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HYPHA DISCOVERY

Metabolites and molecules for tomorrow's drugs

# Solving challenging drug metabolite projects

## Use of Hypha's One-stop Metabolite Shop to deliver 100s of milligrams of three metabolites


- Chemical synthesis
- Recombinant enzymes
- Microbial biocatalysis
- Liver fractions (S9/LMs)
- Extraction & purification
- Structure elucidation
- Certificates of Analysis

*"Hypha has exceeded our expectations and is now a 'go to' lab for biosynthesis/synthesis/purification. Hypha's team was a pleasure to work with and our complicated projects were handled with expertise and professionalism. Their excellent scientific communication and project data were extremely comprehensive and we received updates throughout the process."*

*Head of Toxicology/DMPK*
*US Pharma Company*

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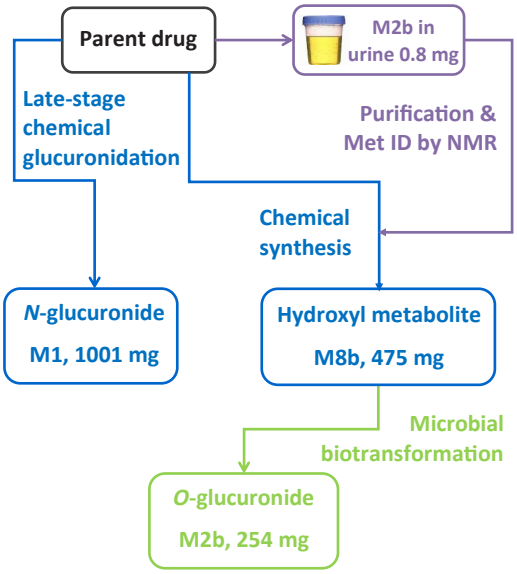
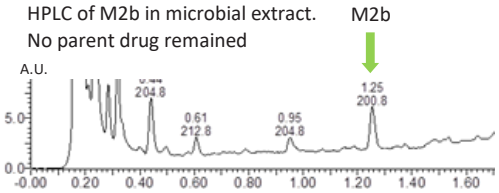
Access to multiple metabolites needed to support clinical development is not always straightforward, and can sometimes mean that more than one technique needs to be applied to fulfil requirements. In one such project, a US pharma client required > 200 mg of three metabolites of a drug; an *N*-glucuronide (M1), an indirect *O*-glucuronide (M2b) and a hydroxylated metabolite (M8b). As part of this project, multiple components of Hypha's one-stop metabolite shop were employed, including chemical synthesis, microbial biotransformation as well as purification and structure elucidation by NMR.

It is our observation that access to *N*-glucuronides is an increasingly common need, as evident in this project where M1, a major *N*-glucuronide, was accessed using chemical synthesis. Key to successful synthesis were the mild deprotection conditions used in the late-stage chemical glucuronidation procedure, resulting in the purification of a gram of M1.

In addition to the *N*-glucuronide, an indirect *O*-glucuronide, M2b, and its aglycone, M8b, were also needed. To make M8b, the position of the hydroxyl group first had to be identified. To achieve this, Hypha chemists purified a small amount of M2b from human urine supplied by the client, and elucidated the structure of the conjugate using cryoprobe NMR spectroscopy. Then, knowing the position of hydroxylation from the structure of the phenolic glucuronide, 100s of mgs of M8b were synthesised. In order to access large amounts of M2b, a different approach was needed as this glucuronide was not amenable to chemical synthesis due to instability and formation of side products.




**Scheme for provision of the three drug metabolites required. Metabolites were supplied to the client at > 95% purity by LC-UV-ELSD.**

Instead, M2b was successfully made through microbial biotransformation of the aglycone M8b. Following a screen to determine the best microbial catalyst, all of the 560 mg of M8b fed to actinomycete Species 45 was metabolised, from which 254 mg of M2b was purified.

Hypha's one-stop metabolite shop allows synthesis and/or purification of all the main types of mammalian phase I and II metabolites. Hypha are able to employ chemical synthesis, microbial biocatalysis, mammalian tissue fractions (multiple species S9s/microsomes) and recombinant enzymes such as PolyCYPs® and Cypex enzymes.

### About Hypha Discovery

Hypha Discovery are experts in metabolite synthesis and purification, as well as specialising in the production of microbially-derived chemicals. We have an extensive client base and work with many of the top pharma and agrochemical companies worldwide, including 7 of the top 10 pharma companies and 4 out of 5 of the top agrochemical companies.