Design, Chemical Synthesis, and Biological Evaluation of Thiosaccharide Analogues of Morphine- and Codeine-6-Glucuronide

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A series of 6-β-thiosaccharide analogues of morphine-6-glucuronide (M6G) and codeine-6-glucuronide (C6G) were synthesized and evaluated with the objective of preparing an analogue of M6G with improved biological activity. The affinity of the thiosaccharide analogues of M6G and C6G was examined by competitive binding assays at μ, δ, and κ opioid receptors. The thiosaccharide compounds in the morphine series **5b**, **5e**, **6a**, and **6c** showed 1.5−2.4-fold higher affinity for the μ receptor than M6G, but were generally less selective than M6G. The functional activity of the M6G and C6G analogues was examined with the [^{35}S]GTP-γ-S assay. Compounds **5b** and **5e** were determined to be full μ agonists, whereas compounds **6a** and **6c** were partial μ agonists. The in vivo antinociceptive activity of compound **5b** was evaluated by the tail flick latency test, giving an ED_{50} of 2.5 mg/kg.

Introduction

Chronic pain represents a major health problem throughout the world. It is estimated that 22% of the primary care patients between the ages of 18 and 65 years suffer from chronic pain at any given time.[1] In the United States there are more than 50 million people who experience chronic pain, and approximately one-half of the population seeks medical help for pain at some point in their lives.[1] Narcotic analgesics in the morphine class remain the accepted treatment for pain due to trauma, surgery, cancer, and for terminal patients. Morphine elicits a number of pharmacological actions mediated by the μ opioid receptor including analgesia, respiratory depression, and inhibition of gastrointestinal transit.[2] The adverse side-effects and concerns due to abuse liability have limited morphine availability and optimal use. Considerable effort has therefore been put forward to develop and understand the appropriate use of narcotic analgesics and to develop new ones with more favorable pharmacological properties.

Morphine-6-glucuronide (M6G, 1, Figure 1) is produced by the UDP glucuronosyl transferase-catalyzed reaction of morphine with UDP glucuronic acid in the liver and is the second most abundant metabolite of morphine in man, accounting for 15% of the total morphine dose.[3] The analgesic potency of M6G is approximately 100 times greater than that of morphine in animals with significantly reduced respiratory depression, nausea, and sedation.[4−7] The reduction in side-effects and enhanced potency observed for M6G make it a promising drug candidate for the treatment of cancer-related pain in man.[8]

Despite this promise, compared with iv M6G, the bioavailability of orally administered M6G is only 11 ± 3%,[3] and this severely limits M6G development as a drug candidate. The hydrolysis of M6G in the gut significantly reduces the available dose. The objective of this work was to test the hypothesis that new pain medications with improved in vivo stability formulated for oral administration could be developed. The *S*-glycoside linkage imparts significantly greater in vivo stability relative to the corresponding *O*-glycoside linkage in metabolic conjugates.[9,10] To determine whether the 6-β-*S*-glycosides (**2**) of M6G and codeine-6-glucuronide (C6G) possess more favorable pharmacological properties than M6G, the compounds **5** and **6** were prepared and tested in vitro and in vivo. The synthesis and the biological evaluation of the resulting compounds are reported herein.

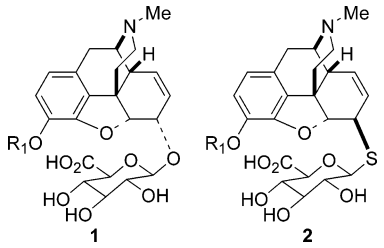

Figure 1. M6G **1** and the thiosaccharide analogue **2**.

Chemistry

The starting compounds for the preparation of the thiosaccharides **5a**−**f** and **6a**−**d** were 6-*O*-tosylmorphine **3a**, 3-*O*-acetyl-6-tosylmorphine **3b**, and 3-*O*-tosylcodeine **3c**.[11] The tosylates **3a**−**c** were prepared in good yield by the reaction of either 3-*O*-acetylmorphine or codeine with *p*-toluenesulfonyl chloride in pyridine at 3 °C overnight.[12] 6-*O*-Tosylmorphine **3a** was prepared in 81% yield by stirring the corresponding acetate **3b** in methanol for 2 h at room temperature with 1 equiv of potassium carbonate. The key step in the synthesis of

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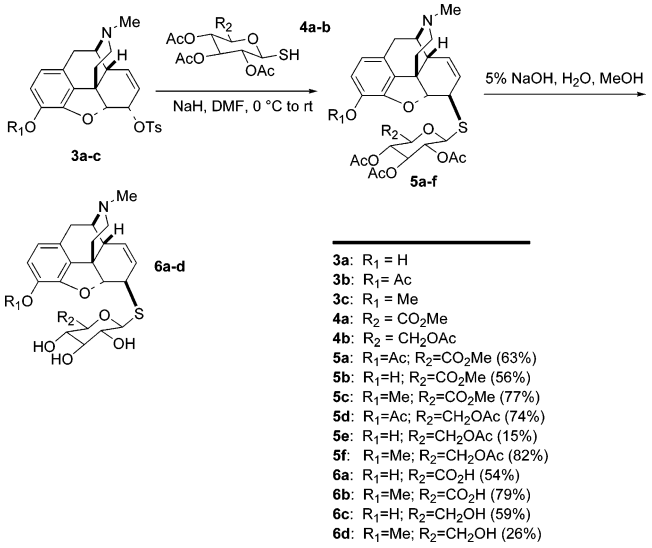
Scheme 1. Synthesis of M6G Thiosaccharide Analogues **5a**−**f** and **6a**−**d**



5a−**f** was the attachment of the thiosaccharide to the phenanthrene nucleus by an S_N2 displacement reaction (Scheme 1). Thus, to prepare the protected sulfur analogue of M6G **5a**, a 0 °C DMF solution of 3-*O*-acetyl-6-*O*-tosylmorphine **3b** was added to 2.7 equiv of a 0 °C DMF solution of the thiolate corresponding to **4a**. The mixture was warmed to room temperature, providing **5a** in 63% overall yield after aqueous workup and purification. In analogous fashion, the morphine derivative **5b** (56%) and the codeine derivative **5c** (77%) were prepared from the tosylates **3a** and **3c**, respectively. It was found that a slight excess of the thiol **4a** (2.7 equiv) relative to sodium hydride (2.6 equiv) was required to decrease loss of the ester protecting groups in the glucuronic acid moiety or the 3-position acetate of **5a**. The use of stoichiometric amounts of the thiol **4a** and sodium hydride in the preparation of compounds **5a**−**c** gave significantly diminished yields.

