High Throughput Synthesis within Flow Reactors

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Continuous ‘Flow’ Reactors

• ‘Micro’ reactors
  • Defined as a series of interconnecting channels formed in a planar surface
  • Channel dimensions of 10-300 μm

• ‘Flow’ reactors
  • Dimensions > 300 μm (up to 5 mm)

• Various pumping techniques available
  • Hydrodynamic flow
  • Electroosmotic flow

• Fabricated from polymers, metals, quartz, silicon or glass

• Why glass?
  • Mechanically strong
  • Chemically resistant
  • Optically transparent
Production Technology

Scale-up:
- Re-optimised at each stage
- Costly and time consuming

Scale-out:
- Numbering-up/replication
- Cost effective and flexible
- Requires reproducibility within single reactors

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Synthesis of $\alpha$-Aminonitriles

- Synthesis of non-proteinogenic $\alpha$-amino acids
  - Novel, efficient syntheses are required
  - Low yields/selectivities often observed

Strecker Reaction:

- Multi-component reaction, useful for the synthesis of thia diazoles, imidazoles, diamines and $\alpha$-amino acids
- Catalysts include: InCl$_3$, BiCl$_3$, KSF clay, Sc(OTf)$_3$, Cs(II)-salt, Pt-salt
- Reaction times in the range of 24 to 48 hr
  - Moderate yields (55 to 95 %)
Disadvantages: Strecker Reaction

Side Reactions:

- Low yields, complex reaction mixtures
  - Laborious purification required

Expensive Catalyst:
- Difficult to recover and recycle
Continuous Flow Synthesis

Aims of Flow Reaction:

- Enable optimisation of imine formation
  - To minimise or prevent cyanohydrin formation
- Employ a stoichiometric quantity of TMSCN and amine
- Recycle catalyst efficiently
  - Reduce degradation due to absence of stirring
Continuous Flow Nucleophilic Addition to Imine

0.2 M Stock Solutions in MeCN:

- Remove solvent and dissolve residue in CDCl₃, analyse by ¹H NMR

Reaction Channel
150 µm (wide) x 50 µm (deep)
Packed-bed
3000 µm (wide) x 300 µm (deep)
x 2.1 cm (long)

0.26 mmol/g
Results: Pre-formed Imine

Reaction Conditions:
- Total flow rate = 100 μl min⁻¹, 41 % conversion (unoptimised)
Reaction Conditions:

- Total flow rate = 5.0 μl min⁻¹, 100 % conversion
- Product also evaluated by MS, IR and CHN analysis to confirm purity
Flow Synthesis of Imines

Reaction Conditions:

- 0.4 M Stock Solutions in MeCN
- Micro Channel Dimensions = 150 μm (wide) x 50 μm (deep)

- Reaction products analysed, off-line, by GC-MS
- Identify optimal conditions for imine formation
Optimisation of Imine Formation

Conversion calculated wrt. residual 4-bromobenzaldehyde
Comparison of Reaction Steps

- Optimal conditions for both reactions = Total flow rate < 5.0 μl min⁻¹
- Conversion calculated wrt. residual imine
Combined Micro Reactor Design

Reaction Conditions:
Total flow rate 5.0 μl min⁻¹, 0.4 M aldehyde and amine, 0.2 M TMSCN
Multi-Step Reaction: NMR Analysis

Flow: Quantitative Conversion (by NMR), 99.9 % Yield, 9.45 mg hr\(^{-1}\) (5.0 \(\mu\)l min\(^{-1}\))

Batch: 64.9 % Conversion, stirred for 24 hr (1.5 eq. TMSCN)
Evaluation of Catalyst Recycling and Leaching in a Micro Reactor

4-Bromo-phenyl-phenethylamino-acetonitrile:

- 0.1 mmol of $\alpha$-aminonitrile synthesised using $2.6 \times 10^{-3}$ mmol of PS-RuCl$_3$
  - Turnover number of 38 for a single reaction
  - In addition to efficient catalyst recycling, it is pharmaceutically important that minimal heavy metals are found in the resulting product

ICP-MS Analysis:

- Stirred Batch Reaction: 440 ppm Ru
  - Micro Reaction: No observable difference from the blank (MeCN)
  - Confirms that minimal catalyst degradation is observed in flow reactors, *cf.* stirred/shaken batch vessels
  - Affording an analytically purer product that obtainable using traditional reactor methodology
Use of Polymer Supported ScOTf₂

Advantages vs. PS-RuCl₃:

- Higher loading commercially available (0.60 mmol g⁻¹)
- Greater tolerance to H₂O
- Effective activation of imines

