Six Charles River rats were used for each determination.

Reverse Passive Arthus Reaction. This assay was performed according to the procedure of Chang and Otterness.²⁴ The animals used were injected intravenously through the tail vein with 1.0 mL of 0.9% saline containing 2.5 mg of Evans Blue and 5.0 mg of ovalbumin, followed immediately by intracutaneous injection of 0.03 mL of rabbit antiovalbumin antiserum diluted with sufficient 0.9% saline to contain 3.65 mg of antibody/mL. The compound to be tested (11, 22, or 53) (30 mg/kg of body weight) was administered orally in a 1:1 N-methyl-D-glucamine and water solution 1 h before the injection of the ovalbumin. The mean index of the reaction was calculated 3-h postinjection as the product of the diameter of the reaction site (indicated by the accumulation of Evans Blue) and the intensity score of the reaction (a subjective score of 1–4). Five Charles River rats were used for each determination.

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(24) Chang, Y.-H.; Otterness, I. G. Eur. J. Pharmacol. 1981, 69, 155-164. performing the pharmaceutical studies and are grateful to the University of North Dakota for a faculty research grant for partial support of this work.

Registry No. 1, 100-47-0; 2, 874-90-8; 3, 2024-83-1; 4, 1885-35-4; 5, 18039-42-4; 6, 6926-51-8; 7, 10493-27-3; 8, 91759-55-6; 9, 51517-88-5; 10, 91759-56-7; 11, 91759-57-8; 12, 21054-65-9; 13, 76765-82-7; 14, 91759-61-4; 19, 91759-62-5; 20, 91759-63-6; 21, 91759-60-3; 18, 91759-61-4; 19, 91759-62-5; 20, 91759-63-6; 21, 91759-64-7; 22, 91759-65-8; 23, 65-85-0; 24, 100-09-4; 25, 93-07-2; 26, 118-41-2; 27, 93-89-0; 28, 94-30-4; 29, 3943-77-9; 30, 6178-44-5; 31, 613-94-5; 32, 3290-99-1; 33, 41764-74-3; 34, 3291-03-0; 35, 13153-01-0; 36, 91759-66-9; 37, 91759-67-0; 38, 70452-38-9; 39, 6596-82-3; 40, 91759-68-1; 41, 91759-67-2; 42, 70452-47-0; 43, 91759-70-5; 44, 91759-71-6; 45, 91759-72-7; 46, 70452-56-1; 47, 54559-45-4; 48, 18204-66-5; 49, 91759-73-8; 50, 18204-69-8; 51, 91759-74-9; 52, 91759-75-0; 53, 91759-76-1; NaN₃, 26628-22-8; ethyl bromoacetate, 105-36-2; phenyl isothiocyanate, 103-72-0; super-oxide, 11062-77-4.

Supplementary Material Available: Tables containing complete spectral and physical data for all compounds synthesized (19 pages). Ordering information is given on any current masthead page.

Probes for Narcotic Receptor Mediated Phenomena. 7.¹ Synthesis and Pharmacological Properties of Irreversible Ligands Specific for μ or δ Opiate Receptors

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Syntheses of affinity reagents for opiate receptors based on the fentanyl, *endo*-ethenotetrahydrooripavine, and etonitazene carbon-nitrogen skeletons are described. The isothiocyanate, bromoacetamido, and methylfumaramido alkylating functions were employed in these compounds, some of which had previously been shown to be μ specific (12, BIT) and δ specific (8, FIT and 19, FAO) in vitro. Antinociceptive activity of the title compounds was determined in the mouse hot-plate test, which revealed that certain compounds in each class showed morphine-like activity. The binding EC₅₀ values against [³H]Dalamid for opiate receptors in NG108-15 (δ receptors) and rat brain membranes ($\mu + \delta$ receptors) are also reported. With this type of experiment, it was possible to independently measure the apparent affinity of the etonitazene congeners 12-14 for the μ and δ receptors.

