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Synthesis and Characterization of a Dual Kappa-Delta Opioid Receptor Agonist Analgesic Blocking Cocaine Reward Behavior

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Supporting Information

ABSTRACT: 3-Iodobenzoyl naltrexamine (IBNtxA) is a potent analgesic belonging to the pharmacologically diverse 6β-amidoepoxymorphinan group of opioids. We present the synthesis and pharmacological evaluation of five analogs of IBNtxA. The scaffold of IBNtxA was modified by removing the 14-hydroxy group, incorporating a 7,8 double bond and various N-17 alkyl substituents. The structural modifications resulted in analogs with picomolar affinities for opioid receptors. The lead compound (**MP1104**) was found to exhibit approximately 15-fold greater antinociceptive potency (ED_{50} = 0.33 mg/kg) compared with morphine, mediated through the activation of kappa- and delta-opioid receptors. Despite its kappa agonism, this lead derivative did not cause place aversion or preference in mice in a place-conditioning assay, even at doses 3 times the analgesic ED_{50}. However, pretreatment with the lead compound prevented the reward behavior associated with cocaine in a conditioned place preference assay. Together, these results suggest the promise of dual acting kappa- and delta-opioid receptor agonists as analgesics and treatments for cocaine addiction.

KEYWORDS: *Opioid analgesics, cocaine addiction, kappa, delta, IBNtxA, MP1104*

Opioids achieve antinociception by activation of mu (MOR-1), kappa (KOR-1), and delta (DOR-1) opioid receptors. However, the desired effect is accompanied by dangerous, potentially life-threatening side effects.[1] In addition, most clinically used opioids possess the liability of substance abuse and cause physical dependence. Since many of the clinically pertinent side effects are mediated through the activation of MOR-1, one approach to diminish the adverse effects is to design and synthesize opioids that interact primarily at DOR-1 and KOR-1.[2] Both DOR-1 and KOR-1 agonists produce antinociception. However, their clinical utility is limited because DOR-1 agonists produce seizures, while KOR-1 agonists cause dysphoria and aversion. Despite these risks, preclinical data indicate that KOR-1 agonists may be useful as treatments for alcohol, opiate, and cocaine abuse.[3]

6β-Amido epoxymorphinans form a class of opioids with diverse pharmacological effects. The scaffold's importance in developing opioids with desirable effects is well established. This scaffold is found in opioid receptor probes,[4] various analgesics[5] (including IBNtxA (**1**)), NNTA,[5] antagonists[6] (such as β-FNA), alcohol cessation agents,[7] and a clinically used antipruritic drug (nalfurafine)[8] (Figure 1). 3-Iodobenzoyl-β-naltrexamine (IBNtxA, **1**), a potent 6β-amido 14-OH-epoxymorphinan derivative recently described by Majumdar and co-workers, binds to exon 11 dependent 6-transmembrane domain (6TM/E11) splice variants of MOR-1 with high affinity. IBNtxA has an advantageous pharmacological profile; its potent antinociception (ED_{50} = 0.39 mg/kg) is not accompanied by respiratory depression, reward, physical dependence, or cross-tolerance to morphine, separating it

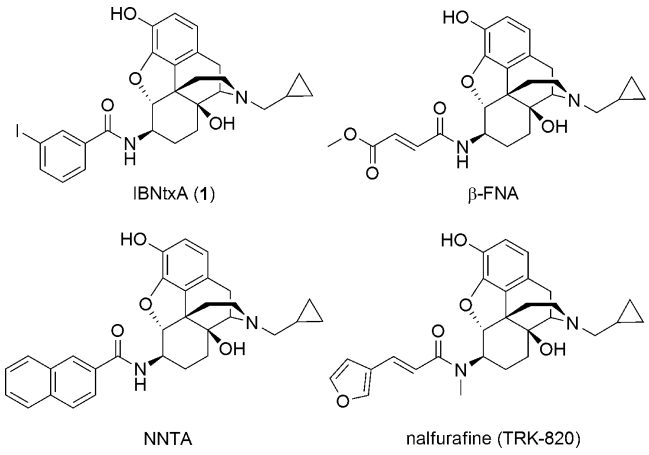

Figure 1. Important 6β-amido epoxymorphinan derivatives.

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from all clinically used mu analgesics.[9] IBNtxA is an effective analgesic in not only acute and thermal pain models, but also neuropathic and inflammatory pain models.[10] However, IBNtxA lacks sufficient selectivity for the primary target site, 6TM/E11, over the traditional opioid receptors. Our initial goal was to design and synthesize IBNtxA analogs to improve selectivity for the 6TM/E11 sites so as to better understand the molecular pharmacology of these sites. We have previously shown that the 6β-(3′-iodobenzoyl)amide moiety is required for high 6TM/E11 affinity. Therefore, no changes in the C-6 position were proposed[11] (Figure 2). All analogs of IBNtxA

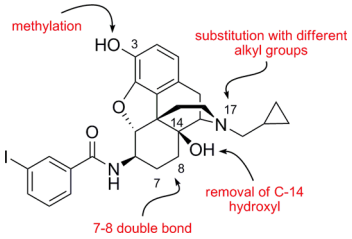

Figure 2. Chemical scope of this study.

that we have reported thus far utilized modifications of the naltrexone/naloxone/oxymorphone backbone (14-OH-epoxymorphinan). To better understand the SAR of 6TM/E11 sites, we examined the morphine/nalorphine backbone (14-*H*-7,8-didehydroepoxymorphinan) and determined whether the incorporation of a 3′-iodo phenyl amido moiety at C-6 would improve selectivity for the 6TM/E11 sites. Following evaluation of the five analogs of IBNtxA on this 14-*H*-7,8-didehydroepoxymorphinan scaffold in radioligand binding and *in vivo* analgesia assays, we found that these analogs showed exceptionally high affinity and selectivity for the traditional opioid receptors over the 6TM/E11 sites. Encouraged by their high affinity for traditional opioid receptors, we decided that detailed investigation of the pharmacology of these compounds was warranted. Compound **16** (**MP1104**) was selected as the lead compound because its affinity for KOR-1 was found to be the highest in the group, and it showed remarkable *in vivo* analgesic potency. Compound **16** (**MP1104**) was found to be a dual agonist at KOR-1 and DOR-1. Since KOR-1 agonists have been reported in the literature to counteract the reinforcing effects of cocaine,[12] we evaluated the ability of our dual KOR-1/DOR-1 agonist **16** (**MP1104**) to prevent the reward behavior associated with cocaine in the conditioned place preference (CPP) test in mice.

RESULTS

Chemistry. In order to achieve the desired structural modifications on the scaffold of IBNtxA (**1**), five analogs were synthesized from codeine (**2**, Scheme 1). The Mitsunobu reaction was used to convert **2** to the phthalimide **3**,[13] which was in turn hydrolyzed to 6β-codeinamine (**4**) by using hydrazine hydrate. Coupling 3-iodobenzoic acid afforded **5**, from which the morphine derivative **6** was synthesized by deprotecting the C-3 phenolic hydroxyl group. Derivatives where the N-17 methyl is replaced with cyclopropylmethyl and phenylethyl groups were synthesized from **5** by N-demethylation and subsequent alkylation followed by deprotection. *N*-allyl derivatives were synthesized from **2** using an alternative pathway due to unfavorable yields in the *N*-allylation of **12**. Compound **2** was first N-demethylated using chloroethyl chloroformate,[14] and the resulting **7** was alkylated with allyl bromide to yield **8**, which was in turn converted to its 6-amino derivative (**10**) using the Mitsunobu reaction and subsequent hydrolysis. Acylation with 3-iodobenzoic acid afforded **11**. Codeine derivatives (**11**, **13**, and **14**) were deprotected with BBr_3 to yield the final compounds **15**−**17**.

Pharmacology. Our initial goals were to remove the C-14 hydroxy, incorporate a 7,8 double bond and various nitrogen substituents at position 17 of IBNtxA (the 7,8-didehydro-14-*H*-epoxymorphinan scaffold) (**1**), and determine how those changes alter the pharmacology of the compounds. The analogs **5**, **6**, and **15**−**17** (Figure 3) were evaluated in competition binding assays against [^{125}I]BNtxA in membranes prepared from either cell lines stably expressing mouse opioid receptors or mouse brain (Table 1). The results were compared with analogous 14-OH-epoxymorphinan derivatives (**1**, **18**−**20**) reported previously by Majumdar et al.[11] The 3-OMe, *N*-methyl analog **5** showed moderate affinity for 6TM/E11 (61 nM) and DOR-1 (49 nM) and high affinity for MOR-1 and KOR-1 (1.2 and 10 nM, respectively). The corresponding analog (**18**) on the 14-OH-epoxymorphinan scaffold had low affinity for all opioid receptors (K_i > 100 nM).

