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Synthesis and biological evaluation of some 6-arylamidomorphines as analogues of morphine-6-glucuronide

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Abstract—A series of 6-β-arylamidomorphines was synthesized and biologically evaluated. Various aryl substituents were introduced into the arylamidomorphines to examine substituent structure–activity relationships. Competition binding assays showed that compounds **10a**–**h** bound to the μ opioid receptor with high affinity (0.2–0.6 nM). Functional assays showed that compounds **10a**–**h** acted as full μ opioid receptor agonists. The ED_{50} of compound **10e**·HCl as an analgesic was 12.6 mg/kg in the tail flick latency test in the rat.

1. Introduction

We recently reported the synthesis and biological evaluation of a small library of thiosaccharide analogues of morphine-6-glucuronide (M6G) **1** with the objective of finding a compound with improved pharmacological activity (Fig. 1).[1] The thiosaccharides were evaluated as opioid receptor ligands by competition binding assays, functional assays and for in vivo antinociceptive activity. Both the 6-β-*S*-glucuronide, **2** and the 6-β-*S*-glucoside, **3** showed high affinity for the μ opioid receptor and functioned as potent agonists (Fig. 1). The evaluation of the thiosaccharides were of interest due to the high analgesic potency and reduced side effects of M6G compared to morphine.[2] However, due to the low bioavailability of M6G it was of interest to determine if simpler more hydrolytically stable analogues could be developed.[3] Two studies have appeared in the literature related to this question. A series of M6G analogues was reported in which the carbohydrate residue, the *N*-substituent and the (*O*)-3 substituent and saturation of the 7,8-double bond were varied.[4] Only the 6-β-glucoside or 6-β-glucuronide showed potent agonism. A series of compounds in which the sugar residue of

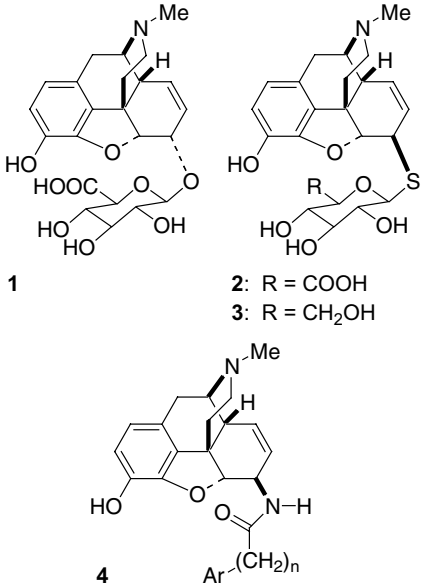

Figure 1. M6G, **1** and analogues **2**, **3**, and **4**.

M6G was replaced with non-saccharide hydrophobic groups introduced through Wittig reactions on dihydrocodeinone was also reported.[5] None of these compounds showed improved analgesic properties relative to codeine. The objective of the present study was to further

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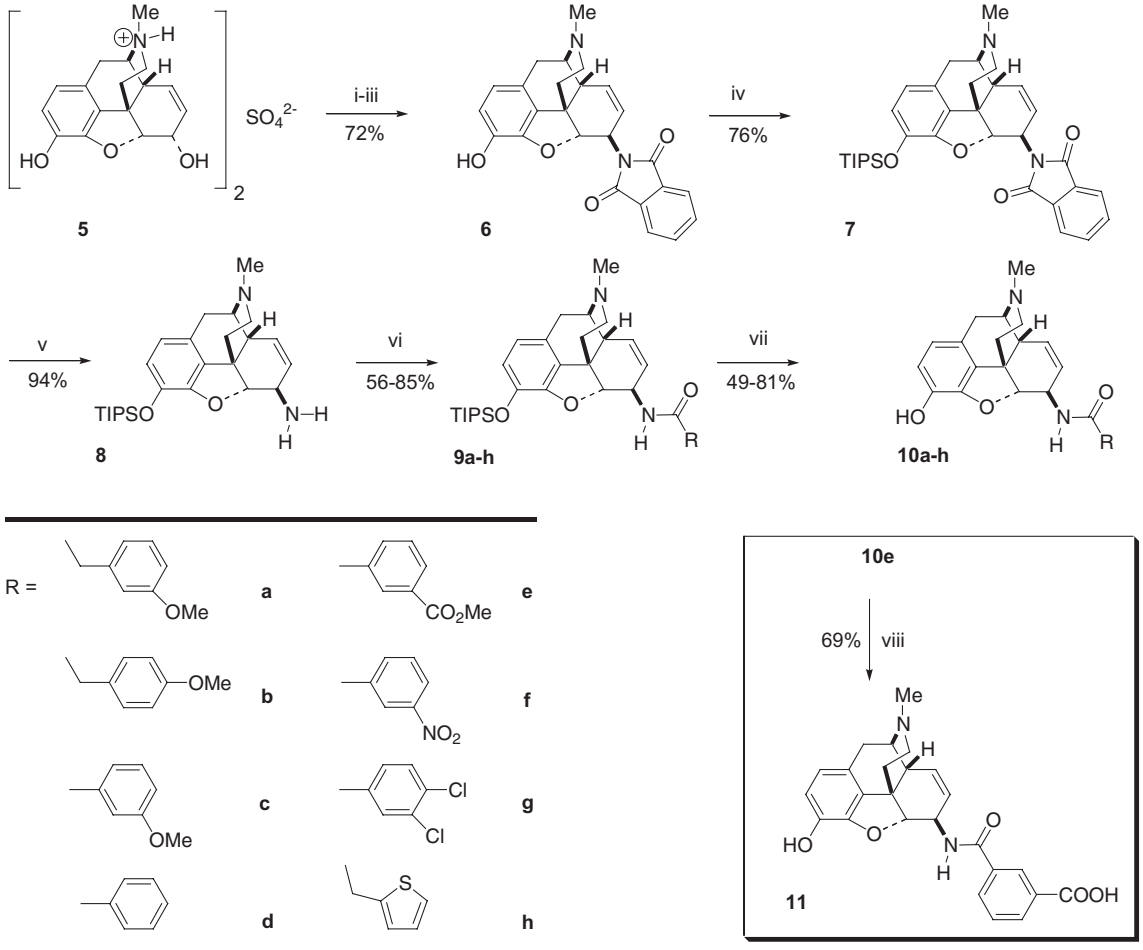
investigate the role of the saccharide portion of M6G on biological activity. In this study, we investigated whether the saccharide portion of M6G could be replaced with simpler functionality, as represented by the general structure **4** (Fig. 1), while retaining a similar pharmacological potency and functional profile-higher affinity for the μ versus δ and κ receptors and μ agonism. The synthesis and biological evaluation of this series of compounds is reported herein.