The concept of multiplicity of opiate receptors, originally proposed by Martin² to account for differing pharmacological effects of several opiates in the spinal dog, has received much support from binding and other pharma-cological studies.³⁻⁸ The three major types of opioid receptors in the CNS are commonly referred to as μ , δ , κ .⁹ Some opioids display considerable selectivity in their interaction with these receptor types. The endogenous opioid peptides enkephalin and dynorphin are relatively selective ligands for δ and κ receptors, respectively.^{9,10} Important questions raised by these observations are whether the receptors are structurally related, and what are the physiological roles of these receptors. Answers to these questions can come from experiments performed with opioids which bind irreversibly to one or another of the receptor types to the exclusion of the others. Many attempts at the preparation of affinity reagents for opioid receptors have been made;¹¹⁻²² some of those based on the 3,14-dihydroxy-4,5-epoxymorphinan system are useful probes for the μ receptor.^{16,17}

With the goal of obtaining opioids specific for opiate receptor subpopulations, we have prepared three classes

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of alkylating opioids closely related to fentanyl, etonitazene, and etorphine which are known to be extremely potent in vivo and have high affinity for opioid receptors in vitro. Several of these derivatives have been found to exhibit the desired selectively for irreversible binding to μ or δ receptor types, and the preparation of one of the δ -specific ligands with high specific activity ³H incorporation has previously permitted our identification of a M_{τ} 58000 subunit of the δ receptor.²³ The δ and μ receptor selective agents described here are, we believe, uniquely useful agents for studying questions related to the structure and function of opioid receptor subtypes, as demonstrated in our pre-liminary communications.^{23,24}

Chemistry. Our synthetic strategy for preparation of each of the three classes of alkylating opioid involved incorporation of a strategically located amino substituent and subsequent conversion to an alkylating function. The alkylating functions employed were methylfumaramido, previously utilized by Portoghese and his collaborators for synthesis of the μ -specific β -FNA,¹⁶ and the bromoacetyl and isothiocyanate functions. The latter two functions were selected since we have previously found that the isothiocvanate function served admirably as the acylating group in the first specific irreversible inhibitor of the benzodiazepine receptor,²⁵ and in a subsequent study with the corresponding bromoacetyl derivative,²⁶ we were able to detect heterogeneity of benzodiazepine receptors by virtue of different reactivities of the two alkylating drugs. In the latter study,²⁶ the bromoacetyl and isothiocyanate functions were found to be particularly suitable candidates for the alkylating moieties since neither reacts to any significant extent with hydroxyl groups as shown by their high stability in 70% ethanol at 25 °C. This property thus confers stability in the aqueous media used for receptor binding studies provided no reactive primary or secondary amine-based buffers are used.

In addition, these two functions have distinctly different reactivities toward amino and sulfhydryl functions in this medium.²⁶ The isothiocyanate function reacted completely with a moderate excess of diethylamine or 1-propanethiol at 25 °C within 5 min. Under the same conditions, the bromoacetyl function required ~ 17 h for complete reaction with diethylamine and little, if any, reaction with 1propanethiol occurred during the same period. Similar reactivity (or lack of) for hydroxyl, amino, and sulfhydryl groups present in the receptor matrix may also be obtained for the bromoacetyl and isothiocyanate functions in vitro or in vivo although changes in the order of reactivity cannot be definitely ruled out since enzymatic-like rate

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enhancements probably occur.

Synthesis of the alkylating fentanyl derivatives was straightforward using a modification of the previously reported route²⁷ to fentanyl and congeners. Reaction of commercially available 4-piperidone hydrochloride with (4-nitrophenyl)acetyl chloride afforded amide 2 in which the protecting (4-nitrophenyl)acetyl function served as precursor for the (4-nitrophenyl)ethyl group (see below). Treatment of 2 with aniline under dehydrating conditions gave Schiff base 3 which was reduced directly with sodium borohydride to 4. Borane reduction of the amide function in 4 then gave diamine 5. Acylation of 5 with propionic anhydride afforded the known²⁸ nitrofentanyl 6. Catalytic reduction of 6 over Pd/C provided the key aminofentanyl 7,²⁸ which was converted to the corresponding isothiocyanate (8, fentanyl isothiocyanate, FIT) or bromoacetamido derivative 9 by reaction with a slight excess of thiophosgene or bromoacetic anhydride, respectively, in a two-phase chloroform-aqueous sodium bicarbonate system. For preparation of isothiocyanate 8, and in general, the thiophosgene should be redistilled in order to remove the dimer²⁹ which accumulates on standing and complicates purification of the isothiocyanate.



