

# Glycosylation in Food Applications

## Introduction

Whereas hydroxylation, methylation, fluorination etc. are in the mainstream of medicinal chemistry and ADMET thinking, attaching carbohydrates to improve molecules is considered rather more exotic or not at all (despite an abundance of examples of natural molecules that gain, alter or lose activity by glycosylation or de-glycosylation).

Even if this is considered, glycoside chemistry is difficult. Chemical glycosylation is not in itself straightforward, and since many interesting molecules have more than one or even many “glycosylatable” side groups (-OH, -COOH, -NH, -SH), all the other groups but the one in question need to be chemically blocked, a very tedious process.

Using small molecule glycosyl-transferase enzymes almost any given molecule (with appropriate side groups) can be glycosylated, most often regio-specifically (thus abolishing the need for side group blocking), in many cases even stereo-specifically and, if needed, with a number of different sugars (glucose, galactose, xylose, glucuronic acid, rhamnose etc.).

## Vanillin

Hansen et al. demonstrated that simple in vivo glycosylation of the aroma aldehyde Vanillin (VAN) to form Vanillin-glucoside (VAN-GLU) would turn this microbially toxic aldehyde into a non-toxic form. It is an attractive proposition to manufacture valuable small molecules by microbial fermentation, but in many cases such efforts stumble upon the toxicity of the molecule in question (often plant-derived) to the production organism. In the case of VAN, this molecule is in principle quite easily made at high titers in Baker's yeast, but it is toxic at low concentrations; a solution was needed. By introducing a specific Vanillin glycosyltransferase in the producing yeast, VAN was turned into VAN-GLU, which turned out to be completely non-toxic and could, therefore, be produced at very high titers. VAN could now be released by contacting with cheap commercial hydrolase enzyme preparations. This process is used in actual VAN manufacturing.

<https://www.ncbi.nlm.nih.gov/pubmed/19286778>

## Stevia

Olsson et al. showed that by utilizing appropriate natural glycosyltransferase enzymes one can successfully enable a manufacturing route not otherwise accessible - one which is rare and inefficient even in nature. Stevia sweeteners (from Stevia leaf) are marred by the fact that while the naturally abundant sweeteners (Rebaudioside A and Stevioside) are high intensity sweeteners, they also confer strong bitterness flavour to most people. Stevia leaf contains many other molecules with “higher degree of glycosylation”, and some of these do not confer bitterness but still sweetness. These molecules are only present in minute quantities in the leaf, however, and therefore extraction is not commercially viable. In this study a combination of Stevia and non-Stevia glycosyltransferase enzymes were combined to arrive at a commercially meaningful pathway for production of rare Stevia sweeteners by fermentation with yeast. The study also gives a good demonstration of how the intricacies of “higher level glycosylation” (2, 3, 4, 5, 6 or even more sugar molecules attached to a small molecule in various configurations) can give rise to widely different functionality.

<https://microbialcellfactories.biomedcentral.com/articles/10.1186/s12934-016-0609-1>



### What is the Gly-it platform?

The Gly-it platform is a library of 380 diverse “Family 1” UDP-glucose dependent glycosyltransferase enzymes (UGTs) plus associated screening, analytical and lab scale production protocols (“Family 1” denotes glycosyltransferases that will glycosylate small molecules).

All the enzymes in Gly-it are found in plants (which have diverse UGTs to work with the diverse range of small molecules that occur in plants or their environment). The kit contains enzymes from all known Family 1 UGT sub-families and sub-sub-families and from a wide set of evolutionarily diverse plants.

The majority of the enzymes will be able to add glucose to small molecule substrates with relevant functional groups. Some enzymes will work with other sugars (such as xylose, rhamnose, galactose or glucuronic acid). We can advise you on the best path for specific sugars.

### Anthocyanins

Osmani et al. investigated the effect of glucuronidation (attaching glucuronic acid) on the stability of natural food coloration molecules called anthocyanins. Anthocyanins are extracted from a large variety of plant sources, but these molecules are often not as stable towards changing storage conditions as needed. Results from this study strongly indicated that glucuronidation increased color stability in response to both heat and light stresses. The study concluded that glucuronidation may be used to stabilize industrially used extracts of natural colorants.

<https://www.ncbi.nlm.nih.gov/pubmed/19281238>

Glycosylation provides remarkable opportunities for improving characteristics, use and production of small molecules that are not currently being taken advantage of. River Stone has significant proprietary knowledge as well as a collection of diverse enzymes that have the potential to help our customers doing just that.

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**We realize that this may be your first step in determining if Gly-it technology will be able to help you in your current project. We are happy to assist you in determining if Gly-It is the right fit.**

To discuss, or for more help, just get in touch. We would like to make sure Gly-it is a proper fit for your current goals.

**To email us directly**  
[contact@gly-it.com](mailto:contact@gly-it.com)

**To call us directly**  
Pharma enquiries: +45 28 717 717  
Other enquiries: +33 7 85 04 84 18