

14 β -[(*p*-Nitrocinnamoyl)amino]morphinones, 14 β -[(*p*-Nitrocinnamoyl)amino]-7,8-dihydromorphinones, and Their Codeinone Analogues: Synthesis and Receptor Activity

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A series of 14 β -[(nitrocinnamoyl)amino]codeinones and morphinones, some of which contain a 5 β -methyl group, were prepared from 14 β -aminocodeinones and 14 β -[*N*-(cyclopropylmethyl)-amino]norcodeinones. The affinities of the target compounds for the μ , δ , and κ opioid receptors were determined by radiolabeled binding experiments using bovine brain membranes. An analogous series of 7,8-dihydrocodeinones and morphinones was prepared and assayed in the same systems. The 3-methoxy derivatives 3 and 4 were more selective than the corresponding morphinones for the μ receptor. The 5 β -methylcodeinones 25 and 27 had lower affinity at all receptors than the corresponding morphinones, but the 5 β -methylmorphinones had affinities similar to the morphinones 5 and 6. A similar pattern was observed in the 7,8-dihydro series. Two compounds, 5 β -methyl-14 β -[(*p*-nitrocinnamoyl)amino]-7,8-dihydromorphinone, 20 (MET-CAMO), and *N*-(cyclopropylmethyl)-14 β -[(*p*-nitrocinnamoyl)amino]-7,8-dihydronormorphinone, 22 (*N*-CPM-MET-CAMO), acted as nonequilibrium ligands in antinociception and membrane binding studies. In mice after icv administration, neither ligand showed any agonist activity but 8–24 h after administration both compounds acted as potent μ antagonists. A Scatchard plot of the effect of *N*-CPM-MET-CAMO on [³H]DAMGO ([³H]D-Ala², (Me)-Phe⁴, Gly(ol)⁵]enkephalin binding to bovine striatal membranes showed that there was a significant decrease in the B_{\max} value and a marginal effect on the K_d value suggesting that the number of binding sites was reduced. When taken together, these results support the view that 20 and 22 bind covalently to the μ receptor. On the other hand, when *N*-acetylcysteine and 22 were allowed to react in a buffered solution, 22 was recovered unchanged. Under these conditions no Michael reaction was observed.

Introduction

Recently, papers have appeared describing the biological activities of some 14 β -[(*p*-halocinnamoyl)amino]- and [(*p*-methylcinnamoyl)amino]-7,8-dihydromorphinones and codeinones.^{1,2} The dihydromorphinones showed long-acting narcotic antagonist properties, and the dihydrocodeinones were partial agonists in the mouse tail-flick assay.¹ One member of this series, 14 β -[(*p*-chlorocinnamoyl)amino]-7,8-dihydro-*N*-(cyclopropylmethyl)normorphinone (clocinnamox, C-CAM), was studied more extensively.² The authors concluded that C-CAM was producing its antagonistic effect via a nonequilibrium mechanism and that the "C-14 substituent probably confers a weak Michael acceptor function to the compound".² In support of this suggestion Burke³ reported that, in a Scatchard analysis, C-CAM caused a reduction in the number of binding sites without affecting the K_d value.

Since a nitro group is more electron-withdrawing than either a halogen or a methyl group, it was anticipated that replacing the *p*-chloro and *p*-methyl substituents on the cinnamoylamino moiety with a *p*-nitro group would make the cinnamoylamino function as a better Michael acceptor.

The synthesis and some biological properties of such compounds are the subject of this paper.

Chemistry

The synthesis of the 14 β -[(*p*-nitrocinnamoyl)amino]-morphinones and 7,8-dihydromorphinones is shown in Scheme I. 14 β -Aminocodeinone (1) prepared from thebaine by the method of Kirby and McLean⁴ was condensed with *p*-nitrocinnamoyl chloride to give 3. Demethylation of 3 with the aid of BBr₃ furnished the morphinone 5. *N*-(Cyclopropylmethyl)northebaine⁵ was converted to 2 by the Kirby–McLean procedure⁴ and was acylated to give 4. Demethylation of 4 furnished the morphinone 6. Catalytic hydrogenation of 1 and 2 gave 7 and 8, respectively, and these 7,8-dihydro intermediates were converted to their respective (*p*-nitrocinnamoyl)amino derivatives 9, 10, 11, and 12 by acylation with *p*-nitrocinnamoyl chloride to give 9 and 10 followed by BBr₃ demethylation to give 11 and 12.

Several years ago it was reported that 5 β -methyl-7,8-dihydromorphinone (metopon) was a more effective oral analgesic than morphine.⁶ Widespread clinical use was hindered by the difficulty in the original synthesis of the compound, particularly with respect to the introduction of the 5 β -methyl group.⁷ Since that time greatly improved procedures have been developed for the direct one-step introduction of the 5 β -methyl group into thebaine.^{8,9} A number of 5 β -methyl-14-[(*p*-nitrocinnamoyl)amino]morphinones and 7,8-dihydromorphinones were prepared by

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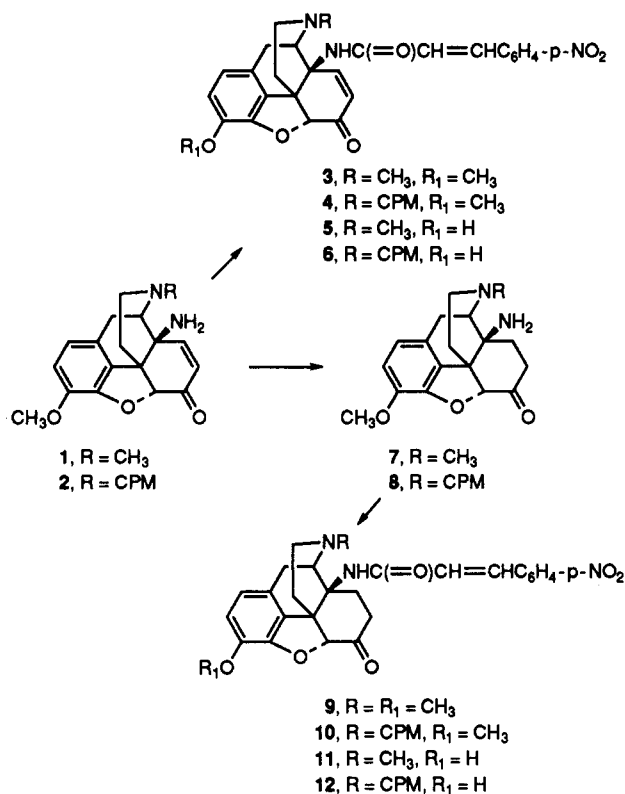
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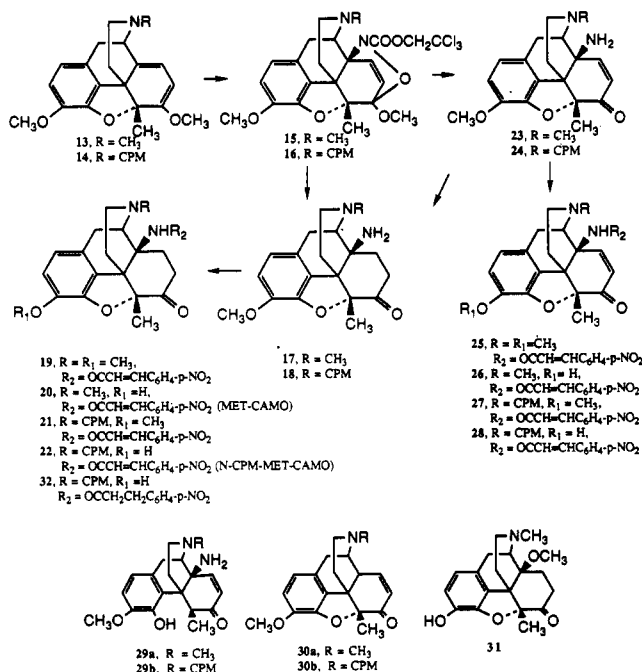
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Scheme I



