High Throughput Synthesis
Of Analytically Pure Compounds
Within Flow Reactors

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Why Miniaturise Synthesis?

- Improving the efficiency of the drug discovery process
- Enhanced methodology for fine chemical synthesis
  - Process control

P. Watts et al., Drug Discovery Today, 2003, 8, 586
Production Technology

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

**Scale-out:**
Numbering-up/replication
Cost effective and flexible
Micro Reactors

- Defined as a series of interconnecting channels formed in a planar surface
- Channel dimensions of 10-300 µm
- Various pumping techniques available
  - Hydrodynamic flow
  - Electroosmotic flow
- Fabricated from polymers, metals, quartz, silicon or glass
- Why glass?
  - Mechanically strong
  - Chemically resistant
  - Optically transparent

Tetrahedron, 2002, 58, 4735
OPRD, 2004, 8, 422
Micro Reactor Construction

- Reactor holders:
  - Standard chips are suitable for both EOF and hydrodynamic pumping
  - LioniX/ChemTriX

- Electroosmotic flow:
  - Application of voltage induced flow

- Hydrodynamic flow:
  - Syringe pump connected using standard fittings
### Aza-Michael Addition

- β-Amino ketones are of synthetic importance as they feature in an array of pharmaceutical compounds.

**Reaction Conditions:**
- 1.0 M in anhydrous MeCN
- Applied fields in the range of 100 to 200 V cm\(^{-1}\) (Reaction length = 4.4 cm)

<table>
<thead>
<tr>
<th>Michael acceptor</th>
<th>Michael Donor</th>
<th>Product</th>
<th>Conversion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl vinyl ketone</td>
<td>Diethylamine</td>
<td><img src="image" alt="Methyl vinyl ketone Diethylamine product" /></td>
<td>100.0</td>
</tr>
<tr>
<td>Acrylonitrile</td>
<td>Diethylamine</td>
<td><img src="image" alt="Acrylonitrile Diethylamine product" /></td>
<td>100.0</td>
</tr>
<tr>
<td>Methyl vinyl ketone</td>
<td>Pyrrolidine</td>
<td><img src="image" alt="Methyl vinyl ketone Pyrrolidine product" /></td>
<td>100.0</td>
</tr>
<tr>
<td>Acrylonitrile</td>
<td>Pyrrolidine</td>
<td><img src="image" alt="Acrylonitrile Pyrrolidine product" /></td>
<td>99.99</td>
</tr>
</tbody>
</table>
Synthesis of Dihydropyrazolines

- Pyrazolines are useful as antibacterial, antifungal, insecticidal and antiparasitic agents.

**Reaction Conditions:**
- 1.0 M in absolute Ethanol
- Applied fields in the range of 100 to 200 V cm\(^{-1}\) (Reaction length = 4.4 cm)

<table>
<thead>
<tr>
<th>α,β-Urnsaturated ketone</th>
<th>Product</th>
<th>Conversion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Chemical Structure" /></td>
<td><img src="image2.png" alt="Chemical Structure" /></td>
<td>100.0</td>
</tr>
<tr>
<td><img src="image3.png" alt="Chemical Structure" /></td>
<td><img src="image4.png" alt="Chemical Structure" /></td>
<td>100.0</td>
</tr>
<tr>
<td><img src="image5.png" alt="Chemical Structure" /></td>
<td><img src="image6.png" alt="Chemical Structure" /></td>
<td>100.0</td>
</tr>
<tr>
<td><img src="image7.png" alt="Chemical Structure" /></td>
<td><img src="image8.png" alt="Chemical Structure" /></td>
<td>100.0</td>
</tr>
</tbody>
</table>
Fine Chemical Synthesis

- New methodology for fine chemical synthesis
- Enhanced yields of more pure products *etc*
- But always require purification
- Generally batch work up required
Knoevenagel Reaction

- Solution phase Knoevenagel reaction
- 1:1 Ratio of reagents (0.5 M) in MeCN
- EOF

