THE ONE STOP METABOLITE SHOP: EMPLOYING MULTIPLE TOOLS TO SOLVE CHALLENGING METABOLITE PROJECTS

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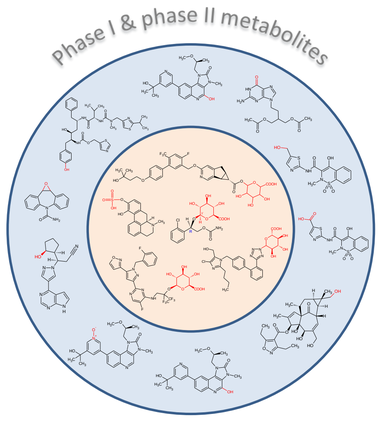


Abstract: Often several strategies are needed to access all key metabolites observed during drug development programs. Hypha has developed a "one-stop metabolite shop" scheme, which utilizes a combination of biological and chemical techniques in order to fulfil requirements to access any type of metabolites. The one-stop metabolite concept offers a parallel or sequential screening step to identify the most productive and cost-effective method to produce target metabolites. Depending on the type and quantity of metabolite required, a combination of chemical synthesis, mammalian S9, microbial biotransformation and recombinant phase I enzymes can be employed. Once the optimal production system is identified, the method can be scaled up to provide up to tens of grams of purified metabolites.

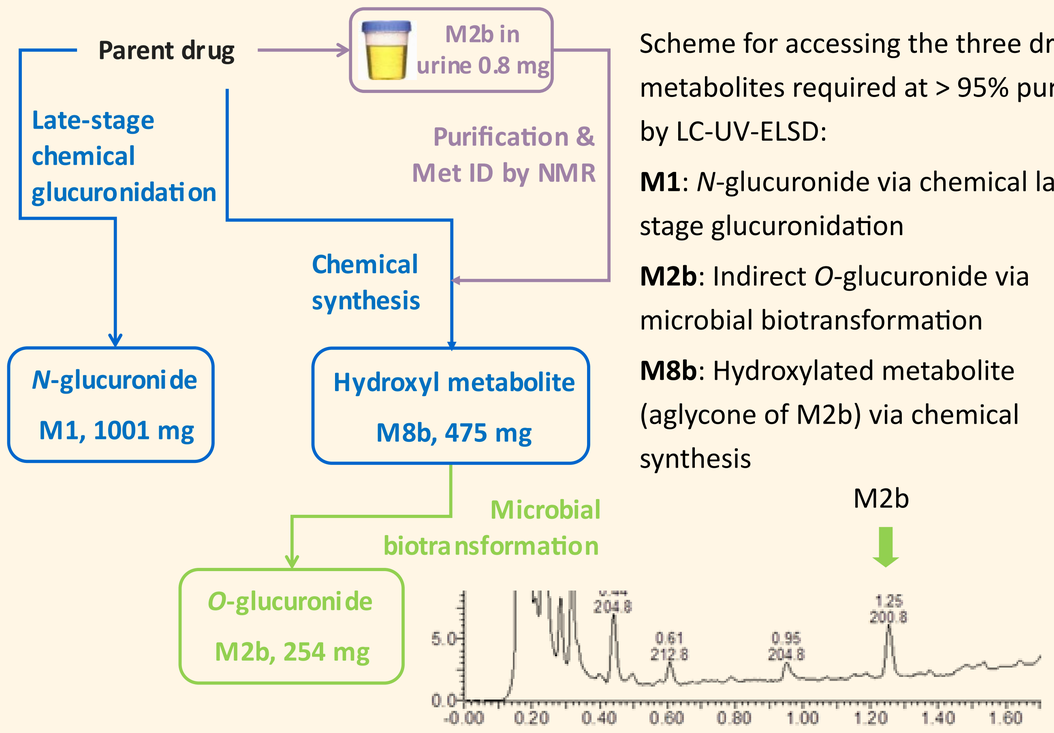
The synergy of using the one-stop shop concept to access metabolites will be exemplified using recent client case studies.

Chemical synthesis | **Mammalian tissue biotransformation** | **Microbial biotransformation** | **PolyCYPs+ Recombinant enzymes** | **Purification / Structure elucidation**

- New to this suite of techniques are an extended panel of PolyCYPs® enzymes, microbial CYPs cloned from some of Hypha's actinomycete strains and expressed in *E.coli* together with redox partners. These enzymes are effective for scalable synthesis of CYP-derived human and other mammalian metabolites. In addition, human AOX and several FMO isoforms have been cloned and expressed in *E.coli* for accessing other phase I human metabolites.

