Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 1583–1586
Bioorganic & Medicinal Chemistry Letters

Synthesis and in vitro biological evaluation of a carbon glycoside analogue of morphine-6-glucuronide

James M. MacDougall,[a,*] Xiao-Dong Zhang,[a] Willma E. Polgar,[b] Taline V. Khroyan,[b] Lawrence Toll[b] and John R. Cashman[a]

[a]*Human BioMolecular Research Institute, 5310 Eastgate Mall, San Diego, CA 92121-2804, USA*
[b]*SRI International, 333 Ravenswood Ave., Menlo Park, CA 94025-3493, USA*

Received 28 October 2004; revised 27 January 2005; accepted 28 January 2005

Abstract—Attachment of a glucose moiety to 6-β-aminomorphine afforded compound **3**, where the glucose moiety was linked to the C-6 nitrogen atom by a two-carbon bridge. The synthesis of **3** was accomplished in eight steps from 3-triisopropylsilyl-6-β-aminomorphine and 2,3,4,6-tetra-*O*-benzyl-D-glucose. The C-glycoside **3** was prepared with the objective of examining a metabolically stable analogue of morphine-6-glucuronide and determining the potency and selectivity of opioid receptor binding. Competition binding assays showed that **3** bound to the μ opioid receptor with a K_i value of 3.5 nM. The C-glycoside **3** exhibited δ/μ and κ/μ selectivity ratios of 76 and 165, respectively. The synthetic intermediate (i.e., benzyl precursor, compound **11**) bound to the μ opioid receptor with a K_i value of 0.5 nM, was less selective for the μ opioid receptor. The [^{35}S]GTPγS assay was used to evaluate the functional properties of compounds **3** and **11**. Compound **3** was determined to be a full agonist at the μ opioid receptor, whereas compound **11** was found to be a partial agonist. Compound **3** was determined to be very stable in the presence of human liver S9, and rat and monkey liver microsomes: no detectable loss of **3** was observed up to 90 min. Compound **3** was also very stable at pH 2 and pH 7.4, suggesting that **3** possessed properties for sustained duration of action.

Morphine-6-glucuronide (M6G) is a phase II metabolic conjugate of morphine with significantly greater analgesic potency than morphine.[1–3] M6G is currently in late stage clinical trials for the treatment of postoperative pain.[4] The oral bioavailability of M6G is only 11%[5] and improvement of the chemical and metabolic stability of M6G could possibly increase its effectiveness as a potential pain medication. A general strategy for improving the in vivo metabolic stability of glycoconjugates involves the replacement of the glycosidic oxygen atom with carbon, nitrogen, or sulfur atoms.[6,7] We previously employed this strategy in designing a library of glucosyl and glucuronosyl analogues of M6G in which the glycosidic oxygen atom was replaced with a sulfur atom.[8] The 6-β sulfur analogues of M6G showed modest improvement in μ opioid receptor affinity and functional efficacy, but showed less selectivity for μ versus δ and κ opioid receptors. Reported herein is an extension of this work detailing the results of an eight-step synthesis and in vitro biological and chemical evaluation of a novel, μ-selective, amide-linked carbon glycoside analogue of M6G (Fig. 1).

The C-β-glycopyranosyl acyl chloride **9** was prepared by a five step procedure from commercially available 2,3,4,6-tetrabenzylglucopyranose **4** (Scheme 1). Compound **4** was oxidized with DMSO/Ac_2O to provide the corresponding lactone **5** (94%).[9] Addition of **5** to a −78 °C THF solution of lithium ethyl acetate,[10] followed by acidic aqueous workup afforded the hemiketal **6**, that was formed by a stereoselective aldol reaction (91%).[11] The reduction of **6** with triethylsilane in the presence of $BF_3{\cdot}OEt_2$ in acetonitrile at 0 °C gave the known ethyl ester **7** (86%).[11,12] Hydrolysis of the ethyl ester group of **7** with LiOH in 1:1 THF/H_2O at reflux and subsequent acidic aqueous workup gave the carboxylic acid **8** (87%). The conversion of **8** to the corresponding acyl chloride **9** (100%, crude yield) was accomplished by stirring in neat thionyl chloride for 18 h. Addition of 1.5 equiv of the crude acid chloride **9** to 3-triisopropylsilyl-6-β-aminomorphine[13] in CH_2Cl_2 in the presence of 2.0 equiv of Et_3N gave the protected morphine glucose analogue **10** (93%). The triisopropylsilyl protecting

