o-Naphthalenedicarboxaldehyde Derivative of 7′-Aminonaltrindole as a Selective *δ*-Opioid Receptor Affinity Label

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Incorporation of a naphthalene-dialdehyde moiety into the *δ* antagonist, 6′-aminonaltrindole afforded a potent, selective, irreversible *δ*-agonist **1**. However, flow cytometry studies revealed no time-dependent specific fluorescence, suggesting that both Lys214 and Cys216 at the recognition site are not involved in covalent binding. Molecular simulation studies suggest that compound **1** may form a Schiff base with the *ϵ*-amino group of Lys214, which could explain its irreversibility and transformation into a *δ*-agonist through a conformational change of TM5.

Introduction

Affinity labels have been employed as tools for the pharmacological and structural characterization of enzymes and receptors. Many of the affinity labels have an electrophilic moiety with covalent binding potential.[1] Electrophilic affinity labels have been used extensively to irreversibly block opioid receptors in vivo and in vitro.[2] However, when employed as receptor probes, such affinity labels generally do not permit a clear distinction between covalent binding and noncovalent pseudo-irreversible binding.

Recently, a new approach to affinity labeling has been developed that employs a fluorogenic moiety such as *o*-phthalaldehyde (OPA[a]) that is capable of specifically cross-linking neighboring lysine and cysteine residues at the recognition site of opioid receptors, with the concomitant formation of a fluorescent isoindole.[3] Such fluorogenic affinity labels were named "reporter affinity labels" because the production of fluorescence reports covalent cross-linking. The specificity of cross-linking with the formation of an easily detectable fluorescent moiety provides an advantage over conventional electrophilic affinity labels because this method is adaptable to detection in cultured cells using flow cytometry. Also, because cross-linking between neighboring residues restricts the translational mobility of the tethered ligand, molecular modeling can be conducted with greater assurance when compared to a ligand bound through only a single residue.

The major disadvantage of the OPA fluorogenic moiety is that laser-induced autofluorescence of cells overlaps partially with the fluorescence of the isoindole fluorophore, thereby reducing the sensitivity of this method. However, the introduction of a naphthalene dicarboxaldehyde (NDA) moiety has been reported to overcome this problem.[4] The reactivity of NDA is similar to that of OPA, and in this regard, the benzo[*f*]isoindole fluorophore formed through cross-linking possesses an excitation maximum of 480 nm as compared to that of 325 nm for the corresponding OPA-derived isoindole.[5] Thus, the excitation and detection of the generated fluorophore is possible without interference from autofluorescence using standard flow cytometers to follow the kinetics of cross-linking.[6] Another advantage is the improved quantum yield of the NDA-derived fluorescence ($ø_f = 0.58$) over that of the OPA-derived fluorescence ($ø_f = 0.42$).[7] For these reasons, NDA is considered superior to OPA as a fluorogenic reagent for the routine detection of amino acids and in labeling enzymes.[5,7] Thus, in the present study, NDA was selected as the fluorogenic moiety for comparing the covalent binding of **1** and the previously reported[3] reference compound **2** (Chart 1) to *δ*-opioid receptors. Here we present details on the synthesis of **1** and its biological properties.

Chemistry

The *δ*-opioid receptor antagonist, naltrindole (NTI), has been widely employed as a pharmacological tool in opioid research as a result of its high selectivity.[8,9] Previous structure−activity relationship (SAR) studies have revealed that substitution at the 7′ position of NTI with an amide functional group is well tolerated.[9−11] Here we describe the synthesis of **1** and its phthalaldehyde analogue **2**. Because the preparation of **2** had not been described in a previous report,[12] it is included together with that of **1**. As shown in Scheme 1, coupling of 7′-aminonaltrindole **5**[13] with 3,4-bis[2-(1,3-dioxolanyl)]-benzoic acid **3**[3]or 5-carboxynaphthalene dialdehyde **4**[4] (Chart 1) in the presence of DCC and HOBt in DMF afforded the protected compounds **6** and **7**, respectively. The aromatic hydroxyl group of **6** was deprotected using potassium carbonate in methanol to give compound **8**. Hydrolysis using 1 N HCl in acetone afforded the *δ*-selective ligands, compound **1**, and the corresponding phthalaldehyde derivative[12] **2**.

Biological Results

Receptor binding of **1** was conducted on *μ*-, *δ*-, and *κ*-opioid receptors singly expressed in human embryonic kidney (HEK-293) cells (Table 1). Compound **1** inhibited [^{3}H]diprenorphine binding to *δ*-, *μ*-, and *κ*-opioid receptors with K_i selectivity ratios of 64 (*μ*/*δ*) and 68 (*κ*/*δ*). The *δ* selectivity was substantially higher for **1** when compared to **2**,[12] whose *μ*/*δ* and *κ*/*δ* K_i ratios were reported[12] to be 7.8 and 10.9, respectively.

