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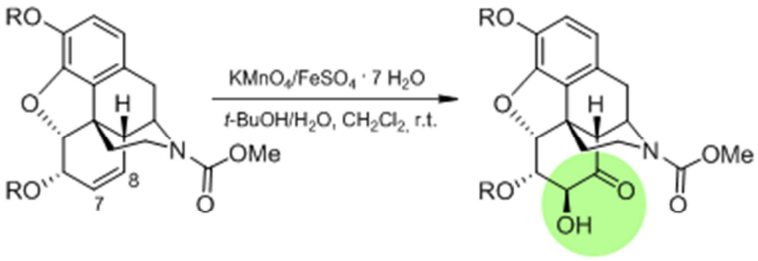

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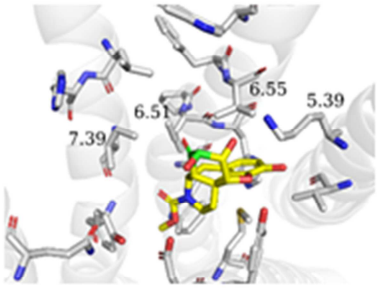

↑ Selectivity at DOR

Synthesis of 7β-hydroxy-8-ketone opioid derivatives with antagonist activity at mu- and delta-opioid receptors

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Abstract

Despite extensive years of research, the direct oxidation of the 7,8-double bond of opioids has so far received little attention and knowledge about the effects of this modification on activity at the different opioid receptors is scarce. We herein report that potassium permanganate supported on iron(II) sulfate heptahydrate can be used as a convenient oxidant in the one-step, heterogeneous conversion of $\Delta^{7,8}$-opioids to the corresponding 7β-hydroxy-8-ketones. Details of the reaction mechanism are given and the effects of the substituent at position 6 of several opioids on the reaction outcome is discussed. The opioid hydroxy ketones prepared are antagonists at the mu- and delta-opioid receptors. Docking simulations and detailed structure-activity analysis revealed that the presence of the 7β-hydroxy-8-ketone functionality in the prepared compounds can be used to gain activity towards the delta opioid receptor. The 7β-hydroxy-8-ketones prepared with this method can also be regarded as versatile intermediates for the synthesis of other opioids of interest.

Keywords: morphine, oxidation, supported permanganate, hydroxy ketone, antagonist, opioid receptor

1. Introduction

Pain is the most common symptom for which patients seek medical attention [1–3]. The impact of chronic pain for the society is dramatic due to the high cost of medical treatment and loss in productivity. Opioids are a group of well-known pain relieving medicines, of which morphine (**1**) is the classical example (Figure 1), used to treat moderate to severe pain conditions such as cancer-related pain, post-surgery pain, chronic low-back pain and pain associated to certain inflammatory diseases like osteoarthritis [4–6]. However, the adverse side effects and the development of tolerance and hyperalgesia, especially in long-term clinical use, hamper the widespread use of opioids for relieving pain. Opioids act by binding to and activating the opioid receptors (ORs) which belong to the superfamily of G protein-coupled receptors [7–9]. The effects of opioids such as morphine, either beneficial (analgesia) or adverse (sedation, respiratory depression, constipation, addiction), are mediated by the mu opioid receptor (MOR), by means of different downstream signalling and regulatory pathways. This receptor has two closely related receptor types (~70% sequence identity) named delta (DOR) and kappa (KOR) receptors.

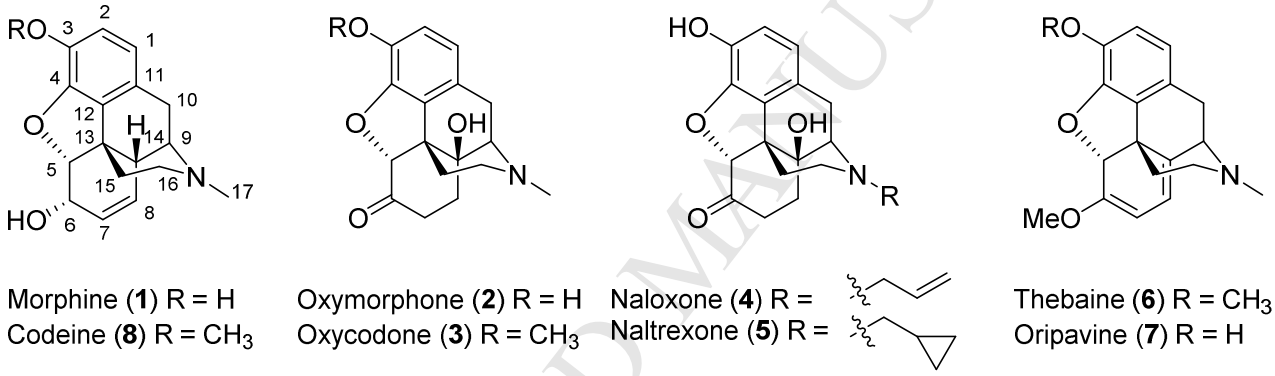


Figure 1. Structures of common opioids **1**-**8**.

Since the isolation of morphine (**1**) in 1806 to the complete elucidation of its chemical structure, concluded in 1927 [10], and the reports on detailed crystal structures of the ORs co-crystallized with their respective ligands in 2012 [7–9], a plethora of semisynthetic derivatives of morphine (**1**) and related opioids has been made aiming at improving the analgesic potential while minimizing the side effects [11–14]. Consequently, solid structure-activity relationships have been built and it is generally accepted that morphine-like activity and addiction liability is associated with the presence of the same stereochemistry as that of morphine (**1**), a relatively small alkyl group on the tertiary nitrogen at position 17 and a free aromatic hydroxy group at position 3 [11–14]. Among the many chemical modifications of opioid compounds, oxidation at position 14 to give 14-hydroxy derivatives has successfully resulted in an increase in analgesic potency as in oxymorphone (**2**) and oxycodone (**3**), and variation of the alkyl substituents at the nitrogen atom has led to the general conclusion that phenylethyl groups improve the analgesic activity whereas allyl or cyclopropylmethyl groups result in compounds such as naloxone (**4**) and naltrexone (**5**) that block the effect of opioids, through antagonistic behavior at the ORs (Figure 1).

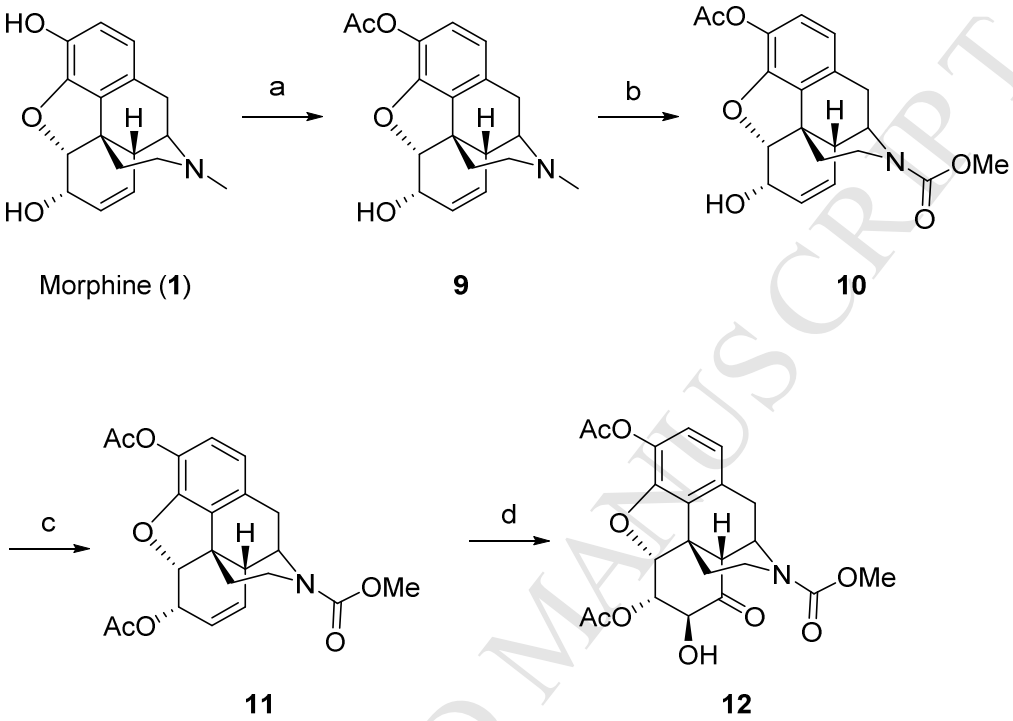
Despite the large number of semisynthetic derivatives of opioid compounds available up to now, which have mainly resulted from derivatization at positions 3, 6 and 17, chemical modification of the 7,8-double bond of opioids is seldom reported in the literature. This double bond has been mostly removed by reduction, in the preparation of some 7,8-dihydro derivatives of interest [15–20]. Attempts to oxidize it using, for instance, peroxy acids yield side products derived from attack on the aromatic ring together with the double bond [21,22]. The epoxidation of morphine proved unsuccessful and a 7β,8β-epoxide could only be obtained from 3-methoxymethylmorphinone, using hydrogen peroxide in aqueous methanol containing sodium hydroxide, revealing the relevance of the C6 substituent on the outcome of this oxidation [23,24]. Oxidations with osmium tetroxide succeeded to give the respective diols [25–28]. However, they were also highly dependent on the substituent present at C6, with more electron withdrawing substituents giving lower product yields. Overall, oxidations of opioids are challenging and almost exclusive to the production of 14-hydroxy derivatives from thebaine (**6**) and oripavine (**7**), in route to the preparation of drugs such as naloxone (**4**) and naltrexone (**5**) (Figure 1) [19,29]. Nonetheless, some oxidized products of opioids, namely 7,8-epoxides, have been suggested to bear less liability to dependence, with activity comparable to the parent compounds both *in vitro* and *in vivo* [23,24]. We therefore envisioned that the establishment of a new protocol for the oxidation of the 7,8-double bond of opioids would be a useful means to produce versatile synthetic intermediates for opioid-related syntheses and to tackle the biological effects that resulted from the oxidation of this particular double bond.

Although the oxidation of codeine (**8**) (Figure 1) with aqueous permanganate has been reported [30], to the best of our knowledge, there are no reports available on the use of solid-supported permanganate-based systems for the oxidation of opioids. These systems are advantageous as they involve heterogeneous reaction conditions, which are typically milder, allowing good to excellent product yields and recovery. In the particular case of potassium permanganate, they avoid handling of this reagent in the presence of water which results in unpractical and tedious work up procedures [31]. In addition, permanganate-mediated oxidations are usually recognized as eco-friendly because the co-product formed, manganese dioxide, can be recycled. Solid supports such as molecular sieves, graphite reagent, polystyrene, alumina, silica gel and copper sulfate pentahydrate have been previously used for permanganate-based oxidation of alcohols to carbonyl compounds, sulfides to sulfones, diols to lactones, and oximes to ketones [32–44]. The oxidation of olefins to diketones, α-hydroxy ketones and epoxides has been described with permanganate-supported on copper sulfate pentahydrate and iron(III) perchlorate hydrate [45,46]. Herein we report the use of potassium permanganate supported on iron(II) sulfate heptahydrate for the oxidation of opioids as a convenient, one-step procedure for the preparation of previously unreported opioid 7β-hydroxy-8-ketones. Their structure and stereochemistry have been determined with the support of 1D and 2D nuclear magnetic resonance (NMR) and X-ray diffraction studies. Moreover, the affinity and activity of the new compounds towards ORs were determined by radioligand binding and receptor activation assays, and herein we provide novel insights into structure-activity relationships resulting from chemical modification at positions 7 and 8.

2. Results and discussion

2.1. Chemistry

Previous research by the authors has shown that potassium permanganate ($KMnO_4$) supported on $Fe(ClO_4)_3{\cdot}nH_2O$ successfully converts Δ^5-steroids to their corresponding steroidal α-hydroxy ketones, in high yields [46]. Therefore, we set out to investigate the use of this same system on the model opioid compound **11**, which was prepared according to the synthesis depicted on Scheme 1.



