2.15 (m, 3 H, C-7 H, C-16 H, and C-3′ H), 2.15–2.4 (m, 2 H, C-15 H and C-3′ H), 2.35 (d, *J* = 6.3 Hz, 2 H, NCH_2-cyclopropyl), 2.5–2.8 (m, 3 H, C-10α H, C-16 H, and C-4′ H), 3.01 (d, *J* = 18.6 Hz, 1 H, C-10β H), 3.07 (d, *J* = 5.4 Hz, 1 H, C-9 H), 3.15–3.3 (m, 1 H, C-6 H), 3.60 (dd, *J* = 4.4 and 10.5 Hz, 1 H, C-1′ H), 3.88 (dd, *J* = 4.1 and 10.6 Hz, 1 H, C-1′ H), 4.52 (d, *J* = 6.3 Hz, 1 H, C-5 H), 4.55–4.65 (m, 1 H, C-2′ H), 5.1–5.25 (m, 2 H, benzylic CH_2), 6.56 (d, *J* = 8.2 Hz, 1 H, C-1 H), 6.76 (d, *J* = 8.1 Hz, 1 H, C-2 H), 7.25–7.45 (m, 5 H, phenyl); FTIR (film) 3382 (OH), 1770, 1606, 1497 cm^{-1}; FAB MS $(M + 1)^+$ 546.

23: ^{1}H NMR ($CDCl_3$) δ 0.05–0.2 (m, 2 H, cyclopropyl CH_2), 0.45–0.6 (m, 2 H, cyclopropyl CH_2), 0.75–0.9 (m, 1 H, cyclopropyl CH), 1.23 (d, *J* = 7.0 Hz, 3 H, C-4′ CH_3), 1.25–1.4 (m, 1 H, C-8 H), 1.4–1.5 (m, 1 H, C-15 H), 1.55–1.8 (m, 3 H, C-7 H, C-8 H, and C-3′ H), 1.85–2.0 (m, 1 H, C-7 H), 2.0–2.15 (m, 1 H, C-16 H), 2.23 (dt, *J* = 5.0 and 12.5 Hz, 1 H, C-15 H), 2.35 (d, *J* = 6.5 Hz, 2 H, NCH_2-cyclopropyl), 2.35–2.45 (m, 1 H, C-3′ H), 2.5–2.7 (m, 3 H, C-10α H, C-16 H, and C-4′ H), 3.01 (d, *J* = 18.5 Hz, 1 H, C-10β H), 3.07 (d, *J* = 5.4 Hz, 1 H, C-9 H), 3.2–3.3 (m, 1 H, C-6 H), 3.67 (dd, *J* = 6.0 and 10.9 Hz, 1 H, C-1′ H), 3.88 (dd, *J* = 3.7 and 11.0 Hz, 1 H, C-1′ H), 4.45–4.6 (m, 1 H, C-2′ H), 4.55 (d, *J* = 6.3 Hz, 1 H, C-5 H), 5.1–5.25 (m, 2 H, benzylic CH_2), 6.56 (d, *J* = 8.0 Hz, 1 H, C-1 H), 6.76 (d, *J* = 8.0 Hz, 1 H, C-2 H), 7.25–7.5 (m, 5 H, phenyl); FTIR (film) 3382 (OH), 1770, 1607, 1497 cm^{-1}; FAB MS $(M + 1)^+$ 546.

