Swiss Summer School 2024

«Chemical Biology»

August 25-29, 2024, Kurhaus Bergün

SCS
Swiss Chemical
Society

Swiss Summer School 2024 on Chemical Biology

The Swiss Summer Schools are multi-day out-of-town courses for students (MSc, PhD & post-doc level) and senior researchers with a strong interest in the fields of chemical biology or organic synthesis. They offer a great platform for the exchange with experts and peers from academia and industry in a wonderful Alpine environment. The students are welocme to actively involve themselves in the program by presenting a poster or a short communication.

For a report on the 2022 Swiss Summer School on Chemical Biology please use the link on the right.

Confirmed Speakers:

Prof. Stefan Arold, King Abdullah University of Science and Technology (SA)
Dr. Guido Koch, Amphilix, SynpleChem, eMolecules (CH)
Prof. Anna Mapp, University of Michigan (USA)
Prof. Brian Paegel, University of California (USA)
Prof. Ben Schumann, Imperial College London (UK)
Prof. Dirk Trauner, University of Pennsylvania (USA)
Dr. Olivia Rossanese, The Institute of Cancer Research, London (UK)
Dr. Jingwn Shi, Space Pharmaceuticals AG, (CH)
Ahmed Mahmoud, Chemspeed (CH)
Dr. Sacha Javor, Space Pharmaceuticals AG (CH)

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Program

Sunday Aug 25, 2024

16.00
Welcome and Summer School Opening
16.30
Prof. Dirk Trauner, University of Pennsylvania (US)
«On the Clinical Relevance of Photopharmacology»
18.30
Dinner
20.00
Get together

Monday, Aug 26, 2024

08.30
Dr. Olivia Rossanese, Institute of Cancer Research, London
«Protein degradation as a therapeutic strategy in oncology: exploring monovalent degraders»
09.30
Prof. Stefan Arold, King Abdullah University of Science and Technology, KAUST (SA)
«Chemical Competition by Biological Design: Function from Noise?»
10.30
Coffee Break
11.00
Prof. Anna Mapp, University of Michigan (USA)
«Strategies for Drugging Transcription Factors: Lessons from Nature Privileged Scaffolds for Modulating Dynamic Protein-Protein Interactions»
12.00
Dr. Guido Koch, Amphilix, SynpleChem, eMolecules (CH)
«Medicinal Chemistry of Kinase Drug Discovery – Case Studies and Future Directions»
13.00
Lunch Break
14.30
Workshop in Teams
16.00
Coffee Break
16.30
Short Communications Session A
18.30
Dinner
20.00
Poster Session A

Program

Tuesday Aug 27, 2024

08.30
Prof. Brian Paegel, University of California, Irvine (USA)
«Scaling Probe Discovery to the Proteome via Selective Translation Modulation»
09.30
Prof. Ben Schumann, Imperial College London (UK)
"From Chemical Precision Tools for O-GalNAc Glycosylation to Cell-specific Biorthogonal Tagging of Glycoproteins"
10.30
Coffee Break
11.00
Dr. Olivia Rossanese, Institute of Cancer Research, London
«Monovalent degraders in cancer therapy: BCL6 as a case study»
12.00
Industry Lecture
Ahmed Mahmoud, Chemspeed
Translating your Processes to Automation for Accelerated Research and Development
13.00
Lunch Break & Excursion
18.30
Dinner
20.00
Poster Session B

Wednesday, Aug 28, 2024

08.30
Prof. Stefan Arold, King Abdullah University of Science and Technology, KAUST (SA)
«The Good, the Bad, and the Fortuitous: Chemical Blobology in Structural Biology»
09.30
Prof. Brian Paegel**,** University of California, Irvine (USA)
«Scaling Pharmacokinetic Analysis to Chart Chemical Space Beyond the Rule-of-5»
10.30
Coffee Break
11.00
Prof. Anna Mapp, University of Michigan (USA)
«Strategies for Drugging Transcription Factors: Lessons from Nature Privileged Scaffolds for Modulating Dynamic Protein-Protein Interactions»
12.00
Dr. Jingwen Shi & Dr. Sacha Javor, Space Pharmaceuticals AG
«tba.»
13.00
Lunch Break

Program

Wednesday, Aug 29, 2024

14.30
Workshop in Teams
16.00
Coffee Break
16.30
Short Communications Session B
18.30
Conference Dinner & Awards

Thursday, Aug 29, 2024

08.30
Prof. Ben Schumann, Imperial College London (UK)
«Xylosyltransferase Bump-and-Hole Engineering to Chemically Manipulate Proteoglycans in Mammalian Cells»
09.30
Prof. Dirk Trauner, University of Pennsylvania (USA)
«On the Clinical Relevance of Total Synthesis»
10.30
Coffee Break
11.00
Closure

TALKS

Bridging Chemistry and Biology: Chemical Biology Strategies for Novel Agrochemical Development

Simone Berardozzi
Principal Scientist Crop Protection Research Chemistry, Lead Generation Insect Control Research
simone.berardozzi@syngenta.com, Schaffhauserstrasse 101, 4332 Stein (CH)

Ensuring global food security for a growing population remains a paramount challenge, underscoring the critical role of modern, science-based crop protection solutions. These solutions safeguard crop yields against emerging pests, evolving resistance mechanisms, and environmental stresses while improving water and nutrient use efficiency, with a focus on safety and sustainability.

Historically, the development of novel crop protection agents has traditionally been an arduous and resource-intensive undertaking, often relying on serendipitous discoveries or laborious screening campaigns.[1]

The integration of chemical biology approaches holds immense potential to revolutionize this process by enabling more efficient target validation and accelerating the discovery of new active ingredients. In crop protection research, chemical biology techniques can be strategically employed to validate the essentiality and druggability of potential targets, providing valuable insights into their biological functions and therapeutic relevance. This knowledge can then guide the design and optimization of lead compounds, ultimately leading to the development of more effective and selective crop protection solutions that meet actual safety and efficacy requirements.

This presentation explores the challenges faced during Lead Generation in crop protection research and highlights how chemical biology tools can aid the development of new active ingredients.

1) G. Berthon, **Where Do Agrochemicals Come From?**, *CHIMIA*, **2020**, *74 (10)*, 822-824.

A high throughput direct-to-biology screening platform for LRRK2 degraders

Ayomipo Adegeye[1], Francesca Cavallo[1], Roland Hjerpe[1], Benoit Gourdet[1]

[1] Sygnature Discovery, Bio City, Pennyfoot St., Nottingham NG1 1GR, UK
adegeye_ayomipo@yahoo.com

Parkinson's disease (PD) is the second most common neurodegenerative disease affecting about 10 million people globally and characterized by the progressive degeneration of dopaminergic neurons in the midbrain resulting in chronic motor disabilities such as tremor, rigidity and bradykinesia. With PD being heavily linked to genetic alterations in about 10 % of cases, mutations in the leucine-rich repeat kinase 2 (LRRK2) gene, especially G2019S variant, represents the most common monogenic cause of PD. The resulting gain of kinase function mutation strongly proposes LRRK2 as a promising kinase drug target for treatment of PD, and LRRK2 degradation by PROTACs as an interesting therapeutic strategy, since protein degradation essentially circumvents accumulation of inhibited LRRK2, thus reducing undesirable side effects caused by standard kinase inhibitors. Recently, the BBB-penetrant VHL-based LRRK2 degrader - XL01126, developed by Ciulli lab, was described as demonstrating potent degradation in multiple cell lines and the selective and potent CRBN-based LRRK2 degrader - ARV-102, developed by Arvinas, was progressed into Phase 1 clinical trials. In this study, we have developed a high throughput compound screening cell-based platform to assess, identify and characterize hit compounds from a PROTAC library of new compounds synthesized at Sygnature Discovery using plate-based chemistry. This screening approach, via Sygnature's proprietary CHARMED® platform, with reference VHL-based tool compound XL01126 as control, afforded two promising CRBN-based LRRK2 degraders (3994-85, 10 nM and 3994-86, 29 nM) using both HTRF and AlphaLISA immunoassays to measure the effect of compounds on LRRK2 degradation in A549 cells. Furthermore, we are currently optimizing and validating key hit compounds in cell-based and neurologically relevant assay models using a plate-based direct-to-biology approach with HTRF/AlphaLISA immunoassays to measure LRRK2 readouts.

[1] X. Liu, A.F. Kalogeropulou, A. Ciulli *et al., J. Am. Chem. Soc.,* **2022**, 144(*37)*, 16930-16952.
[2] C. Bouvier, R. Hjerpe, F. Cavallo *et al., Cells,* **2024,** 13(*7*), 578.

My current research theme in invitro neuroscience focuses on identifying and characterizing hit compounds capable of degrading LRRK2, a protein implicated in Parkinson's disease. This entails developing and optimizing a neuronally relevant cell-based compound screening assay to identify, validate and characterize potent compounds from a compound library of synthesized PROTACs. This requires rational selection of the cellular assay model based on significant LRRK2 expression, preservation of the disease signalling pathway and assay format optimization to generate robust biological data that demonstrates a compound-specific degradative effect on LRRK2 with the design and development of secondary, counter and orthogonal screens. The copious data generated from these experiments by myself and other collaborative lab scientists are combined and carefully evaluated to determine the most active hits based on a cut-off criteria that demonstrates reproducible pharmacological effect on LRRK2. Inferential analysis is employed to suggest improvements in both the assay protocol and the rationally designed compounds to generate more potent hits with improved physicochemical, pharmacokinetic and toxicity profiles. This iterative cycle aims to optimize the structure-activity relationship of tested compounds to fit set compound desirable criteria.

Knowledge gained from this workshop in AI/ML models will be instrumental to my project and career path as ML algorithms will make it easier to analyse volumes of generated data from my experiments and the collaborative lot to quickly identify trends/patterns in choosing and optimizing cellular models for performant assays that best suits the disease and target of interest. The aggregation of pharmacological (selectivity, specificity, potency, binding affinity and stabilization of PROTAC ternary complexes) as well as pharmacokinetic and toxicology data will be better harnessed by a machine learning model to predict which compounds will be potentially good hits at the early stage of drug discovery to minimize drug attrition, thereby speeding up the drug discovery process.

