

Synthesis of [1-³H]Morphine-6-β-D-glucuronide

Bertold D. Berrang*, Christopher D. Wyrick, F. Ivy Carroll and Herbert H. Seltzman

Research Triangle Institute, Organic and Medicinal Chemistry,
P.O. Box 12194, Research Triangle Park, NC 27709, USA

SUMMARY

Tritiated morphine of high specific activity was protected at the 3-position by acetylation and subjected to glucuronidation by Koenigs-Knorr procedure with methyl 2,3,4-tri-O-acetyl-1-bromo-1-deoxy-D-glucopyranuronate and silver carbonate. Fully protected morphine-6-glucuronide was obtained in 42% yield and 95% radiochemical purity as determined by TLC-RAM. Alkali catalyzed solvolysis in two steps furnished [1-³H]morphine-6-β-D-glucuronide in 23% overall radiochemical yield and 99% radiochemical purity determined by HPLC - liquid scintillation counting and TLC-RAM.

KEY WORDS: Tritium Labelled Morphine-6-glucuronide

INTRODUCTION

One of the challenges in modern medicine to this day constitutes the control and management of pain through analgesia in post operative and terminally ill patients. Although much research over many years has been invested in the development of new analgesics it has been difficult to design pain medications with superior qualities to that of morphine. To this day morphine is still frequently used in clinical pain management because of its potency and in spite of a major side effect of inducing chemical dependency. A study (1) of these pharmacological responses of morphine in humans presented compelling evidence that most of the analgesic activity of the drug was actually produced not by the parent drug but by the active metabolite morphine-6-glucuronide which is formed in significant quantities upon administration of morphine in man (2). This striking observation was in part explained by the unexpected lipophilicity of morphine-6-glucuronide (3).

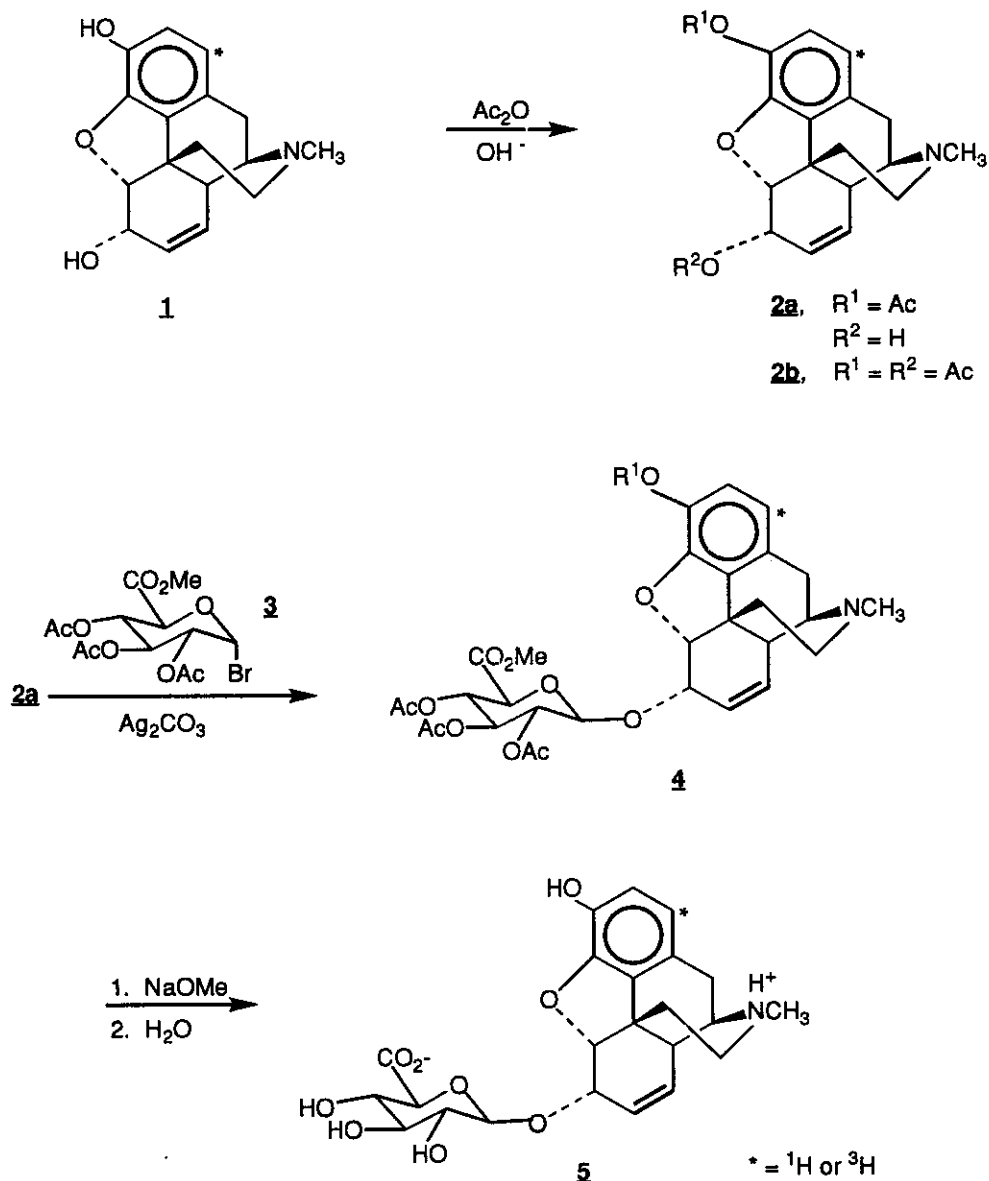
It has long been known that morphine activity is mediated through specific opioid receptors and it has been recognized that different receptor subtypes are responsible for the variety of drug reactions including supraspinal analgesia, respiratory depression, and drug addiction (4). In traditional receptor binding studies the 6-glucuronide of morphine showed a somewhat lower affinity for opioid

