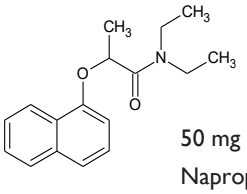
Sequential hydroxylated and glucuronidated metabolites

Mixed metabolic reactions via microbial biotransformation

Hydroxylation and subsequent glucuronidation of pharmaceutical drugs and agrochemical products results from a combination of metabolic pathways. Metabolites arising from these sequential reactions require multi-stage synthesis and are accessible *via* one-step whole cell microbial biotransformation, where both phase I and phase II reactions can be undertaken in the same fermentation as a consequence of the presence of both CYP/oxidative and UGT/glycosylating enzyme systems.

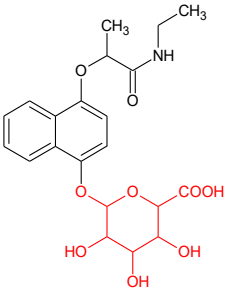
In one client project, a major phase II metabolite was made by one of Hypha's actinomycete strains through sequential phenolic hydroxylation and glucuronidation. The metabolite was produced directly from the parent drug and in very high yields from a synthesized hydroxylated intermediate. The species selected for the glucuronidation reaction achieved >90% molar conversion from the hydroxylated intermediate, resulting in provision of multi-gram quantities of the target metabolite at 99% purity.

In another case study, the herbicide napropamide was screened against a subset of Hypha's biotransforming strains - several hydroxylated and glucuronide metabolites were detected by LC-MS. A fermentation of one strain with the parent compound produced the two major glucuronides relevant to mammalian metabolism through sequential aromatic hydroxylation and glucuronidation, one with and the other without *N*-dealkylation. Structures were confirmed by NMR spectroscopy as those reported previously as animal metabolites (EFSA Scientific Report 2008, 140: 1-74).



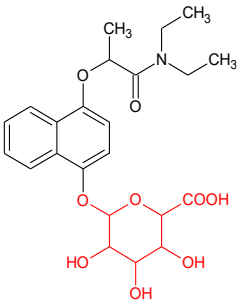
50 mg
Napropamide

Sequential hydroxylation & glucuronidation



21.8 mg
4-OGlu-DE-NPAM

+



17.2 mg
4-OGlu-NPAM

The two known mammalian glucuronides of the herbicide napropamide, isolated from a 0.5L fermentation of one of Hypha's biotransforming strains. Production of further napropamide metabolites is in progress.

We can produce and scale-up mammalian phase I and II metabolites using microbial catalysts, mammalian tissue fractions and recombinant enzymes:

- **For DMPK / ADME / TOX**
- **For Met ID**
- **As standards for quantitation**
- **For bioactivity testing**
- **For stability studies**

Proven Reactions

Methyl hydroxylation
Methylene hydroxylation
Methine hydroxylation
Aromatic hydroxylation
***N*-oxidation**
***N*-methylation**
***N*-dealkylation**
***N*-acetylation**
***O*-dealkylation**
Carbonyl reduction
Heterocycle oxidation via aldehyde oxidase
Aromatic *O*-glucuronidation
Aromatic *N*-glucuronidation
Non-aromatic *O*-glucuronidation
Non-aromatic *N*-glucuronidation
Acyl-glucuronidation
Other glycosidations (AgChem)
***N*-sulfation**
***O*-sulfation**
Thiol conjugation (GSH/NAC)
Transamination
Amino acid conjugations
Sequential reactions e.g. hydroxylation & glucuronidation

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About Hypha Discovery

Hypha Discovery Ltd is a UK-based microbial biotechnology company providing solutions to pharmaceutical and agrochemical R&D partners through the production of mammalian and microbial metabolites, as well as specialising in microbially-derived chemicals. We have an extensive client base and work with many of the top pharma and agrochemical companies worldwide.