Continuous Multistep Chemoenzymatic Synthesis of Chiral, Pyridine-Containing Amines

Pablo Díaz-Kruik[a], David Roura Padrosa[b], Eimear Hegarty[a], Hansjoerg Lehmann[c], Radka Snajdrova,[c] and Francesca Paradisi[a,*]

([a]Department of Chemistry, biochemistry and Pharmaceutical Sciences (DCBP), University of Bern, Freiestrasse 3, Bern, Switzerland; [b]inSEIT AG, Geselleschaftsstrasse 42, 3012 Bern, Switzerland; [c]Biomedical Research, Global Discovery Chemistry,Novartis Pharma AG, 4056 Basel, Switzerland)
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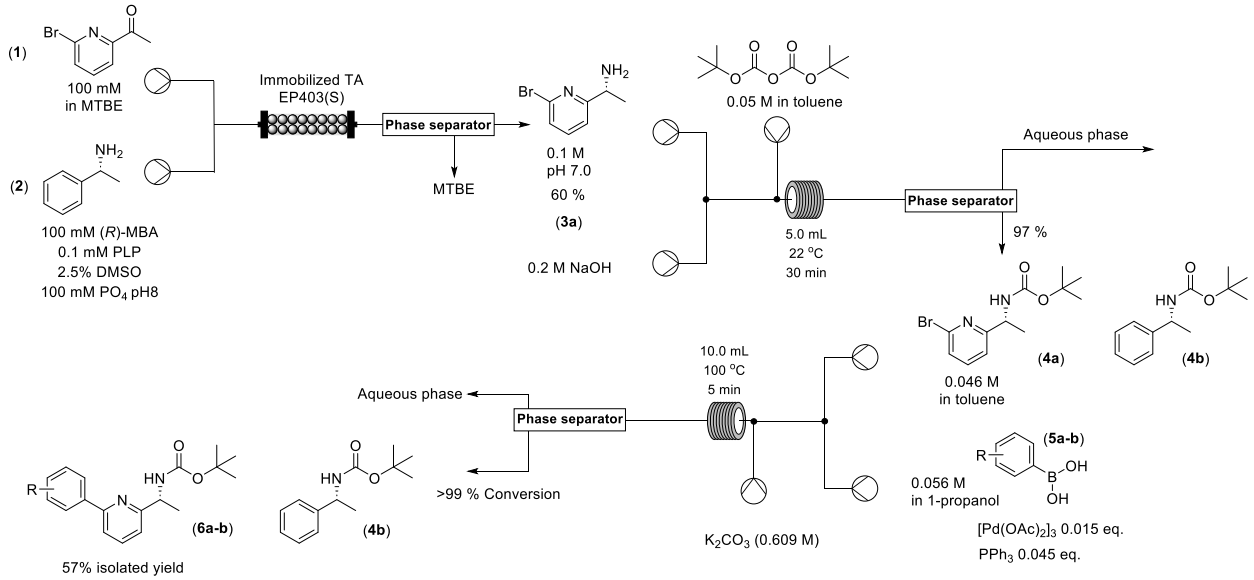
In the last few decades flow chemistry has demonstrated to be a key linker between fundamental research and industrial applications. At the same time the exponential growth of biocatalytic systems is driving synthetic chemistry towards a more efficient and sustainable approach aligning with the 12 principles of green chemistry.[1] As a proof of concept, we synthesized two Chiral α-(hetero)aryl amines (**6a-b**), motifs that are gaining momentum for their biological activities and their use as building blocks towards more complex molecules. Here we report a continuous chemoenzymatic strategy from 2-acetyl-6-bromopyridine (**1**) enabled by careful solvent selection and phase switching.[2,3]



Combining a first biocatalytic transamination reaction performed by an (*R*)-selective transaminase from *Thermomyces stellatus* (TsRTA) in a biphasic system in continuous flow, with in line Boc-protection followed by a Suzuki coupling of a (substituted)phenylboronic acid (**5a-b**), enabled conversions up to >99% towards tert-butyl (R)-(1-(6-(substituted) phenylpyridin-2-yl)ethyl)carbamates as the final products (**6a-b**).[4] This strategy not only constitutes an important example of chemoenzymatic combinations in continuous flow but highlights the importance of the reaction design to minimize waste (through unreacted substrate recirculation), avoid time intensive workups (through inline extractions) and achieve the product in a space time yield of 68 mg·L-1·h-1 with excellent enantiomeric excess (99% ee).

[1] ACS. 12 principles of Green Chemistry, *American Chemical Society*. **1998**.
[2] P. Díaz-Kruik, D.R. Padrosa, E. Hegarty, H. Lehmann, R. Snajdrova, F. Paradisi, *OPRD,* **2024**, https://doi.org/10.1021/acs.oprd.4c00080.
[3] W. Kong,Y. Liu, C. Huang, L. Zhou, J. Gao, N.J. Turner, Y.Jiang, *Angew. Chemie - Int. Ed.,* **2022**, *61* (21), 1–7.
[4] C.M. Heckmann, L.J. Gourlay, B. Dominguez, F. Paradisi, *Front. Bioeng. Biotechnol.,* **2020**, *8*, 1–13

Reshaping the Antibiotic Fidaxomicin

Erik Jung, Tizian Griesser, Anastassia Kraimps, Maja Hunter, Jordan Costafrolaz, Yves Mattenberger, Silvia Dittmann, Andrea Dorst, Alexander Major, Simon Jurt, Tatjana Teofilovic, David Dailler, Daniel Schäfle, Susanne Sievers, Patrick H. Viollier, Rolf Müller, Peter Sander, and Karl Gademann

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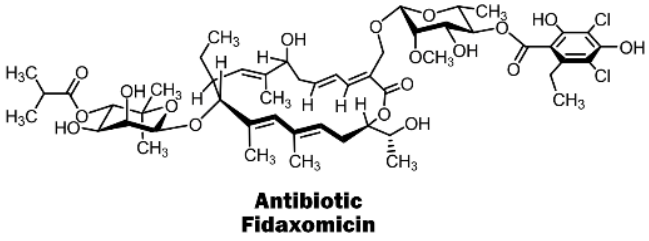
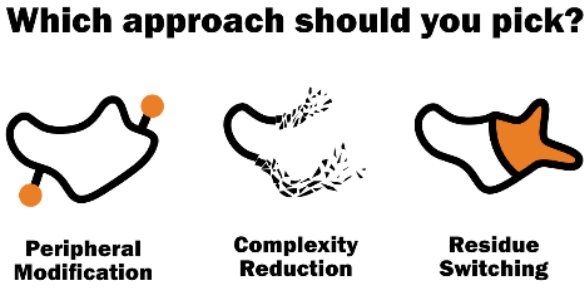
erik.jung@chem.uzh.ch

Natural products become drugs in different ways. The natural product itself can turn out to be sufficiently potent, safe, and bioavailable to treat disease (*e.g.* vancomycin, paclitaxel). However, modification of the natural product structure is often necessary to improve physicochemical properties, spectrum of activity, or counter resistance. We are interested in the natural product antibiotic fidaxomicin, which is used to treat infections in the gut.[1] Exploring several different approaches, such as peripheral modification[2,3], complexity reduction[4], and residue switching[5] we studied how modifying the structure of fidaxomicin affects bioactivity. In this talk we share the lessons learned for the discovery of novel antibiotics.



Antibiotic Fidaxomicin



[1] A. Dorst, E. Jung, K. Gademann, *CHIMIA* **2020**, *74*, 270–273.
[2] E. Jung, A. Kraimps, S. Dittmann, T. Griesser, J. Costafrolaz, Y. Mattenberger, S. Jurt, P. H. Viollier, P. Sander, S. Sievers, K. Gademann, *ChemBioChem* **2023**, *24*, e202300570.
[3] A. Dorst, R. Berg, C. G. W. Gertzen, D. Schäfle, K. Zerbe, M. Gwerder, S. D. Schnell, P. Sander, H. Gohlke, K. Gademann, *ACS Med. Chem. Lett.* **2020**, *11*, 2414–2420.
[4] E. Jung, M. Hunter, A. Dorst, A. Major, T. Teofilovic, R. Müller, K. Gademann, *Helv. Chim. Acta* **2024**, e202400013.
[5] E. Jung, T. Griesser, J. Costafrolaz, Y. Mattenberger, S. Dittmann, A. Dorst, A. Major, D. Dailler, D. Schäfle, S. Sievers, P. H. Viollier, P. Sander, K. Gademann, **2024**, *submitted*.

Expanding the Scope of Extracellular Electron Transfer in *Geobacter Sulfurreducens*: Insights from Organic Salt Reduction

Salem MAJOURI[1], Maksym KARAMASH[1], Giese BERND[1] and Katharina M. FROMM[1]
Department of Chemistry, University of Fribourg, Fribourg, Switzerland
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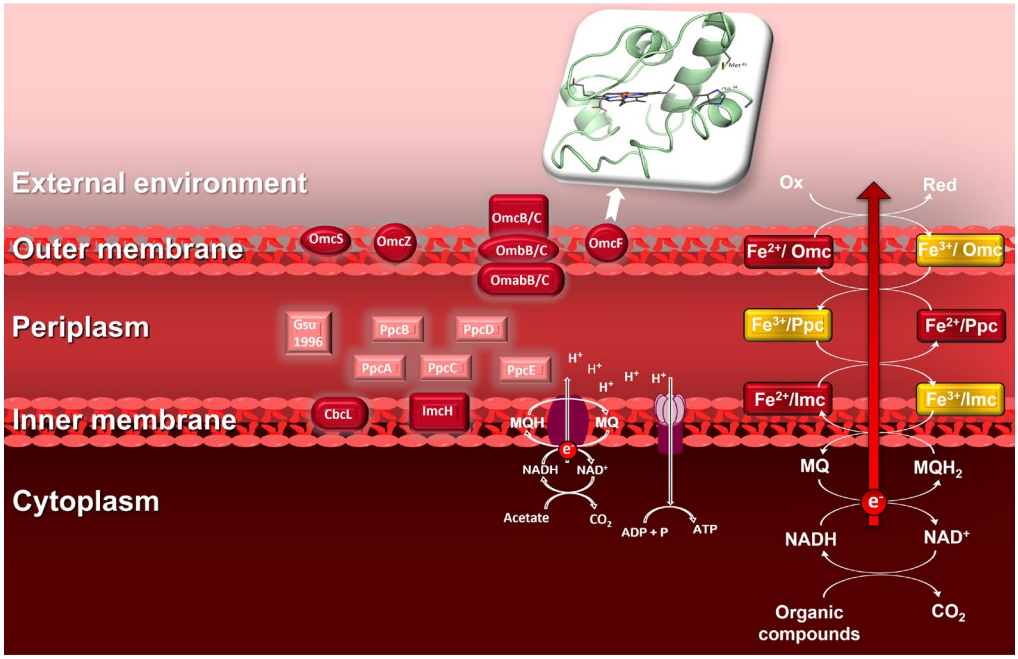
Geobacter sulfurreducens, renowned for its ability to reduce various metal ions through extracellular electron transfer (EET) mediated by cytochromes, remains a subject of intense investigation.[1][2] Previous kinetic studies indicated a constant respiration rate with inorganic salts,[1] prompting us to explore the potential of organic salts to enhance the electron transfer due to their different molecular interactions with the respiratory chain proteins compared to the other options of metal ion-cytochrome interactions.