Scheme 1. Synthesis of compound **12**. Reagents and conditions: (a) $NaHCO_3$, acetic anhydride, r.t., 15 min, 99%. (b) $NaHCO_3$, methyl chloroformate, anhydrous $CHCl_3$, 62 °C, 3 h, 79%. (c) Pyridine, acetic anhydride, 90 °C, 2.5 h, 79%. (d) $KMnO_4$, $FeSO_4{\cdot}7H_2O$, *t*-BuOH, H_2O, CH_2Cl_2, r.t., 45 min, 61%.

Compound **11** was then oxidized using a mixture of $Fe(ClO_4)_3{\cdot}nH_2O$ and $KMnO_4$ with dichloromethane as the solvent, in the presence of water and *tert*-butanol (*t*-BuOH), according to the reaction conditions depicted on Table S1, Entry 1 (Supporting info). However, the reaction mixture became very dense and quickly dried, and the reaction could not be effected even by addition of fresh amounts of solvent to the reaction medium. We next used permanganate-supported on $CuSO_4{\cdot}5H_2O$, according to the reaction conditions in Table S1, Entry 2. This method has been previously reported for the oxidation of olefins to either α-hydroxy ketones, diketones or epoxides [45]. This time, the reaction proceeded in a relatively short time and we were able to isolate a single reaction product, corresponding to the 7β-hydroxy-8-ketone (**12**) (Scheme 1), however at a low yield of 52%. Therefore, we set out to screen the reaction with different metal salts including sulfates, nitrates and acetates in an attempt to improve the yield of desired product.

All metal sulfates tested were able to complete the reaction to give compound **12** as the single product with the exception of Ag_2SO_4, where mixtures of products, with the hydroxy ketone **12** as the major product, were obtained (Table S1, Entries 3-6). High resolution mass spectrometry (HRMS) analysis of the crude reaction mixture revealed the presence of the 7,8-diol derivative and of unreacted starting material. Mixtures of products were also obtained with metal nitrates (Table S1, Entries 7-8) and with $Cu(OAc)_2{\cdot}H_2O$ (Table S1, Entry 9). In the presence of these salts, the isolation of product **12** was more difficult as *t*-BuOH was retained in significant amounts in the crude mixture. The best yield was obtained using $FeSO_4{\cdot}7H_2O$, with mild extension of the reaction time when compared to $CuSO_4{\cdot}5H_2O$ (Table S1, Entry 3). This is the first report on the use of $FeSO_4{\cdot}7H_2O$ supported on $KMnO_4$ for the preparation of α-hydroxy ketones from olefins.

We next investigated which was the minimum amount of $KMnO_4$ that could be used in the presence of $FeSO_4{\cdot}7H_2O$ to give compound **12**, as this would most likely lower the amounts of MnO_2 formed at the end of the reaction and could help to improve the isolation procedure and final yield (Table S1, Entries 10-11). With amounts of oxidant lower than 0.5 g the reaction was slower and could not be completed. Nonetheless, under the conditions described in Table S1, Entry 12, the reaction proceeded well with easier isolation of the final product, small compromise in time, and no compromise in yield. Two final runs were made in the absence of water or *t*-BuOH, under the best conditions given in Table S1 (Entry 12). Without *t*-BuOH, the reaction proceeded much slower and after 3 hours the expected compound **12** could be isolated, in 45% yield (Table S1, Entry 13). However, without water (Table S1, Entry 14), we observed decomposition of the starting material and no product was recovered, even after extension of the reaction time to 3 hours. These observations show that the presence of water is crucial for the success of the reaction and are consistent with a role of *t*-BuOH as a catalyst. 2D NMR experiments (Supporting info, Table S2) and single crystal X-ray diffraction measurements (Figure 2) confirmed the structure and stereochemistry of compound **12**, and provided important information for elucidating the reaction mechanism.

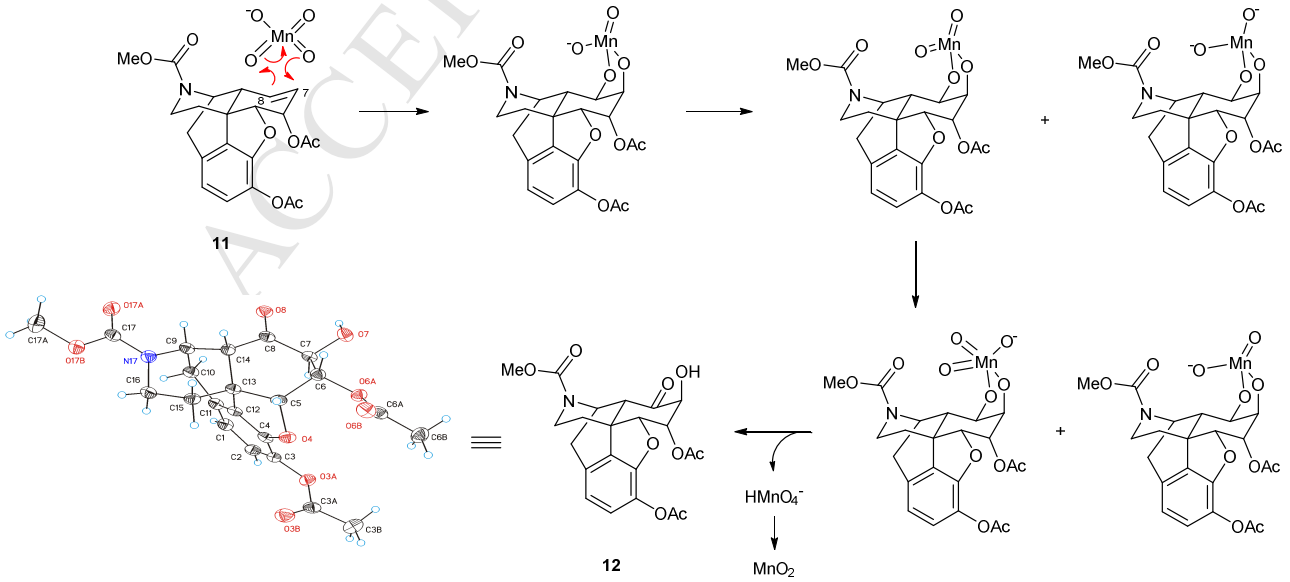
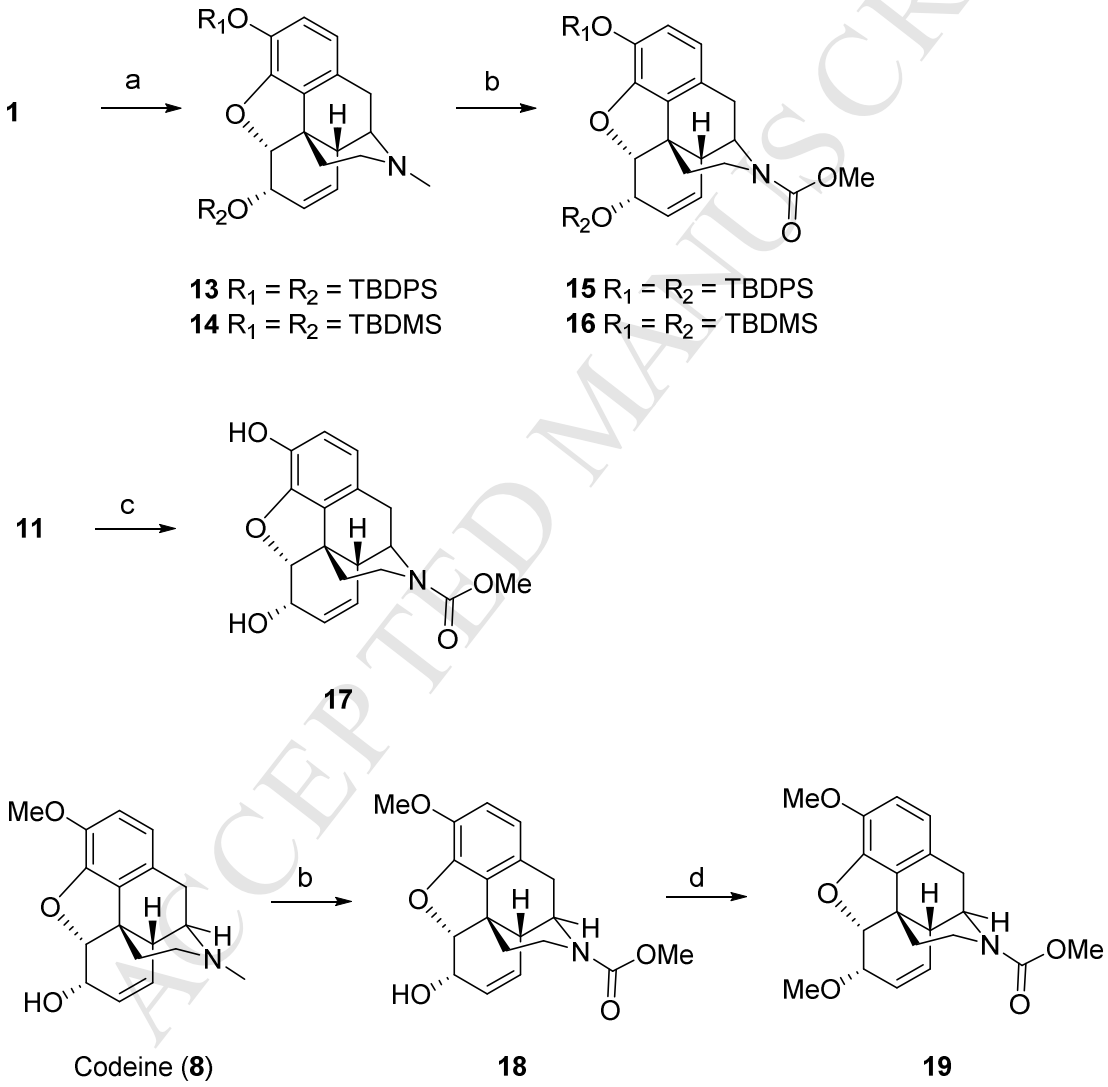


Figure 2. Proposed reaction mechanism for the formation of the 7β-hydroxy-8-ketone **12**.

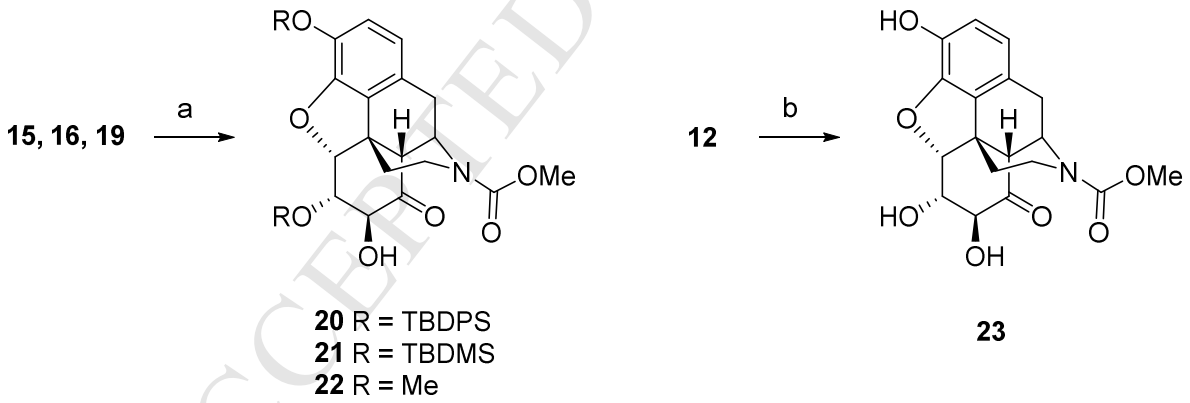
On the newly formed hydroxy ketone **12**, the stereochemistry of the chiral carbon 7 is (*S*) with the hydroxy group assuming an *anti* configuration in relation to the C6 acetoxy group, which suggests that the approximation of the permanganate ion to the double bond occurred on the less hindered face of the opioid **11** (Figure 2). The oxidation of olefins with permanganate has been extensively studied and it is generally accepted that the reaction proceeds by formation of a cyclic manganate(V) intermediate, which is the limiting step (Figure 2) [47].



