Table III. Crystallographic Data for 8a and Structure Determination Details

formula, M	$C_{27}H_{27}NO_6$, 461.52
crystal system, space group	orthorhombic, $P2_12_12_1$ (No. 19)
unit cell dimensions	
a	8.222 (2) Å
b	12.568 (3) Å
c	22.736 (5) Å
packing: V, Z, $F(000)$	2349 (1) Å^3, 4, 976
D_{calcd}, D_{exptl}	1.305, 1.309 g cm^{-3}
reflctns: measd, indep (int R)	5806, 2903 Fp (0.0616)
reflctns used, limit	2276 (1138 Fp) with $I > 1\sigma(I)$
var, ratio Fp/var, last shifts	318, 3.6, <0.03 σ
final R, R_w	0.0908, 0.0501
weighting scheme w^{-1}	$\sigma^2(F) + 0.000179F^2$

chain). Anal. ($C_{27}H_{27}NO_6$) C, H, N.

(*S*,*S*)-(−)-2-Methoxy-2-phenylethyl 2-Methyl-4-[3,4-(methylenedioxy)phenyl]-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (8b). 8b was prepared analogously to 8a above from 1.0 g (4.2 mmol) of **5b**, 0.64 g (4.2 mmol) of **7**, and 0.48 g (4.2 mmol) of **6** in 80 mL of ethanol with addition of 4 mL of 25% ammonia solution and 2 mL of concentrated acetic acid. Colorless crystals from methanol with mp 215 °C. Yield: 0.3 g (15%). $[\alpha]^{20}_D$: −60.5° (c = 1, DMSO). Anal. ($C_{27}H_{27}NO_6$) C, H, N.

(*R*)-(+)-Methyl 2-Methyl-4-[3,4-(methylenedioxy)phenyl]-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (9a). 9a was obtained by dissolving 0.8 g (1.7 mmol) of 8a in 80 mL of methanol, adding 1.0 g (44 mmol) of metallic sodium, and heating the resultant mixture to boiling for 5.5 h. The mother liquor was then concentrated to half its original volume, treated with dilute HCl, and diluted with 70 mL of water. After being allowed to cool, the mixture was extracted repeatedly with dichloromethane, and the combined dichloromethane phases were combined and concentrated to produce a yellowish oil. Crystallization from methanol produced colorless needles with mp 250 °C. Yield: 0.4 g (67%). $[\alpha]^{20}_D$: +148.6° (c = 1.0, DMSO). Anal. ($C_{19}H_{19}NO_5$) C, H, N.

(*S*)-(−)-Methyl 2-Methyl-4-[3,4-(methylenedioxy)phenyl]-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (9b). 9b was prepared analogously to 9a above from 0.28 g (0.6 mmol) of 8b and 0.35 g (15 mmol) of sodium in 50 mL of methanol with a reaction time of 6 h. Workup as described for 9a furnished colorless needles of mp 249 °C. Yield: 0.1 g (49%). $[\alpha]^{20}_D$: −151.7° (c = 1, DMSO). Anal. ($C_{19}H_{19}NO_5$) C, H, N.

3. X-ray Structure Analysis of 8a. Crystal data as well as details of intensity data collection and refinement are given in Table III. The density was obtained from neutral buoyancy (Thoulet solution). The crystal was fixed by glue on a glass fiber and sealed in a glass capillary tube. The quality and symmetry of the crystal was examined by Weissenberg exposures. Integrated intensities were measured by means of $\omega/2\theta$ scans on a CAD4 diffractometer (Enraf-Nonius).

The structure was solved by direct methods (SHELX-86). The refinement (anisotropic temperature factors for all non-hydrogen atoms except the p-phenyl atom C(344)) was by full matrix. Hydrogen positions were considered as riding on carbon atoms except the hydrogen atoms at nitrogen and at the two chiral centers: the latter three hydrogen atoms were refined with isotropic temperature motion. Afterwards, one of the methylene groups of the cyclohexenone rings exhibited a strong thermal motion and a grossly distorted geometry. By means of several difference Fourier syntheses it was possible to split this methylene group in two positions below and above the ring). The ratio of 67/33 for these two positions was chosen by attaining the same isotropic motion in the refinement.

In spite of this consideration and in spite of a careful and long measurement of the Friedel pairs, it was not possible to determine the absolute configuration of 8a without any additional information. This additional information arose from the mandelic acid (R)-center of 8a, and by choosing this center, the second center came out with (R)-configuration as well.

The final refinement came out with a good convergence and an even distribution of the variances. Besides several local written routines, local versions of SHELX-76 and SHELX-86 were used for the calculations and a local version of PLUTO-78 was used for the figure (HB-DPS-8/70 equipment at Zentrum für Datenverarbeitung, Universität Mainz).

Registry No. (*RS*)-1, 611-72-3; 1a, 611-71-2; 1b, 17199-29-0; (*RS*)-2, 7021-09-2; 2a, 3966-32-3; 2b, 26164-26-1; 3a, 17628-72-7; 3b, 66051-01-2; 4, 674-82-8; 5a, 104451-36-7; 5b, 77940-85-3; 6, 504-02-9; 7, 120-57-0; 8a, 139758-84-2; 8b, 139758-85-3; 9a, 139758-86-4; 9b, 139758-87-5.

