Glucuronide Production by Whole-Cell Biotransformation Using Genetically Engineered Fission Yeast Schizosaccharomyces pombe

Călin-Aurel Drăgan, Daniela Buchheit, Daniel Bischoff, Thomas Ebner, and Matthias Bureik

ABSTRACT:

Drug metabolites generated by UDP glycosyltransferases (UGTs) are needed for drug development and toxicity studies, especially in the context of safety testing of metabolites during drug development. Because chemical metabolism synthesis can be arduous, various biological approaches have been developed; however, no whole-cell biotransformation with recombinant microbes that express human UGTs was yet achieved. In this study we expressed human UDP glucose-6-dehydrogenase together with several human or rat UGT isoforms in the fission yeast Schizosaccharomyces pombe and generated strains that catalyze the whole-cell glucuronidation of standard substrates. Moreover, we established two methods to obtain stable isotope-labeled glucuronide metabolites: the first uses a labeled aglycon, whereas the second uses $^{13}$C$_6$-glucose as a metabolic precursor of isotope-labeled UDP-glucuronic acid and yields a 6-fold labeled glucuronide. The system described here should lead to a significant facilitation in the production of both labeled and unlabeled drug glucurononides for industry and academia.

The metabolic steps that lead to drug clearance in the human body are divided into two distinct parts, which encompass chemical modifications of the parent compound (phase I) and conjugations of parent or phase I metabolites with endogenous molecules (phase II). As a huge majority of the 200 most prescribed drugs in the United States are metabolized in the human body (Williams et al., 2004), the synthetic or biosynthetic accessibility of drug metabolites is a prerequisite for drug development and toxicity studies. According to current knowledge, cytochrome P450 (P450) systems are most important for phase I (Bernhardt, 2005; Ingelman-Sundberg et al., 2007) and UDP glycosyltransferases (UGTs) for phase II reactions (Mackenzie et al., 2005), respectively. The human UGT superfamily consists of four families with 22 isoenzymes: the 19 members of the UGT1 and UGT2 families (the latter encompassing subfamilies 2A and 2B) are primarily involved in xenobiotic metabolism and efficiently use UDP glucuronic acid (UDP-GA) for the conjugation of drugs to glucuronic acid (Mackenzie et al., 2005). UGT3A1 was recently shown to be a UDP-N-acetylgalactosaminyltransferase that also appears to have a function in drug metabolism (Mackenzie et al., 2008); the catalytic activity of UGT3A2 is not yet known; and UGT8A1 catalyzes the transfer of galactose from UDP galactose to ceramide (Bosio et al., 1996). Similar to many P450s, some drug-metabolizing UGT isoforms (e.g., UGT1A1, UGT1A6, UGT1A9, and UGT2B15) display polymorphisms with a demonstrated association between genotype and clinical pharmacokinetics (Katz et al., 2008). Many UGTs are expressed in the liver, but other drug entry points such as epithelial surfaces of the nasal mucosa, gut, skin, brain, prostate, uterus, breast, placenta, and kidney also host UGT activity (Tukey and Strassburg, 2000). UGT enzymes are integrally associated with the membranes of the endoplasmic reticulum, possibly with most of the protein oriented toward the luminal side, and may form homo- and hetero-oligomeric structures (Bock and Köhle, 2009).

The formation of glucuronide metabolites may pose toxicological problems to patients via increased drug activity on glucuronidation (Coler et al., 2009) or cause dosage complications as a result of the uncontrolled rerelease of parent aglycones by systemic or enteric adducts to proteins. Such protein adducts have been discussed as a cause for idiosyncratic adverse drug reactions (Bailey and Dickinson, 2003). In addition, acyl glucuronides can exhibit chemical reactivity and can readily form covalently bound adducts to proteins. Such protein adducts have been discussed as a cause for idiosyncratic adverse drug reactions (Bailey and Dickinson, 2003). The identification and structure elucidation of drug glucuronides can be accomplished with milligram amounts, but safety testing may demand gram quantities. Because classic chemical synthesis of glucuronide metabolites can be cumbersome, various biological techniques have been developed to this aim, which include metabolite isolation from animal body fluids, the use of liver homogenates or liver microscale cultures, and whole-cell biotransformation with recombinant microbes that express human UGTs.

This work is part of the following patent application: Dragan C-A, Bureik M, and Buchheit D (2008) Drug metabolism. European patent application EP 08 164 826.3.

