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Efficient synthesis of 1β-*O*-acyl glucuronides via selective acylation of allyl or benzyl D-glucuronate

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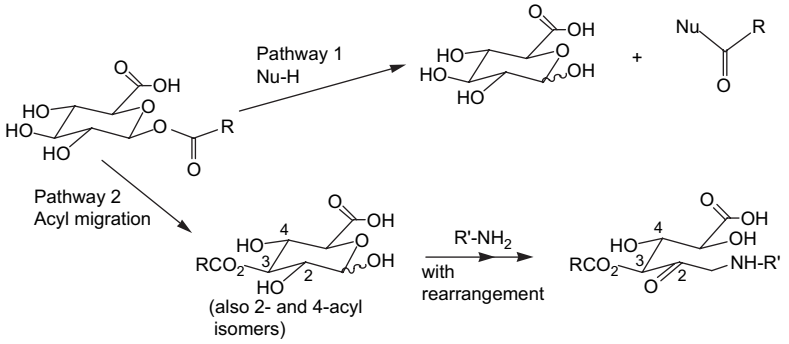
Abstract—Acyl glucuronides are key metabolites for many carboxylic acid-containing drugs, notably those of the non-steroidal anti-inflammatory class. In the processes of drug safety assessment and new drug development, it is essential that acyl glucuronides, if formed in vivo, should be made conveniently available for bioevaluation. We recently showed that selective acylation of allyl glucuronate is a promising method for the synthesis of these metabolites in good yield and with excellent β-anomeric selectivity. We now give fuller details of the allyl ester method and further report that benzyl glucuronate performs at least equally well in the acylation step, offering the advantage of very mild deprotection by catalytic transfer (or conventional) hydrogenation. Depending on the compatibility of other functional groups, as discussed below, this will be the method of choice for many acyl glucuronide syntheses. The value of the method is demonstrated in particular by the synthesis of several acyl glucuronides that are known metabolites of important drugs.

1. Introduction

Glucuronidation is a vital phase 2 metabolic (conjugative) process by which a wide range of drugs and other xenobiotics may be rendered water-soluble, detoxified and excreted. While *O*-glucuronides of alcohols and phenols are well known and generally behave as stable organic molecules,[1,2] acyl (ester) glucuronides (AGs) of carboxylic acids are potentially *reactive* metabolites. In particular, AGs are important phase 2 metabolites for a wide range of carboxylic acid-containing drugs, notably those of the non-steroidal anti-inflammatory class.[3] In a number of cases AGs have been implicated in adverse side effects of such drugs,[4,5] but direct evidence is lacking.

AGs may react (Scheme 1) as acylating agents, by hydrolysis or direct displacement with other nucleophiles, such as those present in body proteins (Pathway 1), or by acyl migration



Scheme 1. Reactivity of acyl glucuronides.

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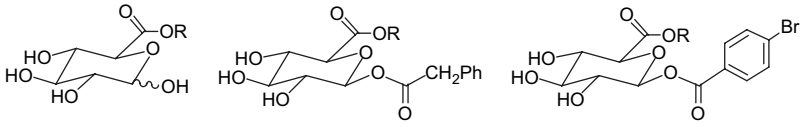
followed by condensation with amines (Amadori rearrangement, Pathway 2).[5] The acyl rearrangement process has been studied in detail by NMR spectroscopy.[6,7] In a recent perspective, the chemistry and biology of AGs has been reviewed in detail, examining in particular their potential as reactive metabolites.[8]

In order to assess the biological activity and potential toxicity of AGs, it is essential that they should be synthesised in pure form, and especially as single 1β-anomers, for evaluation. This is equally important for ongoing assessment of recognised drugs and the development of new ones. Methods for the synthesis of AGs have been reviewed.[2,8] The preference now is for syntheses using minimal rather than global sugar protection, one of the most useful intermediates being allyl glucuronate **1**. Mitsunobu coupling of **1** with a range of carboxylic acids followed by Pd(0) deprotection was shown to be a viable method[9] for the preparation of a range of AGs. Yields were modest, however, and α/β mixtures were invariably generated in the coupling step, requiring careful separation by preparative HPLC.[9,10]

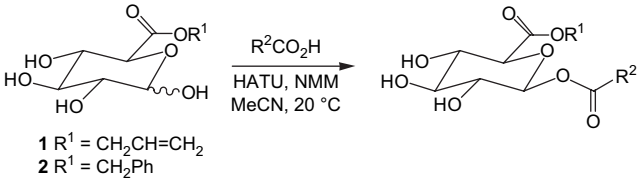


1 R = $CH_2CH{=}CH_2$ (75%)
2 R = CH_2Ph (58–76%)
3 R = H

8b R = CH_2Ph
8d R = H

5c R = $CH_2CH{=}CH_2$
5d R = H

Recently we showed[11] that selective acylation of **1** was an excellent alternative to the Mitsunobu procedure, affording higher yields and essentially pure β-products (Scheme 2). One drawback, when **1** was used, was the persistence of Pd traces in the final products after deprotection of the allyl ester using Pd(0) reagents.[9,12] This is not a major concern for the preparation of analytical standards, but would prohibit biological evaluation of material prepared in this way. While a resin-bound form of the reagent[11] greatly reduced the Pd levels, the use of a different ester seemed to us a promising solution to the problem. We now give full details of the selective acylation method using allyl ester **1** and report the preparation and use of benzyl glucuronate **2** in this method.



1 R^1 = $CH_2CH{=}CH_2$
2 R^1 = CH_2Ph

Scheme 2. 1β-Acyl glucuronide synthesis by selective acylation.

2. Discussion

2.1. Glucuronate esters

Previously **2** was prepared by a three-step synthesis from D-glucuronic acid **3**.[13] We found that direct base-catalysed alkylation of **3** with allyl or benzyl bromide in DMF gave good (up to 76%) yields of crystalline **1** or **2**, after chromatography. Originally DBU was the base used in the preparation of **1** but we found it very difficult to remove all the DBU residues. A resin-bound fluoride[11] (Aldrich art. 387789) was an effective base for either **1** or **2**. Use of TBAF was also possible; this led to products containing variable amounts of glucuronolactone, which were very largely removed by chromatography.[14] Recrystallisation of **1** from methanol gave highly pure product; this was not possible for **2**, but small amounts (viz. 5%) of glucuronolactone did not interfere with the following acylation step. Benzyl chloride did not react satisfactorily, even with iodide catalysis.

2.2. Acylation step

The advantage of the selective acylation method (Scheme 2) using **1** was explained previously.[11a,b] The kinetic anomeric effect was exploited to greatly favour 1β-acylation through the stereoelectronic enhancement of the nucleophilicity of the 1β-alkoxide. There was some precedent in an earlier report of the selective 1β-acylation of glucose derivatives using activated esters.[11c] After considerable experimentation with different carboxyl activators and bases, we found that the combination of HATU for carboxyl activation and *N*-methylmorpholine (NMM) as base gave best results for the acylation of **1**. Bases weaker or stronger than NMM were less effective. This method was successfully applied to the selective acylation of **2** with a range of carboxylic acids **4a**–**14a** (Fig. 1 and Scheme 2), affording products **4b**–**14b** with the yields given in Table 1. Where available the direct comparison with the result when using **1**,[11] yielding allyl esters **5c**, **7**–**9c**, **11c** and **12c**, is given.

An alternative uronium reagent, TBTU, gave a lower yield (entry 7) but, in view of its significantly lower cost compared to HATU, it may be regarded as a satisfactory alternative. Yields with the DIC–HOBt combination[11] (not tabled here) were about 20% lower than with HATU. In cases where there is a direct comparison, **2** performs at least as well as **1**, often giving a cleaner reaction. Selectivity for the β-anomer was essentially complete by ^{1}H NMR (at least 19:1 β/α, typically <2% α-anomer). Characteristic NMR signals for the desired β-products are δ_H 5.5–6.0 (1H, d, J=7.5–8 Hz) for the anomeric proton and δ_C ~95 for the anomeric carbon.

A range of substituted benzoic acids **4a**–**6a** (entries 1–4) gave nearly identical yields, e.g., 4-bromobenzoic acid **5a**, which we used extensively[11] in earlier studies with allyl ester **1**. *p*-Toluic acid **6a** reacted a little more slowly and here 3 equiv of NMM was desirable. The *o*-substituted acid **7a**, entry 4, gave just as good a result, suggesting that steric

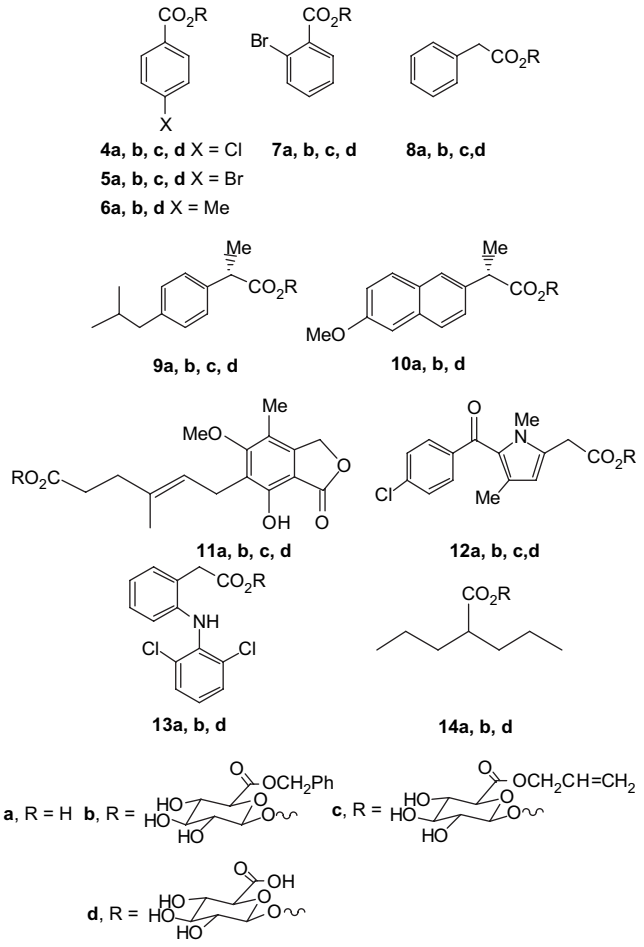


Figure 1. Carboxylic acids used in this study and their acyl glucuronide derivatives.

