

**Supplemental Information for**  
**Characterization of Dibenzo[*a,l*]pyrene-*trans*-11,12-diol**  
**(Dibenzo[*def,p*]chrysene) Glucuronidation by UDP-glucuronosyltransferases**

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Figure S1. Separation of DB[*a,l*]P-*trans*-11,12-diol racemic mixture and circular dichroism analysis.

Figure S2. Kinetic Analysis of individual UGT-over-expressing cell homogenates for DB[*a,l*]P-*trans*-11,12-diol.

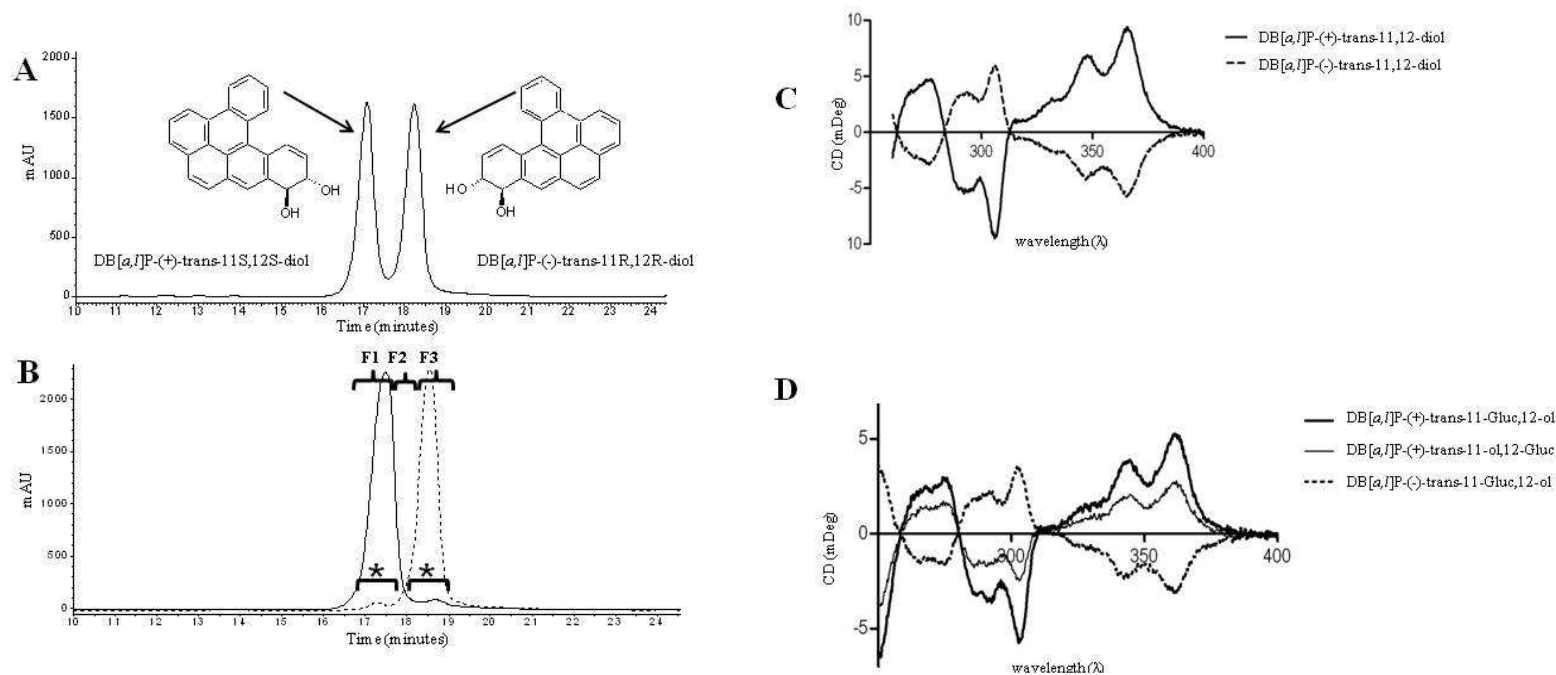
Figure S3. Selective NOE experiment for peak 1.