Irreversible binding assays were carried out on membranes from HEK-293 cells containing stably expressed *μ*-, *δ*-, and *κ*-opioid receptors (Figure 1). Pretreatment of cells containing *δ*-opioid receptors with **1** (1 *μ*M; 37 °C, 60 min incubation in HEPES buffer, pH = 7.5) followed by extensive washing produced no change in the binding of [^{3}H]diprenorphine (0.8 ± 0.6% prewash vs 3.4 ± 3.7% postwash). This was in contrast to the reversible antagonist naloxone (NLX), which exhibited a substantial difference between pre-wash (18.7 ± 1.1%) and

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[a] Abbreviations: OPA, *o*-phthalaldehyde; NDA, naphthalene dicarboxaldehyde; NTI, naltrindole; NTX, naltrexone; NLX, naloxone; MVD, mouse vas deferens; GPI, guinea pig ileum; HEK, human embryonic kidney.

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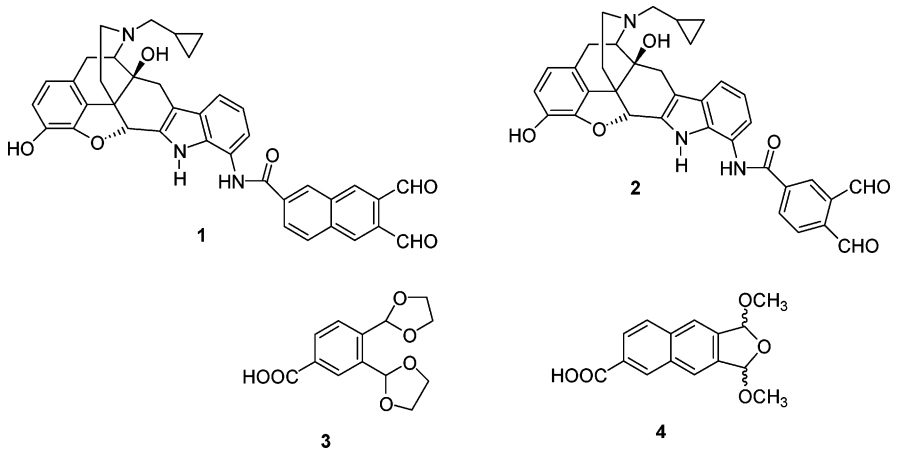
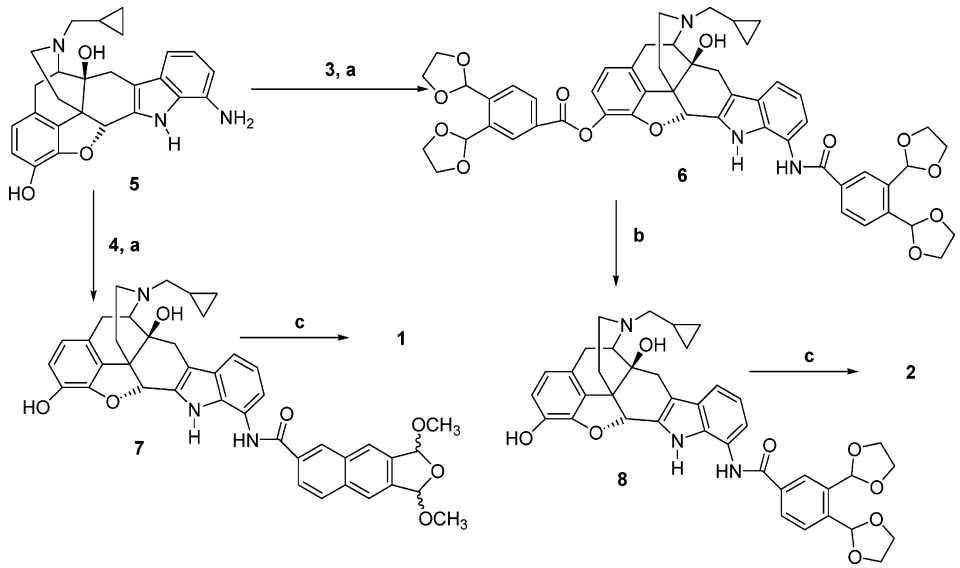
Chart 1. Structures of Compounds **1**−**4**



Scheme 1[a]



[a] Reagents and conditions: (a) DCC, HOBt, DMF, N_2, 0 °C to RT; (b) K_2CO_3, CH_3OH, RT; (c) HCl (1 N), N_2, acetone, RT.

postwash (92.9 ± 8.4%), suggesting that **1** bound irreversibly to the δ-opioid receptor. At μ and κ receptors, the binding of **1** was substantially less than at δ receptors and exhibited no significant differences between the pre- and postwash. The values were 73.9 ± 15.3% prewash versus 78.8 ± 13.1% postwash for μ receptors and 62.0 ± 4.0% prewash versus 67.4 ± 13.6% postwash for κ receptors.