4,5α-Epoxy-3,14-dihydroxy-6β-[(2*R*,4*S*)-2-hydroxy-4-carboxy-*n*-pentoxy]-17-(cyclopropylmethyl)morphinan γ-Lactone (24). To a solution of benzyl ether **22** (30 mg, 0.05 mmol) in ethanol (5 mL) was added 10% Pd on carbon (7 mg) and stirred under an atmosphere of hydrogen gas for 2 h. The mixture was diluted with ethanol (30 mL) and filtered through a 0.5-in. pad of Celite under suction. The solids were rinsed with ethanol (20 mL), and then the combined filtrates were evaporated. The residue was purified by flash chromatography (7.5 g silica gel) eluting with 2% triethylamine–ethyl acetate (90 mL) to give α-methyl-γ-lactone **24** (20 mg, 80% yield) as a viscous oil: ^{1}H NMR ($CDCl_3$) δ 0.05–0.2 (m, 2 H, cyclopropyl CH_2), 0.45–0.6 (m, 2 H, cyclopropyl CH_2), 0.75–0.9 (m, 1 H, cyclopropyl CH), 1.32 (d, *J* = 7.3 Hz, 3 H, C-4′ CH_3), 1.25–1.4 (m, 1 H, C-8 H), 1.4–1.5 (m, 1 H, C-15 H), 1.5–1.65 (m, 1 H, C-8 H), 1.65–1.95 (m, 2 H, C-7 CH_2), 1.95–2.25 (m, 3 H, C-15 H, C-16 H, and C-3′ H), 2.36 (d, *J* = 6.3 Hz, 2 H, NCH_2-cyclopropyl), 2.45–2.65 (m, 3 H, C-10α H, C-16 H, and C-3′ H), 2.99 (d, *J* = 18.2 Hz, 1 H, C-10β H), 3.04 (d, *J* = 6.0 Hz, 1 H, C-9 H), 3.05–3.2 (m, 2 H, C-6 H and C-4′ H), 3.53 (dd, *J* = 2.2 and 12.6 Hz, 1 H, C-1′ H), 3.80 (dd, *J* = 1.2 and 12.7 Hz, 1 H, C-1′ H), 4.36 (d, *J* = 6.7 Hz, 1 H, C-5 H), 4.5–4.6 (m, 1 H, C-2′ H), 6.53 (d, *J* = 8.0 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, 3.88, 9.46, 16.28, 22.58, 23.86, 29.55, 30.35, 32.54, 35.11, 43.79, 47.62, 59.10, 62.24, 70.08, 71.70, 78.07, 84.79, 93.54, 117.57, 118.70, 123.32, 131.13, 140.29, 142.08, 183.08; FTIR (film) 3353 (OH), 1749, 1615, 1507 cm^{-1}; FAB MS $(M + 1)^+$ 456.

4,5α-Epoxy-3,14-dihydroxy-6β-[(2*R*,4*R*)-2-hydroxy-4-carboxy-*n*-pentoxy]-17-(cyclopropylmethyl)morphinan γ-Lactone (25). α-Methyl-γ-lactone **25** was prepared from benzyl ether **23** as described above for the preparation of **24** from **22**. α-Methyl-γ-lactone **25** was obtained (80% yield) as a viscous oil. Alternatively, α-methyl-γ-lactone **25** was prepared from α-methylene-γ-lactone **10** as follows: To a solution of α-methylene-γ-lactone **10** (10 mg, 0.02 mmol) in ethanol (5 mL) was added 10% Pd on carbon (5 mg) and stirred under an atmosphere of hydrogen gas for 90 min. The mixture was diluted with ethanol (30 mL) and filtered through a 0.5-in. pad of Celite under suction. The solids were rinsed with ethanol (20 mL), and the combined filtrates were evaporated. The residue was treated with saturated aqueous $NaHCO_3$ solution (10 mL) and extracted with CH_2Cl_2 (2 × 15 mL). The combined extracts were dried (Na_2SO_4), and the solvent was evaporated to give α-methyl-γ-lactone **25** (10 mg, 100% yield): ^{1}H NMR ($CDCl_3$) δ 0.05–0.15 (m, 2 H, cyclopropyl CH_2), 0.45–0.55 (m, 2 H, cyclopropyl CH_2), 0.75–0.9 (m, 1 H, cyclopropyl CH), 1.25–1.4 (m, 1 H, C-8 H), 1.40 (d, *J* = 7.2 Hz, 3 H, C-4′ CH_3), 1.4–1.5 (m, 1 H, C-15 H), 1.5–1.6 (m, 1 H, C-8 H), 1.65–1.75 (m, 1 H, C-7 H), 1.8–1.95 (m, 1 H, C-7 H), 2.0–2.25 (m, 3 H, C-15 H, C-16 H, and C-3′ H), 2.35 (d, *J* = 6.6 Hz, 2 H, NCH_2-cyclopropyl), 2.35–2.45 (m, 1 H, C-3′ H), 2.5–2.65 (m, 2 H, C-10α H and C-16 H), 2.7–2.85 (m, 1 H, C-4′ H), 2.99 (d, *J* = 18.2 Hz, 1 H, C-10β H), 3.05 (d, *J* = 5.9 Hz, 1 H, C-9 H), 3.15–3.25 (m, 1 H, C-6 H), 3.65 (dd, *J* = 2.3 and 12.9 Hz, 1 H, C-1′ H), 3.71 (dd, *J* = 1.8 and 12.9 Hz, 1 H, C-1′ H), 4.42 (d, *J* = 6.5 Hz, 1 H, C-5 H), 4.45–4.55 (m, 1 H, C-2′ H), 6.53 (d, *J* = 8.1 Hz, 1 H, C-1 H), 6.73 (d, *J* = 8.1 Hz, 1 H, C-2 H); ^{13}C NMR ($CDCl_3$) δ 3.78, 3.91, 9.45, 15.51, 22.58, 24.29, 29.48, 30.39, 30.84, 35.53, 43.84, 47.66, 59.09, 62.21, 69.46, 70.07, 78.42, 84.39, 93.88, 117.43, 118.60, 123.37, 131.28, 140.17, 142.27, 181.50; FTIR (film) 3346 (OH), 1749, 1614, 1503 cm^{-1}; FAB MS $(M + 1)^+$ 456.