To expand the scope of these studies, the 6-β-thioglucose conjugates of 3-*O*-acetylmorphine **5d** (74%) and morphine **5e** (15%), as well as codeine analogue **5f** (82%), were prepared by the reaction of the thiolate of **4b** with **3b** or **3c**, respectively. It was found that sufficient amounts of the phenol **5e** could be obtained for biochemical studies as a side product in the preparation of **5d**, which precluded the preparation of **5d** in a separate reaction. The esters **5a**−**f** were desired for biological evaluation, because they can potentially function as prodrugs analogous to opioids such as heroin. The increased hydrophobicity of the esters **5a**−**f** should impart improved central nervous system availability relative to the deprotected congeners **6a**−**d**. The removal of the ester protecting groups in compounds **5a,c,d,f** was accomplished by treatment with 5% aqueous sodium hydroxide, followed by an acidic workup, giving the deprotected compounds **6a**−**d** in modest yields.[13] A characteristic singlet (δ 5.03−5.22) for the C-5 phenanthrene proton was evident in the ^{1}H NMR spectra of the thiosaccharides **5a**−**f** and **6a**−**d**. This indicated that the dihedral angle with the vicinal C-6 α-disposed proton was approximately 90°. This observation was in agreement with the ^{1}H NMR spectral data from C-6 β-substituted morphine compounds prepared by others.[14] We also attempted to prepare the C-6 α-epimer of compound **5a**. The S_N2 displacement reaction of 3-*O*-acetyl-6-β-*O*-tosylmorphine **7** (Figure 2) with the thiolate of **4a** did not result in sulfide bond formation, as indicated by analysis of the ^{1}H NMR spectrum of the crude reaction mixture. One explanation for this observation is that nucleophilic attack at the C-6 position of 3-*O*-acetyl-6-β-*O*-tosylmorphine **7** via an S_N2 process is prevented by steric interactions.[12,15] This is more clearly illustrated by examining the perspective drawings of the tosylates **7** and **3b** (Figure 2). These drawings suggest that the S_N2 displacement reaction of the C-6 tosyl group in the β-epimer **7** is less favorable than in the α-epimer **3b** due to steric interactions that would occur upon approach of a nucleophile to the bottom face of **7**. The thiosaccharides **4a** (59%) and **4b** (76%) were prepared from the known bromides **8a** and **8b**, respectively (Scheme 2).[16] The syntheses of either **4a** or **4b** was accomplished by a two-step procedure involving an initial S_N2 displacement reaction with potassium thioacetate in DMF, followed by the selective thiolysis of the sulfur acetate group in the intermediate upon treatment with sodium methylthiolate in chloroform/methanol (**4a**) or dichloromethane/methanol (**4b**) at 0 °C.[17]

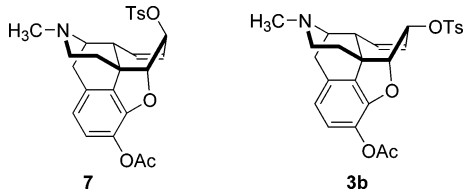
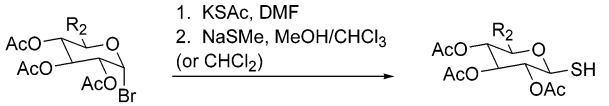


Figure 2. Perspective drawings of 3-*O*-acetyl-6-β-*O*-tosylmorphine **7** and 3-*O*-acetyl-6-*O*-tosylmorphine **3b**.

Scheme 2. Synthesis of Thiols **4a** and **4b**



8a: $R_2 = CO_2CH_3$
8b: $R_2 = CH_2OAc$

4a: $R_2 = CO_2CH_3$ (59%)
4b: $R_2 = CH_2OAc$ (76%)

Table 1. K_i Inhibition Values of μ, δ, and κ Opioid Binding to CHO Membranes by Compounds **5a**−**f** and **6a**−**d**

	K_i (nM) ± SEM			selectivity	
entry	μ	δ	κ	δ/μ	κ/μ
morphine	1.1 ± 0.1	140 ± 2	47 ± 14	127	42
M6G	12.9 ± 0.9	170 ± 1	4060 ± 230	12.5	316
5a	100 ± 2	528 ± 47	189 ± 14	5.3	1.9
5b	5.4 ± 0.1	15.6 ± 2.4	11.0 ± 0.1	2.9	2.0
5c	64 ± 1	438 ± 5	499 ± 7	6.8	7.8
5d	305 ± 66	762 ± 167	1390 ± 200	2.5	4.5
5e	7.8 ± 0.5	19.5 ± 0.3	2970 ± 950	2.5	381
5f	165 ± 28	466 ± 119	2190 ± 880	2.8	13.2
6a	8.7 ± 0.9	31.4 ± 2.3	288 ± 12	3.6	33
6b	463 ± 57	2680 ± 200	>10 000	5.8	>21
6c	5.4 ± 0.8	56.2 ± 2.2	136 ± 17	10.4	25.4
6d	127 ± 23	1140 ± 350	2480 ± 540	9	19.4

Pharmacological Results and Discussion

Table 1 contains the K_i values for compounds **5** and **6**. In the opioid receptor binding assays the following radioligands were used: [^{3}H]DAMGO (μ opioid receptor agonist), [^{3}H]U69593 (κ opioid receptor agonist), and

Table 2. Stimulation of [^{35}S]-GTP-γ-S Binding by Compounds **5a**−**f** and **6a**−**d** Mediated by the μ, δ, and κ Opioid Receptors

	μ		δ		κ	
entry	EC_{50}	E_{max}	EC_{50}	E_{max}	EC_{50}	E_{max}
morphine	15.6 ± 0.5	93 ± 3	ND[b]	ND[b]	ND[b]	ND[b]
M6G	72.3 ± 26.7	45.0 ± 5.0	190 ± 205	80.0 ± 1.0	>10 000	NA[a]
5a	1420 ± 240	37.8 ± 6.7	1320 ± 300	46.8 ± 2.0	NA[a]	NA[a]
5b	43.7 ± 16.5	88.3 ± 19.5	80.2 ± 0.2	104 ± 20	29.1 ± 8.2	23.5 ± 0.9
5c	732 ± 47	19.3 ± 0.3	1010 ± 180	64.3 ± 8.3	2670 ± 60	24.3 ± 4.2
5d	4790 ± 1620	44.1 ± 4.9	3660 ± 1510	39.3 ± 0.8	1720 ± 630	36.9 ± 1.9
5e	33.7 ± 11.3	76.0 ± 3.0	63.8 ± 14.6	45.5 ± 1.5	52.0 ± 4.9	26.5 ± 2.5
5f	994 ± 13	41.9 ± 2.1	872 ± 242	44.9 ± 1.2	>10 000	NA[a]
6a	90.6 ± 22.9	46.6 ± 10.1	50.1 ± 36.7	78.7 ± 0.9	NA[a]	NA[a]
6b	NA[a]	NA[a]	1330 ± 20	44.2 ± 5.4	NA[a]	NA[a]
6c	91.5 ± 23.4	64.5 ± 0.5	192 ± 15	51.5 ± 6.5	321 ± 93	42.5 ± 2.5
6d	1060 ± 20	42.0 ± 6.0	1740 ± 310	27.7 ± 3.3	4630 ± 1770	21.0 ± 1.0

[a] No effect observed. [b] No data available.

