Tetrahedron Letters 50 (2009) 4973–4977

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Convenient syntheses of the in vivo carbohydrate metabolites of mycophenolic acid: reactivity of the acyl glucuronide

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ARTICLE INFO

Article history:
Received 11 May 2009
Revised 4 June 2009
Accepted 12 June 2009
Available online 16 June 2009

ABSTRACT

Following in vivo use of mycophenolic acid, the *O*-aryl and *O*-acyl glucuronides, as well as the recently discovered *O*-aryl glucoside (Scheme 1), are all found as metabolites. We describe convenient preparations of all three derivatives. The phenolic glycosides are obtained by phase-transfer-catalysed alkylation of methyl mycophenolate in very high yield, as an excellent alternative to the Königs-Knorr reaction. We carefully optimised our earlier synthesis of the acyl glucuronide to give a highly pure product in a much improved yield. Finally, we describe the value of a synthetic acyl glucuronide in demonstrating its reactivity towards a known target protein with superior response to the naturally obtained material.

The mould metabolite mycophenolic acid **1** (Scheme 1) was first isolated from a *Penicillium* species in 1896.[1] Its potential as an antibacterial and an antifungal agent was reported in 1946;[2,3] later there was considerable interest in its antiviral and antitumour[4,5] activities and many semi-synthetic analogues were prepared.[6] Recently, **1** and its derivatives have become established as highly effective immunosuppressive[7,8] drugs in the course of transplant surgery. The human metabolic products of **1** are therefore of great importance. Interestingly, a new anticancer indication has recently been found for **1**; its hydroxamic acid derivatives show significant activity as histone deacetylase (HDAC) inhibitors.[9]

Following in vivo administration, the major metabolite of **1** is the *O*-aryl (phenolic) glucuronide **2**,[10] apparently a purely detoxifying metabolite. A much smaller part of the dose (ca. 1.25%)[11] is converted into the *O*-acyl glucuronide **3**; this compound appears to retain activity as an inhibitor of the proliferation of mononuclear leukocytes[12] and it has also been implicated in unfavourable interactions with liver proteins.[13–15] Finally, the aryl glucoside **4** has also been identified as a human metabolite:[16] this Letter also gave evidence for the formation of an acyl glucoside in the kidney, but probably not in therapeutically significant amounts.

It is important that analytical standards of compounds **2**–**4** should be available for analysis and toxicology. The synthesis of **2** via the classical Königs-Knorr procedure is known[17] and an enzymatic preparation of **3** was achieved using homogenised horse liver[18] after removing small amounts of **2** by preparative HPLC; no preparation of **4** has been reported. We now report efficient chemical syntheses of **2**–**4**, avoiding heavy metals[19] for **2** and **4**. Further, the selective acylation method for acyl glucuronide synthesis,[20,21] has been optimised to yield highly pure **3** in very good yield. We also report on the protein reactivity of synthetic **3**.

For the preparation of aryl glucuronide **2**, an ester of **1** is necessary. It was best to heat **1** with methanol and the acid ion-exchange resin IR-120, or treat with tosic acid at 20 °C, to afford methyl ester **5**[17] in high yield and purity, Scheme 2. The use of CH_2N_2 or Me_3SiCHN_2 invariably led to over-reaction, with formation of the phenolic *O*-methyl ether.

Using the Königs-Knorr synthesis[17] of **2**, we found that it was simplest to react **5** with an excess (1.5 equiv) of glycosyl bromide **6**[22] in quinoline with Ag_2CO_3 catalysis. In this way, all the methyl ester **5** was converted to afford the desired conjugate **7** in high yield and purity after recrystallisation: excess **5**, in contrast, could only be fully removed by chromatography.

