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Tetrahedron: *Asymmetry* 11 (2000) 413–416

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TETRAHEDRON:  
*ASYMMETRY*

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## The enzymatic glucuronidation of 3-*O*-protected morphine — a new route to 7,8-dihydromorphine-6-glucuronide

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Received 1 December 1999; accepted 13 December 1999

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### Abstract

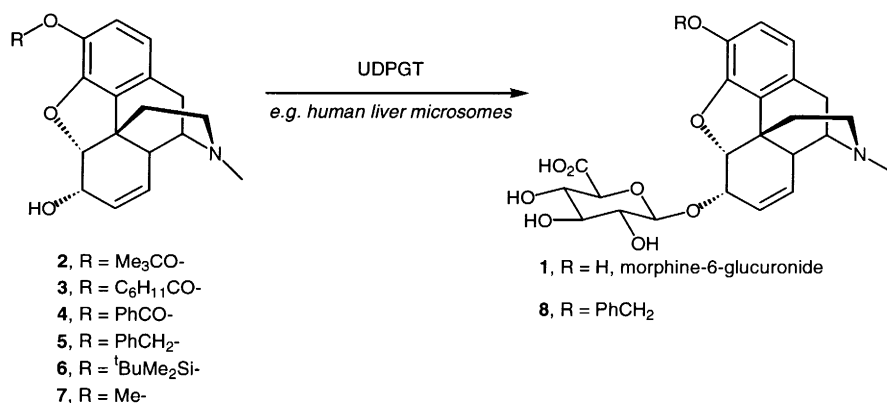
The selective enzymatic glucuronidation of the 6-position of morphine was probed by the glucuronidation of a series of 3-*O*-protected morphine derivatives. 3-*O*-Benzylmorphine **5** was converted to the corresponding 3-*O*-benzylmorphine-6-glucuronide **8** by human liver microsomes. The identity of **8** was confirmed by independent chemical synthesis. The glucuronide **8** was subsequently converted to 7,8-dihydromorphine-6-glucuronide. © 2000 Elsevier Science Ltd. All rights reserved.

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We have been interested in morphine-6-glucuronide **1** (M-6-G) for many years and have previously reported both chemical and enzymatic syntheses.<sup>1,2</sup> Glucuronidation is carried out by the membrane bound enzymes, UDP-glucuronosyl transferases (UGT), which are to be found in a number of tissues but usually have a greater abundance in the liver. In common with the P450 enzymes responsible for primary metabolism, there is a family of UGTs that has evolved to glucuronidate a variety of substrate classes.<sup>3</sup> These have been extensively characterised and a nomenclature system has been developed based upon the DNA sequence of the genes coding for their production. In humans at least 20 different UGTs have been identified and a handful of these have now been cloned and expressed. Of these, one isoform, UGT2B7, has been shown to catalyse morphine glucuronidation at the 3- and 6-positions.<sup>4</sup> The ratio of 3- to 6-glucuronide was 7:1 — similar to the reported ratio for human liver microsomes. This indicates that it is unlikely that there is one UGT catalysing 6-glucuronidation and a separate UGT catalysing 3-glucuronidation. In order to use a UGT enzyme to produce M-6-G **1** selectively, we needed some way to prevent or inhibit this 3-glucuronidation.

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We chose to use a protecting group on the phenolic 3-hydroxy group of morphine and investigate whether this type of substrate would show increased 6-glucuronidation. A range of protecting groups was chosen to include ester, ether and silyl protection (3-pivaloylmorphine **2**, 3-cyclohexanecarbonylmorphine **3**, 3-benzoylmorphine **4**, 3-benzylmorphine **5** and 3-*tert*-butyldimethylsilylmorphine **6**). As a control, 3-methylmorphine **7** (codeine) was also used.

