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ORIGINAL PAPER

Glucosides of morphine derivatives: synthesis and characterization

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Abstract Six 3-*O*- and 6-*O*-glucosides of morphine and codeine derivatives were synthesized by means of glucosylation with acetobromo-α-D-glucose. O-Glucosylation at C6 was carried out by the Koenigs-Knorr method, whereas the 3-*O*-glycoside of morphine was synthesized directly upon stirring morphine with acetobromo-α-D-glucose and aqueous sodium hydroxide in acetone. Complete ^{1}H and ^{13}C NMR assignments are presented for each synthesized compound based on one- and two-dimensional homo- and heteronuclear NMR techniques. Circular dichroism, ultraviolet absorbance, and high-resolution mass spectroscopy data ensure identification and structural characterization of the *O*-glucoside conjugates. The synthesized glucoside conjugates are potential analgesics; the presented spectral and chromatographic data are useful references for various analytical and metabolic studies including samples of biological origin.

Keywords Morphine · Glucoside · NMR spectroscopy · Alkaloids · High-pressure liquid chromatography

Introduction

Morphine, the principal drug of the opioid family, is among the most important agents used for the treatment of pain [1, 2]. Morphine is primarily metabolized by microsomal enzymes in the liver of animals and humans through N-dealkylation, oxidation, and conjugation. Some of the naturally occurring metabolites are pharmacologically active, contributing to the overall analgesic effect [3]. Glucuronide and sulfate conjugates of morphine and its congeners have long been extensively studied. Their pharmacological importance and physicochemical properties are therefore well documented [4–8].

A more recent paper discusses the separation and identification of morphine C3 and C6 *O*-glucoside conjugates in the urine of cancer patients receiving oral morphine treatment. The glucosides were identified by means of high-performance liquid chromatography (HPLC) coupled with mass spectrometry and enzyme digestion [9]. Glucoside conjugates of xenobiotics are rarely found in humans; there are only a limited number of publications on this field [10–16]. Since the physicochemical attributes and the possible clinical relevance of morphine glucosides have not been studied in detail, there is a need to synthesize and characterize glucoside analogous to the identified naturally occurring metabolites. Development of an efficient method for the separation of morphine glucosides and structurally similar conjugates is also necessary. Furthermore, glycosides of pharmacologically important drugs have been shown to possess improved absorption profiles and bioavailability [17–19].

The methods for the synthesis of morphine glycosides have been reviewed. 6-*O*-β-Glucosides were prepared by means of Koenigs-Knorr reaction of the appropriate hydroxyl group of the morphine derivative with the glucose donor acetobromo-α-D-glucose or 2,3,4,6-tetra-*O*-benzoyl-α-D-bromoglucose in the presence of a transition metal activator, usually silver carbonate, followed by the removal of *O*-acyl groups by alkaline hydrolysis [20–23]. Spectroscopic data for the synthesized glucoside conjugates are either missing or incomplete. 6-*O*-α-Rhamnoside of morphine was synthesized by the reaction of 3-*O*-pivaloylmorphine and

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2,3,4,6-tetra-*O*-acetyl-α-rhamnopyranose mediated by trimethylsilyl trifluoromethanesulfonate followed by basic hydrolysis of the esters [24]. Morphine-6-*O*-α-D-mannoside was synthesized by three different reaction pathways. The molecule mimics the pharmacological profile of the natural metabolite morphine-6-*O*-glucuronide. Its analgesic activity 100 times exceeds that of morphine, and it showed markedly fewer side effects in animal models [25].

This article focuses on the synthesis, NMR spectroscopic analysis, and HPLC separation of C3 and C6 *O*-glucoside conjugates of morphine congeners. Selected compounds were studied by circular dichroism (CD) and UV/VIS spectroscopy as well. Most of the reported compounds are novel chemical entities. The presented data may serve as the basis for future pharmacological assays and a powerful tool for the separation and identification of glucoside metabolites in biological samples. The novel glucosides are potential pharmacologically active antinociceptive substances with favorable water solubility.

Results and Discussion

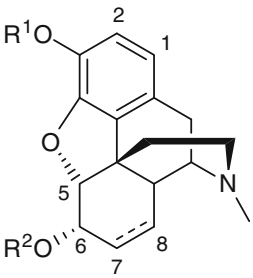
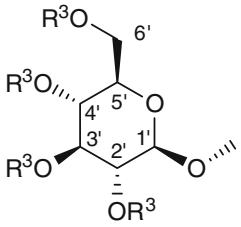
Table 1 lists the synthesized morphine and codeine derivatives. Acetobromo-α-D-glucose was used as glucose donor. O-Glucosylation at C6 was carried out by the Koenigs-Knorr method. Removal of the acetyl protecting groups was achieved upon stirring the tetraacetyl derivatives with a small excess of aqueous lithium hydroxide in methanol [26, 29]. The C3 phenolic hydroxyl group of morphine and dihydromorphine was selectively acetylated prior to the glycosylation step to ensure regioselectivity [30].

The 3-*O*-glucoside of morphine (**3**) was synthesized directly by a modified version of the O-glycosylation reaction of oxyisoflavones upon stirring morphine with acetobromo-α-D-glucose in acetone in the presence of sodium hydroxide and water [31]. The 3-*O*-glycoside of dihydromorphine (**8**) was obtained from tetraacetyl-3-*O*-glucopyranosylmorphine (**2**) by saturation of the Δ7-8 double bond by means of catalytic hydrogenation, since direct C3 O-glucosylation of dihydromorphine afforded the desired product only in low yield. The crude acetylated glucopyranosyl derivatives were purified using column chromatography. The pure product fractions were collected and crystallized from ethanol or diethyl ether (Schemes 1, 2). In summary, six glucoside conjugates were synthesized, including novel chemical entities (**3**, **8**, **10**, and **15**).

