Knoevenagel condensation by flow: solid-supported catalysts

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The Knoevenagel condensation occurs between a compound with an activated methylene carbon and an aldehyde. Typically catalyzed by an organic base such as piperidine or pyridine, the reaction continues to have great importance for C-C bond formation.[1,2]

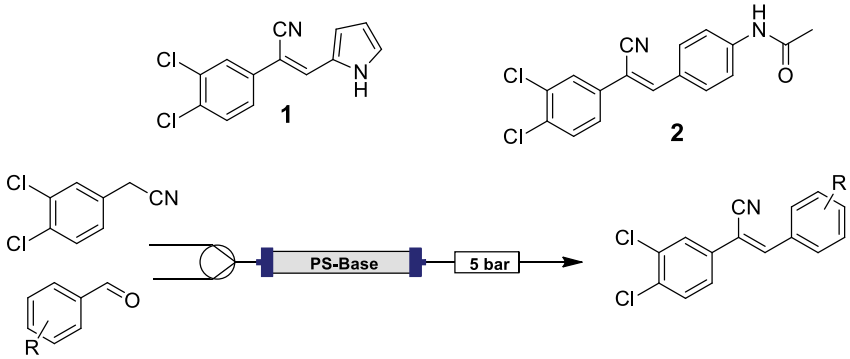
Ongoing projects in our group include the development of small molecule activators of the Arylhydrocarbon Receptor (AhR) pathway, for the treatment of breast cancer.[3] Two compounds that have come out of these studies, ANI-7 (**1**) and its 4-acetamide phenyl derivative (**2**), are both afforded via the Knoevenagel condensation.

We sought to convert this reaction to a flow platform for easy library synthesis. Our aim was that the generation of compounds could be conducted by undergraduate students, with minimal research lab experience.

Initial attempts to convert this reaction to a flow protocol was unsatisfying due to the high equivalents of piperidine required.[4] As such, we sought to explore the preparation and use of a number of organic bases on a silica medium. The use of solid-supported catalysts in flow reactors is well published, and demonstrates many benefits over a homogenous catalyst in solution.[5]

Our requirements were that these heterogenous catalysts were able to be prepared from cheap, readily available starting materials; that their preparation be relatively quick, with little work up required; and that the catalyst be reusable. These requirements ensured that, should the catalyst be exhausted, it could be resynthesized quickly, easily and cheaply. As these solid-supported catalysts would be utilized by undergraduate students, it was essential that their preparation involved mild conditions, and that they were able to be quickly and cheaply prepared. A number of established protocols were utilized,[6-8] as well as some novel syntheses.

Here we report the synthesis of five different silica-supported organic bases, and their use for the synthesis, in a flow instrument, of new ligands for the AhR pathway.



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Intensifying Diffusion-Limited Reactions by Using Static Mixer Electrodes in a Novel Electrochemical Flow Cell

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Surface-mediated reactions require effective mixing for them to be intense and efficient. In electrochemistry where the reactants must contact electrode surfaces for a sufficient time to enable electron tunnelling to occur, exhaustive electrolysis in poorly stirred reactors can take many hours to complete. As a consequence, electrochemical processing in many industries is restricted to batch processing which is more costly and labour intensive than continuous processing and results in significant down time between batches. This is not ideal for bulk production or treatment. Here we describe CSIRO's novel axial flow electrochemical cell which aims to address these mixing problems by using a bespoke static mixer electrode (SME), designed by computational fluid dynamics (CFD) and manufactured using additive manufacturing technology to retain the fidelity of the original design. This electrode was characterized by SEM-EDS and electrosorption measurements. The performance of the electrochemical flow cell was evaluated by probing the ferricyanide ($[Fe(CN)_6]^{3+}$) reduction reaction on a platinum-coated SME under controlled mass transport conditions using cyclic voltammetry (CV), linear sweep voltammetry (LSV) and chronoamperometric measurements. Results affirm that the ferricyanide reduction rate is increased significantly in the novel CSIRO flow electrochemical cell, particularly in dilute solutions ($<10^{-2}$ M) where mass transport by diffusion plays a significant role. The performance of the cell is compared with a rotating disk electrode and a tubular flow cell.

