Conjugate Addition Ligands of Opioid Antagonists. Methacrylate Esters and Ethers of 6α- and 6β-Naltrexol

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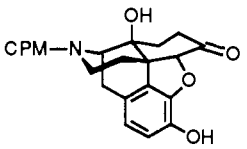
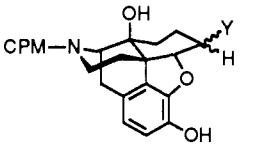
α- and β-naltrexol derived esters **9** and **10** and ethers **11** and **12**, each containing the α,β-unsaturated ester functionality, were prepared as conformationally more flexible analogues of spiro-α-methylene-γ-lactones **5** and **6**. All were active in the opioid radioreceptor binding assay against [^{3}H]bremazocine and more active against [^{3}H]DAGO, indicating μ-subtype selectivity, but only ether **12** showed significant irreversible activity. We conclude that small structural changes, made in very closely related electrophilic opioids, lead to changes in receptor binding. All four compounds were long-acting antagonists to morphine in mice, with ester **10** being approximately equipotent with naltrexone.

Chemoaffinity ligands derived from opioid agonist and antagonist molecules have provided an important approach to aid in characterization of opioid drug receptor interactions.[1] Compounds related to naltrexone (**1**) with

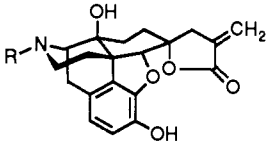


1, naltrexone

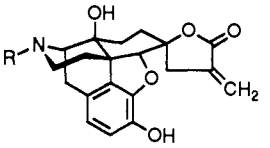


2: Y = N(CH$_2$CH$_2$Cl)$_2$
3: Y = NHCOCH=CHCOOCH$_3$
4: Y = N=C=S

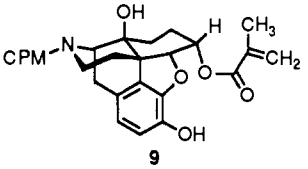
electrophilic functionalities at the C-6 position, e.g., the *N*,*N*-bis(β-chloroethyl) derivatives of 6α- and 6β-naltrexamine (**2**), the fumaramide methyl esters (**3**), the 6α- and 6β-isothiocyanate derivatives (**4**), and other derivatives have shown interesting characteristics in opioid receptor preparations.[1,2] Inactivation of opioid receptor binding by sulfhydryl reagents like *N*-ethylmaleimide suggested that receptor alkylation occurs at or near an opioid binding site.[3] Thus, a sulfhydryl group(s) may serve as a secondary recognition site(s) for certain electrophilic opioid ligands.[4] Of the isomeric spiro-α-methylene-γ-lactones of 6-desoxynaltrexone **5** and **6**, only **6** had irreversible activity in the



5: R = CPM
7: R = Me



6: R = CPM
8: R = Me



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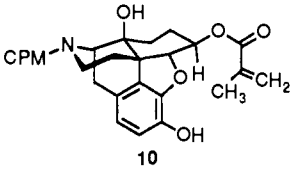

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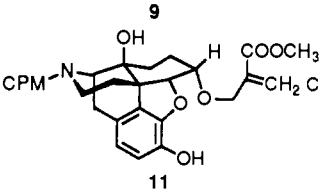

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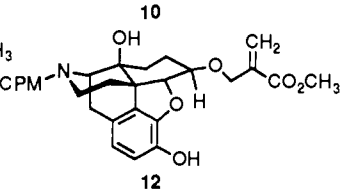

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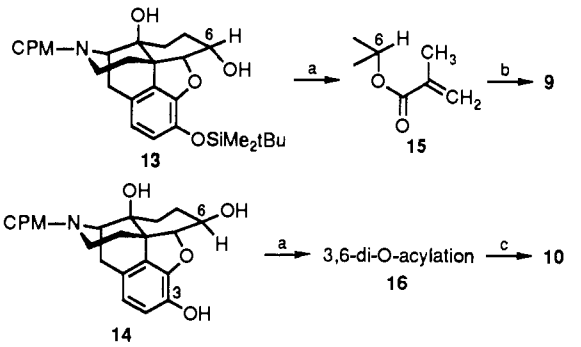
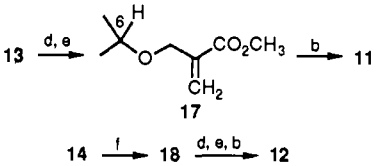
opioid radioreceptor assay, and both diastereoisomers of the corresponding *N*-methylspiro-α-methylene-γ-lactone (**7** and **8**) had no irreversible activity.[5] In addition, the irreversible activity of **6** required the presence of the exocyclic α-methylene unit adjacent to the lactone carbonyl moiety.[6] Endocyclic, α,β-unsaturated or saturated lactones

Scheme I[a]

A. Synthesis of α- and β-Esters **9** and **10**



B. Synthesis of α- and β-Ethers **11** and **12**



[a] Reagents: (a) methacryloyl chloride, DMAP, *N*,*N*-diisopropyl-*N*-ethylamine; (b) *n*-Bu$_4$NF; (c) MeOH, NEt$_3$; (d) KO*t*Bu, *n*BuLi, diisopropylamine, THF; (e) methyl α-(bromomethyl)acrylate; (f) *t*BuMe$_2$SiCl, imidazole, DMF.

