EXPLOITING THE BENEFITS OF CONTINUOUS-FLOW PROCESSING FOR THE DEVELOPMENT OF NOVEL SUSTAINABLE CATALYTIC PROCEDURES

PhD Thesis

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Abbreviations

Ac	acetyl
ACPC	2-aminocyclopentanecarboxylic acid
Asp	aspartic acid
binol	1,1'-bi-2-naphthol
Bn	benzyl
BNS	*E*-β-nitrostyrene
Boc	*tert*-butoxycarbonyl
c	concentration
CF	continuous-flow
cispentacin	(1*R*,2*S*)-2-aminocyclopentanecarboxylic acid or (1*R*,2*S*)-ACPC
conv.	conversion
Cu/C	copper-in-charcoal
CuAAC	Cu(I)-catalyzed azide–alkyne cycloaddition
D	deuterium content
DBAD	dibenzyl azodicarboxylate
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DIEA	*N*,*N*-diisopropylethylamine
DMF	*N*,*N*-dimethylformamide
dr	diastereomeric ratio
EDT	1,2-ethanedithiol
EDTA	ethylenediaminetetraacetic acid
ee	enantiomeric excess
ee_{syn}	enantiomeric excess of *syn* isomer
Fmoc	9*H*-fluoren-9-ylmethoxycarbonyl
Glu	glutamic acid
HATU	1-[*bis*-(dimethylamino)methyliumyl]-1*H*-1,2,3-triazolo[4,5-*b*]pyridine-3-oxide
His	histidine
HMBC	heteronuclear multiple-bond correlation spectroscopy
HPLC	high-performance liquid chromatography
HSQC	heteronuclear single-quantum correlation spectroscopy
ICP-MS	inductively coupled plasma mass spectrometry
*i*Pr	isopropyl
L	channel length
Leu	leucine
MS	mass spectrometry
*n*Bu	normal-butyl
NMM	*N*-methylmorpholine

NMR	nuclear magnetic resonance
NP	normal-phase
*n*Pr	normal-propyl
p	pressure
PEG	polyethylene glycol
Ph	phenyl
Phe	phenylalanine
phen	phenanthroline
PMP	*p*-methoxyphenyl
*p*NBA	*p*-nitrobenzaldehyde
Pro	proline
PS	polystyrene
PS-MBHA	polystyrene resin with a 4-methylbenzhydrylamine linker
quant.	quantitative
RP	reversed-phase
RT	room temperature
sc	supercritical
SPPS	solid-phase peptide synthesis
T	temperature
TBS	*tert*-butyldimethylsilyl
*t*Bu	*tert*-butyl
TentaGel	polyethylene glycol–polystyrene copolymer resin without any linker
TFA	trifluoroacetic acid
TFMSA	trifluoromethanesulfonic acid
TMS	trimethylsilyl
TON	turnover number
Ts	tosyl
Xaa	amino acid with acidic side-chain
y	channel thickness

1. INTRODUCTION AND AIMS

At the dawn of the 21st century, chemical industry has reached the forefront of the transition to more sustainable production. However, progress toward increased sustainability requires developments and novel approaches that imply improved performance and value in chemical production in association with a reduction of the environmental impact through optimization of the use of non-renewable resources, the minimization of waste, safety improvement, etc.

As a novel sustainable alternative for the conventional batch-based synthetic techniques, the concept of continuous-flow (CF) processing has recently emerged in academic and industrial research of fine chemicals. CF-based approaches offer significant advantages over classical segmented unit methodologies.[1, 2] For example, the well-regulated flow reactor concept provides an increased parameter space for chemical synthesis and enables reactions to be performed with an unprecedented level of control due to the greatly enhanced heat and mass transfer and improved mixing properties.[3, 4] This implies higher reaction rates, outstanding selectivity, and safer and greener chemistry as compared with mechanically stirred reaction vessels.[3] The screening of CF reaction conditions is a simple and rapid procedure as there is no need for the large-scale use of reagents and solvents, and the most important reaction parameters (such as, flow rate, pressure, temperature and stoichiometry) can be quickly and precisely adjusted.[5] These advantages and the fundamental differences between batch experiments and CF processing offer a plausible opportunity for automation and excellent transferability between laboratory-based investigations and industrial-scale production and have pushed CF technology to the frontiers of modern sustainable chemistries.[6, 7]

Our major goal was to exploit the benefits of flow processing for reaction optimization and synthesis, and to develop novel sustainable synthetic methodologies with possible usefulness for the pharmaceutical industry. We set out to broaden the chemical space and practical applicability of transition metal-catalyzed and organocatalytic procedures to obtain pharmaceutically relevant intermediates and potentially bioactive compounds in a safe, simple and efficient manner. To achieve our goals, we intended to focus on the following areas in CF: (*i*) heterogeneous catalytic deuterations, (*ii*) Cu(I)-catalyzed azide–alkyne cycloadditions (CuAAC) and (*iii*) organocatalytic asymmetric aldol and conjugate addition reactions.

Enantiomerically pure compounds are crucially important in pharmaceutical research, and the development of enantioselective synthetic methodologies has therefore become one of the frontiers of organic chemistry. Organocatalysis has contributed appreciably to the recent advances in asymmetric syntheses, as it possesses distinct advantages over organometallic

counterparts, and exhibits inherent potential in the synthesis of chiral building blocks for the pharmaceutical industry.[8] However, long reaction times, difficulties in product isolation and selectivity issues often limit the practical applicability of organocatalytic reactions.[9] Accordingly, we aimed to develop a novel sustainable methodology which relies on immobilized peptidic catalysts in a packed-bed CF reactor and eliminates most of the above drawbacks. Utilization of a peptide is highly beneficial, as such a modular catalyst offers an unprecedented level of structural diversity, and facilitates the creation of optimized organocatalysts.[10]

CuAAC is a convenient way to obtain 1,2,3-triazoles with various pharmacological potentials, such as antibacterial, antiviral or antifungal effects.[11] The inherent scalability of flow processing, the facile opportunity for automation and the safety considerations associated with the handling of azides as explosive reactants have recently provoked the development of CF CuAAC procedures. However, most CF examples of CuAAC reactions rely on either costly apparatus or special catalyst types, thereby hampering their sustainable applications.[12, 13] We therefore planned to develop an easily available and inexpensive CF technique for CuAAC reactions, which is at the same time safe and efficient and additionally conveniently adaptable for the preparation of potentially bioactive compounds for the drug discovery.

There are a broad array of pharmacologically interesting molecules among heterocyclic structures, and many of them exhibit various biological properties.[14] Deuteration is widely applied in organic chemistry as deuterium-labeled heterocycles are of considerable importance in drug discovery.[15] However, conventional deuteration methodologies suffer from several drawbacks.[16] For this reason, it was our aim to employ a convenient and safe CF technique with on-demand electrolytic D_2 gas production for the highly selective deuteration of nitrogen-containing heterocycles which are precursors for a series of bioactive compounds, and we proposed to introduce a sustainable alternative for classical batch deuteration approaches.

2. LITERATURE SURVEY

2.1. Flow chemistry – concepts

The petrochemical industry relies on large-scale continuous chemical processes which display high-level efficiency for many years.[17] In contrast, the synthesis of fine chemicals and pharmaceuticals is traditionally performed in well-defined batches both in laboratory practice and on an industrial production-scale. In consequence of the widespread existence of the batch infrastructure and for historical reasons, smaller-scale manufacturing had to face significant challenges to improve the existing conventional methodologies. However, in the past decade the limitations of segmented unit operations have become absolutely clear.[18] The intense need for novel sustainable methodologies to enhance efficiency and reduce environmental factors has finally opened up new routes in compact-scale chemical engineering, and flow chemistry has emerged as a new tool for the synthetic chemistry of fine chemicals.[19, 20]

There are numerous fundamental differences between classical batch experiments and CF reaction technology.[17, 21] First of all, in standard segmentally operated reaction vessels the conversion depends on the reaction time, whereas in a CF apparatus a continuous stream of reactant flows through the reactor channels where the transformations take place, and the conversion to the products becomes a function of the distance covered in the reactor.[22] In other words, the chemical process becomes space-resolved (Figure 1). The control of the flow of

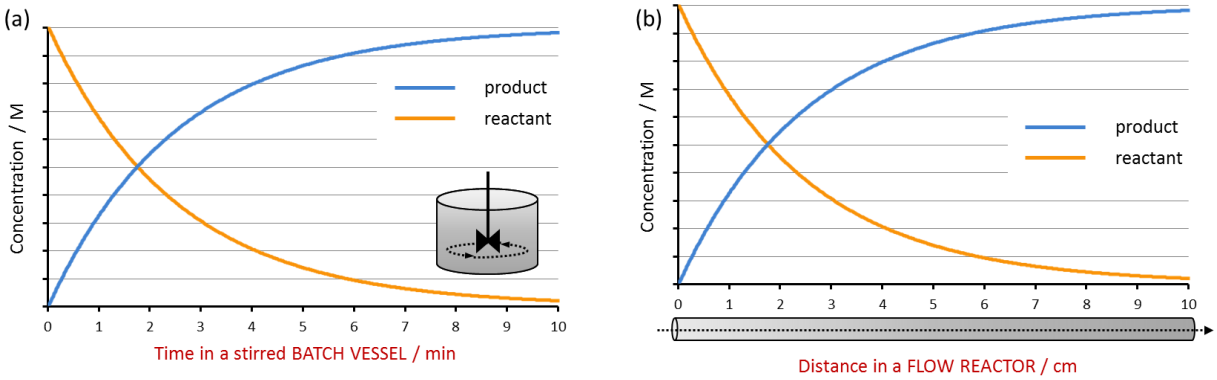


Figure 1. *Comparison of batch and CF processes: a time-resolved transformation in a standard reaction vessel (a), and a space-resolved chemical process in a flow channel (b).*

fluids in CF reactors is most commonly ensured by means of conventional HPLC pumps (hydrodynamic flow), but other approaches, such as electroosmotic or centrifugal flow, are also possible.[23] In a continuous process, the reaction time is assigned as the interval spent by a given molecule in the active reactor zone and is referred as the residence time.[3] The conversion correlates with the residence time, and can easily be fine-tuned through adjustment of the flow rate. In the traditional batch-based synthetic routine, very fast reactions involving highly

reactive species are often difficult or impossible to control without overreaction.[24] In contrast, compact CF reactors offer high-resolution control over the reaction/residence time in the range of milliseconds to seconds and introduce an unprecedented level of selectivity.[25-27] Moreover, the precise residence time control prevents the accumulation of hazardous materials, thereby leading to safer chemistry.[28, 29] In CF production, the stoichiometry is easily controllable through simple adjustment of the flow ratios of the reactants.[3, 22] In contrast, batch stoichiometry depends on the concentration and the volumetric ratio, and fine-tuning is much more complicated. The possibility of facile and rapid reaction parameter screening and the elimination of the need for the large-scale use of reactants and solvents make flow chemistry an ideal tool for high-throughput process development.[30, 31]

Heat and mass transfer in modern compact-scale CF devices are greatly enhanced as compared with conventional batch procedures.[2] Mixing in segmentally operated reaction vessels is mainly achieved by classical mechanical stirring through turbulence at high Reynolds numbers.[4] However, mass transfer is a key element of many chemical transformations and clearly inertial forces often provide insufficient mixing quality (Figure 2a).[1, 32] In contrast, modern flow reactors operate in the range of low Reynolds numbers, where laminar flow conditions predominate.[4, 23] The fluid properties in the capillary-scale ducts of such devices are mainly controlled by viscous forces and fluid-surface interactions, and mixing of the separate reactant streams occurs by diffusion across fluidic interfaces (Figure 2b).[4] According to Fick's law, the velocity of diffusion depends on the channel diameter, which clearly suggests that

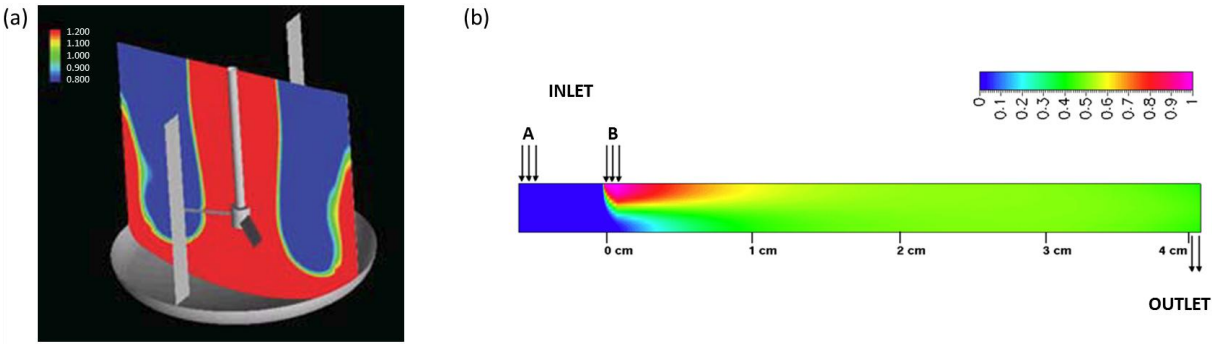


Figure 2. *Concentration profiles in a batch (a) and a CF reactor (b) simulated for a neutralization reaction between HCl (A) and NaOH (B). Levels of concentration equivalents are given according to the color scale.*[32]

miniaturization of the axial dimensions can dramatically enhance the mass transfer in flow reactors.[33] Moreover, the mixing properties in capillary channels can be further improved by using concentric or parallel streams rather than straight lines, and many flow setups include an initial mixing zone prior to the inlet of the actual reactor.[2] In many cases, efficient and rapid mass transfer can dramatically reduce the reaction time and improve the reaction rate relative to conventional batch experiments.[34]

In the course of a chemical reaction, heat is exchanged via the reactor surface, and the surface area-to-volume ratio is therefore a critical factor in efficient reactor design. Compact flow devices have specific surface areas several orders of magnitude larger than those of conventional batch equipment.[1, 3, 35] For example, a 10-mL tubular reactor with a channel diameter of 1 mm has a surface-to-volume ratio around 50 times higher than that of a standard 250-mL round-bottomed flask.[36] Heat transfer in flow reactors is therefore much faster than that in conventional batch units, and reactions can be performed with an unprecedented level of control over temperature (Figure 3).[3, 4, 32] For example, in batch processing, removal of the heat

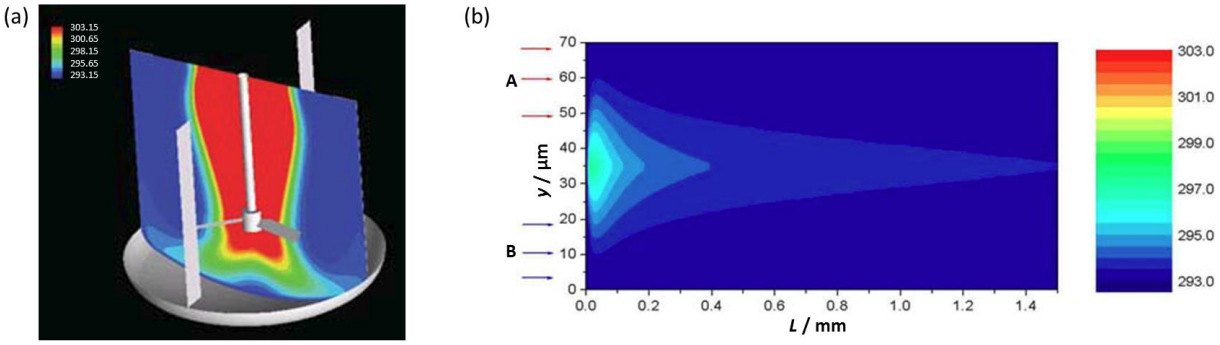


Figure 3. *Temperature profiles in a batch (a) and a CF reactor (b) simulated for an exothermic model reaction (neutralization between HCl (A) and NaOH (B)). Temperature levels are given in Kelvin according to the color scale, y is the channel thickness and L is the channel length.*[32]

of highly exothermic reactions can be challenging, whereas the effective heat-exchanging properties of CF reactors prevent such reactions from thermal runaway and aid the maintenance of isothermal conditions with improved production safety.[2, 37] Even explosive reactants[38] or highly unstable intermediates can be handled readily.[39] On the other hand, the superior heat absorption abilities in flow channels can greatly reduce the reaction times of endothermic transformations. Batch reactors often provide wide temperature profiles, leading to undesired byproduct formation (Figure 4).[32] In contrast, the efficient temperature control in flow results in a narrow temperature distribution and does not permit the formation of hot spots or the accumulation of heat in the reaction mixture, and undesired side-reactions are suppressed, resulting in higher selectivity (Figure 4).[32, 40] Thanks to the effective heat and mass transfer, an additional advantage is the much better reproducibility of CF reactions as compared with those in a conventional apparatus.[22]

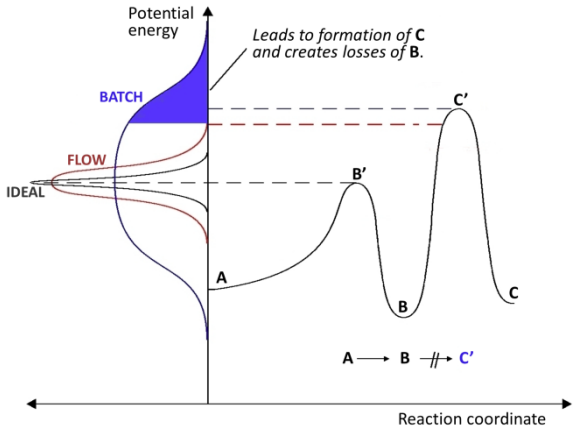


Figure 4. *Broad temperature profile in a batch reactor (blue) leads to the formation of byproduct C. but the narrow heat distribution in a flow reactor (red) restricts undesired side-reactions.*[32]

The application of high pressure in chemical reactions is favorable for many reasons. First of all, as indicated by Le Chatelier's principle, pressurization can result in a rate enhancement in numerous transformations. Typical examples are cycloadditions, Diels–Alder reactions, condensation reactions, etc. On the other hand, pressure increases the miscibility of liquids and the solubility of solids or gases, enhancing synthetic efficiency. High-pressure conditions are widely employed in CF reaction technology, as modular backpressure valves ensure simpler access to harsh conditions than do conventional high-pressure autoclaves.[5, 41] The contained environment and the ease of pressurizing in modern CF reactors remove the boiling point barrier and allow the superheating of solvents in a safe and simple manner, thereby providing novel process windows in an increased parameter space for chemical synthesis.[5, 42, 43] Under very high-temperature/pressure conditions, an unprecedented amount of energy can be supplied to chemical reactions, which leads to improved kinetics and consequently the dramatic shortening of reaction times, even in transformations with very high activation bars.[44, 45] With suitable apparatus, even supercritical (sc) conditions can be achieved for many organic solvents, water or CO_2.[46] Elevated temperatures in CF technology are most commonly attained through the use of external ovens, and built-in Peltier elements can be used to achieve better control over temperature.[47] Microwave dielectric heating has also been integrated into flow devices, and indirect inductive heating techniques were recently introduced.[22]

Among modern bench-top flow reactors, micro- and mesofluidic approaches can be distinguished, depending on the internal channel dimensions, though it is sometimes difficult to draw an exact borderline between these classes.[3] Microfluidic devices (microreactors) are mainly self-contained credit card-sized reactors, which consist of a series of miniaturized channels with specific layouts to promote the mixing of the reactants (Figure 5a).[46] The internal diameters typically range from 10 to 500 μm, and the surface area per unit volume is commonly between 5000 and 50 000 $m^2\ m^{-3}$, which permits the handling of minimal volumes of reactants with superior heat and mass transfer properties.[2, 3] However, the propensity to clogging and major pressure drops are common disadvantages which limit their practical applicability.[3] Mesoreactors are typically coil-type or tubular devices with larger channel diameters (from 500 μm to several mm) and lower surface area per volume values (typically between 100 and 10 000 $m^2\ m^{-3}$), generally designed for higher throughput at the expense of somewhat poorer heat-transfer capabilities (Figure 5b).[3, 22] Mesofluidic devices are usually highly modular and can be combined with packed beds where solid-supported reagents, catalysts or scavengers can be incorporated (Figure 5c).[3, 48] Instead of filled columns, reactors with functionalized inner walls can be utilized.[49] Even micro channels can be coated through such an approach, but in a

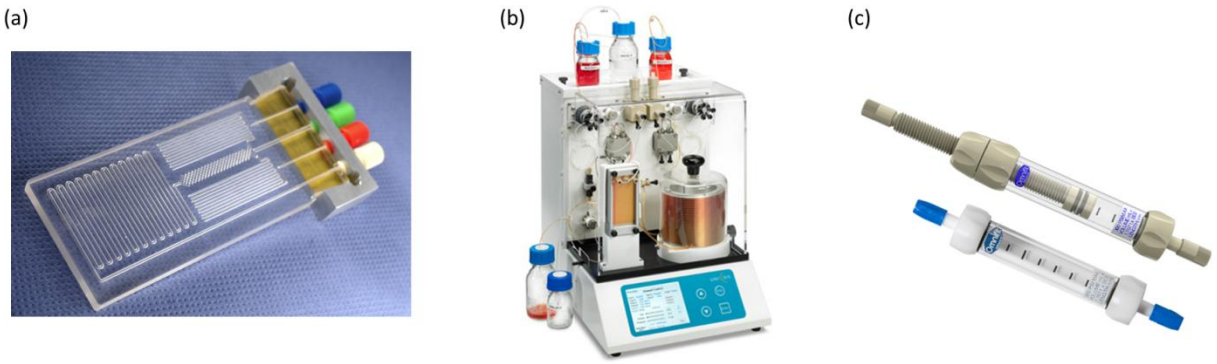


Figure 5. *CF apparatus: a microreactor chip (a),[50] a coil-type mesoreactor (b),[51] and reactor columns (c).[52]*

consequence of issues regarding applicability and ease of use, fixed-bed reactors have become more popular. For heterogeneous catalytic transformations, monolithic flow reactors are also applicable, where the catalyst is employed in the form of a structured material, obtained most commonly by copolymerization.[48] It follows from the nature of these materials that pressure drops along the reactor are avoided; however, their application is still very limited. The use of immobilized materials offers distinct advantages over conventional homogeneous approaches, and this has therefore recently become one of the main driving forces behind the rapid advance of flow processing. Heterogenized reagents and catalysts allow more sustainable chemical synthesis, as such materials can be reused several times, and in many cases regeneration is also possible.[53] Moreover, immobilization reduces the need for time-consuming work-up and purification steps, thereby greatly simplifying the product isolation.[54] The incorporation of hazardous reagents and catalysts into fixed beds ensures excellent ease of use and improved safety because of the lack of direct contact. The local catalyst concentration in filled column reactors is very high, which means that reaction rates can be greatly enhanced. Through the use of immobilized catalysts, reagents and scavengers, multistep consecutive reactions can be adapted into CF synthetic sequences, permitting rapid access to target molecules without the isolation of any intermediates.[55, 56] A combination of supported materials and the recently introduced in-line analytics[57] offers the opportunity of a high level of automation in high-throughput CF synthesis.[7, 58]

Another beneficial feature of CF reaction technology is the inherent scalability.[18, 22] This means that the volume is given as a function of the operation time and flow rate, whereas in conventional segmented units the output depends on the batch size.[3] Increasing the dimensions of a classical reaction vessel after a level makes the process unfeasible, as stirring, heating or cooling become increasingly more difficult, and a further issue arises when noxious materials are to be handled on larger scales. However, even an on-chip microfluidic device can safely produce gram or even kilogram quantities without alteration of the reactor engineering when

continuously operated for hours or even a whole day.[3, 5] Alternatively, scale-up can be further facilitated through the parallel operation of multiple identical reactors (numbering-up concept) or simple extension of the reactor dimensions (length and/or inner diameter).[4, 35] However, if the reactor size is increased above a certain level, reoptimization of the most important reaction parameters may be necessary.[6]

Modern flow technologies satisfy many of the requirements of environmentally benign production (minimal reagent consumption, prevention of waste generation, energy efficiency, atom economy, catalyst reusability, inherently safer chemistry, etc.),[59] thereby contributing considerably to the concept of green and sustainable chemical synthesis.[60] The beneficial features of continuous processing and the rapid developments in this field have motivated the pharmaceutical industry to explore flow chemistry as a novel innovative synthetic alternative from laboratory-based investigations to the subsequent production-scales.[61]

2.2. Applications of flow chemistry*

2.2.1. Heterogeneous catalytic hydrogenations and deuterations

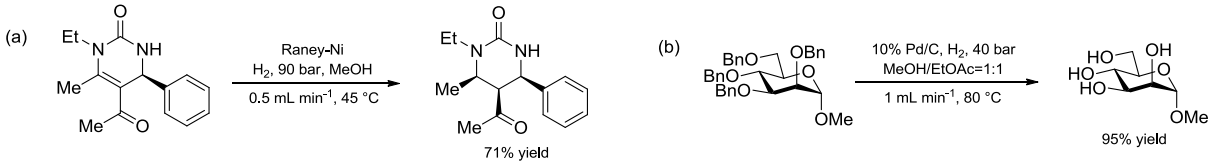
Heterogeneous catalytic hydrogenations are among the most widely investigated transformations in the flow chemistry field, as concerns either academic or industrial applications.[59, 61, 62] In traditional batch-based methodologies, such triphasic catalytic reactions pose a challenge (particularly on a relatively large scale) because of the hazardous and highly explosive nature of the gaseous reactant. Extreme precautions and costly special apparatus, including high-pressure-resistant autoclaves, are therefore required to maintain sufficient operational safety. The direct handling of flammable and pyrophoric catalyst types is another potential contingency, and the exothermic character of these reactions makes efficient heat removal a further issue to consider. Moreover, gas–liquid–solid reaction systems often suffer from long reaction times in conventional batch-based technologies, in consequence of the small interfacial areas between the different phases and the inadequate mixing.[63] Heterogeneous catalytic hydrogenations can furnish significant benefits through the advantageous features of flow processing, and gas–liquid–solid reactions can be carried out with shorter reaction times, improved reaction rates and selectivities, enhanced safety and operational simplicity.[1, 62, 64]

There are several approaches for heterogeneous catalytic hydrogenations in flow. One of the first examples of gas–liquid–solid reactions in a microstructured device was provided by Yeong *et al.*,[65] who developed a falling-film microreactor for the highly exothermic reduction of

*Due to the restrictions of the Doctoral School of Pharmaceutical Sciences regarding the length of dissertations, only the most relevant applications are discussed in this section.

nitrobenzene to aniline. The Pd catalyst was deposited onto microstructured plates on which the thin film (<100 μm) of the reactants was moving under the force of gravity, with a flow of H_2 gas above the liquid film. This reactor set-up facilitated very close interactions between the reacting phases, resulting in very high reaction rates. Fixed-bed or micropacked reactors are also widely applied in heterogeneous catalytic hydrogenations, as illustrated by the publications of a number of groups.[62] For example, Yoswathananont *et al.* carried out the continuous hydrogenation of 4-cyanobenzaldehyde in a tube reactor packed with Pd/C.[66] As an intriguing example of gas–liquid–solid reactions, Kobayashi *et al.* utilized a glass microchannel reactor with a Pd catalyst immobilized on the channel walls.[67, 68] An effective interaction was achieved between the three phases due to the large interfacial areas and the narrow channel diameter. As an improvement, high-pressure conditions were utilized and $scCO_2$ was used as solvent, which resulted in dramatically short reaction times (<1 s).[69] A wide variety of transformations were carried out, such as alkene/alkyne reductions and deprotections, with excellent yields.

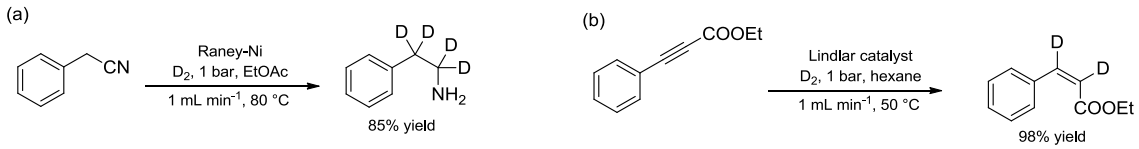
The above-described approaches rely on extraneous H_2 gas sources, which involve potential hazards. The recently introduced H-Cube® (ThalesNano Inc) high-pressure flow hydrogenation mesoreactor eliminates the difficulties in gas handling, greatly improving the operational safety, as H_2 gas is generated *in situ* by the electrolytic decomposition of water. The hydrogenation catalyst is packed into cartridge-like columns, thereby eliminating potentially dangerous direct catalyst handling. Due to its beneficial features, the H-Cube® has gained serious interest over the past years. A wide array of gas–liquid–solid reactions have been fulfilled, such as carbon-carbon double and triple bond hydrogenations. For example, Lou *et al.* utilized an H-Cube® reactor for the selective reduction of carbon-carbon double bonds during the synthesis of highly functionalized tetrahydropyrimidones (Scheme 1a).[70] Nitro, nitrile, carbonyl, oxime and imine reductions and deprotections have also been carried out. For example, Ekholm *et al.* reported the deprotection of benzyl- and/or benzylidene-protected carbohydrates in an H-Cube® system within short reaction times, obtaining excellent yields (Scheme 1b).[71]



Scheme 1. *Selected examples of hydrogenation reactions in the H-Cube® flow reactor.*

Deuteration is widely applied in chemical research, e.g. in tracer studies to follow reaction paths,[72] or as a tool to investigate pharmacokinetics.[15] Moreover, deuterium-labeled compounds are widely used in structural analysis, e.g. as internal standards in mass spectrometry,[73] and in NMR spectroscopy for the promotion of signal assignments and structure elucidations.[74] The

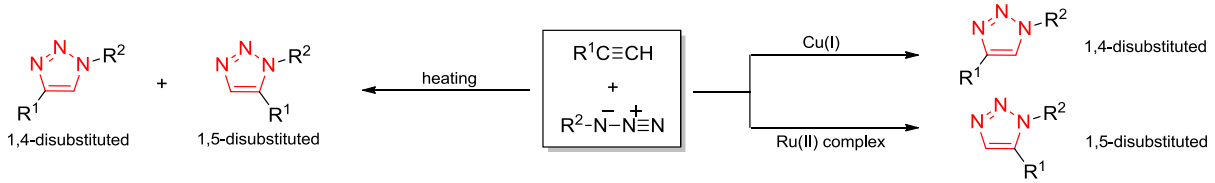
batch synthesis of deuterated compounds suffers from several drawbacks and, in general, lacks sustainability. On the one hand, this is due to the potentially dangerous gas handling, just as in the case of hydrogenations. Another point is that conventional methods for the production of D_2 gas, such as the fractional distillation of liquid hydrogen, pyrolysis of UD_3, and reactions of D_2O with Na, Fe, or Mg, are far from perfect;[16] and as an alternative route, catalytic H–D exchange reactions between H_2 and D_2O do not supply adequately pure D_2 gas.[75, 76] By changing the hydrogen source to deuterated water in an H-Cube® reactor, Mándity *et al.* presented the first approach for CF deuteration reactions (Scheme 2a).[77] The simple, efficient, selective and safe technique eliminated most of the drawbacks of the conventional deuteration procedures and offered high yields, excellent deuterium incorporation ratios and short reaction times for an array of substrates, including β-amino acid derivatives. Most recently, Chandrasekhar *et al.* utilized the above-described methodology in the selective partial reduction of alkynes, resulting in dideuterated olefins in high yields (Scheme 2b).[78]



Scheme 2. *Examples of deuteration reactions in the H-Cube® flow reactor.*

2.2.2. Copper-catalyzed azide–alkyne cycloadditions

The 1,3-diploar cycloaddition of organic azides with alkynes as dipolarophiles is the most direct way to obtain useful 1,2,3-triazoles.[79] The classical Huisgen reaction utilizes only thermal induction and gives an approximately 1:1 mixture of 1,4- and 1,5-disubstituted 1,2,3-triazoles.[80] Due to the high activation energy barrier, these cycloadditions are often very slow, even at elevated temperatures. As a pioneering improvement, Tornøe *et al.* and Rostovtsev *et al.* discovered independently that the application of Cu(I) catalysis leads regioselectively to the formation of the 1,4-disubstituted isomer under mild reaction conditions within shorter reaction times (Scheme 3).[81, 82] The selective formation of the 1,5-disubstituted 1,2,3-triazole isomer was achieved through catalysis with Ru(II) complexes, but the catalytic activity and regioselectivity proved to be sensitive functions of the ligand environment.[83] Thanks to its outstanding efficiency and selectivity, CuAAC has become the definition of the "click chemistry" concept.[84]



Scheme 3. *1,3-Dipolar azide–alkyne cycloadditions.*

In the past decade, the applications of CuAAC have conquered many areas of modern chemistry. In the field of bioconjugation, the CuAAC reaction has emerged as a versatile tool for the labeling of biomolecules with various functionalities.[85] As the 1,2,3-triazole moiety is an excellent mimetic of the amide bond, CuAAC is increasingly found in peptidomimetic chemistry, e.g. for the ligation of peptide fragments, for peptide cyclizations, or for backbone modifications.[86] The field of polymer and materials sciences has also greatly exploited the CuAAC reaction,[87] and it holds great promise for surface modifications,[88] for supramolecular chemistry[89] and for radiolabeling studies.[90] In consequence of their potential biological properties, 1,2,3-triazole-containing compounds have recently become feasible targets for drug discovery.[91] A large number of 1,2,3-triazole-contining compounds exhibit various biological activities, e.g. antiviral, antibacterial, antifungal and anticancer effects (Figure 6).[92] The 1,2,3-triazole moiety is frequently used as a pharmacophore for the modification of known pharmaceutics and to potentiate their biological effects. For example, 1,2,3-triazole analogs of the well-known antiviral cyclic amino acid derivatives oseltamivir and zanamivir have recently been reported (Figure 6).[93, 94] The 1,2,3-triazole skeleton is a constituent part of many modified nucleosides or carbanucleosides with various activities. For instance, the 1,2,3-triazole analog of the bioactive carbanucleoside neplanocin A exhibits notable antiviral effect (Figure 6).[95]

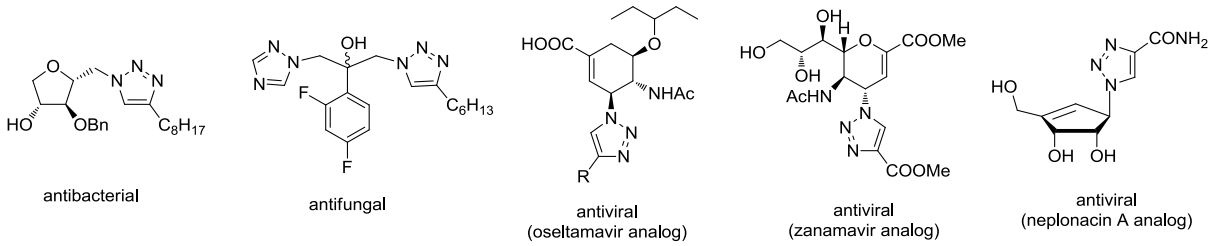
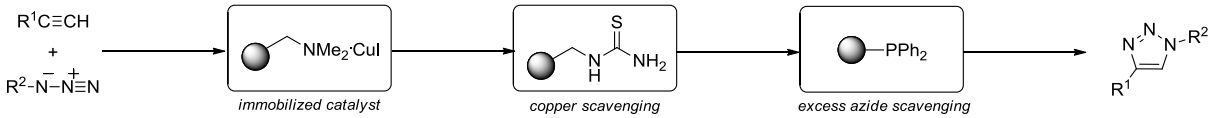


Figure 6. *Some examples of 1,2,3-triazoles with various biological activities.*

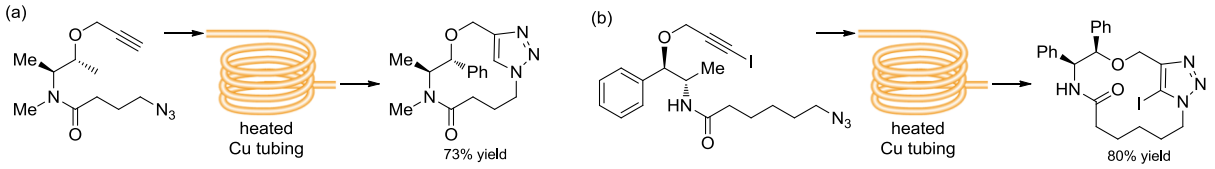
The synthesis of 1,2,3-triazoles via CuAAC comprises an extraordinarily robust and straightforward transformation, which can be performed under a wide array of reaction conditions.[11] For such reactions, the safety aspects associated with the handling of explosive azides and the inherent scalability of CF processing are particularly appealing, thereby reviving CF CuAAC approaches. Smith *et al.* utilized a combination of immobilized catalysts and scavenger resins in a modular flow reactor (Scheme 4).[96] CuI was immobilized in a complex form on a dimethylaminomethyl-grafted polystyrene (PS) support.[97] The copper catalyst was expected to leach from the resin due to the weak coordinative forces, and a subsequent in-line scavenging step was therefore necessary, a thiourea-based metal resin being used to remove the copper contamination from the solution phase. The azide was required in excess to drive the reaction to completion. To obtain the corresponding 1,2,3-triazole products in pure form, the

unreacted azide was removed in-line over a phosphine resin, the azide being captured onto the solid phase via a Staudinger reaction. Triazole products were obtained in short process times, in high yields (up to 93%) and without the need for further purification steps, and gram-scale production was also successfully implemented. Baxendale *et al.* later extended the procedure to a reaction sequence with the *in situ* generation of the alkyne from an aldehyde and the Bestmann–Ohira reagent as the initial step.[98]



Scheme 4. *CuAAC in CF with solid-supported CuI catalyst and scavenger resins.*

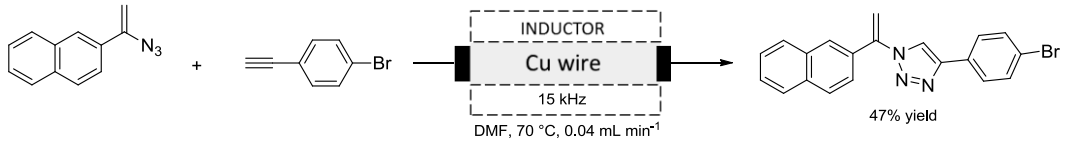
Bogdan and Sach introduced an experimentally more convenient approach for azide–alkyne cycloadditions by developing an on-demand flow reactor made of Cu.[99] The surface of the Cu is covered with non-self-protecting layers of different copper oxides, and the use of Cu tubing ensures catalytically active Cu(I) species without the need for any additional copper catalyst. Furthermore, the reactive organic azides were generated *in situ* from NaN_3 and the corresponding alkyl halides, utilizing one-pot click methodology. Heating was necessary to obtain sufficient reactivity through the CuAAC. 1,4-Disubstituted 1,2,3-triazole isomers were obtained regioselectively in a highly efficient manner, and scale-up experiments were also performed with fewer safety issues than in the corresponding batch process. The scope of the methodology was further extended to the expedient synthesis of drug-like macrocycles based on 1,4-disubstituted and 1,4,5-trisubstituted 1,2,3-triazoles obtained via intramolecular click reactions (Scheme 5).[100, 101] Tu *et al.* later demonstrated that ultrasound promotion can practically eliminate the need for harsh reaction conditions, and 1,2,3-triazoles can be obtained at lower temperatures with Cu flow reactor technology.[102]



Scheme 5. *Synthesis of 1,4-disubstituted (a) and 1,4,5-trisubstituted (b) 1,2,3-triazole-containing macrocycles with Cu flow reactor technology.*

Ceylan *et al.* employed an inductively heated flow reactor solution for CuAAC reactions.[103] A glass column was filled with Cu wiring, which was heated directly and instantly with electromagnetic induction (Scheme 6). With this setup, very high temperatures could be generated inside the Cu, which led to the formation of active Cu(I) species on the surface of the metal without further catalytic source. This extent of reactivity was not attainable with conventionally heated Cu. In order to keep the solvent in the liquid phase even at high

temperatures, a backpressure regulator was introduced into the flow line, and the system was equipped with a metal scavenger cartridge so as to remove copper contamination from the solution phase in-line. Similarly as in the work of Bogdan and Sach,[99] one-pot click methodology was utilized through the *in situ* generation of organic azides, thereby greatly enhancing operational safety. The proposed methodology was successfully applied for the effective synthesis of a small library of vinyl triazoles.[104]



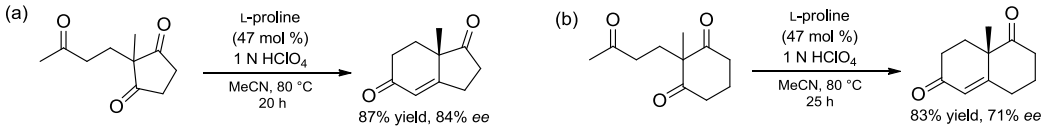
Scheme 6. *CuAAC with an inductively heated CF reactor filled with Cu wiring. (DMF=N,N-dimethylformamide.)*

As a heterogeneous Cu(I) source, copper-in-charcoal (Cu/C) was utilized in a dedicated high-pressure/high-temperature flow reactor by Fuchs *et al.* for the CuAAC reaction between benzyl azide and phenylacetylene.[12] They established that the catalysis through the CF reaction proceeded in the homogeneous phase, due to the leaching of catalytically active copper species. The Cu/C system was originally introduced for azide–alkyne cycloadditions by Lipshutz and Taft as an inexpensive self-stable catalyst, with different types of copper oxides, including Cu_2O, present within the charcoal matrix.[105]

Although the most popular sources of Cu(I) in CF studies are mainly heterogeneous approaches, Varas *et al.* recently reported an efficient approach for CF CuAAC reactions by using a homogeneous copper-complex ($[Cu(phen)(PPh_3)_2]NO_3$) as catalyst.[13] They succeeded in utilizing very low catalyst loadings and short reaction times at elevated temperatures. Copper contamination was efficiently removed via an in-line extraction process, with aqueous ethylenediaminetetraacetic acid (EDTA) as a homogeneous copper-scavenging system.