Figure S4. Selective NOE experiments HPLC peak 2.

#### **SUPPORTING INFORMATION PARAGRAPH**

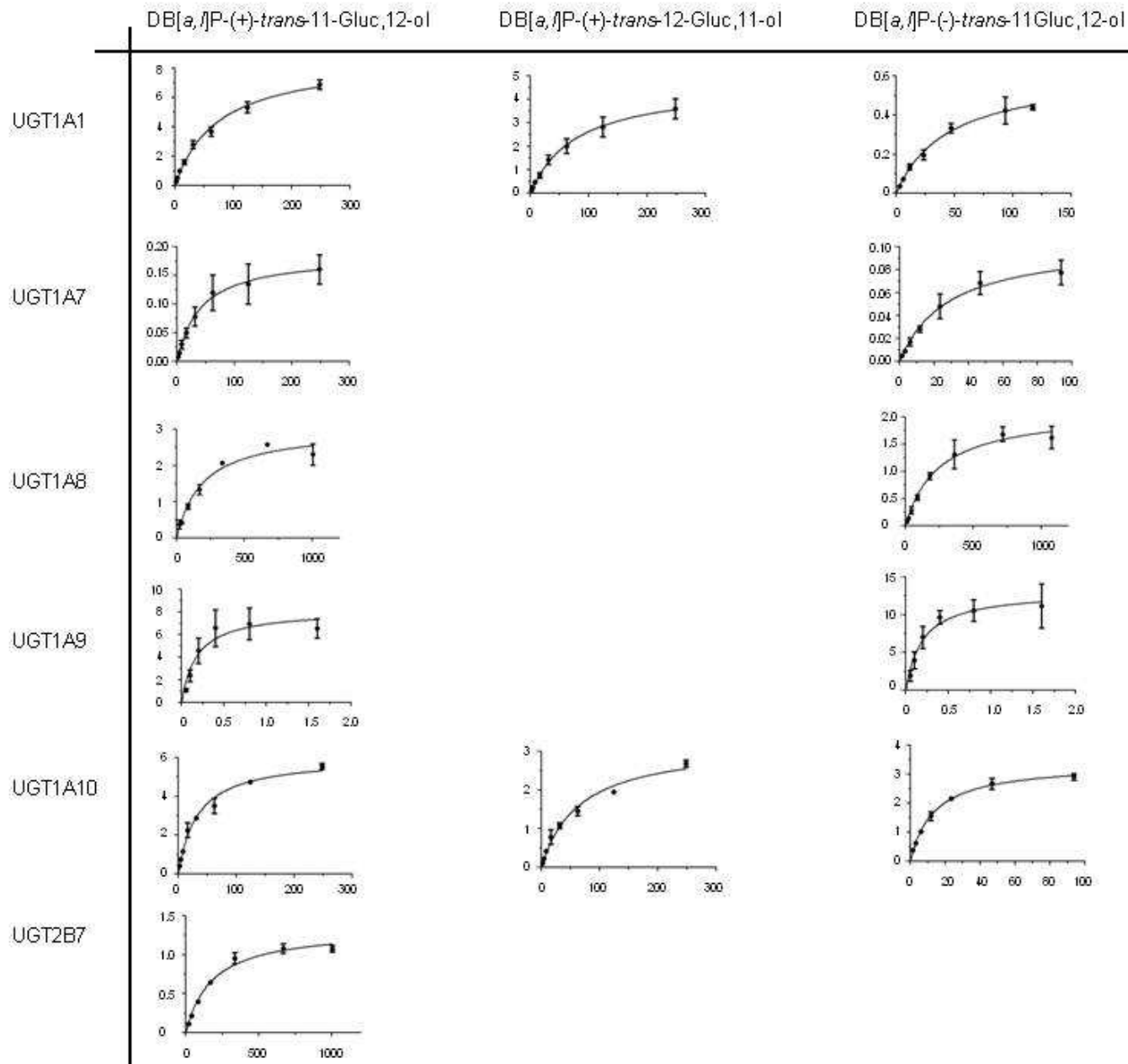
**Separation and identification of DB[*a,l*]P-*trans*-11,12-diol enantiomers.** We separated the racemic DB[*a,l*]P-*trans*-11,12-diol into the individual enantiomers using chiral chromatography (Figure S1A). The enantiomers were collected individually for use in glucuronidation assays (Figure S1B). To identify the stereochemistry of each enantiomer, samples of each were subjected to circular dichroism analysis (Figure S1C). In comparing our circular dichroism data with a previous publication<sup>35</sup>, the first peak was the (+)-*trans*-11*S*,12*S*-diol and the second peak was the (-)-*trans*-11*R*,12*R*-diol.

