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Structure-Activity Relationships of (+)-Naltrexone-Inspired Toll-Like Receptor 4 (TLR4) Antagonists

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Abstract

Activation of Toll-like receptors has been linked to neuropathic pain and opioid dependence. (+)-Naltrexone acts as a Toll-like receptor 4 (TLR4) antagonist and has been shown to reverse neuropathic pain in rat studies. We designed and synthesized compounds based on (+)-naltrexone and (+)-noroxymorphone and evaluated their TLR4 antagonist activities by their effects on inhibiting lipopolysaccharide (LPS) induced TLR4 downstream nitric oxide (NO) production in microglia BV-2 cells. Alteration of the N-substituent in (+)-noroxymorphone gave us a potent TLR4 antagonist. The most promising analog, (+)-*N*-phenethylnoroxymorphone ((4*S*,4a*R*,7a*S*,12b*R*)-4a,9-dihydroxy-3-phenethyl-2,3,4,4a,5,6-hexahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7(7a*H*)-one, **1j**) showed ~ 75 times better TLR-4 antagonist activity than (+)-naltrexone, and the ratio of its cell viability IC_{50}, a measure of its toxicity, to TLR-4 antagonist activity (140 μM /1.4 μM) was among the best of the new analogs. The **1j** was active *in vivo*; it significantly increased and prolonged morphine analgesia.

Graphical Abstract

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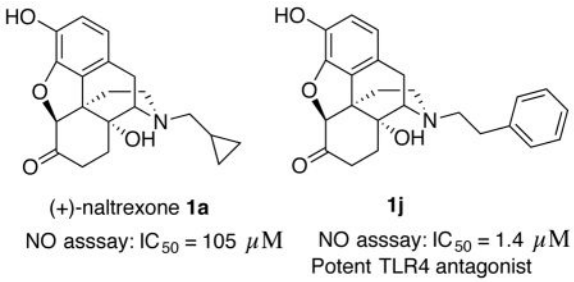
[§]**Author Contributions**

BRS and XW contributed equally to the paper.

The authors declare no competing financial interests.

Supporting Information

^1H and ^{13}C NMRs of novel compounds can be found in the supporting information along with the ^1H NMRs of known compounds. The microanalytical data are presented in tabular form. This material is available free of charge via the Internet at http://pubs.acs.org.



INTRODUCTION

Toll-like receptor 4 (TLR4) detects pathogen-associated molecular patterns,[1] damage-associated molecular patterns,[2, 3] and xenobiotic-associated molecular patterns,[4–6] triggering signal transduction cascades culminating in the production of pro-inflammatory mediators.[1] These pro-inflammatory factors exaggerate neuronal excitability, thereby contributing to neuropathic pain and drug dependence.[7–10] Because of these actions, TLR4 has become an important non-neuronal therapeutic target.[3]

We recently identified naltrexone as a TLR4 antagonist by a variety of biophysical, *in vitro*, *in silico*, and *in vivo* assays.[11–13] Naltrexone was found to non-stereoselectively reverse neuropathic pain in a rat model,[14] which indicates that this action of naltrexone is independent of opioid receptors. Unlike the pharmacologically active (−)-isomer, (+)-naltrexone[15, 16] ((4a*R*,7a*S*,12b*R*)-3-(cyclopropylmethyl)-4a,9-dihydroxy-2,3,4,4a,5,6-hexahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7(7a*H*)-one, **1a**, Figure 1) does not interact with opioid receptors. Since it cannot act as an opioid antagonist, it does not prevent the beneficial analgesic effect of opioids. Further studies have shown that (+)-naltrexone can reduce opioid- and cocaine-induced conditioned place preference, self-administration, drug-primed reinstatement (relapse), and incubation of craving.[4, 17, 18] However, (+)-naltrexone itself does not appear to be the best ligand for treating chronic pain and drug abuse as it has a relatively poor cell viability to TLR4 antagonist ratio (>400/105.5) and is not long-acting *in vivo*, nor is it particularly potent in its action. Because of this, we have explored the design and synthesis of new ligands based on the (+)-naltrexone molecular template in order to obtain a more promising TLR4 antagonist with an improved cell viability to TLR4 antagonist ratio. In this initial study we evaluated the effect of modification or removal of oxygen atoms in (+)-naltrexone, as well as alteration of the N-substituent. We found that the removal of the furan ring and the ketone from (+)-naltrexone afforded an analog that was ~15 times more potent as a TLR4 antagonist. Most gratifying, the replacement of the *N*-cyclopropylmethyl substituent in (+)-naltrexone with an *N*-phenethyl moiety led to analog **1j** with ~75 fold better TLR4 antagonist activity than (+)-naltrexone. *N*-Phenethylnoroxymorphone (4*S*,4a*R*,7a*S*,12b*R*)-4a,9-dihydroxy-3-phenethyl-2,3,4,4a,5,6-hexahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7(7a*H*)-one (**1j**), also had relatively low cell cytotoxicity and *in vivo* efficacy.

RESULTS AND DISCUSSION

Several logical disconnections and modifications to the structure of (+)-naltrexone **1a** (Figure 1) were prepared as structural analogs to establish an initial qualitative SAR. Stereoselective reduction of (+)-naltrexone **1a** afforded (+)-β-naltrexol **2** and (+)-α-naltrexol **3**. The oxide bridge in (+)-naltrexone **1a** was removed to provide (+)-desoxynaltrexone ((4b*S*,8a*R*)-11-(cyclopropylmethyl)-3,8a-dihydroxy-8,8a,9,10-tetrahydro-5*H*-9,4b-(epiminoethano)phenanthren-6(7*H*)-one**, 4)**, and this structure was further simplified by removing the ketone as well, to afford (+)-didesoxynaltrexone (4b*R*,8a*R*)-11-(cyclopropylmethyl)-5,6,7,8,9,10-hexahydro-8a*H*-9,4b-(epiminoethano)phenanthrene-3,8a-diol, **5)**. Further, we examined the effect of the N-substituent in (+)-naltrexone by removing the cyclopropylmethyl group and adding various alkyl (e.g., compound **6**, Figure 1) and phenylalkyl moieties to the secondary amine. Removal of the C-10 carbon from ring B in (+)-naltrexone, a pentacyclic compound, provided the structural analog (+)-10-nornaltrexone (4a*R*,7a*S*,12b*R*)-3-(cyclopropylmethyl)-4a,9-dihydroxy-2,3,4,4a,5,6-hexahydro-1*H*-benzofuro[3,2-*e*]isoquinolin-7(7a*H*)-one, **7**),[19] a somewhat less strained tetracyclic compound. The synthesis of **7** was described previously.[19]

The 12 step synthesis of (+)-naltrexone **1a** (Scheme 1) began with hydrogenation using Pd/C to saturate the double-bond in the enol ether present in sinomenine **8,** followed by treatment of **9** in a $CHCl_3$ - methanesulfonic acid two-phase system to afford (+)-hydrocodone **10**. Formation of the C-6 methyl enol ether in **11** was conducted using the Rapoport *et al.*[20] procedure on our (+)-enantiomer with our critical modification of changing the acid from H_2SO_4 to 5-sulfosalicylic acid for reasons previously described.[15] Bromination of **11** was improved using N-bromosuccinimide (NBS) rather than the reported N-bromoacetamide (NBA) in MeOH[20] to give **12**. Transformation of bromide **12** into one of our needed intermediates, (+)-noroxymorphone **19**, was performed in 7 steps following known procedures,[21] with the modification of 3M HCl instead of H_2SO_4 in the transformation of *N*-cyano **18** to (+)-noroxymorphone **19**. Finally, N-alkylation with cyclopropylmethyl bromide as we have previously described[15] afforded our starting material, (+)-naltrexone **1a**.

Stereospecific reduction of (+)-naltrexone to (+)-naltrexols 2 and 3

We initially attempted to increase the potency of (+)-naltrexone **1a** by determining whether the C-6 keto moiety was necessary for its action; whether a C-6 hydroxyl group might be better. Since it was unpredictable whether that hydroxyl group would be more effective above or below the plane of the ring, we decided to prepare and test both compounds. The stereoselective reduction of (+)-naltrexone **1a** to obtain analogs **2** and **3** was performed using procedures that were known to provide the corresponding (−)-isomers.[22] The use of formamidine sulfinic acid in aqueous sodium hydroxide on (+)-naltrexone **1a** provided (+)-β-naltrexol **2**, while reaction with KH and K-Selectride afforded (+)-α-naltrexol **3** (Scheme 2).

The effect of the (+)-enantiomers **2** and **3** on TLR4 was examined in cell assays. Microglia are the resident cells of the innate immune system in the central nervous system.[23] TLR4 is expressed in microglia and its activation is one of the main mechanisms for

neuroinflammation.[24] We used the microglia BV-2 cell line as the model system of microglia, as they reproduce the primary microglia with high fidelity.[25] TLR4 activation induces the production of the downstream inflammatory factor nitric oxide (NO), which is one of the factors contributing to the development of neuropathic pain[26] and drug addiction.[27, 28] Therefore, NO was used as the readout of lipopolysaccharide (LPS) induced TLR4 activation.[13] As shown in Figure 2, (+)-β-naltrexol **2** and (+)-α-naltrexol **3** each showed reduced TLR-4 antagonism in comparison to (+)-naltrexone **1a**. (+)-Naltrexone **1a** had an IC_{50} = 105.5 ± 10.1 μM in that assay, whereas (+)-β-naltrexol **2** and (+)-α-naltrexol **3** were less potent with IC_{50}'s = 242.8 ± 21.3 and 143.5 ± 20.8 μM, respectively (Table 1). In contrast, (+)-10-nornaltrexone **7**[19] had an IC_{50} = 76.2 ± 27.2 μM. Each of these compounds showed low cell cytotoxicity, their IC_{50} > 400 μM in a cell viability assay. However, the ratio of cytotoxicity to TLR4 antagonism was low (<10) for these analogs, and neither appeared to have great promise.

Removal of Oxygen Functionality

(+)-Desoxynaltrexone **4** (Scheme 3) was prepared using the procedures of Nagase *et al.*,[30, 31] in their synthesis of the (−)-enantiomer of **24** (**1a** → **24**, Scheme 3). This synthesis began with a methylation of the C-3 hydroxy of (+)-naltrexone **1a** to arrive at the 3-methoxyether **20**, which was subsequently treated with activated zinc and ammonium chloride to open the oxide bridge and afford the C-4 hydroxy compound **21** (Scheme 3). The methoxyphenol **21** was converted to the C-4 phenyl ether **22**. Protection of the C-6 ketone in **22** as the cyclic ketal **23** followed by dissolving metal reduction conditions and deprotection of the ketone under acidic conditions provided (+)-O-methyldesoxynaltrexone **25**. Finally, O-demethylation of **25** with BBr_3 gave (+)-desoxynaltrexone **4**.

As shown in Figure 3, in the NO assay (+)-desoxynaltrexone **4** had an IC_{50} = 118.1 ± 22.5 μM with an IC_{50} of cell viability of > 400 μM. (+)-Desoxynaltrexone **4** was a more effective TLR4 antagonist than (+)-naltrexone **1a** at concentrations above 100 μM; at lower concentrations, (+)-naltrexone **1a** was somewhat more potent. These assays indicated that (+)-desoxynaltrexone **4** would not be a better TLR4 antagonist than (+)-naltrexone **1a**.

To determine if the C-6 keto moiety in (+)-desoxynaltrexone **4** had an effect on potency, as measured by the NO assay, we prepared (+)-didesoxynaltrexone **5.** Synthesis of **5** began with a Wolff-Kishner reduction of the O-methyl ether **20** to give alkenyl phenol **26** as described by Wu *et al.*,[32] which was reduced under H_2 atmosphere with PtO_2 to afford **27** (Scheme 4). The same synthetic approach utilized in synthesizing **4** (**21** → **25**, Scheme 3) was then applied to **27** with formation of phenyl ether **28** followed by dissolving metal reduction conditions to provide **29**. Finally, an O-demethylation using BBr_3 afforded (+)-didesoxynaltrexone **5**, the *N*-cyclopropylmethyl analog of (+)-butorphanol.

(+)-Didesoxynaltrexone **5** performed better as a TLR-4 antagonist than (+)-naltrexone **1a** (Figure 4). The increased antagonism difference became more apparent at the 20 μM testing dose. (+)-Didesoxynaltrexone **5** had an IC_{50} value in the NO assay of 48.9 ± 6.8 μM with an IC_{50} of cell viability = 366 ± 78.8 μM. Removal of the C-6 keto moiety from (+)-desoxynaltrexone **4** gave a slightly more potent compound. However, although more potent

than both (+)-naltrexone **1a** and (+)-desoxynaltrexone **4**, the (+)-didesoxynaltrexone **5** was not sufficiently different from either to warrant further work with the compound.

Modification of the N-Substituent

The known strong TLR-4 agonist lipopolysaccharide (LPS) contains a lipid A portion which has 6 unsubstituted linear alkanes (11 carbons and greater). The crystal structure of LPS bound to the TLR4/MD2 complex, where MD-2 is the myeloid differentiation factor 2, reported by Park *et al.*,[33] shows 5 of these lipid chains interacting with MD-2 while one of them interacts with TLR4. We thought we would try to mimic the lipid chain structure by introducing a longer or bulkier alkyl chain on the secondary nitrogen in (+)-noroxymorphone **19** (Scheme 1), with the hope that it might have an effect on the potency of these compounds as TLR4 antagonists. To that end, a four-carbon chain was introduced to obtain (+)-*N*-butylnoroxymorphone **1b**, and five to eight-carbon chains were also synthesized (Scheme 5). The compound with the longest chain was (+)-*N*-octylnoroxymorphone **1f**. In addition, to determine the effect of hydrophobicity and bulk on the alkyl chain, (+)-*N*-(5-phenyl)pentylnoroxymorphone **1g** through (+)-*N*-phenethylnoroxymorphone **1j** were prepared. Also, aryl fluoride **1k** was synthesized to see if adding an electron-withdrawing substituent on the arene would provide any added benefit.

The (+)-*N*-alkyl- and (+)-*N*-arylalkyl-noroxymorphone analogs **1b**–**1k** showed increased TLR4 antagonism versus LPS in the BV2 microglial cell assay ranging from 4–88 times greater than (+)-naltrexone **1b** (Table 2). A representative example from this group of compounds was that of (+)-*N*-hexylnoroxymorphone **1d** (Figure 5). An increase in the length of the carbon chain from 4 carbons (**1b**) through 8 carbons (**1f**) results in an increase in TLR-4 antagonism versus LPS with **1b** showing IC_{50} = 24.8 ± 3.4 μM, and **1f** with IC_{50} = 1.7 ± 0.1 μM (Table 2). This increase in TLR-4 antagonism, however, came at the expense of cell viability where IC_{50} of viability values > 200 μM and 33.4 ± 0.1 μM for **1b** and **1f**, respectively. The *N*-phenylalkyl containing compounds **1g**–**1k** were generally more potent TLR-4 antagonists than the *N*-alkyl chain compounds **1b**–**1f** (e.g., **1j**, Figure 6). The same trend in cell cytotoxicity showing a direct relationship to chain length was observed in this series as well. Although the TLR-4 antagonism remained roughly the same (~ 1.4 μM) for compounds **1g**–**1j**, the ratio of cell toxicity to TLR4 antagonism degenerated as the arylakyl chain length increased. Also, the addition of fluorine to the aromatic ring on the 2-position (**1k**) resulted in an increase in the cell cytotoxicity by approximately two-fold.