receptors (5) although the metabolite was 100-fold more active as analgesic than morphine itself. Furthermore, antagonist studies suggested that morphine-6-glucuronide activity was mediated through opioid μ -receptors in spite of its lower affinity for these receptors. The apparent discrepancy between extremely high analgesic potency and comparatively low binding energy to known opioid receptors suggested the existence of yet another μ -receptor subtype for morphine-6-glucuronide (6). Since the interactions of this morphine metabolite with opioid receptor subtypes are still poorly understood, tritium labelled morphine-6-glucuronide was synthesized in order to facilitate more detailed studies of this potent analgesic drug of significant current interest.

RESULTS AND DISCUSSION

The first synthesis of morphine-6-glucuronide on a one millimole scale was reported by Yoshimura et al (7). Their protocol (Scheme 1) involved the preparation of 3-acetylmorphine **2a** by the method of Welsh (8) followed by a glucuronidation using the Koenigs-Knorr reaction and subsequent deprotection of the adduct **4** by transesterification and hydrolysis to the conjugate **5**. The adaptation of this procedure to a micromolar scale suitable for radiosynthesis presented some interesting challenges. Acetylation according to Welsh of **1** on a 30 micromolar scale with excess acetic anhydride in the presence of aqueous NaHCO₃ surprisingly produced a mixture of **2a** and **2b** in 2:1 ratio based on evidence of comparison with authentic unlabelled materials. After extraction of these products the aqueous phase contained still unreacted **1** which was subsequently treated with more acetic anhydride. Again a mixture of **2a** and **2b** resulted, however with a ratio of 6:1. We concluded from this observation that acetylation at lower pH would prevent formation of **2b**. Conducting acetylation in a phosphate buffer at pH 7 with a large excess of acetic anhydride produced pure **2a** in ~90% yield; no **2b** was detectable by TLC analysis.