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were reversible.[6] These spiro-α-methylene-γ-lactones were very reactive electrophiles to the model nucleophile 4-methoxybenzenethiol as determined by ^{1}H NMR spectroscopy.[7]

In this paper, we report the synthesis and opioid assay data for regioisomeric α- and β-naltrexol-derived esters and ethers, each containing the same α-methylene carboxylic acid ester electrophilic unit in a conformationally less restricted environment. These compounds were prepared to obtain more structure–activity information concerning possible irreversible binding of electrophilic groups attached at the O-6 position.

Chemistry

Routes for preparation of the desired esters and ethers are given in Scheme I. The starting material for ester **9** and ether **11** was the 3-*O*-*t*BuMe$_2$Si ether of α-naltrexol (**13**), obtained from the 3-O-*t*BuMe$_2$Si ether of naltrexone[6] by lithium tri-*sec*-butylborohydride reduction.[8] The starting material for ester **10** and ether **12** was β-naltrexol (**14**), which was obtained by formamidine sulfinic acid reduction of naltrexone (**1**) under aqueous alkaline conditions.[9]

α-Ester **15** was prepared from **13** by selective O-6-esterification using methacryloyl chloride, *N*,*N*-diisopropyl-*N*-ethylamine, and catalytic 4-(dimethylamino)-pyridine in CCl_4–CH_2Cl_2 (3:1). Deprotection of intermediate **15** with tetra-*n*-butylammonium fluoride provided **9**. β-Ester **10** was prepared by 3,6-diesterification of **14** to afford **16**, using 3.5 equiv of methacryloyl chloride, *N*,*N*-diisopropyl-*N*-ethylamine, and catalytic 4-(dimethylamino)pyridine in CH_2Cl_2. Selective transesterification of the phenolic ester was accomplished with MeOH/Et_3N, affording β-ester **10**.

α-Ether **11** was obtained from **13** by treatment with excess potassium diisopropylamide[10] to deprotonate both hydroxyl groups, followed by treatment at −78 °C with methyl α-(bromomethyl)acrylate,[11] affording impure **17**. Removal of the silyl protective group with tetra-*n*-butylammonium fluoride provided the desired ether **11**.

β-Ether **12** was obtained from anhydrous **14**[12] by selective O-3-silylation with 1 equiv of *t*BuMe$_2$SiCl and subsequent treatment of silyl ether **18** with excess potassium diisopropylamide[10] at −78 °C to provide the dialkoxide anion. Selective O-6-alkylation with methyl α-(bromomethyl)acrylate[11] provided **12** after deprotection with tetra-*n*-butylammonium fluoride. Attempts to selectively alkylate the trialkoxide anion generated from **14** with excess KH failed to provide **12**. Instead, the 3-*O*-ether was isolated. The site of O-alkylation was determined on the basis of chemical shift differences in the ^{1}H NMR. The signals of the allylic methylene protons of the 3-*O*-ethers were observed at 4.7–5.0 ppm, whereas those of the 6-*O*-ethers were observed at 4.0–4.5 ppm.

Table I. In Vitro Opioid Receptor Binding Competition against 0.5 nM [^{3}H]Bremazocine and 1 nM [^{3}H]DAGO[a]

compound	IC_{50}, nM	
	[^{3}H]bremazocine	[^{3}H]DAGO
bremazocine	0.5	
6α-ester **9**	20.0	6.8
6β-ester **10**	5.0	1.0
6α-ether **11**	2.4	0.18
6β-ether **12**	12.0	1.5
lactone **6**	6.0	1.8

[a] Values are averages of duplicate determinations (±10–15%) or means of triplicates with standard errors of 10% or less.

Opioid Receptor Binding

Affinity of the 6α- and 6β-naltrexol esters **9** and **10** and ethers **11** and **12** for opioid receptor sites was determined in a crude membrane preparation from bovine caudate nucleus against [^{3}H]bremazocine and [^{3}H]DAGO and compared against the known irreversible antagonist **6**.[5] The results of competition binding experiments are described in Table I. 6β-Ester **10** is about 4 times as potent as 6α-ester **9**. In the ether series, 6α-ether **11** is almost 4 times as potent as is 6β-ether **12**. All are potent selective μ-receptor ligands as noted by the 3–8-fold increase in potency against [^{3}H]DAGO, a highly selective μ-receptor ligand. Similar to results on related spiro lactones, esters, etc., small changes at the 6-position affect potency, but a high degree of activity in the radioreceptor assay is still maintained.[6] The IC_{50}'s in competition with the μ-agonist [^{3}H]DAGO correspond well to the potency observed for **6** against [^{3}H]naltrexone.

To determine whether any of these compounds had irreversible effects on ligand binding in the opioid receptor preparation, concentrations of **6**, **9**, **10**, **11**, and **12**, that were ca. 60–90% inhibitory against 0.5 nM [^{3}H]bremazocine were incubated for 45 min at 25 °C with bovine caudate membranes. The membranes were then washed thoroughly as described in the Experimental Section and assayed again with [^{3}H]bremazocine. Compound **12** and α-methylene lactone **6** were bound irreversibly in this membrane preparation. When binding of **12** was assayed with μ-receptor ligand DAGO, the same amount of binding was observed, suggesting that covalent binding occurred at μ-receptors. Naloxone was protective in both assays (Table II).

