Journal of Medicinal Chemistry

Volume 49, Number 24 November 30, 2006

Perspective

Acyl Glucuronides: Biological Activity, Chemical Reactivity, and Chemical Synthesis

Andrew V. Stachulski,*,† John R. Harding,‡ John C. Lindon,§ James L. Maggs,# B. Kevin Park,# and Ian D. Wilson‡

Department of Chemistry, The Robert Robinson Laboratories, University of Liverpool, Liverpool L69 7ZD, U.K., Drug Metabolism and Pharmacokinetics Department, AstraZeneca U.K. Ltd., Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, U.K., Department of Biomolecular Medicine, Faculty of Medicine, Imperial College London, Sir Alexander Fleming Building, South Kensington, London SW7 2AZ, U.K., and Department of Pharmacology and Therapeutics, University of Liverpool, Liverpool L69 3GE, U.K.

Received May 19, 2006

Introduction: Acyl Glucuronides as Reactive Metabolites

In a definitive review of 1987, Kaspersen and van Boeckel[1] presented a concise account of the chemical synthesis of glucuronide and sulfate conjugates of xenobiotics. At that time, *O*-acyl glucuronides (AGs) had been known as mammalian metabolites for half a century[2] and were briefly discussed in the article, but their syntheses had attracted relatively little interest. While their greater and distinctive chemical reactivity compared to alkyl and aryl glucuronides was appreciated,[3] little attempt had been made to correlate their chemical and biological behavior.[4]

In the years that followed, with the development of precautions for stabilizing AGs in biofluids and the general availability of LC−MS, it became clear that a number of carboxylic acid containing drugs were metabolized extensively to their AGs.[5] The profile of AGs rose dramatically when it was found that many nonsteroidal anti-inflammatory drugs (NSAIDs), withdrawn owing to unacceptable adverse reactions in patients, were metabolized in humans to unstable AGs that formed covalent protein adducts.[5] This led to a growing need to prepare AGs as analytical standards and for toxicological evaluation. Nevertheless, when *O*-glucuronide synthesis was reviewed again in 1998,[6] synthetic methods for AGs were still generally unsatisfactory and many commonly used procedures generated isomeric mixtures. Often incompletely purified conjugates extracted from serum, urine, incubations of hepatic microsomes, or liver homogenate are still employed even when the AGs are required for bioevaluation.[7−9] Also, enzymatic synthesis is still used, with increasing success despite the considerable difficulties of working with the sensitive enzymes involved (see below).

Since the publication of the latter review, AGs of both currently marketed and candidate drugs have continued to be extensively studied, in accord with increasing concern in the pharmaceutical industry regarding reactive metabolites of all kinds. Not the least spur for this activity has been the proposition that the attrition of new drugs by numerous adverse reactions in clinical trials might be reduced if the formation of such reactive metabolites could be restricted through informed design and early bioactivation screening of candidates.[10]

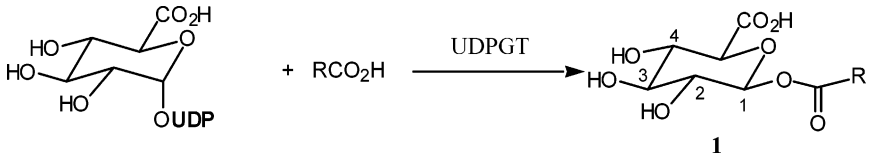
Methods for chemical synthesis of AGs have been greatly improved of late, and interestingly some anomeric esters of other sugars have been detected as in vivo metabolites and also synthesized. Further significant advances in analytical techniques have enabled the quantification of the reactivity of AGs in vitro and identified specific proteins that may be modified by AGs in vivo.

For all these reasons, we feel this Perspective of AGs is now timely. After an initial structural summary, we present a semiquantitative discussion of the chemical reactivity of AGs according to structural type and examine the evidence for their reactivity with biomolecules. We then assess the analytical technologies and procedures applied to AG detection. Finally, we review methods for their synthesis and hope thereby to counter the misconception that AGs are particularly difficult to synthesize. Other reviews[11,12] concentrating mainly on biological aspects of AGs have appeared in recent years.

* To whom correspondence should be addressed. Phone: 44 (0)151 794 3542. Fax: 44 (0)151 794 3588. E-mail: stachuls@liv.ac.uk.
† Department of Chemistry, University of Liverpool.
‡ AstraZeneca U.K. Ltd.
§ Imperial College London.
# Department of Pharmacology and Therapeutics, University of Liverpool.

10.1021/jm060599z CCC: $33.50
Published on Web 11/27/2006

Scheme 1. Biosynthesis of *O*-Acyl Glucuronides



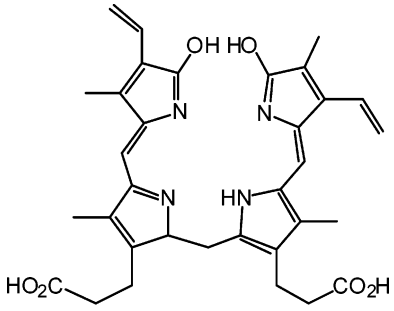
1

Structures and Biosynthesis of AGs and Related Glycosides

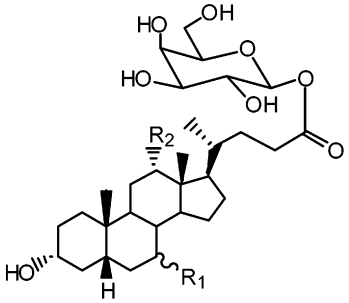
In vertebrates, AGs are synthesized by the conjugation of carboxylic acids with uridine diphosphate glucuronic acid (UDPGA) mediated by various glucuronosyl transferases (UDPGTs)[13] (Scheme 1). Invariably the 1β-isomer shown is the first product generated. At present no single UDPGT isoform is known to glucuronidate only carboxylic acids.

Subsequent rearrangement of the acyl group around the ring, acid- or base-catalyzed, leads to the 2-, 3-, and 4-*O*-AG isomers, all as anomeric α/β mixtures (see below). It has also been shown by NMR studies that a 2-acyl group may migrate to the 1α-OH of the anomeric hemiacetals to generate the 1α-AG isomer.[14] Unless otherwise stated, however, it should be assumed in this review that the first-formed 1β-AG **1** is referred to.

The classic example of an unstable and protein-reactive endogenous AG is the cytotoxic bile pigment bilirubin **2**.[15] Recently both thia and selena analogues of bilirubin have been synthesized and shown to be glucuronidated similarly in rats.[16,17]



2



3 R_1, R_2 = H, OH

Although acyl glycosides other than AGs are known in many plants, only recently have similar conjugates been detected as metabolites of carboxylic acids (endogenous and xenobiotic) in animals. Thus, acyl glucosides[18] and acyl galactosides[19] of bile acids have been isolated from human samples and their structures, e.g., **3**, confirmed by synthesis.[19] Also a 2-deoxy-AG of deoxycholic acid has been prepared as an affinity label,[20] precisely to reduce the possibility of migration and further reaction of the initial adduct. There is no evidence that such molecules occur naturally. Vertebrate UDPGTs employ UDPGA preferentially,[21] but acyl glucoside and galactoside conjugates of xenobiotics have been reported.[22−24] However, two hydroxycarboxylic acid substrates undergo glucosidation exclusively at the phenolic or alcoholic OH group.[25,26] In rare instances, dependent on the species, an acyl glucoside can be a major metabolite.[22] The stability of these glycosides in vivo and their reactivity with proteins do not appear to have been investigated.

In Vivo Fates of Acyl Glucuronides

The chemical and biological reactivity of AGs is of great importance in matters of drug safety and development; Scheme 2 summarizes in general terms the possible in vivo fates of AGs.

The debate over the possible toxicity of AGs is essentially related to the transacylation and rearrangement pathways shown in Scheme 2; AGs, which are either excreted per se or very readily hydrolyzed to regenerate the parent drug or its phase 1 metabolite, are regarded as unlikely to be responsible for toxic effects. The question of the conjugates' "direct" cytotoxicity remains unresolved for lack of detailed information on the intracellular forms and fates of AGs. The partitioning of AGs between the various pathways is critically dependent on their chemical structures as well as their bioenvironment, including genetic factors. We therefore discuss next the chemical reactivity of AGs, followed by their interactions with biomolecules, before considering methods for their characterization and syntheses.

Chemical Reactivity of Acyl Glucuronides

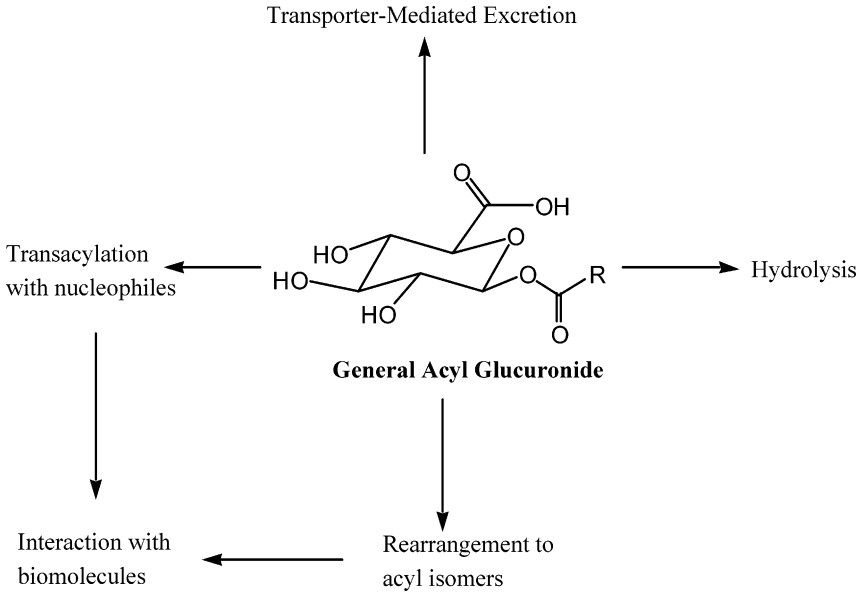
The reactivity of acyl glucuronides is now well understood. In general, two distinct pathways are recognized (Scheme 3).[5] From the initial 1β-isomer, direct reaction with nucleophiles (O, N, or S) may lead to displacement of the acyl residue (pathway 1). Alternatively, migration of the acyl group round the pyranose ring may yield the 2-, 3-, and 4-acyl isomers (pathway 2).

After migration, the acyl group in the 2-, 3-, or 4-position is relatively less liable to direct hydrolysis or displacement, as demonstrated in the greater chemical reactivity of anomeric sugar esters.[27] Reaction with amine nucleophiles then generally takes another course as shown. Reaction at the anomeric carbon (as its aldehyde tautomer), followed by a proton shift, leads to a ring-opened species bearing a stable C−N bond, the so-called Amadori (aldimine−ketimine) rearrangement (see Scheme 4). Evidence for this route has been obtained by trapping experiments with X−NH_2, e.g., in the form of human serum albumin, and either sodium cyanoborohydride[28,29] or, more efficiently, sodium cyanide,[29] which lead to isolable adducts.

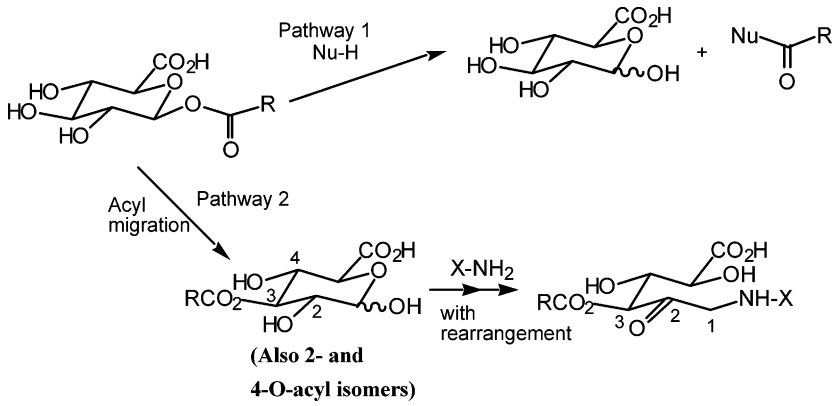
The migration is much more effectively catalyzed by base, even at physiological pH (7.4); in a mildly acidic aqueous environment (pH 4−5), acyl glucuronides are significantly more stable, as shown for diclofenac[28] and probenicid.[30] Stabilizing AGs in biofluids requires careful acidification and cooling.[31]

The 1α-AG isomers such as **4** mentioned earlier arise not by direct anomerization of the 1β-isomer but through the 2-isomer,

Scheme 2. Possible Fates of AGs

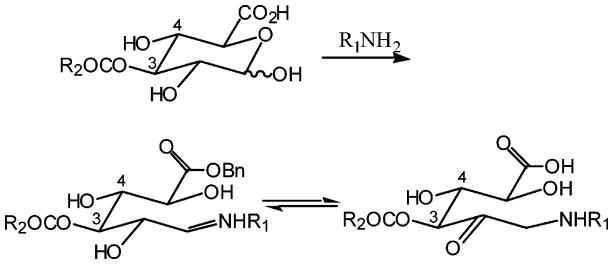


Scheme 3. Reactivity of Acyl Glucuronides[a]

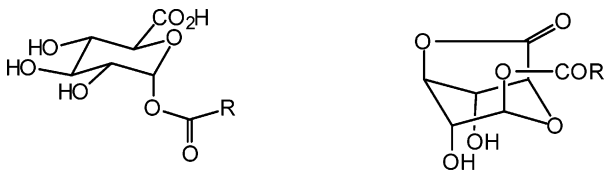


[a] Clearly the rearrangement (pathway 2) requires migration of the acyl group at least as far as O(3).

