JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2002; **45**: 107–113.
DOI: 10.1002/jlcr.540

Research Article

Synthesis of morphine-[*N*-methyl-^{14}C]-6-β-D-glucuronide

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Summary

Protected morphine-6-glucuronide was converted into morphine-[*N*-methyl-^{14}C]-6-glucuronide by a three-step procedure. Methyl (3-pivaloylmorphin-6-yl 2,3,4-tri-*O*-isobutyryl-β-D-glucopyranosid)uronate was N-demethylated by treatment with 1-chloroethyl chloroformate to afford protected normorphine-6-glucuronide as its hydrochloride salt. The normorphine-6-glucuronide derivative was alkylated with iodomethane-[^{14}C] in the presence of potassium carbonate to produce C-14 labelled protected morphine-6-glucuronide. Finally, hydrolysis of the protecting groups using 5% sodium hydroxide solution gave morphine-[*N*-methyl-^{14}C]-6-β-D-glucuronide with a specific activity of 41.8 mCi mmol^{-1} and radiochemical purity of 99.2% (HPLC). Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: carbon-14 labelled morphine-6-glucuronide

Introduction

Morphine-6-glucuronide (M6G) is a metabolite of morphine in the human body and is a more powerful analgesic than morphine itself.[1,2] There have been studies that show M6G to be less toxic than morphine

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Received 9 October 2001
Accepted 24 October 2001

at equi-analgesic doses.[3,4] To facilitate the study of the pharmacokinetics of M6G, the synthesis of M6G-[*N*-methyl-^{14}C] was undertaken.

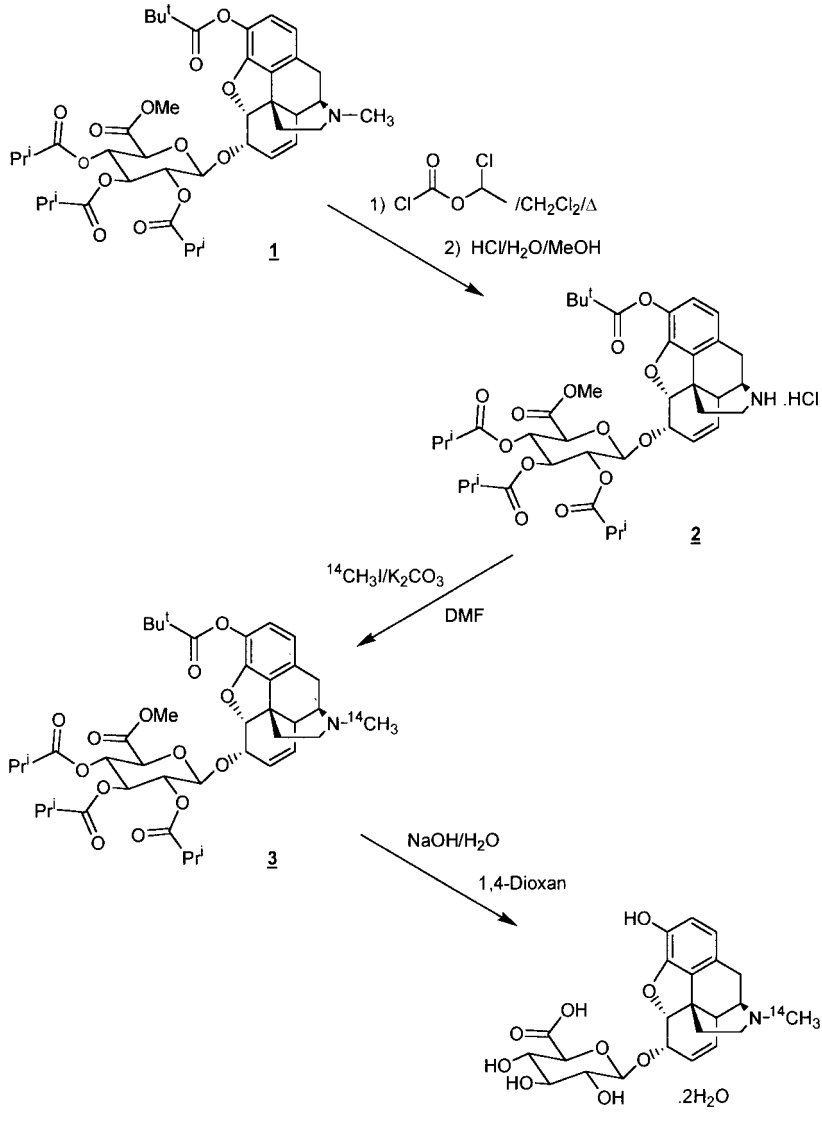
There have been two syntheses of isotopically labelled M6G reported. Berrang *et al.*[5] have described the synthesis of M6G-[1-^{3}H], and Nguyen *et al.*[6] the synthesis of M6G-[*N*-methyl-$^{2}H_3$]. In both cases isotopically labelled morphine was used as the starting material for the synthesis and the method used was based on the Koenigs–Knorr procedure reported by Yoshimura *et al.*[7] in the first synthesis of M6G. The labelled morphine was converted into its 3-acetyl derivative, which was reacted with methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucopyranuronate and silver carbonate, followed by hydrolysis of the protecting groups of the glucuronide intermediate to afford isotopically labelled M6G.