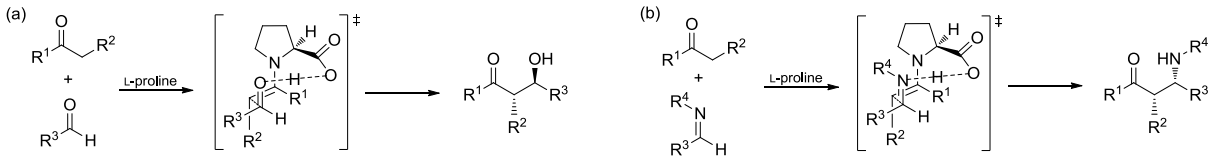
2.2.3. Asymmetric organocatalysis

Organocatalysis uses metal-free low-molecular-weight organic molecules as chiral catalysts for the stereoselective synthesis of valuable compounds. Beneficial features, such as high stability, availability, non-toxicity, and low cost, make the use of organocatalysts extremely attractive as compared with organometallic counterparts. In the 1970s, two industrial research groups independently discovered that the naturally occurring L-proline efficiently catalyzed the enantioselective intramolecular aldol reaction of cyclic triketones (Hajos–Parrish–Eder–Sauer–Wiechert reaction, Scheme 7).[106, 107] However, the potential behind this finding remained undiscovered for almost three decades. The revival of this chemistry is closely related with the discovery of the proline-catalyzed direct asymmetric intermolecular aldol reaction by List



Scheme 7. *Enantioselective intramolecular aldol reactions with L-proline as organocatalyst by Eder, Sauer and Wiechert.*[106] *(Hajos and Parrish investigated the same reactions under slightly modified conditions.*[107]*)*

et al.[108] Since then, L-proline has become a powerful organocatalyst for numerous asymmetric transformations involving enamine intermediates,[9, 109] including Mannich reactions,[110] Michael additions,[111] α-amination reactions,[112] α-aminoxylations,[113] and Diels–Alder reactions.[114] In such transformations, the function of the amine residue of proline is to activate the carbonyl compound by formation of an enamine for reaction with an electrophile, whereas, the carboxylic acid moiety coordinates the stereochemical outcome through hydrogen bonding (Scheme 8).[115, 116] In the golden age of asymmetric catalysis, a wide array of new proline-derived organocatalysts were designed that offered improved catalytic activity and selectivity, and increased substrate scope.[9] In general, proline modifications mean the substitution of the carbon atoms of the pyrrolidine ring,[117] or the replacement of the carboxylic group with various functions, such as amide,[118] tetrazole[119] or siloxy groups.[120] Moreover, apart from proline-derived techniques, alternative efficient organocatalytic approaches have also come to light, such as 1,1'-bi-2-naphthol (binol)-, cinchona alkaloid- or thiourea-based catalysts.[8, 121]



Scheme 8. *Proline-catalyzed aldol (a) and Mannich reactions (b) with transition-state models.*

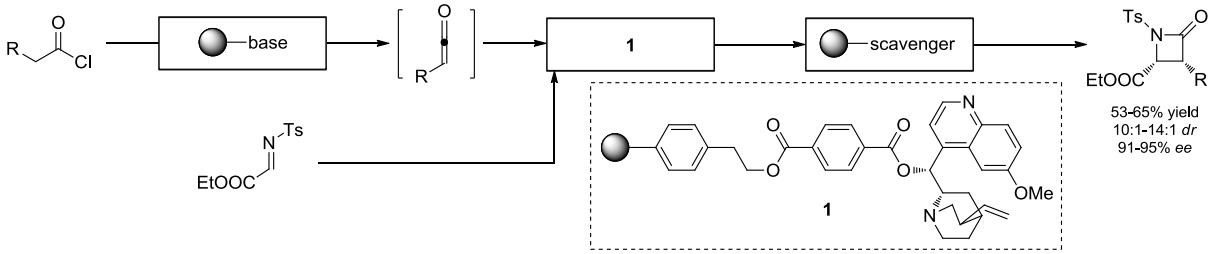
However, the limitation of the above-mentioned techniques (particularly of proline-based catalysis) is that small, rigid organocatalysts offer only restricted structural and functional diversity, which limits the opportunities of creating optimized catalysts. In contrast, the utilization of synthetic peptides, as on-demand organocatalysts, eliminates these drawbacks in consequence of their highly modular nature, readily designable structure and inherent chirality.[10] The earliest examples of peptide-catalyzed asymmetric transformations are an enantioselective cyanohydrin synthesis by Oku and Inoue with a cyclic dipeptide, cyclo(L-Phe-L-His) (Phe=phenylalanine, His=histidine),[122] and the epoxidation of electron-deficient olefins with poly-α-aminoacids by Julia *et al.*[123] It was later shown by several research groups that N-terminal prolyl-peptides are interesting candidates for reactions proceeding via enamine intermediates.[124-126] However, these approaches usually meant only modest procedural enhancements as compared with proline catalysis. Tripeptides with the general formula

H-Pro-Pro-Xaa-NH_2 (Pro=proline), where Xaa is an amino acid with acidic side-chain, were recently employed as effective organocatalysts for the asymmetric aldol and Michael reactions.[127] These structures can be regarded as proline mimetics, as they contain secondary amine and carboxylic acid moieties in a specific orientation due to the turn-conformation of the peptide.[128] These functional groups play similar roles as in conventional proline-mediated transformations.[128, 129] The peptide H-Pro-Pro-Asp-NH_2 (Asp=aspartic acid) was found to be an efficient catalyst for direct aldol reactions,[130] and the closely related peptides H-D-Pro-Pro-Asp-NH_2 and H-D-Pro-Pro-Glu-NH_2 (Glu=glutamic acid) proved to be excellent catalysts for conjugate additions between aldehydes and nitroolefins.[131, 132] There are many further examples of synthetic peptide-mediated asymmetric transformations, including Morita–Baylis–Hillman reactions, Stetter reactions, Friedel–Crafts alkylations, asymmetric acylations, etc.,[10] but their discussion is beyond the scope of this dissertation.

Through the past decade, it was repeatedly demonstrated that flow chemistry is highly beneficial for organocatalytic purposes in terms of productivity, easy automation or facile scale-up; and the development of reliable immobilization techniques for chiral catalysts significantly contributed to this revelation.[133-135] The first (and somewhat forgotten) example of a CF organocatalytic procedure was demonstrated by Cappi *et al.* in 1998.[136] In an improvement of the classical Julia–Colonna asymmetric epoxidation of α,β-unsaturated ketones, they immobilized poly-L-Leu (Leu=leucine) on cross-linked aminomethyl-PS resin, which was packed into a glass column together with H_2O_2 as oxidant. A solution of chalcone and a base (to promote the formation of the reactive peroxy anion) was passed through the packed bed by means of the force of gravity. The resulting chiral chalcone epoxide was obtained as an important synthetic intermediate within a short residence time with excellent conversion (97%) and an enantiomeric excess (*ee*) of 98%. Subsequently, Tsogoeva *et al.* utilized soluble polymer-bound oligo-L-Leu compounds in a continuously operated chemzyme membrane reactor for the asymmetric epoxidation of chalcone, where a nanofiltration membrane retained the polymer-enlarged catalyst, while the resulting epoxide and unconverted chalcone could freely pass through.[137] Kee and Gavriilidis recently designed a dedicated microflow system for homogeneous poly-L-Leu-catalyzed chalcone epoxidation, in which gram-scale production was attainable.[138] With a well-regulated reactor concept, this study implied potential industrial uses.

As another pioneering example, Hafez *et al.* revealed that a combination of multiple "reaction columns" packed with solid-phase reagents, catalysts and scavengers is a powerful tool for the stereoselective multistep synthesis of complex compounds.[139] They applied a Wang-resin-supported quinine derivative (**1**) as chiral organocatalyst in the reactions of imino esters

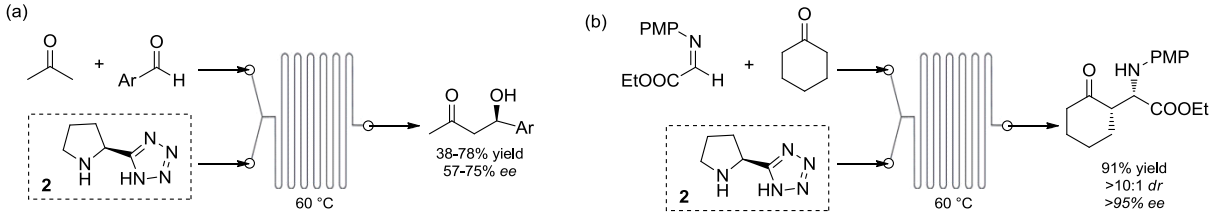
with ketenes, leading to highly enantioenriched β-lactams. The three-step reaction sequence was carried out as a single process in sequentially linked fillable glass columns with gravity-driven flow-through (Scheme 9). To avoid direct handling of reactive intermediates, the ketene was generated *in situ* from the corresponding acid chloride and a polymer-supported base, and then reacted with an imino ester in the presence of immobilized organocatalyst **1**. The excess reactants and byproducts were removed in-line on an immobilized nucleophilic scavenger, thereby greatly simplifying further work-up and purification procedures. The resulting chiral β-lactams were obtained in good yields (53–65%), high diastereoselectivites (up to a diastereomeric ratio (*dr*) of 14:1) and excellent enantioselectivites (up to an *ee* of 94%). Another beneficial feature is that the system proved to be highly robust, as no decrease in activity or selectivity was observed after 60 reaction cycles when the same batch of resins was utilized. Bernstein *et al.* later extended the scope of this concept to the CF stereoselective α-chlorination of acid chlorides with the same immobilized organocatalyst.[140] Besides being the source of stereoselective induction, the cinchona alkaloid-derived catalyst also served as a dehydrohalogenation reagent. Chiral α-chloroester products were generated in good yields (40–61%) and excellent enantioselectivities (up to an *ee* of 94%). Later, the diasteroselective total synthesis of a metalloproteinase inhibitor was successfully fulfilled by utilizing this column-based flow technique, with the α-chlorination reaction as a key step.[141]



Scheme 9. *CF asymmetric synthesis of β-lactams with an immobilized quinine derivative as organocatalyst.*

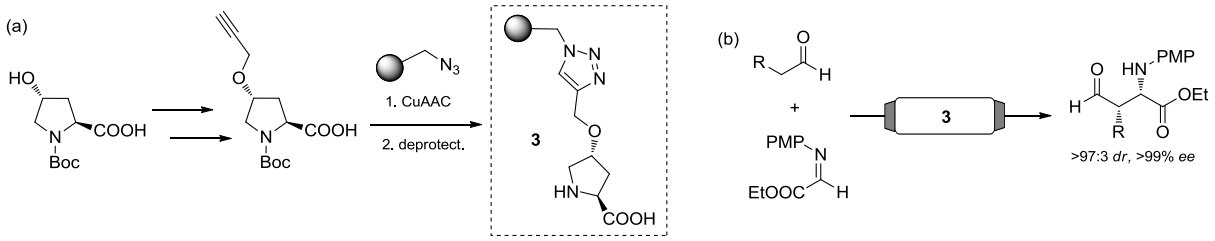
In 2009, Odedra and Seeberger reported the first example of an organocatalytic asymmetric aldol reaction in CF, this being the first successful application of a proline-derived catalyst in a flow system.[142] Homogeneous 5-[(2*S*)-pyrrolidin-2-yl]-1*H*-tetrazole (**2**) was utilized as chiral organocatalyst in a glass microreactor.[119] The solution of the reactants and the catalyst **2** were injected into the heated reactor zone separately through two inlets. Aldol reactions between acetone and various aromatic aldehydes were investigated (Scheme 10a). After thorough optimization of the reaction conditions, it was found that the reaction times could be shortened dramatically under reduced catalyst loading and at higher temperature (60 °C), while the yield and stereoselectivity remained commensurable with the conventional batch results. The scope of the method was further extended to the Mannich reaction between *p*-methoxyphenyl (PMP)-

protected α-iminoglyoxylate and cyclohexanone, yielding the corresponding β-amino ketone product in excellent *ee* and *dr* (Scheme 10b). As an intriguing extension of the homogeneous catalytic concept, Fritzsche *et al.* designed a microfluidic organocatalytic system for the enantioselective vinylogous Mannich reaction with fully integrated on-chip analysis .[143] The CF device was successfully utilized to screen a number of binol-based chiral phosphoric acid catalysts, with a very good correlation with the results of the corresponding batch experiments.



Scheme 10. *CF asymmetric aldol (a) and Mannich reactions (b) with a homogeneous proline-derived organocatalyst in a microreactor.*

As a significant improvement, in 2009 Alza *et al.* took advantage of heterogeneous supporting and reported for the first time the successful utilization of an immobilized proline-based organocatalyst for highly selective Mannich reactions in CF.[144] To avoid alteration of the catalytically active functions of proline, 4-hydroxyproline was converted to an *O*-propargyl derivative, and then connected to an azido-functionalized PS resin by means of a click strategy, leading to immobilized catalyst **3** (Scheme 11a).[145] In fact, the resulting 1,2,3-triazole moiety not only served as a linker between the proline unit and the polymeric backbone, but also contributed to the formation of a highly selective catalytic system. The reactions between various aldehydes and a preformed imine were studied by pumping the solution of the reactants through a glass column containing catalyst **3** (Scheme 11b). Synthetically useful Mannich products were obtained in almost diastereo- and enantiomerically pure form (with *ee* values of >99% and *dr* values of >97:3). Another beneficial feature of this sustainable process is that it proved promisingly rapid, with a residence time on the catalyst bed as low as 6 min. In contrast with the previously mentioned homogeneous approaches,[142, 143] the use of the insoluble polymer-bound catalyst eliminated the need for any further work-up or purification steps, which was highly favorable, as Mannich adducts are rather labile substances. Ayats *et al.* later utilized the same strategy for CF enantioselective aldol reactions between various aromatic aldehydes and cyclohexanone, with PS-supported 4-(1-triazolyl)proline as organocatalyst (**4**, Figure 7a).[146] β-Hydroxyketone products were obtained with excellent enantio- and diastereoselectivities (*ee* values of 95–98% and *dr* values of 96:4–97:4) in gram-amounts with short residence times. Furthermore, the flow process allowed a 6-fold reduction in the effective catalyst loading as compared with the subsequent batch reaction utilizing the same immobilized catalyst.



Scheme 11. *Synthesis of PS-supported organocatalyst **3** (a), and asymmetric Mannich reactions in CF with **3** (b). (Boc=tert-butoxycarbonyl.)*

Massi *et al.* recently employed SiO_2-immobilized organocatalysts in miniaturized HPLC columns for asymmetric aldol reactions. At the beginning of their studies, they utilized SiO_2-supported proline, but this system had serious limitations as the immobilized catalyst deactivated through irreversible decarboxylation, even at room temperature (RT).[147] Later, they reported the use of a SiO_2-supported prolin-derived tetrazole catalyst (**5**, Figure 7b), which was not prone to the above-mentioned deactivation pathway, thereby permitting a more stable catalytic system for aldol reactions.[148] With the improved catalyst, they achieved high conversions (58–>95%) and enantioselectivities (*ee* values of 68–95%) in the reactions of various aromatic aldehydes and cyclohexanone at various temperatures and in various solvents. However, only modest diastereoselectivities (*dr* values of 1:1–3:1) could be reached with any of the reaction conditions. As an interesting example of a heterogeneously catalyzed aldol reaction in a flow reactor, Demuynck *et al.* applied a non-covalently immobilized chiral primary amino acid-derived diamine as organocatalyst (**6**, Figure 7c).[149] A sulfonated fluoropolymer, nafion® NR50, served as solid support, and the chiral diamine was anchored through acid−base interactions. In this approach, the solid acid not only served as catalyst support, but also governed the activity and selectivity of the chiral catalyst. The system was benchmarked with the aldol reaction between 2-butanone and 4-(trifluoromethyl)benzaldehyde, resulting in the corresponding β-hydroxyketone with *ee* values up to 97% and *dr* of 3:1.

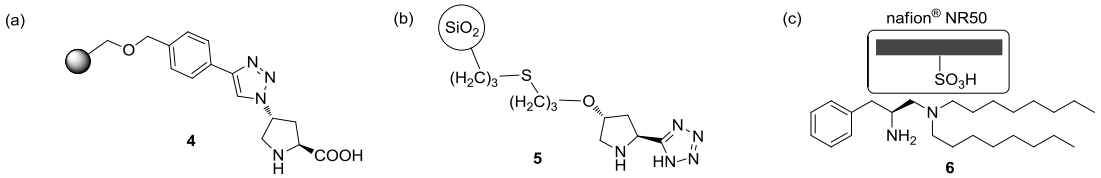
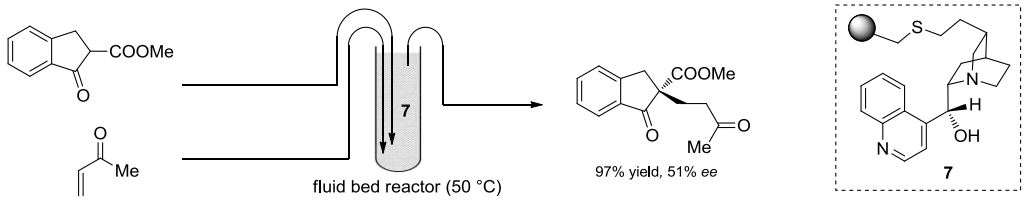


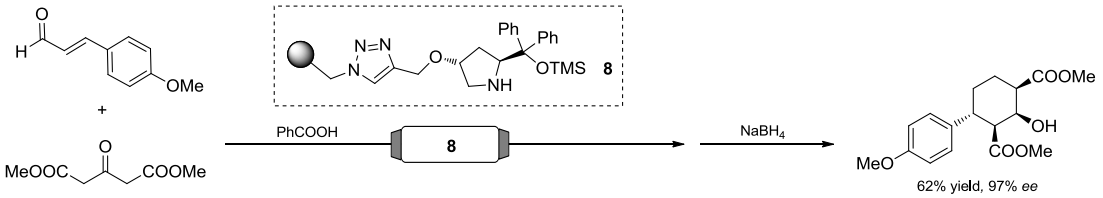
Figure 7. *Immobilized organocatalysts for asymmetric aldol reactions utilized in various CF systems: PS-supported 4-(1-triazolyl)proline catalyst (a), SiO_2-supported prolin-derived tetrazole catalyst (b), and a non-covalently immobilized chiral primary amino acid-derived diamine catalyst (c).*

The first example of a CF Michael reaction was provided by Bonfils *et al.* in 2006, with PS-supported cinchonidine as organocatalyst (**7**).[150] They investigated the reaction between methyl 1-oxo-2-indanecarboxylate and methyl vinyl ketone. The solutions of the reactants were passed through an incubated fluid bed by means of peristaltic pumps. Under optimized reaction

conditions, the corresponding chiral Michael product was obtained within a residence time of 6 h in a yield of 97% and an *ee* of 51% (Scheme 12). Later, Alza *et al.* investigated an enantioselective domino Michael−Knoevenagel reaction in CF operation.[151] A diarylprolinol silyl ether was employed as chiral organocatalyst (**8**), immobilized via a click strategy onto a PS support, and filled into a glass column (Scheme 13). The reaction between 3-(4-methoxyphenyl)acrolein and dimethyl 3-oxoglutarate was investigated. It was found that 1 equivalent of benzoic acid additive was necessary for effective transformation. The immobilized organocatalyst proved to be remarkably robust: after 72 h of continuous use, no significant deterioration of its performance was observed. After a simple $NaBH_4$ reduction, 8.7 g of a synthetically useful highly functionalized cyclohexane derivative was obtained (equivalent to a yield of 62%), with an *ee* of 97%.



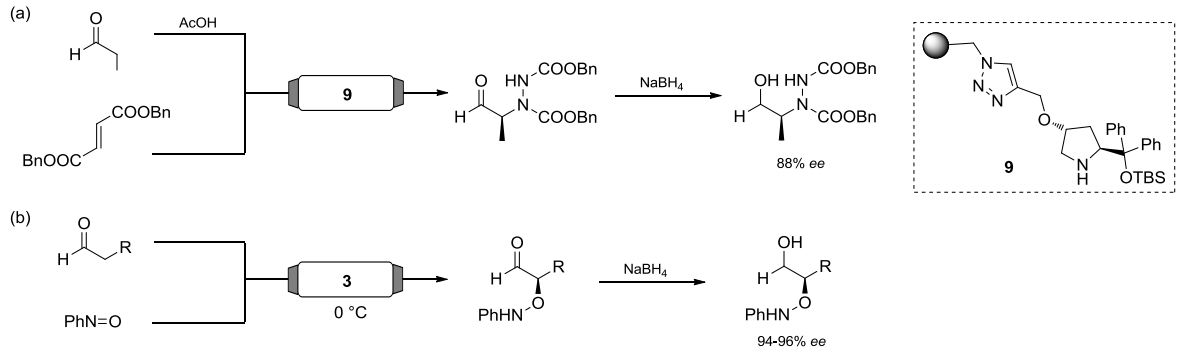
Scheme 12. *CF asymmetric Michael reaction in a fluid bed reactor filled with PS-supported cinchonidine.*



Scheme 13. *Enantioselective domino Michael−Knoevenagel reaction in CF operation with a PS-immobilized diarylprolinol silyl ether as organocatalyst.*

For the stereoselective formation of C−N bonds via the α-amination of aldehydes, Massi *et al.* furnished the first CF example with SiO_2-immobilized proline as organocatalyst.[147] However, besides high conversions, they attained only modest enantioselectivities (up to an *ee* of 58%), even at 0 °C. Fan *et al.* later reported a more sustainable approach for asymmetric α-amination reactions in flow.[152] They employed a PS-supported diphenylprolinol silyl ether catalyst (**9**) similar to that used for Michael reactions.[151, 153] It was found that a high excess of aldehyde was needed to prevent rapid catalyst deactivation, and 0.1 equivalent of AcOH as acidic additive was necessary to accelerate the transformation. The reaction between propanal and dibenzyl azodicarboxylate (DBAD) was studied (Scheme 14a). The swollen heterogeneous catalyst was packed into a glass catalyst bed, which was filled initially with a mixture of the aldehyde and AcOH to promote enamine formation. The circulation of the DBAD solution was subsequently started by another pump. The system was kept in operation continuously for 8 h, with full conversion in the first 6 h. After reduction of the resulting aldehyde, a synthetically

useful α-hydrazino alcohol product was obtained on the gram-scale with an *ee* of 88%. Cambeiro *et al.* utilized a similar concept for the organocatalytic asymmetric α-aminoxylation of aldehydes.[154] PS-immobilized organocatalyst **3** was employed, just as in an earlier study of CF Mannich reactions.[144] Reactions between various aliphatic aldehydes and nitrosobenzene were studied at 0 °C (Scheme 14b). Because of the intrinsic instability of the resulting α-aminoxy aldehydes, the products of the CF reactions were reduced *in situ* to the corresponding alcohols, which were obtained with high productivities and excellent *ee* values (up to 96%) in short reaction times. However, it must be noted that a slow decrease in conversion was observed during continuous operation for hours, which is due to possible catalyst deactivation. Opalka *et al.* reported a different strategy for the same reaction, relying on continuous proline catalysis via leaching of solid proline.[155] By pumping a solution of aldehyde and a thiourea additive through a column filled with solid L-proline, the leaching of the catalyst occurred into the homogeneous phase, thereby giving rise to homogeneous catalytic α-aminoxylation between the aldehyde and nitrosobenzene, which were introduced separately. After the reduction of the α-aminoxy aldehyde products, the corresponding alcohols were obtained in high yields and *ee* values.



Scheme 14. *Asymmetric α-amination (a) and α-aminoxylation (b) of aldehydes in CF with PS-supported organocatalysts.*

3. EXPERIMENTAL SECTION

3.1. CF methodology

CF experiments were carried out in an H-Cube® mesoreactor system containing a stainless steel cartridge as catalyst bed with internal dimensions of 30×4 mm or 70×4 mm.[156] The filled cartridge was embedded in a heating unit controlled by a Peltier system, up to a maximum of 100 °C. This also included a coiled stainless steel reaction line (with an internal diameter of 500 μm) for preheating of the liquid phase before entering the catalyst bed. A backpressure regulator ensured constant pressures up to a maximum of 100 bar, and the CF of the reaction medium was provided by a conventional HPLC pump (Knauer WellChrom K-120).[157] For heterogeneous hydrogenations, the H-Cube® system contains a gas-generation unit consisting of a reservoir for deionized water and a built-in electrolysis cell for the generation of H_2. The *in situ* generated gas is combined via a gas–liquid mixer with the solution of the substrate, and the mixture is then transported to the catalyst bed, where the triphasic reaction takes place.[47] CF deuterations were carried out by changing the hydrogen source to D_2O.[77] In the case of CF organocatalysis and CF CuAAC reactions, the gas generation unit was turned off (i.e. the H-Cube® system was switched to 'no H_2' mode). A brief outline of the CF system is shown in Figure 8.

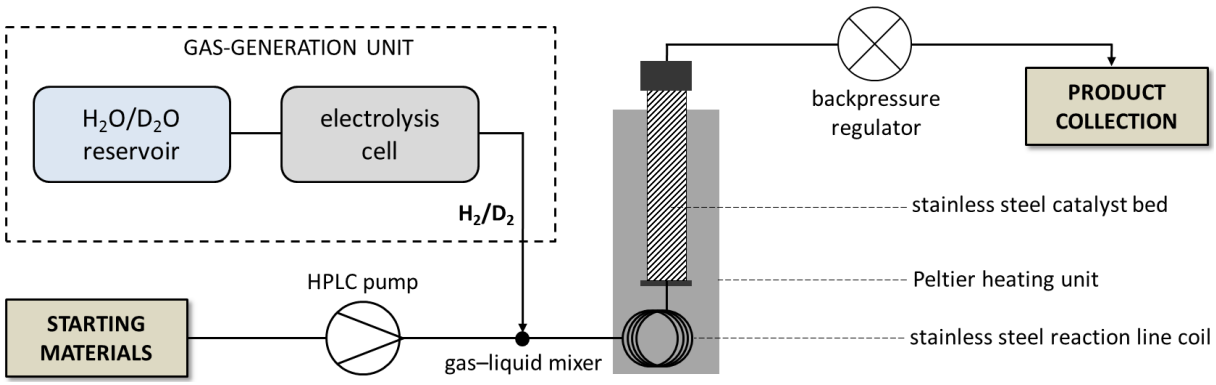


Figure 8. *Experimental setup for the CF reactions.*

To determine the residence time on the filled catalyst bed, a solution of a dye was pumped through the cartridge. The time that elapsed between the first contact of the dye with the catalyst bed and the moment when the colored solution appeared at the column output was measured.

For the CF reactions, the starting materials were dissolved by sonication, and then pumped through the flow reactor. Most important reaction parameters (such as temperature, pressure, flow rate and substrate concentration) were systematically fine-tuned to determine optimal conditions. The crude products were checked by thin-layer chromatography and, if necessary, column chromatographic purification was carried out. The products of the CF reactions were characterized by means of NMR spectroscopy (^{1}H, ^{13}C, ^{13}C HMBC[158] and ^{13}C HSQC[159]), MS

and elemental analysis. In cases of chiral compounds, *ee* was assigned with chiral NP-HPLC, and *dr* was determined from the ^{1}H NMR spectra of the crude material. The deuterium content of the deuterated compounds (i.e. the deuterium incorporation ratio over incidental hydrogen addition) was determined from the relative intensity of ^{1}H NMR indicator signals and by MS analysis. In CF CuAAC reactions, the copper contamination of the resulting materials was determined by means of inductively coupled plasma mass spectrometry (ICP-MS).

3.2. Synthesis and modification of the immobilized peptidic catalysts

The peptidic catalysts were prepared manually by means of solid-phase peptide synthesis (SPPS),[160] utilizing 9*H*-fluoren-9-ylmethoxycarbonyl (Fmoc)/*t*Bu chemistry.[161] Amino-functionalized non-trifluoroacetic acid (TFA)-labile resins were utilized as solid-supports for the SPPS: polyethylene glycol (PEG)–PS copolymer without any linker (TentaGel, Figure 9a),[162] and PS resin with a 4-methylbenzhydrylamine linker (PS-MBHA, Figure 9b).[163] With respect to the different swelling properties of the applied resins,[164] DMF was used as solvent in the case of TentaGel, and DMF/CH_2Cl_2 1:1 was employed for couplings with PS-MBHA. Before any synthetic steps, the resin was swollen thoroughly for 1 h in the appropriate solvent.

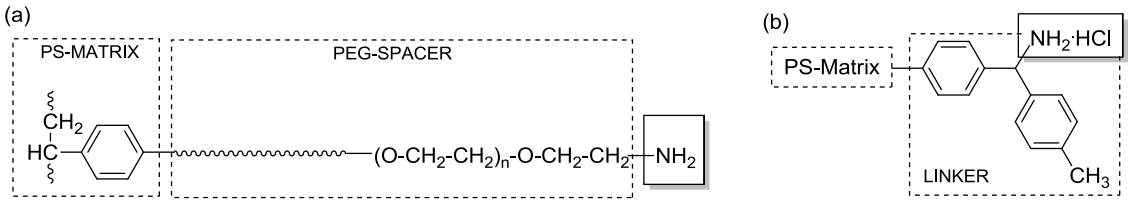
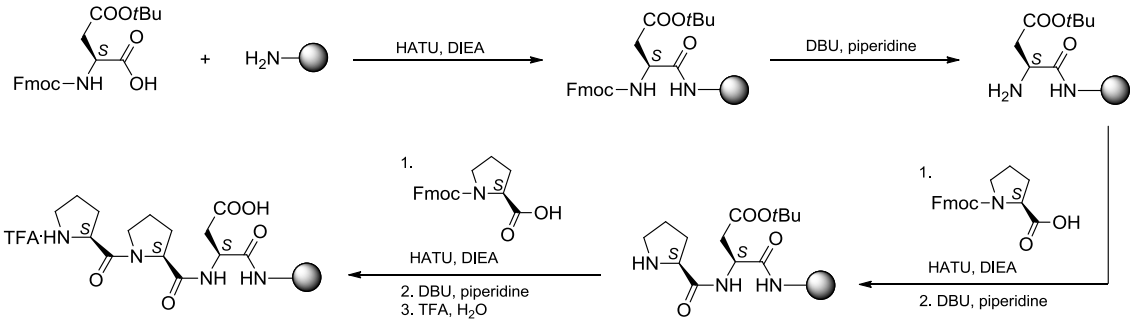


Figure 9. *Solid supports utilized for immobilization of peptidic catalysts: TentaGel (a), PS-MBHA (b).*

A further treatment with 5% *N*,*N*-diisopropylethylamine (DIEA) solution was carried out in the case of PS-MBHA to liberate the amino function from the HCl salt form. 3 equivalents of the Fmoc-protected amino acid and 3 equivalents of 1-[*bis*-(dimethylamino)methyliumyl]-1*H*-1,2,3-triazolo[4,5-*b*]pyridine-3-oxide (HATU)[165] were dissolved, and 6 equivalents of DIEA was added. The solution of the activated amino acid was then poured onto the swollen resin, and the couplings were fulfilled by agitation for 3 h. After that, the incorporation of the amino acid was monitored by means of the ninhydrin or isatin test.[166, 167] Fmoc deprotection was performed in a solution of 2% 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and 2% piperidine with agitation for 2×15 min, and the peptide chain was then elongated with repetition of the coupling and deprotection steps (Scheme 15). After each step, the resin was washed 3 times with CH_2Cl_2, once with MeOH and 3 times with CH_2Cl_2. Finally, the carboxyl side-chain was deprotected without cleavage of the peptide from the solid support by means of agitation



Scheme 15. *An example of the solid-phase synthesis of immobilized peptidic catalysts.*

for 3 h in a mixture of TFA and H_2O (9:1 v/v). After filtration and washing with the same solvents as described previously, the resin-bound peptide was kept at RT for 6 h to dry. The immobilized catalysts were obtained in TFA salt form after the SPPS.

Acetylation of the N-terminus of the immobilized peptide was performed in CF while being loaded into a catalyst cartridge. A mixture of 10% Ac_2O and 2% pyridine in $CHCl_3$ was circulated for 60 min under the following conditions: 0.3 mL min^{-1}, atmospheric pressure and RT. After that, the resin was washed with $CHCl_3$ for 30 min at a flow rate of 1 mL min^{-1}.

TentaGel-bound catalysts were characterized by suspension-phase ^{13}C NMR measurements. The structure of MBHA-immobilized peptides was verified by means of MS and RP-HPLC investigations after cleaving from the resin in a mixture of thioanisole, 1,2-ethanedithiol (EDT), TFA and trifluoromethanesulfonic acid (TFMSA) (2:1:20:2 v/v/v/v) for 0.5 h at –10 °C and then at RT for 1.5 h. Next, the peptide was precipitated with cold Et_2O, collected by filtration and dissolved in TFA. The volume of TFA was reduced to 1 mL by evaporation, and the peptide was precipitated again with Et_2O. After filtration, the peptide was dissolved in 10% AcOH and lyophilized.

3.3. Analytical investigations

NMR measurements were carried out on Bruker Avance DRX 400 or 500 spectrometers in $CDCl_3$ as solvent, using tetramethylsilane as internal standard.[168] ^1H NMR spectra were recorded at 400.1 or 500.1 MHz, ^{13}C NMR at 100.6 or 125.0 MHz, respectively. MS analyses were performed with an Agilent 1100 LC/MSD trap.[169] Microanalysis was carried out on a Perkin–Elmer 2400 elemental analyzer.[170] An analytical HPLC was used with a diode array detector from JASCO.[171] Chiral columns were purchased from Daicel (Chiralcel® OD-H, Chiralpak® IA, Chiralpak® AS),[172] RP-HPLC investigations were performed on a Phenomenex Luna 5μ C18 100A column (250×4.60 mm).[173] The exact conditions for the HPLC analyses can be found in the original papers.[II, IV] ICP-MS investigations were performed on an Agilent 7700x-type instrument equipped with a collision cell, using He as collision gas.[174]

4. RESULTS AND DISCUSSION

4.1. CF deuteration of nitrogen-containing heterocycles[I]

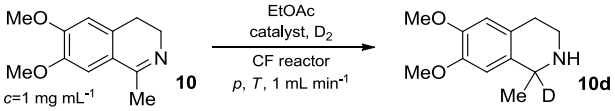
Biologically active compounds are often derived from heterocyclic structures. For example, quinoline, isoquinoline, quinoxaline, and their saturated analogs are commonly used as building blocks in pharmaceutical chemistry.[14] Derivatives of such nitrogen-containing heterocycles exhibit various pharmacological properties, such as antimalarial,[175] antitumor,[176] antibacterial[177] or antidepressant effects,[178] and this structural class has therefore received special attention in the field of drug discovery. Incorporation of deuterium-labeled heterocycles into drugs or drug-like molecules is an ultimate goal for the preparation of tools to investigate pharmacokinetics,[15] but as pointed out in section 2.2.1, conventional methodologies for deuterium labeling suffer from several drawbacks.[16] For this reason, we developed a sustainable CF method for the highly selective deuteration of nitrogen-containing heterocycles.

4.1.1. CF method development

On the basis of earlier results,[77] D_2 was generated *in situ* by means of electrolytic decomposition of D_2O in an H-Cube® reactor (Figure 8).[47] This approach is highly favorable, as the purity of the D_2 gas produced can be as high as 99.99%,[179] and the consumption of D_2O is very low, which implies much higher deuterium efficiency than in earlier methods.[75, 76] Approximately 150 mg of a heterogeneous hydrogenation catalyst was incorporated into the catalyst cartridge with internal dimensions of 30×4 mm. To prevent D–H exchange and maximize deuterium incorporation, EtOAc was employed as aprotic solvent. For an optimization study, deuteration of substituted 3,4-dihydroisoquinoline derivative **10** was selected as a model reaction. 20 mL aliquots of **10** (in 1 mg mL^{-1} solution) were pumped through the CF reactor, maintaining a flow rate of 1 mL min^{-1}, and further reaction conditions were systematically fine-tuned (Table 1). Initially, 5% Pd/$BaSO_4$ was chosen as heterogeneous catalyst over Pd/C, as it is known from the literature that various protic contaminations in the activated charcoal matrix can easily bias the deuterium incorporation ratio.[180] At RT and atmospheric pressure, no conversion was obtained (Table 1, entry 1). As pressurizing apparently increases the solubility of gases, it is straightforward that higher pressures mean higher reaction rates. Accordingly, at 40 bar a conversion of 50% was achieved (Table 1, entry 3), but further elevation to 80 bar did not prove beneficial, as the conversion remained steady at 50% (Table 1, entries 4 and 5). To improve the reaction rates, we tried raising the temperature from RT and simultaneously, increasing the residence time on the catalyst bed by the recirculation of the substrate solution. However, after

three consecutive circulations at 90 °C and 50 bar, the conversion was still below optimal (Table 1, entry 7). Thus, as a more active catalyst, 5% Pt/Al_2O_3 was chosen. At 30 °C and 50 bar, almost quantitative conversion was obtained in a single run (Table 1, entry 8). Moreover, the reduction was highly selective, as the benzene ring remained intact, whereas the heterocycle was successfully deuterated, leading to **10d** with an excellent deuterium incorporation ratio of 97% (Table 2, entry 1). It must be noted that the N–D bond could not be detected in the NMR spectra, as it instantly changed to N–H due to moisture while being exposed to air.

Table 1. Optimization of the reaction conditions in the CF deuteration of **10**.



Entry	Catalyst	*p* (bar)	*T* (°C)	Runs[a]	Conv. (%)[b]
1	5% $Pd/BaSO_4$	1	RT	1	0
2	5% $Pd/BaSO_4$	20	RT	1	0
3	5% $Pd/BaSO_4$	40	RT	1	50
4	5% $Pd/BaSO_4$	60	RT	1	50
5	5% $Pd/BaSO_4$	80	RT	1	50
6	5% $Pd/BaSO_4$	50	70	2	60
7	5% $Pd/BaSO_4$	50	90	3	80
8	5% Pt/Al_2O_3	50	30	1	99

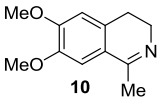
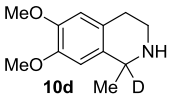
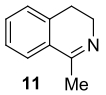
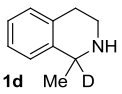
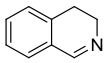
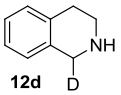
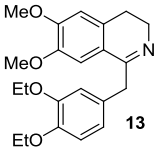
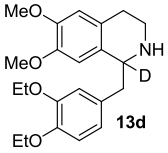
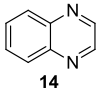
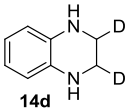
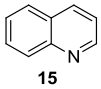
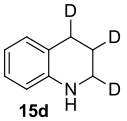
[a]Number of circulations through the CF reactor. [b]Determined by ^1H NMR spectroscopic analysis of the crude material.

4.1.2. Investigation of the scope and applicability of the CF methodology

Deuteration of various nitrogen-containing heterocycles was carried out to generalize the CF methodology. It was found that deuteration of partially unsaturated heterocycles was successful under the previously optimized reaction conditions. In the cases of **11**-**13**, the corresponding deuterated products (**11d**-**13d**) were obtained with excellent conversions (>96%) and deuterium contents (>95%) within a single run (Table 2, entries 2-4). Deuterium incorporation was selective into the carbon-nitrogen double bond as the aromatic ring remained saturated in all cases. It is noteworthy that **13** (drotaverine), a spasmolytic drug, was selectively deuterated on the *endo* position of the double bond. Deuteration of fully aromatic nitrogen-containing heterocycles was also attempted. As was expected, saturation of the heteroaromatic systems required stronger conditions as compared with the 3,4-dihydroisoquinoline derivatives **10**-**13**. In the case of quinoxaline (**14**), utilization of the previous conditions (5% Pt/Al_2O_3, 30 °C, 50 bar) allowed a conversion of 92% (Table 2, entry 5). However, increasing the temperature to 50 °C

resulted in almost quantitative conversion and selective deuterium introduction into the heterocycle with an excellent deuterium content of 95% (Table 2, entry 6). Due to its more stable heteroaromatic system, the efficient deuteration of quinoline (**15**) required more stringent conditions. At 30 °C and 50 bar with 5% Pt/Al_2O_3 as catalyst, a conversion of only 80% could be obtained (Table 2, entry 7). To improve the reaction rate, the temperature was raised to 70 °C and the solution of **15** was circulated consecutively through the flow reactor three times. The incorporation of deuterium into the nitrogen-containing ring was selective even under these conditions. Finally, a deuterium content of 97% and a conversion of 98% could be achieved (Table 2, entry 8). Deuteration of pyrazine was also attempted, but presumably the aromatic ring underwent fragmentation into volatile compounds, and thus no deuterated product could be isolated. Deuterated products **10d**-**15d** were obtained in a sufficiently pure form, and were analyzed after evaporation without further work-up steps.

Table 2. *CF deuteration of selected nitrogen-containing heterocycles.*[a]

Entry	Starting material	Product	*T* (°C)	Runs[b]	Conv. (%)[c]	D (%)[d]
1	 **10**	 **10d**	30	1	99	97
2	 **11**	 **11d**	30	1	99	95
3	 **12**	 **12d**	30	1	97	96
4	 **13**	 **13d**	30	1	96	98
5	 **14**	 **14d**	30	1	92	n.d.
6			50	1	99	95
7	 **15**	 **15d**	30	1	80	n.d.
8			70	3	98	97

[a]Conditions: *c*=1 mg mL^{-1} in EtOAc, 5% Pt/Al_2O_3, 50 bar, flow rate 1 mL min^{-1}. [b]Number of circulations through the CF reactor. [c]Determined by ^1H NMR spectroscopic analysis of the crude material. [d]Deuterium content, which represents the deuterium incorporation ratio over incidental hydrogen addition.

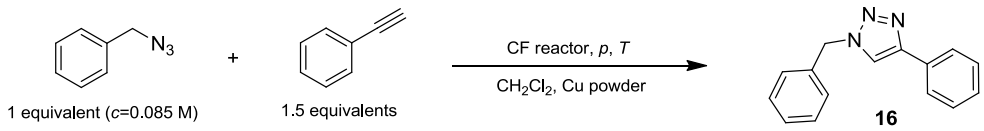
In terms of productivity and selectivity, the above-described CF methodology is highly competitive with the batch results from the literature.[181, 182] Moreover, it means a sustainable alternative for the conventional approaches, as it is safe, simple, rapid and cost efficient, whilst it allows pharmaceutically relevant deuterated products without ponderous purification steps.