4,5α-Epoxy-3,14-dihydroxy-6β-[(2*S*,4*S*)-2-hydroxy-4-carboxy-*n*-pentoxy]-17-(cyclopropylmethyl)morphinan γ-Lactone (26). α-Methyl-γ-lactone **26** was prepared from α-methylene-γ-lactone **11** as described above for the preparation of **25** from **10**. α-Methyl-γ-lactone **26** was obtained (100% yield) as a viscous oil: ^{1}H NMR ($CDCl_3$) δ 0.05–0.2 (m, 2 H, cyclopropyl CH_2), 0.45–0.6 (m, 2 H, cyclopropyl CH_2), 0.75–0.9 (m, 1 H, cyclopropyl CH), 1.28 (d, *J* = 7.0 Hz, 3 H, C-4′ CH_3), 1.25–1.4 (m, 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), 1.67–1.8 (m, 2 H, C-7 H and C-3′ H), 1.8–2.0 (m, 1 H, C-7 H), 2.05–2.15 (m, 1 H, C-16 H), 2.15–2.3 (m, 1 H, C-15 H), 2.36 (d, *J* = 6.5 Hz, 2 H, NCH_2-cyclopropyl), 2.4–2.5 (m, 1 H, C-3′ H), 2.5–2.8 (m, 3 H, C-10α H, C-16 H, and C-4′ H), 3.00 (d, *J* = 18.2 Hz, 1 H, C-10β H), 3.08 (d, *J* = 5.8 Hz, 1 H, C-9 H), 3.2–3.3 (m, 1 H, C-6 H), 3.7–3.8 (m, 2 H, C-1′ CH_2), 4.54 (d, *J* = 6.5 Hz, 1 H, C-5 H), 4.5–4.6 (m, 1 H, C-2′ H), 6.55 (d, *J* = 8.2 Hz, 1 H, C-1 H), 6.71 (d, *J* = 8.0 Hz, 1 H, C-2 H); ^{13}C NMR ($CDCl_3$) δ 3.79, 3.92, 9.43, 15.26, 22.59, 23.75, 29,50, 30.48, 32.74, 35.25, 43.78, 47.80, 59.09, 62.14, 70.04, 70.94, 77.19, 81.67, 95.05, 116.98, 118.77, 124.40, 131.51, 139.30, 142.04, 179.40; FTIR (film) 3356 (OH), 1767, 1618, 1503 cm^{-1}; FAB MS $(M + 1)^+$ 456.

3-(Benzyloxy)-4,5α-epoxy-14-hydroxy-6β-(2-oxoethoxy)-17-(cyclopropylmethyl)morphinan (27). A solution of acetal **16** (300 mg, 0.55 mmol) in 10% aqueous oxalic acid (15 mL) was heated at 50 °C for 30 min. After cooling to ambient temperature, the solution was poured over a mixture of $NaHCO_3$ (3 g) and water (40 mL). The mixture was extracted with CH_2Cl_2 (3 × 20 mL). The combined extracts were dried (Na_2SO_4), and the volatiles were evaporated to give a mixture of aldehyde **27** and its hydrate (260 mg, 99% yield) as a viscous oil. ^{1}H NMR ($CDCl_3$) δ 0.05–0.15 (m, 2 H, cyclopropyl CH_2), 0.45–0.55 (m, 2 H, cyclopropyl CH_2), 0.75–0.9 (m, 1 H, cyclopropyl CH), 1.25–1.5 (m, 2 H, C-8 H and C-15 H), 1.6–1.7 (m, 1 H, C-8 H), 1.75–1.85 (m, 1 H, C-7 CH), 1.95–2.15 (m, 2 H, C-7 H and C-16 H), 2.24 (dt, *J* = 4.9 and 12.4 Hz, 1 H, C-15 H), 2.35 (d, *J* = 6.5 Hz, 2 H, NCH_2-cyclopropyl), 2.5–2.65 (m, 2 H, C-10α H and C-16 H), 3.01 (d, *J* = 18.5 Hz, 1 H, C-10β H), 3.08 (d, *J* = 5.9 Hz, 1 H, C-9 H), 3.25–3.35 (m, 1 H, C-6 H), 4.2–4.35 (m, 2 H, C-6 OCH_2), 4.61 (d, *J* = 6.3 Hz, 1 H, C-5 H), 5.1–5.2 (m, 2 H, benzylic CH_2), 5.28 (s, 2 H, OH), 6.57 (d, *J* = 8.2 Hz, 1 H, C-1 H), 6.75 (d, *J* = 8.2 Hz, 1 H, C-2 H), 7.2–7.5