In general, it is desirable to avoid the use of heavy metal catalysts in glycosidation. Glycosidation of phenols by the trichloroacetimidate method using, for example, BF_3[23] is normally excellent, but here cyclisation of **5** occurs, giving the tetrahydropyran derivative **8**.[24] This side-reaction also prohibits the glucuronidation of **5** using perester **9** or hemiacetal **10** with a strong Lewis acid.[23b] We found that the cyclisation of **5** was much slower with the mild Lewis acid $ZnCl_2$; coupling of **5** with **11** using $ZnCl_2$ as catalyst was therefore attempted, but no conjugate **7** could be obtained and **8** was again formed slowly.

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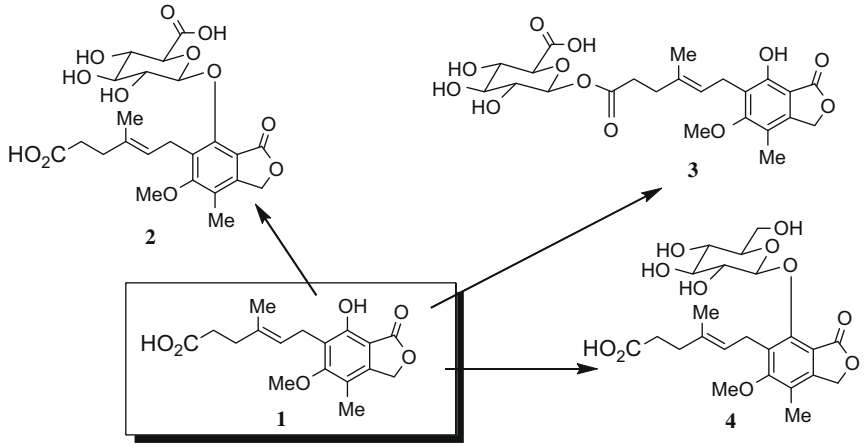
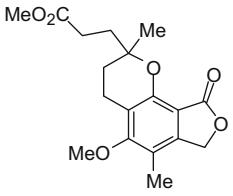
0040-4039/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2009.06.060



Scheme 1. Carbohydrate phase 2 metabolites of mycophenolic acid **1**. It is understood that the same UDPGT isoform, namely UGT1A10, is involved in the biosynthesis of **2** and **3**.



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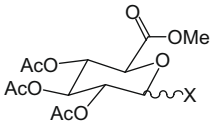


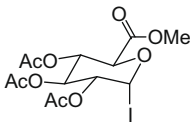
9 X = β-OAc

10 X = α/β-OH

11 X = α-OC(=NH)CCl_3

An excellent alternative method for the transformation of **5** into **7** involves phase-transfer alkylation: initially we used the in situ formed Li phenolate of **5**.[19] Reaction of this salt (Scheme 2) with **6** in a water/$(CH_2Cl)_2$ mixture catalysed by $^nBu_4N^+$ Br^- gave satisfactory conversion into **7**, although it was difficult to drive the reaction to completion: pure **7** was obtained in approximately 40% yield after chromatography. Glycosyl iodide **12**[25] did not give a better yield.

Use of solid phase-transfer catalysis[26] [K_2CO_3 as base in $CHCl_3$ solvent, a minimum amount of water (1:1 molar ratio with the bromosugar) and 2 equiv of **6**] gave a much cleaner reaction: hydrolysis of **5** was avoided and **7** was obtained in 82% yield. We believe that this is the first application of these conditions for glucuronidation: the use of traces of H_2O was mentioned only in a footnote in the original paper[26] but appears crucial and very effective. $PhCH_2N^+Et_3$ Cl^- and $^nBu_4N^+$ Br^- are good catalysts, with $^nBu_4N^+Br^-$ marginally superior, and Cs_2CO_3 is an excellent base. Zemplen deprotection of **7** (using an excess of base since two carboxylates are formed in this step) afforded aryl glucuronide **2** in excellent yield and purity after acidification using IR-120(H^+), filtration, evaporation and trituration with ether. Originally, acetone was used,[17] but MeOH gave a much cleaner product.