Combination of Oxygen Removal and N-Substitution

We incorporated in our designed ligand a combination of the benefits of an extended alkyl chain (e.g., as in (+)-*N*-hexylnoroxymorphone **1d)** with the benefits obtained by removal of the oxide bridge and ketone from (+)-naltrexone (that also increased TLR-4 antagonism, as in (+)-didesoxynaltrexone **5)**, with the hope of obtaining a compound with amplified antagonist potency. Thus, the phenol of (+)-*N*-hexylnoroxymorphone **1d** was methylated (Scheme 6) and the resultant diether **30** was subjected to modified Wolff-Kishner reduction conditions[34] to afford monoether **31**. Attempts to prepare a phenyl ether from the phenolic hydroxyl in **31** like that of **27** → **28** (Scheme 4) were unsuccessful. Instead, the phosphonate ester **32 (**Scheme 6) was synthesized and subsequently treated under dissolving metal

reduction conditions to afford the didesoxy compound **33**. An O-demethylation of **33** followed by hydrogenation of the alkene with Pd/C provided (+)-didesoxy-*N*-hexylnoroxymorphone ((4b*R*,8a*R*)-11-hexyl-5,6,7,8,9,10-hexahydro-8a*H*-9,4b-(epiminoethano)phenanthrene-3,8a-diol**, 6**).

(+)-Didesoxy-*N*-hexylnoroxymorphone **6** did have slightly increased TLR-4 antagonism activity (Figure 7) in comparison to both (+)-*N*-hexylnoroxymorphone **1d** and (+)-*N*-didesoxynaltrexone **5**, with an IC_{50} = 5.2 ± 1.3 μM. However, the increased activity is within the experimental error of the activity of (+)-*N*-hexylnoroxymorphone **1d** itself at 6.7 ± 0.4 μM. In addition, the IC_{50} of viability assay for (+)-didesoxy-*N*-hexylnoroxymorphone **6** was found to be 73.4 ± 4.0 μM, while that of (+)-*N*-hexylnoroxymorphone **1d** was 121.4 ± 7.3 μM. We also examined the *N*-hexyl compound **34** in the BV-2 microglial cell assay, and it was more potent, with an IC_{50} = 22.5 ± 3.2 μM, and less toxic with an IC_{50} of viability = 154.7 ± 5.0 μM, although the ratio (~7) was not as good as desired.

Comparison of Phenol Versus Methyl Ether

All the compounds synthesized and tested up to this point contained a phenol as found in classic agonist and antagonist opiates such as oxymorphone, morphine, and naltrexone. In order to determine if the phenol functionality was necessary for TLR4 activity or whether it had an effect on toxicity, methyl ether **35** ((4a*R*,7a*S*,12b*R*)-4a-hydroxy-9-methoxy-3-phenethyl-2,3,4,4a,5,6-hexahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7(7a*H*)-one) was synthesized (Scheme 7) for comparison with the phenol **1j** in the BV-2 microglia cell assay. The TLR4 antagonist activity of methyl ether **35** was found to be ~4 fold less (IC_{50} = 5.7 ± 1.3 μM) than that of phenol **1j** (IC_{50} = 1.4 ± 1.3 μM). In addition, the cell viability assay showed greater cell cytotoxicity for **35** (IC_{50} of viability = 74.7 ± 3.5 μM) than **1j** (IC_{50} of viability = 140.8 ± 5.5 μM).

Finally, the *in vivo* efficacy of (+)-*N*-phenethylnoroxymorphone (**1j**) was tested on the Hargreaves thermal hyperalgesia assay.[35] Potentiation of morphine analgesia was tested as a clinically relevant outcome, since morphine signaling at TLR4 opposes analgesia in a pro-inflammatory fashion.[5, 36] Here, intrathecal morphine (15 μg plus vehicle; open circle) produced significant analgesia to radiant heat, compared to baseline (Fig. 8; $P < 0.05$). Coadministration of **1j** (60 μg, intrathecal; closed circle) significantly increased and prolonged intrathecal morphine analgesia (Figure 8; Drug x Time: $F_{18,180} = 0.83$, $P = 0.6$; Drug: $F_{1,10} = 16.7$, $P < 0.01$; Time: $F_{18,180} = 3.73$, $P < 0.001$).

CONCLUSION

Stereoselective reduction of the ketone of (+)-naltrexone **1a** to the (+)-naltrexols **2** and **3** resulted in a slight loss of TLR4 antagonist activity, while removal of the oxide bridge and ketone to give (+)-didesoxynaltrexone **5** afforded an ~15 fold increase in activity relative to (+)-naltrexone **1a**. Removal of the bridging benzylic C-10 carbon of (+)-naltrexone resulted in an analog, (+)-10-nornaltrexone **7**, with only a slight improvement in TLR4 antagonism. Interestingly, the replacement of the *N*-cyclopropylmethyl substituent in (+)-naltrexone with a linear alkyl chain (4–8 carbons) resulted in greatly increased activity (~4–62 fold). There

was, however, increased cell cytotoxicity, and a poorer toxicity/activity ratio, associated with longer chain length. The *N*-phenylalkyl analogs were found to be among the most potent TLR-4 antagonists (up to 88 fold compared to (+)-naltrexone). *N*-Phenethyl through *N*-phenylpentyl analogs were found to have similar TLR4 antagonism activity ($IC_{50} \approx 1.4$ μM), while the cell cytotoxicity increased with chain length. Finally, it was found that a methyl ether on the aromatic ring, as in **35**, decreases TLR4 antagonism and increases toxicity compared with the corresponding phenolic compound. The *N*-phenethyl-substituted noroxymorphone (**1j**) was among the most potent compounds found and had the best ratio (~ 100) of TLR4 potent antagonist activity ($IC_{50} = 1.4 \pm 0.3$ μM), and cell cytotoxicity (IC_{50} of viability = 140.8 ± 5.5). This analog **1j** also showed *in vivo* efficacy in potentiating morphine analgesia.

EXPERIMENTAL

Cell based characterization

NO assay—NO assays were performed as described previously.[6] BV-2 microglia cells were grown in DMEM medium supplemented with 10% FBS. BV-2 cells were detached from the flask by trypsin digestion when ~80% confluence was reached. Cells were seeded at a density of 4×10^4 cells/well in 96-well plates. After overnight incubation, the medium was aspirated and changed to DMEM (without FBS). LPS (200 ng/mL) and indicated concentration of compounds were added. Following an additional 24 h treatment, 100 μL of media was removed and added to flat black 96-well microfluor plates (Thermo Scientific, MA, USA). Subsequently, 10 μL of 2,3-diaminonaphthalene (0.05 mg/mL in 0.62 M HCl) was added to each well and incubated for 15 min. The reaction was quenched by addition of 5 μL of 3 M NaOH and the plate was read on a Beckman Coulter DTX880 reader (Fullerton, CA, USA) with excitation at 360 nm and emission at 430 nm. Assay of NO in BV-2 microglia cells, rather than assay of HEK293 TLR4 over-expressing cells, was chosen as the HEK293 TLR4 cells can only report activation of NFkappaB signaling pathways so do not appropriately represent the complexity of TLR4 signaling.[37, 38]

Cell viability assay—Crystal violet staining was used to determine cell viability as described previously.[5, 6] After drug treatment, cells were fixed by 3.7% paraformaldehyde (PFA) for 5 min. and then stained by 0.05% crystal violet for 30 min. The plates were subsequently washed 2 times with tap water and dried for 30 min at room temperature. 200 μL of ethanol was added to each well and the plates were shaken for 15 min at room temperature to dissolve the dye. Absorbance at 540 nm was measured with a Beckman Coulter DTX880 reader (Fullerton, CA, USA)

Data analysis—Origin 7.5 (OriginLab Corporation, Northampton, MA, USA) was used for plotting of the data and statistical analysis. Non-linear logistic regression was used to plot and analyze concentration-response curves and to obtain IC_{50} values. Each experiment was independently repeated at least 3 times with 3–4 biological replicates

In vivo characterization

Animals—Pathogen-free, adult male Sprague Dawley rats were used (300–325 g on arrival; Harlan Labs, Madison, WI), and housed in temperature (23 ± 3 °C) and light (12 h: 12 h light:dark cycle; lights on at 0700) controlled rooms with standard rodent chow and water available *ad libitum*. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Colorado Boulder.

Intrathecal catheter implantation and drug delivery—The method of acute intrathecal drug administration and the construction and implantation of the indwelling intrathecal catheters was based on work described previously.[39] In brief, the day prior to experimentation, intrathecal operations were conducted under isoflurane anesthesia by threading sterile polyethylene-10 tubing (PE-10 Intramedic Tubing; Becton Dickinson Primary Care Diagnostics, Sparks, MD, USA) guided by an 18-gauge needle between the L5 and L6 vertebrae. The catheter was inserted such that the proximal catheter tip lay over the lumbosacral enlargement. The needle was removed and the catheter was sutured to the superficial musculature of the lower back. The catheters were pre-loaded with 60 μg of **1j** in 1.5% DMSO in 0.9% saline + 15 μg morphine in 0.9% saline or vehicle (1.5% DMSO in 0.9% saline + 15 μg morphine in 0.9% saline) at the distal end in a total volume of 10 μL. The catheters were 90 cm in length, allowing remote drug delivery without touching or otherwise disturbing the rats during the testing. On the day of experimentation, drugs were delivered over 20–30 s.

Hargreaves test for analgesia—Rats received at least three 60 min habituations over successive days to the test environment prior to behavioral testing. Latencies for behavioral response to radiant heat stimuli applied to the tail were assessed using a modified Hargreaves test.[35] All testing was conducted blind with respect to group assignment. Briefly, baseline withdrawal values were calculated from an average of three consecutive withdrawal latencies of the tail, measured at 10 min intervals. A short baseline latency (2–3 s) was used to allow quantification of analgesia (lengthening of the latency, relative to baseline, in response to analgesia). A cut-off time of 10 s was imposed to avoid tissue damage. Nociceptive assessments for acute administration experiments were then made at 0 (immediately following remote drug delivery) and every 10 min thereafter for 180 mins.

Statistics and data analysis—The analgesic responses were calculated, as is standard in the pain field, as the % of maximal possible effect (%MPE) using the following equation %MPE = test latency - baseline latency/cutoff – baseline latency x 100. Repeated measures two-way ANOVA with a Holm Sidak posthoc test was used to determine group differences. Repeated measures one-way ANOVA with a Dunnett posthoc test was used to determine changes from baseline. Data are presented as mean ± SEM, and significance was set at $P < 0.05$.

Chemistry

General Methods—Melting points were determined on a Buchi B-545 instrument and are uncorrected. Proton and carbon nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were recorded on a Varian Gemini-400 spectrometer in $CDCl_3$ (unless otherwise noted) with the

values given in ppm (TMS as internal standard) and *J* (Hz) assignments of ^{1}H resonance coupling. Mass spectra (HRMS) were recorded on a VG 7070E spectrometer or a JEOL SX102a mass spectrometer. The optical rotation data were obtained on a PerkinElmer polarimeter model 341. Thin layer chromatography (TLC) analyses were carried out on Analtech silica gel GHLF 0.25 mm plates using various gradients of $CHCl_3$/MeOH containing 1% NH_4OH or gradients of EtOAc:*n*-hexane. Visualization was accomplished under UV light or by staining in an iodine chamber. Flash column chromatography was performed with Fluka silica gel 60 (mesh 220–400). Atlantic Microlabs, Inc., Norcross, GA, or Micro-Analysis, Inc., Wilmington, DE, performed elemental analyses, and the results were within ±0.4% of the theoretical values.

(4*S*,4a*R*,7a*S*,12b*R*)-3-Butyl-4a,9-dihydroxy-2,3,4,4a,5,6-hexahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7(7a*H*)-one (1b): To a stirred solution of (+)-*N*-noroxymorphone dihydrate **19** (0.500 g, 1.54 mmol) in DMF (10 mL) in a screw cap vial was added $NaHCO_3$ (0.502 g, 5.98 mmol) and 1-bromobutane (0.365 mL, 3.39 mmol). The vial was capped (the cap was teflon-lined) and heated at 90 °C for 3 h. The reaction mixture was cooled to room temperature, filtered through Celite, and concentrated *in vacuo*. To the crude residue was added H_2O (10 mL), concentrated aq NH_4OH (2 mL) and $CHCl_3$ (20 mL). The organics were separated and the aqueous layer was extracted with $CHCl_3$ (20 mL). The combined organics were washed with H_2O (5 × 20 mL), dried over Na_2SO_4, filtered through Celite, and concentrated *in vacuo* to afford (+)-*N*-butylnoroxymorphone **1b** as a crude white solid. Purification of this solid by SiO_2 column chromatography with 10% NH_4OH in MeOH/$CHCl_3$ (gradient, 0 → 4%) afforded **1b** (0.151 g, 29%) as a white solid. All spectra reported on the free base. $[\alpha]^{20}_{D}$ = + 179.5 (1.1, $CHCl_3$); mp 150–151 °C; IR (thin film) 3184 $cm^{-1;}$ ^{1}H NMR (400 MHz, $CDCl_3$) δ6.69 (d, *J* = 8.0 Hz, 1H), 6.54 (d, *J* = 8.4 Hz, 1H), 6.08 (br s, 2H), 4.73 (s, 1H), 3.08-2.94 (m, 3H), 2.57-2.38 (m, 5H), 2.26 (m, 1H), 2.13 (td, *J* = 12.0, 3.6 Hz, 1H), 1.86 (m, 1H), 1.61 (td, *J* = 14.0, 3.2 Hz, 1H), 1.52 (m, 1H), 1.48-1.29 (m, 4H), 0.90 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ209.8, 143.5, 138.8, 128.9, 123.9, 119.6, 117.9, 90.3, 70.5, 62.6, 53.9, 50.8, 43.4, 36.0, 31.1, 30.5, 29.6, 22.7, 20.3, 13.9; HRMS (TOF MS ES^{+}) calcd for $C_{20}H_{26}NO_4$ (M + H^{+}) 344.1856, found 344.1857. An HCl salt was prepared by dissolving **1b** free base in hot *i*-PrOH (1.0 mL) followed by the addition of concentrated aq HCl (0.10 mL, 3 equiv) and cooling to 5 °C. The crystals were filtered, rinsed with cold *i*-PrOH, and air-dried to give **1b** as its HCl•2H_2O salt. Anal. Calcd for $C_{20}H_{30}ClNO_6$ (**1b**•HCl•2H_2O): CHN.

(4*S*,4a*R*,7a*S*,12b*R*)-4a,9-Dihydroxy-3-pentyl-2,3,4,4a,5,6-hexahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7(7a*H*)-one (1c): The same procedure used for **1b** was also used for **1c**, with **19** (0.500 g, 1.54 mmol), pentyl bromide (0.421 mL, 3.39 mmol), $NaHCO_3$ (0.502 g, 5.98 mmol), and DMF (10 mL). Purification of the resulting solid by SiO_2 column chromatography with 10% NH_4OH in MeOH/$CHCl_3$ (gradient, 0 → 4%) afforded *N*-pentylnoroxymorphone **1c** (0.506 g, 92%) as a white solid. All spectra reported on the free base. $[\alpha]^{20}_{D}$ = + 173.1 (1.2, $CHCl_3$); mp 168–169 °C; IR (thin film) 3181 $cm^{-1;}$ ^{1}H NMR (400 MHz, $CDCl_3$) δ6.71 (d, *J* = 8.4 Hz, 1H), 6.58 (d, *J* = 8.0 Hz, 1H), 4.70 (s, 1H), 3.11-2.88 (m, 3H), 2.59-2.28 (m, 5H), 2.15 (td, *J* = 12.0, 4.0 Hz, 1H), 1.87 (m, 1H), 1.67-1.45 (m, 3H), 1.37-1.27 (m, 4H), 0.91 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz,

$CDCl_3$) δ 209.7, 143.5, 138.8, 129.0, 124.0, 119.7, 117.8, 90.4, 70.5, 62.7, 54.2, 50.9, 43.4, 36.1, 31.2, 30.5, 29.4, 27.2, 22.7, 22.5, 14.0; HRMS (TOF MS ES^+) calcd for $C_{21}H_{28}NO_4$ ($M + H^+$) 358.2013, found 358.2013. An HCl salt was prepared by dissolving **1c** free base in hot *i*-PrOH (3.5 mL) followed by concentrated HCl (0.34 mL, 3 equiv) and cooling to 5 °C. The crystals were filtered, rinsed with cold *i*-PrOH, and air-dried to give **1c** as the HCl salt. Anal. Calcd for $C_{21}H_{28}ClNO_4$ (**1c**•HCl): CHN.