Reaction Conditions: Amine and aldehyde (0.4 M), TMSCN (0.2 M) in MeCN

<table>
<thead>
<tr>
<th>Entry</th>
<th>Flow Rate a (μl min⁻¹)</th>
<th>Conversion (%) PS-RuCl₃</th>
<th>Conversion (%) PS-ScOTf₂</th>
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<td>2</td>
<td>10</td>
<td>95.2</td>
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<td>3</td>
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<td>4</td>
<td>40</td>
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<tr>
<td>5</td>
<td>100</td>
<td>25.9</td>
<td>36.3</td>
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a Total flow rate (based on delivery of reagents from 3 inputs)
b Calculated based on ¹H NMR integration of the crude product
### 5 x 5 Array of α-Aminonitriles

Using PS-ScOTf$_2$: Amine and aldehyde (0.4 M), TMSCN (0.2 M) in MeCN

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<td><strong>99.90 %</strong>&lt;br&gt; <strong>10.0 µl min$^{-1}$</strong>&lt;br&gt; <strong>0.0125 g hr$^{-1}$</strong></td>
<td><strong>99.85 %</strong>&lt;br&gt; <strong>10.0 µl min$^{-1}$</strong>&lt;br&gt; <strong>0.0142 g hr$^{-1}$</strong></td>
<td><strong>99.69 %</strong>&lt;br&gt; <strong>10.0 µl min$^{-1}$</strong>&lt;br&gt; <strong>0.0150 g hr$^{-1}$</strong></td>
<td><strong>99.97 %</strong>&lt;br&gt; <strong>10.0 µl min$^{-1}$</strong>&lt;br&gt; <strong>0.0133 g hr$^{-1}$</strong></td>
<td><strong>99.87 %</strong>&lt;br&gt; <strong>20.0 µl min$^{-1}$</strong>&lt;br&gt; <strong>0.0223 g hr$^{-1}$</strong>&lt;br&gt; *</td>
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<td><strong>99.90 %</strong>&lt;br&gt; <strong>10.0 µl min$^{-1}$</strong>&lt;br&gt; <strong>0.0189 g hr$^{-1}$</strong></td>
<td><strong>99.74 %</strong>&lt;br&gt; <strong>10.0 µl min$^{-1}$</strong>&lt;br&gt; <strong>0.0197 g hr$^{-1}$</strong></td>
<td><strong>99.93 %</strong>&lt;br&gt; <strong>10.0 µl min$^{-1}$</strong>&lt;br&gt; <strong>0.0181 g hr$^{-1}$</strong>&lt;br&gt; *</td>
<td><strong>99.77 %</strong>&lt;br&gt; <strong>20.0 µl min$^{-1}$</strong>&lt;br&gt; <strong>0.0318 g hr$^{-1}$</strong>&lt;br&gt; *</td>
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<td><strong>99.72 %</strong>&lt;br&gt; <strong>10.0 µl min$^{-1}$</strong>&lt;br&gt; <strong>0.0146 g hr$^{-1}$</strong></td>
<td><strong>99.89 %</strong>&lt;br&gt; <strong>10.0 µl min$^{-1}$</strong>&lt;br&gt; <strong>0.0162 g hr$^{-1}$</strong></td>
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<td><strong>99.94 %</strong>&lt;br&gt; <strong>10.0 µl min$^{-1}$</strong>&lt;br&gt; <strong>0.0154 g hr$^{-1}$</strong></td>
<td><strong>99.73 %</strong>&lt;br&gt; <strong>20.0 µl min$^{-1}$</strong>&lt;br&gt; <strong>0.0264 g hr$^{-1}$</strong>&lt;br&gt; *</td>
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<td><strong>99.99 %</strong>&lt;br&gt; <strong>10.0 µl min$^{-1}$</strong>&lt;br&gt; <strong>0.0188 g hr$^{-1}$</strong></td>
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<td><strong>99.69 %</strong>&lt;br&gt; <strong>20.0 µl min$^{-1}$</strong>&lt;br&gt; <strong>0.0295 g hr$^{-1}$</strong>&lt;br&gt; *</td>
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</table>

Run time = 2.5 hr, except for * where run time = 1.25 hr
Enzymatic Reactions

- Advantages of thermophilic enzymes
  - Less temperature sensitive
  - More stable in organic solvents
  - Longer lasting

- Cloned thermophilic enzyme
  - L-Aminoacylase
    - *Thermococcus litoralis*
    - Optimum temperature 85 °C
    - Hydrolysis of N-acylamino acids

\[
\text{R} \times \text{COOH} \quad \xrightarrow{\text{L-Aminoacylase}} \quad \text{R} \times \text{COOH} + \text{NH}_2 \text{NH} \quad \text{R} \times \text{COOH}
\]