Opioid Antagonist Activity

In experiments performed in mice, the compounds tested were antagonists to morphine. The compounds were 3–10 times less active than the standard naltrexone (0.007 mg/kg), except for β-ester **10**, which was approximately equiactive (Table III). Esters **9** and **10** were more potent than the corresponding ethers **11** and **12** in these experiments by 3–7-fold. The potency difference (esters > ethers) was opposite to the receptor binding affinities (esters < ethers) observed in vitro. This difference could result from the different effects of distribution or metabolism on the two types of agents. Because of the similarity in structures, only small changes in partitioning of the esters and ethers would be expected. If hydrolyzed, esters **9** and **10** would yield the active metabolites α- and β-naltrexol. Ethers **11** and **12** would yield amino carboxylic acids, which may not partition well into the central nervous system. The smaller methyl ester groups in ethers **11** and **12** might be expected to be more rapidly hydrolyzed than the methacrylate esters **9** and **10** of the diastereomeric secondary

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(12) Anhydrous β-naltrexol (**14**) was prepared by dissolving the hydrate obtained by formamidine sulfinic acid reduction[9] in the minimum amount of ethyl acetate (25 mL/g) and drying with $MgSO_4$, followed by evaporation of the solvent.

Table II. Irreversible Inhibition of [^{3}H]Bremazocine Binding[a]

drug (concn)	% control [^{3}H]bremazocine (+NaCl) unwashed	washed	protected
naloxone (1 nM)	0	98	–
6α-ester **9** (100 nM)	22	100	
6β-ester **10** (100 nM)	39	100	
6α-ether **11** (20 nM)	17	79	96
6β-ether **12** (20 nM)	35	60	90
6β-ether **12** (10 nM) against DAGO (1 nM)	20	59	95
lactone **6** (100 nM)	16	50	85

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

Table III. Antagonist Activity Determined in the Tail-Flick Assay Vs Morphine (Sc)[a]

compound	TF vs morphine: AD_{50}, mg/kg (95% CL)
6α-ester **9**	0.02 (0.009–0.07)
6β-ester **10**	0.008 (0.004–0.02)
6α-ether **11**	0.07 (0.06–0.10)
6β-ether **12**	0.06 (0.03–0.12)
naltrexone (1)	0.007 (0.002–0.02)

[a] Compounds were tested as previously described.[13]

O-6-alcohols. Relative agonist/antagonist properties of these various derivatives may also explain the differences between in vivo and in vitro potencies.

Compounds **10–12** were also very potent agents in precipitating the withdrawl syndrome in morphine-dependent monkeys. All compounds had a rapid onset and a long duration of action (>2.5 h), and each was about 5 times as potent as naloxone in this assay conducted by the Committee on Problems of Drug Dependence.[13] Interestingly, **12** precipitated only some of the signs of withdrawal, suggesting less pure antagonist activity.

It is clear that very small changes in structurally similar electrophiles lead to subtle changes in modes of binding which determine whether these ligands bind reversibly or irreversibly in the opioid receptor assay. The irreversibility of only **12** among these flexible-side-chain esters and ethers would seem to be due to suitable alignment of the electrophilic methylene group in this compound with the available receptor nucleophile(s) in the membrane preparations. The observed relative potency differences between the results obtained in vivo and in vitro require additional experiments to examine possible effects of distribution, metabolism, partial agonist/antagonist activity, and receptor binding.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were recorded with a Perkin-Elmer 283 spectrometer or a Perkin-Elmer 1600 series FTIR. Absorptions are expressed in units of frequency (cm^{-1}). NMR spectra were recorded on the Varian VXR-300 spectrometer. Chemical shifts are expressed in parts per million (δ) downfield from tetramethylsilane as an internal standard. Mass spectra were obtained on the VG-7070 and VG-70SEQ mass spectrometers by direct-insertion probe. Optical rotations were measured on a JASCO-DIP-4 digital polarimeter. All reactions were performed in oven- or flame-dried glassware under an argon atmosphere. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl. Dimethylformamide (DMF) was dried by storage over 3-Å molecular sieves. Analytical thin-layer chromatography (TLC) was performed on either Merck EM silica gel 60F-254 or Analtech silica gel HLF glass plates. Merck silica gel 60 (230–400 mesh) was used for preparative flash column chromatography.[14] Microanalyses were performed by Galbraith Laboratories, Knoxville, TN. Where indicated by the symbols of the elements, analyses were within ±0.4% of the theoretical values.