2. Chemistry

The morphine amides **10a**–**h** and **11** used in these studies were prepared in good yields according to the synthetic method outlined in Scheme 1. Morphine sulfate **5** was converted to its 6-β-phthalimide **6** in good yield with a three step procedure involving selective acetylation of the phenolic hydroxyl group utilizing a slight variation of the Welsh procedure,[6] Mitsunobu reaction to replace the C-6 α-disposed hydroxyl group with a β-disposed phthalimido group[7] and then selective removal of the phenolic acetate group by treatment with hydroxylamine hydrochloride in hot ethanol.[7] Triisopropylsilyation of the phenolic hydroxyl group in **6** (76%) was followed by removal of the C-6 phthalimide protecting group in the product **7** by heating in warm ethanol with hydrazine hydrate to give the primary amine **8** (94%). Treatment of **8** with a variety of aryl and heteroaryl acid chlorides in the presence of triethylamine in dichloromethane gave amides **9a**–**h** (60–82%), containing a TIPS group attached to the oxygen atom at the 3-position. Subsequent treatment of the silyl ethers **9a**–**h** with TBAF in THF then gave the desired 6-β-amidomorphines **10a**–**h** (56–82%). Treatment of the methyl ester **10h** with lithium hydroxide in THF/H_2O gave the carboxylic acid **11** (69%). A characteristic singlet (δ 4.85–4.62) for the C-5 phenanthrene proton was evident in the ^{1}H NMR spectra of the amides **10a**–**h** and **11**. 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.[1,8] The ^{1}H and ^{13}C NMR and HRMS spectral data for the compounds **10a**–**h** and **11** were in agreement with the proposed structures.

3. Results and discussion

The IC_{50} values obtained from competition binding assays for compounds **10a**–**h** and **11** were converted into



Scheme 1. Reagents and conditions: (i) Ac_2O, $NaHCO_3$, H_2O; (ii) PPh_3, *i*-$PrO_2CN{=}NCO_2$*i*-Pr, phthalimide; (iii) $NH_2OH{\cdot}HCl$, EtOH, 55 °C; (iv) TIPSCl, imidazole, DMF; (v) NH_2NH_2, EtOH, 55 °C; (vi) RC(O)Cl, NEt_3, CH_2Cl_2; (vii) TBAF, THF; (viii) LiOH, H_2O, AcOH.

K_i values as described in Section 5 and are listed in Table 2. In the binding assays the following radioligands were used: [^{3}H]DAMGO (μ opioid receptor agonist); [^{3}H]U69593 (κ opioid receptor agonist); [^{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 test compounds **10a**–**h** and **11**.[9] With the exception of compound **11** (K_i = 19.92 nM), each of the test compounds **10a**–**h** showed high affinity (K_i = 0.20–0.59 nM) for the μ opioid receptor. Compounds **10a**–**h** possessed 21–64-fold higher affinity for the μ receptor compared with M6G and 1.9–5.5-fold higher affinity for the μ receptor compared with morphine. The rank order of affinity for the most potent compounds at the μ receptor was **10g = 10f = 10e = 10c > 10h = 10a > 10d > 10b ≫ 11**. In general, the compounds **10a**–**h** showed significantly more μ versus δ receptor selectivity than μ versus κ receptor selectivity as indicated by the ratio of δ/μ and κ/μ values (Table 1). An exception to this trend was compound **11**, prepared in order to provide a simple mimic of M6G. Interestingly, the affinity of compound **11** for the μ receptor was reduced by a factor of 87 relative to its methyl ester **10c**. The μ versus δ and μ versus κ selectivity of compound **11** paralleled that observed for M6G, suggesting that a negatively charged group produces a highly unfavorable interaction in the κ binding pocket.[10] Compounds **10a**, **10g**, and **10h** also showed very high affinity for the κ receptor (0.58–0.96 nM). With the exception of compound **10g** (K_i = 0.94 nM), all compounds examined showed significantly less affinity for the δ receptor. Compound **10g** was the least selective of all compounds evaluated, at all three opioid receptors examined.

In order to evaluate the opioid receptor-mediated activation of its associated G protein, test compounds were evaluated using the [^{35}S]GTPγS assay.[11] In this assay, a compound's potency or affinity for the receptor is defined by its EC_{50} for stimulating [^{35}S]GTPγS binding. Agonist efficacy is defined as the degree to which the compound maximally stimulates [^{35}S]GTPγS binding relative to control. The EC_{50} value represents the concentration of a compound that produced 50% maximal stimulation of [^{35}S]GTPγS binding. Full agonists stimulate [^{35}S]GTPγS binding to a maximal extent and partial agonists cause a reduced level of binding. Table 2 gives the E_{max} and EC_{50} values for stimulation of [^{35}S]GTPγS binding at human μ, δ, and κ opioid receptors. The [^{35}S]GTPγS binding data showed that compounds **10a** and **10c**–**h** were full μ agonists, and that compounds **10b** and **11** were partial μ agonists. Compounds **10e**–**g** were found to be full δ agonists, whereas the remaining compounds were found to be partial δ agonists. Compounds **10a**–**f** and **10h** were found to be full κ agonists, whereas compounds **10g** and **11** showed decreased efficacy and were therefore characterized as partial κ agonists. The overall rank order of the EC_{50} values for the functional assay correlated with the K_i values derived from the binding experiments.