Keywords: Morphine-6-glucuronide; Pain; Morphine.
* Corresponding author. Tel.: +1 858 458 9305; fax: +1 858 458 9311; e-mail: jmacdougall@hbri.org

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.01.072

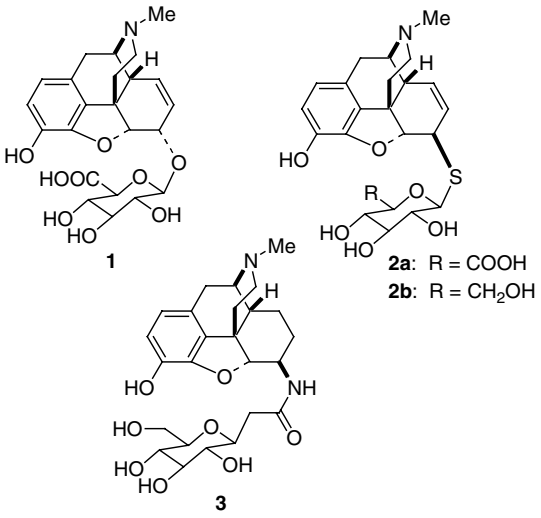


Figure 1. Compound **1** is M6G and **2** and **3** are metabolically stable analogs.

group of **10** was removed by the addition of TBAF in THF to give the phenol **11** (90%). Subsequent catalytic hydrogenation of **11** with activated 10% Pd/C in acidic methanol gave the amide-linked C-β-glycopyranoside analogue of M6G, compound **3** (52%). Hydrogenolysis of **11** with Pd/C in the absence of HCl resulted in the reduction of the phenanthrene carbon double bond and afforded **12** in quantitative yield, but did not result in hydrogenolysis of the glucose benzyl ether groups. Under these conditions, the basic morphinan nitrogen atom in **11** likely inhibited *O*-debenzylation.[14] The spectral data for all the synthetic compounds was in full agreement with the assigned structures or their literature values.[15]

The IC_{50} values obtained from competition binding assays with μ, δ, and κ opioid receptors for compounds **3** and **11** were converted into K_i values as described in the Experimental section. The K_i values for the test compounds and reference materials were listed in Table 1. 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 **3** and **11**.[16] The benzyl derivative **11** and the deprotected congener **3** were both μ receptor selective. Compared to M6G, compound **11** possessed 27-fold greater potency than M6G for the μ opioid receptor. The selectivity of compound **11** for the μ versus δ and μ versus κ receptors were 10-fold and 34-fold, respectively. Compared to M6G, compound **3** showed 3.7-fold greater potency for the μ opioid receptor. This result was consistent with previous studies that have shown that saturation of the 7,8-double bond provided analogues of M6G with increased analgesic potency.[17] The selectivity of compound **3** for the μ versus δ and μ versus κ receptors was 77- and 166-fold, respectively. It is noteworthy that the μ versus δ but not μ versus κ receptor selectivity of compound **3** was considerably improved relative to the value for M6G (i.e., 12.5- and 316-fold selectivity, respectively). Compound **3** showed slightly greater potency toward the μ receptor compared to thiosaccharides **2a** (2.5-fold) and **2b** (1.6-fold) and significantly improved δ/μ and κ/μ receptor selectivity ratios (Table 1).