(m, 5 H, phenyl), 9.74 (s, 0.5 H, CHO); ^{13}C NMR ($CDCl_3$) δ 3.79, 3.92, 9.43, 22.61, 23.93, 29.57, 30.57, 43.71, 47.63, 59.08, 62.07, 69.81, 71.73, 75.38, 81.80, 94.64, 116.80, 118.60, 125.65, 127.34 (2 carbons), 127.65, 128.25 (2 carbons), 132.06, 137.18, 142.40, 143.82, 201.11; FTIR (film) 3600–3200 (OH), 1732, 1634, 1606, 1497 cm^{-1}; FAB MS (M + 1)$^+$ 476.

N,N-Bis[2-[[3-(benzyloxy)-4,5α-epoxy-14-hydroxy-17-(cyclopropylmethyl)morphinan-6β-yl]oxy]ethyl]amine (28). To a solution of aldehyde **27** (530 mg, 1.12 mmol), ammonium acetate (704 mg, 11.2 mmol), and sodium cyanoborohydride (173 mg, 2.24 mmol) in methanol (7 mL) was added 3-Å molecular sieves and stirred for 42 h. The mixture was treated with saturated aqueous NH_4Cl solution (10 mL) and then with 37% aqueous NH_4OH solution (25 mL). After adding 25% ethanol–CH_2Cl_2 (50 mL), the mixture was filtered through a 0.5-in. pad of Celite under suction. The filtrate separated into two layers, and the aqueous layer was extracted with 25% ethanol–CH_2Cl_2 (30 mL). The combined organic layers were dried (Na_2SO_4), and the solvents were evaporated. The residue was purified by flash chromatography (42 g silica gel) eluting with ethyl acetate (250 mL) followed by 4% triethylamine–ethyl acetate (250 mL) to give dimer **28** (90 mg, 17% yield) as a viscous oil: $[\alpha]_D$ −125.5° (c = 1.00, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 0.05–0.2 (m, 4 H, cyclopropyl CH_2), 0.45–0.6 (m, 4 H, cyclopropyl CH_2), 0.75–0.9 (m, 2 H, cyclopropyl CH), 1.25–1.4 (m, 2 H, C-8 H), 1.4–1.5 (m, 2 H, C-15 H), 1.5–1.65 (m, 2 H, C-8 H), 1.7–1.8 (m, 2 H, C-7 CH), 1.8–2.0 (m, 2 H, C-7 H), 2.0–2.1 (m, 2 H, C-16 H), 2.15–2.3 (m, 2 H, C-15 H), 2.34 (d, J = 6.3 Hz, 4 H, NCH_2-cyclopropyl), 2.5–2.65 (m, 4 H, C-10α H and C-16 H), 2.7–2.9 (m, 4 H, C-2′ CH_2), 2.99 (d, J = 18.6 Hz, 2 H, C-10β H), 3.06 (d, J = 5.4 Hz, 2 H, C-9 H), 3.15–3.25 (m, 2 H, C-6 H), 3.55–3.7 (m, 2 H, C-1′ H), 3.7–3.8 (m, 2 H, C-1′ H), 4.54 (d, J = 6.3 Hz, 2 H, C-5 H), 5.0 (br s, 2 H, movable, C-14 H), 5.18 (s, 4 H, benzylic CH_2), 6.54 (d, J = 8.2 Hz, 2 H, C-1 H), 6.73 (d, J = 8.1 Hz, 2 H, C-2 H), 7.2–7.5 (m, 10 H, phenyl); ^{13}C NMR ($CDCl_3$) δ (2 carbons for each signal) 3.77, 3.90, 9.43, 22.62, 23.92, 29.54, 30.59, 43.77, 47.41, 49.44, 59.05, 62.14, 69.05, 69.88, 71.87, 80.59, 94.93, 117.20, 118.20, 125.67, 127.28 (4 carbons), 127.51, 128.14 (4 carbons), 132.36, 137.33, 142.22, 141.19; FTIR (film) 3600–3200 (OH and NH), 1633, 1605, 1495 cm^{-1}; FAB MS (M + 1)$^+$ 936.