With these substrates prepared, we screened for glucuronidation activity using an assay developed by Burchell et al.<sup>5</sup> This is a radiolabel assay using C-14 radiolabelled UDP-glucuronic acid (UDP-GA). The six substrates and a morphine control were all assayed in duplicate and the level of glucuronide formation determined by the HPLC response. The assay was run with 200 nmol UDP-GA and, as the radiolabel scintillation detector used shows only the unreacted UDP-GA and the formation of any glucuronide, quantification of the conversion is possible. The results are reproduced in Table 1.

Table 1

<i>glucuronosyl transferase activity'</i> (nmols/min/mg)			
<i>Compound</i>	<i>3-glucuronide</i>	<i>6-glucuronide</i>	<i>3:6 ratio</i>
3-Piv <b>2</b>	0.109*	0.013*	8.38
3-Cy <b>3</b>	0.102*	0.034*	3.00
3-Bz <b>4</b>	0.134*	nd	
3-Bn <b>5</b>	nd	0.150	
3-TBS <b>6</b>	nd	nd	
3-Me <b>7</b>	nd	0.032	
Morphine	0.328	0.069	4.75

† averaged result over two repeats. Assays ran at 2mM UDP-GA with 0.1 μCi 14-C UDP-GA for 60 minutes.  
Activity determined by integration of unreacted UDP-GA and any detected glucuronides.

\*All ester derivatives (**2** – **4**) were cleaved to give morphine and subsequently its glucuronides

Microsomes contain enzymes other than glucuronosyl transferases including a range of lipases. It was not unexpected, therefore, to observe the formation of 3- and 6-morphine glucuronide from any of the ester derivatives (**2**, **3** and **4**) which were identified by comparison to the morphine control. The TBS derivative **6** is of interest as it showed no glucuronidation and proved to be a weak inhibitor of glucuronidation by human liver microsomes.

The most interesting result was the glucuronidation of 3-*O*-benzylmorphine **5** to give a glucuronidated

species **8** with a retention time of 10.75 min. This compares to 9 min for the morphine glucuronides and 9.75 min for the codeine (3-*O*-methylmorphine) glucuronide **9** (see Fig. 1).

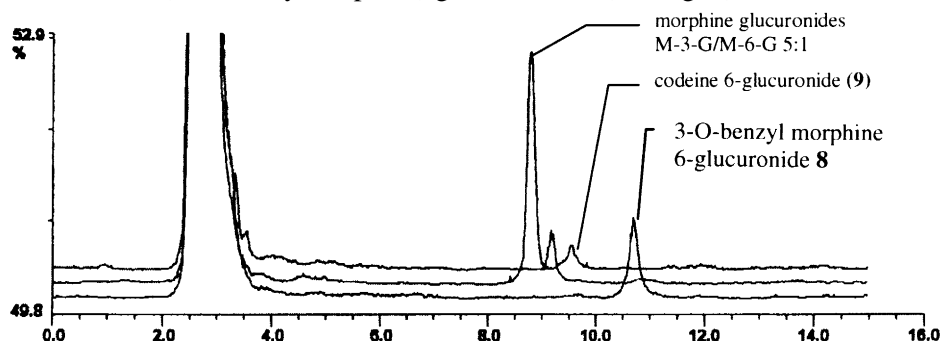
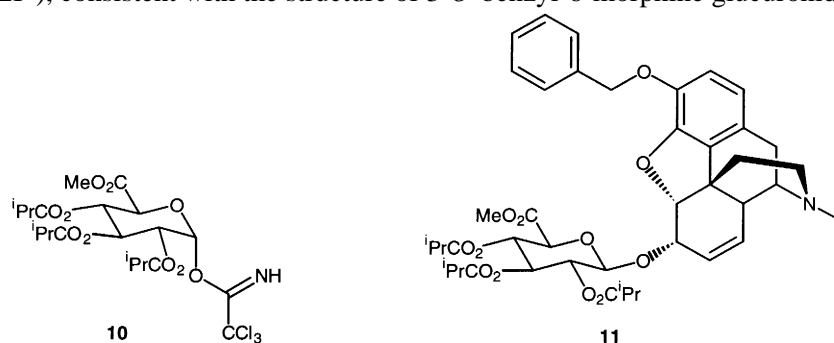
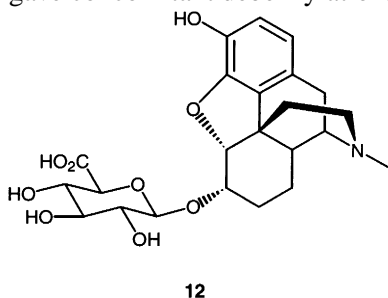


