

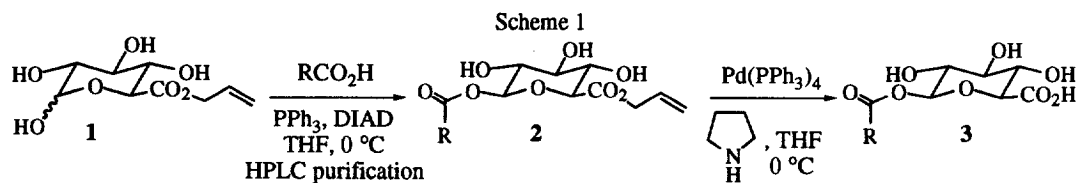
A Convenient Synthesis of  $\beta$ -Acyl Glucuronides

Hélène Juteau,\* Yves Gareau and Marc Labelle

Merck Frosst Centre for Therapeutic Research  
P.O. Box 1005, Pointe-Claire-Dorval, Québec, Canada, H9R 4P8

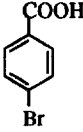


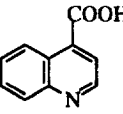
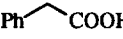
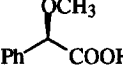
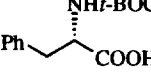
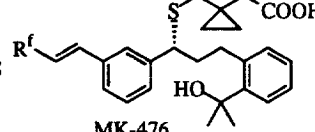
**Abstract:**  $\beta$ -Acyl glucuronides are readily prepared in two steps from allyl D-glucuronate **1** in 14 to 29% overall yields. © 1997 Elsevier Science Ltd. All rights reserved.

Xenobiotics containing carboxylic acid groups are often readily metabolized to acyl glucuronides *in vivo*.<sup>1</sup> As part of our LTD<sub>4</sub> antagonist program, we needed substantial amounts of the acyl glucuronide of MK-476 (Montelukast,<sup>2</sup> Singulair™) for use as a chromatography standard. The preparation of this class of compounds has been reported by enzymatic<sup>3</sup> and chemical<sup>4</sup> methods. In the event where a small amount of  $\beta$ -acyl glucuronide is required, the enzymatic approach is the method of choice. This method becomes tedious and difficult to use on larger scales since decomposition and acyl migration often occur during the isolation step, mainly due to the high pH sensitivity<sup>5</sup> of the desired material. Therefore, when multimilligram amounts of  $\beta$ -acyl glucuronide are needed, a synthetic approach should be envisioned. The syntheses of acyl glucuronides described in the literature<sup>4</sup> require many steps with several protections and deprotections. This prompted us to seek a shorter route and we disclose herein a two-step synthesis of the title compounds without protection of the four hydroxyl groups of the starting allyl glucuronate **1**<sup>6</sup> (Scheme 1).



The first step consists in a selective coupling reaction between carboxylic acid substrate and the allyl glucuronate **1**.<sup>7</sup> In 1986, Smith and co-workers<sup>8</sup> reported a regio and stereoselective synthesis of glycosyl esters from glycoside and carboxylic acid using the Mitsunobu coupling reaction.<sup>9</sup> In the same manner, we are taking advantage of the higher reactivity of the anomeric hydroxyl in the Mitsunobu reaction.<sup>10</sup> The allyl glucuronate **1** was added to a mixture of the desired aromatic carboxylic acid, triphenylphosphine and diisopropylazodicarboxylate (DIAD) in THF at 0 °C for 1 hour.<sup>11,12</sup> Aromatic carboxylic acids gave mixtures of  $\alpha$  and  $\beta$  isomers in a 1 : 5 ratio (entries 1, 2, 3, 4). The  $\beta$ -isomer **2** could be isolated in 25%-40% yield after HPLC purification.<sup>13</sup> The lower yield obtained for 2-bromobenzoic acid (25%, entry 2) may be attributed to steric factors.

