Novel triple mutant of an extremophilic glycosyl hydrolase enables the rapid synthesis of thioglycosides

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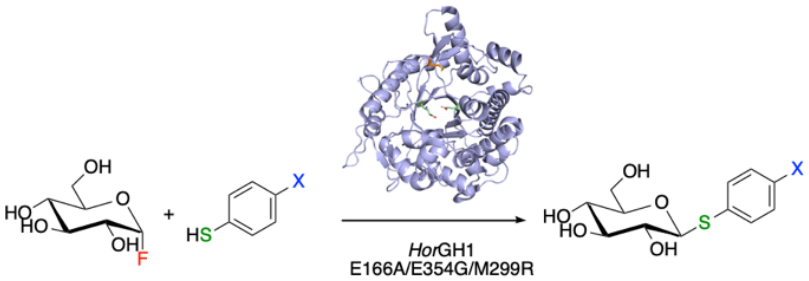
Due to their low susceptibility to hydrolysis, thioglycosides are highly interesting molecules that have been used as enzyme inhibitors and in carbohydrate-based therapeutics. A plethora of chemical syntheses to access thioglycosides have been reported, however, these procedures are complex, offer limited stereochemical control and often require non-sustainable reagents.[1] We previously reported the use of the β-glycosyl hydrolase (GH1; EC 3.2.1.21) from *Halothermothrix orenii* (*Hor*GH1), an extremophilic enzyme with a high tolerance to extreme temperatures, pHs and organic solvents. We also engineered this enzyme towards thioglycosidase activity and highlighted the key role of an arginine residue (M299R mutation) in the recognition of thioglycosides.[3]



Herein, we present the first example of an extremophilic glycosyl hydrolase engineered towards thioglycosynthase activity with a novel combination of mutations.[4] The triple mutant *Hor*GH1 M299R/E166A/E354G gave access to a range of high-value thioglycosides with exquisite stereoselectivity and good to excellent conversions (61-93%). Aside from being easy to handle and cofactor-independent, this robust catalyst remained active for up to 48 hours despite the presence of 30% DMSO. Overall, this works expands the repertoire of mutant glycosidases available for thioglycoside synthesis and provides an innovative, safe, green and profitable synthetic route for the construction of *S*-glycosidic linkages.

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[4] L. Pillet, D. Lim, N. Almulhim, A. I. Benítez-Mateos, F. Paradisi. Novel triple mutant of an extremophilic glycosyl hydrolase enables the rapid synthesis of thioglycosides. *Chem. Commun.*, **2022**, *58*, 12118-12121.