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A Simple Synthesis of Morphine-3,6-di-β-D-glucuronide

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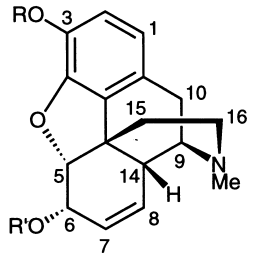
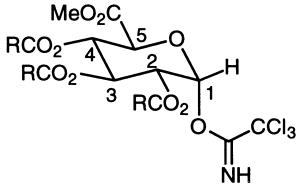
Abstract—A convenient two-step synthesis of morphine-3,6-di-β-D-glucuronide in fair yield from morphine and methyl 2,3,4-tri-*O*-isobutyryl-1-*O*-trichloroacetimidoyl-α-D-glucopyranuronate is reported.

Introduction

The discovery of the analgesic properties of morphine-6-β-D-glucuronide (M6G) **3**, a metabolite of morphine **1**, was of considerable medical interest,[1] and created a demand for appreciable quantities of M6G and related glucuronides for pharmacological and clinical testing. However, synthesis from morphine by standard Koenigs–Knorr coupling or related heterogeneous methods was effectively precluded by both limitation of scale and contamination of the products by heavy-metal ions. As a consequence, we devised and described in a 1993 patent and a subsequent paper, homogeneous procedures suitable for large-scale preparation of various glucuronides of morphine and derivatives by Lewis acid catalysed condensation with glucuronate esters.[2,3] In particular, we gave specific experimental details for a short synthesis (two steps) of morphine-3,6-di-β-D-glucuronide (M3,6diG) **4** from morphine and methyl 2,3,4-tri-*O*-isobutyryl-1-*O*-trichloroacetimidoyl-α-D-glucopyranuronate **5**, which we used to prepare multigram quantities of M3,6diG in 35–50% yield for further research.[2,4] Nevertheless, in a publication of their own synthesis (six steps) of M3,6diG, Berrang, Brine and Carroll apparently found our procedure difficult to reproduce.[5] Their failure was hardly surprising, since, although they mentioned the specified isobutyrate **5** briefly in passing, they actually used the corresponding *acetate* derivative **6** and hence only achieved largely transacetylation, which confirmed our exploratory work[6] on β-glucuronidation of morphine using Schmidt's imidate procedure.[7]



1 R = R' = H
2 R = Ac, R' = H
3 R = H, R' = β-D-glucuronic acid
4 R = R' = β-D-glucuronic acid



5 R = $CHMe_2$
6 R = Me

We had found that attempted Lewis acid catalysed coupling of methyl 2,3,4-tri-*O*-acetyl-1-*O*-trichloroacetimidoyl-α-D-glucopyranuronate **6** with 3-acetylmorphine **2** afforded mostly 3,6-diacetylmorphine and little of the desired 6-glucuronate, and similar poor results with a varied mixture of products were obtained with morphine.[6] It was this experience which led us to propose replacing the methyl of the sugar acetates by a larger group to increase steric hindrance. By this simple means, the rate of nucleophilic attack at the carbonyl, and hence transacylation, would be reduced, whereas the rate of glycosylation should be relatively unaffected. To this end, the *t*-butyl group of pivalate was effective but subsequent hydrolysis proved problematic with formation of unwanted by-products. In the event, the slightly less hindered isobutyrate was found to be the ester