Chromatography

Opiate analgesics and their metabolites are preferably screened by chromatographic methods, especially HPLC [32]. Previously, chromatographic resolvation of morphine

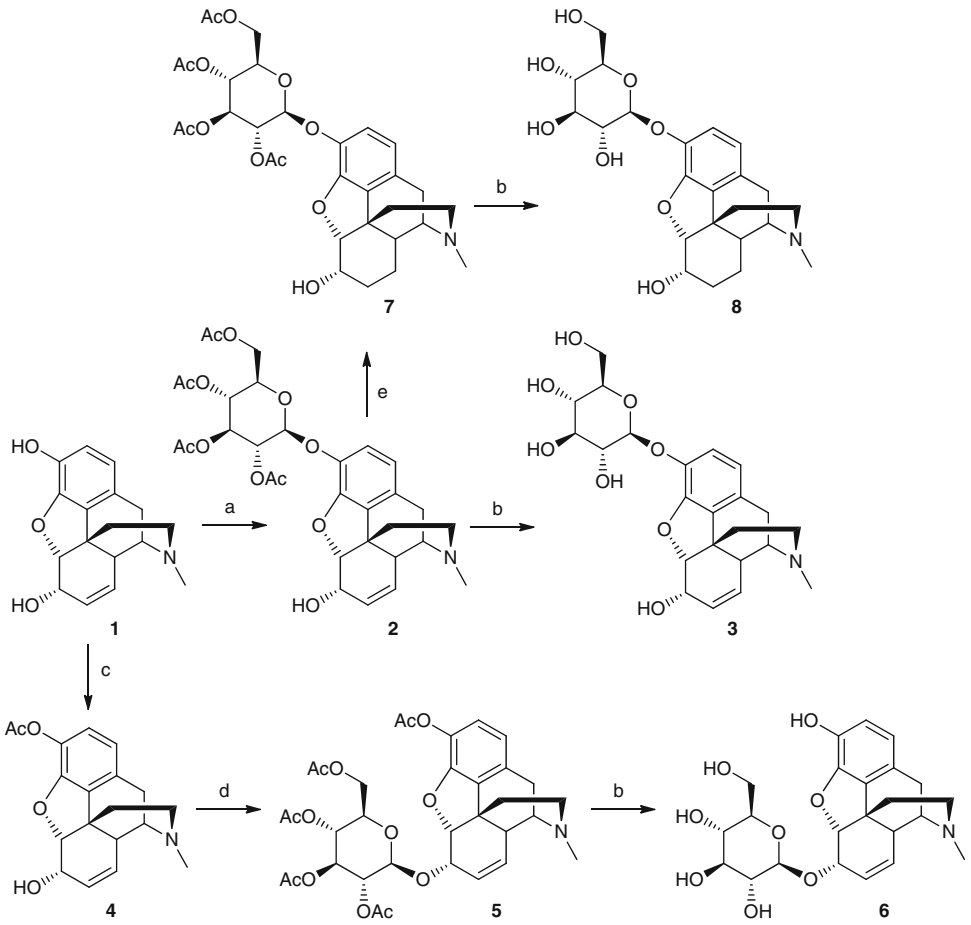
Table 1 Structure and physicochemical data of the synthesized compounds

Compound		R^1 [a]	R^2 [a]	R^3	C7–C8 bond	R_f [b]
	**2**	Glu	H	Acetyl	Double	
	3	Glu	H	H	Double	0.23
	5	Acetyl	Glu	Acetyl	Double	
	6	H	Glu	H	Double	0.30
	7	Glu	H	Acetyl	Single	
	8	Glu	H	H	Single	0.23
	**9**	Acetyl	Glu	Acetyl	Single	
	10	H	Glu	H	Single	0.29
	12	CH_3	Glu	Acetyl	Double	
	13	CH_3	Glu	H	Double	0.40
	14	CH_3	Glu	Acetyl	Single	
	15	CH_3	Glu	H	Single	0.40

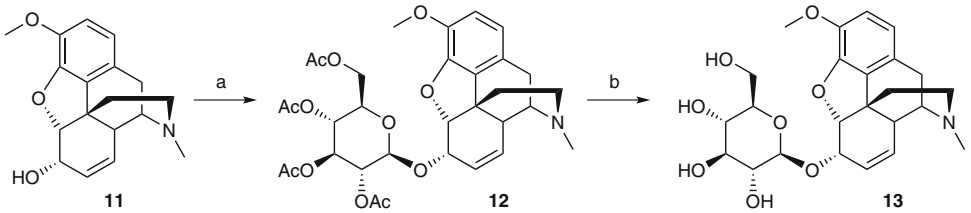
[a] Glu = β-D-glucoside

[b] TLC

Scheme 1



Scheme 2



glucoside and glucuronide conjugates could only be achieved by a tandem combination of C18 and amino HPLC columns [9]. In order to achieve rapid separation of morphine and its less lipophilic metabolites in a single run, the use of a porous graphitized carbon (PGC) column is preferred. The order of retention is irrespective of lipophilicity. Rather, it is controlled by charge interactions on the analyte and the graphite surface. Separation of morphine and its metabolites has previously been carried out by liquid chromatography using a PGC column. That study, however, did not take into account the possible existence of morphine glucoside metabolites [33]. We report the efficient separation of glucoside and glucuronide conjugates of morphine and codeine and their parent compounds using HPLC with PGC stationary phase (Fig. 1). Retention data are shown in Table 2 in conjunction with CD and UV/VIS spectral information.

Optimal retention times and baseline resolution for all chromatography peaks could be achieved by a single isocratic run. The reported HPLC system can be utilized for the rapid analysis of biological samples containing glucoside metabolites of opiates.