Our investigation revealed that certain organic salts indeed exhibited higher respiration rates, aligning with our expectations. Some organic salts were identified as effective redox mediators between cytochromes and metal salts, resulting in a notable increase in the rate of metal salt reduction while reducing its associated toxicity.



This discovery not only expands our understanding of EET mechanisms in *G. sulfurreducens* but also presents a promising avenue for optimizing bacterial metal reduction processes through the strategic use of organic compounds as electron shuttles.

[1] M. Karamash, M. Stumpe, J. Dengjel, C. A. Salgueiro, B. Giese, and K. M. Fromm, *Frontiers in microbiology*, **2022**, 13, 909109.
[2] B. Giese, M. Karamash, and K.M. Fromm, *FEBS Lett*, **2023,** 597, 166-173.

Development of Synthetic Transmembrane Phosphate Transporters

Karolis Norvaisa,[1] Alessio Cataldo,[1] Lau Halgreen,[1] Samantha E. Bodman,[2]
Kristin Bartik,[1] Stephen J. Butler,[2] Hennie Valkenier[1]

[1]Engineering of Molecular NanoSystems (EMNS), Brussels, Belgium; [2]Loughborough University, Department of Chemistry, Loughborough, UK.

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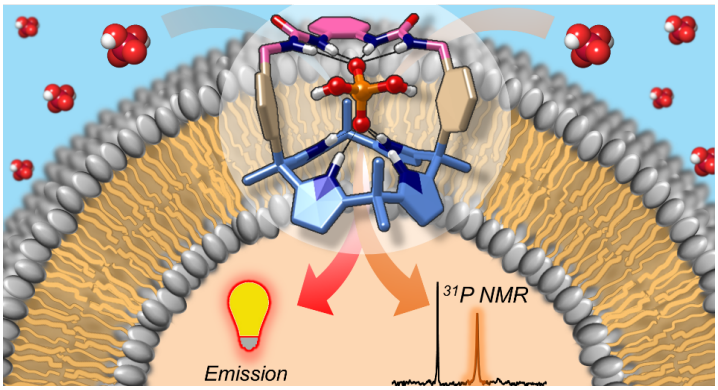
Phosphates and phosphorylated compounds play crucial roles in many biological processes, such as energy transfer in metabolism and enzymatic activity. Because phosphates are anionic and highly hydrophilic, they cannot spontaneously diffuse through cell membranes or lipid bilayers. At the cellular level, transport proteins are essential for moving various charged molecules across cell membranes.[1] While methods exist to purify and reconstitute these transport proteins, along with new assays to track their activity,[2] phosphate carrier proteins still face challenges related to stability and efficient delivery to cell membranes. As an alternative strategy, synthetic compounds can act as mobile carriers or channels in the membrane, typically for transporting chloride as potential therapeutic applications against cystic fibrosis. [3,4]

Until recently, no synthetic transporters for inorganic phosphate were reported. However, in this report, we show a first synthetic carrier based on strapped calix[4]pyrrole scaffold that allows the extraction of strongly hydrated $H_2PO_4^-$ into the lipid bilayer.[5] The deliverable transporter shields negative charges from the lipophilic bilayer interior and transfers inorganic phosphate through the membrane. Phosphate transport was monitored by emission spectroscopy using an encapsulated phosphate sensitive europium(III) probe.[6] Furthermore, ^{31}P-NMR spectroscopy was used to confirm and identify the transported phosphate species.



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[2] Majd, H.; King, M. S.; Palmer, S. M.; Smith, A. C.; Elbourne, L. D.; Paulsen, I. T.; *eLife*, **2018**, *7*, e38821.
[3] Forster, I. C.; Hernando, N.; Biber, J.; Murer, H.; *Mol. Aspects Med.*, **2013**, *34*, 386–395.
[4] Davis, J. T.; Gale, P. A.; Quesada, R.; *Chem. Soc. Rev.*, **2020**, *49*, 6056–6086
[5] Cataldo, A.; Norvaisa, K; Halgreen, L; Bodman, S. E.; Bartik, K.; Butler, S. J.; Valkenier, H.; *J. Am. Chem. Soc.* **2023**, *145*, 16310.
[6] Bodman, S. E.; Breen, C.; Plasser, F.; Butler, S. J.; *Org. Chem. Front.*, **2022**, *9*, 5494.

Optochemical control of Cu(I) homeostasis in mammalian cells

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The redox-active copper atom is a crucial co-factor for the essential cellular processes in all forms of life. Electron exchange by cuproenzymes helps in metabolism, synthesizes hormones, and acts as neurotransmitters. Cellular homeostasis is often required and in the case of copper, it is inevitable. The increase in copper levels evidently enhances the chances of cancer, and Wilson's disease, even lead to neurodegenerative diseases like Alzheimer's.[1] Since copper is an essential element, the complete depletion of copper by the chelators could have adverse effect in many cellular functions. To overcome this issue, an additional layer of control/stimuli over the chelator would be beneficial.[2] In the last several years, light has been used as an external stimulus to control various biological functions due to its non-invasive nature. In this regard, we have designed a photocaged copper chelator that can be activated by light on demand. The efficiency of the copper chelation was confirmed by absorption and fluorescence studies. We have further investigated the copper chelation in HeLa cells in the presence of a turn-on copper specific fluorophore.[3] The copper dependent trafficking of ATPases was studied by immunostaining after incubation with Photocaged chelator for both in presence and absence of light.

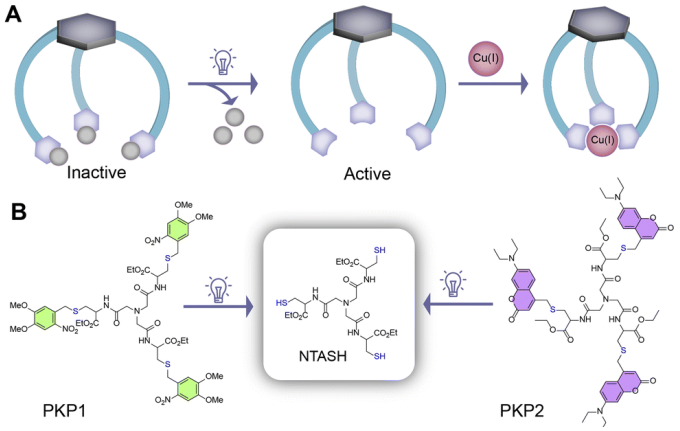

Figure 1 (A) Schematic representation of the photocaged Cu(I) chelator. (B) Cu(I) chelators PKP1 and PKP2 photocaged with NVOC and the coumarin group.

In finding another path to maintain copper homeostasis, we have designed a chelator which can be turned-off in presence of light. A small molecule based tripodal chelator which shows high affinity towards Cu(I), both biochemically and inside mammalian cells. Upon irradiation with light, with copper-specific fluorophore; we have observed increase in the fluorescence leading us to believe that no copper chelation after irradiation is occurring. Mitochondrial activity in cells and copper abundance are intertwined, we are further investigating the changes in mitochondria and cellular activities before and after chelation.[4]

[1] S. Lutsenko, *Curr. Opin. Chem. Biol.,* **2010**, *14,* 211-217.
[2] A. M. Pujol, M. Cuillel, A. S. Jullien, C. Lebrun, D. Cassio, E. Mintz, C. Gateau, P. Delangle, *Angew. Chemie. Int. Ed.,* **2012**, *51*, 7445-7448.
[3] P. K. Pain, D. Palit, M. Shegane, R. P. Singh, D. Manna, *Chem. Commun*., **2023**, *59*, 2315-2318.
[4] P. K. Pain, D. Manna, *Manuscript under preparation*.

Metabolisable linkers as a strategy to reduce non-target uptake of radiolabelled antibodies

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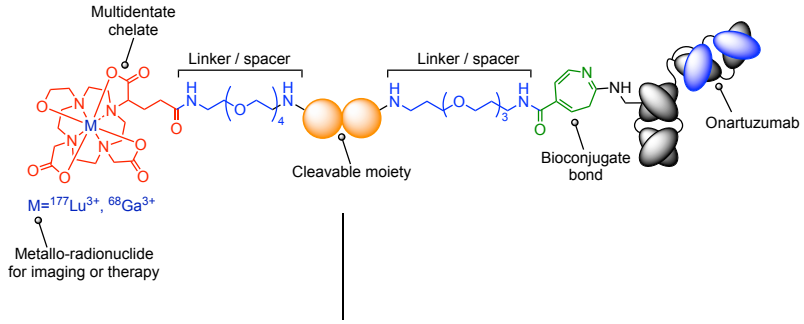
Radioimmunotherapy (RIT) is a fast-emerging field of nuclear medicine. Successful preclinical applications and the subsequent approval of ^{90}Y-ibritumomab tiuxetan (Zevalin®, Bayer, Leverkusen, Germany) and ^{131}I-tositumomab (Bexxar®, GSK, Brentford, UK) opened the door for the development of numerous antibody-based radiopharmaceuticals. Currently, more than 60 radiolabelled antibodies or antibody fragments are reported to enter different phases of clinical trials.[1] Monovalent antibody onartuzumab was used in several studies for imaging of the C-Met receptor, which is significantly overexpressed in several malignancies.[2,3] It shows high affinity to the target receptor and good tumour retention. However, its high uptake into the kidneys hinders its usage as a therapeutic agent.[3]



In this work, we tried to reduce the non-target uptake of onartuzumab by including a cleavable moiety between the radiolabelled chelate and the antibody. Several target enzymes were identified in the kidney, and their corresponding substrates were tested as possible metabolisable linkers in order to accelerate the release of the radioactivity from the kidneys by enabling the fast renal excretion pathway.