**Characterization of DB[*a,l*]P-*trans*-11,12-diol glucuronides.** We found that the CD spectrum of each glucuronide closely matched that of the parent compound from which it originated (Figure S1D), indicating that the glucuronides were generated from their respective parent compounds (Figure S1C).

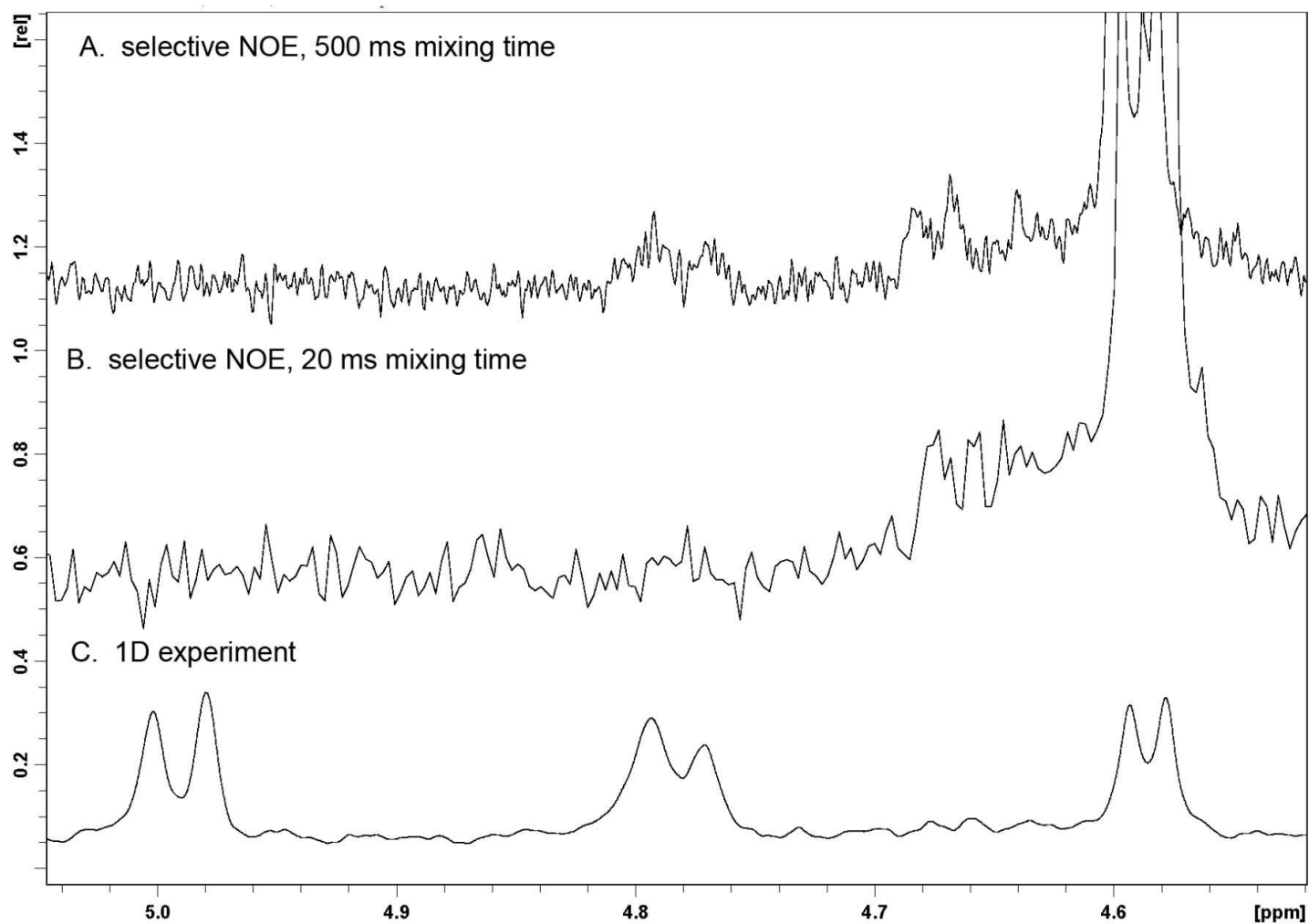


**Figure S1. Separation of DB[a,l]P-trans-11,12-diol racemic mixture and circular dichroism analysis.** A. Shown is a representative HPLC chromatogram of the separation of the DB[a,l]P-trans-11,12-diol racemic mixture using chiral chromatography. The (+)-trans enantiomer elutes first at 16 minutes and subsequently the (-)-trans enantiomer elutes at 17.5 minutes. B. The (+)-trans and (-)-trans enantiomers were collected in three fractions (F1, F2, F3) and reinjected to check for purity. Based on peak area, the contamination of the collected enantiomers was  $\leq 5\%$  (asterisk indicates the contamination peak). C. Circular dichroism analysis was performed for F1 and F3 to verify that they were (+)-trans and (-)-trans, respectively. Each enantiomer was dissolved in DMSO for analysis. D. Circular dichroism analysis of the three glucuronides show the spectrum of (+)-DB[a,l]P-11Gluc and (+)-DB[a,l]P-12Gluc closely follow the spectrum of the (+)-