3-*O*-(*tert*-Butyldimethylsilyl)-4,5α-epoxy-3,6α,14-trihydroxy-17-(cyclopropylmethyl)morphinan (13). To a solution of 3-*O*-(*tert*-butyldimethylsilyl)naltrexone[6] (2.65 g, 5.84 mmol) in dry THF (20 mL), cooled at −78 °C, was added a 1 M solution of lithium tri-*sec*-butylborohydride (L-Selectride) in THF (6.40 mL) over 10 min. The solution was stirred for 25 min at −78 °C and then water (10 mL) was added, initially dropwise. After the cooling bath was removed, the mixture was stirred for 10 min, diluted with water (50 mL), and extracted with CH_2Cl_2 (3 × 60 mL). The combined extracts were washed with water (50 mL), and the volatiles were evaporated. The residue was purified by flash chromatography eluting with 3% triethylamine–10% ethyl acetate–CH_2Cl_2 to give α-alcohol **13** (2.17 g, 82%) as a viscous oil: ^{1}H NMR ($CDCl_3$) δ 0.1–0.2 (m, 2 H, cyclopropyl CH_2), 0.18 (s, 3 H, $SiCH_3$), 0.21 (s, 3 H, $SiCH_3$), 0.5–0.6 (m, 2 H, cyclopropyl CH_2), 0.8–0.9 (m, 1 H, cyclopropyl CH), 1.00 (s, 9 H, $SiC(CH_3)_3$), 1.05–1.15 (m, 1 H, C-7 H), 1.4–1.55 (m, 2 H, C-8 H and C-15 H), 1.6–1.8 (m, 2 H, C-7 H and C-8 H), 2.15–2.25 (m, 2 H, C-15 H and C-16 H), 2.25–2.4 (m, 2 H, NCH_2 cyclopropyl), 2.55–2.7 (m, 2 H, C-10α H and C-16 H), 3.0–3.1 (m, 2 H, C-9 H and C-10β H), 4.1–4.2 (m, 1 H, C-6 H), 4.60 (d, *J* = 4.4 Hz, 1 H, C-5 H), 5.3 (br s, movable, 1 H, OH), 6.50 (d, *J* 8.2 Hz, 1 H, C-1 H), 6.63 (d, *J* 8.1 Hz, 1 H, C-2 H); ^{13}C NMR ($CDCl_3$) δ −4.50, −4.42, 3.77, 3.90, 9.34, 18.18, 22.78, 23.68, 25.58, 28.61, 33.50, 43.06, 46.97, 59.40, 61.96, 66.56, 69.50, 90.15, 118.58, 121.52, 126.40, 131.37, 136.31, 148.08; IR (neat) 3600–3200, 2970, 2940, 2860, 1610, 1500, 1450, 1275, 1265, 850 cm^{-1}.

4,5α-Epoxy-3,14-dihydroxy-6α-(methacryloyloxy)-17-(cyclopropylmethyl)morphinan (9). To a solution of α-alcohol **13** (564 mg, 1.23 mmol), 4-(dimethylamino)pyridine (75 mg, 0.62 mmol), and *N*,*N*-diisopropyl-*N*-ethylamine (650 μL, 3.70 mmol) in CCl_4 (3 mL) and CH_2Cl_2 (1 mL) at 0 °C was added via a cannula an ice-cold solution of methacryloyl chloride (240 μL, 2.47 mmol) in CCl_4 (1 mL). After stirring for 10 h at 10 °C, saturated aqueous $NaHCO_3$ (30 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 60 mL). The combined organic extracts were washed with saturated aqueous NaCl (30 mL) and dried ($MgSO_4$), and the volatiles were evaporated. The residue was purified by flash chromatography eluting with 3% triethylamine–3% ethyl acetate–CH_2Cl_2 to give slightly impure α-ester **15** (425 mg), IR (neat) 1715 cm^{-1}. This material was used directly in the next step.

To a solution of impure α-ester **15** (425 mg, 0.81 mmol) in THF (3.5 mL) was added a 1 M solution of tetra-*n*-butylammonium fluoride in THF (1.21 mL) and stirred for 90 min. The mixture was treated with saturated aqueous $NaHCO_3$ (30 mL) and then extracted with CH_2Cl_2 (3 × 80 mL). The combined organic extracts were washed with saturated aqueous NaCl solution (35 mL) and dried ($MgSO_4$) and the solvents were evaporated. The residue was purified by flash chromatography eluting with 3% triethylamine–3% ethyl acetate–CH_2Cl_2 to give ester **9** (264 mg, 52% from **13**) as an oil which crystallized: mp 176–178 °C (CH_2Cl_2–hexanes); $[\alpha]^{20}_D$ = −128° (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), 0.8–0.9 (m, 1 H, cyclopropyl CH), 1.4–1.7 (m, 4 H, C-7 H, two C-8 H and C-15 H), 1.69 (s, 3 H, methacrylate CH_3), 2.0–2.4 (m, 3 H, C-7 H, C-15 H and C-16 H), 2.39 (d, *J* = 6.3 Hz, 2 H, NCH_2 cyclopropyl), 2.6–2.7 (m, 2 H, C-10α H and C-16 H), 3.04 (d, *J* = 18.1 Hz, 1 H, C-10β H), 3.12 (d, *J* = 5.7 Hz, 1 H, C-9 H), 4.77 (d, *J* = 5.7 Hz, 1 H, C-5 H), 5.4 (br s, movable, 2 H, C-3 OH and C-14 OH), 5.38 (m, 1 H, *E*-vinylic H), 5.4–5.5 (m, 1 H, C-6 H), 5.57 (m, 1 H, *Z*-vinylic H), 6.53 (d, *J* = 8.2 Hz, 1 H, C-1 H), 6.66 (d, *J* = 8.0 Hz, 1 H, C-2 H); ^{13}C NMR ($CDCl_3$) δ 3.86, 3.93, 9.34, 17.94, 22.20, 22.70, 26.32, 31.87, 43.87, 46.81, 59.19, 62.33, 68.53, 70.08, 87.08, 116.77, 118.39, 124.15, 125.78, 130.45, 135.64, 137.63, 144.50, 166.26; IR (KBr) 3300–3500, 2960, 2930, 2825, 1715, 1640, 1510, 1455, 1380, 1325, 1290, 1270, 1165, 1175,