Scheme 2. Preparation of opioid derivatives **15-19**. Reagents and conditions: (a) Compound **13**: TBDPSCl, imidazole, CH_2Cl_2, r.t., 1 h, quant. Compound **14**: TBDMSCl, imidazole, dry DMF, 90 °C, 4 h, 63%. (b) $NaHCO_3$, methyl chloroformate, anhydrous $CHCl_3$, 62 °C, 82% (**15**), 87% (**16**), 97% (**18**). (c) Aq. NaOH sol., MeOH:$CHCl_3$ 4:1, r.t., 30 min, 87%. (d) NaH, MeI, dry THF, r.t., 2 h, 49%.

In reaction media where the basicity is low, disproportionation of this intermediate occurs leading to the formation of ketols. Systems comprising permanganate supported on metal salts such as the one in our work have been reported to proceed by Ω-phase catalysis where the water/*t*-BuOH mixture forms a third phase in the reaction medium, by surrounding the inorganic solids, where the reaction actually takes place [45]. We reason that this effect may account for an overall low basicity in our reaction medium leading the reaction to progress towards the formation of the hydroxy ketone **12**.

To study the effect of the substituent present at C6 on the reaction outcome, we synthesized the opioid derivatives **15**, **16** and **19** (Scheme 2). Compounds **15**, **16** and **19** were then oxidized using the reaction conditions of Table S1, Entry 12 (Scheme 3). The reaction was successful in all opioids and compounds **20**, **21** and **22** were prepared with this method in 36, 34 and 15% yields, respectively, after chromatographic purification. The electron withdrawing effect of the oxygen atom is likely to account for the observed difference in the reactivity. This effect is more pronounced in the methoxy derivative **19** than on the siloxy derivatives **15** and **16**. In compound **11**, bearing an acetoxy group, this effect is the least pronounced due to electron delocalization between the two oxygen atoms. Of note, when the reaction was made using the 6-oxo derivative of compound **10**, we observed degradation of the starting material and no products could be recovered. As a result of deacetylation of compound **12** with sodium hydroxide, compound **23** was obtained, in 90% yield.



Scheme 3. Synthesis of opioid hydroxy ketones. (a) $KMnO_4$, $FeSO_4 \cdot 7H_2O$, *t*-BuOH, H_2O, CH_2Cl_2, r.t., 36% (**20**), 34% (**21**), 15% (**22**). (b) Aq. NaOH sol., MeOH:$CHCl_3$ 4:1, r.t., 2.5 h, 90%.

2.2. Pharmacology

We tested the activity of the new opioid hydroxy ketones **12**, **22** and **23** and the respective 7,8-double bond bearing compounds **11**, **19** and **17** on the human (h) MOR, DOR and KOR. We started by determining the binding of the compounds to hMOR, hDOR and hKOR using membrane preparations obtained from stably transfected cells, by competition binding assay with 1 nM

[^{3}H]diprenorphine as the radioligand. The K_D values for [^{3}H]diprenorphine binding were 0.48 nM to hMOR, 1.1 nM to hDOR and 0.67 nM to hKOR, as previously reported [48,49]. Receptor expression levels, determined as B_{max} were 3.1, 4.7, and 6.9 pmol/mg of protein for hMOR, hDOR, and hKOR receptors, respectively.

Table 1. Inhibition of [^{3}H]diprenorphine binding by opioid 7β-hydroxy-8-ketones (**12**, **22**, **23**) and compounds **11**, **19** and **17**. Values are mean ± SEM from three separate experiments (n=3) performed in triplicate. Statistical significance (*, $p < 0.05$) was determined compared to the 0.05% DMSO control using one-way ANOVA with Dunnett's multiple comparison test.

Compound	MOR % inhibition		DOR % inhibition		KOR % inhibition	
	1 μM	10 μM	1 μM	10 μM	1 μM	10 μM
11	1 ± 2	3 ± 3	1 ± 2	9 ± 2	0 ± 3	1 ± 3
12	7 ± 1	9 ± 2	22 ± 4 *	51 ± 2 *	4 ± 3	8 ± 1
19	3 ± 1	11 ± 2	2 ± 1	10 ± 1	6 ± 3	2 ± 3
22	5 ± 2	5 ± 3	5 ± 2	19 ± 7 *	5 ± 1	0 ± 1
17[a]	16 ±3	58 ± 6 *	21 ± 1 *	59 ± 1 *	7 ± 3	27 ± 3 *
23[b]	8 ± 3	48 ± 6 *	24 ± 2 *	63 ± 1 *	6 ± 1	21 ± 2 *

[a] K_i value of **17** on MOR 0.814–1.72 μM and on DOR 1.69–2.63 μM

[b] K_i value of **23** on MOR 1.08–2.58 μM and on DOR 1.13–1.83 μM

Compounds **17** [File compound17.mol here] and **23** [File compound23.mol here] showed the highest competition to OR binding among the six compounds tested on all three receptor subtypes. They were able to block 48-58% of [^{3}H]diprenorphine binding at the hMOR and 59-63% at hDOR, at 10 μM (Table 1). We further evaluated these two compounds on a concentration range of 0.1 to 50 μM (hMOR) or 0.01 to 50 μM (hDOR) (Figures 3A and B). The K_i values ranged between 0.814–1.72 μM (compound **17**) and 1.08–2.58 μM (compound **23**) for hMOR, and 1.69–2.63 μM (compound **17**) and 1.13–1.83 μM (compound **23**) for hDOR (Table 1). At hKOR the compounds **17** and **23** blocked only 21-27% of radioligand binding at 10 μM, displaying lower binding affinity. As the binding affinity at hKOR was anticipated to be in the high μM range, the K_i was not determined in this case. Compound **12** was also quite effective at hDOR with 51% inhibition of [^{3}H]diprenorphine binding at 10 μM (Table 1), and an estimated K_i value of about 10 μM. To a much lesser extent, compound **22** also showed activity at the hDOR, with 19% inhibition of [^{3}H]diprenorphine binding at 10 μM. Although it is difficult to judge selectivity based on simple screening, preliminary observations can be provided based on Table 1. Compounds **17** and **23** appeared equipotent at the hMOR and hDOR, while having a lower affinity at the hKOR. Compounds **12** and **22** appear more selective for hDOR than for hMOR or hKOR.

We next investigated the agonist or antagonist activities of compounds **17** and **23** using the [^{35}S]GTPγS binding assay. This assay scores the agonist-evoked GDP/GTP exchange that occurs at the G-protein within the receptor-G protein complex, and is classically used to quantify receptor activation. Neither of the compounds acted as agonists in the [^{35}S]GTPγS binding assays on hMOR

and hDOR (Figures 3C and 3E). Conversely they acted as antagonists of DAMGO-mediated activation of hMOR at the concentrations tested, as well as antagonists of SNC80-mediated activation of hDOR at the highest concentration of 50 µM (Figures 3D and 3F).

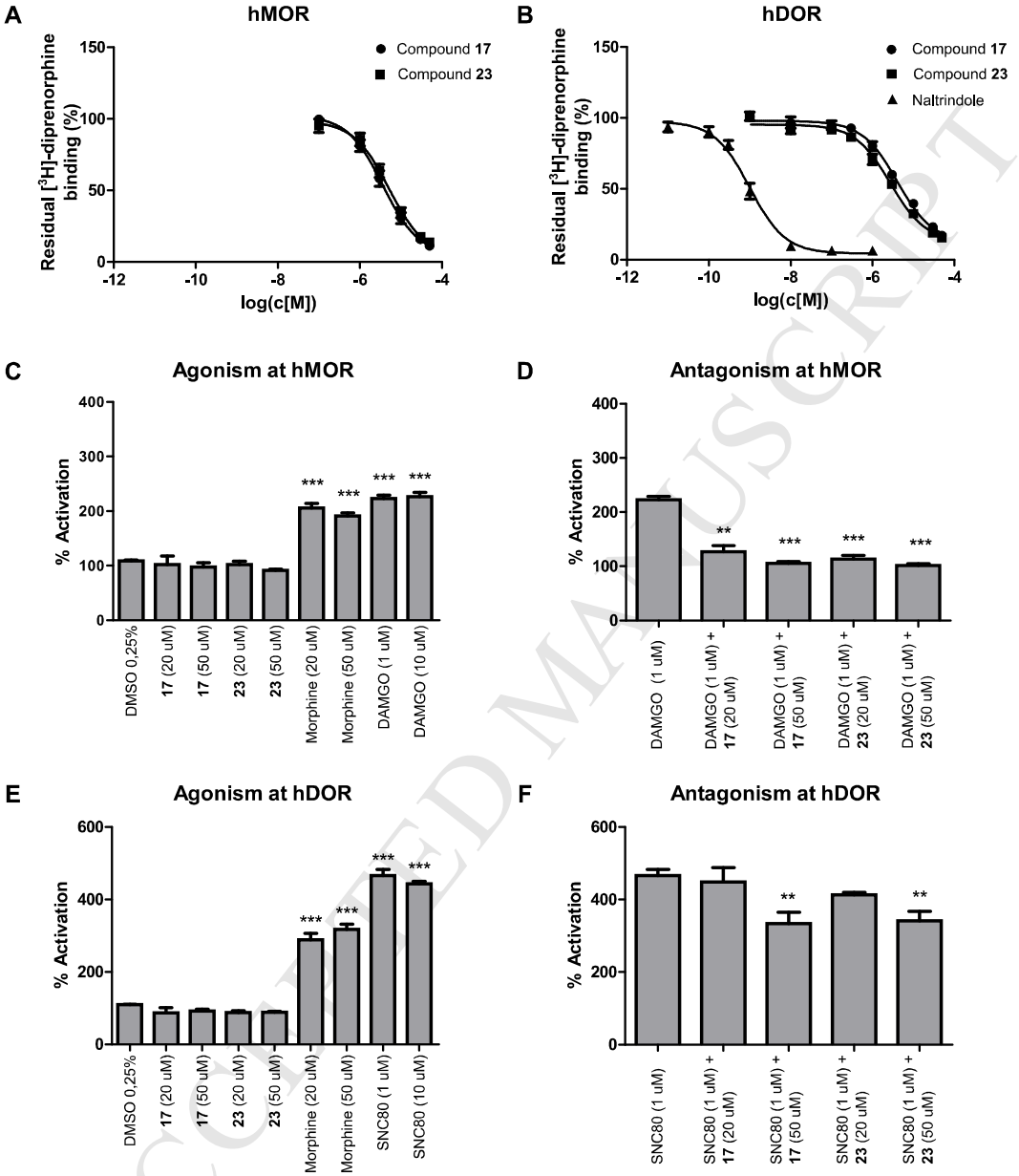


Figure 3. Binding and functional [^{35}S]GTPγS binding assay of **17** and **23** at hMOR and hDOR. A: Competition binding curves of **17** and **23** on hMOR. B: Competition binding curves of **17** and **23** on hDOR. The competition was determined to the general opioid antagonist [^{3}H]diprenorphine. The values are mean ± SEM from three separate experiments (n=3) performed in triplicate. The results were analyzed using non-linear regression analysis. C: No MOR activation was detected either with compound **17** or **23** at 20 or 50 µM concentration, as compared to the MOR agonists DAMGO and morphine. D: Compounds **17** and **23** inhibited MOR activation by DAMGO (1 µM) at 20 and 50 µM concentrations. E: No DOR activation was detected either with compound **17** or **23** at 20 or 50

µM concentration, as compared to the DOR agonist SNC80 or to morphine as the initial starting material of the synthesis. F: Compounds **17** and **23** inhibited DOR activation by SNC80 (1 µM) at the 50 µM concentration.

2.3 Computational chemistry

2.3.1 The orthosteric binding sites of DOR, MOR, and KOR

The ORs belong to the rhodopsin-like GPCR family characterized by seven α-helical transmembrane segments. Crystallographic high-resolution structures have been solved for the three opioid receptor subtypes. The mouse DOR (mDOR) and hKOR have been co-crystallized with selective antagonists, respectively naltrindole (PDB code 4EJ4) and JDTic (PDB code 4DJH) [8,9]. The mouse MOR has been crystallized with β-FNA (PDB code 4DKL), a covalently bound irreversible antagonist [7], as well as with BU72 (PDB code 5C1M), a high efficacy agonist [50]. The three-dimensional structures allow a detailed atomistic view of the orthosteric binding site, formed by a crevice surrounded by the transmembrane segments 3-7, where the ligands are similarly bound (Figures 4A and 4B). The N-terminus is not part of the binding site in the antagonist-bound structures, but in the structure of the active MOR forms a lid on top of the binding cavity and interacts with the co-crystallized ligand [50].

