GLY-KIT

Improving Small Molecules

Glycosylation for Pharma Applications

Introduction

Whereas hydroxylation, methylation, fluorination etc. is 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 or even many "glycolysable" 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 glycosyltransferase 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 steroidal 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 IV 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 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

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

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 metabolization 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 B. *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 attaching by glycosylation glucuronic acid (GLUC) to the molecule. Doxorubicin has serious side effects, e.g. cardiac toxicity at certain concentrations and can therefore only be applied in concentrations not always adequate. 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

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.

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What is Gly-Kit

The Gly-Kit 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-Kit 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 huge 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-Kit will be able to help you in your current project. We are happy to assist you in determining if Gly-Kit is the right fit.

To discuss your order, or for more help, just get in touch. We would like to make sure Gly-Kit is a proper fit for your current goals. Once we connect and assure Gly-kit is the right fit, we will send you pricing options.
Email us directly at sales@rstbio.com

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