[^{3}H]DPDPE (δ opioid receptor agonist). K_i values were determined by measuring the inhibition of binding of these radioligands to the receptor by the ligands **5** and **6**.[18] As with M6G, each ligand tested showed a higher affinity for the μ than the δ or κ receptor. The rank order of affinity for the most active compounds at the μ receptor was **6c** = **5b** > **5e** > **6a** > M6G. Each of these compounds possessed a free phenolic hydroxyl group at the C-3 position. Compared to the affinity of M6G, the affinity of the most active compounds **5b**, **5e**, **6a**, **6c** was increased 1.5−2.4-fold at μ, 2.9−10.3-fold at δ, and 1.4−369-fold at the κ receptor. Thus, both the *S*-glucuronide **6a** and the *S*-glucoside **6c** possessed slightly higher affinity for the μ opioid receptor versus M6G. The μ versus δ and the μ versus κ affinity of the *S*-glucuronide **6a** was decreased 3.5- and 9.6-fold, respectively, compared to M6G, indicating that the stereochemistry at the C-6 position may play a significant role in receptor interactions. The glucose analogue of M6G has been shown to possess moderately higher affinity (5-fold) for the μ opioid receptor compared to M6G,[19] which is consistent with our observations. Compounds **5b** and **5a** that possessed a carbomethoxy group at the C-5′ position showed 1.7−2.7-fold higher affinity for the κ receptor versus the δ receptor. Each of the other ligands showed higher affinity for the δ receptor versus the κ receptor. The replacement of the carbomethoxy group at C-5′ in compound **5b** with an acetoxymethyl group in compound **5e** decreased the affinity for the κ receptor 270-fold. The affinity of the remaining compounds for the μ, δ, and κ receptors possessing either 3-acetoxy groups or 3-methyl ether groups was significantly decreased when compared to M6G. In each instance, removal of the ester functional group in the sugar moiety greatly diminished affinity of the ligands for the κ receptor, while having a less pronounced effect on the affinity of the ligands for the μ and δ receptors.

To evaluate the opioid receptor mediated activation of its associated G protein, the compounds **5** and **6** were evaluated using the [^{35}S]GTP-γ-S assay.[20] In this assay, the compound's potency or affinity for the receptor (defined by its EC_{50} for stimulating [^{35}S]GTP-γ-S binding) was examined in vitro. Agonist efficacy (E_{max}) was defined as the degree to which the compound maximally stimulated [^{35}S]GTP-γ-S binding relative to control. The EC_{50} value represented the concentration of a compound that produced 50% maximal stimulation of [^{35}S]GTP-γ-S binding by that compound. Full agonists stimulated [^{35}S]GTP-γ-S binding to a maximal extent and partial agonists caused a reduced level of binding. As shown in Table 2, with the exception of compound **6b**, all of the compounds examined stimulated [^{35}S]GTP-γ-S binding at the μ and δ receptors. Generally, the compounds were much less efficacious at the κ receptor. Stimulation of [^{35}S]GTP-γ-S binding by M6G was also selective for the μ and δ receptors. The most efficacious compounds at the μ receptor were compounds **5b** (88.0%), **5e** (76.0%), **6c** (64.5%), and **6a** (46.6%). Each of these compounds possessed a free phenolic hydroxyl group. Compounds **5b**, **5e**, **6c**, and **6a** were all more efficacious than M6G in the [^{35}S]GTP-γ-S assay. The same general trend was observed at the δ receptor with compounds possessing a free phenolic hydroxyl showing increased efficacy. The most efficacious compounds at the δ receptor were **5b** (104.4%), **6a** (78.70%), **5c** (64.3%), and **6c** (51.5%). Protection of the phenolic hydroxyl group with an acetate group or a methyl group diminished the efficacy and potency at each opioid receptor. Compounds that effected maximal stimulation may be viewed as agonists at a given receptor. Compounds that elicited less than maximal binding may be viewed as partial agonists or antagonists if no stimulation was observed. Thus, compounds **5b** and **5e** may be viewed as full μ agonists, whereas compounds **5a**, **5c**, **5d**, **5f**, **6a**, **6c**, and **6d** are partial μ agonists. Compounds **5b** and **6a** were also full δ agonists. The remaining compounds **5a**, **5c**, **5d**, **5e**, **5f**, **6b**, **6c**, and **6d** are all partial agonists at the δ receptor. Compounds **5b**, **5c**, **5d**, **5e**, **6c**, and **6d** are partial κ agonists. The compounds **5a**, **5f**, **6a**, and **6b** did not stimulate [^{35}S]GTP-γ-S binding at the κ receptor. The overall rank order of the EC_{50} values for the functional assays correlated with the K_i values derived from the binding experiments.

On the basis of the results of the functional assay and K_i values, the HCl salt of compound **5b** was evaluated for its effect on the tail-flick latency.[21] Administration of **5b**·HCl produced a dose-dependent increase in tail-flick latency, indicative of the analgesic effects of the compound (Figure 3). The overall ANOVA indicated a significant effect of dose [$F(5,64) = 37.26$, $P < 0.0001$]. Averaged across postinjection time, the 3−30 mg/kg doses of compound **5b** produced a significant increase in tail-flick latency relative to controls (Student Newman−Keuls, $p < 0.05$). The two highest doses (10 and 30 mg/kg) of **5b** produced a greater amount of analgesia relative to a 3 mg/kg dose (Student Newman−Keuls, $p < 0.05$) of morphine (Figure 4). The ED_{50} of compound **5b**·HCl for tail flick latency (i.e., 2.5 mg/kg) was slightly

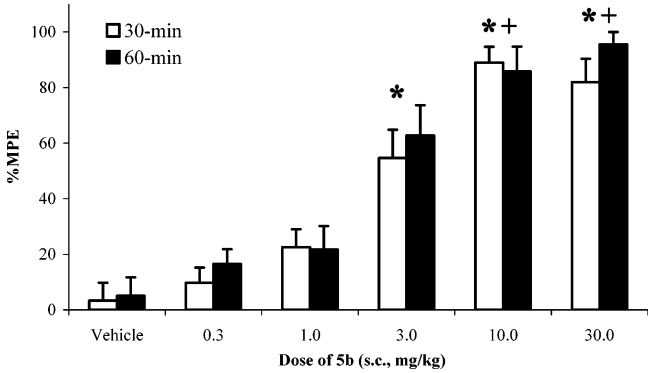


Figure 3. Dose-dependent analgesia induced by administration of **5b** in tail flick assay. Data are mean maximum possible antinociceptive effect (%MPE) (±SEM). Asterisks represent significant differences from vehicle controls (Student Newman−Keuls, $p < 0.05$). Plus signs represent a significant difference from 3 mg/kg **5b** (Student Newman−Keuls, $p < 0.05$).

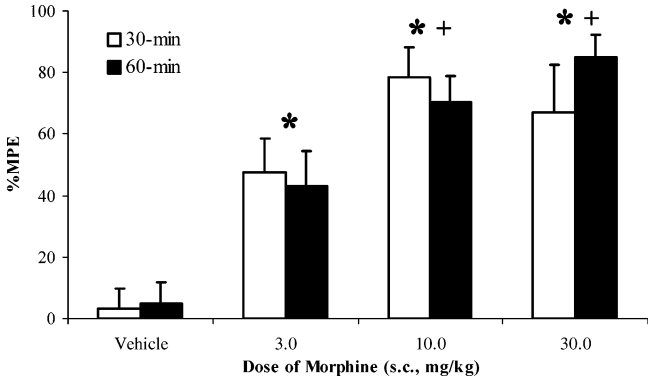


Figure 4. Dose-dependent analgesia induced by administration of morphine in tail flick assay. Data are maximum possible antinociceptive effect (%MPE) (±SEM). Asterisks represent significant differences from vehicle controls (Student Newman−Keuls, $p < 0.05$). Plus signs represent a significant difference from 3 mg/kg morphine (Student Newman−Keuls, $p < 0.05$).

less than that for morphine (i.e., 3.0 mg/kg) (Figure 4). In contrast to morphine, which appeared to be more effective at the 30 min time point, the analgesia of compound **5b** at the 60 min time point was greater than or equal to the analgesia observed at the 30 min time point. It is possible that **5b** has a greater duration of action than morphine. Although compound **5b** had slightly lower binding affinity than morphine, it possessed similar [^{35}S]GTP-γ-S potency and percent stimulation, indicating that the bioavailability of **5b** was also similar to that of morphine. Because this compound is rather nonselective with respect to each of the opioid receptors, it is not certain at this point which receptor mediates the antinociceptive activity. However, the fact that this is a partial agonist at the κ receptors suggests that the antinociceptive activity is likely mediated by the μ receptor.