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1075, 985, 940, 895, 870, 800, 740 cm^{-1}. HREIMS calcd for $C_{24}H_{29}NO_5$ 411.2046, obsd 411.2056. Anal. Calcd for $C_{24}H_{29}NO_5 \cdot 0.5H_2O$: C, H, N.

4,5α-Epoxy-3,6β-(dimethacryloyloxy)-14-hydroxy-17-(cyclopropylmethyl)morphinan (16). To a solution of 6β-naltrexol (14)[9] (2.86 g, 8.34 mmol), 4-(dimethylamino)pyridine (0.51 g, 4.17 mmol), and *N,N*-diisopropyl-*N*-ethylamine (6.54 mL, 37.5 mmol) in CH_2Cl_2 (45 mL) cooled to −20 °C (CCl_4–dry ice) was added a cold solution (−78 °C) of methacryloyl chloride (2.85 mL, 29.2 mmol) in CH_2Cl_2 (20 mL) via a cannula over 10 min. After stirring at −10 °C for 27 h, saturated aqueous $NaHCO_3$ solution (100 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 125 mL). The combined organic extracts were washed with saturated aqueous NaCl solution and dried ($MgSO_4$), and the volatiles were evaporated. The residue was purified by flash chromatography eluting with 2% triethylamine–30% CH_2Cl_2–ethyl acetate to give dimethacrylate ester **16** (4.15 g, 74%) as a white foam: ^1H NMR ($CDCl_3$) δ 1.99 (s, 3 H, acrylate CH_3 *O*-6-ester), 2.14 (s, 3 H, acrylate CH_3 *O*-3-ester), 5.57 (s, 1 H, *E*-vinylic H *O*-6-ester), 5.74 (s, 1 H, *E*-vinylic proton *O*-3-ester), 6.17 (s, 1 H, *Z*-vinylic H *O*-6 ester), 6.32 (s, 1 H, *Z*-vinylic H *O*-3-ester); IR (neat) 1735, 1715, 1635, 1620 cm^{-1}.

4,5α-Epoxy-3,14-dihydroxy-6β-(methacryloyloxy)-17-(cyclopropylmethyl)morphinan (10). A solution of dimethacrylate ester **16** (4.15 g, 8.63 mmol), triethylamine (1.5 mL), THF (10 mL), and methanol (150 mL) was heated at 40 °C for 8 h. After evaporation of the volatiles, the residue was treated with CH_2Cl_2 (150 mL) and saturated aqueous $NaHCO_3$ solution (100 mL). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were washed with saturated aqueous NaCl solution and dried ($MgSO_4$), and the volatiles were evaporated. The residue was purified by flash chromatography eluting with 1% triethylamine–1% methanol–CH_2Cl_2 to give methacrylate ester **10** (2.73 g, 77%) as a foam which crystallized: mp 197.5–199 °C (CH_2Cl_2–hexanes); $[\alpha]^{20}_D$ = −154.1° (c = 1.02, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 0.1–0.2 (m, 2 H, cyclopropyl CH_2), 0.5–0.6 (m, 2 H, cyclopropyl CH_2), 0.8–0.9 (m, 1 H, cyclopropyl CH), 1.4–1.55 (m, 2 H, C-8 H and C-15 H), 1.6–1.7 (m, 1 H, C-8 H), 1.8–2.0 (m, 2 H, 2 C-7 H), 1.95 (s, 3 H, methacrylate CH_3), 2.12 (dt, *J* = 3.1 and 11.8 Hz, 1 H, C-16 H), 2.26 (dt, *J* = 4.7 and 12.3 Hz, 1 H, C-15 H), 2.37 (d, *J* = 6.5 Hz, 2 H, NCH_2 cyclopropyl), 2.55–2.7 (m, 2 H, C-10α H and C-16 H), 3.02 (d, *J* = 18.1 Hz, 1 H, C-10β H), 3.12 (d, *J* = 5.5 Hz, 1 H, C-9 H), 4.65 (d, *J* = 6.8 Hz, 1 H, C-5 H), 4.65–4.8 (m, 1 H, C-6 H), 5.4 (br s, movable, 1 H, OH), 5.58 (m, 1 H, *E*-vinylic H), 6.16 (m, 1 H, *Z*-vinylic H), 6.56 (d, *J* = 8.1 Hz, 1 H, C-1 H), 6.70 (d, *J* = 8.1 Hz, 1 H, C-2 H); ^{13}C NMR ($CDCl_3$) δ 3.77, 3.97, 9.38, 18.31, 22.55, 23.34, 29.33, 30.41, 43.82, 47.93, 59.05, 62.00, 70.08, 75.77, 92.34, 117.08, 118.95, 124.14, 125.65, 130.94, 136.12, 139.52, 141.86, 166.61; IR (KBr) 3300–3500, 2990, 2970, 2930, 2830, 1715, 1635, 1580, 1455, 1400, 1375, 1300, 1240, 1150, 1130, 1020, 985, 945, 925, 895, 870, 835 cm^{-1}. HREIMS calcd for $C_{24}H_{29}NO_5$ 411.2046, obsd 411.2039. Anal. Calcd for $C_{24}H_{29}NO_5 \cdot 0.5H_2O$: C, H, N.

