The synthesis of selected phase II metabolites – *O*-glucuronides and sulfates of drug development candidates

Jens Atzrodt,* Volker Derdau, Wolfgang Holla and Martin Sandvoss

Sanofi-Aventis Deutschland GmbH, R&D, SCP Disposition, Safety & Animal Research (DSAR), Drug Disposition (DD), Isotope Chemistry & Metabolite Synthesis (ICMS), G876, 65926 Frankfurt/Höchst, Germany.
E-mail: Jens.Atzrodt@sanofi.com

Dedicated to Rainer Beckert on the occasion of his 60th anniversary

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Abstract

We have summarized methods for the preparation of phase II metabolites which have proven to be successful in our laboratory for the synthesis of selected *O*-glucuronides and sulfates of recent drug development candidates. The syntheses of the *O*-glucuronides AVE0897 *O*-acyl-glucuronide **7**, AVE2268 *O*-glucuronide **12,** SAR7226 *O*-glucuronide **21** and the preparation of the sulfates **28** of AVE2268 and MDL107292 **33** are described in detail. Analytical aspects related to phase II metabolites and specific chromatographic methods of very polar compounds are discussed.

Keywords: Phase II metabolites, glucuronides, acyl glucuronides, sulfates, metabolite analysis

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1. Introduction

Drugs administered to the body typically undergo biphasic metabolic transformation to facilitate elimination from the body by increased water solubility.[1] During phase I metabolism (functionalization) polar functionalities are either introduced or unmasked e.g. by oxidation, hydrolysis, dealkylation or fragmentation. If the phase I metabolites are sufficiently polar, they may be readily excreted via bile or urine. However, many phase I metabolites are not eliminated rapidly and undergo subsequent phase II transformations in which an endogenous substrate (e.g. glycine, sulfate, or glucuronic acid) interacts with the newly formed functional group to give even more polar conjugates.[2]

Although, in general, metabolism is a detoxification process, phase I metabolism can, in some instances, lead to a chemically reactive or pharmacologically active molecule or even turn a nontoxic compound into a poisonous metabolite.[3] In addition, safety assessments may also be needed for phase II metabolites if the conjugation results in the formation of chemical structures (e.g. acyl glucuronides) known to be linked to a potential toxicity.[4]

In order to assess potential safety concerns related to drug metabolism, the US Food and Drug Administration (FDA) has recently published the industry guidance "Safety Testing of Drug Metabolites"[5] which requires identification[6] of metabolite profiles and further toxicological testing[7] for those metabolites formed at greater than 10% of parent drug systemic exposure at steady state.[8] In the guidance the FDA acknowledges difficulties associated with the synthesis of specific drug metabolites, but nevertheless considers the identification and evaluation of the potential toxicity to be important to ensure patient safety. Consequently, there is a high demand for efficient, robust, and reliable drug metabolite syntheses through from milligram to gram scale. For a drug development candidate syntheses of several different metabolites can be required, not only for pharmacological activity determination and toxicological testing but also for use as authentic samples for: structural proof by NMR, recovery in LC-MS based assays, for protein and receptor binding as well as transporter studies.[9] The latter may require the preparation of a radioactively labeled metabolite. Additionally, stable isotope labeling might be necessary for the validation of bioanalytical LC-MS/MS assays if a relevant metabolite needs to be monitored in clinical studies. In cases where the exposure of a metabolite in animal studies does not approach human exposure (so-called disproportionate metabolites[10]) or when a metabolite is exclusively formed in humans (human unique metabolite[11]) large quantities up to several kilograms may be required for nonclinical toxicological studies.

In principle, metabolite synthesis may be accomplished either by chemical or biological means and both approaches are pursued in the pharmaceutical industry, the choice being dictated by case-specific factors. In particular, the preparation of very polar, conjugated phase II metabolites can have some very challenging aspects, e.g. synthesis, purification, stability, isolation and analysis. Biotechnological approaches for phase II metabolite production, e.g. UGT (uridine diphosphoglucuronosyltransferase) mediated glucuronide preparation[12] have been

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recently reviewed together with whole-cell biotransformation processes for phase I metabolite synthesis.[13] In this paper we would like to summarize specific chemical methods developed for the preparation of phase II metabolites and thereby specifically focus on recent work of our group in this area.

2. *O*-Glucuronides

O-Glucuronides can be divided into three classes: alkyl, aryl, and acyl. 1-*O*-alkyl and -aryl glucuronides generally exhibit good chemical stability. For their synthesis which is sometimes more difficult than the preparation of the corresponding glucosides, acyl and ether protected bromides, chlorides, and imidates are the most common glucuronyl donors.[14]

1-*O*-acyl glucuronides are prone to hydrolysis and rearrangement. In addition to the above mentioned donors, the use of 1-hydroxy sugars has gained increasing popularity. Due to stability issues final deprotection step(s) protective groups which can be removed chemoselectively under mild reaction conditions (e.g. Aloc- or silyl) are becoming increasingly important.[14]

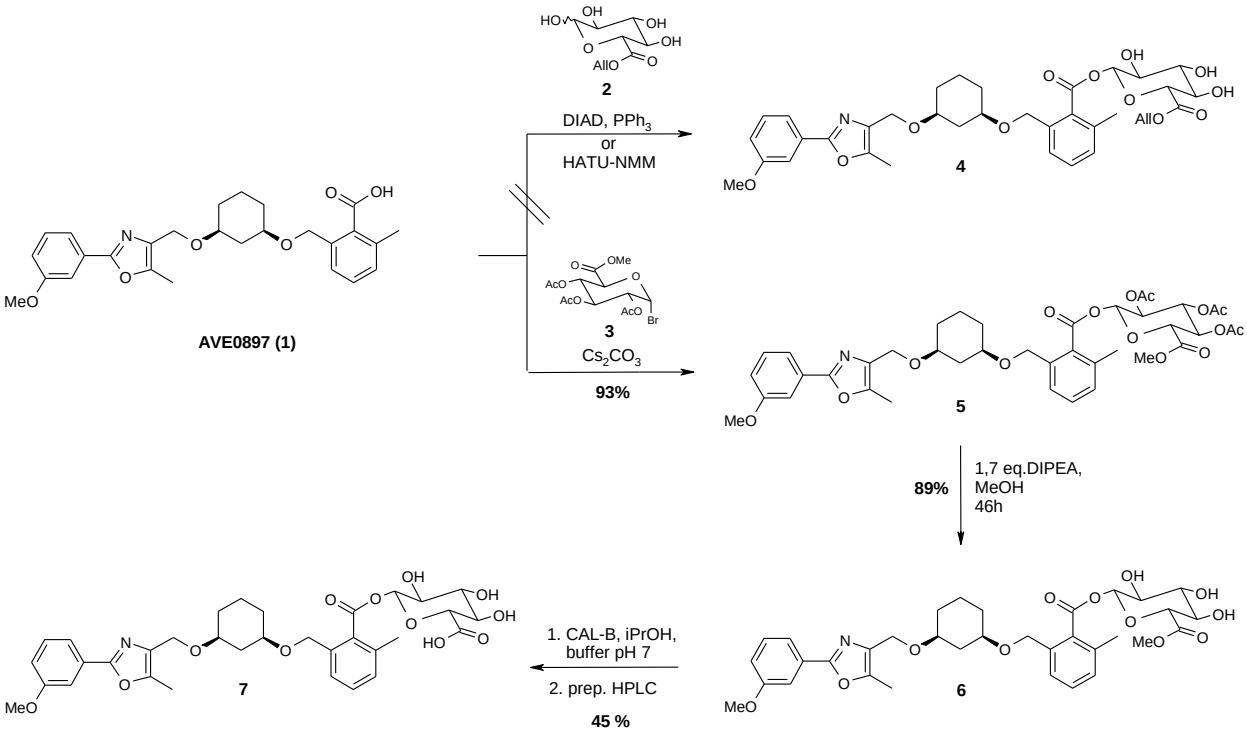
2.1 1-*O*-Acyl glucuronides

Acyl glucuronidation is a major phase II metabolic path for carboxylic acids which can have important toxicological implications for a drug for two reasons: the potential pharmacological activity of the acyl glucuronide and its chemical reactivity.[4,15] This reactivity is due to the electrophilic nature of the acyl glucuronide and can result in: spontaneous hydrolysis to the parent aglycone, pH-dependent intramolecular acyl migration[16] as well as transacylation and glycation reactions with proteins, nucleic acids and other nuclephilic components.[17] The assessment of the potential safety risks associated with acyl glucuronides often requires detailed pharmacokinetic analysis including determination of clearance, systemic exposure and protein binding and consequently in many cases synthesis of metabolite reference material is required.

For all these reasons our group recently received a request for the synthesis of the 1-ß-*O*-acyl glucuronide **7** of the highly potent, orally active mixed peroxisome proliferator-activated receptor (PPAR) α/γ agonist AVE0897 **(1)** developed for treatment of type 2 diabetes. Due to its dual activity, **1** has the potential to improve both glucose control and the atherogenic lipid profile of type 2 diabetics or insulin-resistant patients.