Conclusion

In summary, a series of 10 6-β-thiosaccharide analogues of M6G and C6G were synthesized. The key synthetic step utilized an S_N2 displacement reaction of glucose and glucuronic acid derived 1-β-thiols with 6-*O*-tosylmorphines or 6-*O*-tosylcodeine. The phenols **5b**, **5e**, **6a**, and **6c** showed increased affinity for the μ opioid receptor relative to M6G. The phenol **5e**, possessing a peracetylated thioglucose moiety, showed a 2.5-fold higher affinity for the μ versus δ receptor and a 382-fold higher affinity for the μ versus κ receptor. Functional studies showed that compounds **5b** and **5e** were full μ agonists, whereas compounds **6a** and **6c** were partial μ agonists. The ED_{50} of compound **5b** in the tail flick latency test was 2.5 mg/kg. It can therefore be concluded that the replacement of the C-6 α-disposed glycosidic oxygen atom in M6G and its congeners with a β-disposed sulfur atom is a viable strategy for improving the pharmacological properties of these compounds.

Experimental Section

Synthetic Chemistry. All reactions were run under a positive pressure of nitrogen with magnetic stirring at ambient temperature using oven-dried glassware unless otherwise indicated. Air- and moisture-sensitive liquids were transferred via syringe through rubber septa. Silica gel (230−400 mesh) was used for column chromatography. DMF was dried by filtration through a column of neutral alumina and stored over activated 4 Å molecular sieves under nitrogen prior to use. All other solvents and reagents were used as received. ^{1}H NMR spectra were recorded at 500 MHz using a Varian NMR (NuMega Resonance, San Diego, CA). Chemical shifts were reported in ppm (δ) relative to $CDCl_3$ at 7.26 ppm. Melting points were reported uncorrected. Low resolution mass spectra was done on an Agilent 1100 MSD (HT Labs, San Diego, CA). High-resolution mass spectra was done on a VG 7070 spectrometer with an Opus V3.1 and DEC 3000 Alpha Station data system at the University of California−Riverside. Where combustion analyses are not specified, analytical purities were determined by straight phase HPLC using a Hitachi L74 liquid chromatograph with a L-7400 UV detector, a D7500 integrator, and a Hamilton PRP-I stainless steel column (250 mm × 4.6 mm i.d.).

Methyl 2,3,4-tri-*O*-acetyl-1-β-thio-D-glucopyranosiduronate 4a. The bromide **8a** (2.7 g, 6.8 mmol) was dissolved in DMF (20 mL), and KSAc (0.92 g, 8.1 mmol) was added all at once. The flask was closed and stirred at room temperature for 3.5 h. The DMF was removed by distillation under high vacuum (0.25 mmHg) with the aid of a water bath (55 °C). The residue was dissolved in $CHCl_3$ (100 mL) and washed with water (3 × 10 mL) and brine (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated to a red-brown oil. Absolute EtOH (30 mL) was added to the oil and the mixture was heated to boiling and then slowly cooled to room temperature and then to 0 °C. The resulting crystals were collected on a fritted funnel and washed with EtOH (0 °C, 5 × 4 mL) and dried under high vacuum to provide the intermediate thioacetate as white crystals (1.46 g, 65%): $R_f = 0.61$ (1:1 hexane/EtOAc); mp = 162 °C; ^{1}H NMR ($CDCl_3$) δ 5.32−5.28 (m, 2H), 5.17−5.13 (m, 2H), 4.15 (d, $J = 9.8$ Hz, 1H), 3.71 (s, 3H), 2.37 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H); MS (ESI) *m/z* 415 [M + Na]$^+$.

The thioacetate (100 mg, 0.26 mmol) was dissolved in 1:1 $CHCl_3$/MeOH (2 mL) and cooled to 0 °C. Nitrogen was bubbled through the solution for 5 min, followed by the addition of NaSMe (18 mg, 0.26 mmol). After 5 min, the mixture was poured into 1% concentrated aqueous HCl (10 mL) and extracted with CH_2Cl_2 (2 × 10 mL). The combined CH_2Cl_2 layers were washed with brine (5 mL), dried over anhydrous sodium sulfate, filtered, and concentrated to an orange-white solid that was recrystallized from boiling absolute EtOH (2 mL). After cooling to 0 °C, the crystals were collected on a fritted funnel and washed with small portions of 0 °C EtOH (2 mL total) and dried under high vacuum to give **4a** as white crystals (80 mg, 90%): $R_f = 0.16$ (1:1 hexane/EtOAc); mp = 125 °C; ^{1}H NMR ($CDCl_3$) δ 5.24−5.22 (m, 2H), 4.99 (m, 1H), 4.57 (t, $J = 10.1$ Hz, 1H), 4.04 (d, $J = 9.3$ Hz, 1H), 3.76 (s, 3H), 2.38 (d, $J = 10.3$ Hz, 1H), 2.08 (s, 3H), 2.02 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 170.1, 169.7, 169.5, 166.9, 79.1, 77.5, 73.4, 73.0, 69.5, 53.2, 20.9, 20.7, 20.6; MS (ESI) *m/z* 373 [M + H] $^+$.

2,3,4,6-Tetra-*O*-acetyl-1-*β*-thio-D-glucopyranose 4b. 2,3,4,6-Tetra-*O*-acetyl-1-*S*-acetyl-*β*-D-glucopyranose[10] (496 mg, 1.2 mmol) was dissolved in 1:2 MeOH/CH_2Cl_2 (6 mL) and cooled to 0 °C. Nitrogen was bubbled through the solution for 5 min, followed by the addition of NaSMe (85 mg, 1.2 mmol) in a single portion. The solution was stirred for 5 min under nitrogen, at which time TLC analysis (2:1 hexane/EtOAc) indicated that the reaction was complete. The solution was transferred to a separatory funnel containing 10 mL of 1% aqueous HCl and 10 mL of H_2O and extracted with CH_2Cl_2 (4 × 20 mL). The combined CH_2Cl_2 layers were washed with H_2O (5 mL) and brine (5 mL), dried (Na_2SO_4), filtered, and concentrated. The residue was purified by chromatography (2:1 hexane/EtOAc) to provide **4b** as an orange gel (336 mg, 76%): R_f = 0.2 (2:1 hexane/EtOAc); ^{1}H NMR ($CDCl_3$) δ 5.19 (t, J = 9.5 Hz, 1H), 5.10 (t, J = 9.9 Hz, 1H), 4.97 (t, J = 9.6 Hz, 1H), 4.54 (t, J = 9.9 Hz, 1H), 4.24 (dd, J = 4.8, 12.5 Hz, 1H), 4.12 (dd, J = 2.2, 8.5 Hz, 1H), 3.72 (ddd, J = 2.2, 4.8, 7.1 Hz, 1H), 2.30 (d, J = 9.9 Hz, 1H), 2.09 (s, 3H), 2.08 (s, 3H), 2.02 (s, 3H), 2.00 (s, 3H); MS (ESI) m/z 365 $[M + H]^+$.