3-(Benzyloxy)-4,5α-epoxy-14-hydroxy-6β-(2-oximidoethoxy)-17-(cyclopropylmethyl)morphinan (29). To a mixture of freshly prepared aldehyde **27** (260 mg, 0.55 mmol) and hydroxylamine hydrochloride (76 mg, 1.1 mmol) in methanol (6 mL) was added a solution of $NaHCO_3$ (140 mg, 1.7 mmol) in water (12 mL). After stirring for 2 h, the mixture was extracted with CH_2Cl_2 (3 × 17 mL). The combined extracts were dried ($MgSO_4$), and volatiles were evaporated to give oxime **29** (260 mg, 97% yield) as a glassy solid (mp 57–60 °C): $[\alpha]_D$ = −104.5° (c = 1.00, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 0.05–0.15 (m, 2 H, cyclopropyl CH_2), 0.45–0.55 (m, 2 H, cyclopropyl CH_2), 0.75–0.9 (m, 1 H, cyclopropyl CH), 1.25–1.4 (m, 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 CH), 1.85–2.0 (m, 1 H, C-7 H), 2.0–2.1 (m, 1 H, C-16 H), 2.15–2.3 (m, 1 H, C-15 H), 2.34 (d, J = 6.6 Hz, 2 H, NCH_2-cyclopropyl), 2.5–2.65 (m, 2 H, C-10α H and C-16 H), 3.00 (d, J = 18.6 Hz, 1 H, C-10β H), 3.07 (d, J = 5.4 Hz, 1 H, C-9 H), 3.15–3.3 (m, 1 H, C-6 H), 4.20 (dd, J = 5.6 and 12.9 Hz, 1 H, C-6 OCH), 4.29 (dd, J = 5.5 and 13.0 Hz, 1 H, C-6 OCH), 4.55 (d, J = 6.3 Hz, 1 H, C-5 H), 5.19 (s, 2 H, benzylic CH_2), 6.55 (d, J = 8.2 Hz, 1 H, C-1 H), 6.74 (d, J = 8.2 Hz, 1 H, C-2 H), 7.25–7.45 (m, 5 H, phenyl), 7.45–7.55 (m, 1 H, oxime CH); ^{13}C NMR ($CDCl_3$) δ 3.79, 3.92, 9.40, 22.63, 23.60 and 23.84 (1 carbon), 29.49, 30.55, 43.74, 47.54, 59.07, 62.08, 66.31, 69.96, 71.96, 80.49 and 81.10 (1 carbon), 94.37 and 94.60 (1 carbon), 117.26, 118.41, 125.65, 127.37 (2 carbons), 127.60, 128.19 (2 carbons), 132.14, 137.28, 142.30, 144.02, 148.52; FTIR (film) 3600–3000 (OH), 1633, 1605, 1496 cm^{-1}; FAB MS (M + 1)$^+$ 491.