ABBREVIATIONS: P450, cytochrome P450; UGT, UDP glycosyltransferase; UDP-GA, UDP glucuronic acid; 4MU, 4-methylumbelliferone; T, testosterone; TG, testosterone glucuronide; 4MUG, 4-methylumbelliferone-β-D-glucuronide; HPLC, high-performance liquid chromatography; UGDH, UDP glucose-6-dehydrogenase; EMM, Edinburgh minimal medium; LC/MS, liquid chromatography/mass spectrometry; FTMS, Fourier transform mass spectrometry; EIC, extracted ion currents.
enzyme preparations obtained after recombinant expression of metabolizing enzymes in cell culture or microbial systems (Radominska-Pandya et al., 2005; Khetani and Bhatia, 2008). However, each of these methods has its specific drawbacks. For example, human liver microsomes contain native UGTs, but their use may be hampered by their relative scarce availability, batch-to-batch variations, and the absence of UGTs that are only expressed in other tissues (Tukey and Strassburg, 2000; Jia and Liu, 2007). Heterologous expression of human UGTs in various mammalian or insect cell lines was very helpful for the determination of basic kinetic parameters of the enzymes, but it suffers from low expression levels, low activity normalized to biomass, and stability problems (Radominska-Pandya et al., 2005; Trubetskoy et al., 2008). Although functional UGT expression in baker’s yeast has been shown per se, glucuronide production could only be achieved after preparation of yeast microsomes and addition of UDP-GA. The use of the costly cofactor UDP-GA at considerable concentrations in existing preparative methods seems to be necessary because of the endoplasmic membrane barrier, which limits the entry of UDP-GA in the lumen. In addition, the use of microsomal preparations implies the presence of many UGT isoforms and of other systems that may reduce the yield of the desired glucuronide product by cofactor competition.

Whole-cell biotransformations with recombinant microbes offer many advantages with respect to scalable metabolite production, and corresponding expression systems for human P450s have thus been established in bacteria and yeasts (Ghisalba and Kittelmann, 2007; Pischkeid and Glieder, 2008). In recent years, we showed the usefulness of recombinant strains of the fission yeast Schizosaccharomyces pombe that express human P450s for the production of P450 metabolites of illicit drugs (Peters et al., 2009) and doping substances (Zöllner et al., in press). Because no glucuronidation by whole-cell biotransformation with a unicellular organism that recombinantly expresses human UGTs was yet reported, it was the aim of this study to develop such a system using S. pombe.

Materials and Methods

Fine Chemicals. 4-Methylumbellif erone (4MU), testosterone (T), and testosterone glucuronide (TG) potassium salt were purchased from Sigma-Aldrich (Hamburg, Germany); 4-methylumbellif erone-β-D-glucuronide (4MUG) dihydrate was from Carl Roth (Karlsruhe, Germany); and 13C6-glucose was from Euriso-Top (Saint-Aubin, France). Deuterated T was synthesized by Toromac Organics Ltd. (Saarbrücken, Germany). All the other chemicals used were either from Carl Roth or Sigma-Aldrich. Methanol [high-performance liquid chromatography (HPLC) grade] was from Thermo Fisher Scientific (Waltham, MA).

Coding DNA Sequences. The cDNAs of human UGT1A1, UGT1A9, UGT2A1, UDP-glucose-6-dehydrogenase (UGDH), and/or rat UGT1A7 were synthesized by Entelechon GmbH (Regensburg, Germany); cDNAs of human UGT1A6, UGT1A7, UGT1A8, UGT1A10, UGT2B15, and UGT2B17 were synthesized by GENEART GmbH (Regensburg, Germany).

Media and General Techniques. We used general DNA-manipulating methods, media, and genetic methods for fission yeast as described previously (Drăgan et al., 2005). In addition, we used Edinburgh minimal medium (EMM) containing 100 g/l glucose for biotransformation assays and EMM containing 20 g/l 13C6-glucose for the synthesis of isotope-labeled glucuronides.

