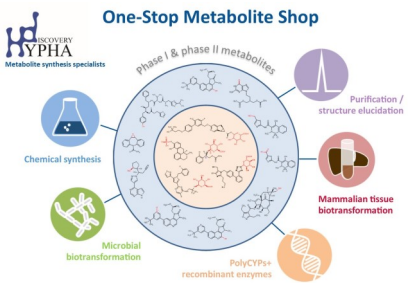
www.hyphadiscovery.com

Metabolites and molecules for tomorrow's drugs

N-glucuronide metabolites

HOW WE MAKE & CHARACTERISE GLUCURONIDES

- **Late-stage chemical synthesis screens tailored to the glucuronide type desired**
- **Microbial biotransformation**
- **Liver fractions (S9/LMs)**
- **Purification from biological matrices e.g. urine**
- **Structure elucidation using a microcryoprobe equipped 700 MHz NMR**



Hypha's One-Stop Metabolite Shop enables synthesis and/or purification of all the main types of mammalian phase I and II metabolites. We use chemical synthesis, microbial biotransformation, mammalian tissue fractions (multiple species of S9s / microsomes) and recombinant enzymes such as PolyCYPs®, and human CYP, AOX and FMO enzymes.

N-glucuronides: on the rise?

We've observed an increase in requests for synthesis of *N*-glucuronides over the last couple of years. We speculate that this may be due to the increasing use of *N*-heterocyclic chemistry in the design of new small molecule drugs, and pan company strategies to reduce CYP metabolism.

The situation is further complicated by the high interspecies variability in formation of some *N*-glucuronides, especially aliphatic tertiary amines and aromatic *N*-heterocycles. UGT1A4 and UGT2B10 are key enzymes responsible for *N*-glucuronidation reactions in humans, rates of which can be much higher than in other animals.

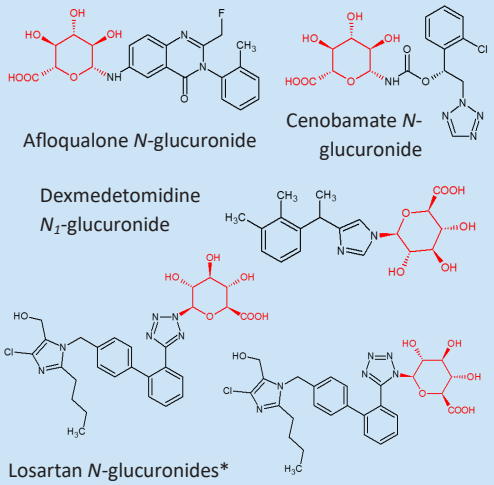
To compound this, synthesis of *N*-glucuronides is not always straightforward, and can be further muddied by metabolite stability issues, complicating interpretation of data.

How do we make *N*-glucuronides?

Microbial biotransformation has broad utility for production of *O*–, acyl, *N*-carbamoyl and some *N*-glucuronides. For the latter, success rates are boosted by application of our proprietary late-stage chemical glucuronidation conditions, as well as utilising liver fractions (S9s or microsomes).

Purification from biological matrices such as urine, where available, is another strategy to access enough metabolite for MetID. Only tens of micrograms are needed for cryoprobe NMR spectroscopy for definitive identification of a metabolite's structure.

Made using late-stage chemical synthesis



Afloqualone *N*-glucuronide

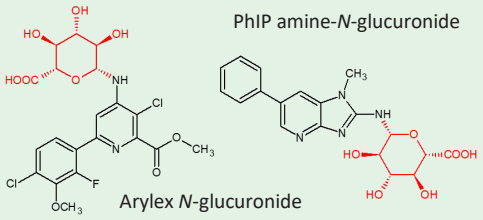
Cenobamate *N*-glucuronide

Dexmedetomidine N_1-glucuronide

Losartan *N*-glucuronides*

*Also made by microbial biotransformation

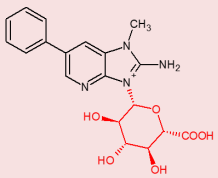
Made using microbial biotransformation



PhIP amine-*N*-glucuronide

Arylex *N*-glucuronide

Made using liver S9



PhiP-N_3-glucuronide

Example *N*-glucuronides made using different techniques in Hypha's one-stop metabolite shop approach

"Hypha Discovery have provided answers to some of our most challenging metabolism and metabolite ID problems. We really appreciate the breadth of expertise available at Hypha Discovery and will definitely reach out for future work."

Director of DMPK, US Pharma Company

***For more information contact us at* enquiries@hyphadiscovery.com**

About Hypha Discovery

Hypha Discovery are experts in metabolite synthesis and purification. We have an extensive client base and work with many of the top pharma and agrochemical companies worldwide, including 8 of the top 10 pharma companies and 5 out of 6 of the top agrochemical companies.