Supplementary Material Available: Table I with noteworthy bond lengths and angles, Table II with fractional atomic coordinates and equivalent isottropic thermal parameters, and tables with anisotropic thermal parameters, H atom coordinates, and a complete listing of bond distances and angles (5 pages); observed and calculated structure factor amplitudes (13 pages). Ordering information is given on any current masthead page.

Selective Reversible and Irreversible Ligands for the κ Opioid Receptor

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Received January 22, 1992

(±)-(5β,7α,8β)-3,4-Dichloro-*N*-methyl-*N*-[3-methylene-2-oxo-8-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-7-yl]benzeneacetamide (**14**) and its (5α,7α,8β) diastereomer **15** have been synthesized from 1,4-cyclohexanedione monoethylene ketal (**1**) in 10 steps. Compound **14**, which we have designated SMBU-1, was found to bind with moderate affinity (K_i = 109 nM) and good selectivity (μ/κ = 29) to the κ opioid receptor, while **15** was only 1/10 as potent as a κ ligand. Preincubation of brain membranes with **14** resulted in wash-resistant inhibition of κ-receptor binding (69 ± 6% of control at 10^{-6} M). The ketone precursor *trans*-*N*-methyl-*N*-[5-oxo-2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide (**12**) showed a higher κ-affinity (K_i = 78 nM) and a much higher κ-selectivity (μ/κ = 166) than **14**. Compound **10**, the ethylene ketal precursor of **12**, exhibited a similar receptor binding profile to **14**, with increased κ-selectivity (μ/κ = 55), while ketal **11**, being a regioisomer of **10** and an oxygen isostere of the κ-selective analgesic spiradoline (U-62,066), demonstrated the highest κ-affinity (K_i = 1.5 nM) and κ-selectivity (μ/κ = 468) observed in this series.

Since the initial proposals of multiple opioid receptors,[1,2] the existence of at least three different types of opioid receptor, namely, μ, κ, and δ, has been well established.[3-5] In recent years, the discovery of irreversible opioid ligands,

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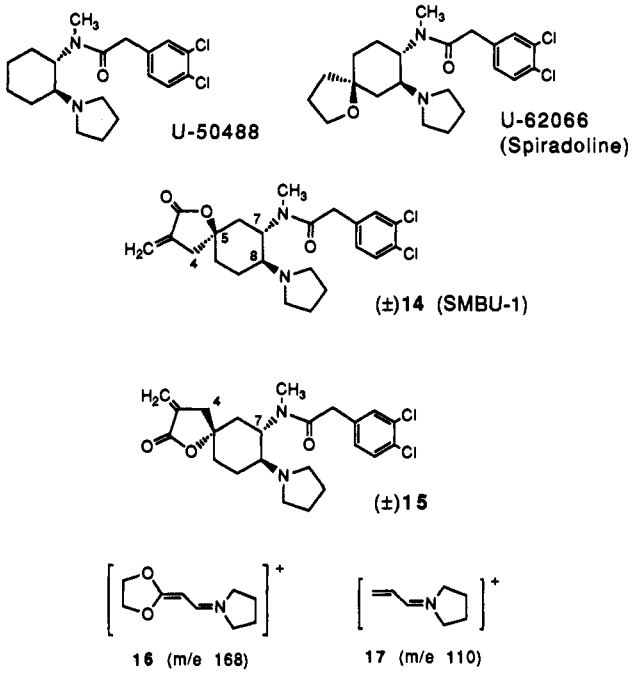
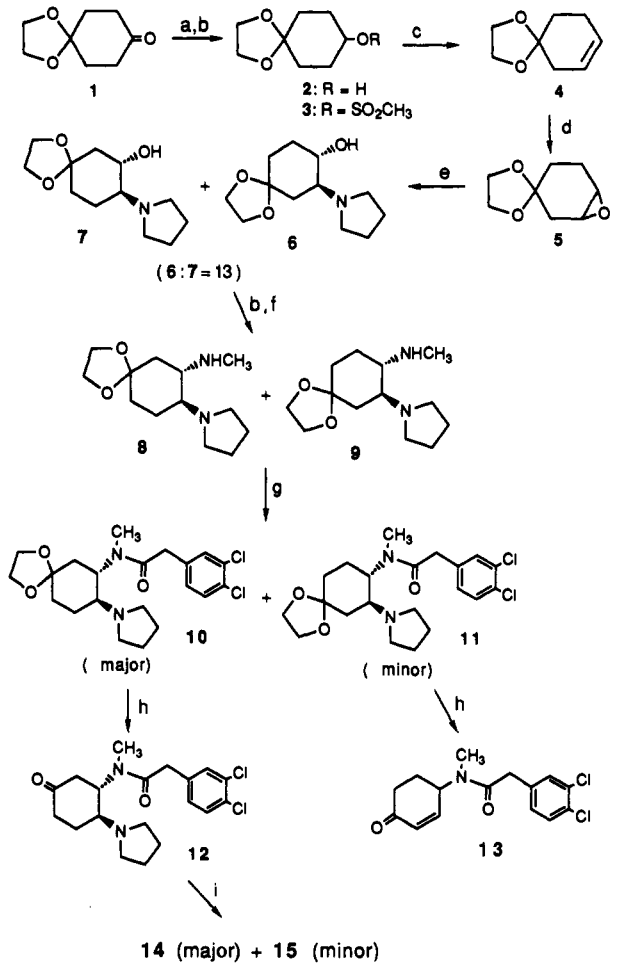
0022-2623/92/1835-2243$03.00/0