Construction of Fission Yeast Strains. UGT-cDNAs were cloned via NdeI and BamHI into the integrative vector pCAD1 (Drăgan et al., 2005) that integrates into the leu1 gene of the fission yeast genome, thereby compensating an ura4 defect. UGDI cDNA was cloned into the expression vector pREP1 (Maundrell, 1993) using NdeI and BamHI yielding pREP1-UGDH. The correctness of all the constructs was verified by automatic sequencing (MWG-Biotech, Ebersberg, Germany). The construction of fission yeast strains expressing functional UGTs was done in two steps. pCAD1-UGT constructs were prepared, before transformation, as reported previously (Drăgan et al., 2005), and used to transform yeast strainNCYC2036 (h ura4-D18). Transformation was done, using competent cells prepared as described elsewhere (Suga and Hatakeyama, 2005), and yielded strains CAD200, DB1, DB3, DB5, DB23, DB24, DB25, DB26, DB32, and DB33. Correct integration into the leu1 locus was verified by selection of leucine auxotrophs on EMM dishes containing 5 μM thiamine but no leucine. Subsequently, strains containing an integrated UGT expression cassette were transformed with pREP1-UGDH as described previously (Okazaki et al., 1990) to yield strains CAD203, DB11, DB13, DB15, DB43, DB44, DB45, DB46, and DB52, and DB53. Both fission yeast expression vectors used in this study contain the strong endogenous nmt1 promoter (Maundrell, 1990, 1993) that permits expression regulation via the presence or absence of thiamine in the medium. Therefore, transformed cells were selected by plating on EMM dishes with 5 μM thiamine to allow better growth under repressed conditions. All the yeast strains used in this study are available from PomBioTech GmbH (Saarbrücken, Germany).

Biomass Production. All the cultures were set up in absence of thiamine to induce expression of the nmt1 promoter. Incubation was carried out at 30°C and 150 rpm. Ten milliliters of EMM containing the appropriate supplements and lacking thiamine was inoculated with cells grown on a plate for 3 days and incubated to stationary phase; these cells were then used to inoculate 100 ml main cultures. Main cultures were incubated for 1 to 2 days for the parental strainsNCYC2036, CAD200, DB1, DB3, DB5, DB23, DB24, DB25, DB26, DB32, and DB33 and 2 to 5 days for the coexpressing strains CAD203, DB11, DB13, DB15, DB43, DB44, DB45, DB46, DB52, and DB53.

Whole-Cell Biotransformation Assay. The biomass was centrifuged (3000g, 5 min, room temperature) and resuspended in 12 ml of EMM with 100 g/l glucose and supplements as required. Substrate was added to a final concentration of 500 μM by adding 600 μl of 10 mM stock solutions in EMM. The biotransformations were carried out in 250-ml wide-neck Erlemeyer flasks at 72 h at 30°C and 150 rpm. Sample volumes of 2 ml were taken at 0 and 72 h and centrifuged (10,000g, 5 min, room temperature). The cell pellets were used to determine the biomass dry weight, whereas the supernatants were centrifuged again and then used for HPLC and liquid chromatography/mass spectrometry (LC/MS) analyses. All the results shown were obtained in at least three independent experiments.

Synthesis of Isotope-Labeled Glucuronide Metabolites. Because of the high cost of the labeled compounds, these experiments were performed at 1-ml scale. For the comparison of the biosynthesis of labeled 4MUG with that of unlabeled 4MUG, the cultivation of the cells was done in EMM containing either 13C6-glucose or unlabeled glucose at a concentration of 20 g/l. Five milliliters of medium without thiamine was inoculated with cells of strain DB13 and incubated for 1 day at 30°C and 150 rpm. One milliliter of this culture was then used to set up a 10-ml main culture, which in turn was incubated for 3 days under the same conditions. The biomass was harvested by centrifugation (3000g, 5 min, room temperature), and cells were resuspended in 1 ml of EMM containing a final concentration of 500 μM 4MUG (50 μl of an ethanolic 10 mM 4MUG stock solution). The assay was carried out in the same manner as described above. For the synthesis of stable isotope-labeled TG, the biotransformation assay was carried out with strain DB53 as described before using T doubly deuterated at C-2, deuterated in β and α positions at C-4 and C-6, respectively, and deuterated at the C-17’s hydroxyl group as substrate. In the acidic fission yeast medium, rapid exchange of the D atom in the OD group at C-17 leads to the 4-fold labeled substrate. All the samples were prepared as described above and analyzed by HPLC and LC/MS.