In the benzimidazole series, etonitazene methylfumaroyl was hydrogenated as the methanesulfonate salt over Pd/Cto afford the amino derivative 11^{30} isolated as the same salt. This salt was converted to the isothiocyanate and bromoacetamido derivatives 12 (benzimidazole isothiocyanate, BIT) and 13, respectively, essentially as described for 8 and 9. Reaction of aminobenzimidazole 11 with methylfumaroyl chloride³¹ in the chloroform-aqueous so-

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dium bicarbonate system afforded the corresponding methylfumaramido 14.



Synthesis of the 7-amino-6,14-endo-ethenomorphinan 15 was accomplished in three steps from thebaine as previously described.³² Selective O-demethylation of the phenolic ether function of 15 proceeded smoothly with boron tribromide as in the conversion of codeine to morphine³³ to afford 16, which was remethylated to 15 with diazomethane in order to insure that no carbon-nitrogen skeleton rearrangement had occurred during O-demethylation. The conversion of 16 to the isothiocyanate, bromoacetamido, and methylfumaramido derivatives 17, 18, and 19 (fumaramidooripavine, FAO), respectively, was then easily accomplished as described above in the fentanyl and benzimidazole series.



15:
$$R_1 = CH_3$$
, $R_2 = NH_2$
16: $R_1 = H$, $R_2 = NH_2$
17: $R_1 = H$, $R_2 = NCS$
18: $R_1 = H$, $R_2 = NHCOCH_2Br$
19: $R_1 = H$, $R_2 = NHCOCH_2Br$

Biological Results. We have previously presented evidence that the alkylating compounds prepared in this study react irreversibly with opioid receptors^{23,24} and that BIT shows specificity for μ receptors and FIT and FAO are specific for δ receptors.²⁴ Direct evidence for covalent binding of [³H]FIT to receptors from neuroblastoma × glioma hybrid NG 108-15 cells was obtained by showing that the complex formed between [³H]FIT and a protein migrating upon electrophoresis in NaDodSO₄ gels with a mobility corresponding to a molecular weight of 58 000 is stable to denaturation by NaDodSO₄ and to boiling in SDS, urea, dithiothreitol mixtures.²³ Formation of this complex could be blocked by active but not inactive enantiomers of opiates and is, therefore, receptor linked. Secondly, we have shown that incubation of membranes isolated from neuroblastoma × glioma hybrid NG108-15 cells with the alkylating ligands followed by thorough washing results in the loss of receptor binding sites without change in the affinity of any remaining opioid binding sites.²⁴ A similar decrease in μ or δ receptor number also occurs in membranes from rat brain after removal of the free alkylating ligand by extensive washing.²⁴ Thus, even through no free alkylating ligand remains after washing, receptor numbers are diminished. These results strongly suggest that covalent bonds have indeed been formed between the receptors and the ligands.