The 3-OH, *N*-methyl (morphine) analog **6** showed moderate affinity for 6TM/E11 (27 nM) versus 41 nM for the corresponding 14-OH-epoxymorphinan analog **19**. Compound **6** had 224-fold higher KOR-1 affinity, 26-fold higher affinity at MOR-1, and 3-fold higher DOR-1 affinity than **19**. Compound **6** had high affinity and some selectivity for MOR-1, which is consistent with previously reported 6β-morphinamides.[5g,15] Compound **6** demonstrated potent antinociception *in vivo* in contrast to **19**, which did not produce antinociception at the highest tested dose (10 mg/kg). This is possibly because **6** has considerably higher affinity for the opioid receptors compared with **19**. Introduction of a 7,8 double bond and removal of 14-OH increased affinities for traditional opioid receptors but decreased selectivity to the 6TM/E11 site labeled by [^{125}I]BNtxA in mouse brain.[9] The presence of a bulkier N-substituent (phenylethyl, compound **15**) dramatically decreased the affinity for the 6TM/E11 site in comparison with **1**, while affinities for MOR-1, DOR-1, and KOR-1 were increased. Compound **15** had 159-, 100-, and 108-fold greater selectivity for MOR-1, KOR-1, and DOR-1 over 6TM/E11 sites, respectively. It also had ∼3-fold higher analgesic potency over morphine when given systemically.[16] The corresponding epoxymorphinan analog has not been reported. The 7,8-didehydro epoxymorphinan analog of **1**, **16** (**MP1104**), was evaluated next. It showed 3-fold lower affinity for 6TM/E11 than **1**, while its affinity for MOR-1, KOR-1, and DOR-1 was 5-, 4.7-, and 3-fold higher, respectively. Compound **16** (**MP1104**) had 22-, 73-, and 6-fold greater selectivity for MOR-1, KOR-1, and DOR-1 over 6TM/E11 sites, respectively. Moreover, **16** (**MP1104**) proved 15-fold more potent than morphine in tail flick analgesia assays (ED_{50} = 0.33 ± 0.09 mg/kg, Figure 4). The 14-*H*-7,8-didehydroepoxymorphinan analog of IBNalA (**20**), **17**, was evaluated next. The 6TM/E11 affinity decreased 4.9-fold and 3.1-fold compared with **1** and **20**, respectively. The MOR-1, KOR-1, and DOR-1 affinities increased 6.5-, 3.6-, and 6.7-fold over **20**. Similar to **16** (**MP1104**), the affinities were 23-, 35-, and 2-fold higher for MOR-1, KOR-1, and DOR-1 receptors over 6TM/E11 for **17**. Compound **17** was equipotent to both **1** and **20** in analgesia assays *in vivo*. Thus, a general trend of enhanced affinities for MOR-1, KOR-1 and DOR-1 was seen over affinity for 6TM/

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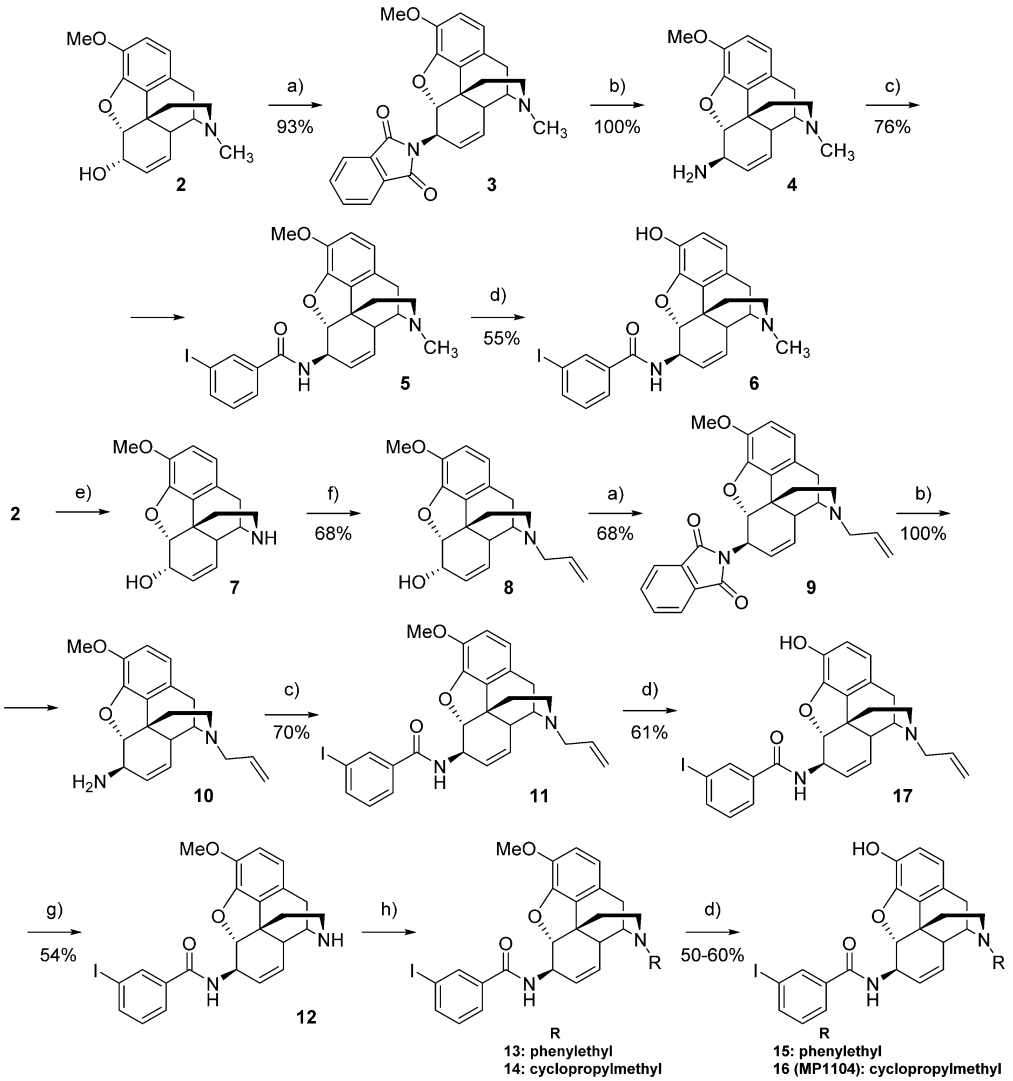
Scheme 1. Synthesis of Derivatives[a]



[a]Reagents and conditions: (a) phthalimide, Ph_3P, dry THF, diisopropyl azodicarboxylate (DIAD) in dry toluene, 16 h, rt; (b) hydrazine hydrate, *cis*-2-penten-1-ol, MeOH, 16 h, rt; (c) 3-iodobenzoic acid, 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate (HATU), *N*,*N*-diisopropylethylamine (DIEA), dimethyl formamide (DMF), 15 min, rt; (d) BBr_3, dichloromethane (DCM), 30 min, 0 °C to rt; (e) chloroethyl chloroformate, $NaHCO_3$, 1,2-dichloroethane, 30 min, rt, then 16 h, 85 °C; (f) allyl bromide, Na_2CO_3, dry DMF, 16 h, 90 °C; (g) DIAD, acetonitrile, 20 h, 65 °C, then pyridine-HCl, 72 h, rt; (h) alkyl halide, Na_2CO_3 or Cs_2CO_3, DMF, 16 h, 90 °C.

E11 with the iodobenzoyl 7,8-didehydroepoxymorphinan scaffold as compared with the corresponding 14-OH-epoxymorphinan scaffold. Despite our findings that these compounds did not have the desired selectivity for the primary target, 6TM/E11, the picomolar affinity of these compounds for the opioid receptors and the corresponding *in vivo* potency intrigued us. Based on our observations of a 6.4 pM affinity for KOR-1 and literature reports noting the potential value of KOR-1 agonists in treating pain and substance abuse, we decided to study the pharmacology of compound **16** (**MP1104**). Furthermore, compound **16** (**MP1104**) had increased *in vivo* analgesic potency (approximately 15-fold higher than morphine).

Compound **16** (**MP1104**) demonstrated full agonist efficacy at MOR-1, DOR-1, and KOR-1 in [^{35}S]GTPγS binding assays. Although highly potent (EC_{50} = 0.027−0.41 nM across receptors) in these functional assays, **16** (**MP1104**) did not demonstrate selectivity among the opioid receptor subtypes (Table 2).

Antinociception was then evaluated in the presence of opioid receptor selective antagonists or using opioid receptor gene-disrupted mice to determine the relative contributions of MOR-1, DOR-1, and KOR-1 to **16** (**MP1104**)-induced analgesia. In CD1 mice, pretreatment with the MOR-1 selective antagonist β-FNA (given 24 h prior to dosing **MP1104** to avoid KOR-mediated analgesia by β-FNA)[18] did not alter antinociception. However, pretreatment with selective antagonists to either DOR-1 (NTI) or KOR-1 (norBNI) attenuated the antinociceptive effects of **16** (**MP1104**) (Figure 5). Pharmacological blockade of DOR-1 and KOR-1 in mice pretreated with both norBNI and NTI fully prevented antinociception induced by **16** (**MP1104**), additionally confirming the lack of MOR-1 involvement in the antinociception. Notably, some opioids are also known to modulate pain response through interaction with the nociception receptor (NOP). No change in the

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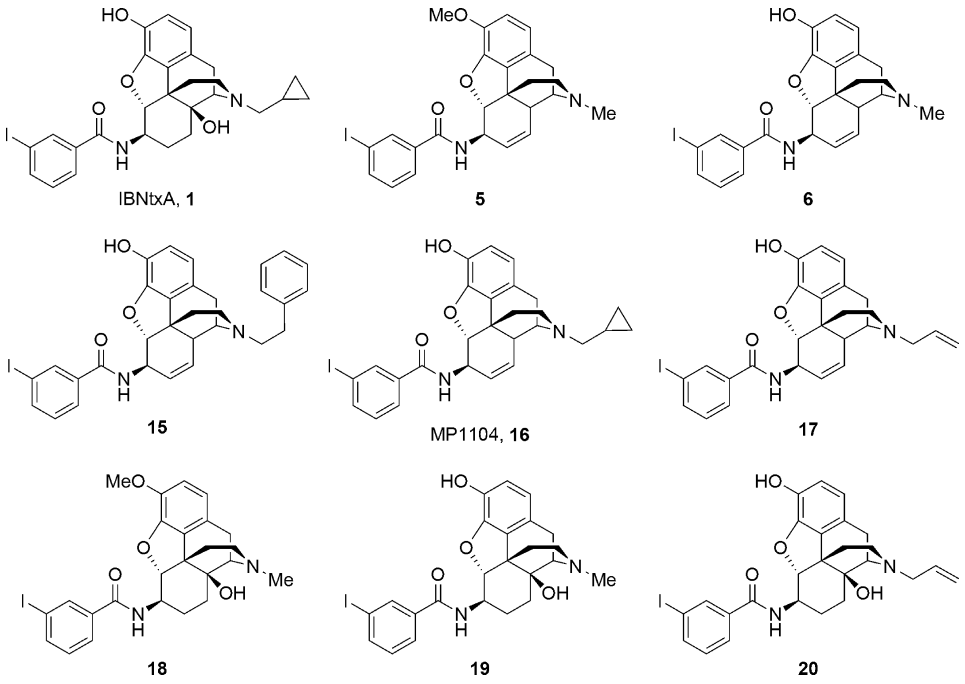

Figure 3. Structures of synthesized 6β-(3′-iodobenzoyl)-7,8-didehydro epoxymorphinans (**5, 6, 15−17**) and their 14-OH-epoxymorphinan analogs (**1, 18−20**) reported by Majumdar et al.[11]