- 100 % conversion
- Reaction very ‘atom efficient’
- BUT product contaminated with base!!
  - Traditional solvent extraction needed
  - This clearly reduces the advantages of flow reactors
Functionally Intelligent Reactors

- Fabricate micro reactors which enable catalysts and/or supported reagents to be spatially positioned

\[ R - R_1 + R_2 - \text{H} \rightarrow + \quad \text{Si} \quad \rightarrow R - R_1 - R_2 - \text{H} \]

- Quantitative conversion to analytically pure product

\( R \) and \( R_1 = \text{COOCH}_3, \text{COCH}_3, \text{CN} \)
\( R_2 = \text{Ph, CH}_3 \)
Key Result - Reproducibility

- Supported reagents deteriorate with time in batch reactions as a result of physical damage and/or loss.

- Reagents last longer in micro reactions as they suffer less damage in flow reactors.

- Throughput ca. 10 mg/hr/channel.

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OPRD, 2004, 8, 942

Tetrahedron, 2004, 60, 8421
# Knoevenagel Reactions

## More Examples

<table>
<thead>
<tr>
<th>Aldehyde</th>
<th>Activated Methylene</th>
<th>Conversion (%)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzaldehyde</td>
<td>Ethylcyanoacetate</td>
<td>99.98</td>
<td>99.70</td>
</tr>
<tr>
<td>4-Bromobenzaldehyde</td>
<td>Ethylcyanoacetate</td>
<td>99.96</td>
<td>99.35</td>
</tr>
<tr>
<td>Methyl-4-formyl benzoate</td>
<td>Ethylcyanoacetate</td>
<td>100.0</td>
<td>99.80</td>
</tr>
<tr>
<td>3,5-Dimethoxybenzaldehyde</td>
<td>Ethylcyanoacetate</td>
<td>99.86</td>
<td>99.89</td>
</tr>
<tr>
<td>4-Benzyloxybenzaldehyde</td>
<td>Ethylcyanoacetate</td>
<td>99.99</td>
<td>99.67</td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td>Malononitrile</td>
<td>99.98</td>
<td>99.40</td>
</tr>
<tr>
<td>4-Bromobenzaldehyde</td>
<td>Malononitrile</td>
<td>99.89</td>
<td>99.79</td>
</tr>
<tr>
<td>Methyl-4-formyl benzoate</td>
<td>Malononitrile</td>
<td>100.0</td>
<td>98.84</td>
</tr>
<tr>
<td>3,5-Dimethoxybenzaldehyde</td>
<td>Malononitrile</td>
<td>99.87</td>
<td>99.17</td>
</tr>
<tr>
<td>4-Benzyloxybenzaldehyde</td>
<td>Malononitrile</td>
<td>100.0</td>
<td>99.73</td>
</tr>
</tbody>
</table>

C. Wiles *et al.*, *Tetrahedron*, 2004, **60**, 8421
Dithiane Synthesis

- Acid catalysed reactions
  - Acetal synthesis and deprotection
- 1 Equivalent of aldehyde and propanedithiol

- ca. 100 % conversion
- > 99 % purity
- But product still contaminated with residual thiol
- Can a scavenger be incorporated to remove this?
### Dithiane Synthesis with Scavenger