Scheme 4. Simulated Amadori Rearrangement with a Model Amine



which after mutarotation at C(1) may transfer the acyl group back to the 1α-OH. This has been conclusively demonstrated by NMR spectroscopy.[14,30]

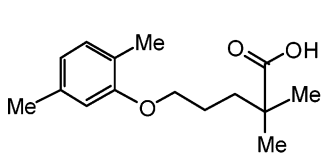


4 **5**

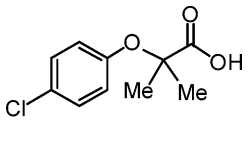
Other rearranged forms of AGs are also known. When the Mitsunobu synthesis[32] (see Synthesis) is used, lactones of type **5** are byproducts, sometimes in appreciable yields. The mechanism of formation is not clear but presumably involves cyclization of the first-formed intermediate ester. If there were an in vivo route to such lactones via free carboxyl acyl glucuronides, they would doubtless be potent acylating agents, transferring in this case the sugar along with the acyl residue.

Concerning 1β-AGs of benzoic acids, the chemical stability/reactivity may be predicted with some confidence by a Hammett correlation,[33] as discussed in detail later (NMR section). Some preliminary studies have been carried out on the computational chemistry modeling of the relative stabilities of the isomers of the AGs of 2-, 3-, and 4-trifluoromethylbenzoic acids.[34] For nonarylcarboxylic acids, the structure−reactivity is more complex. Certainly branching at the α-carbon is a factor leading to increased stability; thus the AGs of gemfibrozil **6**,[35] clofibric acid **7**[36] and valproic acid **8**,[37] all doubly α-substituted, display long half-lives (from about 6−70 h in pH 7.4 buffer[37]).

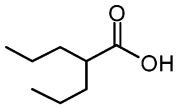
AGs derived from NSAIDs such as naproxen **9** and ibuprofen **10** generally have half-lives from 1 to 4 h under the same conditions.[38] In such compounds, an additional complication is the different rates of rearrangement of the (*R*)- and (*S*)-AG diastereoisomers. In the case of 2-phenylpropanoic acid itself **11**,[38] this rate was found to be about twice as fast for the (2*R*)- as for the (2*S*)-AG epimer, and this ratio is typical of the 2-aryl propanoate class.



6

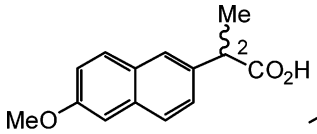


7

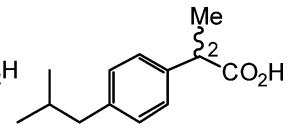


8

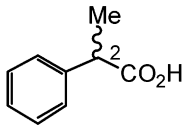
However, it is equally clear that there is no simple correlation between chemical stability and incidence of reported toxicity. Other reports have discussed the relative rates of rearrangement of (*R*)- and (*S*)-isomers of the AGs of the class of NSAIDs.[36,39,40] Interestingly, the relative stabilities of positional AG isomers are affected as well as rates of acyl migration.[41] For ketoprofen, ^{13}C labeling was a useful technique for measuring these effects.[42]



9

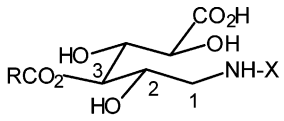


10



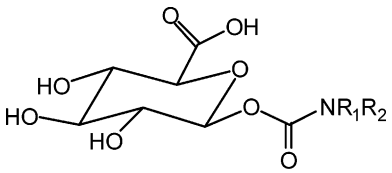
11

It has been proposed by Wang et al.[43] that the reactivity of AGs may be correlated with their rate of formation of Schiff base adducts (see Scheme 4) with a model dipeptide, Lys-Phe. A high correlation, $R^2 = 0.95$, was observed between rate of rearrangement of the primary AG and the trapping (by reaction with HCN or $NaCNBH_3$, leading to **12**) of the Schiff base dipeptide adduct.



12 X = Lys-Phe [assuming acyl migration to O(3)]

The conclusion was that a rate order of acetic acid > propionic acid > benzoic acid was observed, but it is clear that specific structural features may overturn this order; for example, as noted below (in NMR spectroscopy section), the reactivity of substituted benzoyl AGs varies by over 100-fold. Nevertheless, the quantitative nature of this assay is attractive for further testing of concept.



13

Table 1. First-Order Half-Lives of 1-O-AGs of Various Drugs in Aqueous Buffer, pH 7.4, 37 °C (References 37 and 45)

compd	$T_{1/2}$ (h)	structure no. (this review)
tolmetin	0.26	
isoxepac	0.29	
probenecid	0.40	**19**
zenarestat	0.42	
zomepirac	0.45	**18**
diclofenac	0.51	**21**
diflunisal	0.67	
(*R*)-naproxen	0.92	
(*R*)-fenoprofen	0.98	
salicylic acid	1.3	
DMXAA	1.3	**40**
indomethacin	1.4	
(*R*)-carprofen	1.73	
(*S*)-naproxen	1.8	**9**
(*S*)-fenoprofen	1.93	
(*R*)-benoxaprofen	2.0	
(*S*)-carprofen	3.09	
ibuprofen	3.3	**10**
(*S*)-benoxaprofen	4.1	
bilirubin	4.4	**2**
(*R*)-flunoxaprofen	4.5	
furosemide	5.3	
flufenamic acid	7	
clofibric acid	7.3	**7**
(*S*)-flunoxaprofen	8.0	
mefenamic acid	16.5	
(*R*)-beclobric acid	22.4	
(*S*)-beclobric acid	25.7	
telmisartan	26	
gemfibrozil	44	**6**
valproic acid	79	**8**

A closely related group of metabolites, namely, carbamoyl glucuronides such as **13**,[6,44] may be mentioned here; they are significantly more stable than AGs, especially in basic solution.

Two valuable reviews of half-lives of AGs of a number of important drugs have been published.[37,45] They are combined, and the results are shown in Table 1.

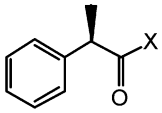
Interaction of Acyl Glucuronides with Biomolecules

Acyl glucuronides are of both pharmacological and toxicological interest.[11,12] Generally speaking, such metabolites are pharmacologically inert. However, AGs represent a considerable concern in drug development because of the propensity of such compounds to rearrange and/or covalently modify proteins. Certain drugs that have caused serious adverse drug reactions in man do indeed form reactive AGs. For example, benoxaprofen, an NSAID marketed in 1982, was withdrawn almost immediately because of a report of fatal cholestatic jaundice. Preclinical evaluation of the drug had not predicted the risk of such a serious reaction. The long-standing association between AG formation and clinical toxicity has not yet been confirmed by either cell-based systems or animal models. Thus, the working hypothesis that AGs per se may cause hepatotoxicity by modification of specific proteins requires further evaluation. The general synthetic availability of AGs, as a result of new methodology, which we emphasize in this review (especially of AGs that are intrinsically unstable), will allow an analysis of the precise relationship between protein modification and protein function. This in turn will provide valuable insight into the potential of a given AG to modify biochemical function and cause cell injury.

The covalent modification of plasma and hepatic proteins by AGs in vivo is now established, though none of the modified proteins have been fully characterized. To prove that the hepato[12] and gastrointestinal toxicity[46] of drugs that form AG

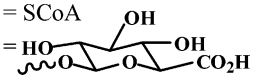
metabolites is due to such interactions is, however, another matter; evidence for such toxicity has been critically reviewed.[45] It is also noteworthy that a quantitative structure−activity study of 21 NSAIDs involving inhibition of glucuronidation revealed that, in general, the AGs were *less* acutely hepatocytotoxic than the parent drugs;[47] furthermore, benzoic and phenylacetic acid types were predicted to behave differently.

As described in the previous section AGs can modify proteins by direct acylation of certain amino acid residues as well as by Schiff base formation with subsequent rearrangement (Scheme 4, pathway 1),[48] though direct evidence for 1-amino-1-deoxy-ketoses is still lacking. It is of value to quantify the reactivity of AGs in comparison to other "natural" acylating agents, and this has been done for thioesters. Taking 2-phenylpropanoic acid as a model NSAID,[49] the −CoA ester **14** and acyl glucuronide **15** were compared as acyl transfer agents. Both species were able to transacylate the SH group of glutathione, and in the case of the thioester, subsequent binding to bovine serum albumin was demonstrated. From experiments with rat hepatocytes,[50] it was concluded that the thiol ester was the more effective "reagent" but that the AG was also able to bind covalently to protein; the higher reactivity of the thioester was effectively balanced by the higher concentration of the AG.



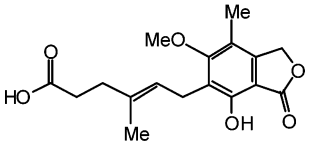
14 X = SCoA

15 X =

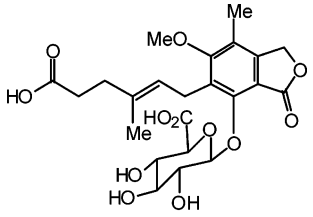


Since an acyl group from an AG may first be transferred to glutathione or a protein SH group, generating a thioester, it is clear that a stepwise rather than a direct acylation by an AG may also be involved. This type of reaction, specifically of clofibric acid **7** with GSH, was first reported by Dickinson et al. in 1995.[51]

The advent of modern proteomic methods is beginning to have an impact on large-scale identification of the protein targets for putative AGs in vivo. Mycophenolic acid **16** is an important natural product used as an immunosuppressant in transplantation and is a substrate for AG formation. It has been shown to form adducts with serum albumin in patients,[52,53] and proteins have been found in rat liver and colon that are posited to be derived from the drug's AG. The hepatic proteins include a protein disulfide isomerase and an ATP synthase. In addition, the AG of **16** has been linked to cytokine release[54] from human leukocytes in vitro and hence to adverse inflammatory reactions associated with use of the drug. However, definitive characterization of the drug-derived moiety of the protein adducts has yet to be reported. The aryl (phenolic) glucuronide **17** of **13** does not appear to undergo bioactivation in vivo.

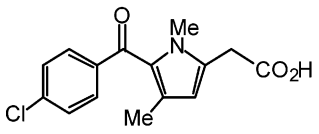


16

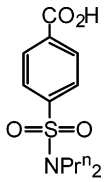


17

By use of immunochemical techniques, hepatic dipeptidyl peptidase IV has been identified as a common target for the AGs of a number of drugs including zomepirac **18**,[55] but otherwise, very few such targets have been described. Here again, there is a question of whether a thiol (acyl-CoA) ester of **18** might be at least as important in binding to liver protein as the AG. Olsen et al. concluded the two had a similar effect.[56]



18

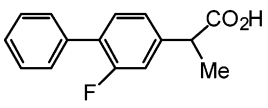


19

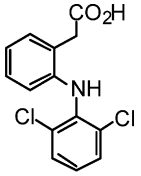
It is rarely clear whether protein modification by AG in tissues occurs via transacylation or imine formation (glycation), but with probenicid **19**,[30] covalent binding to plasma protein occurred mainly by the Schiff base (imine) mechanism, reflecting the short half-life of its AG ($t_{1/2}$ = 0.27 h in buffer; $t_{1/2}$ = 0.17 h in plasma cf. 0.4 h given in Table 1). As noted in the previous section, high chemical stability of AGs is not necessarily associated with low toxicity or the absence of protein modification. Thus, the very stable AG of valproic acid **8** has been shown[57] to interact (by both reversible and irreversible mechanisms) with tubulin and microtubule associated proteins. The far more (chemically) reactive AG of **18** also inhibits tubulin assembly.[58]

As noted above, bile acids are readily glycosidated in vivo, and both they and their conjugates inhibit the glucuronidation of flurbiprofen **20** by hepatic microsomes.[59] It has been suggested that this inhibitory activity might limit the in vivo formation of AGs from drugs and consequently the expression of their hepatotoxicity.

The differential chemical stability of AGs of aryl propanoate NSAIDs referred to in the previous section is paralleled in their biodisposition. Thus, the (2*S*)-AG of the model 2-phenylpropanoic acid showed a significantly greater hepatic extraction than the (2*R*)-isomer **15**.[60] Enantioselective recognition of (*S*)-ibuprofen protein adducts has been demonstrated by using a polyclonal antibody. It was implied that the adduction was caused ultimately by the AG of (*S*)-ibuprofen **10**, mediated via thioesters.[61] This selectivity has also been exploited in an immunoaffinity extraction process.[62] A different kind of selectivity may be seen where the positional AG isomers are relatively long-lived. It was shown that the 2-isomer of (*S*)-naproxen AG is at least as important for protein binding as the initial adduct,[63] though direct transacylation is much slower from the 2-isomer (see previous section**)**. There is evidence that (*S*)-ketoprofen reacts only with Lys residues of HSA in vitro whereas (*R*)-ketoprofen modifies Tyr residues principally and Lys to a lesser extent.[29]



20



21

The interaction of NSAIDs with human serum albumin has in general terms been associated with their AGs for many

years.[64,65] As well as the 2-aryl propanoate class, diclofenac **21** has been extensively studied. It was shown in rats that hepatobiliary transport, by a specific "export pump", is vital to the extracellular modification of hepatic membrane proteins by **21** AG.[66] The link between protein adducts of **21** in intestinal lymphoid tissue and drug hypoallergenicity was suggested earlier[67] without proof of the involvement of metabolites of **21**. In these laboratories we have synthesized the pure 1β-AG of **21** in quantity and demonstrated its conjugation to protein by a trapping experiment,[28] whereas the hydroxy metabolites of **21** (although they can undergo autoxidation to quinoneimines) did not bind. (While the *p*-benzoquinoneimine derivative of 5-hydroxydiclofenac does bind covalently to microsomal proteins, oxidation of the phenol appears to be mediated by metal catalysis,[68] which was evidently not operative in these experiments.)