Table 1. Binding and Analgesia of 5, 6, 15−17, and Their Corresponding 14-OH-Epoxymorphinan Analogs

	K_i [nM][a]				
compd	MOR-1	KOR-1	DOR-1	6TM/E11	tail flick analgesia (CI),[b] ED_{50} [mg/kg]
1	0.11 ± 0.02	0.03 ± 0.001	0.24 ± 0.05	0.16 ± 0.04	0.39 (0.15, 0.58)
5	1.2 ± 0.48	10 ± 3.5	49 ± 6.4	61 ± 7.7	>10
6	0.037 ± 0.0051	0.21 ± 0.046	0.88 ± 0.08	27 ± 18	6.31 (4.6, 8.5)
15	0.088 ± 0.028	0.14 ± 0.022	0.13 ± 0.032	14 ± 3.2	1.3 (1.03, 2.2)
16 (MP1104)	0.021 ± 0.0034	0.0064 ± 0.002	0.08 ± 0.019	0.47 ± 0.009	0.33 ± 0.09
17	0.034 ± 0.006	0.022 ± 0.0043	0.39 ± 0.0041	0.78 ± 0.1	0.53 (0.45, 0.59)
18	>100	>100	>100	>100	>10
19[c]	0.97 ± 0.2	47 ± 14	2.5 ± 1	41 ± 12	>10
20[c]	0.22 ± 0.12	0.08 ± 0.06	2.6 ± 0.18	0.25 ± 0.12	0.6 (0.42, 0.90)
morphine	4.6 ± 1.81[d]			>1000[e]	4.96 ± 0.96[f]
DAMGO[g]	3.34 ± 0.43[d]				
U50,488H		0.73 ± 0.32[d]			
DPDPE[g]			1.39 ± 0.67[d]		

[a][^{125}I]BNtxA (0.1 nM) competition binding assays were performed in membranes prepared from CHO cells expressing mouse MOR-1, DOR-1, or KOR-1 or mouse brain in the presence of CTAP, DPDPE, and U50,488 blockers at 200 nM. K_i was determined by nonlinear regression analysis (GraphPad Prism). [b]Assays were performed at least 2 times, and means ± SEM of replicates or CIs are reported. Groups of mice ($n \geq 10$) received test compound (sc), and analgesia was assessed 30 min later to generate analgesic dose response curves. ED_{50} values (with 95% confidence limits) were determined using nonlinear regression analysis (GraphPad Prism). [c]14-OH-epoxymorphinan analogs. Results from the literature.[11] [d]Value from the literature.[17] [e]Value from the literature.[9] [f]Value from the literature.[16] [g]DAMGO = [D-Ala^2,*N*-MePhe^4,Gly-ol]-enkephalin; DPDPE = [D-Pen^2,D-Pen^5]-enkephalin.

antinociceptive effect of **16** (**MP1104**) was observed when it was administered to mice pretreated with J-113,397, a selective NOP antagonist.[19] The antinociceptive activity of **16** (**MP1104**) was confirmed in the C57BL/6J strain of mouse, which demonstrated an ED_{50} (and 95% confidence interval) value of 0.22 (0.17−0.29) mg/kg, sc. Consistent with the initial pharmacological testing utilizing antagonists in CD1 mice, antinociception induced by **16** (**MP1104**) in this strain of mouse was significantly reduced by DOR-1 antagonism or KOR-1 gene deletion ($F_{(4,59)} = 69.2$, $p < 0.0001$; one-way ANOVA with Tukey's post hoc test; Figure 6). The antinociceptive potency of **16** (**MP1104**) was not significantly altered in mice lacking exon 2 of MOR-1 gene-deleted mice. Taken together, these data indicate that **16** (**MP1104**) antinociception is mediated by KOR-1 and DOR-1 receptors.

Although MOR-1-selective agonists such as morphine are reinforcing and produce significant conditioned place preference, KOR-1-selective analgesics such as U50,488 produce dysphoria and significant conditioned place aversion when compared to preconditioning preferences ($F_{(4,84)} = 3.29$, $p = 0.015$; two-way RM ANOVA with Sidak's multiple comparison post hoc test; Figure 7). Compound **16** (**MP1104**) produced neither conditioned place preference nor aversion in a place conditioning paradigm (ns; Tukey post-hoc test) at a dose

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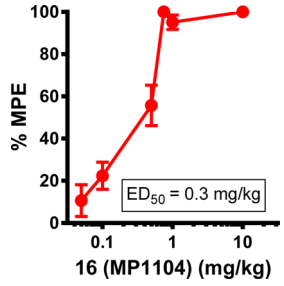

Figure 4. Cumulative dose−response curves were carried out on groups of CD1 mice (n = 10) with **16** (**MP1104**) at the indicated doses (sc), and antinociception was tested 30 min later at peak effect. The ED_{50} value was 0.33 ± 0.09 mg/kg in CD1 mice by using the radiant heat tail-flick assay. All values are expressed as the mean ± SEM of three separate assays.

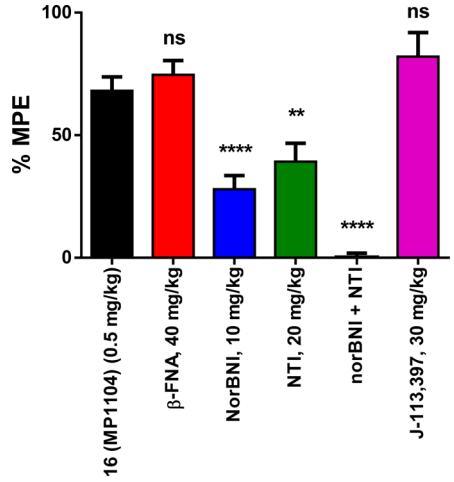

Figure 5. Reversal of antinociception by selective antagonists. Groups of CD1 mice ($n \geq 10$) received **16** (**MP1104**) (0.5 mg/kg sc) and the indicated antagonist. β-Funaltrexamine (β-FNA; 40 mg/kg sc) and norbinaltorphimine (norBNI; 10 mg/kg sc) were administered 24 h before agonist testing. Naltrindole (NTI; 20 mg/kg sc) and J-113,397 (30 mg/kg sc) were administered 15 min before **16** (**MP1104**). All analgesia testing was performed 30 min after the administration of **16** (**MP1104**). Similar results were observed in two independent replications. Compound **16** (**MP1104**) analgesia is insensitive to β-FNA and the NOP antagonist J-113,397, whereas analgesia is partially antagonized by norBNI and NTI. Simultaneous dosing of norBNI and NTI eliminated the antinociceptive response (two-way ANOVA followed by Bonferroni post hoc comparisons test, $p < 0.05$). All values are expressed as the mean ± SEM of two separate assays.

three times greater than the analgesic ED_{50} (1 mg/kg, sc). However, a 10 min pretreatment with **16** (**MP1104**) at this dose prior to cocaine-place conditioning prevented significant place preference produced by cocaine (Figure 7).

DISCUSSION

IBNtxA (**1**) is an opioid analgesic that binds truncated 6TM/E11 associated variants of MOR-1. Compound **1** displays high affinity for this site, as well as for MOR-1, DOR-1, and KOR-1. In an attempt to achieve better selectivity for 6TM/E11 sites, the structure of IBNtxA was modified in four positions: the C-14 hydroxyl group was removed, the 7,8 double bond was incorporated, and the effects of 3-*O*-methylation and the introduction of different N-17 alkyl substituents were studied. All synthesized molecules were evaluated for their relative binding affinity to cloned mouse MOR-1, KOR-1, and DOR-1 receptors in CHO cells and 6TM/E11 sites in mouse brain and for analgesic activity *in vivo* using the radiant heat tail flick assay. Although binding affinity for the 6TM/E11 sites was not improved, the affinity for the traditional opioid receptors was increased. A subnanomolar affinity for DOR-1 affinity was identified, unprecedented for ligands in the 6β-amido epoxymorphinan class. The lead compound, **16** (**MP1104**), was selected for further characterization based on its picomolar receptor affinity and its potent antinociceptive properties *in vivo*. Compound **16** (**MP1104**) was found to be a dual agonist at KOR-1 and DOR-1. However, despite an affinity of 210 pM and full agonism in functional assays at MOR-1, this opioid receptor did not mediate **16** (**MP1104**)-induced antinociception. Although *in vitro* data such as binding affinity often correlates with *in vivo* activity, the relationship is not always consistent.[20] It is possible that *in vivo* metabolism accounts for the differences between the *in vitro* and *in vivo* MOR-1 activity of **16** (**MP1104**). Metabolites are not formed under the conditions of the *in vitro* assays as utilized, thus their effects are not accounted in these assays, even though some contribute significantly to *in vivo* performance attributed to the parent compound. For example, morphine-6-*O*-glucuronide is a metabolite of morphine observed in significant amounts even after parenteral administration of morphine,[21] and possesses superior *in vivo* efficacy over the parent compound.[22] Along a similar fashion, it is possible that metabolism of **16** (**MP1104**) may render a metabolite responsible for the observed *in vivo* activity but without affinity for MOR-1. Examination of metabolism of **16** (**MP1104**) is beyond this initial characterization, but is an important subject for future study.

We used both pharmacological and genetic approaches to determine the relative contributions of opioid receptor families to **16** (**MP1104**) antinociception. The results suggest that **16** (**MP1104**) produces analgesia through agonism at KOR-1 and DOR-1 receptors. The clinical applicability of KOR-1 and DOR-1 opioids has been thought to be limited because DOR-1

Table 2. Opioid Receptor Efficacy of 16 (MP1104)[a]

	EC_{50} [nM]			E_{max} (% stimulation)		
compd	MOR-1	DOR-1	KOR-1	MOR-1	DOR-1	KOR-1
16 (**MP1104**)	0.21 ± 0.03	0.41 ± 0.11	0.027 ± 0.002	103 ± 2.5	88 ± 0.38	104 ± 2.3
DPDPE	*b*	10 ± 2.2	*b*			
DAMGO	19 ± 7.0	*b*	*b*			
U50,488H	*b*	*b*	17 ± 6.1			

[a]Efficacy data were obtained using agonist induced stimulation of [^{35}S]GTPγS binding assay. Efficacy is represented as EC_{50} (nM) and percent maximal stimulation relative to standard agonist DAMGO (MOR-1), DPDPE (DOR-1), or U50,488H (KOR-1) at 100 nM. All values are expressed as the mean ± SEM of three separate assays performed in triplicate. [b]Not determined.