Table 1. K_i inhibition values of μ, δ, and κ opioid binding to CHO membranes by compounds **10a**–**h** and **11**

Compound	K_i (nM) ± SEM			δ/μ	κ/μ
	μ	δ	κ		
Morphine	1.1 ± 0.1	140 ± 2	46.9 ± 14	127	42
M6G	12.85 ± 0.95	160.96 ± 0.73	4058.75 ± 230	13	316
10a	0.35 ± 0.08	9.5 ± 2.6	0.96 ± 0.2	27	2.7
10b	0.59 ± 0.19	8.89 ± 2.1	2.84 ± 0.96	15	5
10c	0.21 ± 0.07	8.96 ± 1.95	2.65 ± 1.25	43	13
10d	0.40 ± 0.03	24.95 ± 2.33	4.13 ± 1.21	62	10
10e	0.23 ± 0.05	3.39 ± 0.03	1.53 ± 0.2	15	6.7
10f	0.20 ± 0.04	18.0 ± 5.63	2.63 ± 1.1	90	13
10g	0.20 ± 0.04	0.94 ± 0.02	0.75 ± 0.2	5	4
10h	0.33 ± 0.02	14.42 ± 1.26	0.58 ± 0.17	44	2
11	19.92 ± 4.29	50.78 ± 8.86	>10K	2.5	>500

Table 2. Stimulation of [^{35}S]GTPγS binding by amides **10a**–**h** and **11** mediated by μ, δ, and κ opioid receptors

Compound	μ		δ		κ	
	EC_{50}	E_{max}	EC_{50}	E_{max}	EC_{50}	E_{max}
M6G	72.3 ± 26.7	45.0 ± 5.0	190.35 ± 22.9	80.0 ± 0	>10K	Flat
10a	1.7 ± 0.2	99.0 ± 4.0	18.8 ± 2.2	51.0 ± 4.0	4.8 ± 0.1	89.0 ± 6.0
10b	4.8 ± 2.1	72.5 ± 14.5	25.2 ± 3.0	59.5 ± 3.5	5.0 ± 1.0	81.5 ± 1.5
10c	2.8 ± 0.2	87.5 ± 6.5	37.8 ± 1.9	65.5 ± 5.5	9.8 ± 0.02	86.0 ± 0.0
10d	5.5 ± 0.9	90.5 ± 9.5	62.7 ± 24.0	61.3 ± 2.3	5.3 ± 1.3	81.1 ± 8.9
10e	2.4 ± 0.3	98.1 ± 7.9	6.20 ± 0.42	99.63 ± 2.19	11.22 ± 3.51	88.6 ± 8.6
10f	1.9 ± 0.2	95.5 ± 7.5	24.5 ± 3.0	94.1 ± 10.9	1.2 ± 0.1	90.3 ± 4.8
10g	0.1 ± 0.0	95.5 ± 10.5	1.3 ± 0.3	106.5 ± 1.5	0.03 ± 0.02	78.5 ± 1.5
10h	6.0 ± 2.1	95.8 ± 19.3	32.4 ± 2.1	57.3 ± 2.8	4.5 ± 0.2	98.0 ± 1.0
11	65.1 ± 21.8	71.0 ± 8.0	74.0 ± 12.7	69.5 ± 3.5	5096	71 ± 12

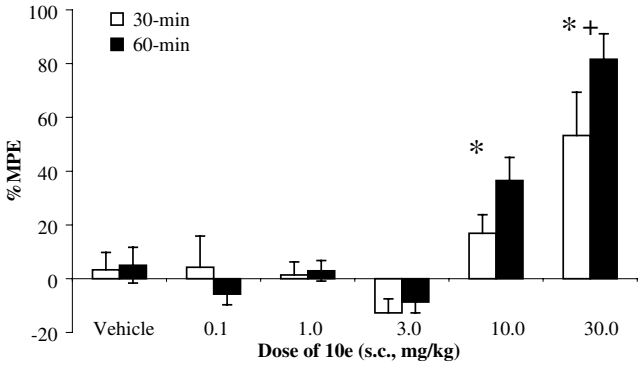


Figure 2. The two highest doses of **10e** produced analgesic effects. Data are mean %MPE (±SEM). Asterisks represent significant differences from vehicle controls (Student Newman–Keuls, $p < 0.05$). Plus signs represent a significant difference between 10 and 30 mg/kg **10e** (Student Newman–Keuls, $p < 0.05$).

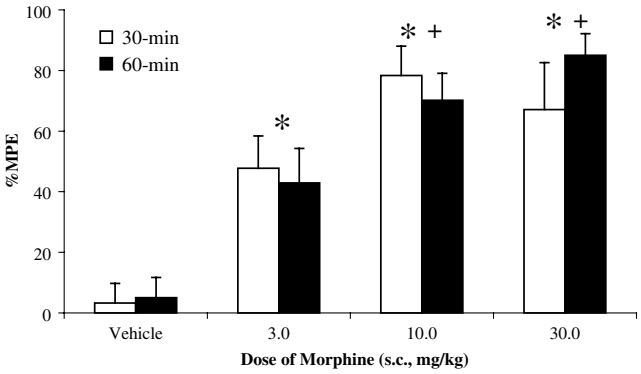


Figure 3. Dose-dependent analgesia induced by administration of morphine. Data are maximum possible antinociceptive effect (%MPE) (±SEM). Asterisks represent significant differences from vehicle controls (Student Newman–Keuls, $p < 0.05$). Plus signs represent a significant difference between 10 and 30 mg/kg morphine (Student Newman–Keuls, $p < 0.05$).

We evaluated compound **10e**·HCl for its analgesic effect on tail flick latency in mice because **10e** possessed a broad spectrum of potency for μ, δ, and κ opioid receptors.[12] Administration of **10e**·HCl produced an increase in tail flick latency that was evident at 30 min and continued to be present at 60 min (Fig. 2). The overall ANOVA indicated a significant effect of dose [$F(5,72) = 14.86$, $P < 0.0001$]. Averaged across post-injection time, the two highest doses of **10e**·HCl produced a significant increase in tail flick latency relative to controls (Student Newman–Keuls, $p < 0.05$). The analgesic effects of 30 mg/kg dose of **10e**·HCl was twofold greater than that produced by the 10 mg/kg dose (Student Newman–Keuls, $p < 0.05$). Compound **10e**·HCl appeared to have a slower onset of action, with significantly increased potency at 60 min over 30 min. Considering its very great potency at μ receptors ($K_i = 0.23$ nM) and full agonist activity at the μ receptor with respect to stimulation of [^{35}S]GTPγS binding ($EC_{50} = 2.4$ nM), compound **10e**·HCl had relatively weak antinociceptive activity, with ED_{50} values of 30.2 and 12.6 mg/kg at 30 and 60 min, respectively. The relatively poor ED_{50} value of **10e**·HCl may indicate poor bioavailability relative to morphine that had an ED_{50} of approximately 3 mg/kg under the same experimental conditions (Fig. 3). We speculate that in vivo hydrolysis of the methyl ester in **10e** to the less active carboxylic acid confounds the functional activity.