A summary of some of the biological activities of the compounds prepared in this study is presented in Table I. Interestingly, compounds in each of the series have morphine-like antinociceptive potencies even though some, like BIT, are μ selective whereas others like FIT and FAO are δ selective in vitro.²⁴ Of course, we cannot say, from these experiments, whether the compounds reach the brain as such or as metabolites of unknown selectively. In binding studies against [³H]Dalamid which binds equally well to μ and δ receptors, the fentanyl and oripavine derivatives bind to membranes of NG108-15 cells with good affinity, whereas the etonitazene derivatives do not. The observations are consistent with our previous finding²⁴ that FIT and FAO and their congeners are δ selective and BIT is μ selective because NG108-15 membranes have only δ receptors.34

With brain membranes it is possible to measure etonitazene congener binding to μ and δ receptors independently against [³H]Dalamid since affinities for these differ by 2 orders of magnitude (or more in the case of the parent compound).²⁴ Thus, BIT and its relatives bind much more avidly to μ than to δ receptors, as expected. It is not possible with this kind of experiment to separate μ and δ binding with the other compounds, but our earlier observations showed that FIT and FAO are strongly δ selective.²⁴ The binding EC₅₀ values measured here with brain membranes for FIT and FAO and their congeners included variable contributions of binding to μ and δ sites.

Experimental Section

Melting points were determined on a Fisher-Johns apparatus and are corrected. NMR spectra were recorded with a Varian 220-MHz spectrometer. Electron-ionization mass spectra (EIMS) were obtained with a Hitachi Perkin-Elmer RMU-6E spectrometer (70 eV). Chemical-ionization mass spectra (CIMS) were obtained with a Finnigan 1015D spectrometer with a Model 6000 data collection system and high-resolution mass spectra were obtained with a V.G. Micromass 7070F spectrometer. Column chromatography was performed with 230-400- mesh EM silica gel. Mass spectra and elemental analysis were obtained from the Section on Analytical Services and Instrumentation, NIADDK. All compounds gave NMR and IR spectra consistent with their structure.

1-[(4-Nitrophenyl)acetyl]-4-piperidone (2). Powdered piperidin-4-one hydrate hydrochloride (1; 19.18 g, 124 mmol) was added to a mechanically stirred biphasic mixture of (4-nitrophenyl)acetyl chloride (24.78 g, 124 mmol) in CH₂Cl₂ (300 mL) and NaHCO₃ (31.88 g, 374 mmol) in H₂O (300 mL). After 1 h the CH₂Cl₂ layer was collected, combined with a CH₂Cl₂ (100 mL) extract of the aqueous phase, washed with 2 N HCl (100 mL), dried (MgSO₄), and evaporated to an oil which crystallized. The crystals were washed with ether (200 mL) to yield pure 2 as a yellow crystalline solid (29.0 g, 89%): mp 102.9–104.3 °C; EIMS,

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compd	antinociceptive act.: ^b ED ₅₀ , µmol/kg, sc	binding EC ₅₀ , ^d nM			
		NG108-15	rat brain		
			μ	δ	
8 (FIT)	$5.0(3.6-7.4)^e$	8	300		
9.HCl	>20	200	500		
12 (BIT)	$2.7 (2.1 - 3.9)^{c}$	280	10 <i>°</i>	800 ^e	
13 `	>102°	200	40	1500	
14	$13.6(9.6-17.6)^{\circ}$	240	10	800	
17 · HCl	3.6 (2.6-5.0)	20	50		
18	$4.7(3.4-6.5)^{c}$	50	70		
19 (FAO)	$3.5(2.2-5.8)^{c}$	50	50		
(-)-morphine sulfate	2.9 (2.5-3.3)	50			

Table I. Properties of Irreversible Opiate Ligands^a

^a See biological methods in the Experimental Section. ^b 95% confidence limits shown in parentheses, as obtained through computerized probit analysis. ^c Dissolved by addition of 1.0 equiv of aqueous HCl and further diluted. ^d The EC₅₀ values are based on two or more determinations and are considered reliable within a factor of 2. ^e Because etonitazene and its congeners bond to μ sites much more tightly than to δ sites, displacement of the nonselective ligand [³H]-D-Ala³-Met⁵-enkephalinamide by these compounds is biphasic and can easily be separated into two classes.²⁴ This is not the case with the other ligands tested, which are primarily directed at δ sites as shown by our earlier studies.²⁴

m/e 262 (M⁺). Anal. (C₁₃H₁₄N₂O₄) C, H, N.