HPLC Analysis. HPLC was performed using a Series II 1090 system (Hewlett Packard, Palo Alto, CA) equipped with a Lichrospher 100 column (50 × 4.6 mm, RP-18, 5 μM; Merck, Darmstadt, Germany) and a diode array detector. The flow rate was 1 ml/min, and the column temperature was 40°C. For the simultaneous detection of 4MU and 4MUG, the initial mobile phase composition was 85% acetic acid (0.1%) (A) and 15% methanol (B). B was linearly increased to 55% from 5 to 10 min, maintained at 55% for further 2 min, and then immediately returned to 15% until the end of the run at 15 min. The eluents were monitored at 320 nm. The retention times of 4MU and 4MUG were 12.4 and 8.4 min, respectively. Quantification of 4MUG was done using an external 4MUG standard, prepared in the initial mobile phase at a concentration of 200 μM.

For the simultaneous detection of T and TG, the initial mobile phase composition was 85% acetic acid (0.1%) (A) and 15% methanol (B). B was...
Although the formation of UDP glucose in yeasts is known, wild-type baker’s yeast does not produce UDP-GA (Oka and Jigami, 2006), and the fission yeast genome contains no open reading frame that shows significant homology to mammalian UGDH enzymes (Wood et al., 2002). Still, the presence of an endogenous UGDH activity as a result of a nonhomologous fission yeast protein could not a priori be ruled out. However, incubation of human UGT-expressing strains (Table 1) with either 4MU (Uchaipichat et al., 2004) or T (Sten et al., 2009) as standard substrate did not lead to detectable glucurondization (data not shown). Therefore, for the development of a whole-cell biotransformation process, an endogenous source of UDP-GA had to be provided by coexpression of UGDH (see Fig. 2). A synthetic cDNA coding for human UGDH was cloned into the autonomously replicating expression vector pREP1 (Maundrell, 1993) to yield the new plasmid pREP1-UGDH. Transformation of all the UGT-expressing strains with this plasmid yielded a new set of 10 UGT- and UGDH-coexpressing strains (Table 1; Fig. 1). These new strains showed significantly reduced growth under induced expression conditions and yielded biomass concentrations between 1 and 2 g/l after 2 to 5 days in contrast to biomass yields between 2 and 3 g/l for strains that only express UGTs (data not shown). Whole-cell biotransformation assays showed that all the coexpressing strains were able to catalyze the glucuronidation of either 4MU or T (Table 2). The identity of the products 4MUG and TG was in each case confirmed by LC/MS analysis (data not shown). As could be expected, the biotransformation activities of the different UGT-expressing strains toward 4MU varied from approximately 1 μM/day for UGT1A1 and UGT1A6, respectively, up to 150 μM/day in the case of UGT1A9. In terms of specific production rates (i.e., normalized to the biomass dry weight), the highest values of almost 20 μmol/day/g were achieved by the UGT1A9- and UGT2A1-expressing strains, respectively, whereas the strains expressing UGT1A1, UGT1A6, and rat UGT1A7, respectively, yielded only values around or even less than 0.1 μmol/day/g. Strain DB53 expressing human UGT2B17 produced TG with a maximum space-time yield of 32.6 mol/day/g. Strain DB53 expressing human UGT1A8 and UGT2A1-expressing strains, respectively, whereas the strains expressing UGT1A1, UGT1A6, and rat UGT1A7, respectively, yielded only values around or even less than 0.1 μmol/day/g. Strain DB53 expressing human UGT2B17 produced TG with a maximum space-time yield of 32.6 mol/day/g and a maximum specific production rate of 3.1 μmol/day/g. To the best of our knowledge, these data show for the first time a successful whole-cell biotransformation using recombinant human UGTs expressed in a unicellular organism.