hindrance is relatively unimportant. The reaction of phenylacetic acid **8a** using 3 equiv of NMM, entry 6, gave an excellent yield, the highest seen for a reaction of **1** or **2** so far. Among other examples, the important drugs ibuprofen **9a**, naproxen **10a**, mycophenolic acid **11a** and zomepirac **12a** all react well (entries 8–11). In the cases of **9a** and **10a** single (2*S*) enantiomers of the starting acids were used; as noted previously,[11] some epimerisation occurs in the acylation step. The major diastereoisomers of the products **9b** and **10b** were isolated and we assume these still have the (2*S*) configuration. Phenolic protection of **11a** was not necessary, but the yield here should be regarded as unoptimised. Zomepirac **12a** is a significant case as its AG has an in vitro half-life of only 0.45 h (pH 7.4, 37 °C)[15] and allergic reactions have led to its withdrawal from the market.[16] Recent investigations[17] have suggested that reactive oxidative metabolites and thioester intermediates as well as the AG of **12a** might haptenate proteins and thereby contribute to the initiation of drug-induced anaphylaxis.

Two further demanding drug examples were studied. Diclofenac **13a**, entry 12, gives a low yield (20%) with much unreacted starting material; nevertheless the method is still preferable to the Mitsunobu procedure[9,10] because of the excellent β/α product ratio. The hindered valproic acid **14a** (entry 13) reacts very slowly and here 1,8-

Table 1. Acyl glucuronide Bn esters **4b**–**14b** by selective acylation of **2**[a,b]

Entry	Carboxylic acid	HATU, NMM (equiv)	Yield (%)	Product	Yield (%) from **1**	Product
1	4-Chlorobenzoic **4a**	1, 3	62	**4b**		
2	4-Bromobenzoic **5a**	1, 2	62	**5b**	59	**5c**
3	4-Methylbenzoic **6a**	1, 3	58	**6b**		
4	2-Bromobenzoic **7a**	1, 2	65	**7b**	52	**7c**
5	Phenylacetic **8a**	1, 2	63	**8b**	66	**8c**
6	Phenylacetic **8a**	1, 3	82	**8b**		
7	Phenylacetic **8a**	1[c], 2	46	**8b**		
8	Ibuprofen **9a**	1, 3	68	**9b**	65	**9c**
9	Naproxen **10a**	1, 2	67	**10b**		
10	Mycophenolic **11a**	1, 3	55	**11b**	44	**11c**
11	Zomepirac **12a**	1, 3	60	**12b**	52	**12c**
12	Diclofenac**13a**	1, 2	20	**13b**		
13	Valproic **14a**	1, 2[d]	40	**14b**		
14	Valproic **14a**	1[e], 2	56[e]	**14b**		

Comparable yields from allyl ester **1** are shown where available.

[a] General conditions: all reagents were combined and stirred at 20 °C in anhydrous acetonitrile for 3–4 h; workup was achieved by addition of Amberlite IR-120 followed by brief stirring, filtration and evaporation.

[b] Except for entry 14, the β/α ratio of the product was >19:1 (^{1}H NMR).

[c] Using TBTU (1 equiv), rather than HATU, with NMM (2 equiv).

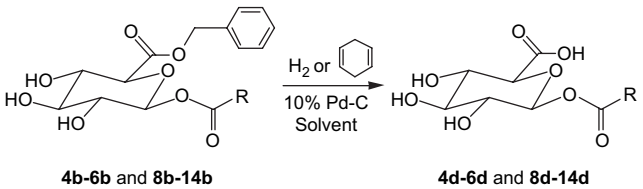
[d] Using DABCO (2 equiv) as base and adding **2** lastly.

[e] Using 1 equiv acid chloride of **14a** and 2 equiv NMM, rather than **14a** and HATU; product was 9:1 β/α.

diazabicyclo[2,2,2]octane (DABCO), a stronger base (pK_a=8.5 vs 7.4 for NMM), is superior to NMM. It is necessary to add **2** slowly to a solution of the other reagents in this case to prevent its self-condensation. In this case we also studied the reaction of the acid chloride of **14a** with NMM as base (entry 15); this procedure gave a better yield of **14b** (56%) but with some loss of selectivity, 10% of the α-anomer (δ 6.3, d, *J*=3.5 Hz) being detected by ^{1}H NMR, and a confirmatory close-eluting peak of equal mass was observed by LC–MS.[18]

2.3. Deprotection

We then addressed the question of deprotection, Scheme 3, bearing in mind that the method should ideally be sufficiently robust to tolerate a range of functionalities in the carboxylic acid.



Scheme 3. Deprotection of benzyl ester intermediates.

Various conditions were studied for the hydrogenolysis of benzyl esters. Using the benzyl ester **8b** of the acyl glucuronide of phenylacetic acid (Table 2) as a model, we were pleased to find that catalytic transfer hydrogenation using cyclohexa-1,4-diene (10% Pd–C, THF+^{i}PrOH, 60 °C, 1.5 h) afforded the free acyl glucuronide **8d** in essentially quantitative yield, entry 1. As expected, cyclohexene (entry 2) was also a feasible donor but required a higher temperature (80 °C); 1,2-dihydronaphthalene (entry 3) was too slow to be useful. Traditional hydrogenation in ^{i}PrOH:

Table 2. Deprotection of benzyl esters **4b–6b** and **8b–14b**, Scheme 3

Entry	Acyl glucuronide Bn ester	Hydrogenation method (see below)	Reaction time (h)	Isolated acyl glucuronide yield (%)	Product
1	Phenylacetic **8b**	A[a]	1.5	100	**8d**
2	Phenylacetic **8b**	B[b]	5	100	**8d**
3	Phenylacetic **8b**	C[c]	4	Low yield	**8d**
4	4-Chlorobenzoic **4b**	A	4.5	95[d]	**4d**
5	4-Methylbenzoic **6b**	A	1.5	100	**6d**
6	4-Bromobenzoic **5b**	A	20	0[e]	**5d**
7	Ibuprofen **9b**	A	2	100	**9d**
8	Naproxen **10b**	A	2	100	**10d**
9	Mycophenolic[f] **11b**	A[g]	2	95[d]	**11d**
10	Zomepirac **12b**	A	4	100	**12d**
11	Diclofenac **13b**	A	2	100	**13d**
12	Valproic **14b**	A	3	100	**14d**

[a] Method A: using cyclohexa-1,4-diene (1 mL) as donor with 10% Pd–C (10% w/w), see Section 4. Conventional hydrogenation with H_2 at 1 atm, other conditions being the same, gave a similar result.
[b] Method B: as method A but using cyclohexene as donor, heated for 5 h at 80 °C.
[c] Method C: using 1,2-dihydronaphthalene as donor, see text.
[d] Purification using reverse-phase chromatography (see text and Section 4) was desirable.
[e] Loss of Br observed (NMR, MS): the reaction was slow and 20 h were required for full deprotection.
[f] Here it was possible to achieve debenzylation without reduction of the trisubstituted double bond, verified by NMR and LC–MS.
[g] Reaction in ethanol was significantly less clean.

THF, 1:1 (10% Pd–C, H_2, 1 atm), not tabulated, was also successful here. Isopropanol was the best solvent, giving cleaner products than ethanol; addition of some THF (up to 1:1 v/v) was often beneficial for improved solubility. One slight drawback of catalytic transfer hydrogenation was that small traces of isopropyl ester (ca. 2% by NMR) were sometimes seen in the final products if the heating in isopropanol was prolonged; such transesterification was not observed when using conventional hydrogenation. For the same reason ethanol is not recommended in this mode.

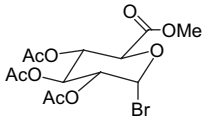
In general highly pure product was obtained simply by filtration, evaporation of solvent and trituration of the residue with a solvent, ether or dichloromethane (see Section 4). If necessary, acyl glucuronides may be purified by conventional chromatography eluting with ethanol–dichloromethane mixtures, preferably containing a little acetic acid (as for material prepared by Pd(0) deprotection of an allyl ester), or by reverse-phase chromatography. Details are given in Section 4.

This method was studied for the other examples prepared in Table 1, including examples with potentially reactive functionalities (aryl halogens, C=C). In the case of 4-bromobenzoic acid derivative **5b** (cyclohexa-1,4-diene, 10% Pd–C, ^{i}PrOH, 60 °C, 5 h), entry 6, the corresponding intermediate was slowly debenzylated together with significant loss of Br. Other donors, or conventional hydrogenation, were no more successful. In the 4-chlorobenzoic acid series, however, entry 5, clean debenzylation of **4b** was observed without dechlorination. Addition of 1 equiv acid ion-exchange resin before hydrogenation was beneficial in this case. This is an important factor as a number of drugs contain aryl–Cl bonds. The benzyl ester intermediate **11b** from mycophenolic acid was also an important test case, since **11a** is widely used (generally as a prodrug ester or the Na salt) in transplant surgery.[19,20] Here (entry 9) no significant reduction of the trisubstituted C=C double bond was seen. The zomepirac **12b** and diclofenac **13b** intermediates, entries 10 and 11, which both contain aryl–Cl bonds, were cleanly deprotected, as was **4b**. The ketone functionality of **12b** was unaffected.