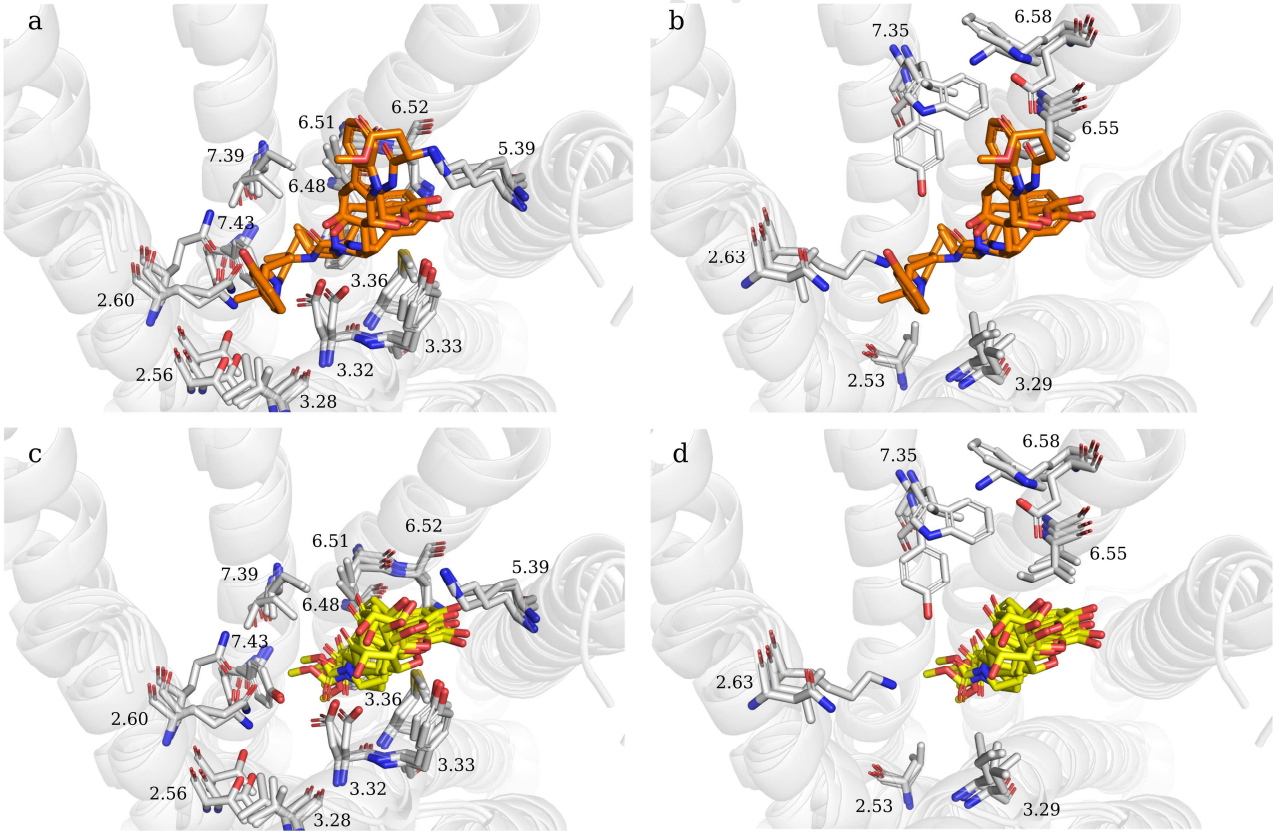


Figure 4. Close-up view of the orthosteric binding cavities for (a, b) co-crystallized ligands and (c, d) docked compounds **17** and **23** in the superimposed MOR, DOR and KOR X-ray structures. Focus is on the binding site conserved amino acids (a, c, message site) and varying amino acids (b, d,

address site). MOR is co-crystallized with β-FNA, DOR with naltrindole and KOR with JDTic. Selected amino acids are labeled using the Ballesteros-Weinstein convention.

The co-crystallized ligands occupy the same region of the binding pocket and accordingly, the amino acids at the binding site are highly conserved among the three OR subtypes (Table 2): out of 19 amino acids at close distance to the co-crystallized ligands, 13 are identical and three conservatively changed. The conserved amino acids are located closer to the intracellular surface of the binding cavity. They form the so-called message site (Figures 4A and 4C) [51,52], which plays a role in the efficacy of ligands. Among those, aspartic acid$^{3.32}$ anchors the positive charge of ligands through a salt bridge, and methionine$^{3.36}$ and tryptophan$^{6.48}$ have hydrophobic contacts. Although amino acids at the message site are conserved, they do not necessarily form the same interactions with ligands: for example, tyrosine$^{3.33}$'s phenolic group forms hydrogen-bonds to naltrindole and β-FNA directly at DOR and MOR, but via a bridging water molecule with JDTic at KOR. At DOR and MOR tyrosine$^{7.43}$ is at hydrogen-bonding distance of aspartic acid$^{3.32}$, holding in place the salt bridge to co-crystallized ligands. In contrast, at the KOR the contact between tyrosine$^{7.43}$ and aspartic acid$^{3.32}$ is not direct but bridged by the ligand JDTic. Polar groups of ligands are in addition to side-chains participating in hydrogen bond networks that involve water molecules [7–9].

Table 2. Amino acid composition of the binding pocket of OR subtypes. Address site differences are in bold. Ballesteros-Weinstein numbering is indicated on the left.

Ballesteros-Weinstein	MOR	DOR	KOR
2.53	A117	A98	**V108**
2.56	T120	T101	T111
2.60	Q124	Q105	Q115
2.63	**N127**	**K108**	**V118**
ECL1	W133	W114	W124
3.28	V143	V124	V134
3.29	**I144**	L125	L135
3.32	D147	D128	D138
3.33	Y148	Y129	Y139
3.36	M151	M132	M142
ECL2	C217	C198	C210
5.39	K233	K214	K227
6.48	W293	W274	W287
6.51	I296	I277	I290
6.52	H297	H278	H291
6.55	V300	V281	**I294**
6.58	**K303**	**W284**	**E297**
7.35	**W318**	**L300**	**Y312**

7.39	I322	I304	I316
7.43	Y326	Y308	Y320

In contrast to the message site, the amino acids located at the binding cavity's extracellular surface are more variable across the three OR subtypes, and consequently likely to play a significant role in selectivity. These amino acids have been named at the address site [51,52]. The most variable positions, differing by size, charge, and polarity, are 2.63 (Lys/Val/Asn), 6.58 (Trp/Glu/Lys) and 7.35 (Leu/Tyr/Trp) (Table 2). Differences at the intracellular surface represent more conservative changes, for example DOR and MOR have alanine$^{2.53}$, but KOR has valine$^{2.53}$. While the co-crystallized ligands occupy fully the message site, whether and how they would occupy the address site differs. At DOR and MOR, co-crystallized ligands with a morphine-like skeleton, naltrindole and β-FNA, are binding towards the transmembrane segments 5-7, in a region surrounded by amino acids 5.39, 6.58, 7.35. Naltrindole bound to DOR is placing indole in a sub-pocket formed by lysine$^{5.39}$, trypthophan$^{6.58}$ and leucine$^{7.35}$. In contrast, JDTic binds to KOR by extending towards transmembrane segment 2 and its carbonyl group directly interacts with tyrosine$^{7.35}$. A similar interaction cannot take place at DOR and MOR that have respectively leucine$^{7.35}$ and trypthophan$^{7.35}$, possibly explaining partly its low affinity for these receptors. Another determinant of the preference of JDTic for the KOR is the small amino acid valine$^{2.63}$ instead of large and polar amino acids in other subtypes, lysine$^{2.63}$ at DOR and asparagine$^{2.63}$ at MOR.

Docking simulations of compounds **17** [File compound17.mol here] and **23** [File compound23.mol here] suggest that the morphine-like skeleton roughly occupies the message site (Figure 4C and D, Supporting info Figure S2). At the MOR [Files complex_compound17_MOR.pse and complex_compound23_MOR.pse] and DOR [Files complex_compound17_DOR.pse and complex_compound23_DOR.pse] subtypes (low μM activity in MOR and DOR), this binding mode is similar to naltrindole/DOR and β-FNA/MOR (Figure 4A and B, Supporting info, Figure S2). In comparison, at KOR the docking simulations suggest a small (~1.5-2.5 Å) shift around the ligand's positions 6 to 8, probably driven by steric clashes with tyrosine$^{7.35}$ (Supporting information, Figure S2). The binding modes are overall in agreement with docking studies of the selective antagonists norbinaltorphimine and 5'-guanidinonaltrindole [8]. Docking simulations of the other four compounds, **11**, **19**, and **22** (mostly inactive) as well as **12** (mid-range μM activity at DOR) to the three subtypes led also to similar binding modes. An exception was found for compounds **11**, **12** and **22** at KOR, where the compounds docked closer to TM2 than classical opioids, which may be due to the larger size of the acetoxy groups in positions 3 and 6 for compounds **11** and **12**.

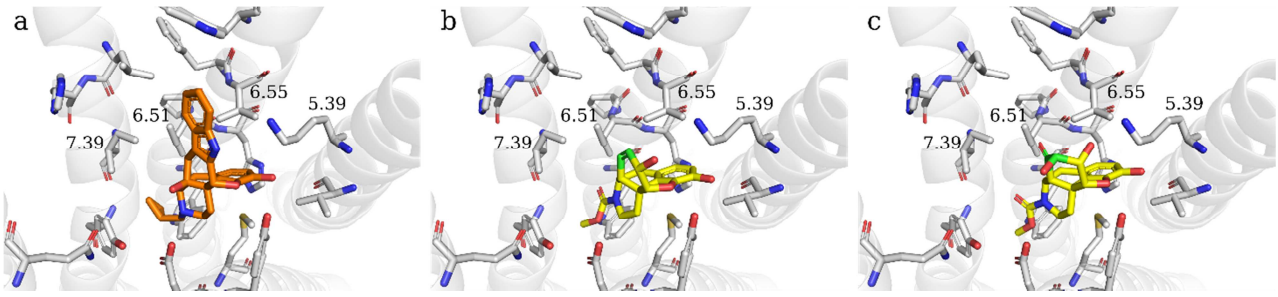


Figure 5. Binding modes at the DOR of (a) co-crystallized naltrindole, (b) docked compound **17** and (c) docked compound **23**. Positions 7 and 8 are shown in green in docked ligands. The 7β-hydroxy-8-ketone is pointing towards the solvent and facing the hydrophobic wall of the binding pocket. Amino acids labeled with Ballesteros-Weinstein numbering, lysine[5.39], isoleucine[6.51], valine[6.55] and isoleucine[7.39].

2.3.2 Structure-activity relationships

We assessed the binding affinities (competition binding assay) of six compounds for the MOR, DOR and KOR in comparison to morphine **1**, with focus on four positions according to Table 3. Positions 7 and 8 had either a double bond or were modified into a 7β-hydroxy-8-ketone moiety whereas positions 3 and 6 had hydroxy, methoxy, or acetoxy substituents. Position 17 had an *N*-methyl carbamate in all cases.

Table 3. Overview of the SARs of the studied compounds. Boxes, estimates of K_i ranges from % binding inhibition. White: high μM, light grey: less than 100 μM, dark grey: less than 10 μM, black: less than 3 μM.