Anomeric stereocontrol is an important aspect in the synthesis of acyl glucuronides, in order to obtain the single ß-anomer observed *in-vivo*.[18] Direct acylation of unprotected glucuronic acid with the *N*-acylimidazoles of carboxylic acids has been restricted to some specific examples, e.g. retinoic acid and reported yields are relatively low.[19] Alternatively fully protected glucuronyl donors can be conjugated with the carboxylic acid using either Mitsunobu conditions[20] or the trichloroacetimidate method.[21] However, multi-step manipulations are required for the preparation of the protected glucuronyl donor[22] and often anomeric mixtures of the 1-*O*-acylglucuronide are observed with this method.

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Scheme 1. Synthesis of 1-*β*-O-acyl glucuronide of AVE0897 (**7**).

Usually much better *β*/*α*-ratios are obtained by acylation of allyl glucuronate **2** under Mitsunobu conditions[23] or the HATU- coupling procedure[24] and therefore, this approach was our first choice for the preparation of protected glucuronate **4** (Scheme 1). Unfortunately, all attempts to achieve coupling under Mitsunobu conditions, by varying coupling reagents e.g. HATU, EDC/HOBT and bases such as DMAP, NMM, DBU, Cs_2CO_3 or even via the acid chloride[25] of AVE0897 failed, most probably due to the sterically hindered position of the carboxyl function. A model reaction with benzoic acid under HATU/DMAP coupling conditions provided the desired glucuronidation product, whereas the same reaction with 2,6-dimethylbenzoic acid failed to work at all.

Selective 1-*β*-*O*-acyl glucuronide formation has been also reported under classical Koenigs-Knorr conditions[26] or in the presence of Cs_2CO_3[27] using commercially available methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-*α*-D-glucopyranuronate **3**. In fact, under the latter conditions both the model substrate 2,6-dimethylbenzoic acid and AVE0897 were converted to their corresponding protected glucuronides and in this way **5** was obtained in very good yield (80 %) as the pure *ß*-anomer.[28]

The next challenge in acyl glucuronide syntheses is the selective removal of the protecting groups without affecting the 1-*β*-*O*-acyl linkage. Recently, a sequential enzyme-catalyzed chemoselective hydrolysis of the *O*-acetyl groups using lipase AS amino (LAS) followed by methyl ester cleavage with PLE (porcine liver esterase) has been reported.[27,29] However, in spite

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of varying incubation conditions, buffer, co-solvent and enzyme concentrations the LAS/PLE method could not be successfully applied to the deprotection of **5**. Selective deacetylation was, however, achieved with Hünig's base in methanol at room temperature.[30] Since acyl-migration and epimerization at the anomeric carbon of **6** could be excluded by NMR analysis, other reported saponification methods e.g. NH_3/MeOH,[31] Zemplén deacylation[32] (catalytic amounts of MeONa in MeOH) or titanate-mediated transesterification[33] were not attempted. Finally the methyl ester group in **6** was removed by an enzymatic cleavage catalyzed by Candida antarctica lipase (CAL-B) in excellent yield. The crude product was purified by preparative HPLC to obtain 1-*β*-*O*-acyl glucuronide **7** of AVE 0897 in 37 % overall yield starting from AVE 0897 **1**. The same sequence was also applied for the synthesis of stable isotope labeled [methoxy-$^{13}C^2H_3$]-7 as well as [carboxyl-^{14}C]-**7** starting from labeled parent drug [methoxy-$^{13}C^2H_3$]-**1** and [carboxyl-^{14}C]-**1**, respectively.

1-*O*-Alkyl and 1-*O*-Aryl glucuronides

For the synthesis of *O*-aryl and *O*-alkyl glucuronides there are basically two approaches:
(1) Glucuronidation by employing a glucuronyl donor and
(2) Synthesis of the corresponding glucoside followed by oxidation of the 6-OH group to the acid function.

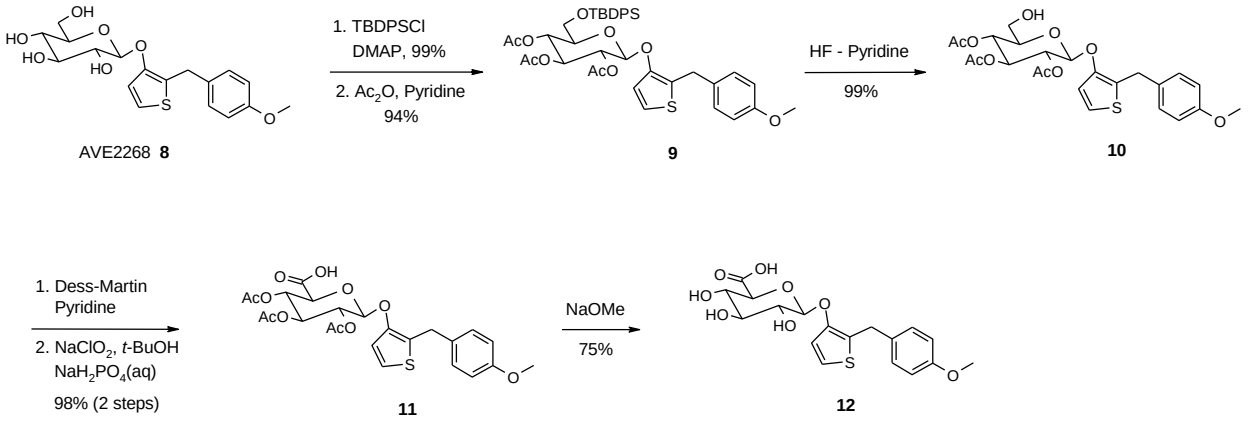
Glucuronidation methods have their parallel in the synthesis of glucosides, but for a given aglycone, glucuronidation needs greater activation. Acyl-protected trichloroacetimidates and bromo sugars are the most often used glucuronyl donors. Both can be synthesized in 3 to 4 steps starting from commercially available D-glucurono-6,3-lactone.[34,35]

For the second synthetic strategy, oxidation of the primary hydroxy group to the acid, a number of reagents are known, e.g. TEMPO/NaOCl[36] or the Dess-Martin periodinane giving the aldehyde, followed by $NaClO_2$ treatment.

For the synthesis of *O*-glucuronide metabolite **12** we initially envisaged a one step synthesis approach starting from parent drug AVE2268 **8**.[37] AVE2268, **8** is a SGLT inhibitor and was developed as a potential treatment for type 2 diabetes. Firstly, we tried to directly oxidize the glucoside moiety to afford the corresponding glucuronide. All attempts using reported methods, e.g. $KMnO_4$, SeO_2, PtO_2/O_2 TEMPO/NaOCl/Oxone,[36] peroxidases, etc. failed. Additionally, all trials to bring about transacetalisation of AVE2268 **8** to **12** by treatment with glucuronic acid in the presence of an acid (e.g. *p*-toluenesulfonic acid) were unsuccessful. Application of an orthogonal protecting group strategy finally allowed the selective oxidation at the C6-position. This route presented several challenges to be overcome (Scheme 2). Any traces of acid had to be avoided in order to prevent acetyl migration during the oxidation step. Attempts to oxidize the acetyl-protected AVE2268, **9** directly to the acid yielded only starting material or decomposition products. Therefore the oxidation was carried out stepwise. In the first oxidation step using the Dess-Martin periodinane, a very weak base had to be used in order to prevent double bond formation by

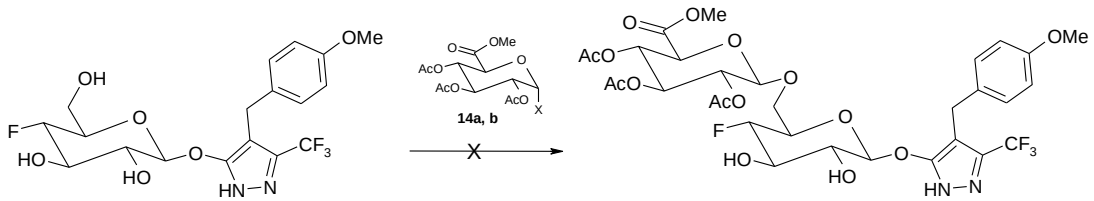
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elimination of acetic acid, and the second oxidation had to be performed immediately on the unstable intermediate aldehyde. Final cleavage of the acetyl groups with sodium methoxide led to **12** after 6 steps in 68 % overall yield.[38]



Scheme 2. Synthesis of *O*-glucuronide of AVE2268 (**12**).