Chart I



especially those which possess selective binding affinity toward one of the three receptors, has contributed significantly to the characterization and isolation of the different opioid receptors. Irreversible opioid ligands described in the literature include the μ-selective 2-(*p*-ethoxybenzyl)-1-[(diethylamino)ethyl]-5-isothiocyanatobenzimidazole hydrochloride (BIT),[6,7] β-funaltrexamine (β-FNA),[8] and the α-methylene-γ-lactone derivative of etorphine;[9] the δ-selective *N*-phenyl-*N*-[1-[2-(*p*-isothiocyanatophenyl)ethyl]-4-piperidinyl]propanamide hydrochloride (FIT)[6,7] and (+)-*cis*-methylfentanyl isothiocyanate (SUPERFIT);[10] the slightly μ-selective 6-desoxy-6-spiro-α-methylene-γ-butyrolactone derivative of naltrexone;[11]

Scheme I[a]



[a] Reagents and conditions: (a) $NaBH_4$, CH_3OH, 0 °C; (b) CH_3SO_2Cl, Et_3N, CH_2Cl_2, 0 °C; (c) DBU, 100 °C; (d) *m*-CPBA, CH_2Cl_2, 0 °C; (e) pyrrolidine, H_2O, 80 °C; (f) CH_3NH_2/CH_3OH, THF, 75 °C; (g) $Cl_2C_6H_5CH_2C(O)Cl$, Et_3N, CH_2Cl_2, 0 °C; (h) 2 N H_2SO_4, CH_3OH, 85 °C; (i) ethyl α-(bromomethyl)acrylate, Zn, THF, 70–75 °C.

and the nonselective β-chlornaltrexamine (β-CNA).[12] Very recently (1*S*,2*S*)-*trans*-2-isothiocyanato-4,5-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide (UPHIT) and related compounds were reported to be selective and irreversible inhibitors of κ receptors.[13,14]

In this paper we describe the synthesis of (±)-(5β,7α,8β)-3,4-dichloro-*N*-methyl-*N*-[3-methylene-2-oxo-

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8-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-7-yl]benzeneacetamide (14) (see Chart I), which we have designated SMBU-1 on the basis of its being a spiro-α-methylene-γ-butyrolactone derivative of the κ-selective opioid U-50,488,[15] and receptor binding data supporting its being a selective and irreversible ligand for the κ opioid receptor. The design rationale for 14 was based on the structure of U-62,066 (Spiradoline),[16] a close analog of U-50,488. We reasoned that replacing the spirotetrahydrofurano group in U-62,066 with a sterically similar spiro-α-methylene-γ-lactone moiety would very likely result in compounds which retain the opioid binding affinity of U-62,066, while the butyrolactone moiety, being a Michael acceptor, might have a chance to form covalent bonds with nearby receptor nucleophiles such as a sulfhydryl (SH) group.

Results and Discussion

Chemistry. Compound 14 and its diastereomer 15 were synthesized according to Scheme I, 1,4-cyclohexanedione monoethylene ketal (1) being the starting material. Thus, 1 was reduced with $NaBH_4$ to give alcohol 2. 2 was converted to mesylate 3, followed by elimination effected with DBU to give olefin 4. Treatment of 4 with *m*-CPBA provided epoxide 5. Epoxide ring-opening of 5 with pyrrolidine resulted in a mixture of *trans*-amino alcohols (6 and 7), which could be separated by column chromatography (6:7 = 13:1). The regioisomeric 6 and 7 were best differentiated by EI mass spectrometry. Thus compound 6 gave the azadienium ion 16 as the base peak (*m/e* 168), while 7 gave a high-intensity peak of ion 17 (m/e 110). The separation of 6 and 7 was found to be not necessary because either 6 or 7 could be treated with mesyl chloride followed by methylamine (steps b and f, Scheme I) to provide the same mixture of regioisomeric *trans*-diamines 8 and 9. Apparently, the above substitution reaction proceeds via the intermediate aziridinium ion formed through intramolecular displacement of the mesyl group by the neighboring pyrrolidine nitrogen. Such anchimeric assistance also renders the resulting diamines to have the trans stereochemistry. 8 and 9, without further separation, were then acylated with 3,4-dichlorophenylacetyl chloride to give a mixture of amides (10 and 11), which was easily separated by flash column chromatography. The major isomer 10 was smoothly deprotected by treatment with dilute H_2SO_4(aq) to give the corresponding ketone 12, while subjection of the minor isomer 11 to the same or other acidic conditions brought about, in addition to ketal hydrolysis, the undesired elimination reaction to give enone 13 as the only identifiable product. Finally, alkylation of 12 with the Reformatsky reagent prepared from ethyl α-(bromomethyl)acrylate[11,17] afforded the desired spiro-α-methylene-γ-lactone derivatives 14 and 15 in a ratio of 2.9 to 1 (cf. Experimental Section). The relative stereochemistry of 14 and 15 was determined by NMR NOESY experiments, which showed strong dipolar coupling (NOE) between H-4 (allylic) and H-7 in 15, thus establishing the spatial proximity or the cis diaxial relationship between C-4 and H-7 in 15; while no NOE was observed between H-4 and H-7 in the major isomer 14. The above assignment of stereochemistry by NMR is in agreement with the general observation that bulky nucleophiles such as the Reformatsky reagent derived from Zn and ethyl α-(bromomethyl)acrylate would react with cyclohexanones, e.g., compound 12, preferentially from the equatorial side.[18]