[1] Rondon A, Rouanet J, Degoul F. Radioimmunotherapy in Oncology: Overview of the Last Decade Clinical Trials. *Cancers (Basel)*. **2021**, 13(21), 5570

[2] Pool M., Terwisscha van Scheltinga A. G. T., Kol A., Giesen D., de Vries E. G. E., Lub-de Hooge M. N. ^{89}Zr-onartuzumab PET imaging of c-MET receptor dynamics. *Eur J Nucl Med Mol Imaging*. **2017**, 44, 1328–1336

[3] Klingler S., Fay R., Holland J. P. Light-Induced Radiosynthesis of 89Zr-DFO-Azepin-Onartuzumab for Imaging the Hepatocyte Growth Factor Receptor. *J Nucl Med*, **2020**, 61 (7), 1072-1078

Pretargeting intracellular oncogenic proteins for click-to-release

Matthias F. Schild, Dennis G. Gillingham

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Systemically administered chemotherapy in the form of cytotoxic agents like doxorubicin is often accompanied by severe side effects. In a similar way to antibody drug conjugates, small molecule drug conjugates (SMDCs) aim to localize the treatment by combining a targeting ligand with a cytotoxic moiety. The localizing part of an SMDC must be selective for a target associated or specific to the cancer tissue in question. For our system we chose the epidermal growth factor receptor (EGFR) a receptor kinase which is overexpressed in several types of cancer, including lung and colorectal cancer, and afatinib a covalent inhibitor targeting the kinase domain of this protein.[1]

The inverse electron-demand Diels-Alder (IEDDA) reaction between tetrazines and trans-cyclooctenes (TCOs) is an emerging biorthogonal reaction. Since its introduction in 2008[2] it has found application in the traditional sense of click reactions of connecting two scaffolds, but moreover it can also act in a dissociative manner called click-to-release,[3] when a suitable leaving group is placed next to the alkene. A biologically active compound can be rendered inactive by placing a bulky TCO on a suitable heteroatom. It will only be activated upon reaction and subsequent release with a tetrazine.

By way of connecting the activating tetrazine to the EGFR targeting afatinib, we aim to localize the cargo release to cells with overexpressed levels of EGFR. Following this treatment, TCO protected MMAE can be introduced which will only be activated in cells with elevated levels of the tetrazine.

[1] Li et al., *Oncogene*, **2008**, *27*, 4702-4711.
[2] Melissa L. Blackman, Maksim Royzen, Joseph M. Fox, *J. Am. Chem. Soc*., **2008**, *130*, 13518-13519.
[3] Versteegen et al., *Angew. Chem. Int. Ed.*, **2013**, *52*, 14112-14116.

Combining comparative metabolomics and molecular networking to study secondary metabolism of *Caenorhabditis elegans* and *briggsae*

Marie-Désirée Schlemper-Scheidt, Stephan H. von Reuss

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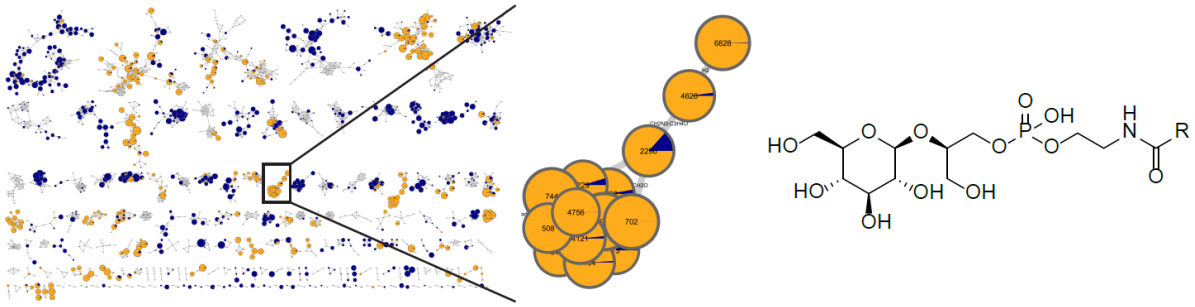
Secondary metabolism in nematodes is characterized by complex modular metabolites that incorporate building blocks from diverse primary biosynthetic pathways [1]. In order to characterize the chemical space of nematode secondary metabolism we employed comparative metabolomics and molecular networking using the hermaphroditic model organism *Caenorhabditis elegans* and the satellite model organism *Caenorhabditis briggsae* [2].
Using GNPS the combined ESI-(–)-MS/MS data from exo- and endometabolome extracts of well-fed and starved cultures were pooled to generate a molecular network of *Caenorhabditis* metabolites. Here, we report the identification of two clusters, which demonstrates the utility of molecular network analysis for the characterization of nematode secondary metabolism. As a proof of concept, we initially focused on the cluster representing ascarosides. Ascarosides represent highly conserved signaling molecules in nematodes, which regulate a wide range of inter- and intraorganismal interactions [1, 3]. Comparative metabolomics using the reference strains *C. elegans* N2 and *C. briggsae* AF16 further enabled us to characterize ascaroside production under different growth conditions.



In addition, a second prominent cluster was identified as amphiphilic *N*-acylethanolamine derivatives, which are highly abundant in *C. elegans* and predominantly excreted into the exometabolome under well-fed conditions. The underlying lipophilic *N*-acylethanolamine building blocks (NAEs) represent a conserved class of signaling molecules in plants, animals and microorganisms [4]. In the nematode *C. elegans* some NAEs have previously been identified, and shown to be retained in the endometabolome [5]. Using a combination of large-scale cultivation, RP-C18 chromatography and NMR spectroscopy a variety of 2-(β-glucosyl)-glyceryl *N*-acyl phosphoethanolamines were unambiguously identified. Their amphiphilic properties, enrichment in the exometabolome, and potential cleavage to known signaling molecules suggests potential roles in intraorganismal interactions.
Taken together, our results demonstrate the utility of comparative metabolomics and molecular networking to explore structural diversity, species-specificity, and growth condition dependency of nematode secondary metabolism.

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Extracellular Peptidases in Wastewater: Specificity and Potential to Biotransform Antimicrobial Peptides

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Anthropogenic chemicals, encompassing pharmaceuticals and personal care products, are integral components of most people's lifes. Post-use, a substantial fraction of these chemicals enters the wastewater system. If not entirely removed during the wastewater treatment, chemicals are released into the environment, where they can have adverse effects on organisms and ecosystem functions. In the case of antibiotics, there is the risk of the emergence of antibiotic resistance genes, amplifying the challenges associated with antimicrobial resistance. While peptide-based chemicals such as antimicrobial peptides (AMPs) have garnered increasing interest in research and development as well as in clinical settings,[1,2] a detailed understanding of their fate in wastewater and WWTPs remains missing. Wastewater inherently harbours a variety of extracellular peptidases. We hypothesize that these peptidases can hydrolyse certain peptide-based micropollutants, thereby preventing their release into the environment. To delve deeper into the fate of peptide-based chemicals in wastewater, we assessed the peptidase activity at different stages of four full-scale WWTPs. Influent samples consistently exhibited highest extracellular peptidase activities compared to later treatment stages.[3] Incubating a set of ten chemically diverse AMPs with extracellular peptidases from influent samples revealed variable biotransformation extents, yet trends across different WWTPs were consistent. Screening the high-resolution mass spectrometry (HRMS) data for transformation products (TPs) of rapidly degraded AMPs, we detected a distinct set of TPs. This finding highlighted the specificity of extracellular peptidases. When comparing extracts from influents from four different full-scale WWTPs, we discovered that almost the same set of TPs was formed, indicating that the specificity is conserved across different wastewaters.[3] To more comprehensively understand peptidase specificity in wastewater, we employed a systematically designed library of 124 tetra-decapeptides[4] spiked into extracellular wastewater extracts from influent samples and monitored peptide concentrations using UHPLC-HRMS. By analysing the formed transformation products, we were able to determine the specificity of extracellular peptidases in wastewater. Collectively, these findings contribute to a holistic understanding of extracellular peptidases in wastewater, enabling predictions about the fate of peptide-based chemicals. Moreover, these findings have the potential to inform the redesign of compounds or aid in developing novel compounds with enhanced biodegradability in wastewater.

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Exploring the Crosstalk Between Tubulin Post-Translational Modifications

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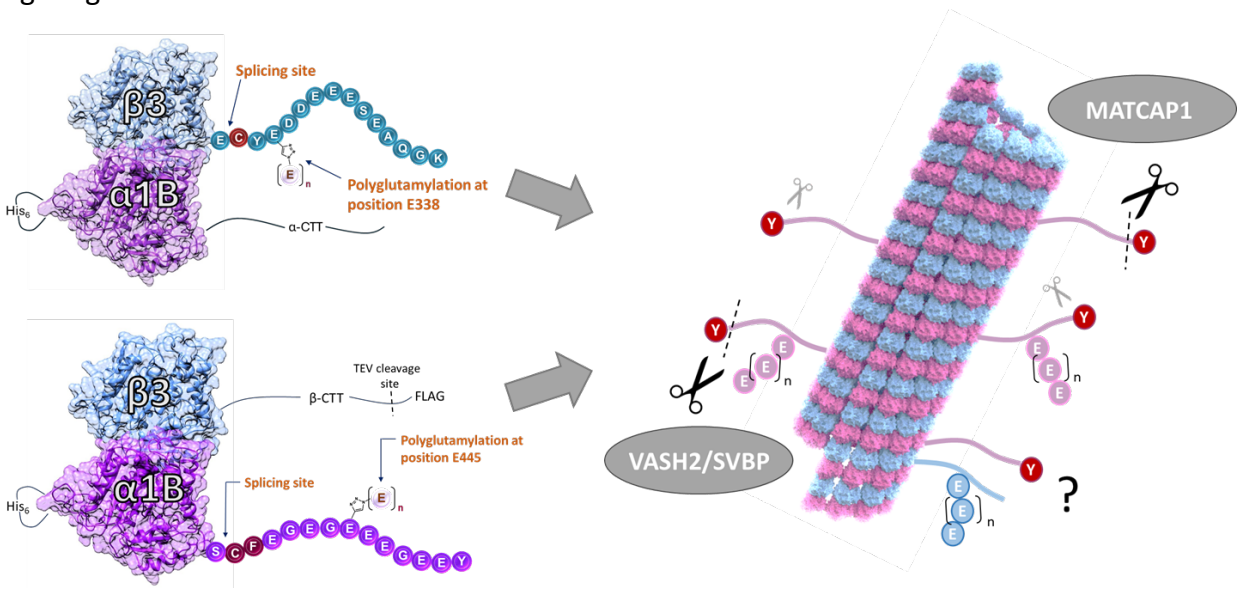
Microtubules are essential protein biopolymers comprised of α- and β-tubulin subunits that constitute the cytoskeleton, centrioles, cilia and other organelles in eukaryotic cells. The biophysical and biochemical properties of microtubules are regulated by a so-called tubulin code: different α/β-tubulin isoforms and various post-translational modifications (PTMs) such as detyrosination/tyrosination, polyglutamylation (polyE) and others. Misregulation of tubulin PTMs is associated with a number of diseases, including neurodevelopmental and respiratory disorders, cardiomyopathies, and cancers.

In our work, we study the role of the tubulin code, in particular the crosstalk between polyE and detyrosination, both critical for cell function and found together on specific microtubules. In earlier work, we developed a method to produce semisynthetic tubulin carrying defined PTMs on α-subunit C-terminal tail (CTT). We showed that polyE upregulates the activity of vasohibins, one of the key enzymes involved in tubulin detyrosination [1].