**Table 1. Mitsunobu Reaction and Removal of the Protecting Group.**

Entry	RCOOH	Compound 2 Ratio of $\beta$ : $\alpha$ <sup>a</sup> ; Yield of $\beta$ -isomer <sup>b</sup>	Compound 3 <sup>b</sup>
1		5 : 1 ; 40% <sup>c</sup>	70% <sup>e</sup>
2		5 : 1 ; 25% <sup>c</sup>	74% <sup>e</sup>
3		5 : 1 ; 33% <sup>c</sup>	88% <sup>e</sup>
4		5 : 1 ; 35% <sup>c</sup>	50% <sup>e</sup>
5		3 : 1 ; 23% <sup>d</sup>	73% <sup>e</sup>
6		2 : 1 ; 25% <sup>d</sup>	69% <sup>e</sup>
7		2 : 1 ; 23% <sup>d</sup>	63% <sup>e</sup>
8		2 : 1 ; 20% <sup>d</sup>	76% <sup>e</sup>

<sup>a</sup>Ratio determined by <sup>1</sup>H NMR on the crude reaction mixture. <sup>b</sup>Isolated yields by HPLC. <sup>c</sup>To a mixture of carboxylic acid and PPh<sub>3</sub> in THF at 0 °C was added DIAD, followed by allyl glucuronate 1. <sup>d</sup>To a mixture of carboxylic acid, PPh<sub>3</sub> and allyl glucuronate 1 in THF at 0 °C was added DIAD. <sup>e</sup>To a mixture of allyl ester 2, Pd(PPh<sub>3</sub>)<sub>4</sub> in THF at 0 °C was added pyrrolidine. <sup>f</sup>R = 7-Chloroquinolinyl.

When this method was applied to aliphatic carboxylic acids, no reaction was observed. We could however get the reaction to proceed by changing the order of mixing of the reagents, namely by adding DIAD to a mixture of aliphatic carboxylic acid, triphenylphosphine and allyl glucuronate 1 in THF at 0 °C.<sup>14</sup> This reaction gave the desired compound 2 although the selectivity for the  $\beta$ -isomer was lower (entries 5, 6, 7, 8). The lower yields (20-25%) obtained for aliphatic carboxylic acids relative to those of the aromatic acids in the Mitsunobu reaction have been previously rationalized<sup>15</sup> by a different reactivity of the carboxylate intermediates.

The last step of the sequence was the removal of the allyl protecting group. This could be readily accomplished with palladium (0) and pyrrolidine in THF at 0 °C for 1 hour<sup>16</sup> to give 3 which is difficult to

handle, because it is acid and base sensitive.<sup>5</sup> The purification was performed by reversed-phase HPLC using acetonitrile and aqueous acetic acid as the eluent. No acyl migration has been observed during the isolation under these conditions. In the case of the acyl glucuronide of MK-476, the structure was further confirmed by comparison with an authentic sample isolated from a rat microsome incubation mixture.<sup>3c</sup> The nature and stereochemistry of the acyl glucuronide was also ascertained by cleavage with  $\beta$ -glucuronidase, which gave a rate of cleavage similar to that of other known glucuronides.<sup>17</sup>

In conclusion,  $\beta$ -acylglucuronides were prepared in only two steps from **1** and an aliphatic or aromatic carboxylic acid. The methodology uses a single protection of glucuronic acid carboxylic group which is deprotected under neutral conditions.