Finally, for comparison (see Section 4), we give details of the deprotection procedure applied to allyl esters **5c** and **11c**, using $Pd(PPh_3)_4$ or polymer-supported Pd(0) reagent,[11] to afford 4-bromobenzoyl acyl glucuronide **5d** and mycophenolic acid acyl glucuronide **11d**. The latter was previously obtained from **11a** by enzymatic synthesis followed by preparative HPLC separation from the co-formed aryl glucuronide.[21]

3. Conclusions

We have demonstrated an effective and versatile two-step synthesis of AGs from readily available glucuronate esters **1** and **2** as single 1β-anomers, using the selective acylation procedure. In the present variation, benzyl glucuronate **2** is shown to acylate at least as well as allyl glucuronate **1** using HATU–NMM. With the single exception of diclofenac **13a**, yields in the acylation step are highly satisfactory, in the range 55–82%. Deprotection is effected under mild conditions, using Pd(0) for allyl esters and catalytic transfer (or conventional) hydrogenation for benzyl esters; these conditions are compatible with a range of functional groups. The examples given include AGs of important drug molecules, some of which are known to have short half-lives in vitro (<1 h in pH 7.4 buffer at 37 °C). We believe this procedure will be of great value in the pharmaceutical industry for evaluation of potentially reactive AG metabolites.



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During the course of this work a new method for AG synthesis has been reported[22] via alkylation of an alkali metal salt

of the carboxylic acid component with the bromosugar **15**. This yields the fully protected acyl glucuronide in good yield and high β-selectivity; deprotection to give the free AG is effected by successive deprotections with enzymes that are able to operate at pH 5. This is clearly a promising method although only three examples have been reported so far.

4. Experimental

4.1. General experimental methods

All organic solvents were anhydrous and of AR grade. Vacuum rotary evaporation was carried out at <30 °C. Analytical thin layer chromatography was performed using Merck Kieselgel 60 F_{254} silica plates. Preparative column chromatography was performed on Merck 938S silica gel. Reverse-phase chromatography was carried out using Lichroprep RP-18, 25–40 μm particle size (Merck). Infrared spectra were obtained either for Nujol mulls in a Perkin–Elmer RX1 FTIR instrument, or using an FTIR-4100 type A instrument (distinguished by suffix FT). Both ^{1}H and ^{13}C NMR spectra were recorded for the solvents noted using either Bruker 250 or 400 MHz instruments (operating at 100 MHz only for ^{13}C spectra) with tetramethylsilane as internal standard. Mass spectra in the chemical ionisation (CI) mode were obtained using a VG7070E mass spectrometer. Both low and high-resolution electrospray mode (ES) mass spectra were obtained using a Micromass LCT mass spectrometer operating in the +ve or −ve ion mode as indicated. Elemental microanalysis was performed by Mr. Steve Apter of the Chemistry Department.

4.2. Allyl α,β-D-glucuronate (1)[8]

A commercial batch of polymer-bound fluoride (Sigma–Aldrich) was stirred in anhydrous DMF at 20 °C for 2 h, filtered, washed with ether and air-dried. This product (15 g, 45 mmol) was stirred in anhydrous DMF (90 mL) under N_2 with D-glucuronic acid (5.82 g, 30 mmol) for 3 h, then allyl bromide (3.99 g, 2.86 mL, 33 mmol) was added and the mixture was stirred at 40 °C for 20 h. The reaction mixture was filtered and the resin was washed with DMF (2×10 mL), then the combined filtrates were evaporated to dryness to give crude product (5.74 g), which was purified by chromatography, eluting with EtOAc–^{i}PrOH–H_2O, 5:3:1 to afford the ester **1**[8] as a solid of variable mp, which was an α/β mixture of varying ratios (5.29 g, 75%). Found: *m/z*, 252.10829. $C_9H_{14}NO_7$ (MNH_4^+) requires *m/z*, 252.10832; ν_{max} (cm^{-1}) 3298 (br) and 1746. A batch of α/β=3:2 showed ^{1}H NMR [$(CD_3)_2CO$]: 3.23 (0.4H, dd, *J*=7.8, 9.2 Hz, 2-Hβ), 3.43 (0.6H, dd, *J*=3.6, 9.4 Hz, 2-Hα), 4.46 (0.4H, t, *J*=9.1 Hz, 4-Hβ), 3.63–3.56 (1H, m, 4-Hα+3-Hβ), 3.73 (0.6H, t, *J*=9.1 Hz, 3-Hα), 3.87 (0.4H, d, *J*=9.7 Hz, 5-Hβ), 4.29 (0.6H, dd, *J*=0.7 and 9.7 Hz, 5-Hα), 4.58 (0.4H, d, *J*=7.8 Hz, 1-Hβ), 4.64 (2H, d, *J*=5.4 Hz, O*CH*$_2$CH=CH$_2$, α and β), 5.16 (0.6H, d, *J*=3.5 Hz, 1-Hα), 5.20 (1H, m, OCH$_2$CH=*CH*$_2$α and β), 5.37 (1H, m, OCH$_2$CH=*CH*$_2$α and β) and 5.87–6.00 (1H, m, OCH$_2$*CH*=CH$_2$α and β). In some runs it was necessary to recrystallise the product from methanol to obtain highly pure material, removing glucuronolactone traces in particular.

4.3. Benzyl α,β-D-glucuronate (2)

Benzyl bromide (1.25 mL, 10.5 mmol) was added for over 1 min to a solution of D-glucuronic acid **3** (1.84 g, 10 mmol) in DMF (10 mL) and 1 M TBAF in THF (11 mL) with stirring at 0 °C. The clear solution was left for 18 h and allowed to warm to 20 °C, whereupon reaction was complete by TLC (20% ^{i}PrOH in CH_2Cl_2). Solvents were evaporated followed by azeotroping with toluene (4×30 mL), then the residue was chromatographed, eluting with 10–20% ^{i}PrOH in CH_2Cl_2. Appropriate fractions (detected by UV and anisaldehyde) were combined and evaporated to afford the anomeric mixture of esters (**2**) as a foam (2.23 g); remaining traces of quaternary ammonium salts were removed by addition of CH_2Cl_2 to afford a solid that was filtered, washed with further CH_2Cl_2 and dried to give **2** as a hygroscopic white solid (1.65 g, 58%). Found: C, 55.0; H, 5.7. $C_{13}H_{16}O_7$ requires C, 54.9; H, 5.5%; ν_{max} (cm^{-1}, FT) 3000–3600 (br), 1722, 1608, 1590 (both vw), 1498 (w) and 1394 (m); ^{1}H NMR [$(CD_3)CO+D_2O$, for a 1:1 α/β mixture]: 3.29 (0.5H, dd, *J*=9.1 and 8.0 Hz, H-2β), 3.49 (0.5H, dd, *J*=9.3 and 3.6 Hz, H-2α), 3.52 (0.5H, t, *J*=9.3 Hz, H-3β), 3.64–3.70 (1H, m, H-4α and β), 3.78 (0.5H, t, *J*=9.3 Hz, H-3α), 3.94 (0.5H, d, *J*=9.6 Hz, H-5β), 4.36 (0.5H, d, *J*=9.7 Hz, H-5α), 4.62 (0.5H, d, *J*=7.8 Hz, H-1β), 5.20 (0.5H, d, *J*=3.6 Hz, H-1α), 5.16–5.24 (2H, m, Ph*CH*$_2$α and β) and 7.30–7.50 (5H, m, ArH); ^{13}C NMR [$(CD_3)CO+D_2O$]: 67.7, 67.8, 72.9, 73.1, 73.3 (2×), 74.3, 75.8, 77.0, 77.3, 94.2, 98.6, 129.3, 129.4, 129.8, 137.2, 137.3, 170.6 and 171.4; *m/z* (CI, NH_3): 302 (MNH_4^+, 45%) and 284 (M^+, 60%). High-resolution mass (CI) calculated for $C_{13}H_{20}NO_7$ (MNH_4^+): 302.12399. Found: 302.12387.

Material prepared in this way typically contained small quantities of glucuronolactone (ca. 5%), which was difficult to remove by crystallisation (cf. **1**) but did not interfere with the next step. The use of polymer-bound fluoride, as described for **1**, was also effective (yield of **2**, 76%).