4,5α-Epoxy-3,6α,14-trihydroxy-6-*O*-(2-carbomethoxyallyl)-17-(cyclopropylmethyl)morphinan (11). To a solution of diisopropylamine (0.82 mL, 5.82 mmol) in THF (7 mL) was added KO*t*Bu (653 mg, 5.82 mmol) and the resulting solution was cooled at −78 °C. A 2.5 M solution of *n*BuLi in hexanes (1.94 mL, 4.85 mmol) was added over 2 min, and after stirring for 15 min at −78 °C, a solution of silyl ether **13** (890 mg, 1.94 mmol) in THF (25 mL) was added over 8 min. The mixture was stirred at −78 °C for 20 min and then a solution of methyl α-(bromomethyl)-acrylate[11] (863 mg, 4.85 mmol) in THF (4 mL) was added over 2 min. The mixture was stirred for 100 min (−78 °C) and treated with saturated aqueous $NaHCO_3$ solution (50 mL). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 25 mL). The combined organic layers were dried (Na_2SO_4) and the volatiles were evaporated. The residue was purified by flash chromatography eluting with 2% triethylamine-CH_2Cl_2 to give slightly impure **17** (960 mg), which was used directly in the next step.

To a solution of impure **17** (1.06 g) in THF (15 mL) was added a 1 M solution of tetra-*n*-butylammonium fluoride in THF (2.9 mL) and it was stirred for 40 min at room temperature. The solution was treated with saturated aqueous $NaHCO_3$ solution (30 mL), and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 × 25 mL), the combined organic layers were dried (Na_2SO_4), and the solvents were evaporated. The residue was purified by flash chromatography eluting with 2% triethylamine–ethyl acetate to give a white foam which was crystallized to give ether **11** (280 mg, 32% from **13**): mp 143–144 °C (CH_2Cl_2–pentane); $[\alpha]^{20}_D$ = −172.5° (c = 1.00, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 0.1–0.2 (m, 2 H, cyclopropyl CH_2), 0.5–0.6 (m, 2 H cyclopropyl CH_2), 0.8–0.9 (m, 1 H, cyclopropyl CH), 1.25–1.4 (m, 1 H, C-7 H), 1.45–1.65 (m, 3 H, two C-8 H and C-15 H), 1.7–1.85 (m, 1 H, C-7 H), 2.23 (d, *J* = 7.7 Hz, 2 H, C-15 H and C-16 H), 2.35 (dd, *J* = 1.7 and 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), 3.07 (d, *J* = 6.3 Hz, 1 H, C-9 H), 3.75 (s, 3 H, CO_2CH_3), 3.99 (dt, *J* = 10.4 and 4.1 Hz, 1 H, C-6 H), 4.27 (d, *J* = 14.3 Hz, 1 H, allylic CH), 4.34 (d, *J* = 14.3 Hz, 1 H, allylic CH), 4.73 (d, *J* = 4.0 Hz, 1 H, C-5 H), 5.1-5.6 (br s, movable, 2 H, C-3 and C-14 OH), 5.75 (d, *J* = 1.7 Hz, 1 H, *E*-vinylic H), 6.22 (d, *J* = 1.5 Hz, 1 H, *Z*-vinylic H), 6.49 (d, *J* = 8.2 Hz, 1 H, C-1 H), 6.68 (d, *J* = 8.2 Hz, 1 H, C-2 H); ^{13}C NMR ($CDCl_3$) δ 3.78, 3.95, 9.38, 20.64, 22.75, 28.15, 33.11, 43.31, 47.39, 51.70, 59.39, 62.10, 67.45, 69.97, 74.68, 88.98, 117.03, 118.38, 124.84, 125.66, 130.64, 136.99, 137.30, 145.45, 166.15; IR (KBr) 3200–3500, 2990, 2915, 2815, 1715, 1630, 1610, 1500, 1460, 1340, 1310, 1280, 1195, 1160, 1115, 1100, 1040, 985, 950, 860 cm^{-1}. HREIMS calcd for $C_{25}H_{31}NO_6$ 441.2151, obsd 441.2146. Anal. Calcd for $C_{25}H_{31}NO_6 \cdot 0.5H_2O$: C, H, N.