3-(Benzyloxy)-4,5α-epoxy-14-hydroxy-6β-(2-aminoethoxy)-17-(cyclopropylmethyl)morphinan (30). To a mixture of $LiAlH_4$ (80 mg, 2.1 mmol) and THF (5 mL) heated at 65 °C was added a solution of oxime **29** (260 mg, 0.53 mmol) in THF (7 mL) over 2 min. The mixture was heated at 80 °C for 2 h. After cooling to ambient temperature, saturated aqueous Na_2SO_4 solution (7 mL) was added dropwise. The mixture was filtered through a 1-in. pad of Celite under suction, and then the solids were rinsed with THF (100 mL). Most of the THF was evaporated, and water (50 mL) was added. The mixture was extracted with CH_2Cl_2 (3 × 15 mL). The combined extracts were dried (Na_2SO_4), and solvents were evaporated. The residue was purified by flash chromatography (18 g silica gel) eluting with 4% triethylamine–CH_2Cl_2 (200 mL) and then with 4% triethylamine–2% methanol–CH_2Cl_2 (100 mL) to give amine **30** (200 mg, 79% yield) as a viscous oil: $[\alpha]_D$ = −112° (c = 1.00, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 0.05–0.15 (m, 2 H, cyclopropyl CH_2), 0.45–0.55 (m, 2 H, cyclopropyl CH_2), 0.75–0.9 (m, 1 H, cyclopropyl CH), 1.25–1.4 (m, 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 CH), 1.8–1.95 (m, 1 H, C-7 H), 2.0–2.15 (m, 1 H, C-16 H), 2.24 (dt, J = 4.9 and 12.3 Hz, 1 H, C-15 H), 2.35 (d, J = 6.6 Hz, 2 H, NCH_2-cyclopropyl), 2.5–2.65 (m, 2 H, C-10α H and C-16 H), 2.8–2.95 (m, 2 H, C-2′ CH_2), 3.01 (d, J = 18.2 Hz, 1 H, C-10β H), 3.08 (d, J = 5.6 Hz, 1 H, C-9 H), 3.15–3.3 (m, 1 H, C-6 H), 3.55–3.7 (m, 2 H, C-1′ CH_2), 4.55 (d, J = 6.3 Hz, 1 H, C-5 H), 5.18 (s, 2 H, benzylic CH_2), 6.56 (d, J = 8.2 Hz, 1 H, C-1 H), 6.75 (d, J = 8.2 Hz, 1 H, C-2 H); ^{13}C NMR ($CDCl_3$) δ 3.79, 3.94, 9.42, 22.64, 23.76, 29.52, 30.63, 41.60, 43.80, 47.44, 59.10, 62.14, 69.94, 70.18, 71.80, 80.52, 94.70, 116.80, 118.41, 125.69, 127.35 (2 carbons), 127.65, 128.26 (2 carbons), 132.22, 137.23, 142.34, 144.10; FTIR (film) 3600–3000 (NH and OH), 1634, 1606, 1498 cm^{-1}; FAB MS (M + 1)$^+$ 477.

4,5α-Epoxy-3,14-dihydroxy-6β-(2-aminoethoxy)-17-(cyclopropylmethyl)morphinan (31). To a solution of amine **30** (450 mg, 0.94 mmol) in 25% acetic acid–water (20 mL) was added 10% Pd on carbon (90 mg) and stirred under 1 atmosphere of hydrogen gas for 90 min. The mixture was diluted with water (50 mL) and filtered through a 0.5-in. pad of Celite under suction. The solids were rinsed with 10% acetic acid–water (30 mL). The combined filtrates were poured over solid $NaHCO_3$ (13 g) and then extracted with 25% ethanol–CH_2Cl_2 (5 × 40 mL). The combined extracts were dried ($MgSO_4$), and then the solvents were evaporated. The residue was triturated with ether (5 mL) to give phenol amine **31** (310 mg, 85% yield) as a beige powder (dec above 170 °C): $[\alpha]_D$ = −111° (c = 1.00, 43% methanol–CH_2Cl_2); ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 0.05–0.2 (m, 2 H, cyclopropyl CH_2), 0.45–0.6 (m, 2 H, cyclopropyl CH_2), 0.75–0.9 (m, 1 H, cyclopropyl CH), 1.3–1.5 (m, 2 H, C-8 H and C-15 H), 1.55–1.65 (m, 1 H, C-8 H), 1.7–1.95 (m, 2 H, C-7 CH_2), 2.05–2.25 (m, 2 H, C-15 H and C-16 H), 2.37 (d, J = 6.3 Hz, 2 H, NCH_2-cyclopropyl), 2.58 (dd, J = 5.8 and 18.6 Hz, 1 H, C-10α H), 2.6–2.7 (m, 1 H, C-16 H), 2.75–2.9 (m, 2 H, C-2′ CH_2), 3.02 (d, J = 18.2 Hz, 1 H, C-10β H), 3.08 (d, J = 5.5 Hz, 1 H, C-9 H), 3.15–3.3 (m, 1 H, C-6 H), 3.5–3.75 (m, 2 H, C-1′ CH_2), 4.46 (d, J = 6.3 Hz, 1 H, C-5 H), 6.55 (d, J = 8.1 Hz, 1 H, C-1 H), 6.68 (d, J = 8.1 Hz, 1 H, C-2 H); ^{13}C NMR ($CDCl_3$) δ 3.78, 3.93, 9.47, 22.59, 23.46, 29.71, 30.49, 40.82, 43.92, 47.61, 59.11, 62.22, 70.11 (2 carbons), 80.59, 93.89, 117.98, 118.80, 122.84, 131.32, 140.94, 142.38; FTIR (KBr) 3600–2400 (NH and OH), 1610 cm^{-1}; FAB MS (M + 1)$^+$ 387.