4.4. Typical acylation procedure using (1)

4.4.1. Allyl 1-*O*-(4-bromo)benzoyl-β-D-glucopyranuronate (5c).[8] 4-Bromobenzoic acid **5a** (0.101 g, 0.5 mmol), allyl glucuronate **1** (0.117 g, 0.5 mmol) and HATU (0.190 g, 0.5 mmol) were stirred in dry acetonitrile (5 mL) with *N*-methylmorpholine (0.110 mL, 0.101 g, 2 equiv) under nitrogen at 20 °C. The reaction was monitored by TLC (10% EtOH–CH_2Cl_2) and after 2 h it was quenched by addition of Amberlyst A-15 (H^+, 2 equiv). After evaporation the residue was chromatographed, eluting with 7% EtOH–CH_2Cl_2. Appropriate fractions were pooled and evaporated, eventually under high vacuum, to afford the title compound **5c** as a foam (0.123 g, 59%), with spectroscopic data as reported.[8] Found: C, 46.2; H, 4.1; *m/z* 438.9992 and 440.9966. $C_{16}H_{17}BrO_8$ requires C, 46.1; H, 4.1%; $C_{16}H_{17}{}^{79}BrO_8Na$ and $C_{16}H_{17}{}^{81}BrO_8Na$ require 439.0004 and 440.9984, respectively; ν_{max} (cm^{-1}) 3386 (br), 1740, 1725 and 1589; ^{1}H NMR [400 MHz, $(CD_3)_2CO$]: 3.63 (2H, m) and 3.72 (1H, m, 2′H, 3′-H and 4′-H), 4.11 (1H, d,

J=9.5 Hz, 5′-H), 4.63 (2H, m, OCH_2CH=CH_2), 5.18 and 5.35 (2H, 2m, OCH$_2$CH=CH_2),5.81 (1H, d, J=7.8 Hz, 1′-H), 5.91 (1H, m, OCH$_2$$CH$=$CH_2$), 7.67 and 8.00 (4H, approx. dd, ArH); ^{13}C NMR [(CD$_3$)CO]: 71.0, 71.1, 72.0, 75.4, 75.8, 94.7, 117.2, 127.8, 128.0, 131.2, 131.5, 131.6, 163.6 and 167.5; m/z (ES$^+$): 439, 441 (MNa$^+$ for ^{79}Br, ^{81}Br, respectively).

Similarly the following compounds were prepared.

4.4.2. 2-Bromobenzoic acid acyl glucuronide, allyl ester (7c).[8] Amorphous white solid. Found: C, 46.3; H, 4.1; m/z 439.0022 and 441.0002. $C_{16}H_{17}BrO_8$ requires C, 46.1; H, 4.1%; $C_{16}H_{17}{}^{79}BrO_8Na$ and $C_{16}H_{17}{}^{81}BrO_8Na$ require 439.0004 and 440.9984, respectively. ν_{max} (cm^{-1}) 3500 (br), 1754, 1720, 1587 and 1564; ^{1}H NMR [200 MHz, (CD$_3$)$_2$CO]: 3.50–3.80 (3H, 3m, 2′H+3′-H+4′-H), 4.17 (1H, d, J=9.4 Hz, 5′-H), 4.66 (2H, m, OCH_2CH=CH_2), 5.21 and 5.37 (2H, 2m, OCH$_2$CH=CH_2), 5.83 (1H, d, J=7.8 Hz, 1′-H), 5.85–6.05 (1H, m, OCH$_2$$CH$=$CH_2$), 7.50–7.60 (2H, m, ArH), 7.75–7.80 and 7.95–8.05 (2H, 2m, ArH); ^{13}C NMR [100 MHz, (CD$_3$)$_2$CO]: 65.9, 72.4, 73.3, 76.9, 77.0, 96.0, 118.1, 122.3, 128.3, 132.6, 132.8, 134.3, 135.2, 164.3 and 168.5; m/z (ES$^+$): 439, 441 (MNa$^+$ for ^{79}Br, ^{81}Br, respectively), also weak 445, 447 (MK$^+$).

4.4.3. Phenylacetic acid acyl glucuronide, allyl ester (8c).[8] Amorphous white solid. Found: C, 57.3; H, 5.8; m/z 375.1073. $C_{17}H_{20}O_8$ requires C, 58.0; H, 5.7%; $C_{17}H_{20}O_8Na$ requires m/z 375.1056; ν_{max} (cm^{-1}) 3396 (br), 1750, 1728 and 1550; ^{1}H NMR [(CD$_3$)$_2$CO]: 3.46, 3.58 and 3.68 (3H, 3m, 2′-H+3′-H+4′-H), 3.76 (2H, s, ArCH_2CO), 4.04 (1H, d, J=9.6 Hz, 5′-H), 4.65 (2H, m, OCH_2CH=CH_2), 5.21 and 5.37 (2H, 2m, OCH$_2$CH=CH_2), 5.61 (1H, d, J=8.1 Hz, 1′-H), 5.90–6.00 (1H, m, OCH$_2$$CH$=$CH_2$) and 7.25–7.35 (5H, m, ArH); ^{13}C NMR [100 MHz, (CD$_3$)$_2$CO]: 41.5, 66.5, 73.0, 73.9, 77.5, 95.9, 118.8, 128.2, 129.6, 130.6, 130.8, 133.4, 135.2, 169.2 and 171.2; m/z (ES$^+$): 375 (MNa$^+$, 100%).

4.4.4. (2*S*)-Ibuprofen acyl glucuronide, allyl ester (9c). White solid, mp 123–124 °C. Found: C, 62.6; H, 7.1; m/z 445.1826. $C_{22}H_{30}O_8$ requires C, 62.6; H, 7.1%; $C_{22}H_{30}O_8Na$ requires m/z 445.1838; ν_{max} (cm^{-1}) 3200–3600 (br), 3538, 1744, 1720 (sh), and 1515; ^{1}H NMR [(CD$_3$)$_2$CO]: 0.90 (6H, d, J=6.6 Hz, (CH_3)$_2$CH), 1.46 (3H, d, J=7.2 Hz, CH_3CHAr), 1.86 (1H, m, (CH$_3$)$_2$$CH$), 2.46 (2H, d, J=7.2 Hz, CHCH_2Ar), 3.45, 3.63 and 3.83 (4H, 3m, 2′-H+3′-H+4′-H+ArCHCH$_3$), 4.03 (1H, d, J=9.4 Hz, 5′-H), 4.64 (2H, m, OCH_2CH=CH_2), 5.19 and 5.34 (2H, 2m, OCH$_2$CH=CH_2), 5.62 (1H, d, J=8.2 Hz, 1′-H), 5.92 (1H, m, OCH$_2$$CH$=$CH_2$), 7.13 and 7.26 (4H, approx. dd, ArH); ^{13}C NMR [100 MHz, (CD$_3$)$_2$CO]: 19.6, 23.1, 31.1, 45.9, 66.5, 73.0, 73.8, 77.5, 95.9, 118.6, 118.8, 128.6, 130.5, 133.5, 139.0, 141.6, 169.1 and 174.1; Mass spec. (ES$^+$): m/z 445 (MNa$^+$, 100%), 867 (weak, 2MNa$^+$).

4.4.5. Mycophenolic acid acyl glucuronide, allyl ester (11c). Amorphous white solid. Found: C, 57.9; H, 5.9. $C_{26}H_{32}O_{12}$ requires C, 58.2; H, 6.0%; ν_{max} (cm^{-1}) 3400 (v br), 1750 (sh), 1732, 1710 (sh) and 1621; ^{1}H NMR [400 MHz, (CD$_3$)$_2$CO]: 1.68 (3H, s, CH$_3$C=C), 2.03 (3H, s, CH$_3$Ar), 2.16, 2.33 (4H, 2t, COCH$_2$CH$_2$), 3.20–3.50 (5H, m, 2′-H+3′-H+4′-H+CH$_2$Ar), 3.66 (3H, s, CH$_3$O), 3.87 (1H, d, J=9.5 Hz, 5′-H), 4.51 (2H, m, OCH_2CH=CH_2), 5.10, 5.25 (2H, approx. 2d, CH$_2$CH=CH_2), 5.17 (3H, m, ArCH_2O+C=CHCH$_2$Ar), 5.40 (1H, d, J=8.1 Hz, 1′-H) and 5.85 (1H, m, CH$_2$$CH$=CH$_2$); ^{13}C NMR [100 MHz, (CD$_3$)$_2$CO]: 11.86, 15.99, 16.64, 23.60, 33.73, 35.20, 61.79, 66.50, 70.91, 72.97, 73.82, 77.40, 95.51, 107.67, 118.07, 118.76, 123.00, 124.48, 133.42, 134.87, 146.43, 154.45, 164.70, 169.19, 172.53 and 173.22; m/z (ES$^+$): 559 (MNa$^+$, 100%) and 575 (MK$^+$, 5%). Found: m/z, 559.1797. $C_{26}H_{32}O_{12}Na$ requires MNa$^+$, 559.1791.

4.4.6. Zomepirac acyl glucuronide, allyl ester (12c). Amorphous white solid. Found: C, 56.3; H, 5.1; N, 2.8; m/z 530.1219. $C_{24}H_{26}ClNO_9$ requires C, 56.7; H, 5.1; N, 2.8%; $C_{24}H_{26}ClNO_9Na$ requires m/z 530.1194; ν_{max} (cm^{-1}) 3200–3600 (br), 1770, 1750 (sh), 1726, 1613, 1580 and 1550 (sh); ^{1}H NMR [400 MHz, (CD$_3$)$_2$CO]: 1.74 (3H, s, pyrrole 4-CH$_3$), 3.46, 3.59 and 3.67 (3H, 3m, 2′-H+3′-H+4′-H), 3.72 (3H, s, NCH$_3$), 3.91 (2H, s, ArCH$_2$CO), 4.06 (1H, d, J=9.5 Hz, 5′-H), 4.68 (2H, m, OCH_2CH=CH_2), 5.23 and 5.38 (2H, 2 m, OCH$_2$CH=CH_2), 5.65 (1H, d, J=8.1 Hz, 1′-H), 5.90–6.00 (1H, m, OCH$_2$$CH$=$CH_2$), 6.03 (1H, s, pyrrole 3-H), 7.55 and 7.69 (4H, approx. dd, ArH); ^{13}C NMR [100 MHz, (CD$_3$)$_2$CO]: 14.87, 33.24, 33.87, 66.55, 72.99, 73.88, 77.56, 77.66, 96.28, 113.74, 118.79, 129.26, 129.85, 130.87, 131.87, 133.48, 134.36, 138.33, 141.22, 169.23, 169.60 and 186.94; m/z (ES$^+$): 530 (MNa$^+$, 100%).