General Procedure for the Reaction of Thiosaccharides with Morphine or Codeine Tosylates. 6-*β*-*S*-(Methyl 2′,3′,4′-tri-*O*-acetyl-*β*-D-glucopyranosyluronate)morphine 5b. A 60% dispersion of NaH (46 mg, 1.2 mmol) was added to a 0 °C DMF solution of **4a** (424 mg, 1.2 mmol) and the resulting red mixture was stirred for 10 min. A 0 °C DMF (8 mL) solution of **3a** (183 mg, 0.42 mmol) was added by syringe over 1 min and the resulting solution was stirred for 4 h while warming to room temperature. The reaction was poured into 20 mL of 0.5% aqueous HCl and the pH was raised to 9 by the addition of solid $NaHCO_3$. The mixture was extracted with CH_2Cl_2 (3 × 50 mL), and the combined organic layers were washed with H_2O (10 mL) and brine (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated to provide a gray solid, which was purified by flash chromatography (SiO_2, 60:1 to 20:1 CH_2Cl_2/EtOH) to provide an off-white solid (144 mg, 56%). An analytical sample of **5b** was obtained by recrystallization from absolute EtOH as a white solid: R_f = 0.10 (20:1 CH_2Cl_2/MeOH); mp = 135 °C (dec); ^{1}H NMR δ 6.63 (d, J = 7.9 Hz, 1H), 6.50 (d, J = 7.9 Hz, 1H), 5.81 (ddd, J = 3.0, 5.6, 9.1 Hz, 1H), 5.52 (dd, J = 8.6, 10.3 Hz, 1H), 5.29−5.21 (m, 2H), 5.05 (d, J = 9.2 Hz, 1H), 5.03 (s, 1H), 4.70 (d, J = 9.6 Hz, 1H), 4.06 (d, J = 9.6 Hz, 1H), 3.74 (s, 3H), 3.71 (d, J = 5.6 Hz, 1H), 3.31 (m, 1H), 3.03−2.99 (m, 2H), 2.62−2.59 (m, 1H), 2.58 (dd, J = 11.7, 4.0 Hz, 1H), 2.43 (s, 3H), 2.38−2.28 (m, 2H), 2.15−2.09 (m, 2H), 2.05 (s, 3H), 2.022 (s, 3H), 2.016 (s, 3H), 1.76 (dd, J = 12.2, 1.8 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 170.3, 169.6, 169.5, 167.0, 144.7, 138.5, 131.8, 130.4, 128.5, 126.7, 119.7, 116.8, 93.7, 84.6, 76.3, 73.4, 70.0, 69.4, 59.2, 53.1, 47.2, 45.0, 44.5, 43.1, 39.8, 35.6, 20.9, 20.8, 20.7; HRMS (ESI) calcd for $C_{30}H_{36}NO_{11}S$ 618.2009, found 618.2011; the average purity of **5b** was found to be ≥99% by analytical HPLC, giving t_R = 3.47 min (55:45:0.01 MeOH/2-propanol/$HClO_4$) and t_R = 3.98 min (45/55/0.01 MeOH/2-propanol/$HClO_4$).

3-*O*-**Acetyl-6-*β*-*S*-(methyl 2′,3′,4′-tri-*O*-acetyl-*β*-D-glucopyranosyluronate)morphine 5a.** According to the general procedure described for **5b**, NaH (53 mg, 1.33 mmol), **4a** (484 mg, 1.38 mmol), and **3b** (246 mg, 0.51 mmol) provided **5a** as an off-white powder (211 mg, 63%): R_f = 0.33 (20:1 CH_2Cl_2/EtOH); mp = 194 °C (dec); ^{1}H NMR ($CDCl_3$) δ 6.74 (d, J = 8.2 Hz, 1H), 6.58 (d, J = 8.2 Hz, 1H), 5.83−5.80 (m, 1H), 5.53 (dd, J = 1.5, 10.2 Hz, 1H), 5.29−5.21 (m, 2H), 5.09 (s, 1H), 5.00 (t, 1H), 4.72 (d, J = 10.3 Hz, 1H), 4.06 (d, J = 9.3 Hz, 1H), 3.76−3.73 (4H), 3.33 (m, 1H), 3.07−3.03 (2H), 2.60−2.58 (m, 1H), 2.44 (s, 3H), 2.37−2.20 (s, 3H over m, 2H), 2.15 (dt, J = 3.7, 11.8 Hz, 1H), 2.04 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 1.80 (d, J = 11.6 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 170.3, 169.5, 169.4, 168.8, 167.0, 149.0, 133.0, 132.6, 132.0, 131.8, 128.0, 121.8, 119.5, 94.5, 85.1, 76.2, 73.3, 70.3, 69.4, 59.0, 53.0, 46.9, 45.6, 44.5, 43.2, 40.0, 35.7, 20.93, 20.90, 20.8, 20.7; MS (ESI) m/z 660 $[M + H]^+$. Anal. Calcd ($C_{32}H_{37}NO_{12}S$): C, 58.26; H, 5.65; N, 2.12; S, 4.86. Found: C, 57.94; H, 5.75; N, 2.07; S, 4.97.

6-*β*-*S*-(Methyl 2′,3′,4′-tri-*O*-acetyl-*β*-D-glucopyranosyluronate)codeine 5c. Following the general procedure described for **5b**, **3c** (231 mg, 0.51 mmol), **4a** (483 mg, 1.38 mmol), and NaH (1.33 mmol) gave **5c** as an off-white solid (249 mg, 77%): R_f = 0.30, 20:1 CH_2Cl_2/EtOH); mp = 172 °C; ^{1}H NMR ($CDCl_3$) δ 6.64 (d, J = 8.1 Hz, 1H), 6.54 (d, J = 8.1 Hz, 1H), 5.82 (ddd, J = 3.0, 6.0, 9.2 Hz, 1H), 5.53 (dd, J = 1.8, 9.4 Hz, 1H), 5.27−5.22 (m, 2H), 5.14 (s, 1H), 4.99 (t, J = 2.1 Hz, 1H), 4.71 (d, J = 10.3 Hz, 1H), 4.02 (d, J = 7.3 Hz, 1H), 3.83 (s, 3H), 3.79 (d, J = 6.0 Hz, 1H), 3.73 (s, 3H), 3.29 (dd, J = 3.3, 5.8 Hz, 1H), 3.05−3.00 (d, J = 19.0 Hz, 1H over m, 1H), 2.55 (dd, J = 4.3, 12.2 Hz, 1H), 2.42 (s, 3H), 2.35 (td, J = 3.4, 12.4 Hz, 1H), 2.29 (dd, J = 6.0, 19.0 Hz, 1H), 2.15 (td, J = 5.0, 12.4 Hz, 1H), 2.02 (s, 3H), 2.016 (s, 3H), 2.00 (s, 3H), 1.77 (dd, J = 1.5, 12.3 Hz, 1H); ^{13}C NMR δ 170.2, 169.5, 169.4, 166.9, 146.3, 142.2, 133.1, 130.7, 127.9, 127.7, 119.1, 112.8, 93.6, 85.2, 76.1, 73.4, 70.3, 69.3, 59.1, 56.4, 53.0, 47.0, 45.5, 44.7, 43.3, 40.0, 36.2, 20.9, 20.8, 20.7, 20.6; MS (ESI) m/z 632 $[M + H]^+$. Anal. ($C_{31}H_{37}NO_{11}S$) C, H, N, S.