Table I. Opioid Receptor Binding Affinity [K_i (nM)][a]

compound	μ	κ	δ	μ/κ
14	3150 ± 175	109 ± 8	9510 ± 1060	29
15	2610 ± 899	1100 ± 62	12300 ± 2670	2.4
10	5210 ± 760	94 ± 27	17500 ± 3820	55
11	702 ± 13	1.5 ± 0.1	1640 ± 520	468
12	13000 ± 860	78 ± 9	>100000	166
U-62,066	252 ± 4	8.6 ± 1.4	9400 ± 980	29
U-50,488	825 ± 219	15 ± 1	>10000	55

[a] Data represents the mean ± SEM of 3 experiments each performed in duplicate.

Table II. Wash-Resistant Binding of 14 and 15 to Opioid Receptors in Guinea Pig Brain Membranes[a]

	% of control		
pretreatment	κ	μ	δ
10^{-7} M 14	92 ± 3	ND[b]	ND
10^{-7} M 15	86 ± 7	89 ± 3	95 ± 3
5×10^{-7} M 14	73 ± 3[c]	ND	ND
10^{-6} M 14	69 ± 6[c]	100 ± 1	100 ± 2
10^{-6} M 15	93 ± 2	83 ± 5	103 ± 2
U-50,488	109 ± 3	91 ± 6	ND
10^{-5} M 14	49 ± 5[d]	73 ± 2[c]	97 ± 1
10^{-5} M 15	74 ± 2[c]	64 ± 2[c]	81 ± 5
U-50,488	105 ± 3	101 ± 1	91 ± 6
10^{-4} M 14	13 ± 4[d]	24 ± 1[d]	66 ± 4[c]
10^{-4} M 15	44 ± 3[d]	36 ± 2[d]	51 ± 2[d]
U-50,488	38 ± 3[d]	78 ± 3[c]	96 ± 7

[a] Data represent binding remaining after preincubation with 14 or 15 followed by four washes. Data represent the mean ± SEM of 3 experiments each performed in duplicate. [b] ND = not determined. [c] $p < 0.05$. [d] $p < 0.01$ (Student *t* test).

Opioid Receptor Binding. The opioid receptor binding affinities (K_i's in nM) of the target compounds 14 and 15 and key intermediates 10–12 are listed in Table I, and those of U-50,488 and U-62,066 are also included for comparison. Numbers in the last column (μ/κ's) are indicators of κ-selectivity. As compared to U-62,066, 14 displayed a 10-fold reduction in κ-affinity and identical κ-selectivity (μ/κ = 29). Compound 15, being diasteromeric to 14 with respect to the spiro C-5, showed further reduction in κ-affinity and virtual loss of κ-selectivity. Ketone 12, the synthetic precursor of 14, showed a higher κ-affinity and a significantly higher κ-selectivity (μ/κ = 166) than 14. Compound 10, the ethylene ketal precursor of 12, gave a receptor binding profile very similar to that of 14, but with increased κ-selectivity (μ/κ = 55). Compound 11, being a regioisomer of 10 and an oxygen analog of U-62,066, demonstrated the highest κ-affinity (K_i = 1.5 nM) and κ-selectivity (μ/κ = 468) observed in this series of compounds. We have also examined the irreversibility of the binding of 14 and 15 to different opioid receptors, with U-50,488 included as a control. It was observed that preincubation of guinea pig brain membranes with 10^{-6} M 14 resulted in a wash-resistant reduction of κ binding to 69% of the control value (Table II), while 15 only inhibits κ and μ binding nonselectively at higher concentrations ($\geq 10^{-5}$ M). At higher concentrations, 14 also displayed wash-resistant binding to both μ and δ receptors. However, the order of potency of 14 as an irreversible inhibitor at the κ, μ, and δ receptors paralleled its potency for displacement of radioligands for binding to these receptors.

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At a high concentration of 10^{-4} M, significant inhibition of κ and μ binding by U-50,488 was also observed in this experiment. But we think the observed inhibition by U-50,488 is due to insufficient washing and time for complete dissociation after treatment of brain membranes with such high concentrations of the agent because we observed less inhibition after four washes than after three washes, while the inhibition of κ binding by 14 after four washes was the same as after three washes.

Conclusion

Compound 14 (SMBU-1) has been synthesized and found to be a selective and irreversible (wash-resistant) ligand at the κ opioid receptor. The reduced κ-affinity of 14 as compared to that of U-62,066 may be due to its less favored regiochemistry since the spiro-γ-lactone moiety in 14 is attached to C-5 of its cyclohexane ring instead of C-4, where the spirotetrahydrofurano group of U-62,066 is located. Ketal 11, which can be viewed as an oxygen analog of U-62,066, was found to possess extremely high affinity and selectivity toward the κ receptor. Unfortunately, compound 11 could not be converted to the desired regioisomers of 14 by the current method, and alternative syntheses have to be sought. It is likely that 14 and related compounds may complement other irreversible opioid ligands as tools in the study of opioid receptors.