Kinetic Control of Aggregation Shape in Micellar Self-Assembly Using Flow Reactors

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The kinetic and thermodynamic processes by which amphiphilic block copolymers (BCPs) self-assemble into nanostructures has been used extensively over the past decades. BCPs can self-assemble into different morphologies such as micelles, vesicles and rods, which have been proven to have a considerable influence in cell-uptake. This process of self-assembly is governed by complex thermodynamic factors such as repulsion of solubilized chains in the hydrophilic corona, stretching of hydrophobic chains in the core and interfacial tensions.[1] However, it remains nontrivial to precisely predict the morphology after self-assembly of amphiphilic BCPs.

Utilizing the benefits of flow chemistry, kinetic control towards micelle shape is investigated using static micromixers and varying flowrates for the aqueous and organic phase.[3] This method allows for micelles to be made with the same block copolymer into ellipsoids (130 nm/33 nm) at low mixing speed to near spherical particles (44 nm /26 nm) solely by altering flowrates of the two phases as shown by small angle neutron scattering (**Figure 1)**. This platform is a first step towards further investigation on morphology control of self-assembled nanostructures using a top-down approach, which will have a significant impact on a plethora of existing drug-delivery applications.

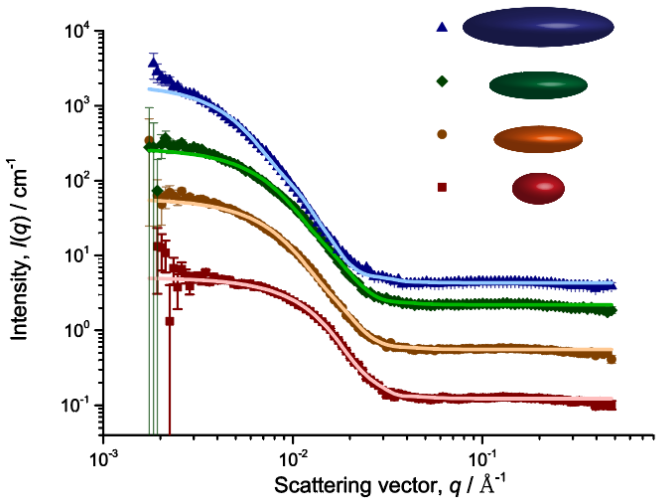

Figure 1. Small-angle neutron scattering data from $PHEA_{30}$-*b*-PS_{100} with a D2O background subtracted (symbols) and theoretical fits (solid lines) of micelles self-assembled at different flow rates. Blue=1mL·min^{-1}, Green=2mL·min^{-1}, Orange=4mL·min^{-1} and Red=8mL·min^{-1}

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Research interests:	Polymers, Self-Assembly, Nanoparticles

Visible-Light Promoted Tandem Photoredox Catalysis: Enabling Aminocarbonylation of Unactivated Alkyl Iodides in Continuous Flow.

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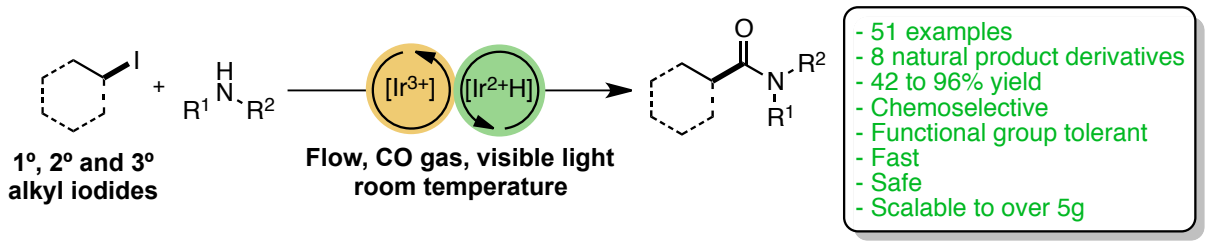
Here we report a method for the radical aminocarbonylation of unactivated 1°, 2° and 3° alkyl iodides using carbon monoxide as C1 building block. The process harnesses our recently discovered tandem photocatalytic cycle of the [Ir(dtbbpy)(ppy)$_2$]PF$_6$ photocatalyst[1] which engaged energy demanding alkyl iodides in combination with flow technology. The synthesis of 51 of alkyl amides is achieved in good to excellent yields and the method is applied to the late-stage functionalisation of a natural product. A bespoke flow chemistry platform was assembled from a HPLC pump, a gas liquid tube-in-tube reactor and blue LED photoreactor, enabling ambient pressures of CO, and short residence times. Mechanistic studies confirm the operation of a tandem photocatalytic cycle of the [Ir(dtbbpy)(ppy)$_2$]PF$_6$ complex and DFT calculations indicates that radical chain propagation has a crucial role in this reaction.