AGs and other *O*-glucuronides are not inert to anabolic transformations (e.g., the monoglucuronide of bilirubin **2** is glucuronidated[69]), and they may be substrates for metabolic activation. Diglucuronide metabolites of hydroxycarboxylic acids are formed in vivo, but whether the ether glucuronide is a substrate for acyl glucuronidation is not known.[70] The AG of **21** undergoes hydroxylation in human liver microsomes, forming a quinoneimine precursor.[71] Gemfibrozil **6** AG is metabolized by CYP2C8 in vitro to a reactive intermediate that inactivates the cytochrome.[72] A special case of molecular activation through acyl glucuronidation is shown in the transformation of the 2,4-diene metabolite of valproic acid **8** into an active Michael acceptor.[73]

The key role of hepatic transport, uptake, and excretion in mediating the biodisposition of AGs in comparison with other organic ions (since carboxylate is present at physiological pH) has also been demonstrated for the AG of gemfibrozil **6**.[74] Uptake of AG in blood by extrahepatic tissues might lead to the adduction of proteins distant from the site of synthesis.

Esters that are in effect prodrugs may lead to AG formation via the free carboxylic acids, formed in vivo, and possibly associated toxicity; thus, the AG derived from dibutyl phthalate has been linked to adverse developmental effects in male rats.[75]

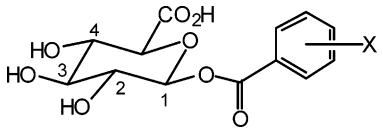
Although studies on the interaction of AGs with biomolecules have concentrated almost exclusively on proteins, it has been suggested that AGs may also damage DNA. Mouse hepatocytes cultured with clofibric acid **7** showed DNA lesions that correlated with the expected rate of formation of AG.[76] The effect was suppressed by addition of borneol, a known glucuronidation inhibitor. Very recently a further study of DNA damage induced by clofibrate **7** and probenecid **19** concluded that iron-dependent oxidative stress could be responsible.[77]

Structural Investigation

a. NMR Spectroscopy. The process of acyl migration in an AG generates a highly complex mixture of products, namely, eight individual acyl isomers plus free glucuronic acid from the competing hydrolysis that occurs concurrently. ^{1}H NMR spectroscopy has been used to characterize this mixture, and the assignment of the NMR chemical shifts of all of the protons from the glucuronic acid moieties of the various isomers has been achieved using two-dimensional TOCSY[a] experiments.[78] An alternative approach has been to allow the reaction to proceed to equilibrium and then to separate the isomers using HPLC directly coupled to an NMR spectrometer.[79−81] By this means, each positional isomer can be isolated and identified. The use of high-field NMR spectroscopy (e.g., at 800 MHz) has been investigated.[82] In addition to these model compounds, namely, substituted benzoic acids, the AG isomers of probenecid **19** have been characterized by this means.[30]

It has also proved possible to carry out HPLC−NMR analysis of AGs in untreated urine.[83] ^{1}H NMR spectroscopy is now routinely employed to characterize isolated AG metabolites.[44] HPLC−NMR has been employed in the analysis of other *O*-glucuronides,[84] and valproic acid AG in human urine has been directly identified by ^{1}H NMR spectroscopy.[85] Direct analysis of metabolites in bile is much more demanding because of its complex multiphasic nature. Nevertheless, following disruption of the biliary micelles on a solid-phase extraction column, the positional isomers of statil AG were detected in rat bile.[86]

When reversed-phase HPLC is coupled with NMR, the elution order of isomers is found to be constant, even with a considerable variation in the structure and size of the aglycone (see the next section for details). Having identified the anomeric proton resonances of the various isomers, the variation in their peak areas can then be used to follow the transacylation reaction as it proceeds to equilibrium. On the basis of these intensity changes, the kinetics of the various reactions can be deconvoluted. However, given the complex competing reactions, it has been found to be easier to interpret the reaction kinetics by determining the reaction rates involving just one isomer at a time through its separation and detection using HPLC−NMR spectroscopy.[87] By this means, a full analysis of the rate constants can in principle be determined. This has been achieved for a number of substituted benzoic acid AGs **22**.[87] For (*S*)-naproxen [(2*S*)-**9**] AG, it was possible to measure individual acyl migration rate constants.[88] That for 1α to 2α/2β was significantly higher than the other rate constants.



22

In some cases, the reactions are too rapid for accurate determination given the finite time required for NMR data acquisition. In this situation, if the isomers can be identified independently, HPLC with UV detection can be used[40] as has been shown for the transacylation of naproxen glucuronide.[88] On the other hand, a good approximation to understanding the transacylation process can be achieved by measuring the decrease in the level of the 1-β-*O*-acyl isomer. In this case, ^{1}H NMR spectroscopy without any prior separation can be used, as has recently been shown for the glucuronides of ketoprofen and some of its metabolites[89] and ibuprofen **10**.[90] This approach has also been used to determine the degradation rates of a series of substituted benzoic acid glucuronides **22** with the ultimate aim of generating QSARs.[91] By following the time-course NMR spectra (specifically the ^{13}C NMR chemical shifts of the benzoyl carbons) of AGs of benzoic acids, type **22**, in pH 7.4 buffer, it is possible to establish kinetic parameters, including half-lives. The data points follow a Hammett-type correlation, with a spread of about 100-fold between the half-lives of 4-nitro and 4-*n*-propoxybenzoyl AGs.[33,34] Thus, in the case of the series of benzoic acid glucuronides, it was shown that the ^{13}C NMR

[a] Abbreviations: HATU, *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; NMM, *N*-methylmorpholine; TOCSY, total correlation spectroscopy.

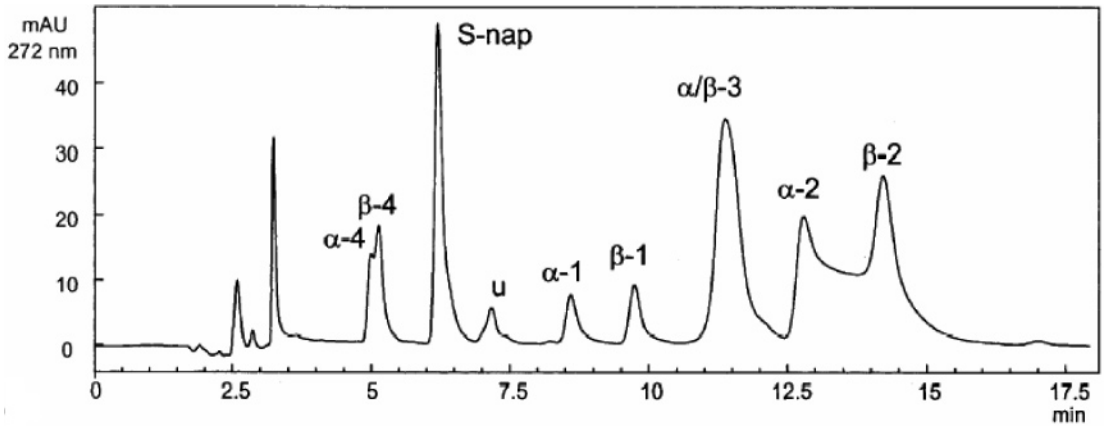
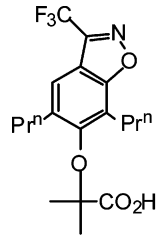


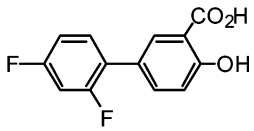
Figure 1. Positional acyl isomers of naproxen AG after equilibration for 6 h, measured by HPLC with UV detection. Each isomer was identified in a separate stopped-flow HPLC−NMR spectroscopy experiment.

chemical shift of the acyl ester carbonyl carbon was correlated with the degradation half-life. This might act as a surrogate marker for the degradation rate and thus remove the necessity of the decomposition measurement at least in a congeneric series. A combined HPLC−NMR study of naproxen AG proved for the first time the existence of 1α-AG isomers[14] and demonstrated their reversible formation from 2-acyl isomers.

b. Mass Spectrometry. The coupling of conventional reversed-phase LC columns to robust electrospray (ES) ionization sources has been the decisive advance in mass spectroscopic techniques applied to the analysis of AGs. For example the detection of a number of ester glucuronides of ibuprofen **10** and its phase I metabolites, extracted from human urine, has been demonstrated in a combined HPLC−NMR−MS study.[92] In-source decomposition is easily minimized, and the sensitive detection of these metabolites in plasma is now routine; for example, a recent paper[93] on the bioanalytical determination of the peroxisome proliferator-activated receptor (PPAR) α/γ agonist **23**, MRL-C, demonstrated that the drug and its AG coexisting in human plasma could be readily separated using a turbo ionspray LCMS technique.



23 MRL-C



24

HPLC−MS/MS with a reversed-phase column at microliter or milliliter flow rates and either a triple quadrupole or 3D ion-trap instrument is now the established combination mass spectrometric technique for analyzing *O*-glucuronides, including AGs, without derivatization. GC−MS analysis of the fully derivatized conjugate[94] is now rarely used, apart from certain quantitative bioanalytical methods.[95]

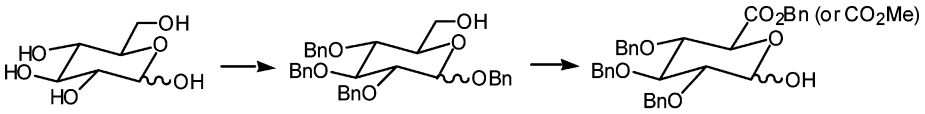
A number of techniques have been used to aid MS identification of glucuronides in general, and these may be anticipated to have value for AGs also. Thus, when the aglycone moiety has more than one OH group, complete derivatization prior to HPLC−MS/MS can aid location of the site of glucuronidation.[96] Capillary electrophoresis MS (CE-MS) is rarely used to analyze *O*-glucuronides.[97,98] Both ES and atmospheric pressure chemical ionization (APCI) sources typically yield abundant protonated molecules, ammonium adducts, or anions of *O*-glucuronides with minimal fragmentation. Although ES ionization is much more efficient than APCI, selective methylation of the glucuronyl carboxylate of a highly polar glucuronide may enhance its detection by APCI-MS.[99,100] Eluants containing ammonium acetate are used frequently in LC−MS and generate $[M + 1]^+$ and $[M + NH_4]^+$ ions simultaneously. High proton affinity of the aglycone favors formation of $[M + 1]^+$ from *O*-glucuronides.[101] In this case, ES positive ion and APCI yield more abundant diagnostic ions than negative ion analysis.

Regarding fragmentation patterns, the behavior of AGs in atmospheric pressure sources,[28] triple quadrupole[102] and ion-trap mass spectrometers,[31,44,70,103] is comparable to the behavior of other *O*-glucuronides; namely, it is dominated by neutral loss of the glucuronic acid moiety as dehydroglucuronic acid. Product ion scans and MS^n ($n = 2$ or 3) fragmentations of AGs allow the identification of both aglycone and glucuronic acid residues with triple quadrupole[103] and 3D ion-trap[104] instruments, respectively.

A limitation of conventional ES sources for quantitative analysis of AGs and other metabolites by LC−MS/MS is the marked inequality of response between parent compound and its metabolites. Recently, it has been shown with a proprietary drug AG that a normalization of the conventional analysis can be approximated by using nanospray HPLC−MS/MS, which yields a more nearly equimolar MS response, as a response calibrator.[105]

MS techniques remain of somewhat limited utility for the study of migrated isomers of AGs because the various transacylated isomers exhibit indistinguishable product ion spectra. However, NMR spectroscopy can determine the structure of the transacylated isomers relatively easily, and in a number of HPLC−NMR investigations it has been noted that the order of elution of the various transacylated glucuronides seems to be conserved regardless of the structure of the aglycone.[14,78−83,87,88] Thus, the 4-*O*-acyl isomers elute first, followed by the 1α-*O*-acyl, 1 *β*-O-acyl, the 3-*O*-acyl isomers, and finally the 2-*O*-acyl isomers. For each isomer, both anomers can sometimes also be resolved, depending on their mutarotation rates. Figure 1 illustrates this order as seen for the isomers of naproxen. Assuming that this general observation holds for other compounds, it would be possible to use this knowledge in combination with HPLC−MS based techniques to investigate transacylation rates.

Scheme 5. Synthesis of a Fully Protected Benzyl Ether Derivative for Acyl Glucuronide Synthesis (Total of Five Steps)

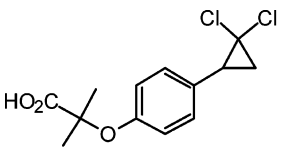


29

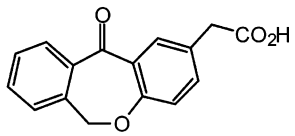
c. Chromatographic and Electrophoretic Techniques. Good separation of the positional isomers of AGs is generally achievable using relatively simple reversed-phase HPLC. Indeed, it was this relatively facile separation that provided the clue that transacylation was a common property of these metabolites, as HPLC was applied to their determination in biological fluids in the 1980s.[106] Earlier literature on quantitative analysis of *O*-glucuronides including AGs has been reviewed.[107]

In addition to the propensity of AGs to form complex mixtures through transacylation (see above) and mutarotation at near-neutral pH, the analysis of AGs in biological matrices is further complicated by the well-known tendency of NSAIDs of the α-phenylpropionic acid class to isomerize at C(2); their AGs typically form as diastereoisomeric mixtures. GC has been used to separate TMS derivatives of positional AG isomers,[94] but LC and capillary electrophoretic techniques predominate. Positional 2-, 3-, and 4-*O*-acyl isomers of many underivatized AGs are separable by reversed-phase HPLC, for example, the AGs of monofluorobenzoic acids[108] and diflunisal **24**.[109] Complete anomeric separation raises different issues (see below). UV spectrophotometry remains the most common method of detecting resolved AG isomers, but directly coupled HPLC−^{1}H NMR spectroscopy (see above) has obvious advantages.[40] Partial anomeric separations of the 1- and 2-*O*-acyl isomers have been accomplished using isocratic elution with a number of synthetic[51] and biogenic[40,81] AGs, but complete resolution of anomers of structurally complex AGs is more challenging.[38] In the absence of chromatographic separation, anomers can be assigned by stopped-flow HPLC−^{1}H NMR spectroscopy[40] (see section a).