Scheme II



the methods shown in Scheme II. 5β-Methylthebaine^{8,9} (13) and *N*-(cyclopropylmethyl)-5β-methylnorthebaine (14) were converted to their respective adducts 15 and 16 by the Kirby-McLean procedure.⁴ Reductive cleavage of these adducts using HCl in ethylene glycol followed by zinc reduction in the presence of NH₄Cl and finally hydrolysis gave not only the desired 14β-amino-5β-methylcodeinone derivatives 23 and 24 but also the dihydrothebainones 29a and 29b as well as the 5β-methylcodeinones 30a and 30b. The structures of the dihydrothebainones, formed by cleavage of the C–O bond, were secured by elemental analyses, IR spectra, and NMR

Table I. Physical Data and Opioid Binding Values of the 14β-[(p-Nitrocinnamoyl)amino]morphinones and Codeinones and Their 5β-Methyl Analogues

compd	R	R ₁	R ₂	IC ₅₀ (nM)		
				μ	δ	κ
3	CH ₃	CH ₃	H	1.01	45.5	68
4	CPM	CH ₃	H	1.45	54.0	5.01
5	CH ₃	H	H	1.45	4.35	4.5
6	CPM	H	H	7.8	106.0	10.8
25	CH ₃	CH ₃	CH ₃	36.2	374	171
26	CH ₃	H	CH ₃	0.80	25.9	184
27	CPM	CH ₃	CH ₃	36.8	542.0	274.0
28	CPM	H	CH ₃	1.88	24.7	25.5

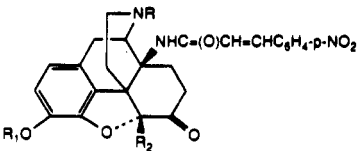
spectra. In the latter, the signal for the 5β-methyl group appeared as a doublet rather than as a singlet as in the case of 30a and 30b showing that ring cleavage occurred in the formation of 29a and 29b. Compounds 30a and 30b resulted from cleavage of the C₁₄–N bond.

Schmidhammer and his colleagues⁹ converted 5β-methylthebaine to 5β-methyl-7,8-dihydrothebainone by catalytic hydrogenation in acetic acid solution using a Pd/C catalyst. If the reduction is carried out in MeOH in the presence of a NaOAc/HOAc buffer, 23 and 24 or preferably the adducts 15 and 16 furnish the codeinone derivatives 17 and 18 without cleavage of the C–O bond. The [(p-nitrocinnamoyl)amino]codeinones 25 and 27 and the corresponding morphinone derivatives 26 and 28 were prepared by the same methods employed to prepare the corresponding analogues shown in Scheme I. The 5β-methyl-7,8-dihydrocodeinones 19 and 21 and the corresponding 5β-methyl-7,8-dihydromorphinone 20 and 22 were prepared the same way.

Biological Results and Discussion

The IC₅₀ values at the μ, δ, and κ receptors for the 14β-(cinnamoylamino)morphinones and codeinones are reported in Table I. The 3-methoxy derivatives 3 and 4 had the same affinities as the morphinone 5 at the μ receptor. Surprisingly, the affinity of 6, a morphinone for all three receptors, was relatively poor. In contrast, the 5β-methylcodeinones 25 and 27 had far lower affinity for all three receptors than the corresponding morphinones 26 and 28. The latter were about as active as the 5β-desmethyl compound 5.

A similar SAR pattern was observed in the 7,8-dihydro series (Table II). The dihydrocodeinones 9 and 10 showed about the same binding affinity for the μ receptor as the dihydromorphinones 11 and 12, but there was a large difference in affinities between the 5β-methyldihydrocodeinones 19 and 21 and the morphinones 20 and 22. The last two compounds were bioassayed in bovine striatal membranes. The binding affinities of 20 and 22 at the μ receptor compared favorably with the morphinone 5 and with the dihydromorphinones 10 and 12, which lack a methyl group at the 5-position of the 4,5-epoxymorphinan ring system. Schmidhammer¹¹ reported that 14β-methoxy-5β-methyl-7,8-dihydromorphinone 31 (14β-methoxymetopon) was 150 times as potent as oxymorphone in the mouse writhing assay and that 14β-methoxynaloxone

Table II. Physical Data and Opioid Binding Values of the 14 β -[(*p*-Nitrocinnamoyl)amino]-7,8-dihydromorphinones and Their 5 β -Methyl Analogues


compd	R	R ₁	R ₂	IC ₅₀ (nM)		
				μ	δ	κ
9	CH ₃	CH ₃	H	0.44	19.7	27.2
11	CH ₃	H	H	0.28	3.5	6.6
10	CPM	CH ₃	H	3.77	248	39.1
12	CPM	H	H	0.87	21.2	12.1
19	CH ₃	CH ₃	CH ₃	104.4	>1 μM	>1 μM
20 ^a	CH ₃	H	CH ₃	0.33 ± 0	50.9 ± 5.2	116 ± 21
21	CPM	CH ₃	CH ₃	32.0	672	>1 μM
22 ^a	CPM	H	CH ₃	0.52 ± 0.07	10.7 ± 0.8	126 ± 2.9
32 ^b	CPM	H	CH ₃	24.0	22.6	162

^a Details of the bioassay methods using bovine striatal membranes are described in ref 10. ^b The 14 β -[(*p*-nitrodihydrocinnamoyl)amino] derivative.

Table III. [³H]DAMGO Saturation Binding to Bovine Striatal Membrane Homogenates Treated in the Absence and Presence of 20 nM of 22

treatment	K _D (nM)	B _{max} (fmol bound)/ mg of protein
none	0.66 ± 0.04	195 ± 3
N-CPM-MET-CAMO	0.82 ± 13	100 ± 18

^a Values are the mean ± SEM of three experiments performed in triplicate.

and 14 β -methoxynaltrexone were substantially less active as narcotic antagonists in the mouse writhing test.

Both 20¹² and 22¹³ behaved as nonequilibrium ligands in bovine striatal membranes. The binding affinities of these ligands were in the same range as that of 31, 14 β -methoxymetopon.¹¹ The latter displayed agonist activity in the mouse writhing assay. In contrast, neither 20 nor 22 produced antinociception in the mouse tail-flick test when administered icv in doses up to 100 nmol, nor did either act as morphine antagonists when given simultaneously with the administration of morphine. However, a 1 nmol dose given by the icv route 8–24 h prior to the administration of 3 nmol of morphine resulted in the complete antagonism of the morphine-induced antinociception.¹² Neither 20 nor 22 antagonized the antinociceptive effects of either δ or κ -mediated antinociception.

A Scatchard plot (Table III) of the effect of 22 on the binding of [³H]DAMGO in bovine striatal membranes resulted in a decrease in the B_{max} without affecting the K_D (Table III). These data are compatible with the hypothesis that 22 binds covalently to the μ receptor.

5 β -Methyl-14 β -[(dihydrocinnamoyl)amino]-7,8-dihydro-*N*-(cyclopropylmethyl)normorphinone, 32, was synthesized from *p*-nitrodihydrocinnamoyl chloride and 18 followed by BBr₃ demethylation. The binding affinities are reported in Table II. This compound behaved as a normal reversible ligand since it washed out of the receptor preparation by normal washing procedures. Since the relevant double bond in 32 was missing, this compound could not behave as a Michael acceptor.