The availability of stable isotope-labeled glucuronides is a prerequisite for the sensitive quantification of glucuronide metabolites in biological matrices by LC/tandem MS, e.g., for the toxicokinetic
monitoring of glucuronides during nonclinical safety studies. Therefore, having established a functional in vivo system for the production of glucuronides, we investigated the possibility to produce isotope-labeled glucuronide metabolites by applying two different strategies. First, either nonlabeled T or 4-fold deuterated T (D4-T) were subjected to whole-cell biotransformation with strain DB53 that coexpresses UGT2B17 and UGDH (Fig. 3a). The comparison of the LC/MS analysis of the extracted ion currents (EICs) of nonlabeled and 4-fold labeled TG, as well as the respective fragmentation spectra, unambiguously shows the successful formation of D4-TG (Fig. 3, **TABLE 2**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Expressed Protein(s)</th>
<th>Substrate</th>
<th>Product</th>
<th>Space-Time Yield</th>
<th>Specific Production Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Min</td>
<td>Max</td>
</tr>
<tr>
<td>DB11</td>
<td>UGT1A1, UGDH</td>
<td>4MU</td>
<td>4MUG</td>
<td>0.5</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.04</td>
<td>0.14</td>
</tr>
<tr>
<td>DB43</td>
<td>UGT1A6, UGDH</td>
<td>4MU</td>
<td>4MUG</td>
<td>0.6</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.04</td>
<td>0.22</td>
</tr>
<tr>
<td>DB44</td>
<td>UGT1A7, UGDH</td>
<td>4MU</td>
<td>4MUG</td>
<td>8.2</td>
<td>18.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.85</td>
<td>2.10</td>
</tr>
<tr>
<td>DB45</td>
<td>UGT1A8, UGDH</td>
<td>4MU</td>
<td>4MUG</td>
<td>5.9</td>
<td>23.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.43</td>
<td>2.50</td>
</tr>
<tr>
<td>CAD203</td>
<td>UGT1A9, UGDH</td>
<td>4MU</td>
<td>4MUG</td>
<td>64.2</td>
<td>151.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12.10</td>
<td>19.70</td>
</tr>
<tr>
<td>DB46</td>
<td>UGT1A10, UGDH</td>
<td>4MU</td>
<td>4MUG</td>
<td>7.0</td>
<td>18.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.56</td>
<td>1.50</td>
</tr>
<tr>
<td>DB13</td>
<td>UGT2A1, UGDH</td>
<td>4MU</td>
<td>4MUG</td>
<td>22.8</td>
<td>114.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.00</td>
<td>19.50</td>
</tr>
<tr>
<td>DB52</td>
<td>UGT2B15, UGDH</td>
<td>4MU</td>
<td>4MUG</td>
<td>3.3</td>
<td>7.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.24</td>
<td>0.63</td>
</tr>
<tr>
<td>DB53</td>
<td>UGT2B17, UGDH</td>
<td>4MU</td>
<td>4MUG</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>DB53</td>
<td>UGT2B17, UGDH</td>
<td>T</td>
<td>TG</td>
<td>15.5</td>
<td>32.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.20</td>
<td>3.10</td>
</tr>
<tr>
<td>DB15</td>
<td>rUGT1A7, UGDH</td>
<td>4MU</td>
<td>4MUG</td>
<td>2.3</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.16</td>
<td>0.19</td>
</tr>
</tbody>
</table>

*Table 2* Glucuronide metabolite formation rates determined in whole-cell biotransformations of fission yeast strains coexpressing human UGDH and mammalian UGT enzymes.

Fig. 1. Fission yeast strain construction scheme. The procedure is exemplarily shown for UGT1A1. The uracil-deficient parental strain NCYC2036 was transformed using the integrative plasmid pCAD1-UGT1A1. The resulting leucine-deficient strain DB1 was in turn transformed with the autosomal plasmid pREPl-UGDH yielding strain DB11. For all the other UGT isoforms, the cloning procedure was done accordingly.

Fig. 2. Schematic representation of the engineered glucuronide biosynthetic pathway in recombinant fission yeast strains. The scheme shows in black the endogenous pathway that leads to the formation of UDP glucose with branching points to several pathways of the central carbon metabolism; reactions depicted in blue indicate the reaction steps introduced into the host by the heterologous coexpression of human UGDH and a mammalian UGT. Glucose (1) enters the cell and is converted to glucose 6-phosphate (2) by hexokinase (HK), which can be interconverted to glucose 1-phosphate (3) by phosphoglucomutase (PGM). The latter is conjugated with UDP by UDP glucose pyrophosphorylase (UGPase) to yield UDP glucose (4). Human UGDH oxidizes 4 to UDP-GA (5). The latter then enters the lumen of the endoplasmic reticulum. Eventually, the heterologously expressed mammalian UGT performs the conjugation of 5 to an aglycon (R) that passes both plasma and endoplasmic membrane to yield a glucuronide metabolite (6). The glucuronide metabolite in turn leaves both the endoplasmic reticulum and the cell.
Second, we intended to show a more general labeling technique by using $^{13}$C isotope-labeled glucuronic acid as substrate and strain DB13 (expressing UGT2A1 and UGDH). Based on the biosynthesis scheme outlined above (Fig. 2), which leads to the endogenous formation of UDP-GA in UGDH-expressing fission yeast strains, we used $^{13}$C-glucose as metabolic precursor of 6-fold labeled UDP-GA (Fig. 3). For this purpose, $^{13}$C-glucose was added throughout all the culturing periods before performing the biotransformation to deplete
Discussion