4.5. Typical acylation procedure using (2)

4.5.1. Benzyl 1-*O*-(2-phenyl)acetyl-β-D-glucopyranuronate (8b). Phenylacetic acid **8a** (0.068 g, 0.5 mmol), benzyl glucuronate **2** (0.142 g, 0.5 mmol) and HATU (0.190 g, 0.5 mmol) were stirred in dry acetonitrile (5 mL) with *N*-methylmorpholine (0.110 mL, 0.101 g, 1 mmol) under nitrogen at 20 °C. The reaction was monitored by TLC (10% EtOH–CH_2Cl_2, Merck Kieselgel analytical plates) and after 2 h workup as described for **5c** but using Amberlite IR-120H resin gave **8b** as a white solid (0.165 g, 82%); mp 119–120 °C. Found: C, 60.2; H, 5.3. $C_{21}H_{22}O_8\cdot H_2O$ requires C, 60.0; H, 5.7%; ν_{max} (cm^{-1}, FT) 3100–3600 (br), 1757 (sh), 1730, 1643 and 1496; ^{1}H NMR [400 MHz, (CD$_3$)$_2$CO]: 3.46, 3.59, 3.69 (3H, 3t, J=9–9.5 Hz, 2-H, 3-H and 4-H), 3.74 (2H, s, ArCH_2CO), 4.08 (1H, d, J=9.6 Hz, 5-H), 4.66–4.76 (3H, m, D$_2$O exch., 3×OH), 5.21 (2H, s, PhCH_2O), 5.61 (1H, d, J=8.1 Hz, 1-H) and 7.25–7.43 (10H, m, ArH); ^{13}C NMR [400 MHz, (CD$_3$)$_2$CO]: 41.0, 67.3, 72.7, 73.5, 77.1, 77.2, 95.5, 127.8, 128.9, 129.0, 129.2, 129.3, 130.4, 134.8, 136.8, 169.0 and 170.1; m/z (ES$^+$): 425 (MNa$^+$, 100%), 827 (2MNa$^+$). High-resolution mass (EI) calculated for $C_{21}H_{22}O_8Na$ (MNa$^+$): 425.1212. Found: 425.1212.

Similarly the following compounds were prepared.

4.5.2. Benzyl 1-(4-chlorobenzoyl)-β-D-glucopyranuronate (4b). White solid, mp 154–155 °C. Found: C, 56.6; H, 4.6. $C_{20}H_{19}ClO_8$ requires C, 56.8; H, 4.5%; ν_{max} (cm^{-1}, FT) 3100–3600 (br), 1740 (sh), 1720, 1593, 1489 and 752; ^{1}H NMR [400 MHz, (CD$_3$)$_2$CO]: 3.64–3.72 (2H, m) and 3.79 (1H, m, 2′-H, 3′-H and 4′-H), 4.19 (1H, d, J=9.6 Hz, 5-H), 5.21 (2H, s, PhCH_2O), 5.84 (1H, d, J=8.0 Hz, 1-H),

7.28–7.42 (5H, 2m, *Ph*CH_2O), 7.56 and 8.06 (4H, 2d, ArH of 4-Cl benzoyl); ^{13}C NMR [100 MHz, $(CD_3)_2CO$]: 67.8, 73.1, 74.0, 77.5, 77.7, 96.5, 129.3, 129.4, 129.5, 129.7, 130.2, 132.9, 137.2, 140.8, 165.1 and 169.4; *m/z* (ES^+): 445 (100%), 447 (MNa^+ for ^{35}Cl and ^{37}Cl). High-resolution mass (ES) calculated for $C_{20}H_{19}{}^{35}ClO_8Na$ (MNa^+): 445.0666. Found: 445.0673.

4.5.3. Benzyl 1-(4-bromobenzoyl)-β-D-glucopyranuronate (5b). White solid, mp 143.5–144.5 °C. Found: C, 51.2; H, 4.1. $C_{20}H_{19}BrO_8$ requires C, 51.4; H, 4.1%; ν_{max} (cm^{-1}, FT) 3100–3600 (br), 1722, 1590, 1485 and 748; ^{1}H NMR [400 MHz, $(CD_3)_2CO$]: 3.66 (2H, m) and 3.77 (1H, m, 2-H, 3-H and 4-H), 4.18 (1H, d, *J*=9.5 Hz, 5-H), 5.21 (2H, s, Ph*C*H_2O), 5.83 (1H, d, *J*=7.8 Hz, 1-H), 7.34 and 7.42 (5H, 2m, *Ph*CH_2O), 7.74 and 8.06 (4H, 2d, ArH of 4-Br benzoyl); ^{13}C NMR [100 MHz, $(CD_3)_2CO$]: 67.7, 73.1, 74.0, 77.4, 77.7, 96.5, 129.3, 129.3, 129.4, 129.7, 129.9, 132.9, 133.2, 137.2, 165.2 and 169.4; *m/z* (ES^+): 489 (100%), 491 (MNa^+ for ^{79}Br and ^{81}Br). High-resolution mass (ES) calculated for $C_{20}H_{19}{}^{79}BrO_8Na$ (MNa^+): 489.0161. Found: 489.0180.

4.5.4. Benzyl 1-(4-methylbenzoyl)-β-D-glucopyranuronate (6b). Amorphous white solid. Found: C, 62.4; H, 5.5. $C_{21}H_{22}O_8$ requires C, 62.7; H, 5.5%; ν_{max} (cm^{-1}, FT) 3100–3600 (br), 1724, 1612, 1500 and 746; ^{1}H NMR [400 MHz, $(CD_3)_2CO$]: 2.41 (3H, s, Ar CH_3), 3.67 (2H, m) and 3.78 (1H, m, 2′-H, 3′-H and 4′-H), 4.16 (1H, d, *J*=9.6 Hz, 5′-H), 5.20 (2H, s, Ph*C*H_2O), 5.83 (1H, d, *J*=7.9 Hz, 1′-H), 7.33 (6H, m, ArH), 7.41 (1H, m, ArH) and 8.00 (2H, m, ArH); ^{13}C NMR [100 MHz, $(CD_3)_2CO$]: 22.0, 39.2, 67.7, 73.1, 74.0, 77.6, 96.2, 128.1, 129.3, 129.5, 129.7, 130.5, 130.9, 137.3, 145.75, 165.9 and 169.5; *m/z* (ES^+): 425 (MNa^+, 100%) and 827 ($2MNa^+$, 12%). High-resolution mass (ES) calculated for $C_{21}H_{22}O_8Na$ (MNa^+): 425.1212. Found: 425.1198.

4.5.5. Benzyl 1-(2-bromobenzoyl)-β-D-glucopyranuronate (7b). Amorphous white solid. Found: C, 52.2; H, 4.4. $C_{20}H_{19}BrO_8$ requires C, 51.4; H, 4.1%; ν_{max} (cm^{-1}, FT) 3100–3600 (br), 1755, 1720, 1587 and 741; ^{1}H NMR [400 MHz, $(CD_3)_2CO$]: 3.61, 3.68 and 3.76 (3H, 3m, 2′-H, 3′-H and 4′-H), 4.20 (1H, d, *J*=9.5 Hz, 5-H), 5.22 (2H, s, Ph*C*H_2O), 5.84 (1H, d, *J*=7.9 Hz, 1-H), 7.20–7.40 (5H, m, *Ph*CH_2O), 7.52 (2H, m, ArH), 7.77 (1H, m, ArH) and 7.98 (1H, m, ArH); ^{13}C NMR [100 MHz, $(CD_3)_2CO$]: 67.7, 73.1, 73.9, 77.5, 77.7, 96.6, 122.9, 128.9, 129.2, 129.3, 129.7, 132.3, 133.1, 134.9, 135.8, 137.3, 164.9 and 169.4. High-resolution mass (ES) calculated for $C_{20}H_{19}{}^{79}BrO_8Na$ (MNa^+): 489.0161. Found: 489.0161.

4.5.6. (2*S*)-Ibuprofen acyl glucuronide, benzyl ester (9b). Amorphous white solid. Found: C, 65.8; H, 6.8; *m/z* 495.1992. $C_{26}H_{32}O_8$ requires C, 66.1; H, 6.8%; ν_{max} (cm^{-1}, FT) 3100–3600 (br), 1739, 1512 and 746; $C_{26}H_{32}O_8Na$ requires *m/z* 445.1995; ^{1}H NMR [$(CD_3)_2CO$]: 0.88 (6H, d, *J*=7.1 Hz, $(CH_3)_2CH$), 1.45 (3H, d, *J*=7.1 Hz, CH_3CHAr), 1.85 (1H, m, $(CH_3)_2CH$), 2.45 (2H, d, *J*=7.2 Hz, CH*C*H_2Ar), 3.43, 3.57 and 3.66 (3H, 3m, 2′-H+3′-H+4′-H), 3.81 (1H, q, *J*=7.1 Hz, Ar*C*HCH_3), 4.04 (1H, d, *J*=9.6 Hz, 5′-H), 5.18 (2H, s, Ph*C*H_2O), 6.00 (1H, d, *J*=7.9 Hz, 1′-H), 7.10 (2H, d, *J*=8.1 Hz, ArH), 7.23 (2H, d, *J*=8.1 Hz, ArH) and 7.30–7.45 (5H, m, ArH of Bn ester); ^{13}C NMR [100 MHz, $(CD_3)_2CO$]: 19.8, 23.0, 31.3, 46.0, 67.6, 73.1, 73.9, 77.60, 77.63, 96.0, 128.6, 129.1, 129.3, 129.7, 130.5, 137.3, 139.0, 141.6, 169.3 and 174.1; Mass spec. (ES^+): *m/z* 495 (MNa^+, 100%), 967 (weak, $2MNa^+$).