(4*S*,4a*R*,7a*S*,12b*R*)-3-Hexyl-4a,9-dihydroxy-2,3,4,4a,5,6-hexahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7(7a*H*)-one (1d): The same procedure used for **1b** was also used for **1d**, with **19** (3.00 g, 9.25 mmol), 1-bromohexane (2.90 mL, 20.4 mmol), $NaHCO_3$ (3.1 g, 37 mmol), and DMF (60 mL) to afford (+)-*N*-hexylnoroxymorphone **1d** (3.0 g, 87%) as an off-white solid that was used without further purification. All spectra reported on the free base. $[\alpha]^{20}_D$ = + 187.3 (1.1, $CHCl_3$); mp 160–162 °C; IR (thin film) 3190 $cm^{-1;}$ ^1H NMR (400 MHz, $CDCl_3$) δ 6.70 (d, *J* = 8.4 Hz, 1H), 6.55 (d, *J* = 8.0 Hz, 1H), 6.24 (br s, 1H), 4.75 (s, 1H), 3.09-2.95 (m, 3H), 2.58-2.39 (m, 5H), 2.29 (d, *J* = 14.8 Hz, 1H), 2.13 (t, *J* = 11.6, 3.2 Hz, 1H), 1.86 (m, 1H), 1.62 (td, *J* = 14.0, 3.2 Hz, 1H), 1.53 (m, 1H), 1.48-1.40 (m, 2H), 1.39-1.21 (m, 6H), 0.87 (t, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 209.7, 143.5, 138.8, 128.9, 123.9, 119.6, 117.8, 90.3, 70.5, 62.6, 54.2, 50.8, 43.3, 36.0, 31.6, 31.1, 30.5, 27.4, 26.8, 22.7, 22.5, 14.0; HRMS (TOF MS ES^+) calcd for $C_{22}H_{30}NO_4$ ($M + H^+$) 372.2169, found 372.2169. An HCl salt was prepared by dissolving **1d** free base in hot *i*-PrOH (21 mL) followed by concentrated HCl (1.9 mL, 3 equiv) and cooling to 5 °C to give **1d** as the HCl salt. Anal. Calcd for $C_{22}H_{30}ClNO_4$ (**1d**•HCl): CHN.

(4*S*,4a*R*,7a*S*,12b*R*)-3-Heptyl-4a,9-dihydroxy-2,3,4,4a,5,6-hexahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7(7a*H*)-one (1e): The same procedure used for **1b** was also used for **1e**, with **19** (0.500 g, 1.54 mmol), 1-bromoheptane (0.533 mL, 3.39 mmol), $NaHCO_3$ (0.502 g, 5.98 mmol), and DMF (10 mL). Purification of this solid by SiO_2 column chromatography with 10% NH_4OH in MeOH/$CHCl_3$ (gradient, 0 → 4%) afforded **1e** (0.319 g) that still contained some DMF. To the impure **1e** was added *i*-PrOH (1.9 mL) followed by concentrated aq HCl (0.20 mL). The suspension was cooled to 5 °C, filtered, rinsed with cold *i*-PrOH (2 × 2 mL), and air-dried to afford pure (+)-*N*-heptylnoroxymorphone **1e** (0.291 g, 45%) as a white HCl•$0.3H_2O$ salt. All spectra reported on the free base. $[\alpha]^{20}_D$ = + 174.7 (1.2, $CHCl_3$); mp 143–145 °C; IR (thin film) 3170 $cm^{-1;}$ ^1H NMR (400 MHz, $CDCl_3$) δ 6.70 (d, *J* = 8.0 Hz, 1H), 6.54 (d, *J* = 8.0 Hz, 1H), 6.03 (br s, 2H), 4.73 (s, 1H), 3.08-2.94 (m, 3H), 2.57-2.38 (M, 4H), 2.28 (d, *J* = 14.4 Hz, 1H), 2.13 (m, 1H), 1.65-1.44 (m, 4H), 1.40-1.20 (m, 6H), 0.86 (t, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 209.8, 143.5, 138.8, 128.9, 124.0, 119.7, 117.9, 90.4, 70.5, 62.6, 54.2, 50.9, 43.4, 36.0, 31.7, 31.2, 30.5, 29.1, 27.5, 27.2, 22.7, 22.5, 14.0; HRMS (TOF MS ES^+) calcd for $C_{23}H_{32}NO_4$ ($M + H^+$) 386.2326, found 386.2327. Anal. Calcd for $C_{23}H_{32.6}ClNO_{4.3}$ (**1e**•HCl•$0.3H_2O$): CHN.

(4*S*,4a*R*,7a*S*,12b*R*)-4a,9-Dihydroxy-3-octyl-2,3,4,4a,5,6-hexahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7(7a*H*)-one (1f): The same procedure used for **1b** was also used for **1f**, with **19** (0.500 g, 1.54 mmol), 1-bromooctane (0.589 mL, 3.39 mmol), $NaHCO_3$ (0.502 g, 5.98 mmol), and DMF (10 mL). Purification of this solid by SiO_2

column chromatography with 10% NH_4OH in MeOH/$CHCl_3$ (gradient, 0 → 4%) afforded **1f** (0.414 g) still containing some DMF. To the impure **1f** was added *i*-PrOH (2.5 mL) followed by concentrated aqueous HCl (0.25 mL). The suspension was cooled to 5 °C, filtered, rinsed with cold *i*-PrOH (2 × 2 mL), and air-dried to afford pure (+)-*N*-octylnoroxymorphone **1f** (0.391 g, 58%) as a white HCl•0.15H_2O salt. All spectra reported on the free base. $[\alpha]^{20}_D$ = + 156.8 (1.2, $CHCl_3$); mp 139–141 °C; IR (thin film) 3184 $cm^{-1;}$ ^1H NMR (400 MHz, $CDCl_3$) δ6.71 (d, *J* = 8.4 Hz, 1H), 6.57 (d, *J* = 8.0 Hz, 1H), 4.71 (s, 1H), 3.10-2.94 (m, 3H), 2.59-2.37 (m, 4H), 2.27 (m, 1H), 2.15 (td, *J* = 12.0, 3.6 Hz, 1H), 1.87 (m, 1H), 1.66-1.44 (m, 3H), 1.30-1.25 (m, 8H), 0.88 (t, *J* = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ209.8, 143.5, 138.8, 128.9, 123.9, 119.6, 117.9, 90.3, 70.5, 62.6, 54.2, 50.8, 43.3, 36.0, 31.7, 31.2, 30.5, 29.4, 29.1, 27.5, 27.2, 22.7, 22.5, 14.0; HRMS (TOF MS ES^+) calcd for $C_{24}H_{34}NO_4$ ($M + H^+$) 400.2482, found 400.2481. Anal. Calcd for $C_{24}H_{34.3}ClNO_{4.15}$ (**1f**•HCl•0.15H_2O): CHN.

(4*S*,4a*R*,7a*S*,12b*R*)-4a,9-Dihydroxy-3-(5-phenylpentyl)-2,3,4,4a,5,6-hexahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7(7a*H*)-one (1g): The same procedure used for **1b** was also used for **1g**, with **19** (0.400 g, 1.24 mmol), 1-bromo-5-phenylpentane (0.504 mL, 2.73 mmol), $NaHCO_3$ (0.402 g, 4.79 mmol), and DMF (8 mL). Purification of this solid by SiO_2 column chromatography with 10% NH_4OH in MeOH/$CHCl_3$ (gradient, 0 → 4%) afforded (+)-*N*-phenylpentylnoroxymorphone **1g** (0.500 g, 89% corrected for DMF) as an off-white solid. All spectra reported on the free base unless otherwise noted. $[\alpha]^{20}_D$ = + 144.3 (1.2, MeOH, HCl salt); mp of HCl salt dec > 276 °C; IR (thin film) 3286, 1723 $cm^{-1;}$ ^1H NMR (400 MHz, $CDCl_3$) δ7.29-7.25 (m, 2H), 7.19-7.16 (m, 3H), 6.73 (d, *J* = 8.4 Hz, 1H), 6.57 (d, *J* = 8.0 Hz, 1H), 6.01 (br s, 2H), 4.70 (s, 1H), 3.09-2.99 (m, 3H), 2.62-2.38 (m, 7H), 2.29 (dt, *J* = 14.0, 2.8 Hz, 1H), 2.16 (td, 12.0, 3.6 Hz, 1H), 1.88 (m, 1H), 1.67-1.49 (m, 6H), 1.41-1.35 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ210.0, 143.6, 142.5, 139.1, 128.9, 128.4, 128.3, 125.7, 123.8, 119.8, 118.1, 90.3, 70.5, 62.6, 54.2, 50.9, 43.6, 36.6, 36.1, 35.8, 31.3, 30.5, 27.3, 26.9, 22.9; HRMS (TOF MS ES^+) calcd for $C_{27}H_{32}NO_4$ ($M + H^+$) 434.23258, found 434.23233. An HCl salt was prepared by dissolving **1g** free base in hot *i*-PrOH (3.5 mL) followed by concentrated aq HCl (0.28 mL, 3 equiv) and cooling to 5 °C. The crystals were filtered, rinsed with cold *i*-PrOH, and air-dried to give the HCl salt. Anal. Calcd for $C_{27}H_{32}ClNO_4$ (**1g**•HCl): CHN.

(4*S*,4a*R*,7a*S*,12b*R*)-4a,9-Dihydroxy-3-(4-phenylbutyl)-2,3,4,4a,5,6-hexahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7(7a*H*)-one (1h): The same procedure used for **1b** was also used for **1h**, with **19** (0.400 g, 1.24 mmol), 1-bromo-4-phenylbutane (0.479 mL, 2.73 mmol), $NaHCO_3$ (0.402 g, 4.79 mmol), and DMF (8 mL). Purification of this solid by SiO_2 column chromatography with 10% NH_4OH in MeOH/$CHCl_3$ (gradient, 0 → 4%) afforded (+)-*N*-phenylbutylnoroxymorphone **1h** (0.398 g, 77%) as an off white solid. All spectra reported on the free base. $[\alpha]^{20}_D$ = + 145.8 (1.1, DMSO); mp 223–225 °C; IR (thin film) 3154, 1724 $cm^{-1;}$ ^1H NMR (400 MHz, DMSO-D_6) δ9.18 (br s, 1H), 7.27-7.12 (m, 5H), 6.54 (d, *J* = 8.4 Hz, 1H), 6.48 (d, *J* = 8.0 Hz, 1H), 4.99 (br s, 1H), 4.72 (s, 1H), 2.97-2.86 (m, 3H), 2.57 (t, *J* = 7.2 Hz, 2H), 2.48-2.41 (m, 3H), 2.28 (m, 1H), 2.06 (d, *J* = 14.0 Hz, 1H), 1.93 (m, 1H), 1.73 (m, 1H), 1.61-1.57 (m, 2H), 1.45-1.38 (m, 2H), 1.26 (d, *J* = 11.2 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-D_6) δ209.1, 143.8, 142.6, 139.8, 129.8,

128.7, 126.1, 123.7, 119.4, 117.6, 89.8, 70.3, 62.4, 53.7, 50.6, 43.6, 36.2, 35.4, 31.5, 30.6, 29.1, 27.0, 22.8; HRMS (TOF MS ES$^+$) calcd for $C_{26}H_{30}NO_4$ (M + H$^+$) 420.21693, found 420.21701. An HCl salt was prepared by dissolving **1h** free base in hot EtOH (2.8 mL) with acetic acid (0.80 mL) and concentrated aq HCl (0.23 mL, 3 equiv) and cooling to 5 °C. The crystals were filtered, rinsed with cold *i*-PrOH, and air-dried to give the HCl•0.5EtOH salt. Anal. Calcd for $C_{27}H_{33}ClNO_{4.5}$ (**1h**•HCl•0.5EtOH): CHN.

(4*S*,4a*R*,7a*S*,12b*R*)-4a,9-Dihydroxy-3-(3-phenylpropyl)-2,3,4,4a,5,6-hexahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7(7a*H*)-one (1i): The same procedure used for **1b** was also used for **1i**, with **19** (0.400 g, 1.24 mmol), 1-bromo-3-phenylpropane (0.415 mL, 2.73 mmol), $NaHCO_3$ (0.402 g, 4.79 mmol), and DMF (8 mL). Purification of this solid by SiO_2 column chromatography with 10% NH_4OH in MeOH/$CHCl_3$ (gradient, 0 → 4%) afforded (+)-*N*-phenylpropylnoroxymorphone **1i** (0.430 g, 50% corrected for DMF) as a light orange oil containing ~ 80 mol % DMF. All spectra reported on the free base unless otherwise noted. $[\alpha]^{20}_D$ = + 152.5 (1.0, MeOH, HCl•0.25H_2O salt); mp of HCl•0.25H_2O salt dec > 276 °C; IR (thin film) 3332, 1724 cm$^{-1;}$ ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28-7.25 (m, 2H), 7.18-7.15 (m, 3H), 6.70 (d, *J* = 8.4 Hz, 1H), 6.53 (d, *J* = 8.4 Hz, 1H), 4.65 (s, 1H), 3.06 (m, 3H), 2.65 (t, *J* = 7.6 Hz, 2H), 2.59-2.46 (m, 4H), 2.38 (t, *J* = 12.6, 5.0 Hz, 1H), 2.25 (dt, *J* = 14.4, 3.0 Hz, 1H), 2.13 (td, *J* = 12.0, 3.6 Hz, 1H), 1.88-1.77 (m, 3H), 1.59 (td, *J* = 14.0, 3.2 Hz, 1H), 1.51 (dd, *J* = 12.8, 2.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 209.7, 143.6, 141.7, 139.2, 128.9, 128.4, 128.3, 125.9, 123.6, 119.7, 118.1, 90.2, 70.5, 62.8, 53.6, 50.8, 43.5, 36.1, 33.4, 31.2, 30.5, 29.1, 22.9; HRMS (TOF MS ES$^+$) calcd for $C_{25}H_{28}NO_4$ (M + H$^+$) 406.20128, found 406.20123. An HCl salt was prepared by dissolving **1i** free base in hot *i*-PrOH (3.0 mL) followed by concentrated aq HCl (0.26 mL, 3 equiv) and cooling to 5 °C. The crystals were filtered, rinsed with cold *i*-PrOH, and air-dried to give the HCl•0.25H_2O salt. Anal. Calcd for $C_{24}H_{28.5}ClNO_{4.25}$ (**1i**•HCl•0.25H_2O): CHN.

(4*S*,4a*R*,7a*S*,12b*R*)-4a,9-Dihydroxy-3-phenethyl-2,3,4,4a,5,6-hexahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7(7a*H*)-one (1j): The same procedure used for **1b** was also used for **1j**, with **19** (0.500 g, 1.54 mmol), 2-bromoethylbenzene (0.458 mL, 3.39 mmol), $NaHCO_3$ (0.502 g, 5.98 mmol), and DMF (10 mL). Purification of this solid by SiO_2 column chromatography with 10% NH_4OH in MeOH/$CHCl_3$ (gradient, 0 → 4%) afforded (+)-*N*-phenethylnoroxymorphone **1j** (0.548 g, 91%) as a white solid. All spectra reported on the free base. $[\alpha]^{20}_D$ = +139.1 (1.1, $CHCl_3$); mp 80–84 °C; IR (thin film) 3165 cm$^{-1;}$ ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (app t, *J* = 7.2 Hz, 2H), 7.25-7.19 (m, 3H), 6.71 (d, *J* = 8.0 Hz, 1H), 6.58 (d, *J* = 8.0 Hz, 1H), 4.68 (s, 1H), 3.08 (d, *J* = 18.4 Hz, 1H), 3.03-2.88 (m, 2H), 2.74-2.54 (m, 6H), 2.39-2.17 (m, 3H), 1.83 (m, 1H), 1.63-1.50 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 209.7, 143.5, 139.7, 138.8, 128.9, 128.5, 128.4, 126.2, 123.8, 119.7, 117.9, 90.2, 70.5, 63.5, 55.9, 50.8, 42.9, 36.0, 34.1, 31.1, 30.3, 23.3; HRMS (TOF MS ES$^+$) calcd for $C_{24}H_{26}NO_4$ (M + H$^+$) 392.1856, found 392.1854. An HCl salt was prepared by dissolving **1j** free base in hot *i*-PrOH (3.8 mL) followed by concentrated aq HCl (0.34 mL, 3 equiv) and cooling to 5 °C. The crystals were filtered, rinsed with cold *i*-PrOH, and air-dried to give the HCl•2H_2O salt. Anal. Calcd for $C_{24}H_{30}ClNO_6$ (**1j**•HCl•2H_2O): CHN.