Compound	position 3	position 6	position 7,8	MOR	DOR	KOR
11	-OAc	-OAc	CH=CH			
12	-OAc	-OAc	-OH =O			
19	-OMe	-OMe	CH=CH			
22	-OMe	-OMe	-OH =O			
17	-OH	-OH	CH=CH			
23	-OH	-OH	-OH =O			

Comparison of the binding results obtained for the pairs of compounds **17** and **23**, **19** and **22**, and **11** and **12** demonstrates that introduction of a 7β-hydroxy-8-ketone is associated with a gain in activity at the DOR, but not at the other OR types. Inhibitory activities at 10 μM increase from 9 to 51% for compounds **11** and **12**, from 10 to 19% for compounds **19** and **22**, and from 59 to 63 % for compounds **17** and **23**. For these two later pairs the change is of lesser magnitude than for the **11** and **12** pair, but the confidence intervals do not overlap. When these compounds (**17**, **19**, **22**, **23**) are docked to the crystal structure of the DOR (as well as in MOR and with the exception at **22** for KOR), positions 7 and 8 are located within an hydrophobic environment surrounded by amino acids isoleucine[6.51], valine[6.55] and isoleucine[7.39], while the extracellular region of the binding cavity funnel

is exposed to the solvent (Figure 5). With the exception of tyrosine$^{7.35}$'s phenolic group in KOR, which is at hydrogen bonding distance from the hydroxy group at position 7, the compound's 7 and 8 newly introduced positions do not seem to form polar interactions with the receptors. Solvent effects may thus be at the source of the inhibitory activity observed with introduction of 7β-hydroxy-8-ketone at DOR, however, these are very difficult to predict. In general, previous work in the literature does not provide a good framework to rationalize the effect of modifying positions 7 and 8. Reduction of the 7,8 double bond in morphine, leading to dihydromorphine, increases activity approximately by two fold at the DOR and MOR, but decreases it at the KOR by 8-fold [53–55]. When comparing dihydrocodeine to codeine (**8**), the activity is in contrast decreasing at DOR and KOR (respectively 6- and 2-fold) and increasing 8.5-fold at MOR [56].

Considering the changes at positions 3 and 6, the hydroxy group is clearly preferred compared to methoxy or acetoxy. This can be seen by comparing the inhibitory activities of compounds **11**, **12**, **19**, and **22** with the activities of compounds **17** and **23**, i.e, at MOR, 3-11% *vs* 48-58%; at DOR 9-51% *vs* 59-63%; and to a lesser extent at KOR 0-10 % *vs* 21-27%. An interesting observation is that the conserved side chain nitrogen of lysine$^{5.39}$ is located near (3-4 Å) position 6 in most docked binding modes at MOR and DOR, where it would potentially form hydrogen bonds that support favoring of the hydroxy groups. Activities of compounds **17** and **23** at all three subtypes are unaffected by introduction of 7β-hydroxy-8-ketone, which may be due to this direct hydrogen bonding of position 6 with lysine$^{5.39}$. For compounds **11**, **12**, **19** and **22**, methoxy and acetoxy groups can be tolerated (or their activities even rescued) at the DOR upon introduction of a 7β-hydroxy-8-ketone, but this mechanism is not clarified by the docking study. Altogether the data presented is in line with the literature, where replacing both hydroxy groups with methoxy groups at positions 3 and 6 (e.g. morphine **1** and thebaine **6**) leads to decreased binding (90-fold at MOR, 20-fold at DOR) [57]. If replacing the hydroxy with methoxy occurs solely at position 3 (morphine **1** and codeine **8**), the decrease in activity is even more noticeable at MOR and KOR (700-fold and 300-fold, respectively), however lower at DOR (3-fold) [58]. In contrast, modifying position 6 (morphine **1** and heterocodeine) leads to increased binding by 3.6-fold at the MOR, 5-fold at the DOR, but is unchanged at KOR [17].

Concerning position 17, compound **1** is easily compared to compound **17**, which are otherwise identical. At MOR, in **17** the loss of the positive charge and the associated loss of salt bridge with aspartate$^{3.32}$ led to a 250-fold increase in K_i and significant loss of radioligand binding inhibition, from 85% at 10 μM for **1** (K_i ~0.004 μM, [54]) to 58 % for **17** (K_i ~1 μM) (Supporting info, Table S3). At KOR K_i has not been determined, but the fold difference is probably even higher than at MOR, since morphine (**1**) displays 80% inhibition of radioligand binding at 10 μM (K_i 0.028 μM) while compound **17** displays only 27% inhibition. At DOR there is surprisingly only limited loss of activity associated with the loss of the positive charge, i.e., only 6-fold: morphine has 63% inhibition (K_i ~0.34 μM) at DOR, while **17** has 59% inhibition (K_i ~2 μM). In addition to charge, in most binding modes, the carbamate fits well into a hydrophobic pocket lined by amino acids tryptophan$^{6.48}$, glycine$^{7.42}$, tyrosine$^{7.43}$ and aspartic acid$^{3.32}$. Altogether *N*-alkylation should thus be associated with a large gain in activity. Nonetheless, we were unable to produce *N*-alkylated compounds within the time frame of the project that supported this work, and especially regulations about opioid substances hindered further work in this direction. Of note, charge is not a requirement

for high activity at ORs. Nanomolar ligands without protonable nitrogen atoms are active at the KOR such as salvinorin A, which partially occupies the orthosteric binding pocket as demonstrated by covalent salvinorin A analogs binding at the KOR [8].

Altogether, concerning determinants of selectivity, the presence of tyrosine$^{7.35}$ in the vicinity of positions 7 and 8 at KOR may be linked to the lower activity at that receptor in comparison to MOR and DOR (respectively tryptophan /leucine$^{7.35}$). Docking simulations suggest that selectivity could be driven through a small shift in ligand binding mode at KOR relative to the other subtypes (see above) that would prevent interactions between the hydroxyl group in position 6 and lysine$^{5.39}$. Glutamate$^{6.58}$ in KOR (lysine/tryptophan$^{6.58}$) is ~10 Å further away but may also play a role.

3. Conclusion

7β-Hydroxy-8-ketone opioids can be prepared in one-step and easily isolated, under heterogeneous conditions, using potassium permanganate supported on metal salts. Our study reveals that the most suitable metal salt for this transformation is iron(II) sulfate heptahydrate and that a number of opioids, bearing different functional groups, can be converted into the expected hydroxy ketones. Better yields are obtained with opioids bearing electron-donating groups at position 6. Mechanistically, the reaction is thought to proceed via formation of a cyclic manganate(V) intermediate that undergoes disproportionation to give the hydroxy ketone. The compounds act as antagonists at MOR and DOR. Overall, our work provides valuable tools for those working in the field of opioids chemistry, as the 7β-hydroxy-8-ketones can be regarded as a good starting point for further syntheses. Herein we have shown that chemical modification at positions 7 and 8 of opioids can lead to increased selectivity at the DOR.

4. Experimental

Chemistry. The reagents were obtained from Sigma Aldrich Co., VWR International Oy, or Merck Reagenzien. For thin layer chromatography (TLC) Silica gel 60 F254 was used. Flash column chromatography (FCC) was made with a Biotage High-Performance Flash Chromatography Sp4-system (Uppsala, Sweden) using a 0.1-mm path length flow cell UV detector/recorder module (fixed wavelength: 254 nm), and 10 g, 25 g or 50 g SNAP cartridges (10–50 mL/min flow rate). The melting points were measured with Stuart SMP40 automated melting point apparatus and are uncorrected. IR spectra were obtained using a Vertex 70 (Bruker Optics Inc., MA, USA) FTIR instrument. The FTIR measurements were made with a horizontal attenuated total reflectance (ATR) accessory (MIRacle, Pike Technology, Inc, WI, USA). The transmittance spectra were recorded at a 4 cm^{-1} resolution between 4000 and 600 cm^{-1} using the OPUS 5.5 software (Bruker Optics Inc., MA, USA). NMR spectra were obtained using a Varian Mercury Plus 300 spectrometer or Bruker Ascend 400 spectrometer, in $CDCl_3$ or CD_3OD, with tetramethylsilane (TMS) as the internal standard. The chemical shifts were reported in parts per million (ppm) and on the δ scale from tetramethylsilane (TMS) as an internal standard. The coupling constants *J* are quoted in Hertz (Hz). If rotamers are observed in the ^{13}C spectrum, the minor rotamer peaks are labelled with *.

Exact mass analysis were performed by Waters UPLC-ESI/QTOF-MS using a Synapt G2 HDMS (Waters, MA, USA) instrument. Purity analyses were executed with Waters Acquity® UPLC system (Waters, Milford MA, USA) attached to Acquity PDA detector and Waters Synapt G2 HDMS mass spectrometer (Waters, Milford MA, USA) via an ESI ion source. The narcotic substances were synthesized under the permission by the Finnish Medicines Agency Fimea (3/2016, 9.3.2016, Helsinki, Finland).

General procedure for oxidations (Table S1)

The respective starting material (0.5 mmol) was dissolved in dichloromethane. The metal salt and $KMnO_4$ were ground to fine powder and added to the stirred solution, followed by water and *t*-BuOH. The mixture was stirred at room temperature and monitored with TLC. When the reaction was complete, the reaction mixture was diluted with diethyl ether (10 mL) and stirred for 3 minutes. The mixture was filtered through a Celite pad and the pad was washed several times with diethyl ether (150 mL in total). The filtrate was washed with water (30 mL), dried with anhydrous Na_2SO_4, filtered and concentrated to dryness.

***N*-Methyl 3,6-di-acetyl-4,5-epoxy-7-hydroxy-8-oxo-(5α,6α,7β)-morphinan carbamate** (**12**). Compound **12** was prepared according to the general procedure described above under the reaction conditions described on Table S1, Entry 12. Colorless solid (137 mg, 61%). The crystals for X-ray analysis were obtained from methanol, directly from the crude. Mp 190 °C (decomp.); FTIR-ATR 3423 (OH), 1770, 1722, 1693, 1446, 1259, 1190, 1109, 766 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ 6.89 (d, J = 8.2 Hz, 1H, H-2), 6.65 (d, J = 8.2 Hz, 1H, H-1), 5.38 (m, 1 H, H-9), 5.15 (d, J = 4.7 Hz, 1H, H-5), 5.08 (m, 1H, H-6), 4.07 (m, 1H, H-16a), 3.75 (brs, 3H, $NCOOCH_3$), 3.57 (d, J = 12.2 Hz, 1H, H-7), 2.89 (m, 4H, H-10a, H-14, H-16b, OH), 2.71 (d, J = 18.9 Hz, 1H, H-10b), 2.31 (s, 3H, 3-$OCO\underline{C}H_3$), 2.18 (s, 3H, 6-$OCO\underline{C}H_3$), 1.98 (m, 2 H, 2 × H-15a and H-15b). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 205.9 (C8), 205.5*, 170.6 (6-$O\underline{C}OCH_3$), 168.4 (3- $O\underline{C}OCH_3$), 155.7 (NCO), 155.5*, 149.2 (C4), 132.4 (C3), 131.9 (C11), 129.3 (C12), 124.4 (C2), 121.0 (C1), 87.5 (C5), 71.4 (C6), 71.1 (C7), 53.1 ($NCOO\underline{C}H_3$), 50.4 (C14), 50.1*, 47.9 (C9), 41.9 (C13), 37.3*, 37.1 (C16), 35.4*, 35.2 (C15), 29.6 (C10), 29.5*, 21.0 ($OCOCH_3$), 20.7 ($OCOCH_3$).. HRMS calcd for $C_{22}H_{24}NO_9$. $[M+1]^+$ 446.1451 found 446.1455. Crystal data: $C_{22}H_{23}NO_9$, M = 445.41 $g{\cdot}mol^{-1}$, crystal dimensions 0.25×0.20×0.05 mm, T = 110 K, orthorhombic, $P2_12_12_1$, a = 7.2918(4), b = 12.5640(7), c = 22.0421(16) Å, V = 2019.4(2) $Å^3$, Z = 4, F(000) = 936, ρ_{calcd} = 1.465 $g{\cdot}cm^{-3}$, μ = 0.971 mm^{-1}, θ range = 4.01–71.30°, reflections collected: 7119, independent: 3771 (R_{int} = 0.0403), R_1 = 0.0448, wR_2 = 0.1035 [$I>2\sigma(I)$], S = 1.036, Flack parameter = 0.09(18).