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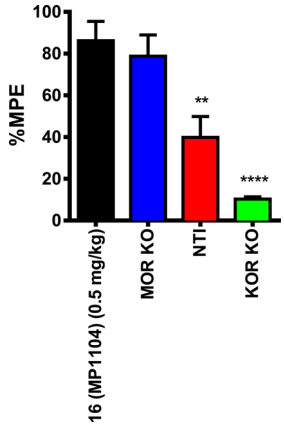

Figure 6. Compound **16** (**MP1104**; 0.5 mg/kg, sc) also produced significant antinociception in C57BL/6J mice or MOR-1 KO mice, but antinociception was significantly attenuated in KOR-1 KO mice and wild-type mice pretreated with NTI (20 mg/kg, sc, 20 min prior to administration of **16** (**MP1104**)). Antinociception was measured 30 min after administration of **16** (**MP1104**). All values are expressed as the mean ± SEM of two separate assays.

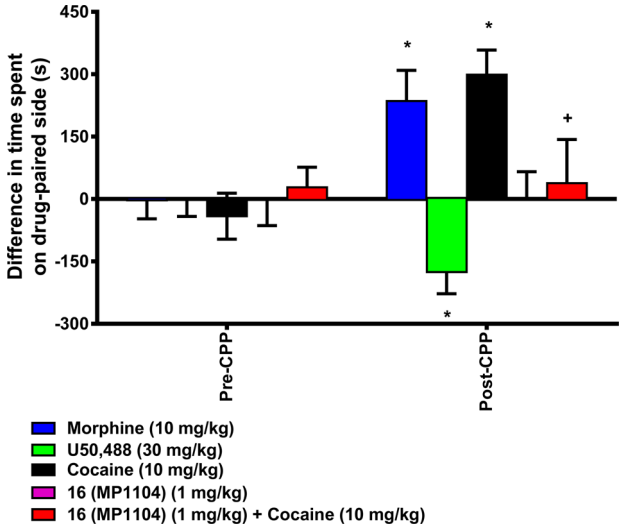

Figure 7. Compound **16** (**MP1104**) alone did not produce conditioned place preference or aversion, but it prevented cocaine-conditioned place preference. After determination of initial preconditioning preferences, mice were place conditioned daily for 2 days with morphine (10 mg/(kg/d), ip), U50,488 (30 mg/(kg/d), ip), cocaine (10 mg/(kg/d) sc), or **16** (**MP1104**) (1 mg/(kg/d) sc). Additional mice received **16** (**MP1104**) (1 mg/(kg/d), sc) 10 min prior to administration of cocaine and place conditioning for each of 2 days. Mean difference in time spent on the drug-paired side ± SEM is presented (n = 15–21). *Significantly different from matching preconditioning preference, $P < 0.05$; + significantly different from cocaine CPP response, $P < 0.05$; two-way repeated measures ANOVA with Sidak's post hoc test.

agonists induce seizures[23] and KOR-1 agonists cause aversion.[24] However, **16** (**MP1104**) was not observed to produce seizures even at doses 30 times its antinociceptive ED_{50} value. Opioid analgesics often alter mood and reward, because MOR-1, DOR-1, and KOR-1 are expressed in the limbic system.[25] Thus, we used a place-conditioning model to determine whether the compound produced place-preference (reward) or aversion. Unlike many opioid clinical analgesics, **16** (**MP1104**) produced neither preference nor aversion for drug-paired chambers in our conditioned place preference (CPP) paradigm at doses 3 times the antinociceptive ED_{50} value. Since acute agonist activation of KOR-1 has been shown in the literature to be capable of counteracting the rewarding effects of cocaine,[10,11,18] we evaluated the ability of our dual KOR-1/DOR-1 agonist **16** (**MP1104**) to prevent the reward behavior associated with cocaine in the conditioned place preference (CPP) test in mice. Interestingly, **16** (**MP1104**) abolished cocaine preference in the CPP paradigm. The involvement of KOR-1 in modulating cocaine reward and reinstatement is well established, and the endogenous kappa opioid system is upregulated in cocaine dependence.[26] Previous studies have found that DOR-1 and KOR-1 selective agonists are rewarding and aversive in CPP models, respectively, when given alone.[27] While DOR-1 agonists such as deltorphins enhanced cocaine reward behavior,[28] numerous studies have demonstrated that acute administration of KOR-1 agonists suppresses the reinforcing effects of abused substances in a number of model systems.[29] Presently, it is plausible that pretreatment with **16** (**MP1104**) prevented cocaine-conditioned place preference through the activation of KOR-1 located on the A10 mesolimbic dopamine pathway and thought to modulate reward signaling. However, the influence of DOR-1 agonist activity in this effect is uncertain. It is established that mixed activity MOR-1/KOR-1 partial agonists might possess therapeutic benefit,[30] and DOR-1 antagonists are reported to reduce alcohol and cocaine intake,[28b,31] but the effect of mixed KOR-1/DOR-1 agonist activity in treating cocaine dependence has not been investigated. To the best of our knowledge, this is the first demonstration of a dual-action DOR-1 and KOR-1 agonist to block cocaine reward, suggesting that additional study of this topic is warranted in the near future.

6-Amido morphinans have been intensively studied in the literature. Review of their SAR highlights that even small changes to their structure can greatly alter their pharmacological profiles. In particular, the nature of the *N*-acyl substituent has a profound effect on receptor subtype selectivity, efficacy, and *in vivo* behavior. In β-FNA, 6β-naltrexamine is acylated with methylfumaric acid. The fumaric acid moiety serves as a Michael acceptor and forms a covalent bond within the MOR-1 binding site, irreversibly antagonizing it.[32] β-FNA also possesses short-acting KOR-1 agonistic properties.[33] Nalfurafine[8] (TRK820) has a similar structure, maintaining the side chain length necessary for KOR-1 binding, but the amide nitrogen is tertiary (*N*-methyl amide), resulting in good KOR-1 selectivity. β-Naltrexamines with a cinnamoyl group have been reported to have high affinity for all opioid receptors, although they all demonstrate KOR-1 agonism with weak efficacy at MOR-1.[5d−f] Multiple research groups have reported series of 6β-aryl amido morphinans. Simple substitutions on the aryl ring of β-naltrexamides lead to analogs with partial agonism at all three opioid receptors,[7b,c] while substitution with halogens (especially iodine) on the aryl ring of β-naloxamides leads to affinity for 6TM/E11sites.[11] NNTA, where the aryl ring of β-naltrexamide is a bulky naphthalene, targets MOR−KOR heteromers and has poorer affinity for DOR-1, while 6′-fluoro-NNTA is somewhat DOR-1 selective.[5a,34] A variety of selective MOR-1 antagonists based on 6β-(4′-pyridyl)-naltrexamide (NAP) have also been reported.[6c,f] While the SAR of naltrexamines has been extensively studied in the literature, only limited information is available on the pharmacology of 6-amido-7,8-didehydro-14-*H*-expoxymorphinans. 6β-Arylamido morphine derivatives were shown to

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be moderately MOR-1 selective, exhibiting full agonism at MOR-1 and KOR-1 with weak DOR-1 efficacy.[15] We recently reported 6β-cinnamoylamidomorphines with moderate selectivity for MOR-1 with full agonism and analgesia being mediated by MOR-1 and DOR-1.[5g] Clearly, small changes in the structure of 6-amidomorphinans have profound impact on opioid subtype selectivity and pharmacology. Further demonstrating this, our lead, **16** (**MP1104**), shows that removal of the 14-OH and incorporation of a 7,8 double bond increases traditional opioid receptor activity and leads to dual kappa-delta agonism. While the KOR-1 activity on this template is not unexpected, the subnanomolar DOR-1 affinity and activity is surprising based on past literature precedence on 6-arylamido morphinans. The only compounds that have been reported in the literature to have dual kappa-delta activity are KDA-16[35] and a diimidazodiazepine peptidomimetic,[36] but these are structurally distinct from 6-amidomorphinans. Thus, further derivatization and optimization of the 6-amido-7,8-didehydro-14-*H*-expoxymorphinans scaffold is an attractive starting point for further studies, the results of which would add to the existing SAR on 6-amidomorphinans and provide molecular tools to study the consequences of stimulating the DOR-1 and KOR-1 systems simultaneously.

In conclusion, incorporation of a 7,8 double bond, removal of the C-14 hydroxyl, and introduction of N-17 substituents on **1** decreased its selectivity for the 6TM/E11 site but significantly increased affinity for MOR-1, DOR-1, and KOR-1. One analog containing an N-17 cyclopropylmethyl group, **16** (**MP1104**), proved to be a nonselective opioid in binding assays and a potent analgesic that utilizes both KOR-1 and DOR-1 receptors to produce antinociception. Compound **16** (**MP1104**) lacks rewarding and dysphoric properties at a dose three times higher than its analgesic ED_{50} value, yet it effectively blocked cocaine-conditioned place preference at the same dose. Our findings suggest dual kappa-delta agonists could be promising novel analgesics and treatments for cocaine addiction.

METHODS

Drugs and Chemicals. Opiates were a gift from the Research Technology Branch of the National Institute on Drug Abuse (Rockville, MD). IBNtxA and [^{125}I]BNtxA were synthesized in our laboratory as previously described.[9,17,37] Na^{125}I and [^{35}S]GTPγS were purchased from PerkinElmer. Miscellaneous chemicals and buffers were purchased from Sigma-Aldrich.