(4*S*,4a*R*,7a*S*,12b*R*)-3-(2-Fluorophenethyl)-4a,9-dihydroxy-2,3,4,4a,5,6-hexahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7(7a*H*)-one (1k): The same procedure used for **1b** was also used for **1k**, with **19** (0.500 g, 1.54 mmol), 2-fluorophenethylbromide (0.476 mL, 3.39 mmol), $NaHCO_3$ (0.502 g, 5.98 mmol), and DMF (10 mL). Purification of this solid by SiO_2 column chromatography with 10% NH_4OH in MeOH/$CHCl_3$ (gradient, 0 → 4%) afforded (+)-*N*-2-fluorophenethylnoroxymorphone **1k** (0.461 g, 73%) as a light yellow solid. All spectra reported on the free base. $[\alpha]^{20}_D$ = +132.6 (0.5, $CHCl_3$); mp 70–74 °C; IR (thin film) 3358 $cm^{-1;}$ ^1H NMR (400 MHz, $CDCl_3$) δ 7.23-7.18 (m, 2H), 7.10 (d, *J* = 7.6 Hz, 1H), 7.05 (t, *J* = 9.2 Hz, 1H), 6.71 (d, *J* = 8.0 Hz, 1H), 6.58 (d, *J* = 8.0 Hz, 1H), 3.09-2.57 (m, 9H), 2.40-2.18 (m, 3H), 1.82 (m, 1H), 1.62-1.54 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 209.9, 162.4, 159.9, 143.6, 139.0, 130.83, 130.79, 128.9, 128.1, 128.06, 126.8, 126.6, 124.1, 123.9, 119.8, 118.1, 115.4, 115.2, 90.3, 70.5, 63.9, 54.7, 50.8, 42.9, 36.0, 31.2, 30.4, 27.7, 23.5; HRMS (TOF MS ES^+) calcd for $C_{24}H_{25}NO_4F$ ($M + H^+$) 410.1762, found 410.1762. An HCl salt was prepared by dissolving **1k** free base in hot *i*-PrOH (3.2 mL) followed by concentrated aq HCl (0.27 mL, 3 equiv) and cooling to 5 °C. The crystals were filtered, rinsed with cold *i*-PrOH, and air-dried to give the HCl•0.5H_2O salt. Anal. Calcd for $C_{24}H_{26}ClFNO_{4.5}$ (**1k**•HCl•0.5H_2O): CHN.

(4*S*,4a*R*,7*S*,7a*S*,12b*R*)-3-(Cyclopropylmethyl)-2,3,4,4a,5,6,7,7a-octahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinoline-4a,7,9-triol (2): The procedures that de Costa *et al.* used on the enantiomer were followed.[22] To a stirring solution of (+)-naltrexone **1a** free base (1.00 g, 2.93 mmol) in 0.533 M NaOH (20 mL) was added a solution of formamidine sulfinic acid (1.01 g, 11.7 mmol) in 0.533 M NaOH (20 mL) dropwise over 15 min. The reaction mixture was warmed to 85 °C and maintained at that temperature for 1 h and 15 min. The reaction mixture was cooled to ~ 5 °C and a saturated NH_4Cl aqueous solution (40 mL) followed by $CHCl_3$ (40 mL) were added. The aqueous fraction was extracted with $CHCl_3$ (3 × 100 mL), washed with H_2O (100 mL), filtered through Celite, and the combined organics were concentrated *in vacuo*. The crude residue was dried under high vacuum at 100 °C for 14 h to give crude **2** (0.805 g, 80%). All spectral data matched those reported by de Costa *et al.* on the enantiomer.[22] $[\alpha]^{20}_D$ = +196.1 (1.0, $CHCl_3$); mp 185–186 °C. To the crude free base **2** (0.805 g) was added EtOAc (8 mL) and (1*R*,3*S*)-(+)-camphoric acid (0.469 g, 2.34 mmol). The mixture was heated to solution, and cooled to room temperature causing salt formation. The salt crystals were filtered and rinsed with EtOAc (8 mL) to afford **2** (0.902 g) as the (1*R*, 3*S*)-(+)-camphoric acid • H_2O. Anal. Calcd for ($C_{30}H_{43}NO_9$) (**2**•camphoric acid•H_2O): CHN.

(4*S*,4a*R*,7*R*,7a*S*,12b*R*)-3-(Cyclopropylmethyl)-2,3,4,4a,5,6,7,7a-octahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinoline-4a,7,9-triol (3): The procedures were followed that de Costa *et al.* used on the enantiomer.[22] To a stirred solution of (+)-naltrexone **1a** free base (1.00 g, 2.93 mmol) in THF (30 mL) was added 35% KH in mineral oil (0.675 mL, 5.86 mmol). The reaction mixture was cooled to 5 °C and K-Selectride (4.40 mL, 4.40 mmol, 1.0M in THF) was added dropwise over 20 min. After stirring at 5 °C for 20 min, the reaction mixture was warmed to room temperature. After stirring at room temperature for 4 h, H_2O (5 mL) was added and the reaction mixture was concentrated *in vacuo* to remove

organics. Additional H_2O (10 mL) was added followed by 3M HCl to arrive at a pH of ~ 3. The aqueous mixture was washed with Et_2O (3 × 20 mL), basified with concentrated aqueous NH_4OH to a pH of ~ 9.5, and extracted with $CHCl_3$ (3 × 30 mL). The combined organics were filtered through Celite and concentrated *in vacuo* to afford a crude solid (0.90 g). This crude solid was recrystallized from EtOAc (6 mL) to give **3** (0.570 g, 57%) as a white solid. All spectral data matched those reported by de Costa *et al.* on the enantiomer.[22] $[\alpha]^{20}_D$ = +202.1 (0.7, MeOH); mp 210–211 °C.

To **3** (0.478 g) was added EtOAc (5 mL) and (1*R*,3*S*)-(+)-camphoric acid (0.279 g, 1.39 mmol). The mixture was heated to solution and subsequently cooled to room temperature, causing salt formation. The salt crystals were filtered and rinsed with EtOAc (5 mL) to afford **3** (0.254 g) as the (1*R*, *3*S)-(+)-camphoric acid • $0.7H_2O$. Anal. Calcd for $C_{30}H_{42.4}NO_{8.7}$ (**3**•camphoric acid•$0.7H_2O$): CHN

(4b*S*,8a*R*,9*S*)-11-(Cyclopropylmethyl)-3,8a-dihydroxy-8,8a,9,10-tetrahydro-5*H*-9,4b-(epiminoethano)phenanthren-6(7*H*)-one (4): The procedure of Nagase *et al*.[30] on the (−)-isomer was followed for the synthesis of **20** from **1a**. To a stirred solution of (+)-naltrexone **1a** free base (2.20 g, 6.45 mmol) was added DMF (60 mL), K_2CO_3 (1.84 g, 13.3 mmol), and MeI (0.484 mL, 7.76 mmol). After stirring at room temperature for 18 h, the reaction mixture was filtered through Celite and concentrated *in vacuo*. To the crude mixture was added H_2O (20 mL) and Et_2O (20 mL). The aq layer was extracted with Et_2O (3 × 20 mL) and the combined organics were washed with H_2O (5 × 20 mL), filtered through Celite, and concentrated *in vacuo* to afford **20** (1.98 g) as a white solid which was used without further purification.

The sequence from **20** → **24** followed the procedures of Nagase *et al*.[31] To a solution of crude **20** (1.98, 5.57 mmol) in MeOH (50 mL) was added NH_4Cl (2.98 g, 55.7 mmol). The reaction mixture was heated to reflux and activated Zn (2.55 g, 39.0 mmol) was added in 3 portions spaced 1 h apart. After refluxing for a total of 3 h, the reaction mixture was cooled to room temperature and filtered through Celite, rinsing with MeOH (50 mL). The filtrate was concentrated *in vacuo* and 3M NaOH (25 mL) was added followed by 10% MeOH in $CHCl_3$ (50 mL). The aqueous layer was extracted with 10% MeOH in $CHCl_3$ (2 × 50 mL), and the combined organics were dried over Na_2SO_4, filtered and concentrated to afford **21** as a white solid which was used without further purification.

To a solution of the crude **21** in pyridine (20 mL) was added concentrated NH_4OH (2.30 g, 16.7 mmol), Cu powder (0.372 g, 5.85 mmol), and bromobenzene (2.93 mL, 27.9 mmol). The reaction mixture was heated to reflux. After refluxing for 14 h, the reaction mixture was cooled to room temperature, filtered through Celite and concentrated *in vacuo* to give crude **22** as an oil. This crude oil was purified by SiO_2 column chromatography with 10% NH_4OH in MeOH/$CHCl_3$ (gradient, 0 → 8%) to afford **22** (0.740 g, 26% from **1a**) as a white solid. Spectral data matched those reported by Nagase *et al.*,[31] on the enantiomer.

To a stirred solution of **22** (0.491 g, 1.13 mmol) in toluene (11 mL) was added ethylene glycol (0.630 mL, 11.3 mmol) and *p*-TsOH•H_2O (0.344 g, 1.81 mmol). The reaction mixture outfitted with a Dean-Stark apparatus was brought to reflux. After refluxing for 4 h, the

reaction mixture was cooled to room temperature and aqueous saturated $NaHCO_3$ (15 mL) followed by K_2CO_3 were added to bring the mixture to pH ~ 9.5. The aqueous layer was extracted with $CHCl_3$ (3 × 15 mL), filtered through Celite, and concentrated *in vacuo* to afford crude **23** (0.512 g) as a white solid, which was used without further purification.

To a stirred solution of crude **23** (0.512 g) in NH_3 (liquid) (6 mL) and THF (6 mL) at −78 °C, was added Na (0.062 g, 2.7 mmol) to give a blue solution. The reaction mixture was maintained at −78 °C and additional Na pieces were added to maintain the blue color over the course of 3 h. After 3 h at −78 °C, EtOH (2 mL) was added and the reaction mixture was warmed to room temperature. Brine (20 mL) and toluene (20 mL) were added to the reaction mixture. The aqueous layer was extracted with toluene (3 × 20 mL) and the combined organics were dried over Na_2SO_4, filtered through Celite and concentrated *in vacuo* to afford crude **24** (0.318 g), which was used without further purification.

To crude **24** (0.318 g) was added 2M HCl (8 mL) and the reaction mixture was heated to reflux. After refluxing for 5 h, the reaction mixture was cooled to room temperature and concentrated aqueous NH_4OH (3 mL) and H_2O (10 mL) were added. The aqueous mixture was extracted with $CHCl_3$ (3 × 25 mL) and the combined organics were dried over Na_2SO_4, filtered through Celite and concentrated *in vacuo* to afford crude **25** (0.270 g) which was used without further purification.

To a stirred solution of crude **25** (0.270 g) in CH_2Cl_2 (8 mL) at 0 °C was bubbled HCl gas until pH ~ 3. A solution of BBr_3 (0.374 mL, 3.95 mmol) in CH_2Cl_2 (2 mL) was added dropwise. The reaction mixture was warmed to room temperature. After stirring at room temperature for 1.5 h, the reaction mixture was cooled to 0 °C and H_2O (10 mL) was added dropwise. The reaction mixture was warmed to room temperature. After stirring 30 min at room temperature, the CH_2Cl_2 was distilled off and the remaining aqueous solution was refluxed for an additional 30 min. The reaction mixture was then cooled to 0 °C and concentrated aq NH_4OH was added to a pH ~ 9.5. The aqueous mixture was extracted with 10% EtOH in $CHCl_3$ (5 × 20 mL) and the combined organics were filtered through Celite and concentrated *in vacuo* to afford **4** as a crude solid. This crude solid was purified by SiO_2 column chromatography with 10% NH_4OH in MeOH/$CHCl_3$ (gradient, 0 → 5%) to afford **4** (0.171 g, 46% from **22**) as a white solid. All spectra reported on the free base. $[\alpha]^{20}{}_D$ = +162.1 (1.1, $CHCl_3$); IR (thin film) 3180 $cm^{-1;}$ ^1H NMR (400 MHz, $CDCl_3$) δ 6.89 (d, J = 8.4 Hz, 1H), 6.86 (s, 1H), 6.63 (d, J = 8.0 Hz, 1H), 6.23 (br s, 2H), 3.13 (d, J = 6.0 Hz, 1H), 3.06 (d, J = 5.6 Hz, 1H), 3.02 (d, J = 10.0 Hz, 1H), 2.83-2.69 (m, 3H), 2.58 (m, 1H), 2.38 (d, J = 6.4 Hz, 2H), 2.17-2.07 (m, 3H), 1.91-1.77 (m, 2H), 1.16 (m, 1H), 0.84 (m, 1H), 0.50 (d, J = 7.6 Hz, 2H), 0.11 (d, J = 4.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 212.1, 155.0, 140.1, 128.5, 126.1, 114.1, 112.4, 69.0, 59.6, 59.1, 46.3, 45.4, 43.2, 37.4, 36.7, 31.6, 24.3, 9.3, 3.8, 3.7; HRMS (TOF MS ES^+) calcd for $C_{20}H_{26}NO_3$ ($M + H^+$) 328.1907, found 328.1909.

To **4** (0.171 g) was added Et_2O (2 mL) and MeOH (15 drops). Anhydrous HCl gas was bubbled through the solution causing salt formation. The salt crystals were filtered, rinsed with Et_2O (2 mL), and air dried to afford **4** (0.090 g) as the HCl • $0.5H_2O$ salt mp 194-200 °C. Anal. Calcd for ($C_{20}H_{27}ClNO_{3.5}$): CHN.

(4b*R*,8a*R*,9*S*)-11-(Cyclopropylmethyl)-6,7,8,8a,9,10-hexahydro-5*H*-9,4b-(epiminoethano)phenanthrene-3,8a-diol (5): To a stirred solution of **28** (0.522 g, 1.24 mmol) in NH_3 (liquid) (6 mL) and THF (6 mL) at −78 °C was added freshly cut Na pieces (0.086 g, 3.72 mmol) during which time a blue solution color persisted. Additional small pieces of Na were added to maintain the blue color of the solution over the course of 4 h while maintaining the temperature at −78 °C. After 4 h, EtOH (2 mL) was added and the reaction mixture was allowed to warm to room temperature. To the reaction mixture was added saturated NaCl (10 mL) and the aq layer was extracted with $CHCl_3$ (3 × 15 mL). The combined organic fractions were dried over Na_2SO_4, filtered through Celite, and concentrated *in vacuo* to afford **29** (0.323 g) as a crude white solid that was used without further purification.