3-*O*-Acetyl-6-*β*-*S*-(2′,3′,4′,5′-tetra-*O*-acetyl-*β*-D-glucopyranosyl)morphine 5d and 6-*β*-*S*-(2′,3′,4′,5′-Tetra-*O*-acetyl-*β*-D-glucopyranosyl)morphine 5e. Following the general procedure described for **5b**, the thiol **4b** (500 mg, 1.37 mmol), NaH (53 mg, 1.32 mmol), and tosylate **3b** (246 mg, 0.51 mmol) gave **5d** as a white foam (255 mg, 74%) together with **5e** (47 mg, 14.6%). Data for **5d**: R_f = 0.2 (20:1 CH_2Cl_2/MeOH); mp = 151.9 °C; ^{1}H NMR ($CDCl_3$) δ 6.73 (d, J = 8.2 Hz, 1H), 6.58 (d, J = 8.2 Hz, 1H), 5.83−5.79 (m, 1H), 5.50 (dd, J = 1.8, 9.7 Hz, 1H), 5.24−5.17 (m, 2H), 5.04 (t, J = 9.9 Hz, 1H), 4.96 (t, J = 9.9 Hz, 1H), 4.69 (d, J = 10.2 Hz, 1H), 4.19 (d, J = 4.2 Hz, 2H), 3.79−3.75 (m, 1H), 3.71 (d, J = 6.0 Hz, 1H), 3.30 (dd, J = 3.3, 5.6 Hz, 1H), 3.06 (s, 1H), 3.04 (d, J = 18.8 Hz, 1H), 2.57 (dd, J = 4.0, 12.2 Hz, 1H), 2.42 (s, 3H), 2.34−2.29 (m, 2H), 2.14−2.11 (m, 1H), 2.07 (s, 6H), 2.05 (s, 6H), 2.00 (s, 3H), 1.80−1.77 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 170.8, 170.1, 169.5, 169.3, 168.5, 148.8, 132.8, 132.1, 131.7, 131.6, 127.9, 121.6, 119.3, 94.4, 85.1, 75.9, 73.8, 70.3, 68.5, 62.4, 58.8, 46.7, 45.8, 44.3, 43.0, 39.7, 35.6, 20.7, 20.6, 20.5; MS (ESI) m/z 674 $[M + H]^+$; HRMS (ESI) calcd for $C_{33}H_{40}NO_{12}S$ 674.2271, found 674.2238; the average purity of **5d** was found to be ≥98% by analytical HPLC giving t_R = 4.86 min (60/40/0.02 MeOH/2-propanol/$HClO_4$ (v:v)) and t_R = 5.66 min (55/45/0.02 MeOH/2-propanol/$HClO_4$ (v:v)). Analytical data for compound **5e**: R_f = 0.08 (20:1 CH_2Cl_2/MeOH); mp = 137.3 °C; ^{1}H NMR δ 6.63 (d, J = 8.2 Hz, 1H), 6.51 (d, J = 8.2 Hz, 1H), 5.79 (ddd, J = 3.6, 5.8, 9.6 Hz, 1H), 5.50 (dd, J = 1.7, 9.6 Hz, 1H), 5.22 (t, J = 9.3 Hz, 1H), 5.09−4.99 (m, 3H), 4.69 (d, J = 10.2 Hz, 1H), 4.27−4.19 (m, 2H), 3.78−3.74 (m, 1H), 3.67 (d, J = 5.8 Hz, 1H), 3.32 (m, 1H), 3.01 (d, J = 18.9 Hz, 1H) over bs (1H), 2.61−2.58 (m, 1H), 2.44 (s, 3H), 2.39−2.27 (m, 2H), 2.16−2.11 (m, 2H), 2.08 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 1.78−1.75 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 171.1, 170.4, 169.69, 169.66, 144.6, 130.3, 131.8, 130.6, 128.3, 126.9, 119.7, 116.7, 94.2, 84.9, 76.1, 74.0, 70.4, 68.7, 62.5, 59.2, 47.2, 45.8, 44.5, 43.2, 39.8, 35.8, 21.0, 20.83, 20.80, 20.6; MS(ESI) m/z 632 $[M + H]^+$; HRMS (ESI) calcd for $C_{31}H_{38}NO_{11}S$ 632.2166, found 632.2136; the average purity of **5e** was found to be ≥99% by analytical HPLC, giving t_R = 3.98 min (60/40/0.02 MeOH/2-propanol/$HClO_4$ (v:v)) and t_R = 4.40 min (55/45/0.02 MeOH/2-propanol/$HClO_4$ (v:v)).

6-*β*-*S*-(2′,3′,4′,5′-Tetra-*O*-acetyl-*β*-D-glucopyranosyl)codeine, 5f. Compound **5f** was prepared according to the general procedure described for **5b**, from thiol **4b** (440 mg, 1.21 mmol), NaH (47 mg, 1.16 mmol), and **3c** (203 mg, 0.448 mmol). Purification was accomplished by flash chromatography (SiO_2, 20:1 CH_2Cl_2/EtOH) to provide **5f** as an off-white foam (237 mg, 82%). An analytical sample was obtained by recrystallization from boiling hexanes: R_f = 0.26 (20:1 CH_2Cl_2/EtOH); mp = 142.5 °C; ^{1}H NMR ($CDCl_3$) δ 6.65 (d, J = 8.2 Hz, 1H), 6.55 (d, J = 8.2 Hz, 1H), 5.85−5.81 (m, 1H), 5.52 (dd, J = 1.9, 9.6 Hz, 1H), 5.22−5.19 (m, 2H), 5.06 (t, J = 9.8 Hz, 1H), 4.97 (dd, J = 9.5, 10.1 Hz, 1H), 4.69 (d, J = 10.1 Hz, 1H), 4.23−4.16 (m, 2H), 3.83 (s, 3H), 3.76 (d, J = 6.0 Hz, 1H), 3.76−3.71 (m, 1H), 3.29 (dd, J = 3.3, 5.8 Hz, 1H), 3.06−3.01 (m, 2H), 2.56 (dd, J = 4.0, 12.2 Hz, 1H), 2.42 (s, 3H), 2.38−2.30 (m, 2H), 2.18−2.15 (m, 1H), 2.12 (s, 3H), 2.09 (s, 6H), 2.00 (s, 3H), 1.99−1.80

(m, 1H); ^{13}C NMR ($CDCl_3$) δ 171.0, 170.4, 169.6, 169.5, 146.3, 142.2, 132.9, 130.7, 127.9, 127.6, 119.1, 112.7, 93.8, 85.5, 76.1, 74.0, 70.5, 68.5, 62.5, 59.1, 56.4, 47.0, 46.1, 44.6, 43.3, 40.0, 36.3, 20.93, 20.92, 20.8, 20.5; MS (ESI) *m/z* 646 $[M + H]^+$; HRMS (ESI) calcd for $C_{32}H_{40}NO_{11}S$ 646.2322, found 646.2304; the average purity of **5f** was found to be ≥99% by analytical HPLC, giving t_R = 5.00 min (60/40/0.02 MeOH/2-propanol/$HClO_4$ (v:v)) and t_R = 5.71 min (55/45/0.02 MeOH/2-propanol/$HClO_4$ (v:v)).