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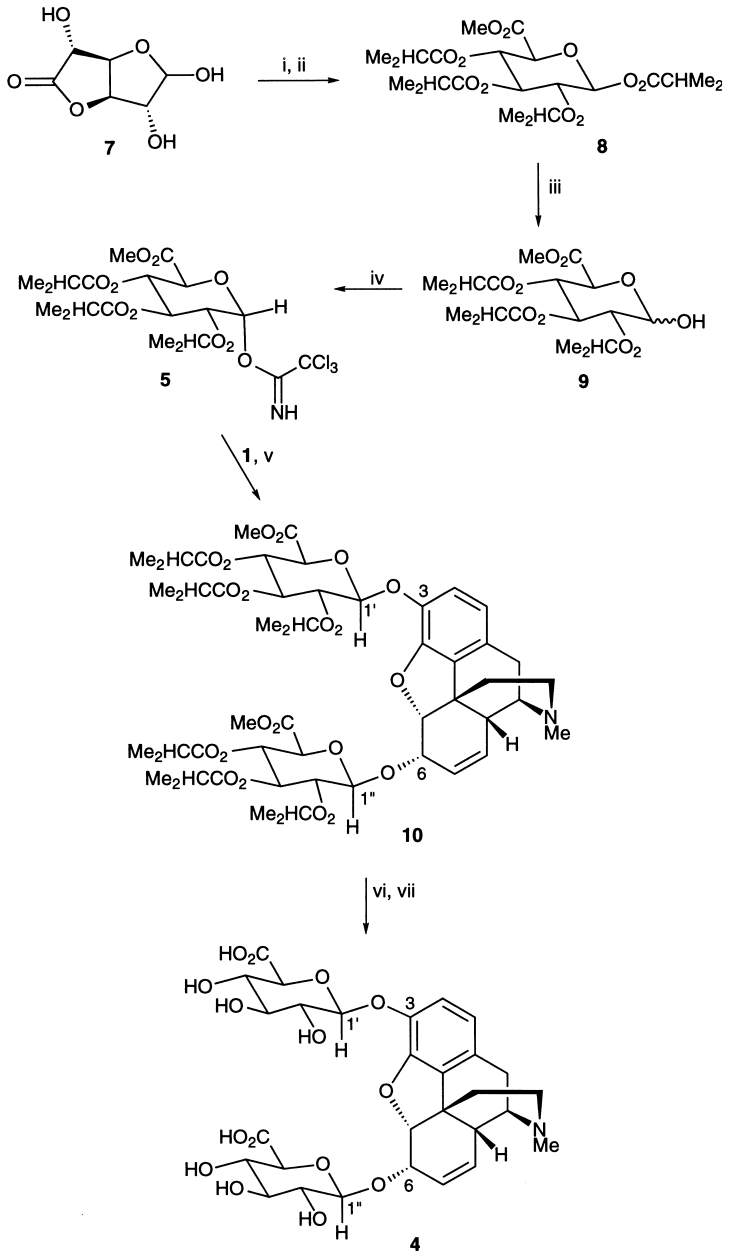

Scheme 1. Reagents: (i) NaOMe/MeOH; (ii) $Me_2CHCOCl$/py; (iii) aq. NH_3/CH_2Cl_2; (iv) $CCl_3CN/CH_2Cl_2/Na_2CO_3$; (v) $BF_3{\cdot}Et_2O/CH_2Cl_2$; (vi) aq. NaOH/MeOH; (vii) AcOH.

of choice, combining minimal transacylation with ease of hydrolysis. Subsequent use of the tri-isobutyrate **5** led to effective preparations of M6G and related derivatives,[2,3,6,8,9] with essentially complete stereoselection for the β-anomers due to participation of the neighbouring C-2 acyl group.[7] We now give a full report of our convenient and expeditious synthesis of M3,6diG (Scheme 1).

Results and Discussion

The readily available D-glucuronolactone **7** was converted with sodium methoxide into a methyl ester, which was then reacted with isobutyryl chloride in pyridine at below 10°C to give methyl tetra-*O*-isobutyrylglucuronate in ca. 50% yield as a 9:1 mixture of the β and α anomers, from which the pure β isomer **8**, mp 126–8°C, could be obtained by recrystallisation from petrol. Control of the temperature during acylation maximised the proportion of the β isomer, which was desirable since it was more readily hydrolysed selectively to the hemi-acetal **9** in the next step. Initially, this was carried out by bubbling gaseous ammonia through a solution of **8** in dichloromethane and methanol, a successful but rather lengthy procedure, but a two-phase system with dichloromethane and saturated aqueous (880) ammonia proved to be more rapid and effective on a large scale, affording methyl 2,3,4-tri-*O*-isobutyrylglucuronate **9** as a mixture of C-1 epimers in 73% yield. The formation of a mixture was not of any material consequence since the epimers interconverted, and hence base-catalysed reaction with trichloro-acetonitrile gave in high yield (85–89%) only the pure α-imidate **5**, mp 80–1°C, characterised by an NMR

singlet for the NH at δ_H 8.71 and a doublet at δ_H 6.66 with J=3.7 Hz for H-1. We found that alkaline metal carbonates could be used as the base instead of the standard sodium hydride,[7] to obvious advantage on a large scale, and that sodium carbonate was more effective than lithium, potassium or caesium carbonates.