In this study, 10 fission yeast strains were cloned that express either one of nine human UGTs or rat UGTA17, respectively (Table 1). As fission yeast does not have an endogenous UGDH enzyme (Wood et al., 2002), neither of these strains displayed glucuronidation activity toward 4MU or T because of a lack of UDP-GA (data not shown). Therefore, a second set of strains was created that coexpress the UGTs mentioned above together with human UGDH. In addition to the intracellular production of UDP-GA as such, its subcellular localization also had to be considered: in human cells, UDP-GA is formed by UGDH in the cytoplasm and subsequently transported by nucleotide sugar transporters into the lumen of the endoplasmic reticulum, where the UGTs are located (Kobayashi et al., 2006). If after expression in fission yeast the subcellular localization of both UGTs and UGDH corresponds to their targeting in mammalian cells, then for biotransformation to occur at least one of the endogenous nucleotide sugar transporters (such as vgs4 or hut1) (Nakanishi et al., 2001) must be able to transport UDP-GA into the endoplasmic reticulum lumen. This seems to be the case, because all the coexpressing strains were able to catalyze the glucuronidation of either 4MU or T (Table 2). Time-space yield of the different UGT-expressing strains toward 4MU varied by roughly 2 orders of magnitude, from 

References


using human cytochrome P450 isozymes heterologously expressed in fission yeast. *Bioanalyti-
sis* 1:821–830.
metabolites: kinetic differences between generated and preformed metabolites. *Toxicol Appl
Pharmacol* 217:143–152.
*Microb Cell Fact* 7:25.
Radominska-Pandya A, Bratton S, and Little JM (2005) A historical overview of the heterolo-
gous expression of mammalian UDP-glucuronosyltransferase isoforms over the past twenty
UDP-glucuronosyltransferases (UGTs) 2B7 and UGT2B17 display converse specificity in
testosterone and epitestosterone glucuronidation, whereas UGT2A1 conjugates both andro-
Suga M and Hatakeyama T (2005) A rapid and simple procedure for high-efficiency lithium
acetate transformation of cryopreserved Schizosaccharomyces pombe cells. *Yeast* 22:799–
804.
Tukey RH and Strassburg CP (2000) Human UDP-glucuronosyltransferases: metabolism,
Turfas SC, Parkin MC, Cowan DA, Halket JM, Smith NW, Braithwaite RA, Elliot SP, Steventon
GB, and Kicman AT (2009) Use of human microsomes and deuterated substrates: an alter-
native approach for the identification of novel metabolites of ketamine by mass spectrometry.
*Drug Metab Dispos* 37:1769–1778.
JO (2004) Human UDP-glucuronosyltransferases: isoform selectivity and kinetics of 4-meth-
ylumbelliferone and 1-naphthol glucuronidation, effects of organic solvents, and inhibition by
Williams JA, Hyland R, Jones BC, Smith DA, Hurst S, Goosen TC, Petikerin V, Kouf JR, and
Ball SE (2004) Drug-drug interactions for UDP-glucuronosyltransferase substrates: a phar-
macokinetic explanation for typically observed low exposure (AUCi/AUC) ratios. *Drug Metab
415:871–880.
Zöllner A, Parr MK, Dragan C-A, Dras S, Schlörer N, Peters F, Maurer HH, Schänzer W, and
Bureik M CYP21-catalyzed production of the long term urinary metandienone metabolite
17β-hydroxymethyl-17α-methyl-18-norandrosta-1,4,13-trien-3-one: a new contribution to the

Address correspondence to: Matthias Bureik, PomBioTech GmbH, Campus Geb.
A1, D-66123 Saarbrücken, Germany. E-mail: matthias.bureik@pombiotech.com

GLUCURONIDE PRODUCTION BY UGT-EXPRESSING FISSION YEAST

515

Downloaded from dmd.aspetjournals.org at ASPET Journals on June 24, 2017

515