1-[(4-Nitrophenyl)acetyl]-4-(phenylamino)piperidine (4). A mixture of 2 (29.0 g, 111 mmol), aniline (11.1 mL, 122 mmol), toluene (450 mL), and a few crystals of p-TsOH was refluxed with a Dean-Stark trap for 5 h. The mixture was cooled, decanted from a residual tar, and evaporated to give crude 3 as a red oil. The oil was taken up in MeOH (200 mL) and stirred for 45 min with NaBH₄ (7.07 g, 186 mmol). The mixture was then diluted with H₂O (300 mL), extracted with CH₂Cl₂ (2 × 300 mL), dried (MgSO₄), and evaporated to a red oil. Trituration with ether (200 mL) gave product 3 as a yellow solid, which was collected, dissolved in MeOH (500 mL). Cooling (-70 °C) yielded pure 4 as yellow crystals: 12.6 g (35% yield); mp 159-160 °C; CIMS (NH₃), m/e 340 (M + 1)⁺. Anal. (C₁₉H₂₁N₃O₃) C, H, N.

1-[2-(4-Nitrophenyl)ethyl]-4-(phenylamino)piperidine (5). A solution of BH₃ (100 mmol) in THF (100 mL) was added slowly to a stirred solution of 4 (11.15 g, 34.3 mmol) in THF (100 mL). The solution was stirred at reflux for 2 h, then diluted with MeOH (20 mL), and stirred an additional 30 min. Evaporation of solvent gave an orange solid, which was heated at reflux for 1 h with a mixture of MeOH (100 mL) and 2 N HCl (100 mL). The solution was made alkaline by addition of concentrated aqueous NaOH, extracted with CH₂Cl₂ (2 × 100 mL), washed with brine (100 mL), dried (MgSO₄), and evaporated to an oil. Crystallization (-70 °C; ether-ligroin, 1:1) gave 5 as yellow crystals (4.4 g). Purification of the filtrate by silica gel flash chromatography (3% MeOH in CH₂Cl₂) gave additional 5 (1.8 g; 6.2 g total yield, 56%): mp 90–92 °C; CIMS (NH₃), m/e 326 (M + 1)⁺. Anal. (C₁₉H₂₃N₃O₂) C, H, N.

N-[1-[2-(4-Nitrophenyl)ethyl]-4-piperidinyl]-N-phenylpropanamide (6). A solution of 5 (5.09 g, 15.7 mmol) and propionic anhydride (4.2 g, 32 mmol) in toluene (30 mL) was refluxed for 3 h, diluted with 30% aqueous NH₄OH (2 mL), stirred at 20 °C for 5 min, then partitioned between saturated aqueous NaH-CO₃ (100 mL) and CH₂Cl₂ (2 × 100 mL), dried (MgSO₄), and evaporated to an orange oil. Crystallization (ether-ligroin) gave 6 as light yellow crystals (4.73 g, 79%), mp 114-116 °C. A sample was recrystallized for analysis: mp 117-119 °C (lit.²⁸ mp 114-119 °C); EIMS, m/e 381 (M⁺). Anal. (C₂₂H₂₇N₃O₃) C, H, N.

N - [1 - [2 - (4 - Aminophenyl)ethyl] - 4 - piperidinyl] - N - phenylpropanamide Hydrochloride (7 · HCl). A solution of 6 (4.5 g, 10.6 mmol) in EtOH (200 mL) was hydrogenated at 40 psi of H₂ over 5% Pd/C (400 mg) for 1.5 h. Filtration through Celite and evaporation of solvent gave a white solid, which was crystallized from ether (-70 °C) to yield 7 (3.30 g) as white crystals, mp 150-153 °C (lit.²⁸ mp 150-151 °C). Conversion to the hydrochloride salt and crystallization from ether-MeOH yielded 7 · HCl (3.4 g, 93%): mp 203-212 °C; high-resolution MS (C₂₂- H₂₉N₃O) calcd 351.2310, found 351.2303.