***N*-methyl 3,6-bis[[(1,1-dimethylethyl)diphenylsilyl]oxy]-4,5-epoxy-7-hydroxy-8-oxo-(5α,6α,7β)-morphinan carbamate** (**20**)**.** Compound **20** was prepared according to the general procedure described above under the reaction conditions described on Table S1, Entry 12. The reaction time was 1.5 h. Purification with automated column chromatography by eluting with an *n*-heptane/ethyl acetate gradient (10 → 25% EtOAc) gave compound **20** as a white solid (150 mg, 36%). Mp 116 °C (decomp.); FTIR-ATR 1694 (C=O), 1498, 1445, 1262, 1174, 1113, 998, 700 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ ppm 7.96 (d, J = 6.8 Hz, 2H), 7.82 (m, 6H), 7.42 (m, 12H), 6.39 (d, J = 8.2 Hz, 1H, aromatic-H), 6.18 (d, J = 8.2 Hz, 1H, aromatic-H), 5.19 (m, 1H), 4.49 (d, J = 4.5 Hz, 1H), 3.90 (m, 1H), 3.88 (dd, J_1 = 11.9 Hz and J_2 = 4.6 Hz, 1H), 3.67 (brs, 3H, $NCOOCH_3$), 3.56

(d, J = 11.9 Hz, 1H), 2.76 (m, 3H), 2.49 (m, 2H), 1.65 (m, 1H), 1.55 (td, J_1 = 12.7 Hz and J_2 = 5.2 Hz, 1H) 1.16 (s, 9H, *t*-Bu), 1.13 (s, 9H, *t*-Bu). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 207.2 (CO), 206.9*, 155.6*, 155.4 (NCO) 148.4, 138.1, 136.4, 136.4, 135.8, 135.8, 134.9, 134.9, 133.9, 133.5, 132.6, 132.4, 130.2, 130.1, 128.8, 128.0, 127.9, 127.8, 126.5*, 126.4, 122.3, 119.9, 89.4, 73.6, 72.6, 53.0, 50.1, 49.8* 47.9, 41.2, 37.3*, 37.1, 36.0*, 35.7, 29.3, 29.1*, 27.0, 26.8, 19.7, 19.6. HRMS calcd for $C_{50}H_{55}NO_7NaSi_2$. $[M+23]^+$ 860.3415 found 860.3417.

***N*-methyl 3,6-bis[[(1,1-dimethyl)-*t*-butylsilyl]oxy]-4,5-epoxy-7-hydroxy-8-oxo-(5α,6α,7β)-morphinan carbamate** (**21**)**.** Compound **21** was prepared according to the general procedure described above under the reaction conditions described on Table S1, Entry 12. The reaction time 50 min. Purification with automated column chromatography by eluting with an *n*-heptane/ethyl acetate gradient (5 → 20% EtOAc) gave compound **21** as a white solid (100 mg, 34%). Mp 167-169 °C; FTIR-ATR 3431 (OH), 1690 (C=O), 1495, 1444, 1254, 1127, 998, 837, 778 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 6.69 (d, J = 8.1 Hz, 1H, aromatic-H), 6.48 (d, J = 8.1 Hz, 1H, aromatic-H), 5.32 (m, 1H), 4.76 (d, J = 4.7 Hz, 1H), 4.02 (m, 2H), 3.74 (brs, 3H, $NCOOCH_3$), 3.43 (d, J = 11.7 Hz, 1H), 2.90 (m, 2H), 2.77 (d, J = 3.3 Hz, 1H), 2.71 (brs, 1H), 2.67 (m, 2H), 1.88 (m, 2H), 0.99 (s, 9H, *t*-Bu), 0.95 (s, 9H (*t*-Bu), 0.22 (s, 3H, $SiCH_3$), 0.19 (s, 3H, $SiCH_3$), 0.16 (s, 3 H, $SiCH_3$), 0.13 (s, 3 H, $SiCH_3$). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 206.7 (CO), 206.5* 155.7*, 155.6 (NCO), 148.4, 137.7, 128.9, 126.8*, 126.6, 123.6, 120.3, 89.9, 73.6, 72.3, 53.1, 50.4, 50.1*, 48.1, 41.4, 37.5*, 37.3 36.3*, 36.0, 29.4, 29.3* 26.0, 25.9, 18.5, 18.6, -4.2 ($SiCH_3$), -4.4 ($SiCH_3$), -4.4 ($SiCH_3$), -4.7 ($SiCH_3$). HRMS calcd for $C_{30}H_{48}NO_7Si_2$. $[M+1]^+$ 590.2969 found 590.2970.

***N*-Methyl 4,5-epoxy-7-hydroxy-3,6-dimethoxy-8-oxo-(5α,6α,7β)-morphinan carbamate** (**22**). Compound **22** was prepared according to the general procedure described above under the reaction conditions described on Table S1, Entry 12. The reaction time 1 h. Purification with automated column chromatography by eluting with *n*-heptane/ethyl acetate (1:5) gave compound **22** as a colorless solid (29 mg, 15%). Mp 125 °C (decomp.); FTIR-ATR 3396 (OH), 1690 (C=O), 1503, 1444, 1274, 1098, 1030, 753, 731 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 6.75 (d, J = 8.3 Hz, 1H, aromatic-H), 6.57 (d, J = 8.3 Hz, 1H, aromatic-H), 5.35 (m, 1H), 5.07 (d, J = 4.1 Hz, 1H), 4.03 (m, 1H), 3.86 (s, 3H, OCH_3), 3.74 (brs, 3H, $NCOOCH_3$), 3.62 (s, 3H, OCH_3), 3.52 (m, 2H), 2.91 (m, 3H), 2.77 (d, J = 3.5 Hz, 1H), 2.65 (d, J = 18.6 Hz, 1H), 1.92 (m, 2H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 205.8 (CO), 205.6*, 155.6*, 155.4 (NCO), 146.1, 142.6, 128.5, 125.9*, 125.7, 120.6, 115.4, 86.1, 78.9, 72.9, 58.1 (OCH_3), 56.6 (OCH_3), 53.0 ($NCOO\underline{C}H_3$), 50.1, 49.8* 48.0, 41.3, 37.3*, 37.2, 35.9*, 35.7, 29.1, 28.9*. HRMS calcd for $C_{20}H_{24}NO_7$. $[M+1]^+$ 390.1553 found 390.1557.

***N*-Methyl 4,5-epoxy-3,6,7-trihydroxy-8-oxo-(5α,6α,7β)-morphinan carbamate** (**23**). Compound **12** (297 mg, 0.667 mmol) was dissolved in a mixture of methanol: chloroform (4:1, 10 mL). A 0.1 M aqueous solution of NaOH (4.5 mL) was added and the resulting white suspension was stirred at room temperature for 2.5 h. The organic solvents were evaporated and the pH of the residue was adjusted to 6 with a 1 M aqueous solution of HCl. The aqueous phase was extracted with a mixture of chloroform: isopropanol 4:1 (3 × 20 mL) and the combined organic phases were washed with brine (18 mL), dried with anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give a light brown solid. The crude product was purified with automated column chromatography by eluting

with dichloromethane/MeOH (19:1) to give **23** as a colorless solid (216 mg, 90%). Mp 145 °C (decomp.); FTIR-ATR 1724, 1693, 1649, 1452, 1396, 1236, 1086, 949, 758 cm^{-1}.^{1}H NMR (Methanol-d_4, 400 MHz): δ ppm 6.70 (d, J = 8.2 Hz, 1H, aromatic-H), 6.53 (d, J = 8.2 Hz, 1H, aromatic-H), 5.24 (m, 1H), 4.35 (d, J = 4.9 Hz, 1H), 4.01 (m, 1H), 3.99 (dd, J_1 = 11.9 Hz and J_2 = 4.9 Hz, 1H), 3.73 (d, J = 14.2 Hz, 3H, $NCOOCH_3$), 3.16 (d, J = 11.9 Hz, 1H), 2.89 (m, 3H), 2.62 (d, J = 18.6 Hz, 1H), 2.04 (td, J_1 = 12.9 Hz and J_2 = 5.3 Hz, 1H), 1.84 (m, 1H). ^{13}C NMR (Methanol-d_4, 101 MHz): δ ppm 208.3 (CO), 157.5 (NCO), 157.2* 146.4, 140.5, 130.5, 126.4, 121.7, 119.9, 91.2, 74.5, 71.3, 53.6*, 53.5 ($NCOO\underline{C}H_3$), 51.4*, 51.2, 49.6, 42.5, 38.6, 38.4*, 36.4, 36.2*, 30.0*, 29.8. HRMS calcd for $C_{18}H_{20}NO_7$. $[M+1]^+$ 362.1240 found 362.1240.

7,8-Didehydro-4,5-epoxy-6-hydroxy-17-methyl-(5α,6α)-morphinan-3-carboxylate (**9**). Morphine (**1**, 0.500 g; 1.75 mmol) was added to a stirred mixture of $NaHCO_3$ (8.00 g; 95.3 mmol) and water (50 mL), at room temperature. Acetic anhydride (4 × 0.83 mL; 8.76 mmol) was added slowly in 4 portions in 10-minute intervals and after the last addition the stirring was continued for 15 min. The reaction mixture was extracted with dichloromethane (3 × 60 mL), washed with brine (40 mL) and dried with anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give **9** as light brown foam (643 mg, 99%), which was used without further purification. FTIR-ATR: 3502 (OH), 1760 ($COOCH_3$), 1614, 1211, 1193, 1033, 941, 784 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ ppm 6.73 (d, J = 8.2 Hz, 1H, aromatic-H), 6.59 (d, J = 8.2 Hz, 1H, aromatic-H), 5.74 (d, J = 10.1 Hz, 1H), 5.28 (d, J = 10.1 Hz, 1H), 4.91 (d, J = 6.9 Hz, 1H), 4.15 (brs, 1H), 3.35 (m, 1H), 3.24 (brs, 1H), 3.05 (d, J = 18.9 Hz, 1H), 2.69 (s, 1H), 2.59 (dd, J_1 = 12.2 and J_2 = 4.5 Hz, 1H), 2.43 (s, 3H, NCH_3), 2.35 (dd, J_1 = 14.4 and J_2 = 4.9 Hz, 2H), 2.28 (s, 3H, $OCOCH_3$), 2.07 (td, J_1 = 12.4 and J_2 = 4.9 Hz, 1H), 1.90 (m, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ ppm 168.5 ($O\underline{C}OCH_3$), 148.7, 134.2, 132.8, 132.3, 131.8, 127.7, 121.0, 119.9, 92.3, 65.9, 58.9, 46.4, 43.0, 42.6, 40.4, 35.2, 20.8, 20.7. HRMS calcd. for $C_{19}H_{22}NO_4$. $[M+1]^+$ 328.1549 found 328.1549.

***N*-Methyl 3-acetyl-7,8-didehydro-4,5-epoxy-6-hydroxy-(5α,6α)-morphinan carbamate** (**10**). Compound **9** (638 mg; 1.95 mmol) was dissolved in dry chloroform (60 mL) under argon. $NaHCO_3$ (2.46 g; 29.2 mmol) and methyl chloroformate (2.56 mL; 33.1 mmol) were added and the mixture was refluxed at 62 °C for 3 hours. The reaction mixture was cooled to room temperature, diluted with dichloromethane (60 mL) and washed with water (40 mL). The aqueous phase was extracted with dichloromethane (3 × 35 mL). The organic phases were combined, washed with brine (40 mL), dried with anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by automated column chromatography by eluting with a gradient of *n*-hexane and ethyl acetate (0 → 100% EtOAc) to give compound **10** as a white solid (574 mg, 79%). Mp 67 °C (decomp.); FTIR-ATR: 3515 (R-OH), 1751 ($COOCH_3$), 1689 (NC=O), 1442, 1213, 1128, 1064, 939, 781 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ ppm 6.77 (d, J = 8.2 Hz, 1H, aromatic-H), 6.61 (d, J = 8.2 Hz, 1H, aromatic-H), 5.81 (d, J = 10.1 Hz, 1H), 5.29 (brs, 1H), 4.91 (m, 1H), 4.91 (dd, J_1 = 7.0 and J_2 = 1.1 Hz, 1H), 4.08 (m, 2H), 3.74 (br s, 3H, $NCOOCH_3$), 3.10 (m, 2H), 2.87 (dd, J_1 = 19.5 and J_2 =5.8 Hz, 1H), 2.74 (d, J = 18.8 Hz,, 1H), 2.56 (m, 1H), 2.29 (s, 3H, $OCOCH_3$), 1.94 (m, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ ppm 168.7 ($O\underline{C}OCH_3$), 156.0 (NCO), 155.7* (NCO), 149.0, 135.5*, 135.3, 132.3, 131.7, 131.6*, 131.3, 126.5, 126.4*, 121.8, 120.4, 92.3, 65.7, 52.9, 50.7*, 50.4, 43.2, 39.6*, 39.5, 37.6, 35.1, 34.8, 30.0*, 29.8, 20.9 ($OCO\underline{C}H_3$). HRMS calcd. for $C_{20}H_{22}NO_6$. $[M+1]^+$ 372.1447 found 372.1447.