The glucuronidation of **2a** by an adapted Yoshimura's procedure on a 30 micromolar scale required treatment of **2a** with 15 mg each of **3** and Ag₂CO₃ to be added over a 9 h period. The tedious addition of these reagents was necessary to avoid any major excess of Ag₂CO₃ in the reaction mixture at any time which would lead to oxidation of morphine to morphinone as described by Rapoport (9) and Reist. The difficulty of adapting this procedure to radiosynthesis was resolved in a study with unlabelled materials in which we found that the undesirable side reaction could be eliminated if the coupling was conducted at 105-110° C in toluene for a much shorter reaction time. Consistent conversions of **2a** to **4** in 80-90% yield were obtained in only 30 min reaction time. No significant amounts of morphinone were detected by TLC even if the Ag₂CO₃ was added in 3-fold



Scheme 1. Synthesis of [1-³H]morphine-6-β-D-glucuronide (³H-5)

excess. The only drawback from using a large excess of the bromosugar **3** and Ag₂CO₃ in the glucuronidation was the concomitant formation of major quantities of extraneous sugar analogs. On a micromolar scale however these could be removed by chromatography on silica gel which furnished **4** in ~95% chemical purity based on TLC comparison with authentic material.

The final step of the synthesis involved removal of the protecting groups from **4** and purification of the resulting **5** by crystallization. Since the reported procedure for removal of the protecting groups (**7**) was not acceptable for radiosynthesis, a modified approach was developed. Treatment of **4** with 2 molar equivalents of methanolic sodium methoxide followed by addition of lithium hydroxide gave **5** without significant formation of by-products. Purification of **5** was accomplished by careful chromatography on microcrystalline cellulose which provided morphine glucuronide **5** in 70% yield (44% based on **1**) and > 95% purity by HPLC analysis.

These reaction conditions were adopted for the synthesis of [1-³H]morphine-6-glucuronide **3H-5**. A solution of **3H-1** (300 mCi, 14 μ moles specific activity 21 Ci/mmol, prepared as reported) (**10**) in a buffer containing triethylamine and acetic acid (from HPLC purification) when treated with acetic anhydride in NaHCO₃ solution at pH 7.5 reacted very sluggishly and furnished only 13% of **3H-2a**. We speculated that the presence of triethylamine in the reaction mixture was interfering with the acetylation of **3H-1**. After addition of excess NaHCO₃ the solution of unreacted **3H-1** was concentrated to remove triethylamine and then treated with acetic anhydride and NaHCO₃ portions over 1 h while maintaining pH ~7-8 and periodically removing **3H-2a** by CH₂Cl₂ extraction. Combined extracts contained 94% of the radioactivity but radiochemical purity was only 75% (TLC-RAM) possibly due to decomposition of **3H-2a** under analysis conditions.

Because of the apparent instability of the crude **3H-2a**, it was directly used in the glucuronide coupling without further purification. Thus, bromosugar **3** and Ag₂CO₃ were applied in large excess (7 molar and 14 molar, respectively) since exploratory test runs indicated that glucuronidation of **3H-2a** was not adversely affected and any remaining water and other impurities were eliminated by sufficient excess of reagents. The reaction was interrupted after 15 min at which time analysis by TLC-RAM indicated the presence of 75% **3H-4** in the crude mixture. Repeated silica gel chromatographies using a gradual gradient of methanol in methylene chloride-ethyl acetate provided a 42% yield of **3H-4** (based on **3H-1**) with a radiochemical purity of 95% which was considered satisfactory for the next step.