A molar excess of the imidate **5** was then reacted with dry morphine in dichloromethane in presence of BF_3 catalyst to give the M3,6diG derivative **10** with exclusive β-stereochemistry at C-1 for both glycosides. Although morphine was virtually insoluble in dichloromethane, it slowly dissolved as it reacted, and after two days essentially only **10** could be detected by TLC, together with excess sugars and a trace of an M3G derivative. Chromatography removed the last and the bulk of the sugars, and recrystallisation of the crude product from ethanol afforded pure morphine-3,6-di(methyl tri-isobutyrylglucuronate) mp 229–230°C $[\alpha]_D=-110$ ($CHCl_3$). The isolated yield of crystalline material was 60%, but analysis of the reaction mixture indicated >90% conversion of morphine. Alternatively, the reaction mixture could be directly triturated with hot methanol to give crystalline product in ca. 35% yield.

The structure was established from its spectroscopic data: inter alia, the UV maxima at 282 and 235 nm did not shift on addition of alkali, hence no free phenol was present, and an ion at *m/z* 1086 (M+1) in the FAB mass spectrum corresponded to the expected morphine diglycoside, which was corroborated by combustion analysis for $C_{55}H_{75}NO_{21}$. Assignment of the ^1H NMR spectrum confirmed the structure as **10**, and in particular, the β-linkages of both glucuronate moieties from doublets for H-1′ and 1″ at δ 5.81 and 4.71 with *trans*-diaxial couplings of 8 Hz for both. Subsequent careful hydrolysis of **10** with alkali and precipitation by acidifying with acetic acid afforded in 89% yield morphine-3,6-di-β-D-glucuronide **4**, which could be recrystallised from aqueous methanol, mp 243–4°C (dec.). It was homogeneous from its 500 MHz ^1H NMR spectrum, which again confirmed the structure and showed both glucuronides to be β-anomers.

We had thus achieved the first synthesis of morphine-3,6-di-β-D-glucuronide in a minimum overall yield from morphine of ca. 50%. However, this could obviously be considerably increased by direct hydrolysis of the crude **10**, rather than recrystallised material, since the only significant impurity is excess sugar, which would remain in solution.

Experimental

General

Melting points were obtained on a Kofler block and are uncorrected. An Optical Activity AA-100 polarimeter was used for optical rotations $[\alpha]_D$. Ultra-violet spectra (λ_{max}) were recorded on a Shimadzu UV-260 spectrometer, infrared spectra (ν_{max}) on a Perkin–Elmer 1710 FT-IR spectrometer, proton magnetic resonance spectra (δ_H) on Varian 500 or 300 MHz spectrometers and mass spectra (*m/z* EI, CI, FAB) on a Kratos Concept IS spectrometer. Molecular formulae were determined by combustion analysis and mass measurement. Merck silica plates F_{254} were used for TLC and Kieselgel 60 for column chromatography. Organic solutions were routinely dried over anhydrous sodium sulfate.

