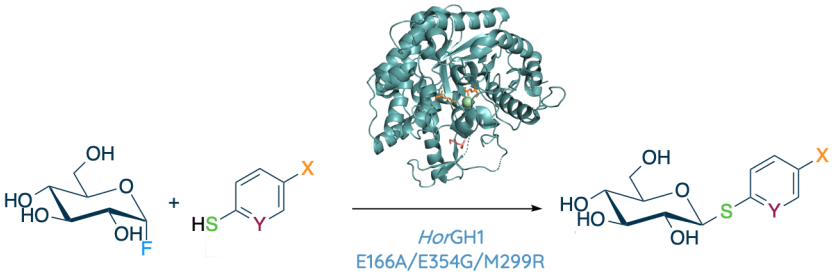
Novel triple mutant of an extremophilic glycosyl hydrolase enables the rapid synthesis of thioglycosides

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Due to their 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 synthesis to access thioglycosides have been reported, however, these procedure are complex, offer limited stereochemical control and often require non-sustainable reagents.[1] Selective and sustainable biosynthetic methods relying on mutant forms of glycosidases which have had their catalytic residues replaced by neutral ones have been reported. While thiooligosaccharides have been synthetized via these strategies,[2] enzymatic syntheses of thioglycoconjuguates are scarce. Moreover, attempts to translate these elegant proof-of-concepts to industrial processes are often plagued by the poor resistance of enzymes to the organic solvents that are often required to dissolve thiols. In order to expand the range of reaction conditions suitable for biocatalytic preparation of thioglycosides, we selected a 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 previously 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] Among the 8 *Hor*GH1 variants resulting from a combination of the E166A (acid/base residue), the E354G (nucleophilic residue) and the M299R mutation, 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 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

[1] K. Pachamuthu, R. R. Schmidt. *Chem Rev,* **2006**, 106, 160–187.
[2] M. Jahn, H. Chen, J. Müllegger, J. Marles, R. A. J. Warren, S. G. Withers. *Chem. Commun.,* **2004**, 4, 274–275.
[3] N. Almulhim, N. R. Moody, F. Paradisi. *Appl. Microbio.l Biotechnol.,* **2020**, 104, 4407–4415.
[4] L. Pillet, D. Lim, N. Almulhim, A. I. Benítez-Mateos, F. Paradisi. *Chem. Commun.,* **2022**, 58, 12118-12121.