4.5.7. (2*S*)-Naproxen acyl glucuronide, benzyl ester (10b). White solid, mp 186–187 °C. Found: C, 64.2; H, 5.9. $C_{27}H_{28}O_9\cdot0.5H_2O$ requires C, 64.1; H, 5.7%; ν_{max} (cm^{-1}, FT) 3573 (sh), 3200–3600 (br), 1728, 1630, 1604, 1502 and 752; ^{1}H NMR [400 MHz, $(CD_3)_2CO$]: 1.55 (3H, d, *J*=7.1 Hz, *C*H_3CH), 3.42, 3.57 and 3.64 (3H, 3m, 2′-H, 3′-H and 4′-H), 3.91 (3H, s, CH_3O), 3.98 (1H, q, *J*=7.1 Hz, CH_3*C*H), 4.05 (1H, d, *J*=9.4 Hz, 5′-H), 5.15 (2H, s, Ph*C*H_2O), 5.63 (1H, d, *J*=8.2 Hz, 1′-H), 7.14 (1H, m), 7.27–7.35 (5H, m), 7.45 (1H, m) and 7.74–7.77 (4H, m, ArH); ^{13}C NMR [100 MHz, $(CD_3)_2CO$]: 19.7, 39.1, 46.2, 56.0, 67.5, 73.0, 73.9, 77.6, 96.0, 106.9, 120.1, 127.3, 127.6, 128.4, 129.1, 129.2, 129.6, 130.3, 130.5, 135.2, 136.8, 137.2, 159.1, 169.3 and 174.0; *m/z* (ES^+): 519 (MNa^+, 100%). High-resolution mass (ES) calculated for $C_{27}H_{28}O_9Na$: 519.1631. Found: 519.1630.

4.5.8. Mycophenolic acid acyl glucuronide, benzyl ester (11b). White solid, mp 54 °C. Found: C, 60.2; H, 5.9. $C_{30}H_{34}O_{12}\cdot0.5H_2O$ requires C, 60.5; H, 5.9%; ν_{max} (cm^{-1}, FT) 3100–3600 (br), 1732, 1620, 1452 and 748 (w); ^{1}H NMR [400 MHz, $(CD_3)_2CO$]: 1.82 (3H, s, CH_3C=C), 2.16 (3H, s, CH_3Ar), 2.30 and 2.47 (4H, 2m, CH_2CH_2CO), 3.40 (2H, d, *J*=6.9 Hz, Ar*C*H_2CH), 3.45, 3.60 and 3.72 (3H, 3m, 2-H, 3-H and 4-H), 3.80 (3H, s, CH_3O), 4.08 (1H, d, *J*=9.6 Hz, 5-H), 5.21 (2H, s, Ph*C*H_2O), 5.28 (2H, s, Ar*C*H_2O, lactone), 5.30 (1H, t, *J*=7.0 Hz, C=*CH*CH_2), 5.57 (1H, d, *J*=8.1 Hz, 1-H) and 7.30–7.43 (5H, m, ArH); ^{13}C NMR [100 MHz, $(CD_3)_2CO$]: 12.0, 16.7, 19.3, 23.7, 33.8, 35.3, 58.3, 61.9, 67.7, 70.6, 73.1, 73.9, 77.5, 95.6, 107.7, 118.1, 123.1, 124.5, 129.4, 129.6, 134.9, 137.2, 146.4, 154.5, 164.8, 169.5, 172.6 and 173.4; *m/z* (ES^+): 609 (100%, MNa^+). High-resolution mass (ES) calculated for $C_{30}H_{34}O_{12}Na$: 609.1948. Found: 609.1968.

4.5.9. Zomepirac acyl glucuronide, benzyl ester (12b). Amorphous white solid. Found: C, 60.1; H, 5.1; N, 2.4. $C_{28}H_{28}ClNO_9$ requires C, 60.3; H, 5.1; N, 2.5%; ν_{max} (cm^{-1}, FT) 3100–3600 (br), 1747, 1610 (sh), 1589, 1452 and 754; ^{1}H NMR [400 MHz, $(CD_3)_2CO$]: 1.71 (3H, s, CH_3Ar), 3.50, 3.63 and 3.73 (3H, 3m, 2-H, 3-H and 4-H), 3.69 (3H, s, CH_3N), 3.86 (2H, narrow AB q, Ar*C*H_2CO), 4.11 (1H, d, *J*=9.6 Hz, 5-H), 5.21 (2H, s, Ph*C*H_2O), 5.67 (1H, d, *J*=8.4 Hz, 1-H), 6.01 (1H, s, pyrrole 3-H), 7.28–7.43 (5H, 2m, *Ph*CH_2O), 7.52 and 7.66 (4H, approx. 2d, ArH of 4-ClPh); ^{13}C NMR [100 MHz, $(CD_3)_2CO$]: 14.9, 33.2, 33.9, 67.8, 72.9, 73.8, 77.4, 77.6, 96.2, 129.2, 129.3, 129.4, 129.7, 129.9, 130.9, 131.9, 134.3, 137.2, 138.4, 141.2, 169.5, 169.6 and 187.0; *m/z* (ES^+): 580 and 582 (MNa^+ for ^{35}Cl and ^{37}Cl). High-resolution mass (ES^+) calculated for $C_{28}H_{28}ClNO_9Na$ [MNa^+]: 580.1350. Found: 580.1340.

4.5.10. Diclofenac acyl glucuronide, benzyl ester (13b). Amorphous white solid. ν_{max} (cm^{-1}, FT) 3100–3600 (br), 1739, 1579, 1502, 1452 (s) and 744; ^{1}H NMR [400 MHz, $(CD_3)_2CO$]: 3.52, 3.61 and 3.71 (3H, 3m, 2-H, 3-H and

4-H), 3.93 (2H, AB q, ArCH_2CO), 4.10 (1H, d, J=9.6 Hz, 5-H), 5.19 (2H, s, PhCH_2O), 5.68 (1H, d, J=8.0 Hz, 1-H), 6.48 (1H, d, J=8.0 Hz, ArH), 6.77 (1H, br s, NH), 6.95 (1H, t, J=7.4 Hz, ArH), 7.10–7.20 (2H, m, ArH), 7.28–7.45 (6H, m, ArH) and 7.46 (2H, d, J=8.1 Hz, ArH); ^{13}C NMR [100 MHz, $(CD_3)_2CO$]: 39.1, 67.7, 73.0, 73.9, 77.3, 77.6, 96.3, 119.1, 123.2, 125.6, 126.2, 129.2, 129.3, 129.3, 129.7, 130.3, 131.1, 132.4, 137.3, 139.2, 144.2, 169.3 and 172.0. High-resolution mass (ES^+) calculated for $C_{27}H_{25}{}^{35}Cl_2NO_8Na$: 584.0855. Found: 584.0864.

4.5.11. Valproic acid acyl glucuronide, benzyl ester (14b). Amorphous white solid. ν_{max} (cm^{-1}, FT) 3100–3600 (br), 1788, 1741, 1601, 1456 and 766; ^{1}H NMR [400 MHz, $(CD_3)_2CO$]: 0.88 (6H, m, 2×CH_3), 1.30 (4H, m, 2×CH_3CH_2), 1.46, 1.60 (4H, 2m, 2×CH_2CH), 2.46 (1H, m, $CH(CH_2)_2$), 3.45, 3.57 and 3.71 (3H, 3m, 2-H, 3-H and 4-H), 4.05 (1H, d, J=9.5 Hz, 5-H), 5.21 (2H, s, PhCH_2O), 5.59 (1H, d, J=8.2 Hz, 1-H) and 7.31–7.41 (5H, m, ArH); ^{13}C NMR [100 MHz, $(CD_3)_2CO$]: 15.7, 21.2, 21.3, 35.3, 35.3, 46.0, 67.4, 73.0, 73.7, 77.4, 77.6, 95.3, 128.9, 129.1, 129.5, 137.2, 169.3 and 175.3; m/z (ES^+): 433 (100%), MNa^+. High-resolution mass (ES^+) calculated for $C_{21}H_{30}O_8Na$: 433.1838. Found: 433.1823.

4.6. Typical deprotection procedure (allyl ester) using $Pd(PPh_3)_4$

4.6.1. 1-*O*-(4-Bromo)benzoyl-β-D-glucopyranuronic acid (5d). $Pd(PPh_3)_4$ (0.014 g, 0.012 mmol) and morpholine (0.011 g, 0.12 mmol) were added to **5c** (0.050 g, 0.12 mmol) with stirring in THF (0.5 mL) at 0 °C for 0.75 h. Evaporation of solvent left a yellow gum, which was purified by chromatography, eluting with EtOH–CH_2Cl_2–CH_3CO_2H, 2:8:0.1, increasing the polarity later to 3:1:0.1. Pooling and evaporation of appropriate fractions (UV and anisaldehyde detection) gave the free acyl glucuronide **5d** (0.034 g, 80%) as a clear gum with spectroscopic data as reported previously.[8] In addition traces of Pd reagent were detected by ^{1}H NMR (excess ArH integral) and MS. For **5d**: ^{1}H NMR [$(CD_3)_2CO$]: 3.55–3.75 (3H, 3m, 2-H+3-H+4-H), 3.87 (1H, d, J=9.2 Hz, 5-H), 5.78 (1H, d, J=7.7 Hz, 1-H), 7.75 (2H, d, J=8.5 Hz, ArH) and 8.06 (2H, d, J=8.6 Hz, ArH).