4.2. Copper-catalyzed azide–alkyne cycloadditions in CF[V,VI]

4.2.1. CF method development

As detailed in the Literature survey (section 2.2.2), there are already a number of CF approaches for CuAAC reactions.[12, 13, 96, 99, 102, 103] However, most examples rely on either costly apparatus or special catalyst types, which limit their sustainable applications. Therefore, we recognized that there would be a huge demand for an easily available, inexpensive CF technique for CuAAC reactions which is at the same time efficient and safe. We envisioned that Cu powder can act as the simplest source for catalytically active Cu(I) species, as Cu undergoes constant oxidation when exposed to air, and non-self-protecting layers of different oxides, including Cu_2O, are formed on its surface and, similarly to cases when heated Cu wiring is employed,[99-101] this can promote CuAAC.[183] In this context, Cu powder can be referred as a readily available 'supported' Cu(I) catalyst, and accordingly, when oxide layers are removed from the surface, the catalytic activity decreases appreciably.[12]

For the CF reactions, 900 mg of Cu powder (with an average particle size of 200 μm) was charged into a catalyst cartridge with internal dimensions of 70×4 mm. The 1,3-dipolar cycloaddition between benzyl azide and phenylacetylene was chosen as a test reaction for an elaborate optimization study (Scheme 16). CH_2Cl_2 was selected as solvent, in which the azide was applied in a concentration of 0.085 M, this proving to be the highest possible concentration which prevented the precipitation of the resulting triazole and a blockage in the channels of the reactor. As small-scale test experiments, 2.5 mL aliquots of a reaction mixture containing 1 equivalent of benzyl azide and 1.5 equivalents of phenylacetylene were pumped through the system in each run, and conversion was determined after each step.



Scheme 16. *1,3-Dipolar cycloaddition between benzyl azide and phenylacetylene as a test reaction for optimization of the CF reaction conditions.*

As the starting point of the optimization study, the test reaction was carried out at atmospheric pressure, RT, and a flow rate of 0.5 mL min^{-1}, resulting in a conversion of only 20%. To improve the reaction rate of the CF CuAAC, we first employed high-pressure conditions, as elevated pressures can expectedly promote triazole formation in accordance with Le Chatelier's principle. On investigation of the pressure dependence (at RT, and maintaining a flow rate of 0.5 mL min^{-1}), it was found that a pressure of at least 80 bar should be employed to obtain any higher conversion (Figure 10). At 100 bar, a conversion of 34% was achieved, and

further elevation would probably have resulted in even higher conversions, but 100 bar was taken as an optimal value as further pressurizing could not be reached with the H-Cube® system.[47] Elevation of the pressure allowed the use of temperatures well above the boiling point of CH_2Cl_2. Thus, as a next step, high-temperature conditions were examined. While the pressure and flow rate were maintained at 100 bar and 0.5 mL min^{-1}, the temperature was increased gradually from RT to 100 °C. Figure 11 shows that the conversion started to increase steeply at 40 °C; at 50 °C, it exceeded 90%, and finally at 100 °C quantitative conversion was achieved. Besides physical chemistry, heating is most likely to enhance the rates of the CuAAC reaction by increasing the solubility of Cu_2O from the surface of the zerovalent Cu matrix.

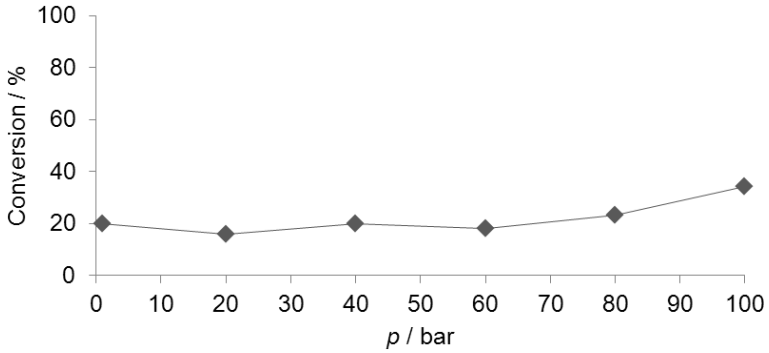


Figure 10. *Pressure dependence of the test CF CuAAC reaction (Scheme 16). Conditions: RT, flow rate 0.5 mL min^{-1}, no additives.*

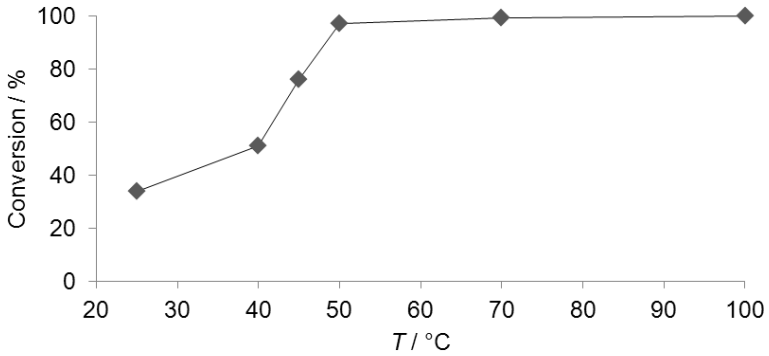


Figure 11. *Temperature dependence of the test CF CuAAC reaction (Scheme 16). Conditions: 100 bar, flow rate 0.5 mL min^{-1}, no additives.*

Under the previously optimized reaction conditions (100 bar, 100 °C), the residence time on the catalyst bed was also fine-tuned. As quantitative conversion was obtained at 0.5 mL min^{-1}, it was unnecessary to employ even lower flow rates; we rather then tried increasing the flow rate to achieve the shortest possible process time. However, it was found that the reaction is very sensitive to the reduction of the residence time, as the conversion decreased dramatically with enhancement of the flow rate (Figure 12). For example, at 1 mL min^{-1}, the conversion was only 31%. Thus, 0.5 mL min^{-1} was regarded as an optimal flow rate, which involved a residence time on the catalyst bed as low as 1.5 min, and a process time of only 5 min to pump the 2.5 mL aliquot of the reaction mixture through the reactor.

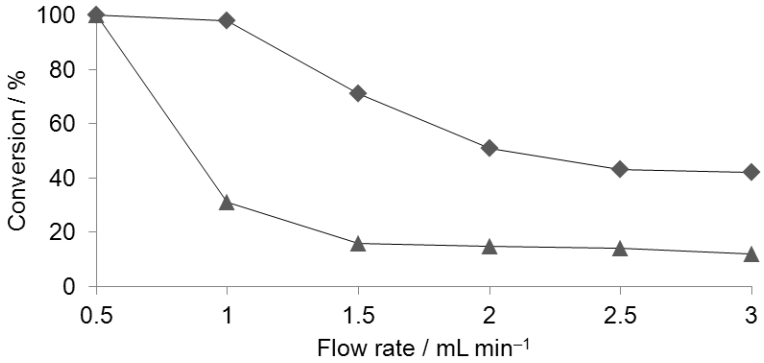
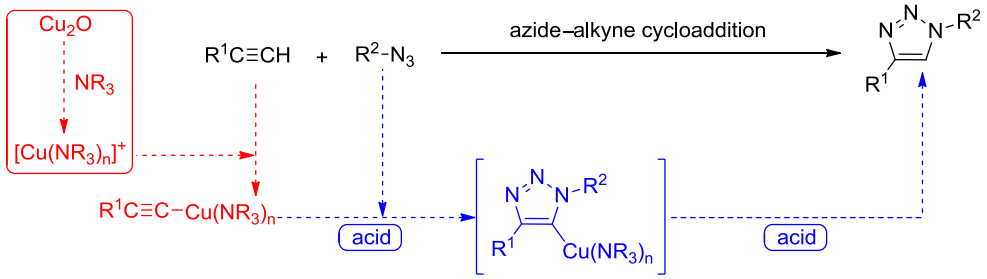


Figure 12. *Fine-tuning of the flow rate in the test CF CuAAC reaction (Scheme 16). ▲: 100 bar, 100 °C, no additives. ♦: 100 bar, RT, 0.04 equivalents of DIEA + 0.04 equivalents of AcOH.*

At 100 bar, 100 °C and a flow rate of 0.5 mL min^{-1}, quantitative conversion was obtained, and due to flow processing the risks that are associated with the handling of azides were successfully lowered as compared with conventional batch approaches,[2, 11] but we envisioned that it would be highly beneficial to avoid harsh reaction conditions completely, with the CF CuAAC procedure at maximum operational safety. Accordingly, we next attempted to improve the rates of the test reaction in the presence of additives, without the use of high temperature. Amines, as basic additives, are known to boost the reactivity of the CuAAC considerably,[11] in particular by coordinating to catalytically active Cu(I) species and promoting their liberation from the Cu matrix, thereby assisting the formation of a distinct Cu–alkyne complex (Scheme 17).[82, 184, 185] Additionally, it was recently shown that catalytic amounts of certain acids can further accelerate the formation of the triazole product through promotion of the conversion of C–Cu bond-containing intermediates by protonation.[186-188] Furthermore, utilization of acidic additives is beneficial as it prevents the formation of unwanted byproducts, such as diacetylenes, bis-triazoles, etc.[185] In contrast, byproduct formation is catalyzed by base. The joint application of a basic and an acidic additive is therefore highly advantageous: this buffer system improves reactivity in CuAAC, even at RT, but without the formation of byproducts.[185]



Scheme 17. *Catalytic cycle of the base–acid jointly promoted CuAAC reaction.*[185]

As basic and acidic additives, DIEA and AcOH were selected, relying on literature data.[185] To investigate the effects of additives, pressure and flow rate was set to 100 bar and 0.5 mL min^{-1}, respectively, and heating was avoided (i.e. RT was utilized). Under these conditions,

a conversion of 34% could be achieved without any additives (Table 3, entry 1). Addition of 0.1 equivalent of AcOH improved the conversion to 56% (Table 3, entry 2), and when 0.1 equivalent of DIEA was employed instead, the conversion rose further to 96% (Table 3, entry 3). The best result was obtained with the joint use of DIEA and AcOH (each in 0.1 equivalent) as additives, allowing the test reaction to proceed quantitatively (Table 3, entry 4). The effects of the amounts of DIEA and AcOH were then scanned on the reaction efficiency. As shown in Table 3, 0.04 equivalents of each additive was sufficient to maintain quantitative conversion (entry 6), but reducing the equivalents further gave poorer results (entries 7 and 8). When the flow rate was increased from 0.5 mL min^{-1} at 100 bar and RT with the joint use of both additives (each in 0.04 equivalents), it was found that the drop in the conversion was not as steep as under the high-pressure/high-temperature conditions (Figure 12). This implies that the joint use of DIEA and AcOH relieves the harsh reaction conditions without heating in CF.

Table 3. Effect of the DIEA/AcOH ratio on the CF reaction between benzyl azide and phenylacetylene with Cu powder (Scheme 16).[a]

Entry	DIEA (equivalent)	AcOH (equivalent)	Conv. (%)[a]	Entry	DIEA (equivalent)	AcOH (equivalent)	Conv. (%)[a]
1	0	0	34	5	0.08	0.08	quant.
2	0	0.1	56	6	0.04	0.04	quant.
3	0.1	0	96	7	0.02	0.02	63
4	0.1	0.1	quant.	8	0.01	0.01	40

[a]Conditions: 1 equivalent of azide (c_{azide}=0.085 M in CH_2Cl_2), 1.5 equivalents of alkyne, 100 bar, RT, flow rate 0.5 mL min^{-1}. [b]Determined by ^1H NMR analysis of the crude material.

As a result of the method development, we determined two distinct parameter sets as optimal conditions: *i*) 100 bar, 100 °C, flow rate of 0.5 mL min^{-1}, without any additives as conditions CF **A**, and *ii*) 100 bar, RT, flow rate of 0.5 mL min^{-1}, with DIEA and AcOH (each in 0.04 equivalents) as conditions CF **B**. Both of them afforded quantitative conversion in the test CuAAC reaction between benzyl azide and phenylacetylene, and selectively gave the 1,4-disubstituted 1,2,3-triazole isomer (**16**) as the product. Moreover, the introduced CF methodologies are safe, simple, and rapid; while, due to the easily available Cu(I) source and cheap additives, they are cost-efficient, enabling sustainable production.

4.2.2. Model reactions

A wide array of model reactions were next carried out under both conditions CF **A** and **B** to obtain a clear comparison between the performances of the two approaches. At first, the azide scope was examined, employing phenylacetylene as dipolarophile. As can be seen in Table 4, excellent results were achieved with either aliphatic or aromatic azides. In the case of aromatic

azides, it was found that either electron-withdrawing (entries 2-7) or electron-donating substituents (entries 8-10) are nicely tolerated, and yields were not distorted significantly upon varying the position of the substituents on the phenyl ring. α-Azido ketones are known to be poor reaction partners in CuAAC reactions, but their 1,3-diploar cycloaddition to different alkynes results in a wide variety of pharmaceutically important compounds.[189] To benchmark our CF methodologies, the reaction of 2-azidoacetophenone with phenylacetylene as dipolarophile was also attempted, and both conditions CF **A** and **B** afforded triazole **28** in outstanding yields (entry 13).

Reactions of a series of alkynes with benzyl azide were also carried out to further broaden the applicability of the methodologies. It was found that, besides phenylacetylene, non-aromatic alkynes, such as pent-1-yne and ethyl propiolate, are nicely tolerated (Table 4, entries 16 and 17). Diethyl acetylenedicarboxylate, as a non-terminal alkyne, was also successfully reacted, thereby resulting in 1,4,5-trisubstituted 1,2,3-triazole **33** in high yields (Table 4, entry 18), which is itself a potent antitubercular agent.[190] Ferrocene–triazole conjugates are widely applied in medicinal chemistry for the labeling and detection of various systems, e.g. in immunoassays, as biosensing probes and in host–guest chemistry,[191] and the incorporation of ferrocene into amino acids or peptides is an efficient tool for secondary structure determinations.[192] Thus, the CuAAC reaction of benzyl azide with ethynyl ferrocene was also attempted, and the corresponding ferrocene–triazole conjugate (**34**) was obtained in excellent yields under both CF conditions (Table 4, entry 19).

Table 4. *Azide–alkyne cycloadditions with Cu powder under optimized conditions in CF.*

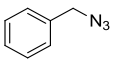
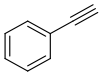
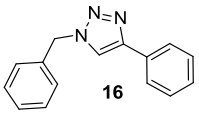
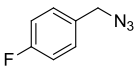
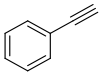
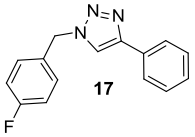
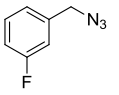
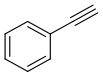
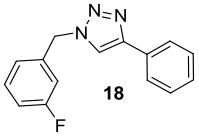
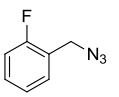
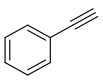
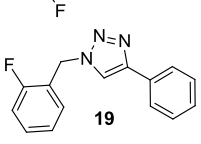
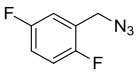
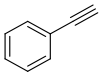
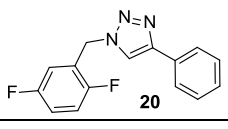
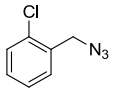
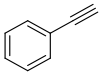
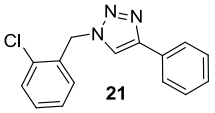
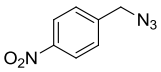
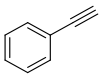
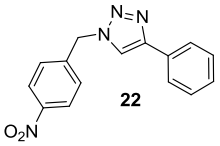
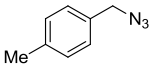
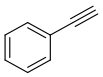
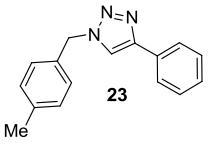
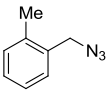
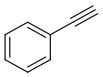
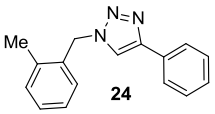
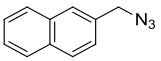
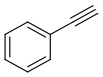
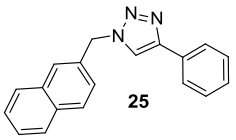
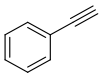
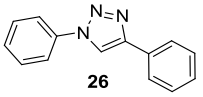
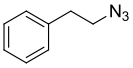
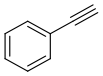
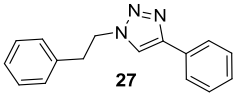
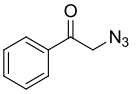
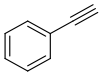
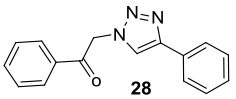
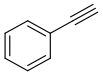
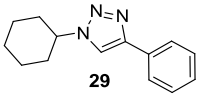
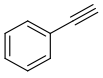
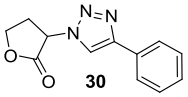
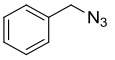
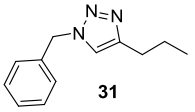
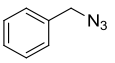
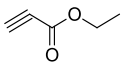
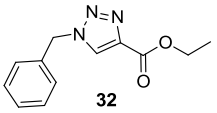
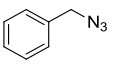
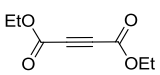
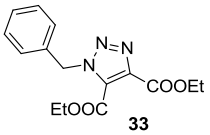
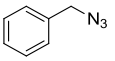
Entry	Azide[a] (1 equivalent)	Alkyne (1.5 equivalents)	Product	Yield (%)[b] CF **A**[c]	CF **B**[d]
1			 **16**	99	99
2			 **17**	98	99
3			 **18**	99	99
4			 **19**	99	99
5			 **20**	93	99

Table 4. *(Continued)*

Entry	Azide[a] (1 equivalent)	Alkyne (1.5 equivalents)	Product	Yield (%)[b] CF **A**[c]	CF **B**[d]
6			 **21**	99	99
7			 **22**	94	99
8			 **23**	83	99
9			 **24**	72	94
10			 **25**	99	99
11			 **26**	94	99
12			 **27**	77	98
13			 **28**	96	99
14			 **29**	99	99
15			 **30**	99	99
16			 **31**	94	91
17			 **32**	99	99
18			 **33**	84	82
19			**34**	99	99

[a]c_{azide}=0.085 M. [b]Yield of isolated product. [c]Conditions CF **A**: CH_2Cl_2 as solvent, 100 bar, 100 °C, flow rate 0.5 mL min^{-1}, without any additives. [d]Conditions CF **B**: CH_2Cl_2 as solvent, 100 bar, RT, flow rate 0.5 mL min^{-1}, with 0.04 equivalents of DIEA + 0.04 equivalents of AcOH.

As can be seen in Table 4, though the application of conditions CF **B** (i.e. the use of additives at RT) resulted in slightly higher yields in some of the model reactions (entries 5, 7-9 and 11-13), significant differences between the efficacies of the two approaches could not be recognized. In the cases of terminal alkynes, the corresponding 1,4-disubstituted 1,2,3-triazole isomers were formed regioselectively. No work-up or purification step was necessary when the conversion was quantitative and any excess alkyne could be volatilized on evaporation (phenylacetylene, pent-1-yne and ethyl propiolate).

4.2.3. Synthesis of 1,2,3-triazole-modified alicyclic β-amino acid derivatives

As a consequence of their pharmacological potential, alicyclic β-amino acids have captured great attention in the past twenty years.[193] One of the most appealing representative of such compounds is cispentacin ((1*R*,2*S*)-ACPC, ACPC=2-aminocyclopentanecarboxylic acid), a naturally occurring antifungal antibiotic (Figure 13).[194] Its synthetic 4-methylene derivative (icofungipen) also exhibits strong antifungal properties,[195] and there are many bioactive compounds amongst highly functionalized cyclohexane-structured β-amino acid derivatives as well, such as oryzoxymycin and tilidine, both exhibiting antiviral properties (Figure 13).[196] Furthermore, alicyclic β-amino acids are important intermediates for synthetic chemistry,[197] and they are widely employed as building blocks of foldamers and peptidic structures.[198]

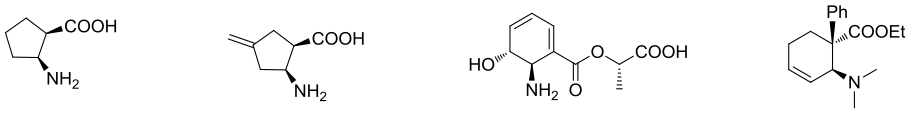


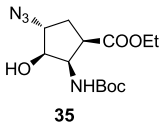
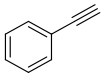
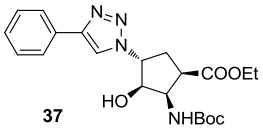
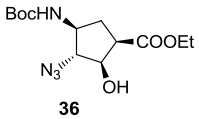
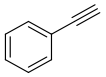
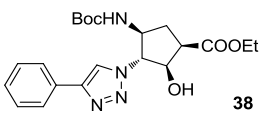
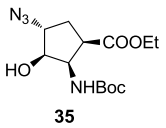
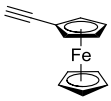
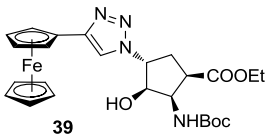
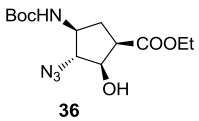
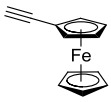
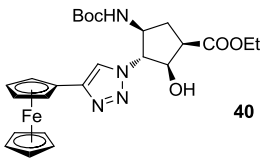
Figure 13. *Bioactive alicyclic β-amino acids.*

The literature findings discussed in section 2.2.2 reveal that the 1,2,3-triazole moiety can be regarded as a pharmacophore, as it is frequently used for the modification of known bioactive products, such as certain cyclic amino acid derivatives (Figure 6).[93, 94] In consequence of these properties, we aimed to employ the newly developed CF CuAAC procedures for the 1,2,3-triazole modification of some alicyclic β-amino acid derivatives, and thereby the sustainable synthesis of a library of potentially bioactive compounds.

To prepare 1,2,3-triazole-modified β-amino acids, azido-substituted cispentacin derivatives **35** and **36** (as racemic compounds) were used initially as reaction partners of phenylacetylene and ethynyl ferrocene.[199] Besides both CF conditions (**A** and **B**), the CuAAC reactions were carried out by employing conventional batch techniques as well to obtain a clear comparison. In the presence of CuI in MeCN under reflux for 12 h, batch reactions of **35** and **36** with phenylacetylene afforded the corresponding 1,2,3-triazole-substituted cispentacin derivatives **37**

and **38** in only moderate yields (69% and 73%, respectively; Table 5, entries 1 and 2). Conditions CF **A** afforded triazoles **37** and **38** in yields comparable with those of the batch reactions (62% and 42%, respectively; Table 5, entries 1 and 2); but with method CF **B**, both **37** and **38** could be isolated in an excellent yield of 99% (Table 5, entries 1 and 2). Conjugates of the azido-functionalized cispentacin derivatives **35** and **36** with ethynyl ferrocene were prepared in flow with outstanding results: both conditions CF **A** and **B** afforded ferrocenyl triazoles **39** and **40** with yields as high as 99% (Table 5, entries 3 and 4). In contrast, in batch, in the presence of CuI under reflux in MeCN, no transformation could be detected at all; and upon changing the catalyst to $CuSO_4$/ascorbic acid, the reaction furnished triazoles **39** and **40** in only moderate yields (51% and 69%, respectively; Table 5, entries 3 and 4) within 14 h.

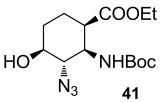
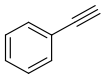
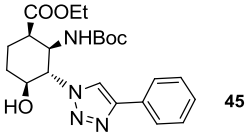
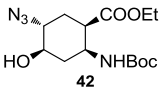
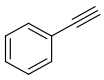
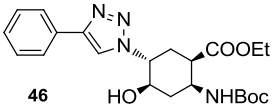
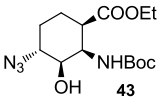
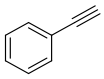
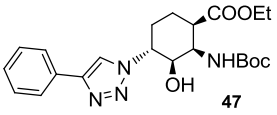
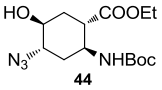
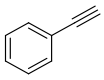
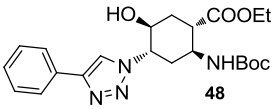
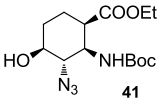
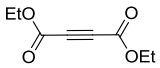
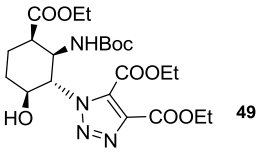
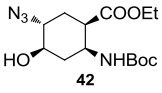
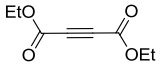
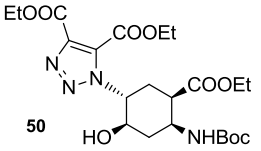
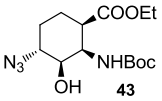
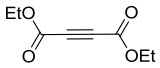
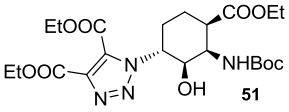
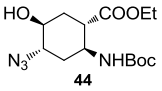
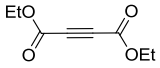
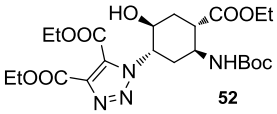
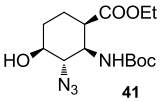
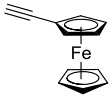
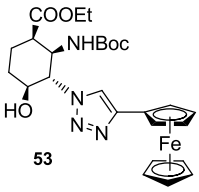
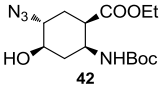
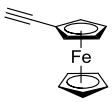
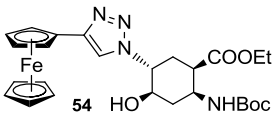
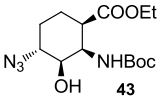
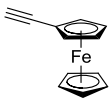
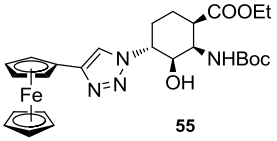
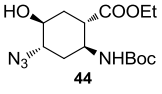
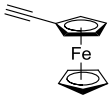
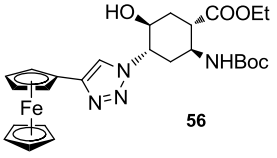
Table 5 *Synthesis of 1,2,3-triazole-substituted cispentacin derivatives with CF CuAAC methods and with conventional batch procedures.*

Entry	Azide[a] (1 equivalent)	Alkyne (1.5 equivalents)	Product	Yield (%)[b] CF **A**[c]	CF **B**[d]	batch
1	 **35**		 **37**	62	99	69[e]
2	 **36**		 **38**	42	99	73[e]
3	 **35**		 **39**	99	99	51[f]
3	 **36**		 **40**	99	99	69[f]

[a] c_{azide}=0.085 M, racemic compounds. [b]Yield of isolated product. [c]Conditions CF **A**: CH_2Cl_2 as solvent, 100 bar, 100 °C, flow rate 0.5 mL min^{-1}, without any additives. [d]Conditions CF **B**: CH_2Cl_2 as solvent, 100 bar, RT, flow rate 0.5 mL min^{-1}, with 0.04 equivalents of DIEA + 0.04 equivalents of AcOH. [e]To a solution of azido ester **35** or **36** (1 equivalent) in MeCN, CuI (1 equivalent) and alkyne (1.1 equivalents) were added and the mixture was stirred under reflux for 12 h. [f]To a solution of azido ester **35** or **36** (1 equivalent) in EtOH/H_2O=12:1, $CuSO_4$ (1 equivalent), ascorbic acid (1 equivalent) and alkyne (1.1 equivalents) were added and the mixture was stirred at RT for 14 h.

Next, azido-substituted β-aminocyclohexanecarboxylates **41**-**44** were employed as reaction partners of three different alkynes (phenylacetylene, diethyl acetylenedicarboxylate and ethynyl ferrocene) to obtain 1,2,3-triazole-modified target compounds.[200] (**41**-**44** were used as racemic compounds.) In reactions with phenylacetylene, method CF **A** afforded triazoles **45**-**48** in only moderate yields (Table 6, entries 1-4). Under conditions CF **B**, **45** and **46** were isolated in excellent yields (96% and 97%, respectively; Table 6, entries 1 and 2), but triazoles **47** and **48** could not be obtained with satisfactory results (yields were 76% and 89%, respectively; Table 6,

Table 6. CF synthesis of 1,2,3-triazole-modified β-aminocyclohexanecarboxylic acid derivatives.

Entry	Azide[a] (1 equivalent)	Alkyne (1.5 equivalents)	Product	Yield (%)[b] CF **A**[c]	CF **B**[d]
1	 **41**		 **45**	61	96
2	 **42**		 **46**	47	97
3	 **43**		 **47**	33	76 (98)[e]
4	 **44**		 **48**	53	89 (98)[e]
5	 **41**		 **49**	98	97
6	 **42**		 **50**	97	98
7	 **43**		 **51**	97	96
8	 **44**		 **52**	97	98
9	 **41**		 **53**	95	97
10	 **42**		 **54**	91	98
11	 **43**		 **55**	96	93
12	 **44**		 **56**	75	97

[a] c_{azide}=0.085 M, racemic compounds. [b]Yield of isolated product. [c]Conditions CF **A**: CH_2Cl_2 as solvent, 100 bar, 100 °C, flow rate 0.5 mL min^{-1}, without any additives. [d]Conditions CF **B**: CH_2Cl_2 as solvent, 100 bar, RT, flow rate 0.5 mL min^{-1}, with 0.04 equivalents of DIEA + 0.04 equivalents of AcOH. [e]Achieved under the following conditions: CH_2Cl_2 as solvent, 100 bar, 100 °C, flow rate 0.5 mL min^{-1}, with 0.04 equivalents of DIEA + 0.04 equivalents of AcOH.

entries 3 and 4). Thus, CF reactions of azides **43** and **44** with phenylacetylene were repeated under high-pressure/high-temperature conditions (100 °C, 100 bar) with the simultaneous use of DIEA and AcOH (each in 0.04 equivalents), resulting in triazoles **47** and **48** in yields of 98% eventually (Table 6, entries 3 and 4). Upon the application of diethyl acetylenedicarboxylate as dipolarophile, trisubstituted 1,2,3-triazole dicarboxylates (**49**-**52**) were obtained in yields of >96% under either of the CF conditions (Table 6, entries 5-8). CuAAC reactions with ethynyl ferrocene gave the corresponding ferrocene–triazole conjugates in excellent results under both CF conditions in the cases of **53**-**55** (Table 6, entries 9-11). Triazole **56** was obtained in a yield of 75% by method CF **A**, but CF **B** resulted in an excellent yield of 97% (Table 6, entry 12).

It can be recognized that the yields of the triazole products formed with phenylacetylene were usually lower than in the reactions with diethyl acetylenedicarboxylate and ethynyl ferrocene, and the differences were significant only in the case of conditions CF **A** (see Table 5, entries 1 and 2 vs. entries 3 and 4; and Table 6, entries 1-4 vs. 5-12). To understand these findings, it must be considered that the carboxylate groups of diethyl acetylenedicarboxylate and the aromatic system of the ferrocenyl group can act as ligands and coordinate Cu(I) effectively from its matrix.[201] Due to this substrate effect, the concentration of the catalytically active Cu(I) and therefore the reactivity is increased as compared with phenylacetylene. In the case of conditions CF **B**, the use of DIEA masks the effect of the alkynes.

1,2,3-Triazole-modified β-amino acid derivatives obtained in reactions with terminal alkynes were selectively formed as 1,4-disubstituted regioisomers. It should be emphasized that epimerization of the triazole products was not detected in the NMR spectra of the crude materials, even under basic conditions, which is due the very short residence time (1.5 min).

Some leaching of copper from the catalyst bed was expected, and thus the trace amounts of copper were determined in the triazole products obtained with both CF methodologies. The analytical data in Table 7 show that the copper contents in the samples obtained under high-pressure/high-temperature conditions without any additives (CF **A**) were lower than those when DIEA and AcOH were jointly applied (CF **B**). After column chromatographic purification, the copper impurities were reduced significantly (entries 1 and 2 vs. 3-16). Copper contents detected in our systems were nicely comparable with the literature CF or batch results.[12, 202]

4.2.4. Scale-up studies

As pointed out in the Literature survey (section 2.1), scale-up in CF processing is straightforward, as volume is a function of time and of flow rate rather than the batch size.[3] Thus, gram-scale syntheses could be performed in a simple, safe and efficient manner.

Table 7. *Contents of copper impurities in the 1,2,3-triazole products obtained in CF.*

Entry	Product	Copper content (μg g^{-1})[a]		Entry	Product	Copper content (μg g^{-1})[a]	
		CF **A**[b]	CF **B**[c]			CF **A**[b]	CF **B**[c]
1	**37**	14.8(±0.8)[d]	70.1(±1.4)[d]	9	**49**	5.2(±0.4)[e]	7.9(±0.4)[e]
2	**38**	12.1(±0.7)[d]	66.2(±1.5)[d]	10	**50**	5.1(±0.3)[e]	7.5(±0.6)[e]
3	**39**	4.4(±0.3)[e]	7.1(±0.6)[e]	11	**51**	4.8(±0.6)[e]	7.7(±0.7)[e]
4	**40**	5.2(±0.5)[e]	7.3(±0.7)[e]	12	**52**	5.3(±0.3)[e]	8.2(±0.6)[e]
5	**45**	4.6(±0.5)[e]	8.4(±0.6)[e]	13	**53**	6.1(±0.5)[e]	8.6(±0.5)[e]
6	**46**	4.2(±0.3)[e]	7.7(±0.6)[e]	14	**54**	4.8(±0.4)[e]	7.7(±0.8)[e]
7	**47**	3.9(±0.5)[e]	8.0(±0.4)[e]	15	**55**	5.4(±0.3)[e]	9.1(±0.4)[e]
8	**48**	4.7(±0.6)[e]	8.2(±0.7)[e]	16	**56**	4.9(±0.6)[e]	7.8(±0.7)[e]

[a]Determined by ICP-MS. [b]Conditions CF **A**: CH_2Cl_2 as solvent, 100 bar, 100 °C, flow rate 0.5 mL min^{-1}, without any additives. [c]Conditions CF **B**: CH_2Cl_2 as solvent, 100 bar, RT, flow rate 0.5 mL min^{-1}, with 0.04 equivalents of DIEA + 0.04 equivalents of AcOH. [d]Without column chromatographic purification. [e]After column chromatographic purification.

For the scale-up studies, a reaction mixture containing 1 equivalent of the azide (c_{azide}=0.085 M) and 1.5 equivalents of the alkyne (and 0.04 equivalents of both additives in the case of conditions CF **B**) in CH_2Cl_2 was continuously pumped through the system under either conditions CF **A** or **B**. During a discrete experiment, the same portion of Cu powder was employed in the catalyst cartridge. First, the CuAAC between benzyl azide and phenylacetylene was scaled up (Scheme 16), utilizing both conditions CF **A** and **B**. In each case, 75 mL of the reaction mixture was pumped through in 150 min. After both experiments, 1.5 g of triazole **16** could be isolated respectively (3 g altogether, which is equivalent to a yield of 99%), without the need for any further work-up but evaporation. Gram-scale synthesis of triazole **52** was then performed under conditions CF **B**. 50 mL of the reaction mixture containing azide **44** and diethyl acetylenedicarboxylate was pumped through the CF reactor in 100 min. After purification, 2.06 g of triazole **52** was obtained, which is equivalent to a yield of 96%.

4.3. CF organocatalysis with solid-supported peptidic catalysts[II-IV]

Organocatalytic aldol reactions and conjugate additions between aldehydes and nitroalkenes are among the most powerful ways for the formation of asymmetric C–C bonds.[8] The resulting chiral β-hydroxy carbonyl compounds and γ-nitroaldehydes are valuable intermediates for various syntheses in pharmaceutical chemistry.[203, 204] Thus, we developed the first CF organocatalytic technique for the stereoselective 1,4-addition of aldehydes to nitroolefins, and then extended the scope of the methodology for asymmetric aldol reactions.

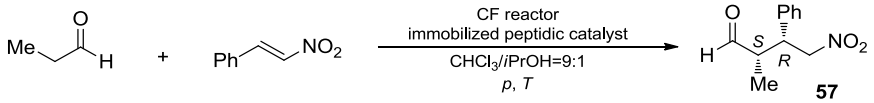
As chiral organocatalysts, N-terminal prolyl-peptides bearing an acidic side-chain at the C-terminus were applied immobilized onto swellable polymer supports. Employing a peptide was highly beneficial, as such modular organocatalysts offered higher structural diversity than small

proline-like counterparts.[10] Moreover, the synthesis and immobilization of the catalyst could easily be combined in SPPS without the need for further synthetic steps. With the use of non-TFA-labile resins as support for the peptide synthesis, the deprotection of the carboxyl side-chain could be fulfilled without cleavage of the peptide after the coupling steps. This provided a highly sustainable methodology, as the heterogeneous support of the SPPS served also as catalyst carrier. The experimental setup was simple, time- and cost-efficient, as it eliminated the need for the peptide work-up and purification steps, and there was no product loss.

4.3.1. CF organocatalytic conjugate additions

4.3.1.1. CF method development

For optimization of the CF reaction conditions, the 1,4-addition of propanal to *E*-β-nitrostyrene (BNS) was chosen as test reaction (Scheme 18). Initially, the peptide H-D-Pro-Pro-Asp-NH-*resin* was employed, immobilized on PS-MBHA resin with a loading of 0.64 mmol g^{-1} (catalyst **58**).[131] For the CF reactions, 150 mg of the heterogeneous catalyst was incorporated into a stainless steel cartridge with internal dimensions of 70×4 mm. To provide accessibility of the immobilized catalyst molecules, the matrix of the supporting polymer should be swollen. Thus, $CHCl_3$/*i*PrOH=9:1 was chosen as an optimal solvent system. This medium ensured appropriate swelling properties for the immobilized catalyst and, at the same time, sufficient solubility of the reactants.[164] In each run, 5 mL aliquots of a reaction mixture were pumped through the CF reactor, and the most important reaction parameters were systematically fine-tuned.



Scheme 18. *Organocatalytic asymmetric 1,4-addition of propanal to BNS as a CF test reaction.*

At a flow rate of 0.5 mL min^{-1}, a rapid screen indicated that a BNS concentration of 8 mg mL^{-1} was favorable at RT and atmospheric pressure, while propanal was applied in 15 equivalents as an initial high excess (Table 8). Utilization of a very high excess of the aldehyde may help the reaction to reach completion, but also makes the procedure less economical. With this in mind, 5 equivalents of propanal was chosen as optimal value.

Table 8. *Fine-tuning of the BNS concentration in the test reaction (Scheme 18) in CF.*[a]

Entry	c_{BNS} (mg mL^{-1})	Yield (%)[b]	Entry	c_{BNS} (mg mL^{-1})	Yield (%)[b]
1	1	0	3	8	54
2	4	35	4	16	45

[a]Conditions: 1 equivalent of BNS and 15 equivalents of propanal in $CHCl_3$/*i*PrOH=9:1, 1 bar, RT, flow rate 0.5 mL min^{-1}, catalyst **58**. [b]Yield of isolated product.

Next, the flow rate was fine-tuned at 60 bar and RT to obtain an optimized residence time on the catalyst bed. At 0.01 mL min^{-1}, a yield of 99% was achieved, but a very long residence time made the process unfeasible. Upon enhancement of the flow rate, the yield decreased almost linearly (Figure 14a). For example, at 0.5 mL min^{-1}, a yield of only 50% could be achieved. As a compromise between throughput and process time, 0.1 mL min^{-1} was set as the optimal flow rate, which involved a residence time on the catalyst bed as low as 7 min, and a process time of 50 min to pump the 5 mL aliquot of the reaction mixture through the system. Particularly

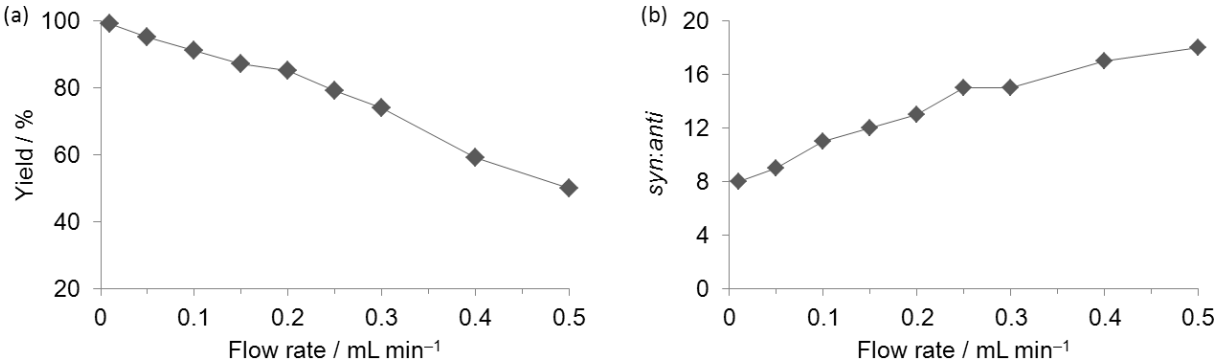
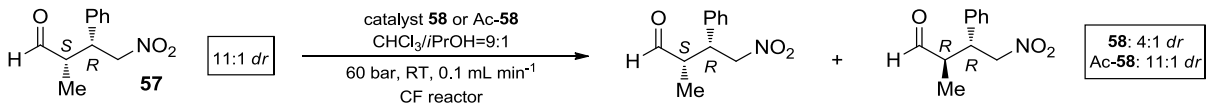


Figure 14. *Optimization of the flow rate in the test CF reaction (Scheme 18). Conditions: 1 equivalent of BNS (c=8 mg mL^{-1}) and 5 equivalents of propanal in $CHCl_3$/iPrOH=9:1, 60 bar, RT, catalyst* ***58****. The enantioselectivity was not dependent on the flow rate: ee was found to be 92–93% in all cases.*

important is that the adjustment of the flow rate influenced not only the productivity, but also the diastereoselectivity. It was found that, the higher the flow rate, the better the *dr* (Figure 14b). For example, at 0.1 mL min^{-1}, **57** could be obtained with a *syn*:*anti* ratio of 11:1, but at 0.01 mL min^{-1} *dr* dropped to 8:1. We hypothesized that the N-terminal secondary amine residue of the peptidic catalyst may catalyze enolization of the γ-nitroaldehyde, and thus the catalyst itself is responsible for the configurational instability of the product.[205] To understand the observed decrease in diastereoselectivity, the isolated γ-nitroaldehyde **57** (8 mg mL^{-1} solution in $CHCl_3$/*i*PrOH=9:1) was recirculated through the bed of catalyst **58** under the following conditions: flow rate of 0.1 mL min^{-1}, 60 bar, RT. It was found that the *syn*:*anti* ratio decreased from 11:1 to 4:1 (Scheme 19). However, when the recirculation of **57** was carried out after the acetylation of the secondary amine of the D-Pro residue of catalyst **58**, no epimerization was detected. This verifies that the decrease in *dr* is induced by the catalyst and consequently, the shorter the residence time, the higher the diastereoselectivity. The enantioselectivity was not dependent on the flow rate; *ee* was 92–93% in all cases.



