Reduction of Toxicity via Glycosylation

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.).

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.

Digitoxin

Langenhan et al. demonstrated how “randomization” of the carbohydrate parts of the known nature-derived pharmaceutical digitoxin (DIG) changes not only its potency but also activity. DIG is a steroidal glycoside from Digitalis, mostly known for its anti-arrhythmic indication (as an inhibitor of the plasma membrane Na^+/K^+-ATPase), but it also shows potent cytotoxicity-based anti-cancer properties. The study shows that when exchanging the natural carbohydrate moiety (a trisaccharide) of DIG with a variety of monosaccharides, one activity disappears altogether (Na^+/K^+-ATPase pump inhibition), whereas the other (cytotoxicity) diverges into a multitude of activity/specificity combinations. As one example, whereas DIG has approximately the same cytotoxicity towards 9 human cancer cell lines, one new DIG-glycoside was much more active against one particular cell line but much less active toward the other 8.
https://www.pnas.org/content/102/35/12305.short

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

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.

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.

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