N-[1-[2-(4-Isothiocyanatophenyl)ethyl]-4-piperidinyl]-N-phenylpropanamide (FIT, 8). A biphasic mixture of 7-HCl (500 mg, 1.18 mmol) and NaHCO₃ (693 mg, 8.25 mmol) in H₂O (10 mL) and CHCl₃ (30 mL) was stirred with ice-bath cooling while

redistilled thiophosgene (108 μ L, 1.41 mmol) was added. After 15 min the CHCl₃ layer was removed, combined with a CHCl₃ extract (15 mL) of the aqueous layer, dried (MgSO₄), and evaporated to a yellow oil. The oil was dissolved in ether (3 mL), diluted with ligroin (20 mL), and filtered to remove an insoluble residue (18 mg). The filtrate was cooled, yielding 8 as a pure crystalline solid (234 mg, 50%): mp 88–91 °C; EIMS, m/e 393 (M⁺). Anal. (C₂₃H₂₇N₃SO) C, H, N.

N - [1 - [2 - [4 - (Bromoacetamido) phenyl]ethyl] - 4piperidinyl]-N-phenylpropanamide Hydrochloride (9·HCl).A biphasic mixture of 7·HCl (424 mg, 1.0 mmol) and NaHCO₃(588 mg, 7.0 mmol) in CHCl₃ (30 mL) and H₂O (10 mL) was stirredwith ice-bath cooling while bromoacetic anhydride (345 mg, 1.33mmol) in CHCl₃ (1 mL) was added. After 20 min the reactionwas worked up as above to yield a foam which solidified upontrituration with ether (-70 °C). The solid was dissolved in MeOH(5 mL) and acidified with ethereal HCl (10 mL), yielding crude9-HCl as a solid. Recrystallization from MeOH-ether gave 9·HClas pure white crystals (135 mg, 26%): mp 221-224 °C; CIMS(CH₄) <math>m/e 472, 474 (M + 1)⁺. Anal. (C₂₄H₃₀BrN₃O₂·HCl) C, H, N.

5-Amino-1-[2-(diethylamino)ethyl]-2-(4-ethoxybenzyl)benzimidazole (11). A solution of 1-[2-(diethylamino)ethyl]-2-(4-ethoxybenzyl)-5-nitrobenzimidazole methanesulfonate (10-CH₃SO₃H, etonitazene methanesulfonate)³⁰ (5.00 g, 10.2 mmol) in H₂O (50 mL) was diluted with EtOH (80 mL) and hydrogenated for 2 h at 45 psi of H₂ over 10% Pd/C (500 mg). Filtration through Celite and evaporation of solvent gave a syrup which was crystallized from 2-propanol (30 mL)-ether (130 mL) to yield 11-CH₃SO₃H as white crystals (4.0 g, 83%): mp 203-206 °C dec; high-resolution MS (C₂₂H₃₀N₄O) calcd 366.2419, found 366.2419. This compound was previously reported as the base-H₂O.³⁰

1-[2-(Diethylamino)ethyl]-2-(4-ethoxybenzyl)-5-isothiocyanatobenzimidazole (BIT, 12). To a biphasic mixture of 11·CH₃SO₃H (2.0 g, 4.33 mmol) and NaHCO₃ (2.96 g, 35.4 mmol) in H₂O (20 mL) and CHCl₃ (50 mL) was added redistilled thiophosgene (512 μ L, 6.7 mmol). After 20 min the CHCl₃ layer was collected, combined with a CHCl₃ extract (50 mL), dried (MgSO₄), and evaporated. Crystallization of the residue from ether (-70 °C) gave 12 as analytically pure crystals (530 mg, 30%): mp 67–68 °C; EIMS, m/e 408 (M⁺). Anal. (C₂₃H₂₈N₄OS) C, H, N.

