

Metabolite purification and structure elucidation

Purification of carisbamate glucuronides from urine and assignment of *R* and *S* epimers by NMR

We produce, scale-up and purify phase I and II metabolites using microbial biotransformation, mammalian tissue fractions, recombinant enzymes and chemical synthesis:

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

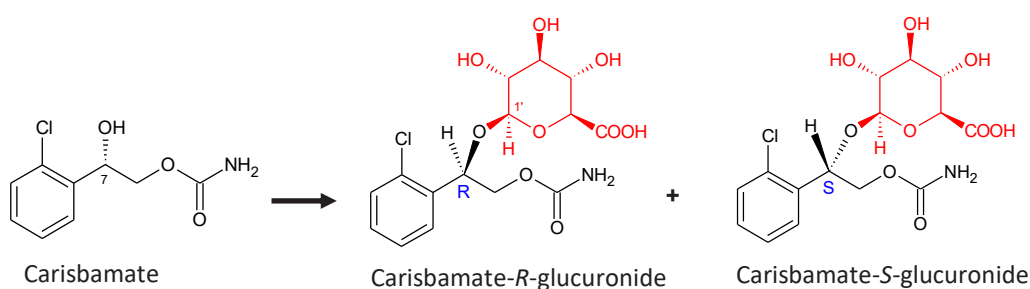
Proven Reactions

- 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

For more information contact us at mail@hyphadiscovery.co.uk

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.



If clearance mechanisms of the test drug results in sufficient quantities of the major metabolites in biological material such as faeces or urine, purification and subsequent identification of metabolites from such matrices is possible.

One such project undertaken at Hypha resulted in tens of milligrams of the *R*- and *S*-O-glucuronides of carisbamate, a neuromodulator developed by SK Life Science, which were purified to >95% purity from 150 ml of rabbit urine using a three-step purification method.

The initial step involved treating the urine with acetonitrile to precipitate proteins and salts, which were removed by centrifugation. After removal of the acetonitrile by evaporation, the supernatant was purified by preparative reversed phase HPLC, initially by applying it directly to an Xbridge C18 OBD column, which was eluted with a shallow water-acetonitrile gradient. The fractions containing the target glucuronides were concentrated and separated by further reversed phase HPLC on a semi-preparative Xbridge prep phenyl column eluted with a very shallow water-methanol gradient. Concentration to dryness of the eluate fractions containing the two target metabolites yielded 95 and 23 mg of the *R*- and *S*-glucuronides, respectively. Confirmation of the structures was obtained by NMR spectroscopy, enabling the use of the purified materials as analytical standards for bioanalysis.

COSY, HSQC and HMBC NMR spectra were obtained for both metabolites, with clear HMBC correlations from the 7-methine protons with the glucuronide carbons and from the glucuronide anomeric protons with the 7-methine

carbons indicating glucuronidation of the 7-hydroxyl group in both instances. The two metabolites were therefore epimers and there were clear differences in their NMR spectra with respect to the chemical shifts and coupling constants for some protons.

In the NOESY spectra (see expansion below of ¹H-¹H NOESY spectra in DMSO-*d*₆) there was one clear nOe correlation between the 7-methine and glucuronide anomeric protons, and this metabolite was proposed to be *R*-carisbamate glucuronide. There was no corresponding correlation evident in the NOESY spectrum of the second metabolite, which was therefore proposed to be *S*-carisbamate glucuronide.

