An ortho $C$-methylation/$O$-glycosylation motif on a hydroxy-coumarin scaffold, selectively installed by biocatalysis

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1. Experimental Section

Solubility of 4,5,7-trihydroxy-3-phenyl-coumarin (1) in presence of DMSO
The solubility of 1 was tested in 50 mM phosphate buffer (pH 6.5) supplemented with 5, 10 or 20% DMSO. At each DMSO concentration 1 was added to final concentrations of 0.5, 1 and 2 mM. After overnight incubation on a thermomixer at 1000 rpm and 35°C, samples were centrifuged for 15 min at 13200 rpm to remove undissolved 1. The concentration of dissolved 1 in the supernatant was determined by HPLC.

Substrate inhibition kinetics
The dependence of NovO and UGT71A15 reaction rates on 1 concentration was fitted by Equation 1 to account for the effect of substrate inhibition. Experimentally determined reaction rates ($r$) at distinct concentrations of 1 ($c_1$) were used. The theoretically attainable maximum rate in absence of substrate inhibition ($r_{\text{max}}$), the Michaelis–Menten constant ($K_M$) and the inhibition constant ($K_I$) were fitted. $IC_{50}$ values represent concentrations of 1 where inhibition by 1 decreased the reaction rate to 50% of the highest experimentally observed rate.

$$r = \frac{r_{\text{max}} + c_1}{K_M + c_1 \left(1 + \frac{c_1}{K_I}\right)}$$

Equilibrium of the UGT71A15 reaction
The equilibrium constant ($K_{eq}$) of 1 glucosylation by UGT71A15 was calculated according to Equation 2. Concentrations of 1 ($c_1$) and 2 ($c_2$) were directly measured by HPLC and those for UDP and UDP-glc were inferred from conversion of 1 and reaction stoichiometry.

$$K_{eq} = \frac{c_2 \cdot c_{\text{UDP}}}{c_1 \cdot c_{\text{UDP-glc}}}$$
2. Results

**Fig. S1** Strep-tag affinity purified enzymes from *E. coli* overexpression cultures were analyzed by SDS-PAGE. S: PageRule™ Prestained Protein Ladder (Thermo Scientific); 1: UGT71A15 (53.9 kDa); 2: NovO (25.4 kDa).

**Fig. S2** Reversed phase C-18 HPLC with UV-detection at 318 nm was used to quantify compounds 1-4.
Fig. S3 The influence of DMSO on methylation of 1 by NovO was studied (100 µM 1, 1 mM SAM, 5 µg mL⁻¹ NovO, pH 6.5). A) Time courses of 2 formation are shown. The DMSO content was 5% (green), 10% (orange), 15% (grey) or 20% (black). Solid lines represent linear fits of the data. B) Relative reaction rates at various DMSO concentrations were calculated from the time courses shown in (A). The solid line represents a linear fit of the data.

Fig. S4 The pH-activity profiles of NovO (A) and UGT71A15 (B) are shown. Either phosphate (circles, solid lines) or HEPES buffer (squares, dashed lines) was used. A) 100 µM 1 were methylated from 1 mM SAM by 5 µg mL⁻¹ NovO. B) 1 mM 1 were glucosylated from 1.5 mM UDP-glc by 1 µg mL⁻¹ UGT71A15.
Fig. S5 $^1$H-NMR of purified 2.

Fig. S6 $^1$H-NMR of purified 3.
Fig. S7 $^{13}$C-NMR of purified 3.

Fig. S8 2D COSY-NMR of purified 3.
Fig. S9 2D HSQC-NMR of purified 3.

Fig. S10 2D HMBC-NMR of purified 3.
Fig. S11 $^1$H-NMR of purified 4.

Fig. S12 $^{13}$C-NMR of purified 4.
Fig. S13 2D COSY-NMR of purified 4.

Fig. S14 2D HSQC-NMR of purified 4.
Fig. S15 2D HMBC-NMR of purified 4.