Methyl 1,2,3,4-tetra-*O*-isobutyryl-β-D-glucopyranuronate 8. To a solution of NaOMe (0.13 g, 2.4 mmol) in MeOH (750 mL) was added glucurono-6,3-lactone (103 g, 0.77 mol) in portions with stirring until dissolved. The solvent was then removed in vacuo, the residue taken up in pyridine (425 mL, 5.4 mol) and the solution cooled to 0°C. Isobutyryl chloride (550 mL, 5.3 mol) in CH_2Cl_2 (350 mL) was then added with mechanical stirring at a rate that kept the temperature below 10°C, and the reaction mixture was left at room temperature overnight. More CH_2Cl_2 (450 mL) was then added and the solution washed with water (400 mL), 2 M HCl (3×200 mL), saturated aq. sodium bicarbonate (5×400 mL) and brine (400 mL). After drying, filtering and evaporating in vacuo, a gum was obtained which crystallised on trituration with petrol (40–60°). Filtration and drying at 40°C in a vacuum oven yielded the title product (150 g, 54%) containing ~10% of the a epimer. Recrystallisation from MeOH or petrol afforded the pure β isomer **8** as needles, mp 126–8°C, $[\alpha]_D=+10$ (*c* 1.7 $CHCl_3$); ν_{max}(film): 1740 cm^{-1}; *m/z* (CI): 506 ($M+NH_4^+$), 418, 401; δ_H (300 MHz, $CDCl_3$): 5.78 (d, *J*=8 Hz, H-1), 5.39 (t, *J*=9.5 Hz, H-3), 5.25 (t, *J*=9.5 Hz, H-4), 5.23 (dd, *J*=9.5, 8 Hz, H-2), 4.19 (d, *J*=9.5 Hz, H-5), 3.75 (s, OMe), 2.65–2.45 (m, 4×C*H*Me_2), 1.17–1.07 (m, 4×CH*Me*$_2$). Found: C, 56.7; H, 7.6. $C_{23}H_{36}O_{11}$ requires: C, 56.6; H, 7.4.

For reference purposes, an alternative procedure with isobutyric anhydride and perchloric acid catalyst, working up with sat. aq. (880) ammonia to destroy excess anhydride, was used to prepare in 13% yield the microcrystalline α-isomer, mp 89–90°C, $[\alpha]_D=+103$ (*c* 0.03 $CHCl_3$); ν_{max}(film): 1750 cm^{-1}; *m/z* (CI): 506 ($M+NH_4^+$), 401, 314; δ_H (300 MHz, $CDCl_3$): 6.41 (d, *J*=3.5 Hz, H-1), 5.57 (t, *J*=10 Hz, H-3), 5.23 (t, *J*=10 Hz, H-4), 5.14 (dd, *J*=10, 3.5 Hz, H-2), 4.39 (d, *J*=10 Hz, H-5), 3.72 (s, OMe), 2.72–2.37 (m, 4×C*H*Me_2), 1.25–1.04 (m, 4×CH*Me*$_2$). Found: C, 56.5; H, 7.4. $C_{23}H_{36}O_{11}$ requires: C, 56.6; H, 7.4.

Methyl 2,3,4-tri-*O*-isobutyryl-D-glucopyranuronate 9. *Procedure A.* A solution of the above crude tetra-isobutyrate (9.0 g, 18 mmol) in CH_2Cl_2 (360 mL) and MeOH (36 mL) was cooled to −4°C, dry ammonia gas was then bubbled through for 2 h, and the solution left to warm up to room temperature overnight. TLC indicated ~50% conversion, so the procedure was repeated until no starting material was observed by TLC. Then after bubbling nitrogen gas through the solution for an hour, it was evaporated, the residue taken up in CH_2Cl_2, loaded on to a pad of silica and eluted with $CHCl_3$/MeOH 9:1. Evaporation of the eluate gave the title product **9** as a glass (6.96 g, 90%) containing a 4:1 mixture of α/β epimers (δ_H 5.54 (d, *J*=3.5 Hz, H-1α)/ 5.78 (d, *J*=8 Hz, H-1β). Recrystallisation from $CHCl_3$/petrol (40–60°) afforded the pure microcrystalline α-epimer, mp 89–90°C, $[\alpha]_D=+76$ (*c* 2.2 $CHCl_3$); ν_{max}(film): 3460, 1750 cm^{-1}; *m/z* (FAB): 418 (M^+); δ_H (300 MHz, $CDCl_3$): 5.65 (t, *J*=10 Hz, H-3), 5.54 (d, *J*=3.5 Hz, H-1), 4.92 (dd, *J*=10,3.5 Hz, H-2), 4.60 (d, *J*=10 Hz, H-5), 3.75 (s, OMe),

2.61–2.43 (m, 4×C*H*Me$_2$), 1.20–1.05 (m, 4×CH*Me$_2$*). Found: C, 54.7; H, 7.4. $C_{19}H_{30}O_{10}$ requires: C, 54.6; H, 7.2.