Spectroscopy

In the NMR spectrum of all compounds values of coupling constant $J_{1'-2'}$ (7.0–8.0 Hz) show that H-1′ hydrogen atoms are in equatorial position; hence, all of the synthesized glucosides are β anomers. There is a 0.4 ppm upfield shift for H-1 and H-2 aromatic protons in the ^{1}H NMR spectra of

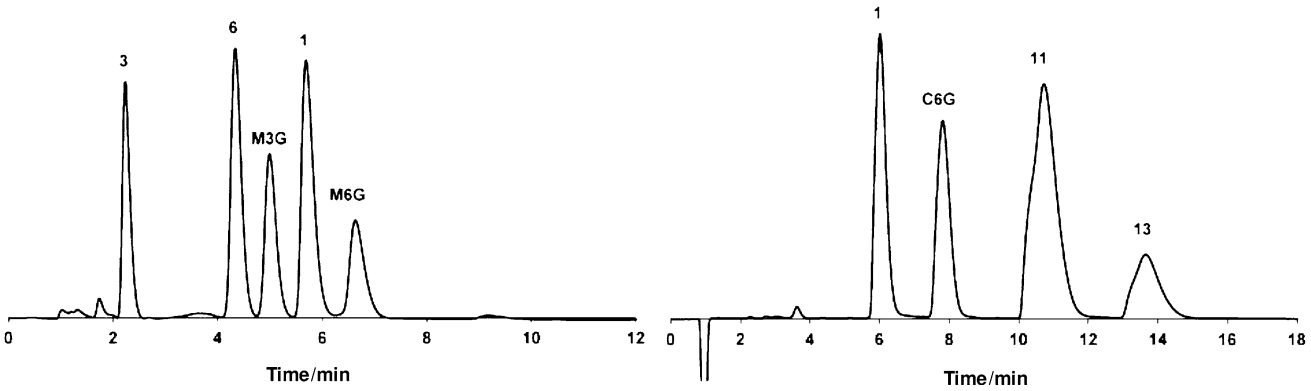


Fig. 1 Chromatograms of the separations: *M3G* morphine-3-*O*-glucuronide, *M6G* morphine-6-*O*-glucuronide, *C6G* codeine-6-*O*-glucuronide

Table 2 HPLC retention and CD/UV spectral data of the compounds studied

Comp.	Retention time/min	Capacity factor/k	Resolution/R_s	CD absorption bands/nm			UV/VIS absorption bands/nm		
				^1L_b	^1L_a	^1B			
3	2.23	1.20		−285.0	242.8	−214.0	282.0	246.0	210.2
6	4.33	3.26	6.61	304.8	252.4	−226.0	297.4	261.8	214.0
M3 G[a]	4.99	3.91	1.73	−286.2	243.4	−215.8	282.2	261.4	211.2
1	5.69	4.60	1.71	−287.6	245.4	−218.2	286.8	250.1	210.8
M6 G[b]	6.63	5.52	2.05	−286.8	245.4	−218.0	284.6	247.6	209.8
1	6.02	5.02		−287.6	245.4	−218.2	286.8	250.1	210.8
C6G[c]	7.82	6.82	2.77	−287.2	246.8	−218.8	286.6	251.6	212.2
11	10.73	9.73	2.80	−287.8	247.4	−218.0	286.2	251.9	211.2
13	13.65	12.65	2.11	−287.4	246.8	−219.0	287.0	250.0	212.2

[a] Morphine-3-*O*-glucuronide

[b] Morphine-6-*O*-glucuronide

[c] Codeine-6-*O*-glucuronide

6-*O*-glucosides of morphine (**6**) and dihydromorphine (**10**). Furthermore, intensive bathochromic shifts for all absorption bands compared to those of **1** in the CD and UV spectra, along with the positive Cotton effect at the ^1L_b band in the CD spectrum of **6**, were detected (Fig. 2; Table 2).

None of these differences could be observed in the spectra of the other compounds studied, suggesting the possible existence of a hydrogen bond between the C3 phenolic hydroxyl and a hydroxyl group on the glucose moiety that alters the local electron distribution in the molecules.

Experimental

The reagents were purchased from Sigma-Aldrich and Alfa Aesar and used without further purification. Solvents were freshly distilled prior to use and were dried over anhydrous Na_2SO_4. HPLC-grade solvents purchased from Merck were used for chromatography. Ultra-pure water was prepared by a Milli-Q Direct 8 Millipore system. Morphine-6-*O*-glucuronide, codeine-6-*O*-glucuronide and morphine-3-*O*-glucuronide were synthesized according to the method desccribed in the literature [26–28].

^1H and ^{13}C NMR spectra were recorded on a 600-MHz Varian VNMRS spectrometer in DMSO-d_6 or chloroform-d_1 solutions; δ is given in ppm relative to Me_4Si as internal standard. ^1H and ^{13}C NMR signals were assigned on the basis of one- and two-dimensional homo- and heteronuclear experiments (COSY, TOCSY, HMBC and HSQC). Melting points were taken on a Stuart SMP-3 apparatus. The high-resolution accurate masses were determined with an Agilent 6230 time-of-flight mass spectrometer. Samples were introduced by an Agilent 1260 Infinity LC system, and the mass spectrometer was operated in conjunction with a Jet Stream electrospray ion source in positive ion mode. Reference masses of $m/z = 121.050873$ and 922.009798 were used to calibrate the mass axis during analysis. Mass spectra were processed using Agilent MassHunter B.02.00 software. CD and UV spectra were

