



Figure S23. Effects of reaction buffer (a), reaction temperature (b), and divalent metal ions (c) on the activities of AmGT44. Maackiain (**2**) was used as the acceptor and UDP-Glc was used as the sugar donor. An optimized reaction time of 4 hour was used. AmGT44 exhibited its maximum activity at pH 7.0 (50 mM Na<sub>2</sub>HPO<sub>4</sub>-NaH<sub>2</sub>PO<sub>4</sub>) and 37°C.

To investigate the enzymatic properties of AmGT44, the pH, temperature and divalent metal ions were studied. All enzymatic reactions were carried out using UDP-Glc as the donor and maackiain (**2**) as the acceptor. The purified enzyme was added separately to the reaction solution and incubated at 37°C for 4 hours. To optimize the reaction pH, various buffers were utilized within different pH ranges: from 4.0-6.0 (citric acid-sodium citrate buffer), 6.0-8.0 (Na<sub>2</sub>HPO<sub>4</sub>-NaH<sub>2</sub>PO<sub>4</sub> buffer), 7.0-9.0 (Tris-HCl buffer), and 9.0-11.0 (Na<sub>2</sub>CO<sub>3</sub>-NaHCO<sub>3</sub> buffer). To determine the optimal reaction temperature, the reactions were carried out at different temperatures (4-70°C). For testing the influence of various divalent cations (Fe<sup>2+</sup>, Ca<sup>2+</sup>, Zn<sup>2+</sup>, Co<sup>2+</sup>, Ba<sup>2+</sup>, Mg<sup>2+</sup>, Ni<sup>2+</sup>), each cation was individually added to the reaction solution at a final concentration of 1 mM. The resulting mixtures were vacuum-dried, and the residue was dissolved in 150 μL methanol. Subsequently, the samples were centrifuged at 15,000 rpm for 30 minutes for UHPLC analysis.