Our method for the synthesis of M6G-[*N*-methyl-^{14}C] uses an approach developed at Ultrafine for the synthesis of N-substituted analogues of M6G. Protected M6G **1**, which can be prepared by the acid catalysed coupling of 3-pivaloylmorphine with glucuronate ester derivatives **5** or **6**,[8,9] undergoes N-demethylation to afford protected normorphine-6-glucuronide **2** by treatment with 1-chloroethyl chloroformate.[10] **2** reacts with a range of alkylating agents to afford, after hydrolysis of the protecting groups, N-substituted analogues of M6G. To use this approach, iodomethane-[^{14}C] was chosen as the alkylating agent for the synthesis of M6G-[*N*-methyl-^{14}C] **4** (Scheme 1).

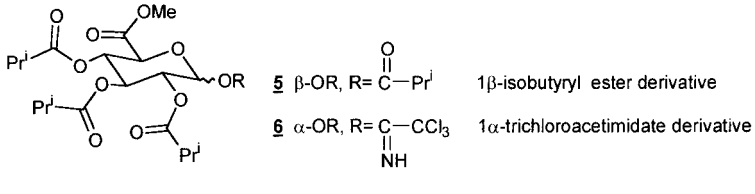
Results and discussion

Protected M6G **1** was N-demethylated using excess 1-chloroethyl chloroformate at reflux in dichloromethane, followed by warming with 1 M hydrochloric acid in methanol to produce protected normorphine-6-glucuronide as its hydrochloride salt **2** in 73% yield after purification by chromatography on silica gel.

To test the viability of our method, **2** was methylated by treatment in DMF solution with iodomethane in the presence of anhydrous potassium carbonate to afford protected M6G **1** in 61% yield, identical by TLC and ^{1}H NMR analysis to the original starting material. The methylation reaction was repeated using iodomethane-[^{14}C] to produce C-14 labelled protected M6G **3** in 37% yield after purification by chromatography on silica gel. To complete the synthesis, hydrolysis of the protecting groups of **3** was achieved by treatment of a dioxan solution of **3** with 5% sodium hydroxide solution. After 4 h, the solution



Scheme 1.



was diluted using methanol and the pH adjusted to 5.5 by dropwise addition of acetic acid. Following removal of the solvent, the product was separated from salts by chromatography on reversed phase silica. M6G-[*N*-methyl-^{14}C] **4** was obtained as an off-white solid in 80% yield with a specific activity of 41.8 mCi mmol^{-1}, radiochemical purity of 99.2% and chemical purity of 97.3%.