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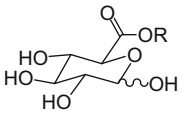
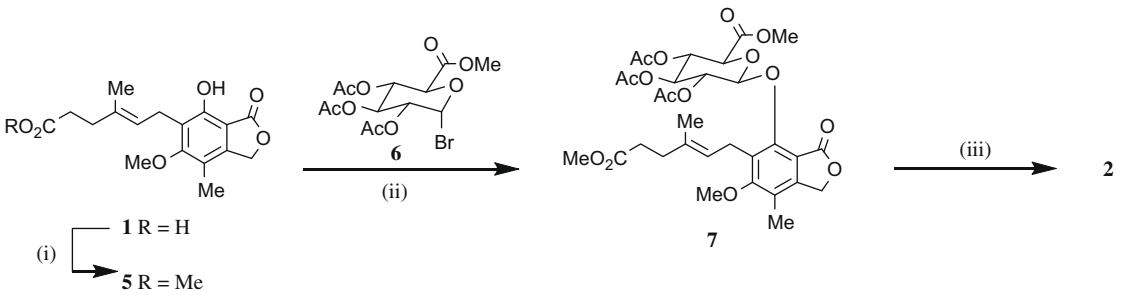


13 R = $CH_2CH{=}CH_2$

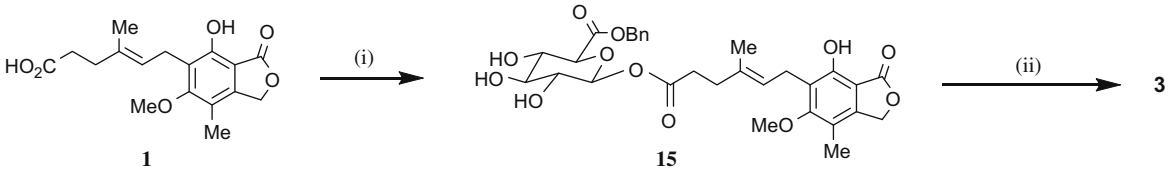
14 R = CH_2Ph

We have already outlined the preparation of acyl glucuronide **3**[20,21] in fair yield, using our selective acylation method, from ester **13** or **14**, and we now report a careful optimisation of this method. The use of the benzyl ester **14** is now preferred, Scheme 3; deprotection of allyl esters requires soluble Pd(0) catalysts[20] and removal of Pd residues may be troublesome.

Thus reaction of **1** with **14** using HATU and either *N*-methylmorpholine (NMM)[20] or *N*-ethylmorpholine (NEM) in acetonitrile afforded benzyl ester conjugate **15** in 55–60% yield after chromatography. It proved best to maintain a 1:1 ratio of **1** to **14** so as to minimise the formation of close-running materials that were difficult to separate fully from **15**. We suspected that these by-products might be due to the free phenolic OH group, and to investigate this, **1** was converted into its *O*-benzyl ether **16**.



Scheme 2. Synthesis of *O*-aryl glucuronide **2**. Reagents and conditions: (i) MeOH, Amberlite IR-120 (H^+), heat, 93%; (ii) quinoline, Ag_2CO_3, 0–20 °C or LiOH, $^nBu_4N^+$ Br^-, $(CH_2Cl)_2$–H_2O, heat, or K_2CO_3, $CHCl_3$, $PhCH_2N^+Et_3$ Cl^-, trace H_2O, 82%; (iii) aq NaOH, MeOH, then IR-120 (H^+), 98%.



Scheme 3. Synthesis of *O*-acyl glucuronide **3**. Reagents and conditions: (i) **14**, HATU, NMM or NEM, MeCN, 3 Å MS, 20 °C, 82%; (ii) H_2, 10% Pd-C, THF–^{i}PrOH, or catalytic transfer hydrogenation, 95%.

Bis-benzylation of **1** using K_2CO_3 and benzyl bromide in DMF gave the bis-benzyl derivative **17**, then hydrolysis of the benzyl ester afforded the protected acid **16**, Scheme 4.