- For glucuronides, a proprietary late-stage chemical screen has been devised, consisting of five robust conditions with deprotection strategies suitable for synthesising all types of glucuronides including acyl glucuronides and *N*-glucuronides. The methodology has proven effective for producing at least one glucuronide from 90% of a panel of commercially available drugs from which glucuronides are known to be formed.

Client case study: Accessing multiple metabolites using late-stage chemical glucuronidation and microbial biotransformation



Scheme for accessing the three drug metabolites required at > 95% purity by LC-UV-ELSD:

M1: *N*-glucuronide via chemical late-stage glucuronidation

M2b: Indirect *O*-glucuronide via microbial biotransformation

M8b: Hydroxylated metabolite (aglycone of M2b) via chemical synthesis

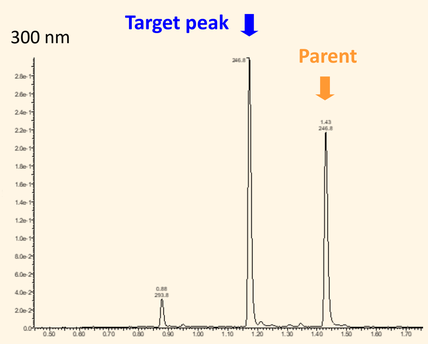
A US pharma client required >200 mg of three metabolites of a drug; an *N*-glucuronide (M1), an indirect *O*-glucuronide (M2b) and a hydroxylated metabolite (M8b).

- 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 one gram of M1.
- To make M8b, the position of the hydroxyl group first had to be identified. To achieve this, a small amount of M2b was purified from human urine supplied by the client, and the structure of the conjugate elucidated 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. 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.

Client case studies: Quick access to oxidised metabolites using PolyCYPs® enzymes

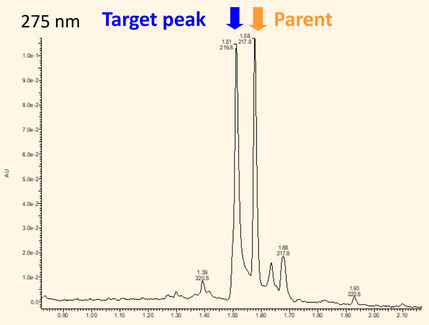
Client case study 1

- Milligram amounts of oxidised metabolites were required of two drug compounds
- Hypha performed screening and scale-up reactions and then provided the scale-up extract to the client for purification and structure elucidation
- Scale-up reactions performed using 500 ml *E.coli* cell pellet of PolyCYPs 196 and 152, dosed at 100 mg/L substrate formulated with a cyclodextrin. Both reactions resulted in > 50% turnover of the parent compound
- PolyCYPs reactions were compared with biotransformation achieved from exposing the parent substrate to a panel of 16 wild type microbes, proven to be talented at metabolising a wide variety of drug compounds



Compound 1

- Biotransformed by multiple microbes and PolyCYPs enzymes
- Several monohydroxylated metabolites formed
- Cleanest biotransformation to the target product by PolyCYP 196

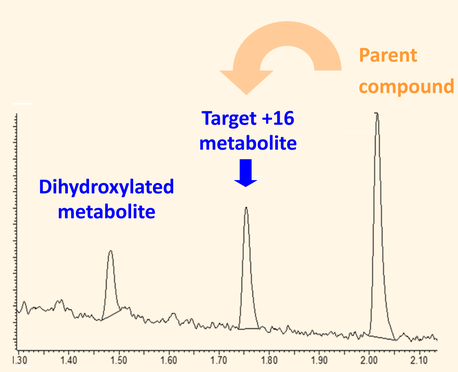


Compound 2

- Single monohydroxylated product produced by PolyCYPs only - PolyCYP 152 best
- Other minor product also of interest
- Glucuronidation and other biotransformation products dominated in microbial whole cell incubations

Client case study 2

- Milligram amounts of a monohydroxylated CYP metabolite required, originally observed in rat LMs
- Drug compound screened vs 22 PolyCYPs
- Best produced by PolyCYP 152 & 359 at 11% conversion
- Scale-up reaction at increased substrate dosing resulted in 35% conversion



- Metabolite purified to > 97% pure by LC-MS-ELSD and confirmed by ^{1}H NMR spectroscopy
- 20.1 mg supplied with a Certificate of Analysis within 3 weeks

About Hypha Discovery

www.hyphadiscovery.co.uk

Hypha Discovery Ltd is a UK-based microbial biotechnology company helping partners in pharmaceutical and agrochemical R&D worldwide succeed through the production of human and other mammalian metabolites, as well as specialising in lead-diversification and production of microbially-derived chemicals. Clients routinely access our biocatalysis technology to generate phase I and II metabolites for MetID, stability testing, use as analytical standards and for producing larger amounts for pharmacological testing.