Procedure B. The tetra-isobutyrate (102 g, 0.21 mol) in CH_2Cl_2 (100 mL) was stirred in a sealed vessel with sat. aq. (880) ammonia (40 mL) overnight. The solution was washed with water (50 mL) and then brine (50 mL), dried, filtered and evaporated to give the gummy hemi-acetal (64 g, 73%) as an α/β mixture similar to that above.

Methyl 2,3,4-tri-*O*-isobutyryl-1-*O*-trichloroacetimidoyl-α-D-glucopyranuronate 5. To a stirred solution of the above α/β mixture of hemiacetals (82.4 g, 0.20 mol) in CH_2Cl_2 (100 mL) was added trichloroacetonitrile (74 mL, 0.73 mol), followed by anhydrous sodium carbonate (12.4 g, 0.12 mol), and the mixture left to stir overnight. Filtration and evaporation in vacuo then yielded the title product **5** as a semicrystalline gum (95.0 g, 85%), which crystallised with dry isopropanol as prisms, mp 80–1°C $[\alpha]_D$=+67 (*c* 5 $CHCl_3$); ν_{max}(film): 3320, 1750, 1680 cm^{-1}; *m/z* (CI): 579 (M^++NH_3), 546, 506, 401, 348; δ_H (300 MHz, $CDCl_3$): 8.72 (s, NH), 6.66 (d, *J*=3.5 Hz, H-1), 5.70 (t, *J*=10 Hz, H-3), 5.30 70 (t, *J*=10 Hz, H-4), 5.20 (dd, *J*=10,3.5 Hz, H-2), 4.51 (d, *J*=10 Hz, H-5), 3.75 (s, OMe), 2.60–2.43 (m, 3×C*H*Me$_2$), 1.17–1.06 (m, 3×CH*Me$_2$*). Found: C, 44.8; H, 5.3; Cl, 18.4; N, 2.6. $C_{21}H_{30}Cl_3NO_{10}$ requires: C, 44.8; H, 5.3; Cl, 18.9; N, 2.5.

Morphine-3,6-di(methyl 2,3,4-tri-*O*-isobutyryl-β-D-glucopyranuronate) 10. *Procedure A.* A suspension of dried morphine (2.0 g, 7.0 mmol) and the above imidate (15.8 g, 28.1 mmol) in dry CH_2Cl_2 (40 mL) containing 4A molecular sieves was stirred under argon at room temperature, while $BF_3{\cdot}Et_2O$ (355 mL, 4.0 g, 28.1 mmol) was added. After 30 min, virtually all of the starting materials had gone into solution and stirring was continued for 2 days. More CH_2Cl_2 (20 mL) was added, the solution washed with saturated aq. sodium bicarbonate (50 mL), water and brine before being dried. Filtration and evaporation in vacuo afforded a semisolid residue, which was taken up in $CHCl_3$/MeOH 40:1 and flash chromatographed on silica. Elution with $CHCl_3$/MeOH 9:1 gave a crude product (8.5 g) consisting (TLC/NMR) of ~80% diglucuronate and ~20% excess sugar. Trituration with EtOH yielded pure morphine-3,6-diglucuronate **10** (4.6 g, 60%) as colourless needles, mp 229–230°C $[\alpha]_D$=−110 (*c* 1.3 $CHCl_3$); ν_{max}(EtOH): 282, 235, 210 nm; ν_{max}(film): 1750 cm^{-1}; *m/z* (FAB): 1086 (MH^+), 686, 401; δ_H (300 MHz, $CDCl_3$): 6.76 (bd, *J*= 8 Hz, H-2), 6.47 (d, *J*=8 Hz, H-1), 5.97 (t, *J*=9.5 Hz, H-3′), 5.81 (d, *J*=8 Hz, H-1), 5.57 (d, *J*=10 Hz, H-8), 5.40 (t, *J*=9.5 Hz, H-3″), 5.27–5.21 (dd, *J*=9.5,8 Hz, H-2′; dd, *J*=9.5,8 Hz, H-2″; d, *J*=10 Hz, H-7), 5.20 (t, *J*=9.5 Hz, H-4′), 5.13 (t, *J*=9.5 Hz, H-4″), 4.72 (d, *J*= 5 Hz, H-5), 4.71 (d, *J*=8 Hz, H-1″), 4.64 (d, *J*=9.5 Hz, H-5′), 4.05 (d, *J*=9.5 Hz, H-5″), 3.98 (dd, *J*=5,1.5 Hz, H-6), 3.75 (s, OMe), 3.69 (s, OMe), 3.34 (dd, *J*=6,3 Hz, H-13), 3.02 (d, *J*=19 Hz, H-12$_{eq}$), 2.60–2.37 (m, H-16$_{eq}$, 6×C*H*Me$_2$), 2.43 (s, NMe), 2.41 (ddd, *J*=13.5,12,4 Hz, H-16$_{ax}$), 2.26 (dd, *J*=19,6 Hz, H-12$_{ax}$), 2.01 (ddd, *J*= 13.5,12,4 Hz, H-15$_{ax}$), 1.84 (bd, *J*=12 Hz, H-15$_{eq}$), 1.34–1.08 (m, 6×CH*Me$_2$*). Found: C, 60.6; H, 6.9; N, 1.3. $C_{55}H_{75}NO_{21}$ requires: C, 60.8; H, 6.9; N, 1.3.