Mice. Male CD1 mice (20−32 g) were obtained from Charles River Laboratories, and C57BL/6J mice (20−32 g each) were obtained from Jackson Laboratories (Bar Harbor, ME). Additional tests used male MOR-1 gene-disrupted "knockout" mice (MOR-1 KO) lacking exon 2 or KOR-1 gene-disrupted "knockout" mice (KOR-1 KO), obtained from breeding colonies established at the Torrey Pines Institute for Molecular Studies from homozygous breeding pairs of mice obtained from the Jackson Laboratory. These mice are on a C57BL/6J genetic background. All mice were maintained on a 12 h light/dark cycle with Purina rodent chow and water available ad libitum and housed in groups of five until testing. All animal studies were preapproved by the Institutional Animal Care and Use Committees of the Memorial Sloan Kettering Cancer Center or Torrey Pines Institute for Molecular Studies, in accordance with the 2002 National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Radioligand Competition Binding Assays. [^{125}I]BNtxA binding in wild-type mice was carried out in the presence of MOR-1 [D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH2 (CTAP)], KOR-1 (U50,488H), and DOR-1 (DPDPE) ligands at 200 nM as previously described as competing agents.[9,17] Assays were performed in whole brain membrane homogenates. All additional binding was carried out in the absence of blockers in membranes prepared from Chinese hamster ovary (CHO) cells stably expressing murine clones of MOR-1, DOR-1, and KOR-1, as previously described.[38] Assays were performed at 25 °C for 90 min in 50 mM potassium phosphate buffer, pH 7.4, containing 5 mM magnesium sulfate. After the incubation, the reaction was filtered through glass-fiber filters (Whatman Schleicher & Schuell, Keene, NH) and washed three times with 3 mL of ice-cold 50 mM Tris-HCl, pH 7.4, on a semiautomatic cell harvester. Nonspecific binding was defined by addition of levallorphan (8 μM) to matching samples and was subtracted from total binding to yield specific binding. K_i values were calculated by nonlinear regression analysis (GraphPad Prism, San Diego, CA). Protein concentrations were determined using the Lowry method with BSA as the standard.[39]

Functional Assays. [^{35}S]GTPγS binding was performed on membranes prepared from stably opioid receptor transfected cells in the presence and absence of the indicated compound for 60 min at 30 °C in the assay buffer (50 mM Tris-HCl, pH 7.4, 3 mM $MgCl_2$, 0.2 mM EGTA, and 10 mM NaCl) containing 0.05 nM [^{35}S]GTPγS, 2 μg/mL each leupeptin, pepstatin, aprotinin, and bestatin, and 30 μM GDP, as previously described.[40] After the incubation, the reaction was filtered through glass-fiber filters (Whatman Schleicher & Schuell, Keene, NH) and washed three times with 3 mL of ice-cold buffer (50 mM Tris-HCl, pH 7.4) on a semiautomatic cell harvester. Filters were transferred into vials with 3 mL of Liquiscent (National Diagnostics, Atlanta, GA), and the radioactivity in vials was determined by scintillation spectroscopy in a Tri-Carb 2900TR counter (PerkinElmer Life and Analytical Sciences). Basal binding was determined in the presence of GDP and the absence of drug. Data was normalized to 100 nM DAMGO, DPDPE, and U50,488 for MOR-1, DOR-1, and KOR-1 binding, respectively. EC_{50} and $\%E_{max}$ values were calculated by nonlinear regression analysis (GraphPad Prism, San Diego, CA).

Tail Flick Analgesia. Antinociception was determined using the radiant heat tail flick technique using an Ugo Basile model 37360 instrument. The intensity was set to achieve a baseline between 2 and 3 s. Baseline latencies were determined before experimental treatments for all mice. Tail flick analgesia was assessed quantally as a doubling or greater of the baseline latency, with a maximal 10 s latency to minimize damage to the tail. Data were also analyzed as percentage maximal percent effect (%MPE), and similar results were observed. At each time point, MPE was calculated according to the formula: % MPE = 100 × (test latency − control latency)/(maximal latency − control latency). Compounds were injected subcutaneously (sc), and analgesia was assessed 30 min later. For antagonism studies, β-FNA (40 mg/kg, sc) and norbinaltorphimine (norBNI, 10 mg/kg, sc) were administered 24 h before **16** (**MP1104**). Naltrindole (NTI, 20 mg/kg, sc) was administered 15 min before **16** (**MP1104**). The 55 °C warm-water tail-withdrawal assay was additionally performed with C57BL/6J, MOR-1 KO, or KOR-1 KO mice as previously described.[41] Briefly, water heated to 55 °C acted as a nociceptive stimulus with the latency to withdraw the tail taken as the end point. Mice showing no response within 5 s during the determination of baseline responses were excluded from the experiment. After determining baseline control responses, mice were administered vehicle or graded doses of morphine or **16** (**MP1104**). All samples were given as single subcutaneous (sc) injections with tail withdrawal latencies measured 30 and 40 min after administration unless otherwise stated. A cutoff of 15 s was used to avoid tissue damage; those mice failing to withdraw their tails within this time were assigned a maximal percent effect (%MPE) of 100%. In the receptor selectivity studies, the DOR-1-selective antagonist naltrindole (20 mg/kg, ip) was administered 20 min prior to administration of **16** (**MP1104**). In vivo experiments were evaluated using GraphPad Prism, San Diego, CA. Statistical significance is indicated as follows: ns, $P > 0.05$; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; ****, $P \leq 0.0001$.

Conditioned Place Preference/Aversion. Mice were conditioned with a counterbalanced place conditioning paradigm using similar timing as detailed previously.[42] The amount of time subjects spent in each of three compartments was measured over a 30 min

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testing period. Prior to place conditioning, the animals (n = 89) did not demonstrate significant differences in their time spent exploring the left (506 ± 14 s) vs right (548 ± 15 s) compartments (p = 0.06; Student's t-test), resulting in a preconditioning response of −5.0 ± 22 s. During each of the next 2 days, mice were administered vehicle (0.9% saline) and consistently confined in a randomly assigned outer compartment for 40 min, half of each group in the right chamber, half in the left chamber. Four hours later, mice were administered morphine (10 mg/kg, ip), U50,488 (30 mg/kg, ip), cocaine (10 mg/kg, sc), **16** (**MP1104**) (1 mg/kg, sc), or cocaine preceded 10 min by **16** (**MP1104**) (1 mg/kg, sc) and confined to the opposite compartment for 40 min. Conditioned place preference data is presented as the difference in time spent in drug- and vehicle-associated chambers and were analyzed via repeated measures two-way ANOVA with the difference in time spent on the treatment- vs vehicle-associated side as the dependent measure and conditioning status as the between-groups factor. Where appropriate, Tukey's HSD or Sidak's multiple comparison post-hoc tests were used to assess group differences. Effects were considered significant when $p < 0.05$. All effects are expressed as mean ± SEM.

Synthesis of Compounds. All chemicals were purchased from Sigma-Aldrich Chemicals and Alfa Aesar and were used without further purification. Reaction mixtures were purified by silica flash chromatography on E. Merck 230−400 mesh silica gel 60 using a Teledyne ISCO CombiFlash R_f instrument with UV detection at 280 and 254 nm. RediSep R_f silica gel normal phase columns were used with a gradient of 0−10% MeOH in DCM. The yields reported are isolated yields. IR spectra were recorded on a Bruker Optics Tensor 27 FTIR spectrometer with peaks reported in cm^{-1}. NMR spectra were recorded on Bruker Avance III 500 or Avance III 600 with DCH CryoProbe instruments. NMR spectra were processed with MestReNova software (ver. 6.1.1.). Chemical shifts are reported in parts per million (ppm) relative to residual solvent peaks rounded to the nearest 0.01 for proton and 0.1 for carbon ($CDCl_3$ ^1H 7.26, ^{13}C 77.3; CD_3OD ^1H 3.31, ^{13}C 49.0; DMSO-d_6 ^{13}C 39.5). Peak multiplicity is reported as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Coupling constants (J) are expressed in Hz. Mass spectra were obtained at the MSKCC Analytical Core Facility on a Waters Acuity SQD LC MS by electrospray (ESI) ionization. High resolution mass spectra were obtained on a Waters Acuity Premiere XE TOF LC-MS by electrospray ionization. Accurate masses are reported for the molecular ion $[M + H]^+$. HPLC was performed with the following: Waters 1525 binary pump, Waters 2489 UV/vis detector, Waters XBridge C18 column (5 μm × 150 mm × 4.6 mm), mobile phase solvent A (water with 0.1% TFA), solvent B (acetonitrile with 0.1% TFA), gradient 5−95% acetonitrile/water, flow rate 1 mL/min. A reversed-phase HPLC using a PerkinElmer LC pump series 200 and a 785A UV/vis detector (214 nM) was used. A Varian microsorb MV 100−5 reversed-phase column (5 μm × 4.6 mm × 250 mm) with the mobile phases being 0.1% TFA in water and 0.1% TFA in ACN with a gradient elution at a flow rate of 1 mL/min was used.

17-Methyl-3-methoxy-4,5α-epoxy-7,8-en-6β-(phthalimido)-morphinan (***3***). To a solution of **2** (1.97 g, 6.58 mmol), phthalimide (1.936 g, 13.16 mmol, 2 equiv), and triphenylphosphine (3.45 g, 13.16 mmol, 2 equiv) in dry THF (40 mL) was added dropwise DIAD (2.59 mL, 13.16 mmol, 2 equiv) solution in dry toluene (8 mL) under argon over a period of 30 min. The reaction mixture was stirred for 7.0 h and up to an overnight period. Water (10 mL) was added at room temperature, and the resulting mixture was stirred for an additional 10 min. The volatiles were removed under reduced pressure. Aqueous 2% citric acid (60 mL) and 1 N HCl (20 mL) were added to the residue. The resulting aqueous layer was extracted with Et_2O/EtOAc 1:2 mixture (3 × 40 mL) to remove most byproducts and excess reagents. The pH of the solution was then adjusted to 10 with 10% aqueous ammonia. The aqueous layer was extracted with DCM (3 × 40 mL), and the organic layer was quickly dried over Na_2SO_4. After filtration over a Buchner funnel, the filtrate was evaporated under reduced pressure, and the resulting residue was dried overnight in vacuum. The crude product was purified by ISO combiflash (3−10% MeOH gradient in DCM). Fractions were collected and concentrated to give an off-white amorphous solid. Yield: 2.186 g (78%). ^1H NMR (600 MHz, $CDCl_3$) delta: 7.86−7.81 (m, 2H), 7.74−7.67 (m, 2H), 6.71 (d, J = 8.2 Hz, 1H), 6.62 (d, J = 8.2 Hz, 1H), 5.70 (d, J = 9.9 Hz, 1H), 5.51 (m, 1H), 5.04 (s, 1H), 4.92−4.84 (m, 1H), 3.89 (s, 3H), 3.43 (s, 1H), 3.33 (s, 1H), 3.06 (d, J = 18.2 Hz, 1H), 2.61 (m, 1H), 2.47 (s, 3H), 2.38−2.22 (m, 3H), 1.75 (d, J = 10.5 Hz, 1H). ESI-MS m/z: 429.0 (MH^+).