**ACKNOWLEDGMENT:** The authors acknowledge Yves Aubin for NMR experiments.

#### REFERENCES AND NOTES:

- G.J. Mulder. *Conjugation Reactions in Drug Metabolism*. Taylor and Francis, NY, 1990, 51-105.
  - G.J. Dutton. *Glucuronidation of Drugs and Other Compounds*. CRC Press, Boca Raton, FL, 1980.
- Labelle, M.; Belley, M.; Gareau, Y.; Gauthier, J.Y.; Guay, D.; Gordon, R.; Grossman, S.G.; Jones, T.R.; Leblanc, Y.; McAuliffe, M.; McFarlane, C.S.; Masson, P.; Metters, K.M.; Ouimet, N.; Patrick, D.H.; Piechuta, H.; Rochette, C.; Sawyer, N.; Xiang, Y.B.; Pickett, C.B.; Ford-Hutchinson, A.W.; Zamboni, R.J.; Young, R.N. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 283-288.
- Fenselau, C.; Pallante, S.; Parikh, I. *J. Med. Chem.* **1976**, *19*, 679-683.
  - Parikh, I.; MacGlashan, D.W.; Fenselau, C. *J. Med. Chem.* **1976**, *19*, 296-299.
  - Nicoll-Griffith, D.; Yergey, J.; Trimble, L.; Williams, H.; Rasori, R.; Zamboni, R. *Drug Metabolism* **1992**, *20*, 383-389.
- DeMesmaeker, A.; Hoffmann, P.; Ernst, B. *Tetrahedron Lett.* **1989**, *30*, 3773-3776. This method gave the  $\beta$ -acyl glucuronide in 3.9 % overall yield in 10 steps.
  - Bugianesi, R.; Shen, T.Y. *Carbohydrate Res.* **1971**, *19*, 179-187. This synthesis yielded the  $\beta$ -acyl glucuronide in 1.7 % overall yield in 8 steps.
- Hansen-Moller, J.; Cornett, C.; Dalgaard, L.; Hansen, S.H. *J. Pharm. Biomed. Anal.* **1988**, *6*, 229-240.
  - Sinclair, K.A.; Caldwell, J. *Biochem. Pharm.* **1982**, 953-957.
- Compound **1** is readily prepared by selective protection of the acid moiety: To a solution of D-glucuronic acid (2.00 g, 10.3 mmol) in 20 ml of DMF at 25 °C, was added DBU (1.70 ml, 11.3 mmol). The mixture was stirred for 15 minutes, then allyl bromide (1.10 ml, 12.3 mmol) was added. The mixture was stirred overnight at 25 °C. The solvent was then removed under high vacuum at 65 °C. The product was purified by flash chromatography over silicic acid gel (8 toluene/2 MeOH/0.1 CH<sub>2</sub>Cl<sub>2</sub>). This yielded the desired allyl ester **1** (1.82 g, 75% yield, ratio 2 : 1,  $\alpha$  :  $\beta$ ). [ $\alpha$ ]<sub>D</sub><sup>25</sup>: +55.9 (c=1.11, MeOH); <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD):  $\beta$ -isomer  $\delta$  3.17 (dd, 1H, *J*= 7.8 and 9.2 Hz); 3.37 (m, 1H); 3.53 (m, 1H); 3.84 (d, 1H, *J*= 9.8 Hz); 4.50 (d, 1H, *J*= 7.8 Hz, CH<sub>β</sub>); 4.65 (m, 2H); 5.22 (dt, 1H, *J*= 10.5 and 1.3 Hz); 5.34 (ddd, 1H, *J*= 17.2, 2.3 and 1.5 Hz); 5.93 (m, 1H);  $\alpha$ -isomer  $\delta$  3.37 (m, 1H); 3.53 (m, 1H); 3.67 (t, 1H, *J*= 9.2 Hz); 4.30 (d, 1H, *J*= 9.8 Hz); 4.65 (m, 2H); 5.11 (d, 1H, *J*= 3.6 Hz, CH<sub>α</sub>); 5.22 (dt, 1H, *J*= 10.5 and 1.3 Hz); 5.34 (ddd, 1H, *J*= 17.2, 2.3 and 1.5 Hz); 5.93 (m, 1H); <sup>13</sup>C NMR of mixture (100 MHz, CD<sub>3</sub>OD):  $\delta$  66.7, 66.8, 72.6, 73.1, 73.3, 73.4, 74.3, 75.8, 77.0, 77.3, 94.4, 98.6, 118.6, 118.7, 133.1, 133.2, 170.4, 171.6. IR (KBr, cm<sup>-1</sup>): 3350, 2940, 1735, 1450, 1400, 1350, 1315, 1255, 1190, 1145, 1125, 1045, 955, 740; HRMS FAB (C<sub>9</sub>H<sub>14</sub>O<sub>7</sub> + Na) calc.: 257.0637; found: 257.0636.
- For review on O-glycosylation methods see: Toshima, K.; Tatsuta, K. *Chem. Rev.* **1993**, *93*, 1503-1531.
- Smith III, A.B.; Hale, K.J.; Rivero, R.A. *Tetrahedron Lett.* **1986**, *27*, 5813-5816.
- Reviews on Mitsunobu reaction: a) Mitsunobu, O. *Synthesis* **1981**, 1-28. b) Hughes, D.L. *Org. Reactions* **1992**, *42*, 335-656.

