

# Glycosylation for Pharma 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.).

## Dexamethasone

Tozer et al. led a pioneering study showing that one can take advantage of the GI tract's distribution of microbiome-derived glycoside hydrolase activities for colon-specific delivery of an anti-inflammatory drug after oral intake. Dexamethasone is a highly efficient steroid anti-inflammatory drug used for a range of indications, including inflammatory bowel disease (and currently being tested for long term use). In this study it was demonstrated how the vast majority of a dexamethasone-glucoside would pass through the stomach and small intestine to arrive at the cecum and colon where it was efficiently hydrolysed by local bacterial populations to liberate the active metabolite Dexamethasone. It was stipulated that this oral delivery method would have a more than 9-fold “selective advantage” as compared to i.v. delivery, based on the higher (colon) luminal and lower blood concentration of the drug.

<https://pubmed.ncbi.nlm.nih.gov/1871038/>

## 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 steroid glycoside from Digitalis, mostly known for its anti-arrhythmic indication (as an inhibitor of the plasma membrane  $\text{Na}^+/\text{K}^+$ -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 ( $\text{Na}^+/\text{K}^+$ -ATPase pump inhibition), whereas the other (cytotoxicity) diverges into a multitude of activity/specification 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>

## Dopamine

Bonina et al. demonstrated activity of Dopamine in Parkinson's models by linking glucose (GLU) to Dopamine (DOM) to form a DOM-GLU prodrug and also by linking galactose (GAL) to form a DOM-GAL prodrug. Normally Parkinson's patients receive L-DOPA which can pass the blood brain barrier (BBB) and then be converted into Dopamine once inside the brain. There are, however, issues with long term L-DOPA treatment, e.g., extensive metabolism and plasma level fluctuations, and therefore, direct uptake of DOM could be attractive. In this study it was shown that DOM-GAL (and possibly also DOM-GLU) were transported across the BBB (likely through the so-called GLUT transporters) and worked better than L-DOPA to reverse symptoms in both of two classical Parkinson's animal models; reserpine-induced hypo-locomotion (in rats) and morphine-induced locomotion (in mice).

<https://www.ncbi.nlm.nih.gov/pubmed/12852438>

## Morphine

Frances et al. demonstrated that morphine-6-glucuronide is a 45-60x more potent analgesic than morphine itself in animal models. Furthermore, morphine-6-glucuronide induced a longer lasting analgesic effect compared to morphine.

<https://europepmc.org/abstract/med/2154808>

## Doxorubicin

Mürdter et al. demonstrated better efficacy of the anti-cancer drug DOXORUBICIN (DOX) by glycosylation with glucuronic acid (GLUC). Doxorubicin has serious side effects, such as cardiac toxicity, and can therefore only be applied in sub-optimal concentrations. This is the case for e.g. certain lung tumors, a problem augmented by the natural tendency of lung cancer tissue to take up less DOX than normal tissue. But the DOX-GLUC prodrug was shown to be taken up in lung cancer tissue at 7-fold increased rate, very likely due to cancer cells expressing high levels of glucuronidase that can cleave off GLUC from DOX-GLY, thus accomplishing improved localized delivery of DOX.

<https://cancerres.aacrjournals.org/content/57/12/2440.short>

## Warfarin

Peltier-Pain et al. showed how one can fundamentally change the mechanism of action of a molecule by glycosylation, in this case causing warfarin to switch from being an anticoagulant to having anti-cancer activity.

<https://onlinelibrary.wiley.com/doi/abs/10.1002/cmdc.201100178>

## Cadalene

Lee et al. demonstrated that glycosylated cadalene derivatives were potentially superior prodrugs by improving solubility and therapeutic efficacy. In vitro cell viability assays confirmed that glycosylated cadalenes were less toxic and more soluble than cadalene itself, and oral administration to mice with xenografted tumours strongly indicated superior therapeutic efficacy, likely due to better uptake of the molecule by the tumour cells.

<https://www.sciencedirect.com/science/article/pii/S0960894X07010281?via%3Dihub>

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.



## 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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