Both steps proceeded in excellent yield,[27] but **16** did not give a better yield than **1** in the conjugation step with **14**, and there were difficulties in the deprotection of the intermediate. Instead (Scheme 3), when the solution of **1**, **14** (1.1 equiv) and NMM (4 equiv) in MeCN was stirred and dried with 3 Å sieves under N_2 for 2 h before addition of HATU (1.1 equiv), ester **15** was obtained in a much improved yield (82%) as the single 1β-anomer (δca. 5.5, d, J = 8 Hz for H-1). For debenzylation of **15**, either conventional hydrogenation or catalytic transfer hydrogenation (1,4-cyclohexadiene, ^{i}PrOH, 60 °C) could be used but it was crucial to use only ^{i}PrOH as solvent, to avoid reduction of the trisubstituted double bond: using THF as co-solvent led to C=C reduction (LC–MS evidence), in proportion to the THF: ^{i}PrOH ratio. Filtration, evaporation and trituration with ether then afforded highly pure product **3**; see the ^{1}H NMR spectrum (Supplementary data).

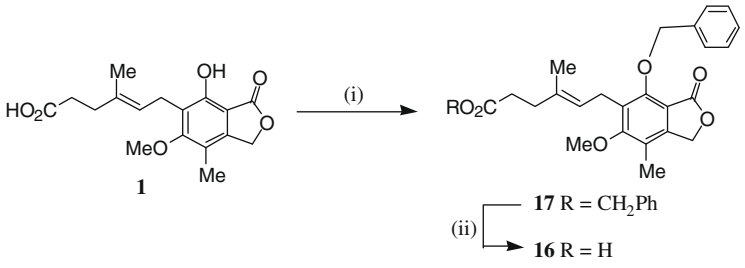
Finally the previously unsynthesised *O*-aryl glucoside **4** was prepared, Scheme 5. Here again the Königs-Knorr procedure[17] or the two-phase alkylation method[19] was viable, but the above two-phase method using minimal added water[26] was best. Thus reaction of glucosyl bromide **18** with methyl ester **5** gave an excellent (92%) yield. The resulting fully protected intermediate **19** finally crystallised from ether-hexane and was subjected to deprotection as described for **2**. Glucoside **4** was obtained in excellent yield and purity.

We now demonstrate the great value of pure synthetic acyl glucuronides in studying protein interactions. Mycophenolic acid **1** is the active metabolite of the immunosuppressive drugs mycophenolate mofetil (MMF) and mycophenolate sodium (MPS), both of which are widely used in immunosuppressant protocols after

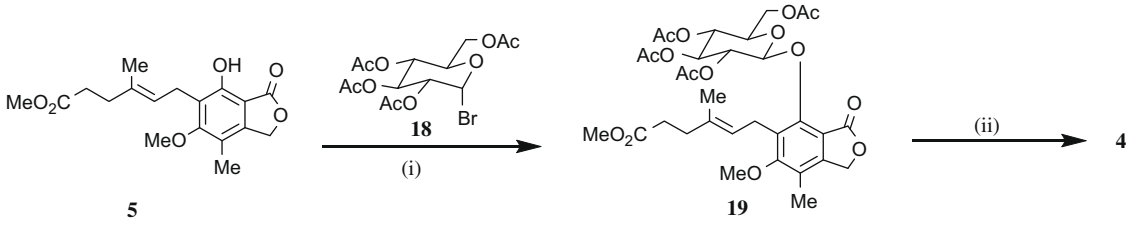
Table 1
Affinity labelling of human r-IMPDH with two different sources of acyl glucuronide **3**

Gel 2 Lane	Concentration and source of 3	Incubation time (h)	Response
(a) No $NaCNBH_3$ (Gel 2)			
1	0 mM S[a]	24	–
2	0 mM N[b]	24	–
3	10 mM S	24	–
4	10 mM N	24	–
5	50 mM S	24	+
6	50 mM N	24	–
7	100 mM S	24	++
8	100 mM N	24	+
9	200 mM S	24	++
10	200 mM N	24	+
Gel 1 Lane			
(b) With $NaCNBH_3$ (Gel 1)			
1	10 mM S	24	+
2	10 mM N	24	+
3	50 mM S	24	++
4	50 mM N	24	++
5	100 mM S	24	+++
6	100 mM N	24	+++
7	200 mM S	24	+++
8	200 mM N	24	+++