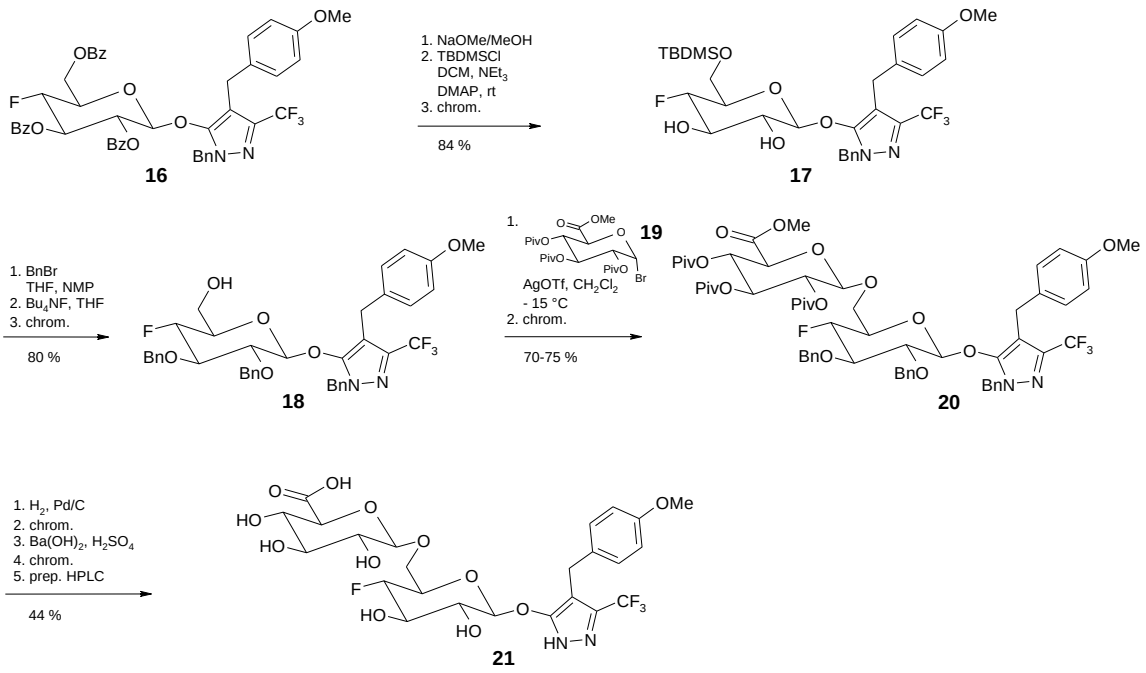
For the synthesis of SAR7226 *O*-glucuronide **21**, we initially investigated the glucuronidation of unprotected SAR7226 (**13**) (Scheme 3). It was hoped to predominantly derivatize the more reactive, primary 6-hydroxy group, but both the reaction of **13** with trichloroacetimidate **14a** catalyzed by TMSOTf and the Ag_2CO_3-mediated reaction with bromo sugar **14b** resulted in only a negligible formation of glucuronides.



Scheme 3. Attempts to glucuronidate unprotected SAR7226 (**13**).

Starting from the fully protected SAR7226 derivative **16**, synthesized from 1-chloromethyl-4-methoxy-benzene, 4,4,4-trifluoro-3-oxo-butyric acid ethyl ester, and benzylhydrazine the synthesis of SAR7226 *O*-glucuronide **21** required 7 linear steps followed by a final purification by preparative HPLC (Scheme 4).

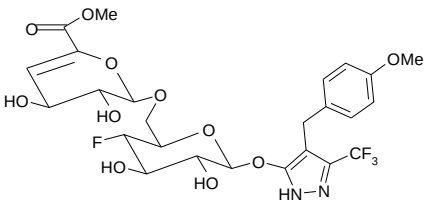
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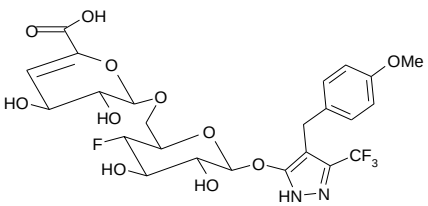
Scheme 4. Synthesis of SAR7226 metabolite **21.**

N-benzyl-, *O*-benzoyl-protected SAR7226 derivative **16** was debenzoylated using sodium methoxide in methanol at ambient temperature, followed by the selective protection of the 6-hydroxy group with TBDMS chloride in dichloromethane in the presence of triethylamine and catalytic amounts of DMAP. Benzylation of **17** employing benzyl bromide and sodium hydride in NMP, and subsequent deprotection of the 6-hydroxy group with TBAF (Bu_4NF) in THF gave the desired glycosyl acceptor **18** in 67 % overall yield. The glucuronidation step was studied with commercially available acetyl-[39] and benzoyl-[40] protected trichloroimidates as well as with acetyl-, benzoyl-, and pivaloyl-protected bromides.[41] The pivaloyl-protected bromide **19,** which turned out to be more stable than the corresponding acetyl compound **14b,** is known to exhibit a lower tendency towards ortho ester formation and gave the best yields of the desired 1-*ß*-glucuronide **20**. AgOTf-catalyzed reaction of glucuronyl donor **19** with the partially protected drug candidate **18** in dichloromethane at – 15°C gave **20** in 70-75 % yield after chromatography. Debenzylation was achieved by hydrogenation in 85 % yield. Hydrolysis of the ester groups was a critical step: depending on the reaction conditions a significant amount of *α,ß*-unsaturated carboxylic ester **22** and/or acid **23** was observed (Scheme 5).

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22



23

Scheme 5. By-products resulting from elimination during basic hydrolysis.

Good results were achieved by using $Ba(OH)_2$[42] for both the hydrolysis of the methyl ester group and the cleavage of the pivaloyl groups. The use of $Ba(OH)_2$ also simplified the work-up: after complete deprotection the reaction mixture was neutralized by addition of an equivalent of sulfuric acid followed by filtration of the resultant sparingly soluble salts. Chromatography on silica gel in dichloromethane/methanol 95:5 and final purification via preparative HPLC gave the desired *O*-glucuronide **9** in about 21 % overall yield.

Sulfates

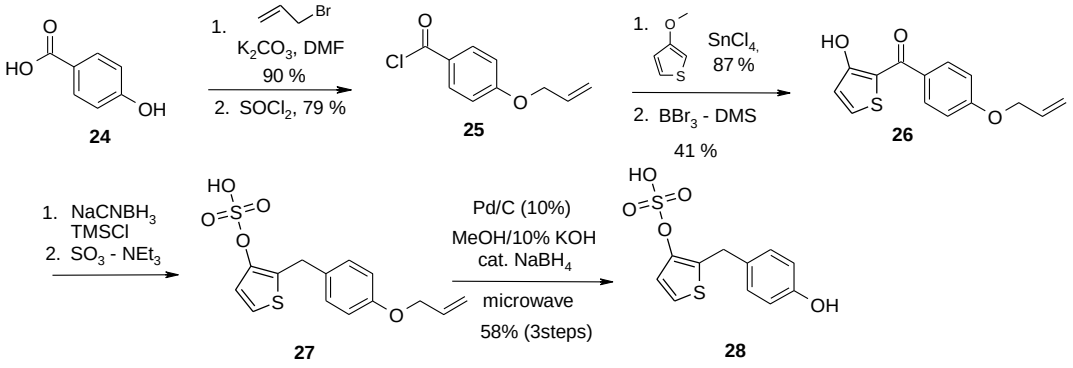
Sulfation, like glucuronidation, is a major reaction in phase II drug and xenobiotic metabolism and was initially identified in the mid-19th century. The reaction involves sulfotransferases (SULTs) which catalyse the transfer of a sulfonate group from 3′-phosphoadenosine 5′phosphosulfate (PAPS) to either a hydroxyl moiety to form a sulfate or to an amine to form a sulfonamide. Sulfate conjugation is, like most metabolism reactions, an inactivation process since most conjugates are biologically inactive owing to the presence of the bulky charged sulfonate group. The sulfonate group has a pKa of approximately 2 and is ionized at physiological pHs which results in rapid excretion of the sulfonates via kidneys and liver.[43]

As described before some of these metabolites are of interest in drug development due to unknown pharmacological or toxicological activity. The syntheses and analytics of sulfates have their own unique challenges. However, only a few examples using enzymatic or chemical examples have been reported.[44] At present, the most convenient synthetic method for the preparation of sulfates applies sulfur trioxide tertiary amine complexes as the sulfation reagent.[45]

