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THE NUVISAN METABOLITE DISCOVERY PLATORM – METABOLITE IDENTIFICATION, SCREENING, SYNTHESIS & CHARACTERIZATION

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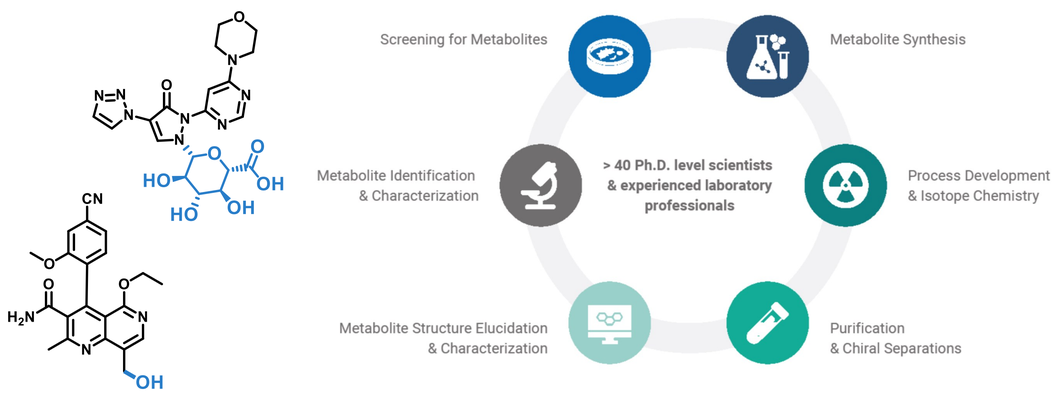
Previously only available within a large pharmaceutical company, our platform combines decades of experience to solve drug metabolite challenges, including biotransformation approaches for milligram to gram scale metabolite generation, complex synthetic chemistry and late-stage diversification. We offer services for isotope chemistry and process development to make labeled drug metabolites, and state-of-the-art analytics for purification, structural elucidation and characterization. Herein, we present selected examples of NUVISAN's diverse activities in this field of synthesis of drug metabolites, isotopically labeled drug candidates, and biomarkers. This includes *de novo* synthesis, method optimization, screening with wild type microorganisms and human and bacterial CYP-enzymes, as well as the application of biotransformation and biocatalysis.

NUVISAN Metabolite Discovery Platform

We support your biotransformation projects and provide metabolites for pharmacological activity testing, for DMPK and toxicological investigations, and as standards for analytical methods. Our services include **metabolite identification** and **structure elucidation** by LC-HRMS, synthesis of **labeled drug metabolites**, **chemoenzymatic synthesis** of biomarkers, and **challenging purifications** and **chiral separations** by normal, reversed phase, and supercritical fluid chromatography (SFC).



Microbiological Chemistry Group

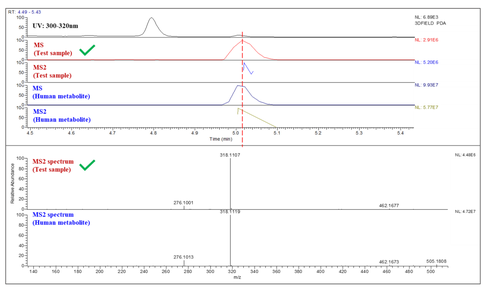
Our Microbiological Chemistry group supports your projects as experts in **redox biocatalysis** and **fermentation**. We make use of nature's diverse toolbox for **production of drug metabolites**, for **biotransformations** on a preparative scale, and for **upscaling of proteins and plasmids**. We synthesize human metabolites in small and large scale and support Life Science Chemistry projects in early research and chemical development.

Our biooxidation toolbox consists of a broad and **unique wild-type strain collection** of more than **2.000 fungi and bacteria**, and more than **750 human and bacterial CYPs**. Our state-of-the art screening and fermentation platform for **protein expression** and **plasmid preparation** in *E. Coli* and insect cells is comprised of 1 and 2 L **Biostat® B-DCU fermenters** for optimization purposes, **5 to 100 L steel fermenters** for scale-up, and **wave-bioreactors**.