Procedure B. A suspension of dried morphine (4.0 g, 14 mmol) and the imidate (36.1 g, 64 mmol) in dry CH_2Cl_2 (100 mL) was stirred under argon at room temperature, while $BF_3{\cdot}Et_2O$ (7.8 mL, 64 mmol) was added. After 90 min, the solution was evaporated in vacuo to a gum, which was crystallised from MeOH to afford the morphine-3,6-diglucuronate (5.4 g, 35%) as needles, mp 229–230°C.

Morphine-3,6-di-β-D-glucuronide 4. Aq. NaOH (1.5 M, 12 mL) was added to a stirred suspension of the above diglucuronate (2.3 g, 2.1 mmol) in MeOH (68 mL), and the mixture left overnight. The solution was then acidified with glacial acetic acid, the precipitate filtered and washed with MeOH (~15 mL). Drying at 60° under high vacuum afforded morphine-3,6-diglucuronide (1.2 g, 89%) as a white solid, which formed microcrystals from aq. MeOH, mp 243–244°C (dec.) $[\alpha]_D$=−188 (*c* 10 H_2O); λ_{max}(H_2O): 282, 236, 208 nm; δ_H (500 MHz, D_2O): 6.99 (d, *J*=8 Hz, H-2), 6.75 (d, *J*=8 Hz, H-1), 5.84 (bd, *J*=10 Hz, H-8), 5.38 (bd, *J*=10 Hz, H-7), 5.28 (d, *J*=6 Hz, H-5), 5.07 (d, *J*=7 Hz, H-1′), 4.70 (d, *J*=8 Hz, H-1″), 4.56 (m, H-6), 4.22 (m, H-13), 3.84 (d, *J*=8 Hz, H-5′), 3.73 (d, *J*=9 Hz, H-5″), 3.61–3.56 (m, H-4′, 3′, 2′), 3.54 (t, *J*=9 Hz, H-3″), 3.51 (t, *J*=9 Hz, H-4″), 3.40 (dd, *J*=9,8 Hz, H-2″), 3.31 (d, *J*=20 Hz, H-12$_{eq}$), 3.06 (ddd, *J*=13,12,4.5 Hz, H-16$_{ax}$), 2.97 (s, NMe), 2.26 (dd, *J*=19,6 Hz, H-12$_{ax}$), 2.01 (ddd, *J*=13.5,12,4 Hz, H-15$_{ax}$), 1.84 (bd, *J*=12 Hz, H-15$_{eq}$). Found: C, 50.5; H, 6.4; N, 1.9. $C_{29}H_{35}NO_{15}{\cdot}3H_2O$ requires: C, 50.4; H, 6.0; N, 2.0.

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