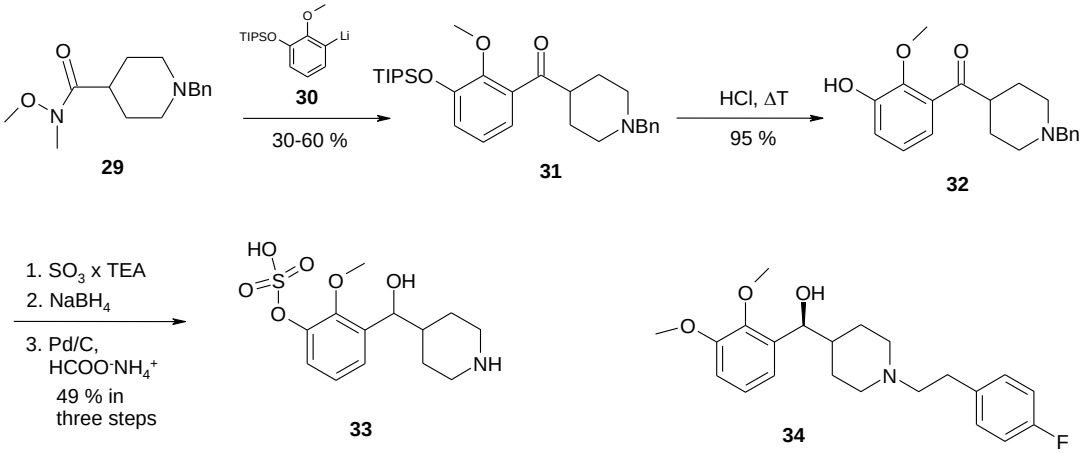
Scheme 6 shows the synthesis of the sulfate metabolite **28** of AVE2268 **8**. 4-Hydroxybenzoic acid **24** was alkylated with allylbromide and converted to the corresponding acyl chloride **25**. Friedel-Crafts acylation with 3-methoxy thiophene and selective methyl ether cleavage resulted in ketone **26**. Carbonyl reduction was followed by the successful formation of the allyl-protected sulfate **27**. After initial unsuccessful attempts to remove the allyl-group we surprisingly succeeded by combining the conditions of Hara[46] and Zhu[47] with the addition of catalytic amounts of $NaBH_4$. Accordingly **27** was heated in the presence of $NaBH_4$-activated Pd/C catalyst in MeOH/KOH under microwave conditions in a closed vial for 80 min. We found that only the combination of high temperature, strong basic conditions and $NaBH_4$-activated Pd/C catalyst gave reasonable yields in the deprotection of **27**. It is

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worthy of note that our first synthesis attempt using a *O*-benzyl protecting group failed because we were unable to remove the protecting group in the last reaction step.



Scheme 6. Synthesis of sulfate **28** of AVE2268.



Scheme 7. Synthesis of sulfate of MDL107292 (**33**).

A further example is shown in scheme **7** wherein sulfate metabolite MDL107292 **33** was synthesized in 5 steps with an overall yield of 15 %.[48] **33** is a metabolite of MDL100907 **34**, a highly selective 5-HT2A antagonist which is being trialed as a potential antipsychotic, antidepressant or as a treatment for insomnia.[49] **33** was prepared starting from Weinreb amide **29** which was reacted with *ortho*-lithiated guajacole derivative **30** to give the resulting ketone **31** in 30-60 % yield. Yield variations were found to be caused by differing purities of reactants and reagents used. After acidic removal of the TIPS-protecting group the phenol **32** was reacted with SO_3×TEA to form the sulfate in 96 % yield. Finally, after non-enantioselective ketone reduction with sodium borohydride, the benzyl protecting group was removed under Pd-catalyzed hydrogenation transfer conditions to yield the final product **33** as a racemic mixture.

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General aspects of the analysis of phase II-metabolites

For analytical synthesis support, in-process control and reliable quality control of phase II metabolites the whole battery of modern small molecule analytics – most importantly HPLC for purity assessment as well as MS and NMR for structural confirmation – is applied.

The one common feature that distinguishes metabolites from their parent molecules is that they are - as a first approximation - *more polar*.

While this does not usually cause problems for NMR (other than potential solubility issues) or MS (where the higher polarity is rather beneficial in electrospray ionization) it requires, in many cases, changes to the HPLC method.

Many metabolites can be analyzed using the chromatographic method applied to their parent molecule – the very polar ones, though, may require changes to the gradient profile or adjustments of the mobile phase composition, buffer type or pH. The target of such optimizations is to obtain sufficient retention on the stationary phase along with narrow peak shapes, resulting in high peak capacities so that any impurities present can be effectively separated from the analyte.

Even though counter ion chromatography is an inexpensive and suitable way to chromatograph very polar and ionic compounds – it is not applicable for preparative purposes and thus is not a good method for synthesis support. The same is true for ion exchange chromatography. It should be noted at this point that certain modifiers, such as TFA, possess counter ion properties without interfering with the isolation of the target compound.

Two examples from our laboratory which have been discussed above are given below:

Acyl glucuronide of AVE0897 (7)

An illustration of a typical case is given in Figure 1: the 1-*β*-*O*-acyl glucuronide of AVE0897 (**7**) is more polar and thus elutes earlier than the parent compound in reverse-phase (RP) mode. The gradient still covers the polarity range so that both compounds can be analyzed and well separated from each other using the same HPLC method.

Sulfate conjugate MDL107292 (33)

In the case of MDL107292 (**33**), a sulfate metabolite of MDL100907 **34**, the gradient method for the parent compound yielded insufficient retention (RP-18 column Waters Symmetry; starting conditions 10% organic, 0.1% TFA, not shown). Even a more drastic modification of the conditions to just 1 % organic eluent and an isocratic hold time of 10 min resulted only in a limited increase of retention (Figure 2a).

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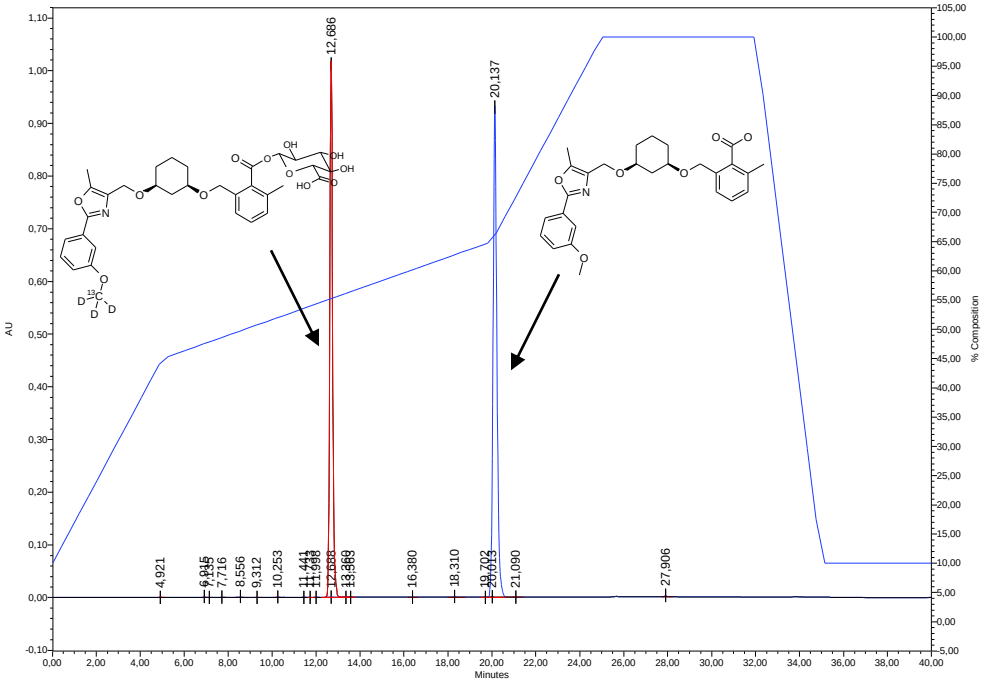


Figure 1. The *O*-acyl glucuronide of AVE0897 **(7)** is more polar and thus elutes earlier than the parent compound, but the gradient still covers the polarity range so that both compounds can be analyzed and well separated from each other using the same HPLC method.

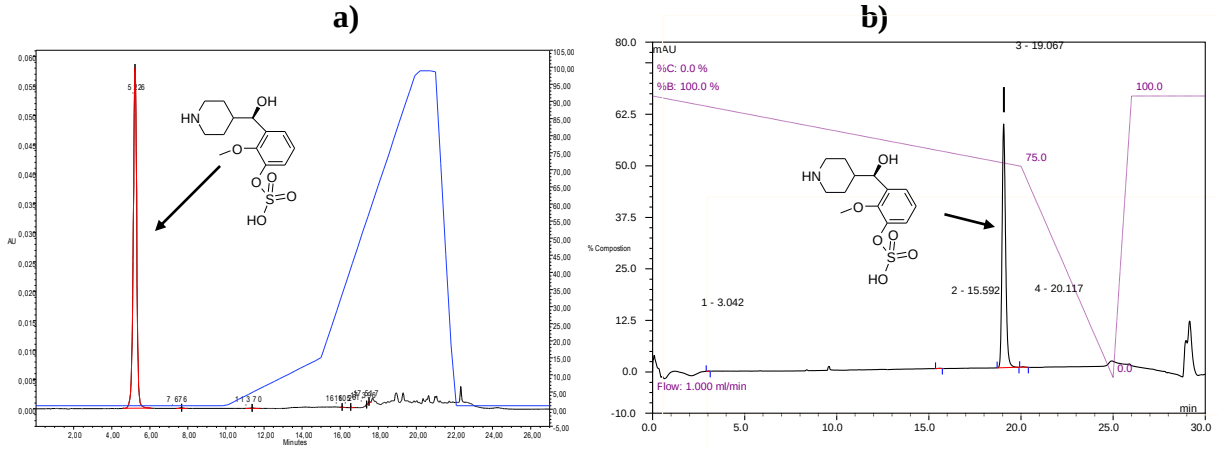


Figure 2. Analysis of MDL107292 (**33**) using two separation modes: **a)** while the modified parent RP18 method yielded only very limited retention, **b**) a HILIC method provided full chromatographic control (peak elutes in a linear section of the HPLC gradient).