Metabolite Identification and Verification

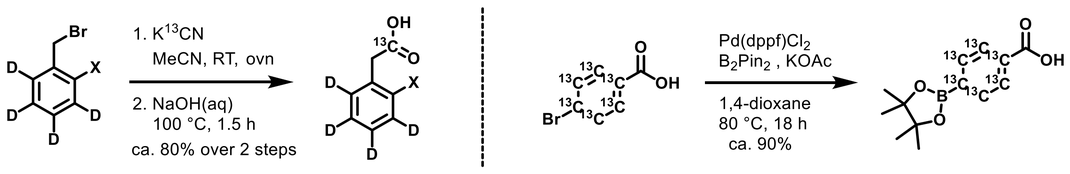
We perform the ***in vitro* generation of human or animal metabolites** with **hepatocytes**, **microsomes**, and **recombinant enzymes** (e.g. CYPs) as references for microbiological synthesis and compare our microbiological synthesis samples with metabolites from *in vitro* samples by UPLC-UV-HRMS.

The **verification of** the desired **metabolite** in the sample of a microbiological screen is carried out by comparison of chromatographic retention time, exact mass and fragment ion mass spectra (e.g. MS2). Afterwards, the metabolite can be synthesized by up-scaling of the **late-stage microbial synthesis without knowing the exact chemical structure**.

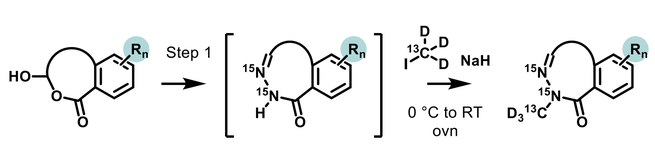


Synthesis of Isotopically Labeled Drugs and Metabolites

Incorporation of labeled carboxylic acid is often the strategy of choice, when stable isotope labeling is required. The synthesis of **isotopically labeled carboxylic acids**, employing readily available starting materials, is frequently performed at NUVISAN and successfully utilized for the synthesis of various labeled amide products.



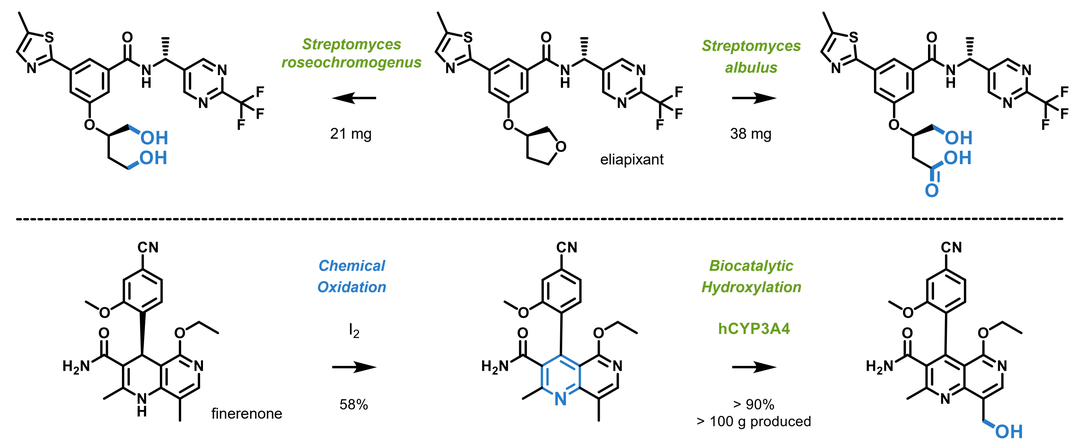
Isotopically labeled products can often be synthesized using established protocols of the non-labeled analogs. However, **method optimization** may be necessary. For example, labeled hydrazine incorporation was optimized using ^{15}N-hydrazine sulfate instead of hydrazine hydrate.