When 22 and *N*-acetylcysteine were allowed to react in an aqueous THF medium at pH 8.0, the ligand 22 was recovered unchanged. In another experiment, the course of the reaction was followed spectrophotometrically by

monitoring the reduction in the λ_{\max} of (*p*-nitrocinnamoyl)-amino UV absorption peak. No change in absorption was observed during the course of the reaction.

Although the biochemical and pharmacological evidence supports the hypothesis that 20 and 22 bind covalently to the μ receptor, the chemical evidence does not. We have reported that 14 β -[(5-azido-2-nitrophenacetyl)amino]-7,8-dihydromorphinone and its N-CPM analogue behave as nonequilibrium ligands at the μ receptor prior to UV irradiation.¹⁵ It is possible that ligands which show very slow dissociation kinetics but do not necessarily bind covalently to the receptor may cause a reduction in the B_{max} value without affecting the K_d values in a Scatchard analysis. In order to determine whether 20 and 22 bind covalently or pseudoirreversibly to the μ receptor we plan to radiolabel 22 and allow it to bind to the receptor in bovine membrane preparations. If the radioactivity persists after extensive washing the membranes will be separated on SDS polyacrylamide gels using DTT to cleave S–S bonds. If the ligand binds covalently to the now denatured receptor it should be visualized easily.

Experimental Section

Melting points were taken on a laboratory Melt-Temp apparatus and are uncorrected. The ¹H-NMR spectra were run on Varian XL-200 MHz and Varian Unity 500 MHz spectrometers. The IR spectra were run on a Perkin-Elmer Model 298 infrared spectrometer. Microanalyses were performed by Atlantic Microlab, Norcross, GA, and are within ±0.4% of the calculated values.

14 β -[(*p*-Nitrocinnamoyl)amino]codeinone (3). A solution of 100 mg of 14 β -aminocodeinone (1)⁵ in 25 mL of CH₂Cl₂ containing 75 μ L of triethylamine was cooled to –30 °C. A solution of *p*-nitrocinnamoyl chloride, freshly prepared from 100 mg of *p*-nitrocinnamic acid and SOCl₂, in 25 mL of CH₂Cl₂ was added dropwise with stirring. The reaction mixture was allowed to warm to room temperature and was stirred for an additional 3 h. At the end of this time, a solution of saturated NaHCO₃ was added carefully, and the organic layer was separated, washed with NaHCO₃ solution and brine, and dried (Na₂SO₄). The solution was taken to dryness, and the residue was chromatographed on a silica gel column using EtOAc/hexane (3:1) as the eluant. There was obtained 110 mg of the desired product, mp 314–316 °C dec. IR (KBr): 3310, 1670, 1620, 1590 cm^{–1}. ¹H NMR (CDCl₃): δ 2.45 (3H, s, NCH₃), 3.08 (1H, d, H₉), 3.20–3.29 (1H, d, H₁₀), 3.83 (3H, s, OCH₃), 4.45 (1H, s, H₅), 6.18–6.23 (2H, q, H₈ and NHC(=O)CH=), 6.57–6.70 (3H, m, H₁, H₂ and H₇), 7.60–7.68 (3H, m, =CHAr and ArH), 8.19–8.23 (2H, d, ArH). Anal. (C₂₇H₂₅N₃O₆·0.5H₂O) C, H.

14 β -[(*p*-Nitrocinnamoyl)amino]-*N*-(cyclopropylmethyl)-norcodeinone (4). A solution of 60 mg of 14 β -amino-*N*-(cyclopropylmethyl)norcodeinone (2)¹⁰ in 15 mL of CH₂Cl₂ containing 40 μ L of triethylamine was cooled to –20 °C with stirring while a solution of *p*-nitrocinnamoyl chloride, prepared from 70 mg of *p*-nitrocinnamic acid and SOCl₂, in CH₂Cl₂ was added dropwise. The reaction mixture was allowed to warm to room temperature, and after being stirred for 1 h, it was treated with a saturated NaHCO₃ solution. The layers were separated, and the organic layer was worked up as described for 3. After chromatography on silica gel using EtOAc/hexane as the eluant there was obtained 35 mg of pure 4, mp 165–167 °C. IR (KBr): 3290, 1670, 1630, 1590 cm^{–1}. ¹H NMR (CDCl₃): δ 3.09–3.18 (1H, d, H₁₀), 3.37–3.39 (1H, d, H₉), 3.82 (3H, s, OCH₃), 4.96 (1H, s, H₅), 6.15–6.28 (2H, q, H₈ and NHC(=O)CH=), 6.51–6.70 (3H, m, H₁, H₂ and H₇), 7.61–7.68 (3H, m, =CHAr and ArH), 8.19–8.23 (2H, d, ArH). Anal. (C₃₀H₂₉N₃O₆·0.5H₂O) C, H.

14 β -[(*p*-Nitrocinnamoyl)amino]morphinone (5). To a cooled (–20 °C) solution of 3 (76 mg, 0.15 mmol) in CH₂Cl₂ was added BBr₃ (63.6 μ L) in CH₂Cl₂ dropwise with stirring. The reaction mixture was slowly brought to room temperature and stirred at this temperature for 4 h. Then 2 mL of MeOH and saturated NaHCO₃ solution were added, and the organic layer

was separated. The aqueous layer was extracted two times with a CHCl₃/MeOH (4:1) mixture. The combined extracts were washed with brine (20 mL) and dried over anhydrous Na₂SO₄, and the solvent was removed under reduced pressure. The residue was chromatographed over silica gel using EtOAc as the eluant to furnish pure **6** (50 mg) as a pale yellow solid, mp 290 °C dec. ¹H NMR (CDCl₃): δ 2.43 (3H, s, NCH₃), 3.1–3.2 (1H, d, H₉), 3.25–3.32 (1H, d, H₁₀), 5.05 (1H, s, H₅), 6.2–6.3 (2H, ABq, H₇ and H₈), 6.58–6.80 (3H, m, H₁, H₂ and NHCOCH=), 7.60–7.75 (3H, m, =CHAr and ArH), 8.20–8.25 (2H, d, ArH). Anal. (C₂₆H₂₃N₃O₆·0.5H₂O) C, H, N.

14 β -[(*p*-Nitrocinnamoyl)amino]-*N*-(cyclopropylmethyl)-normorphinone (6**).** To a cooled solution (–20 °C) of **4** (90 mg, 0.17 mmol) in CH₂Cl₂ was added BBr₃ (63.6 μ L) in CH₂Cl₂ dropwise with stirring. The reaction mixture was stirred at this temperature for 4 h and at room temperature for 30 min. After this period 2 mL of MeOH and saturated NaHCO₃ solution were added, and the organic layer was separated. The aqueous layer was extracted two times with a CHCl₃/MeOH (4:1) mixture. The combined extracts were then washed with brine (20 mL) and dried over anhydrous Na₂SO₄, and the solvent was removed under reduced pressure. The residue was then chromatographed on silica gel with EtOAc/MeOH (4:1) as the eluant to furnish pure **6** (65 mg) as a pale yellow solid, mp 305–310 °C dec. ¹H NMR (DMSO-*d*₆) δ 4.81 (1H, s, H₅), 6.01–6.07 (1H, d, H₈), 6.42–6.57 (1H, m, H₁, H₂ and H₇), 7.14–7.21 (1H, d, NHCOCH=), 7.56–7.58 (1H, d, =CHAr), 7.85–7.89 (2H, d, ArH), 8.25–8.29 (2H, d, ArH), 9.12 (1H, s, HOAr). Anal. (C₂₆H₂₇N₃O₆) C, H, N.