4.7. Typical deprotection procedure (allyl ester) using resin-bound Pd(0)

4.7.1. 1-*O*-Phenylacetyl-β-D-glucopyranuronic acid (8d). PS–PPh_3–Pd(0), purchased from Argonaut Technologies, was stirred in THF–DMF, 1:1 (1 mL per 0.1 g of resin) for 0.5 h, filtered, washed with further THF and dried before use. This material (0.24 g, 0.024 mmol), allyl ester **8c** (0.042 g, 0.12 mmol) and morpholine (0.011 g, 0.12 mmol) were stirred in THF (0.4 mL) and DMF (0.1 mL) at 20 °C under N_2 for 3 h. Amberlite IR-120 (H^+) resin (0.1 g, 0.19 mmol) was added followed by stirring for 0.5 h, then the resin was filtered off and washed with the same solvent (10 mL) followed by evaporation of filtrate and washings to dryness. Chromatography of the crude product, eluting with 15% and then 50% EtOH–CH_2Cl_2, afforded on evaporation of appropriate fractions the product **8d** (0.028 g, 75%) as a foam; see Section 4.8.1. for characterisation of this product.

Similarly the following compound was prepared.

4.7.2. Mycophenolic acid acyl glucuronide (11d).[18] Colourless foam. Found: C, 53.6; H, 5.8. $C_{23}H_{28}O_{12}\cdot H_2O$ requires C, 53.7; H, 5.6%; ν_{max} (cm^{-1}, FT) 2500–3600 (br), 1730, 1620, 1452 and 791 (w); ^{1}H NMR [400 MHz, $(CD_3)_2CO$]: 1.81 (3H, s, CH_3C=C), 2.15 (3H, s, ArCH_3), 2.30, 2.51 (4H, 2m, $COCH_2CH_2$), 3.38 (2H, d, J=6.9 Hz, ArCH_2CH), 3.43, 3.53 and 3.58 (3H, 3m, 2′-H+3′-H+4′-H), 3.70 (1H, d, J=9.5 Hz, 5′-H), 3.78 (3H, s, CH_3O), 5.27 (1H, t, J=6.25 Hz, C=$CH$$CH_2$), 5.30 (2H, s, Ar$CH_2$O) and 5.45 (1H, d, J=8.1 Hz, 1′-H); ^{13}C NMR [$(CD_3)_2CO$]: 11.9, 16.7, 23.6, 33.8, 35.2, 61.9, 64.2, 70.9, 72.9, 73.8, 77.0, 77.5, 95.5, 107.7, 118.1, 123.1, 124.5, 134.9, 146.4, 154.5, 164.8, 172.6 and 173.3. High-resolution mass (ES^-) calculated for $C_{23}H_{27}O_{12}$ $[M-H]^+$: 495.1503. Found: 495.1514.

4.8. Typical deprotection procedure (benzyl ester)

4.8.1. 1-*O*-(2-Phenyl)acetyl-β-D-glucopyranuronic acid (8d). A solution of benzyl ester **8b** (0.121 g, 0.3 mmol) in ^{i}PrOH (5 mL) and THF (5 mL) was stirred at 60 °C with cyclohexa-1,4-diene (1 mL) and 10% Pd–C (10 mg) for 1.5 h. Reaction was then complete by TLC (10% EtOH–CH_2Cl_2): the catalyst was filtered off and the filtrate evaporated to give a residue, which on trituration with ether afforded the product **8d** as an amorphous solid (0.094 g, quant.). Found: C, 54.2; H, 5.4. $C_{14}H_{16}O_8$ requires C, 53.9; H, 5.1%; ν_{max} (cm^{-1}, FT) 3502, 3433 and 3292 superimposed on 2500–3600 (br), 1753, 1620 (sh), 1394 (w) and 768; ^{1}H NMR [$(CD_3)_2CO$]: 3.37, 3.49, 3.57 (3H, 3t, J=9–9.5 Hz, 2′H, 3′-H and 4′-H), 3.62 (2H, s, ArCH_2CO), 3.88 (1H, d, J=9.5 Hz, 5-H), 4.90–5.40 (3H, m, D_2O exch., 3×OH), 5.48 (1H, d, J=8.1 Hz, 1-H) and 7.11–7.22 (5H, m, ArH); ^{13}C NMR [400 MHz, $(CD_3)_2CO$]: 41.5, 73.0, 73.8, 77.1, 77.3, 95.8, 128.3, 129.4, 131.1, 135.2, 170.6 and 171.4; m/z (ES^+): 335 (MNa^+, 100%) and 647 ($2MNa^+$). High-resolution mass (ES) calculated for $C_{14}H_{16}O_8Na$ (MNa^+): 335.0743. Found: 335.0728.

Similarly the following compounds were prepared.

4.8.2. 1-(4-Chlorobenzoyl)-β-D-glucopyranuronic acid (4d). Colourless foam. ν_{max} (cm^{-1}, FT) 3637 (sh), 2500–3650 (br), 1716, 1593, 1431 and 760; ^{1}H NMR [400 MHz, $(CD_3)_2CO$]: 3.56 (2H, m) and 3.62 (1H, m, 2-H, 3-H and 4-H), 3.98 (1H, d, J=9.4 Hz, 5-H), 5.70 (1H, d, J=7.5 Hz, 1-H), 7.44 (2H, d) and 7.96 (2H, d, ArH); ^{13}C NMR [100 MHz, $(CD_3)_2CO$]: 72.9, 73.8, 77.1, 77.4, 96.4, 129.5, 130.2, 132.8, 140.8, 165.2 and 170.4; m/z (ES^-): 331(100%), 333 ($[M-H]^+$ for ^{35}Cl, ^{37}Cl). High-resolution mass (ES) calculated for $C_{13}H_{12}{}^{35}ClO_8$ ($[M-H]^+$): 331.0221. Found: 331.0208.

4.8.3. 1-(4-Methylbenzoyl)-β-D-glucopyranuronic acid (6d). Colourless foam. Found: C, 54.5; H, 6.4. $C_{14}H_{16}O_8\cdot 0.5$ ^{i}PrOH requires C, 54.4; H, 5.8%; ν_{max} (cm^{-1}, FT) 2500–3650 (br), 1703, 1610, 1425 and 750;

^{1}H NMR [400 MHz, $(CD_3)_2CO$]: 2.41 (3H, s, $ArCH_3$), 3.66 (2H, m) and 3.75 (1H, m, 2′-H, 3′-H and 4′-H), 4.10 (1H, d, J=9.4 Hz, 5′-H), 5.82 (1H, d, J=7.6 Hz, 1′-H), 7.35 (2H, m, ArH) and 7.98 (2H, m, ArH); ^{13}C NMR [100 MHz, $(CD_3)_2CO$]: 22.0, 73.0, 73.9, 77.1, 77.6, 96.1, 128.1, 130.5, 131.1, 145.7, 165.9 and 170.5; *m/z* (ES^-): 311 ($[M-H]^+$), 100%. High-resolution mass (ES) calculated for $C_{14}H_{15}O_8$ ($[M-H]^+$): 311.0767. Found: 311.0764.

4.8.4. (2*S*)-Ibuprofen acyl glucuronide (9d). Colourless foam. Found: C, 58.8; H, 7.3. $C_{19}H_{26}O_8\cdot 0.5H_2O$ requires C, 58.3; H, 6.9%; ν_{max} (cm^{-1}, FT) 2500–3650 (br), 1734, 1600 (sh, w), 1512, 1454 and 802 (w); ^{1}H NMR [400 MHz, $(CD_3)_2CO$]: 0.75 [6H, d, J=6.6 Hz, $(CH_3)_2CH$], 1.32 (3H, d, J=7.1 Hz, CH_3CHAr), 1.71 (1H, m, $(CH_3)_2CH$), 2.31 (2H, d, J=7.2 Hz, $ArCH_2CH$), 3.32, 3.46 and 3.53 (3H, 3m, 2-H, 3-H and 4-H), 3.68 (1H, q, J=7.1 Hz, CH_3CHAr), 3.85 (1H, d, J=9.4 Hz, 5-H), 5.47 (1H, d, J=8.1 Hz, 1-H), 6.98 and 7.11 (4H, 2d, ArH); ^{13}C NMR [100 MHz, $(CD_3)_2CO$]: 19.9, 23.1, 35.4, 46.0, 72.9, 73.8, 77.1, 77.6, 95.9, 126.5, 128.7, 130.5, 139.0, 141.6, 170.3 and 174.1. High-resolution mass (ES^-) calculated for $C_{19}H_{25}O_8$: 381.1549. Found: 381.1539.

4.8.5. (2*S*)-Naproxen acyl glucuronide (10d). Colourless foam. ν_{max} (cm^{-1}, FT) 2500–3650 (br), 1747, 1631, 1606, 1508, 1455 and 850 (w); ^{1}H NMR [400 MHz, $(CD_3)_2CO$]: 1.56 (3H, d, J=7.1 Hz, CH_3CH), 3.44, 3.54 and 3.65 (3H, 3m, 2′-H, 3′-H and 4′-H), 3.90 (3H, s, CH_3O), 3.96 (1H, d, J=9.4 Hz, 5′-H), 3.98 (1H, q, J=7.1 Hz, CH_3CH), 5.63 (1H, d, J=8.1 Hz, 1′-H), 7.13 (1H, m, ArH), 7.45 (1H, m, ArH) and 7.76 (4H, m, ArH); ^{13}C NMR [100 MHz, $(CD_3)_2CO$]: 19.7, 46.3, 56.0, 62.4, 73.0, 73.9, 77.7, 96.0, 106.9, 120.1, 127.3, 127.7, 128.4, 128.9, 130.5, 135.2, 136.9, 159.1, 170.3 and 174.1. High-resolution mass (ES^-) calculated for $C_{20}H_{21}O_9$: 405.1186. Found: 405.1177.