In addition to the HPLC−UV and HPLC−NMR separations described above, another convenient HPLC method for separating the diastereoisomers of flurbiprofen **20** AG illustrates the utility of a simple, isocratic reversed-phase system.[110] The AGs of (*R*)- and (*S*)-ibuprofen **10**, synthesized by the Mitsunobu method (see Synthesis) were similarly separated and detected by ESMS.[111] The (*R*)- and (*S*)-ciprofibrate **25** AGs and the positional isomers of naproxen AG can be separated by capillary electrophoresis[112] and micellar electrokinetic capillary chromatography,[113] respectively.



25

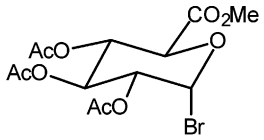


26

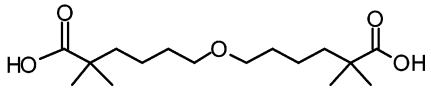
One conclusion from these separations, e.g., of isoxepac **26**,[81,82] is that although both α and β epimers are present in the equilibrium mixtures of the AGs, these anomers are in relatively rapid equilibrium with each other such that they are interconverting on the HPLC time scale. This has the consequence that there is usually incomplete separation between the anomers and often somewhat distorted peak shapes. Importantly, these chromatographic separations can readily be adapted to isolate the individual positional isomers if required, and because in general an acidic mobile phase is used for chromatography (for the effect of pH on acyl migration, see Chemical Reactivity section), there is little interconversion between the 1-, 2-, 3-, and 4-*O*-acyl isomers in such conditions.

Synthesis

Earlier syntheses of AGs[6] will be briefly reviewed first. Formerly, fully protected sugar intermediates were used, and since base-catalyzed deprotection of esters would generally also cleave the ester link to the aglycone, other protecting groups were employed. A recent exception was a traditional Konigs−Knorr synthesis, using bromosugar **27**, of the mono-AG of the lipid-modulating agent **28**.[114] Here, Zemplen deprotection (^{i}PrNEt$_2$−MeOH) was used in the final step. The added hydrolytic stability of the product owing to steric hindrance (tertiary α-carbon) was doubtless decisive.



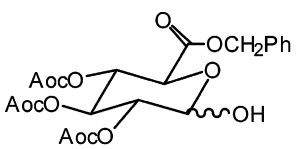
27



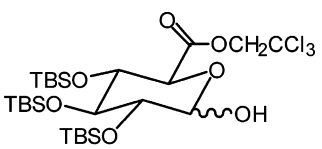
28

Other fully protected sugars call for multistep preparations, e.g., five steps from glucose or laevoglucosan for the fully benzylated derivative **29**[115−117] (Scheme 5), which is usually coupled by the Mitsunobu reaction followed by deprotection using catalytic hydrogenation. For instance, the AGs of the bile acids (cf. **3**) have been prepared in this way (Bn ester in **29**).[118] Coupling via the imidate method is also possible for **29** but again introduces extra steps.[116]

Allyl or allyloxycarbonyl protection has also been used, since mild deprotection using Pd^0 reagents is then feasible. Thus, intermediate **30** was again coupled by the Mitsunobu reaction followed by deprotection using Pd(PPh$_3$)$_4$.[119] Another fully protected sugar intermediate that has been used is the tris-silyl ether **31**.[120] Here, deprotection using HF was possible after coupling without disturbing the acyl glucuronide; use of tetra-*n*-butylammonium fluoride (TBAF) led to degradation. Clearly the use of a relatively strong anhydrous acid is not too harsh for the ester glucuronide link. Multistep syntheses are required for both intermediates.



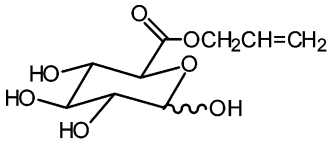
30 Aoc = Allyloxycarbonyl



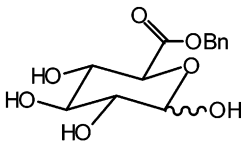
31 TBS = t-butyldimethylsilyl

Unprotected glucuronic acid may be coupled to the carboxylic acid in special cases, as summarized in the earlier review,[6] particularly for retinoic acid derivatives,[121,122] using acyl fluoride or imidazolide activation.

In general, syntheses requiring many steps are undesirable, and modern AG syntheses employ monoesters available from glucuronic acid in one step. Following the seminal paper by Juteau et al.,[32] the Mitsunobu procedure using allyl ester **32** has become popular. In these laboratories, we have used **32** to prepare the acyl glucuronide of diclofenac in 100−200 mg amounts.[28] Nevertheless, the Mitsunobu procedure does not solve the anomeric problem because 1β/1α mixtures, typically from 5:1 to 2:1, are generated; complete separation normally requires preparative HPLC.



32



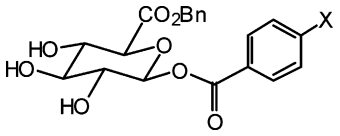
34

An excellent alternative, applicable to a range of carboxylic acids, is to employ selective acylation of **32** following activation of RCO_2H using Carpino's uronium reagent HATU and NMM.[123] Here, the kinetic anomeric effect[124] greatly favors the 1β-product **33** (Scheme 6) because the 1β-alkoxide of **32** is much more reactive than its 1α-counterpart; <5% (and typically <2%) α-anomer could be detected by NMR. In contrast, the Mitsunobu procedure eventually involves an S_N2 displacement by RCO_2^- at the anomeric center.

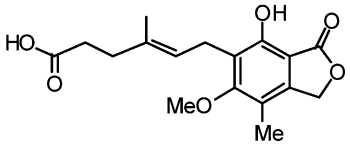
Yields by the acylation method were critically dependent on the strength of base used. With NMM, satisfactory to good yields of 43−66% were obtained for a range of carboxylic acids using a 1:1 molar ratio of reactants. Examples included the important drugs ibuprofen **10**, mycophenolic acid **16**, and zomepirac **18**. The products were simply isolated by brief column chromatography.

Removal of the allyl ester from intermediates **33** calls for Pd(0) deprotection in the presence of a secondary amine, e.g., pyrrolidine or morpholine.[28,32,125] It may be difficult to remove Pd traces from the final product, however, even after chromatography. One solution is to employ a resin-bound Pd catalyst[123] or a Pd scavenger, but these reagents are expensive. Recently we have been evaluating the benzyl ester **34** with very promising results.[126] Yields and anomeric selectivities, using the selective acylation protocol, were in all comparable cases at least as good as those obtained with **32**. Here, deprotection may be effected using insoluble Pd/C with either catalytic transfer or conventional hydrogenation.

Under these conditions Ar−Cl bonds as in **35** were unaffected, and the trisubstituted C=C in mycophenolic acid **16** was not reduced, but the Ar−Br in **35**, X=Br, was cleaved. This is an important detail because a number of active drugs contain Ar−Cl bonds. Yields were essentially quantitative in the absence of further functionality.



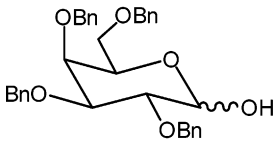
35 X= Cl, Br



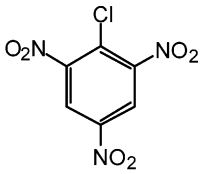
16

Monoesters **32** and **34** are convenient intermediates, but their preparation is not trivial. The original procedure[32] calls for alkylation of glucuronic acid with allyl bromide and DBU in DMF, but the DBU residues are very difficult to remove fully. We have found a resin-bound fluoride base to be a simpler alternative.[123] Even here, however, recrystallization is necessary to obtain the highly pure ester. The same is true when TBAF is used as base, though this is a rapid reaction. A less expensive alternative is to preform a quaternary ammonium salt of glucuronic acid using $Et_4N^+OH^-$ followed by alkylation with the halide, giving a similar yield after chromatography.[127]

The acyl galactosides mentioned above[19] were prepared from the fully protected sugar **36** by activation of the protected bile acid using 2-chloro-1,3,5-trinitrobenzene **37**. Conceptually this is also a selective acylation of the anomeric OH, but the β/α selectivity appears to be much lower than seen in the HATU−NMM method; the different electronic character of the sugar may be a factor.



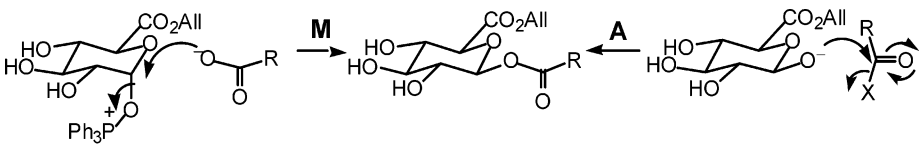
36



37

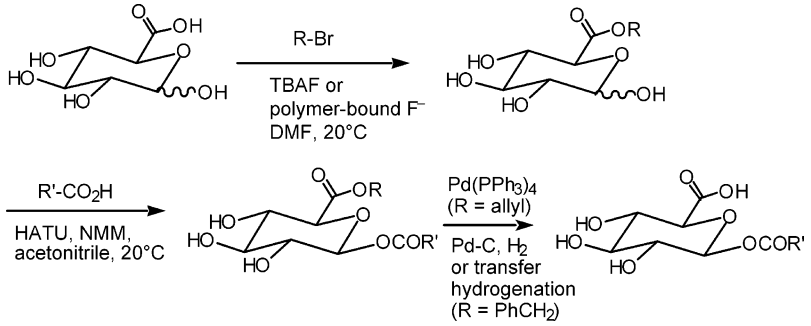
Enzymatic syntheses were discussed in the earlier review[6] and are still employed. An interesting case was the AG of **16**, obtained by the action of horse liver homogenate.[128] A mixture of aryl glucuronide and AG was obtained and separated by preparative HPLC. Enantiomerically pure AGs of ^{14}C-labeled 2-phenylpropanoic acid **11** were obtained via a perfused rat liver preparation.[129] Also, further details have been published of an enzyme membrane reactor procedure that eases the handling of UDPGTs by immobilization, greatly improving their isolated lifetime, and has been applied to AG synthesis.[130]

Scheme 6. Synthesis of AGs by the Mitsunobu (M) and Acylation (A) Routes, All = Allyl



33

Scheme 7. Synthesis of AGs by Selective Acylation[a]



[a] R = allyl or benzyl.

In conclusion, the recommended method for chemical synthesis of AGs is now the selective acylation of a monoprotected glucuronate ester with the parent drug or other carboxylic acid. The method shows excellent regio- and stereoselectivity for the 1β-product and proceeds in good yield; deprotection may be achieved under mild conditions. The overall synthetic route is summarized in Scheme 7, employing either allyl or benzyl ester protection.

Reported AGs of New Drugs

We finally present a compilation (Table 2 and Chart 1) of biogenic and synthetic AGs that have been identified as metabolites or prepared during the evaluation of new drugs between 1998 and 2006.[23,24,44,70,94,103,104,131−148] It is hoped this will increase the value of the review to all involved in the drug discovery process.

