Gram-scale chemoenzymatic synthesis of human milk oligosaccharides using crude cell lysate

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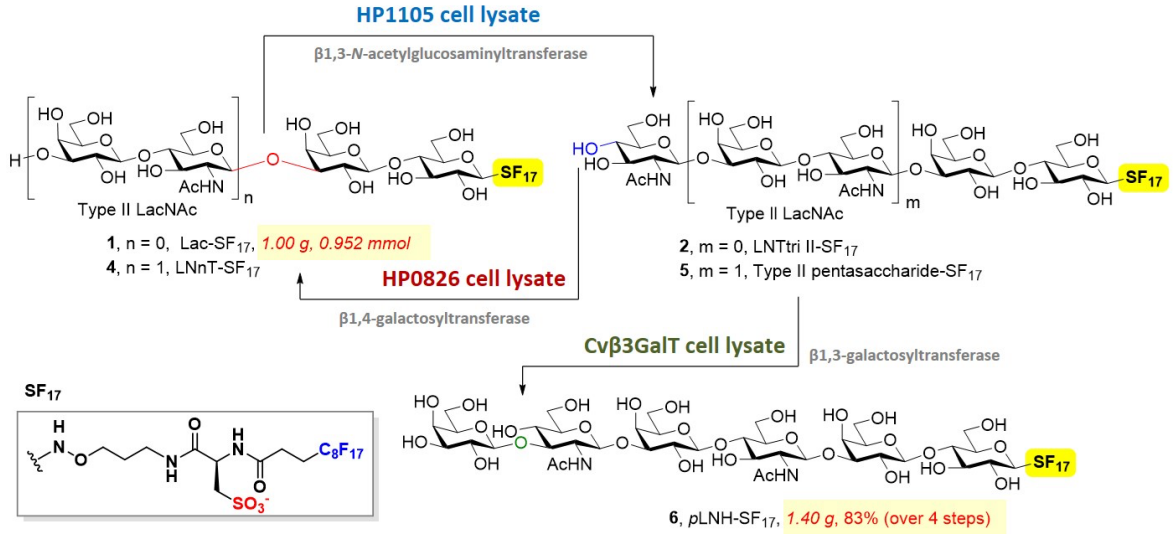
Human milk oligosaccharides (HMOs) are known to play an important role in living system such as prebiotic activity. The demand of pure materials for further studies rise approaches toward the synthesis of HMOs by chemical methods, microbial fermentations and enzymatic strategies. We recently reported a convenient enzymatic synthesis of fucosylated HMOs via appending a readily removable sulfo-fluorous affinity tag (**SF_{17}**) at the reducing end of glycan, which allows facile purification after enzymatic glycan extension.[1] Herein, we report a new function of the **SF_{17}**-tag that shows a glycosyl hydrolase-resistant property, which allow the glycosyltransferase-catalyzed reaction proceeding in the crude cell lysate without glycans decomposition. The preparative-scale synthesis (> 1 gram) of *para*-lacto-*N*-hexose (*p*LNH) and its derivatives including fucosyl *para*-lacto-*N*-hexose I, fucosyl *para*-lacto-*N*-hexose IV, difucosyl *para*-lacto-*N*-hexose I isomer, difucosyl *para*-lacto-*N*-hexose II and trifucosyl *para*-lacto-*N*-hexose I) were achieved, and so was the synthesis of A-antigen series HMOs (A antigen tetrasaccharide, hexasaccharide and heptasaccharide). The availability of these well-defined structures will provide valuable standards for the characterization and quantification of complex glycans isolated from nature, enables comprehensive screens for new prebiotic activities and the advances in exploring the HMO–gut microbiome relationship, those can significantly expedite the progress of tailor-made formula production in the future.



Gram-scale synthesis of para-human milk oligosaccharides by crude cell lysate of glycosyltransferases using the glycosyl hydrolase-resistant SF17-tagg

Bibliographic references:

[1] Y.-T. Huang, Y.-C. Su, H.-R. Wu, H.-H. Huang, E. C. Lin, T.-W. Tsai, H.-W. Tseng, J.-L. Fang, C.-C. Yu, (2021) ACS Catal. (11) 2631–2643.