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Autonomous Macromonomer Production with Flow Chemistry

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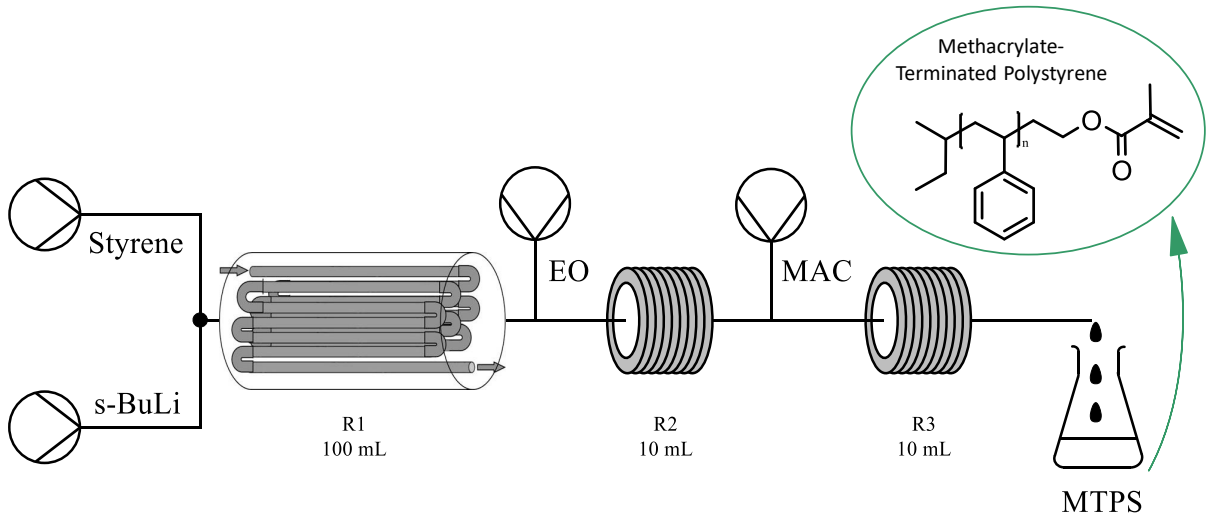
Continuous flow chemistry was utilized for the autonomous manufacturing of methacrylate-terminated polystyrene (MTPS) macromonomers. Anionic polymerization of styrene was followed by ethylene oxide chain extension and subsequent methacryloyl chloride termination by sequential reagent addition along an integrated continuous flow path. Methacrylate-terminated polystyrene macromonomers of defined molecular weight were produced with greater than 99% styrene conversion and over 97% methacrylate end group functionality in under 20 minutes.



NANOMACHINE BIOCATALYSTS: TOOLS FOR CONTINUOUS FLOW BIOCATALYSIS

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Key Words: continuous flow biocatalysis, nanomachines, cofactor recycling.

Assembling cell-free, cascading multi-enzyme enzyme reactions continuous flow systems for the conversion of low value renewable feedstocks into high value products represents a fourth wave of biocatalysis for renewable green chemistry and synthetic biology applications [1]. However, major limitations to both applications include the cost of producing multiple purified enzymes and of providing a continuous supply of diffusible cofactors or cosubstrates [2]. We have applied synthetic biology principles to produce fusion proteins between enzymes and their cofactor-recycling partner enzymes, with concomitant *in situ* recycling of a modified tethered cofactor, with an added conjugation protein element to allow immobilization of the nanomachines to a surface. This has enabled the construction of nanomachine flow reactors which can be combined in an interchangeable, "plug-and-play" manner to construct complex synthetic continuous flow networks or Nanofactories [3]. Synthesis of the anti-diabetic drug, D-fagomine, reductive amination to produce various chiral or conjugated amines (Fig. 1) and deracemization of alcohols have been used to exemplify the principles, and we have demonstrated tethered cofactor recycling of ATP, $NAD(H)^+$ and $NADP(H)^+$, as well as ligand-directed immobilization of a variety of enzymes to illustrate the use of these nanomachine biocatalysts as tools for the *de novo* construction of continuous flow systems for synthetic biology and fine chemical synthesis. Our research is currently exploring the use of frugal innovation principles to integrate reactor design with on-line analytics for real-time reaction monitoring, and, subsequently, dynamic control over fluidics via feedback loops.