**Blue CuSO₄ turns yellow**

**Self-indicating scavenger**

<table>
<thead>
<tr>
<th>Aldehyde</th>
<th>Flow Rate/µl min⁻¹</th>
<th>Conversion/%</th>
<th>Yield/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-Bromobenzaldehyde</td>
<td>61.4</td>
<td>99.99 (0.005)ᵃ</td>
<td>99.92</td>
</tr>
<tr>
<td>4-Chlorobenzaldehyde</td>
<td>61.7</td>
<td>99.99 (0.003)</td>
<td>99.91</td>
</tr>
<tr>
<td>4-Cyanobenzaldehyde</td>
<td>65.4</td>
<td>99.99 (0.002)</td>
<td>99.94</td>
</tr>
<tr>
<td>4-Biphenylcarboxaldehyde</td>
<td>63.0</td>
<td>99.99 (0.001)</td>
<td>99.97</td>
</tr>
<tr>
<td>4-Methylbenzaldehyde</td>
<td>70.0</td>
<td>99.99 (0.001)</td>
<td>99.97</td>
</tr>
<tr>
<td>4-Benzyloxybenzaldehyde</td>
<td>61.1</td>
<td>99.99 (0.002)</td>
<td>99.22</td>
</tr>
<tr>
<td>2,4-Dihydrobenzaldehyde</td>
<td>58.9</td>
<td>99.99 (0.003)</td>
<td>99.90</td>
</tr>
<tr>
<td>Methyl-4-formyl benzoate</td>
<td>60.4</td>
<td>99.99 (0.008)</td>
<td>99.82</td>
</tr>
<tr>
<td>2-Furaldehyde</td>
<td>67.9</td>
<td>99.99 (0.001)</td>
<td>99.92</td>
</tr>
</tbody>
</table>

ᵃ Numbers in parentheses represent % RSD
Synthesis of Thioketals

- Kinetically less favourable than acetalisation

**Batch (24 hrs)**

**Flow Reactor**

**Flow Reactor Results:**
- % RSD = 0.004
- n = 6
- Flow rate = 40.4 µl min⁻¹
- Conversion = 99.94 %
# Thioketalisation Results

![Diagram: Thioketalisation Reaction](image)

<table>
<thead>
<tr>
<th>Ketone</th>
<th>Flow Rate/$\mu$l min$^{-1}$</th>
<th>Conversion/%</th>
<th>Yield/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetophenone</td>
<td>41.5</td>
<td>99.99 (0.004)$^a$</td>
<td>99.57</td>
</tr>
<tr>
<td>Cyclohexanone</td>
<td>42.2</td>
<td>99.99 (0.003)</td>
<td>99.62</td>
</tr>
<tr>
<td>Butyrophenone</td>
<td>41.6</td>
<td>99.99 (0.005)</td>
<td>99.90</td>
</tr>
<tr>
<td>4-Methylacetophenone</td>
<td>42.0</td>
<td>99.99 (0.001)</td>
<td>99.91</td>
</tr>
<tr>
<td>4-Nitroacetophenone</td>
<td>40.9</td>
<td>99.99 (0.004)</td>
<td>99.95</td>
</tr>
<tr>
<td>2-Methylcyclohexanone</td>
<td>41.6</td>
<td>99.99 (0.001)</td>
<td>99.96</td>
</tr>
<tr>
<td>Cyclopentanone</td>
<td>43.0</td>
<td>99.99 (0.001)</td>
<td>99.91</td>
</tr>
<tr>
<td>4-Aminoacetophenone</td>
<td>40.3</td>
<td>99.99 (0.001)</td>
<td>99.93</td>
</tr>
<tr>
<td>4-Hydroxyacetophenone</td>
<td>41.9</td>
<td>99.99 (0.0004)</td>
<td>99.90</td>
</tr>
</tbody>
</table>

$^a$Numbers in parentheses represent % RSD
Chemoselective Thioacetalisation

Question: What would happen if the compound to be protected contained both a ketone and an aldehyde?

- Chemoselective protection can be achieved by using reagents such as boron trifluoride etherate.