Chart 1. Structures for Table 2

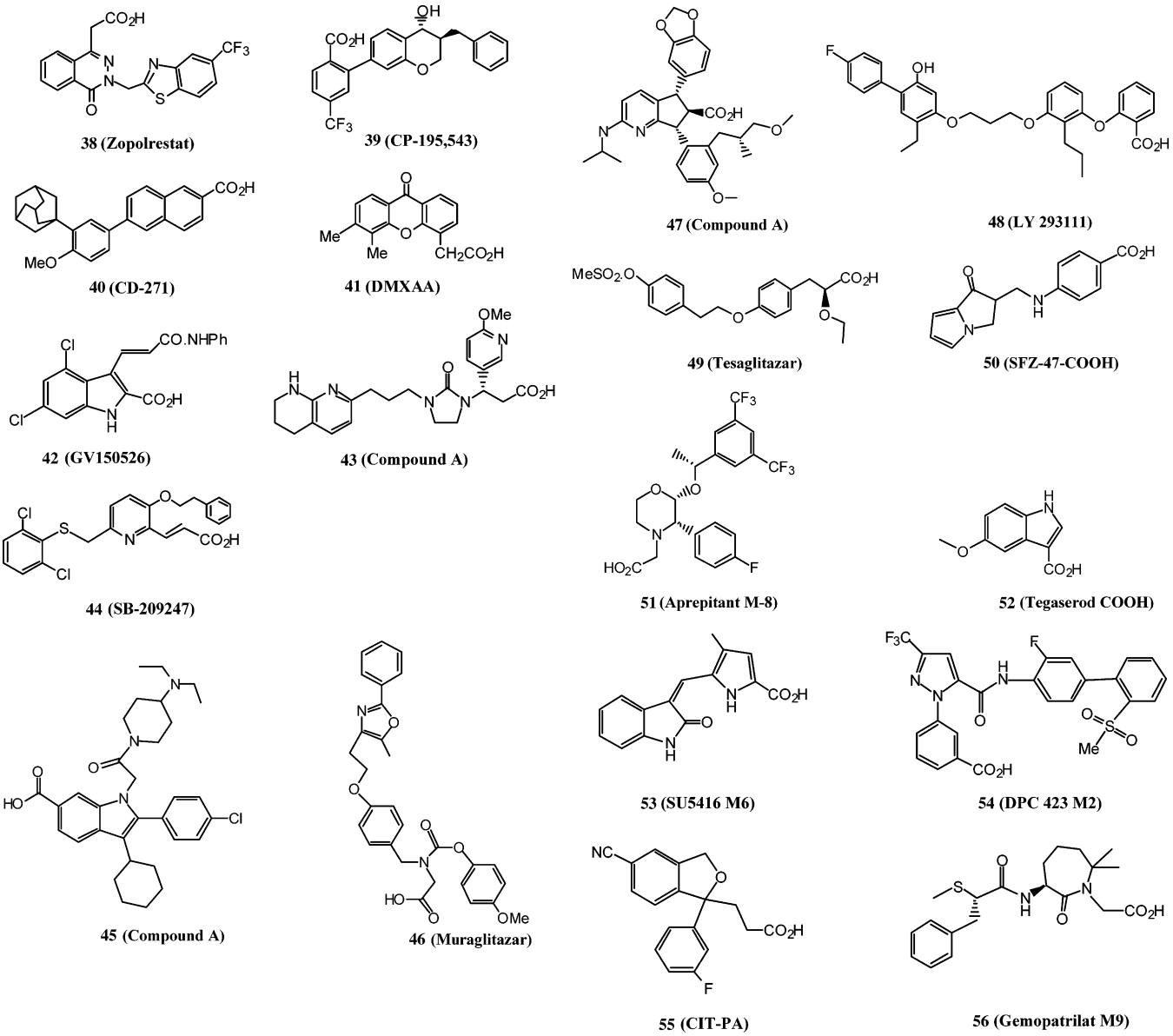


Table 2. Biogenic and Synthetic AGs of New Drugs, 1998−2006

carboxylic acid[a]	therapeutic class	comment	ref
38	antidiabetic (aldose reductase inhibitor)	major biliary and urinary metabolite in dogs	131
39	antiinflammatory (LT-B4 antagonist)	biliary metabolite (rats, monkeys); aryl glucuronide also formed	132
40	acne therapy (retinoid)	synthetic material, potential prodrug	133
41	anticancer/cytokine−chemokine activator	major metabolite in human hepatic microsomes, plasma, and urine	134−136
42	stroke (Gly antagonist)	major biliary metabolite (rats, dogs); metabolite in human liver microsomes	137,138
43	osteoporosis (integrin receptor antagonist)	metabolite in rat, dog, monkey, and human hepatocytes	139
44	antiinflammatory (LT-B4 antagonist)	major biliary metabolite in rats; metabolite in human liver microsomes; AG of both sulfide and sulfoxide seen	140
23	antidiabetic (PPAR-α/γ agonist)	major metabolite in rat, dog, monkey, and human hepatocytes	94, 141
45	HCV NS5B polymerase inhibitor	metabolite in rat, dog, and monkey	142
46	PPAR-α/γ agonist	major biliary metabolite in mice and humans	31, 103
47	endothelin ET(A) receptor antagonist	metabolite in human liver microsomes	23, 24
48	eicosanoid pathway modulator	major plasma metabolite in rats; aryl and diglucuronide also found	70
49	PPAR-α/γ agonist	major urinary metabolite in humans	143
50 (M)	NSAID	urinary conjugate of product of oxidative metabolism in rabbits	104
51 (M)	substance P antagonist	biliary conjugate of product of oxidative metabolism in dogs but not rats	144
52 (M)	5-HT_4 receptor partial agonist	major plasma metabolite of product of metabolism in humans	145
53 (M)	Flk-1 receptor antagonist	plasma metabolite of product of oxidative metabolism in rats and dogs	146
54 (M)	coagulation factor Xa inhibitor	urinary metabolite of product of oxidative metabolism in mice, rats, dogs, and humans	44
55 (M)	SSRI	urinary metabolite of product of oxidative metabolism in humans	147
56 (M)	vasopeptidase inhibitor	urinary metabolite of product of metabolism in rats, dogs, and humans	148

[a] M = carboxylic acid metabolite of drug. Structures are shown in Chart 1.

Note Added in Proof. During the preparation of this Perspective, a new synthetic method for acyl glucuronides has been reported.[149] This calls for the alkylation of an alkali metal salt (preferably cesium) of the carboxylic acid component with bromosugar **27**. Good yields of the protected 1β products were obtained in high anomeric purity: deprotection was effected in two stages using a number of esterases or lipases that could operate at pH5. This represents a four (or five) step synthesis but appears to be a promising new synthetic approach; three examples were given.

Biographies

Andrew V. Stachulski graduated from the University of Cambridge in 1971 and remained there for his Ph.D. (1974), supervised by Prof. Alan Battersby. He then held postdoctoral fellowships with the Medical Research Council and at Jesus College, Oxford, U.K. In 1978 he joined Beecham Pharmaceuticals (later SmithKline Beecham), working on β-lactam antibiotics, and in 1991 he moved to Ultrafine Chemicals, Manchester, becoming research manager. In 2001 he joined the University of Liverpool, where he is now a senior lecturer. His research interests are in natural product chemistry, especially carbohydrates, and drug discovery and in applying chemical synthesis to issues of drug metabolism. He has authored or coauthored over 50 research papers and became an FRSC in 1998.

John R. Harding received his D.Phil. in organic chemistry from the University of Oxford in 1977. Since that time he has specialized in synthetic isotope chemistry at The Radiochemical Centre, Amersham, ICI, and Zeneca and is currently Head of Isotope Chemistry at AstraZeneca, Alderley Park, where his team supports both drug discovery and development programmes. His research interests also include chemical aspects of drug metabolism, particularly in the field of reactive metabolites.

John C. Lindon obtained his B.Sc. (1966), Ph.D. (1969), and D.Sc. (1993) degrees from Birmingham University, U.K. Following postdoctoral research at Columbia University, New York, he joined the University of Southampton, studying liquid crystals using NMR spectroscopy and managing the NMR laboratory. In 1976 he joined the Wellcome Foundation Ltd., ultimately becoming Head of Spectroscopy in 1995. He returned to academia in 1995 and is now a Visiting Professor and Senior Research Investigator at Imperial College London, U.K. He is Editor-in-Chief of the Encyclopedia of Spectroscopy and Spectrometry and sits on several editorial boards. He has authored some 400 research papers, review articles, and chapters. His current research interests are in NMR, the use of computer modeling methods, and the application of analytical techniques to metabonomics.

James L. Maggs received his Ph.D. in 1980 from the University of Wales at University College, Cardiff, U.K., working in the Department of Biochemistry under the late Prof. K. S. Dodgson. Subsequently, he has held postdoctoral and Experimental Officer appointments in the Department of Pharmacology and Therapeutics, University of Liverpool, and is currently Principal Experimental Officer in the Bioanalytical Laboratory responsible for metabolic studies.

B. Kevin Park became a Lecturer in 1976 in the Department of Pharmacology and Therapeutics at the University of Liverpool, where he is now a Professor of Pharmacology and Head of Department. He was awarded Honorary Membership of the Royal College of Physicians in 1995 and is a Commissioner on Human Medicines, an Executive Officer in the British Toxicology Society, a member of the MRC Physiological Medicines and Infections Board, and a Fellow of the Academy of Medical Sciences. He has authored over 400 peer-reviewed publications. He was a Wellcome Principal Research Fellow from 1989 to 1999. Other distinctions include the Sandoz Prize for Pharmacology, the Pfizer Medal for Innovative Science, the SmithKline and Beecham Prize for Clinical Pharmacology, and The Werner Kalow Lectureship (Canada).

Ian D. Wilson is a Principal Scientist in the Department of Drug Metabolism and Pharmacokinetics within AstraZeneca Research (Alderley Park, U.K.). He graduated from University of Manchester Institute of Science and Technology, then obtained his Ph.D. (1978) and D.Sc. (1998) degrees from Keele University. He has authored some 300 papers or reviews and received a number of awards from the Royal Society of Chemistry and the Chromatographic Society, most recently the 28th SAC Gold Medal from Analytical Division of the RSC. He serves on numerous editorial boards. He is currently a Visiting Professor at the Universities of York, Keele, Sheffield Hallam, and Manchester and Imperial College. His current research is directed toward the further development of combination chro-

matographic techniques and their application to drug metabolism and metabonomics.

References

(1) Kaspersen, F. M.; Van Boeckel, C. A. A. A review of the methods of chemical synthesis of sulphate and glucuronide conjugates. *Xenobiotica* **1987**, *17*, 1451−1471.

(2) Goebel, W. F. Derivatives of glucuronic acid. VIII. The structure of benzoylglucuronic acid. *J. Biol. Chem.* **1938**, *122*, 649−653.

(3) van Breemen, R. B.; Fenselau, C. C. Reaction of 1-*O*-acyl glucuronides with 4-(*p*-nitrobenzyl)pyridine. *Drug Metab. Dispos.* **1986**, *14*, 197−201.

(4) Faed, E. M. Properties of acyl glucuronides: implications for studies of the pharmacokinetics and metabolism of acidic drugs. *Drug Metab. Rev.* **1984**, *15*, 1213−1249.

(5) Spahn-Langguth, H.; Benet, L. Z. Acyl glucuronides revisited: is the glucuronidation process a toxification as well as a detoxification mechanism? *Drug Metab. Rev.* **1992**, *24*, 5−48.

(6) Stachulski, A. V.; Jenkins, G. N. The synthesis of *O*-glucuronides. *Nat. Prod. Rep.* **1998**, *15*,173−188.

(7) Bougie, D.; Aster, R. Immune thrombocytopenia resulting from sensitivity to metabolites of naproxen and acetaminophen. *Blood* **2001**, *97*, 3846−3850.

(8) Spencer, J. P.; Rice-Evans, C.; Williams, R. J. Modulation of pro-survival Akt/protein kinase B and ERK1/2 signaling cascades by quercetin and its in vivo metabolites underlie their action on neuronal viability. *J. Biol. Chem.* **2003**, *278*, 34783−34793.

(9) Yang, J. H.; Hsia, T. C.; Kuo, H. M.; Chao, P. D.; Chou, C. C.; Wei, Y. H.; Chung, J. G. Inhibition of lung cancer cell growth by quercetin glucuronides via g2/m arrest and induction of apoptosis. *Drug Metab. Dispos.* **2006**, *34*, 296−304.

(10) Kalgutkar, A. S.; Gardner, I.; Obach, R. S.; Shaffer, C. L.; Callegari, E.; Henne, K. R.; Mutlib, A. E.; Dalvie, D. K.; Lee, J. S.; Nakai, Y.; O'Donnell, J. P.; Boer, J.; Harriman, S. P. A comprehensive listing of bioactivation pathways of organic functional groups. *Curr. Drug Metab.* **2005**, *6*, 161−225.

(11) Bailey, M. J.; Dickinson, R. G. Acyl glucuronide reactivity in perspective: biological consequences. *Chem.-Biol. Interact.* **2003**, *145*, 117−137.

(12) Boelsterli, U. A. Xenobiotic acyl glucuronides and acyl CoA thioesters as protein-reactive metabolites with the potential to cause idiosyncratic drug reactions. *Curr. Drug Metab.* **2002**, *3*, 439−450.

(13) Sakaguchi, K.; Green, M.; Stock, N.; Reger, T. S.; Zunic, J.; King, C. Glucuronidation of carboxylic acid containing compounds by UDP-glucuronosyltransferase isoforms. *Arch. Biochem. Biophys.* **2004**, *424*, 219−225.

(14) Corcoran, O.; Mortensen, R. W.; Hansen, S. H.; Troke, J.; Nicholson, J. K. HPLC−^{1}H NMR spectroscopic studies of the reactive α-1-*O*-acyl isomer formed during acyl migration of *S*-naproxen β-1-*O*-acyl glucuronide. *Chem. Res. Toxicol.* **2001**, *14*, 1363.

(15) Ohkawa, T.; Norikura, R.; Yoshikawa, T. Rapid LC−TOFMS method for identification of binding sites of covalent acylglucuronide−albumin complexes. *J. Pharm. Biomed. Anal.* **2003**, *31*, 1167−1176.

(16) Tipton, A. K.; Lightner, D. A.; McDonagh, A. F. Synthesis and metabolism of the first thia-bilirubin. *J. Org. Chem.* **2001**, *66*, 1832.

(17) Ghosh, B.; Lightner, D. A.; McDonagh, A. F. Synthesis, conformation and metabolism of a selenium bilirubin. *Monatsh. Chem.* **2004**, *135*, 1189−1199.

(18) Iida, T.; Nakamori, R.; Yabuta, R.; Yada, S.; Takagi, Y.; Mano, N. Ikegawa, S.; Goto, J.; Nambara, T. Potential bile acid metabolites. 24. An efficient synthesis of carboxyl-linked glucosides and their chemical properties. *Lipids* **2002**, *37*, 101−110.

(19) Kakiyama, G.; Sadakiyo, S.; Iida, T.; Mushiake, K.; Goto, T.; Mano, N.; Goto, J.; Nambara, T. Chemical synthesis of 24-beta-D-galactopyranosides of bile acids: a new type of bile acid conjugates in human urine. *Chem. Phys. Lipids* **2005**, *134*, 141−150.

(20) Mano, N.; Nishijima, A.; Saito, S.; Ikegawa, S.; Goto, J. Synthesis and characterisation of deoxycholyl 2-deoxyglucuronide: A water-soluble affinity labelling reagent. *Lipids* **2003**, *38*, 873−879.

(21) Bock, K.W. Vertebrate UDP-glucuronosyltransferases: functional and evolutionary aspects. *Biochem. Pharmacol.* **2003**, *66*, 691−696.