14 β -[(*p*-Nitrocinnamoyl)amino]-7,8-dihydrocodeinone (9**).** A solution of 200 mg of dihydrocodeinone (**7**) and triethylamine (95 μ L) in 40 mL of dry CH₂Cl₂ was cooled to –20 °C. To this solution was added *p*-nitrocinnamoyl chloride, freshly prepared from 193 mg of *p*-nitrocinnamic acid and SOCl₂, in 15 mL of CH₂Cl₂ dropwise with stirring. The reaction mixture was slowly brought to room temperature and stirred for 2.5 h. The solution of saturated NaHCO₃ was added, and the organic layer was separated. It was washed with saturated NaHCO₃ solution (15 mL) and water (2 \times 15 mL) and dried (Na₂SO₄), and the solvent was evaporated under reduced pressure. The solid residue was chromatographed on silica gel using EtOAc as the eluant to furnish 150 mg of the desired product **9**, mp 278–280 °C. IR (KBr): 3350, 2920, 1710, 1670, 1620, 1500 cm^{–1}. ¹H NMR (CDCl₃): δ 2.44 (3H, s, NCH₃), 3.06–3.07 (1H, d, H₉), 3.23–3.27 (1H, d, H₁₀), 3.89 (3H, s, ArOCH₃), 4.95 (1H, s, H₅), 6.68–6.77 (3H, m, H₁, H₂, and NHCOCH=), 7.70–7.73 (3H, m, =CHAr and ArH), 8.25–8.27 (2H, d, ArH). Anal. (C₂₇H₂₇N₃O₆·0.25H₂O) C, H, N.

14 β -[(*p*-Nitrocinnamoyl)amino]-7,8-dihydro-*N*-(cyclopropylmethyl)norcodeinone (10**).** A solution of 250 mg of **8**¹⁴ and triethylamine (100 μ L) in dry CH₂Cl₂ was cooled to –20 °C. A solution of *p*-nitrocinnamoyl chloride, freshly prepared from *p*-nitrocinnamic acid (200 mg) and SOCl₂, in CH₂Cl₂ was added dropwise with stirring. The reaction mixture was slowly brought to room temperature and stirred for 1 h. After this period the reaction mixture was washed with saturated NaHCO₃ solution and water and dried (Na₂SO₄). The solvent was evaporated to leave a solid residue, which was chromatographed on silica gel using EtOAc/hexane (3:2) as the eluant. There was obtained 230 mg of **10**, mp 130–133 °C. IR (KBr): 3400, 2910, 2810, 1710, 1660, 1620, 1600, 1500 cm^{–1}. ¹H NMR (CDCl₃): δ 3.10–3.14 (1H, d, H₁₀), 3.35–3.36 (1H, d, H₉), 3.88 (3H, s, OCH₃), 4.93 (1H, s, H₅), 6.63–6.76 (3H, m, H₁, H₂ and NHCOCH=), 7.67–7.72 (3H, m, =CHAr and ArH), 8.25–8.26 (2H, d, ArH). Anal. (C₃₀H₃₁N₃O₆·H₂O) C, H, N.

14 β -[(*p*-Nitrocinnamoyl)amino]-7,8-dihydromorphinone (11**).** To a cooled (–20 °C) solution of **9** (120 mg) in dry CH₂Cl₂ (40 mL) was added BBr₃ (93 μ L) dissolved in CH₂Cl₂ dropwise with stirring. The mixture was slowly brought to room temperature and stirred for 1.5 h. After this period MeOH (5 mL) and saturated NaHCO₃ solution were added, and the organic layer was separated. The aqueous layer was further extracted twice with CHCl₃/MeOH (3:1) (2 \times 40 mL). The combined extracts were washed with saturated NaHCO₃ solution (15 mL) and water (15 mL) and dried, and the solvent was removed under reduced pressure. The solid residue was chromatographed on silica gel using EtOAc/MeOH (19:1) as the eluant to yield 50 mg of **11** as a light yellow solid, mp 245–247 °C. IR (KBr): 3350,

2910, 1705, 1660, 1620, 1500 cm^{–1}. ¹H NMR (CDCl₃): δ 2.42 (3H, s, NCH₃), 5.07 (1H, s, H₅), 6.59–6.75 (3H, m, H₁, H₂ and NHCOCH=), 7.49–7.51 (2H, d, ArH), 7.62–7.65 (1H, d, =CHAr), 8.16–8.18 (2H, d, ArH). Anal. (C₂₆H₂₅N₃O₆·0.5H₂O) C, H, N.

5 β -Methylthebaine (13**).** A solution of 10 g of thebaine in 500 mL of dry THF was stirred and cooled to –78 °C in a nitrogen atmosphere, and to it was added a hexane solution of 3.08 g of *n*-BuLi. After the solution was stirred for 20 min, 6.1 g of (CH₃)₂SO₄ was added in one portion, and the reaction mixture was allowed to warm to room temperature over a 3-h period. Water was added, and the THF was removed under reduced pressure to leave a suspension which was extracted with CHCl₃. The extract was washed with H₂O and dried. The residue left after evaporation of the CHCl₃ was crystallized from EtOH to give 7.5 g (72%) of the desired product, mp 159–160 °C (lit.⁸ mp 154–157 °C).

***N*-(Cyclopropylmethyl)-5 β -methylnorthebaine (**14**).** This compound was prepared from 9.1 g of *N*-cyclopropylnorthebaine, 2.5 g of BuLi, and 4.9 g of dimethyl sulfate by the same procedure described above in 95% yield, mp 116 °C, after crystallization from EtOH. IR (KBr): 3000, 2900, 2800, 1600, 1450, 1250 cm^{–1}. ¹H NMR (CDCl₃): δ 1.72 (3H, s, 5 β -CH₃), 2.43–2.46 (2H, d, NCH₂), 3.53 (3H, s, C₅-OCH₃), 3.80 (3H, s, C₃-OCH₃), 4.88–4.92 (1H, d, H₉), 5.51–5.59 (1H, d, H₇), 6.55–6.63 (2H, m, H₁ and H₂). Anal. Calcd for (C₂₃H₂₇N₃O₃) C, H, N.

14 β -[(*p*-Nitrocinnamoyl)amino]-7,8-dihydro-*N*-(cyclopropylmethyl)normorphinone (12**).** To a cooled (–20 °C) solution of **10** (100 mg) in CH₂Cl₂ was added BBr₃ (72 μ L) dissolved in CH₂Cl₂ dropwise with stirring. The reaction mixture was slowly brought to room temperature and stirred for 2 h. After this period MeOH (3 mL) and a saturated solution of NaHCO₃ were added, and the organic layer was separated. The aqueous layer was extracted again with CHCl₃/MeOH (3:1) (2 \times 25 mL), the combined extracts were washed with saturated NaHCO₃ solution (15 mL) and water (15 mL) and dried (Na₂SO₄), and the solvent was evaporated. The residue was chromatographed on silica gel using EtOAc/hexane (3:2) as the eluant to give the desired product **12** (45 mg), mp 260–263 °C. IR (KBr): 3250, 2910, 1710, 1660, 1620, 1500 cm^{–1}. ¹H NMR (CDCl₃): δ 4.9 (1H, s, H₅), 6.58–6.73 (3H, m, H₁, H₂ and NHCOCH=), 7.64–7.67 (3H, m, =CHAr, ArH), 8.22–8.23 (2H, d, ArH). Anal. (C₂₉H₂₉N₃O₆·0.75H₂O) C, H, N.