To the crude **29** (0.323 g) was added CH_2Cl_2 (10 mL), and anhydrous HCl was bubbled through the solution for 5 min. The reaction mixture was cooled to 0 °C and a solution of BBr_3 (0.51 mL, 5.4 mmol) in CH_2Cl_2 (5 mL) was added dropwise. After addition, the reaction mixture was warmed to room temperature, stirred for 1.5 h and cooled to 0 °C. To the mixture was added H_2O (10 mL), the CH_2Cl_2 was distilled off, and the reaction mixture was refluxed for 30 min. The solution was cooled to 0 °C, concentrated NH_4OH (1 mL) was added and the aqueous layer was extracted with $CHCl_3$/EtOH (3 × 10 mL, 5:1). The combined organics were dried over Na_2SO_4, filtered through Celite, and concentrated *in vacuo* to afford **5** as a crude white solid. Purification of this crude solid by SiO_2 column chromatography with 10% NH_4OH in MeOH/$CHCl_3$ (gradient, 0 → 8%) afforded **5** (0.254 g, 65% from **28**) as a white solid. $[\alpha]^{20}_D$ = +98.0 (1.0, $CHCl_3$); mp 80–86 °C; IR (thin film) 3275 $cm^{-1;}$ ^1H NMR (400 MHz, CD_3OD) δ 6.93 (d, J = 7.2 Hz, 1H), 6.73 (s, 1H), 6.60 (d, J = 6.8 Hz, 1H), 4.94 (s, 2H), 3.04 (d, J = 18.4 Hz, 1H), 2.91 (s, 1H), 2.58 (d, J = 8.8 Hz, 1H), 2.52 (m, 1H), 2.49-2.32 (m, 2H), 2.15-1.76 (m, 5H), 1.50-1.37 (m, 5H), 1.03 (d, J = 12.0 Hz, 1H), 0.87 (m, 1H), 0.53 (d, J = 6.8 Hz, 2H), 0.15 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.8, 144.1, 130.2, 129.0, 115.2, 113.5, 72.2, 62.9, 61.1, 46.6, 43.4, 39.0, 33.5, 32.1, 26.3, 23.6, 23.5, 11.2, 5.3, 5.1; HRMS (TOF MS ES^+) calcd for $C_{20}H_{28}NO_2$ ($M + H^+$) 314.2115, found 314.2114.

Anhydrous HCl gas was bubbled through a solution of **5** (0.254 g) in a mixture of Et_2O (2 mL) and MeOH (0.2 mL). The HCl salt of **5** precipitated almost immediately. It was filtered and rinsed with Et_2O (3 mL) to give **5** as an HCl•0.5 MeOH salt, mp (dec) >270 °C. Anal. Calcd for $C_{20.5}H_{30}ClNO_{2.5}$ (**5**•HCl•0.5 MeOH): CHN.

(4b*R*,8a*R*,9*S*)-11-Hexyl-6,7,8,8a,9,10-hexahydro-5*H*-9,4b-(epiminoethano)phenanthrene-3,8a-diol (6): To a solution of **34** (0.31 g, 0.90 mmol) in EtOH (8 mL) and AcOH (1 mL) was added 5% Pd/C Escat 103 (0.030 g). The reaction mixture was subjected to an atmosphere of H_2 (33 psi) for 14 h on a Parr hydrogenation apparatus. After 14 h, H_2O (10 mL) and concentrated aq NH_4OH (2 mL) were added, followed by $CHCl_3$ (15 mL). The aqueous layer was extracted with $CHCl_3$ (3 × 15 mL), and the combined organics were dried over Na_2SO_4, filtered through Celite, and concentrated *in vacuo* to afford **6** as a crude yellow oil. Purification of this oil by SiO_2 column chromatography with 10% NH_4OH in MeOH/$CHCl_3$ (gradient, 0 → 3%) afforded **6** (0.206

g, 67%) as a light yellow foam. All spectra reported on the free base. $[\alpha]^{20}_{D}$ = +67.7 (1.0, $CHCl_3$); mp 108–110 °C; IR (thin film) 3289 $cm^{-1;}$ ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.93 (d, *J* = 8.0 Hz, 1H), 6.77 (s, 1H), 6.64 (d, *J* = 7.6 Hz, 1H), 5.49 (br s, 2H), 3.02 (d, *J* = 17.6 Hz, 1H), 2.77-2.68 (m, 2H), 2.43-2.40 (m, 3H), 2.10-1.75 (m, 5H), 1.51-1.20 (m, 12H), 1.02 (d, *J* = 12.8 Hz, 1H), 0.86 (t, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.7, 142.5, 128.3, 127.7, 113.2, 112.0, 70.3, 61.4, 54.3, 44.3, 41.5, 37.0, 31.7, 31.5, 30.2, 27.6, 27.0, 24.8, 22.6, 21.7, 21.6, 14.0; HRMS (TOF MS ES^+) calcd for $C_{22}H_{34}NO_2$ ($M + H^+$) 344.2584, found 344.2584. An HCl salt was prepared by dissolving **6** free base in hot *i*-PrOH (1.4 mL) followed by concentrated aq HCl (0.15 mL, 3 equiv) and cooling to 5 °C. The resultant salt was filtered, rinsed with cold *i*-PrOH, and dried in a vacuum oven at room temperature. Anal. Calcd for $C_{22}H_{34}ClNO_2$ (**6**•HCl): CHN.

(4b*R*,8a*R*)-11-(Cyclopropylmethyl)-3-methoxy-4-phenoxy-6,7,8,8a,9,10-hexahydro-5*H*-9,4b-(epiminoethano)phenanthren-8a-ol (28): To a stirred solution of **20** (1.63 g, 4.59 mmol) in diethylene glycol (16 mL) was added hydrazine hydrate (1.10 mL, 22.7 mmol). The reaction mixture was heated to 70 °C and maintained at that temperature for 1 h. After 1 h, a solution of KOH (1.80 g, 32.1 mmol) in H_2O (0.9 mL) was added and the solution was heated to 130 °C. After heating at 130 °C for 2 h, the reaction mixture was cooled to room temperature and H_2O (15 mL) was added. The mixture was extracted with Et_2O (3 × 15 mL), washed with saturated NaCl (30 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford **26** as a crude oil. Purification of this oil by SiO_2 column chromatography with 10% NH_4OH in MeOH/$CHCl_3$ (gradient, 0 → 3.5%) afforded **26** (0.859 g, 55%) as a colorless oil. Spectra matched that reported by Wu *et al.*[40]

To a solution of **26** (0.808 g, 2.37 mmol) in EtOH (20 mL) and AcOH (2 mL) was added PtO_2 (150 mg, 0.661 mmol). The reaction mixture was subjected to an atmosphere of H_2 (33 psi) while shaking for 3 h in a Parr hydrogenator. After 3h, H_2O (20 mL) was added followed by concentrated aqueous NH_4OH to obtain a pH of ~ 9.5. The reaction mixture was concentrated *in vacuo* to remove the organics and the aqueous layer was extracted with $CHCl_3$/EtOH (3 × 20 mL, 10:1), dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford crude **27** (0.675 g), which was used without further purification.

To the stirred solution of crude **27** (0.675 g) in pyridine (20 mL) was added K_2CO_3 (0.814 g, 5.88 mmol), Cu powder (0.132 g, 2.06 mmol), and bromobenzene (1.03 mL, 9.80 mmol). The reaction mixture was refluxed for 36 h, cooled to room temperature, filtered through Celite rinsing with $CHCl_3$, and concentrated *in vacuo* to give a crude tacky orange solid. Purification of the crude solid by SiO_2 column chromatography with 10% NH_4OH in MeOH/$CHCl_3$ (gradient, 0 → 5%) afforded **28** (0.522 g, 53% from **26**) as a tacky orange solid. $[\alpha]^{20}_{D}$ = +56.6 (1.6, $CHCl_3$); IR (thin film) 3426 $cm^{-1;}$ ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (app t, *J* = 7.8 Hz, 2H), 6.97 (d, *J* = 8.4 Hz, 1H), 6.93 (t, *J* = 8.0 Hz, 1H), 6.83 (d, *J* = 8.4 Hz, 1H), 6.70 (d, *J* = 8.0 Hz, 1H), 4.47 (br s, 1H), 3.65 (s, 3H), 3.01 (m, 1H), 2.93-2.88 (m, 2H), 2.67 (d, *J* = 13.6 Hz, 1H), 2.51 (dd, *J* = 11.6, 4.0 Hz, 1H), 2.34 (m, 1H), 2.33 (t, *J* = 6.4 Hz, 1H), 2.09 (td, *J* = 11.8, 2.4 Hz, 1H), 1.87-1.73 (m, 3H), 1.33 (m, 1H), 1.15 (d, *J* = 13.2 Hz, 1H), 0.82 (m, 1H), 0.49 (d, *J* = 8.0 Hz, 2H), 0.10 (d, *J* = 4.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.8, 150.8, 141.3, 135.4, 131.0, 129.0, 124.5, 120.9, 114.5, 110.5,

69.5, 60.3, 59.2, 55.8, 44.7, 42.6, 33.8, 32.0, 29.9, 25.4, 22.9, 21.9, 9.4, 3.8, 3.7; HRMS (TOF MS ES$^+$) calcd for $C_{27}H_{34}NO_3$ (M + H$^+$) 420.2533, found 420.2533.

(4*S*,4a*R*,7a*S*,12b*R*)-3-Hexyl-4a-hydroxy-9-methoxy-2,3,4,4a,5,6-hexahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7(7a*H*)-one (30): To a stirred solution of (+)-*N*-hexylnoroxymorphone **1d** (3.0 g, 8.08 mmol) in DMF (50 mL) was added K_2CO_3 (2.50 g, 18.1 mmol) and iodomethane (0.659 mL, 10.6 mmol). The reaction mixture was stirred at room temperature for 48 h, filtered through Celite, and concentrated *in vacuo*. To the crude residue was added H_2O (50 mL), concentrated aq NH_4OH (5 mL) and $CHCl_3$ (50 mL). The organics were separated and the aqueous layer extracted with $CHCl_3$ (50 mL). The combined organics were washed with H_2O (5 × 40 mL), dried over Na_2SO_4, filtered through Celite, and concentrated *in vacuo* to afford **30** (2.98 g, 96%) as a colorless oil that was used without further purification. An analytical sample was prepared by purification with SiO_2 column chromatography with 10% NH_4OH in MeOH/$CHCl_3$ (gradient, 0 → 4%). $[\alpha]^{20}_D$ = + 161.9 (2.0, $CHCl_3$); IR (thin film) 3377 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.66 (d, *J* = 8.4 Hz, 1H), 6.58 (d, *J* = 8.0 Hz, 1H), 5.10 (br s, 1H), 4.62 (s, 1H), 3.85 (s, 3H), 3.06 (d, *J* = 18.4 Hz, 1H), 2.97 (td, *J* = 14.4, 4.8 Hz, 1H), 2.91 (m, 1H), 2.58-2.31 (m, 1H), 2.25 (dt, *J* = 14.4, 3.0 Hz, 1H), 2.10 (td, *J* = 12.4, 3.6 Hz, 1H), 1.83 (m, 1H), 1.62-1.42 (m, 4H), 1.34-1.23 (m, 6H), 0.86 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 208.4, 144.8, 142.8, 129.4, 124.9, 119.2, 114.8, 90.2, 70.1, 62.7, 56.7, 54.2, 50.7, 43.3, 36.0, 31.6, 31.3, 30.6, 27.4, 26.8, 22.7, 22.5, 13.9; HRMS (TOF MS ES$^+$) calcd for $C_{23}H_{32}NO_4$ (M + H$^+$) 386.2326, found 386.2326.

(4b*S*,8a*R*,9*S*)-11-Hexyl-3-methoxy-8,8a,9,10-tetrahydro-7*H*-9,4b-(epiminoethano)phenanthren-8a-ol (33): To a stirred solution of **30** (2.783 g, 7.22 mmol) in $CHCl_3$ (60 mL) in a screw-cap vial was added 1,2-bis(*tert*-butyldimethylsilyl)hydrazine[34] (4.87 g, 18.8 mmol) and the vial was capped (teflon-lined). After stirring at room temperature for 18 h, hexanes (120 mL) were added and the resultant suspension was filtered through Celite rinsing with hexanes (2 × 60 mL). The filtrate was concentrated *in vacuo* and hexanes (120 mL) were added to the residue (part 1). This was added to a DMSO/tBuOH/KOtBu mixture that had been prepared in a separate flask (DMSO (60 mL) and *t*-butanol (1.4 mL) were added to potassium *tert*-butoxide (16.2 g, 144 mmol) and stirred for 30 min), and the mixture was stirred for 30 min. The reaction mixture was then added to a chilled saturated $NaHCO_3$/H_2O (1:1, 500 mL) solution. The aqueous mixture was extracted with 1:1 EtOAc/hexanes (3 × 500 mL). The organics were combined and washed with H_2O (5 × 250 mL), dried over Na_2SO_4, filtered through Celite, and concentrated *in vacuo* to give crude **31** (1.31 g) that was used without further purification.

To a stirred suspension of 60% NaH in mineral oil (0.282 g, 7.05 mmol) in THF (30 mL) at 5 °C was added dropwise a solution of crude **31** (1.31 g) in THF (5 mL). The reaction mixture was stirred at 5 °C and diethylchlorophosphate (0.918 mL, 6.35 mmol) was added dropwise. After stirring at 5 °C for 1 h, saturated NH_4Cl (35 mL) and $CHCl_3$ (60 mL) were added. The aqueous layer was extracted with $CHCl_3$ (3 × 60 mL) and the combined organic solution was dried over Na_2SO_4, filtered through Celite, and concentrated *in vacuo* to afford crude **32** (2.35 g), which was used without further purification.

To a stirred solution of crude **32** (2.35 g) in NH_3 (liquid) and THF (15 mL) at −78 °C was added freshly cut Na (0.90 g, 3.9 mmol). After stirring 30 min at −78 °C, an additional 0.90 g of Na was added and the reaction continued to stir for another 15 min, at which time the starting material was consumed as indicated by TLC. MeOH (1 mL) was added to the reaction mixture, the solution was warmed to room temperature, and H_2O (20 mL) and concentrated aq NH_4OH (1 mL) were added. The aqueous layer was extracted with $CHCl_3$ (3 × 20 mL). The organic solution was dried over Na_2SO_4, filtered through Celite, and concentrated *in vacuo* to afford **33** (1.36 g) as a yellow oil. Purification of this oil by SiO_2 column chromatography with 10% NH_4OH in MeOH/$CHCl_3$ (gradient, 0 → 4%) afforded **33** (0.675 g, 26% from **30**) as a light yellow oil. All spectra reported on the free base. $[\alpha]^{20}_D$ = +25.7 (1.1, $CHCl_3$); IR (thin film) 3418 $cm^{-1;}$ ^1H NMR (400 MHz, $CDCl_3$) δ 6.96 (d, *J* = 8.4 Hz, 1H), 6.71 (d, *J* = 2.4 Hz, 1H), 6.66 (dd, *J* = 8.4, 2.4 Hz, 1H), 5.95 (d, *J* = 10.0 Hz, 1H), 5.84 (dt, *J* = 7.2, 3.2 Hz, 1H), 3.76 (s, 3H), 3.07 (d, *J* = 18.0 Hz, 1H), 2.82 (d, *J* = 5.6 Hz, 1H), 2.76 (dd, *J* = 18.0, 6.0 Hz, 1H), 2.51-2.38 (m, 4H), 2.23-1.98 (m, 3H), 1.69 (m, 1H), 1.54-1.43 (m, 3H), 1.38, 1.23 (m, 7H), 0.88 (t, *J* = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.8, 145.8, 131.5, 127.6, 127.0, 126.0, 112.4, 111.3, 68.7, 61.5, 55.0, 54.0, 44.1, 41.5, 35.0, 31.6, 27.8, 27.5, 26.9, 24.6, 22.8, 22.5, 14.0; HRMS (TOF MS ES^+) calcd for $C_{23}H_{34}NO_2$ ($M + H^+$) 356.2584, found 356.2584.