(22) Arima, N.; Kato, Y. Species differences in absorption, metabolism and excretion of pranoprofen, a 2-arylpropionic acid derivative, in experimental animals. *J. Pharmacobio-dyn.* **1990**, *13*, 739−744.

(23) Tang, C.; Hochman, J. H.; Ma, B.; Subramanian, R.; Vyas, K. P. Acyl glucuronidation and glucosidation of a new and selective endothelin ET(A) receptor antagonist in human liver microsomes. *Drug Metab. Dispos.* **2003**, *31*, 37−45.

(24) Tang, C.; Ma, B. Glycosidation of an endothelin ET(A) receptor antagonist and diclofenac in human liver microsomes: aglycone-dependent UDP-sugar selectivity. *Drug Metab. Dispos.* **2005**, *33*, 1796−1802.

(25) Shipkova, M.; Armstrong, V. W.; Wieland, E.; Niedmann, P. D.; Schutz, E.; Brenner-Weiss, G.; Voihsel, M.; Braun, F.; Oellerich, M. Identification of glucoside and carboxyl-linked glucuronide conjugates of mycophenolic acid in plasma of transplant recipients treated with mycophenolate mofetil. *Br. J. Pharmacol.* **1999**, *126*, 1075−1082.

(26) Nakano, K.; Ando, H.; Sugawara, Y.; Ohashi, M.; Harigaya, S. Hopantenic acid β-glucoside as a new urinary metabolite of calcium hopantenate in dogs. *Drug Metab. Dispos.* **1986**, *14*, 740−745.

(27) Dickinson, R. G.; King, A. R. Studies on the reactivity of acyl glucuronides-II. Interaction of diflunisal acyl glucuronide and its isomers with human serum albumin in vitro. *Biochem. Pharmacol.* **1991**, *42*, 2201−2206.

(28) Kenny, J. R.; Maggs, J. L.; Meng, X.; Sinnott, D.; Clarke, S. E.; Park, B. K.; Stachulski, A. V. Syntheses and characterisations of the acyl glucuronide and hydroxy metabolites of diclofenac. *J. Med. Chem.* **2004**, *47*, 2816−2825.

(29) Presle, N.; Lapicque, F.; Fournel-Gigleux, S.; Magdalou, J.; Netter, P. Stereoselective irreversible binding of ketoprofen glucuronides to albumin. Characterization of the site and the mechanism. *Drug Metab. Dispos.* **1996**, *24*, 1050−1057.

(30) Akira, K.; Uchijima, T.; Hashimoto, T. Rapid internal acyl migration and protein binding of synthetic probenecid glucuronides. *Chem. Res. Toxicol.* **2002**, *15*, 765−772.

(31) Li, W.; Zhang, D.; Wang, L.; Zhang, H.; Cheng, P. T.; Zhang, D.; Everett, D. W.; Humphreys, W. G. Biotransformation of carbon-14 labeled muraglitazar in male mice: interspecies difference in metabolic pathways leading to unique metabolites. *Drug Metab. Dispos.* **2006**, *34*, 807−820.

(32) Juteau, H.; Gareau, Y.; Labelle, M. A convenient synthesis of β-acyl glucuronides. *Tetrahedron Lett.* **1997**, *38*, 1481−1484.

(33) Vanderhoeven, S. J; Troke, J.; Tranter, G. E.; Wilson, I. D.; Nicholson, J. K.; Lindon, J. C. Nuclear magnetic resonance (NMR) and quantitative structure−activity relationship (QSAR) studies on the transacylation reactivity of model 1β-*O*-acyl glucuronides. II: QSAR modelling of the reaction using both computational and experimental NMR parameters. *Xenobiotica* **2004**, *34*, 889−900.

(34) Nicholls, A. W.; Akira, K.; Lindon, J. C.; Farrant, R. D.; Wilson, I. D.; Harding, J.; Gillick, D. A.; Nicholson, J. K. NMR spectroscopic and theoretical chemistry studies on the internal acyl migration of the 1-*O*-acyl-β-D-glucopyranuronate (glucuronide) conjugates of 2-, 3- and 4-trifluoromethylbenzoic acids. *Chem. Res. Toxicol.* **1996**, *9*, 1414−1424.

(35) Sallustio, B. C.; Foster, D. J. R. Reactivity of gemfibrozil 1-*O*-beta-acyl glucuronide. Pharmacokinetics of covalently bound gemfibrozil−protein adducts in rat. *Drug Metab. Dispos.* **1995**, *23*, 892−899.

(36) Fenselau, C. In *Conjugation−Deconjugation Reactions in Drug Metabolism and Toxicity*; Kaufmann, F. C., Ed.; Springer-Verlag: Berlin, 1994; p 367.

(37) Ebner, T.; Heinzel, G.; Prox, A.; Beschk, K.; Wachsmuth, H. Disposition and chemical stability of telmisartan 1-*O*-acylglucuronide. *Drug Metab. Dispos.* **1999**, *27*, 1143−1149.

(38) Hasegawa, H.; Akira, K.; Shinohara, Y.; Kasuya, Y.; Hashimoto, T. Kinetics of intramolecular acyl migration of 1β-O-acyl glucuronides of (*R*)- and (*S*)-2-phenylpropionic acids. *Biol. Pharm. Bull.* **2001**, *24*, 852−855.

(39) Mortensen, R. W.; Corcoran, O.; Cornett, C.; Sidelmann, U. G.; Troke, J.; Lindon, J. C.; Nicholson, J. K.; Hansen, S. H. LC−^{1}H NMR used for determination of the elution order of *S*-naproxen glucuronide isomers in two isocratic reversed-phase LC-systems. *J. Pharm. Biomed. Anal.* **2001**, *24*, 477−485.

(40) Akira, K.; Hasegawa, H.; Shinohara, Y.; Imachi, M.; Hashimoto, T. Stereoselective internal acyl migration of 1 beta-*O*-acyl glucuronides of enantiomeric 2-phenylpropionic acids. *Biol. Pharm. Bull.* **2000**, *23*, 506−510.

(41) Mortensen, R. W.; Sidelmann, U. G.; Tjornelund, J.; Hansen, S. H. Stereospecific pH-dependent degradation kinetics of *R*- and *S*-naproxen-l-*O*-acyl-glucuronide. *Chirality* **2002**, *14*, 305−312.

(42) Akira, K.; Taira, T.; Hasegawa, H.; Sakuma, C.; Shinohara, Y. Studies on the stereoselective internal acyl migration of ketoprofen glucuronides using ^{13}C labeling and nuclear magnetic resonance spectroscopy. *Drug Metab. Dispos.* **1998**, *26*, 457−464.

(43) Wang, J.; Davis, M.; Li, F.; Azam, F.; Scatina, J.; Talaat, R. A novel approach for predicting acyl glucuronide reactivity via Schiff base formation: development of rapidly formed peptide adducts for LC/MS/MS measurements. *Chem. Res. Toxicol.* **2004**, *17*, 1206−1216.

(44) Mutlib, A. E.; Shockcor, J.; Chen, S. Y.; Espina, R. J.; Pinto, D. J.; Orwat, M. J.; Prakash, S. R.; Gan, L. S. Disposition of 1-[3-(aminomethyl)phenyl]-*N*-[3-fluoro-2′-(methylsulfonyl)-[1,1′-biphenyl]-4-yl]-3-(trifluoromethyl)-1*H*-pyrazole-5-carboxamide (DPC 423) by novel metabolic pathways. Characterization of unusual metabolites by liquid chromatography/mass spectrometry and NMR. *Chem. Res. Toxicol.* **2002**, *15*, 48−62.

(45) Shipkova, M.; Armstrong, V. W.; Oellerich, M.; Wieland, E. Acyl glucuronide drug metabolites: toxicological and analytical implications. *Ther. Drug Monit.* **2003**, *25*, 1−16.

(46) Boelsterli, U. A. Reactive acyl glucuronides: Possible role in small intestinal toxicity induced by nonsteroidal anti-inflammatory drugs. *Toxic Subst. Mech.* **1999**, *18*, 83−100.

(47) Siraki, A. G.; Chevaldina, T.; O'Brien, P. J. Application of quantitative structure−toxicity relationships for acute NSAID cytotoxicity in rat hepatocytes. *Chem.-Biol. Interact.* **2005**, *151*, 177−191.

(48) Qiu, Y.; Burlingame, A. L.; Benet, L. Z. Mechanisms for covalent binding of benoxaprofen glucuronide to human serum albumin. Studies by tandem mass spectrometry. *Drug Metab. Dispos.* **1998**, *26*, 246−256.

(49) Li, C.; Benet, L. Z.; Grillo, M. P. Studies on the chemical reactivity of 2-phenylpropionic acid 1-*O*-acyl glucuronide and *S*-acyl-CoA thioester metabolites. *Chem. Res. Toxicol.* **2002**, *15*, 1309−1317.

(50) Li, C.; Benet, L. Z.; Grillo, M. P. Enantioselective covalent binding of 2-phenylpropionic acid to protein in vitro in rat hepatocytes. *Chem. Res. Toxicol.* **2002**, *15*, 1480−1487.

(51) Shore, L. J.; Fenselau, C.; King, A. R.; Dickinson, R. G. Characterization and formation of the glutathione conjugate of clofibric acid. *Drug Metab. Dispos.* **1995**, *23*, 119−123.

(52) Shipkova, M.; Armstrong, V. W.; Weber, L.; Niedmann, P. D.; Wieland, E.; Haley, J.; Tonshoff, B.; Oellerich, M. German study group on mycophenolate mofetil therapy in pediatric renal transplant recipients. Pharmacokinetics and protein adduct formation of the pharmacologically active acyl glucuronide metabolite of mycophenolic acid in pediatric renal transplant recipients. *Ther. Drug Monit.* **2002**, *24*, 390−399.

(53) Shipkova, M.; Beck, H.; Voland, A.; Armstrong, V. W.; Grone, H. J.; Oellerich, M.; Wieland, E. Identification of protein targets for mycophenolic acid acyl glucuronide in rat liver and colon tissue. *Proteomics* **2004**, *4*, 2728−2738.

(54) Wieland, E.; Shipkova, M.; Schellhass, U.; Schutz, E.; Niedmann, P. D.; Armstrong, V. W.; Oellerich, M. Induction of cytokine release by the acyl glucuronide of mycophenolic acid: a link to side effects? *Clin. Biochem.* **2000**, *33*, 107−113.

(55) Wang, M.; Gorrell, M. D.; Abbott, C. A.; Jaggi, R.; Marguet, D.; Dickinson, R. G. Hepatic covalent adduct formation with zomepirac in the CD26-deficient mouse. *J. Gastroenterol. Hepatol.* **2002**, *17*, 66−71.

(56) Olsen, J.; Li, C.; Bjornsdottir, I.; Sidenius, U.; Hansen, S. H.; Benet, L. Z. In vitro and in vivo studies on acyl-coenzymeA-dependent bioactivation of zomepirac in rats. *Chem. Res. Toxicol.* **2005**, *18*, 1729−1736.

(57) Cannell, G. R.; Bailey, M. J.; Dickinson, R. G. Inhibition of tubulin assembly and covalent binding to microtubular protein by valproic acid glucuronide in vitro. *Life Sci.* **2002**, *71*, 2633−2643.

(58) Bailey, M. J.; Worrall, S.; de Jersey, J.; Dickinson, R. G. Zomepirac acyl glucuronide covalently modifies tubulin in vitro and in vivo and inhibits its assembly in an in vitro system. *Chem.-Biol. Interact.* **1998**, *115*, 153−166.

(59) Mano, N.; Goto, T.; Nikaido, A.; Narui, T.; Goto, J. Inhibition of the rat hepatic microsomal flurbiprofen acyl glucuronidation by bile acids. *J. Pharm. Sci.* **2003**, *92*, 2098−2108.

(60) Shackleford, D. M.; Nation, R. L.; Milne, R. W.; Hayball, P. J.; Evans, A. M. Stereoselective hepatic disposition of model diastereomeric acyl glucuronides. *J. Pharmacokinet. Pharmacodyn.* **2004**, *31*, 1−27.

(61) Ito, H.; Ishiwata, S.; Kosaka, T.; Nakashima, R.; Takeshita, H.; Negoro, S.; Maeda, M.; Ikegawa, S. Enantioselective immunorecognition of protein modification with optically active ibuprofen using polyclonal antibody. *J. Chromatogr., B* **2004**, *806*, 11−17.

(62) Ikegawa, S.; Isriyanthi, N. M. R.; Nagata, M.; Yahata, K.; Ito, H.; Mano, N.; Goto, J. The enantioselective immunoaffinity extraction of an optically active ibuprofen-modified peptide fragment. *Anal. Biochem.* **2001**, *296*, 63−72.

(63) Iwaki, M.; Ogiso, T.; Inagawa, S.; Kakehi, K. In vitro regioselective stability of β-1-*O*- and 2-*O*-acyl glucuronides of naproxen and their covalent binding to human serum albumin. *J. Pharm. Sci.* **1999**, *88*, 52−57.

(64) Georges, H.; Jarecki, I.; Netter, P.; Magdalou, J.; Lapicque, F. Glycation of human serum albumin by acylglucuronides of nonsteroidal anti-inflammatory drugs of the series of phenylpropionates. *Life Sci.* **1999**, *65*, PL151−PL156.

(65) Chiou, Y.; Tomer, K. B.; Smith, P. C. Effect of nonenzymatic glycation of albumin and superoxide dismutase by glucuronic acid and suprofen acyl glucuronide on their functions in vitro. *Chem.-Biol. Interact.* **1999**, *121*, 141−159.