5 β -Methyl-19-[(2,2,2-trichloroethoxy)carbonyl]-6,14-dihydro-6,14-(epoxyimino)thebaine (15**).** A solution of 6.0 g of 5 β -methylthebaine (**13**) in 200 mL of EtOAc was added to a cooled (0 °C) solution of 5.89 g of NaIO₄ in 150 mL of 0.5 M NaOAc solution at pH 6.0. To the vigorously stirred solution there was added 3.8 g of 2,2,2-trichloroethyl *N*-hydroxycarbamate in small portions over a period of 30 min. The mixture was stirred at 0 °C for 3 h and then treated with excess aqueous NaHCO₃. The organic layer was separated, washed with a solution of Na₂S₂O₃ and brine, and dried (Na₂SO₄). Evaporation of the EtOAc solution left an oily residue which was covered with a small amount of MeOH and stored at 0 °C. The adduct, which crystallized, was filtered and dried, 7.2 g (mp 157–158 °C). IR (KBr): 2910, 1710, 1610 cm^{–1}. ¹H NMR (CDCl₃): δ 1.69 (3H, s, 5 β -CH₃), 3.48 (3H, s, OCH₃), 3.79 (3H, s, ArOCH₃), 4.59–4.62 (2H, q, OCH₂-CCl₃), 5.97–6.17 (2H, ABq, H₅ and H₇), 6.52–6.56 (2H, H₁ and H₂). Anal. (C₂₃H₂₅Cl₃N₂O₆) C, H, N.

***N*-(Cyclopropylmethyl)-5 β -methyl-19-[(trichloroethoxy)carbonyl]-6,14-dihydro-6,14-(epoxyimino)northebaine (**16**).** Using the procedure described above, 6 g of 5 β -methyl-*N*-(cyclopropylmethyl)northebaine (**14**) afforded 5.7 g of the adduct **16**, mp 132–134 °C. IR (KBr): 2990, 2915, 1720 cm^{–1}. ¹H NMR (CDCl₃): δ 1.67 (3H, s, 5 β -CH₃), 3.57 (3H, s, OCH₃), 3.77 (3H, s, ArOCH₃), 4.95–5.12 (2H, ABq, OCH₂CCl₃), 6.01–6.17 (2H, ABq, H₅ and H₇), 6.51–6.95 (2H, ABq, H₁ and H₂). Anal. (C₂₆H₂₉Cl₃N₂O₆·0.5H₂O) C, H, N.

14 β -Amino-5 β -methylcodeinone (23**, and **29a**, and **30a**).** To a solution of 0.26 M HCl in 40 mL of ethylene glycol was added 2.0 g of the adduct **15**. After the solution was stirred for 3 h at room temperature there was added slowly 900 mg of (NH₄)₂CO₃ and 1.7 g of zinc powder, and the suspension was stirred at 70 °C for 1 h. The excess zinc was filtered off, and the filtrate was made alkaline with NaHCO₃ solution. After dilution with H₂O, the reaction mixture was extracted with 3 \times 50 mL of CHCl₃.

The combined extracts were washed with brine, dried, and evaporated. The residue was dissolved in 20 mL of MeOH, 10 mL of H₂O, and 1 mL of 6 N HCl and heated under reflux for 30 min. After cooling, the reaction mixture was made alkaline with NaHCO₃ solution, washed with H₂O, and dried. Evaporation of the CHCl₃ left an oil which was chromatographed on a silica gel column using EtOAc/MeOH (9:1) as the eluant. There was obtained 327 mg (25%) of 23, mp 149–150 °C, 528 mg (40%) of 29a, mp 170–172 °C, and 62 mg (5%) of 30a, mp 175–177 °C. Spectral data for 27. IR (KBr): 3350, 2950, 1675, 1600 cm⁻¹. ¹H NMR (CDCl₃): δ 1.72 (3H, s, 5β-CH₃), 2.40 (3H, s, NCH₃), 2.83–2.86 (1H, d, H₉), 3.16–3.25 (1H, d, H₁₀), 3.80 (3H, s, ArOCH₃), 5.99–6.04 (1H, d, H₈), 6.58–6.66 (3H, m, H₇, H₁ and H₂). Anal. (C₁₉H₂₂N₂O₃·0.25H₂O) C, H, N.

For 29a. IR (KBr): 3480, 2910, 1655 cm⁻¹. ¹H NMR (CDCl₃): δ 1.51–1.55 (3H, d, 5β-CH₃), 2.32 (3H, s, NCH₃), 3.81 (3H, s, OCH₃), 4.52–4.55 (1H, q, H₅), 5.75–5.8 (1H, d, H₈), 6.63–6.64 (1H, d, H₇), 6.67–6.69 (2H, m, H₁, H₂). Anal. (C₁₉H₂₀N₂O₃·0.25H₂O) C, H, N.

For 30a. IR (KBr) 2900, 1665, 1600 cm⁻¹. ¹H NMR (CDCl₃): δ 1.61 (3H, s, 5β-CH₃), 2.45 (3H, s, NCH₃), 3.82 (3H, s, OCH₃), 5.98–6.04 (1H, dd, H₈), 6.56–6.67 (3H, m, H₇, H₁ and H₂).

14β-Amino-5β-methyl-7,8-dihydrocodeinone (17). A solution of 500 mg of the adduct 15 in 15 mL of MeOH and 25 mL of a 2 N HOAc/1.5 N NaOAc solution was hydrogenated in the presence of 150 mg of 5% Pd/C at 50 psi. After 10 h, the catalyst was filtered off and washed with a small amount of 2 N HOAc. The combined filtrates were made alkaline with NH₄OH and extracted with 3 × 25 mL of CHCl₃. The extracts were combined, washed with H₂O, dried, and evaporated. The residue was chromatographed on a silica gel column using EtOAc/MeOH (8:1) as the eluant. There was obtained 220 mg of pure 17, mp 168–169 °C. IR (KBr): 3380, 3340, 1710 cm⁻¹. ¹H NMR (CDCl₃): δ 1.63 (3H, s, 5β-CH₃), 2.33 (3H, s, NCH₃), 3.03–3.12 (2H, m, H₉, H₁₀), 3.82 (3H, s, OCH₃), 6.51–6.64 (2H, m, H₁, H₂). Anal. (C₁₉H₂₂N₂O₃): C, H, N.

14β-Amino-5β-methyl-7,8-dihydro-N-(cyclopropylmethyl)norcodeinone (18). A solution of 2 g of the adduct 16 dissolved in 20 mL of MeOH and 25 mL of 2 N AcOH/1.3 N NaOAc was hydrogenated at 50 psi in the presence of 5% Pd/C (500 mg) as the catalyst. After 8 h, the catalyst was filtered off, washed with 2 N AcOH, treated with concentrated NH₄OH solution, and extracted with CHCl₃ (3 × 30 mL), and the extract was washed with water (2 × 20 mL) and dried. The solvent was removed in vacuo. The residue was chromatographed on silica gel using EtOAc as the eluant. There was obtained 700 mg of 18, mp 92–95 °C. IR (KBr): 3340, 3000, 2940, 1710, 1600 cm⁻¹. ¹H NMR (CDCl₃): δ 1.68 (1H, s, 5β-CH₃), 3.86 (3H, s, OCH₃), 6.56–6.58 (1H, d, H₂), 6.64–6.66 (1H, d, H₁). Anal. (C₂₂H₂₈N₂O₃) C, H, N.