Experimental

Elemental analysis was performed by the Microanalytical Laboratory of the Department of Chemistry, University of Manchester, Manchester, UK. The melting point of **2** was determined using a Büchi 530 melting point apparatus. NMR spectra were recorded on a Bruker AC-250 or a Varian Gemini 200 spectrometer. Mass spectrometry was performed on a Micromass Trio 2000 mass spectrometer. The IR spectrum of **2** was recorded using a Bruker Vector 22 spectrometer. For TLC, aluminium foil or glass plates pre-coated with silica gel were employed. The results of elution of the plates were visualized under UV light and by staining with iodoplatinate solution. HPLC analysis was carried out using a Prodigy ODS(3) 5 μm 250 × 4.6 mm column at 35°C eluting with 0.1% TFA in water:acetonitrile (3:1 v/v) at 1 ml min^{-1}. The M6G-[*N*-methyl-^{14}C] was detected using UV (280 nm) and an LB509 radio-detector. The radiochemical yield was determined by liquid scintillation counting.

*Methyl (3-pivaloylnormorphin-6-yl 2,3,4-tri-O-isobutyryl-β-*D*-glucopyranosid)uronate hydrochloride* **2**

1-Chloroethyl chloroformate (0.23 ml, 2.13 mmol) was added to a stirred solution of **1** (1.17 g, 1.52 mmol) in anhydrous dichloromethane (8 ml) and the solution was heated under reflux for 6 h. The reaction was monitored by TLC (MeOH:CH_2Cl_2, 1:9 v/v). After 2 and 4 h, a further portion of 1-chloroethyl chloroformate (0.12 ml) was added. The reaction mixture was poured into ether (40 ml)–water (40 ml) and 1-butanol (*ca* 3 ml) was added to break up the emulsion which formed. The layers were separated and the aqueous layer was extracted with ethyl acetate (2 × 15 ml). The combined organic solution was washed with brine (20 ml), dried ($MgSO_4$) and the solvent evaporated. The residue was dissolved in methanol (23 ml) and 1 M hydrochloric acid

(1.6 ml) was added. The mixture was stirred and heated (oil bath at 45°C) for 2 h. The solvent was evaporated and the remaining water was removed by evaporation of ethanol and toluene from the residue. The crude product was purified by chromatography on silica gel (46 g) eluting with 5% methanol in dichloromethane to afford **2** as an off-white solid (0.88 g, 73%), m.p. 254–257°C dec. (methanol:water). FT-IR ν_{max} 1747 cm^{-1} (C═O). ^{1}H NMR (250 MHz, $CDCl_3$) δ_H 10.01 (2H, br, s, NH_2); 6.81 (1H, d, $J = 8$ Hz, H-2); 6.65 (1H, d, $J = 8$ Hz, H-1); 5.85 (1H, br, d, $J = 10$ Hz, H-8); 5.2–5.35 (3H, m, H-3′, H-4′, H-7); 5.13 (1H, m, H-2′); 5.00 (1H, d, $J = 5.5$ Hz, H-5); 4.94 (1H, d, $J = 7$ Hz, H-1′); 4.40, 4.31 (2H, m, H-6, H-9); 4.14 (1H, m, H-5′); 3.74 (3H, s, CO_2CH_3); 3.3–3.45, 2.95–3.2 (5H, m, 2H-10, H-14, 2H-16); 2.4–2.6 (4H, m, $3 \times COCH(CH_3)_2$, H-15$_{ax}$); 2.17 (1H, br, d, $J = 12$ Hz, H-15$_{eq}$); 1.38 (9H, s, $COC(CH_3)_3$); 1.06–1.15 (18H, m, $3 \times COCH(CH_3)_2$). MS (EI) m/z 755 $[M]^+$ (free base), 756 $[M+H]^+$; (CI, NH_3) 756 $[M+H]^+$, 773 $[M+NH_4]^+ \cdot C_{40}H_{53}NO_{13} \cdot HCl \cdot \frac{1}{2}H_2O$ (801.32) requires: C, 59.95; H, 6.92; N, 1.75%. Found: C, 59.86; H, 6.69: N, 1.67%.