(66) Seitz, S.; Kretz-Rommel, A.; Elferink, R. P. J. O.; Boelsterli, U. A. Selective protein adduct formation of diclofenac glucuronide is critically dependent on the rat canalicular conjugate export pump (Mrp2). *Chem. Res. Toxicol.* **1998**, *11*, 513−519.

(67) Ware, J. A.; Graf, M. L. M.; Martin, B. M.; Lustberg, L. R.; Pohl, L. R. Immunochemical detection and identification of protein adducts of diclofenac in the small intestine of rats: possible role in allergic reactions. *Chem. Res. Toxicol.* **1998**, *11*, 164−171.

(68) Shen, S.; Marchick, M. R.; Davis, M. R.; Doss, G. A.; Pohl, L. R. Metabolic activation of diclofenac by human cytochrome P-450 3A4: role of 5-hydroxydiclofenac. *Chem. Res. Toxicol.* **1999**, *12*, 214−222.

(69) Crawford, J. M.; Ransil, B. J.; Narciso, J. P.; Gollan, J. L. Hepatic microsomal bilirubin UDP-glucuronosyltransferase. The kinetics of bilirubin mono- and diglucuronide synthesis. *J. Biol. Chem.* **1992**, *267*, 16943−16950.

(70) Perkins, E. J.; Cramer, J. W.; Farid, N. A.; Gadberry, M. G.; Jackson, D. A.; Mattiuz, E. L.; O'Bannon, D. D.; Weiss, H. J.; Wheeler, W. J.; Wood, P. G.; Cassidy, K. C. Preclinical characterization of 2-[3-[3-[(5-ethyl-4′-fluoro-2-hydroxy[1,1′-biphenyl]-4-yl)oxy]propoxy]-2-propylphenoxy]benzoic acid metabolism: in vitro species comparison and in vivo disposition in rats. *Drug Metab. Dispos.* **2003**, *31*, 1382−1390.

(71) Kumar, S.; Samuel, K.; Subramanian, R.; Braun, M. P.; Stearns, R. A.; Chiu, S.; Evans, D. C.; Baillie, T. A. Extrapolation of diclofenac clearance from in vitro microsomal metabolism data: role of acyl glucuronidation and sequential oxidative metabolism of the acyl glucuronide. *J. Pharmacol. Exp. Ther.* **2002**, *303*, 969−978.

(72) Ogilvie, B. W.; Zhang, D.; Li, W.; Rodrigues, A. D.; Gipson, A. E.; Holsapple, J.; Toren, P.; Parkinson, A. Glucuronidation converts gemfibrozil to a potent, metabolism-dependent inhibitor of CYP2C8: implications for drug−drug interactions. *Drug Metab. Dispos.* **2006**, *34*, 191−197.

(73) Tang, W.; Abbott, F. S. Bioactivation of a toxic metabolite of valproic acid, (*E*)-2-propyl-2,4-pentadienoic acid, via glucuronidation. LC/MS/MS characterization of the GSH−glucuronide diconjugates. *Chem. Res. Toxicol.* **1996**, *9*, 517−526.

(74) Sabordo, L;. Sallustio, B. C.; Evans, A. M.; Nation, R. M. Hepatic disposition of the acyl glucuronide1-*O*-gemfibrozil-D-glucuronide: Effects of dibromosulfophthalein on membrane transport and aglycone formation. *J. Pharmacol. Exp. Ther.* **1999**, *288*, 414−420.

(75) Fennell, T. R.; Krol, W. L.; J. Sumner, S. C.; Snyder, R. W. Pharmacokinetics of dibutylphthalate in pregnant rats. *Toxicol. Sci.* **2004**, *82*, 407−418.

(76) Ghaoui, R.; Sallustio, B. C.; Burcham, P. C.; Fontaine, F. R. UDP-glucuronosyltransferase-dependent bioactivation of clofibric acid to a DNA-damaging intermediate in mouse hepatocytes. *Chem.-Biol. Interact.* **2003**, *145*, 201−211.

(77) Sallustio, B. C.; DeGraaf, Y. C.; Weekley, J. S.; Burcham, P. C. Bioactivation of carboxylic acid compounds by UDP-glucuronosyltransferases to DNA-damaging intermediates: Role of glycoxidation and oxidative stress in genotoxicity. *Chem. Res. Toxicol.* **2006**, *19*, 683−691.

(78) Farrant, R. D.; Spraul, M.; Wilson, I. D.; Nicholls, A. W.; Nicholson, J. K.; Lindon, J. C. Assignment of the 750 MHz ^{1}H NMR resonances from a mixture of transacylated ester glucuronic acid conjugates with the aid of oversampling and digital filtering during acquisition. *J. Pharm. Biomed. Anal.* **1995**, *13*, 971−977.

(79) Sidelmann, U.; Gavaghan, C.; Carless, H. A. J.; Farrant, R. D.; Lindon, J. C.; Wilson, I. D.; Nicholson, J. K. Identification of the novel positional isomers of 2-fluorobenzoic acid 1-*O*-acyl-glucuronide by directly coupled HPLC−NMR. *Anal. Chem.* **1995**, *67*, 3401−3404.

(80) Sidelmann, U.; Gavaghan, C.; Carless, H. A. J.; Spraul, M.; Hofmann, M.; Lindon, J. C.; Wilson, I. D.; Nicholson, J. K. 750 MHz directly coupled HPLC−^{1}H NMR: Application for sequential characterisation of the positional isomers and anomers of 2-, 3- and 4-fluorobenzoic acid glucuronides in equilibrium mixtures. *Anal. Chem.* **1995**, *67*, 4441−4445.

(81) Sidelmann, U.; Lenz, E. M.; Sanderson, P. N.; Hofmann, M.; Spraul, M.; Lindon, J. C.; Wilson, I. D.; Nicholson, J. K. 750 MHz HPLC−NMR spectroscopic studies on the separation and characterization of the positional isomers of the glucuronides of 6,11-dihydro-11-oxobenz[*b*,*e*]oxepin-2-acetic acid. *Anal. Chem.* **1996**, *68*, 106−110.

(82) Lenz, E. M.; Greatbanks, D.; Wilson, I. D.; Spraul, M.; Hofmann, M.; Lindon, J. C.; Troke, J.; Nicholson, J. K. Direct characterisation of drug glucuronide isomers in human urine by HPLC−NMR spectroscopy: Application to the positional isomers of 6,11-dihydro-11-oxo-dibenz[*b*,*e*]oxepin-2-acetic acid glucuronide. *Anal. Chem.* **1996**, *68*, 2832−2837.

(83) Sidelmann, U. G.; Braumann, U.; Hofmann, M.; Spraul, M.; Lindon, J. C.; Nicholson, J. K.; Hansen, S. H. Directly-coupled 800 MHz HPLC−NMR spectroscopy of urine and its application to the identification of the major phase II metabolites of tolfenamic acid. *Anal. Chem.* **1997**, *69*, 607−612.

(84) Fisher, M. B.; Jackson, D.; Kaerner, A.; Wrighton, S. A.; Borel, A. G. Characterization by liquid chromatography−nuclear magnetic resonance spectroscopy and liquid chromatography−mass spectrometry of two coupled oxidative−conjugative metabolic pathways for 7-ethoxycoumarin in human liver microsomes treated with alamethicin. *Drug Metab. Dispos.* **2002**, *30*, 270−275.

(85) Imbenotte, M.; Azaroual, N.; Cartigny, B.; Vermeersch, G.; Lhermitte, M. Identification and quantitation of xenobiotics by ^{1}H NMR spectroscopy in poisoning cases. *Forensic Sci. Int.* **2003**, *133*, 132−135.

(86) Lenz, E. M.; Wilson, I. D.; Wright, B.; Partridge, E. A.; Rodgers, C. T.; Haycock, P. R.; Lindon, J. C.; Nicholson, J. K. A comparison of quantitative NMR and radiolabelling studies of the metabolism and excretion of statil (3-(4-bromo-2-fluorobenzyl)-4-oxo-3*H*-phthalazin-1-ylacetic acid) in the rat. *J. Pharm. Biomed. Anal.* **2002**, *28*, 31−43.

(87) Sidelmann, U. G.; Hansen, S. H.; Cavaghan, C.; Carless, H. A. J.; Lindon, J. C.; Farrant, R. D.; Wilson, I. D.; Nicholson, J. K. Measurement of internal acyl migration reaction kinetics using directly-coupled HPLC−NMR: Application for the positional isomers of synthetic 2-fluorobenzoyl-D-glucopyranuronic acid. *Anal. Chem.* **1996**, *68*, 2564−2572.

(88) Mortensen, R. W.; Corcoran, O.; Cornett, C.; Sidelmann, U. G.; Lindon, J. C.; Nicholson, J. K.; Hansen, S. H. *S*-Naproxen-1-*O*-acyl glucuronide degradation kinetic studies by stopped-flow high-performance liquid chromatography−^{1}H NMR and high-performance liquid chromatography−UV. *Drug Metab. Dispos.* **2001**, *29*, 375−380.

(89) Skordi, E.; Wilson, I. D.; Lindon, J. C.; Nicholson, J. K. Kinetic studies on the intramolecular acyl migration of β-1-*O*-acyl glucuronides: application to the glucuronides of (*R*)- and (*S*)-ketoprofen, (*R*)- and (*S*)-hydroxy-ketoprofen metabolites, and tolmetin by ^{1}H-NMR spectroscopy. *Xenobiotica* **2005**, *35*, 715−725.

(90) Vanderhoeven, S. J.; Lindon, J. C.; Troke, J.; Nicholson, J. K.; Wilson, I. D. NMR spectroscopic studies of the transacylation reactivity of ibuprofen 1-β-*O*-acyl glucuronide. *J. Pharmaceut. Biomed. Anal.* **2006**, *41*, 1002−1006.

(91) Vanderhoeven, S. J.; Lindon, J. C.; Troke, J.; Tranter, G. E.; Wilson, I. D.; Nicholson, J. K. NMR and QSAR studies on the transacylation reactivity of model 1β-*O*-acyl glucuronides. I: Design, synthesis and degradation rate measurement. *Xenobiotica* **2004**, *34*, 73−85.

(92) Clayton, E.; Taylor, S.; Wright, B.; Wilson, I. D. The application of high performance liquid chromatography, coupled to nuclear magnetic resonance spectroscopy and mass spectrometry (HPLC−NMR−MS), to the characterisation of ibuprofen metabolites from human urine. *Chromatographia* **1998**, *47*, 264−270.

(93) Riffel, K. A.; Groff, M. A.; Wenning, L.; Song, H.; Lo, M.-W. Fully automated liquid−liquid extraction for the determination of a novel insulin sensitizer in human plasma by heated nebulizer and turbo ionspray liquid chromatography−tandem mass spectrometry. *J. Chromatogr., B* **2005**, *819*, 293−300.

(94) Kobayashi, T.; Ando, H.; Sugihara, J.; Harigaya, S. Metabolism of ethyl 2-(4-chlorophenyl)-5-(2-furyl)-oxazole-4-acetate, a new hypolipidemic agent, in the rat, rabbit, and dog. Glucuronidation of carboxyl group and cleavage of furan ring. *Drug Metab. Dispos.* **1987**, *15*, 262−266.

(95) Leis, H. J.; Fauler, G.; Raspotnig, G.; Windischhofer, W. Quantitative gas chromatographic/mass spectrometric analysis of morphine glucuronides in human plasma by negative ion chemical ionization mass spectrometry. *J. Mass Spectrom.* **2002**, *37*, 395−400.

(96) Watanabe, Y.; Nakajima, M.; Ohashi, N.; Kume, T.; Yokoi, T. Glucuronidation of etoposide in human liver microsomes is specifically catalyzed by UDP-glucuronosyltransferase 1A1. *Drug Metab. Dispos.* **2003**, *31*, 589−595.

(97) Meier, H.; Blaschke, G. Capillary electrophoresis-mass spectrometry, liquid chromatography−mass spectrometry and nanoelectrospray−mass spectrometry of praziquantel metabolites. *J. Chromatogr., B* **2000**, *748*, 221−231.

(98) Wey, A. B.; Caslavska, J.; Thormann, W. Analysis of codeine, dihydrocodeine and their glucuronides in human urine by electrokinetic capillary immunoassays and capillary electrophoresis-ion trap mass spectrometry. *J. Chromatogr., A* **2000**, *895*, 133−146.

(99) Keski-Hynnila, H.; Luukkanen, L.; Taskinen, J.; Kostiainen, R. Mass spectrometric and tandem mass spectrometric behavior of nitrocatechol glucuronides: a comparison of atmospheric pressure chemical ionization and electrospray ionization. *J. Am. Soc. Mass Spectrom.* **1999**, *10*, 537−545.

(100) Ohta, M.; Kawakami, N.; Yamato, S.; Shimada, K. Analysis of acetaminophen glucuronide conjugate accompanied by adduct ion production by liquid chromatography−atmospheric pressure chemical ionization-mass spectrometry. *J. Pharm. Biomed. Anal.* **2003**, *30*, 1759−1764.

(101) Kuuranne, T.; Vahermo, M.; Leinonen, A.; Kostiainen, R. Electrospray and atmospheric pressure chemical ionization tandem mass spectrometric behavior of eight anabolic steroid glucuronides. *J. Am. Soc. Mass Spectrom.* **2000**, *11*, 722−730.

(102) Zhang, J. Y.; Wang, Y.; Dudkowski, C.; Yang, D.; Chang, M.; Yuan, J.; Paulson, S. K.; Breau, A. P. Characterization of metabolites of celecoxib in rabbits by liquid chromatography/tandem mass spectrometry. *J. Mass Spectrom.* **2000**, *35*, 1259−1270.