The in vitro pharmacological profile of **1** was investigated on the electrically stimulated mouse vas deferens[14] (MVD; receptor density delta > mu ≫ kappa) and the guinea-pig ileal longitudinal muscle[15] (GPI) preparations (receptor density, mu > kappa ≫ delta), as previously described.[16] No significant change in the agonism of morphine in the presence of **1** (100 nM) was observed in the GPI (morphine IC_{50} was 23.4 ± 5.5 nM vs 19.5 ± 3.5 nM in the presence of **1**). In the MVD, **1** behaved as an agonist (IC_{50} = 0.46 nM) and was in the same potency range as the standard δ agonist [D-Ala[2],D-Leu[5]]-enkephalin (DADLE), and the phthalaldehyde, an analogue of **2** (0.12 nM; Figure 2).[12] Compound **1** was potently antagonized by the δ antagonist, NTI (IC_{50} ratio, 215).

Experiments were carried out to study the specific generation of the benzo[*f*]isoindole fluorophore upon incubation with δ-opioid receptor using flow cytometry. This was accomplished using a Becton-Dickinson FACS Vantage equipped with an

Table 1. Binding of Compound **1** to μ-, δ-, and κ-Opioid Receptors[a]

opioid receptor	apparent K_i values[b] (nM)
μ	144 ± 10
δ	2.25 ± 1.07
κ	153 ± 51

[a] [^{3}H]Diprenorphine was employed as radioligand. [b] The apparent K_i reflects both the reversible and the irreversible binding components.

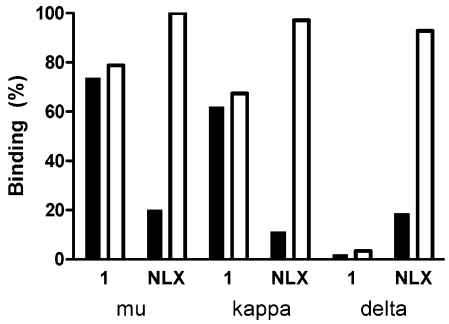


Figure 1. Irreversible binding of **1** to cloned opioid receptors expressed in HEK-293 cells. Membranes of HEK cells stably expressed with either μ-, δ-, or κ-opioid receptors were pretreated with 1 μM of either **1** or NLX at 37 °C for 60 min. Unbound receptors were determined using [^{3}H]diprenorphine before (closed bars) and after washing (open bars). The values represent means of duplicate experiments.

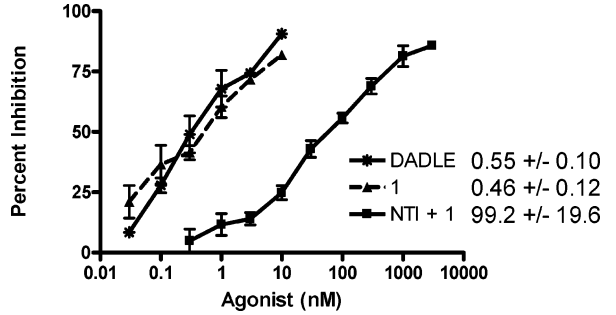


Figure 2. Dose−response curves of DADLE and compound **1** in the presence and absence of NTI in the MVD.

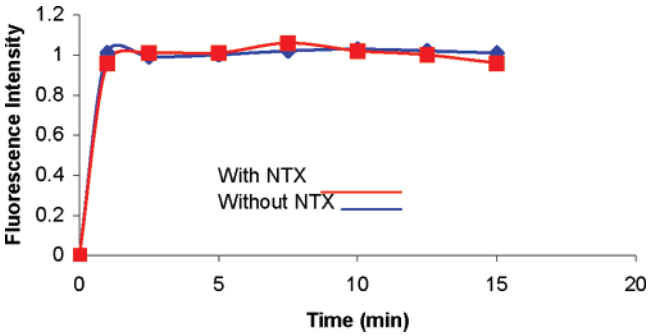


Figure 3. Comparison of the change in specific fluorescence intensity (emission wavelength = 488 nm) of δ-opioid receptors expressed in HEK cells upon incubation of compound **1** (100 nM) in the presence and absence of the opioid antagonist NTX (1 μM). Experiments were performed in triplicates for each receptor and cell line. Variations between experiments were less than 5%. The values represented here are the average of the three data sets.

argon laser for excitation at 488 nm using a band-pass filter of 530 ± 15 nm for detection. A baseline autofluorescence was measured for the HEK cells containing stably expressed δ-opioid receptors suspended in HEPES buffer (pH = 7.5). To determine if the increase in fluorescence was due to a specific covalent cross-linking at the receptor (specific fluorescence), control studies were performed by pretreating the HEK cells with the opioid antagonist naltrexone (NTX, 1 μM). When incubated with compound **1**, there was an increase in fluorescence intensity, but this increase appeared to be nonspecific because it was not significantly blocked by NTX (Figure 3). Hence, the increase in fluorescence intensity may be due to nonspecific cross-linking of both Lys and Cys residues at sites other than that of the receptor recognition locus.

Discussion

Compound **1** irreversibly inhibited [^{3}H]diprenorphine binding to the δ-opioid receptor, with an apparent K_i value that was comparable to that of its analogue, PNTI[3] (**2**). Significantly, **1** functioned as a full δ agonist similar in potency to that of PNTI **2** in the MVD preparation. Studies on the covalent binding of **1** to δ-opioid receptors expressed in HEK-293 cells using flow cytometry showed a time-dependent increase of fluorescence (Figure 3), suggesting the cross-linking of lysine and cysteine residues. However, in view of the finding that the increase in fluorescence was not affected by pretreatment with the opioid antagonist, NTX, it appears that cross-linking of both lysine and cysteine residues at the recognition site did not occur.