However, vasohibins are not alone responsible for tubulin detyrosination. Indeed, the recently discovered enzyme, MATCAP1 [2] represents a second carboxypeptidase providing tubulin detyrosination activity. Here, we use semisynthetic microtubule substrates, carrying various degrees of polyE to demonstrate that α-tubulin polyglutamylation downregulates MATCAP1 activity, as opposed to vasohibins.

So far, our semisynthetic approach has been limited to the α-tubulin subunit. Of note, cryoEM structural data [2] indcate that MATCAP might be as well sensitive to PTMs on β-tubulin CTT. To test this hypothesis, I am working on developing novel chemical methods to also introduce PTMs, in particular polyE, into β-tubulin. Moreover, the establishment of these methods would complement the toolkit for investigating the tubulin code in molecular detail.



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POSTERS

Stereochemistry of Cell Penetrating Peptides

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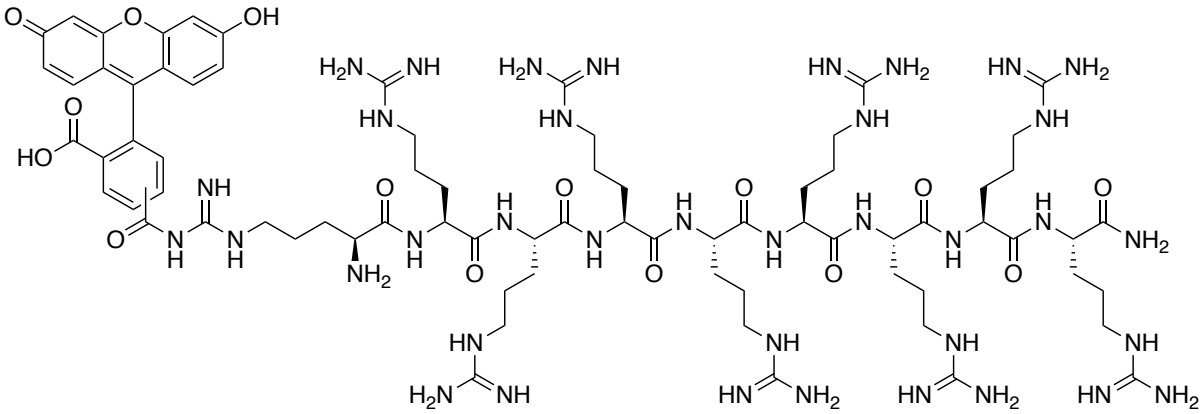
Peptides are linear oligomers of amino acids, almost exclusively of L-amino acids. Nevertheless, mixed-chirality sequences also occur, either in natural products mostly from microbial origin, such as the cyclic peptide Gramicidin S,[1] or in designed peptides such as our recently reported antimicrobial undecapeptide **ln69**.[2] Both of these peptides are membrane disruptors and the mixed chirality appears to play a key role in their activity/toxicity profile. Here we asked the question whether mixed chirality might also affect the properties of cell penetrating peptides (CPPs), focusing on the case of the arginine nonapeptide which is well-known as a drug delivery peptide.[3] We prepared a series of nona-arginine diastereomers by solid-phase peptide synthesis which we labeled at their N-terminus with 5(6)-carboxyfluorescein to trace cellular uptake.[4] The results of this study will be presented in the poster.



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Synthesis of GBD Derived Bicyclic Diamine as Interesting Scaffold for Medicinal Chemistry

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The Generated Data Base (GDB), curated by the Reymond group, offers stable, feasible, 3D-shaped chiral molecules, enabling exploration a vast chemical space, providing promising scaffolds for Medicinal Chemistry. These molecules are innovative due to their complex polycyclic system, high sp^3-hybridized carbon fraction and the presence of many quaternary carbons. [1,2] This project exploits a subset of the GDB, listing 1139 mono- and bicyclic saturated diamine scaffolds containing 5, 6 or 7 membered rings, including 680 novel scaffolds. Herein I discuss initial synthetic steps for preparing a target subset of these diamines based on Diels-Alder and ring expansion reactions *(Figure 1A).* The amine functional group within the cyclic scaffolds grants straightforward functionalisation with desired moieties. Many small molecule cores in literature features this interesting structural motifs, namely, Tofacitinib *(Figure 1B),* an anti-inflammatory drug, bears one edo and one exo cyclic amine while mCMY416, an anti-infective lead, contains a bicyclic scaffold. [3]

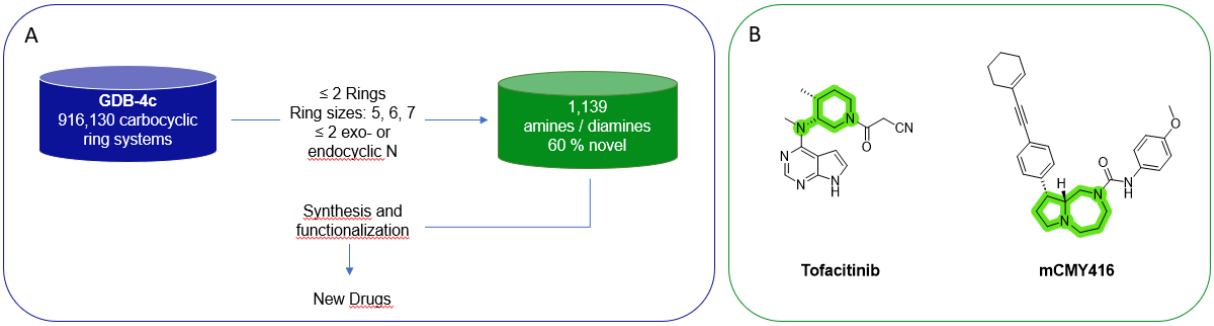

Figure 1

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Enzymatic cascade for the production of halogenated API precursors

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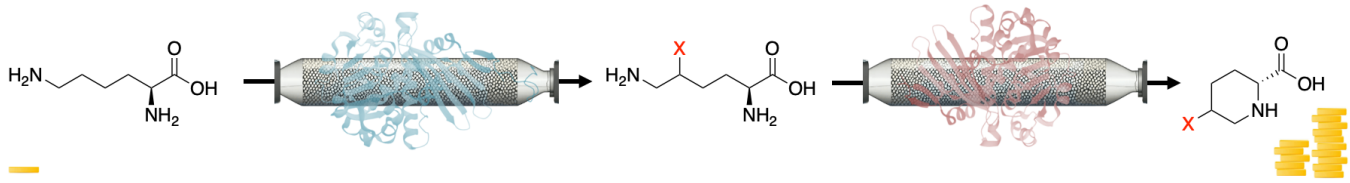
The synthesis of pipecolic acid derivatives is critical for the development of bioactive molecules and pharmaceutical compounds, particularly local anaesthetics and antibiotics. Halogens are known to enhance the pharmacokinetic and pharmacodynamic profiles of APIs and facilitate cross-coupling reactions essential for complex drug synthesis.[1] However, traditional synthetic methods struggle with the regioselective halogenation of unactivated sp^3 C-H bonds, a challenge overcome by our biocatalytic approach. We report the usage of engineered variants of Lysine Cyclodeaminase and a Hydroxylase in a two-enzyme cascade to produce chiral halogenated pipecolic acid from cost-effective starting materials.[2] The process is designed for scalability and high efficiency by utilising immobilised enzymes, with the potential of future integration in continuous flow reactors for enhanced performance.



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Predicting Off-targets from ChEMBL Data Using the Polypharmacology Browser

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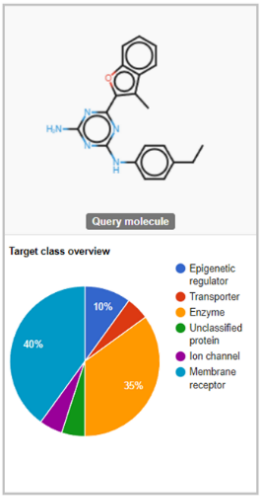
The public archive ChEMBL, which collects bioactive compounds and their associated targets from the literature, has been used by many groups to build models predicting the possible targets of small molecules to guide the experimental search for off-targets. In our group we have developed the polypharmacology browsers (PPB and PPB2),[1,2] which assign possible targets to a query molecule based on molecular fingerprint similarities to ChEMBL molecules, and provided critical insights in several practical case studies such as the identification of LPAATβ as the actual target of a putative kinase inhibitor (Figure).[3,4]



Rank	ChEMBL ID	Common name	Nearest neighbours
1	CHEMBL4772	1-acylglycerol-3-phosphate O-acyltransferase beta	Show NN
2	CHEMBL253	Cannabinoid CB2 receptor	Show NN
3	CHEMBL1904	Glutamate [NMDA] receptor subunit epsilon 2	Show NN
4	CHEMBL1800	Corticotropin releasing factor receptor 1	Show NN
5	CHEMBL224	Serotonin 2a (5-HT2a) receptor	Show NN
6	CHEMBL228	Serotonin transporter	Show NN
7	CHEMBL2916	Telomerase reverse transcriptase	Show NN
8	CHEMBL1293222	Nucleotide-binding oligomerization domain-containing protein 1	Show NN
9	CHEMBL2971	Tyrosine-protein kinase JAK2	Show NN
10	CHEMBL3764	Urotensin II receptor	Show NN
11	CHEMBL3833	Trace amine-associated receptor 1	Show NN
12	CHEMBL204	Thrombin	Show NN
13	CHEMBL3018	Matriptase	Show NN
14	CHEMBL3286	Urokinase-type plasminogen activator	Show NN
15	CHEMBL244	Coagulation factor X	Show NN

However, our PPB and PPB2 models are mostly associated only a single target per ChEMBL molecule and one protein type of targets which is a single protein. To better integrate the existing polypharmacology information available in ChEMBL, we are updating our PPB to handle multi-target information for ChEMBL molecules, using various machine learning models into account, and exploiting the latest version of the database featuring a total of 1.9 million molecule-target associations.