5β-Methyl-14β-[(p-nitrocinnamoyl)amino]-7,8-dihydrocodeinone (19). A solution of 14β-amino-5β-methyl-7,8-dihydrocodeinone 17 (400 mg, 1.21 mmol) in dry CH₂Cl₂ (50 mL) and triethylamine (203 μL) was cooled to –60 °C with stirring. A solution of p-nitrocinnamoyl chloride, freshly prepared from the acid (353 mg) and thionyl chloride in dry CH₂Cl₂, was added dropwise to the stirred solution, and the mixture was slowly brought to room temperature and stirred for 3 h. Aqueous saturated NaHCO₃ was added, and the separated organic layer was washed with NaHCO₃ solution (2 × 15 mL) and water (2 × 15 mL) and dried (Na₂SO₄). The crude product obtained after the evaporation of the solvent was chromatographed on silica gel using EtOAc/hexane (9:1) as the eluant to furnish 350 mg (57%) of 19, which was further crystallized from MeOH, mp 283–285 °C. IR (KBr): 3240, 2920, 1700, 1665, 1625, 1590 cm⁻¹. ¹H NMR (CDCl₃): δ 1.63 (3H, s, 5β-CH₃), 2.42 (3H, s, NCH₃), 3.86 (3H, s, OCH₃), 6.67–6.70 (3H, m, H₁, H₂ and NHCOCH=), 7.64–7.71 (m, 3H, COCH=CH and ArH), 8.21–8.26 (2H, d, ArH). Anal. (C₂₈H₂₈N₂O₆) C, H, N.

5β-Methyl-14β-[(p-nitrocinnamoyl)amino]-7,8-dihydromorphinone (20). The procedure described for 26 was employed for preparing 20. A 130-mg portion of 19 gave 105 mg of 20 after chromatography over silica gel using EtOAc as the eluant: mp 296–300 °C dec. IR (KBr): 1700, 1660 cm⁻¹. ¹H NMR (DMSO-d₆): δ 1.56 (3H, s, 5β-CH₃), 2.25 (3H, s, NMe), 3.02–3.07 (1H, d,

H₉), 6.51–6.57 (2H, ABq, H₁, H₂), 7.82–7.86 (2H, d, ArH), 8.23–8.28 (2H, d, ArH). Anal. (C₂₇H₂₇N₃O₆·0.25H₂O) C, H, N.

5β-Methyl-14β-[(p-nitrocinnamoyl)amino]-7,8-dihydro-N-(cyclopropylmethyl)norcodeinone (21). This compound was prepared by the procedure described for 19; 350 mg (0.953 mmol) of 18 was treated with the acid chloride obtained from 280 mg of p-nitrocinnamic acid to furnish 300 mg of 21 after chromatography over silica gel with EtOAc/hexane (7:3) as eluant, mp 265–268 °C. IR (KBr): 3250, 1700, 1660, 1620 cm⁻¹. ¹H NMR (CDCl₃): δ 1.63 (3H, s, 5β-CH₃), 2.36 (2H, d, NCH₂), 3.81 (3H, s, OCH₃), 6.64–6.77 (3H, m, H₁, H₂ and NHCOCH=), 7.60–7.64 (3H, m, =CHAr and ArH), 8.17–8.21 (2H, d, ArH). Anal. (C₃₁H₃₃N₃O₆) C, H, N.

5β-Methyl-14β-[(p-nitrocinnamoyl)amino]-7,8-dihydro-N-(cyclopropylmethyl)normorphinone (22). The procedure described for 20 was used to prepare 22. One hundred milligrams of 21 was treated with BBr₃ (52 μL). The yellow residue obtained after evaporation of the solvent was purified by chromatography on silica gel with EtOAc/hexane (7:3), which was crystallized from CHCl₃ to yield 57 mg of 22, mp 210–215 °C. IR (KBr): 1700, 1665 cm⁻¹; ¹H NMR (DMSO-d₆): δ 1.4 (3H, s, 5β-CH₃), 2.77–2.88 (1H, d, H-9), 6.32–6.46 (2H, ABq, H₁, H₂), 7.07–7.14 (1H, d, NHCOCH=), 7.36–7.44 (1H, d, =CHAr), 7.60–7.64 (2H, d, ArH), 8.05–8.09 (2H, d, ArH). Anal. (C₃₀H₃₁N₃O₆·0.25H₂O) C, H, N.

5β-Methyl-14β-[(nitrodihydrocinnamoyl)amino]-7,8-dihydro-N-(cyclopropylmethyl)normorphinone (32). Using the procedure described for the preparation of 19, 150 mg of the codeinone 18 and 130 mg of p-nitrodihydrocinnamoyl chloride furnished 60 mg of the p-nitrodihydrocinnamoyl derivative. Demethylation of the amide with 45 μL of BBr₃ in 30 mL of dry CHCl₃ at –20 °C gave a crude product which was purified on a preparative TLC plate using EtOH/hexane (3:2) as the developing solvent. There was obtained 15 mg of pure 32, mp 264–266 °C dec. ¹H NMR (CDCl₃): δ 1.55 (3H, s, 5β-CH₃), 6.54–6.56 (1H, d, H₁), 6.71–6.73 (1H, d, H₂), 7.54–7.56 (1H, d, ArH), 8.00–8.10 (1H, d, ArH). Anal. (C₃₀H₃₁N₃O₆·0.5H₂O) C, H, N.

14β-Amino-5β-methyl-N-(cyclopropylmethyl)norcodeinone (24), 29, and 30b). Using the same procedure employed for the preparation of 23, there was obtained 170 mg (20%) of 24, mp 150–153 °C, 300 mg (30%) of 29b, mp 178–180 °C, and 85 mg (10%) of 30b, mp 85–86 °C. Spectral data for 24. IR (KBr): 3350, 2920, 1660, 1630 cm⁻¹. ¹H NMR (CDCl₃): δ 1.77 (3H, s, 5β-CH₃), 3.8 (3H, s, ArOCH₃), 6.04–6.06 (1H, d, H₈), 6.59 (1H, d, H₇), 6.63–6.66 (2H, m, H₁ and H₂). Compound 24 was used without further purification for the preparation of 27.

For 29b. IR (KBr): 3400, 2910, 1660 cm⁻¹. ¹H NMR (CDCl₃): δ 1.51–1.53 (3H, d, 5β-CH₃), 3.78 (3H, s, OCH₃), 4.50–4.52 (1H, q, 5-H), 5.75–5.77 (1H, d, H₈), 6.47–6.49 (1H, d, H₇), 6.60–6.66 (2H, m, H₁ and H₂). Anal. (C₂₂H₂₈N₂O₃): C, H, N.

For 30b. IR (KBr): 2910, 1665, 1600 cm⁻¹; ¹H NMR (CDCl₃): δ 1.60 (3H, s, 5β-CH₃), 3.80 (3H, s, ArOCH₃), 5.98–6.01 (1H, dd, H₈), 6.53–6.62 (3H, m, H₁, H₂ and H₇). Anal. (C₂₂H₂₈N₂O₃) C, H, N.