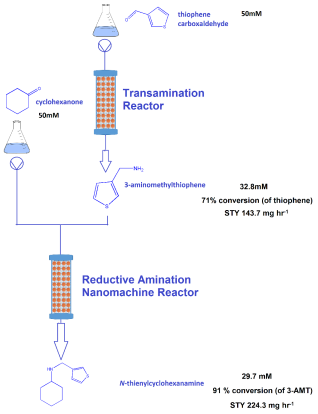

Figure 1: Continuous flow biocatalysis system combining transamination and reductive amination for the formation of conjugated amines.

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The Application of Homogeneous and Heterogeneous Ring-Opening Polymerisation into Flow

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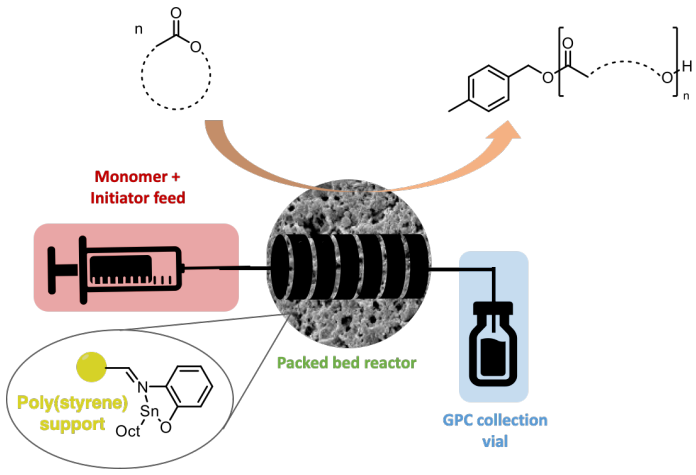
A significant amount of research has gone towards the development of sustainable alternatives to fossil fuel derived polymers; this has been driven in part by the increased awareness of the effect of plastics on the environment. Polymers from lactones such as *L*-lactide have received particular attention due to their biogenic properties. The use of heterogeneous catalysts in the production of these polymers has remained largely unexplored, despite significant study of homogeneous analogues. In fact, the former could offer both a means of producing polymers of high purity with low metal content without any extra purification. Further, the chance to recover and recycle the catalysts offers a significant economic benefit, and there is an added advantage of using the catalysts in a flow system to enable continuous polymer synthesis. Flow systems are well known for addressing many issues that plague batch processes; thereby improving mixing, heat transfer, and offering a viable way to scale up reactions. In particular, ring-opening polymerisation (ROP) of lactones in flow has previously been shown to produce polymers with excellent control over the dispersity and molecular weight (M_n).[1,2]

In this work, we present the development of heterogeneous metal complexes for use in the ROP of lactide, leading up to initial studies incorporating ROP into a flow set-up. A series of metal complexes were immobilised onto inert poly(styrene) (PS) supports and utilised in the ROP of various lactones in bulk conditions. **PS-L^{H}ZnOAc**, **PS-L^{H}SnOct** and **PS-L^{Cl}SnOct** were identified as the most successful heterogeneous catalysts for the ROP of *L*-lactide. Investigations by *in situ* ATR-FT-IR revealed conversions reaching *ca.* 90% in 55 minutes, with excellent molecular weight control and dispersities ($Đ_M$ 1.15–1.17). Catalyst reuse was also possible, with up to 7 reuse cycles, albeit accompanied by a progressive reduction in conversion. Due to the reusability of the catalysts, we are developing the application of these catalysts into flow systems, where the catalyst is immobilised into a packed bed, and continuous production of polymer is possible. Initial investigations of the homogeneous flow system also offer promising results, demonstrating the applicability of ROP into flow.