In this regard, it has been proposed that fluorophore formation arising from the interaction of PNTI **2** with δ-opioid receptors is due to covalent linking of both lysine 214 and cysteine 216 residues in TM5.[12] The covalently induced activation of the δ receptor by **2** was suggested to be mediated through perturbation of TM5. Given the irreversible binding of **1** and its inability to produce specific fluorescence in cultures cells, the potent δ agonist activity may be due to Schiff base formation between one of its carboxaldehyde groups and the ϵ-amino group pf lysine 214 without cross-linking to cysteine 216. As both **1** and **2** function as potent agonists, this covalently induced effect could arise through cross-linking with lysine 214, which would promote torsional perturbation in TM5, which would lead to a concomitant conformational change of intracellular loop 3 involved in G protein activation. As illustrated in Figure 4, simulated docking of **1** to the δ-receptor recognition site suggests that one of the carboxaldehyde groups is close enough to lysine 214 to form a Schiff's base, but due to the greater length of its naphthalene moiety compared to that of the benzene moiety in **2**, cross-linking to cysteine would be less likely to occur. This is consistent with the irreversibility of **1** without specific fluorescence upon interaction with δ receptors.

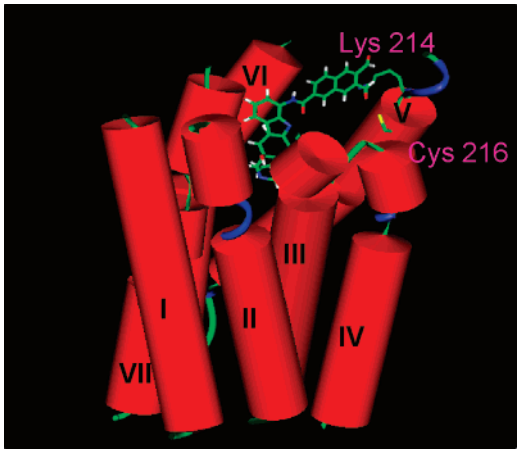


Figure 4. Docking of **1** in a δ-opioid receptor model[12] using Glide.[17] The energy minimizations were carried out using Insight II (Accelrys, Inc., San Diego, CA).

Conclusions

In conclusion, a new, selective affinity label **1** has been synthesized for the δ-opioid receptor. Although **1** has high affinity and selectivity for δ-opioid receptors, the positioning of its naphthalene dialdehyde moiety at the recognition site prevents efficient formation of the fluorogenic benzo[*f*]isoindole moiety through cross-linking both lysine 214 and cysteine 216. We propose that unlike PNTI **2**, which is reported to be fluorogenic, **1** binds covalently only to lysine 214. It is proposed that Schiff base formation with the ϵ-amino group of lysine 214 promotes rotation of TM5, which induces a conformational change in intracellular loop 3, which leads to the potent δ agonist activity of both **1** and **2**.

Experimental Section

General. Materials. NTX hydrochloride was obtained from Mallinckrodt and was used to synthesize 7′-aminonaltrindole, as previously reported.[13] All other reagents were purchased from either Aldrich Chemical Co. or Lancaster. Reactions sensitive to air were performed under a nitrogen atmosphere in oven-dried glassware. ^{1}H NMR spectra were taken on a Varian Inova 300 MHz instrument using DMSO-d_6 as a solvent, unless otherwise noted, and the chemical shifts are expressed in ppm on the δ scale. All spectra were recorded at ambient temperature. Gravity and low-pressure chromatography were performed over silica gel (200−400 mesh, Aldrich Chemical Co.) as the stationary phase under nitrogen. TLC was performed on Analtech silica gel GF. Low and high-resolution

mass spectra were obtained on a VG-707EHF spectrometer in the chemistry department.

Transient Transfection. HEK-293 cells in DMEM (Gibco, BRL) supplemented with 10% bovine calf serum (Hyclone) and 1% penicillin/streptomycin (Gibco, BRL) were maintained at 37 °C and in 5% CO_2. Cells were seeded at 16% for 24 h prior to transfection. Fresh media was added 2 h prior to transfection. Cells were transfected with plasmid DNA (20 μg/100 mm plate) of either wild-type or mutant receptor cDNA using the calcium phosphate precipitation method.[18] Media was changed 5 h after transfection. Transfected cells were harvested 48−72 h after tranfection for binding studies.