10. a) Beaupere, D.; Uzan, R.; Meslouti, A.E.; Aouad, M.E.A. *Tetrahedron Lett.* **1994**, *35*, 6279-6282. b) Penades, S.; Corrales, G.; Sharma, S.K. *Tetrahedron Lett.* **1995**, *36*, 5627-5630. c) Schmidt, R.R.; Schmidt, C.; Huchel, U. *Tetrahedron Lett.* **1995**, *36*, 9457-9460.
11. Coupling reaction of aromatic carboxylic acids **2**: To a solution of 4-bromobenzoic acid (206 mg, 1.02 mmol), PPh<sub>3</sub> (270 mg, 1.02 mmol) in 4 ml of THF at 0 °C was added DIAD (200 μL, 1.02 mmol). After 5 min, a solution of allyl glucuronate **1** (120 mg, 0.51 mmol) in 1 ml of THF was added slowly (over 10 minutes). The solution turned dark. After 1h at this temperature, the solvent was removed under vacuum. <sup>1</sup>H NMR spectrum on the crude reaction mixture showed a 5 : 1 ratio of β : α isomers. The product was purified by filtration over silica gel (95 CH<sub>2</sub>Cl<sub>2</sub>/5 MeOH) and by HPLC (Novapak C-18, 25 x 100mm, 12 ml/min, 35% CH<sub>3</sub>CN/65% H<sub>2</sub>O, rt= 14.7 min). This reaction yielded the desired product **2** (80 mg, 40% yield). [α]<sub>D</sub><sup>25</sup>: +2.5 (c=0.96, MeOH); <sup>1</sup>H NMR (400 MHz, acetone): δ 3.65 (m, 2H); 3.74 (m, 1H); 4.13 (d, 1H, J= 9.5 Hz); 4.64 (d, 2H, J= 5.5 Hz); 5.18 (dd, 1H, J= 1.2 and 10.5 Hz); 5.34 (dd, 1H, J= 1.5 and 17.2 Hz); 5.81 (d, 1H, J= 7.6 Hz); 5.93 (m, 1H); 7.73 (d, 2H, J= 8.5 Hz); 8.00 (d, 2H, J= 8.5 Hz). <sup>13</sup>C NMR (100 MHz, acetone): δ 70.3, 76.7, 77.6, 81.1, 81.3, 100.2, 122.5, 133.2, 133.6, 136.6, 136.9, 137.1, 168.9, 172.9. IR (neat, cm<sup>-1</sup>): 3400, 1725, 1675, 1585, 1420, 1315, 1290, 1255, 1175, 1125, 1065, 1010, 850, 755; HRMS FAB (C<sub>16</sub>H<sub>17</sub>O<sub>8</sub>Br + Na) calc.: 439.0005; found: 439.0004.
12. Changes in the stoichiometry of the reagents, temperature and time of the reaction afforded no better result. Different phosphines and azodicarboxylates were also used but did not provide any improvement on the yield of the reaction.
13. A third major product could be isolated during the reaction, in a 1 : 1 ratio with the α-isomer. We tentatively assign the following structure for **4** based on NMR analysis: [α]<sub>D</sub><sup>25</sup>: +33.6 (c=0.72, MeOH); <sup>1</sup>H NMR (400 MHz, acetone): δ 4.55 (s, 1H); 4.67 (d, 1H, J= 6.0 Hz); 5.01 (d, 1H, J= 4.2 Hz); 5.13 (dd, 1H, J= 6.0 and 4.2 