4. Conclusion

In summary, a series of nine morphine arylamides was synthesized by the reaction of the 6-β-aminomorphine, **8** with aryl and heteroaryl acid chlorides. Compounds **10a**–**h** were found to bind to the μ opioid receptor with significant potency (0.20–0.59 nM). Functional assays showed that compounds **10a**–**h** were full μ opioid receptor agonists. The carboxylic acid derivative, **11** possessed significantly diminished affinity for the μ receptor and functional efficacy. Compounds **10a**, **10g**, and **10h** also showed high affinity and functioned as full agonists at the κ opioid receptor. These studies showed the effect on pharmacological activity observed by replacing the saccharide portion of M6G with arylamide groups.

5. Experimental

5.1. Chemistry

5.1.1. General information. All reactions were conducted under a positive pressure of dry 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. The term brine refers to a saturated solution of sodium chloride. Silica gel (230–400 mesh) was used for column chromatography. DMF was dried through a column of neutral alumina and stored over activated 4 Å molecular sieves under nitrogen prior to use. CH_2Cl_2 and THF were distilled from CaH_2 immediately prior to use. All other solvents and reagents were used as received. ^{1}H NMR spectra were recorded at 18 °C on a 500 MHz Varian NMR (NuMega Resonance, San Diego, CA). Chemical shifts were reported in ppm (δ) relative to $CDCl_3$ at 7.26 ppm unless indicated otherwise. 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 Opus V3.1 and DEC 3000 Alpha Station data system at the University of California Riverside. Melting points were reported uncorrected. Where combustion analyses are not specified, analytical purities were determined by straight phase HPLC using a Hitachi L74 liquid chromatograph with a D7500 integrator and a Hamilton PRP-I stainless steel column (250 mm × 4.6 mm id). For determination of analytical purities: mobile phase A = 60/40/0.02 MeOH/2-propanol/$HClO_4$ (v:v); mobile phase B = 55/45/0.018 MeOH/2-propanol/$HClO_4$ (v:v).

5.1.2. 3-*O*-Triisopropylsilyl-6-β-phthalimidomorphine 7. 6-β-Phthalimidomorphine,[7] **6** (1.89 g, 4.55 mmol) and imidazole (0.74 g, 10.9 mmol) were dissolved in CH_2Cl_2 (20 mL) and TIPSCl (1.17 mL, 5.45 mmol) was added

by syringe. After 2 h, the solution was diluted with CH_2Cl_2 (25 mL) and poured into saturated aqueous $NaHCO_3$ (25 mL). The CH_2Cl_2 layer was removed and the aqueous layer was extracted with additional CH_2Cl_2 (2×10 mL). The combined CH_2Cl_2 layers were washed with brine (10 mL), dried over Na_2SO_4, filtered, and concentrated. Flash chromatography of the residue (20:1 CH_2Cl_2/MeOH) provided **7** as a white foam (1.98 g, 76%): $R_f = 0.3$ (20:1 CH_2Cl_2/MeOH); mp = 84.9 °C; ^{1}H NMR δ 7.85–7.83 (m, 2H, Pht-H), 7.72–7.70 (m, 2H, Pht-H), 6.66 (d, $J = 8.1$ Hz, 1H, Ar-H), 6.51 (d, $J = 8.1$ Hz, 1H, Ar-H), 5.70–5.68 (m, 1H, C_7H), 5.48–5.46 (m, 1H, C_8H), 4.98 (s, 1H, C_5H), 4.88–4.86 (m, 1H, C_6H), 3.42 (m, 1H), 3.32–3.30 (m, 1H), 3.02 (d, $J = 18.4$ Hz, 1H), 2.61–2.58 (m, 1H), 2.46 (s, 3H, NMe), 2.36–2.25 (m, 3H), 1.72–1.69 (m, 1H), 1.29 (heptet, $J = 7.7$ Hz, 3H), 1.12–1.08 (18H); MS (ESI) *m/z* 571 $[M + H]^+$.

5.1.3. 3-*O*-Triisopropylsilyl-6-β-aminomorphine 8. The phthalimide **7** (1.72 g, 3.03 mmol) was dissolved in 95% aqueous EtOH (26 mL) and hydrazine hydrate (0.7 mL) was added by syringe. The solution was heated at 55 °C for 80 min, during which time a white precipitate formed. The mixture was concentrated. Water (50 mL) and 3% aqueous NH_4OH (4 mL) were added and the mixture was extracted with $CHCl_3$ (5×50 mL). The $CHCl_3$ extracts were dried over Na_2SO_4, filtered, and concentrated to a yellow oil. Flash chromatography (SiO_2, 15:1 CH_2Cl_2/MeOH with 0.1% NEt_3) provided **8** as a yellow oil (1.24, 94%): $R_f = 0.16$; ^{1}H NMR δ 6.60 (d, $J = 8.2$ Hz, 1H, Ar-H), 6.44 (d, $J = 8.2$ Hz, 1H, Ar-H), 5.84 (ddd, $J = 3.0$, 5.4, 9.4 Hz, 1H, C_7H), 5.45 (dd, J = 1.8, 9.4 Hz, 1H, C_8H), 4.56 (s, 1H, C_6H), 3.43 (d, $J = 5.4$ Hz, 1H), 3.32 (dd, $J = 3.2$, 5.5 Hz, 1H), 3.02 (s, 1H), 2.98 (s, 1H), 2.59 (dd, $J = 4.1$, 12.1 Hz, 1H), 2.45 (s, 3H, NMe), 2.39–2.31 (m, 2H), 2.07 (dt, $J = 5.0$, 12.4 Hz, 1H), 1.80 (dd, $J = 2.0$, 12.6 Hz, 1H), 1.27–1.20 (heptet, $J = 7.6$ Hz, 3H), 1.09–1.06 ($3 \times$ d, $J = 7.6$ Hz, 18H); MS (ESI) *m/z* 441 $[M + H]^+$.