The functional activity of compounds **3** and **11** was evaluated using the [^{35}S]GTPγS assay (Table 2).[18] The [^{35}S]GTPγS assay measures the ability of the test compound to activate the G protein associated with either the μ, δ, or κ opioid receptor. In this assay, the compound's potency or affinity for the receptor (defined by its EC_{50} for stimulating [^{35}S]GTPγS binding) was examined in vitro. Agonist efficacy (E_{max}) was defined as the degree to which the compound maximally stimulated [^{35}S]GTPγS binding relative to control. The EC_{50} value represented the concentration that produced 50% maximal stimulation of [^{35}S]GTPγS binding by that compound. Full agonists stimulated [^{35}S]GTPγS binding to a maximal extent and partial agonists caused a decreased level of binding. Based on the E_{max} values for stimulating [^{35}S]GTPγS binding, compound **3** was determined to be a full agonist at the μ and δ receptors and a partial agonist at the κ receptor. Compound **11** was determined to be a full agonist at the δ receptor and a partial agonist at the μ and κ receptors. The efficacy (E_{max}) of compound **3** at the μ receptor (75%) was substantially higher than M6G (45%) and the thiosaccharide analogues **2a** and **2b** (i.e., 46.6% and 36.0%, respectively).

In order to evaluate metabolic stability, test compounds **3**, **11**, and **12** were incubated with hepatic microsome preparations from rat (RLM) and monkey (MLM) supplemented with NADPH.[15] In addition, stability in the

Table 1. Competitive inhibition of μ, δ, and κ opioid receptors by compounds **2a**, **2b**, **3**, and **11**

Entry	K_i (nM) ± SEM[a]			Receptor selectivity	
	μ	δ	κ	δ/μ	κ/μ
M6G[b]	12.9 ± 0.9	170 ± 1	4060 ± 230	12.5	316
2a[b]	8.7 ± 0.9	31.4 ± 2.3	288 ± 12	3.6	33
2b[b]	5.4 ± 0.8	56.2 ± 2.2	136 ± 17	10.4	25.4
3	3.5 ± 0.4	266.3 ± 47.3	574.9 ± 7.8	76.7	166
11	0.5 ± 0.2	7.2 ± 1.2	15.8 ± 0.4	10.3	33.7

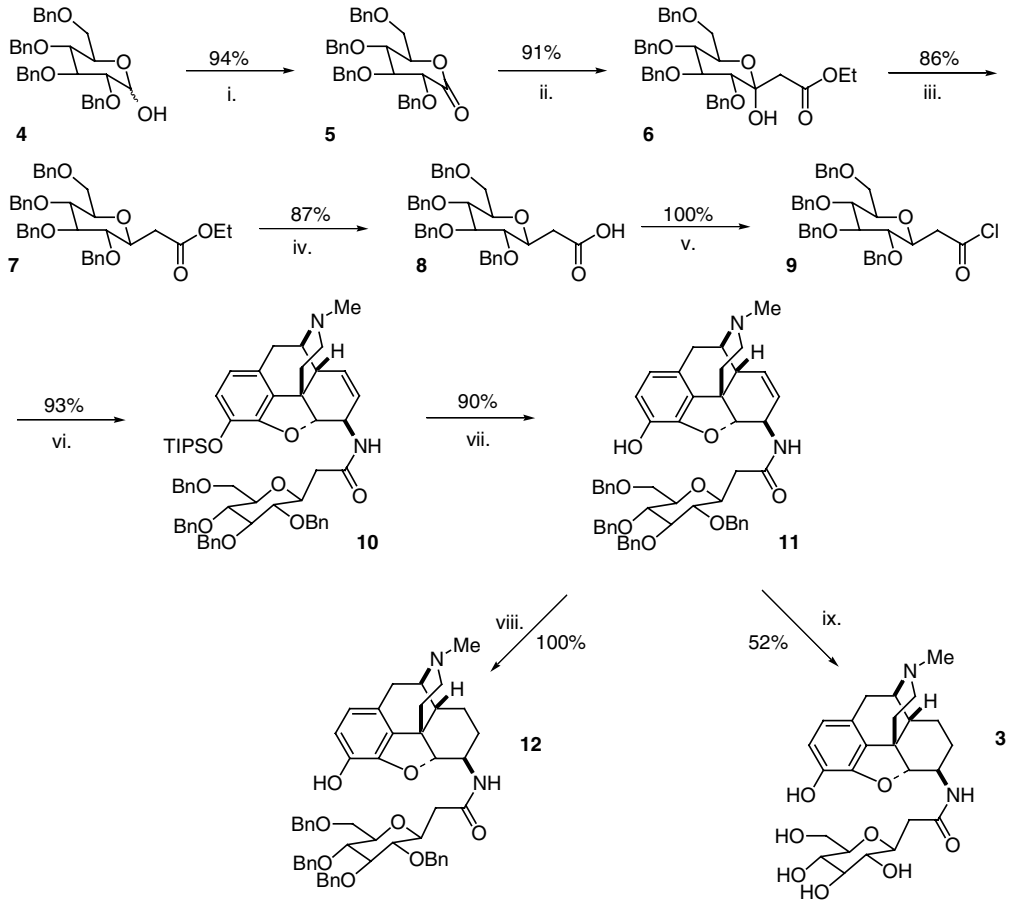
[a] SEM, standard error of the mean. Each value is the mean of at least three independent determinations ± SEM.
[b] Data taken from Ref. 6. The assay conditions used in the evaluation of test compounds **3** and **11** were the same as those used in the evaluation of M6G, **2a**, and **2b**.