17-Methyl-3-methoxy-4,5α-epoxy-7,8-en-6β-(amino)morphinan (***4***). To the solution of **3** (1.117 g, 2.61 mmol) and *cis*-2-penten-1-ol (3.95 mL, 39.10 mmol, 15 equiv) in MeOH was added hydrazine aqueous solution (35%; 2.36 mL, 26.06 mmol, 10 equiv). The reaction mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure. To the remaining residue were added aqueous 1.5 N HOAc and aqueous 1 N HCl until the pH was about 3, and the aqueous mixture was filtered to remove the white precipitate. The filtrate was neutralized with 10% aqueous NH_4OH to pH 9−10, and the compound was extracted with DCM. The organic phase was washed with water and brine and dried over Na_2SO_4. The crude product was purified by flash chromatography (87% EtOAC/10% MeOH/3% NH_4OH) to give a white amorphous solid. Yield: 722 mg, 93%. ^1H NMR (600 MHz, $CDCl_3$) delta: 6.67 (d, J = 8.1 Hz, 1H), 6.56 (d, J = 8.1 Hz, 1H), 5.94−5.84 (m, 1H), 5.47 (d, J = 9.8 Hz, 1H), 4.62 (s, 1H), 3.84 (s, 3H), 3.51 (d, J = 4.7 Hz, 1H), 3.42−3.32 (m, 1H), 3.12 (s, 1H), 3.05 (d, J = 18.5 Hz, 1H), 2.66 (b, 1H), 2.48 (s, 3H), 2.43−2.35 (m, 2H), 2.15−2.12 (m, 1H), 1.85 (d, J = 10.6 Hz, 1H). ESI-MS m/z: 298.9 (MH^+).

17-Methyl-3-methoxy-4,5α-epoxy-7,8-en-6-β-[(3′-iodo)-benzamido]-morphinan (***5***). To the solution of **4** (1.507 g, 5.05 mmol) and 3-iodobenzoic acid (1.628 g, 6.56 mmol, 1.3 equiv) in dry DMF (50 mL) was added HATU (2.50 g, 6.57 mmol, 1.3 equiv). After stirring at room temperature for 5 min, DIEA (2.64 mL, 15.15 mmol, 3 equiv) was added to the reaction mixture, and it was stirred at room temperature for 15 min. The solvent was mostly removed under reduced pressure with heating to give an oily residue. EtOAc was then added to dilute the residue, and then it was washed with brine 5 times to remove DMF. The organic phase was dried over Na_2SO_4 and evaporated to give a crude product. The crude product was purified by flash chromatography (1−6% MeOH in DCM gradient) to afford an off-white amorphous solid. Yield: 2.032 g, 76%. ^1H NMR (600 MHz, $CDCl_3$) delta 8.11 (s, 1H), 7.83 (d, J = 7.9 Hz, 1H), 7.72 (d, J = 7.8 Hz, 1H), 7.17 (t, J = 7.8 Hz, 1H), 6.72 (d, J = 8.2 Hz, 1H), 6.60 (d, J = 8.2 Hz, 1H), 6.15 (s, 1H), 5.96−5.87 (m, 1H), 5.67 (d, J = 9.7 Hz, 1H), 4.92 (s, 1H), 4.61 (t, J = 6.3 Hz, 1H), 3.88 (s, 3H), 3.62 (s, 1H), 3.30 (s, 1H), 3.09 (d, J = 18.8 Hz, 1H), 2.82 (d, J = 9.3 Hz, 1H), 2.62 (s, 3H), 2.59−2.47 (m, 2H), 2.23 (dd, J = 12.3, 8.5 Hz, 1H), 1.89 (dd, J = 17.1, 8.5 Hz, 1H). ^{13}C NMR (151 MHz, $CDCl_3$) delta 165.9, 146.1, 142.7, 140.8, 136.2, 136.2, 132.9, 130.5, 130.2, 129.3, 126.5, 126.3, 119.3, 114.1, 94.5, 92.3, 59.6, 56.9, 50.6, 47.4, 44.0, 43.2, 40.3, 35.8, 20.6. ESI-MS m/z: 529.0 (MH^+). HRMS calcd for $C_{25}H_{26}N_2O_3I$ (MH^+), 529.0988; found, 529.0998. HPLC: 7.163 min. Purity: 98.01%.

17-Methyl-3-hydroxy-4,5α-epoxy-7,8-en-6-β-[(3′-iodo)-benzamido]-morphinan (***6***). The solution of **5** (100 mg, 0.189 mmol) in dry DCM (4 mL) was cooled to 0 °C, followed by addition of 1 M BBr_3 (1.325 mL, 1.325 mmol, 7 equiv) in the dark, and the mixture was slowly warmed to room temperature and stirred for 30 min. Aqueous ammonia (1.3 mL) was added into the reaction mixture, and the mixture was stirred for 1 h. Aqueous saturated sodium bicarbonate was added, and the product was extracted with DCM. The organic phase was washed with brine, dried over Na_2SO_4, and evaporated to give a crude product. The crude product was purified by flash chromatography (1−15% MeOH in DCM gradient) to afford **6** as a white amorphous solid. Yield: 67 mg, 69%. ^1H NMR (600 MHz, $CDCl_3$) delta: 8.08 (s, 1H), 7.83 (d, J = 7.8 Hz, 1H), 7.70 (d, J = 7.8 Hz, 1H), 7.15 (t, J = 7.8 Hz, 1H), 6.69 (d, J = 8.1 Hz, 1H), 6.54 (d, J = 8.1 Hz, 1H), 6.05 (d, J = 5.2 Hz, 1H), 5.92−5.83 (m, 1H), 5.72 (d, J = 9.8 Hz, 1H), 4.85 (s, 1H), 4.60 (t, J = 6.2 Hz, 1H), 3.44 (s, 1H), 3.12 (s, 1H), 3.06 (d, J = 18.5 Hz, 1H), 2.67 (d, J = 8.7 Hz, 1H), 2.48 (s, 3H), 2.45−2.37 (m, 2H), 2.14−2.06 (m, 1H), 1.84 (d, J = 12.4 Hz, 1H). ^{13}C NMR (151 MHz, $CDCl_3$) delta: 171.4, 166.1, 144.6, 140.9, 138.6, 136.2, 136.0, 130.5, 129.8, 128.7, 126.4, 119.7, 117.0, 94.6, 93.0,

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60.6, 59.5, 50.8, 47.3, 44.2, 43.1, 21.3, 20.5, 14.4. ESI-MS m/z: 515.2 (MH^+). HRMS calcd for $C_{24}H_{24}N_2O_3I$ (MH^+), 515.0832; found, 515.0831. HPLC: 6.564 min. Purity: 91.74%.

*17-Nor-3-methoxy-4,5α-epoxy-7,8-en-6-α-OH-morphinan (**7**).* To an ice-cold mixture of **2** (1.00 g, 3.34 mmol) and sodium bicarbonate (1.684 g, 20.04 mmol, 6 equiv) in absolute 1,2-dichloroethane (20 mL) was added dropwise 1-chloroethyl chloroformate (1.44 mL, 13.36 mmol, 4 equiv). The solution mixture was stirred at room temperature for 30 min then heated at 85 °C overnight. The reaction mixture was cooled to room temperature, and then it was filtered. The filtrate was evaporated under reduced pressure to give a residue, which was directly used for the alcoholysis without further purification. The residue was added absolute methanol (20 mL) under argon, and the stirred solution was heated at 90 °C for 1 h. The solvent was removed under reduced pressure to yield a solid, which was taken up in 100 mL of DCM and neutralized with saturated Na_2CO_3 solution (100 mL). The organic layer was separated, the water layer was extracted with DCM (100 mL), and the combined organic layer was evaporated under reduced pressure to give a solid with light yellow color. The product was carried over to the next step without further purification.

*17-Allyl-3-methoxy-4,5α-epoxy-7,8-en-6-α-OH-morphinan (**8**).* To the solution of crude **7** and the allyl bromide (202 μL, 2.34 mmol, 0.7 equiv) in dry DMF (20 mL) was added Na_2CO_3 (354 mg, 3.34 mmol, 1 equiv). The reaction mixture was stirred under argon at 90 °C overnight. Mass spectrometry showed that there was still some starting material left, so more allyl bromide (40 μL, 0.468 mmol, 0.14 equiv) was added to the reaction, and it was stirred at 90 °C for 2 h. The reaction mixture was cooled, and DMF was mostly removed under reduced pressure. Water and aqueous 1 N NaOH was added to the oily residue to reach pH 8−9, and the compound was extracted with DCM. The organic phase was dried over Na_2SO_4 and purified by flash chromatography (1−5% MeOH in DCM) to give a light brown amorphous solid. Yield: 743 mg, 2 steps combined, 68%. ^{1}H NMR (600 MHz, $CDCl_3$) delta 6.66 (d, J = 8.2 Hz, 1H), 6.57 (d, J = 8.2 Hz, 1H), 5.93−5.82 (m, 1H), 5.74−5.66 (m, 1H), 5.29 (dt, J = 9.9, 2.6 Hz, 1H), 5.25 (dd, J = 3.0, 1.4 Hz, 1H), 5.17 (dd, J = 10.1, 1.6 Hz, 1H), 4.89 (dd, J = 6.5, 1.1 Hz, 1H), 4.21−4.14 (m, 1H), 3.84 (s, 3H), 3.46 (dd, J = 6.2, 3.2 Hz, 1H), 3.24−3.14 (m, 2H), 2.98 (d, J = 18.5 Hz, 1H), 2.88 (d, J = 10.1 Hz, 1H), 2.72−2.62 (m, 2H), 2.37 (td, J = 12.3, 3.5 Hz, 1H), 2.29 (dd, J = 18.6, 6.3 Hz, 1H), 2.05 (td, J = 12.5, 5.1 Hz, 1H), 1.91−1.83 (m, 1H). ESI-MS m/z: 326.2 (MH^+).