5β-Methyl-14β-[(p-nitrocinnamoyl)amino]codeinone (25). A solution of 100 mg of 23 in 20 mL of dry CH₂Cl₂ and 55 μL of triethylamine was cooled to –60 °C. A solution of p-nitrocinnamoyl chloride prepared from 118.5 mg of p-nitrocinnamic acid and SOCl₂ in 10 mL of CH₂Cl₂ was added dropwise with stirring. The reaction mixture was allowed to warm to room temperature, and after 5 h a solution of saturated NaHCO₃ was added. The layers were separated, and the organic layer was washed with H₂O and dried (Na₂SO₄). The solvent was evaporated, and the residue was chromatographed on a column of silica gel using EtOAc/hexane (9:1) as the eluant. There was obtained 70 mg of 25, mp 310 °C dec, after crystallization from MeOH. IR (KBr): 3280, 1670, 1660, 1590 cm⁻¹. ¹H NMR (CDCl₃): δ 1.73 (3H, s, 5β-CH₃), 2.51 (3H, s, NCH₃), 3.82 (3H, s, OCH₃), 6.34 (2H, d, H₈, NHCOCH=), 6.59–6.71 (3H, m, H₇, H₁, H₂), 7.58–7.69 (3H, m, ArH, =CHAr), 8.21–8.25 (2H, d, ArH). Anal. (C₂₈H₂₇N₃O₆) C, H, N.

5β-Methyl-14β-[(p-nitrocinnamoyl)amino]normorphinone (26). To a cooled (–20 °C) solution of 25 (100 mg) in CH₂Cl₂ was added a solution of BBr₃ (75 μL) in CH₂Cl₂ dropwise with stirring. The mixture was slowly brought to room temperature and stirred for 1.5 h; after this period 2 mL of MeOH

and NaHCO₃ solutions were added and the organic layer separated. The aqueous layer was extracted further with CHCl₃/MeOH (3:1) (2 \times 25 mL), the combined extracts were washed with water and dried, and the solvent was evaporated using a rotary evaporator. The residue obtained was chromatographed on silica gel using EtOAc/hexane (3:2) as the eluant to furnish pure **26** (75 mg), mp 315 °C dec. IR (KBr): 3350, 2910, 1660, 1630, 1590 cm⁻¹. ¹H NMR (DMSO-*d*₆): δ 1.59 (3H, s, 5 β -CH₃), 6.04–6.06 (1H, d, H₈), 6.49–6.51 (2H, m, H₁ and H₂), 6.88–6.90 (1H, d, H₇), 7.26–7.29 (1H, d, =CHAr), 7.46–7.49 (1H, d, NHCOCH=), 7.86–7.88 (1H, d, ArH), 8.26–8.28 (1H, d, ArH). Anal. (C₂₇H₂₅N₃O₆·0.75H₂O) C, H, N.

5 β -Methyl-14 β -[(p-nitrocinnamoyl)amino]-N-(cyclopropylmethyl)norcodeinone (27). The method described for the preparation of **25** was used. Two hundred milligrams of **24** furnished 120 mg of the desired amide **27**, mp 293–295 °C dec. IR (KBr): 3280, 1670, 1630 cm⁻¹. ¹H NMR (CDCl₃): δ 1.71 (3H, s, 5 β -CH₃), 3.81 (3H, s, OCH₃), 6.30–6.71 (5H, m, H₁, H₂, H₇, H₈ and COCH=), 7.61–7.71 (3H, m, ArH, =CHAr), 8.19–8.20 (2H, d, ArH). Anal. (C₃₁H₃₁N₃O₆·H₂O) C, H, N.

5 β -Methyl-14 β -[(p-nitrocinnamoyl)amino]-N-(cyclopropylmethyl)normorphine (28). To a solution of 60 mg of the codeinone **27** in 40 mL of CH₂Cl₂ was added dropwise with stirring a solution of 83 mg of BBr₃ in 20 mL of CH₂Cl₂. The mixture was allowed to warm to room temperature and was stirred for 1 additional h. A solution of saturated NaHCO₃ was added cautiously, and the organic layer was separated. It was washed with NaHCO₃ solution and H₂O and dried. After chromatography on a silica gel column using EtOAc/hexane (3:2) as the eluant there was obtained 20 mg of pure **28**, mp 265–270 °C dec. IR (KBr): 3250, 1660 cm⁻¹. ¹H NMR (CDCl₃): δ 1.67 (3H, s, β -CH₃), 3.05–3.15 (1H, d, H₁₀), 6.26–6.31 (1H, d, H₈), 6.44–6.66 (4H, m, H₇, NHCOCH=, H₁ and H₂), 7.59–7.61 (3H, m, =CHAr, ArH), 8.17–8.22 (2H, d, ArH). Anal. (C₃₀H₂₉N₃O₆·1.2H₂O) C, H, N.

Addition Reaction of N-Acetylcysteine to 22. Twenty-five milligrams of **22** was dissolved in 3 mL of THF and 3 mL of tissue buffer pH 8.0 and stirred at 37 °C. To this solution was added N-acetylcysteine (7.8 mg) dissolved in buffer and the resulting solution stirred at 37 °C for 24 h. The reaction mixture was then neutralized with pH 5.5 buffer and extracted with CHCl₃. The CHCl₃ extracts were washed with water, and the residue obtained after the evaporation of the solvent was found to be the starting material **22** (17 mg, 76% recovery) from TLC. The aqueous layer was evaporated, and the residue was dissolved in MeOH. Analysis (TLC, IR) of the residue after evaporation of the MeOH showed that no adduct between N-acetylcysteine and **22** was formed.

The addition of N-acetylcysteine to **22** was followed spectrophotometrically. A 10⁻³ M stock solution of N-acetylcysteine and **22** were made in 50% THF and buffer (pH 7.4). One mL of each were then mixed and diluted to give a 10⁻⁴ M solution. The λ_{\max} of **22** was 314 nm. Ultraviolet spectra of this solution were taken periodically and compared to the spectra of starting materials. There was no shift in the absorption peaks due to (p-nitrocinnamoyl)amino group after 24 h indicating that no Michael addition had occurred.

Radioligand Binding Assays. Calf frontal cortex or calf caudate tissue was obtained by dissection of calf brain over ice and was immediately homogenized. Two grams of tissue was suspended in 30 mL of 50 mM Tris-HCl buffer, pH 7.7, at 25 °C containing 10 mM MgSO₄ and 0.5 mM EDTA. Following centrifugation for 15 min at 14000g the samples were resuspended in buffer and incubated at 37 °C for 15 min. The homogenates were centrifuged for 10 min at 14000g, resuspended in buffer, and recentrifuged and stored as a pellet at -30 °C until use.

Radioligand binding assays were performed in triplicate in a final volume of 2 mL of 50 mM Tris-HCl, pH 7.5, 10 mM MgSO₄, and 0.5 mM EDTA. The tissue concentrations used were 10 mg wet weight of calf frontal cortex for μ and κ binding sites and 12 mg wet weight of calf caudate for the δ binding site; tissue was added last. Nonspecific binding was determined using 1 μ M naloxone for [³H]DAMGO,¹⁶ [³H]DPDPE,¹⁷ and [³H]U-69,593.¹⁸ Incubation times used were 30 min for the μ -selective peptide [³H]DAMGO and the κ -selective ligand [³H]U-69,593 at 37 °C and 4 h for the δ -selective peptide [³H]DPDPE at 25 °C. The IC₅₀ values for all the compounds, except for **20** and **22** (Table

I and Table II), were determined by incubating membranes with eight concentrations of the ligands in the presence of either 0.8 nM [³H]DAMGO, 1 nM [³H]DPDPE, or 1 nM [³H]U-69,593 to measure their affinity for μ , δ , and κ receptor sites, respectively. The binding was terminated by rapid filtration through Whatman GF/B glass fiber filters presoaked in 0.1% polyethyleneimine for 30 min for [³H]DPDPE and [³H]U-69,593 binding and rinsed with two 2-mL aliquots of cold 50 mM Tris-HCl buffer, pH 7.5. Filters were counted in 5 mL of Ecoscint A scintillation fluid.