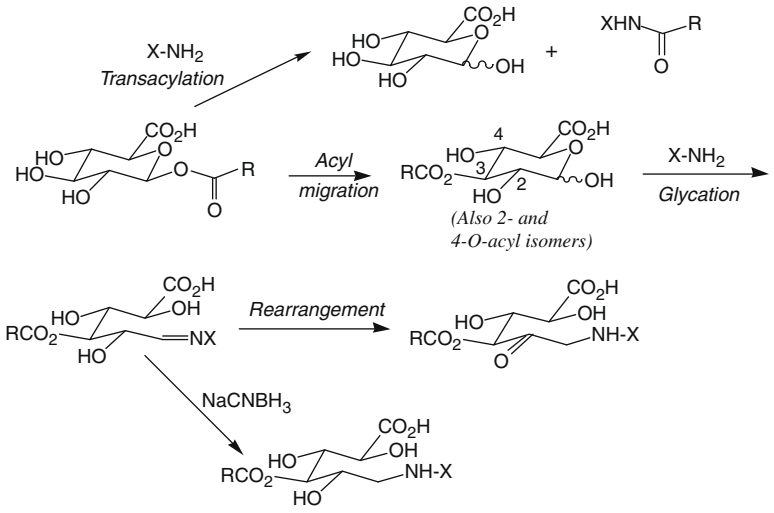
[a] S = synthetic **3** prepared as in text.
[b] N = naturally obtained material (biochemically synthesised).



Scheme 4. Synthesis of mycophenolic acid *O*-benzyl ether. Reagents and conditions: (i) $PhCH_2Br$, K_2CO_3, DMF, 96%; (ii) NaOH, aq EtOH, then H^+, 98%.



Scheme 5. Synthesis of mycophenolic acid *O*-glucoside. Reagents and conditions: (i) quinoline, Ag_2CO_3, 0–20 °C or LiOH, ^{n}Bu$_4$N$^+$ Br$^-$, $(CH_2Cl)_2$–H_2O, heat, or K_2CO_3, $CHCl_3$, $PhCH_2N^+Et_3$ Cl^-, trace H_2O, 92%; (ii) aq NaOH, MeOH, then IR-120 (H^+), 98%.



Scheme 6. Rearrangement and reduction of acyl glucuronide–protein adducts. Here X–NH_2 is a protein Lys side-chain.

solid organ transplantation.[28] The immunosuppressant action of **1** resides in the uncompetitive, selective and reversible inhibition of inosine-5′-monophosphate dehydrogenase (IMPDH), resulting in a decreased de novo synthesis of guanine nucleotides with consequent impairment of nucleic acid synthesis.[28] MPA **1** is primarily metabolised in the liver to the inactive metabolite **2**, but also to a lesser extent to the chemically reactive metabolite **3**.[29] Covalent binding of acyl glucuronides to proteins is considered an initiating event for the organ toxicity of drugs containing a carboxylic acid group.[14,15] In a previous study we showed that **3** is an inhibitor of IMPDH,[12] a key enzyme in the de novo synthesis of purine nucleotides, and is also capable of forming protein adducts in vivo.[13,30,31]

Using a previously described method[32] for the analysis and characterisation of **3**, the above synthetic material was compared with a biochemically synthesised sample regarding their modification of IMPDH. By HPLC analysis, the two samples of **3** had virtually identical purity (1–1.5% aglycone present). We have previously shown[8] that the acyl glucuronide isolated from transplant recipients treated with mycophenolate mofetil, although apparently homogeneous according to HPLC, is in fact a mixture of acyl isomers by ester group migration to *O*-2, 3 and 4. It is feasible to improve the stability of **3** by acidification,[33] in common with other *O*-acyl glucuronides.[15] The synthetic material showed a superior ability to conjugate with protein in the absence of $NaCNBH_3$ trapping, but the two showed very similar, concentration-dependent responses with $NaCNBH_3$ present [Table 1; see Supplementary data for the Western blot analysis (figure)].