- We proposed that by simply optimising the residence time within the reactor, product selectivity could be achieved:
  - Remove product before second thiolation occurs.
Stirred Reactor vs. Flow reactor

<table>
<thead>
<tr>
<th>Reactor Type</th>
<th>Mono-</th>
<th>Di-</th>
<th>Un-reacted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Flow</td>
<td>✓</td>
<td>✗</td>
<td>✗</td>
</tr>
</tbody>
</table>

Using 1 eq. 1,3-propanedithiol
Two-Step Synthesis

- Spatially position different ‘incompatible’ reagents (e.g. acid and base)
- Model reaction:

**Step One:**

\[
\begin{align*}
\text{H}_3\text{CO} & \quad \text{OCH}_3 \\
\text{H} & \quad \text{H}^+ \\
\text{H} & \quad \text{H} + \text{MeOH}
\end{align*}
\]

**Step Two:**

\[
\begin{align*}
\text{H}^+ & \quad \text{N=C=NN} \\
\text{N=C=NN} & \quad \text{Base}
\end{align*}
\]
### Multi-Step Results: Ethyl cyanoacetate

![Reaction Scheme](image)

<table>
<thead>
<tr>
<th>Aldehyde</th>
<th>Flow Rate (µl min⁻¹)</th>
<th>Conversion (%)</th>
<th>Actual Yield (g)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzaldehyde</td>
<td>0.50</td>
<td>99.99</td>
<td>0.0150 g</td>
<td>99.4</td>
</tr>
<tr>
<td>4-Bromobenzaldehyde</td>
<td>0.80</td>
<td>99.99</td>
<td>0.0338 g</td>
<td>99.8</td>
</tr>
<tr>
<td>4-Chlorobenzaldehyde</td>
<td>0.65</td>
<td>99.99</td>
<td>0.0277 g</td>
<td>99.6</td>
</tr>
<tr>
<td>4-Cyanobenzaldehyde</td>
<td>0.84</td>
<td>99.99</td>
<td>0.0284 g</td>
<td>99.7</td>
</tr>
<tr>
<td>2-Naphthaldehyde</td>
<td>0.84</td>
<td>99.99</td>
<td>0.0298 g</td>
<td>99.8</td>
</tr>
<tr>
<td>Methyl-4-formyl benzoate</td>
<td>0.65</td>
<td>100.0</td>
<td>0.0253 g</td>
<td>99.7</td>
</tr>
<tr>
<td>4-Benzyloxybenzaldehyde</td>
<td>0.48</td>
<td>99.99</td>
<td>0.0219 g</td>
<td>99.1</td>
</tr>
<tr>
<td>Nitrothiophenecarboxaldehyde</td>
<td>0.75</td>
<td>99.99</td>
<td>0.0238 g</td>
<td>99.7</td>
</tr>
<tr>
<td>3,5-Dimethoxybenzaldehyde</td>
<td>0.55</td>
<td>99.99</td>
<td>0.0213 g</td>
<td>99.5</td>
</tr>
<tr>
<td>4-Methylbenzaldehyde</td>
<td>0.89</td>
<td>99.99</td>
<td>0.0284 g</td>
<td>99.3</td>
</tr>
</tbody>
</table>
Protection of Alcohols: Tetrahydropyranyl Ethers (THP)

How are they synthesised?

![Chemical structure](image)

**Reaction Conditions:**

- 1.2 to 2.0 eq. 3,4-dihydro-2H-pyran (DHP)
- Catalysed by:
  - $p$-Toluenesulfonic acid, pyridinium $p$-toluenesulfonate, zirconium tetrachloride, bromodimethylsulfonium bromide
- Stirred at room temperature:
  - Yields 55-97 %
- Purified by column chromatography:
  - To remove unreacted alcohol, acid catalyst and excess DHP
Batch Investigation

Reaction Conditions:

- 1.0 M Benzyl alcohol/3,4-dihydro-2H-pyran in MeCN
  - 5 x 10^{-3} mmol Acid catalyst (5 x 10^{-3} mol %)
- Stirred at room temperature
  - Analysed every 30 min

Disadvantages:

- Incomplete conversion after 24 hrs
  - Deprotection occurred

<table>
<thead>
<tr>
<th>Time (Hrs)</th>
<th>Conversion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02</td>
<td>52.7</td>
</tr>
<tr>
<td>0.5</td>
<td>58.7</td>
</tr>
<tr>
<td>1</td>
<td>54.0</td>
</tr>
<tr>
<td>2</td>
<td>42.4</td>
</tr>
<tr>
<td>3</td>
<td>1.4</td>
</tr>
</tbody>
</table>