Keywords: Computer-aided drug design, Polypharmacology, Target prediction, Web-based tool, Cheminformatics

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Phosphorylation of alcohol sidechains

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MYC is an intrinsically disordered protein (IDP) that acts as a regulator of gene transcription and is deregulated in over 50% of human cancers.[1–3] Its activity is largely modulated by phosphorylation and the protein-protein interactions (PPIs) they induce.[4] Changes in phosphorylation could be responsible for switching from a controlled (normal) state to a deregulated (cancer) state.[5] To date, mainly small fragments of MYC with minimal phosphorylations have been isolated and studied.[6,7]

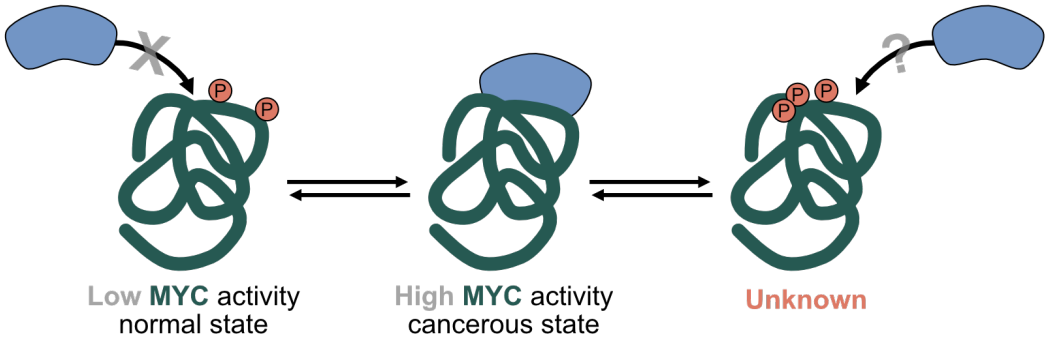

Figure 1. Phosphorylation dependence of MYC activity on cancer.

It is challenging to obtain peptides with multiple selective phosphorylations using the typical methods of a phosphorylated building block or P(III) global phosphorylation. The SPPS building block approach suffers from incomplete couplings that lead to decreased yield, limiting the number of phosphorylated residues that can be incorporated, and high costs. Meanwhile, P(III) phosphoamidite methods require a harsh oxidation step that can be incompatible with oxidation prone residues and destructive to the peptide.[8,9] To address this issue, a novel method for the on-resin (poly)phosphorylation of Ser, Thr & Tyr sidechains has been developed. This approach utilizes previously discovered, inexpensive and commercially available P(V) reagents, eliminating the need for oxidation and expensive SPPS building blocks.

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Discovery of indolicidin derived antimicrobials by diastereomeric optimization

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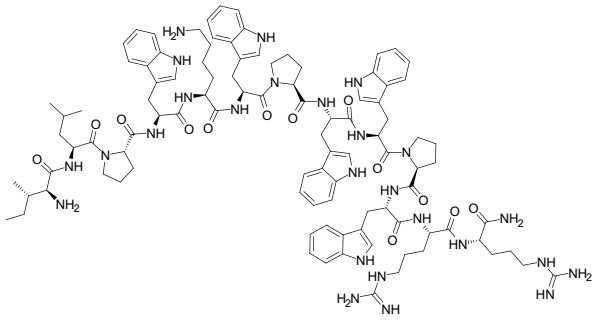
Antimicrobial peptides (AMPs) are regarded as reliable resources for anti-bacteria agents[1]. Indolicicin (ILPWKWPWWPWRR-NH_2) is a linear AMP displaying moderate and broad antibacterial activities towards gram-negative bacteria. However, severe hemolysis and limited antibacterial ability are the obstacles in therapeutical application[2]. As a result, discovering indolicidin analogues with increasing anti-bacteria efficiency and hemolytic safety has arose wide attention as a feasible method.



Recently, several interesting works in our group confirmed that diastereomeric optimization is an available strategy to modify peptides bioactivities and lead to optimized AMPs. Siriwardena *et al.* demonstrated stereochemical purity plays a critical role in the properties of AMPs[3]. Stereorandomization preserves antibacterial effect and decrease hemolysis. Personne *et al.* introduced D-residues in a α-helical linear undecapeptide to obtain stereoisomeric analogues with improved antibacterial effect and reduced toxicity[4]. Based on these works, diastereomeric optimization might provide a promising method to obtain effective and safe indolicidin derivatives. This project focuses on replacing the L amino acids with D amino acids to obtain diastereomeric indolicidin analogues. Preferred peptides will be tested at further biological and pharmaceutical experiments to investigate cellular mechanism and drug-like property.

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Analysis of InsP1 and InsP2 in biosamples by CE-MS

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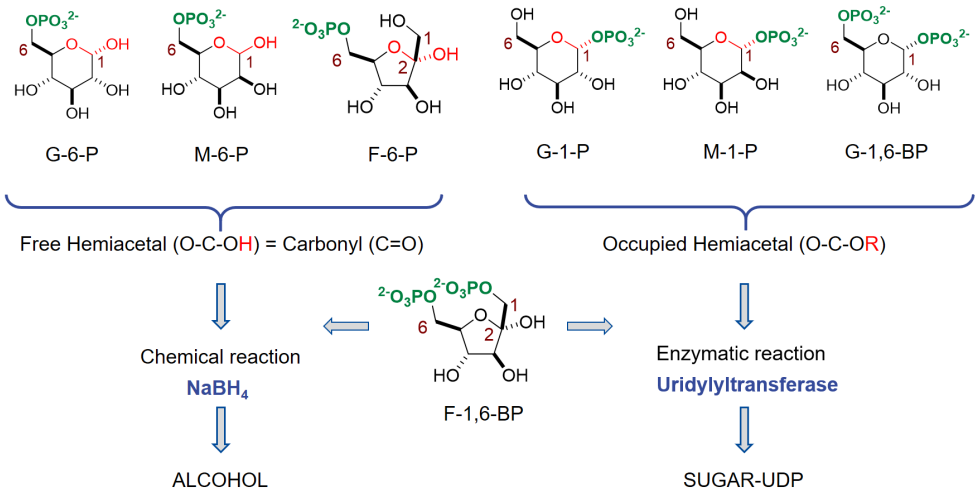
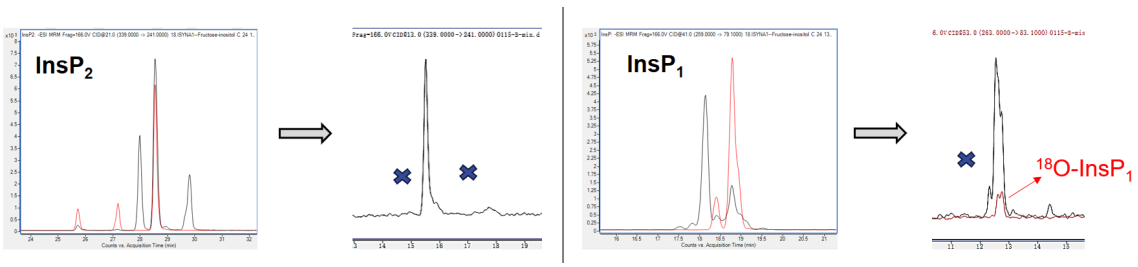
Inositol phosphates play an essential role in the biology world and they are a type of molecule with numerous isomers, chromophore-free and a high charge density structures. Capillary electrophoresis mass spectrometry (CE-MS) has been shown to be a powerful analysis approach for InsP3-InsP8.[1-3] However, for InsP1 and InsP2, it is common to find mixtures of inositol phosphates and sugar phosphates in biosamples, which share the same mass and become problematic for the assignment by CE-MS.

Here we introduce the pre-treatment method to remove sugar phosphates and enable the accuracy of the measurement of InsP1 and InsP2, using uridylyltransferase and sodium borohydride (NaBH4). Uridylyltransferase converts occupied hemiacetal sugar phosphates to UDP-bonded products (sugar-UDP), e.g., glucose-1-phosphate to glucose-1-UDP. NaBH4 reacts with free hemiacetal sugar phosphates and generated corresponding alcohols, e.g., glucose-6-phosphate to sorbitol. The comparison between the biosample before and after the treatment showed that sugar phosphates were removed in the fragmentation and only inositol phosphates were reserved, ready for further analysis.





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A chemical degron system for exploring PALB2 synthetic lethal combinations

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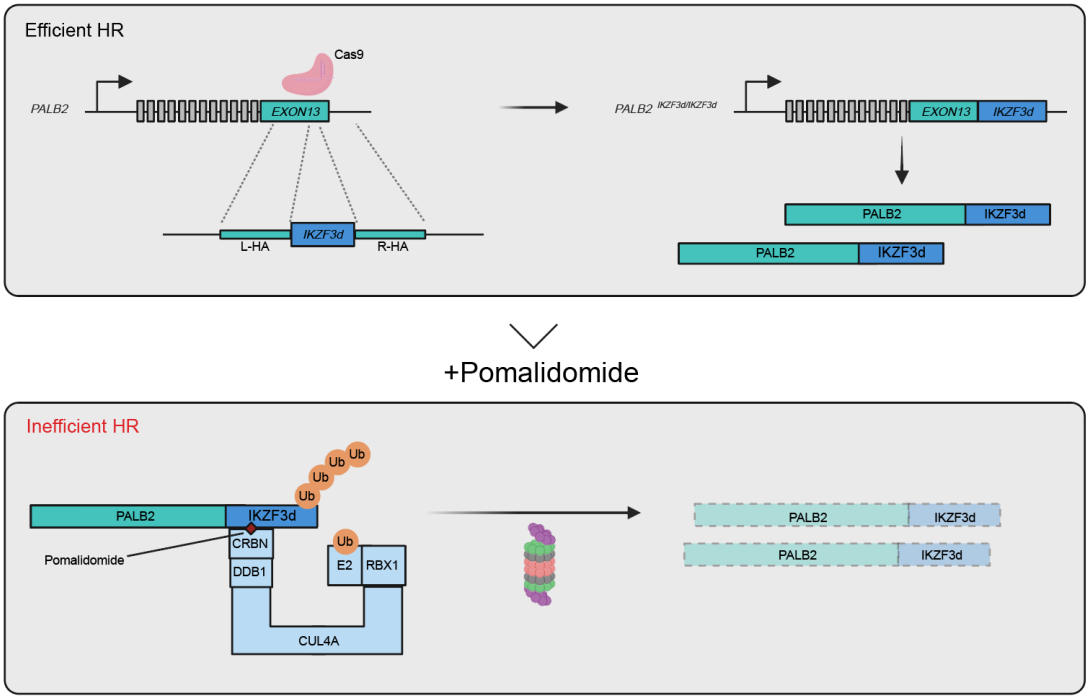
Cells with poorly functioning homologous recombination (HR) are extremely sensitive to poly (ADP-ribose) polymerase (PARP) inhibition. Current approaches to exploit this vulnerability focus exclusively on PARP inhibition in a mutated HR genetic background. If HR factors could be inhibited or degraded in normal cells with drugs, then combination treatments of these agents with PARP inhibitors could open new patient populations to this well-validated combination. Here we build a model system which fully recapitulates PARP/HR synthetic lethality by installing a small-molecule responsive zinc-finger degron in the HR factor partner and localizer of BRCA2 (PALB2). We also then test a battery of peptide ligands for PALB2 based on its natural binding partner. Taken together these our studies validate PALB2 as a target for drug development and provide the tools for identifying small molecule binders.