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Organocatalytic Decarboxylation of Amino Acids in Continuous Flow: A Scalable Route to 3-Hydroxypyrrolidine

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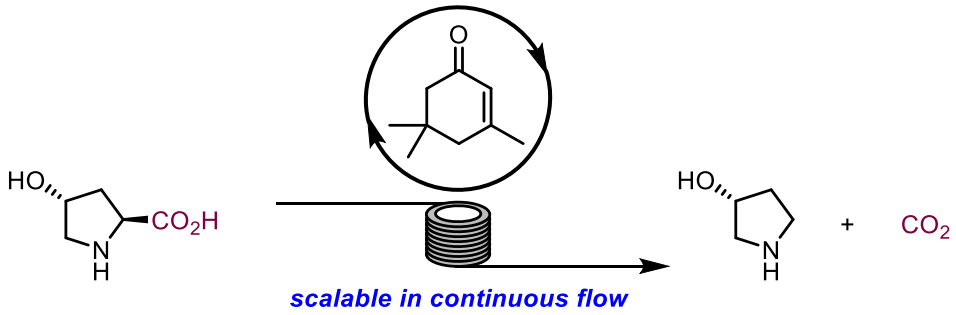
The decarboxylation of amino acids represents a straightforward route to the production of versatile amines from inexpensive and abundant bio-feedstock. In the presence of an enone organocatalyst, the thermal decarboxylation of α–amino acids readily occurs at high temperatures. However, this reaction must be performed under pressurised 'bomb' conditions or under microwave irradiation, preventing effective transferal to scales beyond the laboratory bench.

For the first time, homogenous continuous flow methods enable the rapid, high-throughput decarboxylation of α-amino acids. The process is mediated by an inexpensive and recyclable enone, yielding product amines in high purity after aqueous extraction. This methodology has been applied to the preparation of valuable (*R*)-3-hydroxypyrrolidine from *trans*-4-hydroxyproline.



Multistep Synthesis of Benzhydrylamines via Photoredox Catalysed Transfer Hydrogenation in Continuous Flow

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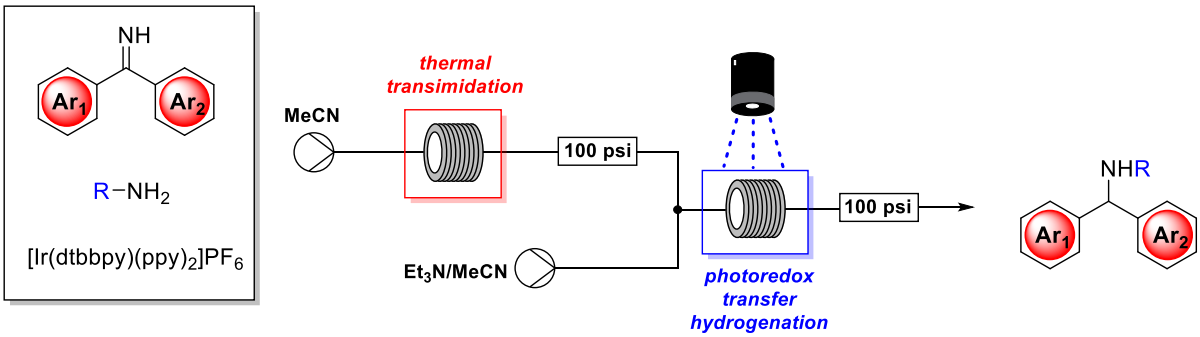
The multistep synthesis of biologically relevant benzyhdrylamines has been demonstrated combining principles of flow chemistry and photoredox catalysis. The synthesis was conducted using commercially available reagents via thermal transimination to afford N-substituted imines. Using Et_3N as both a single electron donor and hydrogen atom donor, the transfer hydrogenation of the diarylimines in the presence of the photocatalyst $[Ir(dtbbpy)(ppy)_2]PF_6$ was readily achieved. The reaction scope of this multistep flow process is broad and we demonstrate the practicality of this method through facile scale-up and synthesis of an active pharmaceutical ingredient.