5-(Bromoacetamido)-1-[2-(diethylamino)ethyl]-2-(4-ethoxybenzyl)benzimidazole (13). To a biphasic mixture of 11-CH₃SO₃H (924 mg, 2.0 mmol) and NaHCO₃ (1.17 g, 13.9 mmol) in H₂O (20 mL) and (50 mL) was added with ice-bath cooling a solution of bromoacetic anhydride (728 mg, 2.8 mmol) in CHCl₃ (2 mL). After 45 min the CHCl₃ layer was removed, combined with a CHCl₃ extract of the aqueous phase (25 mL), dried (Mg-SO₄), and evaporated, yielding a foam. Trituration with ether gave 13 as a white solid (935 g, 80%): mp 96 °C, resolidifying, then decomposing above 220 °C. Anal. (C₂₄H₃₁BrN₄O₂) C, H, N. This compound forms dimeric and trimeric quaternary salts after standing in solution or prolonged storage as crystalline material.

1-[2-(Diethylamino)ethyl]-2-(4-ethoxybenzyl)-5-(methylfumaramido)benzimidazole (14). A solution of 11·CH₃SO₃H (1.38 g, 3 mmol) and NaHCO₃ (1.76 g, 21 mmol) in H₂O (20 mL) was stirred on an ice bath with CHCl₃ (30 mL) and methylfumaroyl chloride³¹ (660 mg, 4.5 mmol) in CHCl₃ (5 mL) was added over 10 min. After 20 min the CHCl₃ layer was removed, combined with a CHCl₃ (40 mL) extract of the aqueous layer, washed with saturated aqueous NaHCO₃ (40 mL) and brine (30 mL), decolorized with charcoal, dried (MgSO₄), and evaporated to a brown foam. The foam was mixed with ether, decanted from an insoluble residue, and allowed to stand at 20 °C, yielding 14 as white crystals (340 mg, 25%): mp 143-146 °C; high-resolution MS (C₂₇H₃₄N₄O₄) calcd 478.2580, found 478.2550. Anal. (C27H34N4O4) C, H, N.

 7α -Amino-6,14-endo-ethenotetrahydrooripavine (16). A solution of 7α -amino-6,14-endo-ethenotetrahydrothebaine (15³²) (4.95 g, 14 mmol) was added during 2 min to a well-stirred solution of BBr₃ (21.25 g, 84.8 mmol) in CHCl₃ (80 mL) maintained at 23-24 °C. After 15 min the reaction mixture was poured into a well-stirred mixture of 30% aqueous NH4OH (50 mL) and ice (200 g). The CHCl₃ layer was removed, reduced in volume to 20 mL, and shaken with the aqueous phase. The resulting crystalline material was filtered and washed with H₂O. The crude product was dissolved in a minimum volume of MeOH, filtered, and evaporated to an oil which was crystallized from cold acetone to yield 16 as colorless prisms (3.24 g, 68%): mp 139-140 °C; CIMS (NH_3) , m/e 341 $(M + 1)^+$. The dioxalate was crystallized from a MeOH-acetone mixture for analysis: mp 246-248 °C dec. Anal. (C₂₀H₂₄N₂O₃·2(CO₂H)₂) C, H, N. The hydrochloride salt was crystallized from EtOH as colorless prisms; mp >300 °C dec.

 7α -Isothiocyanato-6,14-endo-ethenotetrahydrooripavine Hydrochloride (17·HCl). A solution of 16·HCl (413 mg, 1 mmol) in H₂O (15 mL), CHCl₃ (30 mL), and NaHCO₃ (504 mg, 6 mmol) was stirred for 5 min and then treated with redistilled thiophosgene (126 mg, 1.1 mmol). After 15 min the $CHCl_3$ layer was removed, washed successively with dilute aqueous NH_4OH (2 × 25 mL) and H₂O (2 \times 25 mL), dried (Na₂SO₄), treated with methanolic HCl, and evaporated. Crystallization from MeOHacetone gave 17 HCl as colorless prisms (265 mg, 63%); mp >300 °C dec; CIMS (NH₃), m/e 383 (M + 1)⁺. Anal. (C₂₁H₂₂N₂O₃·H-Cl·2H₂O) C, H, N.