Receptor Binding Assays. At 60 to 72 h after transfection, HEK cells were washed three times with 25 mM HEPES buffer (pH 7.4) and were resuspended with 8−12 mL of 25 mM HEPES/100 mm plate. Saturation binding assays were performed in triplicate. Nonselective binding was determined using 10 μM NTX. Assays were incubated at room temperature for 90 min in a total binding volume of 0.5 mL and were terminated by filtration through a Whatman GF/B filter that had been presoaked in 0.25% poly-(ethyleneimine) immediately prior to filtration. Filters were washed three times with 4 mL of ice-cold 25 mM HEPES buffer, and scintillation counting was performed with a Beckman 3801 LS scintillation counter. Protein concentrations were determined by the method of Bradford.[19] Raw binding data was analyzed with RADLIG and LIGAND (G. A. McPherson, Biosoft, Cambridge, U.K.). Inhibition constants (K_i) were determined from IC_{50} values with the Cheng−Prusoff equation.[20]

Irreversible Binding. Cloned cells were harvested and diluted in 0.25 mM HEPES buffer (pH = 7.4) and incubated with 1 μM **2**, NLX, or β-FNA for 60 min at room temperature. Aliquots (400 μL) were used for binding experiments with [^{3}H]-diprenorphine, as usual (prewash). The remaining suspension was centrifuged (3000× for 10 min), and the supernatant was discarded and resuspended in 1.0 mL HEPES. This procedure was repeated for a total of three washes. Aliquots of the suspension were then tested for binding as above (postwash). Results are expressed as % total binding (B − NS/TB × 100), where B is the activity bound in the sample, NS is the nonspecific binding (defined by 10 μM NLX), and TB is the total activity bound without any compound present.

Flow Cytometry. This was accomplished using a Becton−Dickinson FACS Vantage equipped with an argon laser for excitation at 488 nm using a band-pass filter of 530 ± 15 nm for detection. The kinetics of cross-linking was followed in δ receptor transfected HEK cells suspended in HEPES buffer. A total of 100 nM of **1** was added to the suspension, and the fluorescence intensity was monitored at various time points over a 15 min period when the fluorescence intensity began to level off. Control studies were performed by pretreating the HEK cells with the opioid antagonist NTX (1 μM) to determine whether the increase in fluorescence is indeed due to a covalent cross-linking (specific fluorescence).

7′-{3,4-Bis[2-(1,3-dioxolanyl)]-benzoylamino}-3-{3,4-bis[2-(1,3-dioxolanyl)]-benzyloxy}-17-(cyclopropylmethyl)-6,7-didehydro-4,5α-epoxy-14-hydroxyindolo[2′,3′:6,7]-morphinan (6). DCC (0.86 g, 4.2 mmol, 3.6 equiv) was added to a solution of 7′-aminonaltrindole (**5**; 0.5 g, 1.16 mmol, 1 equiv), HOBt (0.56 g, 4.20 mmol, 3.6 equiv), and 3,4-bis[2-(1,3-dioxolanyl)]-benzoic acid[3] (1.02 g, 3.80 mmol, 3.3 equiv) in DMF (15 mL). The mixture was warmed to room temperature and stirring was continued for 5 days. The precipitate was removed by filtration, the filtrate was poured into an aqueous saturated solution of sodium hydrogen carbonate (150 mL), and the mixture was extracted with ethyl acetate. The organic extracts were combined and washed with water and dried over sodium sulfate. Evaporation of the solvent gave a yellow solid, which was subjected to silica gel flash column chromatography ($CHCl_3$/NH_4OH 99:1) to afford 0.72 g (67%) of the desired amido ester **6**. R_f 0.50 ($CHCl_3$/CH_3OH/NH_4OH 97:1:2). ^{1}H NMR (DMSO-d_6): δ 11.08 (br s, 1H), 10.07 (br s, 1H), 8.16 (s, 1H), 7.97−8.07 (m, 3H), 7.61−7.70 (m, 2H), 7.33 (d, J = 7.5 Hz, 1H), 7.23 (d, J = 7.5 Hz, 1H), 6.93 (m, 2H), 6.73 (d, J = 8.4 Hz, 1H), 6.07 (s, 3H), 6.04 (s, 1H), 5.52 (s, 1H), 4.78 (br s, 1H), 3.94−4.07 (m, 16H), 3.37 (m, 1H), 3.20 (m, 1H), 2.65−2.95 (m, 3H), 2.30−2.55 (m, 4H), 2.10−2.25 (m, 1H), 1.61 (m, 1H), 0.90 (m, 1H), 0.50 (m, 2H), 0.15 (m, 2H). IR (KBr) 1740, 1652 cm^{-1}.