Multicomponent condensations as an easy and accessible method for creating bioactive compounds

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Many substances used in medicinal chemistry, agrochemicals, and dyes have a nitrogen-containing heterocyclic framework. For example, essramycin **1** is the first triazolopyrimidine antibiotic with strong antibacterial activity. Just 6.25 µg/ml of the triazolopyrimidine-6-carboxylic acid derivative **2** is enough for 92% inhibition of the growth of Mycobacterium *tuberculosis* H37Rv. The 6-hydrazidetriazolopyrimidine derivative **3** has demonstrated potent activity against Gram-positive and Gram-negative bacterial strains, and the thiosemicarbazide derivative **4** has potent activity against Gram-positive strains [1].

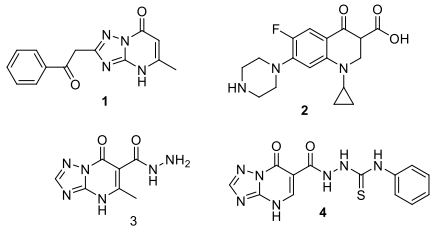

Figure 1. Biologically active pyrimidine derivatives

We have previously synthesized a series of 3-amino-1,2,4-triazolopyrimidine derivatives [2]. In order to diversify the structure of these derivatives and study their biological activity, we carried out a number of modifications, including methylation, acetylation, reduction, and aromatization reactions.

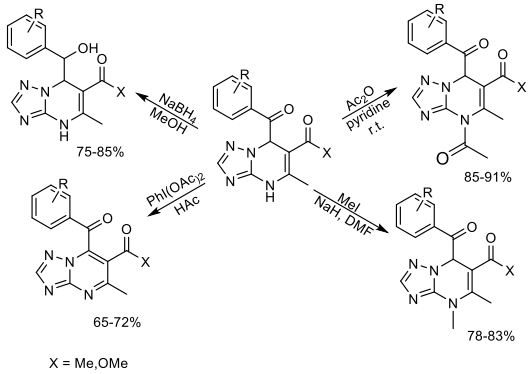

Figure 2. Scheme of the modification of 3-amino-1,2,4-triazole derivatives

It turned out that methylation with methyl iodide in the presence of KOH in acetonitrile does not lead to the formation of a product, unlike the reaction with NaH in dimethylformamide in an inert medium. Acetylation occurs both for compounds containing an acetylacetone fragment and for compounds with an acetoacetic ester residue. Aromatization occurs only at high temperatures - boiling in acetic acid - while the reduction of the aryl fragment is possible at 0°C in methanol. The structures of the obtained compounds were confirmed by ^{1}H and ^{13}C NMR, as well as COSY, HMBC, NOESY spectroscopy, and mass spectrometry.

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Synthesis of modified carbohydrates as glyco-donors aiming complex glycans synthesis and glycopeptides

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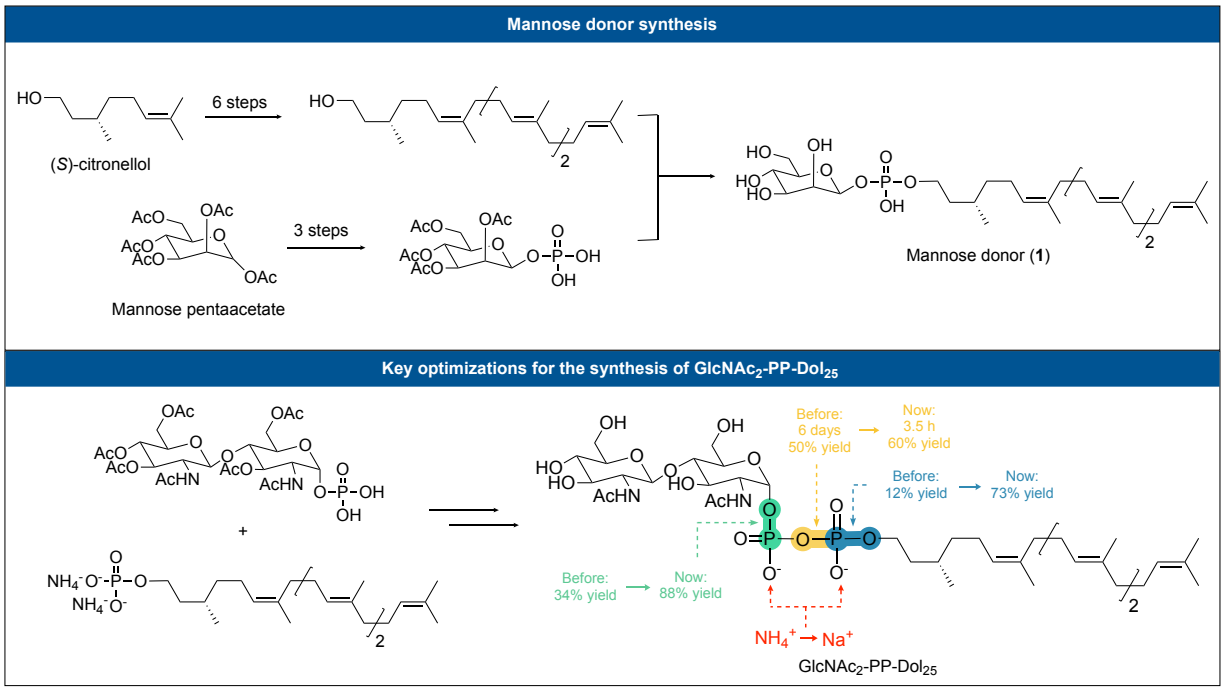
Lipid-linked oligosaccharides (LLOs) are pivotal in *N*-protein glycosylation, a crucial post-translational modification facilitating a broad spectrum of *N*-glycan structures.[1] In eukaryotes, this biological process is governed by various enzymes such as ALG (asparagine-linked glycosylation) and OST (oligosaccharyltransferase), which use LLO as substrates.[2] Our laboratory has developed simplified LLOs precursors, which are converted to synthetic LLOs through enzymatic synthesis employing glyco donors such as dolichyl phosphomannose (**1**).[3] Recently, we have also optimized the synthesis and purification of dolichyl diphosphochitobiose ($GlcNAc_2$-PP-Dol_{25}), providing a reliable method for preparing new LLO analogues.[4]

Extending on this work, we aim to synthesize modified glyco substrates capable of being recognized by Alg enzymes to produce complex oligosaccharides. Ultimately, these synthesized glycans hold the potential for producing glycopeptides, offering promising avenues for further exploration in glycoscience and biotechnology.



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Design, Synthesis and Characterization of a Selective Tritium-labeled $P2Y_{12}$ Receptor Antagonist

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The $P2Y_{12}$ receptor expressed on platelets is a target of antithrombotic drugs (e.g. clopidogrel and ticagrelor).[1] In addition, it is expressed in the brain on microglial cells and involved in neuroinflammation.[2] Radioligands, e.g. positron emission tomography (PET) tracers, targeting $P2Y_{12}$ receptors have potential for diagnostic imaging and therapeutic monitoring providing a non-invasive method to study receptor expression and distribution as well as monitoring activated microglial cells in vivo.[3-4]

In this study, we set out to develop a non-nucleotidic, potent and selective radioligand for the labeling of $P2Y_{12}$ receptors, with potential to penetrate into the brain. A comprehensive data analysis of published $P2Y_{12}$ receptor antagonists was conducted and key parameters such as binding affinity, selectivity, pharmacokinetic properties, and properties related to brain bioavailability were evaluated. This led to the selection of a promising $P2Y_{12}$ receptor antagonist scaffold,[5] and the design and synthesis of a tritium-labeled $P2Y_{12}$ receptor antagonist, termed [^{3}H]PSB-22219. The new radioligand displayed high-affinity binding to membrane preparations recombinantly expressing the human $P2Y_{12}$ receptor (K_D = **4.57** nM) and low non-specific binding (< 10 % of total binding), while non-transfected cells were devoid of specific binding sites for the radioligand. Selectivity of [^{3}H]PSB-22219 was confirmed versus the closely related receptor subtypes $P2Y_1$ and $P2Y_{13}$. The established radioligand binding assay was employed to characterize $P2Y_{12}$ receptors natively expressed in human platelets (K_D = **2.53** nM) and rat brain cortical membrane preparations (K_D = **5.35** nM). This new, superior radioligand is expected to become a useful pharmacological tool. It will contribute to the future development of PET ligands and therapeutics targeting brai $P2Y_{12}$ receptors.

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Evaluating Lysyl Oxidase Activity with Turn-On Fluorescent Probes

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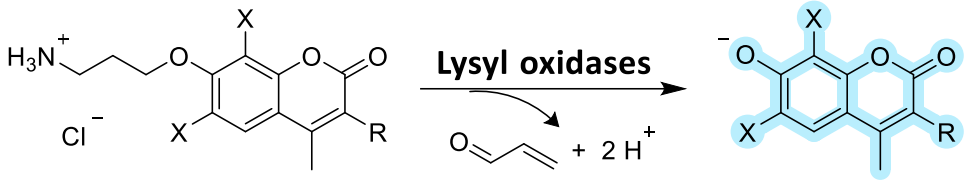
Remodelling and maturation of collagen, the dominant structural protein in mammals, is crucial for the integrity of organs and wound healing.[1,2] These processes include post-translational cross-linking of collagen strands triggered by the oxidation of lysine residues through lysyl oxidases (LOXs). This enzyme family consists of five isoforms - lysyl oxidase and four lysyl oxidase-like enzymes. LOXs catalyze the oxidative deamination of lysine residues in the telopeptide domain of collagen and are important for the mechanical properties of the extracellular matrix (ECM).[1] Excessive LOX activity is, however, associated with fibrotic and malignant diseases which are estimated to account for around 45% of deaths in developed countries.[3]

A comprehensive investigation of LOX activity is therefore important for a deeper understanding of normal physiological versus pathological processes. The current standard activity assay detects hydrogen peroxide, the by-product of the oxidative deamination reaction, and lacks specificity.[4] Our group has recently developed an enzyme-reactive sensor that detects LOX *in vitro*, *in vivo* and in tissue sections.[5]



In this work, we developed a quick and straightforward assay for measuring LOX activity, based on the turn-on of a coumarin-based sensor. We have examined various analogs of the activity-based probe and evaluated their selectivity for LOX isoforms over related amine oxidases. We anticipate that our tools will be valuable for the screening of drug candidates targeting LOXs and deciphering the role of LOXs' in healthy and diseased states.

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Synthesis of novel bicyclic diamine scaffolds derived from tropinone

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The generated databases (GDBs) are a large collection of generated chemical scaffolds which adhere to principles of synthetic feasibility and chemical stability. Interestingly, most of the compounds in the database are novel which make the GDB an important resource for discovering novel chemical targets and allows us to explore previously unknown chemical space.