This confirms the value of the synthetic material. The significant difference in response between the two batches of **3** and IMPDH in the absence of reducing agent is interesting, and may reflect the two possible modes of reaction (in the absence of $NaCNBH_3$) shown in Scheme 6. Thus both direct transacylation of the acyl residue and glycation [reaction of an amine at C(1)], forming initially an imine, then a rearranged (Amadori) product are possible. If the initial purity of synthetic **3** is higher (i.e., less acyl migration has occurred), it could more readily undergo transacylation as this pathway is known to occur much more readily with an anomeric ester rather than a 2, 3 or 4-ester group after migration.[15] On the other hand, the glycation pathway requires acyl migration to occur first.

In conclusion, we have achieved convenient high-yielding preparations of the phase 2 metabolites of mycophenolic acid, compounds **2**–**4**. The value of pure synthetic **3** in studying protein reactivity is clearly shown. These procedures will be most valuable in providing analytical standards for all who are engaged in research on the therapeutic and toxic effects of mycophenolic acid **1** and add to our understanding of the important topic of acyl glucuronide–protein reactivity and potential toxicity.[15]

Acknowledgements

We are most grateful to Analytical Services International Ltd. for financial support to A.E.J., H.K.W. and P.M.; to Novartis plc for a generous gift of mycophenolic acid; and to Roche Palo Alto LLC for a biochemical sample of mycophenolic acid acyl glucuronide.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.06.060.

References and notes

1. Gosio, B. *Rivista Igiene Sanita Pubblica Ann.* **1896**, *7*, 825.
2. Florey, H. W.; Gilliver, K.; Jennings, M. A.; Sanders, A. G. *Lancet* **1946**, *1*, 46.
3. Gilliver, K. *Ann. Botany* **1946**, *10*, 271.
4. Williams, R. H.; Lively, D. H.; De Long, D. C.; Cline, J. C.; Sweeney, M. J.; Poore, G. A.; Larsen, S. H. *J. Antibiot.* **1968**, *21*, 463.
5. Carter, S. B.; Franklin, T. J.; Jones, D. F.; Leonard, B. J.; Mills, S. D.; Turner, R. W.; Turner, W. B. *Nature* **1969**, *233*, 848.
6. Jones, D. F.; Mills, S. D. *J. Med. Chem.* **1971**, *14*, 305; none of the semi-synthetic analogues had superior activity.
7. Mitsui, A.; Suzuki, S. *J. Antibiot.* **1969**, *22*, 358.
8. Shipkova, M.; Armstrong, V. W.; Wieland, E.; Niedmann, P. D.; Schütz, E.; Brenner-Weiss, G.; Voihsel, M.; Braun, F.; Oellerich, M. *Brit. J. Pharmacol.* **1999**, *126*, 1075.
9. Chen, L.; Wilson, D.; Jayaram, H. N.; Pankiewicz, K. W. *J. Med. Chem.* **2007**, *50*, 6685.
10. (a) Franclin, T.; Jacobs, V.; Jones, G.; Ple, D.; Bruneau, P. *Cancer Res.* **1996**, *56*, 984; (b) Sweeney, M. J.; Hoffmann, D.; Esterman, M. A. *Cancer Res.* **1972**, *32*, 1803.
11. Shipkova, M.; Wieland, E.; Schütz, E.; Wiese, C.; Niedmann, P.; Oellerich, M.; Armstrong, V. W. *Transplant Proc.* **2001**, *33*, 1080.
12. Schütz, E.; Shipkova, M.; Armstrong, V. W.; Wieland, E.; Oellerich, M. *Clin. Chem.* **1999**, *45*, 419.
13. Shipkova, M.; Beck, H.; Voland, A.; Armstrong, V. W.; Grone, H. J.; Oellerich, M.; Wieland, E. *Proteomics* **2004**, *4*, 2728.
14. Spahn-Langguth, H.; Benet, L. Z. *Drug Metab. Rev.* **1992**, *24*, 5.
15. Stachulski, A. V.; Harding, J. R.; Lindon, J. C.; Maggs, J. L.; Park, B. K.; Wilson, I. D. *J. Med. Chem.* **2006**, *49*, 6931.
16. Shipkova, M.; Strassburg, C. P.; Braun, F.; Streit, F.; Grone, H. J.; Armstrong, V. W.; Tukey, R. H.; Oellerich, M.; Wieland, E. *Brit. J. Pharmacol.* **2001**, *132*, 1027.