β-1′,3′-dimethoxybenzylphthalan-7′-aminonaltrindole (7). 1,3-Dimethoxy-1,3-dihydro-naphtho[2,3-*c*]furan-6-carboxylic acid (0.5 g, 1.8 mmol, 3.3 equiv), HOBt (0.27 g, 1.98 mmol, 3.6 equiv), and 7′-aminonaltrindole **5** (0.24 g, 0.55 mmol, 1 equiv) were dissolved in 10 mL of anhydrous DMF. This solution was cooled to 0 °C and stirred for 15 min. DCC (0.41 g, 1.98 mmol, 3.6 equiv) was added, and the reaction was sealed under nitrogen and allowed to rise to ambient temperature. The mixture was stirred for 4 days at room temperature until the reaction was completed. The precipitate was filtered off and the reaction mixture was dissolved in 200 mL of water and extracted with ethyl acetate several times. The ethyl acetate layer was washed with water and brine, dried over anhydrous sodium sulfate, filtered, and concentrated to a brown glassy foam. This was purified over silica gel column using dichloromethane/methanol/ammonia, D/M/A (94.5:5:0.5) to yield 0.09 g of desired product. ^{1}H NMR (DMSO-d_6): δ 10.95 (s, 1H, exchangeable in D_2O), 10.14 (s, 1H, exchangeable in D_2O), 8.83 (s, 1H, exchangeable in D_2O), 8.62 (s, 1H), 8.01 (m, 3H), 7.34 (d, 1H, J = 7.5 Hz), 7.11 (d, 1H, J = 8.1 Hz), 6.84 (t, 1H, J = 8.1 Hz), 6.36 (m, 3H), 6.10 (m, 1H), 5.39 (s, 1H), 4.63 (br s, 1H, exchangeable in D2O), 3.26 (m, 6H), 2.93 (m, 1H), 2.64 (m, 2H), 2.25 (m, 6H), 2.02 (m, 1H), 1.44 (m, 1H), 0.75 (m, 1H), 0.36 (m, 2H), 0.01 (m, 2H). FABHRMS [M + H]$^+$ for $C_{41}H_{39}N_5O_7$: calcd, 686.2788; found, 686.2883.

7′-{3,4-Bis[2-(1,3-dioxolanyl)]-benzoylamino}-17-(cyclopropylmethyl)-6,7-didehydro-4,5α-epoxy-14-hydroxyindolo[2′,3′:6,7]-morphinan (8). Potassium carbonate (0.44 g, 3.20 mmol, 5 equiv) was added to a solution of **6** (0.60 g, 0.64 mmol, 1 equiv) in methanol (10 mL). The suspension was allowed to stir at room temperature for 1 h. The mixture was poured into water (150 mL) and extracted with ethyl acetate. The combined organic extracts were washed with water and dried over sodium sulfate. Evaporation of the solvent gave a yellow solid, which was subjected to silica gel column chromatography ($CHCl_3$/CH_3OH/NH_4OH 96:2:2) to afford 0.21 g (48%) of the desired amide **8**. R_f 0.32 ($CHCl_3$/CH_3OH/NH_4OH 93:5:2). ^{1}H NMR (DMSO-d_6): δ 10.98 (br s, 1H), 10.15 (br s, 1H), 8.90 (br s, 1H), 8.14 (s, 1H), 8.05 (m, 1H), 7.67 (d, J = 8.7 Hz, 1H), 7.35 (d, J = 7.5 Hz, 1H), 7.20 (d, J = 7.5 Hz, 1H), 6.91 (t, J = 7.5 Hz, 1H), 6.46 (m, 2H), 6.12 (s, 2H), 5.48 (s, 1H), 4.74 (br s, 1H), 3.94−4.09 (m, 8H), 3.30 (m, 1H), 3.05 (m, 1H), 2.60−2.82 (m, 3H), 2.30−2.48 (m, 4H), 2.09−2.20 (m, 1H), 1.57 (m, 1H), 0.88 (m, 1H), 0.47 (m, 2H), 0.12 (m, 2H). IR (KBr) 1652 cm^{-1}. FABHRMS m/z 534.6244 (M + H)$^+$.

17-(Cyclopropylmethyl)-7′-(3,4-diformylbenzoylamino)-6,7-didehydro-4,5α-epoxy-14-hydroxyindolo[2′,3′:6,7]-morphinan (2). A 0.5 N aqueous solution of HCl (2.3 mL, 1.15 mmol, 6 equiv) was added dropwise to an ice cold solution of compound **8** (0.13 g, 0.19 mmol, 1 equiv) in acetone (10 mL). The mixture was allowed to warm to room temperature, and stirring was continued under nitrogen for 5 days. After cooling, the solution was adjusted to pH 8−9 with 10% aqueous sodium hydrogen carbonate. The solvent was removed under reduced pressure at 25 °C, and the mixture was extracted with chloroform. The combined extracts were washed with water and dried over sodium sulfate. Evaporation of the solvent afforded a solid, which was triturated with ethyl ether. The resulting precipitate was collected by filtration and washed with a small amount of cold ethyl ether, affording 0.076 g of the dialdehyde in 67% yield. R_f 0.50 ($CHCl_3$/CH_3OH 80:20). ^{1}H NMR (DMSO-d_6): δ 11.07 (s, 1H), 10.55 (s, 2H), 10.44 (s, 1H), 8.93 (s, 1H), 8.58 (s, 1H), 8.45 (d, 1H, J = 7.8 Hz), 8.12 (d, 1H, J = 7.8 Hz), 7.34 (d, 1H, J = 7.5 Hz), 7.23 (d, 1H, J = 7.8 Hz), 6.94 (t, 1H, J = 7.8 Hz), 6.43−6.50 (m, 2H), 5.48 (s, 1H), 4.72 (br s, 1H), 3.28 (m, 1H), 3.05 (m, 1H), 2.63−2.76 (m, 3H), 2.25−2.46 (m, 4H), 2.10−2.19 (m, 1H), 1.56 (m, 1H), 0.85 (m, 1H), 0.47 (m, 1H), 0.12 (m, 2H). IR (KBr) 1650 cm^{-1} (broad). FABHRMS m/z 590.2318 [M + H]$^+$, $C_{35}H_{31}N_3O_6$ requires 589.2212. Anal. Calcd for $C_{35}H_{31}N_3O_6$·$2H_2O$: C, 67.18; H, 5.63; N, 6.71. Found: C, 66.93; H, 5.59; N, 6.56.