(103) Wang, L.; Zhang, D.; Swaminathan, A.; Xue, Y.; Cheng, P. T.; Wu, S.; Mosqueda-Garcia, R.; Aurang, C.; Everett, D. W.; Humphreys, W. G. Glucuronidation as a major metabolic clearance pathway of C-14 labeled muraglitazar in humans: metabolic profiles in subjects with or without bile collection. *Drug Metab. Dispos.* **2006**, *34*, 427−439.

(104) Dong, Q.; Gu, J.; Zhong, D.; Fawcett, P. J.; Chu, D. Isolation and identification of the glucuronide of 4-(3*H*-1,2-dihydro-1-pyrrolizinone-2-methylamino)benzoic acid from rabbit urine. *J. Chromatogr., B* **2002**, *772*, 369−372.

(105) Valaskovic, G. A.; Utley, L.; Lee, M. S.; Wu, J. T. Ultra-low flow nanospray for the normalization of conventional liquid chromatography/mass spectrometry through equimolar response: standard-free quantitative estimation of metabolite levels in drug discovery. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 1087−1096.

(106) Illing, H. P. A.; Wilson, I. D. pH dependant formation of β-glucuronidase resistant conjugates from the biosynthetic ester glucuronide of isoxepac. *Biochem. Pharmacol.* **1981**, *30*, 3381−3384.

(107) Bedford, C. T. Glucuronic acid conjugates. *J. Chromatogr., B* **1998**, *717*, 313−326.

(108) Sidelmann, U.; Hansen, S. H.; Gavaghan, C.; Nicholls, A. W.; Carless, H. A.; Lindon, J. C.; Wilson, I. D.; Nicholson, J. K. Development of a simple liquid chromatographic method for the separation of mixtures of positional isomers and anomers of synthetic 2-, 3- and 4-fluorobenzoic acid glucuronides formed via acyl migration reactions. *J. Chromatogr., B* **1996**, *685*, 113−122.

(109) King, A. R.; Dickinson, R. G. Studies on the reactivity of acyl glucuronides-I. Phenolic glucuronidation of isomers of diflunisal acyl glucuronide in the rat. *Biochem. Pharmacol.* **1991**, *42*, 2289−2299.

(110) Mano, N.; Nikaido, A.; Narui, T.; Yamasaki, D.; Goto, J. Rapid and simple quantitative assay method for diastereomeric flurbiprofen glucuronides in the incubation mixture. *J. Chromatogr., B.* **2002**, *776*, 125−131.

(111) Ikegawa, S.; Murao, N.; Oohashi, J.; Goto, J. Separatory determination of diastereomeric ibuprofen glucuronides in human urine by liquid chromatography electrospray ionization mass spectrometry. *Biomed. Chromatogr.* **1998**, *12*, 317−321.

(112) Huttemann, H.; Blaschke, G. Achiral and chiral determination of ciprofibrate and its glucuronide in human urine by capillary electrophoresis. *J. Chromatogr., B* **1999**, *729*, 33−41.

(113) Hansen, S. H.; Christiansen, I. Separation of isomers of acyl glucuronides using micellar electrokinetic capillary chromatography in dynamically coated capillaries. *Electrophoresis* **2004**, *25*, 3277−3281.

(114) Akin, A.; Curran, T. T. Preparation of CI-1027 glucuronide: Metabolite of a remarkable lipid-modulating agent. *Synth. Commun.* **2005**, *35*, 1649−1661.

(115) Pravdic, M.; Keglevic, D. The synthesis of the fully benzylated C-1 hydroxyl free glucuronic acid. *Tetrahedron* **1965**, *21*, 1897−1901.

(116) Methyl ester: Schmidt, R. R.; Rucker, E. Stereoselective glycosidations of glucuronic acids. *Tetrahedron Lett.* **1980**, *21*, 1421−1424. Imidate activation: Schmidt, R. R.; Grundler, G. Einfache synthese von β-D-glucopyranosyluronaten (Simple synthesis of β-D-glucopyranuronates). *Synthesis* **1981**, 885−887.

(117) Panfil, I.; Lehman, P. A.; Zimniak, P.; Ernst, B.; Franz, T.; Lester, R.; Radominska, A. Biosynthesis and chemical synthesis of carboxyl-linked glucuronide of lithocholic acid. *Biochim. Biophys Acta* **1992**, *1126*, 221−228.

(118) Goto, J.; Murao, N.; Oohashi, J.; Ikegawa, S. Synthesis of bile acid 24-acyl glucuronides. *Steroids* **1998**, *63*, 180−185.

(119) De Mesmaeker, A.; Hoffmann, P.; Ernst, B. A new protected form of glucuronic acid for the synthesis of labile 1-*O*-acyl-β-D-glucuronides. *Tetrahedron Lett.* **1989**, *30*, 3773−3776.

(120) Tanaka, M.; Okita, M.; Yamatsu, I. Synthesis of glucuronides of α,β-unsaturated carboxylic acids. *Carbohydr. Res.* **1993**, *241*, 81−88.

(121) Becker, B.; Barua, A. B.; Olson, J. A. All-trans- retinoyl β-glucuronide. *Biochem. J.* **1996**, *314*, 249−252 and references therein.

(122) Barua, A. B.; Huselton, C. A.; Olson, J. A. Synthesis of novel glucuronide conjugates of retinoid carboxylic acids. *Synth. Commun.* **1996**, *26*, 1355−1361.

(123) Perrie, J. A.; Harding, J. R.; Holt, D. W.; Johnston, A.; Meath, P.; Stachulski, A. V. Effective synthesis of 1β-acyl glucuronides by selective acylation. *Org. Lett.* **2005**, *7*, 2591−2594.
(124) Schmidt, R. R. New methods for the synthesis of glycosides and oligosaccharides. Are there alternatives to the Koenigs−Knorr method? *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 212−235.
(125) Kunz, H.; Dombo, B. Solid phase synthesis of peptides and glycopeptides on polymeric supports with allylic anchor groups. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 711−713.
(126) Bowkett, E. R.; Park, B. K.; Stachulski, A. V. Manuscript in preparation.
(127) Meng, X.; Stachulski, A. V. Unpublished results.
(128) Kittlemann, M.; Rheinegger, U.; Espigat, A.; Oberer, L.; Aichholz, R.; Francotte, E.; Ghisalba, O. Preparative enzymatic synthesis of the acyl glucuronide of mycophenolic acid. *Adv. Synth. Catal.* **2003**, *345*, 825−829.
(129) Shackleford, D. M.; Hayball, P. J.; Reynolds, G. D.; Hamon, D. P. G.; Evans, A. M.; Milne, R. W.; Nation, R. L. A small-scale synthesis and enantiomeric resolution of (*RS*)-[1-^{14}C] 2-phenylpropionic acid and biosynthesis of its diastereomeric acyl glucuronides. *J. Labelled Compd. Radiopharm.* **2001**, *44*, 225−234.
(130) Pfaar, U.; Gygax, D.; Gertsch, W.; Winkler, T.; Ghisalba, O. Enzymatic synthesis of beta-D-glucuronides in an enzyme membrane reactor. *Chimia* **1999**, *53*, 590−593.
(131) Schneider, R. P.; Fouda, H. G.; Inskeep, P. B. Tissue distribution and biotransformation of zopolrestat, an aldose reductase inhibitor, in rats. *Drug Metab. Dispos.* **1998**, *26*, 1149−1159.
(132) Dalvie, D. K.; O'Donnell, J. P. Metabolism of CP-195,543, a leukotriene B4 receptor antagonist, in the Long-Evans rat and cynomolgus monkey. *Xenobiotica* **1999**, *29*, 1123−1140.
(133) Ruhl, R.; Tiel, R.; Lacker, T. S.; Strohschein, S.; Albert, K.; Nau, H. Synthesis, high-performance liquid chromatography−nuclear magnetic resonance characterization and pharmacokinetics in mice of CD271 glucuronide. *J. Chromatogr., B* **2001**, *757*, 101−109.
(134) Zhou, S. F.; Paxton, J. W.; Tingle, M. D.; McCall, J.; Kestell, P. Determination of two major metabolites of the novel anti-tumour agent 5,6-dimethylxanthenone-4-acetic acid in hepatic microsomal incubations by high-performance liquid chromatography with fluorescence detection. *J. Chromatogr., B* **1999**, *734*, 129−136.
(135) Zhou, S. F.; Kestell, P.; Baguley, B. C.; Paxton, J. W. Preclinical factors influencing the relative contributions of phase I and II enzymes to the metabolism of the experimental anti-cancer drug 5,6-dimethylxanthenone-4-acetic acid. *Biochem. Pharmacol.* **2003**, *65*, 109−120.
(136) Zhou, S. F.; Paxton, J. W.; Tingle, M. D.; Kestell, P.; Jameson, M. B.; Thompsom, P. I.; Baguley, B. C. Identification and reactivity of the major metabolite (beta-1-glucuronide) of the anti-tumor agent 5,6-dimethylxanthenone-4-acetic acid (DMXAA) in humans. *Xenobiotica* **2001**, *31*, 277−293.
(137) Gilissen, R. A. H. J.; Ferrari, L.; Barnaby, R. J.; Kajbaf, M. Human hepatic metabolism of a novel 2-carboxyindole glycine antagonist for stroke: in vitro−in vivo correlations. *Xenobiotica* **2000**, *30*, 843−856.
(138) Kajbaf, M.; Barnaby, R. J.; Bottacini, M.; Bertolotti, L.; Ismail, I. M.; Cholerton, T. J.; Pellegatti, M. Pharmacokinetics, metabolism and excretion of the glycine antagonist GV150526A in rat and dog. *Xenobiotica* **2003**, *33*, 415−428.
(139) Cui, D. H.; Subramanian, R.; Shou, M. G.; Yu, X.; Wallace, M. A.; Braun, M. P.; Arison, B. H.; Yergey, J. A.; Prueksaritanont, T. In vitro and in vivo metabolism of a potent and selective integrin $\alpha_v\beta_3$ antagonist in rats, dogs, and monkeys. *Drug Metab. Dispos.* **2004**, *32*, 848−861.
(140) Kenny, J. R.; Maggs, J. L.; Tettey, J. N. A.; Harrel, A. W.; Parker, S. G.; Clarke, S. E.; Park, B. K. Formation and protein binding of the acyl glucuronide of a leukotriene B_4 antagonist (SB-209247): relation to species differences in hepatotoxicity. *Drug Metab. Dispos.* **2005**, *33*, 271−281.
(141) Kochansky, C. J.; Xia, Y. Q.; Wang, S.; Cato, B.; Creighton, M.; Vincent, S. H.; Franklin, R. B.; Reed, J. R. Species differences in the elimination of a peroxisome proliferator-activated receptor agonist highlighted by oxidative metabolism of its acyl glucuronide. *Drug Metab. Dispos.* **2005**, *33*, 1894−1904.
(142) Giuliano, C.; Fiore, F.; Di Marco, A.; Padron Velazquez, J.; Bishop, A.; Bonelli, F.; Gonzalez-Paz, O.; Marcucci, I.; Harper, S.; Narjes, F.; Pacini, B.; Monteagudo, E.; Migliaccio, G.; Rowley, M.; Laufer, R. Preclinical pharmacokinetics and metabolism of a potent non-nucleoside inhibitor of the hepatitis C virus NS5B polymerase. *Xenobiotica* **2005**, *35*, 1035−1054.
(143) Ericsson, H.; Hamren, B.; Bergstrand, S.; Elebring, M.; Fryklund, L.; Heijer, M.; Ohman, K. P. Pharmacokinetics and metabolism of tesaglitazar, a novel dual-acting peroxisome proliferator-activated receptor α/γ agonist, after a single oral and intravenous dose in humans. *Drug Metab. Dispos.* **2004**, *32*, 923−929.
(144) Huskey, S. E.; Dean, B. J.; Doss, G. A.; Wang, Z.; Hop, C. E.; Anari, R.; Finke, P. E.; Robichaud, A. J.; Zhang, M.; Wang, B.; Strauss, J. R.; Cunningham, P. K.; Feeney, W. P.; Franklin, R. B.; Baillie, T. A.; Chiu, S. H. The metabolic disposition of aprepitant, a substance P receptor antagonist, in rats and dogs. *Drug Metab. Dispos.* **2004**, *32*, 246−258.
(145) Vickers, A. E.; Zollinger, M.; Dannecker, R.; Tynes, R.; Heitz, F.; Fischer, V. In vitro metabolism of tegaserod in human liver and intestine: assessment of drug interactions. *Drug Metab. Dispos.* **2001**, *29*, 1269−1276.
(146) Zhao, Y.; Yang, C. Y.; Haznedar, J.; Antonian, L. Simultaneous determination of SU5416 and its phase I and phase II metabolites in rat and dog plasma by LC/MS/MS. *J. Pharm. Biomed. Anal.* **2001**, *25*, 821−832.
(147) Dalgaard, L.; Larsen, C. Metabolism and excretion of citalopram in man: identification of *O*-acyl- and *N*-glucuronides. *Xenobiotica* **1999**, *29*, 1033−1041.
(148) Wait, J. C. M.; Vaccharajani, N.; Mitroka, J.; Jemal, M.; Khan, S.; Bonacorsi, S. J.; Rinehart, J. K.; Iyer, R. A. Metabolism and disposition of [^{14}C]gemopatrilat after oral administration to rats, dogs and humans. *Drug Metab. Dispos.* **2006**, *34*, 961−970.
(149) Baba, A.; Yoshioka, T. Sythesis of 1β-acyl glucuronides of diclofenac, nafenamic acid and (*S*)-naproxen by the chemo-selective enzymatic removal of protecting groups from the corresponding methyl acetyl derivatives. *Org. Biomol. Chem.* **2006**, *3*, 3303−3310.

JM060599Z