Experimental Section

Synthesis. Melting points were taken in a capillary tube by using a Yamato MP-21 melting point apparatus and are uncorrected. IR spectra were determined with a Perkin-Elmer 1760-X FT-IR spectrometer. NMR spectra were recorded on a Bruker AM-300 or AM-80 spectrometer; chemical shifts were recorded in parts per million downfield from Me_4Si. 2D NOESY spectra were obtained on a Bruker AM-500 spectrometer. Mass spectra were recorded on a JEOL JMS-D300 mass spectrometer; HRMS was obtained with a JEOL JMS-HX110 spectrometer. Elemental analyses were performed with a Perkin-Elmer 240C instrument. TLC was performed on Merck (Art. 5717) silica gel plates and visualized with UV light (254 nm) or upon heating after treatment with 2% phosphomolybdic acid in ethanol. Flash column chromatography was performed with Merck 40–63-μm silica gel. Reagent-grade THF was distilled from sodium benzophenone prior to use. Other anhydrous solvents were distilled from CaH_2 and stored over 4-Å molecular sieves until use.

(A) 1,4-Dioxaspiro[4,5]decan-8-ol (2). To a stirred solution of 1,4-cyclohexanedione monoethylene ketal (25 g, 160 mmol) in CH_3OH (150 mL) was added $NaBH_4$ (12.1 g, 320 mmol) in portions. The resulting mixture was stirred for 2.5 h, and the methanol was evaporated. To the residue was added dilute NaOH(aq), followed by extraction with $CHCl_3$/2-propanol = 4:1. The combined organic extracts were washed with brine, dried with $MgSO_4$, and evaporated to give 2 as an oil (25.2 g, quantitative): IR (neat) 3400, 2950–2890, 1100 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.90 (m, 4 H), 3.75 (m, 1 H), 1.80 (m, 4 H), 1.55 (m, 4 H); MS (EI) m/e 158 (M^+), 99 (base peak), 86; HRMS m/e (M^+) calcd 158.0943, obsd 158.0947.

(B) 1,4-Dioxaspiro[4,5]decan-8-yl Methanesulfonate (3). To a stirred solution of 2 (25.4 g, 160.5 mmol) and Et_3N (32 mL, 224 mmol) in dry CH_2Cl_2 (250 mL) at 0 °C was added slowly CH_3SO_2Cl (15 mL, 190 mmol). The mixture was stirred for 2.5 h and treated with saturated $NaHCO_3$(aq). The organic layer was separated and the aqueous layer extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried ($MgSO_4$), and evaporated to give 3 as a yellow solid (39.2 g), which was crystallized from n-hexane to give white crystals (32.2 g, 90%): mp 61–63 °C; IR (KBr) 2970–2900, 1350, 1180, 1120 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.76 (m, 1 H), 3.88 (m, 4 H), 2.96 (s, 3 H), 2.0–1.5 (m, 8 H); MS m/e 236 (M^+), 141, 140, 129, 125, 99 (base peak); HRMS m/e (M^+) calcd 236.0718, obsd 236.0717.

(C) 1,4-Dioxaspiro[4,5]dec-7-ene (4). A stirred mixture of 3 (37.9 g, 160.5 mmol) and 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU, 39.0 g, 256 mmol) was heated at 100–110 °C under N_2 for 26 h. The mixture was then cooled, treated with brine, and extracted with ether. The combined ether extracts were washed with brine, dried ($MgSO_4$), and evaporated to give 4 as an oil (21.4 g): IR (neat) 3028, 2950–2882, 1653 (st, C=C), 1115 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.59 (m, 2 H), 3.90 (s, 4 H), 2.19 (m, 4 H), 1.70 (m, 2 H); MS m/e 140 (M^+), 99 (base peak), 86.

(D) (±)-1,4-Dioxaspiro[4,5]dec-7-ene Oxide (5). To a stirred solution of 4 (21.2 g, 151 mmol) in CH_2Cl_2 (400 mL) at 0 °C was added m-chloroperbenzoic acid (39.0 g, 227 mmol). The mixture was stirred for 4 h, treated with 10% Na_2SO_3 (180 mL), and stirred for another 1 h. The organic layer was separated, washed with Na_2CO_3(aq) and H_2O, dried ($MgSO_4$), and evaporated to give 5 as an oil (22.5 g), which could be purified by Kugel-rohr distillation: IR (neat) 2962–2884, 1110 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.85 (m, 4 H), 3.09 (t, 2 H), 2.1–1.4 (m, 6 H); MS m/e 156 (M^+), 139, 111, 99 (base peak), 86.