Methyl (3-pivaloylnormorphin-[N-methyl-^{14}C]-6-yl 2,3,4-tri-O-isobutyryl-β-D-glucopyranosid)uronate **3**

Anhydrous potassium carbonate (385 mg, 2.79 mmol) was added to a stirred solution of **2** (486 mg, 0.61 mmol) in anhydrous DMF (5 ml). The contents of the flask were cooled to −196°C (liquid nitrogen) and the flask was evacuated. Iodomethane-[^{14}C] (99 mg, 0.69 mmol, 41.78 mCi mmol^{-1}) was introduced *via* bulb-to-bulb distillation. The reaction mixture was allowed to warm to room temperature on the vacuum line and then stirred for a further 3 h. The reaction flask vacuum was slowly released by admitting nitrogen (no volatile radioactivity was detected) and the reaction mixture analysed by TLC (CH_2Cl_2:MeOH, 9:1 v/v). No starting material **2** remained. DMF and residual iodomethane-[^{14}C] were removed by bulb-to-bulb distillation, and the residue partitioned between water (25 ml) and ethyl acetate (30 ml). The two phases were separated and the aqueous layer extracted with ethyl acetate (4×15 ml) until no radioactivity remained. The combined organic extracts were dried ($MgSO_4$) and the solvent removed under reduced pressure. The crude product (459 mg) was purified by column chromatography on silica gel (25 g) eluting with 3% methanol in dichloromethane to give the product **3** as an off-white solid (173 mg, 37%, 41.8 mCi mmol^{-1}, 9.37 mCi), identical by TLC and ^{1}H NMR analysis to unlabelled protected M6G **1**.

Morphine-[N-methyl-^{14}C]-6-β-D-glucuronide dihydrate **4**

To a solution of **3** (170 mg, 0.22 mmol) in dioxan (1.5 ml), cooled in a cold water bath, was added 5% w/v sodium hydroxide solution (1.4 ml). After stirring at room temperature for 4 h, TLC analysis (*n*-BuOH:acetone:AcOH:1.7% NH_4OH, 45:15:10:30 v/v/v/v) showed complete hydrolysis of the protecting groups. The solution was diluted with methanol (7.5 ml) and the pH adjusted to 5.5 by dropwise addition of glacial acetic acid. The solvent was removed by freeze-drying and the crude product purified using a short column of reversed phase silica (10 g). Salts were removed from the retained crude product by eluting with water (150 ml) and **4** was eluted using 90% aqueous acetonitrile (75 ml). Removal of the solvent under reduced pressure, followed by drying on a vacuum line for 48 h, gave **4** as a white solid (88 mg, 80%, 41.8 mCi mmol^{-1}, 7.39 mCi). The results of analyses (TLC, ^{1}H NMR, HPLC with UV detection at 280 nm) obtained for **4** were identical to those obtained for unlabelled M6G. M6G-[*N*-methyl-^{14}C] co-chromatographed with unlabelled M6G on HPLC analysis, R_t 5.3 min. M6G-[*N*-methyl-^{14}C] had chemical purity by integration of 97.3% and a radiochemical purity of 99.2%.

Conclusion

Morphine-[*N*-methyl-^{14}C]-6-glucuronide **4** has been successfully synthesized from protected M6G **1** by a three-step procedure in 22% overall chemical yield. The radiochemical yield from iodomethane-[^{14}C] was 30%.

Acknowledgements

The authors would like to thank Mr Peter Hannah (BioDynamics) for his valuable assistance and advice with the synthesis of iodomethane-[^{14}C].

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