Deprotection of **3H-4** was accomplished by treatment with 10 molar excess of sodium methoxide followed by addition of water for hydrolysis of the methyl glucuronate functional group. (Exploratory tests had shown that performing the deprotection in two steps gave a purer product and less decomposition than one step hydrolysis with sodium hydroxide). Crude **3H-5** was obtained in 86% radiochemical yield and was purified by repeated reversed phase silica gel chromatography which proved to be more efficient than cellulose chromatography applied previously in the isolation of

unlabelled **5**. Thus [1-³H]morphine-6-glucuronide was obtained in 57% yield (based on **3H-4**) with a specific activity of 21 Ci/mmol and radiochemical purity of 99% (TLC-RAM and HPLC-RAM). Stored in aqueous methanol, **3H-5** was found to be stable for more than one year.

EXPERIMENTAL

Reactions were conducted using anhydrous solvents under nitrogen atmosphere unless otherwise noted. HPLC analysis was performed on a Rainin system equipped with a Rheodyne injector, Rabbit HP pump, and JN/US β-RAM detector with a 525 μL liquid scintillator cell. TLC radioscan were obtained with a Bioscan System 200 imaging scanner. For TLC analysis E. Merck 250 μm silica gel plates with F254 UV indicator were used. Radioactivity was determined on a Packard Minaxi Tricarb Series 4000 liquid scintillation counter.

3-Acetyl [1-³H]morphine, 3H-2a. A solution of [1-³H]morphine (**3H-1**) (300 mCi, 21 Ci/mmol, 0.014 mmol) (10) in HPLC eluant (62% MeOH, 36% H₂O, 1.2% HOAc, 0.8% Et₃N) was concentrated to 3 mL with a nitrogen stream. The concentrate was diluted with water (10 mL) and the pH was adjusted to 7.5 with aqueous NaHCO₃. Acetic anhydride (0.40 mL, 4.2 mmol) was added with rapid stirring over a 15 min period. The reaction mixture was extracted with CH₂Cl₂ (2 x 4 mL) to remove **3H-2a**. The aqueous layer contained 262 mCi of unchanged **3H-1** and was now at pH 6. Solid NaHCO₃ (1.0 g, 11.9 mmol) was added to the aqueous reaction mixture and concentrated with a nitrogen stream to 5 mL, to further remove residual MeOH, diluted with water (15 mL), and treated with a further portion of acetic anhydride (0.4 mL, 4.2 mmol) over a 15 min period. The reaction mixture was extracted with CH₂Cl₂ (2 x 3 mL) to remove further **3H-2a** that formed. Two additional cycles of acylation and extraction were conducted: NaHCO₃ (1.5 g, 17.9 mmol), acetic anhydride (0.56 mL, 5.9 mmol) over 15 min, extraction, NaHCO₃ (0.6 g, 7.1 mmol), acetic anhydride (0.56 mL, 5.9 mmol) over 15 min, extraction. At this point, the aqueous phase contained only 17.3 mCi of unreacted **3H-1**. The combined organic extracts were concentrated and diluted and re-concentrated with toluene. TLC-radioscan (SiO₂, CHCl₃-MeOH-NH₄OH 9:1:0.1) revealed a product with a purity of 75% and with the identical R_f (0.69) to that of authentic material (8). The product, which appeared to decompose on the TLC plate, was used without further purification.

Methyl (3-Acetyl [1-³H]morphine-6-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosid) Uronate, 3H-4. A toluene solution of **3H-2a** (assumed 282 mCi, see above) was concentrated to 700 μL and treated with the bromosugar **3** (11) (42 mg, 0.11 mmol) in one portion. The clear solution was heated to 105-110 °C in a septum capped flask under nitrogen and treated with freshly prepared

Ag₂CO₃ (12) (55 mg, 0.2 mmol) in one portion. The resulting suspension was heated at 105-110 °C with vigorous stirring under a slow nitrogen flow for 15 min while replenishing toluene to maintain a volume of 400-500 µL. The suspension was then filtered and analyzed by TLC-radioscan (SiO₂, CHCl₃-MeOH-NH₄OH, 9:1:0.1) R_f 0.80 [identical with authentic material (4)] indicating a radiochemical purity of 75%. Concentration to 300 µL and chromatography on 4 g of silica gel eluting with a gradient of 1-7% MeOH in CH₂Cl₂-EtOAc, 4:1 readily removed sugar byproducts and raised the purity to 95% after two further elutions with a more gradual gradient. Yield 119 mCi (42%).