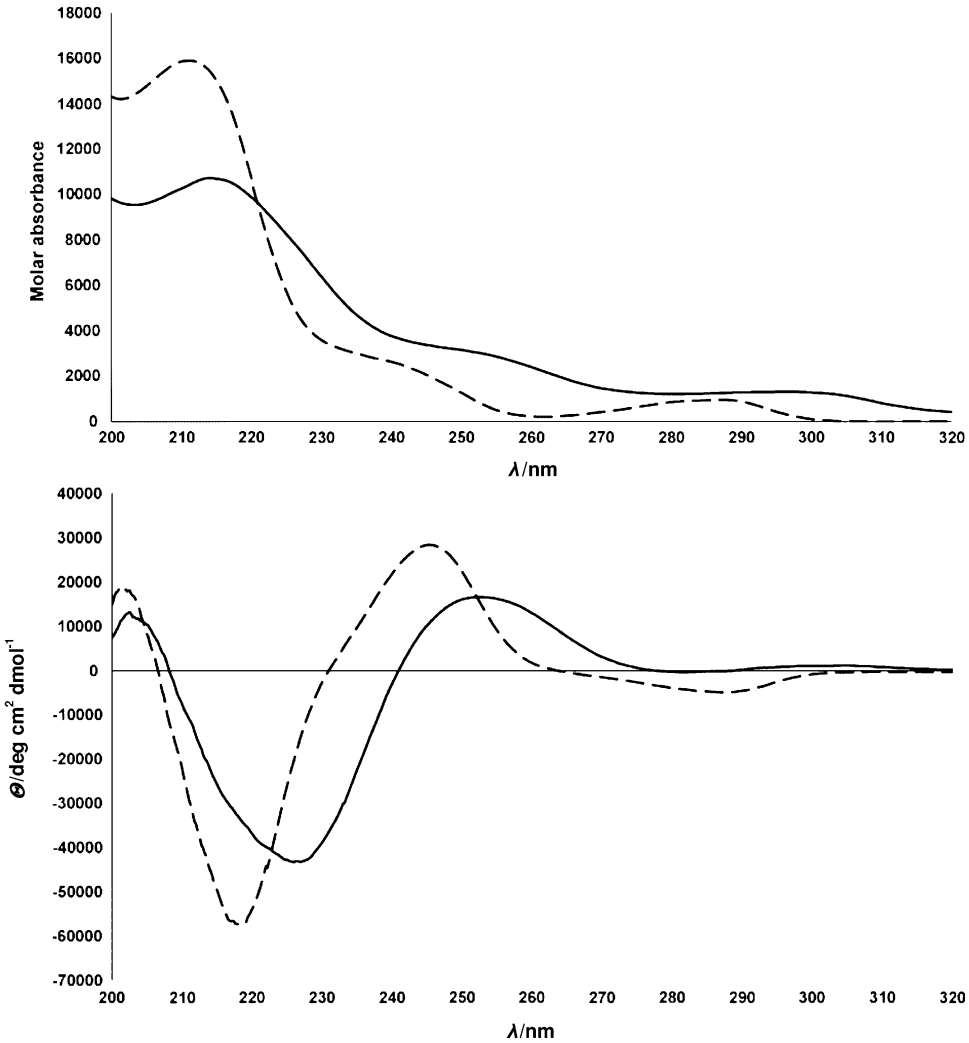


Fig. 2 UV and CD absorption spectra of **1** (*dashed line*) and **6** (*solid line*)

registered on a Jasco J-720 spectropolarimeter. The HPLC consisted of a Jasco PU-980 Intelligent HPLC pump with a Rheodyne 7725i injector and a Jasco UV-975 Intelligent UV/VIS detector. Stationary phase was a Hypercarb (100 × 4.6 mm, 5 μm) column purchased from Thermo Fischer Scientific.

Chromatography

Analyte solutions for HPLC and CD/UV were prepared by dissolving 1 mg of each substance in 10 cm^3 acetonitrile. Injection volume: 20 mm^3. Mobile phase: methanol 43 v/v %; acetonitrile 17 v/v %; aqueous ammonium acetate/ammonia buffer (pH 9.2) 40 v/v %. Ammonium acetate concentration was 0.1 mol dm^{-3}. The separations were performed isocratically; eluent flow rate was 1.5 cm^3 min^{-1}. UV/VIS detector wavelength was set to 220 nm. The temperature was set to 25 °C. Hold-up time (t_R) was taken as the retention time of the solvent disturbance peak. Chromatograms were registered by Borwin chromatography software (v. 1.21). Retention parameters were calculated according to the definitions of European Pharmacopoeia 7. Thin layer chromatography: mobile phase: 10:25:25:50 mixture of water, methanol, cc acetic acid and dichloroethane. Stationary phase: Merck Silica Gel 60 F254. Spray solution for visualization: thymol (0.5 g) dissolved in a mixture of 5 cm^3 cc sulfuric acid and 95 cm^3 ethanol. TLC plates were heated for 15 min at 130 °C after spraying.

General method for Koenigs-Knorr glucosylation: (5α,6α)-7,8-didehydro-4,5-epoxy-3-methoxy-17-methyl-morphinan-6-yl-β-D-2,3,4,6-tetraacetylglucopyranoside (tetraacetyl-6-O-glucopyranosylcodeine) (**12**, $C_{32}H_{39}NO_{12}$) Codeine (**11**, 1.00 g, 3.34 mmol) was dissolved in 150 cm^3 dry benzene, and 2.50 g Ag_2CO_3 was added. The mixture was vigorously stirred, and 2.80 g acetobromo-α-D-glucose (6.81 mmol) was added in small portions over 3 h. The resulting slurry was refluxed for 20 h. The precipitate was filtered and the filtrate evaporated to dryness under reduced pressure. The crude product was purified using silica gel

column chromatography with a chloroform/methanol gradient mixture from 95:5 to 90:10 as eluent. Codeinone, resulting from the simultaneous oxidation reaction by Ag_2CO_3 present in the reaction mixture of the Koenigs-Knorr glucosylation of **11**, could be isolated from crude **12**. The pure product fractions were collected and recrystallized from hot ethanol to yield 0.50 g of **12** (32 %) as white crystals. M.p.: 196–197 °C; ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 6.52$ (d, $J = 8.1$ Hz, H-2), 6.63 (d, $J = 8.1$ Hz, H-1), 5.69 (d, $J = 9.5$ Hz, H-7), 5.32 (d, $J = 9.5$ Hz, H-8), 5.27 (t, $J = 9.4$ Hz, H-3′), 5.12 (t, $J = 9.4$ Hz, H-4′), 5.07 (dd, $J = 9.4$, 7.5 Hz, H-2′), 4.90 (d, $J = 6.0$ Hz, H-5), 4.90 (d, $J = 7.5$ Hz, H-1′), 4.32 (d, $J = 6.0$ Hz, H-6), 4.28 and 4.16 (2 dd, $J = 15.0$ Hz, H-6′), 3.77 (ddd, $J = 4.5$, 2.5 Hz, H-5′), 2.16 (s, 3H, 2′-acetyl), 2.08 (s, 3H, 6′-acetyl), 2.03 (s, 3H, 4′-acetyl), 2.02 (s, 3H, 3′-acetyl) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 130.5$ (CH), 128.8 (CH), 118.9 (CH), 113.4 (CH), 98.2 (CH), 88.3 (CH), 72.8 (CH), 72.1 (CH), 71.9 (CH), 71.2 (CH), 68.6 (CH), 62.1 (CH_2), 20.8 (CH_3), 20.6 (CH_3), 20.6 (CH_3), 20.5 (CH_3) ppm; HRMS: *m/z* calcd. for $[M + H]^+$ ($[C_{32}H_{40}NO_{12}]^+$) 630.2541, found 630.2535.