N-Acetyl-DL-amino acid   L-Amino Acid   N-Acetyl-D-amino acid
Methods of Enzyme Immobilisation

- Adsorption
- Covalent Binding
- Entrapment
- Encapsulation
- Cross linking
Cross Linked Enzyme Aggregates

- **Preparation:**

  - Enzyme in Solution
  - Aggregation
  - Precipitant
  - Cross Linking
  - Glutaraldehyde
  - CLEA
  - Particle size 5-100 μm

- **Advantages**
  - Self supported (no cost for external support)
  - Does not require intensive purification and crystallisation
  - High stability
  - No leaching
  - Easy separation
  - Cost effective
  - Universally applicable to other enzymes
Aminoacylase CLEAs
Experimental Set-up

Collection (HPLC analysis)

Syringe Pump → Packed Aminoacylase CLEA → Silica frit

Reactors

N-benzoyl-L-phenylalanine \[\text{L-aminoacylase} \quad 0.1 \text{ mol/L Tris-HCL pH 8.0} \quad \text{H}_2\text{N-phenylalanine} + \text{Benzoic acid}\]
Batch Reaction of CLEAs

- Experimental conditions
  - 2 mg CLEA
  - Room temperature
  - Substrate 10 mmol/L benzoyl-L-phenylalanine

- Reaction reaches completion after ca. 5 hours
Packed Flow CLEA Reactor

- Experimental conditions
  - 2 mg CLEA in reactor
  - Room temperature
  - Substrate 10 mmol/L benzoyl-L-phenylalanine
  - Flow rate 2 μl/min (ca. residence time 10 minutes)
CLEA Summary

• CLEAs provide an easy way to incorporate enzymes into flow reactors
• High conversion at room temperature demonstrated
  • ca. 95 % continuous conversion
  • Effective high enzyme concentration
• However back pressure is very high leading to irreproducibility
Immobilisation onto Monoliths

Monoliths fabricated by the polymerisation of monomers in the presence of porogenic solvents and an initiator.

• Advantages
  • Easy to prepare directly inside flow reactors
  • Adjust the porosity and pore diameter
  • Low backpressure
  • UV initiation directs location of monolith

• Disadvantages
  • No universal immobilisation monolith functionality
Effect of Flow Rate on Conversion

- Experimental conditions
  - Room temperature
  - Substrate 10 mmol/L benzoyl-L-phenylalanine
Enzyme Stability

- Experimental conditions
  - Room temperature and 50 °C
  - Substrate 10 mmol/L benzoyl-L-phenylalanine
  - Flow rate 1 μl/min
### Substrate Screening

- **Experimental conditions**
  - Room temperature and 50 °C
  - Substrate 10 mmol/L concentration
  - Flow rate 1 μl/min

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Substrate conversion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-Benzoyl-L-phenylalanine</td>
<td>100.0</td>
</tr>
<tr>
<td>N-Benzoyl-D,L-phenylalanine</td>
<td>50.0</td>
</tr>
<tr>
<td>N-Acetyl-L-phenylalanine</td>
<td>100.0</td>
</tr>
<tr>
<td>N-Chloroacetyl-L-phenylalanine</td>
<td>100.0</td>
</tr>
<tr>
<td>N-Benzoyl-L-threonine</td>
<td>68.3</td>
</tr>
<tr>
<td>N-Benzoyl-L-leucine</td>
<td>52.2</td>
</tr>
<tr>
<td>N-Acetyl L tyrosine</td>
<td>33.3</td>
</tr>
<tr>
<td>N-Acetyl-L-tryptophan</td>
<td>7.0</td>
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Conclusions

- Micro reactors allow the rapid optimisation of reactions
  - High-throughput synthesis
  - Combinatorial and library synthesis
- Immobilised reagents and enzymes allow the synthesis of analytically pure compounds
- Substrate screening
- Integration of solution phase and catalysed reactions
- Micro reactors generate products in:
  - Higher purity
  - Higher conversion
  - Higher selectivity
- *In situ* formation of reagents

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Research Workers and Collaborators

- **Current group members**
  - Dr. Charlotte Wiles
  - Dr. Bongkot Ngansom
  - Dr. Joe Dragavon
  - Gareth Wild
  - Tamsila Nayyar
  - Julian Hooper
  - Linda Woodcock
  - Haider Al-Lawati
  - Ben Wahab

- **Past group members**
  - Dr. Nikzad Nikbin
  - Dr. Ping He
  - Dr. Victoria Ryabova
  - Dr. Vinod George
  - Dr. Leanne Marle
  - Mairead Kelly

- **Funding**
  - EPSRC
  - Sanofi-Aventis
  - LioniX
  - Astra Zeneca
  - EU FP6
  - Yorkshire Concept