3-*O*-(*tert*-Butyldimethylsilyl)-4,5α-epoxy-3,6β,14-trihydroxy-17-(cyclopropylmethyl)morphinan (18). A solution of anhydrous 6β-naltrexol[12] (2.50 g, 7.30 mmol), *tert*-butyldimethylsilyl chloride (1.21 g, 8.0 mmol), and imidazole (1.09 g, 16.0 mmol) in DMF (20 mL) was stirred for 23 h and then treated with half-saturated aqueous $NaHCO_3$ solution (30 mL). The mixture was extracted with CH_2Cl_2 (3 × 25 mL), the combined extracts were dried (Na_2SO_4), and the volatiles were evaporated. The residue was purified by flash chromatography eluting with 4% triethylamine–20% CH_2Cl_2–hexanes to give silyl ether **18** (1.49 g, 45%) as a white foam: $[\alpha]_D$ = −117.5° (c = 1.00, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 0.1–0.2 (m, 2 H, cyclopropyl CH_2), 0.19 (s, 6 H, $Si(CH_3)_2$), 0.5–0.6 (m, 2 H, cyclopropyl CH_2), 0.75–0.9 (m, 1 H, cyclopropyl CH), 0.99 (s, 9 H, $SiC(CH_3)_3$), 1.3–1.5 (m, 2 H, C-8 H and C-15 H), 1.5–1.7 (m, 2 H, C-7 H and C-8 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), 2.35 (d, *J* = 6.6 Hz, 2 H, NCH_2 cyclopropyl), 2.5–2.7 (m, 2 H, C-10α H and C-16 H), 3.01 (d, *J* = 18.3 Hz, 1 H, C-10β H), 3.08 (d, *J* = 5.6 Hz, 1 H, C-9 H), 3.5–3.6 (m, 1 H, C-6 H), 4.44 (d, *J* = 5.5 Hz, 1 H, C-5 H), 5.17 (br s, movable, 1 H, OH), 6.51 (d, *J* = 7.9 Hz, 1 H, C-1 H), 6.64 (d, *J* = 8.0 Hz, 1 H, C-2 H); ^{13}C NMR ($CDCl_3$) δ −4.49, −4.41, 3.81, 3.88, 9.41, 18.23, 22.69, 25.36, 25.65, 29.07, 31.37, 43.64, 46.98, 59.17, 62.06, 69.99, 71.88, 94.75, 118.21, 121.64, 125.59, 131.75, 138.10, 145.83. FTIR (neat) 3700–3200, 2929, 2857, 1632, 1608, 1496, 1271, 1038, 865, 841 cm^{-1}. HREIMS calcd for $C_{26}H_{39}NO_4Si$ 457.2648, obsd 457.2651.

4,5α-Epoxy-3,6β,14-trihydroxy-6-*O*-(2-carbomethoxyallyl)-17-(cyclopropylmethyl)morphinan (12). To a solution of diisopropylamine (0.84 mL, 6.02 mmol) in THF (10 mL) was added KO*t*Bu (676 mg, 6.02 mmol) and the solution was cooled at −78 °C. A 2.5 M solution of *n*BuLi in hexanes (2.2 mL, 5.54 mmol) was added over 4 min and the solution was stirred at −78 °C for 12 min. A solution of silyl ether **18** (1.10 g, 2.41 mmol) in THF (10 mL) was added over 7 min, the mixture was stirred at −78 °C for 22 min, and then a solution of methyl α-(bromomethyl)acrylate[11] (986 mg, 5.54 mmol) in THF (1.5 mL) was added over 3 min. The mixture was stirred at −78 °C for 30 min, saturated aqueous $NaHCO_3$ solution (30 mL) was added, and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 × 15 mL), the combined organic layers were dried (Na_2SO_4), and the volatiles were evaporated. To a solution of the residue in THF (15 mL) was added a 1 M solution of tetra-*n*-butylammonium fluoride in THF (3.6 mL) and stirred for 40 min at room temperature. The solution was treated with saturated aqueous $NaHCO_3$ solution (30 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The combined extracts were dried (Na_2SO_4) and the solvents were evaporated. The residue was purified by flash chromatography eluting with 4% triethylamine–ether to give β-ether **12** (500 mg, 47%) as a white foam: $[\alpha]^{20}_D$ = −110.5° (c = 1.00, CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 0.1–0.2 (m, 2 H, cyclopropyl CH_2), 0.5–0.6 (m, 2 H, cyclopropyl CH_2), 0.75–0.9 (m, 1 H, cy-

clopropyl CH), 1.32 (dt, J = 2.7 and 13.4 Hz, 1 H, C-8 H), 1.4-1.5 (m, 1 H, C-15 H), 1.61 (dt, J = 3.3 and 13.6 Hz, 1 H, C-8 H), 1.7-1.8 (m, 1 H, C-7 H), 1.92 (dq, J = 2.3 and 12.9 Hz, 1 H, C-17 H), 2.11 (dq, J = 3.2 and 11.7 Hz, 1 H, C-16 H), 2.21 (dt, J = 4.4 and 12.2 Hz, 1 H, C-15 H), 2.35 (d, J = 6.5 Hz, 2 H, NCH_2-cyclopropyl), 2.5-2.7 (m, 2 H, C-10α H and C-16 H), 3.00 (d, J = 18.2 Hz, 1 H, C-10β H), 3.06 (d, J = 5.5 Hz, 1 H, C-9 H), 3.15-3.25 (m, 1 H, C-6 H), 3.83 (s, 3 H, CO_2CH_3), 4.14 (d, J = 13.6 Hz, 1 H, allylic CH), 4.45 (d, J = 6.6 Hz, 1 H, C-5 H), 4.48 (d, J = 13.7 Hz, 1 H, allylic CH), 5.7 (br s, movable, 1 H, OH), 5.80 (s, 1 H, E-vinylic CH), 6.26 (s, 1 H, Z-vinylic CH), 6.53 (d, J = 8.2 Hz, 1 H, C-1 H), 6.70 (d, J = 8.2 Hz, 1 H, C-2 H); ^{13}C NMR ($CDCl_3$) δ 3.79, 3.90, 9.44, 22.57, 23.63, 29.74, 30.35, 43.77, 47.66, 52.15, 59.07, 62.16, 68.09, 70.09, 79.04, 94.48, 117.07, 118.60, 123.82, 127.24, 131.51, 137.69, 139.84, 141.99, 167.63; FTIR (KBr) 3700-3100, 2948, 1718, 1636, 1452, 1321, 1097 cm^{-1}. FABMS calcd for $[M + H]^+$ $C_{25}H_{32}NO_6$ 442.2226, obsd 442.2244. Anal. Calcd for $C_{25}H_{31}NO_6$: C, H, N.