(5α,6α)-3-Acetyloxy-7,8-didehydro-4,5-epoxy-17-methylmorphinan-6-yl-β-D-2,3,4,6-tetraacetylglucopyranoside (tetraacetyl-6-O-glucopyranosyl-3-O-acetylmorphine) (**5**, $C_{33}H_{39}NO_{13}$)

Yield: 34 %; m.p.: 99–100 °C; ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 6.73$ (d, $J = 8.0$ Hz, H-2), 6.55 (d, $J = 8.0$ Hz, H-1), 5.70 (d, $J = 9.4$ Hz, H-7), 5.29 (d, $J = 9.4$ Hz, H-8), 5.24 (t, $J = 9.5$ Hz, H-3′), 5.12 (t, $J = 9.5$ Hz, H-4′), 5.08 (dd, $J = 9.5$, 7.7 Hz, H-2′), 4.88 (d, $J = 6.0$ Hz, H-5), 4.82 (d, $J = 7.7$ Hz, H-1′), 4.26 and 4.15 (2 dd, $J = 15.3$ Hz, H-6′), 4.23 (d, $J = 6.0$ Hz, H-6), 3.76 (ddd, $J = 4.5$, 2.4 Hz, H-5′), 2.31 (s, 3H, 3-acetyl), 2.10 (s, 3H, 2′-acetyl), 2.08 (s, 3H, 4′-acetyl), 2.05 (s, 3H, 6′-acetyl), 2.03 (s, 3H, 3′-acetyl) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 130.7$ (CH), 128.7 (CH), 121.9 (CH), 119.2 (CH), 99.9 (CH), 89.8 (CH), 74.1 (CH), 72.8 (CH), 71.9 (CH), 71.2 (CH), 68.4 (CH), 62.0 (CH_2), 20.7 (4 CH_3), 20.7 (CH_3) ppm; HRMS: *m/z* calcd. for $[M + H]^+$ ($[C_{33}H_{40}NO_{13}]^+$) 658.2494, found 658.2487.

(5α,6α)-3-Acetyloxy-4,5-epoxy-17-methylmorphinan-6-yl-β-D-2,3,4,6-tetraacetylglucopyranoside (tetraacetyl-6-O-glucopyranosyl-3-O-acetyldihydromorphine) (**9**, $C_{33}H_{41}NO_{13}$)

Yield: 32 %; ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 6.81$ (d, $J = 7.8$ Hz, H-2), 6.63 (d, $J = 7.8$ Hz, H-1), 5.19 (t, $J = 9.5$ Hz, H-3′), 5.07 (dd, $J = 9.5$, 7.7 Hz, H-2′), 4.90 (t, $J = 9.5$ Hz, H-4′), 4.76 (d, $J = 7.7$ Hz, H-1′), 4.67 (d, $J = 6.0$ Hz, H-5), 4.23 and 4.13 (2 dd, $J = 15.0$ Hz, H-6′), 3.96 (d, $J = 6.0$ Hz, H-6), 3.75 (ddd, $J = 4.5$, 2.5 Hz, H-5′), 2.32 (s, 3H, 3-acetyl), 2.13 (s, 3H, 2′-acetyl), 2.07 (s, 3H, 6′-acetyl), 2.02 (s, 3H, 4′-acetyl), 1.99 (s, 3H, 3′-acetyl) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 122.8$ (CH), 119.1 (CH), 99.1 (CH), 88.6 (CH), 74.7 (CH), 72.5 (CH), 71.6 (CH), 71.2 (CH), 68.4 (CH), 62.9 (CH_2), 20.7 (4 CH_3), 20.6 (CH_3) ppm; HRMS: *m/z* calcd. for $[M + H]^+$ ($[C_{33}H_{42}NO_{13}]^+$) 660.2651, found 660.2648.

(5α,6α)-4,5-Epoxy-3-methoxy-17-methylmorphinan-6-yl-β-D-2,3,4,6-tetraacetylglucopyranoside (tetraacetyl-6-O-glucopyranosyldihydrocodeine) (**14**, $C_{32}H_{41}NO_{12}$)

Yield: 47 %; m.p.: 190–192 °C; ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 6.69$ (d, $J = 8.0$ Hz, H-2), 6.58 (d, $J = 8.0$ Hz, H-1), 5.18 (t, $J = 9.5$ Hz, H-3′), 5.08 (t, $J = 9.5$ Hz, H-4′), 4.82 (dd, $J = 9.5$, 7.5 Hz, H-2′), 4.91 (d, $J = 7.5$ Hz, H-1′), 4.65 (d, $J = 6.0$ Hz, H-5), 4.25 and 4.14 (2 dd, $J = 15.1$ Hz, H-6′), 4.09 (d, $J = 6.0$ Hz, H-6), 3.77 (ddd, $J = 4.4$, 2.5 Hz, H-5′), 2.08 (s, 3H, 6′-acetyl), 2.07 (s, 3H, 2′-acetyl), 2.02 (s, 3H, 4′-acetyl), 1.98 (s, 3H, 3′-acetyl) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 118.6$ (CH), 113.6 (CH), 98.4 (CH), 88.1 (CH), 72.9 (CH), 72.5 (CH), 71.7 (CH), 71.6 (CH), 68.7 (CH), 62.2 (CH_2), 20.8 (CH_3), 20.7 (CH_3), 20.7 (CH_3), 20.6 (CH_3) ppm; HRMS: *m/z* calcd. for $[M + H]^+$ ($[C_{32}H_{42}NO_{12}]^+$) 632.2702, found 632.2698.