(4b*S*,8a*R*,9*S*)-11-Hexyl-8,8a,9,10-tetrahydro-7*H*-9,4b-(epiminoethano)phenanthrene-3,8a-diol (34): To a solution of **33** (0.575 g, 1.62 mmol) in $CHCl_3$ (14 mL) at −78 °C was added a solution of BBr_3 (0.77 mL, 8.1 mmol) in $CHCl_3$ (2 mL) dropwise. The reaction mixture was warmed to room temperature over the course of 1h and then H_2O (15 mL) was added dropwise. The $CHCl_3$ layer was distilled off and the resulting aqueous solution was refluxed for 30 min. The reaction mixture was cooled to ~ 5 °C, and concentrated aqueous NH_4OH (1mL) and $CHCl_3$ (20 mL) were added. The aqueous layer was extracted with $CHCl_3$ (3 × 20 mL) and the combined organics were dried over Na_2SO_4, filtered through Celite, and concentrated *in vacuo* to afford **34** (0.479 g, 87%) as an off-white solid. All spectra reported on the free base. $[\alpha]^{20}_D$ = +35.6 (1.0, $CHCl_3$); mp 137-139 °C; IR (thin film) 3293 $cm^{-1;}$ ^1H NMR (400 MHz, $CDCl_3$) δ 6.88 (d, *J* = 8.4 Hz, 1H), 6.67 (d, *J* = 2.0 Hz, 1H), 6.60 (dd, *J* = 8.2, 2.0 Hz, 1H), 5.90 (d, *J* = 10.0 Hz, 1H), 5.80 (d, *J* = 9.6 Hz, 1H), 5.43 (br s, 2H), 3.04 (d, *J* = 18.4 Hz, 1H), 2.84 (d, *J* = 5.6 Hz, 1H), 2.75 (dd, *J* = 18.0, 5.8 Hz, 1H), 2.50-2.37 (m, 4H), 2.21-2.09 (m, 2H), 1.99 (m, 1H), 1.70 (m, 1H), 1.53 (dd, *J* = 12.8, 6.4 Hz, 1H), 1.37-1.19 (m, 7H), 0.87 (t, *J* = 6.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.3, 145.7, 131.5, 127.7, 126.9, 125.4, 113.8, 113.3, 69.3, 61.5, 54.1, 44.1, 41.4, 35.0, 31.6, 27.7, 27.6, 26.9, 24.6, 22.7, 22.6, 14.0; HRMS (TOF MS ES^+) calcd for $C_{22}H_{32}NO_2$ ($M + H^+$) 342.2428, found 342.2428. An HCl salt was prepared by dissolving **34** free base in hot *i*-PrOH (3.4 mL) followed by concentrated aq HCl (0.34 mL, 3 equiv) and cooling to 5 °C. The resultant salt was filtered, rinsed with cold *i*-PrOH, and dried in a vacuum oven at room temperature. Anal. Calcd for $C_{22}H_{31}ClNO_2$ (**34**•HCl): CHN.

(4a*R*,7a*S*,12b*R*)-4a-Hydroxy-9-methoxy-3-phenethyl-2,3,4,4a,5,6-hexahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7(7a*H*)-one (35): The same procedure as **30** with **1j** HCl•$2H_2O$ (0.200 g, 0.431 mmol), K_2CO_3 (0.298 g, 2.16 mmol), iodomethane (0.040 mL,

0.65 mmol), and DMF (4 mL) afforded **35** (0.164 g, 94%) as a white solid. All spectra reported on the free base. $[\alpha]^{20}_{D}$ = + 164.4 (1.3, $CHCl_3$); mp 146–148 °C; IR (thin film) 3404 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.35-7.32 (m, 2H), 7.25-7.20 (m, 3H), 6.70 (d, *J* = 8.4 Hz, 1H), 6.62 (d, *J* = 8.0 Hz, 1H), 4.78 (br s, 1H), 4.64 (s, 1H), 3.90 (s, 3H), 3.10 (d, *J* = 18.4 Hz, 1H), 2.98 (td, *J* = 14.0, 5.0 Hz, 1H), 2.91 (d, *J* = 6.0 Hz, 1H), 2.84-2.58 (m, 5H), 2.38-2.17 (m, 3H), 1.82 (m, 1H), 1.63-1.51 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 208.3, 144.8, 142.7, 139.6, 129.3, 128.4, 128.3, 126.1, 124.7, 119.2, 114.7, 90.1, 70.1, 63.5, 56.6, 55.8, 50.5, 42.7, 35.9, 34.0, 31.2, 30.3, 23.2; HRMS (TOF MS ES^+) calcd for $C_{25}H_{28}NO_4$ ($M + H^+$) 406.20128, found 406.20109. An HCl salt was prepared by dissolving **35** free base in THF (1.1 mL) followed by concentrated aq HCl (0.10 mL, 3 equiv) and cooling to 5 °C, *i*-PrOH (1.1 mL) was added and gave crystals that were filtered, rinsed with cold *i*-PrOH, and air-dried to give the HCl•1.5H_2O. Anal. Calcd for $C_{25}H_{31}ClNO_{5.5}$ (**35**•HCl•1.5H_2O): CHN.

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Abbreviations

TLR4	Toll-like receptor 4
NO	nitric oxide
LPS	lipopolysaccharides
DMEM	Dulbecco’s Modified Eagle Medium
FBS	fetal bovine serum

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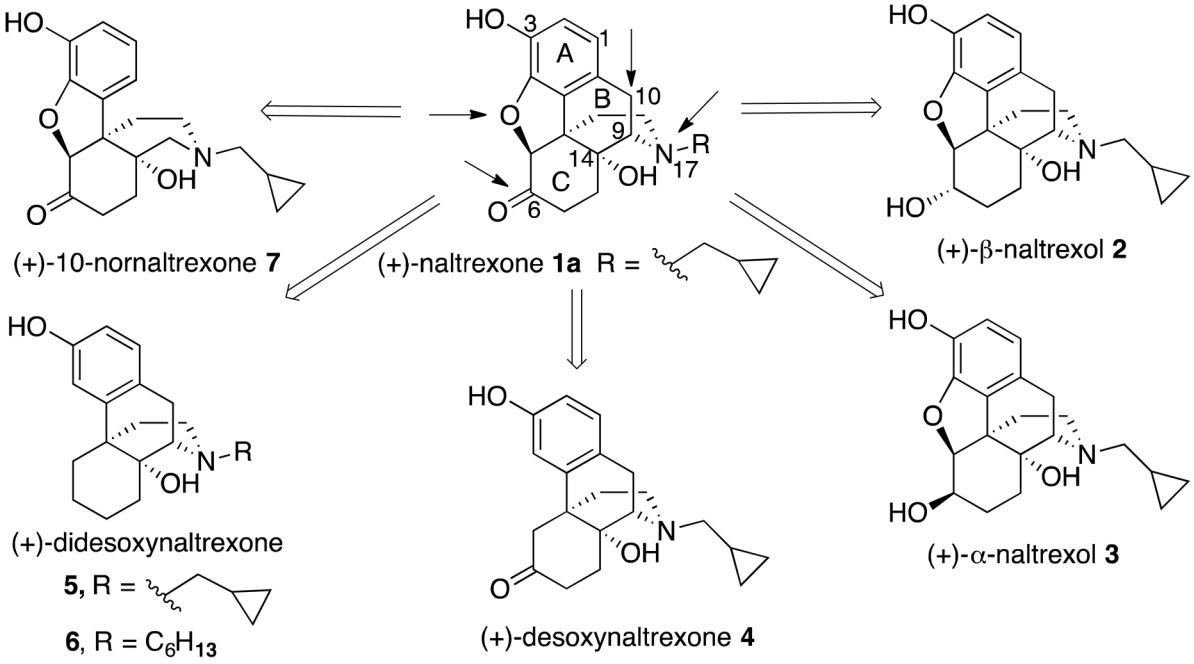


Figure 1.
Synthetic targets **2** – **7**

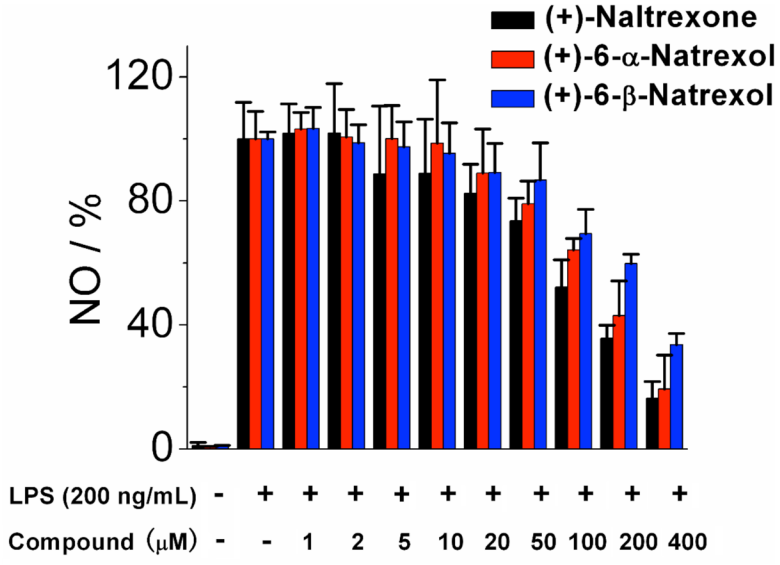


Figure 2.

(+)-Naltrexone **1a**, (+)-β-naltrexol **2** and (+)-α-naltrexol **3** inhibit LPS induced NO over-production in a dose-dependent manner. BV-2 cells were treated with 200 ng/mL LPS and various concentrations of compounds for 24 h. NO in the supernatant was measured by the 2,3-diaminonaphthalene method.[6, 29] The NO in the LPS (200 ng/mL) control group was normalized as 100%.

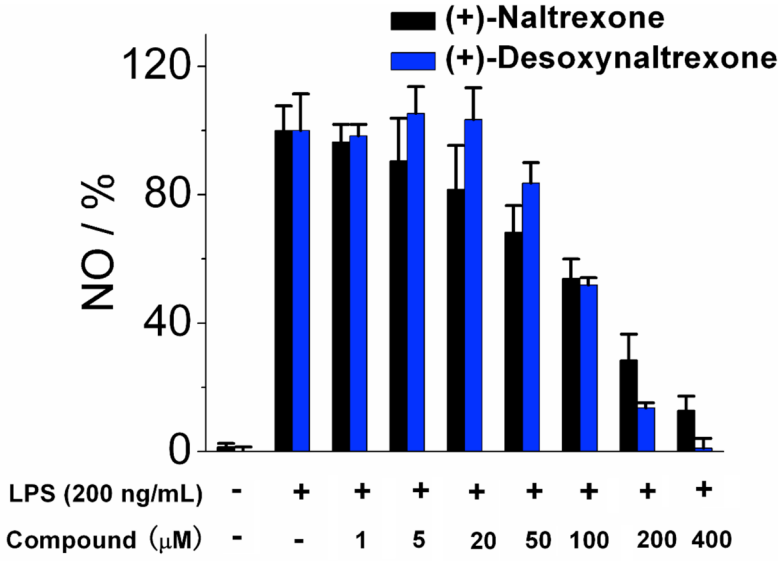


Figure 3.

(+)-Desoxynaltrexone **4** inhibits LPS induced NO over-production in a dose-dependent manner. (+)-Naltrexone **1a** served as a control. BV-2 cells were treated with 200 ng/mL LPS and various concentrations of the test compounds for 24 h. NO in the supernatant was measured by the 2,3-diaminonaphthalene method.[6, 29] The NO in the LPS (200 ng/mL) control group was normalized to 100%.

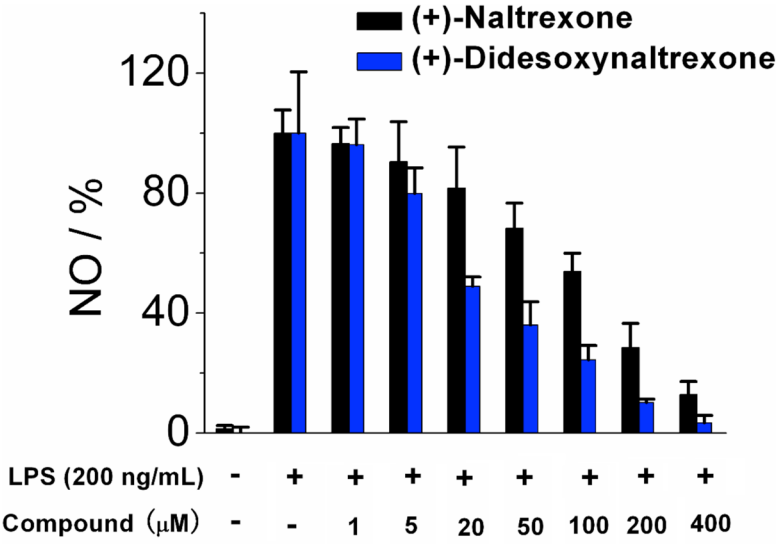


Figure 4.

(+)-Didesoxynaltrexone **5** inhibits LPS induced NO over-production in a dose-dependent manner. (+)-Naltrexone **1a** served as the control compound for comparison. BV-2 cells were treated with 200 ng/mL of LPS and various concentrations of test compounds for 24 h. NO in the supernatant was measured by the 2,3-diaminonaphthalene method.[6, 29] The NO in the LPS (200 ng/mL) group was normalized as 100%.

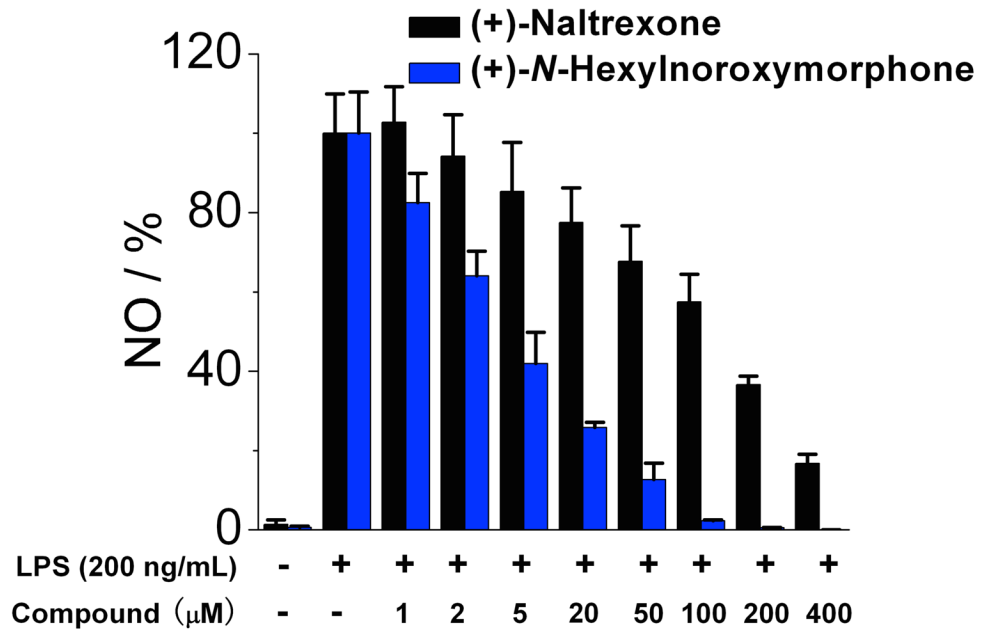


Figure 5.

(+)-*N*-Hexylnoroxymorphone **1d** inhibits LPS induced NO over-production in a dose-dependent manner. (+)-Naltrexone **1a** served as the control compound for comparison. BV-2 cells were treated with 200 ng/mL LPS and various concentrations of test compounds for 24 h. NO in the supernatant was measured by the 2,3-diaminonaphthalene method.[6, 29] The NO in the LPS (200 ng/mL) control group was normalized to 100%.