As the reversed-phase C-18 ligands of the stationary phase are prone to collapse in such highly aqueous conditions, an "AQ"-type of reversed-phase stationary phase, which can be operated even in 100 % aqueous eluents was also tried as an alternative, but with very similar results (not shown).

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Even though limited retention could be achieved using the two chromatographic systems described above for initial analytical support of the synthesis, the fact that RP-chromatography had been taken to its limits with no room for further variation left (i.e. an almost totally aqueous eluent already in isocratic conditions) made a new method with better retention control necessary. Instead of further types of RP-material, an orthogonal separation mechanism was chosen: Hydrophilic Interaction Chromatography, HILIC.[50] Here, a partition of the analytes between a less polar mobile phase (typically acetonitrile) and a more polar, aqueous solvation sphere around the stationary phase takes place. Polar analytes are eluted with increasing amounts of aqueous eluent. The elution order is inversed compared to reversed-phase chromatography. Apolar compounds elute prior to polar ones. It has been demonstrated that HILIC shows very high orthogonality to the RP separation mechanism.[51]

The results of such a HILIC chromatogram are shown in Figure 2 (b). The phase II metabolite MDL107292 **33** elutes with a linear gradient of increasing levels of aqueous eluent after 19 min at an aqueous eluent level of approx. 25 %. Full chromatographic control is obtained: The gradient profile can be adjusted in order to vary retention times or in case a potential impurity needs to be separated from the main peak.

Under the conditions of HILIC method (6.5 mM ammonium acetate buffer) the analyte MDL 107292 is fully ionized. This is usually beneficial for good HILIC retention, the HILIC interaction taking place between approx. 2.5 % and 30 % [52] aqueous content. It should be noted that other types of separation mechanisms may contribute to the separation as well, most notably ionic exchange interactions.

Conclusions

Difficulties associated with the preparation of specific phase II drug metabolites can be varied and challenging. However, to ensure patient safety, the potential toxicological implications of phase II metabolites have to be evaluated, as requested by the FDA, and thus these synthetic challenges need to be overcome. We have summarized methods for the preparation of phase II metabolites which have proved to be successful in our laboratory for the synthesis of selected *O*-glucuronides and sulfates of recent drug development candidates. The *O*-acyl-glucuronide **7** of AVE0897 **1** was prepared under Königs-Knorr conditions followed by basic deacylation and enzymatic saponification. *O*-Glucuronide **12** of AVE2268 **8** was synthesized by a two step oxidation strategy. The synthesis of SAR7226 *O*-glucuronide **21** encompassed 8 linear steps and used a divergent protecting group strategy. In the synthesis of sulfate **28** a $NaBH_4$-activated palladium-catalyzed reductive deallylation process was developed in order to overcome catalyst deactivation resulting from traces of sulfur impurities. Sulfate MDL107292 **33,** a metabolite of MDL100907, was synthesized in 9 steps, demonstrating the possibility for applying a reductive debenzylation as final deprotection step. Analytical release of phase II metabolites proved to be highly challenging due to the typical very polar nature of these compounds. Different analytical

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approaches were discussed based on the examples 1-*ß*-*O*-acyl glucuronide of AVE 0897 **7** and sulfate MDL107292 **33**.

Experimental Section

General. ^{1}H (300, 500 MHz) and ^{13}C (75, 125 MHz) NMR spectra were obtained on Bruker spectrometers in the solvents indicated. All experiments were carried out under argon in dried glassware, using syringe-septum cap techniques. Column chromatography was carried out using Merck Kieselgel 60 silica gel (particle size: 63-200 µm). The purity of the products was determined by a LC-MS system with a symmetry Shield RP18 column, 3,9 x 150 mm with gradient program; conditions: mobile phase: A: water (900 mL), acetonitrile (100 mL), TFA (1 mL) mobile phase B: water (100 mL), acetonitrile (900 mL), TFA (0.75 mL), flow 1,5 mL/min, detection UV at 254 nm and 210 nm. Commercially available chemicals and solvents were used as obtained.
HRMS spectra were recorded on a Bruker µOTOF-QII instrument equipped with an ESI source. The sample was introduced in 0.1% formic acid / acetonitrile or water / acetonitrile using a syringe pump at 3-4 µL / min. Standard resolution MS spectra were recorded on a Bruker Esquire 3000 ion trap instrument at identical conditions.
Analytical HPLC was performed either on a Waters Alliance 2695 or a Dionex Summit instrument equipped with variable wavelength UV- or DAD-detectors using the conditions given at the individual compound sections below.

[Methoxy-$^{13}C^{2}H_3$]-methyl 1-*O*-[2-[(1*R*,3*S*)-3-[2-(3-methoxyphenyl)-5-methyloxazol-4-yl-methoxy]-cyclohexyloxymethyl]-6-methylbenzoic acid]-2,3,4-tri-*O*-acetyl-β-D-glucopyran-uronate (5). In a flask filled with argon, **[methoxy-$^{13}C^{2}H_3$]-AVE0897 (1)** (469 mg, 1.00 mmol) was dissolved in DMSO (8 mL). Cesium carbonate (326 mg, 1.00 mmol) and acetobromo-α-D glucuronic acid methyl ester (532 mg, 1.34 mmol) were added with stirring. The reaction mixture was stirred for 3.5 h at 30 °C. After complete conversion (LC-MS and TLC, ethyl acetate/heptane 3:2) water (8 mL) was added and the mixture extracted three times with ethyl acetate (15 mL). The combined organic layers were washed with water (20 mL), sodium bicarbonate solution (8 wt% in water, 20 mL) and brine (20 mL). The organic phase was dried over Na_2SO_4 and evaporated *in vacuo* to obtain **[methoxy-$^{13}C^{2}H_3$]-5** (729 mg, 0.93 mmol, 93% yield) as a colorless oil; ^{1}H-NMR (300 MHz, ACN-d_3 - D_2O 3:1): δ = 0.96-1.08 (m, 4H), 1.64 (m, br, 1H), 1.86 (m br, 2H), 1.96-2.0 (m, 9H), 2.22 (s, 3H), 2.32 (s, 3H), 2.37 (m, 1H), 3.17-3.34 (m, 2H), 3.63 (s, 3H), 4.38 (m, 2H), 4.48 (s, 2H), 5.08-5.19 (m, 2H), 5.42 (t, *J* = 9.4 Hz, 1H), 6.11(d, *J* = 8.1 Hz, 1H), 7.0 (dd, *J* = 9.0, *J* = 2.5 Hz, 1H), 7.19 (m, 2H), 7.32 (t, *J* = 7.6 Hz, 1H) 7.38 (t, *J* = 8.1 Hz, 7.46 (s, 1H), 7.50 (t, *J* = 7.7 Hz, 1H) ppm; ^{13}C-NMR (75 MHz, ACN-d_3 - D_2O 3:1): δ = 10.42, 14.42, 19.70, 20.75, 20.85, 20.99, 21.28, 32.20, 32.24, 39.02, 53.78, 55.40 (sept. *J* = 21.7 Hz, O$^{13}CD_3$), 61.76, 68.36, 69.97, 70.90, 72.51, 73.18, 76.56, 76.95, 92.64,

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101.00, 111.75 (d, *J* = 4.5 Hz), 117.35 (d, *J* = 4.5 Hz), 119.25, 127.29, 129.40, 130.95, 131.31, 131.41, 131.80, 134.06, 136.66, 137.70, 148.38, 169.32, 160.87, 168.14, 168.33, 170.97, 171.40, 171.59 ppm; MS (ESI^+) *m/z* (%): 786.3 [$M+H^+$] (100), 808.3 [$M+Na^+$] (60); HRMS (ESI^+): *m/z* = 808.3067 [calc. for $C_{39}{}^{13}CH_{44}{}^{2}H_3NO_{15}$ + Na: 808.3059].