(5α,6α)-7,8-Didehydro-4,5-epoxy-6-hydroxy-17-methylmorphinan-3-yl-β-D-2,3,4,6-tetraacetylglucopyranoside (tetraacetyl-3-O-glucopyranosylmorphine) (**2**, $C_{31}H_{37}NO_{12}$)

Morphine (**1**, 2.80 g, 9.82 mmol) was suspended in 40 cm^3 acetone. Acetobromo-α-D-glucose (5.00 g, 12.16 mmol) and 6 cm^3 2 M aqueous NaOH solution were added and stirred for 24 h at room temperature. The precipitate was filtered, and the filtrate was evaporated to dryness under reduced pressure, suspended in water and basified (pH 9) with 10 % aqueous NaOH. After extraction with chloroform and evaporation of the solvent, the crude product was purified using silica gel column chromatography with a chloroform/methanol isocratic mixture of 80:20 as eluent. The pure fractions were collected and crystallized from diethyl ether to yield 0.76 g of **2** (12 %) as white crystals. M.p.: 160–161 °C; ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 6.77$ (d, $J = 8.0$ Hz, H-2), 6.53 (d, $J = 8.0$ Hz, H-1), 5.67 (d, $J = 9.4$ Hz, H-7), 5.28 (t, $J = 9.4$ Hz, H-3′), 5.27 (d, $J = 9.4$ Hz, H-8), 5.25 (d, $J = 7.5$ Hz, H-1′), 5.20 (t, $J = 9.4$ Hz, H-4′), 5.16 (dd, $J = 9.4$, 7.5 Hz, H-2′), 4.87 (d, $J = 6.0$ Hz, H-5), 4.17 (d, $J = 6.0$ Hz, H-6), 4.27 and 4.19 (2 dd, $J = 15.0$ Hz, H-6′), 3.84 (ddd, $J = 4.5$, 2.5 Hz, H-5′), 2.08 (s, 3H, 4′-acetyl), 2.05 (s, 3H, 6′-acetyl), 2.03 (s, 3H, 2′-acetyl), 2.03 (s, 3H, 3′-acetyl) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 133.8$ (CH), 128.4 (CH), 120.2 (CH), 119.7 (CH), 100.1 (CH), 91.8 (CH), 72.8 (CH), 72.4 (CH), 71.9 (CH), 68.5 (CH), 66.4 (CH), 62.0 (CH_2), 20.8 (CH_3), 20.7 (3 CH_3) ppm; HRMS: *m/z* calcd. for $[M + H]^+$ ($[C_{31}H_{38}NO_{12}]^+$) 616.2389, found 616.2382.

(5α,6α)-4,5-Epoxy-6-hydroxy-17-methylmorphinan-3-yl-β-D-2,3,4,6-tetraacetylglucopyranoside (tetraacetyl-3-O-glucopyranosyldihydromorphine) (**7**, $C_{31}H_{39}NO_{12}$)
Tetraacetyl-3-*O*-glucopyranosylmorphine (**2**, 0.90 g, 2.00 mmol) was dissolved in 40 cm^3 ethanol, and 0.20 g Pd/C catalyst was added. The mixture was shaken under hydrogen atmosphere using a shaker device. After the completion of the reaction the catalyst was filtered and washed with ethanol, and the filtrate was evaporated to dryness under reduced pressure. The remaining semi-crystalline solid was recrystallized from diethyl ether to yield 0.70 g of **7** (78 %) as white crystals. M.p.: 115–117 °C; ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 6.89$ (d, $J = 8.1$ Hz, H-2), 6.69 (d, $J = 8.1$ Hz, H-1), 5.59 (d, $J = 7.6$ Hz, H-1′), 5.31 (t, $J = 9.5$ Hz, H-3′), 5.18 (t, $J = 9.5$ Hz, H-4′), 5.15 (dd, $J = 9.4$, 7.6 Hz, H-2′), 4.62 (d, $J = 6.0$ Hz, H-5), 4.14 (d, $J = 6.0$ Hz, H-6), 4.25 and 4.05 (2 dd, $J = 15.0$ Hz, H-6′), 3.90 (ddd, $J = 4.5$, 2.5 Hz, H-5′), 2.08 (s, 3H, 2′-acetyl), 2.04 (s, 3H, 6′-acetyl), 2.04 (s, 3H, 2′-acetyl), 2.04 (s, 3H, 3′-acetyl) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 120.8$ (CH), 119.6 (CH), 99.1 (CH), 89.2 (CH), 72.2 (CH), 72.0 (CH), 71.3 (CH), 68.0 (CH), 66.0 (CH), 61.8 (CH_2), 20.8 (CH_3), 20.6 (3 CH_3) ppm; HRMS: *m/z* calcd. for $[M + H]^+$ ($[C_{31}H_{40}NO_{12}]^+$) 618.2545, found 618.2542.

General method for hydrolysis of acetyl protecting groups: (5α,6α)-7,8-didehydro-4,5-epoxy-3-methoxy-17-methylmorphinan-6-yl-β-D-glucopyranoside (6-O-glucopyranosylcodeine) (**13**, $C_{24}H_{31}NO_8$)
Tetraacetyl-6-*O*-glucopyranosylcodeine (**12**, 60 mg, ~ 0.1 mmol) was dissolved in 10 cm^3 methanol, and 0.6 cm^3 1 M aqueous LiOH was added and stirred for 3 h at room temperature. The solvent was removed under reduced pressure to yield **13** (quantitative) as an amorphous solid. ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 6.62$ (d, $J = 7.9$ Hz, H-2), 6.48 (d, $J = 7.9$ Hz, H-1), 5.69 (d, $J = 9.5$ Hz, H-7), 5.30 (d, $J = 9.5$ Hz, H-8), 4.98 (d, $J = 6.0$ Hz, H-5), 4.44 (d, $J = 7.5$ Hz, H-1′), 4.35 (d, $J = 6.0$ Hz, H-6), 3.65 and 3.43 (2 dd, $J = 15.0$ Hz, H-6′), 3.19 (dd, $J = 9.4$, 7.5 Hz, H-2′), 3.13 (ddd, $J = 4.5$, 2.5 Hz, H-5′), 3.07 (t, $J = 9.4$ Hz, H-4′), 3.03 (t, $J = 9.4$ Hz, H-3′) ppm; ^{13}C NMR (150 MHz, DMSO-d_6): $\delta = 131.3$ (CH), 129.4 (CH), 119.1 (CH), 113.7 (CH), 102.1 (CH), 89.5 (CH), 77.5 (CH), 77.1 (CH), 74.3 (CH), 73.2 (CH), 70.4 (CH), 61.4 (CH_2) ppm; HRMS: *m/z* calcd. for $[M + H]^+$ ($[C_{24}H_{32}NO_8]^+$) 462.2122, found 462.2118.