Table 2. Stimulation of [^{35}S]GTPγ*S* binding by compounds **2a**, **2b**, **3**, and **11** mediated by the μ, δ, and κ opioid receptors

Entry	μ		δ		κ	
	EC_{50}[a]	E_{max}[b]	EC_{50}	E_{max}	EC_{50}	E_{max}
M6G[d]	72.3 ± 26.7	45.0 ± 5.0	190 ± 20	80.0 ± 1.0	>10 K	ND[c]
2a[d]	90.6 ± 22.9	46.6 ± 10.1	50.1 ± 36.7	78.7 ± 0.9	ND[c]	ND[c]
2b[d]	91.5 ± 23.4	64.5 ± 0.5	192 ± 15	51.5 ± 6.5	321 ± 93	42.5 ± 2.5
3	37.2 ± 0.5	75.2 ± 3.6	334.9 ± 123.2	73.9 ± 7.8	1717.5 ± 24.5	38.5 ± 5.5
11	622.6 ± 9.2	36.0 ± 11.2	2.2 ± 1.1	65.4 ± 1.9	1.8 ± 0.6	44.5 ± 16.3

[a] EC_{50} (nM): The EC_{50} value represents the concentration of compound that produced 50% stimulation of [^{35}S]GTPγS binding.
[b] E_{max} (% stimulation): Agonist efficacy is defined as the degree to which the compound maximally stimulates [^{35}S]GTPγS binding relative to control.
[c] ND: No detectable activity.
[d] Data from Ref. 6. The assay conditions used in the evaluation of **2a** and **2b** were the same as those used in the evaluation of M6G, **3**, and **11**.

presence of the post-mitochondrial supernatant from human liver (HLS9) was examined.[15] HPLC was used to evaluate the disappearance of starting material over time. The benzyl derivative **11** possessed half-lives of 24.6 and 30.7 min in the presence of RLM and MLM, respectively. Mass spectral analysis of metabolic extracts of **11** showed a prominent metabolite ion that was consistent with loss of a benzyl group. In the presence of HLS9, no detectable metabolism of **11** was observed in incubations run for 90 min. The C7/C8 dihydro derivative **12** possessed half-lives of 42.9 and 19.8 min in the presence of HLS9 and RLM, respectively, but was completely stable for 90 min in the presence of MLM. The glucose derivative **3** was quite stable in the presence of all three hepatic preparations from these species and no detectable loss of 3 was observed for 90 min. The chemical stability of **3**, **11**, and **12** was examined at pH 2.0 and pH 7.4 by HPLC analysis. No detectable loss of **3** was observed at pH 7.4. At pH 7.4, compounds **11** and **12** had half-lives of 1419 and 1903 min, respectively. At pH 2.0, compounds **3**, **11**, and **12** had half-lives of 3311, 1229, and 1900 min, respectively.