Scheme 19. *The N-terminal secondary amine of the peptidic catalyst epimerizes the γ-nitroaldehyde product.*

The pressure dependence of the CF reaction was then investigated at RT and a flow rate of 0.1 mL min^{-1}. It was observed that enhancement of the pressure from atmospheric to 60 bar improved the reaction rate, but further elevation proved not to be beneficial (Figure 15a). The diastereoselectivity showed a slight dependence on the pressure. For example, at 1 bar a *syn*:*anti* ratio of 9:1 was detected, but increasing the pressure to an optimal 60 bar resulted in a *dr* of 11:1 (Figure 15b). The enantioselectivity did not change upon pressurizing; *ee* was constant at around 92–93% in all cases. To improve productivity further, we tried elevating the temperature (at 60 bar and a flow rate of 0.1 mL min^{-1}). It was found that heating gave rise to higher yields (Figure 16a), but also dramatically reduced the diastereo- and enantioselectivity (Figure 16b). For example, at 90 °C a yield of 99% was achieved, but the *syn*:*anti* ratio was only 1.5:1 and *ee* was only 77%. Consequently, RT was regarded as optimal temperature.

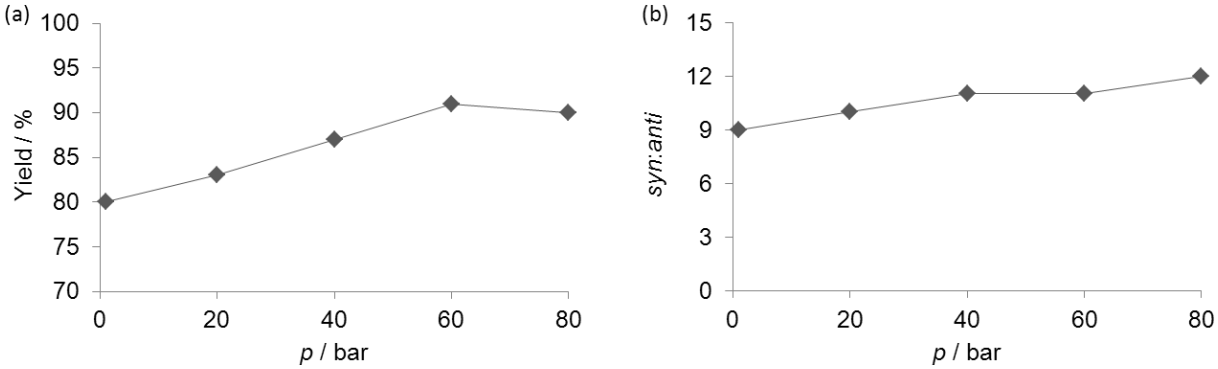


Figure 15. *Pressure dependence of the test CF reaction (Scheme 18). Conditions: 1 equivalent of BNS (c=8 mg mL^{-1}) and 5 equivalents of propanal in $CHCl_3$/iPrOH=9:1, RT, flow rate 0.1 mL min^{-1}, catalyst* ***58****. The enantioselectivity was not dependent on the pressure: ee was found to be 92–93% in all cases.*

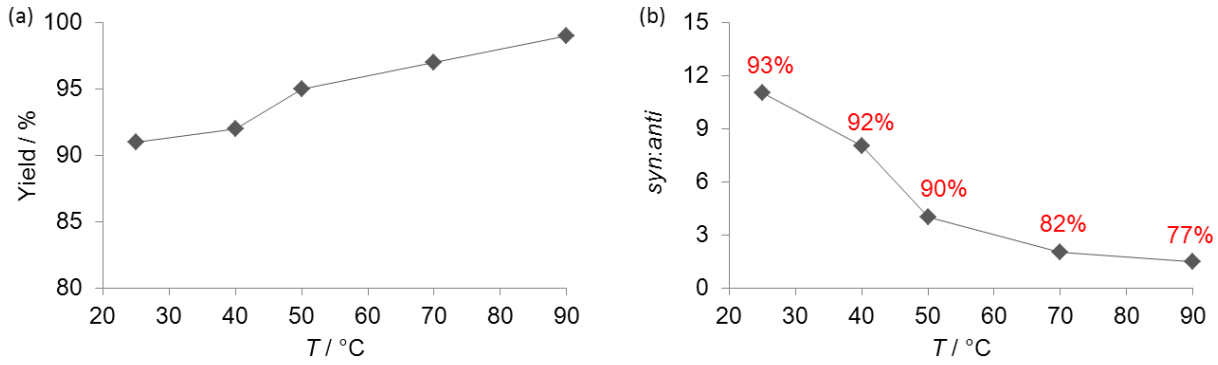


Figure 16. *Temperature dependence of the test CF reaction (Scheme 18). Conditions: 1 equivalent of BNS (c=8 mg mL^{-1}) and 5 equivalents of propanal in $CHCl_3$/iPrOH=9:1, 60 bar, flow rate 0.1 mL min^{-1}, catalyst* ***58****. On the right side, ee values are presented in red.*

The supported peptidic catalyst was obtained in a TFA salt form after the SPPS, and the effect of a basic additive was next examined to promote the liberation of the N-terminal secondary amine and maybe facilitate catalysis.[129] On the basis of literature data, *N*-methylmorpholine (NMM) was chosen as base,[131] and the effects of different amounts of NMM were then investigated under the previously optimized CF reaction conditions. It emerged that

the diastereoselectivity decreased dramatically upon addition of the base, whereas the yield and enantioselectivity remained intact (Table 9). These results suggested that NMM causes epimerization of the γ-nitroaldehyde product, and thus it proved best to avoid basic additives and leave the peptide in the TFA salt form.

Table 9. Investigation of the effect of NMM as an additive on the 1,4-addition of propanal to BNS (Scheme 18) under the previously optimized CF reaction parameters.[a]

Entry	NMM (equivalent)	Yield (%)[b]	*syn*:*anti*[c]	ee_{syn} (%)[d]	Entry	NMM (equivalent)	Yield (%)[b]	*syn*:*anti*[c]	ee_{syn} (%)[d]
1	0	91	11:1	93	3	0.02	90	7:1	91
2	0.01	92	9:1	92	4	0.04	91	6:1	92

[a]Conditions: 1 equivalent (c=8 mg mL^{-1}) and 5 equivalents of propanal in $CHCl_3$/*i*PrOH=9:1, 60 bar, RT, flow rate 0.1 mL min^{-1}, catalyst **58**. [b]Yield of isolated product. [c]Determined by ^{1}H NMR analysis of the crude material. [d]Determined by chiral-phase HPLC analysis.

As a final step of the optimization study, the peptidic catalyst was also fine-tuned. First, the effect of the exchange of the C-terminal amino acid from aspartic acid to the homologous glutamic acid (bearing an additional methylene group on the acidic side-chain) was examined.[132] H-D-Pro-Pro-Glu-NH-*resin* was synthetized on PS-MBHA with a loading of 0.64 mmol g^{-1} (**59**). In the 1,4-addition of propanal to BNS under the previously optimized reaction conditions, catalyst **59** furnished comparable diastereo- and enantioselectivities to **58**, but lower yield (Table 10, entries 1 and 2). Next, the influence of the resin loading was investigated. Thus, H-D-Pro-Pro-Asp-NH-*resin* and H-D-Pro-Pro-Glu-NH-*resin* were immobilized on PS-MBHA with a loading of 0.32 mmol g^{-1} (catalysts **60** and **61**). The lower loadings resulted in lower yields, but *ee* and *dr* were not affected (Table 10, entries 3 and 4). TentaGel resin with a loading of 0.29 mmol g^{-1} was also tested as solid support for the former peptide sequences (catalysts **62** and **63**). Despite the poorer swelling properties of TentaGel in $CHCl_3$,[164] the results were comparable with the cases when catalysts **60** and **61** were employed (Table 10, entries 5 and 6). We next explored the effects of the peptide conformation-directing role of the bioinspired substitution of the central proline unit with β-amino acids.[206] Thus, novel tripeptide catalysts containing various ACPC isomers were synthetized on PS-MBHA resin (with a loading of 0.64 mmol g^{-1}). Only the application of (1*R*,2*R*)-ACPC (**64**) gave comparable results with the catalysts containing the central proline unit (Table 10, entry 7). Insertion of (1*S*,2*S*)- (**65**), (1*S*,2*R*)- (**66**) or (1*R*,2*S*)-ACPC(=cispentacin) (**67**) into the sequence gave notably lower yields, but in some cases, higher *dr* (Table 10, entries 8 and 10). These results suggest that exchange of the central proline residue distorts the peptide conformation significantly in the cases of catalysts **65**, **66** and **67**. In the case of (1*R*,2*R*)-ACPC (catalyst **64**), however, the peptide conformation remains similar to that of the parent catalyst, which allows efficient catalysis. The structures of the utilized catalysts are shown in Figure 17.

Table 10. Fine-tuning of the immobilized peptidic catalyst in the 1,4-addition of propanal to BNS (Scheme 18) under the previously optimized reaction conditions in CF.[a]

Entry	Peptide sequence	Resin	Loading (mmol g^{-1})[b]	#	Yield (%)[c]	*syn*:*anti*[c]	ee_{syn} (%)[d]
1	H-D-Pro-Pro-Asp-NH-*resin*	PS-MBHA	0.64	**58**	91	11:1	93
2	H-D-Pro-Pro-Glu-NH-*resin*	PS-MBHA	0.64	**59**	79	12:1	91
3	H-D-Pro-Pro-Asp-NH-*resin*	PS-MBHA	0.32	**60**	78	11:1	92
4	H-D-Pro-Pro-Glu-NH-*resin*	PS-MBHA	0.32	**61**	69	11:1	91
5	H-D-Pro-Pro-Asp-NH-*resin*	TentaGel	0.29	**62**	81	11:1	92
6	H-D-Pro-Pro-Glu-NH-*resin*	TentaGel	0.29	**63**	73	12:1	91
7	H-D-Pro-(1*R*,2*R*)-ACPC-Asp-NH-*resin*	PS-MBHA	0.64	**64**	83	10:1	90
8	H-D-Pro-(1*S*,2*S*)-ACPC-Asp-NH-*resin*	PS-MBHA	0.64	**65**	41	15:1	93
9	H-D-Pro-(1*S*,2*R*)-ACPC-Asp-NH-*resin*	PS-MBHA	0.64	**66**	70	9:1	70
10	H-D-Pro-(1*R*,2*S*)-ACPC-Asp-NH-*resin*	PS-MBHA	0.64	**67**	35	19:1	89

[a]Conditions: 1 equivalent (c=8 mg mL^{-1}) of BNS and 5 equivalents of propanal in $CHCl_3$/*i*PrOH=9:1, 60 bar, RT, flow rate 0.1 mL min^{-1}. [b]Loading of the resin. [c]Yield of isolated product. [c]Determined by ^1H NMR analysis of the crude material. [d]Determined by chiral-phase HPLC analysis.

Thus, the best results were given with catalyst **58**. Under the overall optimized reaction conditions (c_{BNS}=8 mg mL^{-1}, 5 equivalents of propanal, flow rate 0.1 mL min^{-1}, 60 bar, RT), γ-nitroaldehyde **57** was obtained in a yield of 91%, with a *syn*:*anti* ratio of 11:1, and an *ee* of 93%. These results compare well with those of the literature batch procedure utilizing the same catalyst in the homogeneous phase.[131] However, the batch reaction required a reaction time of 24 h, whereas the flow process proved much more rapid with a short residence time on the catalyst bed, and the ease of product isolation further enhanced sustainability of the CF method.

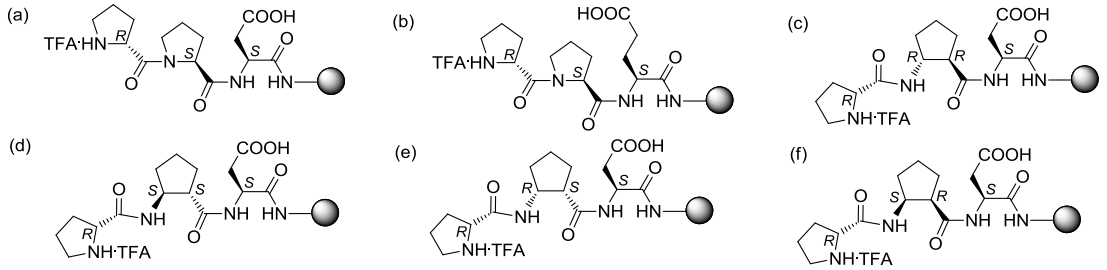


Figure 17. *Structures of the immobilized peptidic catalysts. H-D-Pro-Pro-Asp-NH-resin (a), H-D-Pro-Pro-Glu-NH-resin (b), H-D-Pro-(1R,2R)-ACPC-Asp-NH-resin (c), H-D-Pro-(1S,2S)-ACPC-Asp-NH-resin (d), H-D-Pro-(1S,2R)-ACPC-Asp-NH-resin (e), H-D-Pro-(1R,2S)-ACPC-Asp-NH-resin (f).*

4.3.1.2. Testing of the catalyst reusability

Catalyst reusability was investigated by repetition of the test reaction between BNS and propanal under the previously optimized reaction conditions, the same portion of catalyst **58** being recycled. In each run, a 5 mL aliquot of the reaction mixture was pumped through the system in 50 min. It was found that the yield decreased slowly through the consecutive reaction cycles, but *ee* remained constant around at 93% (Table 11). Interestingly, a slight increase in the

diastereoselectivity could be observed. The decrease in the yield and the enhancement of *dr* may equally be explained in terms of some kind of irreversible blocking of the N-terminal secondary amine of the catalyst. The turnover number (TON) of the immobilized catalyst in the CF process was 46, which could be calculated from the data in Table 11.

Table 11. *Investigation of the reusability of catalyst **58** in the 1,4-addition of propanal to BNS (Scheme 18) under the previously optimized CF reaction conditions.*[a, b]

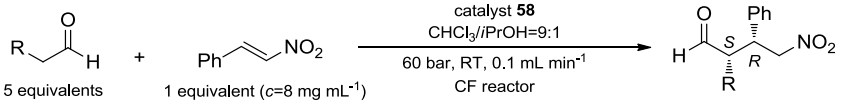
Cycle	Yield (%)[c]	*syn*:*anti*[d]	ee_{syn} (%)[e]	Cycle	Yield (%)[c]	*syn*:*anti*[d]	ee_{syn} (%)[e]
1.	91	11:1	93	6.	76	15:1	93
2.	86	12:1	93	7.	74	15:1	93
3.	84	12:1	94	8.	72	16:1	93
4.	83	12:1	93	9.	67	15:1	94
5.	82	13:1	93	10.	66	15:1	94

[a]In each run, 5 mL of the reaction mixture was pumped through the system in 50 min. [b]Conditions: 1 equivalent (*c*=8 mg mL^{-1}) of BNS and 5 equivalents of propanal in $CHCl_3$/*i*PrOH=9:1, 60 bar, RT, flow rate 0.1 mL min^{-1}.[c]Yield of isolated product. [d]Determined by ^1H NMR analysis of the crude material. [e]Determined by chiral-phase HPLC analysis.

4.3.1.3. Investigation of the scope and applicability of the CF method

A number of conjugate additions between various aldehydes and BNS were studied under the previously optimized reaction conditions with catalyst **58** to explore the applicability of the CF process. As can be seen in Table 12 (entries 1-5), excellent results were obtained with linear aldehydes, affording the corresponding γ-nitroaldehyde products in good yields (60–91%), high diastereoselectivites (up to a *dr* of 36:1) and excellent enantioselectivities (up to an *ee* of 93%). A branched aldehyde bearing a substituent in the β-position was also tested as Michael donor, but a significantly lower yield resulted (entry 6). In the case of α-branched aldehydes, no conversion was observed due to steric hindrance of the formyl group.

Table 12. *Investigation of the scope and applicability of the optimized CF organocatalytic procedure*.[a]



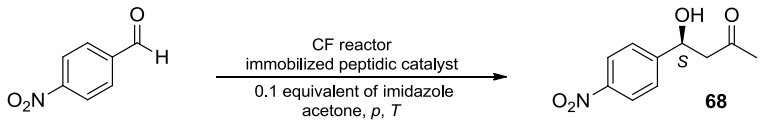
Entry	R	Yield (%)[b]	*syn*:*anti*[c]	ee_{syn} (%)[d]
1	Me	91	11:1	93
2	Et	60	22:1	91
3	*n*Pr	68	15:1	91
4	*n*Bu	87	14:1	91
5	Bn	65	36:1	92
6	*i*Pr	22	20:1	91

[a]A 5 mL aliquot of the reaction mixture was pumped through in 50 min. [b]Yield of isolated product. [c]Determined by ^1H NMR analysis of the crude material. [d]Determined by chiral-phase HPLC analysis.

4.3.2. CF organocatalytic aldol reactions

4.3.2.1. CF method development

To extend the scope of the CF organocatalytic procedure to asymmetric aldol reactions, reoptimization of the reaction conditions was necessary. The aldol addition between *p*-nitrobenzaldehyde (*p*NBA) and acetone resulting in β-hydroxyketone **68** was set out as a test reaction (Scheme 20). Acetone was employed not only as reactant, but also as solvent. 0.1 equivalent of imidazole was used as a base to prevent the formation of any elimination side-product. According to the literature, the most effective peptidic catalyst for aldol reactions is H-Pro-Pro-Asp-NH_2.[130] We therefore chose this catalyst sequence for an initial optimization study. H-Pro-Pro-Asp-NH-*resin* was synthetized on TentaGel (with a loading of 0.27 mmol g^{-1}, catalyst **69**), as it swells better in polar solvents than PS-MBHA.[164] For the CF experiments, 300 mg of the solid-supported peptide was filled into a catalyst cartridge with internal dimensions of 70×4 mm. In each run, 5 mL of the reaction mixture was pumped through the system in 50 min.



Scheme 20. *Organocatalytic asymmetric aldol reaction between pNBA and acetone as a CF test reaction.*

The aldehyde concentration was rapidly fine-tuned at a flow rate of 0.5 mL min^{-1}, RT and atmospheric pressure. It was found that the lower the aldehyde concentration, the higher the conversion (Table 13), and 4 mg mL^{-1} was assigned as the optimal value. Under these conditions, a conversion of 71% could be achieved, but it was observed that increasing the pressure improves the reaction rates further. Similarly to the earlier results with CF conjugate additions, it was found that pressurizing improved conversions up to an optimal 60 bar, but further elevation to 100 bar was not beneficial, as the conversion remained steady at around 80% (Figure 18). It was noted that *ee* was not dependent on pressurizing: it was 79–81% in all cases. On investigation of the temperature dependence (at a flow rate of 0.5 mL min^{-1} and 60 bar), it emerged that heating enhanced the reaction rates, but also dramatically decreased the

Table 13. *Fine-tuning of the pNBA concentration in the test reaction (Scheme 20) in CF.*[a]

Entry	$c_{p\text{NBA}}$ (mg mL^{-1})	Conversion (%)[b]	Entry	$c_{p\text{NBA}}$ (mg mL^{-1})	Conversion (%)[b]
1	0.25	83	5	4	71
2	0.5	81	6	8	67
3	1	77	7	16	61
4	2	73			

[a]Conditions: 1 equivalent of *p*NBA and 0.1 equivalent of imidazole in acetone, 1 bar, RT, flow rate 0.5 mL min^{-1}, catalyst **69**. [b]Determined by ^{1}H NMR spectroscopic analysis of the crude material.

enantioselectivity. For example, at 80 °C, nearly quantitative conversion was achieved, but *ee* dropped to 50% (Figure 19). Thus, RT was assigned as optimal temperature. To improve conversion at RT and 60 bar, the residence time on the catalyst bed was extended. Thus, the flow rate was decreased systematically from 0.5 mL min^{-1}. 0.1 mL min^{-1} was found optimal, resulting in quantitative conversion within a residence time on the catalyst bed as low as 6 min (Figure 20). It is worth mentioning that, even at a flow rate of 1 mL min^{-1}, the conversion was still nearly 60%. The enantioselectivity did not change in the investigation of the flow rate dependence: *ee* was 79–81% in all cases.

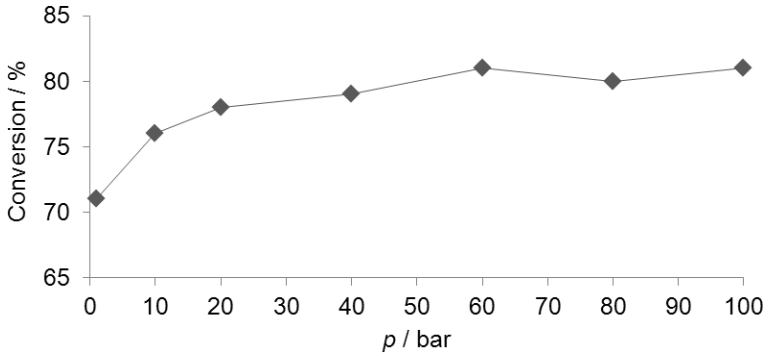


Figure 18. *Pressure dependence of the test aldol reaction (Scheme 20) in CF. Conditions: 1 equivalent of pNBA and 0.1 equivalent of imidazole in acetone, RT, flow rate 0.5 mL* min^{-1}*, catalyst* ***69****. The enantioselectivity was not dependent on the pressure: ee was found to be 79–81% in all cases.*

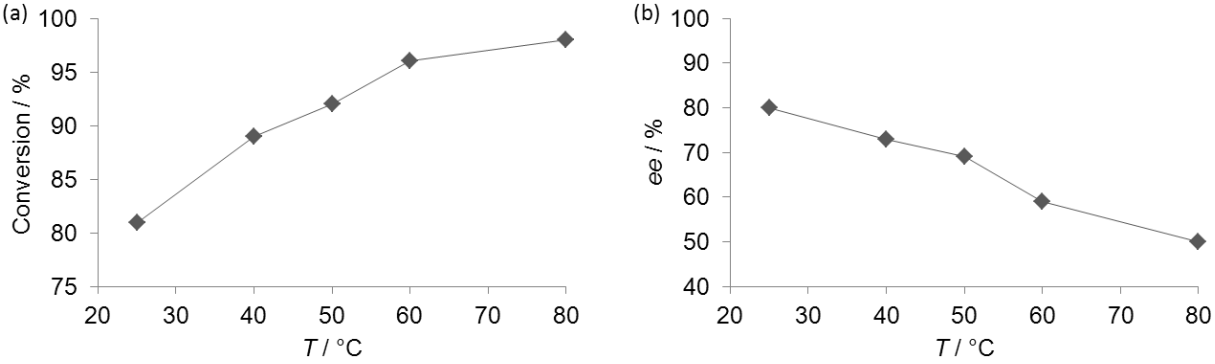


Figure 19. *Temperature dependence of the test aldol reaction (Scheme 20) in CF. Conditions: 1 equivalent of pNBA and 0.1 equivalent of imidazole in acetone, 60 bar, flow rate 0.5 mL* min^{-1}*, catalyst* ***69****.*

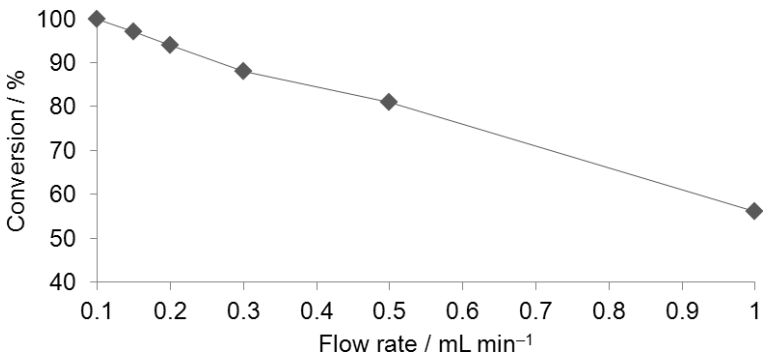


Figure 20. *Fine-tuning of the flow rate in the test aldol reaction (Scheme 20) in CF. Conditions: 1 equivalent of pNBA and 0.1 equivalent of imidazole in acetone, 60 bar, RT, catalyst* ***69****. The enantioselectivity was not dependent on the flow rate: ee was found to be 79–81% in all cases.*

As a solid support for the H-Pro-Pro-Asp-NH-*resin* peptide sequence, PS-MBHA with a loading of 0.64 mmol g^{-1} (catalyst **70**) was also tested under the previously optimized reaction conditions. However, due to its poorer swelling properties in acetone, lower conversion and *ee* were obtained than with catalyst **69** (Table 14, entries 1 and 3). We were interested in the activities of the conjugate addition catalysts **62** and **58** in aldol reactions too. It was found that neither of the D-proline N-terminal catalysts gave comparable results to **69** or **70**. The conversion and *ee* were dramatically decreased, and it must be noted that the absolute configuration of the β-hydroxyketone product **68** inverted to *R* (Table 14, entries 4 and 5).

Table 14. *Fine-tuning of the immobilized peptidic catalyst in the aldol reaction between pNBA and acetone (Scheme 20) under the previously optimized reaction conditions in CF.*[a]

Entry	Peptide sequence	Resin	Loading (mmol g^{-1})[b]	#	Conv. (%)[c]	Yield (%)[d]	*ee* (%)[e]
1	H-Pro-Pro-Asp-NH-*resin*	TentaGel	0.27	**69**	quant.	>99	80
2[f]	H-Pro-Pro-Asp-NH-*resin*	TentaGel	0.27	**69**	90	89	85
3	H-Pro-Pro-Asp-NH-*resin*	PS-MBHA	0.64	**70**	71	68	62
4	H-D-Pro-Pro-Asp-NH-*resin*	TentaGel	0.27	**62**	29	27	–26[g]
5	H-D-Pro-Pro-Asp-NH-*resin*	PS-MBHA	0.64	**58**	19	16	–18[g]

[a]Conditions: 1 equivalent of *p*NBA and 0.1 equivalent of imidazole in acetone, 60 bar, RT, flow rate 0.1 mL min^{-1} [b]Loading of the resin. [c]Determined by ^{1}H NMR analysis of the crude material. [d]Yield of isolated product. [e]Determined by chiral-phase HPLC analysis. [f]Carried out at 4 °C. [g]The absolute configuration of the β-hydroxyketone inverted to *R*.

Under the overall optimized reaction conditions (c_{pNBA}=4 mg mL^{-1}, flow rate 0.1 mL min^{-1}, 60 bar, RT) with catalyst **69**, the test aldol reaction afforded β-hydroxyketone **68** in a yield of >99% with an *ee* of 80% (Table 14, entry 1). No work-up or purification step was necessary: **68** could be isolated in a pure form after evaporation. When the reaction was carried out at 4 °C instead of RT, *ee* increased to 85%, whereas the yield remained at around 90% (Table 14, entry 2). It should be noted that, under the optimal reaction conditions, no self-aldol or H_2O-eliminated side-product was detected. These results are nicely comparable with those of the literature batch procedure. However, when the same solid-supported catalyst was employed for the same aldol reaction in a simple flask, a reaction time of 6 h was needed for completion,[207] whereas the flow process relies on short residence times on the catalyst bed, and thus, prominently short process times.

4.3.2.2. Testing of the catalyst reusability

Reusability of catalyst **69** was evaluated through the repetition of the aldol reaction between *p*NBA and acetone under the previously optimized reaction conditions. In each cycle, a 5 mL aliquot of the reaction mixture was pumped through the reactor in 50 min, and the same portion of catalyst was reused for the whole study. No decrease in activity or selectivity was observed

after 20 consecutive runs. The conversion was still quantitative and *ee* was at around 80%, just as in the first run (Table 15). This mean that, after nearly 17 h of persistent use in flow, the immobilized catalyst was still as active as initially. The described CF methodology is therefore extremely robust. To determine the TON of the immobilized catalyst for the flow procedure, the experiment was run further after the 20th cycle. The solution of the starting materials was continuously pumped through the catalyst bed, but after 80 h of continuous use the immobilized catalyst was still not totally deactivated. TON was calculated to be 710, which is an extraordinarily high value.

Table 15. Testing of the reusability of catalyst **69** in the aldol reaction between *p*NBA and acetone (Scheme 20) under the previously optimized CF reaction conditions.[a, b]

Cycle	Conv. (%)[c]	Yield (%)[d]	*ee* (%)[e]	Cycle	Conv. (%)[c]	Yield (%)[d]	*ee* (%)[e]
1–5	98–quant.	97–>99	79–80	11–15	quant.	>99	79–80
6–10	quant.	97–>99	78–81	16–20	99–quant.	98–>99	79–80

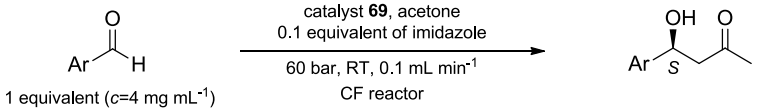
[a]In each run, 5 mL of the reaction mixture was pumped through the system in 50 min. [b]Conditions: 1 equivalent of *p*NBA and 0.1 equivalent of imidazole in acetone, 60 bar, RT, flow rate 0.1 mL min^{-1}. [c]Determined by ^{1}H NMR analysis of the crude material. [d]Yield of isolated product. [e]Determined by chiral-phase HPLC analysis.

Due to the extremely reusable peptidic catalyst, the straightforward technique of catalyst synthesis/immobilization, the ease of product isolation and the short process times, the CF approach emerged as highly sustainable methodology.

4.3.2.3. Investigation of the scope and applicability of the CF method

To extend the applicability of the described procedure, a wide array of aromatic aldehydes were tested in aldol reactions with acetone under the previously optimized conditions with catalyst **69**. As can be seen in Table 16, aldehydes with an electron-withdrawing group on the aromatic ring performed as excellent reaction partners. The corresponding β-hydroxyketone products were obtained in excellent yields (68–>99%) and high enantioselectivities (up to an *ee* of 80%, entries 1-8). It was observed that the reactivity increased with the electron-withdrawing capability of the substituting residue and it was also dependent on the position of the electron-withdrawing group. In the event of quantitative conversion, no work-up or purification was necessary after evaporation. Lower yields (27–59%), but still good enantioselectivities (up to an *ee* of 76%), were obtained with aldehydes bearing an electron-donating substituent on the aromatic ring or no substituent at all (entries 9-12). Productivities were determined as a benchmark of the flow reactions, and the results compared well with CF references from the literature.[146, 147] Self-aldol product formation was not observed in the above experiments; but, in some cases, H_2O elimination occurred from the corresponding β-hydroxyketone.

Table 16. *Investigation of the scope and applicability of the optimized CF organocatalytic procedure.*[a]



Entry	Ar	Productivity[b]	Conv. (%)[c]	Yield (%)[d]	*ee* (%)[e]
1	*p*-$NO_2C_6H_4$	1.96	quant.	>99	80
2	*o*-$NO_2C_6H_4$	1.96	quant.	>99	79
3	*p*-NCC_6H_4	2.26	quant.	>99	74
4	*o*-ClC_6H_4	2.11	quant.	>99	79
5	*m*-ClC_6H_4	1.50	78	71 (5)[f]	76
6	*p*-ClC_6H_4	1.48	80	70 (7)[f]	78
7	*p*-BrC_6H_4	1.09	76	68 (6)[f]	80
8	*p*-FC_6H_4	2.05	quant.	86 (13)[f]	79
9	*o*-$MeOC_6H_4$	0.81	39	37	76
10	C_6H_5	1.65	76	59 (13)[f]	70
11	2-naphthyl	0.57	37	30 (5)[f]	75
12	1-naphthyl	0.51	35	27 (4)[f]	71

[a]A 5 mL aliquot of the reaction mixture was pumped through the reactor in 50 min. [b]In mmol of pure isolated product ($mmol_{resin}^{-1}$ h^{-1}). [c]Determined by ^1H NMR analysis of the crude material. [d]Yield of isolated product. [e]Determined by chiral-phase HPLC analysis. [f] Yield of the corresponding dehydration product.

4.3.3. Exploring the role of pressure in the CF reactions

It was observed through the CF method development that pressurizing improved the rates of both organocatalytic reactions (Figures 15 and 18). The question can arise whether the conversion is dependent on the catalyst activity itself (kinetic regime) or is limited by the transport phenomena of the reactants in the matrix of the catalyst carrier (diffusion regime). In the case of the kinetic regime, the rate of the catalytic reaction is proportional to the catalyst loading in the packed bed. To probe the diffusion dependence, the Koros–Nowak test was performed.[208, 209] Thus, the loading in the catalyst bed was halved, and the test reactions (Schemes 18 and 20) were repeated. It was found for both CF organocatalytic systems that the decrease in conversion (and in yield) was not proportional to the loading decrease. For 1,4-addition, see Table 10, entries 1 vs. 3. For aldol reaction, the diagnostic test was performed under the following conditions: 60 bar, RT, flow rate of 0.5 mL min^{-1} with catalyst **69**, and the conversion decreased from 81 to 52%. This means that, under the present conditions, the CF reactions are diffusion-controlled, and the role of pressure is to aid the diffusion of the reactants into the swollen polymer matrix. It can be concluded that pressurizing is definitely beneficial when swellable resins are to be used as heterogeneous catalyst support.

5. SUMMARY

Novel, sustainable heterogeneous catalytic methodologies were developed for the CF synthesis of pharmaceutically relevant intermediates and potentially bioactive compounds.

For the CF deuteration of nitrogen-containing heterocycles, on-demand electrolytic D_2 production was applied, which afforded much higher deuterium efficiency and improved safety as compared with the conventional batch techniques.

EtOAc was chosen as aprotic solvent to prevent D–H exchange and to maximize deuterium incorporation. 5% $Pd/BaSO_4$ proved insufficiently active even at high temperatures, and 5% Pt/Al_2O_3 was therefore chosen as optimal heterogeneous catalyst. It was observed that the utilization of pressures as high as 50 bar was necessary to obtain high reaction rates.

Besides heterocyclic model compounds and substituted derivatives, a spasmolytic drug, drotaverine, was also deuterated. The deuterium-labeled products were obtained in excellent conversions (96–99%) and deuterium contents (95–98%). It was found that deuterium incorporation was highly selective, as the benzene ring remained intact in all cases.

The described CF deuteration procedure lacks most of the drawbacks of the classical batch techniques, while it is convenient, time- and cost-efficient and safe.

A simple, inexpensive and rapid CF technique was developed for 1,3-dipolar cycloaddition reactions between organic azides and acetylenes, which eliminated the need for costly special apparatus and applied Cu powder as a readily available Cu(I) source.

Initially, the merits of flow processing were exploited by increasing the reaction rates with high-pressure/high-temperature conditions. Subsequently, it was demonstrated that the harsh reaction conditions can be relieved through the joint use of DIEA and AcOH as basic and acidic additives, the CF CuAAC thereby being accomplished with maximum operational safety at RT.

The applicability of the CF methodology was found extremely wide, for different azides and alkynes. Excellent yields (72–99%) were obtained with either aliphatic or aromatic starting materials. In some of the cases, the joint use of basic and acidic additives (each in 0.04 equivalents) at RT resulted in higher yields than at 100 bar and 100 °C.

The scope of the CF CuAAC process was extended to the preparation of 1,2,3-triazole-substituted alicyclic β-amino acid derivatives, as novel potentially bioactive compounds. Phenylacetylene, diethyl acetylenedicarboxylate and ethynyl ferrocene were reacted as dipolarophiles. Yields were as high as 99% in some of the cases, and the copper impurities detected in our systems compared well with CF or batch results from the literature. No epimerization of the triazole products were observed even upon applying basic conditions.

Large-scale synthesis was implemented simply and safely as a function of process time. 3 g of triazole **16** was isolated in 3 h, and 2.06 g of triazole **52** was prepared in 100 min.

In all reactions with terminal alkynes, the 1,4-disubstituted regioisomers were formed selectively due to the efficient Cu(I) catalysis.

We introduced the first CF organocatalytic methodology for asymmetric 1,4-addition of aldehydes to nitroolefins, and extended the scope of the sustainable procedure to aldol reactions.

Solid supported peptidic catalysts containing a proline unit at the N-terminus and an acidic side-chain at the C-terminus were applied. The catalysts were readily synthetized and immobilized in the same step by SPPS without the cleavage of the peptide from the resin.

For both organocatalytic transformations, appropriate test reactions were chosen and the most important reaction conditions were thoroughly fine-tuned. In the case of conjugate additions, it was observed that the diastereoselectivity decreased continuously when the flow rate was reduced. It was verified that the catalyst itself epimerized the product.

Pressurizing improved reaction rates in both transformations up to an optimal value of 60 bar. It was established that the CF reactions are diffusion-controlled and the role of pressure is to promote the transport of the reactants into the matrix of the catalyst carrier.

It was found that heating also enhanced the reaction rates, but it proved best to employ RT, as higher temperatures dramatically lowered stereoselectivities.

The effect of a basic additive was examined to improve the catalytic activity in cases of 1,4-additions. However, the best result was obtained without the base, as it epimerized the product.

It emerged that for 1,4-additions the catalyst H-D-Pro-Pro-Asp-NH-*resin* on PS-MBHA was the most favorable, and exchange of the central proline unit gave satisfactory results only if the substituting amino acid did not generate significant distortion in the conformation of the peptide. For aldol reactions, H-Pro-Pro-Asp-NH-*resin* on TentaGel performed best.

In aldol reactions, the reusability of the peptidic catalyst was extremely high, as it did not lose any of its activity or selectivity during 17 h of continuous use in the flow reactor. In cases of conjugate additions, the heterogenous catalyst was reusable, but its activity decreased slowly in the course of the consecutive reaction cycles.

With the CF method, chiral γ-nitroaldehydes were obtained in yields up to 91% with high diastereoselectivites (up to a *dr* of 36:1) and excellent enantioselectivites (up to an *ee* of 93%). β-Hydroxyketones, as aldol adducts, were furnished with excellent yields (up to 91%) and high enantioselectivities (up to an *ee* of 85%). The CF results were nicely comparable with those of the literature batch procedures. The batch reactions required hours of reaction time, whereas the CF procedures were rather rapid, with residence times on the catalyst bed as short as 7 or 6 min.

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Appendix

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FULL-LENGTH PAPER

Highly selective deuteration of pharmaceutically relevant nitrogen-containing heterocycles: a flow chemistry approach

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Abstract A simple and efficient flow-based technique is reported for the catalytic deuteration of several model nitrogen-containing heterocyclic compounds which are important building blocks of pharmacologically active materials. A continuous flow reactor was used in combination with on-demand pressure-controlled electrolytic D_2 production. The D_2 source was D_2O, the consumption of which was very low. The experimental set-up allows the fine-tuning of pressure, temperature, and flow rate so as to determine the optimal conditions for the deuteration reactions. The described procedure lacks most of the drawbacks of the conventional batch deuteration techniques, and additionally is highly selective and reproducible.

Keywords Deuteration · Flow chemistry · Heterogeneous catalysis · Nitrogen-containing heterocycles · Reduction

Introduction

The replacement of one or more atom by one of its isotopes has proven to be unique in its efficacy [1]. In organic chemistry, deuteration has frequently been used, especially in tracer studies, to follow reaction paths [2], and in kinetic studies to determine the effects of the isotope on reaction rates [3]. Furthermore, the stability of this nuclide and its nuclear properties have contributed to the development of its use in structural analysis. For instance, deuterated compounds are widely used in NMR spectroscopy to facilitate signal assignment and structure determination [4, 5], and they are general internal standards in mass spectrometry (MS) [6].

Conventional batch techniques for the synthesis of deuterated compounds utilize D_2 gas as deuterium source [7]. The general methods of producing D_2 gas suffer from several drawbacks on a laboratory scale, such as difficulties in gas handling, high costs, etc. Other methods have been employed to overcome these difficulties, such as catalytic H–D exchange reactions. However, these methods are far from being perfect. They do not produce adequately pure D_2, and they also require high pressure, the use of a special catalyst or an excess amount of a strong base or acid [8, 9].

Flow chemistry approaches have recently emerged as a new productive organic synthetic methodology [10]. As pointed out below they offer many useful advantages over conventional batch techniques. For example, mixing in continuous flow (CF) is much more efficient than conventional mechanical stirring: it can be achieved rapidly, which leads to shorter reaction times [11]. Moreover, CF reactors are able to tolerate high pressure, and thus the reaction temperature can be far above the solvent's boiling point, which is another gain from the aspect of reaction time. Multistep reactions can be arranged in a continuous sequence, which is especially beneficial if intermediate compounds are unstable, since they will exist only momentarily and in very small quantities. Furthermore, reactions which involve gaseous reagents can be safely handled, whereas in batch a complicated high-pressure autoclave is necessary. Multiphase liquid reactions can be performed well with high reproducibility over a range of conditions, because CF systems allow the use of fixed-bed catalysts. In such reactors, residence times can be easily fine-tuned with the flow rate, and consequently all compounds can be sufficiently exposed to the catalyst [12].

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Among flow chemistry techniques, the best-characterized and most widely used reaction is heterogeneous hydrogenation, although flow-based deuterium labeling is currently gaining in interest as it is time- and cost-efficient and far more convenient than the conventional batch deuteration [13].

Nitrogen-containing heterocycles such as quinoline, isoquinoline, quinoxaline, and their saturated derivatives are commonly used building blocks in pharmaceutical chemistry [14]. They are of high importance among both naturally occurring and synthetic pharmacologically active compounds. Quinoline, isoquinolines, and their tetrahydro derivatives are present in the structures of many biologically interesting materials. This family of heterocycles has been used as HIV protease inhibitors [15], antimalarial drugs [16], antitumor agents [17], antidepressants [18], and drugs for the treatment of asthma [19] and they have potential as antibacterial agents [20]. Examples of pharmaceutically interesting quinoxalines include broadly active antitumor agents [21], antitrypanosomal drugs [22], an angiotensin II receptor antagonist [23] and an adenosine receptor antagonist [24]. As the above examples clearly show, substituted heterocyclic compounds can offer a high degree of structural diversity and have proven to be broadly useful therapeutic agents. Because of their potential biological effects and wide-ranging synthetic applicability, the preparation and transformations of these compounds have recently acquired considerable significance in heterocyclic chemistry [25]. Moreover, considerable interest has recently been demonstrated in this type of compounds for the synthesis of huge combinatorial libraries, which offers an opportunity for the rapid synthesis of drug-like molecules [14].