Fig. 1.

The assay was repeated with unlabelled UDP-GA and the supernatants analysed by LC-MS to confirm the identity of the glucuronide **8** formed with 3-*O*-benzylmorphine **5**. The retention time of 3-*O*-benzylmorphine **5** was 11.74 min and the LC of the production of the glucuronidation of 3-*O*-benzylmorphine showed a small peak at 11.28 min corresponding to the glucuronide **8** peak we had observed with the radiolabel study. The MS corresponding to this peak at 11.28 min showed a molecular ion of 552.18 (EI<sup>+</sup>), consistent with the structure of 3-*O*-benzyl-6-morphine glucuronide **8**.



The structure of the enzymatic product was corroborated by chemical synthesis and conversion to a known compound. Thus, reaction of 3-*O*-benzylmorphine **5** with the tri-isobutyryl imidate **10**<sup>6</sup> [BF<sub>3</sub>·Et<sub>2</sub>O, (CH<sub>2</sub>Cl)<sub>2</sub>] afforded glucuronate ester **11** in 48% yield after chromatography; use of the stronger Lewis acid Me<sub>3</sub>SiO<sub>3</sub>SCF<sub>3</sub> gave concomitant debenzylation.



This provides another example<sup>1</sup> of the value of **10** in the glucuronidation of morphine derivatives, despite erroneous reports to the contrary.<sup>7</sup> Hydrolysis of **11** using NaOH in aq. Pr<sup>i</sup>OH followed by neutralisation, then purification by reverse-phase silica chromatography, afforded the free glucuronide **8** (66%) which proved identical (MS, HPLC) with the enzymatic product.

Finally, hydrogenolysis of **8** (10% Pd–C, H<sub>2</sub>O–MeOH–AcOH) gave both debenylation and reduction of the 7,8-double bond, leading to 7,8-dihydromorphine-6-glucuronide **12**. We have reported previously<sup>8</sup> that this derivative is a more potent analgesic than either morphine or morphine-6-glucuronide.

*Selected spectroscopic data:* compound **11**:  $\delta_{\text{H}}$  (300 MHz, CDCl<sub>3</sub>), inter alia, 2.80 (3H, s), 3.79 (3H, s), 4.20 (1H, d), 4.45 (1H, m), 5.00 (2H, m), 5.15–5.50 (6H, m), 5.85 (1H, br d), 6.52 and 6.73 (2H, 2 d);  $m/z$  (E.I.) 775 (M<sup>+</sup>, 10%). Compound **8**:  $\delta_{\text{H}}$  [300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO+D<sub>2</sub>O], inter alia, 2.85 (3H, s), 4.05 (1H, m), 4.45 (1H, m), 4.56 (1H, d), 5.05 (2H, s), 5.16 (1H, narrow d), 5.25 and 5.75 (2H, 2 d), 6.60 and 6.78 (2H, 2 d), and 7.20–7.40 (5 H, m);  $m/z$  (E.S.+ve mode) 574 (MNa<sup>+</sup>, 20%) and 552 (MH<sup>+</sup>, 100%).

## Acknowledgements

We are very grateful to Prof. Brian Burchell for his advice and allowing us to carry out the radiolabel experiment in his laboratories. This work was partially funded by the Teaching Company Scheme.

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