Towards the Continuous Flow Synthesis of Alkyl Citrate Natural Products

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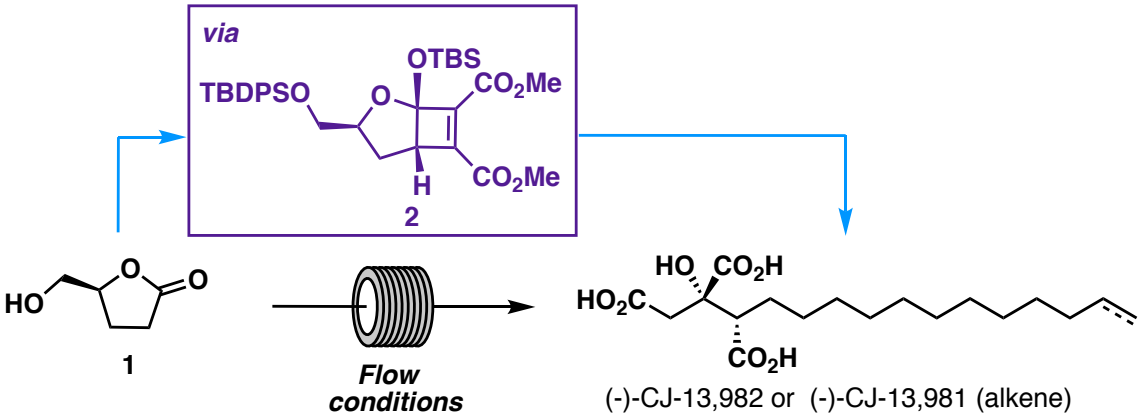
The alkyl citrate family of complex fungal metabolites are potent inhibitors of squalene synthase (SSase), a key enzyme implicated in the biosynthesis of cholesterol. This presentation will detail our efforts toward the continuous flow total synthesis of citrates (-)-CJ-13982 and (-)-CJ-13981 via the cyclobutene diester **2**, from biomass derived (*S*)-(+)-γ-hydroxymethyl-γ-butyrolactone **1**.



Absolute control over molecular weight distributions through autonomous polymer synthesis

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Fully self-optimizing autonomous reactors for organic synthesis have emerged in the last few years. The role of automation in chemical synthesis can be categorized into three distinct categories; *autonomous high throughput screening, autonomous optimization* and *enhanced process control.* A typical autonomous reactor consists out of an external controlled continuous flow reactor platform coupled with spectroscopy based online analysis tools. The online analysis tools provide direct information on the progress of the reaction and permits for a direct steering to the intended reaction trajectory. While these reactors are well developed in the field of organic transformation, no such system exists for polymer synthesis yet.

In our research we designed successfully an autonomous platform for polymerization reactions whereby a conventionally calibrated online SEC system is coupled to an external controlled continuous flow reactor platform. A generic algorithm was designed to optimize the average molecular weight to a predefined target under optimal conditions (low dispersities and high monomer conversion).[1] In short, the automated synthesis platform is able to target series of average molecular weights of (meth)acrylate polymers with an accuracy of less than 2.5 % while requiring no human interaction beyond the start-up phase. The developed technology is used to synthesize artificial molecular weight distributions by precisely combining multiple discrete polymer samples with targeted molecular weight. This way the autonomous polymerization setup is able to control and tune both the molecular weight and dispersity of the final polymer sample.[2]

The automated flow platform is a first step in synthesizing systematic polymer libraries with unprecedented control over the materials properties in a reproducible fashion. Such accessible tailor-made libraries, both in molecular weight and dispersity, are anticipated to have an impact in the domains of self-assembly, drug-delivery, and materials design.

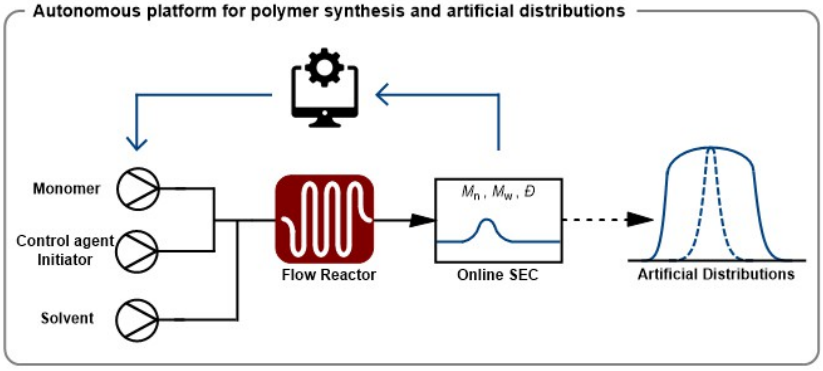

Figure 1. Schematic overview of experimental setup. The product stream is injected into an online SEC system and subsequently analysed and processed by the optimization algorithm.