Morphine 6-*β*-*S*-D-glucuronide 6a. To a solution of **5a** (50.0 mg, 0.08 mmol) in MeOH (1.6 mL) was added 5% aqueous NaOH (0.12 mL). The golden solution was stirred at room temperature for 18.5 h, acidified to a pH of 5 with 10 drops of glacial AcOH, and concentrated. The resulting white, amorphous solid was purified by flash chromatography on silica gel (16 × 1 cm, 5:1 to 1:1 CH_3CN/MeOH) and then dried for 4 h at 100 °C (0.25 mmHg) to provide **5b** as a white powder (19.4 mg, 54%): R_f = 0.26 (1:1 CH_3CN/MeOH with 0.2% AcOH); mp > 300 °C (dec); ^{1}H NMR (D_2O) δ 6.65 (d, J = 8.1 Hz, 1H), 6.61 (d, J = 8.1 Hz, 1H), 5.92 (m, 1H), 5.61 (d, J = 9.6 Hz, 1H), 5.19 (s, 1H), 4.69 (d, J = 9.9 Hz, 1H), 3.87 (d, J = 5.9 Hz, 1H), 3.77 (d, J = 9.0 Hz, 1H), 3.64 (m, 1H), 3.57−3.51 (m, 2H), 3.38 (t, J = 9.2 Hz, 1H), 3.13−3.09 (m, 2H), 2.83 (dd, J = 3.7, 12.3 Hz, 1H), 2.59−2.54 (s, 3H over m, 2H), 2.19 (dt, J = 8.5, 13.2 Hz, 1H), 1.87 (d, J = 12.0 Hz, 1H); ^{13}C NMR (D_2O) δ 174.6, 144.3, 139.6, 129.8, 128.8, 127.7, 123.3, 119.0, 116.9, 91.7, 85.0, 79.3, 76.1, 71.3, 70.7, 58.0, 45.6, 43.3, 42.6, 40.3, 37.2, 33.0, 19.7; MS (ESI) *m/z* 478 $[M + H]^+$. Anal. Calcd ($C_{23}H_{27}NO_8S$): C, 57.85; H, 5.70; N, 2.93; S, 6.71. Found: C, 57.75; H, 5.95; N, 2.73; S, 6.58.

Codeine 6-*β*-*S*-D-glucuronide 6b. Compound **6b** was prepared according to the procedure described for **6a. 5c** (60.4 mg, 0.095 mmol), MeOH (2 mL), and 5% aqueous NaOH (0.28 mL) provided **6b** (38 mg, 79%) as a white solid: R_f = 0.08 (1:1 MeCN/MeOH with 0.2% AcOH); mp > 300 °C (dec); ^{1}H NMR (D_2O) 6.82 (d, J = 8.2 Hz, 1H), 6.69 (d, J = 8.2 Hz, 1H), 5.83 (ddd, J = 2.8, 6.0, 9.5 Hz, 1H), 5.22 (s, 1H), 4.66 (d, J = 9.9 Hz, 1H), 3.84 (d, J = 6.0 Hz, 1H), 3.81 (s, 3H), 3.76 (d, J = 6.0 Hz, 1H), 3.57−3.50 (m, 2H), 3.42 (dd, J = 3.3, 5.7 Hz, 1H), 3.40−3.34 (m, 1H), 3.08 (d, J = 19.0 Hz, 1H), 3.01 (m, 1H), 2.60 (dd, J = 4.0, 12.4 Hz, 1H), 2.43−2.37 (s, 3H overlapping with m, 1H), 2.30 (td, J = 3.4, 12.6 Hz, 1H), 2.12 (td, J = 4.8 Hz, 13.0, 1H), 1.75 (d, J = 12.0 Hz, 1H); ^{13}C NMR (D_2O) δ 174.5, 144.4, 140.5, 131.0, 129.5, 127.3, 127.1, 119.0, 112.9, 92.8, 85.1, 79.3, 76.1, 71.3, 70.8, 57.1, 55.7, 45.1, 43.2, 43.0, 40.5, 37.7, 33.8, 19.3; HRMS (ESI) calcd for $C_{24}H_{28}NO_8S$ 490.1536, found 490.1525; the average purity of **6b** was found to be >99% by analytical HPLC, giving t_R = 1.62 min (70:30 CH_3CN/MeOH (v:v)) and t_R = 1.46 min (MeOH).

Morphine 6-*β*-*S*-D-Glucose, 6c. To an amber solution of **5d** (50 mg, 0.074 mmol) in MeOH (6 mL) was added 5% aqueous NaOH (0.4 mL). A white precipitate formed within 5 min. The mixture was stirred for 18 h and then AcOH (1.5 N, 15 drops to a pH of 7) was added. The solution was stirred for 5 min and then saturated $NaHCO_3$ (15 drops to a pH of 8.5) was added. The mixture was concentrated and the residue was dissolved in 0.5 mL of water with 3 drops of 13% NH_4OH and purified by preparative TLC (SiO_2, 200:40:5:0.05 CH_2Cl_2/MeOH/H_2O/13% NH_4OH). After drying for 4 h at 100 °C (0.25 mmHg), **6c** was obtained as a white powder (20 mg, 59%): R_f = 0.21; mp > 300 °C (dec); ^{1}H NMR (CD_3OD) δ 6.55 (d, J = 8.0 Hz, 1H), 6.46 (d, J = 8.0 Hz, 1H), 5.90 (ddd, J = 2.9, 5.8, 9.0 Hz, 1H), 5.52 (dd, J = 1.6, 9.7 Hz, 1H), 5.22 (s, 1H), 4.54 (d, J = 9.8 Hz, 1H), 3.90 (d, J = 11.2 Hz, 1H), 3.77 (d, J = 6.0 Hz, 1H), 3.70 (dd, J = 5.2, 11.9 Hz, 1H), 3.42 (dd, J = 3.2, 5.6 Hz, 1H), 3.42−3.41 (m, 3H), 3.24 (dd, J = 8.4, 9.6 Hz, 1H), 3.07 (bs, 1H), 3.05 (d, J = 18.7 Hz, 1H), 2.68 (dd, J = 4.0, 12.3 Hz, 1H), 2.49 (s, 3H), 2.49−2.39 (m, 2H), 2.18 (dt, J = 7.9, 12.8 Hz, 1H), 1.81−1.79 (m, 1H); ^{13}C NMR (CD_3OD) δ 146.3, 140.4, 131.5, 131.4, 130.3, 126.7, 120.4, 117.9, 95.3, 88.4, 82.3, 79.9, 74.8, 71.6, 63.2, 60.7, 48.2, 46.3, 45.3, 43.0, 40.4, 36.7, 24.2; MS (ESI) *m/z* 464 $[M + H]^+$; HRMS (ESI) calcd for $C_{23}H_{30}NO_7S$ 464.1743, found 464.1727; the average purity of **6c** was found to be 97.0% by analytical HPLC, giving t_R = 3.94 min (60/40/0.02 MeOH/2-propanol/$HClO_4$ (v:v)) and t_R = 4.42 min (55/45/0.02 MeOH/2-propanol/$HClO_4$ (v:v)).