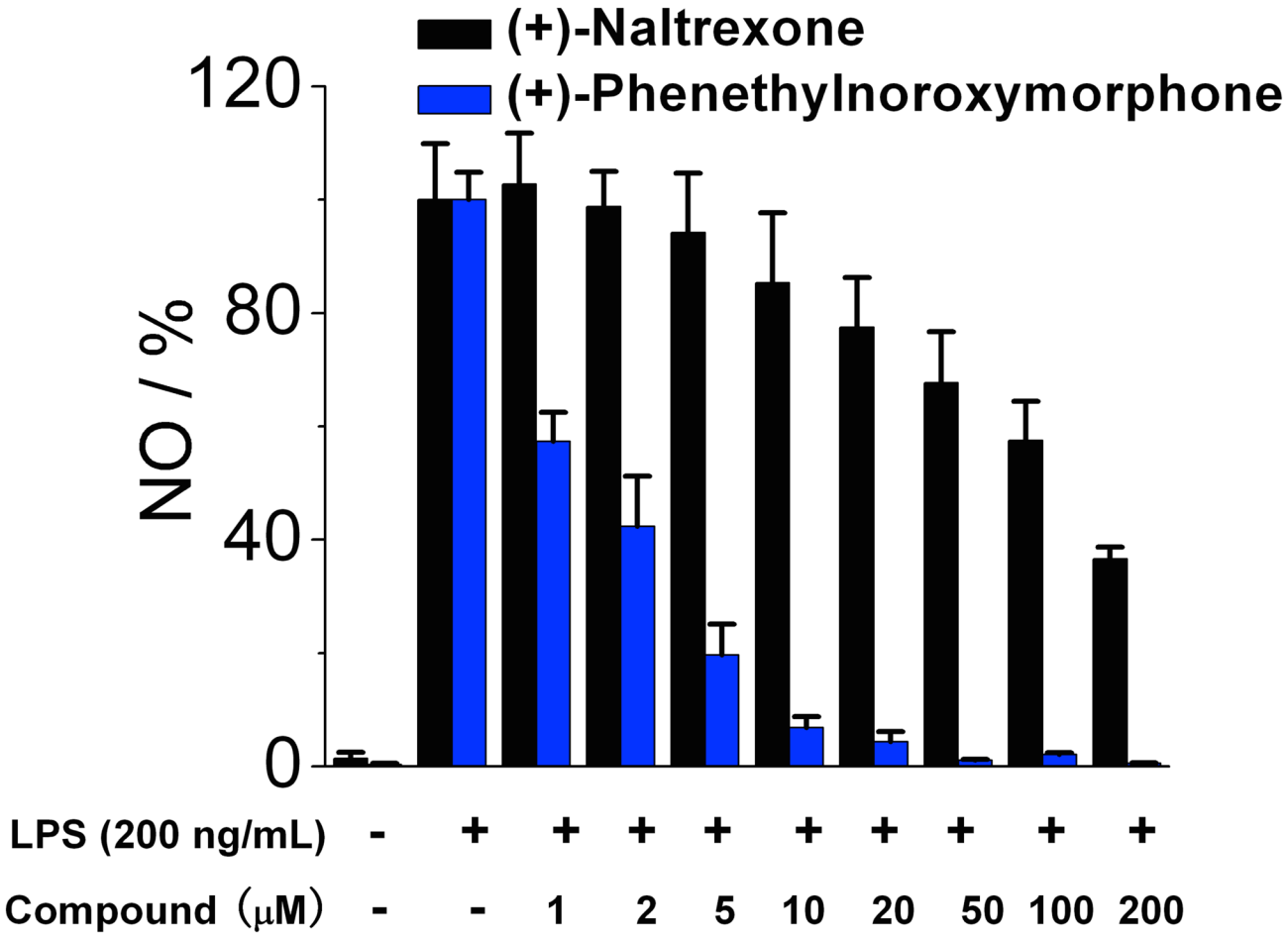


Figure 6.

(+)-*N*-Phenethylnoroxymorphone **1j** inhibits LPS induced NO over-production in a dose-dependent manner. (+)-Naltrexone **1a** served as the control compound for comparison. BV-2 cells were treated with 200 ng/mL LPS and various concentrations of test compounds for 24 h. NO in the supernatant was measured by the 2,3-diaminonaphthalene method.[6, 29] The NO in the LPS (200 ng/mL) group was normalized to 100%.

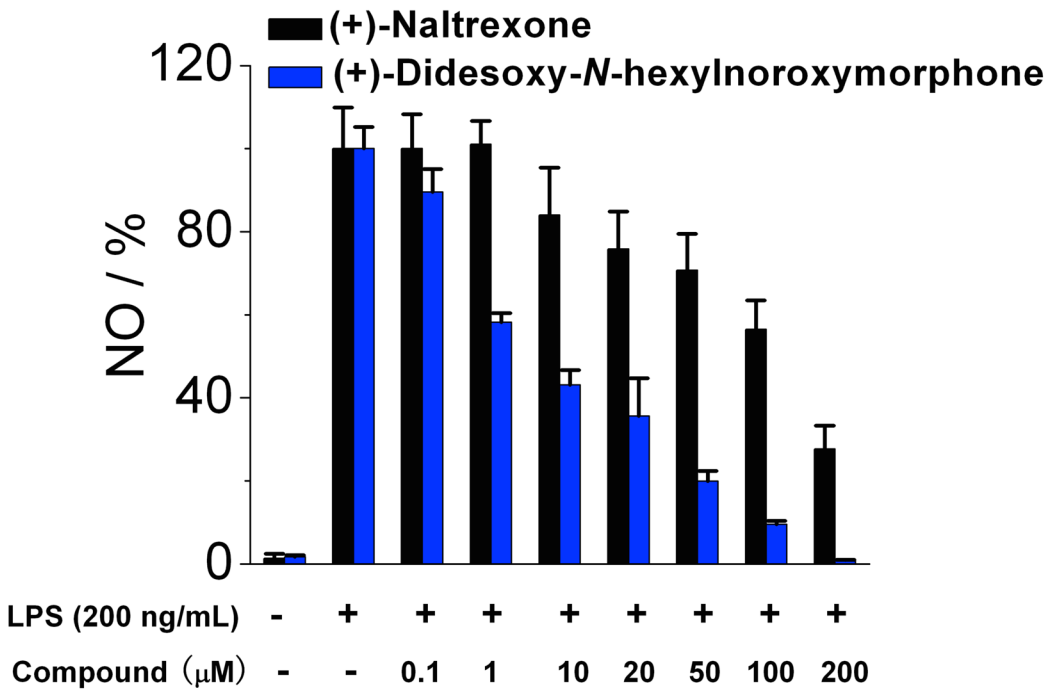


Figure 7.

(+)-Didesoxy-*N*-hexylnoroxymorphone **6** inhibits LPS induced NO over-production in a dose-dependent manner. (+)-Naltrexone **1a** served as the control compound for comparison. BV-2 cells were treated with 200 ng/mL LPS and various concentrations of test compounds for 24 h. NO in the supernatant was measured by the 2,3-diaminonaphthalene method.[6, 29] The NO in the LPS (200 ng/mL) group was normalized to 100%.

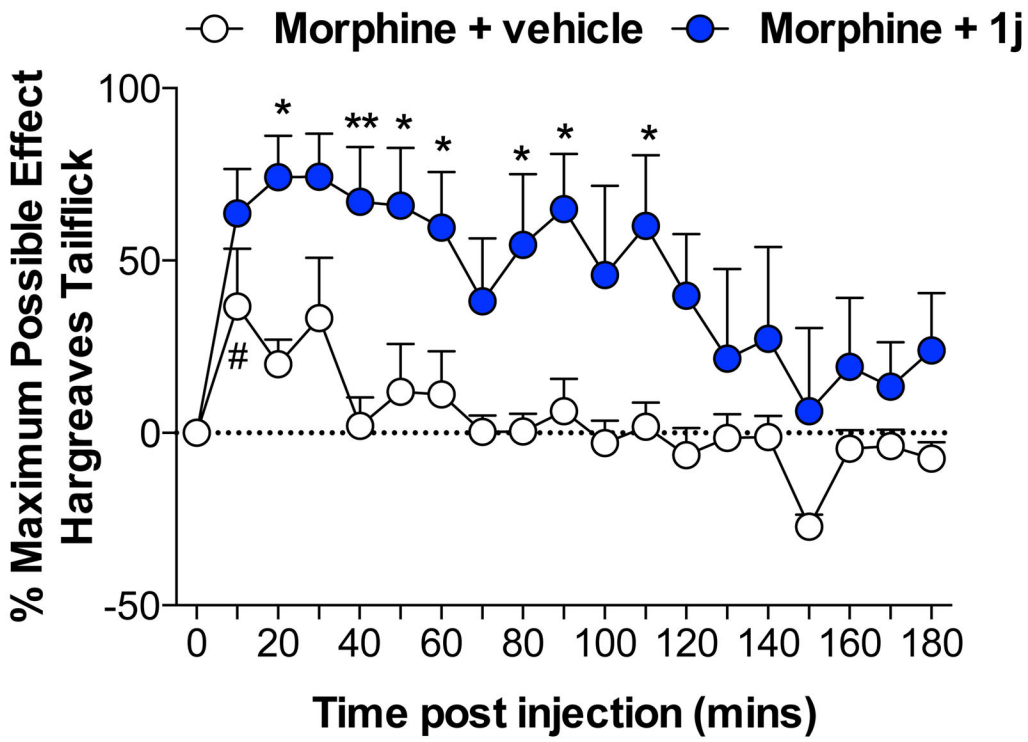
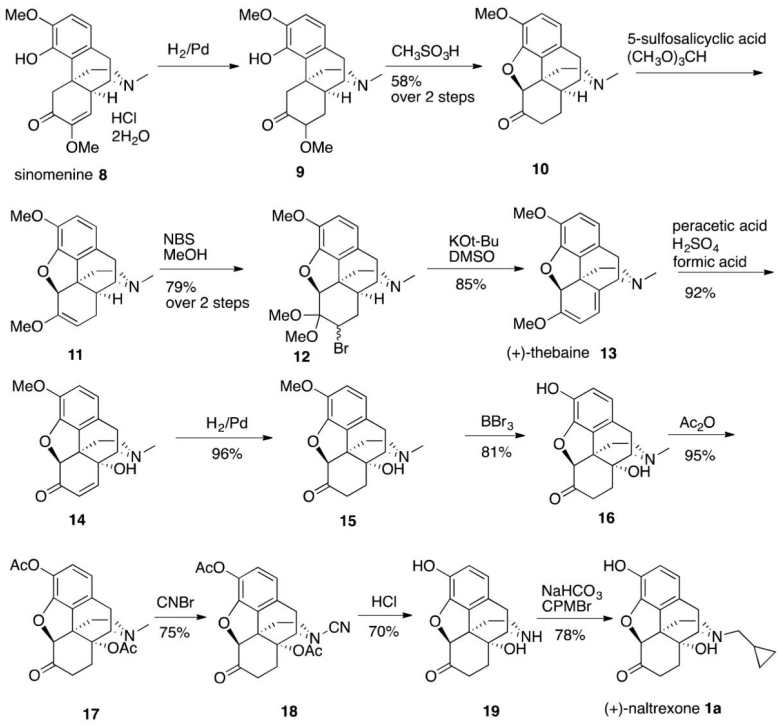


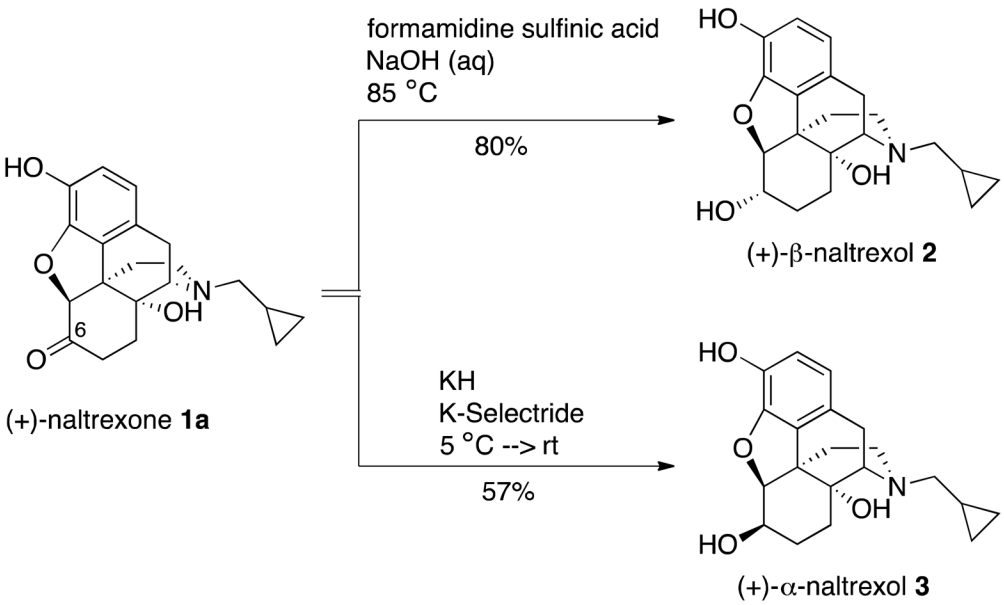
Figure 8.

Intrathecal coadministration of morphine (15 μg) with **1j** (60 μg) produced a significant potentiation of morphine tailflick analgesia. n = 6/group *$P < 0.05$, **$P < 0.01$: relative to vehicle; $^{\#}P < 0.05$: relative to baseline.

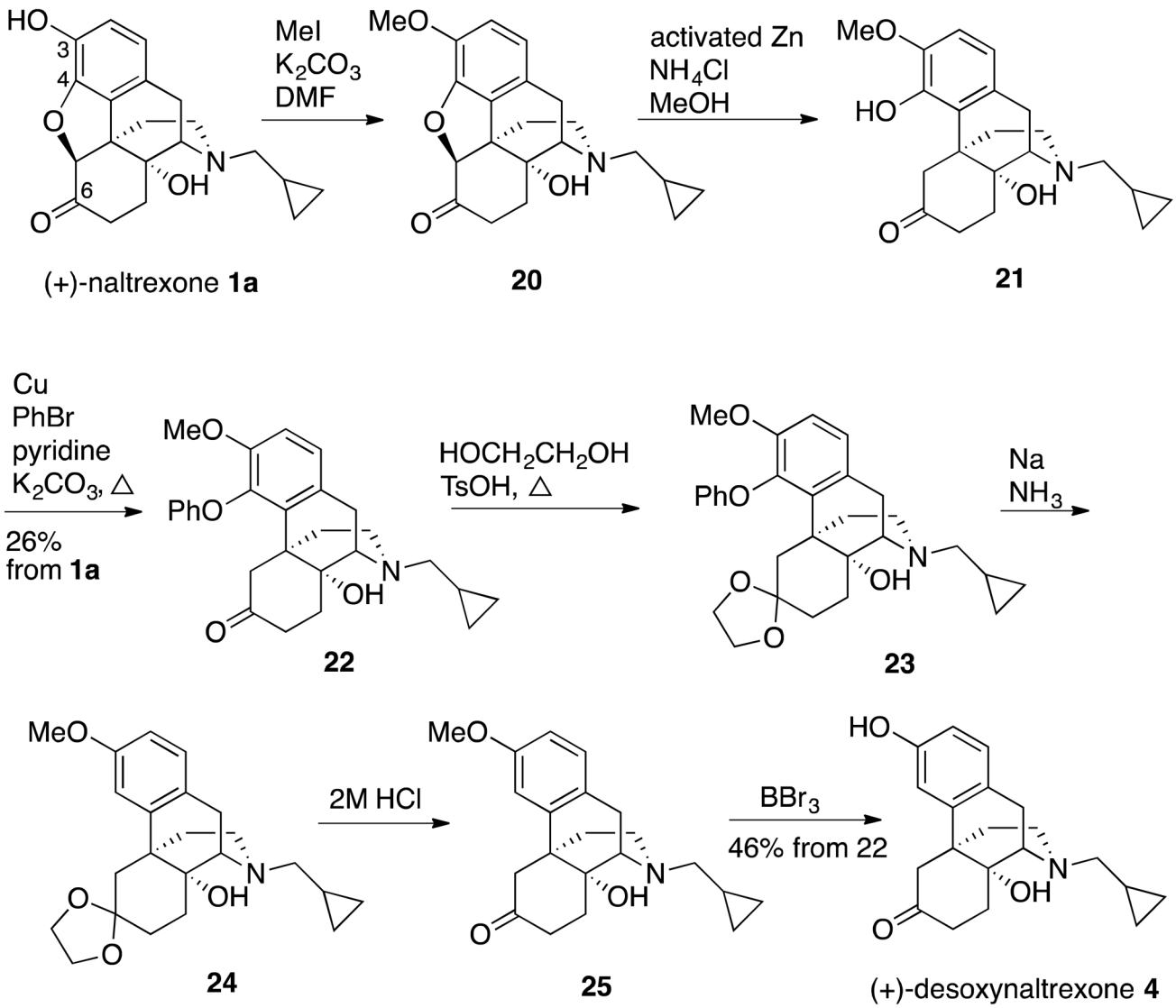


Scheme 1.