Although the demand for isotopically labeled compounds is continuously growing, and the search for rapid, convenient, catalytic procedures leading to enhanced isotope incorporation is a hot topic [26], the CF synthesis of deuterated products has rarely been applied. However, conventional batch techniques for the synthesis of highly deuterated heterocyclic compounds are quite popular, in spite of their several drawbacks. For instance, the transfer deuteration of unsaturated heteroaromatic compounds has been developed in recent years, where deuterated ammonium formate has been used as in situ deuterium source and mild catalytic conditions have been applied [27]. Additionally, several new substitutional H–D exchange methods have been reported [28, 29]. All of these techniques provide moderate yields and quite good deuterium contents and they are constantly being improved; however, most of them are highly time- and cost-demanding and require special apparatus. In this situation, flow-based techniques can be of advantage in the field of isotopically labeled heterocyclic synthesis. For this reason, we propose here a convenient flow-based route for the highly selective deuteration of certain nitrogen-containing heterocycles and some of their substituted derivatives (Fig. 1).

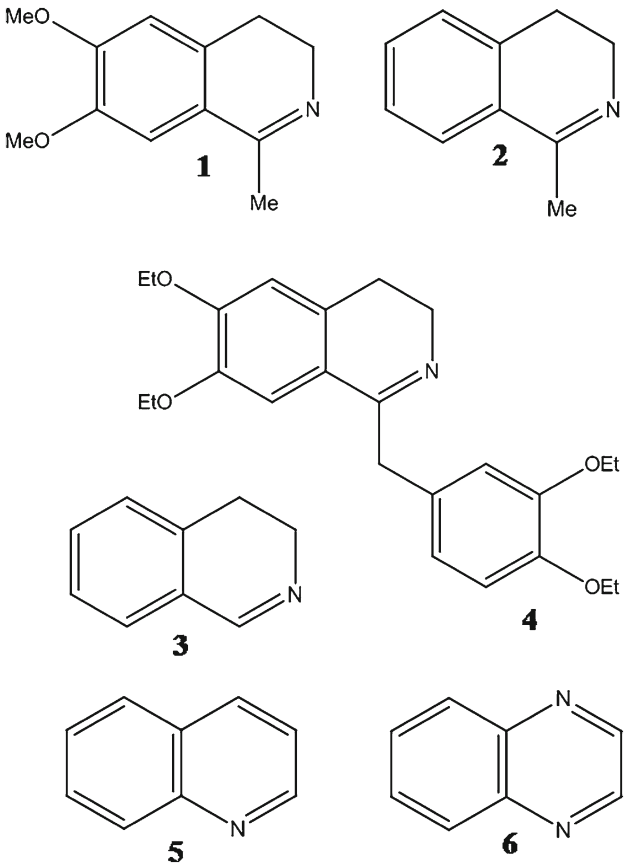


Fig. 1 Compounds to be deuterated

Experimental

CF methodology

Deuteration reactions were performed in a flow system (H-Cube®, ThalesNano) combined with a built-in electrolytic cell [12]. The D_2 gas necessary for the reactions was generated in situ by the electrolytic decomposition of D_2O, no other external gas source being needed. In contrast with the earlier methods, the purity of the D_2 gas produced can be as high as 99.9%, and the D_2O consumption is rather low. The flow of substrate is combined with D_2 gas, and the mixture is then transported to the catalyst bed, where the reaction takes place. The appropriate heterogeneous catalyst is contained in a cartridge-like stainless steel tube, as in conventional HPLC columns, which eliminates potentially dangerous direct catalyst handling and ensures excellent ease of use. The process of flowing a deuterium/substrate mixture through the catalyst bed vastly increases the interaction between the three phases (solid catalyst, liquid substrate, deuterium gas). This interaction is important due to the limited solubility of deuterium in solvent. The catalyst cartridge, with a linear dimension of 3 cm and internal diameter of

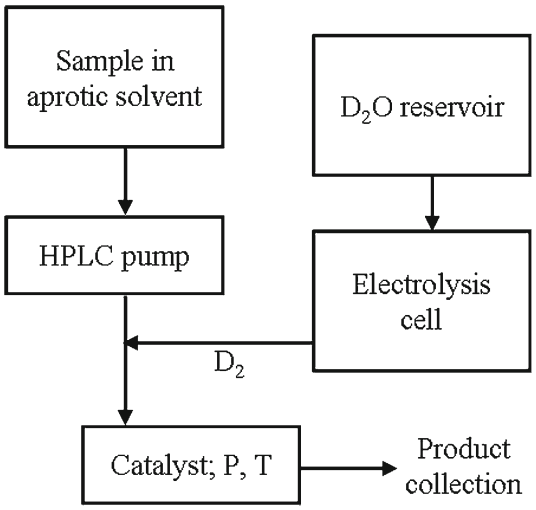


Fig. 2 Experimental scheme for CF deuteration

4 mm, encompasses approximately 150 mg of catalyst, which is reusable after thorough washing with solvent until deactivation. The comparatively small amounts of excess gas are continually exhausted by the system, eliminating the need for many of the special precautions normally required for the handling of dangerous and flammable gases. The system can be pressurized and heated. The heater unit is a Peltier heating system which consists of a stainless steel reaction line coil contained within a stainless steel block in which the catalyst holder cartridge is placed as well. The application of pressure not only helps to shift reaction equilibria, but also means that a far greater proportion of gas will be in solution during the reaction than in a conventional autoclave. So as to prevent D–H exchange and maximize deuterium incorporation, an aprotic solvent was used. Reaction conditions such as catalyst type, pressure, temperature, and flow rate were systematically adjusted in order to determine the optimal conditions for the deuteration reactions. A schematic outline of the CF deuteration reactor is presented in Fig. 2.

General procedure of the synthesis and analysis of the deuterated compounds

20 mg of substrate **1**–**6** was dissolved, respectively, in 20 mL ethyl acetate with 10 min sonication. After that, the deuteration reactions were carried out under the previously optimized conditions with the CF reactor (the optimization procedure is outlined in Table 1, for the optimal conditions see Tables 2 and 3). Before further analytical investigations TLC analyses were performed routinely, $CHCl_3$:MeOH=9:1 was used as eluent. Reagents and catalysts were purchased from Sigma-Aldrich and were used without further purification.

After evaporation of the solvent and drying of the samples, the crude deuterated products were characterized by MS, ^{1}H-NMR spectroscopy, and ^{13}C-HSQC and ^{13}C-HMBC experiments. The deuterium content (which represents the deuterium incorporation rate over incidental hydrogen addition) was determined from the relative intensity of the ^{1}H-NMR indicator signals and by MS analysis.

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker Avance DRX 400 spectrometer, in $CDCl_3$ as applied solvent, with TMS as internal standard, at 400.1 and 100.6 MHz, respectively. MS analysis was carried out with an Agilent 1100 LC/MSD trap.

Characterization data of the deuterated products

*1-Deutero-6,7-dimethoxy-1-methyl-1,2,3, 4-tetrahydroisoquinoline (**1d**)*

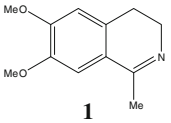
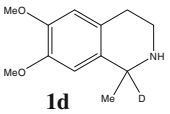
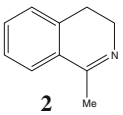
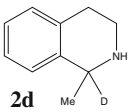
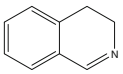
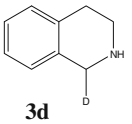
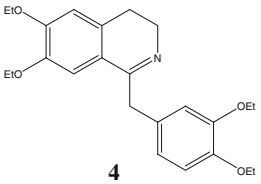
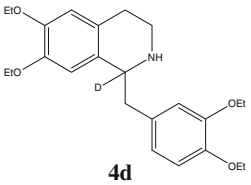
^{1}H-NMR (400.1 MHz, $CDCl_3$)δ_H: 1.47 (3H, s, CH_3), 2.63–2.72, 2.76–2.87 (1H, m, CH), 2.98–3.07, 3.24–3.32 (1H, m, CH–N), 3.88 (6H, s, $2CH_3$-O), 6.60, 6.66 (1H, s, H–Ar). ^{13}C-NMR (100.6 MHz, $CDCl_3$)δ_C: 22.6 (CH_3), 29.7 (CH_2), 42.0 (CH_2–N), 51.0 (CD–N), 56.2 ($2CH_3$–O), 109.2, 111.8 (CH–Ar),126.3, 131.8 (C–Ar), 147.3 (O–C–Ar). MS m/z: 209.3 $(M+1)^+$.

Table 1 Optimization of reaction conditions for the deuteration of compound **1** at 1 mg/mL, with a flow rate of 1 mL/min, in ethyl acetate as solvent

Entry	Catalyst	p/atm	T/°C	Runs[a]	Conv./%
1	5% $Pd/BaSO_4$	1	RT	1	0
2	5% $Pd/BaSO_4$	20	RT	1	0
3	5% $Pd/BaSO_4$	40	RT	1	50
4	5% $Pd/BaSO_4$	60	RT	1	50
5	5% $Pd/BaSO_4$	80	RT	1	50
6	5% $Pd/BaSO_4$	50	70	2	60
7	5% $Pd/BaSO_4$	50	90	3	80
8	5% Pt/Al_2O_3	50	30	1	99

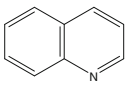
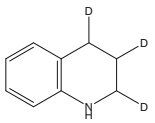
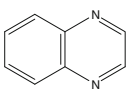
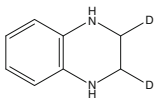
[a] Number of discrete circulations via the CF reactor

Table 2 Deuteration of selected isoquinolines containing an unsaturated hetero ring

Entry	Starting material	Product	Optimal conditions	Conv./%	D[b]/%
1	 **1**	 **1d**	5% Pt/Al_2O_3, 50 atm, 30 °C, 1 run[a]	99	97
2	 **2**	 **2d**	5% Pt/Al_2O_3, 50 atm, 30 °C, 1 run[a]	99	95
3	 **3**	 **3d**	5% Pt/Al_2O_3, 50 atm, 30 °C, 1 run[a]	97	96
4	 **4**	 **4d**	5% Pt/Al_2O_3, 50 atm, 30 °C, 1 run[a]	96	98

[a] Number of discrete circulations via the CF reactor
[b] Deuterium contents (represent the deuterium incorporation rate over incidental hydrogen addition)

Table 3 Deuteration of selected compounds containing a heteroaromatic ring

Entry	Starting material	Product	Optimal conditions	Conv./%	D[b]/%
5	 **5**	 **5d**	5% Pt/Al_2O_3, 50 atm, 70 °C, 3 runs[a]	98	97
6	 **6**	 **6d**	5% Pt/Al_2O_3, 50 atm, 50 °C, 1 run[a]	99	95

[a] Number of discrete circulations via the CF reactor
[b] Deuterium contents (represent the deuterium incorporation rate over incidental hydrogen addition)

*1-Deutero-1-methyl-1,2,3,4-tetrahydroisoquinoline (**2d**)*

^{1}H-NMR (400.1 MHz, $CDCl_3$)δ_H: 1.49 (3H, s, CH_3), 2.73–2.82, 2.86–2.96 (1H, m, CH), 3.02–3.11, 3.26–3.35 (1H, m, CH–N), 7.09–7.23 (4H, m, 4H–Ar). ^{13}C-NMR (100.6 MHz, $CDCl_3$)δ_C: 22.3 (CH_3), 29.7 (CH_2), 41.4 (CH_2–N), 50.7 (CD–N), 125.7, 128.9 (2CH–Ar),134.7, 139.6 (C–Ar). MS m/z: 149.2 $(M+1)^+$.

*1-Deutero-1,2,3,4-tetrahydroisoquinoline (**3d**)*

^{1}H-NMR (400.1 MHz, $CDCl_3$)δ_H: 2.84 (2H, t, J = 6.0 Hz, CH_2), 3.18 (2H, t, J = 6.0 Hz, CH_2–N), 4.01–4.06 (1H, m, CHD–N), 7.01–7.18 (4H, m, 4H–Ar). ^{13}C-NMR (100.6 MHz, $CDCl_3$)δ_C: 28.8 (CH_2), 43.9 (CH_2–N), 47.2 (CHD–N), 125.7, 126.1, 127.1, 129.1 (CH–Ar), 134.4 (2C–Ar). MS m/z: 135.2 $(M+1)^+$.

*1-Deutero-1-(3,4-diethoxybenzyl)-6,7-diethoxy-1,2,3,4-tetrahydroisoquinoline (**4d**)*

^{1}H-NMR (400.1 MHz, $CDCl_3$)δ_H: 1.41–1.50 (12H, m, $4CH_3$), 2.63–2.76 (2H, m, CH_2–N), 2.84–2.98, 3.11–3.26 (2H, m, CH_2), 4.03–4.15 (8H, m, $2CH_2$–O), 6.63, 6.72 (1H, s, H–Ar), 6.76–6.80 (2H, m, 2H–Ar), 6.86 (H, m, H–Ar). ^{13}C-NMR (100.6 MHz, $CDCl_3$)δ_C: 14.5 ($4CH_3$), 29.1, 40.7 (CH_2), 42.0 (CH_2–N), 56.2 (CD–N), 64.6 ($4CH_2$–O), 111.2, 113.1, 113.7, 114.7, 121.5 (CH–Ar),127.6, 129.9, 131.2 (C–Ar), 146.5, 147.0, 147.9, 148.3 (O–C–Ar). MS m/z: 401.7 $(M+1)^+$.

*2,3,4-Trideutero-1,2,3,4-tetrahydroquinoline (**5d**)*

^{1}H-NMR (400.1 MHz, $CDCl_3$)δ_H: 1.90–2.00 (1H, m, CHD), 2.74–2.82 (1H, m, CHD), 3.28–3.36 (1H, m, CHD–N), 6.50 (1H, d, $J = 7.7$ Hz, H–Ar), 6.64 (1H, t, $J = 7.7$ Hz, H–Ar), 6.94–7.03 (2H, m, 2H–Ar). ^{13}C NMR (100.6 MHz, $CDCl_3$)δ_C: 22.3, 27.1 (CHD), 42.3 (CHD–N), 114.7, 117.6, 127.0, 130.5 (CH–Ar), 121.8 (C–Ar), 144.7 (N–C–Ar). MS m/z: 137.2 $(M+1)^+$.

*2,3-Dideutero-1,2,3,4-tetrahydroquinoxaline (**6d**)*

^{1}H-NMR (400.1 MHz, $CDCl_3$)δ_H: 3.40–3.47 (2H, m, 2CHD), 6.50–6.56 (2H, m, 2H–Ar), 6.59–6.65 (2H, m, 2H–Ar). ^{13}C-NMR (100.6 MHz, $CDCl_3$)δ_C: 41.1 (2CHD–N), 115.3, 118.9 (2CH–Ar), 133.6 (2N–C–Ar). MS m/z: 137.2 $(M+1)^+$.

Results and discussion

The present starting compounds (Fig. 1) can be divided into two main groups: **1**, **2**, **3** and **4** are partially unsaturated, whereas **5** and **6** contain a fully aromatic hetero ring condensed to a benzene ring. It may be expected that the reduction of the C=N bond of the unsaturated starting materials requires milder conditions than those in the case of the fully aromatic hetero rings.

Substrate **1** was chosen as subject for optimization of the reaction conditions (Fig. 3), initially with 5% $Pd/BaSO_4$ as catalyst because activated charcoal as carrier is not suitable: it can contain various protic contaminants which can easily lead to H–D scrambling [30, 31]. In an attempt to maximize deuterium incorporation, ethyl acetate was used as aprotic solvent. At room temperature and atmospheric pressure, no conversion was achieved. At 40 atm, the conversion reached 50%, but further pressurizing was completely ineffective, as in the range 40–80 atm at room temperature the conversion remained at 50%. We next tried increasing the temperature while moderate pressure was applied, so as to keep the deuterium dissolved. When this proved to be insufficient, we tried circulating the reaction mixture repeatedly. After three circulations at 90 °C, the methyl signals of the methoxy groups in the ^{1}H-NMR spectrum indicated that the conversion was still below optimal. At this point, we changed to the more active 5% Pt catalyst on $A1_2O_3$ carrier. This led to almost complete conversion at 30 °C in point of the deuteration of the hetero ring, whereas the protons of the benzene ring remained intact, as it is indicated in the ^{1}H and ^{13}C-NMR spectra by the presence of the aromatic protons (at 6.60 and 6.66 ppm) and aromatic carbons (at the region of 109.2–147.3 ppm). This result is highly competitive as compared with the literature batch methods mentioned above [27, 28]. Throughout the whole optimization procedure, the concentration and flow rate were maintained constant at 1 mg/mL and 1 mL/min, respectively. The fine tuning of the reaction conditions is outlined in Table 1.

On the basis of the promising results obtained with **1**, the partially unsaturated **2**, **3**, and **4** were also subjected to deuteration in the CF system, with ethyl acetate as solvent and 5% $Pt/A1_2O_3$ as catalyst. Under the previously optimized reaction conditions, almost full conversion and more than 95% deuterium content could be achieved in all three cases (Table 2). Moreover, the deuterium incorporation is proved to be highly selective in each case, since the benzene ring remained unsaturated as it is indicated by the aromatic signals of the NMR spectra. Drotaverine (**4**), a spasmolytic drug, has two structural isomers specified by the *endo* and *exo* position of the non-aromatic double bond. The presence of the multiplet signal of the two protons of the bridging carbon atom in the ^{1}H NMR spectra at 3.11–3.26 ppm gave evidence of the selective deuterium incorporation at the C-1 *(endo)* position, though the N–D bond is not evident as it instantly changes to

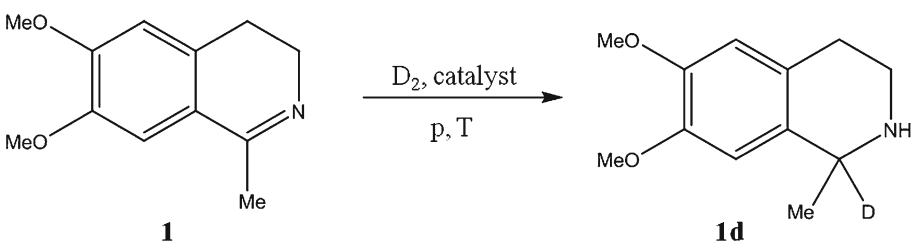


Fig. 3 Deuteration of compound **1** (the N–D bond instantly changes to N–H while being exposed to air)

N–H from H_2O vapor while being exposed to air (Table 2). Consequently, we can conclude that with CF under optimal conditions substitution did not influence the deuterium incorporation at the non-aromatic hetero rings.

As expected, the saturation of the aromatic hetero rings required more stringent conditions. It was pointless to attempt their deuteration with a Pd catalyst, so we tried the previously optimized conditions with 5% $Pt/A1_2O_3$ as catalyst. Because of the considerable electronegativity of the nitrogen atoms, the symmetry of the electron distribution and the aromatic stability of the hetero ring are lower as compared with the non-heterocyclic rings, and consequently the reactivity is higher. Quinoline (**5**) contains only one nitrogen atom, whereas quinoxaline (**6**) contains two nitrogens in the 1,4-positions. Accordingly, it is not at all surprising that selective saturation of the hetero ring of quinoline requires a higher temperature and repeated runs, whereas for quinoxaline milder conditions and a single circulation were sufficient. As may be seen in Table 3, almost full conversion and an outstanding deuterium incorporation ratio were achieved in both cases. Moreover, the reactions proved to be highly selective, as the NMR spectra clearly indicate that the aromatic rings remained intact, whereas the hetero rings became saturated as the signals of their hydrogen and carbon atoms can be found in the aliphatic region, at 1.90–3.47 ppm and 22.3–42.3 ppm, respectively. Selective deuteration of pyrazine was also attempted, but proved unsuccessful. It is probable that the aromatic ring underwent fragmentation into volatile compounds, irrespective of the catalyst type.

To summarize, we have developed a simple flow-based method for the deuterium labeling of model unsaturated heterocyclic compounds, which are precursors of a series of pharmacologically active materials. As expected, the saturation of the heteroaromatic rings required more stringent conditions than for the C=N bonds; nevertheless, we succeeded in finding optimal conditions for the highly selective deuteration of all six starting materials. Our set of model compounds contained several substituted derivatives and a drug molecule. It emerged that under the optimal conditions, substitution did not influence the deuterium incorporation and conversion appreciably; consequently, this technique appears to offer the opportunity to arrange a large number of related compounds rapidly into huge combinatorial libraries. We should emphasize that the proposed CF-based method lacks most of the drawbacks of the conventional batch deuteration techniques, while additionally it is convenient, cost- and time-efficient and safe, besides being highly selective and reproducible.

Acknowledgments We are grateful to the Hungarian Research Foundation (OTKA NK81371) and TAMOP 4.2.2-08/1-2008-0013 for financial support.

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DOI: 10.1002/cssc.201100332

Highly Efficient 1,4-Addition of Aldehydes to Nitroolefins: Organocatalysis in Continuous Flow by Solid-Supported Peptidic Catalysts

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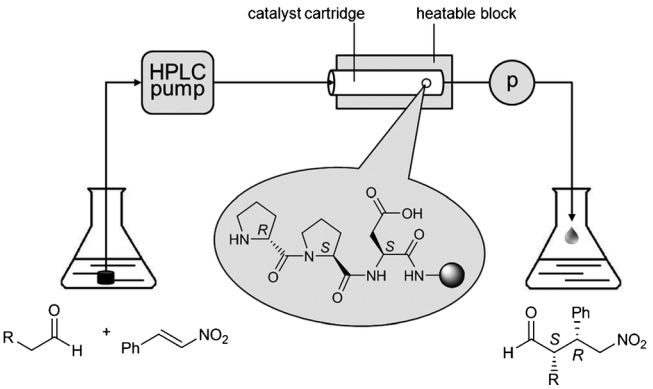
Continuous flow (CF) approaches have recently gained in significance among synthetic techniques, in consequence of the massive number of advantages they offer over conventional batch procedures, for example, the efficient mixing of substrates, faster heat and mass transfer, and shorter reaction times.[1] Modern flow technologies eliminate the need for the large-scale use of reagents and solvents, and the most important reaction parameters (such as flow rate, temperature, and pressure) can be fine-tuned and monitored quickly and precisely. This implies time- and cost-efficient reaction condition screening, facilitating the generation of large libraries of drug-like molecules and easy scale-up.[2] Heterogenizing homogeneous catalysts on a solid support is a trend towards the increase of the efficiency of synthetic techniques. By incorporating immobilized catalysts, the scope of chemical processes involving flow can be further broadened.[3]

A variety of C–C bond-forming reactions have been achieved through asymmetric organocatalysis in recent years.[4] Proline (Pro) has been described as a catalyst for reactions involving enamines, in general with moderate yield or enantioselectivity.[5] Small rigid organocatalsyts offer only a limited number of sites for structural and functional diversity; in contrast, peptides have a modular nature which allows the creation of optimized catalysts.[6] In recent years, a large number of peptidic catalysts have been designed,[7] and it has been shown that *N*-terminal prolyl-peptides efficiently catalyze reactions proceeding via enamine intermediates.[8] Wennemers et al. introduced tripeptide-containing proline and carboxylic acid moieties (proline mimetics) as organocatalysts for the 1,4-addition of aldehydes to nitroolefins,[9] leading to γ-nitroaldehydes, which are valuable intermediates for the synthesis of various compounds.[10] These peptides provide higher catalytic activity and stereoselectivity than proline itself, under mild conditions and at low catalyst loadings.[11]

We present here the first CF approach for the selective asymmetric synthesis of γ-nitroaldehydes, utilizing solid-supported peptidic catalysts, readily synthesized and immobilized in one single step. The simple and efficient technique permitted catalyst reusability, facile scale up, and ease of product isolation. Reaction condition optimization led to dramatically shortened reaction times, high yields and stereoselectivities comparable with those in the batch process.[9c] The peptidic catalysts were synthesized by using Fmoc/*t*Bu protocols on solid supports with different swelling properties: polyethylene glycol–polystyrene copolymers (TentaGel) without any linker and polystyrene resins with 4-methylbenzhydrylamine (MBHA). These experimental set-ups allowed catalyst synthesis and immobilization in a single step; for example, after the coupling steps, the side-chain protecting groups were removed by the use of trifluoroacetic (TFA) acid without cleavage of the peptide. This eliminated the need for further peptide work-up and purification steps and made the whole procedure highly simple and efficient. The heterogenized catalyst was incorporated into a cartridge-like stainless steel tube. The reactions were performed in an H-Cube system in the 'no H_2' mode; the cartridge was placed in a stainless steel block, which contained a Peltier heating system. Constant pressure was ensured by using a back pressure valve, and the CF of the reaction medium was provided by using an HPLC pump. A schematic outline of the packed bed reactor is presented in Scheme 1.[12]



Scheme 1. Experimental set-up for the CF organocatalysis.

For an initial reaction condition optimization study, the peptide H-D-Pro-Pro-Asp-NH-resin (**1a**, Asp = aspartic acid) was used as catalyst in the test reaction of the 1,4-addition of propanal to *E*-β-nitrostyrene (BNS). The catalyst was immobilized on MBHA resin with a loading of 0.64 mmol g^{-1}. The solvent system used ($CHCl_3$/*i*PrOH = 9:1) was chosen on the basis of literature data, this medium ensuring appropriate swelling properties for the immobilized catalysts.[9c] A rapid screen indicated that 8 mg mL^{-1} of BNS was suitable at room temperature and atmospheric pressure, while the aldehyde was applied in an initial high excess (Table S1 in the Supporting Information). In further parameter screening, the aim was to make the procedure efficient while maintaining high yield and selectivity. Adjustment of the flow rate means fine-tuning of the residence time on the catalyst bed; the longer the residence time, the

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higher the yield. With a flow rate as low as 0.01 mL min^{-1}, a quantitative yield was achieved, but more than 8 h was required to pump 5 mL of the starting material through the system. In contrast, when a flow rate of 0.5 mL min^{-1} was used, 10 min was sufficient for a single run, but the yield dropped to 50%. As a compromise between reaction time and throughput, the flow rate was set to 0.1 mL min^{-1} to achieve a yield >90% (Figure 1 and Table S2 in the Supporting Information).

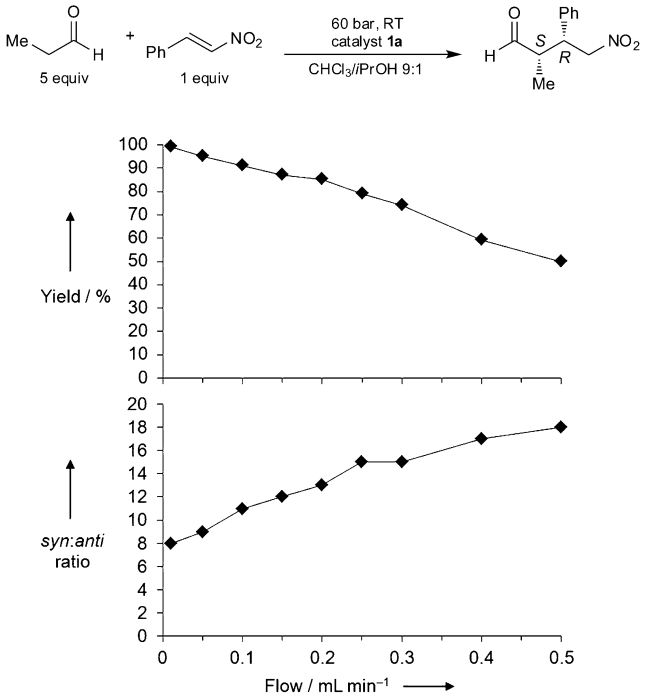


Figure 1. Fine-tuning of the flow rate in the conjugate addition of propanal to BNS.

The fine-tuning of the flow rate influenced not only the yield, but also the diastereoselectivity: the lower the flow rate, the lower the diastereomeric ratio (*dr*). To explain this phenomenon, a short epimerization study was performed. When the resulting γ-nitroaldehyde was recirculated on the bed of catalyst **1a** at 0.1 mL min^{-1}, the *syn*/*anti* ratio decreased from 11:1 to 4:1, whereas when the recirculation of γ-nitroaldehyde was repeated after the acetylation of the secondary amine of the D-Pro residue of catalyst **1a**, no epimerization occurred. This suggested that the catalyst itself can affect the epimerization of the product, and consequently, the longer the residence time, the lower the diastereoselectivity. Application of a high excess of the aldehyde may push the reaction equilibrium to completion, resulting in higher yields, but also makes the procedure less economical. With this in mind, 5 equivalents of propanal were chosen as optimum (Table S2 in the Supporting Information). Next, we examined the pressure dependence of the flow process. Increasing the pressure from atmospheric to 60 bar (1 bar = 10^5 Pa) led to higher yields;

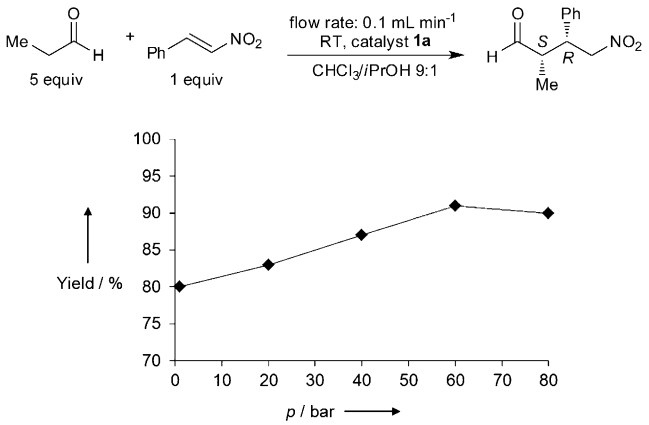
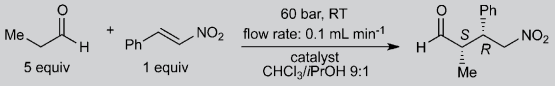


Figure 2. Pressure dependence of the conjugate addition of propanal to BNS.

further elevation proved to be not beneficial (Figure 2 and Table S3 in the Supporting Information). This phenomenon raises the question of whether the conversion is dependent on the catalyst activity itself or is influenced by the transport phenomena of the reactants in the polymer. To investigate the diffusion dependence, we performed the Koros–Nowak test.[13] The loading of the MBHA resin was halved by acetylation, and the same reaction was performed with catalyst **1b**. The decrease in the yield was not proportional to the decrease in loading (Table 1, entries 1 and 2). Consequently, the reaction is under diffusion control, and pressure is necessary to virtually raise the active surface of the catalyst. Increase of the temperature provided higher yields, but also dramatically lowered the selectivity. For example, at 90 °C a quantitative yield was obtained, but with 77% *ee* and a *syn*/*anti* ratio of 1.5:1 (Table S3 in the Supporting Information).

The effects of different additives have been investigated previously.[11b] The role of *N*-methylmorpholine (NMM) in the reac-

Table 1. Fine-tuning of the peptidic catalyst for the conjugate addition of propanal to BNS under optimum flow conditions.



Entry	Immobilized catalyst	Yield[a] [%]	*syn*/*anti*[b]	*ee*(*syn*)[c] [%]
1	H-D-Pro-Pro-Asp-NH-resin[d] (**1a**)	91	11:1	93
2	H-D-Pro-Pro-Asp-NH-resin[e] (**1b**)	78	11:1	92
3	H-D-Pro-Pro-Glu-NH-resin[d] (**2a**)	79	12:1	91
4	H-D-Pro-Pro-Glu-NH-resin[e] (**2b**)	69	11:1	91
5	H-D-Pro-(1*R*,2*R*)-ACPC-Asp-NH-resin[d] (**3**)	83	10:1	90
6	H-D-Pro-(1*S*,2*S*)-ACPC-Asp-NH-resin[d] (**4**)	41	15:1	93
7	H-D-Pro-(1*S*,2*R*)-ACPC-Asp-NH-resin[d] (**5**)	70	9:1	70
8	H-D-Pro-(1*R*,2*S*)-ACPC-Asp-NH-resin[d] (**6**)	35	19:1	89
9	H-D-Pro-Pro-Asp-NH-resin[f] (**7**)	81	11:1	92
10	H-D-Pro-Pro-Glu-NH-resin[f] (**8**)	73	12:1	91

[a] Yield of isolated product. [b] Determined by performing ^{1}H NMR spectroscopic analysis of the crude material. [c] Determined by performing chiral-phase HPLC analysis. [d] Resin: MBHA with a loading of 0.64 mmol g^{-1}. [e] Resin: MBHA with a loading of 0.32 mmol g^{-1}. [f] Resin: TentaGel with a loading of 0.29 mmol g^{-1}.

tion mixture was tested by utilizing catalyst **1a** under optimum flow conditions. The higher the NMM amount added, the lower the diastereoselectivity observed because of possible epimerization, whereas the yield and enantioselectivity remained intact (Table S4 in the Supporting Information). This raises the question of whether NMM itself epimerized the product. Therefore, we incubated the product in an NMM-containing solvent and investigated the *syn*/*anti* ratio before and after this procedure. The diastereoselectivity decreased dramatically. Thus, it proved best to not use NMM at all and to leave the peptides in the TFA salt form, in which they are present after the solid-phase peptide synthesis (SPPS).

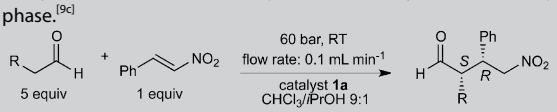
To achieve optimum conditions, we also fine-tuned the peptide catalyst. We first tested the effect of the exchange of the C-terminal amino acid from aspartic acid to the homologous glutamic acid (Glu).[9b] H-D-Pro-Pro-Glu-NH-resin was synthesized on the MBHA support with two different loadings (0.64 and 0.32 mmol g^{-1}, **2a** and **2b**). These furnished comparable selectivities to catalyst **1a**, but lower yields (Table 1, entries 3 and 4). Wennemers et al. recently applied the same solid-supported catalyst with Glu as C-terminal utilizing a standard batch technique. They achieved quantitative yields and high selectivities, but very long reaction times were needed.[9a] The effect of the peptide conformation-directing role of the bioinspired substitution of the central proline unit with β-amino acids was also investigated.[14] Insertion of (1*R*,2*R*)-2-aminocyclopentane-carboxylic acid (ACPC) (**3**) led to comparable results with catalyst **1a**, whereas application of (1*S*,2*S*)- (**4**), (1*S*,2*R*)- (**5**), or (1*R*,2*S*)-ACPC (**6**) resulted in significantly lower yields (Table 1, entries 5–8). This suggests that exchange of the proline linker gives satisfying results, if the substituting residue does not induce significant distortion in the conformation of the peptide. H-D-Pro-Pro-Asp-NH-resin and H-D-Pro-Pro-Glu-NH-resin were also synthesized on TentaGel (with a loading of 0.29 mmol g^{-1}, **7** and **8**). The different swelling properties and the lower loading of the resin gave rise to lower yields, whereas the selectivity of the reaction remained comparable to that obtained with MBHA (Table 1, entries 9 and 10).

Catalyst reusability was verified by repetition of the test reaction under optimum flow conditions by using catalyst **1a**. In each run, 50 mg of crude product was collected in 50 min. During the first five consecutive reactions, the yield remained >80%, and after the 10th run it was still nearly 70%. Notably, the enantioselectivities remained practically constant, whereas the diastereoselectivities increased slightly (Table S5 in the Supporting Information). The increase in the *syn*/*anti* ratio can be explained by blocking of the secondary amine moiety of the D-Pro motif of the catalyst, which leads to epimerization of the product.

Under the optimum conditions for catalyst **1a**, the corresponding γ-nitroaldehyde product of the test reaction was obtained in a yield of 91%, a *syn*/*anti* ratio of 11:1, and 93% *ee*. These results compare well with those of the batch procedure, in which the same catalyst was applied in the homogeneous phase.[9c] However, it should be noted that the batch reaction required 24 h, whereas at the optimum flow rate of 0.1 mL min^{-1}, the residence time on the catalyst bed with the CF method was only 7 min, which can be regarded as the reaction time. This considerable decrease in reaction time is attributable to the beneficial features of the technique: the use of CF, the high local catalyst concentration in the catalyst bed, and the high pressure.[1]

To investigate the scope and applicability of the described method, a number of conjugate addition reactions between various aldehydes and BNS were explored. The reaction conditions were set at the previously optimized values, and the immobilized catalyst **1a** was used. As seen in Table 2, excellent results were obtained with linear aldehydes (Table 2, entries 1–5). The corresponding γ-nitroaldehyde products were formed in good yields (between 60 and 90%), the enantioselectivities were >90%, and the diastereoselectivities were also high. A branched aldehyde–bearing substituent in the β-position was also tolerated, but a lower yield was acquired (Table 2, entry 6). The selectivities obtained with the CF technique compared well with those achieved with the batch procedure, but the yields are somewhat lower (Table 2). However, taking into account that the CF reactions took place in 7 min, whereas the batch reactions required 12–24 h, the CF results are thought-provoking.[9c] 1,4-Addition of a series of α-branched aldehydes (2-methylpropanal, 2-methylbutanal, 2-phenylpropanal, phenylacetaldehyde, and diphenylacetaldehyde) to BNS was also attempted, but no conversion was obtained under the utilized reaction conditions, probably caused by steric hindrance.

Table 2. Demonstration of the scope and applicability of the CF organocatalytic technique, and comparison of the results with the batch data reported by Wennemers et al. utilizing the same catalyst in the homogeneous phase.[9c]



Entry	R	Technique	Yield[a] [%]	*syn*/*anti*[b]	*ee*(*syn*)[c] [%]
1	Me	CF	91	11:1	93
		batch ref.	98	9:1	91
2	Et	CF	60	22:1	91
		batch ref.	93	25:1	95
3	*n*Pr	CF	68	15:1	91
		batch ref.	94	15:1	92
4	*n*Bu	CF	87	14:1	91
		batch ref.	quant	15:1	92
5	Bn	CF	65	36:1	92
		batch ref.	89	15:1	95
6	*i*Pr	CF	22	20:1	91
		batch ref.	88	50:1	92

[a] Yield of isolated product. [b] Determined by performing ^{1}H NMR spectroscopic analysis of the crude material. [c] Determined by using chiral-phase HPLC analysis.

Thus, we have developed the first CF method for the asymmetric organocatalytic conjugate addition of aldehydes to nitroolefins. A solid-supported peptide has been utilized as catalyst, which has been synthesized and immobilized in the same step. Ignoring the peptide cleavage means no work-up, no purification, and no product loss. The technique is rapid, simple, and efficient while it lacks most of the drawbacks of the con-

ventional batch procedure. After optimization of the reaction conditions, synthetically useful chiral γ-nitroaldehydes have been obtained in excellent yields and stereoselectivities. The results are nicely comparable with those of the batch technique. As a consequence of the beneficial features of the CF method, the reaction times have been markedly shorter than with the batch process, whereas the catalyst reusability, the ease of product isolation and the possibility of facile scale up enhance the efficiency of the CF technique. Immobilized peptidic organocatalysts are, therefore, useful for broadening the scope of flow chemical processes.

Acknowledgements

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Keywords: continuous flow reactors · immobilization · michael addition · organocatalysis · peptides

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III

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SZTE Gyógyszerkémiai Intézet

Organokatalízis folyamatos áramú reaktorban[1]

Az áramlásos technológiákat már régóta alkalmazza az olajipar, az utóbbi években viszont a szerves szintetikus kémiában is elkezdetek meghonosodni, köszönhetően annak, hogy a hagyományos szakaszos eljárásokhoz képest számos előnnyel rendelkeznek, ezek közül a legfontosabbak: a reagensek gyors és hatékony keveredése, hatékony hőátadás és rövid reakcióidők [1, 2]. A modern áramlásos berendezések nagyon kis anyagmennyiségeket is tudnak kezelni (mikroreaktorok), továbbá a legfontosabb reakcióparaméterek (áramlási sebesség, nyomás és hőmérséklet) pontosan szabályozhatók, így az optimális reakciókörülmények gyorsan és könnyedén beállíthatók [3]. Nemcsak homogén fázisú reakciók kivitelezhetők folyamatos áramban, az olajiparhoz hasonlóan állóágyas reaktorok is elterjedtek, amelyekben tetszőleges immobilizált reagensek, katalizátorok helyezhetőek el, sőt egyes reaktortípusok gáz halmazállapotú reagensek kezelésére is alkalmasak, akár szuperkritikus körülmények között is [4–6]. Mivel a technológiai alapok a nagyiparban gyökereznek, a méretnövelési és automatizálási lehetőségek is adottak, így a laboratóriumi kísérletek köréből kilépve a gyógyszeripar számára is vonzóvá tehető a technika.