[Methoxy-$^{13}C^2H_3$]-methyl 1-*O*-[2-[(1*R*,3*S*)-3-[2-(3-methoxyphenyl)-5-methyloxazol-4-yl-methoxy]-cyclohexyloxymethyl]-6-methylbenzoic acid]-β-D-glucopyranuronate (6). In a flask filled with argon, **[methoxy-$^{13}C^2H_3$]-5** (726 mg, 0.93 mmol) was dissolved in dry methanol (8 mL). 220 µL N,N-diisopropylethylamine (1.1 mmol, 1.72 eq.) was added and the reaction mixture stirred for 72 h at room temperature. After complete conversion (LC-MS and TLC, dichloromethane/methanol/glacial acetic acid 950:50:1), the solvent was removed *in vacuo* and the residue purified by column chromatography on silica gel eluting with dichloromethane/methanol/glacial acetic acid 950:50:1. Selected fractions containing the product were pooled and concentrated *in vacuo* to obtain **[methoxy-$^{13}C^2H_3$]-6** (549 mg, 0.83 mmol, 89 % yield) as a colorless solid; ^{1}H-NMR (300 MHz, ACN-d_3 - D_2O 3:1): δ = 0.9-1.1 (m, 4H), 1.65 (m, br, 1H), 1.78-1.96 (m, 2H), 2.24 (s, 3H), 2.28 (s, 3H), 2.32 (m, 1H), 3.18-3.28 (m, 2H), 3.50-3.57 (m, 3H), 3.69 (s, 3H), 4.03-4.09 (m, 1H), 4.36 (s, 2H), 4.52 (q, *J* = 8.3 Hz, 2H), 5.74 (d, *J* = 7.98 Hz, 1H), 6.98 (dd, *J* = 8.2 Hz, *J* = 1.98 Hz, 1H), 7.13-7.20 (m, 2H), 7.24-7.30 (m, 1H), 7.37 (t, *J* = 8.07 Hz, 1H), 7.42 (m, 1H), 7.46 (d, 7.74, 1H) ppm; ^{13}C-NMR (75 MHz, ACN-d_3 - D_2O 3:1): δ = 10.37, 19.73, 21.11, 31.93, 32.02, 36.66, 53.58, 55.43 (sept. *J* = 21.75 Hz, $O^{13}CD_3$), 61.49, 68.45, 72.04, 72.86, 76.19, 76.41, 76.51, 76.93, 95.41, 111.77 (d, *J* = 4.5 Hz), 117.42 (d, *J* = 4.5 Hz), 127.35, 129.03, 131.04, 131.21, 131.34, 132.35, 133.58, 136.93, 137.01, 148.63, 160.40, 160.66, 169.02, 170.54 ppm; MS (ESI^+) *m/z* (%): 660.3 [$M+H^+$] (100), 682.3 [$M+Na^+$] (50); HRMS (ESI^+): *m/z* = 660.2939 [calc. for $C_{33}{}^{13}CH_{38}{}^{2}H_3NO_{12}$ + H: 660.2923].

[Methoxy-$^{13}C^2H_3$]-1-*O*-[2-[(1*R*,3*S*)-3-[2-(3-methoxyphenyl)-5-methyloxazol-4-yl-methoxy]-cyclohexyloxymethyl]-6-methylbenzoic acid]-β-D-glucupyranuronic acid (7). **[methoxy-$^{13}C^2H_3$]-6** (549 mg, 0.83 mmol) was dissolved in isopropanol (4 mL), 2.5 mM pH 7 phosphate buffer (24 mL) and subsequently Candida antarctica lipase (CAL-B) (820 mg) were added. The reaction mixture was stirred for 36 h at room temperature. After complete conversion (LC-MS and TLC, dichloromethane/methanol/glacial acetic acid 950:50:1), isopropanol was removed *in vacuo*. Water (10 mL) was added and the mixture was extracted with ethyl acetate (3x10 mL). The combined organic layers were washed with water (10 mL) and brine (10 mL). The organic phase was dried over Na_2SO_4 and evaporated *in vacuo* to obtain crude **[methoxy-$^{13}C^2H_3$]-7** (491 mg, (0.76 mmol, 91% yield) as a colorless solid with a purity of 94% (LC-MS). The crude product was further purified by preparative HPLC (column Phenomenex Gemini C18, 250 x 50 mm; 10 µm particle size; flow rate: 100 ml/min; eluent A: TFA 0.05%, eluent B acetonitrile, gradient program). Fractions containing the product (LC-MS) were pooled and neutralized by addition of bicarbonate solution. The product was precipitated by evaporation of the organic solvent, filtered off and dried over $CaCl_2$ *in vacuo* to afford **[methoxy-$^{13}C^2H_3$]-7** (239 mg, 0.37 mmol, 45% yield) as a colorless amorphous solid with a purity of 99.8%, (analytical HPLC conditions: HPLC-column: Uptisphere, 150x 4.6mm, 5µm particle size. eluent

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A: water/acetonitrile/TFA 900:100:1 (v/v/v). eluent B: water/acetonitrile/TFA 100:900:0.75 (v/v/v); flow rate: 1 ml/minute; UV detection wavelength: 276 nm; injection volume: 10 µl. sample solvent: acetonitrile; column temperature: 30 °C; sampler temperature: 15 °C; gradient profile: 0 min/ 10% eluent B, 5 min/ 45% eluent B, 20 min/ 65% eluent B, 25 min/ 100% eluent B, 32 min/ 100% eluent B, 35 min/ 10% eluent B, 40 min/ 10% eluent B); ^{1}H-NMR (300 MHz, ACN-d_3 - D_2O 3:1): δ = 0.96-1.14 (m, 4H), 1.68 (m, 1H), 1.88 (m, 1H), 1.94 (m, *J* = 2.4 Hz, 1H), 2.29 (s, 3H), 2.33 (s, 3H), 2.37 (m, 1H), 3.28 (m, 2H), 3.40-3.60 (m, 3H), 3.98 (m, 1H), 4.40 (s, 2H), 4.56 (q, *J* = 13.2 Hz, 2H), 5.76 (d, *J* = 7.9 Hz, 1H), 7.02 (dd, *J* = 8.2 Hz, *J* = 2.4 Hz, 1H), 7.20 (m 2H), 7.32 (t, *J* = 7.6 Hz, 1H), 7.39 (t, *J* = 8.9 Hz, 1H, 7.46 (s, 1H), 7.50 (t, *J* = 7.7 Hz, 1H) ppm; ^{13}C-NMR (75 MHz, ACN-d_3 - D_2O 3:1): δ = 10.40, 19.82, 21.19, 32.09, 32.15, 39.03, 55.45 (sept, *J* = 21.7 Hz, O^{13}CD$_3$), 61.64, 68.57, 72.13, 72.99, 76.48, 76.77, 77.17, 95.53, 111.80 (d, *J* = 4.5 Hz), 117.53 (d, *J* = 4.5 Hz), 119.35, 127.42, 129.27, 131.03, 131.17, 131.37, 132.63, 133.82, 137.00, 137.32, 148.54, 160.48, 160.84, 169.01 ppm; MS (ESI$^+$) *m/z* (%): 646.3 [M+H$^+$] (100)**;** MS (ESI$^-$) *m/z* (%): 644.2 [M-H$^+$] (100); HRMS (ESI$^+$): *m/z* = 646.2773 [calc. for $C_{32}{}^{13}CH_{36}{}^{2}H_3NO_{12}$ + H: 646.2767].

2-Benzyl-4-(4-methoxybenzyl)-5-trifluoromethyl-2*H*-pyrazol-3-(4-desoxy-4-fluoro-6-*O*-tert-butyl-dimethylsilyl)-β-D-glucopyranosid (17). Tri-*O*-benzoyl-protected compound **16**, (7.2 g, 8.6 mmol) MeOH (150 mL) and 30% NaOMe in MeOH (1.5 mL) were stirred at 20-23 °C for 2 h. During the reaction the white suspension turned to a clear colorless solution. After complete conversion (tlc, ethyl acetate/heptane 2:1) saturated aqueous NH_4Cl solution (50 mL) was added; the resultant mixture was filtered and evaporated *in vacuo*. Addition of water and threefold extraction with ethyl acetate, drying of the combined organic phases with Na_2SO_4 and evaporation to dryness yielded 7.3 g of crude *N*-benzyl-protected SAR7226 (along with benzoic acid methyl ester). For regioselective silylation, this material was dissolved in dichloromethane (75 mL). Then triethylamine (2.4 mL, 17.1 mmol) and a catalytic amount of 4-dimethylamino-pyridine (DMAP) were added. The mixture was stirred at at 20-23 °C and a solution of TBDMS chloride (2.5 g, 16.5 mmol) in dichloromethane (4 mL) was added. After 30 h the conversion of the starting material was almost complete. Water (25 mL) was added and the pH was adjusted to 3–4 with saturated aqueous $KHSO_4$. The phases were separated; the organic phase was washed with brine and water. Drying of the combined organic phases with Na_2SO_4 and evaporation *in vacuo* gave 9 g of crude **17**. Chromatography on silica gel (n-heptane/ethyl acetate 1:0 → 1:2) followed by evaporation gave **17** (4.65 g, 7.3 mmol, 84% yield) as a sticky, almost colorless foam; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.05 (s, 6H), 0.89 (s, 9H), 2.52 (br, 1H), 2.73 (br, 1H), 3.11 (m, 1H), 3.53 (t, *J* = 8.6 Hz, 1H), 3.62 (dt, *J* = 15.1, 9.1 Hz, 1H), 3.73 (s, 2H), 3.78 (s, 3H), 3.90 (m, 2H), 4.43 (dt, *J* = 50.5, 9.1 Hz, 1H), 4.50 (d, *J* = 7.7 Hz, 1H), 5.30 (d, *J* = 15.3 Hz, 1H), 5.41 (d, *J* = 15.3 Hz, 1H), 6.81 (d, *J* = 8.7 Hz, 2H), 7.05 (d, *J* = 8.7 Hz, 2H), 7.22 (d, *J* = 6.9 Hz, 2H), 7.28-7.36 (m, 3H) ppm; MS (ESI$^-$): *m/z* (%) = 360.9 (31), 685.1 (100) [M+HCOO$^-$]; HRMS (ESI$^+$): *m/z* = 663.2479 [calc. for $C_{31}H_{40}F_4N_2O_6Si$ + Na: 663.2489].