4,5α-Epoxy-3,14-dihydroxy-6β-(2-isothiocyanatoethoxy)-17-(cyclopropylmethyl)morphinan (12). To a solution of amine **31** (34 mg, 0.09 mmol) in CH_2Cl_2 (2 mL) was added $NaHCO_3$ (16 mg, 0.019 mmol) followed by thiophosgene (7 μL, 0.09 mmol) dropwise. After stirring for 45 min, the mixture was treated with half-saturated aqueous $NaHCO_3$ solution (10 mL). The mixture was extracted with CH_2Cl_2 (3 × 8 mL). The combined extracts were dried (Na_2SO_4), and the volatiles were evaporated. The residue was purified by flash chromatography (2.5 g silica gel) eluting with ether (50 mL) to give isothiocyanate **12** (20 mg, 53% yield) as a white solid foam: $[\alpha]_D$ = −60.5° (c = 0.33, CH_2Cl_2). The HCl salt was recrystallized (isopropanol–ether) (dec above 190 °C): ^{1}H NMR (free amine) ($CDCl_3$) δ 0.0–0.2 (m, 2 H, cyclopropyl CH_2), 0.45–0.6 (m, 2 H, cyclopropyl CH_2), 0.75–0.9 (m, 1 H, cyclopropyl CH), 1.34 (dt, J = 3.0 and 13.5 Hz, 1 H, C-8 H), 1.4–1.5 (m, 1 H, C-15 H), 1.6–1.7 (m, 1 H, C-8 H), 1.75–1.85 (m, 1 H, C-7 H), 1.95 (dq, J = 2.6 and 12.9 Hz, 1 H, C-7 H), 2.10 (dt, J = 3.5 and 11.9 Hz, 1 H, C-16 H), 2.25 (dt, J = 4.8 and 12.3 Hz, 1 H, C-15 H), 2.37 (d, J = 6.4 Hz, 2 H, NCH_2-cyclopropyl), 2.5–2.7 (m, 2 H, C-10α H and C-16 H), 3.02 (d, J = 18.5 Hz, 1 H, C-10β H), 3.09 (d, J = 5.5 Hz, 1 H, C-9 H), 3.2–3.3 (m, 1 H, C-6 H), [3.66 (t, J = 5.0 Hz, 2 H) and 3.78 (t, J = 4.9 Hz, 2 H) C-1′ CH_2 and C-2′ CH_2], 4.56 (d, J = 6.4 Hz, 1 H, C-5 H), 6.57 (d, J = 8.2 Hz, 1 H, C-1 H), 6.72 (d, J = 8.0 Hz, 1 H, C-2 H); ^{13}C NMR ($CDCl_3$) δ 3.69, 3.81, 9.26, 22.48, 23.61, 29.37, 30.32, 43.66, 45.29, 47.79, 58.96, 62.02, 67.57, 69.92, 81.38, 94.75, 116.54, 118.69, 124.26, 131.33,

131.95, 139.11, 141.75; FTIR (film) 3600–3000 (OH), 2108 (NCS), 1621, 1502 cm^{-1}; HRFABMS $(M + 1)^+$ calcd for $C_{23}H_{29}N_2O_4S$ 429.1848, obsd 429.1836. Anal. ($C_{23}H_{28}N_2O_4S \cdot HCl$) C, H, N.

Opioid Receptor Binding. Radioreceptor binding assays were carried out as described previously using guinea pig brain homogenate.[12] The radioligand used was [^{3}H]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 [^{3}H]DAGO [D-Ala^2-NMePhe^4-Gly-ol^5-enkephalin] (Amersham) (60.0 Ci/mmol) was used. For κ-binding sites, 0.5 nM [^{3}H]bremazocine was used in the presence of 100 nM unlabeled DAGO and 100 nM unlabeled DPDPE to block μ and δ sites, respectively. For δ-binding sites 1.0 nM [^{3}H]DPDPE [D-Pen^2-D-Pen^5-enkephalin] (New England Nuclear) (34.3 Ci/mmol) was used. Nonspecific binding was determined using naloxone (10 μM). Stock solutions of each test compound were prepared immediately prior to the assay by dissolving the free amine in 50% acetic acid (200 μL) and then were serially diluting with water. Nine concentrations of each ligand to be tested were examined in competition experiments with each radioligand. The samples were incubated in 50 mM Tris-HCl buffer (pH 7.4) at 25 °C for 1 h and then rapidly filtered through Whatman GF/B filters, which were rinsed twice 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.