*17-Allyl-3-methoxy-4,5α-epoxy-7,8-en-6β-(phthalimido)-morphinan (**9**).* To the solution of **8** (200 mg, 0.615 mmol), phthalimide (181 mg, 1.229 mmol, 2 equiv), and triphenylphosphine (322 mg, 1.229 mmol, 2 equiv) in dry THF (5 mL) was added dropwise DIAD (242 μL, 1.229 mmol, 2 equiv) solution in dry toluene (1 mL) under argon over a period of 30 min. The reaction mixture was stirred for 7.0 h up to an overnight period. Water (1 mL) was added at room temperature, and the resulting mixture was stirred for an additional 10 min. The volatiles were removed under reduced pressure. Aqueous 2% citric acid (8 mL) and 1 N HCl (2 mL) were added to the residue. The resulting aqueous layer was extracted with Et_2O/EtOAc 1:1 mixture (4 × 15 mL) to remove most byproducts and excess reagents. The pH of the solution was then adjusted to 10 with 10% aqueous ammonia. The aqueous layer was extracted with DCM (3 × 40 mL), and the organic layer was quickly dried over Na_2SO_4. After filtration over a Buchner funnel, the filtrate was evaporated under reduced pressure, and the resulting residue was dried overnight in vacuum. The crude product was purified by ISO combiflash (3−10% MeOH gradient in DCM). Fractions were collected and concentrated to give an off-white amorphous solid. Yield: 191 mg, 68%. ^{1}H NMR (500 MHz, CDCl3) delta: 7.86−7.82 (m, 2H), 7.74−7.70 (m, 2H), 6.71 (d, J = 8.2 Hz, 1H), 6.61 (d, J = 8.2 Hz, 1H), 5.91 (m, 1H), 5.69 (d, J = 10.0 Hz, 1H), 5.52−5.48 (m, 1H), 5.25 (dd, J = 17.1, 1.6 Hz, 1H), 5.15 (d, J = 10.2 Hz, 1H), 5.04 (s, 1H), 4.91−4.86 (m, 1H), 3.89 (s, 3H), 3.43−3.39 (m, 2H), 3.25−3.15 (m, 1H), 2.98 (d, J = 18.3 Hz, 1H), 2.71−2.67 (m, 1H), 2.38−2.22 (m, 3H), 1.76−1.70 (m, 1H). ESI-MS m/z: 455.2 (MH^+).

*17-Allyl-3-methoxy-4,5α-epoxy-7,8-en-6β-(amino)morphinan (**10**).* To the solution of **9** (190 mg, 0.418 mmol) and *cis*-2-penten-1-ol (675 μL, 6.688 mmol, 16 equiv) in MeOH was added hydrazine aqueous solution (35%; 379 μL, 4.18 mmol, 10 equiv). The reaction mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure. To the remaining residue was added aqueous 1.5 N HOAc and aqueous 1 N HCl until the pH was about 3, and the aqueous mixture was filtered to remove the white precipitate. The filtrate was neutralized with 10% aqueous NH_4OH to pH 9−10, and the compound was extracted with DCM. The organic phase was washed with water and brine and dried over Na_2SO_4. The crude product was purified by flash chromatography (87% EtOAC/10% MeOH/3% NH_4OH) to give a white amorphous solid. Yield: 140 mg, quantitative. ^{1}H NMR (600 MHz, $CDCl_3$) delta: 6.66 (d, J = 8.2 Hz, 1H), 6.54 (d, J = 8.1 Hz, 1H), 5.94−5.82 (m, 2H), 5.48 (dd, J = 9.8, 1.8 Hz, 1H), 5.24 (dd, J = 17.1, 1.6 Hz, 1H), 5.16 (dd, J = 10.1, 1.4 Hz, 1H), 4.60 (s, 1H), 3.84 (s, 3H), 3.50 (d, J = 5.6 Hz, 1H), 3.41 (dd, J = 5.7, 3.2 Hz, 1H), 3.26−3.12 (m, 2H), 2.99−2.87 (m, 2H), 2.66 (dt, J = 12.2, 6.0 Hz, 1H), 2.35−2.26 (m, 2H), 2.09−1.99 (m, 1H), 1.82 (dd, J = 12.5, 1.7 Hz, 1H). ESI-MS m/z: 325.1 (MH^+).

*17-Allyl-3-methoxy-4,5α-epoxy-7,8-en-6-β-[(3′-iodo)benzamido]-morphinan (**11**).* To the solution of the **10** (137 mg, 0.422 mmol) and 3-iodobenzoic acid (136 mg, 0.549 mmol, 1.3 equiv) in dry DMF (1.7 mL) was added HATU (209 mg, 0.549 mmol, 1.3 equiv). After stirring at room temperature for 5 min, DIEA (221 μL, 1.267 mmol, 3 equiv) was added to the reaction mixture, and it was stirred at room temperature for 15 min. The solvent was mostly removed under reduced pressure with heating to give an oily residue. EtOAc was added to dilute the residue, and then it was washed with brine 5 times to further remove DMF. The organic phase was dried over Na_2SO_4 and evaporated to give a crude product. The crude product was purified by flash chromatography (1−4% MeOH in DCM gradient) to afford an off-white amorphous solid. Yield: 163 mg, 70%. ^{1}H NMR (500 MHz, $CDCl_3$) delta: 8.10 (t, J = 1.5 Hz, 1H), 7.84 (ddd, J = 7.9, 1.4, 1.0 Hz, 1H), 7.71 (d, J = 8.0 Hz, 1H), 7.17 (t, J = 9.4 Hz, 1H), 6.71 (d, J = 8.2 Hz, 1H), 6.58 (d, J = 8.2 Hz, 1H), 6.01−5.87 (m, 3H), 5.69 (dd, J = 9.7, 1.7 Hz, 1H), 5.32 (d, J = 17.2 Hz, 1H), 5.26 (d, J = 10.1 Hz, 1H), 4.91 (s, 1H), 4.62 (t, J = 6.4 Hz, 1H), 3.88 (s, 3H), 3.61 (s, 1H), 3.36−3.27 (m, 2H), 3.18−3.11 (m, 1H), 3.03 (d, J = 18.6 Hz, 1H), 2.44 (dd, J = 18.3, 5.5 Hz, 2H), 2.11 (ddd, J = 22.9, 15.4, 4.8 Hz, 1H), 1.87 (dd, J = 17.0, 15.4 Hz, 2H). ESI-MS m/z: 555.0 (MH^+).

*17-Nor-3-methoxy-4,5α-epoxy-7,8-en-6-β-[(3′-iodo)benzamido]-morphinan (**12**).* The solution of **5** (1.29 g, 2.441 mmol) and DIAD (865 μL, 4.395 mmol, 1.8 equiv) in acetonitrile (20 mL) was heated at 65 °C for 20 h. The reaction mixture was cooled to room temperature, followed by the addition of pyridine HCl (564 mg, 4.883 mmol, 2 equiv), and the reaction mixture was stirred at room temperature for 3 days. The solvent was removed under reduced pressure to yield the crude product, which was then purified by ISCO combiflash (1−15% MeOH in DCM) to give a light brown amorphous solid. Yield: 676 mg, 54%. ^{1}H NMR (600 MHz, $CDCl_3$) delta: 9.92 (b, 2H), 8.17 (s, 1H), 7.80 (d, J = 7.9 Hz, 1H), 7.77 (d, J = 7.8 Hz, 1H), 7.15 (t, J = 7.8 Hz, 1H), 6.76 (d, J = 8.3 Hz, 1H), 6.64 (d, J = 8.2 Hz, 1H), 6.58 (s, 1H), 5.97−5.87 (m, 1H), 5.62 (d, J = 10.0 Hz, 1H), 4.99 (s, 1H), 4.61 (t, J = 6.2 Hz, 1H), 4.27 (s, 1H), 3.89 (s, 3H), 3.59 (s, 1H), 3.35−3.30 (m, 2H), 3.03−2.97 (m, 2H), 2.43 (td, J = 13.5, 4.7 Hz, 1H), 2.09−1.93 (m, 1H), 1.68 (s, 2H). ESI-MS m/z: 515.0 (MH^+).

*17-Phenethyl-3-methoxy-4,5α-epoxy-7,8-en-6-β-[(3′-iodo)-benzamido]-morphinan (**13**).* To the solution of **12** (200 mg, 0.389 mmol) in dry DMF (4 mL) was added Cs_2CO_3 (190 mg, 0.583 mmol, 1.5 equiv) and (2-iodoethyl)benzene (67.5 μL, 0.467 mmol, 1.2 equiv). The reaction mixture was stirred and heated under argon at 90 °C overnight. The solvent was mostly removed under reduced pressure. The remaining oily residue was diluted with EtOAc and washed with brine 5 times to further remove DMF. The organic phase was dried over Na_2SO_4, and the crude product was purified by flash chromatography (1−3% MeOH in DCM) to give a light brown amorphous solid (40 mg, 20% yield), which was not pure. The impurity could not be separated from the product, so it was carried over to the next step without further purification. ^{1}H NMR (600 MHz,

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$CDCl_3$) delta 8.10 (s, 1H), 7.85 (d, J = 7.9 Hz, 1H), 7.71 (d, J = 7.8 Hz, 1H), 7.31 (t, J = 7.5 Hz, 1H), 7.20−7.16 (m, 4H), 6.70 (d, J = 8.1 Hz, 1H), 6.56 (d, J = 8.1 Hz, 1H), 5.91 (dd, J = 11.8, 6.2 Hz, 1H), 5.89−5.81 (m, 1H), 5.73−5.68 (m, 1H), 4.90 (s, 1H), 4.61 (s, 1H), 3.89 (s, 3H), 3.50 (b, 1H), 3.04−3.00 (m, 2H), 2.88−2.75 (m, 2H), 2.43−2.34 (b, 2H), 2.11−2.00 (b, 1H), 2.00−1.93 (m, 1H), 1.90−1.86 (m, 1H), 1.26 (t, J = 7.1 Hz, 1H). ESI-MS m/z: 619.2 (MH^+).