2-Benzyl-4-(4-methoxybenzyl)-5-trifluoromethyl-2*H*-pyrazol-3-(2,3-di-*O*-benzyl-4-desoxy-4-fluoro)-β-D-glucopyranosid (18). 6-*O*-TBDMS-protected compound **17** (4.6 g, 7.2 mmol)

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was dissolved in THF (5 mL), and NaH (1.70 g, 42.5 mmol) was added and the mixture stirred for 45 min at 20-23 °C. To the resulting white suspension, benzyl bromide (3.5 mL, 29.4 mmol) was added over a period of 10 min. After stirring for 4 h saturated aqueous NH_4Cl was added, the phases were separated, the aqueous phase was extracted several times with ethyl acetate and the combined organic phases were dried (Na_2SO_4) and evaporated *in vacuo*. The crude 6-*O*-TBDMS-2,3-*O*-benzyl-protected compound (9.5 g, $\leq$ 7.1 mmol) was used for the next step without further purification and characterization. For desilylation this material was dissolved in THF (100 mL) and treated with Bu_4NF (2.4 g, 7.6 mmol) with stirring at 20-23 °C. After 90 min saturated aqueous NH_4Cl solution was added, the phases were separated, the organic phase was washed with water, dried (Na_2SO_4), evaporated *in vacuo* and purified by chromatography on silica gel (ethyl acetate/n-heptane 1:3 → 1:0) yielding **18** (4.2 g, 5.8 mmol, 80%) as a colorless foam; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.89 (m, 1H), 3.47 (s, 2H), 3.51 (t, *J* = 8.3 Hz, 1H), 3.56 (dt, *J* = 15.3, 8.9 Hz, 1H), 3.76 (s, 3H), 3.82 (d, *J* = 16.6 Hz, 1H), 3.94 (d, *J* = 16.6 Hz, 1H), 4.46 (dt, *J* = 50.2, 9.1 Hz, 1H), 4.68 (d, *J* = 7.5 Hz, 1H), 4.71-4.85 (m, 4 H), 5.27 (d, *J* = 15.6 Hz, 1H), 5.30 (d, *J* = 15.6 Hz, 1H), 6.79 (d, *J* = 8.7 Hz, 2H), 7.01 (d, *J* = 8.7 Hz, 2H), 7.16-7.23 (m, 4H), 7.26-7.39 (m, 11H) ppm; MS (ESI$^-$): *m/z* (%) = 360.9 (11), 571.0 (29), 751.2 (100) [M+HCOO$^-$]; HRMS (ESI$^+$): *m/z* = 729.2544 [calc. for $C_{39}H_{38}F_4N_2O_6$ + Na: 729.2558].

2-Benzyl-4-(4-methoxybenzyl)-5-trifluoromethyl-2*H*-pyrazol-3-(2,3-di-*O*-benzyl-4-desoxy-4-fluoro)-β-D-glucopyranosyl-(2,3,4-tri-*O*-pivaloyl)-β-D-glucopyranosiduronic acid (20). The 6-OH-deprotected compound **18** (740 mg, 1.1 mmol) dissolved in dichloromethane (5 mL) was added under argon to a solution of glucuronyl donor **19** (890 mg, 1.6 mmol) in dichloromethane (5 mL) over 4Å molecular sieves. After stirring for 30 min at 20-23 °C the slightly yellow solution was cooled to -12 °C (ice/ethanol) and AgOTf (400 mg, 1.5 mmol) added. The reaction mixture was stirred for 5 h at -12 to -10 °C the color changing from slightly yellow to blue-purple. After filtration and purification of the filtrate by chromatography on silica gel (elution with ethyl acetate/toluene 1:8) the fully protected disaccharide **20** (870 mg; 0.75 mmol) was isolated by evaporation as a colorless solid; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.05 (s, 9H), 1.07 (s, 9H), 1.10 (s, 9H), 3.54 (t, *J* = 8.3 Hz, 1H), 3.62 (s, 3H), 3.68 (s, 3H), 3.60-3.93 (m, 6H), 4.43-4.76 (m, 6H), 4.85 (m, 2H), 4.99 (t, *J* = 9.6 Hz, 1H), 5.13 (d, *J* = 7.5 Hz, 1H), 5.26 (d, *J* = 15.5 Hz, 1H), 5.36 (d, *J* = 15.5 Hz, 1H), 5.44 (m, 1H), 6.78 (d, *J* = 8.5 Hz, 2H), 6.96 (d, *J* = 8.5 Hz, 2H), 7.05. (d, *J* = 7.5 Hz, 2H), 7.14-7.38 (m, 13H) ppm; MS (ESI$^+$): *m/z* (%) = 1149.4 (100) [M+H$^+$]; HRMS (ESI$^+$): *m/z* = 1149.4942 [calc. for $C_{61}H_{72}F_4N_2O_{15}$ + H: 1149.4948].

4-(4-Methoxybenzyl)-5-trifluoromethyl-2*H*-pyrazol-3-(4-desoxy-4-fluoro)-β-D-glucopyranosyl β-D-glucopyranosiduronic acid (21). Fully protected disaccharide **20 (**460 mg, 0.4 mmol) was dissolved in MeOH (5 mL), treated with 5-10% Pd/C (140 mg , water content 54%, Degussa), and the reaction mixture was hydrogenated at 35 °C/5 bar for 15 h. The catalyst was removed by filtration, washed with MeOH and dichloromethane, and the combined organic solutions were evaporated under reduced pressure. 350 mg of a white foam were isolated.

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Chromatography on silicagel (elution with ethyl acetate/n-heptane 2:1) gave the pure debenzylated product (290 mg) as a colorless solid.

140 mg of this debenzylated ester-protected disaccharide were dissolved in methanol (15 mL) and water (15 mL). $Ba(OH)_2$ (200 mg, 1.2 mmol) was added and the reaction mixture was stirred at 20-23 °C for 2 days. To completely remove the pivaloyl groups, further $Ba(OH)_2$ (100 mg), methanol (30 mL) and water (10 mL) were added and the reaction mixture was stirred at 50 °C for 2 days. After complete saponification, 1 N H_2SO_4 (1.2 mL) was added (pH ~ 5-6) and the resulting precipitate filtered off, then washed with water and methanol. Filtrate and washings were combined and evaporated *in vacuo* yielding crude **21** (160 mg). Chromatography on silica gel (elution with dichloromethane/methanol 9:1 → 4:1 → 2:1) yielded a colorless solid (110 mg) which was further purified by preparative HPLC using a YMC Pro C18 RS column (5 µm, 150 × 20 mm; flow rate 10 mL/min; gradient: 15 min, water-CH_3CN 90:10 –> 50:50). Two batches of **21** were isolated: 52 mg with a purity of 98.6% and 44 mg with 93.2% purity; ^{1}H NMR (500 MHz, CD_3CN : D_2O 1:1 + 0.1% DCOOD) δ = 3.21 (dd, *J* = 9.2, 8.0 Hz, 1H), 3.36 (t, *J* = 9.1 Hz, 1H), 3.45 (m, 2H), 3.67 (d, *J* = 9.8 Hz, 1H), 3.7-3.8 (m, 8H), 4.02 (m, 1H), 4.35 (d, *J* = 7.9 Hz, 1H), 4.38 (dt, *J* = 50.5, 8.9 Hz, 1H), 5.04 (d, *J* = 7.9 Hz, 1H), 6.82 (d, *J* = 8.7 Hz, 2H), 7.10 (d, *J* = 8.7 Hz, 2H) ppm; ^{13}C NMR (75 MHz, CD_3CN : D_2O 1:1 + 0.1% DCOOD) δ = 26.41, 56.04, 68.70, 72.67, 73.36, 73.48, 73.63, 73.96, 74.34, 74.58, 76.18, 76.52, 88.15, 90.55, 102.63, 103.87, 106.23, 114.80, 130.15, 130.39, 133.13, 158.65 ppm; MS (ESI^+): *m/z* (%) = 273.0 (20), 613.3 (100) [$M+H^+$], 635.3 (28) [$M+Na^+$], 651.3 (11) [$M+K^+$]; HRMS (ESI^+): *m/z* = 635.1465 [calc. for $C_{24}H_{28}F_4N_2O_{12}$ + Na: 635.1471].