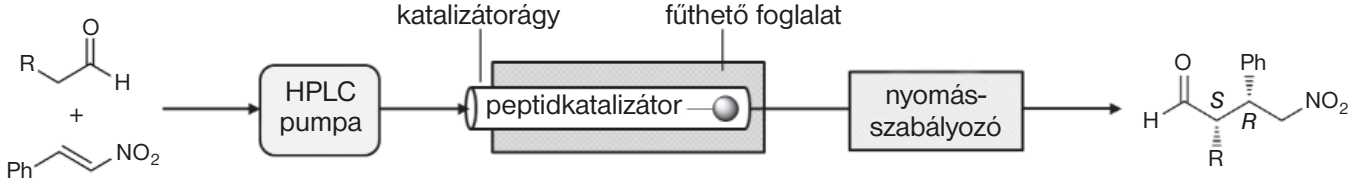
Az organokatalízis olyan újszerű megközelítés a szerves kémiában, amely fématomokat nem tartalmazó szerves molekulákat alkalmaz katalizátorként. E tudományterület virágzása az ezredforduló környékén indult, egy egyszerű aminosav, a prolin mint organokatalizátor elterjedésével. A prolin a C–C kötések kialakítására szolgáló népszerű Michael-, Mannich- és aldolreakciók királis katalizátorává vált, jó hozam- és szelektivitásértékek elérése mellett [7]. Mivel a szintézismódszerek hatékonyságának növelése iránt folyamatosan nőtt az igény, a prolin módosításával számos aktívabb és/vagy szelektívebb organokatalizátort fejlesztettek ki [8, 9]. Nem nehéz belátni azonban, hogy a kisméretű, prolinszerű katalizátorok továbbfejlesztése, módosítása egy adott szint elérése után korlátozott a funkciós csoportok limitált száma miatt. Ugyanakkor ha olyan moduláris felépítésű molekulákat alkalmazunk katalizátorként, mint a peptidek, akkor az előző akadályon könnyedén túllépve, az építőelemek változtatásával (finomhangolás) kedvező tulajdonságokkal rendelkező katalizátorokat állíthatunk elő. A peptidek mellett szóló érv az is, hogy királis információtartalmuk nem merül ki az aminosavak aszimmetriájában, hanem másodlagos és harmadlagos szerkezetükből is jelentős királis indukció adódhat. Az utóbbi években számos peptid, illetve peptidszerű organokatalizátort fejlesztettek ki, és megszületett a felismerés, miszerint egyes peptidek a prolin katalitikus tulajdonságait imitálják, sőt túl is mutatnak azon [10].

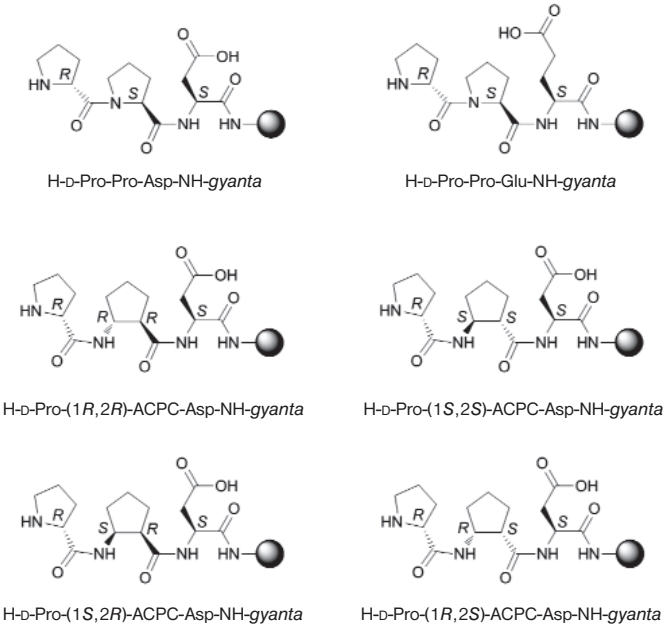
Munkánk során kidolgoztuk az első organokatalitikus áramlásos módszert aldehidek nitroolefinekre történő konjugált addíciójához, szilárd hordozós peptidkatalizátor alkalmazásával [6]. Választásunk azért esett a peptidekre, mert szilárd fázisú peptidszintézis alkalmazásával a katalizátor szintézise és immobilizációja egy lépésben megvalósítható. Az Fmoc/*t*Bu protokoll szerint történő peptidszintézishez olyan speciális gyantákat kerestünk hordozóként, amelyekről a védőcsoport-eltávolítási lépések során nem hasad le a katalizátor. Végül két hordozóra esett a választásunk: (*i*) politelinglikol–polisztirol koplimer TentaGel-re és (*ii*) 4-metilbenzhidrilamin linkerrel rendelkező térhálósított polisztirol gyantára (MBHA). Ez a kísérleti elrendezés meglehetősen egyszerű és praktikus, ugyanis megspórolhatók az idő- és energiaigényes peptidtisztítási és feldolgozási lépések, az immobilizált katalizátort pedig rozsdamentes acél oszlopokba töltve közvetlenül felhasználhattuk a peptidszintézis után. A folyamatos áramú reakciókat egy H-Cube® készülék segítségével hajtottuk végre „no H_2” üzemmódban. A katalizátort tartalmazó rozsdamentes acél oszlop (katalizátorágy) egy fűthető foglalatba került, a reagensek folyamatos áramát ezen keresztül egy HPLC pumpa biztosította. A berendezés egy nyomásszabályzó egységet is tartalmazott. A kísérleti elrendezés vázlatát az **1. ábrá**n szemléltetjük [11].

Kiindulásként, a folyamatos áramú módszer finomhangolásához irodalmi megfontolások alapján az alábbi peptidkatalizátort választottuk: H-D-Pro-Pro-Asp-NH_2 (Pro = prolin, Asp = aszpara-

[1] A cikk Ötvös Sándor PhD-hallgatónak a 2011-es Szegedi Ifjú Szerves Kémikusok Támogatásáért Alapítvány tudományos előadóülésén elhangzott, első helyezett előadása alapján készült.

1. ábra. A folyamatos áramú berendezés vázlata

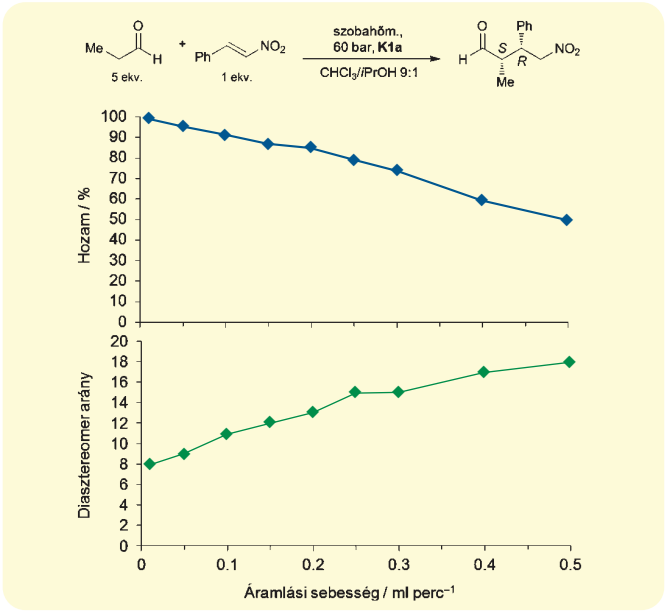


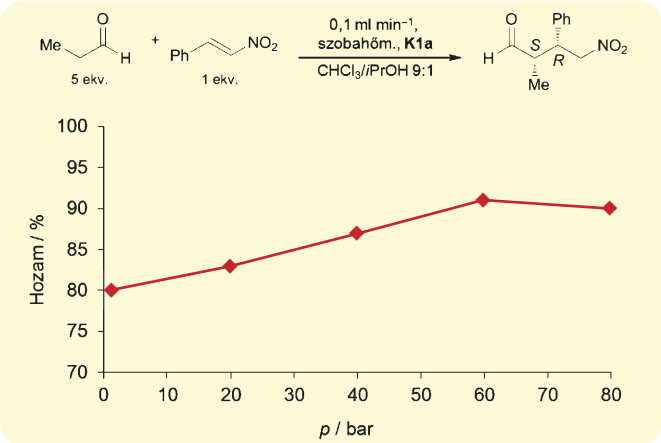


2. ábra. Szilárd hordozós peptidkatalizátorok

ginsav) [12]. E tripeptid prolinmimetikumnak tekinthető, ugyanis szekunder amin- és karboxil-funkciót tartalmaz speciális orientációban **(2. ábra)**. A katalitikus aktivitásért az említett funkciós csoportok, a szelektivitásáért pedig elsősorban a peptid másodlagos szerkezete felelős. A peptidet kezdetben 0,64 mmol · g^{-1} töltöttségű MBHA gyantán immobilizáltuk (**K1a**). Az oldószer körültekintő kiválasztása fontos volt, ugyanis figyelembe kellett vennünk a reagensek oldhatósága mellett a porózus szerkezetű hordozó duzzadási tulajdonságait is. Mivel alapvetően mind a kétféle gyanta apoláros oldószerekben duzzad jól, viszont a kiindulási anyagok inkább poláros oldószerekben oldhatók, egy oldószerelegyre, a $CHCl_3$/*i*PrOH 9 : 1-re esett a választásunk. A reakcióparaméterek optimálásához a propionaldehid *E*-β-nitrosztirolra (BNS) történő konjugált addícióját választottuk tesztreakcióként, a BNS koncentrációját 8 mg · ml^{-1} konstans értéken tartva. A továbbiakban az alábbi reakcióparaméterek változtatásának hatását vizsgáltuk: áramlási sebesség, reagensfelesleg, nyomás és hőmérséklet. A célunk az volt, hogy a módszert a lehető leghatékonyabbá tegyük, azaz a minimális reakcióidőt érjünk el minimális reagensfelesleg alkalmazásával, magas hozam és szelektivitásértékek elérése mellett. Egy áramlásos eljárás esetében a reakcióidőt a reagensek reaktorban töltött tartózkodási ideje jelenti (esetünkben a „reaktor" alatt csak a töltetes oszlopot értjük). Minél nagyobb az áramlási sebesség, annál kisebb a tartózkodási idő és annál nagyobb az eljárás kapacitása, viszont a túl nagy áramlási sebesség (azaz túl rövid reakcióidő) a konverzió rovására mehet. Ezt jól példázza, hogy 60 bar nyomáson, szobahőmérsékleten, 5 ekv aldehidfelesleggel 0,01 ml · $perc^{-1}$ áramlási sebesség mellett közel teljes hozamot értünk el, viszont az áramlást 0,5 ml·$perc^{-1}$-re növelve a termelés a felére esett vissza **(3. ábra)**. Érdemes ugyanakkor azt is mérlegelni, hogy az előbbi esetben 5 ml reakcióelegyet több mint 8 órába tartott átpumpálni a reaktoron, viszont 0,5 ml · $perc^{-1}$ áramláson ugyanez 10 percbe telt. Ezért kompromisszumként 0,1 ml · $perc^{-1}$ áramlási sebességet választottunk optimálisnak, amelyen 91%-os termelés adódott. Az áramlási sebesség változtatása nemcsak a termelésre van hatással, hanem a diasztereomer arányra (*dr*) is: minél kisebb az áramlási sebesség, annál alacsonyabb a *dr (3. ábra)*. Ezt azzal magyarázhatjuk, hogy maga a katalizátor kiválthatja a termék epimerizációját. Hogy ezt bebizonyítsuk, a termékként kapott γ-nitroaldehid oldatát újra átpumpáltuk a katalizátorágyon 0,1 ml · $perc^{-1}$ áramlási sebességgel, és azt tapasztaltuk, hogy ekkor a *dr* 11 : 1-ről 4 : 1-re romlik. Ha azonban a peptid N-terminális D-prolinját először acetilcsoporttal védtük, és csak utána pumpáltuk át rajta a γ-nitroaldehid terméket, nem változott a *dr*. Ez a megfigyelés megerősítette, hogy a lehető legkisebb tartózkodási időt célszerű választani a katalizátorágyon. Az aldehid nagy feleslege a reakciót ugyan a termékképződés irányába mozdíthatja, viszont a túl nagy reagensfelesleg a módszer hatékonyságát csökkenti. 60 bar nyomáson, szobahőmérsékleten, 0,1 ml · $perc^{-1}$ áramlás mellett 15 ekv propionaldehid-felesleg közel teljes hozamot eredményezett, azonban az optimális értéket 5 ekv-ben maximáltuk. Ezután megvizsgáltuk a nyomás növelésének hatását is (szobahőmérsékleten, 0,1 ml · $perc^{-1}$ áramlási sebesség mellett). Azt tapasztaltuk, hogy atmoszférikusról 60 barra növelve a nyomást a termelés 80-ról 91%-ra nő, további emelés azonban hatástalannak bizonyult **(4. ábra).** Felvetődik a kérdés, hogy a reakciót a katalizátor aktivitása mellett a reagensek katalizátorhordozó mátrixán belül történő transzportja korlátozhatja-e. Hogy kijelenthessük: a reakció az általunk megadott körülmények között diffúziókontrollált, elvégeztük a Körös–Nowák-tesztet [13]. Ehhez az amin-végződések acetilezésével az MBHA gyanta töltöttségét megfeleztük, majd az így kapott 0,32 mmol · g^{-1} kapacitású gyantán immobilizáltuk a D-Pro-Pro-Asp-NH_2 peptidet (**K1b**). A tesztreakciót megismételve **K1b**-vel 78%-os termelést kaptunk, míg ugyan ilyen körülmények között **K1a**-val 91%-ot sikerült elérni. Mivel a termelés csökkenése nem arányos a katalizátortöltöttség csökkenésével, kijelenthető, hogy a reakció az általunk szabott feltételek mellett diffúziókontrollált, és a magas nyomás a reagensek transzportját hivatott elősegíteni a katalizátorhordozó duzzadt mátrixán belül. A hőmérséklet változtatásának hatásait vizsgálva arra a következtetésre jutottunk, hogy nem érdemes szobahőmérséklet fölé növelni a reaktor hőmérsékletét, mert a termelés növekedése mellett drasztikus mértékben csökken a sztereoszelektivitás.

3. ábra. Az áramlási sebesség finomhangolása a propionaldehid BNS-re történő folyamatos áramú konjugált addíciója során

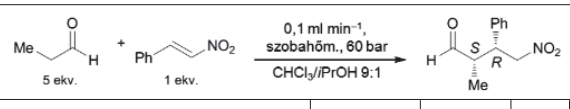




4. ábra. A nyomásnövelés hatásának vizsgálata a propionaldehid BNS-re történő folyamatos áramú konjugált addíciója során

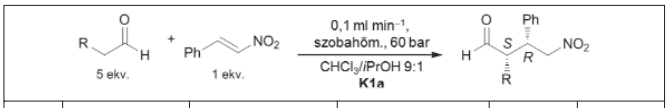
Az áramlásos reakció főbb paramétereinek optimálása után a peptidkatalizátor szerkezeti finomhangolását is elvégeztük. Vizsgáltuk a C-terminális aszparginsav cseréjének hatását a vele homológ glutaminsavra (Glu). A H-D-Pro-Pro-Glu-NH_2 peptidszekvenciát 0,32 és 0,64 mmol · g^{-1} kapacitású MBHA gyantán is immobilizáltuk (**K2a** és **K2b**). A propionaldehid és BNS tesztreakcióját az optimális körülményeken megismételve azt tapasztaltuk, hogy a glutaminsavat tartalmazó katalizátorok ugyan **K1a**-val és **K1b**-vel összemérhető sztereoszelektivitást nyújtanak, viszont katalitikus aktivitásuk kisebb, ugyanis alacsonyabb termeléseket értünk el velük **(1. táblázat)**. Ezután izoszter-helyettesítéseket hajtottunk végre a katalizátor középső prolinrészének β-aminosavakra történő cseréjével, majd vizsgáltuk a peptidkonformáció változásának katalitikus aktivitásra gyakorolt hatásait [14]. Azt tapasztaltuk, hogy (1*R*,2*R*)-2-aminociklopentánkarbonsavra (ACPC) történő csere (**K3**) gyakorlatilag **K1a**-val összemérhető eredményeket ad, azonban az (1*S*,2*S*)- (**K4**), (1*S*,2*R*)- (**K5**) és (1*R*,2*S*)-ACPC (**K6**) izomerekkel történő helyettesítés esetén a katalizátor aktivitása és/vagy szelektivitása jelentősen csökken *(1. táblázat)*. Ezek az eredmények azt bizonyítják, hogy hatékony katalízishez a katalizátor aktív csoportjainak (*szek*-amin és karboxil) megfelelő orientációja, és ezt biztosító peptidkonformáció szükséges. A D-Pro-Pro-Asp-NH_2 és D-Pro-Pro-Glu-NH_2 peptidszekvenciákat 0,27 mmol · g^{-1} töltöttségű TentaGel gyantához kötve is előállítottuk (**K7** és **K8**). Mivel a TentaGel gyanta duzzadási tulajdonságai valamivel kedvezőtlenebbek, sőt a töltöttsége is alacsonyabb, nem meglepő, hogy az MBHA hordozóhoz képest alacsonyabb termeléseket sikerült elérnünk *(1. táblázat)*. Az előállított peptidkatalizátorokat a *2. ábrán* szemléltetjük.

1. táblázat. Az immobilizált peptidkatalizátor finomhangolása a propionaldehid BNS-re történő folyamatos áramú konjugált addíciója során



Immobilizált katalizátor	Gyanta-töltöttség/ mmol · g^{-1}	Termelés/ %	*dr*	*ee*/ %
K1a: H-D-Pro-Pro-Asp-NH-MBHA	0,64	91	11:1	93
K1b: H-D-Pro-Pro-Asp-NH-MBHA	0,32	78	11:1	92
K2a: H-D-Pro-Pro-Glu-NH-MBHA	0,64	79	12:1	91
K2b: H-D-Pro-Pro-Glu-NH-MBHA	0,32	69	11:1	91
K3: H-D-Pro-(1R,2R)-ACPC-Asp-NH-MBHA	0,64	83	10:1	90
K4: H-D-Pro-(1S,2S)-ACPC-Asp-NH-MBHA	0,64	41	15:1	93
K5: H-D-Pro-(1S,2R)-ACPC-Asp-NH-MBHA	0,64	70	9:1	70
K6: H-D-Pro-(1R,2S)-ACPC-Asp-NH-MBHA	0,64	35	19:1	89
K7: H-D-Pro-Pro-Asp-NH-TentaGel	0,27	81	11:1	92
K8: H-D-Pro-Pro-Glu-NH-TentaGel	0,27	73	12:1	91

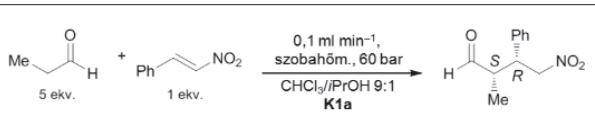
Az optimális reakciókörülményeket az alábbiakban foglalhatjuk össze: 0,1 ml · $perc^{-1}$ áramlási sebesség, 5 ekv aldehidfelesleg, 60 bar nyomás, szobahőmérséklet, **K1a** katalizátor. Ezeket a paramétereket alkalmazva a tesztreakció eredményéül (2*S*,3*R*)-2-metil-4-nitro-3-fenilbutanalt 91%-os termelés, 11 : 1 *dr* és 93% *ee* elérésével sikerült előállítanunk. Ezek az eredmények jó egyezést mutatnak azokkal az irodalmi értékekkel, amelyeket hagyományosan lombikban kevertetve hordozómentes H-D-Pro-Pro-Asp-NH_2 peptid katalizátor alkalmazásával értek el **(2. táblázat)** [12]. Fontos azonban hangsúlyozni, hogy esetünkben a katalizátorágyon töltött tartózkodási idő, ami a reakcióidőnek felel meg, mindössze 7 perc volt, ezzel szemben a hagyományos szakaszos eljárás alkalmazásával 24 óra szükséges. A reakcióidő ilyen drasztikus csökkenése az alkalmazott módszer előnyös tulajdonságainak köszönhető: (*i*) áramlásos kémia, (*ii*) nagy nyomás és (*iii*) magas lokális katalizátorkoncentráció.



R	Módszer	Reakcióidő	Termelés/%	*dr*	*ee*/%
Me	áramlás	7 perc	91	11:1	93
	batch ref.	24 óra	98	9:1	91
Et	áramlás	7 perc	60	22:1	91
	batch ref.	12 óra	93	25:1	95
*n*Pr	áramlás	7 perc	68	15:1	91
	batch ref.	12 óra	94	15:1	92
*n*Bu	áramlás	7 perc	87	14:1	91
	batch ref.	12 óra	99	15:1	92
Bn	áramlás	7 perc	65	36:1	92
	batch ref.	12 óra	89	15:1	95
*i*Pr	áramlás	7 perc	22	20:1	91
	batch ref.	24 óra	88	50:1	92

2. táblázat. A kidolgozott folyamatos áramú módszer felhasználhatóságának kiterjesztése és az eredmények összehasonlítása irodalmi batch referenciával [12]

Tanulmányoztuk az immobilizált katalizátor újrahasználhatóságát is. Ezért az optimális körülményeken a tesztreakciót egymás után többször végrehajtottuk ugyanazon a katalizátortölteten. Minden egyes reakció során 5 ml kiindulási elegyet pumpáltunk át a reaktoron 50 perc alatt. Az első öt reakció után a termelés 80% fölött maradt, és a 10. újrahasználás után is még 70% körül értékek adódtak **(3. táblázat)**. A kísérletsorozat során az *ee* gyakorlatilag nem változott, azonban a *dr* nőtt. Ez azzal magyarázható, hogy a D-prolin *szek*-amin-csoportja a katalizátor aktív részét képezi, ugyanakkor a termék epimerizációját is okozhatja. A katalizátor kimerülésével blokkolódnak ezek a funkciós csoportok, azaz csökken az aktivitás, viszont epimerizáció sem történik.



Felhasználás	Termelés/%	*dr*	*ee*/%
1.	91	11:1	93
2.	86	12:1	93
3.	84	12:1	94
4.	83	12:1	93
5.	82	13:1	93
6.	76	15:1	93
7.	74	15:1	93
8.	72	16:1	93
9.	67	15:1	94
10.	66	15:1	94

3. táblázat. Az immobilizált organokatalizátor újrahasználhatóságának vizsgálata

A kidolgozott módszer alkalmazhatóságát kiterjesztettük számos további aldehid és BNS konjugált addíciójára. A reakciókat a korábban optimalizált körülmények között hajtottuk végre, **K1a** alkalmazásával. Elágazást nem tartalmazó aldehidek nagyon jó reakciópartnernek bizonyultak, ugyanis a megfelelő γ-nitroaldehid termékeket magas hozam (60–91%), *dr* (11 : 1–36 : 1) és *ee* (91–93%) elérésével állítottuk elő *(2. táblázat)*. A β-elágazást tartalmazó izovaleraldehid esetében alacsonyabb termelést kaptunk, viszont a sztereoszelektivitás magas volt. A *2. táblázatban* saját eredményeink mellett hagyományos batch referenciák is találhatók [12]. Jól látható, hogy az általunk folyamatos áramban elért sztereoszelektivitások jó egyezésben vannak az irodalmi értékekkel, bár a termelések rendszerint valamivel alacsonyabbak. Azonban azt is figyelembe kell venni, hogy az általunk kidolgozott módszer nagyon gyors, reakcióideje mindössze 7 perc, viszont a hagyományos eljárás lassú, 12–24 órás kevertetést igényel.

Munkánkat röviden összefoglalva elmondható, hogy kidolgoztuk az első folyamatos áramú módszert organokatalitikus 1,4-addíciós reakciókhoz. Katalizátorként szilárd hordozóhoz kötött peptidet alkalmaztunk, amelynek szintézise és immobilizációja praktikusan egy lépésben történt. A módszer célszerűségét fokozza, hogy a reakcióidőt sikerült jelentősen csökkentenünk a hagyományos szakaszos eljáráshoz képest, továbbá, hogy az immobilizált katalizátor újrahasználható.

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Bruckner-termi előadás

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Arany(I)tartalmú szupramolekulák szintézise és egykristály-röntgendiffrakciós szerkezetmeghatározása

A *szupramolekuláris kémia* még mindig fiatal területe a kémiának, amely a biológiából vett molekuláris önszerveződés elvének felhasználásával a fizika, az elektronika, a kémiai technológia, a nanotechnológia számára potenciálisan hasznos anyagokat képes létrehozni. A szupramolekuláris kémia a nem kovalens kötések kémiája, és definíció szerint ez a típusú kémia már a „molekulákon túli" rendszerek kémiája. Éppen ezért nem véletlen, hogy a korszerű szupramolekuláris kémia csak a múlt század nyolcvanas éveiben kezdett el kibontakozni, amikor a modern szerkezetvizsgálati módszerek is rohamos fejlődésnek indultak és alkalmazásukkal lehetővé vált a szupramolekulák szerkezetmeghatározása. Az 1987-ben Donald J. Cram, Jean-Marie Lehn és Charles J. Pedersen számára odaítélt kémiai Nobel-díj már a szupramolekuláris kémia fontosságának az elismerését jelentette. [1]

A lumineszcens, katalitikus, redoxaktív és/vagy biológiai szempontból jelentős fémcentrumoknak a szupramolekulákba történő beépítésével változatos alakzatú és méretű, sokszor szokatlan tulajdonságú rendszerek állíthatók elő. Az arany(I)tartalmú szupramolekulák vizsgálatának részeként arra kerestük a választ, hogy az arany(I)ionok és megfelelően megválasztott ligandumok önszerveződési reakciója milyen egyedi szerkezetű és sajátságú szupramolekulákat eredményez. A nagyméretű szupramolekulák

IV

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Asymmetric aldol reaction in a continuous-flow reactor catalyzed by a highly reusable heterogeneous peptide

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ABSTRACT

A solid-supported peptide-catalyzed continuous-flow (CF) process was developed for asymmetric aldol reactions. The catalyst was readily synthesized and immobilized by solid-phase peptide synthesis (SPPS) on a swellable polymer support in one single step. Ignoring the peptide cleavage from the resin means no work-up, no purification, and no product loss. After thorough optimization of the reaction conditions, synthetically useful β-hydroxyketone products were obtained in high yields and stereoselectivities. It was found that the heterogeneous catalytic reaction is diffusion-controlled under the present conditions; thus, elevation of the pressure is necessary to maximize conversion of the flow process. Besides being simple and efficient, the described method is also rapid and promisingly productive, with short residence times on the catalyst bed. The immobilized peptidic catalyst is highly recyclable, while further advantageous features are the ease of product isolation and the possibility of facile scale-up, furnishing sustainable catalytic methodology.

1. Introduction

Asymmetric C–C bond formation has always been a cardinal subject in organic chemistry. Around the turn of the century, proline (Pro) was introduced as a promising new catalyst for reactions involving enamine intermediates [1–3]. In the golden age of asymmetric catalysis, a huge number of Pro-derived organocatalysts were developed that demonstrated enhanced catalytic activity and selectivity [4–14]. Short peptides and peptide-like molecules (as Pro mimetics) proved to be excellent catalysts for asymmetric transformations [15–22]. Small, rigid catalysts offer only a limited number of sites for structural and functional diversity, whereas synthetic peptides have the advantage of designable modularity as they are made up from the same chiral building blocks (amino acid residues) as enzymes [23,24]. The most reactive peptidic organocatalysts developed to date for the aldol [25] and Michael [26,27] reactions were reported by Wennemers et al. These tripeptides contain Pro and carboxylic acid moieties in a specific orientation to each other [28]. It has been shown that both the secondary amine residue and the carboxyl group are crucial for effective catalysis [29,30], while immobilization of the peptide on a solid support does not weaken its effectivity, but ensures excellent ease of use, and catalyst reusability [31,32].

CF technologies have captured attention in modern synthetic chemistry as they offer a massive number of advantages over conventional batch procedures, for example, the efficient mixing of substrates, faster heat and mass transfer, and shorter reaction times [33–39]. The well-regulated CF reactor concept enables reactions to be performed with an unprecedented level of control. The most important reaction parameters (such as flow rate, pressure, and temperature) can be adjusted and monitored quickly and precisely [35,40–43]. The need for the large-scale use of reagents and solvents is eliminated, so that the screening of reaction conditions becomes simple and time- and cost-efficient, which implies even rapid library synthesis and an opportunity for automatization [44–47]. Heterogenizing homogeneous catalysts on a solid support is a trend toward the increase in the efficiency of synthetic techniques [48–51]. Through the incorporation of immobilized catalysts and reagents, the scope of flow chemical processes can be further broadened [52–62]. In consequence of these benefits, the conversion of laboratory-based flow chemistry experiments to the subsequent production scale is straightforward [63,64].

A literature search reveals the thought-provoking finding that aldol reactions in standard batch mode involving the use of peptides or other Pro mimetics as catalysts usually demand many hours or even days if high yields and high stereoselectivities are to be attained (Table S1, see Supporting information). In the last few years, several CF approaches have also been described for organocatalytic aldol reactions; but in most cases, these have a number of

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drawbacks such as (i) long process times [65], (ii) difficulties of product isolation [66], and (iii) lower conversions [67] that limit their practical applicability yet. It must be noted here that, around the final stage of the preparation of this manuscript, Pericas et al. reported a promising CF method for aldol reactions with an immobilized proline derivative as organocatalyst [68].

With the aim of creating a more sustainable and industrially reliable catalytic procedure for stereoselective aldol reactions, we have developed a simple and efficient CF method in which a solid-supported peptide is utilized as chiral organocatalyst. The described technique permits outstanding catalyst reusability, ease of product isolation, and the opportunity of facile scale-up. Reaction condition optimization led to high yields, stereoselectivities, and productivities. Short residence times were utilized on the catalyst bed, this being the fastest CF technique to date to the best of our knowledge.

2. Experimental

2.1. General information

The materials and reagents used were of the highest commercially available grade and were applied without any further purification steps. Flash column chromatography was performed on Merck silica gel 60, particle size 63–200 μm, and analytical thin-layer chromatography (TLC) on Merck silica gel 60 F_{254} plates. Compounds were visualized by means of UV or $KMnO_4$. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance DRX 400 spectrometer, in $CDCl_3$ as solvent, with TMS as internal standard, at 400.1 and 100.6 MHz, respectively. MS analysis was carried out with an Agilent 1100 LC/MSD Trap. HPLC analyzes were performed on an analytical HPLC with a diode array detector from JASCO. An H-Cube® system was utilized as CF reactor.

2.2. Synthesis of catalysts

The peptidic catalysts were synthetized manually by a solid-phase technique, utilizing 9H-fluoren-9-ylmethoxycarbonyl/*tert*-butyl (Fmoc/*t*Bu) chemistry on two solid supports: polyethylene glycol (PEG)–polystyrene (PS) copolymer without any linker (TentaGel, with a loading of 0.27 mmol g^{-1}), and PS resin with a 4-methylbenzhydrylamine linker (PS-MBHA, with a loading of 0.64 mmol g^{-1}) (Fig. 1). When TentaGel resin was utilized, the whole peptide synthesis procedure was carried out in DMF; for PS-MBHA, DMF/CH_2Cl_2 1:1 was used as solvent. Before any synthetic steps, the resin was swollen by agitation for 1 h in the applied solvent. In the case of PS-MBHA, further treatment with 5% *N*,*N*-diisopropylethylamine (DIEA) solution was necessary to liberate the amino function from the HCl salt form. DIEA (6 eq) was added to a solution of Fmoc-protected amino acid (3 eq) and 1-[*bis*-(dimethylamino)methyliumyl]-1*H*-1,2,3-triazolo[4,5-*b*]pyridine-3-oxide (HATU, 3 eq). The activated amino acid was then added to the amino-functionalized resin, and the mixture was agitated for 3 h. After coupling, the resin was washed (CH_2Cl_2 3×, MeOH 2×, CH_2Cl_2 3×), and the amino acid incorporation was checked by means of the Kaiser or isatin test [69,70]. Fmoc deprotection was carried out in a solution of 2% 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and 2% piperidine with agitation for 2 × 15 min. After filtration, the resin was washed, and the coupling and deprotection steps were repeated. Finally, the *t*Bu side-chain protecting group was removed from the aspartic acid (Asp) residue in a mixture of trifluoroacetic acid (TFA) and H_2O (9:1 v/v) at room temperature (RT) for 3 h (Scheme 1). After removal of TFA in vacuo, the resin was washed thoroughly (CH_2Cl_2 6×, MeOH 5×, CH_2Cl_2 6×) and was then kept at RT for 6 h to dry. The immobilized catalyst was utilized as TFA salts after the SPPS.

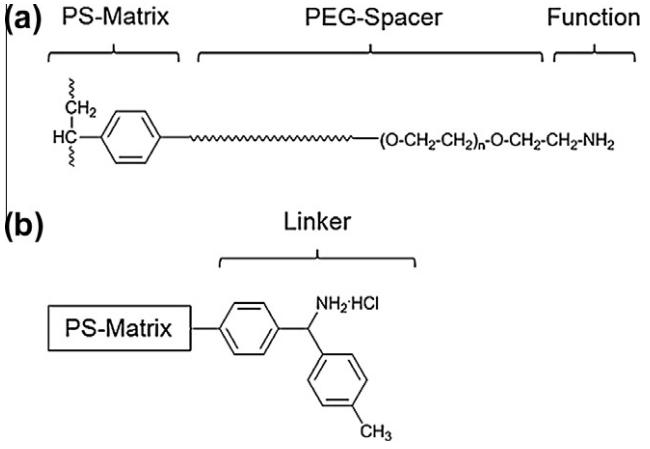


Fig. 1. Solid supports utilized in this study for catalyst immobilization: (a) TentaGel, (b) PS-MBHA.

2.3. Analysis of catalysts

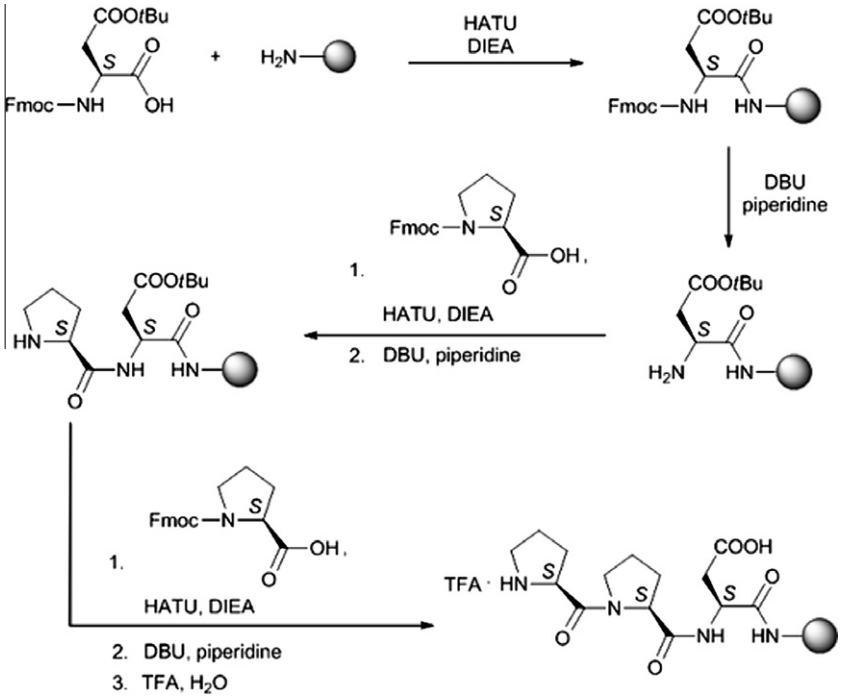
The structure of the TentaGel-immobilized catalysts was checked by means of suspension-phase ^{13}C NMR measurements. In the case of PS-MBHA-immobilized catalysts, the swelling properties of the resin made suspension-phase NMR unfeasible. MS and RP-HPLC investigations were therefore carried out after cleavage from the resin in a mixture of thioanisole, 1,2-ethanedithiol (EDT), TFA, and trifluoromethanesulfonic acid (TFMSA) (2:1:20:2 v/v/v/v) for 0.5 h at −10 °C and then at RT for 1.5 h. The peptide was next precipitated by the addition of cold Et_2O, collected by filtration and dissolved in TFA. After reduction of the TFA volume to 1 mL by evaporation, the peptide was precipitated with Et_2O, collected by filtration, dissolved in 10% AcOH and lyophilized. The detailed analytical data are presented in Supporting information.

2.4. CF methodology

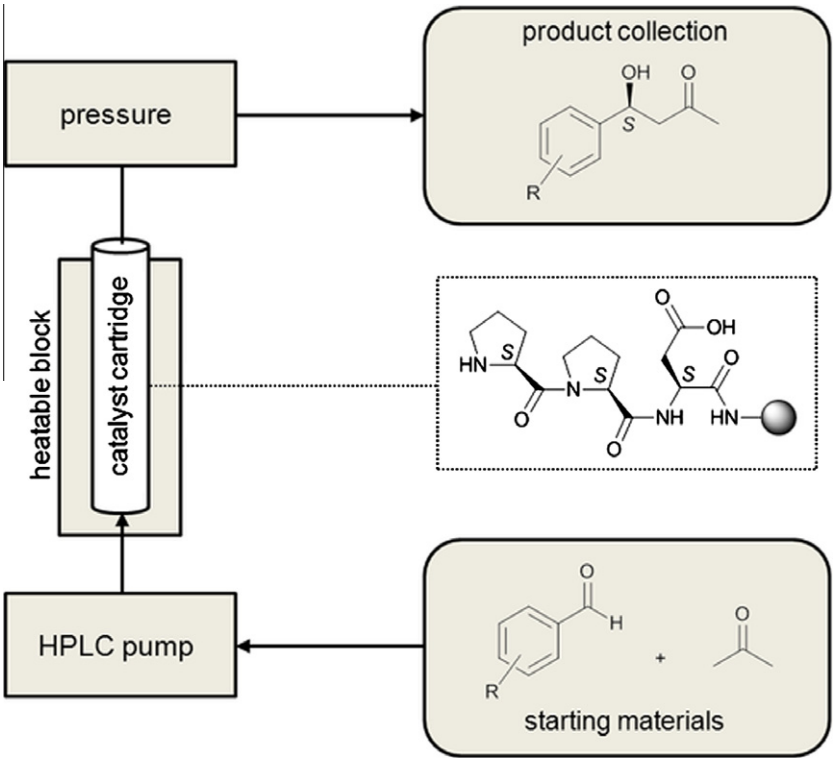
Flow experiments were performed in a dedicated high-pressure CF reactor with a fixed catalyst bed (H-Cube®, operated in "no H_2" mode). For the experiments 300 mg of the solid-supported peptide was incorporated into a replaceable stainless steel cartridge with internal dimensions of 70 × 4 mm. The filled cartridge was placed into a stainless steel block, which contains a Peltier heating system that can be heated to 100 °C. A back pressure valve was built in to ensure constant pressures up to 100 bar. The reaction mixture was pumped through the cartridge by means of an HPLC pump (Knauer WellChrom HPLC-pump K-120) at flow rates of 0.01–1.0 mL min^{-1}. This experimental setup allowed the systematical adjustment of the most important reaction parameters such as catalyst type, pressure, temperature, and flow rate in order to determine the optimal conditions. A brief outline of the CF catalytic system is presented in Scheme 2 [71].

2.5. General aspects of the preparation of aldol products in CF

For the CF method development, 20 mg (0.13 mmol, 1 eq) *p*-nitrobenzaldehyde (*p*NBA) and 0.9 mg (0.013 mmol, 0.1 eq) imidazole were dissolved in 5 mL acetone. The solution was homogenized by sonication for 3 min and was then pumped through the CF reactor under the appropriate conditions. The completion of the reaction was checked by TLC with a mixture of *n*-hexane/EtOAc as eluent. The crude aldol products were evaporated and then, if necessary, purified by column chromatography with a



Scheme 1. Synthesis of the heterogeneous catalyst H-Pro-Pro-Asp-NH-*resin*.



Scheme 2. A brief outline of the CF organocatalytic procedure.

mixture of *n*-hexane/EtOAc as eluent. The β-hydroxyketones were characterized by NMR spectroscopy and chiral NP-HPLC. The detailed analytical data can be found in Supporting information. Between two reactions in the CF reactor, the catalyst bed was washed for 10 min with acetone at 1 mL min^{-1}.

2.6. Measurement of the residence time on the catalyst bed

The residence time on the catalyst bed was determined by pumping an acetone solution of ink through the system and measuring the time that elapsed between the first contact of the

dye with the resin and the moment when a blue color appeared at the column output.

3. Results and discussion

3.1. Catalyst synthesis and immobilization

Employing a peptide as catalyst was the best possible choice as the catalyst synthesis and the immobilization can easily be combined in SPPS, thereby eliminating the need for further synthetic steps, and it offers the highest structural diversity. After the coupling steps, the deprotection of the carboxyl side chain was carried without cleavage of the peptide, as non-TFA-labile resins were used as support for the SPPS, which served further on as catalyst carrier (Fig. 1 and Scheme 1). This experimental setup is simple and reasonably economical, as there is no need for the time-consuming peptide work-up and purification steps. After thorough washing of the resin, the heterogenized organocatalyst was ready to use in CF. Currently, the most effective peptidic organocatalyst for aldol reactions is the tripeptide H-Pro-Pro-Asp-NH_2 [25], and we therefore chose this catalyst initially for CF method development.

3.2. Optimization of the reaction conditions

For optimization of the initial reaction conditions, the aldol reaction between *p*NBA and acetone was chosen as test reaction, with imidazole as base to prevent the formation of elimination side product. Acetone served not only as reagent, but also as solvent. As concerns the catalyst accessibility, appropriate swelling of the solid support is a crucial factor. In polar solvents, TentaGel resin swells better than PS-MBHA [72]; hence, the H-Pro-Pro-Asp-NH-*resin* catalyst was initially synthetized on TentaGel (catalyst **1**) (Fig. 2). For rapid fine-tuning of the aldehyde concentration in the reaction mixture, an initially high flow rate of 0.5 mL min^{-1}, RT and atmospheric pressure were applied. This quick screening indicated that the lower the amount of aldehyde in the reaction mixture, the higher the conversion (Fig. 3). A concentration of 4 mg mL^{-1} seemed to be a good compromise between conversion and productivity.

There are a number of examples in the literature where elevation of the pressure in organocatalytic procedures led to increased

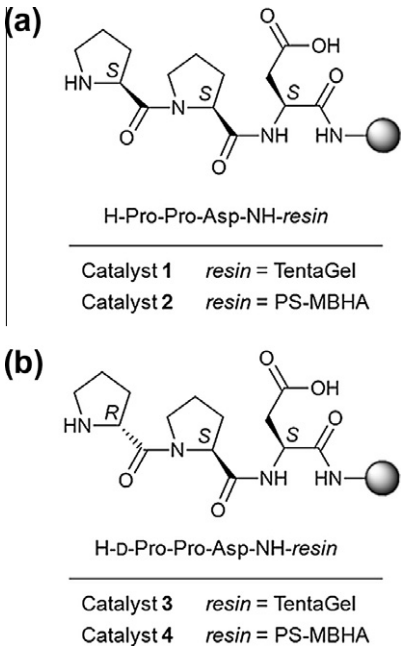


Fig. 2. Heterogeneous peptidic catalysts.