Codeine 6-*β*-*S*-D-Glucose 6d. 5f (120 mg, 0.19 mmol) was dissolved in MeOH (4 mL) and 5% aqueous NaOH (0.6 mL) was added. The mixture was stirred at room temperature for 18 h and then treated with saturated aqueous NH_4Cl (1 mL). The mixture was concentrated and the residue was purified by preparative TLC (SiO_2, 200:40:5:0.05 CH_2Cl_2/MeOH/water/13% concentrated NH_4OH). After drying for 4 h at 100 °C, **6d** was obtained as a white powder (23 mg, 26%): R_f = 0.17; mp = 201.6 °C; ^{1}H NMR (CD_3OD) δ 6.79 (d, J = 8.3 Hz, 1H), 6.68 (d, J = 8.3 Hz, 1H), 6.02 (ddd, J = 3.0, 5.9, 9.1 Hz, 1H), 5.55 (dd, J = 1.8, 9.8 Hz, 1H), 5.36 (s, 1H), 4.57 (d, J = 9.8 Hz, 1H), 4.10 (dd, J = 3.0, 6.2 Hz, 1H), 3.91 (dd, J = 1.6, 12.1 Hz, 1H), 3.83 (d, J = 6.1 Hz, 1H), 3.81 (s, 3H), 3.70 (dd, J = 5.1, 12.0 Hz, 1H), 3.39−3.23 (complex m, 6H), 3.01−3.00 (m, 1H), 2.98 (s, 3H), 2.90 (dd, J = 6.6, 19.8 Hz, 1H), 2.41 (dt, J = 4.8, 13.8 Hz, 1H), 2.04 (dd, J = 2.7, 14.0 H, 1H); ^{13}C NMR (CD_3-OD) δ 147.8, 144.5, 131.7, 130.1, 128.9, 125.3, 121.1, 116.2, 94.5, 88.3, 82.3, 79.9, 79.6, 74.8, 71.6, 63.1, 62.3, 57.3, 45.7, 44.3, 41.9, 38.8, 34.8, 23.0; MS (ESI) *m/z* 478 $[M + H]^+$; HRMS (ESI) calcd for $C_{24}H_{32}NO_7S$ 478.1899, found 478.1886; the average purity of **6d** was found to be ≥99% by analytical HPLC, giving t_R = 4.79 min (60/40/0.02 MeOH/2-propanol/$HClO_4$ (v:v)) and t_R = 5.64 min (55/45/0.02 MeOH/2-propanol/$HClO_4$ (v:v)).

Receptor Binding. Binding to cell membranes was conducted in a 96-well format, as described previously.[22] Cells were removed from the plates by scraping with a rubber policeman, homogenized in Tris buffer using a Polytron homogenizer, and then centrifuged once and washed by an additional centrifugation at 27 000*g* for 15 min. The pellet was resuspended in 50 mM Tris, pH 7.5, and the suspension incubated with [^{3}H]DAMGO, [^{3}H]DPDPE, or [^{3}H]U69593, for binding to μ, δ, or κ opioid receptors, respectively. The total volume of incubation was 1.0 mL, and samples were incubated for 60−120 min at 25 °C. The amount of protein in the binding reaction varied from approximately 15 to 30 μg. The reaction was terminated by filtration using a Tomtec 96 harvester (Orange, CT) with glass fiber filters. Bound radioactivity was counted on a Pharmacia Biotech beta-plate liquid scintillation counter (Piscataway, NJ) and expressed in counts per minute. IC_{50} values were determined using at least six concentrations of test compound and calculated using Graphpad/Prism (ISI, San Diego, CA). K_i values were determined by the method of Cheng and Prusoff.[23]

[^{35}S]GTP-γ-S Binding. [^{35}S]GTP-γ-S binding was conducted basically as described by Traynor and Nahorski.[20] Cells were scraped from tissue culture dishes into 20 mM Hepes, 1 mM EDTA and then centrifuged at 500*g* for 10 min. Cells were resuspended in this buffer and homogenized using a Polytron homogenizer. The homogenate was centrifuged at 27 000*g* for 15 min and the pellet resuspended in buffer A, containing 20 mM Hepes, 10 mM $MgCl_2$, 100 mM NaCl, pH 7.4. The suspension was recentrifuged at 27 000*g* and suspended once more in buffer A. For the binding assay, membranes (8−15 μg protein) were incubated with [^{35}S]GTP-γ-S (50 pM), GDP (10 μM), and the appropriate compound, in a total volume of 1.0 mL, for 60 min at 25 °C. Samples were filtered over glass fiber filters and counted as described for the binding assays. Statistical analysis was conducted using the program Prism.

Determination of Analgesic Activity of Morphine Analogues 5b·HCl.[21] Male ICR mice weighing 20−25 g at the start of the experiment were used. Animals were group-housed under standard laboratory conditions and were kept on a 12:12 h day−night cycle (lights on at 08:00). Animals were handled for 1−2 days prior to conducting the experiments. Morphine·HCl or **5b**·HCl were dissolved in water. Drugs were injected at a volume of 0.1 mL. Nociception was assessed using the tail flick assay with an analgesia instrument (Stoelting) that uses radiant heat. This instrument is equipped with an automatic quantification of tail flick latency and a 15 s cutoff to prevent damage to the animal's tail. During testing, the focused beam of light was applied to the lower half of the

animal's tail, and tail flick latency was recorded. Baseline values for tail flick latency were determined before drug administration in each animal. Basal tail flick latency was between 3.2 and 8.0 s (average 5.86 ± 0.16 SEM). Immediately after testing, animals were injected subcutaneously with the test compound or saline as a vehicle control. Following injections, animals were tested for tail flick latencies at 30 and 60 min postinjection. Antinociception was quantified by the following formula: % antinociception = 100 × [(test latency − baseline latency)/(15 − baseline latency)]. If the animal did not respond prior to the 15 s cutoff, the animal was assigned a score of 100%. Behavioral results were analyzed using ANOVAs with morphine and **5b**·HCl as between-group variables and postdrug treatment time (30, 60 min) as the repeated measure followed by Student Newman−Keuls post-hoc tests where appropriate. The level of significance was set at $p < 0.05$.

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Supporting Information Available: Elemental analysis of **5a**, **5c**, and **6a**; HPLC purity results for **5b**, **5d**−**f**, **6b**−**d**; and HRMS data for **5b**, **5d**−**f**, **6b**−**d**. This material is available free of charge via the Internet at http://pubs.acs.org.

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