5.1.4. General procedure for the synthesis of 6-β-amidomorphines. 6-β-(3′-Methoxy)benzylamidomorphine 10a. The amine **8** (80 mg, 0.18 mmol) was dissolved in CH_2Cl_2 (4 mL) and NEt_3 (72 μL, 0.52 mmol) and then 3-methoxyphenylacetyl chloride (80 μL, 0.51 mmol) were added by syringe. The resulting pale yellow solution was stirred at rt in a closed vial. After 2 h, the solution was concentrated and the residue was purified by flash chromatography (20:1 $CHCl_3$/MeOH) to provide **9a** as a white foam (78 mg, 73%): $R_f = 0.18$ (20:1 CH_2Cl_2/MeOH); MS (ESI) *m/z* 589 $[M + H]^+$. Compound **9a** (64 mg, 0.11 mmol) was dissolved in 5% aqueous THF (2.2 mL). TBAF (0.18 mL, 1.0 M solution in THF) was added by syringe and the pale yellow solution was stirred at rt for 2 h. The solution was concentrated and 1% HCl (2 mL) was added. The mixture was stirred for 2 min and then transferred to a separatory funnel with the aid of water (20 mL). The mixture was made basic (pH 8.5) with solid $NaHCO_3$ and extracted with $CHCl_3$ (5×10 mL). The combined $CHCl_3$ extract was washed with brine (4 mL), dried (Na_2SO_4), filtered, and concentrated. Flash chromatography (20:1 $CHCl_3$/MeOH) provided **10a** as a white solid (38 mg, 81%): $R_f = 0.14$ (20:1 $CHCl_3$/MeOH); mp = 144 °C; ^{1}H NMR δ 7.24 ($J = 7.9$ Hz, 1H, Ar-H), 6.82–6.79 (m, 3H, Ar-H), 6.6 4 (d, $J = 8.2$ Hz, 1H, Ar-H), 6.49 (d, $J = 8.2$ Hz, 1H, Ar-H), 5.67 (ddd, $J = 3.6$, 6.1, 9.1 Hz, 1H, C_7H), 5.58–5.53 (m, 2H, C_8H, NH), 4.65 (s, 1H, C_5H), 4.37 (t, $J = 6.4$ Hz, 1H, C_6H), 3.79 (s, 3H, OMe), 3.54 (s, 2H), 3.34 (m, 1H), 3.00 (d, $J = 18.6$ Hz, 1H), 2.89 (s, 1H), 2.61 (dd, $J = 4.0$, 12.2 Hz, 1H), 2.43 (s, 3H, NMe), 2.38–2.29 (m, 2H), 1.99 (dt, $J = 9.6$, 12.2 Hz, 1H), 1.76 (dd, $J = 1.9$, 12.6 Hz, 1H); ^{13}C NMR δ 171.1, 160.2, 144.5, 138.9, 136.2, 132.8, 130.3, 129.9, 128.6, 125.8, 121.7, 119.6, 117.1, 115.1, 113.1, 93.0, 59.3, 55.4, 50.5, 47.3, 44.1, 43.9, 43.0, 39.8, 35.5, 20.5; MS (ESI) *m/z* 433 $[M + H]^+$; HRMS (ESI) calcd for $C_{26}H_{29}N_2O_4$ 433.2127, found 433.2115 $[M + H]^+$; the average purity of **10a** was found to be $\geqslant 99\%$ by analytical HPLC giving $t_R = 4.38$ min (mobile phase A) and $t_R = 5.09$ min (mobile phase B).

5.1.5. 6-β-(4′-Methoxy)benzylamidomorphine 10b. Compound **10b** was prepared according to the general procedure described for **10a**, from **8** (78.4 mg, 0.178 mmol), NEt_3 (72 μL, 0.517 mmol), and 4-methoxyphenylacetyl chloride (80 μL, 0.523 mmol) giving **9b** as a white foam (70 mg, 67%): $R_f = 0.11$ (20:1 $CHCl_3$/MeOH); MS (ESI) *m/z* 589 $[M + H]^+$. The intermediate silyl ether **9b** (65 mg, 0.11 mmol) and TBAF (0.18 mL, 0.18 mmol) gave **10b** (29 mg, 61%) as a white solid: $R_f = 0.14$ (20:1 $CHCl_3$/MeOH); mp = 247 °C (dec); ^{1}H NMR δ 7.15 (d, $J = 8.6$ Hz, 2H, Ar-H), 6.86 (d, $J = 8.6$ Hz, 2H, Ar-H), 6.64 (d, $J = 8.1$ Hz, 1H, Ar-H), 6.48 (d, $J = 8.1$ Hz, 1H, Ar-H), 5.67 (ddd, $J = 3.0$, 5.6, 9.5 Hz, 1H, C_7H), 5.56 (dd, $J = 1.5$, 9.5 Hz, 1H, C_8H), 5.45 (d, $J = 6.4$ Hz, 1H, NH), 4.65 (s, 1H, C_5H), 4.36 (t, $J = 6.3$ Hz, 1H, C_6H), 3.79 (s, 3H, OMe), 3.50 (s, 2H), 3.34 (m, 1H), 3.00 (d, $J = 18.6$ Hz, 1H), 2.87 (s, 1H), 2.61 (dd, $J = 3.8$, 11.9 Hz, 1H), 2.44 (s, 3H, NMe), 2.38–2.28 (m, 2H), 1.98 (dt, $J = 4.9$, 12.5 Hz, 1H), 1.77 (d, $J = 10.7$ Hz, 1H); ^{13}C δ 171.5, 158.8, 144.4, 138.7, 132.6, 130.4, 129.7, 128.3, 126.5, 125.6, 119.3, 117.0, 114.5, 92.8, 59.1, 55.3, 50.3, 47.0, 43.9, 42.9, 42.7, 39.6, 35.4, 20.2; MS (ESI) *m/z* 433 $[M + H]^+$; HRMS (ESI) calcd for $C_{26}H_{29}N_2O_4$ 433.2127, found 433.2136 $[M + H]^+$; the average purity of **10b** was found to be $\geqslant 99\%$ by analytical HPLC giving $t_R = 4.44$ min (mobile phase A) and $t_R = 5.24$ min (mobile phase B).