From the GDB we have found an interesting novel family of tricyclic and bicyclic chemical scaffolds which can be accessed synthetically from tropinone.
In this poster we describe a simple synthesis to obtain novel bi-cyclic diamine scaffolds from tropinone which can be easily functionalized to explore novel medicinal chemistry space.

Small molecule drugs often consist of rigid scaffolds equipped with reactive handles, typically amino groups which can be functionalized. To explore previously unknown chemical space, we can use generated databases (GDBs), which are a large collection of generated chemical scaffolds adhering to principles of synthetic feasibility and chemical stability. Comparing the generated databases (GDB) with biologically active small molecules in ChEMBL reveals that many scaffolds, even structurally simple ones, have never been synthesized (1).

Here we discuss the synthesis of a novel family of tricyclic and bicyclic amino containing chemical scaffolds which can be accessed synthetically from tropinone and can be easily functionalized to explore differentiated medicinal chemistry space.

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Synthesis of Chiral Tricyclic Piperazine Scaffolds from the GDB Database

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The generated databases (GDBs) are a collection of possible molecules up to a certain size which are filtered by rules of synthetic feasibility and chemical stability. Interestingly, a large number of these molecules are novel, intrinsically chiral, 3D-shaped and have never been synthesized. (1) As such the GDBs are a valuable source of new scaffolds for medicinal chemistry, which is why we are interested in exploring these databases synthetically. In the past this has already yielded interesting scaffolds such as triquinazine which has been used to discover a nanomolar and selective inhibitor of Janus Kinase 1 (see Figure 1). (2)

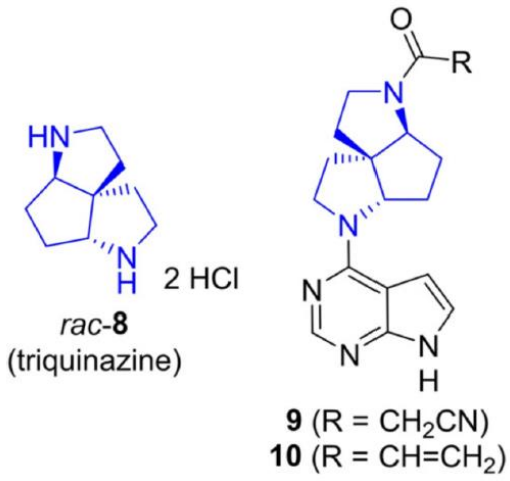

Figure 1 Structure of triquinazine and the nanomolar and selective inhibitor of Janus Kinase 1 utilizing it. Taken from (2)

Building on this success, in our ongoing research we have further explored the GDBs to identify additional interesting cases of novel chiral tricyclic scaffolds. These scaffolds are synthesized to be used in a medicinal chemistry context.

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Pretargeting intracellular oncogenic proteins for click-to-release

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Systemically administered chemotherapy in the form of cytotoxic agents like doxorubicin is often accompanied by severe side effects. In a similar way to antibody drug conjugates, small molecule drug conjugates (SMDCs) aim to localize the treatment by combining a targeting ligand with a cytotoxic moiety. The localizing part of an SMDC must be selective for a target associated or specific to the cancer tissue in question. For our system we chose the epidermal growth factor receptor (EGFR) a receptor kinase which is overexpressed in several types of cancer, including lung and colorectal cancer, and afatinib a covalent inhibitor targeting the kinase domain of this protein.[1]

The inverse electron-demand Diels-Alder (IEDDA) reaction between tetrazines and trans-cyclooctenes (TCOs) is an emerging biorthogonal reaction. Since its introduction in 2008[2] it has found application in the traditional sense of click reactions of connecting two scaffolds, but moreover it can also act in a dissociative manner called click-to-release,[3] when a suitable leaving group is placed next to the alkene. A biologically active compound can be rendered inactive by placing a bulky TCO on a suitable heteroatom. It will only be activated upon reaction and subsequent release with a tetrazine.

By way of connecting the activating tetrazine to the EGFR targeting afatinib, we aim to localize the cargo release to cells with overexpressed levels of EGFR. Following this treatment, TCO protected MMAE can be introduced which will only be activated in cells with elevated levels of the tetrazine.

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Regiodivergent Ring-Expansion of Oxindoles to Quinolinones

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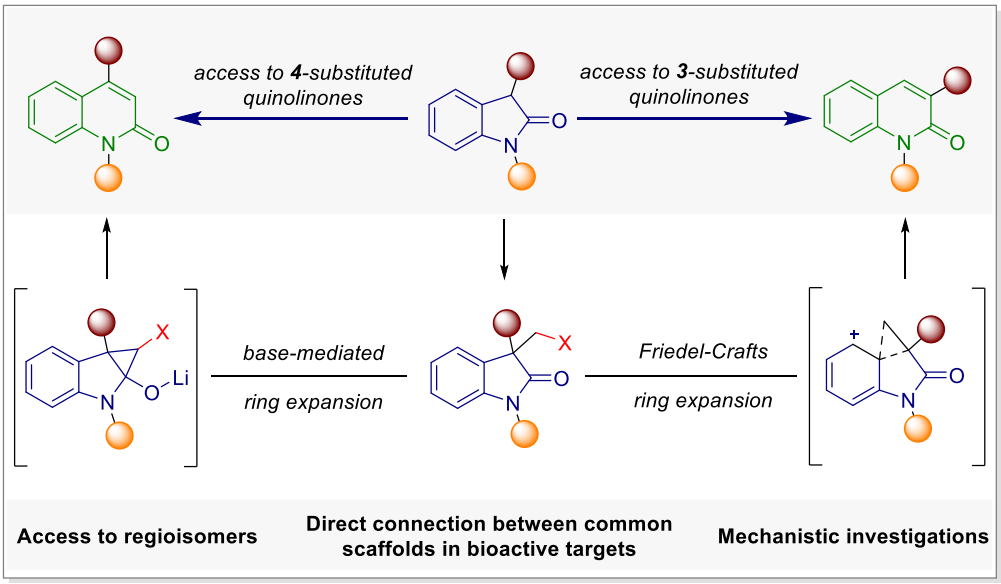
The exploration of diverse molecular structures is pivotal for uncovering innovative pharmaceuticals, pesticides, and other tailor-made compounds. Particularly in studies concerning structure-activity relationships (SAR), the investigation of variations in the core structures of lead compounds holds considerable interest.[1,2] However, to obtain these diverse molecular architectures, usually *de novo* multistep syntheses are required, obstructing access to otherwise desirable analogues. Recently, novel techniques like cut-and-sew and skeletal editing have emerged to directly modify core scaffolds.[1,3] However, these methods often focus on linking two skeletal structures, overlooking the importance of obtaining isomers, which could be particularly valuable in SAR studies.

Here, we present a divergent approach that allows for the interconversion of oxindoles to quinolinone regioisomers.[4] This not only enables the rapid synthetic connection between these two prominent scaffolds, but also offers access to isomers, mitigating the need for *de novo* syntheses in SAR studies.

The initially disclosed method for the conversion of oxindoles to 4-substituted quinolinones comprises a LiHMDS-mediated pathway, allowing for a broad range of functional groups to be tolerated. To investigate mechanistic details of this transformation a variety of experiments are presented, e.g. Hammet-plot analysis and KIE studies. Complementing this initial discovery, we designed a transformation of oxindoles to the isomeric 3-substituted quinolinones, through a Friedel-Crafts type mechanism from the same starting materials. Together, both methods are applied to a variety of oxindole drugs, such as the cognitive enhancer linopiridine, or the epilepsy drug doliracetam. Further, the synthesis of an analogue of the quinolinone drug tipifarnib is achieved, giving access to the drug in the least number of steps as compared to the literature, while also offering a straightforward route to its regioisomer.



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Potent Inducers of Paraptosis Through Electronic Tuning of Michael Acceptors

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Paraptosis is a non-apoptotic programmed cell death characterized by cytoplasmic vacuolation due to endoplasmic reticulum (ER) and mitochondria swelling. This process may lead to protein and Ca^{2+} homeostasis disruption and activation of the unfolded protein response of the endoplasmic reticulum (UPR^{ER}). [1,2] Paraptotic cells do not exhibit DNA fragmentation or caspase activation. [1] The mechanisms that lead to paraptosis are not well understood. However, specific targets have been reported to induce paraptosis. These targets include the insulin-like growth factor I receptor (IGFIR), [1,2] GDP-dissociation inhibitor beta (GDI2), [3] and ubiquitin-specific peptidase 10 (USP10).[4] Due to defective apoptosis, some cancer cells exhibit resistance to current therapies. Therefore, non-apoptotic programmed cell death mechanisms such as paraptosis have gained significance in cancer therapy.

In this study, we developed a set of Michael acceptors, one of which exhibited paraptosis induction in HEK293, HeLa, and MDA-MB-231 cell lines. This process was characterized by the formation of vacuoles arising from the ER and did not involve caspase activation. The compound caused swelling of the ER and mitochondria, increased superoxide production, and exhibited reactivity towards cysteines. Furthermore, our initial proteomic analysis revealed specific proteins that differ from known paraptosis-inducing targets. These proteins could be potential targets for paraptosis activation.

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Development of dual MGMT-proteasome inhibitors

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O6-methylguanine-DNA methyltransferase (MGMT) is a DNA repair protein that rescues cells from guanine O6-alkylation. This mechanism protects cells from malignant transformation, but in already existing tumours it decreases the efficacy of chemotherapy based on DNA-alkylating agents [1]. Another more widely used cancer therapy is based on proteasome inhibition. The proteasome is a sophisticated protein complex that plays a central role in protein degradation in eukaryotic cells. Since cancer cells strongly rely on high protein turnover, proteasomal degradation becomes vital for tumours [2].

In this work we aimed for the development of bispecific molecules that can do both: inhibit proteasome and cause MGMT degradation. Two series of molecules which comprise O6-benzyl guanine attached to the proteasome targeting scaffold were created. O6-Benzylguanine should covalently modify C145 of MGMT, thus causing its degradation, and the proteasome targeting part should covalently bind to the beta-5 subunit of the proteasome 20S core particle. First, compound activity was measured with western blotting. Then we developed a fluorescent cell model that allowed us to assess proteasome inhibition in a more productive way with flow cytometry. Furthermore, we investigated if the proteasome inhibition caused by some of our molecules was MGMT-dependent. For this, molecules were tested in a cell line expressing mutated C145A MGMT that should not be modified by benzylguanine moiety. Bispecific molecules appeared to inhibit proteasome in an MGMT-independent manner. Hence, we found molecules that function as dual proteasome-MGMT inhibitors, even though the two mechanisms are performed independently within the cell.

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