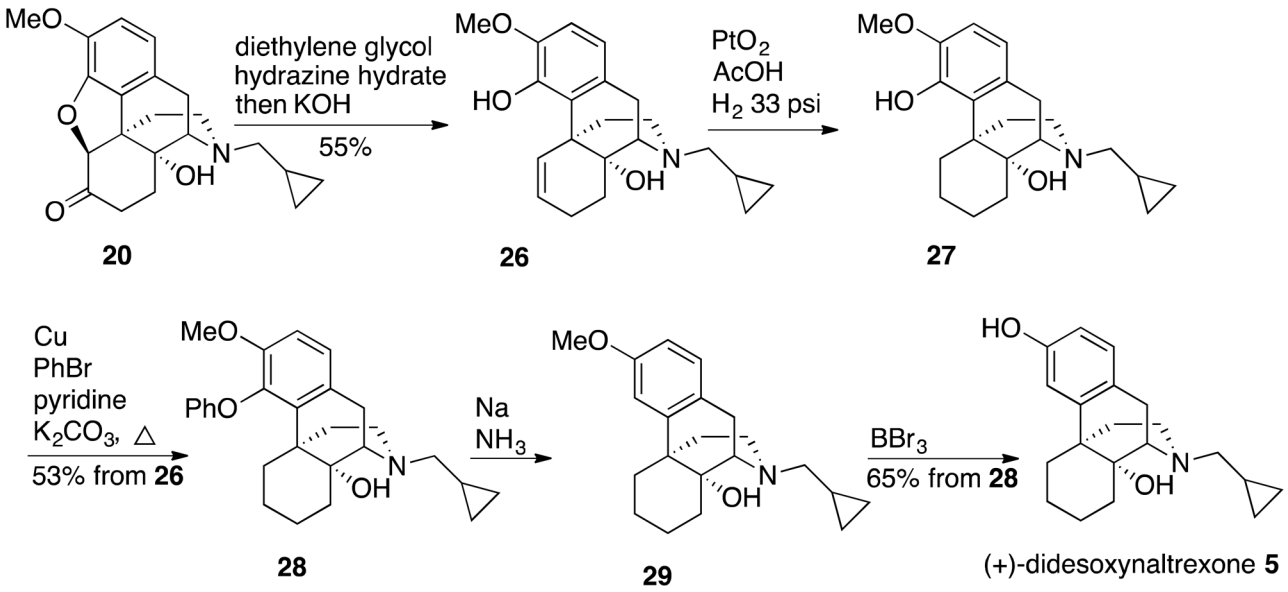
Synthesis of (+)-Naltrexone **1a** from Commercially Available Sinomenine **8**



Scheme 2.
Stereoselective Reduction of (+)-Naltrexone **1a**

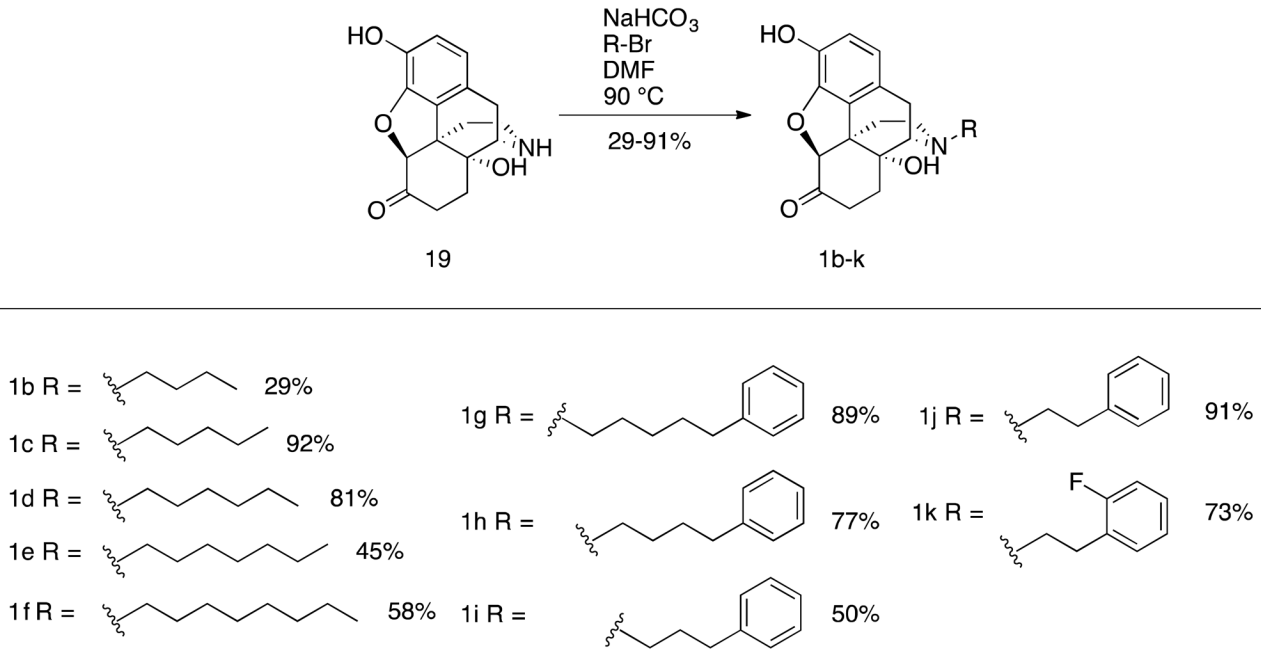


Scheme 3.
Synthesis of (+)-Desoxynaltrexone **4** from (+)-Naltrexone **1a**



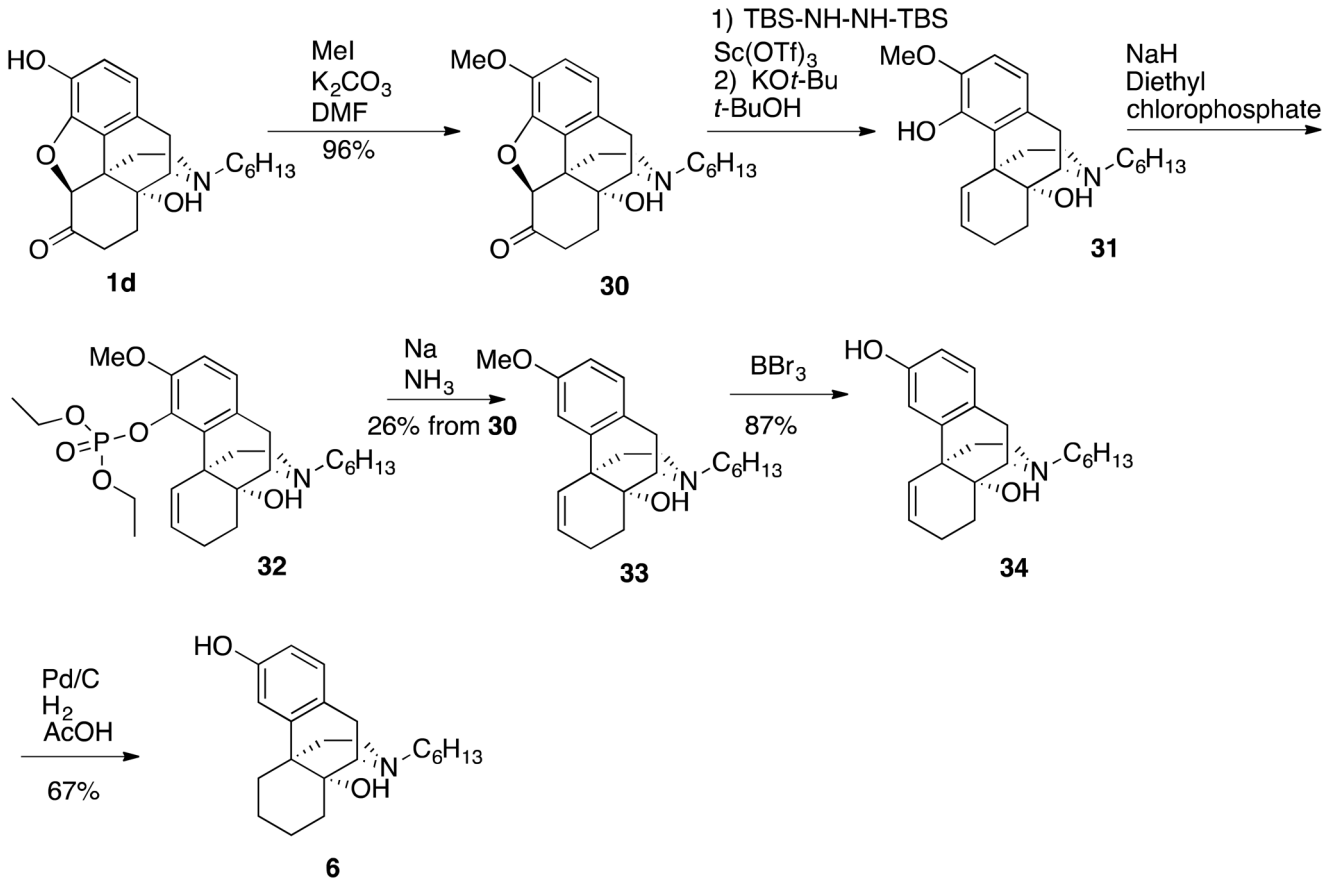
Scheme 4.

Synthesis of (+)-Didesoxynaltrexone **5** from (+)-O-Methylnaltrexone **20**



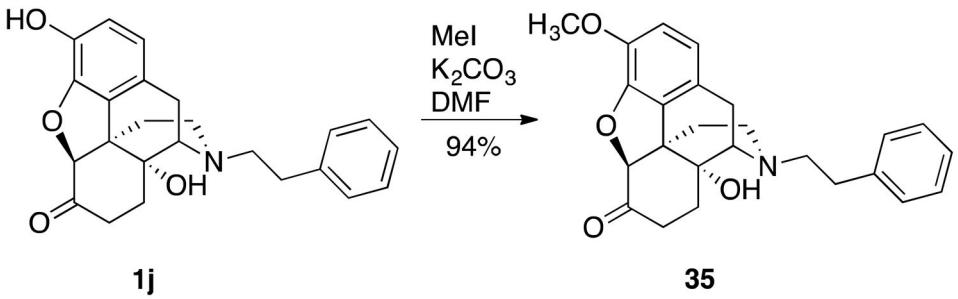
Scheme 5.

N-Alkyl and *N*-Arylalkyl Substituents in (+)-Noroxymorphone **1b**–**1k**



Scheme 6.

Synthesis of (+)-Didesoxy-*N*-hexylnoroxymorphone **6** from (+)-*N*-Hexylnoroxymorphone **1d**



1j **35**

Scheme 7.
Synthesis of (+)-*N*-Phenethylnoroxycodone **35**

Table 1

IC_{50} Values of (+)-Naltrexone **1a**, (+)-β-Naltrexol **2**, (+)-α-Naltrexol **3**, and (+)-10-Nornaltrexone **7**

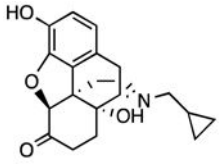
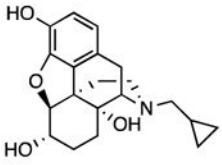
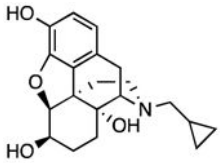
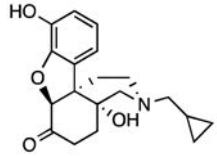
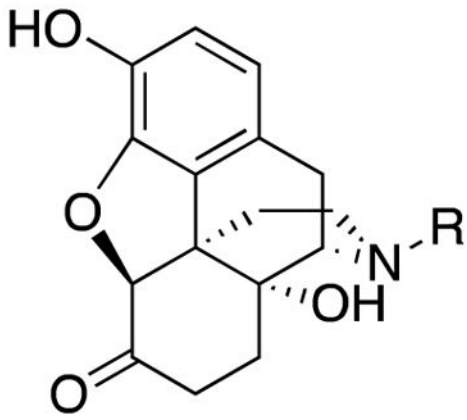
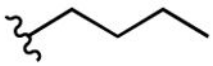
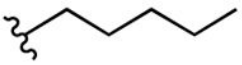
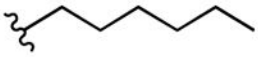
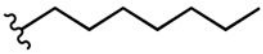
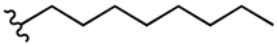
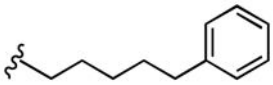
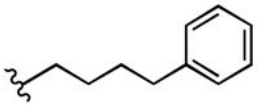
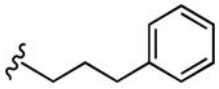
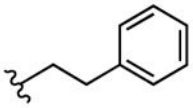
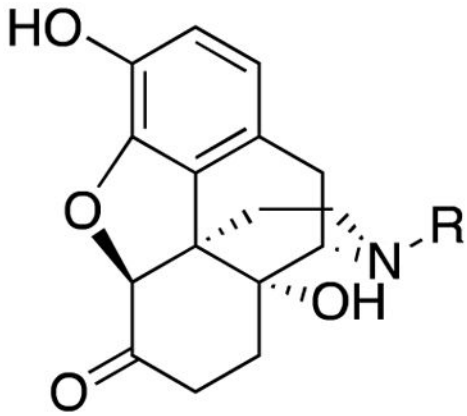
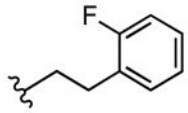
Compound	IC_{50} of NO (μM)	IC_{50} of viability (μM)
 (+)-naltrexone **1a**	105.5 ± 10.1	> 400
 (+)-β-naltrexol **2**	242.8 ± 21.3	> 400
 (+)-α-naltrexol **3**	143.5 ± 20.8	> 400
 (+)-10-nornaltrexone **7**	76.2 ± 27.2	> 400

Table 2

IC_{50} Values of (+)-*N*-Alkylnoroxymorphone Analogs **1b**–**1k**.

	IC_{50} of NO (μM)	IC_{50} of viability (μM)
		
1b R = 	24.8 ± 3.4	> 200
1c R = 	7.5 ± 1.7	> 200
1d R = 	6.7 ± 0.4	121.4 ± 7.3
1e R = 	3.0 ± 0.2	68.5 ± 2.8
1f R = 	1.7 ± 0.1	33.4 ± 1.0
1g R = 	1.7 ± 0.4	26.8 ± 0.4
1h R = 	1.2 ± 0.2	37.1 ± 1.9
1i R = 	1.4 ± 0.2	69.3 ± 3.4
1j R = 	1.4 ± 0.3	140.8 ± 5.5

	IC_{50} of NO (μM)	IC_{50} of viability (μM)
		
1k R = 	1.8 ± 0.2	65.7 ± 3.3