Scheme 1. Reagents and conditions: (i) $CH_3S(O)CH_3$, Ac_2O; (ii) $LiCH_2CO_2Et$, THF then HCl, −78 °C to rt; (iii) Et_3SiH, $BF_3{\cdot}OEt_2$,CH_3CN, 0 °C to rt; (iv) NaOH, H_2O, THF, reflux then HCl; (v) $SOCl_2$; (vi) 3-*O*-triisopropylsilyl-6-β-aminomorphine, Et_3N, CH_2Cl_2; (vii) Bu_4NF, THF, H_2O; (viii) H_2, 10% Pd–C, MeOH; (ix) H_2, 10% Pd–C, MeOH, HCl.

In summary, the C-glycoside **3** was prepared by an eight-step convergent synthesis from 2,3,4,6-tetra-*O*-benzyl-D-glucose, **4**, and 3-triisopropylsilyl-6-β-aminomorphine. Compound **3** showed a 3.7-fold greater affinity for the μ opioid receptor compared to M6G. The selectivity ratios of compound **3** for the δ versus μ and κ versus μ receptors were 76.7 and 166, respectively. The δ/μ selectivity for compound **3** (i.e., 76.7) was significantly improved relative to the value for M6G, which was 12.5. Compound **3** was quite stable in the presence of human liver S9, and rat and monkey liver microsomes supplemented with NADPH. Compound **3** was also very stable at pH 2 and pH 7.4. Together, the data suggests that **3** has the properties necessary for sustained pharmacological activity. Further work is in progress to investigate the biological properties of these compounds. Increasing the chemical and metabolic stability of M6G may provide a new class of longer-lived, potent opioid agonists with greater bioavailability.

Acknowledgments

We thank Andy Liu for synthesis of actone **5**.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2005.01.072.