The IC₅₀ values for **20** and **22** were determined as described previously.¹⁰

[³H]DAMGO Saturation Binding to Bovine Striatal Membranes Treated in the Absence and Presence of 20 nM N-CPM-MET-CAMO. In a final volume of 2 mL of 50 mM Tris-HCl, pH 7.5, 10 mg of bovine striatal membrane protein was incubated with 20 nM **22** for 60 min at 25 °C. Control membranes were treated under identical conditions except for the omission of **22**. The reaction was terminated by diluting the contents of the tubes to 40 mL with cold 50 mM Tris-HCl, pH 7.5. The membranes were centrifuged at 39000g for 15 min at 4 °C, and the wash step was repeated four times. The membranes were resuspended in 2 mL of 50 mM Tris-HCl, pH 7.5, and were used in [³H]DAMGO saturation binding assays.

[³H]DAMGO saturation binding was measured by incubating 200 μ L of resuspended membranes with [³H]DAMGO at concentrations ranging from 0.05 to 6.4 nM in a final volume of 1 mL of 50 mM Tris-HCl, pH 7.5. After a 60-min incubation at 25 °C, the membranes were filtered through Schleicher & Schuell No. 32 glass fiber filters. The filters were washed three times with 4 mL of cold 50 mM Tris-HCl, pH 7.5. Bound radioactivity was determined by liquid scintillation spectrophotometry in 2 mL of Ecoscint A scintillation fluid (National Diagnostics). Nonspecific binding was measured by inclusion of 10 μ M naloxone. Protein concentration was determined by the method of Bradford.¹⁹ Saturation data were analyzed by nonlinear regression analysis using the LIGAND program.²⁰ The experiment was performed in triplicate and was replicated three times.

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References

- Aceto, M. D.; Bowman, E. R.; May, E. L.; Woods, J. H.; Smith, C. B.; Medzhradsky, F.; Jacobson, A. E. Very Long-acting Narcotic Antagonists: The 14 β -p-Substituted Cinnamoylamino Morphinones and Their Partial μ Agonist Codeinone Relatives. *Arzneim-Forsch/Drug Res.* 1989, 39, 570–575.
- Comer, S. D.; Burke, T. F.; Lewis, J. W.; Woods, J. H. CLOCINNAMOX: A Novel, Systemically Active Irreversible Opioid Antagonist. *J. Pharmacol. Exp. Ther.* 1992, 262, 1051–1056.
- Burke, T. F. Symposium on the Pharmacology of Irreversible Opioid Antagonists, The College of Problems of Drug Dependence, June 21, 1992, Keystone, CO.
- Kirby, G. W.; McLean, D. An Efficient Synthesis of 14 β -aminocodeinone From Thebaine. *J. Chem. Soc., Perkin Trans. 1* 1985, 1443–1445.
- Bentley, K. W.; Bower, J. D.; Lewis, J. W. Novel Analgesics and Molecular Rearrangements in the Morphine Thebaine Group. Part XVI. Some Derivatives of 6,14-endo-Etheno-7,8-dihydromorphine. *J. Chem. Soc. C* 1969, 2569–2572.
- Eddy, N. B.; Halbach, H.; Braendton, V.-S. Synthetic Substances with Morphine-like Effect. *Bull. Wild Health Org.* 1957, 17, 569–583. Later studies of metopon in patients with moderate to severe pain revealed that although metopon was indeed more potent than morphine, the oral to parenteral ratio of the two analgesics was about the same; i.e., the ratio was about 6:1 for both drugs. Houde, R. W. Private communication.
- Small, L. F.; Fitch, H. M.; Smith, W. E. Addition of Organomagnesium Halides to pseudocodeine Types. II. Preparation of Nuclear Acylated Morphine Derivatives. *J. Am. Chem. Soc.* 1936, 58, 1457–1463.
- Boden, R. M.; Gates, M.; Ho, S. P.; Sundaraman, P. Derivatives of the Thebaine Anion. 1. Structure of Metopon. A Direct Demonstration. *J. Org. Chem.* 1982, 47, 1347–1349.

- (9) Schmidhammer, H.; Fritsch, F. (-)-N-[(Cyclopropyl)methyl]-3,4-dimethoxy-5-methylmorphinan-6-one. *Helv. Chim. Acta* 1988, 71, 642-647.
- (10) Jiang, Q.; Seyed Mozaffari, A.; Bidlack, J. M.; Archer, S. 14 β -(Dithioglycolamido)-bis-7,8-dihydromorphinone (TAMO) and 14 β -(dithioglycolamido)-bis-7,8-dihydro-N-(cyclopropylmethyl)normorphinone (N-CPM-TAMO): Chemistry and Opioid Binding Properties. Manuscript in preparation.
- (11) Schmidhammer, H.; Schratz, A.; Mitterdorfer, J. Synthesis and Biological Evaluation of 14-Alkoxymorphinans. *Helv. Chim. Acta* 1990, 73, 1784-1787.
- (12) Jiang, Q.; Sebastian, A.; Archer, S.; Bidlack, J. M. MET-CAMO: 5 β -Methyl-14 β -(p-nitrocinnamoylamino)-7,8-dihydromorphinone: a Long-Lasting μ Opioid Receptor Antagonist Devoid of Agonist Properties. *Eur. J. Pharmacol.* 1993, 230, 129-130.
- (13) Jiang, Q.; Sebastian, A.; Archer, S.; Bidlack, J. M. MET-CAMO and N-CPM-MET-CAMO: Pharmacology and Binding Properties. Manuscript in preparation.
- (14) Lewis, J.; Smith, C.; McCarthy, P.; Walter, D.; Kobylecki, R.; Myers, M.; Haynes, A.; Lewis, C.; Waltham, K. New 14-Aminomorphinones and Codeinones. *NIDA Research Monograph No. 90*, 1988, 136-143.
- (15) Simon, E. J.; Fan, L. Q.; Hiller, J. M.; Seyed-Mozaffari, A.; Archer, S. Photoaffinity Ligands for the μ Receptor. *Life Sci.*, in press.
- (16) Titeler, M.; Lyon, R. A.; Kuhar, M. J.; Frost, J. F.; Dannals, R. F.; Leonhardt, S.; Bullock, A.; Rydelek, L. T.; Price, L. D.; Struble, G. R. μ -Opiate Receptors are Selectively Labelled by [3 H]Carfentanil in Human and Rat Brain. *Eur. J. Pharmacol.* 1989, 167, 221-228.
- (17) Clark, J. A.; Itzhak, Y.; Hruby, V. J.; Yamamura, H. I.; Pasternak, G. W. [D-Pen 2 ,D-Pen 6]enkephalin (DPDPE): a δ -Selective Enkephalin with Low Affinity for μ 1 Opiate Binding Sites. *Eur. J. Pharmacol.* 1986, 128, 303-304.
- (18) Lahti, R. A.; Mickelson, M. M.; McCall, J. M.; Von Voigtlander, P. F. [3 H]U-69593 A Highly Selective Ligand for the Opioid κ Receptor. *Eur. J. Pharmacol.* 1985, 109, 281-284.
- (19) Bradford, M. M. A Rapid Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal. Biochem.* 1976, 72, 248-253.
- (20) Munson, P. J.; Rodbard, D. Ligand: A Versatile Computerized Approach for Characterization of Ligand-Binding Systems. *Anal. Biochem.* 1980, 107, 220-239.