*17-Cyclopropylmethyl-3-methoxy-4,5α-epoxy-7,8-en-6-β-[(3′-iodo)benzamido]-morphinan (**14**).* To the solution of **12** (400 mg, 0.778 mmol) in dry DMF (8 mL) was added Na_2CO_3 (123.6 mg, 1.167 mmol, 1.5 equiv) and (bromomethyl)cyclopropane (90.5 μL, 0.933 mmol, 1.2 equiv). The reaction mixture was stirred and heated under argon at 90 °C overnight. The solvent was mostly removed under reduced pressure. The remaining oily residue was diluted with EtOAc and washed with brine 5 times to further remove DMF. The organic phase was dried over Na_2SO_4, and the crude product was purified by flash chromatography (3−5% MeOH in DCM) to give a light brown amorphous solid. Yield: 347 mg, 78%. ^{1}H NMR (600 MHz, $CDCl_3$) delta: 8.08 (s, 1H), 7.84 (d, J = 7.9 Hz, 1H), 7.70 (d, J = 7.9 Hz, 1H), 7.18 (t, J = 7.8 Hz, 1H), 6.69 (d, J = 8.2 Hz, 1H), 6.55 (t, J = 7.4 Hz, 1H), 5.94 (m, 1H), 5.84 (d, J = 5.1 Hz, 1H), 5.73 (dd, J = 9.7, 1.8 Hz, 1H), 4.90 (s, 1H), 4.63 (t, J = 6.4 Hz, 1H), 3.88 (s, 3H), 3.71 (s, 1H), 3.07 (s, 1H), 2.96 (d, J = 18.4 Hz, 1H), 2.84 (d, J = 9.0 Hz, 1H), 2.51−2.40 (m, 2H), 2.39−2.26 (m, 2H), 2.14- 0.05 (m, 1H), 1.86 (d, J = 12.6 Hz, 1H), 0.90 (s, 1H), 0.56 (d, J = 7.3 Hz, 2H), 0.17 (d, J = 3.9 Hz, 2H). ESI-MS m/z: 569.2 (MH^+).

*17-Phenethyl-3-hydroxy-4,5α-epoxy-7,8-en-6-β-[(3′-iodo)-benzamido]-morphinan (**15**).* The solution of **9** (40 mg, 0.0647 mmol) in dry DCM (2 mL) was cooled to 0 °C, followed by addition of 1 M BBr_3 (453 μL, 0.453 mmol, 7 equiv) in the dark, and the mixture was slowly warmed to rt and stirred for 30 min. Aqueous ammonia (420 μL) was added into the reaction mixture, and the mixture was stirred for 1 h. Aqueous saturated sodium bicarbonate was added, and the product was extracted with DCM. The organic phase was washed with brine, dried over Na_2SO_4, and evaporated to give a crude product. The crude product was purified by flash chromatography (1−5% MeOH in DCM gradient) to afford a light yellow amorphous solid. Yield: 21.6 mg, 55%. ^{1}H NMR (600 MHz, $CDCl_3$) delta: 8.08 (s, 1H), 7.85 (d, J = 7.9 Hz, 1H), 7.72 (d, J = 7.8 Hz, 1H), 7.30 (t, J = 7.5 Hz, 2H), 7.23 (d, J = 7.4 Hz, 3H), 7.18 (t, J = 7.8 Hz, 1H), 6.69 (d, J = 8.1 Hz, 1H), 6.52 (d, J = 8.1 Hz, 1H), 5.92 (dd, J = 15.1, 4.5 Hz, 1H), 5.91−5.85 (m, 1H), 5.77−5.67 (m, 1H), 4.87 (s, 1H), 4.58 (t, J = 6.3 Hz, 1H), 3.53 (s, 1H), 3.03−2.98 (m, 2H), 2.90−2.75 (m, 2H), 2.42−2.33 (m, 3H), 2.06−2.02 (m, 2H), 1.8−1.81 (d, J = 13.4 Hz, 1H), 1.3−1.17 (m, 1H). ^{13}C NMR (151 MHz, $CDCl_3$) delta: 166.0, 144.5, 140.9, 136.2, 136.1, 130.6, 128.9, 128.6, 126.4, 119.6, 116.7, 94.6, 93.1, 57.7, 57.5, 50.80, 45.5, 45.0, 39.0, 36.1, 34.8, 21.3. ESI-MS m/z: 605.1 (MH^+). HRMS calcd for $C_{31}H_{30}N_2O_3I$ (MH^+), 605.1301; found, 605.1298. HPLC: 8.020 min. Purity: 95.64%.

*17-Cyclopropylmethyl-3-hydroxy-4,5α-epoxy-7,8-en-6-β-[(3′-iodo)benzamido]-morphinan (**16**, **MP1104**).* The solution of **10** (227 mg, 0.399 mmol) in dry DCM (8 mL) was cooled to 0 °C, followed by addition of 1 M BBr_3 (2.8 mL, 2.80 mmol, 7 equiv) in the dark, and the mixture was slowly warmed to room temperature and stirred for 30 min. Aqueous ammonia (2.6 mL) was added into the reaction mixture, and the mixture was stirred for 1 h. Aqueous saturated sodium bicarbonate was added, and the product was extracted with DCM. The organic phase was washed with brine and dried over Na_2SO_4 and evaporated to give a crude product. The crude product was purified by flash chromatography (1−7% MeOH in DCM gradient) to afford a light yellow amorphous solid. Yield: 162 mg, 73%. ^{1}H NMR (600 MHz, $CDCl_3$) delta: 8.08 (s, 1H), 7.84 (d, J = 7.9 Hz, 1H), 7.71 (d, J = 7.8 Hz, 1H), 7.16 (t, J = 7.8 Hz, 1H), 6.68 (d, J = 8.1 Hz, 1H), 6.51 (d, J = 8.1 Hz, 1H), 5.96 (s, 1H), 5.90 (m, 1H), 5.75 (d, J = 9.8 Hz, 1H), 4.87 (s, 1H), 4.59 (t, J = 6.2 Hz, 1H), 3.74 (s, 1H), 3.08 (s, 1H), 2.94 (d, J = 18.4 Hz, 1H), 2.87 (s, 1H), 2.47 (d, J = 28.9 Hz, 2H), 2.40−2.22 (m, 2H), 2.09 (s, 1H), 1.83 (d, J = 11.6 Hz, 1H), 0.98−0.86 (m, 1H), 0.56 (d, J = 7.1 Hz, 2H), 0.18 (s, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) delta: 166.0, 144.7, 140.9, 136.2, 136.1, 130.5, 126.4, 119.6, 94.6, 93.1, 60.6, 60.1, 56.9, 50.6, 45.7, 45.0, 21.3, 20.9, 14.4, 4.2, 4.2. ESI-MS m/z: 555.1 (MH^+). HRMS calcd for $C_{27}H_{28}N_2O_3I$ (MH^+), 555.1145; found, 555.1132. HPLC: 7.034 min. Purity: 98.01%.

*17-Allyl-3-hydroxy-4,5α-epoxy-7,8-en-6-β-[(3′-iodo)benzamido]-morphinan (**17**).* The solution of **8** (30 mg, 0.054 mmol) in dry DCM (1.2 mL) was cooled to 0 °C, followed by addition of 1 M BBr_3 (379 μL, 0.379 mmol, 7 equiv) in the dark, and the mixture was slowly warmed to room temperature and stirred for 30 min. Aqueous ammonia (360 μL) was added into the reaction mixture, and the mixture was stirred for 1 h. Aqueous saturated sodium bicarbonate was added, and the product was extracted with DCM. The organic phase was washed with brine, dried over Na_2SO_4, and evaporated to give a crude product. The crude product was purified by flash chromatography (1−5% MeOH in DCM gradient) to afford a white amorphous solid. Yield: 18 mg, 61%. ^{1}H NMR (600 MHz, $CDCl_3$) delta: 8.07 (s, 1H), 7.79 (d, J = 7.9 Hz, 1H), 7.68 (d, J = 7.8 Hz, 1H), 7.10 (t, J = 7.8 Hz, 1H), 6.67 (d, J = 8.1 Hz, 1H), 6.51 (d, J = 8.1 Hz, 1H), 6.26 (s, 1H), 5.89−5.85 (m, 1H), 5.85−5.78 (m, 1H), 5.70 (d, J = 9.9 Hz, 1H), 5.26 (t, J = 18.2 Hz, 1H), 5.17 (d, J = 10.2 Hz, 1H), 4.85 (s, 1H), 4.56 (t, J = 6.3 Hz, 1H), 3.52 (s, 1H), 3.29−3.12 (m, 2H), 3.07 (s, 1H), 2.98 (d, J = 18.5 Hz, 1H), 2.71 (dd, J = 11.8, 3.6 Hz, 1H), 2.38−2.24 (m, 2H), 2.13−1.97 (m, 1H), 1.77 (d, J = 11.8 Hz, 1H). ^{13}C NMR (151 MHz, $CDCl_3$) delta: 166.2, 144.6, 140.8, 138.6, 136.3, 136.1, 135.3, 133.6, 130.5, 130.2, 128.5, 126.5, 126.1, 119.6, 118.5, 117.2, 94.5, 92.9, 58.5, 56.8, 50.9, 45.5, 44.8, 40.2, 35.7, 20.9. ESI-MS m/z: 541.2 (MH^+). HRMS calcd for $C_{26}H_{26}N_2O_3I$ (MH^+), 541.0988; found, 541.1004. HPLC: 6.837 min. Purity: 96.43%.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acschemneuro.5b00153.

HPLC chromatograms of compounds (PDF)

■ AUTHOR INFORMATION

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Author Contributions

§A.V. and G.F.M. contributed equally to this work. A.V., G.F.M., J.P.M., and S.M. designed the experiments; A.V., G.F.M., S.O.E., M.L.G., V.L.R., A.H., and J.J.S. performed the experiments; A.V., G.W.P., and J.J.S. contributed reagents and analytical tools; A.V., G.F.M., G.W.P., J.P.M., and S.M. analyzed data; and A.V., G.F.M., J.P.M., and S.M. wrote the paper.

Notes

The authors declare no competing financial interest.

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