(E) (±)-*trans*-7-(1-Pyrrolidinyl)-1,4-dioxaspiro[4,5]decan-8-ol (6) and (±)-*trans*-8-(1-Pyrrolidinyl)-1,4-dioxaspiro[4,5]decan-7-ol (7). A mixture of 5 (22.5 g, 144 mmol), pyrrolidine (18 mL, 216 mmol), and H_2O (13 mL) was heated in a sealed vessel at 80–84 °C for 18 h. The mixture was then cooled, treated with H_2O, and extracted with CH_2Cl_2. The combined CH_2Cl_2 extracts were washed with H_2O and brine, dried ($MgSO_4$), and evaproated to give a crude mixture of 6 and 7 (33.15 g). A portion of the mixture (1.1 g) was separated by flash chromatography (silica gel; 10–15% CH_3OH and 1% NH_4OH in CH_2Cl_2) to give 6 (0.91 g, 82.7%) and 7 (0.07 g, 6.4%). 6: brownish crystals, mp 47–51 °C; IR (Nujol) 3412, 2954–2819, 1081 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.75 (m, 4 H), 3.65 (s, br, 1 H), 3.20 (dt, J = 10.1 and 4.4 Hz, 1 H), 2.60 (td, J = 10.1 and 3.6 Hz, 1 H), 2.55–2.36 (m, 4 H), 1.84–1.28 (m, 10 H); MS m/e 227 (M^+), 168 (base peak), 113, 97, 86; ^{13}C NMR (20 MHz, $CDCl_3$) δ 109.09, 69.68, 64.15, 64.00, 61.65, 46.96, 32.40, 30.09, 28.83, 23.46; HRMS m/e (M^+) calcd 227.1521, obsd 227.1520. 7: oil; ^1H NMR ($CDCl_3$) δ 3.88 (m, 4 H), 3.80 (br, 1 H), 3.57 (td, J = 9.4 and 4.4 Hz, 1 H), 2.65–2.41 (m, 5 H), 2.15 (m, 1 H), 1.79–1.20 (m, 9 H); MS m/e 227 (M^+), 196, 182, 168, 117 (base peak), 110, 97; HRMS m/e (M^+) calcd 227.1521, obsd 227.1521.

(F) (±)-*trans*-*N*-Methyl-8-(1-pyrrolidinyl)-1,4-dioxaspiro[4,5]decan-7-amine (8) and (±)-*trans*-*N*-Methyl-7-(1-pyrrolidinyl)-1,4-dioxaspiro[4,5]decan-8-amine (9). CH_3SO_2Cl (5.4 mL, 70 mmol) was added slowly to a stirred solution of a mixture of 6 and 7 (13.2 g, 58.0 mmol) and Et_3N (10.5 mL, 75.5 mmol) in dry CH_2Cl_2 (200 mL) at 0 °C. The mixture was stirred for 3 h at 0 °C and then treated with cold brine and saturated $NaCO_3$(aq). The organic layer was separated, while the aqueous layer was extracted with CH_2Cl_2. The combined organic layer and extracts were washed with cold brine, dried ($MgSO_4$), and evaporated under reduced pressure to give a mixture of crude methanesulfonates (17.71 g, 100%). Without further purification, the crude mixture was dissolved in THF (130 mL) and treated with a solution of CH_3NH_2 in CH_3OH (22.5 mL, 40%). The resulting mixture was heated under N_2 at 75 °C overnight and then evaporated to remove solvents. The residue was redissolved in a solution of 20% 2-propanol in $CHCl_3$. The solution was then washed with brine, dried ($MgSO_4$), and evaporated to give a crude product, which was purified by flash chromatography (silica gel, 10% CH_3OH and 1% NH_4OH in CH_2Cl_2) to provide a mixture of 8 and 9 (9.91 g, 71%): ^1H NMR ($CDCl_3$) δ 3.9 (m, 4 H), 2.73 (s, 1 H), 2.55 (m, 6 H), 2.3 (3 H), 2.1–1.2 (m, 10 H); ^{13}C NMR (20 MHz, $CDCl_3$) δ 109.34, 108.50, 64.02, 63.92, 61.16, 60.63, 59.08, 58.39, 47.41, 46.57, 38.67, 34.03, 33.67, 32.99, 32.83, 30.19, 26.97, 23.65, 18.20. A portion of the mixture (2.4 g) was purified by MPLC to give pure 8 (the major isomer, 1.49 g, 60%): IR (neat) 3398 (br, NH), 2952, 2877, 2800; ^1H NMR (300 MHz, $CDCl_3$) δ 3.9 (m, 4 H), 2.75 (s, 1 H), 2.55 (m, 6 H), 2.33 (s, 3 H), 2.1–13 (m, 10 H; MS m/e 240 (M^+), 197, 110, 97, 87 (base peak); ^{13}C NMR (20 MHz, $CDCl_3$) δ 107.80, 63.36, 63.29, 60.56, 57.76, 46.83, 38.00, 33.05; 32.35, 23.06, 17.62; HRMS m/e (M^+) calcd 240.1837, obsd 240.1837.

(G) (±)-*trans*-3,4-Dichloro-*N*-methyl-*N*-[8-(1-pyrrolidinyl)-1,4-dioxaspiro[4,5]dec-7-yl]benzeneacetamide (10) and (±)-*trans*-3,4-Dichloro-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1,4-dioxaspiro[4,5]dec-8-yl]benzeneacetamide (11). To a stirred solution of 3,4-dichlorophenylacetic acid (10.15 g, 49.5 mmol) in dry benzene (130 mL) was added slowly oxalyl chloride (10.8 mL, 123 mmol). After stirring for 2 h, evaporation