(1-Benzylpiperidin-4-yl)-(2-methoxy-3-triisopropylsilanyloxyphenyl)methanone (31). The lithiated TIPS-protected guajacole **30** was prepared by adding tert-butyllithium (1.5 M in pentane, 14.3 mL, 21.6 mmol) to a solution of TIPS-protected guajacole (2.00 g, 7.14 mmol) in diethylether at 0 °C. The solution was stirred for 45 min at 0 °C and a solution of Weinreb amide **29** dissolved in diethylether (10 mL) was added via cannula. The reaction mixture was allowed to warm to room temperature and was quenched with ammonium chloride solution (25 mL) after 16 hours. The organic phase was washed with brine (2 x 25 mL) and dried over magnesium sulfate. Filtration, followed by evaporation of solvent gave the crude product as a yellow oil which was purified by column chromatography on silica gel, eluted with heptane/ethyl acetate 3:1) to give **31 (**1.1 g; 2.28 mmol, 32%) as a colorless oil. ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.80-0.82 (m, 18H), 1.01-1.07 (m, 6H), 1.23-1.41 (m, 4H), 2.23-2.37 (m, 2H), 2.81-2.88 (m, 2H), 3.48 (s, 3H), 7.16-7.23 (m, 8H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 12.83, 17.86, 27.80, 29.12, 29.92, 36.03, 37.23, 52.98, 63.10, 76.57, 120.75, 123.03, 124.08, 127.12, 128.22, 129.17, 129.25, 137.81, 148.75, 149.41, 213.53 ppm; MS (ESI^+) *m/z* (%): 482 [$M+H^+$] (100); HRMS (ESI^+): *m/z* = 482.3085 [calc. for $C_{29}H_{44}NO_3Si$ + H: 482.3012]

(1-Benzylpiperidin-4-yl)-(2-methoxy-3-hydroxyphenyl)methanone (32). Compound **31 (**1.1 g, 2.28 mmol) was dissolved in ethanol (4 mL) and conc. HCl (2 mL) was added at room temperature. The mixture was heated to 90 °C for 2 h and then evaporated to dryness *in vacuo*. The residue was dissolved in ethanol and again evaporated to dryness *in vacuo*. The residue was

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washed with n-heptane (20 mL) and adjusted to pH 5 with 2N NaOH. The product was extracted with dichloromethane (3 x 30 mL), and the combined organic phases were dried over Na_2SO_4 and the solvent was evaporated *in vacuo* to give **32 (**704 mg, 2.17 mmol, 95%) as a colorless solid. ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.78-0.0.97 (m, 2H), 1.00-1.23 (m, 2H), 1.72-1.87 (m, 3H), 2.12-2.35 (m, 2H), 2.85-2.90 (m, 2H), 3.00-3.09 (br s, 1H, OH), 3.75 (s, 3H, OMe), 6.86 (dd, *J* = 7.4, 2.1 Hz, 1H), 6.93 (dd, *J* = 8.0, 7.4 Hz, 1H), 6.98 (dd, *J* = 8.0, 2.1 Hz, 1H), 7.21-7.32 (m, 5H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 29.10, 51.47, 62.61, 62.87, 77.20, 100.00, 118.82, 120.19, 124.87, 127.79, 128.46, 129.71, 132.47, 145.15, 149.37, 205.17 ppm; MS (ESI^+.) *m/z* (%): 326 [$M+H^+$] (100); HRMS (ESI^+): *m/z* = 326.1751 [calc. for $C_{20}H_{24}NO_3$ + H: 326.1678].

Sulfuric acid mono-[2-methoxy-3-(1-piperidin-4-yl-ethyl)phenyl]ester (33). Phenol **32** (913 mg, 2.80 mmol) was dissolved in dichloromethane (15 mL) and triethylamine (7.6 mL). At room temperature, sulfurtrioxide triethylamine complex (2.56 g, 14.0 mmol) was added in portions as a solid. The reaction mixture was stirred at room temperature for two days and the progress of the reaction was followed by LC-MS. Then the solvent was evaporated *in vacuo* and the residue was purified by chromatography on silica gel, eluted with dichloromethane/methanol (5:1) to give) the sulfate (1.09 g, 2.69 mmol) as an orange oil. This material was used without further purification in the next reaction step.

The above sulfate (1.09 g, 2.69 mmol) was dissolved in methanol (10 mL) and sodium borohydride (1.07 g, 26.9 mmol) was added in portions. The mixture was stirred for 16 h at room temperature (LC-MS control). After complete conversion, water (10 mL) was added and the mixture was extracted three times with dichloromethane/n-butanol (5:1, 3 x 40 mL) . The combined organic phases were dried over Na_2SO_4 and the solvent removed *in vacuo* to give the crude product as a colorless oil which was directly used in the next reaction step.

Under argon atmosphere Pd/C (5% Pd, 40 mg, Degussa) and ammonium formate (1.55 g, 24.5 mmol) were suspended in methanol (10 mL) and at room temperature the crude oil dissolved in methanol (2 mL) was added dropwise. The reaction mixture was stirred for 3 h at room temperature and 5 h at 40 °C. The catalyst was removed by filtration and the filtrate was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel eluted with dichloromethane/methanol/NEt_3 (3:1:0.1) to give **33 (**435 mg, 1.37 mmol) asa colorless solid. Final purification and removal of salts were accomplished by HPLC under RP conditions: HPLC-column Luna-C18, 150 x 25 mm, 5µm particle size. Eluent A: water. Eluent B: acetonitrile; flow rate: 14 ml/minute; UV detection wavelength: 210 nm.

Analytical HPLC: modified reversed phase method: column: Waters Symmetry C18, 150x 3mm, 5µm particle size. Eluent A: 0.1% TFA in water. Eluent B: water/acetonitrile/TFA 100:900:0.75 (v/v/v). Flow rate: 0.8 ml/minute. UV detection wavelength: 210 and 269 nm. Injection volume: 10 µl. Sample solvent: eluent A. Column temperature: 20 °C. Sampler temperature: 15 °C.

Gradient profile: 0 min/ 1% eluent B, 10 min/ 1% eluent B, 15 min/ 15% eluent B, 20 min/ 99% eluent B, 21 min/ 99% eluent B, 22 min/ 1% eluent B, 27 min/ 1% eluent B. HILIC-method: column: TSOH, TSKgel Amide-80, 250x 4,6mm, 5µm particle size. Eluent A: 6.5 mM

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ammonium acetate in water / acetonitrile 900:100. Eluent B: acetonitrile. Flow rate: 1 ml/minute. UV detection wavelength: 210 and 269 nm. Injection volume: 10 µl. Sample solvent: acetonitrile/water 1:1 (v:v). Column temperature: 30 °C. Sampler temperature: 20 °C. Gradient profile: 0 min/ 100% eluent B, 20 min/ 75% eluent B, 25 min/ 0% eluent B, 26 min/ 100% eluent B, 30 min/ 100% eluent B. ^{1}H-NMR (500 MHz, DMSO-d_6): δ = 1.40-1.47 (m, 2H), 1.72-1.75 (m, 1H), 1.80-1.84 (m, 1H), 2.75-2.81 (m, 2H), 3.19-3.30 (m, 2H), 3.81 (s, 3H), 4.66 (d, *J* = 6 Hz, 1H), 5.18 (br s, 1H, OH), 6.95 (dd, *J* = 7.8, 3.9 Hz, 1H), 7.02 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.43 (dd, *J* = 7.9, 1.6 Hz, 1H), 8.25 (s, 1H) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 24.12, 25.12, 43.17, 50.97, 52.77, 60.46, 69.15, 119.88, 121.23, 122.40, 136.86, 145.84, 147.38 ppm; MS (ESI$^+$) *m/z* (%): 318 [M+H$^+$] (100); HRMS (ESI$^+$): *m/z* = 340.0832 [calc. for $C_{13}H_{19}NO_6S$ + Na: 340.0831].

Glossary

Ac	Acetyl,
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DMAP	Dimethylaminopyridine
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimid
HATU	2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluoro-phosphate
HOBT	1-Hydroxybenzotriazole
NMM	N-Methyl-morpholine
SGLT	Sodium dependent Glucose transport
TEMPO	2,2,6,6-Tetramethylpiperidin-1-yl)oxyl
Oxon	Potassium peroxymonosulfate
TBDPS	tert-Butyl-diphenylsilyl
TBDMS	tert-Butyl-dimethylsilyl
TIPS	Triisopropylsilyl
TFA	Trifluoroacetic acid
TEA	triethylamine

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