17-(Cyclopropylmethyl)-7′-(3,4-diformylnaphthoylamino)-6,7-didehydro-4,5α-epoxy-14-hydroxyindolo[2′,3′:6,7]-morphinan (1). β-1′,3′-Dimethoxybenzylphthalan-7′-aminonaltrindole (**5**; 0.065 g, 1 equiv) was dissolved in 10 mL of dry acetone. To this was added 0.8 mL of a 1 N solution of HCl. This was stirred under nitrogen for 3 days at room temperature. After about 2 h of stirring, the solution changed from faint yellow to a deep canary yellow. After 3 days, the solution was added dropwise to an acetone/ether (1:1) mixture to precipitate the desired product. The product was filtered to yield 0.035 g of pure compound. ^{1}H NMR (DMSO-d_6): δ 11.39 (s, 1H), 10.57 (s, 1H), 10.56 (s, 1H), 10.52 (s, 1H), 9.28 (s, 1H, exchangeable in D_2O), 8.97 (s, 1H), 8.78 (s, 1H), 8.71 (s, 1H), 8.42 (d, 1H, J = 9.0 Hz), 8.38 (d, 1H, J = 9.0 Hz), 7.50 (d, 1H, J = 7.8 Hz), 7.24 (d, 1H, J = 7.5 Hz), 7.01 (t, 1H, J = 7.8 Hz), 6.65 (d, 1H, J = 8.4 Hz), 6.58 (d, 1H, J = 8.4 Hz), 6.44 (s, 1H), 5.69 (s, 1H), 4.11 (m, 1H), 3.40 (m, 1H), 3.23 (m, 1H), 3.07 (m, 2H), 2.95 (m, 2H), 2.62 (m, 4H), 1.80 (m, 1H), 1.09 (m, 1H), 0.69 (m, 1H), 0.62 (m, 1H), 0.45 (m, 2H). FABHRMS $[M + H]^+$ for $C_{39}H_{33}N_3O_6$: calcd, 640.2369; found, 640.2487.

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Supporting Information Available: Experimental details are included for the synthesis of compounds **3** and **4**. This information is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Coleman, R. F.; Creighton, T. E. *Protein Function*; IRL Press: Oxford, U.K., 1997; p 155. (b). Oppenheimer, C. L.; Czech, M. P. Affinity Labeling of Receptors. *Growth Maturation Factors* **1984**, *2*, 193−221. (c) Sokolovsky, M. Affinity and Photoafinity Labeling of Receptors. *Brain Recept. Methodol*. **1984**, *A*, 153−167.

(2) Takemori, A. E.; Portoghese, P. S. Development of Non-Equilibrium Opioid Receptor Probes. *New Methods Drug Res.* **1985**, *1*, 113−128.

(3) Le Bourdonnec, B.; Kouhen, R. E.; Lunzer, M. M.; Law, P. Y.; Loh, H. H.; Portoghese, P. S. Reporter Affinity Labels: An *o*-Phthalaldehyde Derivative of β-Naltrexamine as a Fluorogenic Ligand for Opioid Receptors. *J. Med. Chem.* **2000**, *43* (13), 2489−2492.

(4) McCurdy, C. R.; Le Bourdonnec, B.; Metzger, T. G.; Kouhen, R. E.; Zhang, Y.; Law, P. Y.; Portoghese, P. S. Naphthalene Carboxaldehyde As An Electrophilic Moiety for Affinity Labeling: Application to Opioid Receptor Affinity Labels With Greatly Improved Fluorogenic Properties. *J. Med. Chem.* **2002**, *45* (14), 2887−2890.

(5) (a) Chiu, D. T.; Lillard, S. J.; Scheller, R. H.; Zare. R. N.; Rodriguez-Crz, S. E.; Williams, E. R.; Orwar, O.; Sandberg, M.; Lundqvist, J. A. Probing Single Secretory Vesicles with Capillary Electrophoresis. *Science* **1998**, *279*, 1190−1193. (b) Gilman, S. D.; Ewing, A. G. Post-Column Derivatization For Capillary Electrophoresis Using Naphthalene-2,3-dicarboxaldehyde and 2-Mercaptoethanol. *Anal. Methods Instrum.* **1995**, *2*, 133−141. (c) Lai, F.; Sheehan, T. Matrix Effects in the Derivatization of Amino Acids with Naphthalene Dicarboxaldehyde, 9-Fluorenylmethyl Chloroformate and Phenylisothiocyanate. *BioTechniques* **1993**, *14*, 642−644. (d) De Montigny, P.; Stobaugh, J. F.; Givens, R. S.; Carlson, R. G.; Srinivasachar, K.; Sternson, L. A.; Higuchi, T. Naphthalene-2,3-dicarboxaldehyde/Cyanide Ion: A Rationally Designed Fluorogenic Reagent for Primary Amines. *Anal. Chem.* **1987**, *59*, 1096−1101. (e) Roach, M. C.; Harmony, M. D. Determination of Amino Acids at Subfemtomole Levels by High Performance Liquid Chromatography with Laser-Induced Fluorescence Detection. *Anal. Chem.* **1987**, *59*, 411−415. (f) Miura, T.; Kashiwamura, M. A Sensitive Fluorometric determination of Erythrocyte and Serum Argininosuccinate Lyase Activities with 2,3- Naphthalenedicarboxaldehyde. *Anal. Sci*. **1986**, *2*, 473−476.