***N*-Methyl 3,6-diacetyl-7,8-didehydro-4,5-epoxy(5α,6α)-morphinan carbamate** (**11**). Compound **10** (0.100 g, 0.269 mmol) was dissolved in pyridine (0.62 mL) and acetic anhydride (0.11 mL, 1.13 mmol) was slowly added. The reaction mixture was stirred at 90 °C for 2.5 h, after which the solvent was co-evaporated with toluene. The residue was dissolved in dichloromethane (30 mL) and washed with aqueous saturated $NaHCO_3$ solution (10 mL). The aqueous phase was extracted with dichloromethane (3 × 10 mL), the organic phases were combined and washed with brine (8 mL), dried with anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give brown oil. The crude product was purified with automated column chromatography by eluting with an *n*-hexane/ethyl acetate gradient (3:2 to 1:1) to give **11** as a white solid (88.0 mg, 79%). Mp 181-182 °C; FTIR-ATR 1684, 1587, 1313, 1217, 1175, 1067, 831, 754 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ ppm 6.80 (d, *J* = 8.2 Hz, 1 H, aromatic-H), 6.59 (d, *J* = 8.2 Hz, 1 H, aromatic-H), 5.69 (d, *J* = 10.1 Hz, 1 H), 5.45 (d, *J* = 8.9 Hz, 1 H), 5.11 (m, 2 H), 4.89 (m, 1 H), 4.06 (m, 1H), 3.74 (br s, 3 H, $NCOOCH_3$), 2.91 (m, 2 H), 2.75 (d, *J* = 18.8 Hz, 1 H), 2.61 (m, 1H), 2.27 (s, 3 H, $OCOCH_3$), 2.13 (s, 3 H, $OCOCH_3$), 1.92 (m, 2 H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ ppm 170.5 ($O\underline{C}OCH_3$), 168.4 ($O\underline{C}OCH_3$), 156.0 (NCO), 149.6, 132.2, 131.1, 131.0*, 130.4, 129.8*, 129.5, 128.2, 128.1*, 122.6, 119.9, 88.5, 67.7, 52.9, 50.7*, 50.4, 43.2, 39.7*, 39.6, 37.8, 34.9, 34.7*, 29.9*, 29.7, 20.7 ($OCO\underline{C}H_3$), 20.7 ($OCO\underline{C}H_3$). HRMS calcd for $C_{22}H_{24}NO_7$. $[M+1]^+$ 414.1553 found 414.1554.

7,8-Didehydro-3,6-bis[[(1,1-dimethylethyl)diphenylsilyl]oxy]-4,5-epoxy-17-methyl-(5α,6α)-morphinan (**13**). Compound **13** was prepared according to the literature [59]. ^1H NMR ($CDCl_3$, 300 MHz): δ ppm 7.85 (t, *J* = 6 Hz, 4H), 7.75 (dd, J_1 = 24 and J_2 = 7.2 Hz, 4H), 7.40 (m, 12H), 6.26 (d, *J* = 8 Hz, 1H, aromatic-H), 6.12 (d, *J* = 8 Hz, 1H, aromatic-H), 5.79 (d, *J* = 10.7 Hz, 1H), 5.15 (d, *J* = 9.5 Hz, 1H), 4.52 (d, *J* = 5.6 Hz, 1H), 4.14 (brs, 1H), 3.22 (brs, 1H), 2.89 (d, *J* = 18.5 Hz, 1H), 2.45 (m, 1H), 2.35 (s, 3H, NCH_3), 2.16 (d, *J* = 19.1 Hz, 1H), 1.72 (brs, 2H), 1.12 (d, *J* = 3.3 Hz, 18H, 2 × *t*-Bu). HRMS calculated for $C_{49}H_{56}NO_3Si_2$. $[M+1]^+$ 732.3799 found 762.3803.

7,8-Didehydro-3,6-bis[[(1,1-dimethyl)-*t*-butylsilyl]oxy]-4,5-epoxy-17-methyl-(5α,6α)-morphinan (**14**). Morphine **1** (0.600 g; 2.10 mmol) and imidazole (726 mg; 10.7 mmol) were dissolved in dry dimethylformamide (3.6 mL) under argon. *t*-Butyldimethylsilyl chloride (1.20 g; 7.96 mmol) was added and the reaction mixture was heated at 90 °C for 4 hours. The reaction mixture was diluted with dichloromethane (40 mL), washed with water (2 × 20 mL), dried with anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified using automated column chromatography by eluting with a chloroform → methanol gradient (0 → 4%) to give compound **14** as light brown solid (677 mg, 63%). Mp 117-119 °C ; FTIR-ATR 1494, 1444, 1253, 1124, 1033, 979, 835, 773 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ ppm 6.56 (d, J = 8.0 Hz, 1H, aromatic-H), 6.41 (d, *J* = 8.0 Hz, 1H, aromatic-H), 5.59 (m, 1H), 5.23 (m, 1H), 4.66 (dd, J_1 = 5.7 Hz, J_2 = 1.4 Hz 1H), 4.21 (m, 1H), 3.34 (dd, J = 6.4, 3.2 Hz, 1H), 3.02 (d, J = 18.6 Hz, 1H), 2.59 (m, 2H), 2.44 (m, 1H), 2.44 (s, 3H, NCH_3), 2.30 (dd, J = 18.6, 6.4 Hz, 1H), 2.02 (td, J = 12.3, 5.3 Hz, 1H), 1.89 – 1.80 (m, 1H), 0.97 (s, 9H, *t*-Bu), 0.93 (s, 9H, *t*-Bu), 0.21 (s, 3H, $SiCH_3$), 0.14 (s, 3H, $SiCH_3$), 0.13 (s, 3H, $SiCH_3$), 0.10 (s, 3H, $SiCH_3$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ ppm 150.2, 137.2, 133.9, 131.3, 128.5, 127.6, 121.4, 118.6, 92.9, 69.6, 59.0, 46.6, 44.2, 43.3, 41.6, 36.2, 26.1, 26.0, 21.0, 18.5, 18.4, -4.2, -4.5, -4.6, -4.7. HRMS calcd. for $C_{29}H_{47}NO_3Si_2$. $[M+1]^+$ 514.3173 found 514.3173.

***N*-Methyl 7,8-didehydro-3,6-bis[[(1,1-dimethylethyl)diphenylsilyl]oxy]-4,5-epoxy-(5α,6α)-morphinan carbamate** (**15**). Compound **13** (0.760 g; 0.997 mmol) was dissolved in dry chloroform (32 mL) under argon. $NaHCO_3$ (1257 mg; 15.0 mmol) and methyl chloroformate (1.31 mL; 17.0 mmol) were added and the mixture was refluxed at 62 °C for 2 h 20 min. The reaction mixture was diluted with dichloromethane (20 mL) and washed with water (35 mL). The aqueous phase was extracted with dichloromethane (2 × 20 mL). The organic phases were combined, washed with brine (30 mL), dried with anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by automated column chromatography by eluting with *n*-heptane/ethyl acetate (5:1) to give compound **15** as a colorless solid (659 mg, 82%). Mp 75 °C (decomp.); FTIR-ATR 1697 (C=O), 1498, 1448, 1324, 1269, 1172, 1108, 1087, 981, 825, 698 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ ppm 7.84 (m, 4H), 7.78 (m, 2H), 7.70 (m, 2H), 7.40 (m, 12H), 6.30 (d, *J* = 8.1 Hz, 1H, aromatic-H), 6.13 (d, *J* = 8.2 Hz, 1H, aromatic-H), 5.83 (d, *J* = 9.8 Hz, 1H), 5.15 (m, 1H), 4.75 (m, 1 H), 4.52 (d, *J* = 5.5 Hz, 1H), 4.15 (m, 1H), 3.94 (m, 1H), 3.67 (br s, 3H, $NCOOCH_3$), 2.96 (m, 1H), 2.75 (m, 1H), 2.59 (d, *J* = 18.6 Hz, 1H), 2.24 (brs, 1H), 1.63 (m, 2H), 1.13 (s, 9H, *t*-Bu), 1.10 (s, 9H, *t*-Bu). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 155.8 (NCO), 155.5*, 150.2, 137.9, 136.1, 135.9, 135.9, 135.8, 134.4, 134.1, 133.9, 133.6, 133.3, 130.6, 130.0, 129.9, 129.9, 129.8, 127.9, 127.8, 127.8, 127.8, 126.3, 126.1, 120.7, 118.7, 92.4, 69.8, 52.8, 50.4*, 50.1, 44.6, 40.4*, 40.2, 37.4, 35.7, 35.4*, 30.0*, 29.8, 27.0, 26.9, 19.7, 19.4. HRMS calcd. for $C_{50}H_{55}NO_5NaSi_2$. $[M+23]^+$ 828.3516 found 828.3515.

***N*-Methyl 7,8-didehydro-3,6-bis[[(1,1-dimethyl)-*t*-butylsilyl]oxy]-4,5-epoxy-(5α,6α)-morphinan carbamate** (**16**). Compound **14** (538 mg; 1.05 mmol) was dissolved in dry chloroform (25 mL) under argon. $NaHCO_3$ (1.32 g; 15.7 mmol) and methyl chloroformate (1.37 mL; 17.8 mmol) were added and the reaction mixture was refluxed at 62 °C for 4 hours. The reaction mixture was diluted with dichloromethane (60 mL) and washed with water (40 mL). The aqueous phase was extracted with dichloromethane (2 × 50 mL), the organic phases were washed with brine (40 mL), dried with anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by automated column chromatography by eluting with *n*-hexane/ethyl acetate (5:1) to give compound **16** as a pale yellow solid (508 mg, 87%). Mp 160-162 °C; FTIR-ATR 1697 (C=O), 1496, 1444, 1249, 1128, 1091, 979, 838, 775 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ ppm 6.59 (d, *J* = 8.1 Hz, 1H, aromatic-H), 6.41 (d, *J* = 8.1 Hz, 1H, aromatic-H), 5.65 (d, *J* = 9.7 Hz, 1H), 5.23 (m, 1H), 4.87 (m, 1H), 4.66 (dd, *J* = 5.7, 1.4 Hz, 1H), 4.07 (m, 2H), 3.73 (brs, 3H, $NCOOCH_3$), 3.06 (m, 1H), 2.86 (m, 1H), 2.71 (d, *J* = 18.6 Hz, 1H), 2.47 (brs, 1H), 1.89 (m, 2H), 0.97 (s, 9H, *t*-Bu), 0.93 (s, 9H, *t*-Bu), 0.21 (s, 3H, $SiCH_3$), 0.14 (s, 3H, $SiCH_3$), 0.12 (s, 3H, $SiCH_3$), 0.10 (s, 3H, $SiCH_3$) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ ppm 156.0 (NCO), 155.6*, 150.3, 137.5, 135.0*, 134.7, 130.3, 127.4, 127.3*, 126.4, 126.2*, 121.9, 119.0, 92.8, 69.3, 52.8, 50.6*, 50.4, 44.7, 40.6*, 40.4, 37.5, 36.0, 35.7*, 30.1*, 30.0, 26.1, 26.0, -4.2, -4.5, -4.6, -4.7. HRMS calcd. for $C_{30}H_{47}NO_5Si_2Na$. $[M+1]^+$ 580.2890 found 580.2889.