5.1.6. 6-β-(3′-Methoxy)benzamidomorphine 10c. Compound **10c** was prepared according to the general procedure described for **10a**, from **8** (82 mg, 0.185 mmol), NEt_3 (76 μL, 0.545 mmol), and anisoyl chloride (71 μL, 0.521 mmol) giving **9c** as a white foam (88 mg, 82%); $R_f = 0.28$ (10:1 CH_2Cl_2/MeOH); MS (ESI) *m/z* 575 $[M + H]^+$. The intermediate silyl ether **9c** (73 mg, 0.174 mmol) and TBAF (0.21 mL, 0.21 mmol) then gave **10c** as a white solid (42.5 mg, 58%): $R_f = 0.15$ (15:1 DCM/MeOH); mp = 212.9 °C (dec); ^{1}H NMR δ 7.34–7.33 (m, 1H, Ar-H), 7.27–7.23 (m, 2H, Ar-H), 7.02–7.00 (m, 1H, Ar-H), 6.68 (d, $J = 8.1$ Hz, 1H, Ar-H), 6.51 (d, $J = 8.1$ Hz, 1H, Ar-H), 6.27 (d, $J = 6.8$ Hz, 1H, NH), 5.84 (ddd, $J = 3.0$, 5.8, 9.2 Hz, 1H, C_7H), 5.68

(dd, $J = 1.2$, 9.9 Hz, 1H, C_6H), 4.85 (s, 1H, C_5H), 4.59 (t, $J = 6.4$ Hz, 1H, C_6H), 3.81 (s, 3H, OMe), 3.47 (dd, $J = 3.0$, 5.0 Hz, 1H), 3.19–3.16 (m, 1H), 3.04 (d, $J = 18.5$ Hz, 1H), 2.68 (dd, $J = 4.0$, 12.0 Hz, 1H), 2.49 (s, 3H, NMe), 2.43–2.38 (m, 1H), 2.11 (dt, $J = 4.8$, 12.6 Hz, 1H), 1.80 (d, $J = 10.9$ Hz, 1H); ^{13}C NMR δ 167.5, 160.0, 144.7, 139.1, 135.6, 132.7, 129.9, 129.8, 128.9, 125.5, 119.7, 119.0, 118.2, 117.4, 112.6, 93.0, 59.5, 55.7, 50.9, 47.4, 44.1, 43.0, 39.9, 35.4, 20.6; MS (ESI) m/z 419 $[M + H]^+$; HRMS (ESI) calcd for $C_{25}H_{27}N_2O_4$ 419.1971, found 419.1984 $[M + H]^+$; the average purity of **10c** was found to be $\geqslant 99\%$ by analytical HPLC giving $t_R = 4.41$ min (mobile phase A) and $t_R = 5.05$ min (mobile phase B).

5.1.7. 6-β-Benzamidomorphine 10d. Compound **10d** was prepared according to the general procedure described for **10a**, from **8** (82 mg, 0.23 mmol), NEt_3 (76 μL, 0.55 mmol), and benzoyl chloride (61 μL, 0.52 mmol) giving **9d** as a white foam (79 mg, 78%); $R_f = 0.23$ (20:1 $CHCl_3$/MeOH); MS (ESI) m/z 545 $[M + H]^+$. The intermediate silyl ether **9d** (65 mg, 0.110 mmol) and TBAF (0.18 mL, 0.18 mmol) then gave **10d** as a white solid (29 mg, 61%): $R_f = 0.15$ (15:1 CH_2Cl_2/MeOH); mp = 184.4 °C (dec); ^{1}H NMR δ 7.78 (m, 2H, Ar-H), 7.53–7.50 (m, 1H, Ar-H), 7.42–7.41 (m, 1H, Ar-H), 6.72 (d, $J = 8.1$ Hz, 1H, Ar-H), 6.55 (d, $J = 8.1$ Hz, 1H, Ar-H), 6.18 (d, $J = 6.2$ Hz, 1H, NH), 5.89 (ddd, $J = 3.0$, 5.3, 9.5 Hz, 1H, C_7H), 5.69 (dd, $J = 1.5$, 9.5 Hz, 1H, C_8H), 4.86 (s, 1H, C_5H), 4.64 (t, $J = 6.3$ Hz, 1H, C_6H), 3.54 (br s, 1H), 3.31 (br s, 1H), 3.06 (d, $J = 18.7$ Hz, 1H), 2.77 (m, 1H), 2.57 (s, 3H, NMe), 2.50–2.48 (m, 3H), 2.23–2.19 (m, 1H), 1.86 (dd, $J = 2.2$, 12.9 Hz, 1H); ^{13}C NMR δ 167.4, 144.5, 138.8, 133.9, 132.9, 131.7, 129.8, 128.6, 128.6, 127.0, 125.7, 119.4, 117.1, 92.8, 59.1, 50.6, 47.0, 44.0, 42.9, 39.9, 35.5, 20.5; MS (ESI) m/z 389 $[M + H]^+$; HRMS (ESI) calcd for $C_{24}H_{25}N_2O_3$ 389.1865, found 389.1867 $[M + H]^+$; the average purity of **10d** was found to be 99% by analytical HPLC giving $t_R = 4.43$ min (mobile phase A) and $t_R = 5.16$ min (mobile phase B).

5.1.8. 6-β-(3′-Carbomethoxy)benzamidomorphine 10e. Compound **10e** was prepared according to the general procedure described for **10a**, from **8** (159 mg, 0.36 mmol), NEt_3 (151 μL, 1.09 mmol), and 3-carbomethoxybenzoyl chloride (217 mg, 1.01 mmol) giving **9e** as a white foam (122 mg, 56%); $R_f = 0.22$ (20:1 $CHCl_3$/MeOH); MS (ESI) m/z 603 $[M + H]^+$. The intermediate silyl ether **9e** (102 mg, 0.17 mmol) and TBAF (0.47 mL, 0.47 mmol) then gave **10e** as a white solid: $R_f = 0.14$ (30:1 DCM/MeOH); mp = 166.0 °C (dec); ^{1}H NMR δ 8.32 (s, 1H, Ar-H), 8.14 (d, $J = 7.8$ Hz, 1H, Ar-H), 8.00 (d, $J = 7.8$ Hz, 1H, Ar-H), 7.49 (t, $J = 7.8$ Hz, 1H, Ar-H), 6.68 (d, $J = 8.1$ Hz, 1H, Ar-H), 6.52 (d, $J = 8.1$ Hz, 1H, Ar-H), 6.33 (d, $J = 7.0$ Hz, 1H, NH), 5.85 (ddd, $J = 9.4$, 5.6, 3.1 Hz, 1H, C_7H), 5.73 (d, $J = 9.9$ Hz, 1H, C_8H), 4.85 (s, 1H, C_5H), 4.62 (t, $J = 6.2$ Hz, 1H, C_6H), 3.93 (s, 3H, OMe), 3.42 (dd, $J = 3.2$, 5.2 Hz, 1H), 3.10 (br s, 1H), 3.05 (d, $J = 18.5$ Hz, 1H), 2.63 (dd, $J = 12.1$, 4.2 Hz, 1H), 2.46 (s, 3H, NMe), 2.40–2.34 (m, 2H), 2.08 (dt, $J = 12.5$, 4.9 Hz, 1H), 1.81 (d, $J = 10.8$ Hz, 1H); ^{13}C δ 166.4, 166.2, 144.3, 138.7, 134.2, 133.0, 132.7, 131.9, 130.5, 129.8, 128.9, 128.3, 127.6, 125.7, 119.5, 117.0, 92.9, 59.1, 52.4, 50.9, 47.1, 44.0, 42.9, 39.9, 35.4, 20.2; MS (ESI) m/z 447 $[M + H]^+$; HRMS (ESI) calcd for $C_{26}H_{27}N_2O_5$ 447.1920, found 447.1930 $[M + H]^+$; the average purity of **10e** was found to be $\geqslant 99\%$ by analytical HPLC giving $t_R = 4.39$ min (mobile phase A) and $t_R = 5.06$ min (mobile phase B).