17. Ando, K.; Suzuki, S.; Arita, M. *J. Antibiot.* **1970**, *23*, 408.
18. Kittleman, M.; Rheinegger, U.; Espigat, A.; Oberer, L.; Aichholz, R.; Francotte, E.; Ghisalba, O. *Adv. Synth. Catal.* **2003**, *345*, 825.
19. Walsh, J. S.; Patanella, J. E.; Halm, K. A.; Facchine, K. L. *Drug Metab. Dispos.* **1995**, *23*, 869.
20. Perrie, J. A.; Harding, J. R.; Holt, D. W.; Johnston, A.; Meath, P.; Stachulski, A. V. *Org. Lett.* **2005**, *7*, 2591.
21. Bowkett, E. R.; Harding, J. H.; Maggs, J. L.; Perrie, J. A.; Park, B. K.; Stachulski, A. V. *Tetrahedron* **2007**, *63*, 7596.
22. Bollenback, G. N.; Long, J. W.; Benjamin, D. G.; Lindquist, J. A. *J. Am. Chem. Soc.* **1955**, *77*, 3310.
23. (a) Schmidt, R. R. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 212; For examples, in the glucuronic acid series, see: (b) Fischer, B.; Nudelman, A.; Ruse, M.; Herzig, J.; Gottlieb, H. E.; Keinan, E. *J. Org. Chem.* **1984**, *49*, 4988.
24. McCorkindale, N. J.; Baxter, R. L.; Turner, W. B. *Tetrahedron* **1981**, *37*, 2131.
25. Brown, R. T.; Scheinmann, F.; Stachulski, A. V. *J. Chem. Res. (S)* **1997**, 370.
26. Hongu, R. M.; Saito, K.; Tsujihara, K. *Synth. Commun.* **1999**, *29*, 2775.
27. Jones, A. E., M. Phil. Thesis, University of Liverpool, in preparation. A similar procedure was used to prepare the phenolic allyl ether of 1: Meath, P.; Stachulski, A. V., unpublished observations.
28. Sollinger, H. W. *Clin. Transplant.* **2004**, *18*, 485.
29. Shipkova, M.; Armstrong, V. W.; Oellerich, M.; Wieland, E. *Expert Opin. Drug Metab. Toxicol.* **2005**, *1*, 505.
30. Shipkova, M.; Armstrong, V. W.; Weber, L.; Niedmann, P. D.; Wieland, E.; Haley, J.; Tönshoff, B.; Oellerich, M. *Ther. Drug Monit.* **2002**, *24*, 390.
31. Asif, A. R.; Armstrong, V. W.; Voland, A.; Wieland, E.; Oellerich, M.; Shipkova, M. *Biochimie* **2007**, *89*, 393.
32. Shipkova, M.; Schutz, E.; Armstrong, V. W.; Niedmann, P. D.; Oellerich, M.; Wieland, E. *Clin. Chem.* **2000**, *46*, 365.
33. Mino, Y.; Naito, T.; Matsushita, T.; Kagawa, Y.; Kawakami, J. *J. Pharm. Biomed. Anal.* **2008**, *46*, 603.