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The Visible Light Mediated Radical Decarboxylative α-Alkylation of Amines in Continuous Flow

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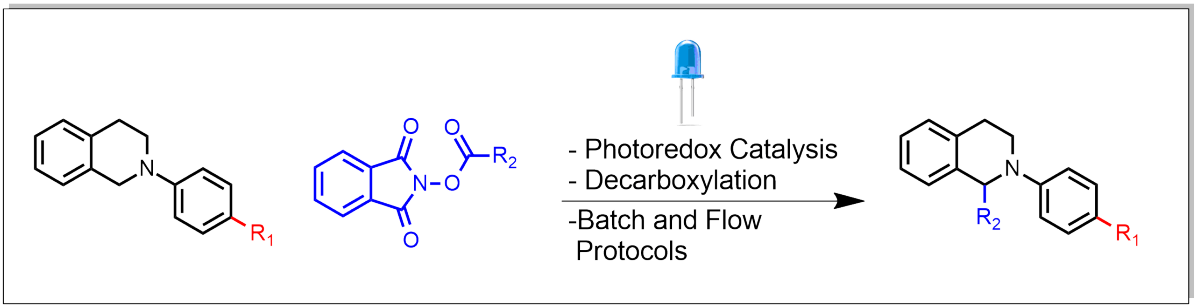
Fused bicyclic amines are an important structural motif due to their presence in a variety of pharmaceuticals and natural products. Thus, there is an ongoing demand for improvement in the synthesis and functionalization surrounding fused bicyclic amines. Photoredox chemistry has stimulated a renaissance in radical based methodology[1], with the visible light mediated α-functionalization of amines having been reported previously[2]; however, the methodology is typically limited to nucleophilic addition of the iminium ion[3] or radical addition to a Michael acceptor[4].

This work presents the α-alkylation of one class of fused bicyclic amines, the tetrahydroisoquinolines (THIQ), via a visible-light mediated radical-radical coupling process. N-Hydroxyphthalimide (NHP) esters derived from commercially available carboxylic acids were employed as an alkyl radical source to undergo coupling with α-amino radicals generated via photocatalytic methods. The development of a flow protocol is reported.



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Automated Polymer Synthesis Platform for Integrated Conversion Targeting Based on Inline Benchtop NMR

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The implementation of analytical devices inline into the synthesis stream allows close monitoring of chemical reactions. Flow chemistry can benefit especially from in- and online analysis as flow rates can be adapted dynamically, allowing for precise reaction control. By adding self-optimizing feedback loops and machine learning principles to the process, a precision and accuracy can be obtained that is hard or even impossible to achieve by human experience and intuition. Batch polymerisations are known to be prone to batch-to-batch variations and their offline analyses are often time-consuming. The combination of flow chemistry, online/inline monitoring and computer-guided software resolves these issues and leads to an increased reproducibility and efficiency in polymer synthesis. A benchtop NMR spectrometer was coupled to the outlet of a flow reactor for inline analysis of monomer conversion during control radical polymerizations.[1] Timesweep experiments – these are experiments in which residence times are screened rapidly in a single experiment – were performed in order to acquire the whole kinetic profile of thermal RAFT polymerisations in less than one hour. Additionally, an algorithm was programmed that allows targeting pre-defined monomer conversion by choosing the appropriate residence time independently and adjusting the flowrate accordingly. Offline analysis of the synthetised polymer samples reveals a high accuracy and precision in molecular weight distributions with deviations of only 3%. As an upgrade of the platform, also the synergistic coupling of inline NMR and online gel permeation chromatography will be discussed.

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A clean and green photochemical protocol for the di- & tri-fluoromethylation of heteroarenes

The selective incorporation of fluorinated motifs, in particular CF_3 and CF_2H groups, into organic compounds has emerged increasing attention since organofluorine molecules are of the utmost importance in the areas of pharmaceutical, agrochemical, and material sciences. Herein we successfully report the use of CH_2FSO_2Na and CH_3SO_2Na reagents as the fluorinating source in batch and continues flow. A simple, green and transition-metal-free protocol has been applied in challenging di- and tri-fluoromethylation of electron-rich heteroarenes. Additionally, this protocol is distinguished by utilising readily available, cheap reagent diacetyl as the blue light absorber and solvent sensitizer which initiate the single electron transfer processes. Generality of this reaction was demonstrated with the substrates that are important building blocks for pharmaceuticals such as indoles, pyrroles and pyrazines.