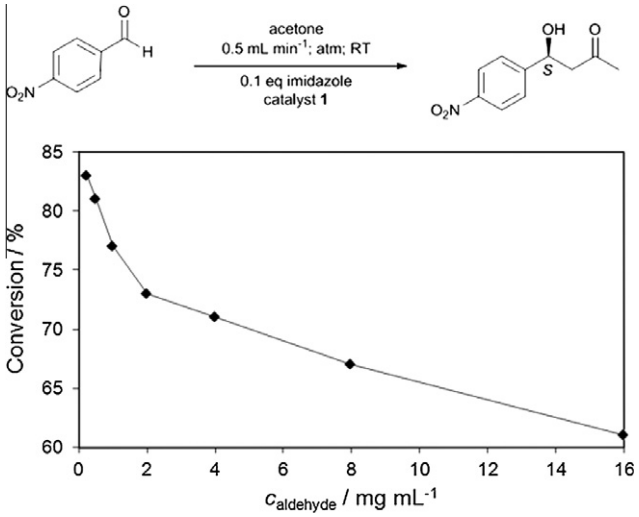


Fig. 3. Fine-tuning of the concentration of *p*NBA in the CF test reaction.

conversion and selectivity [73–76]. Hence, we also investigated the pressure dependence of the aldol reaction. Even at atmospheric pressure ($c_{aldehyde}$ = 4 mg mL^{-1}; flow rate = 0.5 mL min^{-1}; RT), a conversion of >70% and an *ee* of 80% could be achieved, but increase in the pressure resulted in still higher conversions up to an optimal 60 bar (Fig. 4). Further elevation to 100 bar was not beneficial, and the conversion remains steady at around 80%. (It is noteworthy that *ee* was not dependent on the pressure.) This phenomenon raises the question of whether the conversion is dependent on the catalyst activity itself or is influenced by the transport phenomena of the reactants in the matrix of the polymer. To probe the diffusion dependence, the Koros–Nowak test was performed [77,78]. When the catalyst loading in the cartridge was halved by simply using a mixture of 150 mg blank TentaGel resin and 150 mg catalyst **1**, the conversion of the same reaction decreased from 81% to 52%. The fall in the conversion is not proportional to the loading decrease. Consequently, the reaction is diffusion-controlled, and the effect of the elevated pressure is equivalent to an increase in the surface area of the catalyst.

To improve the conversion further, we tried elevating the temperature. It emerged that heating led to higher conversions, but also dramatically lowered *ee*. For example, at 80 °C, 60 bar and a flow rate of 0.5 mL min^{-1}, the conversion was nearly quantitative,

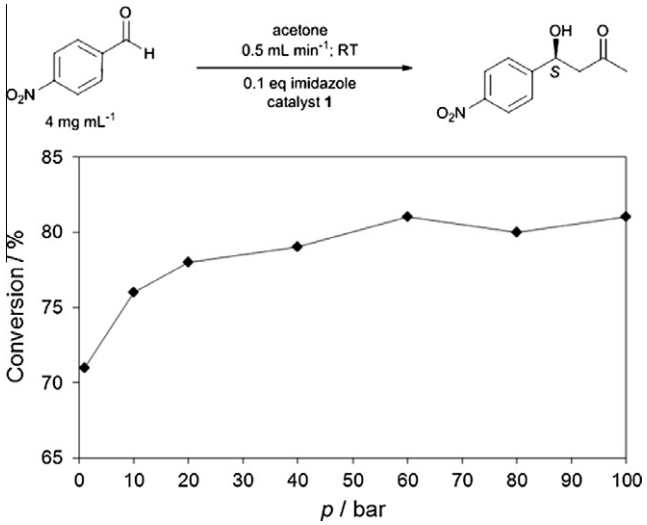


Fig. 4. Investigation of the pressure dependence of the test aldol reaction in CF.

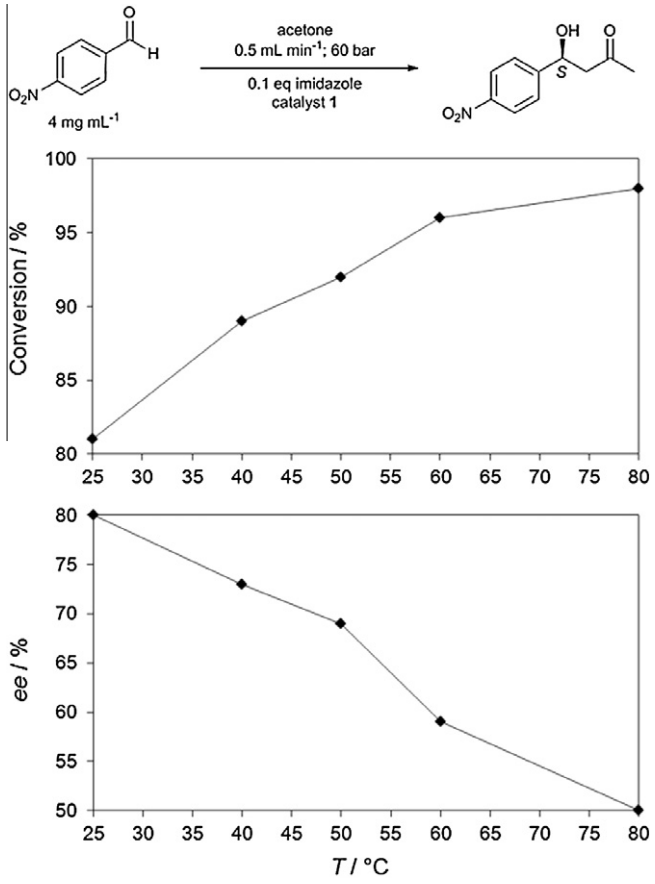


Fig. 5. Investigation of the temperature dependence of the test aldol reaction in CF.

but *ee* fell to 50% (Fig. 5). Thus, RT was regarded as optimal temperature.

In further parameter screening, the aim was to make the procedure efficient while maintaining high conversion and selectivity. Under the previously optimized reaction conditions, the flow rate was also fine-tuned for optimization of the residence time on the catalyst bed; the longer the residence time, the higher the conversion. When the flow rate was reduced to the optimal 0.1 mL min^{-1}, the residence time was long enough to achieve quantitative conversion and 80% *ee*. Even at 1 mL min^{-1}, the conversion was still nearly 60% (Fig. 6). It is worth mentioning that the *ee* was not dependent on the flow rate.

For additional optimization, the peptidic catalyst too was fine-tuned. As a solid support of the H-Pro-Pro-Asp-NH-*resin*, PS-MBHA (catalyst **2**) was tested under the previously optimized conditions. Its poorer swelling properties in acetone resulted in lower conversion and *ee* than with catalyst **1** (Table 1, entry 4). H-D-Pro-Pro-Asp-NH_2 is an effective catalyst for the 1,4-addition of aldehydes to nitroolefins [26]. We were interested in its efficiency in aldol reactions and tested the effect of replacement of the N-terminal L-Pro by D-Pro. H-D-Pro-Pro-Asp-NH-*resin* was synthetized on TentaGel (catalyst **3**) and also on PS-MBHA (catalyst **4**) as solid support, but in both cases, the conversion and *ee* were dramatically less than with catalyst **1** (Table 1, entries 5 and 6). The structures of the utilized catalysts are depicted in Fig. 2.

3.3. Results of the test reaction and comparison with the batch data

Under the overall optimized reaction conditions, with catalyst **1**, the corresponding β-hydroxyketone product of the test reaction was obtained in a yield of >99% with 80% *ee* (Table 1, entry 2).

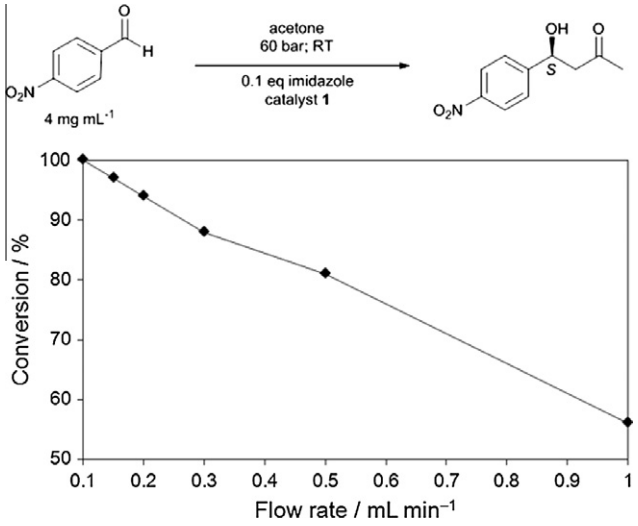


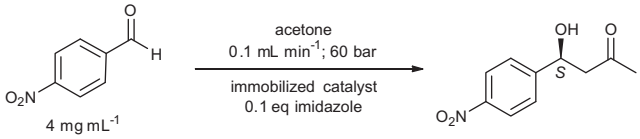
Fig. 6. Fine-tuning of the flow rate in the test CF reaction.

When the reaction was performed at 4 °C instead of RT, *ee* increased to 85%, while the isolated yield was still around 90% (Table 1, entry 3). It is noteworthy that no self-aldol or H_2O-eliminated side product was observed at all. At the optimal flow rate of 0.1 mL min^{-1}, the residence time on the catalyst bed was only 6 min, and it took 50 min to pump through a 5-mL aliquot of the reaction mixture, leading to around 27.5 mg crude product. When the same solid-supported catalyst was used for the aldol reaction between *p*NBA and acetone in a simple flask, a reaction time of 6 h was needed for completion (Table 1, entry 1), and a somewhat lower yield and *ee* were achieved than in CF [32]. When other Pro-derived organocatalysts were employed in batch, the reaction times were even longer (Table S1, entries 1–14). It must be noted here that literature batch reactions were usually carried out in a larger volume than in our small-scale test experiments, but scaling up in CF is straightforward through extension of the reactor size. On collection of the solution of the crude product material, no work-up or purification steps were needed, which further enhances the efficacy of the described method. These promising results are due to the beneficial features of the utilized technique: the application of CF, the swellable polymer-supported peptidic catalyst, and the high local catalyst concentration in the catalyst bed and the high pressure.

3.4. Testing of the catalyst reusability

The efficacy of a reaction mediated by a solid-supported catalyst may be characterized by the degree of reusability of the catalyst. Accordingly, the test aldol reaction between *p*NBA and acetone was repeated under the optimized reaction conditions, the same portion of catalyst **1** loaded in the cartridge being recycled. It was found that after the 20th consecutive experiment, the conversion was still quantitative and *ee* was 80%, just as in the first reaction (Table 2). In each run, a 5-mL aliquot of the reaction mixture was pumped through the system in 50 min, leading to around 27.5 mg crude product without further purification. When all of the test reactions involved in the catalyst recycling study were taken into account, the finding was that, after nearly 17 h of persistent use under optimal flow conditions, the immobilized peptidic catalyst was still as active as initially. The described CF technique is therefore prominently robust. In order to determine the turnover number (TON) of the immobilized catalyst, the experiment was run further after the recycling study, utilizing the same

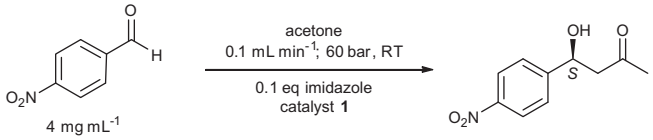
Table 1
Fine-tuning of the catalyst for the aldol reaction between *p*NBA and acetone under the optimized flow conditions, and comparison of the CF results with a batch reference from the literature.



Entry	Process	Catalyst	*T* (°C)	Time (min)[a]	Conv. (%)[b]	Yield (%)[c]	*ee* (%)[d]
1[e]	Batch	**1**	RT	360	n.d.	94	78
2	CF	**1**	RT	6	Quant.	>99	80
3	CF	**1**	4	6	90	89	85
4	CF	**2**	RT	6	71	68	62
5	CF	**3**	RT	6	29	27	−26[f]
6	CF	**4**	RT	6	19	16	−18[f]

[a] Reaction time of the batch experiment, residence time of the CF reactions.
[b] Determined by ^{1}H NMR spectroscopic analysis of the crude material.
[c] Yield of isolated product.
[d] Determined by chiral-phase HPLC analysis.
[e] Batch reference from the literature [32].
[f] Absolute configuration of the resulting β-hydroxyketone inverted to *R*.

Table 2
Testing of the reusability of catalyst **1** in CF under optimal conditions.



Entry	Cycle	Conv. (%)[a]	Yield (%)[b]	*ee* (%)[c]
1	1–5	98-quant.	97–>99	79–80
2	6–10	Quant.	97–>99	78–81
3	11–15	Quant.	>99	79–80
4	16–20	99-quant.	98–>99	79–80

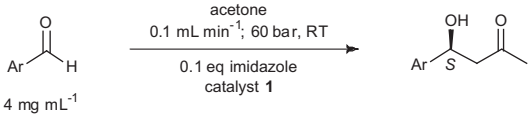
[a] Determined by ^{1}H NMR spectroscopic analysis of the crude material.
[b] Yield of isolated product.
[c] Determined by chiral-phase HPLC analysis.

portion of resin and the optimized CF conditions, with continuous pumping-through of a 4 mg mL^{-1} solution of *p*NBA and 0.1 eq imidazole in acetone [79]. The CF conditions resulted in a TON of 710 [80], whereas the TON calculated from the batch data was 472 [32].

3.5. Investigation of the scope and applicability of the method

In order to verify the scope and applicability of the described CF method, a number of further aldol reactions between various aromatic aldehydes and acetone were carried out under the optimized conditions with catalyst **1**. The data in Table 3 (entries 1–8) demonstrate that, for aldehydes with an electron-withdrawing group on the aromatic ring, good or excellent yields (68–>99%) and high *ee* (75–80%) were obtained. When the conversion was quantitative, no further work-up or purification steps were needed (Table 3, entries 1–4). The yield increased with the electron-withdrawing capability of the substituting residue and was dependent on the position of the electron-withdrawing group: in the aldol reaction between *o*-chlorobenzaldehyde and acetone, the yield was >99%, but for *m*- and *p*-chlorobenzaldehyde, it was lower. Aldehydes bearing an electron-donating substituent on the aromatic ring or no substituent at all proved to be weaker reaction partners in the aldol reaction with acetone. Lower yields (27–59%), but still high

Table 3
Investigation of the scope and applicability of the CF organocatalytic procedure under the overall optimized reaction conditions.



Entry	Ar[a]	Productivity[b]	Conv. (%)[c]	Yield (%)[d]	*ee* (%)[e]
1	*p*-$NO_2C_6H_4$	1.96	Quant.	>99	80
2	*o*-$NO_2C_6H_4$	1.96	Quant.	>99	79
3	*p*-NCC_6H_4	2.26	Quant.	>99	74
4	*o*-ClC_6H_4	2.11	Quant.	>99	79
5	*m*-ClC_6H_4	1.50	78	71 (5)[f]	76
6	*p*-ClC_6H_4	1.48	80	70 (7)[f]	78
7	*p*-BrC_6H_4	1.09	76	68 (6)[f]	80
8	*p*-FC_6H_4	2.05	Quant.	86 (13)[f]	79
9	*o*-$MeOC_6H_4$	0.81	39	37	76
10	C_6H_5	1.65	76	59 (13)[f]	70
11	2-Naphthyl	0.57	37	30 (5)[f]	75
12	1-Naphthyl	0.51	35	27 (4)[f]	71

[a] A 5-mL aliquot of the solution of the starting material was pumped through in 50 min.
[b] In mmol of pure isolated product (mmol$^{-1}_{resin}$ h^{-1}).
[c] Determined by ^{1}H NMR spectroscopic analysis of the crude material.
[d] Yield of isolated product.
[e] Determined by chiral-phase HPLC analysis.
[f] Yield of the corresponding dehydration product.

ee (70–76%), were obtained. No side reaction of self-aldol product formation occurred, but in several cases, H_2O elimination from the resulting β-hydroxyketone was observed. These yield and *ee* values are competitive with those of the standard batch procedures (for comparison, a number of batch references are listed in Table S1), and the productivities were excellent in almost all cases (Table 3).

4. Conclusions

We have developed a heterogeneous catalytic CF method for asymmetric aldol reactions utilizing a solid-supported peptide as organocatalyst. The peptide was synthetized by SPPS and immobilized in the same step. The lack of peptide cleavage has the results

that no work-up and no purification are necessary, and there is no product loss. After optimization of the reaction conditions and the peptidic catalyst, β-hydroxyketone products were obtained in high yields and stereoselectivities comparable with literature batch results. The residence time on the catalyst bed was as low as 6 min and, due to further beneficial features of the technique, promisingly high productivities were achieved. The peptidic catalyst is highly recyclable, so that the procedure is exceedingly robust, while the ease of product isolation and the possibility of facile scale-up further enhance the efficacy of the described method. Heterogeneous catalysis is therefore a useful tool for broadening the scope of flow chemistry.

Acknowledgments

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jcat.2012.08.006.

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[80] The immobilized catalyst was not totally deactivated after 80 h of persistent use. Thus, TON was estimated by fitting a second-order function to the 'time'–'mmol of pure isolated product' data set relationship.

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Alkyne–Azide Cycloadditions with Copper Powder in a High-Pressure Continuous-Flow Reactor: High-Temperature Conditions versus the Role of Additives

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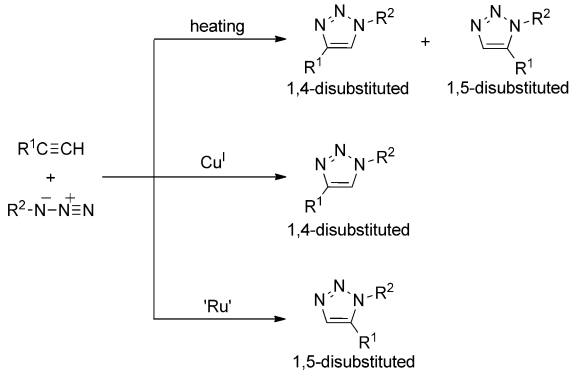
Abstract: A safe and efficient flow-chemistry-based procedure is presented for 1,3-dipolar cycloaddition reactions between organic azides and acetylenes. This simple and inexpensive technique eliminates the need for costly special apparatus and utilizes Cu powder as a plausible Cu^{I} source. To maximize the reaction rates, high-pressure/high-temperature conditions are utilized; alternatively, the harsh reaction conditions can be moderated at room temperature by the joint application of basic and acidic additives. A comparison of the performance of these two approaches in a series of model reactions has resulted in the formation of useful 1,4-disubstituted 1,2,3-triazoles in excellent yields. The risks that are associated with the handling of azides are lowered, thanks to the benefits of flow processing, and gram-scale production has been safely implemented. The synthetic capability of this continuous-flow technique is demonstrated by the efficient syntheses of some highly functionalized derivatives of the antifungal cispentacin.

Keywords: alkynes · azides · click chemistry · continuous-flow reactors · copper

Introduction

The Huisgen 1,3-dipolar cycloaddition reaction of organic azides with acetylenes is a simple route to useful 1,2,3-triazoles.[1] Following thermal induction, this reaction results in an approximate 1:1 mixture of 1,4- and 1,5-disubstituted triazole isomers.[2] Owing to the pioneering work of Sharpless and co-workers[3] and Meldal and co-workers[4] on copper catalysis, the Huisgen reaction has captured enormous interest in the field of synthetic chemistry. In contrast with the thermal route, the Cu^{I}-catalyzed azide–alkyne cycloaddition (CuAAC) reaction is regioselective, thereby exclusively giving the 1,4-regioisomer within a short reaction time (Scheme 1). The selective formation of 1,5-disubstituted 1,2,3-triazoles through ruthenium catalysis has also been described,[5] but the broad applicability, reliability, and efficiency of CuAAC has led to it becoming the standard of the "click chemistry" concept. Applications of CuAAC can increasingly be found in many areas of modern chemistry, such as polymer and materials sciences,[6] supramolecular chemistry,[7] bioconjugation,[8] and combinatorial chemistry.[9] The CuAAC reaction is also significant from the viewpoint of drug discovery,[10] because 1,2,3-triazoles possess a wide range of biological properties, such as antiviral (**1**),[11] antibacterial (**2**),[12] antifungal (**3**),[13] and anticancer activities (**4**; Scheme 2).[14] The 1,2,3-triazole moiety is extensively used in medicinal chemistry as a pharmacophore to modify known bioactive molecules and to potentiate their biological activities. For example, the 1,2,3-triazole analogues of antiviral agent zanamivir (**5**)[15] and the well-known bioactive carbanucleoside neplanocin A (**6**) exhibit notable antiviral activity (Scheme 2).[16]



Scheme 1. 1,3-Dipolar cycloaddition reactions between organic azides and acetylenes, which result in disubstituted triazoles.

Flow-chemistry techniques are currently enjoying an upsurge in interest because they offer great advantages over traditional batch-based synthetic procedures.[17] The well-regulated continuous-flow (CF) reactor concept allows the efficient mixing of substrates and faster heat and mass transfer,[18] so that reactions can be performed with an unprecedented level of control within short reaction times.[19] Be-

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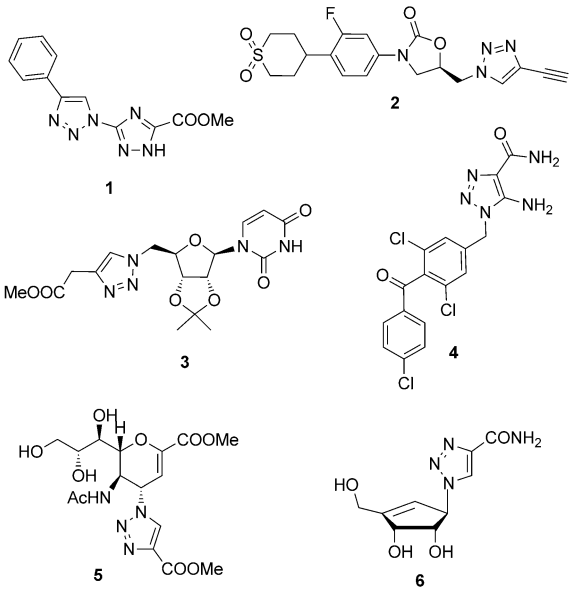


Scheme 2. Some examples of bioactive 1,2,3-triazoles.

cause the need for the large-scale use of reagents and solvents is eliminated, the screening of the reaction conditions becomes simple, time- and cost-efficient, and safe.[20] These benefits facilitate rapid library synthesis and afford an opportunity for automatization and excellent transferability between laboratory-based investigations and subsequent production scales.[21]

A number of CF strategies for CuAAC reactions have been reported. The most popular sources of Cu^I for CF methods are heterogeneous approaches that employ copper-in-charcoal (Cu/C),[22] immobilized Cu^I species,[23] or copper flow-reactor technology without extraneous Cu^I salts,[24] etc. In contrast, Hessel and co-workers recently reported an efficient homogeneous approach for CF CuAAC reactions, by using $[Cu(phen)(PPh_3)_2]NO_3$ as a catalyst.[25]

To improve the practical applicability of CF CuAAC procedures, we set out to develop a simple and inexpensive technique that could eliminate the need for costly apparatus or special catalyst types, whilst at the same time being safe and efficient. Thus, we used Cu powder as the cheapest available source of Cu^I and exploited the merits of flow processing by increasing the reaction rates with high-pressure/high-temperature conditions; we were subsequently able to moderate these harsh reaction conditions through the use of both basic and acidic additives at room temperature. The results that were obtained by using these two methods were compared for a series of model CuAAC reactions. The risks that are associated with the handling of explosive azides were minimized as a consequence of the beneficial features of CF technology. We also applied this method to the synthesis of highly functionalized derivatives of the antifungal cispentacin, (1*R*,2*S*)-2-aminocyclopentanecarboxylic acid, which is an interesting precursor for new triazole carbanucleosides. Scale-up experiments were also performed, thus achieving gram-scale syntheses.

Results and Discussion

Method

The CF experiments were performed in a dedicated high-pressure/high-temperature reactor (H-Cube, operated in "no H_2" mode) that consisted of a replaceable stainless steel cartridge (internal dimensions: 70 mm × 4 mm) as a catalyst bed, a Peltier heating system to heat the cartridge up to 100 °C, and a pressure-control unit to ensure constant pressures of up to 100 bar (Figure 1).[26] The reaction mixture was pumped through the cartridge by using a HPLC pump (Knauer WellChrom HPLC-pump K-120). The experimental setup allowed the safe application of high-pressure/high-temperature conditions for unstable reactants, such as azides, without the potential hazard of an explosion, because only limited amounts of the reactants were exposed to the zone of harsh reaction conditions and, even then, only for a safely controllable, short amount of time. Fine-tuning of the most important reaction parameters, such as pressure, temperature, and flow rate, is simple and efficient, thus making the whole screening process promisingly rapid.

Cu powder was utilized as a simple and cheap source of Cu^I. When exposed to air, Cu metal undergoes constant oxidation, with the formation of non-self-protecting layers of different Cu oxides, such as Cu_2O.[27] Thus, Cu powder can be regarded as a "supported" Cu^I catalyst. Accordingly, if the oxide layers are removed from the Cu surface, the catalytic activity decreases significantly.[22b] For the flow experiments, Cu powder (900 mg) with an average particle size of about 200 μm was incorporated into the catalyst cartridge.

Optimization of the Reaction Conditions

To achieve the maximum conversion within the shortest possible process time, the most important reaction conditions (pressure, temperature, flow rate, and additives) were systematically fine-tuned. For the optimization studies, the 1,3-dipolar cycloaddition reaction between benzyl azide and phenylacetylene was chosen as a test reaction (Scheme 3).

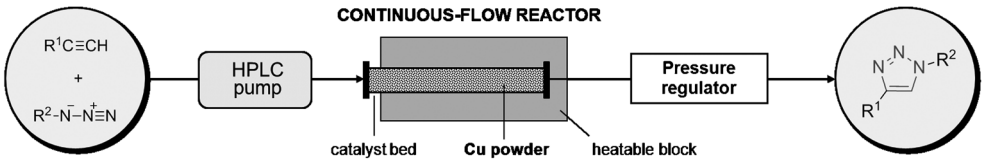
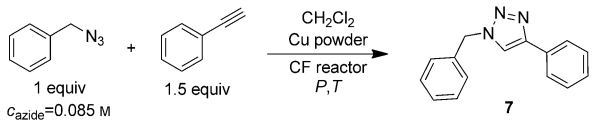


Figure 1. Experimental setup for the CF CuAAC procedure.



Scheme 3. 1,3-Dipolar cycloaddition reaction between benzyl azide and phenylacetylene as a test reaction for optimization of the CF reaction conditions.

On the basis of literature data, CH_2Cl_2 was used as the solvent.[28] To achieve maximum productivity, we planned to apply the highest possible azide concentration and 0.085 M was found to be the most appropriate: Higher concentrations led to the precipitation of the triazole product in the CF reactor. In small-scale test experiments, aliquots (2.5 mL) of a reaction mixture that contained 1 equivalent of benzyl azide and 1.5 equivalents of phenylacetylene were pumped through the system in each run.

First, we set out to maximize the rate of the test reaction under high-pressure/high-temperature conditions. Thus, the pressure was increased from atmospheric pressure to 100 bar, whilst the temperature and flow rate were kept constant at room temperature and 0.5 mL min^{-1}, respectively. At atmospheric pressure, a conversion of only 20% could be achieved and the pressure had to be increased to >80 bar to obtain higher conversion (Figure 2). At 100 bar, a conversion of 34% was achieved, which was taken as the optimal value. Elevation of the pressure not only increased the rate of triazole production, in accordance with Le Chatelier's principle, but also allowed the use of higher temperatures without the solvent boiling over. In this way, the system could be heated to well above the boiling point of CH_2Cl_2, whilst the pressure and flow rate were maintained at 100 bar and 0.5 mL min^{-1}, respectively. Figure 3 shows that the conversion started to increase sharply at about 40 °C; at 50 °C, the conversion exceeded 90% and, at 100 °C, practically quantitative conversion was obtained.

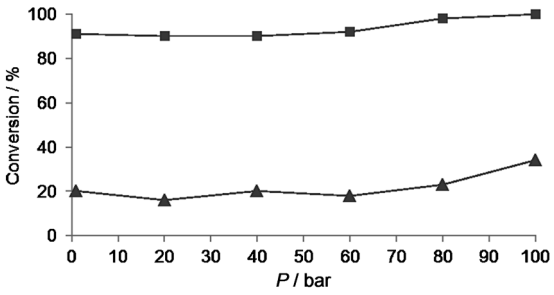


Figure 2. Investigation of the pressure dependence of the test reaction between benzyl azide and phenylacetylene under CF: ▲ room temperature, flow rate: 0.5 mL min^{-1}, no additives; ■ room temperature, flow rate: 0.5 mL min^{-1}, DIEA (0.04 equiv)+HOAc (0.04 equiv).

The fine-tuning of the flow rate is related to the optimization of the residence time on the catalyst bed. Longer residence times lead to higher conversions, but also involve longer process times. Because quantitative conversion was obtained under the previously optimized conditions (100 bar, 100 °C) at a flow rate of 0.5 mL min^{-1}, it was unnecessary to decrease the flow rate. However, when we attempted to decrease the residence time on the catalyst bed by increasing the flow rate, we found that the conversion decreased dramatically. For example, at a flow rate of 1 mL min^{-1}, the conversion was only 31% and, when the flow rate was increased to as high as 3 mL min^{-1}, the conversion fell sharply to about 10% (Figure 4).

Figure 3. Investigation of the temperature dependence of the test reaction at 100 bar between benzyl azide and phenylacetylene in CF (flow rate: 0.5 mL min^{-1}) without any additives.

It is well-known that the application of amines as basic additives can boost the reactivity of the CuAAC reaction considerably.[29] The amine serves as a ligand to liberate the catalytically active Cu^I species from its matrix by coordinating to it and accelerating the formation of a distinct Cu–alkyne complex (Scheme 4).[3,28,30] It was recently demonstrated that catalytic amounts of certain acids could further improve the rate of the reaction through acceleration of the conversion of intermediates that contained C–Cu bonds by protonation (Scheme 4).[28,31] With the aim of moderating the harsh reaction conditions, we set out to check the effects of both basic and acidic additives on the rate of the test CuAAC reaction at room temperature. On the basis of literature data, *N*,*N*-diisopropylethylamine (DIEA) and HOAc were chosen as the basic and acidic additives, respectively.[28,31] With no additives, at 100 bar, room temperature, and

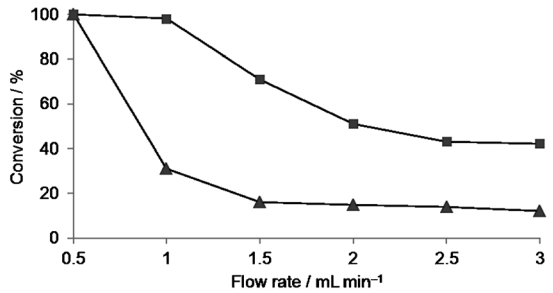
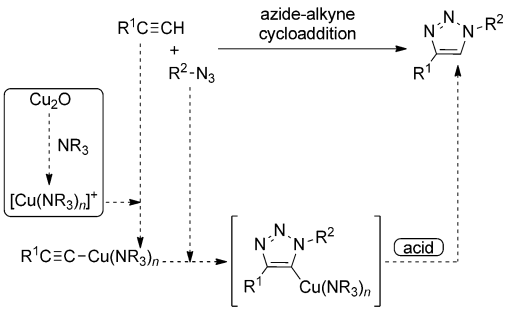


Figure 4. Fine-tuning of the flow rate in the test reaction between benzyl azide and phenylacetylene under high-pressure/high-temperature and high-pressure/RT conditions with additives in CF: ▲ 100 bar, 100 °C, no additives; ■ 100 bar, room temperature, DIEA (0.04 equiv)+HOAc (0.04 equiv).



Scheme 4. Catalytic cycle of the CuAAC reaction, jointly promoted by an acid and a base.[3,28,30,31]

a flow rate of 0.5 mL min^{-1}, a conversion of 34% could be achieved (Table 1, entry 1). Upon the addition of 0.1 equivalents of HOAc, with maintenance of the same pressure, temperature, and flow rate, the conversion improved to 56% (Table 1, entry 2) and, when 0.1 equivalents of DIEA were used, the conversion rose further to 96% (Table 1, entry 3). The best result was obtained with both additives together: With DIEA (0.1 equiv)+HOAc (0.1 equiv), the reaction proceeded quantitatively (Table 1, entry 4). Next, we examined the effect of the amount of DIEA+HOAc on the reaction rate. The addition of 0.04 equivalents of each additive was sufficient to obtain quantitative conversion (Table 1, entries 5 and 6); however, lower amounts gave lower conversions (Table 1, entries 7 and 8). The pressure dependence of the reaction with the use of additives was also investigated. We found that lowering the pressure from 100 bar to atmospheric pressure led to a moderate decrease in conversion (Figure 2). Consequently, elevated pressure is needed to obtain good conversions. When the flow rate was increased, to decrease the residence time on the catalyst bed, at 100 bar and room temperature, with DIEA (0.04 equiv)+HOAc (0.04 equiv), we found that the drop in the conversion was not as steep as under the high-pressure/high-temperature conditions (Figure 4). This result means that the joint use of basic and acidic additives can successfully relive the harsh reaction conditions and appreciably enhance the efficiency of the flow process.

Table 1. Effect of the DIEA/HOAc ratio on the reaction between benzyl azide and phenylacetylene in a CF reactor.[a]

Entry	DIEA [equiv]	HOAc [equiv]	Conversion[b] [%]
1	0	0	34
2	0	0.1	56
3	0.1	0	96
4	0.1	0.1	quantitative
5	0.08	0.08	quantitative
6	0.04	0.04	quantitative
7	0.02	0.02	63
8	0.01	0.01	40

[a] 100 bar, room temperature, flow rate: 0.5 mL min^{-1}. [b] Determined by ^{1}H NMR spectroscopy of the crude material.

As a result of this screening process, we selected two distinct parameter sets as our optimal conditions: 1) 100 bar, 100 °C, flow rate: 0.5 mL min^{-1}, without any additives (conditions CF **A**) and 2) 100 bar, room temperature, flow rate: 0.5 mL min^{-1}, with DIEA (0.04 equiv)+HOAc (0.04 equiv) (conditions CF **B**). Both sets of conditions afforded quantitative conversion in the test CuAAC reaction between benzyl azide and phenylacetylene and selectively gave the 1,4-disubstituted 1,2,3-triazole isomer (**7**) as the product. These small-scale test experiments afforded about 50 mg of triazole **7** in each run, without the need for further purification after evaporation. At the optimal flow rate of 0.5 mL min^{-1}, the residence time on the catalyst bed was as low as 1.5 min and a process time of only 5 min was needed to pump through the 2.5 mL aliquot of the reaction mixture.

Scale-Up Experiments

One of the most appealing advantages of flow processing is its inherent scalability, which means that, in CF production, the volume is given as a function of time and flow rate, whereas, in standard flask-based (batch) processes, the output depends on the batch size.[21b,d,e] The batch scale-up of processes that involve unstable reactants, such as azides, can be dangerous, because the accumulation of high concentrations of these materials can give rise to an explosive hazard, whereas, in CF processes, this risk is minimized because the residence time in the active zone of the reactor is nicely controllable and the procedure remains simple and safe, even upon scale-up.

To probe the preparative abilities of our above-described CF methods, the CuAAC reaction between benzyl azide and phenylacetylene was scaled-up under both conditions CF **A** and CF **B**. A reaction mixture of the azide (1 equiv, $c_{azide} = 0.085$ M), the alkyne (1.5 equiv), and DIEA (0.04 equiv)+HOAc (0.04 equiv, only under conditions CF **B**) in CH_2Cl_2 was continuously pumped through the system. In both experiments, 75 mL of the reaction mixture was pumped through in 150 min, thus leading to the formation of about 1.5 g of triazole **7**, which was equivalent to a yield of 99%. This result meant that, altogether, almost 3 g of the triazole product could be isolated (after evaporation) within 5 h, without the need for any further work-up or purification steps, in a simple and safe manner.

Investigation of the Scope and Applicability of these CF Methods

To determine the scope of these CF procedures, a series of model reactions were performed, some of which afforded possible precursors of biologically useful compounds. These reactions were performed under both sets of optimal conditions (CF **A** and CF **B**) and the efficacies of these methods were then compared. In all cases, when terminal alkynes were used, their corresponding 1,4-disubstituted 1,2,3-triazole isomers were formed selectively.

Outstanding results were obtained in the reactions between phenylacetylene and various azides. As shown in Table 2, both aromatic and aliphatic azides were found to be excellent substrates for the CF CuAAC reaction. In the case of aromatic azides, we found that both electron-withdrawing (Table 2, entries 2–7) and electron-donating groups (Table 2, entries 8–10) on the phenyl rings were nicely tolerated and the yields were not significantly affected by the position of the substituent(s). α-Azido ketones are known to be less reactive than simple azides in CuAAC reactions, but their cycloaddition reactions with different alkynes can lead to a diverse range of compounds of pharmaceutical importance.[32] Thus, the reaction of 2-azidoacetophenone with phenylacetylene was also studied (Table 2, entry 13) and we found that both CF methods afforded triazole **19** in excellent yields. Interestingly, the use of additives (conditions CF **B**) gave slightly better results in terms of the azide scope and, in certain cases, somewhat higher yields were afforded than with method CF **A** (Table 2, entries 2, 5, 7–9, and 11–13).

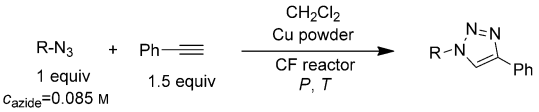
As shown in Table 3, a series of alkynes were also tested as reaction partners of benzyl azide. We found that, besides phenylacetylene, non-aromatic alkynes, such as pent-1-yne and ethyl propiolate, performed well in the CF CuAAC reaction (Table 3, entries 1 and 2). A non-terminal alkyne, diethyl acetylenedicarboxylate, was also successfully utilized as a dipolarophile, thus giving a high yield of 1,4,5-trisubstituted triazole **24** (Table 3, entry 3), which is known to be a potent antitubercular agent.[33] Ferrocene-substituted biomolecules are of increasing interest in medicinal chemistry:[34] The labeling of biomolecules with ferrocene has been extensively used for electrochemical detection or for immunoassays[35] and the incorporation of ferrocene into amino acids or peptides has received considerable attention in the investigation of the secondary structures of different peptides.[36] Thus, the scope of this method was further broadened by the reaction between benzyl azide and ethynyl ferrocene. The corresponding ferrocenyl triazole model compound (**25**) was obtained in excellent yield (99%) with both CF methods (Table 3, entry 4). In general, conditions CF **A** and CF **B** performed equally well in tests of the alkyne scope.

Notably, when the conversion was quantitative and any excess alkyne could be volatilized on evaporation (phenylacetylene, pent-1-yne, and ethyl propiolate), no further work-up or purification step was needed.

Batch and CF Syntheses of Highly Functionalized Cispentacin Derivatives

Cispentacin is a naturally occurring carbocyclic β-amino acid that possesses strong antifungal properties.[37] Several other related alicyclic β-amino acid derivatives (e.g., icofungipen, oryzoxymycin, etc.) have also been reported to be antibacterial agents.[38] Carbocyclic and heterocyclic β-amino acids are key elements of a series of bioactive products with antitumoral, antibacterial, or antiviral activities.[39]

Table 2. Model reactions between phenylacetylene and various azides under optimized conditions in a CF reactor.



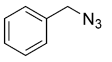
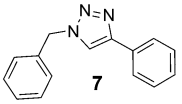
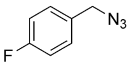
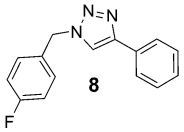
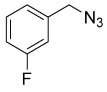
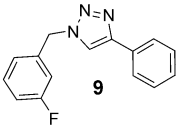
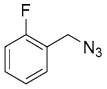
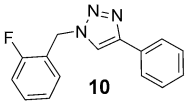
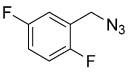
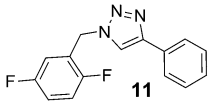
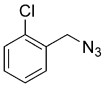
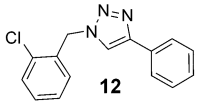
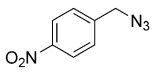
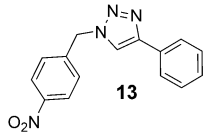
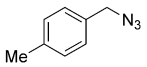
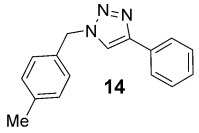
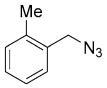
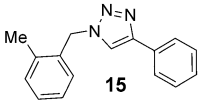
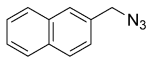
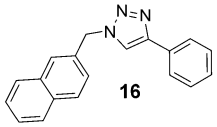
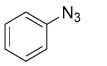
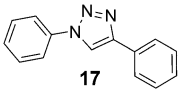
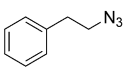
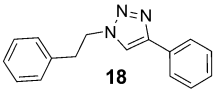
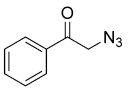
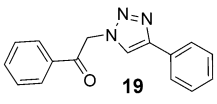
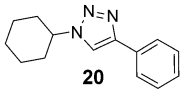
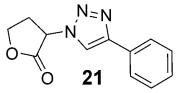
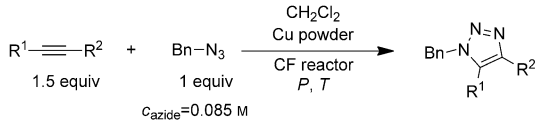
Entry	Azide	Product	Yield [%][a] CF **A**[b]	CF **B**[c]
1		 **7**	99	99
2		 **8**	98	99
3		 **9**	99	99
4		 **10**	99	99
5		 **11**	93	99
6		 **12**	99	99
7		 **13**	94	99
8		 **14**	83	99
9		 **15**	72	94
10		 **16**	99	99
11		 **17**	94	99
12		 **18**	77	98
13		 **19**	96	99

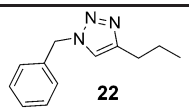
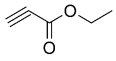
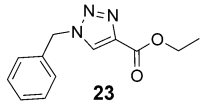
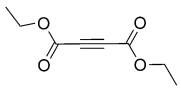
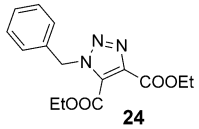
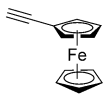
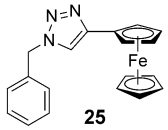
Table 2. (Continued)

Entry	Azide	Product	Yield [%][a] CF **A**[b]	CF **B**[c]
14		 **20**	99	99
15		 **21**	99	99

[a] Yield of isolated product. [b] Conditions CF **A**: 100 bar, 100 °C, flow rate: 0.5 mL min^{-1}, without any additives. [c] Conditions CF **B**: 100 bar, room temperature, flow rate: 0.5 mL min^{-1}, with DIEA (0.04 equiv)+HOAc (0.04 equiv).