5.1.9. 6-β-(3′-Nitro)benzamidomorphine 10f. Compound **10f** was prepared according to the general procedure described for **10a**, from **8** (80 mg, 0.18 mmol), NEt_3 (76 μL, 0.55 mmol), and 3-nitrobenzoyl chloride (95 mg, 0.51 mmol) giving **9f** as a white foam (87 mg, 81%); $R_f = 0.17$ (20:1 $CHCl_3$/MeOH); MS (ESI) m/z 590 $[M + H]^+$. The intermediate silyl ether **9f** (73 mg, 0.168 mmol) and TBAF (0.20 mL, 0.20 mmol) then gave **10f** as a light yellow solid (36 mg, 49%): $R_f = 0.11$ (20:1 $CHCl_3$/MeOH); mp 208.3 °C (dec); ^{1}H NMR δ 8.54 (t, $J = 1.4$ Hz, 1H, Ar-H), 8.26 (dd, $J = 1.4$, 8.2 Hz, 1H, Ar-H), 8.11 (d, $J = 8.0$ Hz, 1H, Ar-H), 7.55 (t, $J = 8.0$ Hz, 1H, Ar-H), 6.85 (d, $J = 6.4$ Hz, 1H, NH), 6.65 (d, $J = 8.1$ Hz, 1H, Ar-H), 6.51 (d, $J = 8.1$ Hz, 1H, Ar-H), 5.84 (ddd, $J = 3.0$, 5.5, 9.2 Hz, 1H, C_7H), 5.71 (d, $J = 10.3$ Hz, 1H, C_8H), 4.88 (s, 1H, C_5H), 4.63 (t, $J = 6.3$ Hz, 1H, C_6H), 3.45 (t, $J = 3.2$ Hz, 1H), 3.18 (s, 1H), 3.04 (d, $J = 18.6$ Hz, 1H), 2.68 (dd, $J = 3.8$, 11.8 Hz, 1H), 2.47 (s, 3H, NMe), 2.41–2.34 (m, 2H), 2.15 (dt, $J = 7.6$, 12.4 Hz, 1H), 1.78 (d, $J = 11.5$ Hz, 1H); ^{13}C NMR δ 165.2, 148.0, 144.4, 138.9, 135.5, 133.3, 132.7, 129.8, 129.7, 128.2, 126.1, 125.4, 122.0, 119.5, 117.3, 92.5, 59.2, 51.1, 47.1, 43.9, 42.8, 39.6, 35.1, 20.3; MS (ESI) m/z 434 $[M + H]^+$; HRMS (ESI) calcd for $C_{24}H_{24}N_3O_5$ 434.1716, found 434.1719 $[M + H]^+$; the average purity of **10f** was found to be $\geqslant 99\%$ by analytical HPLC giving $t_R = 4.11$ min (mobile phase A) and $t_R = 4.78$ min (mobile phase B).

5.1.10. 6-β-(3′,4′-Dichloro)benzamidomorphine 10g. Compound **10g** was prepared according to the general procedure described for **10a**, from **8** (81 mg, 0.18 mmol), NEt_3 (77 μL, 0.55 mmol), and 3,4-dichlorobenzoyl chloride (108 mg, 0.52 mmol) giving **9g** as a white foam (96 mg, 85%); $R_f = 0.2$ (20:1 CH_2Cl_2/MeOH); MS (ESI) m/z 614 $[M + H]^+$. The intermediate silyl ether **9g** (81 mg, 0.18 mmol) and TBAF (0.22 mL, 0.22 mmol) then gave **10g** as a white solid (48 mg, 49%): $R_f = 0.17$ (15:1 CH_2Cl_2/MeOH); mp = 228.5 °C (dec); ^{1}H NMR δ 7.82 (d, $J = 2.1$ Hz, 1H, Ar-H), 7.54 (dd, $J = 2.1$, 8.4 Hz, 1H, Ar-H), 7.39 (d, $J = 8.4$ Hz, 1H, Ar-H), 6.67 (d, $J = 8.0$ Hz, 1H, Ar-H), 6.56 (d, $J = 6.2$ Hz, 1H, Ar-H), 6.52 (d, $J = 8.0$ Hz, 1H, Ar-H), 5.82 (ddd, $J = 2.9$, 5.3, 9.5 Hz, 1H, C_7H), 5.68 (dd, $J = 1.2$, 9.5 Hz, 1H, C_8H), 4.83 (s, 1H, C_5H), 4.58 (t, $J = 6.3$ Hz, 1H, C_6H), 3.47 (dd, $J = 3.0$, 5.3 Hz, 1H), 3.19 (m, 1H), 3.05 (d, $J = 18.6$ Hz, 1H), 2.70 (dd, $J = 3.8$, 11.9 Hz, 1H), 2.50 (s, 3H, NMe), 2.43–2.37 (m, 2H), 1.77 (d, $J = 11.0$ Hz, 1H), ^{13}C NMR δ 165.4, 144.4, 138.9, 136.1, 133.7, 133.0, 132.5, 130.5, 129.6, 129.3, 128.4, 126.3, 125.2, 119.5, 117.4, 92.5, 59.2, 52.4, 50.8, 47.2, 43.8, 42.7, 39.5, 35.1, 29.7, 25.8, 20.3, 20.5, 13.6; MS (ESI) m/z 457 $[M + H]^+$; HRMS (ESI) calcd for $C_{24}H_{23}Cl_2N_2O_3$ 457.1086, found 457.1087 $[M + H]^+$; the average purity

of **10g** was found to be ⩾99% by analytical HPLC giving t_R = 3.93 min (mobile phase A) and t_R = 4.43 min (mobile phase B).