(5α,6α)-7,8-Didehydro-4,5-epoxy-6-hydroxy-17-methylmorphinan-3-yl-β-D-glucopyranoside (3-O-glucopyranosylmorphine) (**3**, $C_{23}H_{29}NO_8$)
Yield: 94 %; ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 6.67$ (d, $J = 8.0$ Hz, H-2), 6.67 (d, $J = 8.0$ Hz, H-1), 5.54 (d, $J = 9.5$ Hz, H-7), 5.26 (d, $J = 9.5$ Hz, H-8), 4.99 (d, $J = 7.5$ Hz, H-1′), 4.70 (d, $J = 6.0$ Hz, H-5), 4.11 (d, $J = 6.0$ Hz, H-6), 3.64 and 3.42 (2 dd, $J = 15.1$ Hz, H-6′), 3.24 (ddd, $J = 4.5$, 2.5 Hz, H-5′), 3.23 (t, $J = 9.4$ Hz, H-3′), 3.16 (dd, $J = 9.4$, 7.5 Hz, H-2′), 3.12 (t, $J = 9.4$ Hz, H-4′) ppm; ^{13}C NMR (150 MHz, DMSO-d_6): $\delta = 133.4$ (CH), 128.4 (CH), 118.4 (CH), 116.2 (CH), 100.1 (CH), 91.7 (CH), 76.6 (2 CH), 73.2 (CH), 69.6 (CH), 60.5 (CH_2) ppm; HRMS: *m/z* calcd. for $[M + H]^+$ ($[C_{23}H_{30}NO_8]^+$) 448.1966, found 448.1960.

(5α,6α)-7,8-Didehydro-4,5-epoxy-3-hydroxy-17-methylmorphinan-6-yl-β-D-glucopyranoside (6-O-glucopyranosylmorphine) (**6**, $C_{23}H_{29}NO_8$)
Yield: 97 %; ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 6.16$ (d, $J = 7.9$ Hz, H-2), 6.09 (d, $J = 7.9$ Hz, H-1), 5.56 (d, $J = 9.3$ Hz, H-7), 5.27 (d, $J = 9.3$ Hz, H-8), 4.80 (d, $J = 6.0$ Hz, H-5), 4.50 (d, $J = 7.5$ Hz, H-1′), 4.27 (d, $J = 6.0$ Hz, H-6), 3.65 and 3.44 (2 dd, $J = 15.0$ Hz, H-6′), 3.32 (t, $J = 9.4$ Hz, H-3′), 3.13 (ddd, $J = 4.5$, 2.5 Hz, H-5′), 3.09 (t, $J = 9.4$ Hz, H-4′), 3.07 (dd, $J = 9.4$, 7.5 Hz, H-2′) ppm; ^{13}C NMR (150 MHz, DMSO-d_6): $\delta = 130.8$ (CH), 128.3 (CH), 118.8 (CH), 118.6 (CH), 101.2 (CH), 86.3 (CH), 76.9 (CH), 76.8 (CH), 74.0 (CH), 70.3 (CH), 61.1 (CH_2) ppm; HRMS: *m/z* calcd. for $[M + H]^+$ ($[C_{23}H_{30}NO_8]^+$) 448.1966, found 448.1961.

(5α,6α)-4,5-Epoxy-6-hydroxy-17-methylmorphinan-3-yl-β-D-glucopyranoside (3-O-glucopyranosyldihydromorphine) (**8**, $C_{23}H_{31}NO_8$)
Yield: 94 %; ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 6.71$ (d, $J = 8.0$ Hz, H-2), 6.52 (d, $J = 8.0$ Hz, H-1), 5.09 (d, $J = 7.5$ Hz, H-1′), 4.47 (d, $J = 6.0$ Hz, H-5), 3.85 (d, $J = 6.0$ Hz, H-6), 3.66 and 3.42 (2 dd, $J = 15.0$ Hz, H-6′), 3.27 (ddd, $J = 4.5$, 2.5 Hz, H-5′), 3.25 (t, $J = 9.4$ Hz, H-3′), 3.17 (dd, $J = 9.5$, 7.5 Hz, H-2′), 3.11 (t, $J = 9.5$ Hz, H-4′) ppm; ^{13}C NMR (150 MHz, DMSO-d_6): $\delta = 118.2$ (CH), 117.1 (CH), 100.3 (CH), 90.3 (CH), 76.8 (CH), 76.7 (CH), 73.4 (CH), 69.8 (CH), 65.7 (CH), 60.6 (CH_2) ppm; HRMS: *m/z* calcd. for $[M + H]^+$ ($[C_{23}H_{32}NO_8]^+$) 450.2122, found 450.2126.

(5α,6α)-4,5-Epoxy-3-hydroxy-17-methylmorphinan-6-yl-β-D-glucopyranoside (6-O-glucopyranosyldihydromorphine) (**10**, $C_{23}H_{31}NO_8$)
Yield: 95 %; ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 6.19$ (d, $J = 8.0$ Hz, H-2), 6.15 (d, $J = 8.0$ Hz, H-1), 4.40 (d, $J = 6.0$ Hz, H-5), 4.24 (d, $J = 7.5$ Hz, H-1′), 3.89 (d, $J = 6.0$ Hz, H-6), 3.62 and 3.38 (2 dd, $J = 15.0$ Hz, H-6′), 3.19 (t, $J = 9.4$ Hz, H-3′), 3.06 (ddd, $J = 4.5$, 2.5 Hz, H-5′), 3.02 (t, $J = 9.5$ Hz, H-4′), 2.84 (dd, $J = 9.5$, 7.5 Hz, H-2′) ppm; ^{13}C NMR (150 MHz, DMSO-d_6): $\delta = 119.1$ (CH), 118.4 (CH), 104.3 (CH), 85.3 (CH), 76.9 (CH), 76.7 (CH), 76.4 (CH), 74.0 (CH), 69.8 (CH), 61.2 (CH_2) ppm; HRMS: *m/z* calcd. for $[M + H]^+$ ($[C_{23}H_{32}NO_8]^+$) 450.2122, found 450.2122.