Hz); 6.46 (s, 1H); 7.67 (dt, 2H, J= 8.8 and 2.3 Hz); 7.88 (dt, 2H, J= 8.8 and 2.0 Hz). <sup>13</sup>C NMR (100 MHz, acetone): δ 70.3, 78.6, 81.4, 82.9, 103.7, 128.7, 129.8, 132.3, 132.5, 164.6, 175.4. IR (neat, cm<sup>-1</sup>): 3400, 1800, 1715, 1705, 1590, 1265, 1130, 1120, 1110, 1070, 1060, 1030, 1010, 980, 940, 750; HRMS FAB (C<sub>13</sub>H<sub>13</sub>O<sub>8</sub>Br + Na) calc.: 380.9586; found: 380.9587. We also ran <sup>1</sup>H, <sup>13</sup>C, HMQC, HMBC and NOE NMR experiments on the acetylated analogue.
14. Coupling reaction of aliphatic carboxylic acids **2**: To a solution of 2-bromophenylacetic acid (1.08 g, 5.0 mmol), PPh<sub>3</sub> (1.31 g, 5.0 mmol) and allyl glucuronate **1** (585 mg, 2.5 mmol) in 25 ml of THF at 0 °C was added DIAD (1.0 ml, 5.0 mmol) over 10 minutes. The dark solution was stirred 1 h at this temperature. The isolation and purification are the same as described for aromatic carboxylic acids.
15. Hughes, D.L.; Reamer, R.A. *J. Org. Chem.* **1996**, *61*, 2967-2971.
16. Deprotection of allyl ester **3**: To a solution of allyl-(4-bromophenyl-1-O-acyl)-β-D-glucuronate **2** (100 mg, 0.24 mmol) in 1 ml of THF at 0 °C was added Pd(PPh<sub>3</sub>)<sub>4</sub> (28 mg, 0.024 mmol) followed by pyrrolidine (19 μL, 0.23 mmol). The mixture was stirred 30 minutes at this temperature and the solvent was removed under vacuum. The product was first purified by filtration over silica gel (8 CH<sub>2</sub>Cl<sub>2</sub>/2 MeOH/0.1% HOAc) followed by HPLC (Novapak C-18, 25 x 100mm, 12 ml/min, 20% CH<sub>3</sub>CN/80% H<sub>2</sub>O/0.1% HOAc, rt= 8.8 min). This gave 60 mg (69% yield) of the acyl glucuronide of 4-bromobenzoic acid **3**. [α]<sub>D</sub><sup>25</sup>: -5.5 (c=0.70, MeOH); <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>CN): δ 3.54 (m, 3H); 4.00 (d, 1H, J= 9.5 Hz); 5.72 (d, 1H, J= 7.5 Hz); 7.70 (d, 2H, J= 8.6 Hz); 7.97 (d, 2H, J= 8.6 Hz). <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>CN): δ 72.1, 72.9, 75.8, 76.4, 95.7, 129.3, 129.4, 132.6, 133.0, 165.2, 169.9. IR (neat, cm<sup>-1</sup>): 3350, 1720, 1695, 1590, 1480, 1400, 1270, 1065, 1010, 840, 750; HRMS FAB (C<sub>13</sub>H<sub>13</sub>O<sub>8</sub>Br + Na) calc.: 398.9691; found: 398.9690.
17. Levvy, G.A.; Marsh, C.A. *The Enzymes*. Academic Press, New York, 2<sup>nd</sup> ed., Vol. 4, 1960, p.397-407.

