



Supplementary Figure S4: Enzymatic glycosylation reactions using *pNP-α-glucoside* (top) and *pNP-β-glucoside* (bottom) as acceptor substrate were performed in volumes of 20 μl (consisting of 1 mM of the respective *pNP*-glucoside, 10 mM of glycosyl fluoride **4a** or **4b**, and recombinant glycosynthases StspBGlcE383A or AgtuBGlcE358S (5 mg/mL) in 100 mM sodium phosphate buffer, pH 7.0). After 16 h at 37 $^{\circ}\text{C}$, the reactants were analyzed using a HPLC system equipped with a reversed-phase column (Cosmosil 5C18 MS-II 4.6 \times 250 mm, Nacalai Inc., Japan). The UV absorbance was measured at 300 nm. Solvent A was 50 mM aqueous ammonium formate buffer (pH 4.5), and solvent B was acetonitrile. The analytes were separated using a linear gradient between 25–50% of solvent B from 0–10 min at a flow rate set to 0.5 mL/min. Peak integration and other chromatographic calculations were performed using the Shimadzu Labsolutions software package (version 5.91).