of benzene excess oxalyl chloride provided crude 3,4-dichlorophenylacetyl chloride. The crude was dissolved in CH_2Cl_2 (50 mL) and added slowly to a stirred solution of a mixture of 8 and 9 (9.91 g, 41.23 mmol) and Et_3N (17.25 mL, 123 mmol) in CH_2Cl_2 (100 mL) at 0 °C. The resulting mixture was stirred for another 1.5 h, treated with H_2O and $NaHCO_3$(aq), and extracted with CH_2Cl_2. The combined extracts were washed with H_2O and brine, dried ($MgSO_4$), and evaporated to give a crude product mixture, which was chromatographed (silica gel, 10% CH_3OH in CH_2Cl_2) to give 10 (9.5 g, 54%) and 11 (1.1 g, 6.0%). Both 10 and 11 were recrystallized from acetonitrile. 10: mp 95–97 °C; IR (KBr) 2957–2872, 2806, 1622 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.3–7.0 (m, 3 H), 4.0–3.7 (m, 5 H), 3.65 (m, 2 H), 2.77 (s, 3 H), 2.71–2.4 (m, 5 H), 1.9–1.4 (m, 10 H); MS *m/e* 426 (M^+), 316, 209, 159, 123, 110, 99 (base peak); ^{13}C NMR ($CDCl_3$) δ 169.94, 169.40, 135.71, 132.22, 131.06, 130.71, 130.52, 130.11, 128.58, 128.24, 108.30, 107.98, 64.31, 59.06, 52,69, 48.44, 47.21, 40.51, 39.75, 39.26, 37.68, 33.82, 33.54, 29.93, 27.09, 23.86, 20.64, 18.27; HRMS *m/e* (M^+) calcd 426.1477, obsd 426.1475. Anal. ($C_{21}H_{28}O_3N_2Cl_2$) C, H, N. 11: mp 90–92 °C; IR (KBr) 3058, 2960–2820, 1625 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.3–7.0 (m, 3 H), 3.9 (m, 5 H), 3.6 (dd, *J* = 13.5 and 15.6 Hz, 2 H), 3.1–2.5 (m, 8 H), 1.9–1.5 (m, 10 H); MS *m/e* 427 ($M^+ + 1$), 344, 312, 209, 168 (base peak), 159, 86; ^{13}C NMR ($CDCl_3$) δ 169.01, 135.51, 131.60, 130.20, 129.85, 129.58, 127.85, 108.35, 63.72, 63.59, 54.73, 53.37, 46.31, 39.75, 32.93, 30.73, 29.21, 24.43, 23.35; HRMS *m/e* (M^+) calcd 426.1477, obsd 426.1472. Anal. ($C_{21}H_{28}O_3N_2Cl_2$) C, H, N.

(H) **(±)-*trans*-3,4-Dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-5-oxocyclohexyl]benzeneacetamide (12).** A mixture of 10 (3.02 g, 7.07 mmol), CH_3OH (85 mL), and 2 N H_2SO_4 (32 mL) was heated under reflux for 24 h. The mixture was then evaporated to remove CH_3OH, neutralized with $NaHCO_3$(aq), and extracted with CH_2Cl_2. The combined extracts were washed with H_2O, dried ($MgSO_4$), and evaporated to give crude 12 (2.78 g), which was purified by flash chromatography (silica gel; 10% CH_3OH in CH_2Cl_2) to provide 12 (2.1 g, 77.5%): IR (neat) 2960–2807, 1718 (ketone C=O), 1636 (amide C=O); ^1H NMR ($CDCl_3$) δ 7.3–7.0 (m, 3 H), 4.75 (q, 1 H), 3.65 (dd, *J* = 15.6 and 15.6 Hz, 2 H), 3.0 (t, 1 H), 2.85 (s, 3 H), 2.6–2.4 (m, 6 H), 2.35–1.5 (m, 8 H); MS *m/e* 382 (M^+), 165, 159, 137, 110 (base peak), 96; ^{13}C NMR ($CDCl_3$) δ 207.59, 169.65, 135.26, 132.31, 130.69, 130.21, 128.23, 57.89, 54.45, 47.85, 43.04, 40.37, 38.96, 30.72, 23.73, 20.02; HRMS *m/e* (M^+) calcd 382.1214, obsd 382.1196. HBr salt of 12: white crystals (from 2-propanol), mp 202.5–203.5 °C. Anal. ($C_{19}H_{25}O_2N_2Cl_2Br$) C, H, N.