References and notes

1. Osborne, R.; Thompson, P.; Joel, S.; Trew, D.; Patel, N.; Slevin, M. *Br. J. Clin. Pharmacol.* **1992**, *34*, 130–138.
2. Osborne, R.; Joel, S.; Trew, D.; Slevin, M. *Lancet* **1988**, *i*, 828.
3. Carrupt, P. A.; Testa, B.; Bechalany, A.; el Tayar, N.; Descas, P.; Perrissoud, D. *J. Med. Chem.* **1991**, *34*, 272–1275.
4. Gutman, A. L.; Nisnevitch, G.; Yudovitch, L.; Rokhman, I. U.S. Patent 6,737,518, 2004 and references cited therein.
5. Penson, R. T.; Joel, S. P.; Roberts, M.; Gloyne, A.; Beckwith, S.; Slevin, M. L. *Br. J. Clin. Pharmacol.* **2002**, *53*, 347–354.
6. Postema, M. H. D.; Piper, J. L. *Org. Lett.* **2003**, *5*, 1721–1723.
7. Kiefel, M. J.; Thomson, R. T.; Radocanovi, M.; von Itzstein, M. *J. Carbohydr. Chem.* **1999**, *18*, 937–959.
8. MacDougall, J. M.; Zhang, X.-D.; Polgar, W. E.; Khroyan, T. V.; Toll, L.; Cashman, J. R. *J. Med. Chem.* **2004**, *47*, 5809–5815.
9. Kuzuhara, H.; Fletcher, H. G. *J. Org. Chem.* **1967**, *32*, 2531–2534.
10. Rathke, M. W. *J. Am. Chem. Soc.* **1970**, *92*, 3222–3223.
11. Lewis, M. D.; Cha, J. K.; Kishi, Y. *J. Am. Chem. Soc.* **1982**, *104*, 4976–4978.
12. Tiedemann, R.; Turnbull, P.; Moore, H. W. *J. Org. Chem.* **1999**, *64*, 4030–4041.
13. MacDougall, J. M.; Zhang, X.-D.; Polgar, W. E.; Khroyan, T. V.; Toll, L.; Cashman, J. R. *Bioorg. Med. Chem.* **2004**, *12*, 5983–5990.
14. Czech, B. P.; Bartsch, R. A. *J. Org. Chem.* **1984**, *49*, 4076–4078.
15. New compounds **3**, **10**, **11**, and **12** showed satisfactory ^{1}H NMR, ^{13}C NMR, and MS data. Analytical purity for compound **11** was determined by straight phase HPLC using a Hitachi L74 liquid chromatograph with a L-7400 UV–vis detector (254 nm), a D7500 integrator, and an Axxi-chrom silica column (250 mm × 4.6 mm, i.d.) (Richard Scientific, Novato, CA). For determination of analytical purities a mobile phase A = 60/40/0.02, MeOH/2-propanol/$HClO_4$ (v/v) or mobile phase B = 55/45/0.01, MeOH/2-propanol/$HClO_4$ (v/v) was used. The average purity of **11** was found to be ⩾99% by analytical HPLC giving t_R = 3.24 min (mobile phase A) and t_R = 4.66 min (mobile phase B). The purity of **3** was determined with straight phase HPLC as described above. The purity of **3** was found to be ⩾98% by analytical HPLC giving t_R = 3.41 min (mobile phase A) and t_R = 3.74 min (mobile phase B). Metabolic stability was evaluated by incubating the test compound (0.4 mM) in the presence of MLM or HLM (1.2 mg/mL) or HLS9 (3.2 mg/mL), 50 mM potassium phosphate buffer (pH 7.4), 0.5 mM $NADP^+$, 0.5 mM glucose-6-phosphate, 5 IU/mL glucose-6-phosphate dehydrogenase, 1 mg/mL diethylenetriaminepentaacetic acid, and 7 mM $MgCl_2$ (0.1 mL total volume). Each time course incubation was run for 0, 10, 20, 30, 60, or 90 min at 37 °C. For compounds **11** and **12**, the reaction was stopped at the appropriate time by the addition of CH_2Cl_2/2-propanol (1 mL, 3:1 v/v), 10 mg Na_2CO_3 and mixed thoroughly. After centrifugation to separate the layers, the organic solvent was removed under a stream of argon. MeOH (0.2 mL) was added to the residue and the resulting material was thoroughly mixed, centrifuged, and an aliquot was analyzed by HPLC using the conditions described above. The extraction efficiency for **11** and **12** was calculated to be 85%. For compound **3**, a solid phase extraction procedure was used. The metabolic incubations were stopped by the addition of cold Na_2CO_3 (0.02 mL, 2 M) and centrifuged. The supernatant was placed on an Oasis solid phase extraction cartridge (Waters Corp., Bedford, MA), washed with H_2O (0.7 mL), followed by 5:95 MeOH/H_2O (0.7 mL) and eluted with 80:20 CH_3CN/MeOH (0.7 mL). The organic solvent was removed under a stream of argon. To the residue MeOH (0.2 mL) was added and the resulting material was analyzed by HPLC using the reverse phase conditions described above. The extraction efficiency was 45%. Selected metabolic extracts were also analyzed with an M-8000 ion trap mass spectrometer (Hitachi, San Jose, CA) equipped with an electrospray ionization source. Sample delivery was carried out in the flow injection analysis mode using a LaChrom separation module with an isocratic solvent system of MeOH/water/HCOOH (50:50:0.17, v/v) running at 0.2 mL/min.
16. Zaveri, N.; Polgar, W.; Olsen, C.; Kelson, A. B.; Grundt, P.; Lewis, J. W.; Toll, L. *Eur. J. Pharmacol.* **2001**, *428*, 29–36.
17. Stachulski, A. V.; Scheinmann, F.; Ferguson, J. R.; Law, J. L.; Lumbard, K. W.; Hopkins, P.; Patel, N.; Clarke, S.; Gloyne, A.; Joel, S. P. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1207–1214.
18. Traynor, J. R.; Nahorski, S. R. *Mol. Pharmacol.* **1995**, *47*, 848–854.