7α-(Bromoacetamido)-6,14-endo-ethenotetrahydrooripavine (18). In a manner similar to above, 16-HCl (413 mg, 1 mmol) was treated with bromoacetic anhydride (286 mg, 1.1 mmol) in a mixture of H₂O (15 mL), CHCl₃ (30 mL), and NaHCO₃ (504 mg, 6 mmol). The crude product was purified by flash chromatography and crystallized from ether to afford 18 as colorless prisms (280 mg, 61%): mp 297-301 °C dec; CIMS (NH₃), m/e 461, 463 (M + 1)⁺. Anal. (C₂₂H₂₅N₂O₄Br¹/₂H₂O) C, H, N.

 7α -(Methylfumaramido)-6.14-endo-ethenotetrahydrooripavine (FAO, 19). In a manner similar to above, reaction

of 16-HCl (413 mg, 1 mmol), NaHCO₃ (504 mg, 6 mmol), and methylfumaroyl chloride³¹ (148 mg, 1 mmol) in H_2O (15 mL) with CHCl₃ (30 mL) afforded a crude product (494 mg), which was purified by silica gel flash chromatography to afford pure material. Crystallization from CH_2Cl_2 -ligroin gave 19 as colorless flakes (375 mg, 82%); mp 165–166 °C; CIMS (NH₃), m/e 453 (M + 1)⁺. Anal. $(C_{25}H_{28}N_2O_6^{,1}/_2H_2O)$ C, H, N.

Biological Methods. Antinociceptive activities of the compounds were measured after subcutaneous injection in the mouse hot-plate assay as previously described.³⁴⁻³⁶

Opiate binding was measured to membranes of neuroblastoma \times glioma hybrid cell NG108-15 membranes⁸⁷ which contain only δ receptors³⁸ and to rat brain membranes³⁹ which contain both μ and δ receptors. In all experiments the radiolabeled ligand used was [³H]-D-Ala²-Met⁵-enkephalinamide (Dalamid), a ligand with equal affinities for μ and δ sites.⁶ Membranes were incubated with 2 nM [³H]Dalamid in 10 mM Tris-Cl, pH 8, at 37 °C in the presence of competing ligand at concentrations between 10⁻¹⁰ and 10^{-5} M. At the end of the incubation, the samples were rapidly cooled to 4 °C in ice and membrane-bound ligand was separated from free with the aid of prepacked, 9-mL columns of Sephadex G-25 as described previously. The membranes with bound ligand emerge in the first 4.5 mL eluted from these columns and are collected directly into scintillation vials and assayed for radioactivity after addition of 12 mL of Aquasol. Etonitazene and its derivatives displace Dalamid from brain membranes in a biphasic manner, and the curves are easily dissected into μ and δ components as shown earlier²⁴ and as indicated here. Other evidence for type specificity of these compounds was presented earlier.²⁴

Registry No. 1, 41979-39-9; 2, 91742-66-4; 3, 91742-67-5; 4, 91742-68-6; 5, 91742-69-7; 6, 1640-11-5; 7, 1169-73-9; 7·HCl, 91742-70-0; 8, 85951-63-9; 9, 91742-72-2; 9-HCl, 91742-71-1; 10-CH₃SO₃H, 58-75-3; 11, 91742-73-3; 12, 85951-65-1; 13, 91742-80-2; 14, 91742-81-3; 15, 24501-03-9; 16, 87453-63-2; 16.2(CO₂H)₂, 91742-74-4; 16·HCl, 91742-75-5; 17, 91742-77-7; 17·HCl, 91742-76-6; 18, 91742-78-8; 19, 91742-79-9; (4-nitrophenyl)acetyl chloride, 50434-36-1; aniline, 62-53-3; propionic anhydride, 123-62-6; thiophosgene, 463-71-8; bromoacetic anhydride, 13094-51-4; methylfumaroyl chloride, 17081-97-9.

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