[1-³H]Morphine-6-β-D-glucuronide, ³H-5. Combined chromatography fractions of ³H-4 (119 mCi) were concentrated to 100 µL and immediately diluted with methanol (4 mL) and treated with freshly prepared 0.22 M NaOMe (300 µL). After standing for 4 h at ambient temperature, the pale yellow mixture was treated with water (0.5 mL) and allowed to stand overnight. TLC-radioscan showed the reaction to be complete and contain ³H-5 in 86% radiochemical purity. Adjustment of the mixture to pH 6 with 2.5% methanolic acetic acid was followed by concentration to 200 µL, dilution with water (1.5 mL) and reconcentration to 1 mL under a nitrogen stream to remove volatile organic solvent prior to chromatography. The crude product was chromatographed successively on 1.3 g then 1.4 g of Whatman LRP-2 reversed phase C18 bonded silica gel eluting with H₂O, 3% MeOH-H₂O, and 6% MeOH-H₂O and then rechromatographed with H₂O, 2% MeOH-H₂O, and 4% MeOH-H₂O to yield 67.7 mCi (57%) of the title compound. The radiochemical purity was 99% by TLC-radioscan (SiO₂, CHCl₃-MeOH-H₂O-NH₄OH, 2:3:1:0.07) R_f 0.66 and HPLC (Rainin Microsorb 5 µ C18 250 x 4.6 mm reversed phase silica gel column, 28% CH₃CN - 0.01 M NaH₂PO₄-0.001 M dodecanesulfonic acid buffer to pH 2.5 with H₃PO₄, β-RAM detection, 1 mL/min) R_t 4.0 min. The product was stored in 50 mL MeOH - H₂O at -70 °C.

ACKNOWLEDGEMENT

This work was supported by NIDA Contract No. N01DA-6-7054

REFERENCES

1. Abbott, F.; Palmour, R. — *Life Sci.* **43**: 1685 (1988)
2. Osborne, R.; Joel, S.; Trew, D.; Slevin, M. — *Clin. Pharmacol. Ther.* **47**: 12 (1990)
3. Carrupt, P.-A.; Testa, B.; Bechalany, A.; El Tayar, N.; Descas, P.; Perrissoud, D. — *J. Med. Chem.* **34**: 12721 (1991)
4. Wolozin, B. L.; Pasternak, G. W. — *Proc. Natl. Acad. Sci.* **78**: 6181 (1981)
5. Paul, D.; Standifer, K. M.; Inturrisi, K. M.; Pasternak, G. W. — *J. Pharmacol. Exp. Ther.* **251**: 477 (1989)

6. Rossi, G. C.; Pan, Y.-X.; Brown, G. P.; Pasternak, G. W. — *FEBS Let.* **369**: 192 (1995)
7. Yoshimura, H.; Oguri, K.; Tsukamoto, H. — *Chem. Pharm. Bull.* **16**: 2114 (1968)
8. Welsh, L. H. — *J. Org. Chem.* **19**: 1409 (1954)
9. Rapoport, H.; Reist, H. H. — *J. Am. Chem. Soc.* **72**: 490 (1950)
10. Seltzman, H. H.; Roche, M. J.; Laudeman, C. P.; Wyrick, C. D.; Carroll, F. I. — *J. Label. Compound and Radiopharm.*, in press.
11. Bollenback, G. N.; Long, T. W.; Benjamin, D. G.; Lindquist, J. A. — *J. Am. Chem. Soc.* **77**: 3310 (1955)
12. Wolfrom, M. L.; Lineback, D. R. — *Methods in Carbohydrate Chemistry*, Whistler, R. L.; Wolfrom, M. L. Eds.; Academic Press, New York, Vol 2, p. 341, 1963