(6) Zhang, Y.; McCurdy, C. R.; Metzger, T. G.; Portoghese, P. S. Specific Cross-Linking of Lys 233 and Cys 235 in the Mu Opioid Receptor By A Reporter Affinity Label. *Biochemistry* **2005**, *22* (44), 2271−2275.

(7) Orrwar, O.; Fishman, H. A.; Ziv, N. W.; Scheller, R. H.; Zare, R. N. Use of 2,3-Naphthalenedicarboxaldehyde Derivatization for Single-Cell Analysis of Glutathione by Capillary Electrophoresis and Histochemical Localization by Fluorescence Microscopy *Anal. Chem.* **1995**, *67*, 4261−4268.

(8) Takemori, A. E.; Portoghese, P. S. Selective Naltrexone-Derived Opioid Receptor Antagonists. *Annu. Rev. Pharmacol. Toxicol*. **1992**, *32*, 239−269.

(9) Portoghese, P. S.; Sultana, M.; Takemori, A. E.; Design of Peptidomimetic δ Opioid Receptor Antagonists Using the Message-Address Concept. *J. Med. Chem*. **1990**, *33*, 1714−1720.

(10) Ananthan, A.; Johnson, C. A.; Carter, R. L.; Clayton, S. D.; Rice, K. C.; Xu, H.; Davis, P.; Porreca, F.; Rothman, R. B. Synthesis, Opioid Receptor Binding, and Bioassay of Naltrindole Analogs Substituted in the Benzene Indolic Moiety. *J. Med. Chem.* **1998**, *41*, 2872−2881.

(11) Kshirsagar, T.; Nakano, A. H.; Law, P. Y.; Elde, R.; Portoghese, P. S. NTI4F: A Non-Peptide Fluorescent Probe Selective for Functional Delta Opioid Receptors. *Neurosci. Lett.* **1998**, *249*, 83−86.

(12) Le Bourdonnec, B.; El Kouhen, R.; Poda, G.; Law, P. Y.; Loh, H. H.; Ferguson, D. M.; Portoghese, P. S. Covalently Induced Inactivation of the δ Opioid Receptor Using Fluorogenic Affinity Label, 7-(Phthalaldehydecarboxamido)naltrindole (PNTI). *J. Med. Chem.* **2001**, *44*, 1017−1020.

(13) Portoghese, P. S.; Sultana, M.; Nelson, W. L.; Klein, P.; Takemori, A. E. δ Opioid Antagonistic Activity and Binding Studies of Regioisomeric Isothiocyanate Derivatives of Naltrindole: Evidence for δ Receptor Subtypes. *J. Med. Chem.* **1992**, *35*, 4086−4091.

(14) Henderson, G.; Hughes, J.; Kosterlitz, H. W. New Example of a Morphine-Sensitive Neuro-effector Junction: Adrenergic Transmission in the Mouse Vas Deferens. *Br. J. Pharmacol.* **1972**, *46*, 764−766.

(15) Rang, H. P. Stimulant Actions of Volatile Anesthetics on Smooth Muscles. *Br. J. Pharmacol.* **1964**, *22*, 356−365.

(16) Portoghese, P. S.; Takemori, A. E. Tena A Selective Kappa Opioid Receptor Antagonist. *Life Sci.* **1985**, *36*, 801−805.

(17) Friesner, R. A.; Banks, J. L.; Murphy, R. B.; Halgren, T. A.; Klicic, J. J.; Mainz, D. T.; Repasky, M. P.; Knoll, E. H.; Sheely, M.; Perry, J. K.; Shaw, D. E.; Francis, P.; Shenkin, P. S. Glide: A New Approach for Rapid, Accurate Docking and Scoring. 1. Method and Assessment of Docking Accuracy. *J. Med. Chem.* **2004**, *47*, 1739−1749.

(18) Chen, C.; Okayama, H. High-Efficiency Transformation of Mammalian Cells by Plasmid DNA. *Mol. Cell. Biol.* **1987**, *7*, 2745−2752.

(19) Bradford, M. M. A Rapid and Sensitive Method for Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal. Biochem.* **1976**, *72*, 248−254.

(20) Cheng, Y. C.; Prusoff, W. H. Relationship Between Inhibition Constant (K_i) and the Concentration of Inhibitor Which Causes 50% Inhibition (I_{50}) of An Enzymic Reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099−3108.

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