58.7 % Conversion to THP Ether (30 min)
Synthesis of THP Ethers in a Flow Reactor

Reaction conditions:
- 1.0 M Benzyl alcohol and 3,4-dihydro-2H-pyran in MeCN
- 5 mg Silica-supported sulfonic acid
- 1.00 µl min⁻¹ 100% conversion
- Products analysed after 10 min runs

<table>
<thead>
<tr>
<th>Applied Field (V cm⁻¹)</th>
<th>333</th>
<th>250</th>
<th>167</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow Rate (µl min⁻¹)</td>
<td>4.50</td>
<td>2.40</td>
<td>1.00</td>
</tr>
<tr>
<td>Conversion (%)</td>
<td>58.0</td>
<td>62.9</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Cleavage of THP ethers

- Efficient removal of THP ethers
  - Acid catalysed alcoholysis or hydrolysis
- Equilibrium dependant

Traditional Approach:
- Bismuth triflate, A-15, p-TsOH, Iron sulfate
- Reaction times range 5 min to 24 hr

Flow Reaction Conditions:
- 1.0 M Benzyloxy-tetrahydropyran in MeOH
- 5 mg of Silica-supported sulfonic acid
- 1.00 μl min⁻¹ (300 V cm⁻¹)
- The ‘protection group’ can be removed in vacuo
Oxidations

- Selective oxidation of primary alcohols to aldehydes

*Flow reactor*

Reaction Conditions:

- Pressure-driven flow
- 0.01 M benzyl alcohol in DCM

Can the product selectivity be controlled by varying the residence time?
Flow Reactor Results

### Chromatogram Plot

**Batch**

**Fast Flow**

**Slow Flow**

<table>
<thead>
<tr>
<th>Alcohol</th>
<th>Flow Rate/µl min⁻¹</th>
<th>Aldehyde</th>
<th>Carboxylic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzyl alcohol</td>
<td>650</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Benzyl alcohol</td>
<td>≤50</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

**Note:** These flow rates are too fast for EOF pumping!!
More Examples: Selective Oxidations

<table>
<thead>
<tr>
<th>Alcohol</th>
<th>Flow Rate/µl min⁻¹</th>
<th>Aldehyde</th>
<th>Carboxylic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,5-Dimethoxybenzyl alcohol</td>
<td>650</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>3,5-Dimethoxybenzyl alcohol</td>
<td>50</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>4-Bromobenzyl alcohol</td>
<td>650</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>4-Bromobenzyl alcohol</td>
<td>50</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>4-Chlorobenzyl alcohol</td>
<td>650</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>4-Chlorobenzyl alcohol</td>
<td>50</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>4-Cyanobenzyl alcohol</td>
<td>650</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>4-Cyanobenzyl alcohol</td>
<td>50</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

- No Chromium residues are released into the environment
- No purification required
- The use of a co-oxidant enables the supported oxidant to be recycled
Conclusions

- Micro reactors allow the rapid optimisation of reactions
  - High-throughput synthesis
  - Combinatorial and library synthesis
- Immobilised reagents allow the synthesis of analytically pure compounds
- Micro reactors are suitable for a wide range of reactions
- Micro reactors generate products in:
  - Higher purity
  - Higher conversion
  - Higher selectivity
  - \textit{In situ} formation of reagents
  - \textit{In situ} purification of products

Research Workers and Collaborators

- Dr. Charlotte Wiles
- Dr. Nikzad Nikbin
- Dr. Ping He
- Dr. Victoria Ryabova
- Dr. Vinod George
- Dr. Leanne Marle
- LioniX
- Astra Zeneca
- Novartis
- Mairead Kelly
- Gareth Wild
- Tamsila Nayyar
- Julian Hooper
- Linda Woodcock
- Haider Al-Lawati
- Ben Wahab
- EPSRC
- Sanofi-Aventis