(I) **(±)-(5β,7α,8β)-3,4-Dichloro-*N*-methyl-*N*-[3-methylene-2-oxo-8-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-7-yl]benzeneacetamide (14) and (±)-(5α,7α,8β)-3,4-Dichloro-*N*-methyl-*N*-[3-methylene-2-oxo-8-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-7-yl]benzeneacetamide (15).** To a stirred solution of 12 (0.94 g, 2.4 mmol) in dry THF (10 mL) under N_2 was added activated Zn powder (0.24 g, prepared from Zn powder by treatment with dilute HCl followed by washing with acetone/ether and drying). The resulting mixture was heated to 40–45 °C, while ethyl α-(bromomethyl)acrylate (0.53 g, 2.9 mmol) was slowly added. After further stirring for 3 h, the reaction mixture was allowed to cool to room temperature. The cooled solution was filtered through Celite and evaporated to remove THF. The residue was treated with H_2O and Na_2CO_3(aq) and extracted with CH_2Cl_2. The combined extracts were washed with H_2O, dried ($MgSO_4$), and evaporated to give a crude mixture, which contained 14 and 15 in a ratio of 2.9 to 1 on the basis of HPLC analysis. The crude product was purified by flash chromatography (silica gel; 3–8% CH_3OH in CH_2Cl_2) to give 14 (0.60 g, 56%), 15 (0.23 g, 21%), and a fraction of "14 + 15" (0.17 g). 14: TLC (CH_3OH:CH_2Cl_2 = 1:12) R_f 0.36; HPLC (RP-Select B, 5 μm; 45% H_2O in MeOH buffered at pH 3.5 with 0.005 M heptanesulfonic acid as ion-pairing agent; 1 mL/min; UV detection at 280 nm) t_R 10.7 min; mp 142–150 °C (from acetonitrile); IR (HBr) 2936–2805, 1757 (lactone C=O), 1645 (amide C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.4–7.1 (m, 3 H), 6.2 (dt, *J* = 14.0 and 2.5 Hz, 1 H), 5.6 (dt, *J* = 10.9 and 2.2 Hz, 1 H), 4.0 (m, 1 H), 3.65 (m, 2 H), 2.8–2.4 (m, 10 H), 2.0–1.4 (m, 10 H); MS *m/e* 450 (M^+), 233, 220, 110 (base peak), 97; ^{13}C NMR ($CDCl_3$) δ 169.85, 169.46, 168.76, 168.61, 135.59, 135.46, 134.69, 134.15, 131.96, 130.57, 130.37, 130.24, 130.09, 129.93, 128.77, 128.21, 122.63, 121.92, 82.37, 82.19, 58.47, 57.12, 55.83, 47.82, 47.06, 41.39, 40.40, 39.90, 39.58, 39.30, 36.26, 35.96, 26.85, 23.68, 19.54, 17.89; HRMS *m/e* (M^+) calcd 450.1477, obsd 450.1648. Anal. ($C_{23}H_{28}O_3N_2Cl_2$) C, H, N. 15: TLC R_f 0.47; HPLC t_R 9.0 min; IR (neat) 2935, 1759 (lactone C=O), 1628 (amide C=O) cm^{-1}, ^1H NMR ($CDCl_3$) δ 7.4–7.3 (m, 2 H), 7.1–7.05 (m, 1 H), 6.2 (t, *J* = 2.5 Hz, 1 H), 5.6 (t, *J* = 2.5 Hz, 1 H), 4.6 (t, br, 1 H), 3.7 (m, 2 H), 3.0 (d, *J* = 15 Hz, 1 H), 2.84–2.76 (m, 5 H), 2.57 (m, 2 H), 2.47 (m, 2 H), 1.82–1.64 (m, 9 H), 1.35 (m, 1 H); MS *m/e* 451 ($M^+ + 1$), 233, 110 (base peak), 97; ^{13}C NMR ($CDCl_3$) δ 170.3, 169.8, 169.1, 135.3, 134.5, 132.3, 131.1, 130.7, 130.5, 130.2, 128.6, 128.2, 123.1, 82.7, 57.4, 53.3, 51.3, 47.1, 42.2, 40.2, 39.9, 37.5, 35.7, 26.4, 23.8, 17.5; HRMS *m/e* (M^+) calcd 450.1477, obsd 450.1468.

Opioid Receptor Binding Assays. Determination of κ, μ, and δ opioid receptor affinities was performed as described before.[19] Guinea pig brain membranes (about 1 mg of protein) were incubated with unlabeled drugs and radioligands in a final volume of 1 mL of buffer containing 100 mM NaCl and 50 mM Tris-HCl, pH 7.4. The final concentrations of the radioligands used to label the opioid receptors were as follows: 1 nM (–)-[^3H]ethylketocyclazocine (28.1 Ci/mmol) in the presence of 500 nM 2-D-Ala-5-D-Leu-enkephalin (DADLE) and 20 nM sufentanil (κ binding); 0.5 nM [^3H]naloxone (30.5 Ci/mmol) (μ binding); and 1 nM [^3H]DADLE (30.0 Ci/mmol) in the presence of 4 nM sufentanil (δ binding). Samples were incubated at room temperature for 45 min, quickly filtered through Whatman GF/C glass fiber filters under negative pressure, and washed 3 times with 5 mL of ice-cold Tris buffer. Nonspecific binding was determined in the presence of 10 μM ethylketocyclazocine (κ) and naloxone (μ and δ). Radioactivity was determined by liquid scintillation counting. Protein was determined by the method of Lowry et al.[20]

Wash-Resistant Binding. Guinea pig brain membranes were prepared by the method of Tam.[21] Washed brain membranes were resuspended (1 mg of protein/mL) in 50 mM Tris-HCl, pH 7.4, divided into equal aliquots, and incubated in the presence or absence of various concentrations of the test compound (14 or 15) at 25 °C for 60 min. The incubation was terminated by centrifugation at 57000*g* for 20 min. The pellets were resuspended in 50 mM Tris-HCl, pH 7.4, incubated at 25 °C for 20 min, and washed three more times by repeated centrifugation, resuspension, and incubation. This procedure was sufficient to remove all washable 14 (or 15) because additional washings did not further decrease the inhibition of binding by 14 (or 15).

Acknowledgment. We thank Dr. Ling-Kang Liu at the Institute of Chemistry, Academia Sinica, for assistance in obtaining the 500-MHz NOESY spectra. This research was supported by a grant (NSC78-0412-B002-17) from the National Science Council of the R.O.C.

Supplementary Material Available: 2D NMR data for 14 and 15 (2 pages). Ordering information is given on any current masthead page.

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