Table 3. Model reactions between benzyl azide and various alkynes under optimized conditions in a CF reactor.



Entry	Acetylene	Product	Yield [%][a] CF **A**[b]	CF **B**[c]
1		 **22**	94	91
2		 **23**	99	99
3		 **24**	84	82
4		 **25**	99	99

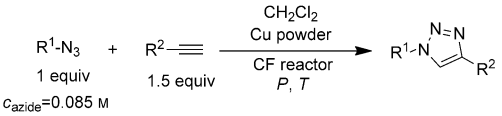
[a] Yield of isolated product. [b] Conditions CF **A**: 100 bar, 100 °C, flow rate: 0.5 mL min^{-1}, without any additives. [c] Conditions CF **B**: 100 bar, room temperature, flow rate: 0.5 mL min^{-1}, with DIEA (0.04 equiv)+HOAc (0.04 equiv).

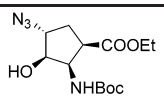
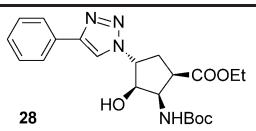
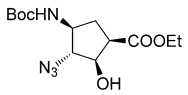
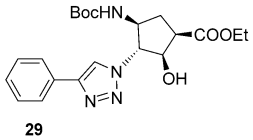
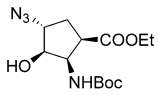
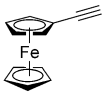
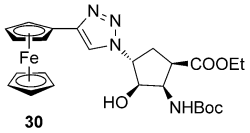
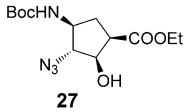
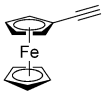
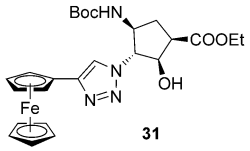
We investigated the application of our CF CuAAC procedures (CF **A** and CF **B**) for the synthesis of potentially bioactive 1,2,3-triazole-modified cispentacin derivatives, in comparison with the results that were obtained by using standard batch techniques. In all cases, the 1,4-disubstituted triazole isomers were formed selectively. To prepare triazole-modified cispentacins, either under batch conditions or by using CF methods, the previously synthetized azido-substituted cispentacins (**26** and **27**) were used as reaction partners of phenylacetylene or ethynyl ferrocene.[40] Compounds **26** and **27** were racemates; the structures in Table 4 show their relative stereochemistry. In contrast to the reactions with ethyl propiolate or diethyl acetylenedicarboxylate,[41] the batch reactions of phenylacetylene with azido esters **26** or **27** under thermal conditions did not result in the desired triazole derivatives (**28** and **29**, respectively). Thus, the use of a Cu^{I} catalyst was necessary. In the presence of CuI in MeCN at reflux for 12 h, the reactions afforded triazole-substituted cispentacin derivatives **28** and **29** in only moderate yields (69% and 73%, respectively; Table 4, entries 1 and 2). For both triazoles, method CF **A** resulted in yields that were comparable with those of the batch processes, but, under conditions CF **B**, the yields were significantly higher (99% in both cases) and both triazoles **28** and **29** could be obtained in their pure form without the need for any further work-up steps after evaporation (Table 4, entries 1 and 2). We measured and compared the copper content in unpurified triazoles **28** and **29** that were synthetized by using both methods (CF **A** and CF **B**). The analytical data in Table 5, entries 1–4, showed that the level of copper contamination in the samples that were obtained without any additives (method CF **A**) were lower than those when DIEA+HOAc were jointly applied (method CF **B**).

Next, azido-substituted cispentacin derivatives **26** and **27** were subjected to dipolar cycloaddition with ethynyl ferrocene in batch processes in the presence of CuI at reflux in MeCN. Surprisingly, no transformations were detected under these conditions. On changing the catalyst from CuI to $CuSO_4$/ascorbic acid, the reaction furnished ferrocenyl triazole derivatives **30** and **31** in moderate yields (51% and 69%, respectively) within 14 h (Table 4, entries 3 and 4). In contrast, under conditions CF **A** or CF **B**, triazoles **30** and **31** were obtained in excellent yields (99%) in both cases (Table 4, entries 3 and 4). Because the excess ethynyl ferrocene was not volatilized on evaporation, purification of the crude product by column chromatography on silica gel was required after both CF reactions. In the cases of triazoles **30** and **31**, their copper content was determined after purification. As shown in Table 5, entries 5–8, the levels of copper impurities in both methods decreased considerably after flash column chromatography.

Thus, method CF **B** was found to be more efficient for the synthesis of triazole-modified cispentacin derivatives. However, a direct comparison with the batch results is difficult, because the local copper concentration in the flow technique is much higher than that in a simple flask reaction and the batch reactions were performed in larger volumes than the CF experiments. Nonetheless, the short process times, the excellent yields and efficacy, the ease of product isolation, and the opportunity for the simple and safe scale-up of the reaction demonstrate the outstanding synthetic capabilities of this CF method. This CF method may also be useful for the synthesis of products that are derived from large biomolecules (such as peptides, dendrimers, and sugars),[42] although its applicability to hydrophilic structures is limited, owing to the non-polar reaction medium that is used. The presence of trace amounts of copper in the isolated reaction products must be acknowledged as another potential drawback. However, it should be emphasized that

Table 4. Synthesis of highly functionalized cispentacin derivatives in a CF reactor and under standard batch conditions.



Entry	Azide	Acetylene	Product	Yield [%][a] CF **A**[b]	CF **B**[c]	Batch
1	 **26**		 **28**	62	99	69[d]
2	 **27**		 **29**	42	99	73[d]
3	 **26**		 **30**	99	99	51[e]
4	 **27**		 **31**	99	99	69[e]

[a] Yield of isolated product. [b] Conditions CF **A**: 100 bar, 100 °C, flow rate: 0.5 mL min^{-1}, without any additives. [c] Conditions CF **B**: 100 bar, room temperature, flow rate: 0.5 mL min^{-1}, with DIEA (0.04 equiv)+HOAc (0.04 equiv). [d] To a solution of azido ester **26** or **27** (1 equiv) in MeCN were added CuI (1 equiv) and alkyne (1.1 equiv) and the mixture was stirred under reflux for 12 h. [e] To a solution of azido ester **26** or **27** (1 equiv) in EtOH/water (12:1) were added $CuSO_4$ (1 equiv), ascorbic acid (1 equiv), and alkyne (1.1 equiv) and the mixture was stirred at room temperature for 14 h.

Table 5. Copper content in the triazole-modified cispentacins that were obtained by using various CF methods.

Entry	Triazole product	Method	Copper content [μg g^{-1}][a]
1	**28**	CF **A**	14.8(±0.8)[b]
2	**28**	CF **B**	70.1(±1.4)[b]
3	**29**	CF **A**	12.1(±0.7)[b]
4	**29**	CF **B**	66.2(±1.5)[b]
5	**30**	CF **A**	4.4(±0.3)[c]
6	**30**	CF **B**	7.1(±0.6)[c]
7	**31**	CF **A**	5.2(±0.5)[c]
8	**31**	CF **B**	7.3(±0.7)[c]

[a] Determined by ICP-MS. [b] Without chromatographic purification. [c] After purification by column chromatography on silica gel.

copper is a biogenic substance, unlike many other heavy metals, and simple chromatographic purification can decrease its content substantially, as shown in Table 5. The copper content in our triazole products compare well with other literature results of CF[22b] or batch processes.[43]

Conclusions

A simple and inexpensive CF method was implemented for the 1,3-dipolar cycloaddition of organic azides and acetylenes, which utilized Cu powder as the cheapest available Cu^I source. The need for costly apparatus and special catalyst species is eliminated and the high-pressure/high-temperature conditions were successfully moderated through the joint use of basic and acidic additives to maximize the reaction rates. The benefits of flow processing meant that the risks that were associated with the handling of dangerous azides were significantly reduced and gram-scale syntheses were successfully achieved in a safe and simple manner. This CF technique has a wide scope for azides and alkynes and selectively results in synthetically useful 1,4-disubstituted 1,2,3-triazole compounds in excellent yields within short process times, some of which have notable biological activity. The utilization of additives in flow processes not only improved the safety but also efficacy of these reactions and typically afforded higher yields than under the high-temperature conditions. Some highly functionalized derivatives of the antifungal cispentacin were also effectively synthetized with this CF methodology and also with conventional batch procedures, thus demonstrating the outstanding synthetic capabilities of flow processing. The cispentacin derivatives are interesting precursors for new triazole carbanucleosides with possible biological activities.

Experimental Section

General Information

The reagents and materials were of the highest commercially available purity and were used without any further purification steps. Flash column chromatography was performed on Merck silica gel 60 (particle size: 63–200 μm) and analytical thin-layer chromatography (TLC) was performed on Merck silica gel 60 F254 plates. Compounds were visualized by UV irradiation or by staining with $KMnO_4$. ^{1}H NMR, ^{13}C HSQC, and ^{13}C HMBC NMR spectra were recorded on a Bruker Avance DRX 400 spectrometer in $CDCl_3$ with TMS as an internal standard (^{1}H: 400.1, ^{13}C: 100.6 MHz). Microanalysis was performed on a Perkin–Elmer 2400 elemental analyzer. A H-Cube system was used as a CF reactor.

Determination of the Copper Content of the Triazole Products

Copper concentrations were determined by inductively coupled plasma mass spectrometry on an Agilent 7700x-type instrument that was equipped with a collision cell. Determination of the copper content was performed on the ^{63}Cu isotope by using He as a collision gas. The standard solutions for external calibration were prepared from a stock solution (Certipur, Merck), by dilution with doubly deionized water (Millipore MillQ, Merck). All glassware and plastic utensils that were used during the determination were pre-cleaned by alternately soaking in solutions of trace-quality nitric acid and hydrochloric acid (Suprapur, Merck), followed by rinsing with copious amounts of doubly deionized water.

Preparation of Benzylic and Aliphatic Azides

NaN_3 (25 mmol, 1.5 equiv) was added to a stirring solution of the corresponding bromide (17 mmol, 1 equiv) in DMSO (25 mL) and the resulting mixture was stirred at 60 °C for 4 h. Then, brine and Et_2O were added to the solution and the organic layer was washed thoroughly with brine. Next, the combined organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure. The corresponding azides were obtained in a purity of >99% and were used in subsequent reactions without further work-up.[44]

Preparation of Phenyl Azide

Aniline (10 mmol, 1 equiv) was suspended in 17% hydrochloric acid (60 mL) at RT and EtOH (5 mL) was added. The mixture was cooled to 0 °C and $NaNO_2$ (15 mmol, 1.5 equiv) was added in small portions. After stirring at 0 °C for 30 min, NaN_3 (15 mmol, 1.5 equiv) was slowly added and the mixture was stirred for an additional 2 h at RT. The reaction mixture was extracted with Et_2O and the combined organic layers were washed with a saturated solution of $NaHCO_3$ and with brine.[44] After drying over Na_2SO_4, the solvent was evaporated and phenyl azide was obtained in a purity of 98%. The product was used in subsequent reactions without further work-up.[44]

General Procedure for the CF Reactions

For the CF reactions, the azide (0.21 mmol, 1 equiv) and the alkyne (0.32 mmol, 1.5 equiv), as well as DIEA (0.0084 mmol, 0.04 equiv) and HOAc (0.0084 mmol, 0.04 equiv) in method CF **B**, were dissolved in CH_2Cl_2 (2.5 mL). The solution was homogenized by sonication for 1 min and then pumped through the CF reactor under the appropriate conditions. The crude product was checked by TLC (*n*-hexane/EtOAc) and the solvent was removed under reduced pressure. If necessary, the products were purified by column chromatography on silica gel (*n*-hexane/EtOAc). The triazole products were characterized by elemental analysis and NMR spectroscopy. For detailed analytical data, see the Supporting Information. Between two reactions in the CF reactor, the catalyst bed was washed for 5 min with CH_2Cl_2 at a rate of 1 mL min^{-1}.

General Procedure for the CuI-Catalyzed Batch Reaction

CuI (1.95 mmol, 1 equiv) and alkyne (2.15 mmol, 1.1 equiv) were added to a solution of azido ester **26** or **27** (1.95 mmol, 1 equiv) in MeCN and the mixture was stirred under reflux for 12 h. The solvent was then removed under vacuum and the residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc, 2:1).

General Procedure for the $CuSO_4$/Ascorbic-Acid-Catalyzed Batch Reaction

$CuSO_4$ (1.95 mmol, 1 equiv), ascorbic acid (1.95 mmol, 1 equiv), and alkyne (2.15 mmol, 1.1 equiv) were added to a solution of azido ester **26** or **27** (1.95 mmol, 1 equiv) in EtOH (6 mL) and water (0.5 mL) and the mixture was stirred at RT for 14 h. The solvent was removed under vacuum and the residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc, 1:1).

Measurement of the Residence Time on the Catalyst Bed

A solution of a blue ink in CH_2Cl_2 was pumped through the system and the time that elapsed between the first contact of the ink with the catalyst bed and the moment when the blue color appeared at the column output was measured to determine the residence time.

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VI

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Efficient continuous-flow synthesis of novel 1,2,3-triazole-substituted β-aminocyclohexanecarboxylic acid derivatives with gram-scale production

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Abstract

The preparation of novel multi-substituted 1,2,3-triazole-modified β-aminocyclohexanecarboxylic acid derivatives in a simple and efficient continuous-flow procedure is reported. The 1,3-dipolar cycloaddition reactions were performed with copper powder as a readily accessible Cu(I) source. Initially, high reaction rates were achieved under high-pressure/high-temperature conditions. Subsequently, the reaction temperature was lowered to room temperature by the joint use of both basic and acidic additives to improve the safety of the synthesis, as azides were to be handled as unstable reactants. Scale-up experiments were also performed, which led to the achievement of gram-scale production in a safe and straightforward way. The obtained 1,2,3-triazole-substituted β-aminocyclohexanecarboxylates can be regarded as interesting precursors for drugs with possible biological effects.

Introduction

In recent years, triazole-containing compounds have become potential targets for drug discovery [1,2]. A large number of 1,2,3-triazoles exhibit various biological effects [3], e.g., antiviral (**1**), antibacterial (**2**), antifungal (**3**) and anticancer (**4**) activities [4-7] (Figure 1). The 1,2,3-triazole skeleton is frequently used as a pharmacophore for the modification of known pharmaceuticals. Triazole analogues of several bioactive compounds have recently been reported. Examples are those of the well-known highly functionalized antiviral cyclic amino acid derivatives oseltamivir and zanamivir (**5** and **6** in Figure 1) [8,9]. The 1,2,3-triazole moiety is a constituent part of many modified nucleosides or carbanucleosides with antiviral, anti-HIV or cytostatic activities [10-12]. However, the scope of triazole chemistry is not confined to drug discovery. There are

an increasing number of applications in numerous other areas of modern chemical sciences, such as bioconjugation [13], supramolecular chemistry, [14] and polymer sciences [15].

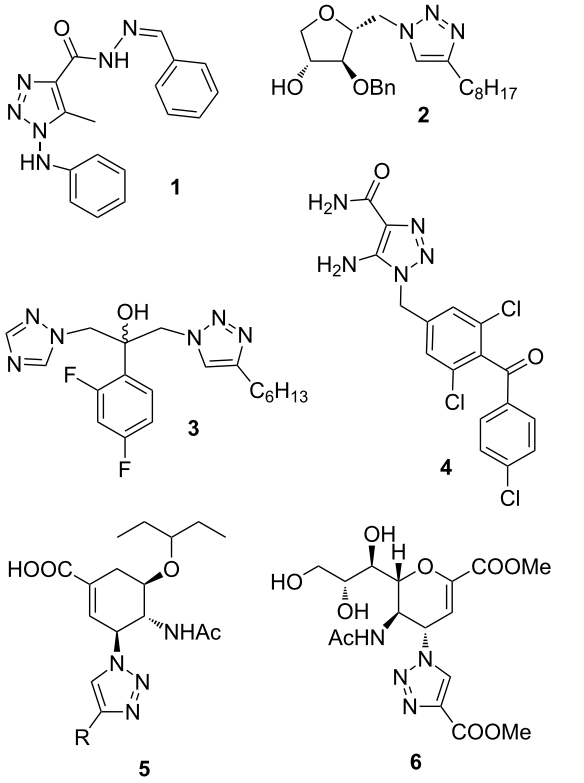
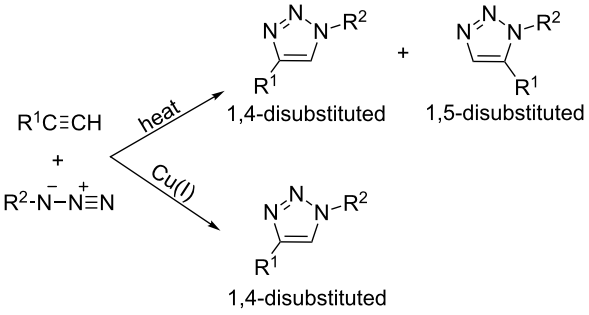


Figure 1: Examples of 1,2,3-triazoles with various biological activities.

Probably the most useful and powerful procedure for the synthesis of 1,2,3-triazoles is the Huisgen 1,3-dipolar cycloaddition of organic azides with acetylenes [16]. The classical Huisgen reaction, thermally induced, gives an approximate 1:1 mixture of 1,4- and 1,5-disubstituted 1,2,3-triazole isomers (Scheme 1) [17]. However, when Cu(I) catalysis is applied, the reaction becomes regioselective, exclusively yielding the 1,4-regioisomer within a relatively short reaction time [18-20]. Recently, Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC) has become the basis of the so-called click chemistry concept due to its wide applicability and efficiency.

Over the past twenty years, alicyclic β-amino acids have attracted great interest among synthetic chemists, thanks to their massive pharmacological potential [21,22]. For example, cispentacin ((1*R*,2*S*)-2-aminocyclopentanecarboxylic acid, **7**) is a widely investigated naturally occurring carbocyclic β-amino acid with strong antifungal properties against *Candida* species (Figure 2) [23]. Its synthetic 4-methylene derivative icofungipen (**8**), also an antifungal agent, is now proceeding



Scheme 1: 1,3-Dipolar azide–alkyne cycloadditions.

through clinical development for the oral treatment of yeast infections (Figure 2) [24]. Certain multi-substituted cyclohexane amino acid derivatives, such as oryzoxymycin (**9**) and tilidine (**10**), are also well-known bioactive agents with anticancer, antibacterial, antiviral or analgesic effects (Figure 2) [25,26]. The alicyclic β-amino acids are key intermediates for the synthesis of a series of pharmaceutically relevant products [27], such as amino esters, amino alcohols, azides and heterocycles. Moreover, they are frequently used as building blocks for the synthesis of new peptides and foldamers with possible biological effects [28].

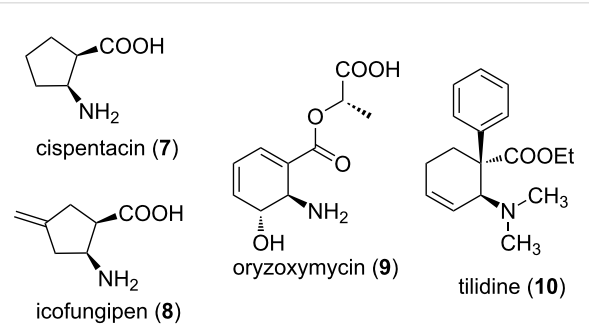


Figure 2: Selected bioactive alicyclic β-amino acids.

Modern continuous-flow (CF) technologies offer many advantages over classical batch-based procedures [29-32], including efficient mixing quality [33], excellent heat and mass transfer [34], shorter reaction times [35-37], reduced reagent consumption [38-40], improved safety [41,42], and operational simplicity [43]. Furthermore, CF methodologies provide opportunities for a simple and rapid scale-up [44,45] and automation [46,47] of chemical processes. They also tend to be environmentally benign technologies [48]. In consequence of these benefits, flow chemistry-based techniques have exerted a significant impact on modern synthetic chemistry, ranging from laboratory-based experiments to industrial-scale production.

Here, we describe a safe and efficient CF synthesis of a series of novel 1,2,3-triazole-modified β-aminocyclohexanecarboxylic acid derivatives as potential biologically active compounds. Gram-scale production is also reported, which predicts a possible usefulness for the pharmaceutical industry.

Results and Discussion

Several approaches are to be found in the literature for the Cu(I)-catalysed flow synthesis of triazoles. Heterogeneous Cu(I) sources are most popular, such as copper-in-charcoal (Cu/C) [49,50], solid supported Cu(I) species [51-54], and heated copper wirings [55-58], but a homogeneous technique has also recently emerged [59]. The main driving forces behind these CF methodologies are the safety aspects associated with the handling of azides and the inherent scalability of flow processing. Moreover, when organic azides are formed in situ, operational safety can be further improved [55,57]. We envisioned that it would be simplest to make use of copper powder as a catalytic source [60]. Similarly to cases when heated rings of copper wire are employed, a copper surface acts as a source of active copper species. Copper is constantly oxidized when exposed to air, and non-self-protecting layers of different oxides, including Cu_2O, are formed on its surface [61], which can promote CuAAC. Thus, we utilized copper powder in a stainless steel column, which served as a catalyst bed later on. The catalyst bed was placed into a stainless steel block with a Peltier heating system, which could heat the column up to 100 °C. A backpressure regulator was also integrated to ensure pressures up to 100 bar. The mixture of the reactants was pumped through the system continuously by means of an HPLC pump. This experimental setup is practical and cheap, as it does not require costly catalysts or special apparatus. At the same time this setup is safe, even with unstable reactants such as azides (Figure 3).

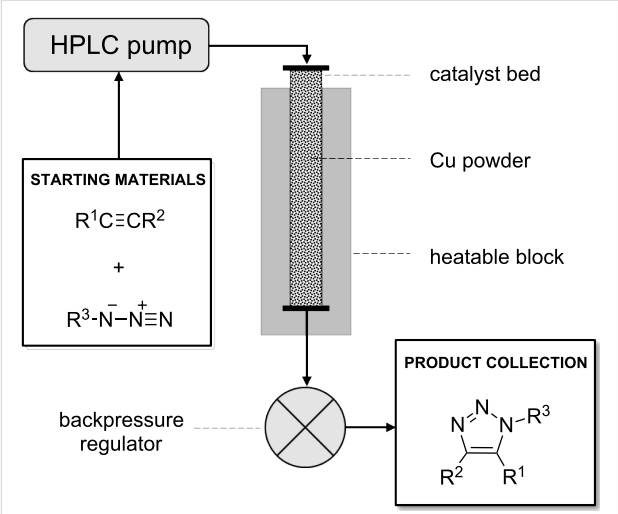


Figure 3: Experimental setup for the CF reactions.

To maximize the CF triazole synthesis reaction rates, it appeared easiest to use high-temperature conditions initially. The application of elevated pressure in CuAAC is also beneficial, as it can promote the product formation in accordance with Le Chatelier's principle [60] and also prevents the solvent from boiling over when high temperature is used. Thus, 100 °C and 100 bar were selected as conditions **A** for the CF synthesis. However, when azides are reacted, it is important to minimize the explosion hazard. Accordingly, we attempted to improve the rates of the reaction in the presence of additives, without the use of high temperature [60]. Amines are known to accelerate CuAAC, in particular by coordinating to catalytically active Cu(I) species and promoting their liberation from the copper matrix [62,63]. It was recently shown that the use of certain acids as additives is also beneficial, as this can further accelerate the formation of the triazole product [64-66] and also prevents the accumulation of unwanted byproducts, such as diacetylenes, bistriazoles, etc. [67]. At the same time, byproduct formation is catalysed by a base, and the joint utilization of a basic and an acidic additive is therefore favourable. This buffer system gives rise to a high reactivity in CuAAC, even at room temperature (rt), but without byproduct formation [60,67]. This system thus greatly improves the safety relative to the high-temperature conditions. The literature data led us to select *N*,*N*-diisopropylethylamine (DIEA) as a base and HOAc as an acid [67], which were used jointly as additives, each in 0.04 equivalents, at 100 bar and rt as conditions **B** [60].

As starting materials for the CF CuAAC reactions, azido-substituted β-aminocyclohexanecarboxylates **11**–**14** were prepared previously by a diastereoselective epoxidation of the corresponding 2-aminocyclohexenecarboxylates, followed by a regioselective oxirane ring opening with NaN_3 [68]. Three different alkynes (phenylacetylene, diethyl acetylenedicarboxylate and ethynyl ferrocene) were employed as dipolarophiles to yield a library of novel 1,2,3-triazole-modified cyclic β-amino acid derivatives. Compounds **11**–**14** were racemates, the structures in Table 1 show their relative stereochemistry. The CF syntheses were carried out under both conditions **A** and **B** in order to obtain a clear comparison between the performances of the two approaches. CH_2Cl_2 was used as a solvent, and the starting azides were used in a concentration of 0.085 M. A higher concentration of the starting azides led to the precipitation of the triazole product and a blockage in the CF reactor. Aliquots of 2.5 mL of a reaction mixture containing 1 equivalent of the azide and 1.5 equivalents of the acetylene were pumped through the reactor in each run with a a flow rate of 0.5 mL min^{-1}. At this flow rate the residence time on the catalyst bed was as low as 1.5 min and it took only 5 min of process

time to pump the 2.5 mL aliquots through the system. This resulted in around 100 mg of crude product, depending on the conversion and the molecular masses of the reactants.

In the Cu(I)-catalysed reactions between phenylacetylene and the azido-substituted β-amino acid derivatives **11**–**14**, 1,4-disubstituted 1,2,3-triazole isomers (**15**–**18**) were regioselectively formed. The high-pressure/high-temperature conditions **A** led to only medium yields (Table 1, entries 1–4), but under conditions **B** the yields of triazoles **15** and **16** were excellent, and those of triazoles **17** and **18** were high (76% and 89%, respectively; Table 1, entries 1–4). When the CF reactions of azides **13** and

Table 1: CF synthesis of 1,2,3-triazole-substituted alicyclic β-amino acid derivatives.

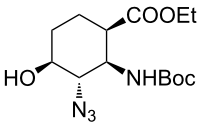
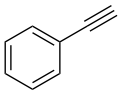
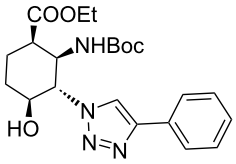
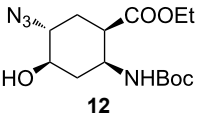
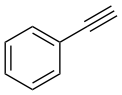
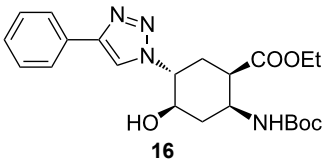
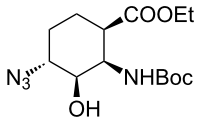
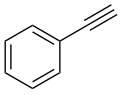
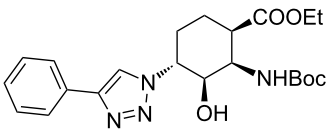
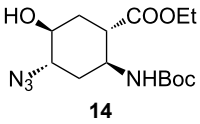
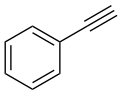
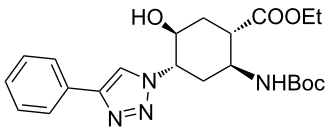
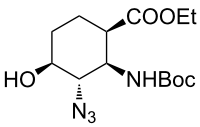
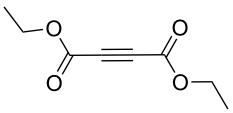
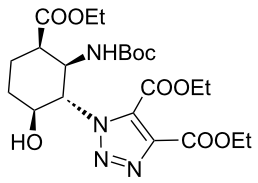
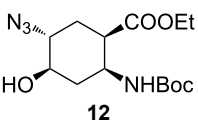
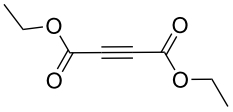
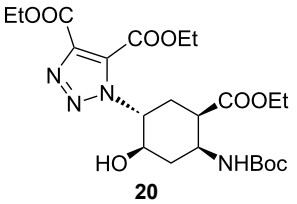
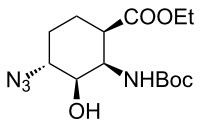
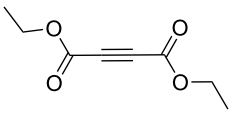
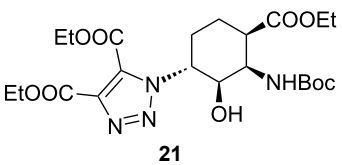
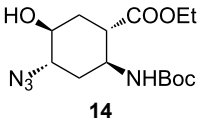
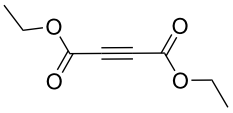
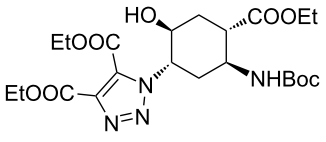
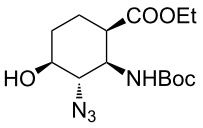
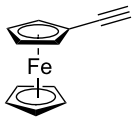
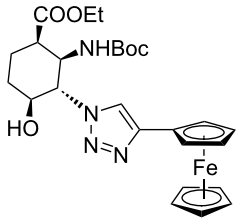
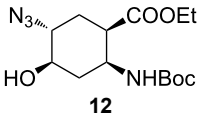
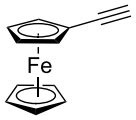
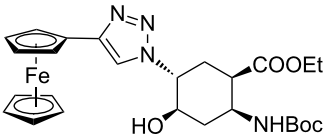
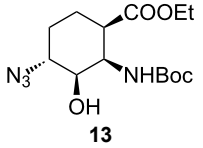
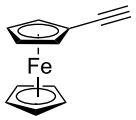
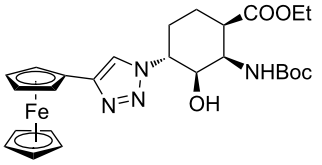
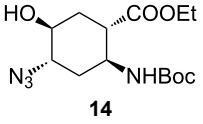
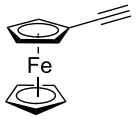
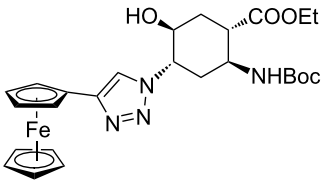
Entry	Azide[a] (1 equivalent)	Acetylene (1.5 equivalents)	Product	Yield[b] (%)	
				A[c]	**B**[d]
1	 **11**		 **15**	61	96
2	 **12**		 **16**	47	97
3	 **13**		 **17**	33	76 (98)[e]
4	 **14**		 **18**	53	89 (98)[e]
5	 **11**		 **19**	98	97
6	 **12**		 **20**	97	98
7	 **13**		 **21**	97	96

Table 1: CF synthesis of 1,2,3-triazole-substituted alicyclic β-amino acid derivatives. (continued)

8	 **14**		 **22**	97	98
9	 **11**		 **23**	95	97
10	 **12**		 **24**	91	98
11	 **13**		 **25**	96	93
12	 **14**		 **26**	75	97

[a]c_{azide} = 0.085 M. [b]Yield of isolated product. [c]Conditions **A**: CH_2Cl_2 as solvent, 100 bar, 100 °C, flow rate 0.5 mL min^{-1}, without any additives. [d]Conditions **B**: CH_2Cl_2 as solvent, 100 bar, rt, flow rate 0.5 mL min^{-1}, with 0.04 equivalents of DIEA + 0.04 equivalents of HOAc. [e]Achieved under the following conditions: CH_2Cl_2 as solvent, 100 bar, 100 °C, flow rate 0.5 mL min^{-1}, with 0.04 equivalents of DIEA + 0.04 equivalents of HOAc.

14 with phenylacetylene were repeated under high-pressure/high-temperature conditions with the simultaneous use of additives (100 bar, 100 °C, 0.04 equivalents each of DIEA and HOAc; further conditions were not modified), triazoles **17** and **18** were obtained in very high yields (98% in both cases; Table 1, entries 3 and 4).

1,4,5-Trisubstituted 1,2,3-triazoles are of notable importance in drug discovery. For example, several 1,2,3-triazole-4,5-dicarboxylates display significant antituberculotic activity in vitro [69]. Thus, a nonterminal alkyne, diethyl acetylenedicarboxylate, was subjected to CF CuAAC with the azido-functionalized β-amino acid derivatives **11**–**14** as reaction partners. 1,4,5-Trisubstituted 1,2,3-triazole dicarboxylates **19**–**22** were obtained in excellent yields (>96%) under both conditions **A** and **B** (Table 1, entries 5–8). In this set of CF syntheses, no significant difference was observed between the performances of the two methods.

Ferrocene-triazole conjugates play a crucial role in the labelling and detection of various systems, such as biomolecules, polymers, nanomaterials and supramolecular assemblies [70]. They also have potential applications in medicinal chemistry and drug discovery as biosensing probes, in immunoassays and in host–guest chemistry [71]. Ferrocene-substituted amino acids have been of significant importance in the investigation of the secondary structures of different peptides and foldamers [72]. Thus, conjugates of the azido-functionalized β-amino acid

derivatives **11**–**14** were prepared with ethynylferrocene as a dipolarophile. Both conditions **A** and **B** afforded ferrocenyltriazoles **23**–**25** in excellent yields (>91%; Table 1, entries 9–11). However, in the case of ferrocenyltriazole **26** the high-pressure/high-temperature conditions **A** led to a yield of only 75%, whereas the use of additives at rt (conditions **B**) proved more efficient, with a yield of 97% (Table 1, entry 12). Triazoles **23**–**26** were obtained selectively as 1,4-disubstituted regioisomers.

To understand the differences between the results obtained with the three different dipholarophiles, it must be taken into account that the carboxylate groups of diethyl acetylenedicarboxylate and the aromatic system of the ferrocenyl group as ligands can probably coordinate copper from its matrix. Therefore, the concentration of the catalytically active Cu(I) species is increased as compared to the reactions with phenylacetylene [73-75]. Accordingly, the yields were usually higher in the reactions with diethyl acetylenedicarboxylate and ethynylferrocene than with phenylacetylene (Table 1, entries 5–12 versus entries 1–4). These differences can mainly be observed between the results obtained under conditions **A**. This is because the base, as an additive, evolves the same effect and improves the reactivity through the CuAAC, thus in the case of conditions **B** (the use of additives) the influence of the alkyne is practically masked.

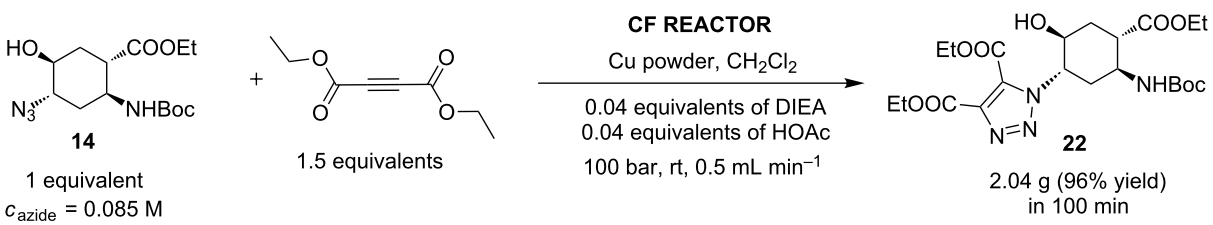
The presence of trace amounts of copper in the chromatographically purified triazole products was determined by means of inductively coupled plasma mass spectrometry (ICP–MS). The analytical data in Table 2 show that the contents of copper impurities in the products were appropriately low, i.e., amounts of 3.9–9.1 μg g^{-1} were detected. It should be noted that the samples obtained with the joint use of DIEA + HOAc (conditions **B**) contained more copper than those obtained under conditions **A** (high-temperature/high-pressure without additives). The levels of copper contamination detected in our triazole products compare well with literature results relating to CF [50] and conventional batch experiments [76].

In conventional batch-based chemistry, the scale-up of chemical reactions can be a challenge because the output depends on the batch size. The situation becomes even more complicated when unstable reactants such as azides are handled on a large scale. However, the scalability of CF procedures is a straightforward function of time and the flow rate, and the risks associated with the accumulation of hazardous species are minimized, because the solution of the reactants is eluting continuously from the active zone of the reactor [33,34,44,45,60]. The CF CuAAC between azide **14** and diethyl acetylenedicarboxylate was scaled up in a simple, safe and efficient manner to achieve gram-scale production (Scheme 2). Methods **A** and **B** proved equally efficient in the small-scale CF syntheses of triazole **22** (Table 1, entry 8). However, we performed the large-scale experiment at 100 bar and rt in the presence of the additives (conditions **B**) so as to ensure maximum safety throughout the procedure. A CH_2Cl_2 solution of the reaction mixture containing 1 equivalent of the azide (c_{azide} = 0.085 M), 1.5 equivalents of the acetylene and 0.04 equivalents of each additive was pumped continuously through the system at a flow

Table 2: Copper contents in the triazole products after column chromatographic purification on silica gel.

Entry	Product	Copper content (μg g^{-1})[a]	
		A[b]	**B**[c]
1	**15**	4.6 (±0.5)	8.4 (±0.6)
2	**16**	4.2 (±0.3)	7.7 (±0.6)
3	**17**	3.9 (±0.5)	8.0 (±0.4)
4	**18**	4.7 (±0.6)	8.2 (±0.7)
5	**19**	5.2 (±0.4)	7.9 (±0.4)
6	**20**	5.1 (±0.3)	7.5 (±0.6)
7	**21**	4.8 (±0.6)	7.7 (±0.7)
8	**22**	5.3 (±0.3)	8.2 (±0.6)
9	**23**	6.1 (±0.5)	8.6 (±0.5)
10	**24**	4.8 (±0.4)	7.7 (±0.8)
11	**25**	5.4 (±0.3)	9.1 (±0.4)
12	**26**	4.9 (±0.6)	7.8 (±0.7)

[a]Determined by ICP–MS. [b]Conditions **A**: CH_2Cl_2 as solvent, 100 bar, 100 °C, flow rate 0.5 mL min^{-1}, without any additives. [c]Conditions **B**: CH_2Cl_2 as solvent, 100 bar, rt, flow rate 0.5 mL min^{-1}, with 0.04 equivalents of DIEA + 0.04 equivalents of HOAc.



Scheme 2: Gramm-scale CF synthesis of triazole **22** under conditions **B**.

rate of 0.5 mL min^{-1}. During the whole scale-up procedure, the same portion of copper powder was used in the catalyst bed. The solution of the crude product was collected for 100 min, and after purification 2.06 g of triazole **22** was obtained, which is equivalent to a yield of 96%.

Conclusion

Twelve highly functionalized 1,2,3-triazole-substituted β-aminocyclohexanecarboxylic acid racemates were successfully prepared in CF mode as a small library of novel compounds with possible biological effects. The CF syntheses were first performed under high-pressure/high-temperature conditions with copper powder as a readily accessible Cu(I) source. Subsequently, to moderate the harsh reaction conditions, the reaction temperature could be lowered to rt in the presence of additives. The joint use of a base and an acid dramatically improved the reactivity in the CuAAC, while it completely eliminated unwanted byproduct formation. These conditions ensured enhanced safety and typically higher yields than those attained under the harsh reaction conditions. Simple, efficient and safe gram-scale production was also implemented in a short processing time, which can be important for potential industrial applications.

Experimental

General Information

The reagents and materials were of the highest commercially available purity grade and were used without any further purification. Flash column chromatography was performed on Merck silica gel 60, particle sizes ranged from 63 to 200 μm, and analytical thin-layer chromatography (TLC) on Merck silica gel 60 F254 plates. Compounds were visualized with UV light or $KMnO_4$. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance DRX 500 spectrometer, in $CDCl_3$ as a solvent, with TMS as internal standard, and at 500.1 and 125.0 MHz, respectively. Microanalyses were performed on a Perkin-Elmer 2400 elemental analyser.

Determination of the copper contents of the triazole products

Copper concentrations were determined by ICP–MS by using an Agilent 7700x instrument equipped with a collision cell. The determination was carried out on the isotope ^{63}Cu, with He as collision gas. The standard solutions for external calibration were prepared from a stock solution (Certipur, Merck) by dilution with doubly deionized water (Millipore MillQ, Merck). All glassware and plastic utensils used during the determination were precleaned by soaking in solutions of trace-metal-grade nitric acid and hydrochloric acid (Suprapur, Merck), followed by rinsing with copious amounts of doubly deionized water.

General procedure for the CF reactions

An H-Cube® system was used as a CF reactor in the "no H_2" mode. For the CF reactions, the catalyst bed (internal dimensions: 70 mm × 4 mm) was filled with ~900 mg of copper powder with an average particle size of 200 μm. 70 mg (0.21 mmol, 1 equivalent) of the corresponding azide and 0.32 mmol (1.5 equivalents) of the alkyne, and (only in method **B**) 1.5 μL (0.0084 mmol, 0.04 equivalents) of DIEA and 0.5 μL (0.0084 mmol, 0.04 equivalents) of HOAc were dissolved in 2.5 mL of CH_2Cl_2. The solution was homogenized by sonication, and then pumped through the CF reactor under the appropriate conditions. Between two reactions in the CF reactor, the catalyst bed was washed at rt for 5 min with CH_2Cl_2 at a flow rate of 1 mL min^{-1}. The crude product was checked by TLC with a mixture of *n*-hexane/EtOAc as an eluent, and the solvent was next evaporated off under vacuum. Column chromatographic purification was carried out on silica gel with a mixture of *n*-hexane/EtOAc as an eluent. The 1,2,3-triazole-modified compounds were characterized by elemental analysis and NMR experiments. For detailed analytical data see Supporting Information File 1.

Measurement of the residence time on the catalyst bed

To determine the residence time, a CH_2Cl_2 solution of a blue ink was pumped through the catalyst bed. The time that elapsed between the first contact of the ink with the bed and the moment when the blue colour appeared at the column outlet was measured.

Supporting Information

Supporting Information File 1

Detailed analytical data of the prepared compounds and a collection of NMR spectra.
[http://www.beilstein-journals.org/bjoc/content/supplementary/1860-5397-9-172-S1.pdf]

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