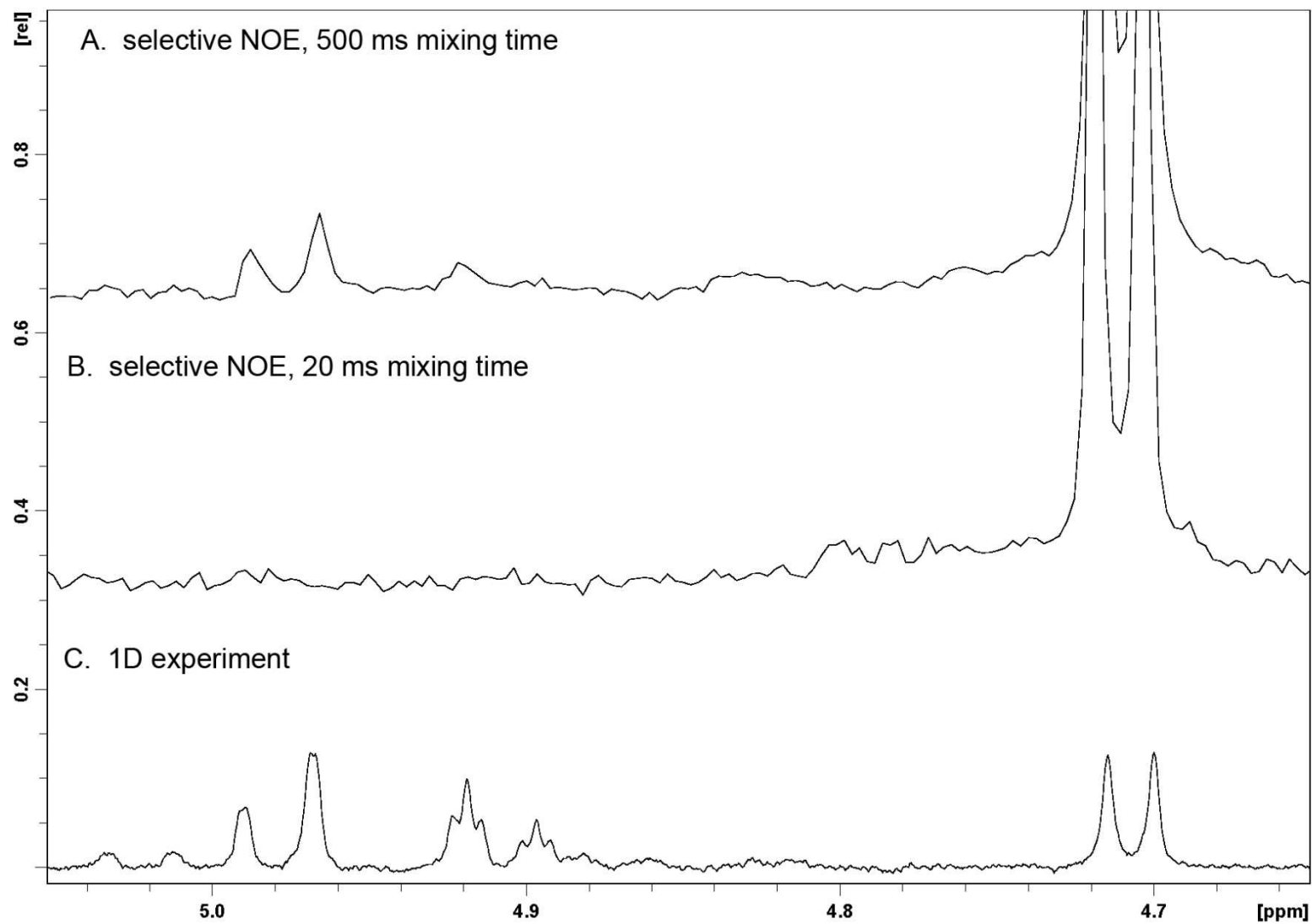
trans enantiomer from which they were derived and (-)-DB[*a,l*]P-11Gluc closely follows the spectrum of the (-)-trans enantiomer from which it was derived. The glucuronides were dissolved in ethanol for analysis.



**Figure S2. Concentration curves for DB[a,l]P-*trans*-11,12-diol-glucuronide formation with UGT-over-expressing cell homogenates.** Shown are Michaelis-Menten curves to obtain  $V_{\max}$  and  $K_M$  values for the formation of each glucuronide by individual UGTs. The x-axis represents the concentration of DB[a,l]P-11,12-diol ( $\mu\text{M}$ ) and the y-axis represents velocity of the reaction ( $\text{pmol}\cdot\text{min}^{-1}\cdot\mu\text{g}^{-1}$ ). Experimental conditions are described in the Materials and Methods section. All reactions were performed in triplicate.  $k_{\text{cat}}$  was calculated by adjusting for UGT expression according to western blot analysis.



**Figure S3. Selective NOE experiment for peak 1.** A. Irradiation of the 1'-proton at 4.59 ppm, with 500 ms mixing time. B. Irradiation of the 1'-proton at 4.59 ppm, with 20 ms mixing time. C. 1D experiment showing the chemical shifts of the 11, 12, and 1' protons. The increase in intensity of the peaks at 4.8 ppm in panel A versus panel B indicates that the 1' proton is closer to the protons at the 12-position than the proton at the 11-position.



**Figure S4. Selective NOE experiments HPLC peak 2** A. Irradiation of the 1'-proton at 4.74 ppm, with 500 ms mixing time. B. Irradiation of the 1'-proton at 4.74 ppm, with 20 ms mixing time. C. 1D experiment to show the chemical shifts

of the 11, 12, and 1' protons. The increase in intensity of the peaks at 4.98 ppm in panel A versus panel B indicates that the 1' proton is closer to the protons at the 11-position than the proton at the 12-position.