4.8.6. Zomepirac acyl glucuronide (12d). Colourless foam. ν_{max} (cm^{-1}, FT) 2500–3600 (br), 1747, 1610 (sh), 1589, 1452 and 754; ^{1}H NMR [400 MHz, $(CD_3)_2CO$]: 1.72 (3H, s, $ArCH_3$), 3.52, 3.65 and 3.72 (3H, 3m, 2-H, 3-H and 4-H), 3.71 (3H, s, CH_3N), 3.89 (2H, s, $ArCH_2CO$), 4.05 (1H, d, J=9.4 Hz, 5-H), 5.67 (1H, d, J=8.0 Hz, 1-H), 6.01 (1H, s, pyrrole 3-H), 7.52 and 7.67 (4H, 2d, ArH); ^{13}C NMR [100 MHz, $(CD_3)_2CO$]: 14.9, 31.2, 33.3, 72.9, 73.8, 77.1, 77.5, 96.1, 113.8, 126.5, 129.4, 129.9, 131.9, 134.4, 138.4, 141.2, 169.6, 170.4 and 187.0. High-resolution mass (ES^-) calculated for $C_{21}H_{21}NO_9{}^{35}Cl$: 466.0905. Found: 466.0885.

4.8.7. Diclofenac acyl glucuronide (13d). Colourless foam. ν_{max} (cm^{-1}, FT) 3639 (sh, w), 2500–3650 (br), 1734, 1581, 1504, 1450 (s), 769 and 744; ^{1}H NMR [400 MHz, $(CD_3)_2CO$]: 3.52, 3.59 and 3.68 (3H, 3m, 2-H, 3-H and 4-H), 3.94 (2H, s, $ArCH_2CO$), 4.03 (1H, d, J=9.5 Hz, 5-H), 5.68 (1H, d, J=7.9 Hz, 1-H), 6.47 (1H, d, J=7.8 Hz, ArH), 6.78 (1H, br s, NH), 6.94 (1H, m, ArH), 7.07–7.19 (2H, m, ArH), 7.30 (1H, m, ArH) and 7.44 (2H, m, ArH); ^{13}C NMR [100 MHz, $(CD_3)_2CO$]: 38.8, 73.0, 73.9, 77.0, 77.3, 96.5, 119.1, 123.5, 125.6, 126.6, 126.7, 129.2, 130.3, 131.0, 132.5, 139.1, 144.3, 170.2 and 171.9; *m/z* (ES^-): 470 (85%), 472 and 474 [$(M-H)^+$ for $^{35}Cl_2$, $^{35}Cl/^{37}Cl$ and $^{37}Cl_2$)]. High-resolution mass (ES^-) calculated for $C_{20}H_{18}{}^{35}Cl_2NO_8$: 470.0409. Found: 470.0388.

4.8.8. Valproic acid acyl glucuronide (14d). Hard colourless gum. ν_{max} (cm^{-1}, FT) 2500–3700 (br), 1734 and 1460; ^{1}H NMR [400 MHz, CD_3CN+D_2O]: 0.85–0.90 (6H, 2m, $2\times CH_3CH_2$, non-eq.), 1.25–1.32 (4H, m), 1.40–1.50 and 1.50–1.60 (4H, m), 2.40–2.50 (1H, m, $COCH(CH_2)_2$), 3.38, 3.48 and 3.56 (3H, 3m, 2-H, 3-H+4-H), 3.91 (1H, d, J=9.5 Hz, 5-H) and 5.48 (1H, d, J=8.2 Hz, 1-H); ^{13}C NMR [100 MHz, THF-d_8]: 13.0, 13.0, 19.7, 19.8, 33.6, 33.7, 44.6, 71.3, 72.1, 75.6, 76.5, 93.7, 168.8 and 173.3; *m/z* (ES^-): 319 (100%), $(M-H)^+$. High-resolution mass (ES^-) calculated for $C_{14}H_{23}O_8$: 319.1393. Found: 319.1404.

4.9. Purification of acyl glucuronides

In general acyl glucuronides of high purity are obtained from benzyl esters by the preceding method. If further purification is necessary, we recommend either conventional chromatography as applied to **5d** and **8d** above (allyl ester method), or the use of reverse-phase silica 'LiChroprep'. For the latter, on a 0.1 g scale of product, the crude product is applied to a column of LiChroprep (2 g) and eluted with a gradient of acetonitrile (typically from 20 to 60% for most AGs) in 0.1% AcOH–H_2O. Appropriate fractions are then pooled and evaporated. This procedure makes use of the greatly enhanced stability of AGs at mildly acidic pH; similarly 0.1% aq formic acid has been used, see Ref. 21.

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References and notes

1. Kaspersen, F. M.; Van Boeckel, C. A. A. *Xenobiotica* **1987**, *17*, 1451–1471.
2. Stachulski, A. V.; Jenkins, G. N. *Nat. Prod. Rep.* **1998**, *15*, 173–188.
3. Bailey, M. J.; Dickinson, R. G. *Chem.-Biol. Interact.* **2003**, *145*, 117–137.
4. Shipkova, M.; Armstrong, V. W.; Oellerich, M.; Wieland, E. *Ther. Drug Monit.* **2003**, *25*, 1–16.
5. Spahn-Langguth, H.; Benet, L. Z. *Drug Metab. Rev.* **1992**, *24*, 5–47.
6. Vanderhoeven, S. J.; Lindon, J. C.; Troke, J.; Tranter, G. E.; Wilson, I. D.; Nicholson, J. K. *Xenobiotica* **2004**, *34*, 73–85.
7. Vanderhoeven, S. J.; Troke, J.; Tranter, G. E.; Wilson, I. D.; Nicholson, J. K.; Lindon, J. C. *Xenobiotica* **2004**, *34*, 889–900.
8. Stachulski, A. V.; Harding, J. R.; Lindon, J. C.; Maggs, J. L.; Park, B. K.; Wilson, I. D. *J. Med. Chem.* **2006**, *49*, 6931–6945.
9. Juteau, H.; Gareau, Y.; Labelle, M. *Tetrahedron Lett.* **1997**, *38*, 1481–1484.
10. Kenny, J. R.; Maggs, J. L.; Meng, X.; Sinnott, D.; Clarke, S. E.; Park, B. K.; Stachulski, A. V. *J. Med. Chem.* **2004**, *47*, 2816–2825.
11. (a) Perrie, J. A.; Harding, J. R.; Holt, D. W.; Johnston, A.; Meath, P.; Stachulski, A. V. *Org. Lett.* **2005**, *7*, 2591–

2594; (b) Schmidt, R. R. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 212–235; (c) Plusquellec, D.; Roulleau, F.; Bertho, F.; Lefeuvre, M. *Tetrahedron* **1986**, *42*, 2457–2467.
12. Kunz, H.; Dombo, B. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 711–713.
13. Polakova, M.; Joniak, D.; Duris, M. *Monatsh. Chem.* **2000**, *131*, 1197–1205.
14. Meng, X.; Stachulski, A. V. Unpublished results.
15. Ebner, T.; Heinzel, G.; Prox, A.; Beschk, K.; Wachsmuth, H. *Drug Metab. Dispos.* **1999**, *27*, 1143–1149.
16. Levy, D. B.; Vasilomanolakis, E. C. *Drug Intell. Clin. Pharm.* **1984**, *18*, 983–984.
17. (a) Chen, Q.; Doss, G. A.; Tung, E. C.; Liu, W.; Tang, Y. S.; Braun, M. P.; Didolkar, V.; Strauss, J. R.; Wang, R. W.; Stearns, R. A.; Evans, D. C.; Baillie, T. A.; Tang, W. *Drug Metab. Dispos.* **2006**, *34*, 145–151; (b) Olsen, J.; Li, C.; Bjornsdottir, I.; Sidenius, U.; Hansen, S. H.; Benet, L. Z. *Chem. Res. Toxicol.* **2005**, *18*, 1729–1736.
18. The anomeric mixture of AG benzyl esters of **14** was eluted at 0.9 mL/min from a C_8 column with a gradient of MeCN (30–50% over 20 min) in 10 mM NH_4OAc, monitored at 254 nm and their positive-ion mass spectra obtained by electrospray ionisation in a Waters Quattro II instrument. Retention times were 18.3 and 19.7 min.
19. Shipkova, M.; Armstrong, V. W.; Weber, L.; Niedmann, P. D.; Wieland, E.; Haley, J.; Tonshoff, B.; Oellerich, M. *Ther. Drug Monit.* **2002**, *24*, 390–399.
20. (a) Shipkova, M.; Beck, H.; Voland, A.; Armstrong, V. W.; Grone, H. J.; Oellerich, M.; Wieland, E. *Proteomics* **2004**, *4*, 2728–2738; (b) Asif, A. R.; Armstrong, V. W.; Voland, A.; Armstrong, V. W.; Wieland, E.; Oellerich, M.; Shipkova, M. *Biochemie* **2007**, *89*, 393–402.
21. Kittlemann, M.; Rheinegger, U.; Espigat, A.; Oberer, L.; Aichholz, R.; Francotte, E.; Ghisalba, O. *Adv. Synth. Catal.* **2003**, *345*, 825–829.
22. Baba, A.; Yoshioka, T. *Org. Biomol. Chem.* **2006**, *4*, 3303–3310.