Nanomachine Biocatalysts: multi-enzyme cascades for sustainable industrial chemistry

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Continuous flow chemistry offers many advantages over traditional batch reactions, including, reaction flexibility due to the modular nature of flow reactors, improved scalability and yield, facile isolation of the product and greater safety. Biocatalysis offers complementary advantages, particularly with respect to high regio- and stereo-specificity. Continuous flow-reactors containing immobilized enzymes may offer many of the same advantages of modularity, scalability and yield and provide exquisite regio- and stereo-selectivity: however, for enzymes requiring cofactors the cost of continuously supplying cofactor can be prohibitive. When recycling cofactors in a flow system, the enzyme, the cofactor recycling enzyme and the cofactor must all be immobilized in such a way that the cofactor is free to participate in reactions with both enzymes. We have produced engineered enzymes that retain and recycle their cofactors, and can be immobilised to a surface, which enable their use in continuous flow reactors. Multiple enzyme-based continuous flow reactors can be connected in a modular fashion to achieve multistep transformations.

We set out to produce fusion proteins between synthetic enzymes and their cofactor-recycling partner enzymes with concomitant *in situ* recycling of a modified tethered cofactor, thus combining the advantages of biocatalysis and *in situ* cofactor-recycling. We have developed fit-for-purpose multi-enzyme fusion proteins with tethered cofactors to catalyse these reactions, with each fusion protein comprising a synthetic domain and a cofactor-recycling domain (**Fig. 1a**).

Furthermore, we have produced a prototype reactor, based on our modular design, to synthesise the anti-diabetic drug D-fagomine. With three stereocentres the chemical synthesis of D-fagomine is complex, we have shown it can be produced enzymatically in a flow reactor from glycerol *via* two regiospecific, cofactor-dependent steps (an ATP-dependent phosphorylation and an NAD-dependent oxidation) and a stereospecific aldol condensation to produce the precursor to D-fagomine (3*S*,4*R*-dihydroxyketone), the final chemical step requires simultaneous deprotection and reductive cyclisation (**Fig. 1b**).

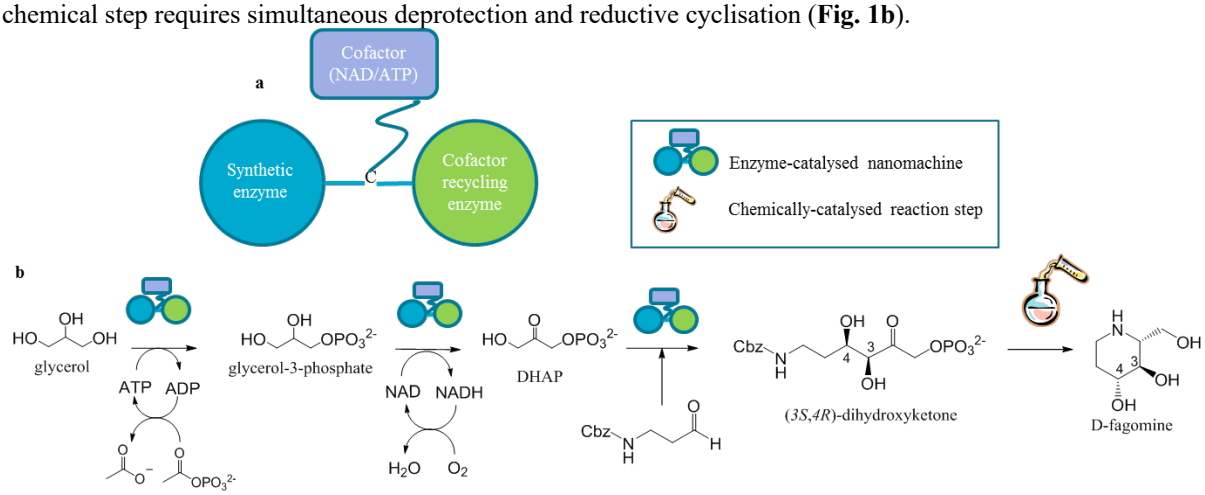

Figure 1: a) Bi-enzymatic fusion protein with tethered cofactor; **b)** Multi-enzyme cascade demonstrating synthesis of D-fagomine