5.1.11. 6-β-(Thiophen-2′-yl)acetamidomorphine 10h. Compound **10h** was prepared according to the general procedure described for **10a**, from **8** (86 mg, 0.2 mmol), 2-thiopheneacetyl chloride (0.68 μL, 0.55 mmol), and NEt_3 (82 μL, 0.59 mmol) giving **9h** as a white foam (66 mg, 60%); R_f = 0.23 (20:1 $CHCl_3$/MeOH); MS (ESI) *m/z* 565 $[M + H]^+$. The intermediate silyl ether **9h** (56 mg, 0.1 mmol) and TBAF (0.120 mL, 0.12 mmol) then gave **10h** as a white solid (32 mg, 79%): R_f = 0.10 (20:1 $CHCl_3$/MeOH); mp = 154.4 °C; ^{1}H NMR δ 7.18 (d, *J* = 4.5 Hz, 1H, Ar-H), 6.94–6.91 (m, 2H, Ar-H), 6.64 (d, *J* = 8.1 Hz, 1H, Ar-H), 6.49 (d, *J* = 8.1 Hz, 1H, Ar-H), 5.82 (d, *J* = 7.1 Hz, 1H, NH), 5.69 (ddd, *J* = 3.5, 5.5, 9.4 Hz, 1H, C_7H), 5.59 (dd, *J* = 1.2, 9.9 Hz, 1H, C_8H), 4.65 (s, 1H, C_5H), 4.39 (t, *J* = 6.4 Hz, 1H, C_6H), 3.77 (s, 2H), 3.37 (dd, *J* = 3.3, 5.8 Hz, 1H), 3.01 (d, *J* = 18.5 Hz, 1H), 2.93 (m, 1H), 2.64 (dd, *J* = 4.1, 12.1 Hz, 1H), 2.45 (s, 3H, NMe), 2.39–2.31 (m, 2H), 2.02 (dt, *J* = 7.6, 12.6 Hz, 1H), 1.77 (dd, *J* = 10.8, 1.7 Hz, 1H); ^{13}C NMR δ 170.2, 144.6, 139.0, 136.0, 132.9, 129.9, 128.4, 127.6, 125.9, 125.6, 119.6, 117.3, 92.9, 59.2, 50.4, 47.3, 44.1, 43.0, 39.7, 37.7, 35.5, 20.5; MS (ESI) *m/z* 409 $[M + H]^+$; HRMS (ESI) calcd for $C_{23}H_{25}N_2O_3S$ 409.1586, found 409.1572 $[M + H]^+$; the average purity of **10h** was found to be ⩾99% by analytical HPLC giving t_R = 4.31 min (mobile phase A) and t_R = 5.02 min (mobile phase B).

5.1.12. 6-β-(3′-Carboxy)benzamidomorphine 11. Compound **10e** (31 mg, 0.067 mmol) was dissolved in 4 mL of 1:1 THF/H_2O and LiOH·H_2O (27 mg, 0.643 mmol) was added. The colorless solution was stirred at rt for 3.75 h, glacial AcOH was added (15 drops), and the solution was concentrated. The residue was purified by flash chromatography on SiO_2 (5:1–1:1 $CHCl_3$/MeOH). The appropriate fractions were concentrated and the residue was stirred with 10 mL of 10:1 $CHCl_3$/MeOH and filtered through paper to remove residual SiO_2. The filtrate was concentrated to provide **11** as a white solid, 20 mg (69%): R_f = 0.17 (1:1 $CHCl_3$/MeOH with 0.2% AcOH), mp = 217.1 °C (dec); ^{1}H NMR (CD_3OD) δ 8.41–8.40 (m, 1H, NH), 8.13–8.12 (m, 1H, Ar-H), 7.90–7.88 (m, 1H, Ar-H), 7.47 (t, *J* = 7.7 Hz, 1H, Ar-H), 6.62 (d, *J* = 8.2 Hz, 1H, Ar-H), 6.55 (d, *J* = 8.2 Hz, 1H, Ar-H), 5.81–5.78 (m, 1H, C_7H), 5.69 (d, *J* = 9.9 Hz, 1H, C_8H), 4.82 (s, 1H, C_5H), 4.51–4.50 (m, 1H), 3.65 (dd, *J* = 3.2, 5.4 Hz, 1H), 3.27 (br s, 1H), 3.12 (d, *J* = 18.9 Hz, 1H), 2.87 (dd, *J* = 4.0, 12.9 Hz, 1H), 2.64 (s, 3H, NMe), 2.62–2.57 (m, 2H), 2.20 (dt, *J* = 4.8, 12.9 Hz, 1H), 1.81 (dd, *J* = 2.4, 12.9 Hz, 1H); ^{13}C NMR (CD_3OD) δ 180.5, 170.2, 145.6, 141.2, 139.4, 135.5, 133.6, 131.0, 130.9, 130.5, 130.3, 129.5, 129.2, 125.6, 120.7, 118.5, 93.8, 61.3, 52.8, 42.6, 39.9, 35.1, 30.9, 22.1; MS (ESI) *m/z* 431 $(M - H)^-$; HRMS (ESI) calcd for $C_{25}H_{25}N_2O_5$ 433.1758, found 433.1749; the average purity of **11** was found to be 99% by analytical HPLC giving t_R = 3.93 min (mobile phase A) and t_R = 4.43 min (mobile phase B).

6. Biological methods

6.1. Receptor binding

Binding to cell membranes was conducted in a 96-well format, as described previously.[9] Cells were removed from the plates by scraping with a rubber policeman, homogenized in Tris buffer using a Polytron homogenizer, 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.[13]

6.2. [^{35}S]GTPγS binding

[^{35}S]GTPγS binding was conducted basically as described by Traynor and Nahorski.[11] Cells were scraped from tissue culture dishes into 20 mM Hepes, 1 mM EDTA, 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]GTPγS (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.

6.3. Determination of analgesic activity of morphine analogue 10e·HCl[12]

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 **10e·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 post-injection. 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, **10e**·HCl as between group variables and post-drug 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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