Irreversibility and Protection Studies. The studies were carried out as described previously.[12] Membrane preparations were incubated with drug to be tested for 1 h at 25 °C. For protection studies, naloxone was added at a concentration of 1 μM (recovery was checked with naloxone alone). After incubation, the samples were diluted 4-fold with buffer and centrifuged for 15 min at 20000*g*. The supernatant was removed, and the pellet was resuspended in 3 times the original volume of buffer and incubated at 37 °C for 15 min, centrifuged again, and resuspended in the original volume of buffer. A binding assay using [^{3}H]-bremazocine, [^{3}H]DAGO, or [^{3}H]DPDPE was carried out as described above.

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Ring Substituted and Other Conformationally Constrained Tyrosine Analogues of [D-Pen^2,D-Pen^5]enkephalin with δ Opioid Receptor Selectivity[1]

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The conformationally restricted, cyclic disulfide-containing δ opioid receptor selective enkephalin analogue [D-Pen^2,D-Pen^5]enkephalin (DPDPE) was modified by 2′ (CH_3) and 3′ (I, OCH_3, NO_2, NH_2) ring substitutions and by β-methyl conformationally constrained β-methyltyrosine derivatives in the 1 position. The potency and selectivity of these analogues were evaluated by bioassay in the mouse vas deference (MVD, δ receptor assay) and guinea pig ileum (GPI, μ receptor assay) assays and by radioreceptor binding assays in the rat brain using [^{3}H]CTOP (μ ligand) and [^{3}H][*p*-ClPhe^4]DPDPE (δ ligand). The analogues showed highly variable potencies in the binding assays and in the bioassays. Aromatic ring substituents with positive Hammett constants had decreased potency, while substituents with negative Hammett constants has increased potency for the opioid receptor. The most potent and most selective compound based on the binding was [2′-MeTyr^1]DPDPE (IC_{50} = 0.89 nM and selectivity ratio 1310 in the binding assays). The 6-hydroxy-2-aminotetralin-2-carboxylic acid-containing analogue, [Hat^1]DPDPE, also was highly potent and selective in both assays, demonstrating that significant modifications of tyrosine in enkephalins are possible with maintenance of high potency and δ opioid receptor selectivity. Of the β-methyl-substituted Tyr^1 analogues, [(2*S*,3*R*)-β-MeTyr^1]DPDPE was the most potent and δ receptor selective. The results with substitution of β-MeTyr or Hat instead of Tyr also demonstrate that topographical modification in a conformationally restricted ligand can significantly modulate both potency and receptor selectivity of peptide ligands that have multiple sites of biological activity.

Since the discovery of enkephalins in 1975[2] numerous studies have been made to elucidate the structure–activity relationship of enkephalins and other opioid peptides. Although several thousand peptide analogues have been synthesized (for reviews[3-6]), highly receptor selective *and* potent peptide analogues only have been obtained during the last 10 years which confirm the postulate of a multiplicity of opioid receptors.[7,8]

One approach for the design of highly selective ligands involves the incorporation of conformational constraints.[9] In our laboratory, this approach has led to the development of highly selective ligands for both μ and δ opioid receptors.[10,11] One of the most selective analogues for the δ opioid receptors was the cyclic analogue [D-Pen^2,D-

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(1) Symbols and abbreviations are in accord with the recommendations of the IUPAC–IUB Commission on Nomenclature (*J. Biol. Chem.* 1972, *247*, 977–983). All optically active amino acids are of L variety unless otherwise noted. Other abbreviations used are β-MeTyr, β-methyltyrosine; Hat, 6-hydroxy-2-aminotetralin-2-carboxylic acid; Pen, penicillamine; CTP, D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH_2; DPDPE, [D-Pen^2,D-Pen^5]enkephalin; CTOP, D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2; [*p*-ClPhe^4]DPDPE, Tyr-D-Pen-Gly-*p*-ClPhe-D-Pen; GPI, guinea pig ileum; MVD, mouse vas deferens; FAB-MS, fast atom bombardment mass spectrometry; TFA, trifluoroacetic acid; HPLC, high-performance liquid chromatography; D-Tic, tetrahydroisoquinoline-2-carboxylic acid; Ad, adamantyl; SPPS, solid-phase peptide synthesis.

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