Opioid Receptor Binding. The binding assay was carried out essentially as described by Lin and Simon.[15] Crude membranes were prepared from bovine striatum and stored at -70 °C until needed. The labeled ligands used were [^{3}H]bremazocine (18.5 Ci/mmol) for total opioid receptors and [^{3}H]DAGO (33.8 Ci/mmol) for μ-receptors. The concentrations of labeled ligand were 0.5 nM for [^{3}H]bremazocine and 1 nM for [^{3}H]DAGO. Five concentrations of each drug to be tested were used for competition against labeled ligands. Nonspecific binding was measured in the presence of 10 μM naloxone. The samples were incubated in 50 mM Tris-HCl or potassium phosphate buffer, pH 7.4, containing 1 mM EDTA for 45 min at 25 °C. Samples were rapidly filtered through Whatman GF/B filters, rinsed twice with 4 mL of cold buffer, dried, and counted in a toluene-based scintillation cocktail in a scintillation counter.

Values reported are averages of duplicate determinations (±10-15%) or means of triplicates with standard deviations of 15% or less.

Irreversibility and Protection Studies. Membrane preparations were incubated with drug to be tested for 45 min 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 20000g. 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 (0.5 nM) was carried out as described above.

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Registry No. 9, 124154-46-7; 10, 117332-64-6; 11, 124154-47-8; 12, 124154-48-9; 13, 124154-49-0; 13 ketone, 96453-52-0; 14, 49625-89-0; 15, 124154-50-3; 16, 124154-51-4; 17, 124154-52-5; 18, 124266-28-0; methacryloyl chloride, 920-46-7; methyl α-(bromomethyl)acrylate, 4224-69-5.

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Functionalized Congener Approach for the Design of Novel Muscarinic Agents. Synthesis and Pharmacological Evaluation of *N*-Methyl-*N*-[4-(1-pyrrolidinyl)-2-butynyl] Amides

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A functionalized congener approach was used to design ligands for muscarinic cholinergic receptors (mAChRs). A series of ω-functionalized alkyl amides of *N*-methyl-4-(1-pyrrolidinyl)-2-butynamine (**22**) were prepared as functionalized analogues of UH 5 [*N*-methyl-*N*-[4-(1-pyrrolidinyl)-2-butynyl]acetamide], a muscarinic agonist related to oxotremorine. Intermediate **22** was coupled to a series of Boc-protected ω-amino acids, and the resulting amides were deprotected and acylated. Intermediate **22** was also acylated with succinic anhydride and derivatized. The synthetic intermediates and final compounds were evaluated in vitro for their effects on the turnover of phosphatidylinositides in SK-N-SH human neuroblastoma cells that express m_3AChRs, and on the production of cyclic AMP in NG108-15 neuroblastoma x glioma cells that express only m_4AChRs. The displacement of [^{3}H]-*N*-methylscopolamine was also measured in membrane preparations from each of these cell lines. Conjugates of glycine and β-alanine were agonists at m_4AChRs, having little or no activity at m_3AChRs. The potency in displacement of [^{3}H]-*N*-methylscopolamine from both m_3- and m_4AChRs generally increased with increasing chain lengths of the ω-aminoalkyl congeners. The amides of 7-aminoheptanoic acid and 8-aminooctanoic acid, and their Boc-protected derivatives, had comparable affinities to UH 5 (K_i = 5.0 and 4.5 μM at m_3AChRs and at m_4AChRs, respectively) at both receptors but lacked any agonist effects.

The potential therapeutic benefit of central muscarinic cholinergic agonists in the treatment of Alzheimer's disease (AD) has recently received considerable attention.[1,2] However, the currently available therapeutic cholinergic agents suffer from serious side effects, toxicity, and narrow therapeutic windows.[1,2] Recent molecular biological studies of muscarinic cholinergic receptors[3,4] (mAChRs) have now raised the possibility of designing subtype-specific agonists that, by virtue of their improved selectivity, should be devoid of many of these side effects. Although several selective muscarinic antagonists have been defined pharmacologically, relatively few selective agonists are known. In the current work we describe a new series of muscarinic agents and their pharmacological properties.

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