(5α,6α)-4,5-Epoxy-3-methoxy-17-methylmorphinan-6-yl-β-D-glucopyranoside (6-O-glucopyranosyldihydrocodeine) (**15**, $C_{24}H_{33}NO_8$)

Yield: 98 %; ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 6.73$ (d, $J = 8.0$ Hz, H-2), 6.58 (d, $J = 8.0$ Hz, H-1), 4.72 (d, $J = 6.0$ Hz, H-5), 4.31 (d, $J = 7.5$ Hz, H-1′), 3.94 (d, $J = 6.0$ Hz, H-6), 3.63 and 3.40 (2 dd, $J = 15.0$ Hz, H-6′), 3.14 (t, $J = 9.4$ Hz, H-3′), 3.08 (ddd, $J = 4.5$, 2.5 Hz, H-5′), 3.01 (t, $J = 9.5$ Hz, H-4′), 2.87 (dd, $J = 9.5$, 7.5 Hz, H-2′) ppm; ^{13}C NMR (150 MHz, DMSO-d_6): $\delta = 118.3$ (CH), 113.5 (CH), 102.4 (CH), 87.9 (CH), 76.8 (CH), 76.4 (CH), 74.7 (CH), 73.9 (CH), 69.9 (CH), 60.9 (CH_2) ppm; HRMS: *m/z* calcd. for $[M + H]^+$ ($[C_{24}H_{34}NO_8]^+$) 464.2279, found 464.2275.

References

1. Trescot A, Glaser SE, Hansen H, Benyamin R, Patel S, Manchikanti L (2008) Pain Phys 11:S181
2. Vallejo R, Barkin RL, Wang VC (2011) Pain Phys 14:E343
3. Smith HS (2011) Clin J Pain 27:824
4. Váradi A, Gergely A, Béni S, Jankovics P, Noszál B, Hosztafi S (2011) Eur J Pharm Sci 42:65
5. Mori M, Oguri K, Yoshimura H, Shimomura K, Kamata O, Ueki S (1972) Life Sci 11:525
6. Yoshimura H, Ida S, Oguri K, Tsukamoto H (1973) Biochem Pharmacol 22:1423
7. Pasternak GW, Bodnar RJ, Clark JA, Inturrisi CE (1987) Life Sci 41:2845
8. Paul D, Standifer KM, Inturrisi CE, Pasternak GW (1989) J Pharmacol Exp Ther 251:477
9. Chen XY, Zhao LM, Zhong DF (2003) Br J Clin Pharmacol 55:570
10. Matern H, Matern S (1987) Biochim Biophys Acta. Lipids Lipid Metab 921:1
11. Paibir SG, Soine WH, Thomas DF, Fisher RA (2004) Eur J Drug Metab Pharmacokinet 29:51
12. Shipkova M, Armstrong VW, Wieland E, Niedmann PD, Schütz E, Brenner-Weiß G, Voihsel M, Braun F, Oellerich M (1999) Br J Pharmacol 126:1075
13. Tang BK (1990) Pharmacol Ther 46:53
14. Tang BK, Kalow W, Grey AA (1978) Res Commun Chem Pathol Pharmacol 21:45
15. Tang BK, Kalow W, Grey AA (1979) Drug Metab Dispos 7:315
16. Tjornelund J, Hansen SH, Cornett C (1989) Xenobiotica 19:891
17. Biasutto L, Marotta E, Bradaschia A, Fallica M, Mattarei A, Garbisa S, Zoratti M, Paradisi C (2009) Bioorg Med Chem Lett 19:6721
18. Hirpara KV, Aggarwal P, Mukherjee AJ, Joshi NJ, Burman AC (2009) Anticancer Agent Med Chem 9:138
19. Zhao X, Tao X, Wei D, Song Q (2006) Eur J Med Chem 41:1352
20. Casparis P, Kuhni E, Leinzinger E (1949) Pharm Acta Helv 24:145
21. Kováč P, Rice KC (1995) Heterocycles 41:697
22. Lacy C, Sainsbury M (1995) Tetrahedron Lett 36:3949
23. Stachulski AV, Jenkins GV (1998) Nat Prod Rep 15:173
24. Stachulski AV, Scheinmann F, Ferguson JR, Law JL, Lumbard KW, Hopkins P, Patel N, Clarke S, Gloyne A, Joel SP (2003) Bioorg Med Chem Lett 13:1207
25. Arsequell G, Salvatella M, Valencia G, Fernández-Mayoralas A, Fontanella M, Venturi C, Jiménez-Barbero J, Marrón E, Rodríguez RE (2009) J Med Chem 52:2656
26. Berrang B, Twine CE, Hennessee GL, Carroll FI (1975) Synth Commun 5:231
27. Brown RT, Carter NE, Lumbard KW, Scheinmann F (1995) Tetrahedron Lett 36:8661
28. Yoshimura H, Oguri K, Tsukamoto H (1968) Chem Pharm Bull 16:2114
29. Mertz AAH (1993) Method for synthesizing glucuronides of 4,5-epoxy morphinanes. PCT Int Appl WO1993005057; March 18, 1993
30. Welsh LH (1954) J Org Chem 19:1409
31. Bognár R, Lévai A (1973) Acta Chim Acad Sci Hung 77:435
32. Bosch ME, Sánchez AR, Rojas FS, Ojeda CB (2007) J Pharm Biomed Anal 43:799
33. Barrett DA, Pawula M, Knaggs RD, Shaw PN (1998) Chromatographia 47:667