Entry	Conditions Step 1	Yield (over 2 steps)
1	$^{15}N_2H_4{\cdot}H_2O$, AcOH	ca. 5%
2	$^{15}N_2H_4{\cdot}H_2SO_4$, $NaHCO_3$	ca. 65%

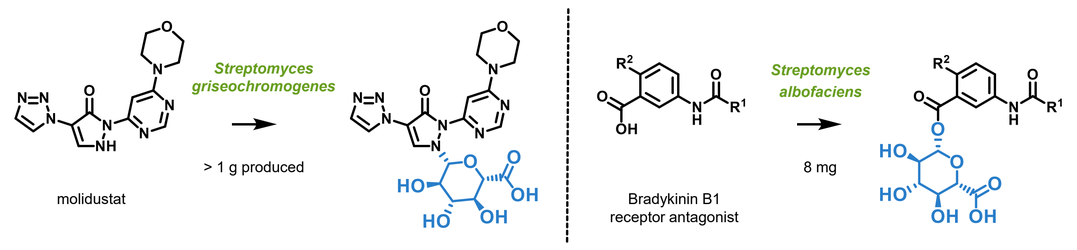
Oxidized Metabolites for Clinical Development

Oxidized metabolites are the most important human metabolites and are needed frequently during clinical development of novel clinical candidates. Their **synthesis with** our **wild-type microbial strains or CYP variants** as **late-stage derivatization** is highly efficient as in the cases of the P2X3 inhibitor eliapixant (WO2020/260463) or the MR antagonist finerenone (ChemMedChem 2012, 7, 1385; Drug Metab Dispos 46: 1546, 2018).



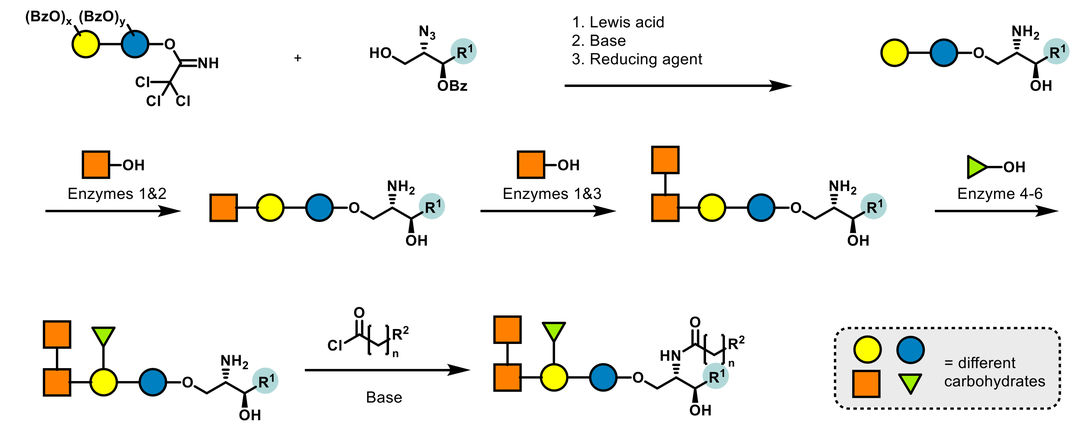
Glucuronides for Clinical Development

Glucuronides are the most important phase II human metabolites and are increasingly needed during clinical development. Our ***Streptomyces* strains** allow the efficient ***N*- and *O*-glucuronidation** of drugs like the HIF-PH inhibitor molidustat (ChemMedChem 2018, 13, 988) or a Bradykinin B1 receptor antagonist (Research Disclosure journal 2023, #RD 710023).



Chemoenzymatic Synthesis of Complex Carbohydrates

A highly efficient, **chemoenzymatic total synthesis** of **complex carbohydrates as biomarkers** was successfully performed. Initially, a sphingosine derivative was coupled with a glycosyl trichloroacetimidate, followed by deprotection and azide reduction. **Three enzymatic steps using six in-house produced enzymes** form a pentasaccharide without need for protecting groups completely regio- and stereoselectively. Final acylation with fatty acid chlorides provides several complex carbohydrates.



Conclusion

With over 60 years of experience in biotransformation within pharmaceutical and agricultural settings, we excel in producing **drug metabolites**, conducting preparative-scale **biotransformations**, and upscaling of **proteins** and **plasmids**. We provide **isotope chemistry** and process development, as well as state-of-the-art analytics for purification, structural elucidation and characterization of metabolites. Our biotransformation approaches are performed in milligram to gram scale including **complex synthetic chemistry** and **late-stage diversification**.

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