***N*-Methyl 7,8-didehydro-4,5-epoxy-3,6-dihydroxy-(5α,6α)-morphinan carbamate** (**17**). Compound **11** (127 mg, 0.307 mmol) was dissolved in a mixture of methanol: chloroform (7:2, 4.5 mL). A 0.1 M aqueous solution of NaOH (2.2 mL) was added to the solution and the resulting white suspension was stirred at room temperature for 30 min. The organic solvents were evaporated, water (5 mL) was added to the residue, and the pH of the aqueous phase was adjusted to 5 with a 1

M aqueous solution of HCl. The aqueous phase was extracted with dichloromethane (3 × 30 mL) and the combined organic phases were washed with brine (15 mL), dried with anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give yellowish oil. The crude product was purified with automated column chromatography by eluting with *n*-heptane/ethyl acetate (1:2) to give **17** as a colorless solid (87.9 mg, 87%). Mp 88 °C (decomp.); FTIR-ATR 3283, 1666, 1450, 1232, 1126, 785, 725 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ ppm 6.67 (d, J = 8.1 Hz, 1 H, aromatic-H), 6.49 (d, J = 8.1 Hz, 1 H, aromatic-H), 5.71 (m, 1 H), 5.26 (d, J = 9.8 Hz, 1 H), 5.87 (m, 2 H), 4.21 (m, 1 H), 4.06 (m, 1H), 3.73 (br s, 3 H, $NCOOCH_3$), 3.04 (m, 1 H), 2.84 (m, 1 H), 2.71 (d, J = 18.6 Hz, 1 H), 2.51 (m, 1H), 1.92 (m, 2 H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ ppm 156.1 (NCO), 155.8*, 145.5, 138.3, 133.9*, 133.6, 129.9, 127.6, 127.3*, 125.5, 125.3*, 120.5, 117.5, 91.5, 66.5, 53.0, 50.7*, 50.4, 43.7, 40.0*, 39.8, 37.7, 35.5, 35.2*, 29.8*, 29.6. HRMS calcd for $C_{18}H_{20}NO_5$. $[M+1]^+$ 330.1341 found 330.1342.

***N*-Methyl 7,8-didehydro-4,5-epoxy-6-hydroxy-3-methoxy-(5*α*,6*α*)-morphinan carbamate** (**18**). Codeine (**8**, 0.500 g; 1.67 mmol) was dissolved in dry chloroform (42 mL) under argon. $NaHCO_3$ (2.11 g; 25.1 mmol) and methyl chloroformate (2.19 mL; 28.4 mmol) were added and the mixture was refluxed at 62 °C for 2 h 15 min. The reaction mixture was cooled to room temperature, diluted with dichloromethane (60 mL) and washed with water (30 mL). The aqueous phase was extracted with dichloromethane (3 × 20 mL). The organic phases were combined, washed with brine (35 mL), dried with anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by automated column chromatography by eluting with *n*-hexane and ethyl acetate (1:1) to give compound **18** as a white solid (555 mg, 97%). Mp 120 °C (decomp.); FTIR-ATR 3439 (OH), 1688 (C=O), 1503, 1443, 1130, 1052, 942, 784 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ ppm 6.69 (d, J = 8.1 Hz, 1H, aromatic-H), 6.57 (d, J = 8.1 Hz, 1H, aromatic-H), 5.77 (d, J = 9.9 Hz, 1H), 5.29 (m, 1H), 4.88 (m, 1H), 4.88 (dd, J_1 = 6.6 Hz and J_2 = 1.2 Hz, 1H), 4.08 (m, 2H), 3.85 (s, 3H, OCH_3), 3.74 (brs, 3H, $NCOOCH_3$), 3.02 (m, 1H), 2.86 (m, 2H), 2.73 (d, J = 18.6 Hz, 1H), 2.53 (m, 1H), 1.94 (m, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ ppm 156.0 (NCO), 155.7*, 146.6, 142.7, 134.7*, 134.5, 130.1, 127.1, 126.9*, 126.0, 125.9*, 120.2, 113.7, 91.2, 66.21, 56.55, 52.9, 50.7*, 50.5, 43.5, 39.9*, 39.8, 37.7, 35.7, 35.4*, 29.7*, 29.6. HRMS calcd for $C_{19}H_{22}NO_5$. $[M+1]^+$ 344.1498 found 344.1498.

***N*-Methyl 7,8-didehydro-4,5-epoxy-3,6-dimethoxy-(5*α*,6*α*)-morphinan carbamate** (**19**). A 60% dispersion of NaH in mineral oil (175 mg, 4.37 mmol) was suspended in dry THF (20 mL) under argon. A solution of compound **18** (0.500 g, 1.46 mmol) in dry tetrahydrofuran (20 mL) was added to the stirred suspension over 90 min, after which the stirring was continued for additional 70 min. Iodomethane (413 mg, 2.19 mmol) was added and the mixture was stirred for 2 h. The reaction was quenched by slow addition of a 25 wt-% solution of NaOMe in methanol (2.0 mL, 8.7 mmol). The mixture was diluted with water (30 mL) and the organic solvents were evaporated. The remaining aqueous phase was extracted with chloroform (3 × 50 mL). The combined organic phases were washed with water (30 mL), dried with anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by automated column chromatography by eluting with *n*-hexane and ethyl acetate (2:1) to give compound **19** as a white solid (256 mg, 49%). Mp 158 °C (decomp.); FTIR-ATR 1688 (C=O), 1502, 1444, 1174, 1057, 941, 789 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ ppm 6.66 (d, J = 8.2 Hz, 1H, aromatic-H), 6.51 (d, J = 8.2 Hz, 1H, aromatic-H), 5.78 (d, J = 9.9 Hz, 1H), 5.32 (m, 1H), 4.98 (dd, J_1 = 6.0 Hz and J_2 = 1.3 Hz, 1H), 4.88 (m, 1H), 4.06 (m, 1H), 3.83 (s,

3H, OCH_3), 3.75 (m, 1H) 3.73 (brs, 3H, $NCOOCH_3$), 3.52 (s, 3H, OCH_3), 3.04 (m, 1H), 2.88 (m, 1H), 2.72 (d, J = 18.6 Hz, 1H), 2.51 (brs, 1H), 1.92 (m, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ ppm 156.0 (NCO), 155.7*, 147.8, 142.6, 131.9*, 131.6, 129.9, 127.6, 127.5*, 125.8, 125.7*, 119.4, 114.1, 89.5, 75.9, 57.3, 56.7, 52.9, 50.7*, 50.5, 44.0, 40.3*, 40.2, 37.7, 35.9, 35.6*, 29.8*, 29.7. HRMS calcd for $C_{20}H_{24}NO_5$. $[M+1]^+$ 358.1654 found 358.1656.

Computational modeling

We studied possible binding modes of compounds **17**, **23**, **19**, **22**, **11**, **12** computationally by docking them to inactive forms of the MOR, (PDB code 4DKL), DOR (PDB code 4EJ4), and KOR (PDB code 4DJH). Docking was conducted with Schrödinger Maestro 10.3 [60], Glide XP. The docking protocol was validated by trying to recreate the binding modes of co-crystallized ligands. Grid generated was centered on the centroid of the co-crystallized ligands that indicates well the center of the orthosteric binding pocket. For DOR and MOR, the ligand docking method was based on a rigid protein. For KOR the rigid protein did not lead to reasonable solutions (KOR ligands located very high in the binding pocket) but reasonable solutions were delivered by the induced-fit protocol; induced fit dockings at the KOR are therefore presented in this manuscript. Prior to docking ligand preparation was performed with standard parameters with LigPrep (pH 7.0 ± 2). The poses were selected based on the best docking score, but alternative poses were also considered. Water molecules were not considered during the docking runs. PyMOL was used for visualizing the results [61].

Biology

Materials - DAMGO, naltrindole, naloxone and GDP were obtained from Sigma. GTP was from Thermo Scientific. [^{3}H]Diprenorphine (specific activity, 37 Ci/mmol) and [^{35}S]GTPgS (specific activity, 1250 Ci/mmol) were obtained from PerkinElmer Life Sciences, SNC80 and U50488 from Tocris (Bio-Techne, Lille, France), and morphine from Francopia (Gentilly, France). Stably transfected cells expressing human (h) MOR (HEK), DOR (HEK) and KOR (CHO) were obtained and cultured as described [48,49]. K_D, K_i and B_{max} values were determined using the Prism software (GraphPad, San Diego, CA).

Cell viability - The toxicity of the compounds **12**, **17**, **22** and **23** was evaluated on HEK-293 cells by cell viability assay. The cells 9000 cells/well) were incubated in the presence of the compounds (0.1-100 µM) for 24 hours. The growth medium was changed to Opti-MEM prior to the addition of the compounds. CellTiterGlo reagent was used for cell lysis and determination of the ATP levels of the lysate from luminescence. The results were analyzed using Prism software (GraphPad, San Diego, CA). No effect on cell viability was seen at concentrations lower than 100 µM (Supporting info, Figure S3), showing that these compounds do not have any inherent properties that would interfere strongly with the basic functions of living cells.

Cell Membrane Preparations – Membranes from transfected cells were obtained as previously described [48,49]. Briefly, cells were washed with phosphate-buffered saline (PBS, pH 7.4), scrapped off the plates with PBS, pelleted by centrifugation at 1500 rpm for 10 min at room temperature and frozen at -80 °C. All the following steps were performed at 4 °C. The pellet was

thawed and suspended in 30 mL of cold 50 mM Tris-HCl, pH 7.4, 1 mM EDTA and 0.1 mM phenylmethylsulfonyl fluoride (added extemporaneously). The cell lysate was Dounce-homogenized (30 ×) and centrifuged at 2500 rpm for 10 min. The pellet was resuspended in the buffer (15 mL), Dounce-homogenized (30 ×), and centrifuged again at 2500 rpm 10 min. Both supernatants were combined and ultracentrifuged at 40,000 rpm for 25 min. The pellet was re-suspended in 5 mL of 50 mM Tris-HCl, pH 7.4, 1 mM EDTA and 0.32 M sucrose and Dounce-homogenized (10 ×). The membrane preparations were aliquoted and stored at -80 °C. Protein concentration was determined using the Bradford assay.

Receptor Binding Assay - For saturation experiments, a range of [^{3}H]diprenorphine concentrations (from 7.8×10^{-11} to 5×10^{-9} M) was used. Nonspecific binding was determined in the presence of 10 µM naloxone. For competition experiments, membrane proteins were diluted in 50 mM Tris-HCl pH 7.4 and 1 mM EDTA, and incubated with [^{3}H]diprenorphine (1 nM), and several concentrations of competitor ligand (1×10^{-7} to 5×10^{-5} M, for hDOR naltrindole 1×10^{-11} to 1×10^{-9} M) in a total volume of 0.2 mL at 23 °C for 1 h. Incubation mixtures were washed (8 ×) using a cell harvester (PerkinElmer FilterMate Harvester) with cold 50 mM Tris-HCl, pH 7.5 on GF/B filters (Whatman) presoaked with 50 mM Tris HCl pH 7.4 and 0.1% polyethyleneimine. Bound radioactivity was determined by scintillation counting using TopCount (Perkin Elmer). K_i and K_D values were determined using the Prism software (GraphPad, San Diego, CA).

[35S]GTPgS Binding Assay – From each hMOR- and hDOR-transfected cell type, 5 µg of membrane preparations were incubated at 23 °C for 1 h in 50 mM Tris-HCl, pH 7.3, 5 mM $MgCl_2$, 100 mM NaCl, 1 mM EDTA, 30 µM GDP, 0.2 nM [35S]GTPgS, and ligands (1 or 10 µM for the reference opioid ligand, and 20 or 50 µM for the competitor ligands) in a final volume of 0.2 mL. Nonspecific binding was determined in the presence of 10 µM GTP. Incubation mixtures were washed (6 ×) using a cell harvester (PerkinElmer FilterMate Harvester) with cold 50 mM Tris-HCl, pH 7, 5 mM $MgCl_2$, 50 mM NaCl on H_2O-presoaked GF/B filters. Bound radioactivity was determined by scintillation counting. DAMGO and SNC80 were used as MOR and DOR activators, respectively, because DAMGO and SNC80 are full high-affinity MOR and DOR agonists and are classically used for examining antagonism by new compounds.

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Highlights

- A convenient, one-step procedure to oxidize $\Delta^{7,8}$-opioids into the corresponding 7β-hydroxy-8-ketones is reported
- The prepared compounds are antagonists at the mu- and delta-opioid receptors
- 7β-hydroxy-8-ketone can lead to increased selectivity at the delta opioid receptor
- The hydroxy ketone functionality is a good starting point for further opioid synthesis